Calcium phosphate orthodontic adhesives: An *in vitro* investigation of bond strength and calcium ion release

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Abstract

Aims

The aim of this study was to compare the orthodontic bond strength of an experimental tri-calcium phosphate (TCP) composite manufactured in house with commercially available bonding agents.

Methods

Orthodontic brackets were bonded to 420 previously extracted premolar teeth using Transbond XT (a conventional composite), Fuji Ortho (a resin-modified glass ionomer), Aegis Ortho (an amorphous calcium phosphate (ACP) composite) and experimental composites containing 0%, 1%, 5% and 10% TCP. Bond strength and the mechanism of bond failure was recorded following wet and dry storage of the specimens. The calcium ion release of the 10% TCP composite was measured compared to Aegis Ortho using an ion-selective electrode.

Results

All adhesives showed reduction in bond strength following wet storage. Transbond XT produced the greatest bond strength following both dry (15.29 $\pm$ 4.30 MPa) and wet (12.30 $\pm$ 2.18 MPa) storage compared to the other adhesives ($P<0.05$). Fugi Otho LC, Aegis Ortho and experimental composite 1 (0% TCP) produced clinically acceptable bond strengths following dry (11.76 $\pm$ 3.16 MPa, 11.89 $\pm$ 3.08 MPa and 12.41 $\pm$ 3.24 MPa respectively) and wet storage (9.55 $\pm$ 4.35 MPa, 10.08 $\pm$ 4.09 MPa and 9.21 $\pm$ 2.44 MPa respectively),

which were not statistically different from each other. The experimental composites containing TCP (experimental composites 2 (1% TCP), 3 (5% TCP), and 4 (10% TCP)) produced statistically lower ($P< 0.05$) but clinically acceptable bond strengths following dry storage (8.84 ± 2.24 MPa, 8.37 ± 2.14 MPa and 8.07 ± 1.89 MPa respectively). However the bond strength of the TCP containing experimental composites (experimental composites 2 (1% TCP), 3 (5% TCP) and 4 (10% TCP)) were clinically unacceptable following aqueous storage (2.26 ± 0.57 MPa, 1.93 ± 0.46 MPa and 1.46 ± 0.54 MPa respectively) ($P<0.05$). All the adhesives showed a significant reduction in bond strength following aqueous storage compared to dry storage ($P<0.05$). Experimental composites (2,3 and 4) that contained TCP showed an increase in cohesive bond failures ($P<0.05$) following aqueous storage.

The 10% TCP composite (experimental composite 4) was found to release a significantly greater concentration of calcium ions ($89.2 \times 10^{-5} \pm 18.6 \times 10^{-5}$) M compared to Aegis Ortho ($11.9 \times 10^{-5} \pm 4.5 \times 10^{-5}$ M) ($P<0.05$) after a 6 week period.

Conclusions

All the orthodontic adhesives investigated suffered from hydrolytic degradation which affected their bond strength. The addition of a leachable calcium phosphate to composite reduced the mechanical integrity of the composite which lead to a reduction in bond strength and an increase in cohesive bond failures.

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Abbreviations used

ACP - Amorphous calcium phosphate

ARI - Adhesive Remnant Index

Bis-EMA - Ethoxylated bisphenol A glycol dimethacrylate

Bis-GMA - Bisphenol A glycol dimethacrylate

CaP 0 - Experimental composite with 0% TCP

CaP 1 -Experimental composite with 1% TCP

CaP 5 - Experimental composite with 5% TCP

CaP 10 - Experimental composite with 10% TCP

HEMA - Hydroxyethylmethacrylate

RMGIC - Resin-modified glass-ionomer cement

TCP - Tri-calcium phosphate

TEGDMA - Triethylene glycol dimethacrylate

UDMA - Urethane dimethacrylate

Chapter One

Literature review and aims of study

Chapter one: Literature review and aims of study

1.1 Introduction

Contemporary fixed orthodontic appliance treatment relies on the ability to bond orthodontic brackets to surface tooth enamel.

Buonocore (1955) showed that application of phosphoric acid to tooth enamel would produce an etched enamel surface. The etched enamel surface allows micromechanical retention of the most commonly used adhesives in orthodontics which are composite resins. Other adhesives that have been used to bond orthodontic brackets to teeth include glass ionomer cements (GIC), resin-modified glass-ionomer cements (RMGIC) and compomers.

Factors affecting the choice of adhesive used include the ease of use of the adhesive, the bond strength of the adhesive and any additional beneficial effect that the adhesive can provide. Adhesives that require no mixing and set on command are more preferable for clinical reasons and composites adhesives are currently able to provide these features.

The ideal bond strength of orthodontic adhesives should be less than the tensile bond strength of enamel which was reported to be approximately 14.5 MPa by Bowen and Rodriguez (Bowen and Rodriguez, 1962), this is to reduce the risk of enamel fracture on bracket removal. However, the bond strength needs to be great enough so that the brackets

can withstand daily occlusal forces and remain attached to the teeth throughout the course of orthodontic treatment, as frequent debonding of brackets will delay treatment.

There are risks associated with fixed orthodontic appliance treatment and in particular to the enamel surface of the teeth around the orthodontic brackets. The enamel can undergo demineralisation. Clinically, this results in visible white or brown patches of enamel around the bracket.

The risk of demineralisation damage has been addressed by the application of dentrifices containing enamel remineralising agents and fluoride to varying levels of success (Marinho *et al*, 2005b, 2007). However the patients at most risk of demineralisation and the development of white spot lesions are those who are the least compliant with oral hygiene instructions, diet instructions and the use of these beneficial dentrifices. So although these patients would benefit the most from the use of these dentrifices they are also the least likely to do so (Zachrisson and Zachrisson, 1971b). In order to overcome this issue of compliance, demineralising agents and fluoride have been incorporated into orthodontic adhesives to provide a reservoir of remineralising ions (calcium and phosphate) and fluoride which would reduce the degree of demineralisation resulting from orthodontic treatment.

Studies have shown that the highest bond strength of orthodontic adhesives is achieved with composite adhesives (Rock and Abdullah, 1997; Bishara *et al*, 1999; Bishara *et al* 2001; Bishara *et al,* 2002; Littlewood *et al,* 2000; Grandhi *et al,* 2001; Summers *et al,*

2004; Dunn *et al,* 2007; Foster *et al,* 2008; Uysal *et al,* 2010). However conventional resin composites are not capable of leaching any ions that could promote remineralisation. On the the other hand, glass ionomer cements and resin-modified glass ionomer cements are capable of leaching fluoride but have been shown to produce an inferior bond strength in comparison to composite adhesives (Bishara *et al*, 1999; Valente *et al,* 2002; Millett *et al,* 2003). Composite adhesives containing leachable remineralising ions have been developed. Studies on these materials have focused on the bond strength of these materials (Dunn *et al,* 2007; Foster *et al* 2008; Uysal *et al,* 2010) and there is a lack of studies that quantify the remineralising or beneficial potential of these modified composites.

1.2 Demineralisation

A major risk of fixed appliance orthodontic treatment is that of demineralisation of the tooth enamel around the bonded orthodontic brackets. Demineralisation is due to attack of the enamel surface from the acidic by-products of plaque metabolism. For demineralisation to occur, four elements are required (Kidd and Smith, 1991):

1. Plaque - streptoccous mutan counts have been shown to be higher in patients undergoing fixed appliance orthodontic treatment and a positive correlation shown between oral hygiene and caries incidence in orthodontic patients (Zachrisson and Zachrisson, 1971b).

2. Substrate - this depends on the diet, the greater the frequency of cariogenic substrate in the diet, the higher the risk of decalcification.

3. Susceptible tooth surface - this will depend on patient variability.

4. Time - the longer the acidic by-products of plaque metabolism are in contact with the tooth surface the greater the decalcification that will occur.

Figure 1.1 Equilibrium between hydroxyapatite formation and dissolution

$$Ca_{10}(PO4)_6(OH)_2(s) + 8H^+(aq) \longrightarrow 10Ca^{2+}(aq) + 6HPO_4^{2-}(aq) + 2H_2O(l)$$

Evidence shows that caries is a dynamic process of events involving both demineralisation and remineralisation (Figure 1.1). Since caries requires 4 processes described above to be occurring there are 4 ways of preventing it. When sufficient acid is produced by plaque bacteria to reduce the pH of the tooth environment by 5.5 (Cury and Tenuta, 2008), the acid dissolves the carbonated hydroxyapatite which is the main component of the tooth enamel. This demineralisation of enamel causes porosity of the enamel to occur and enlarge with further demineralisation. This porosity leads to the appearance of enamel white spots.

Saliva is a remineralising solution and the process of remineralisation is where the dissolved minerals are returned to the tooth surface. Cavities result when the rate of demineralisation exceeds the rate of remineralisation and the latticework is destroyed.

Studies have shown that the incidence of decalcification following fixed appliance orthodontic treatment is high. Gorelick *et al* (1992), found 50% of patients had at least one white spot during orthododontic treatment. 23% of patients had these white white spots on the maxillary incisors and 6.6% of patients had cavitation present. Al Maaitah *et al* (2011),

found the incidence was higher with almost 72% of post treatment orthodontic patients having at least one white spot lesion present.

A shift in the distribution of caries compared to the general population has been shown for patients undergoing orthodontic treatment (Zachrisson and Zachrisson, 1971a). This shift is from the posterior teeth to the anterior teeth and from interproximal surfaces to the smooth surfaces of the teeth. A positive correlation has also been demonstrated to exist between poor oral hygiene and the incidence of caries in fixed orthodontic appliance patients (Zachrisson and Zachrisson, 1971b).

Typical courses of fixed orthodontic appliance treatment last between 18 months to 24 months and the incidence of decalcification is often related to the length of treatment (Gorelick *et al*, 1982).

1.3 Preventive measures and treatment for demineralisation

Decalcification of tooth enamel results in white spot lesions which are unsightly and can be the basis of patient dissatisfaction with treatment. The lesions can resolve to a certain extent spontaneously following treatment.

Currently the recommended treatment for decalcification is good oral hygiene for a period of 2 to 3 months without fluoride supplementation. Removal of plaque after fixed appliance treatment results in arrest of further demineralisation and gradual regression of the lesion (Årtun and Thylstrup, 1989). The reason for not using fluoride supplementation is that remineralisation of the surface enamel lesions is different to that of the subsurface lesions (Ogaard *et al*, 1988). The surface lesions remineralise quicker and more completely than the subsurface lesions, this is likely due to lesion arrest by fluoride use. This arrest of the surface lesion prevents complete repair from occurring and limits remineralisation to the surface but does not reduce the appearance of the white spots (Årtun and Thylstrup, 1989).

Fluoride therapy is a method used to promote remineralisation. Fluoride does not prevent caries but it does control the rate of caries progression. When fluoride ions and dissolved hydroxyapatite are present in plaque fluid at a pH above 4.5 the fluoride replaces the hydroxide in the hydroxyapatite to form fluoroapatite. The fluoroapatite surface of the enamel is more acid-resistant than the original hydroxyapatite and it is formed faster than ordinary remineralised enamel would be.

Fluoride also plays an important role in enhancing the chemical reactions that lead to precipitation of calcium phosphate. In the oral cavity, an equilibrium exists between calcium phosphate in the enamel and calcium and phosphate ions in the saliva. Fluoride shifts this equilibrium to favour the formation of calcium phosphate in the enamel.

The process of remineralisation is dependent on calcium phosphate penetrating into the enamel and crystallising. This crystallisation of calcium phosphate fills the porous spaces that were produced by demineralisation. Remineralisation occurs on the remaining mineral if calcium and phosphate are present in the fluid phase surrounding the enamel crystals.

The normal vehicle for delivery of calcium and phosphate ions is saliva. Saliva contains enough calcium and phosphate ions to keep the oral environment saturated, but it does not cause extra remineralisation to occur as there are inhibitors present in saliva to prevent unwanted precipitation. It is not the concentration of calcium and phosphate that changes the most in saliva but the pH. When the enamel pH is allowed to drop below 5.5, a point of undersaturation is reached and demineralisation occurs. When the pH rises above this level, saturation or supersaturation is reached and so some remineralisation occurs (Lata *et al,* 2010; Madan *et al,* 2011).

1.3.1 Fluoride

Fluoride is available in many forms and most forms have been found to be effective in reducing enamel demineralisation. These forms include:

1. Toothpastes - These have been shown to be effective for reducing enamel demineralisation (Marinho *et al,* 2005a).

2. Rinses - These have also been shown to be effective. However, they rely on patient compliance (Marinho *et al,* 2005b). A Cochrane review in 2008 concluded that daily rinsing with a fluoride mouth rinse reduces the severity of enamel demineralisation surrounding a fixed orthodontic appliance (Benson *et al,* 2008).

3. Varnishes - These are also effective however due to the practicalities in their application they have not become routinely used (Marinho *et al,* 2005b).

4. Cements - Fluoride in glass-ionomer cements and compomers may be effective in reducing enamel demineralisation but the evidence for this is weak. Fluoride has not successfully been added to composites in a manner that would be effective for reducing demineralisation. Compomers have a lower fluoride release profile compared to resin modified glass-ionomer cements which in turn have a lower fluoride release profile compared to glass-ionomer cements. The addition of fluoride containing compounds to composite have provided an initial release of fluoride which then rapidly falls away. The

initial release of fluoride is due to the presence of the fluoride releasing compound near the surface of the compomer. As this source gets depleted, the release of fluoride rapidly falls as the fluoride compound within the resin cannot diffuse through the matrix at sufficient speed to maintain release of fluoride. Water sorption of compomers allows an acid-base reaction between the glass and the the polycarboxyl groups on the modified resin. To aid the diffusion of water into the material and fluoride ions out of the matrix, some of the matrix resins used have been more hydrophilic than those normally used (e.g glycerol dimethacrylate). Compomers however do not show the ability to reabsorb fluoride form the oral environment as glass-ionomers cements can (Van Noort, 2007).

5. Fluoride releasing modules - There is limited evidence of their effectiveness and the addition of fluoride to modules adversely affects their physical properties (Banks *et al,* 2000).

6. Sustained release devices - These devices are attached to molar tubes and the fluoride is released at a slow rate over several months, however there is limited evidence they are effective (Curzon and Toumba, 2004; Marini *et al*, 1999).

1.3.2 Calcium Phosphate

Calcium phosphate products have been used for the treatment of enamel demineralisation. The aim of these products are to promote remineralisation of the tooth enamel by replacing the calcium phosphate ions lost during demineralisation.

Most products containing calcium phosphate act as salivary enhancers. They provide more calcium phosphate than would normally come from saliva. These products work best in patients with poor salivary flow who do not maintain the right level of calcium phosphate. However, in patients with normal salivary flow, a system is needed that delivers calcium phosphate to the site it is needed and then remains there long enough to be used when the conditions are favourable. As the process of demineralisation and remineralisation is dynamic and one event occurs over the other when the equilibrium is balanced in favour of it, additional calcium phosphate is not needed when remineralisation is occurring as there is a saturated or supersaturated saliva environment present. Additional calcium phosphate is needed most when demineralisation is occurring and an undersaturated calcium phosphate saliva environment exists. The additional calcium phosphate can then act as a common ion and stop or inhibit part of the demineralisation that occurs when the pH drops below 5.8. Therefore, calcium phosphate products do not only remineralise, they also inhibit demineralisation.

Calcium phosphate comes in a variety of products including dentrifices, hypersensitivity products, varnishes, chewing gum, sealants and prophy pastes.

Different types of calcium phosphate technologies also exist, these include amorphous calcium phosphate (ACP), Tri-calcium phosphate (TCP), calcium sodium phosphosilicate (CSP) and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) (Table 1.1).

calcium phosphate	mechanism of action
Amorphous calcium phosphate (ACP)	Releases calcium and phosphate ions to convert to apatite and remineralise when it comes into contact with saliva.
Tri-calcium phosphate (TCP)	Releases calcium and phosphate ions to convert to apatite and remineralise when it comes into contact with saliva.
Calcium sodium phosphosilicate (CSP)	Reacts with saliva, releasing calcium, phosphate and sodium into the oral environment. The sodium buffers the acid and then the calcium and phosphate ions saturate saliva precipating into demineralised areas to form a new layer of hydroxyapatite filling the demineralised lesions.
Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP)	CPP is an organic molecule that is able to bind calcium and phosphate ions and stabilise ACP. The calcium and phosphate contained in the milk-derived peptide bind to the tooth surface. ACP is released during acidic challenges.

Table 1.1 Forms of calcium phosphate technology available.

Further treatment for unresolved white spots is microabrasion of the enamel surface with acid and pumice. This is the optimal way to remove superficial enamel opacities with minimal enamel loss (Welbury and Carter, 1993).

The effectiveness of calcium phosphate technologies to treat enamel demineralisation has been demonstrated. Calcium phosphate can be incorporated into different products for application.

1.3.2.1 Dentrifices

There are commercially available dentrifices containing calcium phosphate aimed at reducing demineralisation. These are:

Recaldent - A casein phosphopeptide stabilised amorphous calcium phosphate (CPP-ACP) that has been shown to inhibit demineralisation of enamel *in vitro* (Reynolds *et al,* 1995) and *in vivo* (Reynolds, 1987; Reynolds, 1998; lijuna *et al*, 2004; Morgan *et al*, 2008; Shen *et al*, 2011).

Enamelon - An unstabilized amorphous calcium phosphate (ACP) that has been shown to inhibit demineralisation of enamel *in vitro* (Schemehom *et al,* 1999b; Hicks and Flaitz, 2000) and *in vivo* (Schemehom *et al,* 1999a; Papas *et al,* 1999).

Novamin - A bioactive glass containing calcium sodium phosphosilicate (CSP) that is commercially available as a dentrifice.

Clinpro 5000 and vanish (3M) - Tri-calcium phosphate (TCP) containing dentrifices that have been shown to reduce demineralisation and promote remineralisation *in vitro* (Hogan *et al,* 2010; Karlinsey *et al,* 2010)

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) solutions have also been shown to promote enamel remineralisation effectively (Reynolds, 1997; Aimutis,

2004) and inhibit demineralisation (Nasab *et al,* 2007). Walker *et al* (2006), found that although milk contains casein phosphate, addition of CPP-ACP to milk, resulted in enhanced remineralisation. A dose of 5 grams of CPP-ACP produced 148% more remineralisation compared to 2 grams of CPP-ACP per litre of milk.

1.3.3 Composites containing remineralising ions

Although dentrifices have been shown to provide a beneficial effect, they are reliant upon patient compliance to use them. The patients who are at greatest risk of decalcification are those who are least compliant with oral hygiene instructions given. This group of patients are also more likely to be less compliant in the use of dentrifices. Geiger *et al* (1992), showed that only 13% of patients comply with using topical fluoride. A solution to reduce patient reliance on use of dentrifices is to incorporate calcium phosphate into the adhesive used for bracket attachment. Composite adhesives have been modified to incorporate calcium phosphate remineralising ions, however there is a lack of evidence to quantify the level of ions that these materials are capable of leaching.

1.3.3.1 ACP

Amorphous calcium phosphate (ACP) has been shown to be capable of reducing enamel decalcification. Skrtic *et al* (1996), showed that artificially produced caries like lesions in bovine teeth coated with ACP-filled composites recovered 71% of their lost mineral content. When the pH of the environment drops below 5.8, calcium and phosphate ions were released from the ACP material (Antonucci and Skrtic, 2010). Uysal *et al*. (2010), conducted an *in vitro* study to compare the microhardness of the enamel of human premolar subjected to cycles of demineralisation. They found that the microhardness of the enamel around the brackets bonded with ACP containing composite was almost twice as hard as the brackets bonded with conventional composite. A similar *in vitro* investigation which assessed the level of remineralisation by laser fluorescence also concluded that an ACP containing composite was capable of remineralising enamel (Usyal *et al,* 2009).

1.3.3.2 TCP

TCP has also been shown to be capable of reducing enamel decalcification. Karlinsey *et al* (2010b), demonstrated that a application of a TCP material produced greater microhardness of enamel when subjected to cycles of demineralisation and therefore greater remineralisation of the surface.

1.3.4 Bond strength of calcium phosphate composites

Studies have shown that the bond strength of conventional composite to be superior to glass-ionomer cement, resin-modified glass-ionomer cement, compomers and to composites that have been modified to incorporate remineralising ions (Rock and Abdullah, 1997; Bishara *et al,* 1999; Bishara *et al,* 2001; Bishara *et al,* 2002; Littlewood *et al,* 2000; Grandhi *et al,* 2001; Summers *et al,* 2004; Dunn, 2007; Foster *et al,* 2008; Uysal *et al,* 2010).

ACP composite bond strength

An orthodontic composite containing amorphous calcium phosphate (ACP) is commercially available as Aegis Ortho (Bosworth Co., Skokie, Illinois). Aegis Ortho (ACP containing composite) has shown a reduced bond strength in comparison to conventional composite adhesives (Dunn *et al,* 2007; Foster *et al,* 2008; Uysal *et al,* 2010).

Foster *et al* (2008), conducted an *in vitro* investigation to compare the bond strength of Aegis Ortho (ACP) composite against a conventional composite (Transbond XT) and a resin-modified glass ionomer cement (Fugi Ortho LC). The study showed that the ACP composite (Aegis Ortho) produced an inferior bond strength compared to the conventional composite (Transbond XT) but produced a bond strength that was comparable to the resin-

modified glass ionomer cement (Fugi Ortho LC). The conclusion of the study was that the ACP composite produced a clinically satisfactory bond strength of 8.3 +/- 2.8 MPa.

Dunn (2007) conducted an *in vitro* investigation comparing the bond strength of ACP composite (Aegis Ortho) against a conventional composite (Transbond XT). This study showed that brackets bonded with the ACP composite (Aegis Ortho) failed at significantly lower forces compared to brackets bonded with conventional composite. The bond strength of the ACP composite (Aegis Ortho) produced was clinically unacceptable at 14.2 +/- 3.1 N (approximately 1.2 MPa).

Uysal *et al* (2010), also conducted an *in vitro* investigation comparing the bond strength of ACP composite (Aegis Ortho) against a conventional composite (Transbond XT) and found that the ACP composite (Aegis Ortho) produced significantly lower bond strengths compared to the conventional composite (Usyal *et al,*2010b). The bond strength of the ACP composite (Aegis Ortho) produced was clinically acceptable at 24.2 +/- 5.4 MPa. This study was conducted using ceramic brackets which produced higher bond strengths.

Composites modified to contain leachable calcium and phosphate exhibit inferior mechanical properties, durability and water sorption characteristics compared to commonly used conventional composites (O'Donnell *et al*, 2006). Uncontrolled aggregation of calcium phosphate particulates and poor interfacial interaction play key roles in adversely affecting the mechanical properties of ACP composites (Schumacher *et al,* 2007). The clinical use of leachable ACP composites may be compromised by the

mechanical integrity of the material. The relatively poor filler and matrix interfacial adhesion and excessive water sorption that occurs in both resin and filler phases of these composites leads to poorer mechanical integrity of these materials compared to conventional composites (Reynolds, 2008; Skrtic *et al,* 2000).

A solution to the problem of the ACP composites losing their mechanical integrity due to utilising the ACP is to hybridise the ACP to various components in the composite. Hybridisation of the calcium phosphate with standard filler components such as silicate and zirconia results in a decrease in internal hydroxyapatite formation and therefore improved mechanical integrity of the composite (Skrtic *et al,* 2003). However, this hybridisation of calcium phosphate to improve the mechanical integrity of the composite also reduces the ability of the material to leach calcium phosphate.

In order to develop a composite adhesive that would be capable of leaching sufficient calcium phosphate and also maintaining mechanical integrity, composites containing tri-calcium phosphate (TCP) were developed for this investigation. TCP is relatively more stable and does not precipitate to form hydroxyapatite as readily as ACP in an aqueous environment.

1.4 Remineralisation measurements

Studies reporting on the remineralisation or demineralisation inhibition effect of calcium phosphates and fluorides on enamel have measured the effect in several ways:

1. Microhardness Testing - This method uses a hardness testing machine to make an indentation on the surface of enamel. The indentation is made at a known force and speed. The hardness of the enamel can then be determined by examination of the indented surface. Demineralised enamel will show lower hardness values.

2. Quantitative Light-Induced Fluorescence - This method uses fluorescent light to illuminate enamel. The fluorescence of the enamel is related to the mineral content of the enamel. Computer software can be used to quantify the fluorescence of the enamel and determine the mineral content.

An additional indirect method to determine the potential effect of remineralising agents is to measure the concentration of calcium and phosphate or fluoride leached by the material of interest. In the case of calcium phosphate, studies have used ion-selective calcium electrodes to measure the concentration of calcium leached from the calcium phosphate materials. This is an *in vitro* method of measurement of the ability of a material to leach calcium and phosphate.

1.5 Enamel

1.5.1 Formation and composition of enamel

Enamel consists of 96% inorganic mineral, 1% organic material and 3% organic water by percentage weight. By volume it is composed of 88% inorganic mineral, 2% organic material and 12% water. It is the most highly mineralised tissue in the body. The inorganic component is a crystalline calcium phosphate hydroxyapatite. This is also found in bone, calcified cartilage, dentine and cementum.

Enamel formation involves two phases, an initial secretory phase where partially mineralised enamel is formed and a later maturation phase where influx of additional mineral occurs with the removal of organic material and water. Amelogenesis (the formation of enamel) begins early in the crown stage of tooth development. It is formed when cells of the internal dental epithelium differentiate into ameloblasts. The trigger for this is when the cells of the internal dental epithelium induce the adjacent dental papillal cells to differentiate into odontoblasts and produce dentine. The formation of dentine then initiates the further differentiation of the cells of the internal enamel epithelium into ameloblasts.

The secretory phase

In the secretory phase the ameloblasts are polarised columnar cells. Enamel proteins are released into the surrounding area to form the enamel matrix. The matrix consists of the enamel proteins and an array of enzymes. The matrix is partially mineralised by the enzyme alkaline phosphatase.

After this first layer has been formed the ameloblasts move away form the dentine allowing for the development of Tomes' processes at the apical pole of the cell. Enamel formation continues around adjoining ameloblasts resulting in a walled area, or pit, that carry the Tomes' process and also around the end of each Tomes' process, resulting in a deposition of enamel matrix inside each pit. The enamel matrix that is within the pit, eventually becomes enamel rods and the walls between the Tomes' processes eventually become interrod enamel. The two are only distinguishable by the orientation of the calcium phosphate crystals. The interrod enamel is formed slightly in advance of the rod enamel.

The maturation phase

In the maturation phase, the ameloblasts undergo significant structural change in preparation of the maturation phase. The ameloblasts transport substances used in the formation of enamel. The ameloblasts become involved in a cyclical process where water and organic material are selectively removed and inorganic material is introduced in alternate bursts of activity. Prior to tooth eruption the ameloblasts are broken down, therefore enamel cannot regenerate or repair after it had been formed.

1.5.2 Structure of enamel

The basic unit of enamel is the enamel rod. Enamel rods are 4-8 micrometers in diameter and 2-2.5mm in length. They are tightly packed masses of hydroxyapatite crystals in an organised pattern. The hydroxyapatite crystals are needle shaped structures that are hexagonal in cross section. The hydroxyapatite crystals along the central axis of the rods run mostly parallel to the longitudinal axis of the rod and the crystals more distant form the central axis flare laterally. The hydroxyapatite crystals in the interrod region are orientated in a different direction to those in the rods. In cross-section the rods are the shape of a keyhole, with the head orientated towards the crown of the tooth and the tail orientated towards the root of the tooth. The rods span from the enamel surface to the enamel-dentine junction (Sharaway and Yeager, 1990).

At the enamel surface the rod structure is irregular or absent as the Tomes' processes are lost as amelogenesis comes to an end. A distinct prismless layer of enamel is commonly found in the outermost 30 micrometers of all primary teeth and in the gingival third of the enamel of all permanent teeth. The crystals in these regions are perpendicular to the surface of the enamel (Band and Lobjojie, 1966; Gwinnett, 1966; Crabb, 1964; Ripa *et al,* 1966). It has been reported that an increased etching time is required for bonding further posteriorly in the mouth (Marshall *et al,* 1975), which may be due to an increase in the thickness of the prismless layer (Whittaker, 1982) or due to surface enamel being more highly mineralised than the subsurface enamel.

1.5.3 Physical properties of enamel

Enamel is extremely hard and it has a hardness that is comparable to mild steel. The high mineral content of enamel is what makes it so hard, but this also makes enamel brittle. The integrity of enamel is maintained by the underlying layer of dentine. The thickness of enamel varies from a maximum of 2.5 mm over the occlusal surfaces to an knife-edge at the cervical margin. The thickness of the enamel has an impact on the colour as the underlying dentine colour is seen more in thinner sections of enamel.

1.5.4 Surface enamel

The surface enamel is harder and less porous than the subsurface enamel. The electron microscope has shown that the surface structure of enamel changes over time with age. In unerupted teeth a structureless layer 0.5 to 1.5 micrometers thick is present. Below this, lies a layer of loosely packed crystallites 5 nanometers thick, with undermineralised material between these layers. In and among the fine crystallites are randomly distributed large platelike crystals. The fine crystallite layer merges into the subsurface enamel layer, where the crystals are tightly packed. In erupted teeth, the structureless surface layer of enamel is lost due to attrition, abrasion and erosion. It has been proposed that the presence of prismless enamel may limit the development of an etch pattern, resulting in a weaker bond strength due to reduction of resin tag penetration (Gwinnett, 1973; Whittaker, 1982).

An organic deposit forms on the enamel surface of erupted teeth, this is called the salivary pellicle. Plaque bacteria accumulate on this pellicle layer.

Enamel becomes less permeable with age as the pores diminish as the crystals aquire more ions and grow. Younger enamel acts like a semipermeable barrier and allows the passage of water and small molecule substances through the pores between the crystals. The reduction in permeability with age leads to a reduction in the water content of enamel over an increasing time.

1.5.5. Enamel surface preparation

1.5.5.1 Prophylaxis of enamel surface

Pumice prophylaxis has been recommended prior to enamel etching since direct bonding of orthodontic brackets first became popular (Gwinnett, 1988). The purpose of prophylaxis is to remove the pellicle layer on the enamel surface. Salivary proteins adhere to the surface of enamel and form the pellicle layer which is approximately 1-10 micrometers thick. Buonocore (1955) included an initial step to remove the pellicle layer prior to acid etching the enamel. Miura *et al* (1973), first presented evidence to support the necessity of prophylactic cleaning for improved bond strength. Further scanning electron microscopy studies have shown that pumice prophylaxis prior to etching removes organic material from the enamel surface (Hosoya Y and Goto G, 1990) .

However, studies have also been conducted which have shown that omitting the pumice prophylaxis phase prior to etching does not affect the bond strength of adhesives. Main *et al* (1983) found that etching alone was adequate for removing the acquired pellicle layer from enamel and did not reduce the bond strength of adhesion to enamel. Donnan and Ball (1988) found no difference in retention rates of pit and fissure sealants where pumice prophylaxis was performed and when this step was omitted. Lindauer *et al* (1997) found that omitting the pumice prophylaxis step did not affect the bond strength of brackets in an *in vitro* study.

Numerous pastes have been used for prophylaxis of the enamel surface including pumice, silica, and zirconium silicate. The ideal paste should be capable of removing the organic pellicle layer form the enamel without being abrasive to the enamel surface. As all pastes used for prophylaxis are however harder than the surface of enamel, some enamel loss is inevitable. This can be reduced by the use of a rubber cup as apposed to a brush for the prophylaxis procedure (Thompson and Way, 1981). A preparation of water and pumice flour has been recommended as it avoids introducing any contaminants that may be present in commercial pastes (Gwinnett, 1981).

1.5.5.2 Acid-etching of enamel

The standard protocol for successful bonding to the enamel surface has been to acid etch the surface since 1955 when Buonocore used phosphoric acid as an enamel conditioner and discovered that this increased the retention of acrylic resin restorations (Buonocore, 1955). Acid etching dissolves the hydroxyapatite crystals and allows for the penetration of resin tags into the etched enamel surface to provide micromechanical retention.

Acid etching selectively erodes certain hydroxyapatite formations and produces different patterns of etch (Poole and Johnson, 1967, Silverstone *et al,* 1975; Brannstrom *et al,*1978; Galil and Wright, 1979). It has been reported that the ideal etch pattern occupies less than 5% of the etched enamel surface and there are differences between tooth types (Mattick and Hobson, 1997).

Scanning electron microscopes have shown that three etching patterns predominate, these are:

Type 1 - This is the most common pattern and is characterised by the preferential removal of the rod core. This produces a honeycomb pattern.

Type 2- The rod periphery is preferentially removed and the core remains intact. This produces a cobblestone pattern.

Type 3- This patten occurs less frequently and is irregular and indiscriminate.

Hobson and McCabe in a clinical study found that the etch pattern was not essential in order to produce a strong bond (Hobson and McCabe, 2002).

1.5.5.3 Etchant and etch duration

Buonocore first used 85% phosphoric acid for 30 seconds (Buonocore, 1955). Since then he reduced the concentration to 50% (Buonocore, 1970). Other etchants that have been used and include hydrochloric acid, ethylene diamine tetra-acetic acid (EDTA), pyruvic acid, nitric acid, citric acid and polyacrylic acid. The most commonly used enamel etchant for bonding composites is phosphoric acid. Currently the most consistent and suitable etch patterns are achieved with 30% to 40% phosphoric acid. A concentration of phosphoric acid above 27% precipitates monocalcium phosphate which is readily soluble and easily washed away. Concentrations of phosphoric acid below 27% precipitates dicalcium phosphate dihydrate which is less soluble. Deposits of dicalcium phosphate dihydrate remaining on the enamel surface can affect the bond strength of composite adhesives.

An etch time of 15 seconds with 37% phosphoric acid has been recommended for anterior teeth and premolars (Kinch *et al,* 1998). An etch time of 30 seconds has been shown to significantly increase the bond strength for molars (Johnson *et al,* 1998). Prolonged etching over 90 seconds destroys the etch pattern and results in reduced bond strength. This is because prolonged exposure to the etch results in a smooth enamel surface as the whole surface is eroded, this then reduces the ability of the resin tags to penetrate the enamel (Wang, 1991). Gardner and Hobson (2001), recommend the use of 37% phosphoric acid and a 30 second etch time for routine orthodontic bonding.

Phosphoric acid etch is available in liquid or gel form. The liquid form is more easily washed away, but it is more difficult to precisely control placement. The use of liquid or gel etch has been shown not to affect the bond strength (Maijer, 1982; Mixson *et al,* 1988).

The method of etch application affects the etch pattern produced. It has been shown that rubbing (Bates *et al,* 1982) obscures the prism boundaries and stroking can lead to better etch patterns (Oliver, 1988).

1.5.5.4 Enamel conditioning

Enamel conditioning with polyacrlic acid has been proposed for use to reduce the enamel loss that occurs from the use of phosphoric acid. Polyacrylic acid produces some etching of the enamel surface and forms calcium sulphate dihydrate crystals which bond to the enamel surface. Polyacrylic acid can produce adequate bond strength albeit 30% lower than that achieved with phosphoric acid. Polyacrylic acid is recommended for enamel conditioning prior to the use of glass-ionomer cements. It has been shown to increase the bond strength of glass-ionomer cements used for bracket bonding (Bishara *et al,* 2000).

1.5.5.5 Washing and drying enamel

The etchant must be washed off the enamel surface prior to bonding. The council on Dental Material Instruments and Equipment (Gwinnett, 1982) recommend 10 to 15 seconds of rinsing with copious amounts of water per quadrant of the mouth and if an acid gel is used than washing for twice this length of time (Rock *et al,* 1990). After the etch has been washed off, the enamel must be thoroughly dried for the bonding of resin adhesives. For the bonding of glass ionomer cements, it is important that the enamel is not thoroughly dried but remains damp.

1.6 Bonding Systems

Prior to the use of diacrylate resin bonding agents, low viscosity primers are placed on the enamel surface. Primers are placed to ensure good wetting of the etched enamel surface prior to the use of higher viscosity filled diacrylate bonding agents. Primers contain bifunctional molecules, or coupling agents. One end of these bifunctional molecules is hydrophilic and this allows it to have good penetration into the tooth surface, the other end is hydrophobic and allows it to polymerise to the composite resin.

Several generations of bonding systems have been developed:

First generation - These systems contained N-phenylglycine and glycidyl methacrylate (NPG-GMA as the bifunctional molecule (Bowen, 1965)). The bond strengths of these systems were only 1-3 MPa.

Second generation - These bonding systems were introduced in the late 1970's and incorporated halophosphorous esters of unfilled resins such as bisphenol-A glycidyl methacrylate (Bis-GMA), or hydroxyethyl methacrylate (HEMA). They improved the bond strength to dentine.

Third generation - This generation of bonding system introduced etching of the dentine to remove or modify the smear layer. Then a primer containing hydrophilic resin monomers, which include hydroxyethyl trimellitate anhydride (4-META), hydroxyethyl methacrylate

(HEMA) and biphenyl dimethacrylate(BPDM) is applied. Hydrophilic groups in the primer infiltrate the smear layer, modifying it and promoting the adhesion to dentine. The hydrophobic groups of the primer create adhesion to the resin by polymerisation. This generation of bonding system usually use a hydrophilic dentine resin primer. After primer application, an unfilled resin is placed on the enamel and dentine surface.

Fourth generation - One of the main characteristics of fourth-generation bonding systems is the use of the total etch technique (Kanca, 1991; Gwinnett, 1993). This technique allows for the etching of enamel and dentine at the same time using phosphoric acid for 15-20 seconds. The surface must be left moist order to prevent the collapse of the collagen network in dentine. The hydrophilic primer can penetrate the exposed collagen network to form a hybrid layer in dentine.

Fifth generation- This generation of bonding system aimed to reduce the number of bonding steps and therefore improve clinical efficiency. There are two different types of bonding systems.

1. Self-etch primer (SEP) bonding systems - These combine the etching and priming phases into one. Self-etching primers are comprised of aqueous solutions of methacrylated phosphoric esters. Prophylactic pumicing of the enamel surface prior to the application of self-etch primers (SEP) improves the bond strength (Burgess et al 2006). The phosphate group on the methacrylated phosphoric acid ester dissolves the calcium and removes it from the hydroxyapatite. This calcium then forms a complex

with phosphate group of the acid and is incorporated into the methacrylate resin network when the primer polymerises, this neutralises the acid. This way, enamel etching and resin penetration of the primer into the etched enamel occurs in one step. The SEP solution should be agitated on the enamel surface for approximately 3 to 5 seconds to move the calcium ions away from the enamel surface and allow unused phosphate groups to come to the enamel surface (Ostby *et al,* 2007). The solvent must then be dispersed by a gentle blast of dry oil free air in order to evaporate the solvent. Dorminey *et al* (2003) showed that leaving this step out has a detrimental effect on the bond strength.

2. One-bottle systems - These combine the primer and adhesives into one solution. The solution is applied after etching enamel and dentine together using the total etching technique with 35-37% phosphoric acid for 15-20 seconds (Ferarri *et al,* 1997). This bonding system creates a mechanical interlock with etched dentine by means of resin tags and formation of a hybrid layer. They have shown high bond strength values to enamel and dentine (Tay *et al,* 1994; Mason *et al,* 1998).

Sixth generation - These bonding systems use only one solution to achieve a bond to enamel and dentine. The first evaluations of these systems show that they have a sufficient bond to conditioned dentine but the bond to enamel is less effective. The reason for this may be due to the sixth-generation systems being composed of an acidic solution that is not of sufficient strength to adequately etch the enamel.

1.6.1 Bond strength of the different bonding systems.

Studies on the first five generations of bonding systems where phosphoric acid was used to etch enamel showed a uniform etch pattern. When phosphoric acid has not been used or when self-etching primers (fifth and sixth generations) have been used, the bonding to enamel has been less effective. Bishara *et al* (2001), found that the bond strength of SEP (Transbond plus SEP, 7.1 MPa) were significantly lower compared to conventional etch and bond (Transbond XT, 10.4 MPa). Velo *et al* (2002), also showed that SEP (Prompt L-Pop, 11.55 MPa) produced lower bond strength compared to conventional etch and bond (Transbond XT, 15.71 MPa). Ireland *et al* (2003), reported similar findings from a clinical study comparing SEP against conventional etch and bond.

1.7 Adhesives

1.7.1 Resin based composites (RBCs)

RBC's are synthetic resins that are used as adhesives and restorative materials. They are composed of two or more components. Typically they contain an organic resin monomer and an inorganic filler. The filler particles are normally coated with a coupling agent so they can bond to the monomers (known as hybridisation of the filler). The material sets through polymerisation of the monomers, this can be achieved through either chemical reaction or light activation via a photo-initiator added to the composite or a combination of the two.

Components of RBCs

1. Priniciple Monomers - The most common principal monomers are based on the aromatic dimethacrylate monomer Bis-GMA (also known as Bowen's resin, Bowen 1962). The methacrylate monomer undergoes free radical addition polymerisation, which results in shrinkage of the resin matrix, typically this is 10% (Glen, 1982). Side chains on the molecule are capable of undergoing cross-linking and this reduces polymerisation shrinkage of the material. Bis-GMA is a large molecular structure and therefore a highly viscous material. An alternative lower viscosity monomer which is commonly used as a substitute for Bis-GMA is urethane dimethacrylate (UDMA). As it has a lower viscosity

compared to Bis-GMA there is not a need for the addition of diluent monomers (Watts, 2001).

2. Diluent Monomers - To reduce the viscosity of principal monomer Bis-GMA, diluent monomers of lower molecular weight are added. Common diluent monomers are triethyleneglycoldimethacrylate (TEGDMA) and hydroxyethylmethacrylate (HEMA) which are low molecular weight monomers that are more reactive than Bis-GMA (Peutzfeldt, 1997; Schulze *et al,* 2003). The dilution effect of these monomers allows greater filler incorporation into the RBC. The concentration of diluent monomers affects the viscosity of the RBC. A greater concentration leads to a less viscous composite.

3. Fillers - The filler content of RBC's is at least 50% by mass but varies between 50-80%. The filler particles can consist of glass, aluminium silicate, barium, strontium and borosilicate glasses. Fillers reduce the degree of polymerisation shrinkage, improve the wear resistance, reduce the coefficient of thermal expansion, improve viscosity and the ease of handling of RBC's. The filler particles are usually coated with a coupling agent such as silane to improve the adhesion to the dimethacrylate resin. The most commonly used silane is y-methacryloxypropltrimethoxysilane. The bonding of the filler to the resin matrix (hybridisation) improves the young's modulus, tensile strength, compressive strength and wear resistance of the RBC. RBC's can be classified according to the particle size of the filler:

Macrofilled - These typically have filler sizes greater than 1 micron. Although macrofilled composite resins are still available, their use is restricted due as their limited properties lead to suboptimal performance. They are subject to greater roughness, staining, wear and discolouration.

Most RBC's can be classified into two categories:

A. Microfilled composites - These are typically filled 35-50% by weight with pre-polymerised filler particles 0.02-0.04 microns in size. These RBC's are highly polishable and produce good aesthetics. They are mainly used in anterior restorations for these aesthetic reasons. They are not used in areas where the restoration would undergo heavy stresses because they are prone to fracture (Ritter, 2005). Their lower filler content makes them physically inferior to hybrid composites. The microfilled RBC's exhibit higher coefficients of thermal expansion, greater water sorption, greater polymerization shrinkage, a lower modulus of elasticity, lower tensile strength, and lower fracture toughness compared to hybrid composites (Ferracane, 1995). There are reinforced microfilled RBC's available for use in posterior restorations and positive clinical studies have been reported on their use. (Rasmusson, 1995).

B. Hybrid composites - These contain a heterogeneous aggregate of filler particles. They typically contain 70-80% filler by weight. The filler size is usually between 0.04-1 microns to 5 microns. On average the filler size is usually greater than 1 micron. This mixture of filler particles gives hybrid RBC's good physical properties and better polishability than

macrofilled RBC's. The majority of hybrid RBC's have medium viscosity. The viscosity of these RBC's can be altered by altering the filler content. Increasing the filler content, typically above 80% in volume produces a high viscosity RBC's (also known as packable RBC's). High viscosity RBC's are more wear resistant and have better sculptability. In addition the increased filler load reduces polymerisation shrinkage. Reducing the filler content, typically to 50% in volume produces low viscosity RBC's (also known as flowable RBC's). These RBC's have inferior mechanical properties and exhibit greater polymerisation shrinkage than high viscosity RBC's. Low viscosity RBC's are useful in regions of difficult access as it is easily flows and adapts to cavity preparations. These properties has lead to their use in fissure sealants, repairing restorations, bonding splints and as luting agents for bonding veneers and crowns. There is evidence however that the use of low viscosity RBC's does not improve the marginal seal of adhesive restorations (Lindberg *et al,* 2005, Baratieri *et al,* 2003).

Nanofilled - composites are available and consist of very small filler particles between 5-75 nanometers. The nano filler particles also agglomerate into clusters of 0.6-1.4 microns. These RBC's have similar physical properties to hybrid RBC's but have better polish and gloss.

4. Coupling Agents - The filler particles are coated with a coupling agent, typically silane, in order to bond the filler to the resin matrix. One end of the silane molecules bonds to the filler while the other end couples with the resin matrix. The use of silane reduces the

loss of filler particles from the composite and also prevents water diffusion through the matrix-filler interface.

5. Initiator -RBC's can be chemically cured, light cured or dual cured by a combination of these.

Chemical cured - Chemically cured composites are available in either twin paste or no-mix forms. Twin paste systems have one activator paste and one initiator paste. When these pastes are mixed, free radicals are produced for addition polymerisation to occur. No-mix systems require the initiator to be applied to the tooth surface prior to the application of the composite containing the activator. The application of the composite onto the initiator sets of the polymerisation reaction. The disadvantage of chemically cured systems is that they cannot be set on command and have a limited working time.

Light cured - Light cured composites contain an alpha-diketone, typically camphorquinone (CQ). CQ absorbs high intensity photons from light of 440-480 nanometer wavelength. This leads to an excited state of CQ which produces radicalised ketones that initiate the polymerisation reaction. Light curing is possible beneath metal brackets due to transillumination through enamel. The advantages of light cured composites are that they can be command set and so reduces the chance of moisture contamination during bracket placement. It also allows for an unlimited working time and it is easier to clean excess composite around the bracket up. Light sources to cure the composites include halogen,

high performance halogen, light emitting diode (LED) and plasma arc lights. Studies have shown that bond strengths achieved with LED lights with a 10 second exposure are comparable to those using a conventional halogen light for 40 seconds (Mavropoulos *et al,* 2005).

Dual cured - Dual cured composites are cured by both light and chemical agents. The advantages are they allow command set by being light activated and provide an assurance that complete polymerisation will take place in thick section due to the chemical curing. The use of dual cure adhesives have been shown to give similar bond strength to chemically and light cured materials (Smith and Shivapuja, 1993).

6. Inhibitor - To prevent premature polymerisation of the composite during storage an inhibitor is added to composite. The inhibitor is typically a monomethyl ester of hydroquinone.

1.7.2. Glass-ionomer cements (GICs)

Glass-ionomer cements were introduced in 1972 (Wilson and kent, 1972). They have two components. A liquid component which consists of an aqueous solution of an organic acid, typically polyacrylic acid, and a powder component which consists of an ion-leachable glass, typically aluminofluorosilicate glass. When the two components are mixed, an acid-base reaction occurs between the polyacrylic acid and the aluminofluorosilicate glass, this releases calcium and aluminium ions from the glass surface. The polyacrylic acid can also be incorporated into the powder, in which case mixing the powder with water activates the setting reaction.

In the setting reaction calcium ions are released first and calcium polyacrylate chains form, this leads to the cement changing to a gel consistency. Later aluminium ions are released and become incorporated into the gel and form a cross-linked calcium-aluminium carboxylate gel. The set cement consists of a heterogeneous material of glass particles coated in a siliceous gel surrounded by a polysalt hydrogel matrix. Glass ionomer cement can take weeks or months to set. It has been shown that the bond strength increases over the first month (Choo *et al,* 2001).

GIC's are capable of bonding directly to the enamel surface. The carboxyl group in the polyacrylic acid forms ionic bonds with calcium ions (McClean, 1996). Enamel conditioning is recommended by GIC manufacturers as cleaning and roughening the enamel surface decreases the surface energy.

GIC's exposed to aqueous environments are capable of leaching ions. Fluoride present in the matrix of GIC's can be leached into the local environment and also absorbed (Forsten, 1991). GIC's have the advantage of reducing demineralisation due to this fluoride leaching ability. However as it is only the fluoride in the matrix that can be leached, this is a very small percentage of the overall fluoride in the cement (Wilson and Groffamn, 1985). The level of fluoride leached is higher immediately after setting of the cement, but over time this reduces until an equilibrium is reached between the cement and the environment. The setting reaction takes 24 hours and GIC's are sensitive to moisture during the early setting reaction and to desiccation as the cement sets.

1.7.3. Resin modified glass-ionomer cements (RMGICs)

Resin-modified glass-ionomer cements (RMGICs) are GIC's with an added resin component. The advantages of adding resin composite technology to glass-ionomer cements has been the ability to command set the material, improved strength and toughness, reduced desiccation and improved resistance to acid attack. The resin component can form up to 10% of the cement. RMGIC's can be chemically cured or light-activated for curing. These cements undergo an acid-base reaction between the polyacrylic acid and glass and also undergo polymerisation of the resin component. As GICs are water based, the resin incorporated needs to be water soluble and typically hydroxyethyl methacrylate (HEMA) is used for this reason.

High bond failure rates for RMGIC's have been reported where the enamel has been dried excessively prior to the bond placement (Cacciafesta, 1999), therefore it is important that the enamel remains moist prior to RMGIC placement. The resin polymerisation in the modified materials reduces the sensitivity of these cements to water compared to GIC, but the properties of these materials are still affected by exposure to water.

1.7.4. Compomers

Compomers are polyacid modified resin composites which have been modified so as to be able to release fluoride over an extended period of time. They are essentially composite resins with the technology of glass-ionomer cements incorporated into them. They differ from RMGIC's in the size of the resin component, which is typically 30-50% (Gladys *et al,* 1997). Compomers come as single component materials. The resin component undergoes free radical addition polymerisation after light curing. Water is not mixed into the cement but must diffuse from the oral environment into the polymeric matrix to trigger acid-base reaction of the polyacid and glass in the cement, this limits the extent of the acid-base reaction and therefore the extent of fluoride that is leachable from the cement.

1.8. Water sorption of adhesives

RBC's

All composite resins absorb water from the oral environment and undergo hygroscopic expansion (Cook *et al,* 1984). The effect of water sorption leads to the leaching of components from the composite. The sorption of water is dependent on factors such as the monomer composition, the degree of polymerisation and porosity of the resin matrix. A linear expansion by water uptake of 0.09-0.72% has been reported (Bowen *et al,* 1982). Studies have shown that water sorption is due to the Fickian diffusion process. This is a process where water molecules from the environment diffuse through the polymer system (Martin *et al,* 2003; Sideridou *et al,* 2003). This diffusion is also dependent on the polarity of the monomer system and porosity within the composite matrix. Water molecules are attracted to the polar regions on hydrophilic monomers and form hydrogen bonds to these regions. This then allows further separation of the polymer network, creating more pores and spaces that allow sorption of more water. Water also sorbs into composite due to the presence of voids on the composite surface that develop during the polymerisation process. Water sorption leads to hydrolytic degradation which breaks down various components within the composite (Ferracane, 2006). This reduces the mechanical properties of the composite and allows components of the composite to be leached into the environment (Peutzfeldt, 1997).

GIC's

Water plays a critical role in the setting of glass ionomer cements. It provides the medium for the acid-base setting reaction to occur. Then it slowly hydrates the cross linked agents to produce a stable gel structure that is stronger and less susceptible to moisture contamination. Newly mixed glass ionomer cement exposed to air will craze and crack as a result of desiccation. Additionally any water contamination of newly mixed glass ionomer cement can cause dissolution of the matrix forming cations and anions to the surrounding areas. Therefore glass ionomer cements are susceptible to both desiccation and contamination by water during the initial setting of the material.

1.9. Bond strengths and failure rates of adhesives

RBC's have shown the greatest bond strength of orthodontic adhesives generally ranging between 10-18 MPa (Rock and Abdullah, 1997; Bishara *et al,* 1999; Bishara *et al,* 2001; Bishara *et al,* 2002; Littlewood *et al,* 2000; Grandhi *et al,* 2001; Summers *et al,* 2004). The bond strength of GIC's is relatively low ranging between 3-7 MPa (Fajen *et al,* 1990; Voss *et al,* 1993; Wiltshire, 1994), this bond strength is a reflection of the tensile strength of GIC. Failure is usually cohesive within the GIC, thus bond failure commonly leaves cement residues to the tooth. Millet and McCabe (1996) found little evidence to support the use of GIC for orthodontic bracket bonding due to unreliable bracket retention. The bond strength of RMGIC has been reported to range from 5-19 MPa (McCourt *et al,* 1991; Ewoldsen *et al,* 1995; Cacciafesta *et al,* 1998; Fricker, 1998; Lippitz *et al,* 1998; Meehan *et al,* 1999). Bond strengths reported for RMGIC are generally lower than that of RBC's (Owens and Miller, 2000; Movahhed *et al,* 2005; Justus *et al,* 2010), however in the presence of moisture they have shown a higher bond strength (Cheng *et al,* 2011). Compomers have been shown to produce bond strengths comparable to RBC's (Millett *et al,* 2000), however they do not leach as much fluoride as GIC's or RMGIC's. The reported bond strength range of compomers is 7-12 MPa (Ashcraft *et al,* 1997; Rock and Abdullah, 1997; Meehan *et al,* 1999)

GIC's have also been shown to have greater bond failure rates than RBC's (Miguel *et al,* 1995; Miller *et al,* 1996; Norevall *et al,* 1996). Millett (1999) investigated bond failure rates and showed RBC to have a significantly lower bond failure rate than RMGIC, and

GIC to have the worst bond failure rate. Other studies have also shown a higher bond failure rate for RMGIC adhesives compared to RBC adhesives (Fricker, 1998; Oliveria *et al,* 2004; Summers *et al,* 2004).

1.10. Bond strength

There is little research to quantify what the ideal bond strength of an orthodontic adhesive should be. The tensile bond strength of enamel is approximately 14.5 MPa (Bowen and Rodriguez, 1962) and fractures in enamel can occur with bond strengths as low as 13.5 MPa (Retief, 1974).

Suggestions have been made on what bond strength orthodontic adhesives should achieve. These include:

- 1.38 MPa or above (Newman, 1965).
- 4.90-7.85 MPa (Reynolds, 1975).
- 2.86-7.59 MPa (Keizer *et al,* 1976)

The bond strength of the orthodontic adhesives need to be high enough to deal with intra oral forces that the bracket will be subjected to on a daily basis. Theses forces include soft tissue pressure, the component of occlusal force that is directed onto the bracket, forces from the orthodontic appliance and iatrogenic forces from the orthodontist.

1.11. Bond strength testing

The reported bond strengths of orthodontic adhesives is dependent on the method of testing and measurement. Bond strengths have been evaluated *in vitro* and *in vivo*. *In Vitro* testing involves laboratory debonding of orthodontic brackets bonded to tooth samples using a mechanical device where the force used to debond the bracket can be measured. Typically an Instron Universal tesing machine is used to achieve this (Figure 2.3). *In Vivo* testing involves clinical investigations where the incidence of bracket failure is measured over a period of time.

1.12. Application of debonding force

There have been differing methods of measuring bond strength (Fox *et al,* 1994). The type of force applied to the adhesive will depend on the direction that the force is applied to the bracket. The direction of force will affect the bond strength measurement. A shear force will only be applied to the adhesive if the direction of force is parallel to the surface of the enamel and applied at the junction of the bracket and adhesive. Any force that is not applied at this point will also introduce a peel force (figure 1.2) (Fox *et al,* 1994).

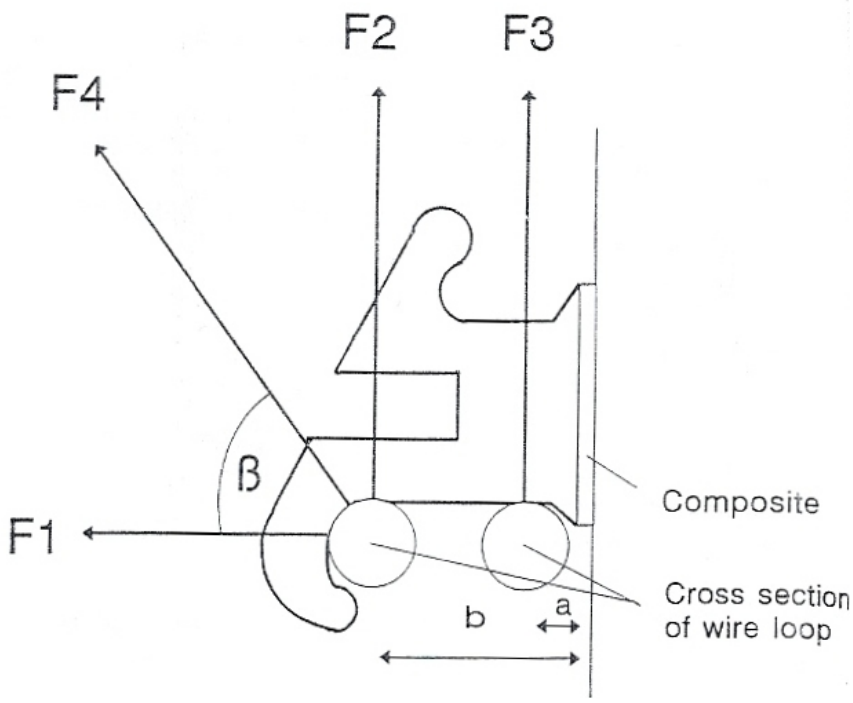


Figure 1.2. A diagrammatic representation of the relationship of the displacing wire loop to the bracket and bonding agent.

F1 = force vector often used for a 'tensile' test. F2 and F3 = force vectors used for a 'shear' test. F4= the more likely force vector which will be at an angle ß away from the long axis of the bracket. a and b represent the varying distances that a 'shear' force vector is away from the bracket/ composite interface (Fox *et al,* 1994). The shorter distance 'a' is the greater the 'shear' component of debonding force. Generally as the wire loop will be at a perpendicular distance 'a' from the composite adhesive surface, some element of peel force will also be applied on debonding.

Fox *et al* (1994), published a critique of bond strength testing in orthodontics. They found that the majority of bond strength testing was conducted using the wire loop method. This is where the shear force is transmitted to the bracket by a wire loop placed around the bracket. As there is no standardised method of bond strength testing they made suggestions for a protocol for future bond strength testing in orthodontics. These suggestions included:

1. The surface of premolar enamel should be used on teeth extracted from adolescent patients for orthodontic reasons
2. Teeth should be used after 1 month, but before 6 months from extraction and stored in distilled water prior to bonding
3. Debonding should take place on an Instron or equivalent machine at a cross-head speed of 0.1mm per minute.
4. Site of failure should be reported.
5. Care should be taken to ensure the point of application and direction of the debonding force is the same for all specimens.
6. Bond strengths should be quoted in either Newtons or Megapascals.
7. At least 20 and preferably 30 specimens should be used per test.

Although Fox *et al* (1994), recommend a cross-head sped of 0.1mm per minute, Eliades and Brantley (2000) reported the most common cross-head speed used for orthodontic bond strength testing to be 0.5mm per minute. Studies have shown that cross-head speeds of 0.1 mm/min, 0.5 mm/min, 1.0 mm/min or 5.0 mm/min do not have any significant effect

on bond strength measurements (Reis *et al,* 2004; Klocke and Kahl-Nieke, 2005). Other factors that affect the bond strength are the adhesive product, the quality of the enamel surface and the storage of the test specimen prior to debonding and the test method (Olio, 1993).

1.13. Bond failure site

The adhesive can fail in 3 ways:

1. Adhesive failure - this is where the adhesive fails at the interface of the enamel or bracket. In this situation all the remnants of the adhesive would remain on either the tooth or the bracket.

2. Cohesive failure - this is where the adhesive fails internally. In this situation the remnants of the adhesive would be present equally on the tooth and on the bracket.

3. Combination of adhesive and cohesive failure - in this situation remnants of the adhesive would be present on the tooth and the bracket but in a more unequal distribution.

Clinically an adhesive failure at the enamel surface is favourable as it would reduce the necessity to remove adhesive at debond. However a failure at the tooth surface also carries a higher risk of enamel fracture on debond, especially if the bond strength of the adhesive is close to the tensile strength of enamel. Årtun and Bergland (1984) devised an index to record the method of bond failure. This is the known as the Adhesive Remnant Index (ARI).

1.14 Aims of study

The aim of this study was to develop an orthodontic adhesive which would be capable of leaching calcium and phosphate ions that would promote remineralisation of tooth enamel. There is currently a commercially available orthodontic composite resin adhesive containing amorphous calcium phosphate (ACP) (Aegis Ortho, Bosworth Co., Skokie, Illinois) which claims to provide the benefit of promoting remineralisation through its ability to leach calcium and phosphate ions. Studies investigating the bond strength achieved with this adhesive have shown it to have an inferior bond strength compared to conventional composites (Dunn, 2007; Foster *et al,* 2008; Uysal 2010).

There are a lack of studies to quantify the amount of calcium and phosphate that Aegis Ortho is capable of leaching, or to quantify the remineralisation potential of this adhesive. An aim of this study was to develop composite adhesives containing tri-calcium phosphate (TCP) as opposed to amorphous calcium phosphate (ACP) which is used in Aegis Ortho. The reason for doing this, is that studies have shown that amorphous calcium phosphate is a relatively unstable molecule and readily forms hydroxyapatite in an aqueous environment (Skrtic *et al,* 2003; Skrtic and Antonucci, 2006). This instability of amorphous calcium phosphate would compromise the mechanical integrity of the composite. Tri-calcium phosphate (TCP) is more stable in an aqueous environment and therefore would potentially not have as significant an effect on the mechanical integrity of the composite into which it is incorporated.

In summary the aims of this study were:

1. To compare the bond strength of developed experimental tri-calcium phosphate composites against a commercially available amorphous calcium phosphate composite (Aegis Ortho, Bosworth Co., Skokie, Illinois) and other commercially used orthodontic adhesives including a composite (Transbond XT, 3M Unitek, Monorovia, California) and resin-modified glass ionomer cement (Fugi Ortho LC, GC corporation, Tokyo).
2. To determine the effect of water on bond strength and mechanism of bond failure of these adhesives.
3. To determine if the percentage of TCP in the experimental composites have any effect on the bond strength.
4. To quantify the concentration of the remineralising ions released from the experimental tri-calcium phosphate (TCP) composite and the commercial amorphous calcium phosphate (ACP) composite adhesive.

1.14.1 Null hypotheses

The null hypotheses for this study were:

1. There is no difference between the bond strength of any of the orthodontic adhesives tested.

2. 7 day water immersion does not affect the bond strength of the adhesives. Therefore, there is no difference in bond strength between the orthodontic adhesives when stored in wet or dry conditions.

3. The bond strength of the experimental tricalcium phosphate (TCP) composite is not affected by the percentage of TCP in the composite.

4. There is no difference in the ARI score or mechanism of bond failure between the adhesives.

5. There is no difference between the experimental TCP composite and the amorphous calcium phosphate (ACP) composite (Aegis Ortho, Bosworth Co., Skokie, Illinois) in their ability to leach calcium phosphate.

Chapter Two

Materials and method

Chapter Two: Materials and method

2.1 Key tests

There were three key areas of laboratory testing:

1. Bond strength testing of commercial orthodontic adhesives and experimental composite adhesives.

2. Assessment of the site of bond failure of the adhesives.

3. Calcium ion release from the amorphous calcium phosphate (ACP - Aegis Ortho) composite and the tricalcium phosphate (TCP) experimental composite (10% TCP).

2.2 Synthesis of experimental composites

2.2.1 Synthesis of the co-monomer resin

The co-monomer resin for the experimental composites was mixed according to the weight percentage ratios of the components listed in table 2.1.

Table 2.1. The weight percentage ratios of the components of the co-monomer resin.

Acronym	Material	Weight Percentage	Supplier
Bis-GMA	Bisphenyl-A diglycidyl ether	60	Sigma-Aldrich, UK
TEGMA	Triethylene glycol dimethacylate	40	Sigma-Aldrich, UK
CQ	Camphorquinone	0.2	Sigma-Aldrich, UK
DMAEMA	Dimethylaminoethylmethacrylate	0.3	Sigma-Aldrich, UK
BHT	Butylated hydroxytoluene	0.1	Sigma-Aldrich, UK

Formula 1.

$$\frac{\text{weight of Bis-GMA (g)}}{\text{Weight \% of Bis-GMA}} = \text{Weight of 1 \%}$$

Formula 2.

Weight of 1% of Bis-GMA X Weight % of component required = Weight of component required (g)

The resin was synthesis by the following method:

1. An empty glass beaker was placed on a balance (Mettler AE 163, Toledo Ltd, UK) accurate to 10^{-4}g.
2. The balance was then reset to the baseline reading (in order to calibrate).
3. Bis-GMA monomer was then added to the beaker and the beaker was placed back on the balance to obtain the weight of Bis-GMA.
4. The weight of the Bis-GMA was used as the 60 % weight percentage of the co-monomer combination.
5. The weight of each of the other components of the resin were then calculated using formula 1 and formula 2.
6. The calculated weight of each of the components was measured out separately and then added to the resin mixture.
7. The weight percentages of the components used are listed in table 2.1.
8. The final resin was composed of 60 weight% of Bis-GMA, 40 weight % of TEGMA, 0.2 weight % CQ, 0.3 weight % DMAEMA and 0.1 weight % BHT.
9. The initiator, co-initiator and inhibitor (CQ, DMAEMA and BHT) were placed on a hot plate at room temperature (23^{o}C) at 350 RPM for 20 minutes to ensure through dissolution of the components. This mixture was then added to the co-monomers Bis-GMA and TEGMA.
10. The whole mixture was then placed on a hot plate that was heated up to 60^{o}C + 1^{o}C for 60 minutes and a magnetic stirrer was used to mix the solution (the 60^{o}C temperature was below the activation temperature of all the resin components used).

11. The completed resin was then placed in a plastic container and wrapped in aluminium foil in order to prevent any light causing premature activation. The containers of resin were then kept in a refrigerator until being used to synthesise the composites.

2.2.2 Synthesis of the composite

The experimental composites were synthesised using the previously synthesised resin mixture (30% weight), silanated barium glass filler and Tri-calcium phosphate filler. Table 2.2 shows the fillers used in the experimental composites.

Table 2.2. The fillers used in the experimental composites.

Acronym	Filler	Particle size (um)	Supplier
Ba	Silanated Barium glass	1.5	Ivoclar, Vivadent Ltd, UK.
TCP	Tricalcium phosphate	11	University of Birmingham, Biomaterial Department.

Formula 3.

$$\frac{\text{weight of resin (g)}}{\text{Weight \% of resin}} = \text{Weight of 1 \%}$$

Formula 4.

Weight of 1% of resin X Weight % of component required = Weight of component required (g)

Table 2.3. Experimental composites

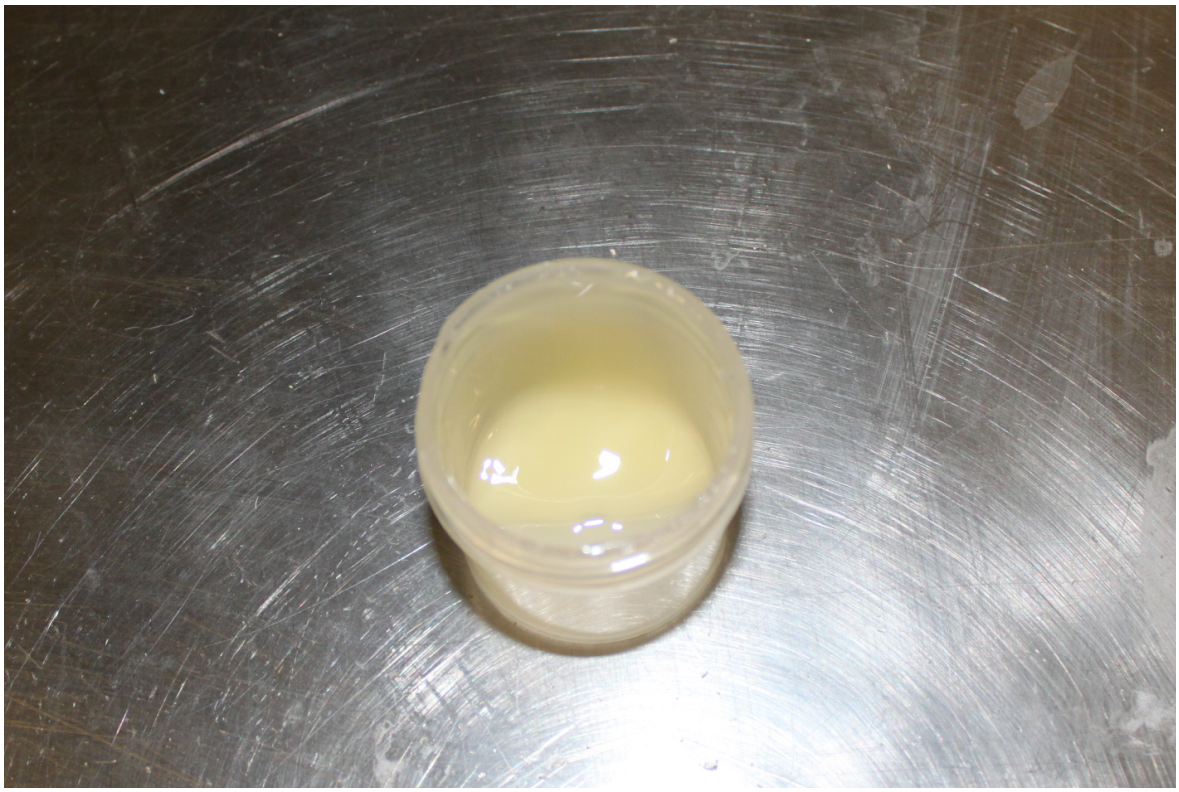
Experimental Composite (EC)	TCP weight %	Filler weight %	Filler particle size	Resin weight %
1	0	70	1.5	30
2	1	69	1.5	30
3	5	65	1.5	30
4	10	60	1.5	30

Four combinations of composites were composed and are listed in table 2.3. The method of synthesis was as follows:

1. An empty plastic container was placed on a balance (Mettler AE 163, Toledo Ltd, UK) accurate to 10^{-4}g. The balance was then reset to the baseline reading (in order to calibrate).
2. Resin previously synthesised was then added to the plastic container and the container was placed back on the balance to obtain the weight of the resin.
3. The weight of the resin was used as the 30 % weight percentage of the composite.
4. The weight of each of the other components of the composite were then calculated using formula 3 and formula 4.
5. The calculated weight of each of the components was measured out separately and then added to the resin mixture.
6. The weight percentages of the components used are listed in table 2.3.
7. The container with the composite mixture was then placed into a SPEED mixer (Siemens, DAC 150 FVZ-K) for 3 minutes at 3000 RPM to ensure a thorough mix and dissolution of all the components.

8. The completed composite container was then wrapped in aluminium foil in order to prevent any light causing premature activation. The containers of composite were then kept in a refrigerator until being used (Figure 2.1).

Figure 2.1. Experimental composite specimen



2.2.3 Composition of commercial adhesives

Table. 2.4 Commercial materials tested

Adhesive	Manufacturer	Batch Number
Transbond XT	3M Unitek, Monorovia, California	LOT 9GT
Fugi Ortho LC	GC Corporation Tokyo, Japan	LOT 1102241
Aegis Ortho	Bosworth Co., Skokie, Illinois	LOT 1001-031

Table. 2.5 Composition of Transbond XT

Transbond XT	Composition	%
	Silane treated quartz	70-80
	Bisphenol A diglycidyl ether dimethacrylate (BISGMA)	10-20
	Bisphenol A bis(2-hydroxyethyl ether) dimethacrylate (Bis-EMA)	5-10
	Silane treated silica	< 2
	Diphenyliodoniumhexaflurophosphate	< 0.2

Table. 2.6 Composition of Fugi Ortho LC

Fugi Ortho LC	Powder	Powder %	Liquid	Liquid %
	Alumino-silicate glass	100	Polyacrylic acid	20-22
			2-Hydroxyethyl methacrylate	35-40
			Proprietary Ingredient	5-15
			2,2,4, Trimethyl hexamethylene dicarbonate	5-7
			Triethylene glycol dimethacrylate	4-6

Table. 2.7 Composition of Aegis Ortho

Aegis Ortho	Composition	%
	Amorphous calcium phosphate (ACP)	38
	Glass filler	25
	Urethane dimethacrylate	37

2.3 Bond strength testing

2.3.1 Power calculation for bond strength testing

The number of specimens required per group for bond strength testing was calculated using data from Sunna and Rock (1999) where they reported a bond strength of 18MPa with a standard deviation of 2.4MPa. A clinically significant difference was set at 3 MPa to produce a standardised difference of 3/2.4 = 1.25. This produces a sample size of 28 for 80% power at $P<0.01$ using Altman's nomogram (Altman, 1991). Therefore 30 samples were made per group in order to achieve this level of power.

2.3.2 Tooth specimens used

The tooth specimens used in this study were prepared from extracted human maxillary premolar teeth. This study was registered under generic ethical approval obtained by the University of Birmingham for the use of human tooth tissue for research purposes.

REC REFERENCE NO: 09/H0405/33 (for Birmingham Dental School Tooth Bank)

These teeth were relatively easily available due to high frequency of premolar extractions for orthodontic treatment. These teeth were obtained from the tooth bank at the Birmingham Dental Hospital and it is likely that they were sourced from the local population. This means that these teeth would have been exposed to fluoride as the West Midlands is a fluoridated water area with one part per million of fluoride ion.

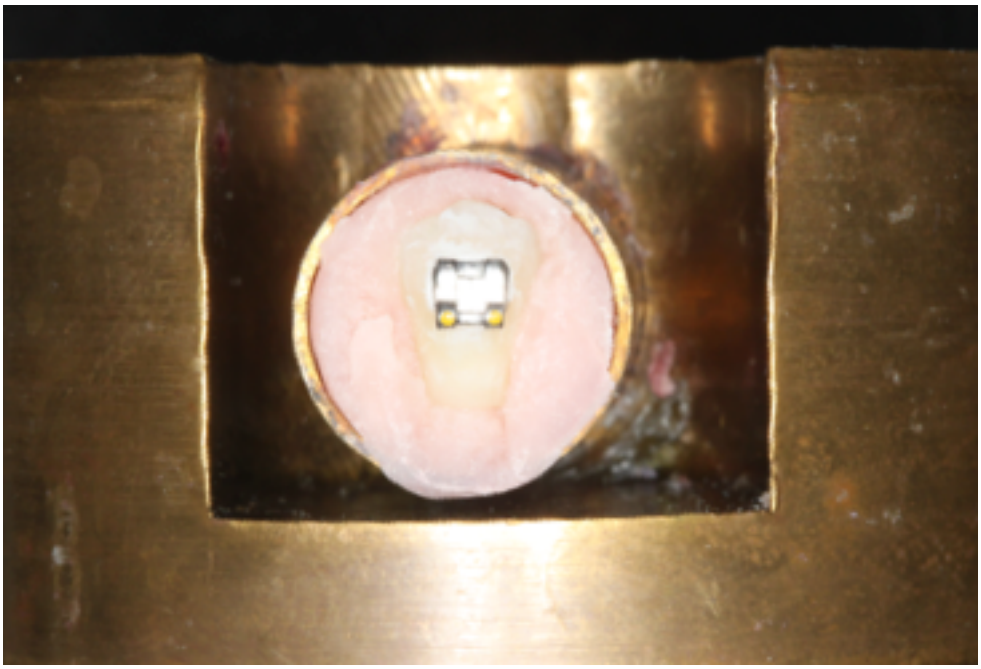
The maxillary premolar teeth were stored in tubes containing distilled water with thymol crystals added (0.1% weight/volume) to inhibit bacterial growth (Silverstone, 1976) and stored in the dark at $10^{o}C \pm 5^{o}C$ (Fox *et al,* 1994). These teeth were stored for a maximum of 6 months prior to use. All the teeth were examined visually for their suitability. Only sound premolar teeth were included. Teeth were rejected if they had evidence of caries, restorations enamel defects, pronounced cracking detectable by direct visual inspection (Zachrisson *et al,* 1980) or if they had been exposed to any chemicals post extraction. The tubes containing the teeth were numbered and randomly allocated to experimental groups using a random number table.

2.3.3 Preparation of the teeth

The roots of the maxillary premolar teeth were sectioned just below the cement-enamel junction with a motorised circular bone saw. Water irrigation was used during the sectioning of the roots. Grooves were placed on the mesial and distal surfaces of the premolar crowns. This was to allow mechanical retention of the tooth crowns which would be held within brass cylinders with self-curing orthodontic acrylic resin. The maxillary premolar crowns were subsequently stored in tubes of distilled water in order to prevent dehydration.

The sectioned maxillary premolar crowns were then embedded in brass cylinders (8mm radius, 43mm length) using self-curing orthodontic acrylic resin to a depth of 20mm. The premolar crown was embedded into the brass cylinder so that the buccal surface of the premolar projected out of the brass cylinder and the buccal surface was perpendicular to the long axis of the brass cylinder. These specimens were then stored in tubes of distilled water at room temperature to avoid dehydration of the enamel (Abdullah and Rock, 1996) (Figure 2.2).

Figure 2.2. The prepared tooth specimens embedded into self-curing acrylic in brass cylindrical tubes.



2.3.4 Preparation of the enamel surface

A standardised method was used for the preparation of the enamel surface for all specimens prior to bracket bonding. This was as follows:

1. The buccal enamel surface was polished for 10 seconds with a fluoride free pumice slurry using a rubber prophylactic cup in a slow hand piece.

2. The pumice was rinsed away with water and air spray for 10 seconds and the buccal enamel surface was then dried with a stream of oil-free compressed air for 10 seconds.

2.3.5 Brackets used and placement technique

The brackets used were uncoated 'Victory Series' straight wire, upper premolar twin brackets with a standard mesh base (3M Unitek, Monorovia, California).

These brackets were bonded on the buccal surface of the premolar crowns according to Andrews (1976) recommendation for bracket positioning. This is at the intersection of the long axis of the clinical crown (LACC) and the clinical crown long axis midpoint (LA point).

All brackets were placed firmly into the correct position with a force of 700g for 5 seconds and a force gauge was used to monitor this force (Correx Co, Bern, Switzerland).

Excess adhesive flash that appeared around the bracket during placement was removed with a sharp probe and then pressure was reapplied to the bracket for another 5 seconds.

2.3.6 Curing

Each bracket was light cured for 20 seconds with an Ortholux LED Curing Light (3M Unitek, Monrovia) in accordance with the manufacturer's specifications.

The curing light had a 8.3mm diameter curing tip with a light intensity of 740mW/cm^2 and a wavelength of 470-480nm. As variations in exposure times affect bond strength (Bishara *et al,* 1998; Wang and Meng, 1992; Oesterle *et al,* 1995) the curing tip was calibrated before use, using an in-built light intensity meter.

2.3.7 Bond strength test groups

Table 2.8. Table of bond strength test groups.

Bond Strength Group (BSG)	Adhesive	Storage
1	Transbond XT	Dry
2	Transbond XT	Wet
3	Fugi Ortho LC	Dry
4	Fugi Ortho LC	Wet
5	Aegis Ortho (ACP)	Dry
6	Aegis Ortho (ACP)	Wet
7	EC 1 (0% TCP)	Dry
8	EC 1 (0% TCP)	Wet
9	EC 2 (1% TCP)	Dry
10	EC 2 (1% TCP)	Wet
11	EC 3 (5% TCP)	Dry
12	EC 3 (5% TCP)	Wet
13	EC 4 (10% TCP)	Dry
14	EC 4 (10% TCP)	Wet

*EC - Experimental composite
ACP - Amorphous calcium phosphate
TCP - Tricalcium phosphate

2.3.8 Preparation of the group specimens for bond strength testing

The specimens were prepared as follows:

1. The enamel was etched for 30 seconds with 37% phosphoric acid. This was applied with a brush and agitated onto the enamel surface. The exception to this was group 3 and 4 (Fugi Ortho LC groups) where the enamel surface of the tooth specimens were conditioned with GC Fugi ORTHO CONDITIONER (GC corporation, Tokyo) for 20 seconds
2. The etch/conditioner was then rinsed away with water and air spray for 10 seconds and then dried with a stream of oil-free compressed air until a frosty appearance of the etched enamel surface could be seen visually.
3. Groups 1,2,7-14 (Transbond XT and experimental composite group) specimens then had Transbond XT (3M Unitek, Monorovia, California) primer applied to the buccal enamel surface with a brush. Groups 5 and 6 (Aegis Ortho groups) had Aegis Ortho (Bosworth Co., Skokie, Illinois) primer applied and groups 3 and 4 (Fugi Ortho LC groups) did not have any primer applied. A stream of oil-free compressed air was applied to the specimens where primer was applied to the surface in order to obtain a thin layer of primer. The primer was then light cured for 10 seconds with an Ortholux LED Curing Light (3M Unitek, Monrovia).
4. The adhesives were then applied to the mesh surface of an uncoated Victory Series straight wire, upper premolar twin brackets (3M Unitek, Monorovia, California).
5. The brackets were placed onto the buccal surface of the premolar tooth as outlined earlier.

6. The brackets were light cured for 20 seconds using an Ortholux LED Curing Light (3M Unitek, Monrovia).
7. The specimens were then then stored in either dry specimen tubes or wet specimen tubes filled with distilled water at 37^0C for 1 week prior to being debonded. Although a one week time span does not reflect typical orthodontic practice, it is a more convenient time span for laboratory testing and allows sufficient time for the adhesive cements to mature to the optimal bond strength (Chamda and Stein, 1996).

2.3.9. Bond strength testing

The bond strength of the adhesives was measured using an Instron Universal Testing Machine (Model 5544, Instron Inc, Canton, Massachusetts, USA) (Figure 2.3, 2.4).

Figure 2.3. Instron Universal Testing Machine.

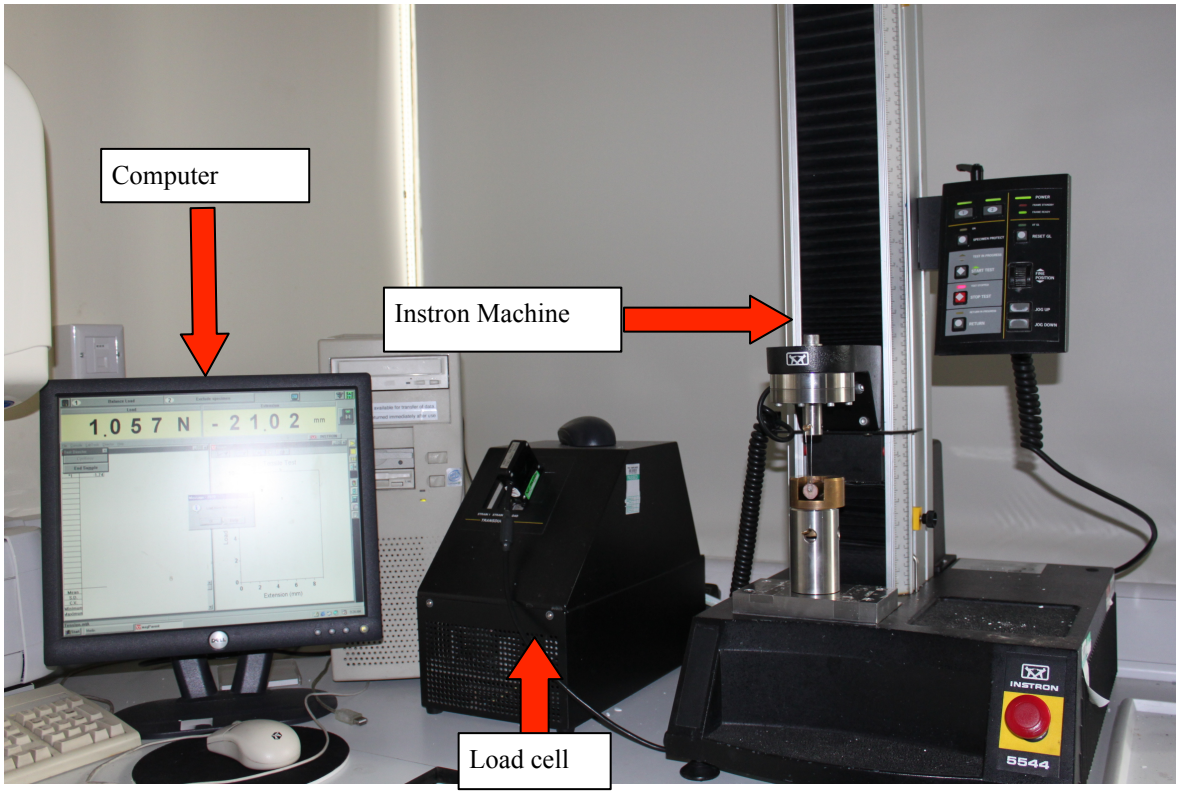
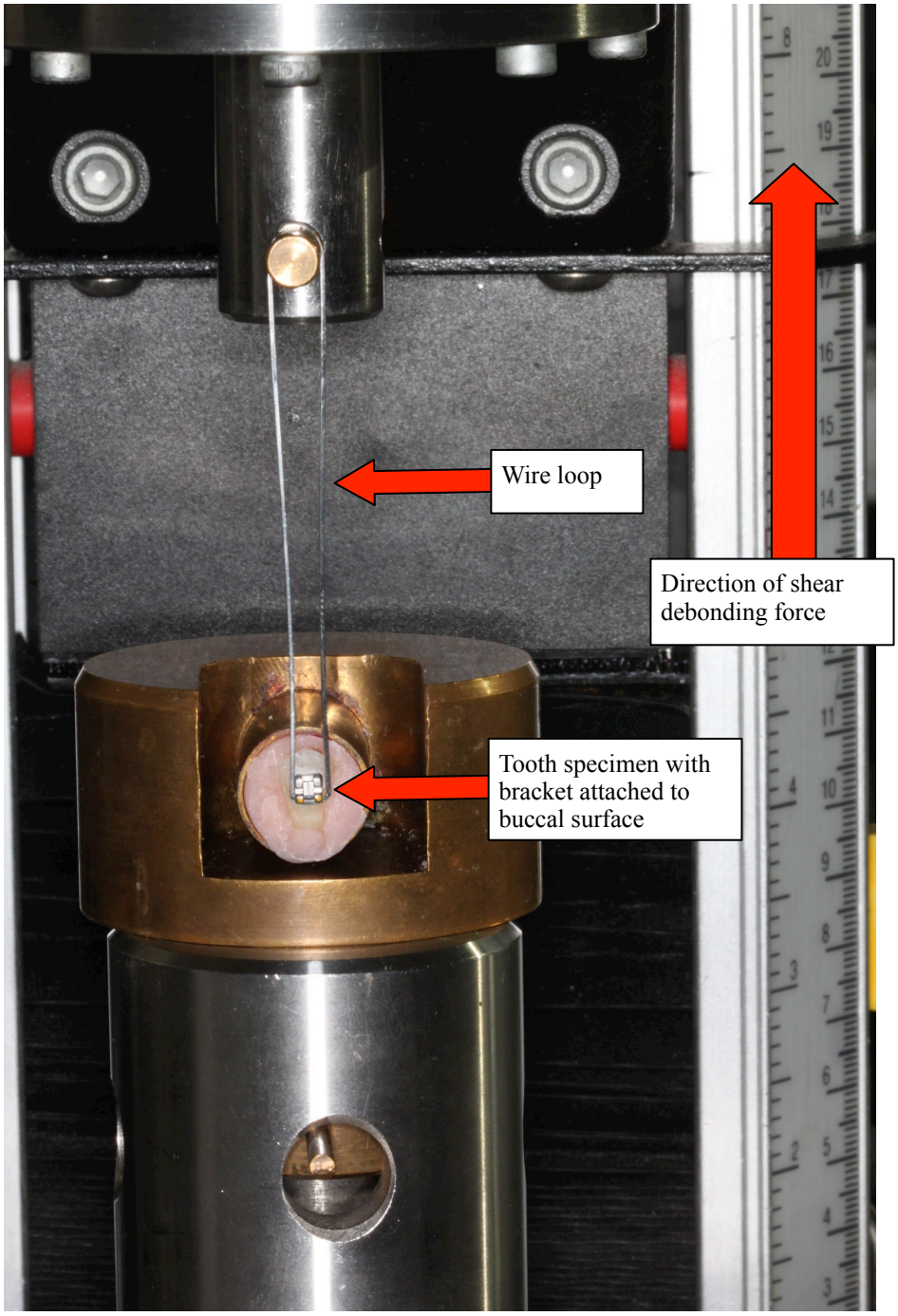


Figure 2.4. Wire loop method of debonding.



The brass cylinders containing the premolar crowns with brackets bonded were fitted into a customised jig which was attached to the lower cross head of the Instron Universal Testing Machine. The brass cylinder could rotate when attached to the customised jig and could also move in and out. This allowed for positional control of the brackets in relation to the wire loop used to debond the brackets. The specimen brackets were positioned so that shear forces could be applied at right angle to the long axis of the bracket body. The direction of force for debonding the brackets was gingivo-occlusal and parallel to the buccal surface of the tooth. A stainless steel wire loop (0.016" x 0.016", 5 mm wide and 67mm long) was placed so that it was engaged above from the fixed upper crosshead of the Instron Universal Testing machine and below from the gingival tie wings of the premolar bracket (Figure 2.4). The wire dimension was chosen as it would completely fill the space between the bracket base and the tie-wings. This feature ensures that the wire loop is always the same fixed distance from the adhesive/bracket interface in all specimens, therefore making the testing process reproducible. Katona (1997) showed that an increase in the distance from the tooth surface to the point of application would increase the bond strength. The wire loop applied a shear-peel force to the adhesive/bracket interface as it is not possible to apply a pure shear load to a bracket. This is due to the presence of an unavoidable inherent bending moment and so the term shear-peel is used to describe this phenomenon (Katona, 1997). The stresses generated in the laboratory setting are varied and complex and unlikely to be truly reflective of the stresses generated in the clinical setting (Tavas and Watts, 1979). The Instron testing machine was set at a cross-head speed of 0.5mm per minute (Eliades *et al,* 2000) and a 2 KN load cell was used to measure the

shear-peel strength. The load at bracket failure was recorded in newtons by Bespoke Merlin software connected to the Instron machine (Figure 2.3).

The loads were then converted to megapascals using the formula:

$$\text{MPa } (N/mm^2) = \frac{\text{Load (N)}}{\text{Bracket base area } (mm^{2)}}$$

The bracket base area was 9.10mm^2 according to the bracket manufacturers (3M Unitek, Monorovia, California).

2.4. Adhesive remnants post debond and mechanism of bond failure

Following the bond strength testing, all the specimens were all analysed under 10 X magnification with a stereo optical microscope and calibrated graticle to assess the adhesive remnants on the tooth surface.

The adhesive remnant index (ARI) was used to score the adhesive remnants on the tooth specimens following debond (Årtun and Bergland, 1984) (Table 2.9).

Table 2.9. The adhesive remnant index scores (Årtun and Bergland, 1984).

0	No adhesive left on the tooth
1	Less than half of the adhesive left on the tooth
2	More than half of the adhesive left on the tooth
3	All of the adhesive left on the tooth, with distinct impression of the bracket mesh

To then compare the mechanism of bond failure the ARI scores would be grouped into adhesive or cohesive bond failures as follows:

Adhesive failure = ARI score 0 or 3

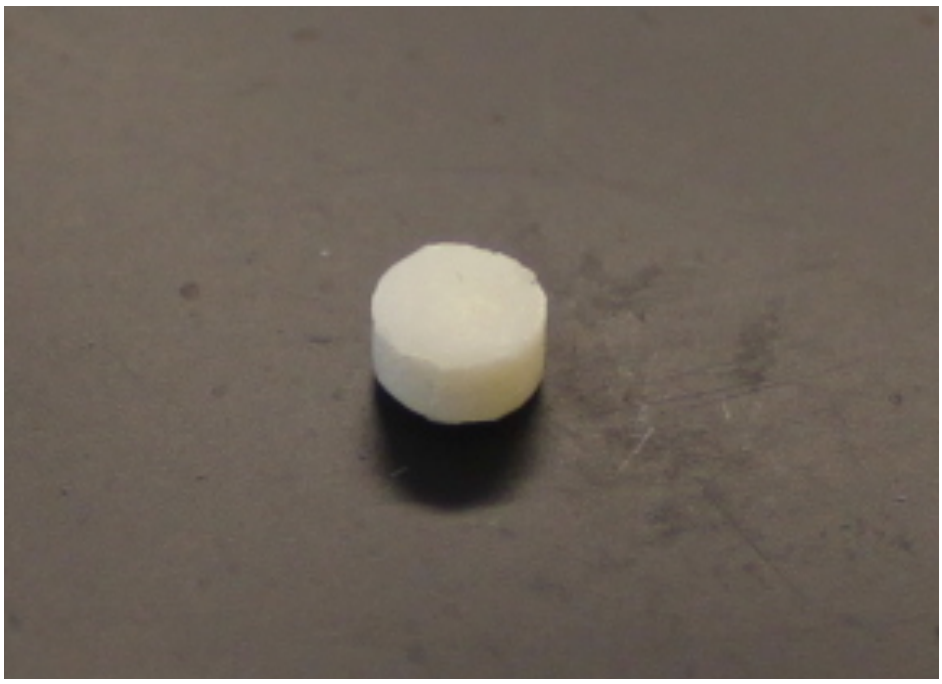
Cohesive failure = ARI score 1 or 2

2.5. Calcium ion release testing

2.5.1 Calcium ion release samples

Discs of the Aegis Ortho (ACP) composite and experimental composite 4 (10% TCP) were constructed. A mould was used to construct these discs of composite. These discs were 4 mm in diameter and 2 mm in thickness (Figure 2.5).

Figure 2.5. Image of composite disc constructed for the calcium ion release test.



20 discs of each composite were placed into a specimen tube and 10ml of distilled and de-ionised water was added. The tubes were wrapped in aluminium foil to prevent any light

exposure and stored in an incubator (Laboratory thermal equipment Ltd) at 37^{0C} for a 6 week time period. 10 tubes for each composite were constructed.

This process was repeated over a 5 week time period. Therefore in total there were 50 specimens constructed for each composite (Table 2.10). After 6 weeks of incubation the calcium concentration of the solutions were measured to detect the level of calcium release from the two composites.

Table 2.10. ACP (Aegis Ortho) and TCP (experimental composite 4 (10%TCP)) groups constructed.

WEEK	AEGIS ORTHO (ACP)	Experimental Composite 4(TCP)
1	ACP 1 (10 samples, 20 discs in each)	TCP 1 (10 samples, 20 discs in each)
2	ACP 2 (10 samples, 20 discs in each)	TCP 2 (10 samples, 20 discs in each)
3	ACP 3 (10 samples, 20 discs in each)	TCP 3 (10 samples, 20 discs in each)
4	ACP 4 (10 samples, 20 discs in each)	TCP 4 (10 samples, 20 discs in each)
5	ACP 5 (10 samples, 20 discs in each)	TCP 5 (10 samples, 20 discs in each)

2.5.2 Calcium ion release measurement

A calcium selective electrode (Jenway meter, model 3510) was used for the measurement of the calcium ion release from the ACP composite (Aegis Ortho) and the TCP experimental composite (experimental composite 4) groups. These electrodes use a replaceable membrane cap which has a solid, polymeric membrane containing a selective ion exchanger. The electrode potential of solutions is measured by their effect on the ion exchange material.

The electrode was calibrated each week using using calcium chloride calibration solutions to ensure consistency and accuracy of readings (Rundle, 2000). The calibration solutions were made by dilution of calcium chloride of known molecular weight. Solutions of calcium ion concentration of 0.1M, 0.01M, 0.001M and 0.0001M were constructed.

The composite samples were taken out of the incubator after 6 weeks of storage. The solutions were throughly shaken and then the ion selective calcium electrode was placed into the sample for 10 minutes until a stable electrode reading could be obtained (Figure 2.6, 2.7).

Figure 2.6. Calcium ion release measurement using calcium ion-selective electrode.

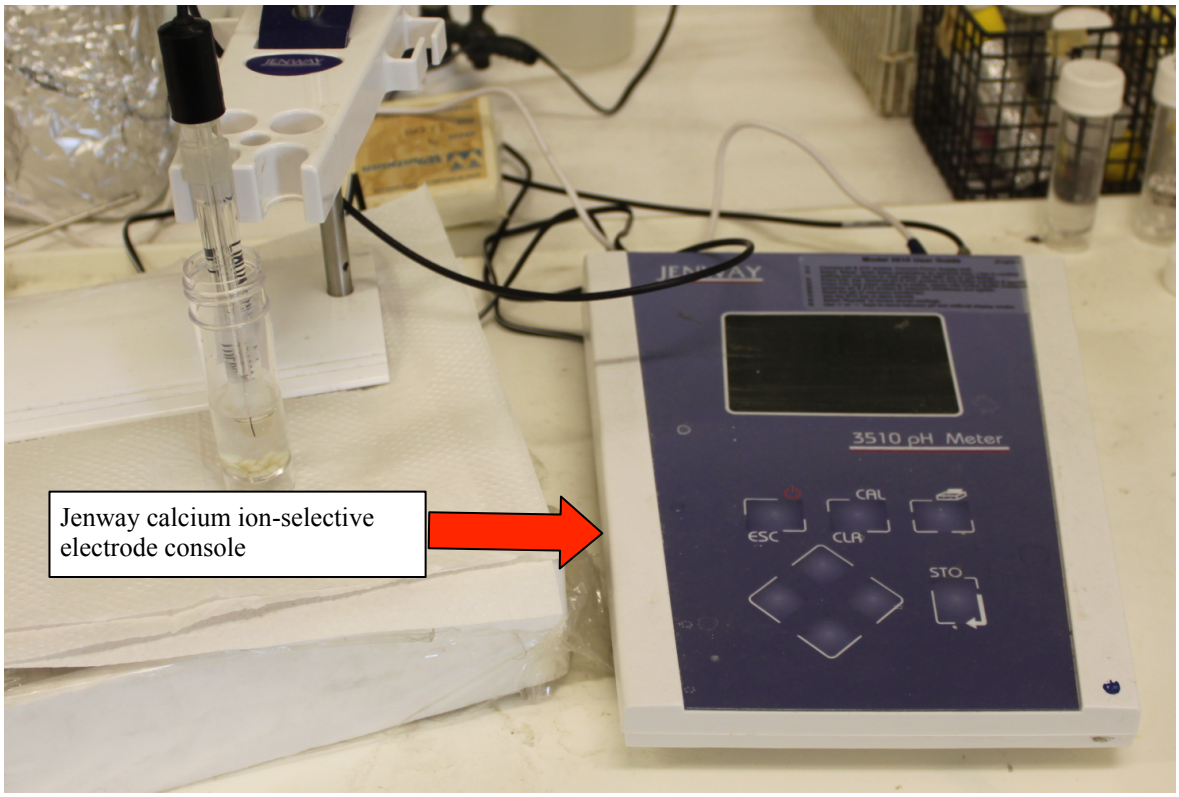
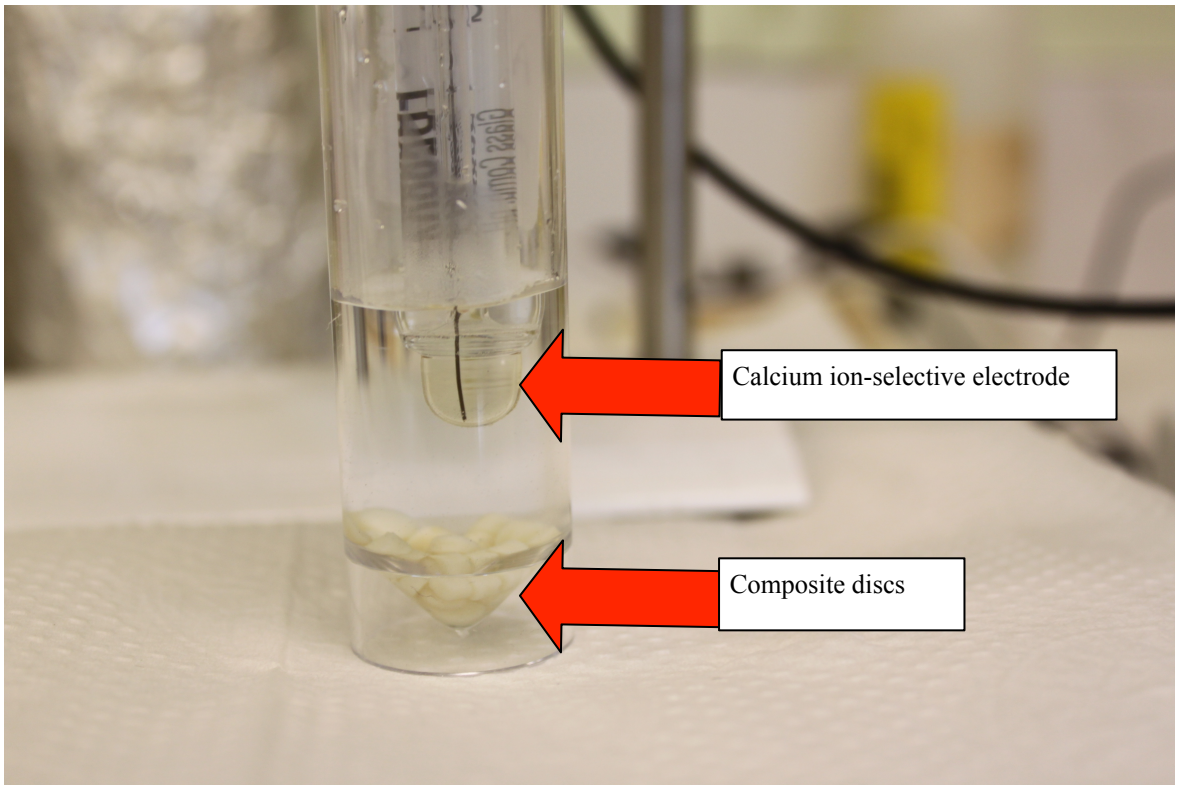


Figure 2.7. Calcium ion-selective electrode used to measure calcium ion concentration.



2.5.3 Calculating the calcium ion concentration from the electrode readings

The ion-selective electrode uses an ion selective membrane to allow only calcium ions to penetrate to electrode. A potential drop is developed between the two sides of the sensing membrane. This potential is proportional to the logarithm of the concentration of the calcium ion in accordance with the Nernst equation:

$$E = X + S\log(C)$$

Where:

E is the measured voltage

X is the reference potential

S is the slope

C is calcium ion concentration.

The method used to obtain quantitative results from the readings of the ion-elective electrode was to prepare calibration solutions of known calcium ion concentration. The voltage readings of these calibration solutions of known concentration were then used to plot a graph to obtain the Nernst equation. The electrode potential of each of the test specimens of unknown calcium ion concentration was then measured with the ion-selective electrode. The calcium ion concentration of the test solutions could then be calculated using the Nernst equation.

2.6 Statistical analysis

Statistical analysis of the results obtained were carried out using Minitab Release 15 (Minitab Ltd, UK).

2.6.1 Bond strength test results

To test the normality of the data, a Kolomogrov-Smirnov test was performed on sample sets using a significance value of P= 0.05. Since the data varied significantly from the pattern expected from a population with a normal distribution, data analysis was performed with distribution free test methods. Non-parametric, Kruskal-Wallis and Mann-Whitney tests were subsequently performed with a significance level of P= 0.05.

Box and whisker plots were produced to highlight the median and inter-quartile ranges of the bond strength data.

2.6.2 ARI and mechanism of bond failure results

The ARI scores were graphically represented and Kruskal-Wallis and Mann-Whitney non-parametric statistical tests were used to analyse the ordinal ARI score results. A significance level of P=0.05 was used. The ARI scores were then categorised into adhesive

or cohesive mechanism of bond failures and a Chi-square statistical test (X^2) was performed on the nominal data. A significance level of P=0.05 was used.

2.6.3 Calcium ion release results

As the results of the calcium ion release test was found to vary significantly from a distribution pattern expected from a population with a normal distribution, data analysis was performed with distribution free test methods. Non-parametric, Kruskal-Wallis and Mann-Whitney tests were performed with a significance level of P= 0.05.

A power calculation was performed retrospectively using the data from the 10 groups (5 ACP and 5 TCP groups) each having 10 specimens (10 ACP tubes each ACP group and 10 TCP tubes in each TCP group). Therefore there were in total 100 tubes used in the test.
The largest standard deviation was seen in the TCP group at 18.6 X 10^{-5}. A required difference of 11.9 X 10^{-5} was chosen as being significant as a change of this magnitude would be equivalent to a 100% change in the mean concentration of calcium ion release from the ACP composite groups (table 3.17). A standardised difference of 11.9 X 10^{-5} /18.6 X 10^{-5} = 0.64 produced a sample size of 100 for 80% power at P<0.01 and a sample size of 75 at P<0.05 using Altman's nomogram (Altman, 1991). Therefore no further samples were needed for this test.

2.7 Materials used

1. 420 Maxillary premolars
2. 420 uncoated victory series premolar brackets (3M Unitek, Monorovia, California)
3. 420 Specimen tubes
4. Thymol crystals
5. Distilled water
6. Circular bone saw
7. High speed handpiece
8. Slow speed handpiece
9. Self-curing orthodontic resin
10. Brass cylinders
11. Wax
12. Rubber cup
13. Pumice
14. 37% phosphoric acid etch
15. Ortholux LED Curing Light (3M Unitek, Monrovia)
16. Transbond XT primer (3M Unitek, Monorovia, California)
17. Transbond XT composite (3M Unitek, Monorovia, California)
18. Fugi Ortho Conditioner (GC corporation, Tokyo)
19. Fugi Ortho LC (GC corporation, Tokyo)
20. Aegis Ortho primer (Bosworth Co., Skokie, Illinois)
21. Aegis Ortho composite (Bosworth Co., Skokie, Illinois)

22. Bisphenyl-A diglycidyl ether (Sigma-Aldrich, UK)

23. Triethylene glycol dimethacylate (Sigma-Aldrich, UK)

24. Camphorquinone (Sigma-Aldrich, UK)

25. Dimethylaminoethylmethacrylate (Sigma-Aldrich, UK)

26. Butylated hydroxytoluene (Sigma-Aldrich, UK)

27. Silanated Barium glass (Ivoclar, Vivadent Ltd, UK)

28. Tri-calcium phosphate

29. SPEED mixer (Siemens, DAC 150 FVZ-K)

30. Balance (Mettler AE 163, Toledo Ltd, UK)

31. Hot plate

32. Magnetic Stirrer

33. Specimen tubes

34. Foil

35. Refrigerator

36. Incubator

37. Instron Universal Testing Machine (Model 5544)

38. Wire loop

39. Brass cylinder instron jig

40. Force Gauge (Correx Co, Bern, Switzerland)

41. Stereo optical Microscope X 10 magnification

42. Calcium release specimen template mould

43. Calcium ion electrode (Jenway meter, model 3510)

44. Calcium chloride

45. Microsoft Excel

46. Minitab 15 statistical program

Chapter Three

Results

Chapter Three: Results

3.1 Bond strength test

3.1.1 Results summary

Table 3.1. Results of bond strength test groups

Bond Strength Test Group	Adhesive	Storage	Mean Bond Strength (MPa)	Standard Deviation (MPa)
1	Transbond (composite)	Dry	15.29	4.30
2	Transbond (composite)	Wet	12.30	2.18
3	Fugi Ortho LC (RMGIC)	Dry	11.76	3.16
4	Fugi Ortho LC (RMGIC)	Wet	9.55	4.35
5	Aegis Ortho (ACP composite)	Dry	11.89	3.08
6	Aegis Ortho (ACP composite)	Wet	10.08	4.09
7	Experimental 1 (CaP-0)	Dry	12.41	3.24
8	Experimental 1 (CaP-0)	Wet	9.21	2.44
9	Experimental 2 (CaP-1)	Dry	8.84	2.24
10	Experimental 2 (CaP-1)	Wet	2.26	0.57
11	Experimental 3 (CaP-5)	Dry	8.37	2.14
12	Experimental 3 (CaP-5)	Wet	1.93	0.46
13	Experimental 4 (CaP-10)	Dry	8.07	1.89
14	Experimental 4 (CaP-10)	Wet	1.46	0.54

3.1.2 Frequency distribution of bond strength test groups

Figure 3.1. Box and whisker plot of dry bond strength (Tukey, 1977): The plot illustrates bond strengths based on the median, quartiles and outlying values. The box represents the interquartile range (that contains 50% of values), the whisker is the range of lowest to highest values, the black line is the median score and an asterix marks an outlying value.

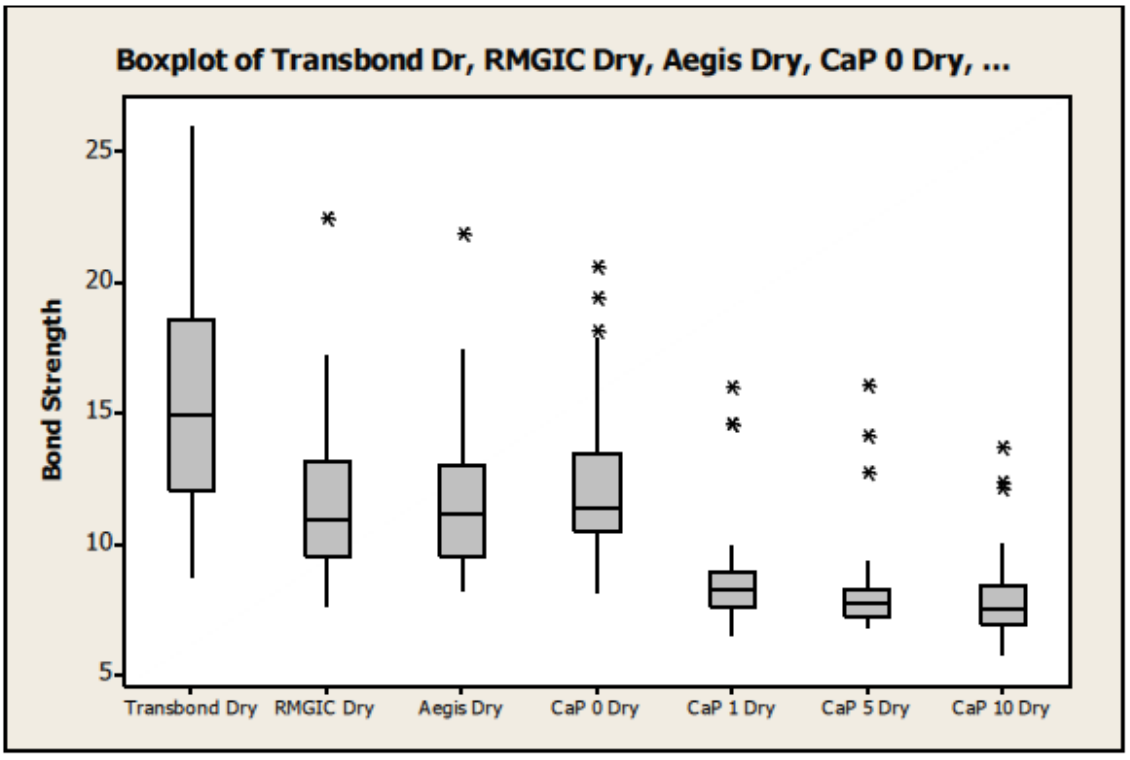
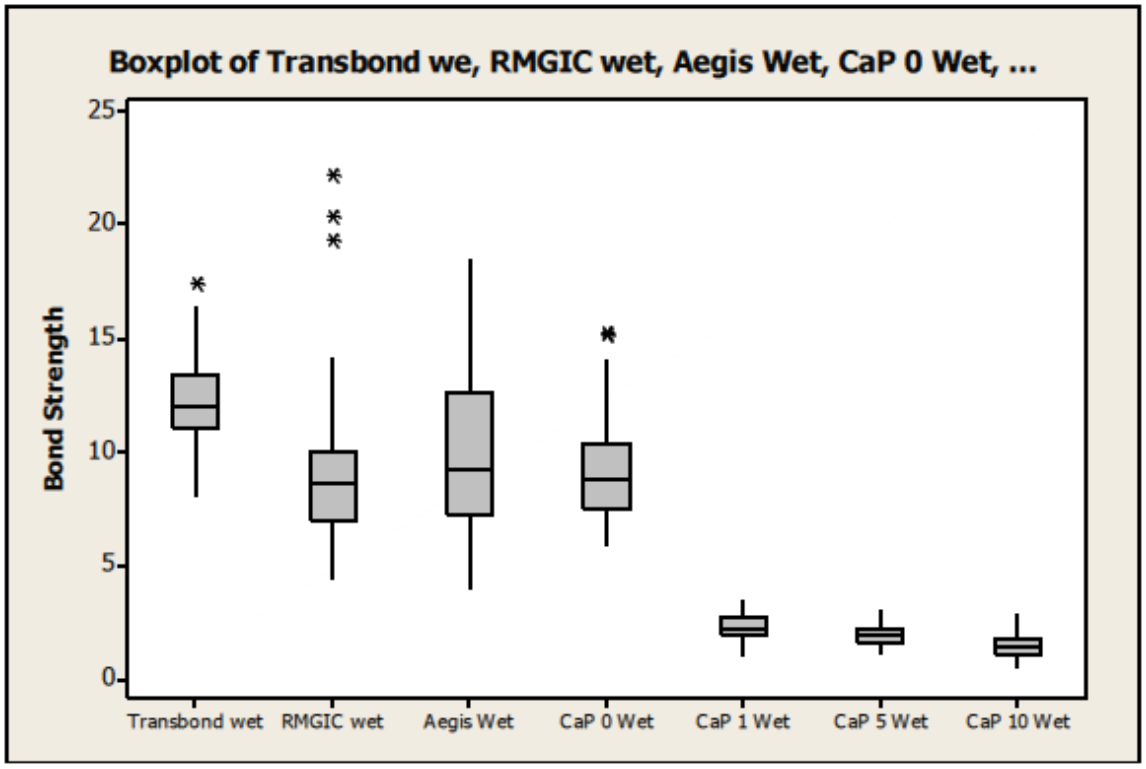
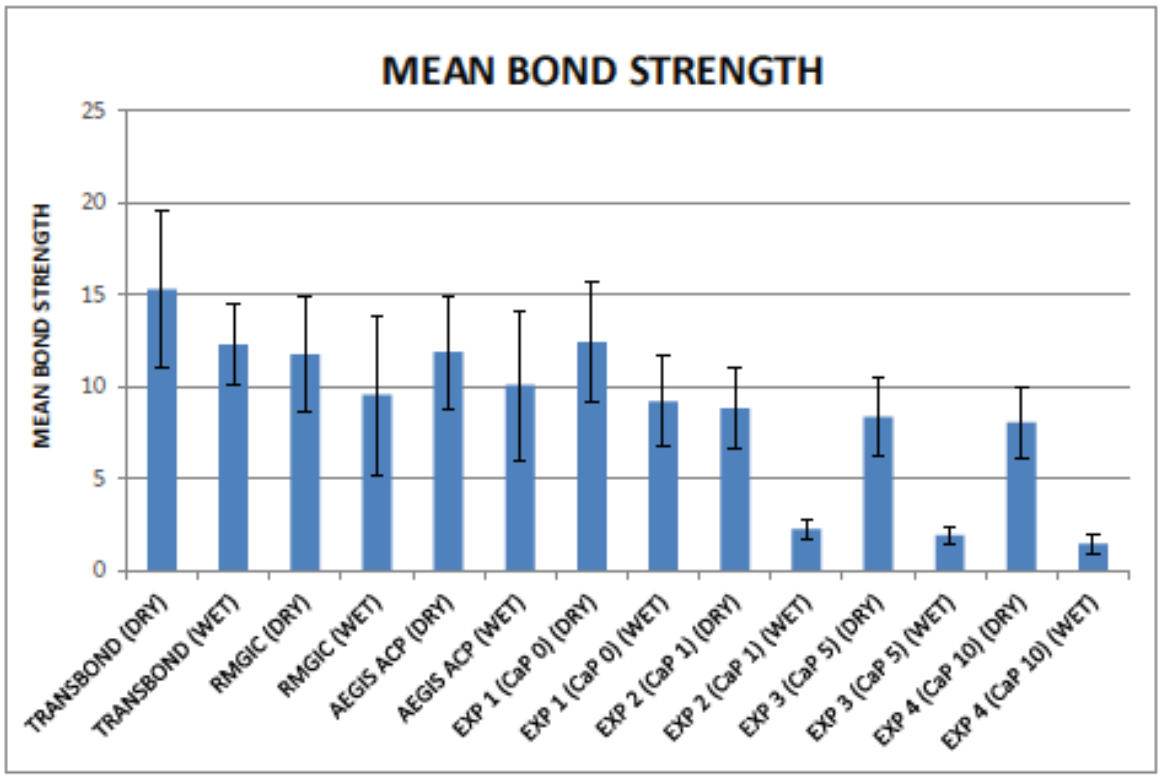


Figure 3.2. Box and whisker plot of the wet bond strength: The plot illustrates bond strengths based on the median, quartiles and outlying values. The box represents the interquartile range (that contains 50% of values), the whisker is the range of lowest to highest values, the black line is the median score and an asterix marks an outlying value.



3.1.3. Bar chart of mean bond strengths of test groups

Figure 3.3. Bar chart of mean bond strengths and standard deviation of all materials tested in dry and wet storage.



3.1.4 Statistical analysis of bond strength test results

3.1.4.1. Bond strengths -Dry

Kruskal-Wallis Test

Table 3.2 Kruskal-Wallis test of dry bond strengths.

Adhesive	N	Median	Ave Rank	Z
Transbond	30	14.976	167.4	6.02
RMGIC	30	10.994	129.1	2.29
Aegis	30	11.159	132.0	2.58
CaP 0	30	11.424	138.9	3.26
CaP 1	30	8.325	69.1	-3.54
CaP 5	30	7.771	53.4	-5.07
CaP 10	30	7.574	48.6	-5.54
Overall	210		105.5	

H = 109.49 DF = 6 P= 0.000

The Kruskal-Wallis test showed that there was statistically significant differences between the bond strengths of the adhesives following dry storage. Therefore a Mann-Whitney test was performed to determine which groups had statistically significant differences.

Mann-Whitney Test (P values)

Table 3.3 Mann-Whitney test of dry bond strengths.

	Transbond	RMGIC	Aegis	CaP 0	CaP 1	CaP 5	CaP 10
Transbond	x	x	x	x	x	x	x
RMGIC	0.0007*	x	x	x	x	x	x
Aegis	0.0010*	0.7958	x	x	x	x	x
CaP 0	0.0044*	0.3403	0.4553	x	x	x	x
CaP 1	0.0000*	0.0000*	0.0000*	0.0000*	x	x	x
CaP 5	0.0000*	0.0000*	0.0000*	0.0000*	0.0451*	x	x
CaP 10	0.0000*	0.0000*	0.0000*	0.0000*	0.0232*	0.3632	x

The Mann-Whitney test showed that following dry storage:

- The bond strength of Transbond was statistically significantly greater than all the other adhesives tested.
- There was no statistical difference between the bond strength of RMGIC (Fugi Ortho LC) and Aegis Ortho or experimental composite 1 (containing 0% TCP).
- The bond strength of the experimental composites containing TCP at 1%, 5% and 10% were statistically significantly lower than that achieved with all other materials.
- As the concentration of TCP was increased in the experimental, a statistically significant reduction in bond strength was seen.

3.1.4.2.Bond Strengths -Wet

Kruskal-Wallis Test

Table 3.4 Kruskal-Wallis test for wet bond strengths.

Adhesive	N	Median	Ave Rank	Z
Transbond	30	12.007	176.2	6.88
RMGIC	30	8.649	138.3	3.19
Aegis	30	9.249	147.9	4.13
CaP 0	30	8.770	139.6	3.32
CaP 1	30	2.165	61.2	-4.31
CaP 5	30	1.935	48.5	-5.55
CaP 10	30	1.437	26.8	-7.66
Overall	210		105.5	

H= 166.06 DF = 6 P = 0.000

The Kruskal-Wallis test showed that there was statistically significant differences between the bond strengths of the adhesives following wet storage. Therefore a Mann-Whitney test was performed to determine which groups had statistically significant differences.

Mann-Whitney Test (P values)

Table 3.5 Mann-Whitney test for wet bond strength

	Transbond	RMGIC	Aegis	CaP 0	CaP 1	CaP 5	CaP 10
Transbond	x	x	x	x	x	x	x
RMGIC	0.0000*	x	x	x	x	x	x
Aegis	0.0073*	0.4290	x	x	x	x	x
CaP 0	0.0000*	0.5895	0.4643	x	x	x	x
CaP 1	0.0000*	0.0000*	0.0000*	0.0000*	x	x	x
CaP 5	0.0000*	0.0000*	0.0000*	0.0000*	0.0256*	x	x
CaP 10	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0004*	x

The Mann-Whitney test showed that following wet storage:

- The bond strength of Transbond was statistically significantly greater than all the other adhesives tested.
- There was no statistical difference between the bond strength of RMGIC (Fugi Ortho LC), Aegis Ortho or experimental composite 1 (containing 0% TCP).
- The bond strength of the experimental composites containing TCP at 1%, 5% and 10% were statistically significantly lower than that achieved with all other materials.
- As the concentration of TCP was increased in the experimental composite, a statistically significant reduction in bond strength was seen.

3.1.4.3 Dry vs Wet bond strength

Mann-Whitney Test (P values)

Table 3.6 Mann-Whitney test for dry vs wet bond strength

	Transbond	RMGIC	Aegis	CaP 0	CaP 1	CaP 5	CaP 10
Transbond	0.0046*	x	x	x	x	x	x
RMGIC	x	0.0005*	x	x	x	x	x
Aegis	x	x	0.0436*	x	x	x	x
CaP 0	x	x	x	0.0000*	x	x	x
CaP 1	x	x	x	x	0.0000*	x	x
CaP 5	x	x	x	x	x	0.0000*	x
CaP 10	x	x	x	x	x	x	0.0000*

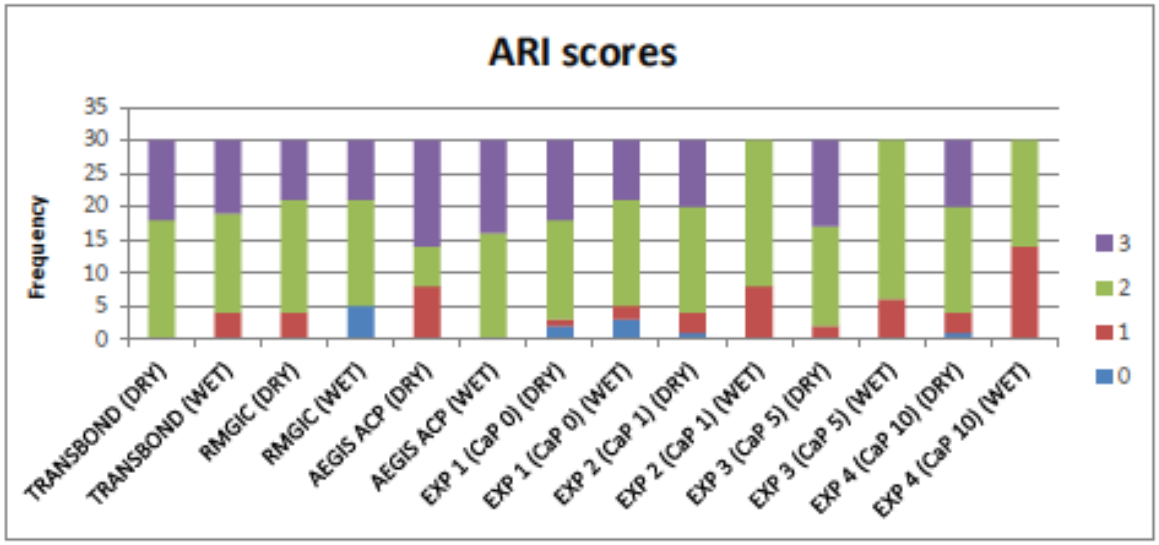
The Mann-Whitney test showed:

- All adhesives suffered a statistically significant reduction in bond strength following wet storage.

3.2. Results of ARI (Adhesive Remnant Index) scores

3.2.1. Graphical representation of ARI scores

Figure 3.4. Graphical representation of ARI scores.



ARI scores

0	No adhesive left on the tooth
1	Less than half of the adhesive left on the tooth
2	More than half of the adhesive left on the tooth
3	All of the adhesive left on the tooth, with distinct impression of the bracket mesh

3.2.2. Table of results of ARI

Table 3.7 Results of ARI scores.

ARI	1	2	3	4	5	6	7	8	9	10	11	12	13	14
0	0	0	0	5	0	0	2	3	1	0	0	0	1	0
1	0	4	4	0	8	0	1	2	3	8	2	6	3	4
2	18	15	17	16	6	16	15	16	16	22	15	24	16	16
3	12	11	9	9	16	14	12	9	10	0	13	0	10	0

* ARI - Adhesive Remnant Index Score (0-3)
* 1-14 - test groups

3.2.3 Statistical analysis of ARI scores

3.2.3.1 Dry ARI

Kruskal-Wallis Test

Table 3.8 Kruskal-Wallis test of dry ARI scores.

Adhesive	N	Median	Ave Rank	Z
Transbond	30	2.000	114.0	0.83
RMGIC	30	2.000	96.5	-0.88
Aegis	30	3.000	109.8	0.42
CaP 0	30	2.000	107.0	0.14
CaP 1	30	2.000	99.2	-0.62
CaP 5	30	2.000	113.0	0.73
CaP 10	30	2.000	99.2	-0.62
Overall	210		105.5	

H= 2.52 DF = 6 P=0.866
H= 3.07 DF = 6 P= 0.800 (adjusted for ties)

The Kruskal-Wallis test showed that there was no statistically significant differences between the ARI scores of the adhesives following dry storage. Therefore it was not necessary to perform a Mann-Whitney test as this test suggested there were no statistically significant differences. A Mann-Whitney test was performed just for completion below.

Mann-Whitney Test (P values)

Table 3.9 Mann-Whitney test for dry ARI scores.

	Transbond	RMGIC	Aegis	CaP 0	CaP 1	CaP 5	CaP 10
Transbond	x	x	x	x	x	x	x
RMGIC	0.2340	x	x	x	x	x	x
Aegis	0.8650	0.4733	x	x	x	x	x
CaP 0	0.6952	0.5059	0.8073	x	x	x	x
CaP 1	0.3329	0.8766	0.5395	0.6256	x	x	x
CaP 5	0.9705	0.2838	0.9000	0.7227	0.3790	x	x
CaP 10	0.3329	0.8766	0.5395	0.6256	1.0000	0.3790	x

The Mann-Whitney test showed:

- There was no statistical significant differences in the ARI scores of the adhesives following dry storage.

3.2.3.2 Wet ARI

Table 3.10 Kruskal-Wallis test for wet ARI scores

Adhesive	N	Median	Ave Rank	Z
Transbond	30	2.000	125.2	1.92
RMGIC	30	2.000	113.5	0.77
Aegis	30	2.000	144.2	3.77
CaP 0	30	2.000	114.8	0.91
CaP 1	30	2.000	83.8	-2.11
CaP 5	30	2.000	89.1	-1.60
CaP 10	30	2.000	67.9	-3.66
Overall	210		105.5	

H = 34.04 DF = 6 P = 0.000
H = 43.86 DF = 6 P = 0.000 (adjusted for ties)

The Kruskal-Wallis test showed that there was statistically significant differences between the ARI scores of the adhesives following wet storage. Therefore a Mann-Whitney test was performed to determine which groups had statistically significant differences.

Mann-Whitney test (P values)

Table 3.11 Mann-Whitney test for wet ARI

	Transbond	RMGIC	Aegis	CaP 0	CaP 1	CaP 5	CaP 10
Transbond	x	x	x	x	x	x	x
RMGIC	0.5059	x	x	x	x	x	x
Aegis	0.2581	0.0905*	x	x	x	x	x
CaP 0	0.5444	0.9470	0.0905	x	x	x	x
CaP 1	0.0076*	0.0679	0.0001*	0.0519	x	x	x
CaP 5	0.0170*	0.1120	0.0001*	0.0933	0.6627	x	x
CaP 10	0.0004*	0.0112*	0.0000*	0.0061*	0.1858	0.0773	x

The Mann-Whitney test showed that following wet storage:

- There was statistically significant differences between the ARI scores of the tricalcium phosphate (TCP) experimental adhesives containing 1%, 5% and 10% TCP compared to Transbond, Aegis Ortho and the experimental adhesive with no TCP (CaP 0).
- RMGIC showed a statistically significant difference in ARI score compared to Aegis Ortho and the experimental adhesive containing 10% TCP.

3.2.3.3. Dry Vs Wet ARI scores

Mann-Whitney Test (P values)

Table 3.12 Mann-Whitney test for dry vs wet ARI scores.

	Transbond	RMGIC	Aegis	CaP 0	CaP 1	CaP 5	CaP 10
Transbond	0.4553	x	x	x	x	x	x
RMGIC	x	0.7675	x	x	x	x	x
Aegis	x	x	0.6204	x	x	x	x
CaP 0	x	x	x	0.3953	x	x	x
CaP 1	x	x	x	x	0.0144*	x	x
CaP 5	x	x	x	x	x	0.0014*	x
CaP 10	x	x	x	x	x	x	0.0010*

The Mann-Whitney test showed:

- The ARI scores following dry and wet storage for Transbond, RMGIC, Aegis Ortho and experimental composite 1 (0% tricalcium phosphate (TCP)) were not statistically significantly different.
- The experimental composites containing TCP (1%, 5% and 10%) did show a statistically significant difference in ARI score between dry and wet storage.

3.2.4 Mechanism of bond failure of adhesives

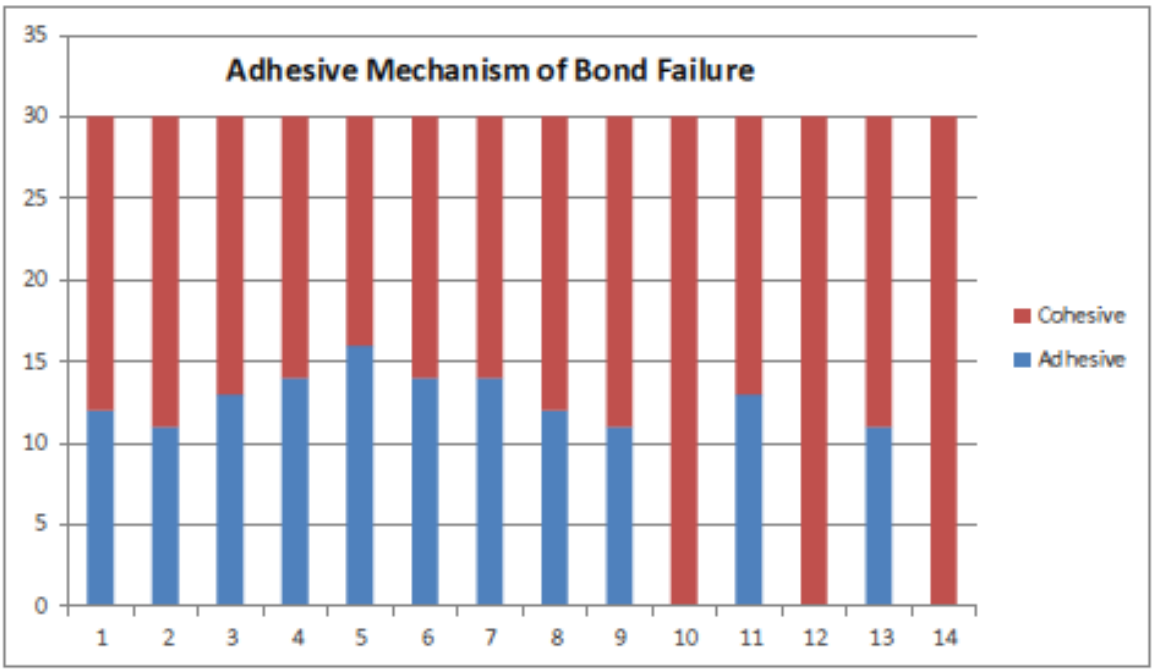
In order to determine if there were differences between the mechanism of bond failure between the materials the adhesive remnant index (ARI) scores were categorised into adhesive and cohesive failure groups. So an ARI score of 0 or 3 would equal an adhesive failure and an ARI score of 1 or 2 would equal an cohesive failure.

Adhesive failure = ARI score 0 or 3

Cohesive failure = ARI score 1 or 2

3.2.4.1. Graphical representation of the mechanism of bond failure of the adhesives

Figure 3.5. Graphical representation of bond failure mechanism of all adhesives.



* Groups 1-14 (30 specimens per group).

3.2.4.2 Table of results of the mechanism of bond failure

Table 3.13 Results of the mechanism of bond failure.

Bond Failure	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Adhesive (ARI 0 or 3)	12	11	9	14	16	14	14	12	11	0	13	0	11	0
Cohesive (ARI 1 or 2)	18	19	21	16	14	16	16	18	19	30	17	30	19	30

*Groups 1-14 (30 specimens per group)

3.2.4.3. Statistical analysis of the mechanism of bond failure of adhesives

3.2.4.3.1. Dry mechanism of bond failures

Chi-Square (P values)

Table 3.14 Chi-Square test results of dry mechanism of bond failures.

	Transbond	RMGIC	Aegis	CaP 0	CaP 1	CaP 5	CaP 10
Transbond	x	x	x	x	x	x	x
RMGIC	0.417	x	x	x	x	x	x
Aegis	0.301	0.067*	x	x	x	x	x
CaP 0	0.602	0.184	0.606	x	x	x	x
CaP 1	0.791	0.584	0.194	0.432	x	x	x
CaP 5	0.793	0.284	0.438	0.795	0.598	x	x
CaP 10	0.791	0.584	0.194	0.432	1.000	0.598	x

The Chi-Square test showed:

- That following dry storage, the only statistically significant difference seen in the mechanism of failure was between RMGIC (Fugi Ortho LC) and Aegis Ortho.

3.2.4.3.2. Wet mechanism of bond failures (P values)

Table 3.15 Chi-Square test results of wet mechanism of bond failures.

	Transbond	RMGIC	Aegis	CaP 0	CaP 1	CaP 5	CaP 10
Transbond	x	x	x	x	x	x	x
RMGIC	0.432	x	x	x	x	x	x
Aegis	0.432	1.000	x	x	x	x	x
CaP 0	0.791	0.602	0.602	x	x	x	x
CaP 1	0.000*	0.000*	0.000*	0.000*	x	x	x
CaP 5	0.000*	0.000*	0.000*	0.000*	1.000	x	x
CaP 10	0.000*	0.000*	0.000*	0.000*	1.000	1.000	x

The Chi-Square test showed:

- That following wet storage, the mechanism of bond failure was statistically significantly different for the experimental adhesives containing tricalcium phosphate (TCP - 1%, 5% and 10%) compared to all the other adhesives.
- The experimental adhesives containing TCP (experimental composites 2,3 and 4) showed a statistically significantly greater percentage of cohesive failure in bonding compared to Transbond XT, Fugi Ortho LC, Aegis Ortho or the experimental composite with no TCP (experimental composite 1).

3.2.4.3.3. Dry Vs Wet mechanism of bond failures (P values)

Table 3.16 Chi-Square test results of dry vs wet mechanism of bond failures.

	Transbond	RMGIC	Aegis	CaP 0	CaP 1	CaP 5	CaP 10
Transbond	0.791	x	x	x	x	x	x
RMGIC	x	0.184	x	x	x	x	x
Aegis	x	x	0.606	x	x	x	x
CaP 0	x	x	x	0.602	x	x	x
CaP 1	x	x	x	x	0.000*	x	x
CaP 5	x	x	x	x	x	0.0000*	x
CaP 10	x	x	x	x	x	x	0.0000*

The Chi-Square test showed:

- That following wet storage, the mechanism of bond failure was statistically significantly different for the experimental composites containing tricalcium phosphate (TCP - 1%, 5% and 10%).
- The experimental composites containing TCP (experimental composites 2,3 and 4) showed a statistically significantly greater percentage of cohesive failure in bonding following wet storage.
- Transbond, Fugi Ortho LC, Aegis Ortho and the experimental composite with no TCP (experimental composite 1) showed no statistically significant difference in the mechanism of bond failure between dry and wet storage.

3.3. Calcium ion release results

3.3.1. Overall results summary

Table 3.17 Overall calcium ion release results summary.

Adhesive	Mean calcium ion release concentration (M)	Standard Deviation (M)
Aegis Ortho (ACP composite)	11.9×10^{-5}	4.5×10^{-5}
Experimental 4 (TCP composite, CaP-10)	89.2×10^{-5}	18.6×10^{-5}

The results of the calcium ion release testing of the amorphous calcium phosphate (ACP) composite (Aegis Ortho) and the experimental tricalcium phosphate (TCP) composite (containing 10% calcium phosphate), showed that the TCP composite released almost eight times a greater level of calcium ions compared to the ACP composite.

3.3.2. Calcium ion release results for Aegis Ortho (ACP composite)

Table 3.18 Calcium ion release results for Aegis Ortho (ACP composite)

Calcium ion release group	Adhesive	Mean calcium ion concentration (M)	Standard Deviation (M)
ACP1	Aegis Ortho	7.8×10^{-5}	2.9×10^{-5}
ACP2	Aegis Ortho	7.5×10^{-5}	*2.3×10^{-5}*
ACP3	Aegis Ortho	17.7×10^{-5}	4.0×10^{-5}
ACP4	Aegis Ortho	11.5×10^{-5}	4.9×10^{-5}
ACP5	Aegis Ortho	1.15×10^{-5}	4.4×10^{-5}
OVERALL		11.9×10^{-5}	4.5×10^{-5}

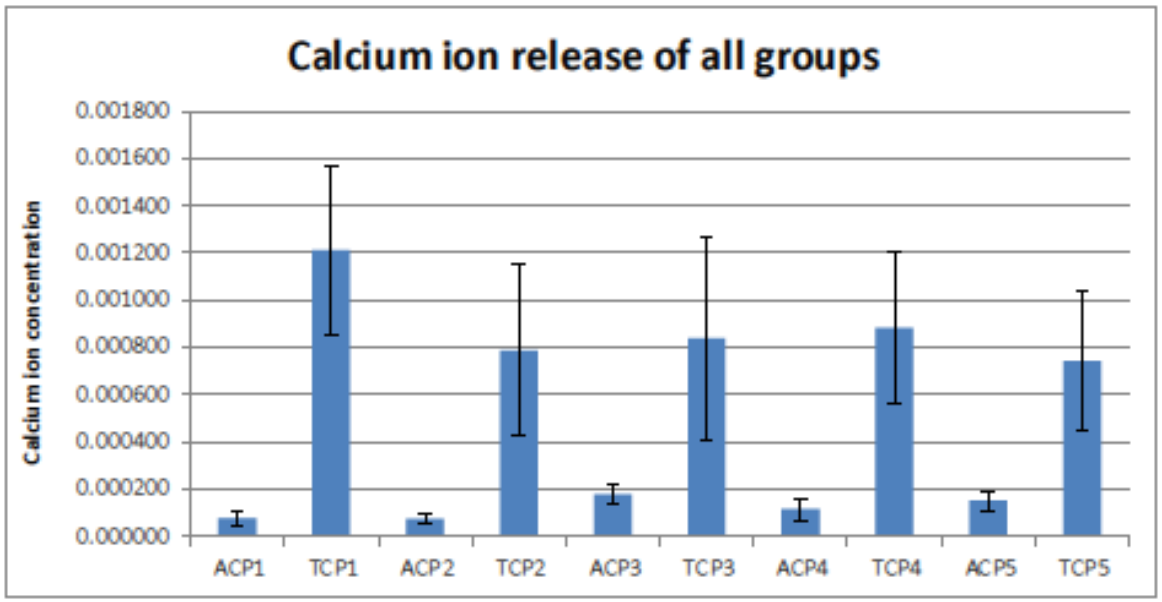
3.3.3. Calcium ion release results for experimental composite 4 (TCP)

Table 3.19 Calcium ion release results for experimental composite 4 (10% TCP).

Calcium ion release group	Adhesive	Mean calcium ion concentration (M)	Standard Deviation (M)
TCP1	Experimental 4 (CaP-10)	121.2×10^{-5}	35.5×10^{-5}
TCP2	Experimental 4 (CaP-10)	78.6×10^{-5}	36.2×10^{-5}
TCP3	Experimental 4 (CaP-10)	83.9×10^{-5}	42.9×10^{-5}
TCP4	Experimental 4 (CaP-10)	88.1×10^{-5}	32.0×10^{-5}
TCP5	Experimental 4 (CaP-10)	74.3×10^{-5}	29.6×10^{-5}
OVERALL		89.2×10^{-5}	18.6×10^{-5}

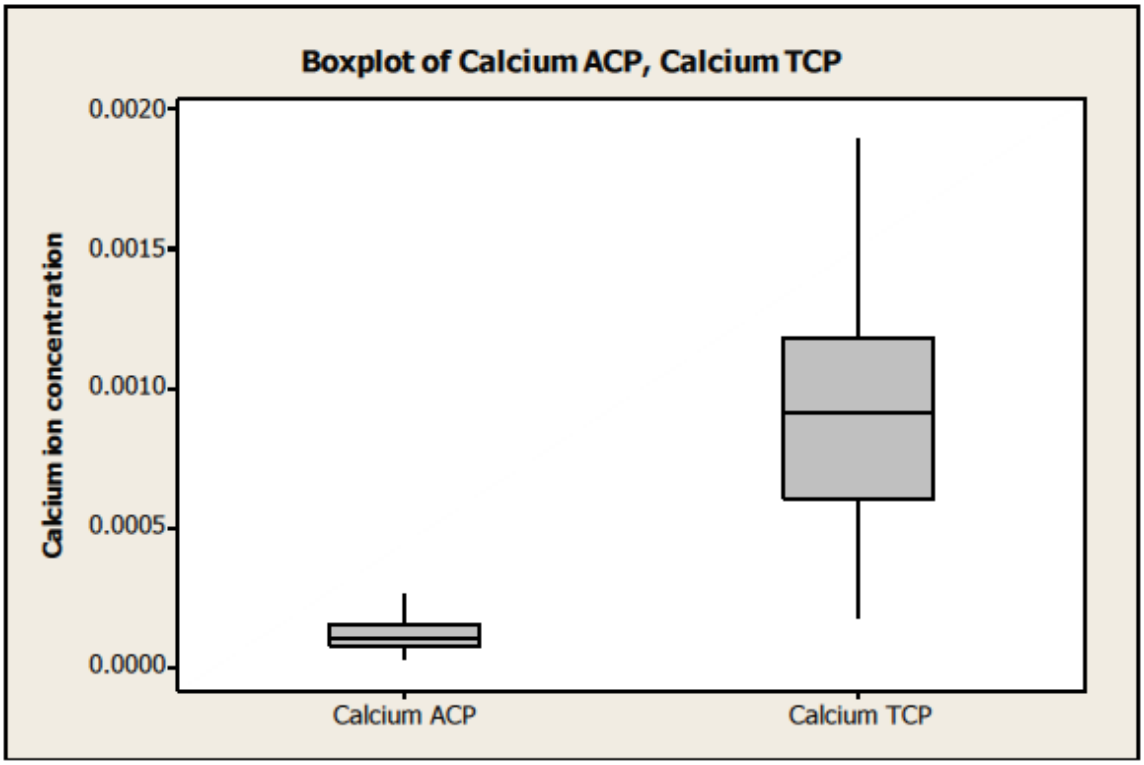
3.3.4 Bar chart of calcium ion release of all groups

Figure 3.6. Calcium ion release of all groups. Bar chart showing the mean and standard deviation of the concentration of calcium ions released after 6 weeks for samples of Aegis Ortho (ACP 1-5) and experimental composite 4 (TCP 1-5).



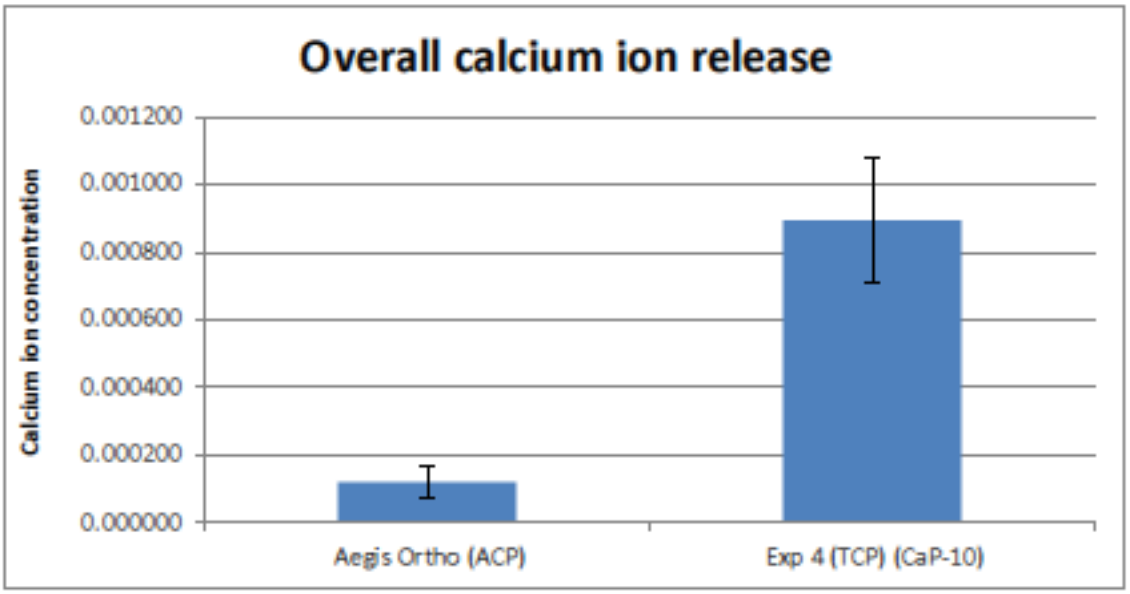
3.3.5 Frequency distribution of the overall calcium ion release

Figure 3.7 Box and whisker plot of overall ACP and TCP calcium ion release (Tukey 1977): The plot illustrates bond strengths based on the median, quartiles and outlying values. The box represents the interquartile range (that contains 50% of values), the whisker is the range of lowest to highest values and the black line is the median score.



3.3.6. Bar chart of overall calcium ion release of ACP and TCP composites

Figure 3.8. Bar chart of overall calcium ion release of ACP and TCP composites. Overall mean and standard deviation of the concentration of calcium ion released from the Aegis Ortho groups (ACP 1-5) and the experimental composite 4 groups (TCP 1-5) after 6 weeks.



3.3.7. Statistical analysis of calcium ion release results

Kruskal-Wallis Test

Table 3.20 Kruskal-Wallis test for calcium ion release.

Adhesive	N	Median	Ave Rank	Z
ACP	50	0.0001099	25.7	-8.54
TCP	50	0.0009162	75.3	8.54
Overall	100		50.5	

H = 73.02 DF = 1 P = 0.000

H = 73.08 DF =1 P = 0.000 (adjusted for ties)

The Kruskal-Wallis test showed that the tricalcium phosphate (TCP) experimental composite containing 10% TCP released a statistically significantly greater concentration of calcium ions compared to amorphous calcium phosphate (ACP) containing Aegis Ortho composite.

Mann-Whitney test results (P value)

Table 3.21 Mann-Whitney test for calcium ion release.

Mann-Whitney Test	TCP
ACP	P=0.0000

The Mann-Whitney test showed that the calcium release from the tricalcium phosphate (TCP) experimental composite, containing 10% TCP was statistically significantly greater (P= 0.0000) than the calcium ion release from the amorphous calcium phosphate (ACP) containing Aegis Ortho composite.

Chapter Four

Discussion

Chapter Four: Discussion

4.1 Bond strengths

4.1.1 Dry Bond Strengths

The bond strength test results (Table 3.1) showed that Transbond (15.29 + 4.30 MPa) produced the greatest bond strength of all the adhesives tested. This was statistically significantly greater than the bond strength produced by all the other adhesives.

Fugi Ortho LC (RMGIC) (11.76 + 3.16 MPa), Aegis Ortho (11.89 + 3.08 MPa) and experimental composite 1 which contained no tricalcium phosphate (TCP) (12.41 + 3.24 MPa) showed no statistical differences in bond strength.

The experimental composites 2 (8.84 + 2.24 MPa), 3 (8.37 +2.14 MPa) and 4 (8.07 +1.89 MPa) which contained 1%, 5% and 10% TCP respectively showed statistically significantly lower bond strengths compared to all the other adhesives. In addition, as the percentage of TCP was increased from 1% to 5% and from 5% to 10%, a statistically significant reduction in bond strength was seen.

Conventional composite has been shown to produce greater shear bond strength compared to GIC and RMGIC (Rock and Abdullah, 1997; Bishara *et al,* 1999; Bishara *et al,* 2001; Bishara *et al,* 2002; Littlewood *et al,* 2000; Grandhi *et al,* 2001; Summers *et al,* 2004).

Conventional composite bonds to enamel via a physical interlock (micromechanical retention of resin tags) this is able to provide a greater bond strength compared to the ionic bonding of GICs and RMGICs to enamel. The RMGIC does not obtain the micromechanical interlock that is achieved by the composite adhesives.

Differences in the composition of the composite adhesives may have affected the observed differences in bond strength. The greater the proportion of filler in the composite, the less the polymerisation shrinkage of the material will be on setting, this therefore would lead to less stress concentration within the material on setting (Van Noort, 2007). Transbond XT is composed of 70-80% quartz filler , whereas Aegis Ortho is composed of an estimated 77% filler in total, of which 38% is amorphous calcium phosphate (ACP) filler. The experimental tricalcium phosphate (TCP) composites were composed of 70% filler in total (including the percentage of TCP filler). The TCP filler in the experimental adhesives were not bonded to the resin matrix via any coupling agent (i.e they were not silanated), therefore the lack of bond at the resin and TCP filler interface may have lead to inefficient stress distribution through the material and areas of stress concentration and crack propagation. Due to the propriety nature of Aegis Ortho, it is not known if the ACP filler is bonded to the resin matrix via a coupling agent. If the ACP is not bonded to the resin then this may create areas of stress concentration and crack propagation. If the ACP is bonded to the resin matrix then this may not allow the ACP to be released at an effective rate in order to promote enamel remineralisation.

The resins used in the composite adhesives also vary. Transbond XT is composed of 10-20% Bis-GMA and 5-10% Bis-EMA, Aegis Ortho is composed of an estimated 27% UDMA resin and the experimental composites were composed of 30% Bis-GMA and TEGDMA in a 60:40 mix ratio. The use of TEGDMA in the experimental adhesive may have been responsible for the reduced bond strength as TEGDMA undergoes greater polymerisation shrinkage compared to Bis-GMA and UDMA monomers. (Floyd and Dickens, 2005). Aegis Ortho may also undergo greater polymerisation shrinkage compared to Transbond XT as it has been shown that UDMA undergoes greater polymerisation shrinkage compared to Bis-GMA resins (Atai *et al,* 2005).

4.1.2 Wet Bond Strengths

The bond strength test results (Table 3.1) following wet storage showed that Transbond (12.30 + 2.18 MPa) produced the greatest bond strength which was statistically significantly greater than that of any other adhesive.

Fugi Ortho LC (RMGIC) (11.76 + 4.35 MPa), Aegis Ortho (10.08 + 4.09 MPa) and experimental composite 1 (9.21 + 2.44 MPa) which contained no tricalcium phosphate (TCP) produced clinically acceptable bond strengths that were not statistically significantly different from each other.

The experimental composites 2 (2.26 + 0.57 MPa) , 3 (1.93 + 0.46 MPa) and 4 (1.46 + 0.54 MPa) containing TCP at 1%, 5% and 10% respectively showed a statistically significantly reduced bond strength compared to the other adhesives. In addition, as the percentage of TCP was increased from 1% to 5% and from 5% to 10%, a statistically significant reduction in bond strength was seen. The significant reduction in bond strength seen following wet storage may be attributed to the hydrolytic degradation suffered by the TCP containing experimental composites. An increased susceptibility to hydrolytic degradation would correlate to the the increased incidence of cohesive bond failures of the TCP containing experimental composites (experimental composites 2,3 and 4) that was found following storage in aqueous solution.

4.1.3 Dry and wet storage effects on bond strengths

The bond strengths of the adhesives investigated showed a similar pattern of bond strength when they were stored dry as when they were stored wet (table 3.1). When stored dry and wet, conventional composite (Transbond XT) produced the statistically greatest bond strength followed by the amorphous calcium phosphate (ACP) composite (Aegis Ortho), RMGIC (Fugi Ortho LC) and experimental composite 1 (containing 0% tricalcium phosphate (TCP)) which did not statistically differ from each other.

The bond strengths of the experimental adhesives containing TCP (experimental composites 2,3 and 4) were statistically significantly lower than the other adhesives. Additionally, as the percentage of TCP increased, the bond strength decreased.

The bond strength of all the adhesives reduced after they were stored in aqueous solution for one week compared to when they were stored dry. The bond strength of the composite (Transbond XT) reduced by 2.99 MPa, the ACP composite (Aegis Ortho) bond strength reduced by 1.81 MPa, the bond strength of the RMGIC (Fugi Ortho LC) reduced by 2.21 MPa, experimental composite 1 (0% TCP) bond strength reduced by 3.20 MPa, experimental composite 2 (1% TCP) bond strength reduced by 6.58 MPa, experimental composite 3 (5% TCP) bond strength reduced by 6.44 MPa and experimental composite 4 (10% TCP) bond strength reduced by 6.61 MPa. The reduction in bond strength seen when the adhesives were stored wet were all statistically significant.

The reduction in bond strength seen in aqueous storage suggests that all the adhesives undergo hydrolytic degradation. In order for composites to have acceptable mechanical properties it is important that the filler and the resin are strongly bonded to each other. The resin component is hydrophobic whereas the glass filler is hydrophilic, therefore a silane coupling agent is commonly used to achieve a bond between the two components. This bond is important to ensure that there is efficient stress transfer between the resin and the glass filler. If there is no bond between the resin and the filler then this would likely create crack initiation sites resulting in excessive creep and eventually fracture of the composite. There is evidence that the quality of this interface influences the extent to which the composite is affected by solvents. The glass filler in Transbond XT is presilanated, thus creating a bond between the filler and the resin. Due to the propriety nature of Aegis Ortho, the manufacturers did not state whether the glass filler was presilanated, however as this material did not show a significant deterioration in bond strength following aqueous storage, it may be that the filler is bonded to the resin via a coupling agent to maintain the mechanical integrity of the composite. This bonding of the filler to the resin may be one of the reasons why Transbond XT and Aegis Ortho did not undergo as significant a deterioration in bond strength as the TCP containing experimental composite adhesives. The experimental composites did not have a coupling agent to bond the tricalcium phosphate (TCP) to the resin matrix. This lack of bonding may have produced insufficient stress transfer between the resin and the filler and therefore been a site of crack initiation.

Differences in the glass filler in the composites may have also have contributed to the greater hydrolytic degradation seen in the TCP experimental composites. A greater

proportion of filler will mean a reduced volume of absorbing resin polymer (Oysaed and Ruyter,1986; Ferracane, 1997). The experimental composites developed consisted of 70% filler by weight, this was lower than that of Transbond XT which is between 70-80% and that of Aegis Ortho which is estimated to be around 73%. Solderholm (1983; 1990), Solderholm *et al* (1984) and Oysaed and Ruyter (1986) showed that the mechanism of hydrolytic degradation was increased if there were metallic ions in the filler particles. This is due to the electropositive nature of the metallic ions that tend to react with water. The charge balance following the loss of these metallic ions into water is reestablished by the penetration of hydrogen ions from the water. This increase in concentration of hyroxy ions results in breakdown of the siloxane bonds of the silica network and this leads to an autocatalytic cycle of surface degradation (Martos *et al,* 2003). The experimental composites were produced with silanated barium glass compared to quartz filler used in Transbond XT. Composites containing silica or quartz fillers have been found to be comparatively inert in water compared to composites containing radiopaque glasses (Ferracane, 2006).

Venz and Dickens (1991) showed differences in long term water sorption (6 months) for polymer networks composed of various monomers. They showed TEGDMA to have greater water sorption than Bis-GMA, which in turn showed greater water sorption than UDMA. The differences were due to hydrophilic ester linkages in TEGDMA, hydroxyl groups in Bis-GMA, urethane linkages in UDMA and the presence of ester groups in all the polymer networks. Water enters the polymer network through intermolecular spaces and the extent of water uptake is dependent on the density of the polymer network,

potential for hydrogen bonding and polar interactions. Sideridou *et al,* (2003) compared resins produced with BIS-GMA, TEGDMA, UDMA and Bis-EMA. They showed that TEGDMA produced the most dense polymer network, which was however the most flexible and absorbed the highest amount of water. Bis-GMA formed the most rigid network which absorbed lower water than the resin made by TEGDMA but higher than the resin made by UDMA and Bis-EMA. Bis-EMA absorbed the lowest amounts of water. They also showed that gradual replacement of TEGDMA in copolymers of Bis-GMA with UDMA or Bis-EMA resulted in more flexible resins with lower water sorption. The experimental composite was a Bis-GMA/TEGDMA copolymer compared to Transbond XT which is a Bis-GMA/Bis-EMA copolymer and Aegis Ortho which is a UDMA polymer. These differences in the material constituents may be responsible for the differences in bond strength following aqueous storage seen between these materials.

Fugi Ortho LC showed a deterioration in bond strength following aqueous storage. Glass-ionomer cements are susceptible to hygroscopic expansion and desiccation in the initial setting time as loosely bound water can move in or out of the setting material. The addition of resin to RMGIC has provided some protection for the setting reaction, but these cements still remain sensitive to water uptake and loss. The resin used commonly for RMGICs and in Fugi Ortho LC is HEMA which is a hydrophilic resin monomer and this makes the cement susceptible to greater water uptake compared to the adhesives with more hydrophobic monomers (Mount, 2002). Studies have shown that RMGICs have a greater level of water sorption compared to compomers and composite (Musanje, 2001; Toledano, 2003).

It has been shown that composite adhesives and RMGIC's are porous materials and over time absorb moisture (Forsten, 1991; Peutzfeldt, 1997; Ferracane, 2006). Clinically, orthodontic adhesives will be used in an aqueous environment and the bond strengths of all the commercially available adhesives (Transbond, Fugi Ortho LC (RMGIC) and Aegis Ortho) and experimental composite 1 (0% TCP) in wet storage showed clinically acceptable bond strengths.

Experimental composite 1, which contained no TCP showed a comparable bond strength to the ACP composite (Aegis Ortho) and RMGIC (Fugi Ortho LC) in dry and wet storage. The addition of TCP produced a reduction in the bond strength of the experimental composite. However, the bond strength of the 1%, 5% and 10% experimental TCP composites after dry storage were clinically adequate, but the bond strength significantly deteriorated to clinically unacceptable levels after aqueous storage.

The experimental composite with no TCP added (Experimental Composite 1) showed a bond strength that was lower than that produced with the commercial composite adhesive (Transbond XT). The differences are likely to be due to the manufacturing of the composite adhesives. The commercial composite was made in a specialised laboratory where the manufacturing is more refined and controlled compared to the laboratory where the experimental adhesives were developed.

The significant reduction in bond strength of the experimental composites containing TCP (experimental composites 2,3 and 4 (Bond Strength Test Groups 10,12 and 14)) in aqueous

storage suggests that these adhesives are more susceptible to water degradation compared to the commercially available Transbond, Fugi Ortho LC, Aegis Ortho adhesives and the experimental composite developed with no TCP (experimental Composite 1 (Bond Strength Test Group 8)).

4.2 ARI scores and mechanism of bond failure of adhesives

4.2.1 ARI scores and mechanism of bond failure of adhesives stored dry

The ARI scores of all the adhesives tested following dry storage showed no statistically significant differences between them. Comparison of the mechanism of bond failure also showed that there was no statistically significant differences between any of the adhesives tested except between RMGIC (Fugi Ortho LC) and Aegis Ortho, where the RMGIC showed a statistically greater number of cohesive bond failures compared to Aegis Ortho.

4.2.2 ARI scores and mechanism of bond failure of adhesives stored wet

The ARI scores and mechanism of failure analysis following wet storage of the adhesives showed the experimental composites containing tricalcium phosphate (TCP - experimental composites 2,3 and 4) produced a statistically significantly greater number of cohesive bond failures compared to all the other adhesives.

There was no statistically significant differences in ARI scores between Transbond, RMGIC, Aegis Ortho or experimental composite 1 (containing 0% TCP).

4.2.3 Differences in ARI scores and mechanism of bond failure between wet and dry storage

The ARI scores and mechanism of bond failure analysis showed that the experimental composites containing tricalcium phosphate (TCP - experimental composite 2,3 and 4) had a statistically significantly greater number of cohesive bond failures after wet storage compared to dry storage. This increase in cohesive bond failure can be attributed to the fact that the experimental composites containing TCP (experimental composites 2,3 and 4) were more susceptible to hydrolytic degradation than the other adhesives. These materials were shown to be more susceptible to hydrolytic degradation as a result of their ability to leach calcium phosphate, as shown by the results of the calcium ion release tests.

The other adhesives did not show any statistically significant changes in the ARI score or mechanism of bond failure between wet and dry storage.

4.3 Comparison of findings to other research/ studies

There is little research to quantify what the ideal bond strength of orthodontic adhesives should be. Suggestions on clinically acceptable bond strength have been between 4.90 - 7.85 MPa (Reynolds, 1975) and 2.86-7.59 MPa (Keizer *et al,* 1976). The tensile bond strength of enamel is approximately 14.5 MPa (Bowen and Rodriquez, 1962) and so bond strengths below this have been recommended to avoid the risk of enamel damage on debond.

The bond strength testing of the commercially available orthodontic adhesives revealed that the composite (Transbond XT) adhesive produced a higher bond strength than RMGIC (Fugi Ortho LC) adhesive and amorphous calcium phosphate (ACP) composite (Aegis Ortho) as has been shown by other studies.

The ACP composite (Aegis Ortho) produced bond strengths of very similar magnitude to the RMGIC (Fugi Ortho LC) but lower than that of the conventional composite (Transbond XT). This suggests that the addition of ACP to composite compromises the bond strength of the material.

Other studies have found Aegis Ortho (ACP composite) to have a statistically significantly lower bond strength compared to Transbond XT (conventional composite) adhesive. The bond strengths of the commercially available orthodontic adhesives used in this study compared to that found in some other studies are shown in table 4.1.

Table 4.1 Bond strength comparison to other studies.

Bond Strength Group	Adhesive	This study	Dunn et al 2007	Foster et al 2008	Uysal et al 2010	Justus et al 2010	Movahhed et al 2005	Owens and Miller 2000
2	Composite (Transbond XT) stored wet	12.3 +/- 2.18 MPa	119 +/- 9.1 N (approx 10 MPa)	15.2 +/- 3.6 MPa	36.7 +/- 6.8 MPa	8.12 ± 3.10 MPa	11 ± 1.6 MPa	7.9 +/- 2.1
4	RMGIC (Fugi Ortho LC) stored wet.	9.55 +/- 4.35 MPa		8.3 +/- 2.8 MPa		5.71 ± 3.87 MPa	9.6 ± 1.6 MPa	5.3 +/- 1.2
6	ACP composite (Aegis Ortho) stored wet.	10.08 +/- 4.09 MPa	14.2 +/- 3.1 N (approx 1.2 MPa)	6.6 +/- 1.5 MPa	24.2 +/- 5.4 MPa			

The variation in bond strengths reported by these studies are likely to be due to differences in bond strength testing methodology used.

The studies which investigated the bond strength of Aegis Ortho (ACP composite) conducted by Dunn *et al* (2007) , Foster *et al* (2008) and Uysal *et al* (2010) varied in the crosshead speed of the Instron machine and Uysal *et al* (2010) used ceramic brackets which may explain the higher bond strengths reported. Dunn *et al* (2007) and Uysal *et al* (2010) used a chisel rod to debond the brackets as opposed to the wire loop method which is most commonly used in orthodontic bond strength testing (Fox *et al,* 1994).

It would appear from the results that the bond strength of the ACP composite (Aegis Ortho) are inferior to that of the conventional dental composite (Transbond XT).

Studies which investigated the bond strength of Fugi Otho LC (RMGIC) and Transbond XT (conventional composite) (Fricker, 1998; Larmour and Stippups, 2001; Oliveria *et al,* 2004; Summers *et al,* 2004; Reicheneder *et al,* 2009; Brauchli *et al,* 2010) have shown Fugi Ortho LC (RMGIC) to have inferior bond strength compared to Transbond XT (conventional composite) as has been found in this study. Again theses studies all vary in the methodology used for bond strength testing, which could explain differences in the reported bond strengths.

4.4 Bond Strength testing method critique

There is no standardised method of bond strength testing for orthodontic adhesives. Fox *et al* (1994) has made suggestions for a protocol for bond strength testing. The bond strength testing method used in this study was based on these suggestions, and included:

1. The use of surface premolar enamel from teeth extracted from adolescent patients for orthodontic reasons.
2. Teeth used within 6 months from extraction and stored in distilled water prior to debonding.
3. After bonding the teeth were stored at 37^0C
4. Brackets were debonded with an Instron machine.
5. Care was taken to ensure that the point of application and direction of the debonding force was the same for all specimens.
6. 30 specimens were used in each group.
7. The site of failure was recorded.
8. Bond strengths was calculated in megapascals.

Fox *et al* (1994) suggested a cross-head speed of 0.1mm/min of the Instron machine, but the most commonly used cross-head speed in orthodontic bond strength testing is 0.5mm/ min (Eliades and Brantley, 2000) and this speed was chosen for this study. The cross-head speed is not so important as it has been shown that cross-head speeds of 0.1, 0.5, 1.0, and 5.0 mm/min do not influence debonding force measurements (Reis *et al,* 2004; Klocke and

Kahl-Nieke, 2005). The lack of a standardised orthodontic bond strength testing method makes comparison between studies on bond strength testing difficult and intra-study comparisons are more accurate due to all groups of interest being tested by the same method.

All the variables that can affect the bond need to be controlled to produce comparable studies. These factors include:

- The manufacturer of the adhesive used.
- The bracket used for bonding.
- The tooth specimen used and storage method.
- The surface treatment of the enamel prior to bonding.
- The method of setting the adhesive.
- The method of bond strength testing (a standardised method).

4.5 Calcium ion release of experimental TCP composite vs ACP composite

The mean concentration of calcium ions released from the 5 groups (TCP groups 1-5) of tricalcium phosphate (TCP) experimental composite 4 (10% TCP) after 6 weeks was 89.2 x 10^{-5} ($\pm$ 18.6 x 10^{-5}) M. In comparison, the 5 groups (ACP groups 1-5) of amorphous calcium phosphate (ACP) composite (Aegis Ortho) released a concentration of calcium ions after 6 weeks of 11.9 x 10^{-5} ($\pm$ 4.5 x 10^{-5}) M.

These results showed that experimental composite 4 (10% TCP) released almost up to eight times the concentration of the ACP composite (Aegis Ortho). A difference that was shown to be statistically significant.

This significantly greater ability of the TCP experimental composite to leach calcium ions also explains why these materials showed greater hydrolytic degradation and hence more cohesive bond failures following aqueous storage and a significantly lower bond strength.

4.6 Calcium ion release measurement method

The measurement method chosen to detect the release of calcium phosphate from the adhesive composites was to measure the calcium ion release with a calcium ion selective electrode. The accuracy and reproducibility of this method of calcium ion concentration measurement is highly dependent on accurately determining the electrode slope (Rundle, 2000). Therefore, to ensure accuracy of readings, the electrode was calibrated with solutions of known calcium ion concentration prior to each time the calcium ion concentration of the amorphous calcium phosphate (ACP) composite (ACP groups 1-5) and the tricalcium phosphate (TCP) composite (TCP groups 1-5) specimen groups were measured. New calibration solutions were made to calibrate the calcium electrode each time a pair of groups (ACP group 1 and TCP group 1) were to be measured. 5 pairs of groups (ACP and TCP groups 1-5) were measured to obtain sufficient power of the investigation.

4.7 Interplay of aqueous solution storage, calcium ion release and bond strength.

Water sorption is due to the Fickian diffusion process, a process where water molecules from the environment diffuse through the polymer system (Martin *et al,* 2003; Sideridou *et al,* 2003). Water molecules attracted to the polar regions on hydrophilic monomers form hydrogen bonds to these regions. This allows further separation of the polymer network creating more pores and spaces that allow further sorption of water. The presence of voids on the composite surface that develop during the polymerisation process also allows water sorption to occur that leads to hydrolytic degradation which breaks down various components within the composite (Ferracane, 2006). This reduces the mechanical properties of the composite but allows components of the composite to be leached into the environment (Peutzfeldt, 1997).

This explains the results found in this study which have shown the bond strength of the experimental tricalcium phosphate (TCP) composites to deteriorate significantly in aqueous solution storage but release a concentration of calcium ions up to 8 times greater compared to the amorphous calcium phosphate (ACP) composite (Aegis Ortho).

Even though the ACP composite (Aegis Ortho) has a calcium phosphate weight content of 38% compared to the TCP experimental composite (experimental composite 4) which was composed of a 10% calcium phosphate, the experimental composite released a significantly greater concentration of calcium ions.

The bond strength of the ACP composite (Aegis Ortho) did not deteriorate when these specimens were stored in aqueous solution. This suggests that the ACP in Aegis Ortho is locked within the composite via a coupling agent to hybridise the ACP filler to the resin matrix and so the ACP is not able to be leached out. The calcium phosphate fillers in ACP composites have utilised tetraethoxysilane (TEOS) and zirconyl chloride ($ZrOCl_2$) as hybridising agents (Skrtic *et al,* 1996). This allows them to bond to the resin matrix, thus maintaining the mechanical integrity of the composite. This improved mechanical integrity however comes at the expense of limiting the leachability of the calcium phosphate as it is hybridised into the matrix.

TCP is a more stable than ACP which readily forms hydroxyapatite in aqueous solution and it was hypothesised that TCP would therefore maintain better mechanical integrity of the composite. The TCP experimental composites showed comparable bond strengths to the ACP composite in dry storage, demonstrating that the composite had good mechanical integrity in dry storage. The leaching of calcium phosphate seems to be responsible for significantly reducing the mechanical integrity of the TCP experimental composites in aqueous solution. The ARI scores for the TCP experimental composites following aqueous storage support this and showed a significantly greater number of cohesive bond failures.

The experimental composite with no TCP added (experimental composite 1) showed a bond strength that was comparable to the ACP composite (Aegis Ortho) and RMGIC (Fugi Ortho LC) in dry and wet storage. The glass filler used for the experimental composites was presilanated and was therefore hybridised to the resin matrix, thus maintaining the

mechanical integrity of the composite. However, the TCP filler that was used in the TCP containing experimental adhesives (experimental composites 2,3, and 4) did not have any coupling agent to hybridise it to the resin matrix. It is possible that if the TCP was hybridised to the resin matrix then the composite may have better stability in aqueous solution and therefore produce a higher bond strength and fewer cohesive bond failures. However, as mentioned earlier, hybridising the calcium phosphate to the resin matrix would then limit the ability of the composite to leach calcium phosphate.

4.8 Conclusions of the null hypotheses

The null hypotheses proposed for this study were therefore:

1. False for there being no differences between the bond strength of any of the orthodontic adhesives tested. As statistically significant differences were found in the bond strengths of the adhesives tested.

2. False for water not affecting the bond strength of the adhesives. As there were statistically significant reductions in bond strength for all the orthodontic adhesives following aqueous storage.

3. False for the bond strength of the tricalcium phosphate (TCP) experimental composites (experimental composite 2,3, and 4) not being affected by the percentage of TCP (1%, 5% and 10%) in the adhesives. As statistically significant reductions in bond strength were seen as the percentage of TCP was increased.

4. False for there being no difference in the ARI score or mechanism of bond failure between the adhesives. As the TCP containing experimental adhesives (experimental adhesives 2,3 and 4) showed a statistically significantly greater number of cohesive bond failures following aqueous storage.

5. False for there being no difference between the ability of the TCP experimental composite (experimental composite 4 containing 10% TCP) and ACP composite (Aegis Ortho) to leach calcium phosphate. As a statistically significantly greater concentration of calcium ions was leached by the TCP experimental adhesive.

Chapter Five

Conclusion

Chapter Five: Conclusion

This study has shown that orthodontic adhesives suffer from hydrolytic degradation. The bond strengths of composite (Transbond XT), RMGIC (Fugi Ortho LC), amorphous calcium phosphate (ACP) composite (Aegis Ortho) and the tricalcium phosphate (TCP) experimental composite adhesives were all reduced when they were exposed to an aqueous environment.

The highest bond strength was achieved by the conventional composite adhesive (Transbond XT, 15.29 ± 4.30 MPa dry and 12.30 ± 2.18 MPa wet) followed by the ACP composite adhesive (Aegis Ortho, 11.89 ± 3.08 MPa dry and 10.08 ± 4.09 MPa wet) and RMGIC adhesive (Fugi Ortho LC, 11.76 ± 3.16 MPa dry and 9.55 ± 4.35 MPa wet). These adhesives showed clinically acceptable bond strengths in aqueous solution.

Experimental composite adhesive with no TCP (experimental composite 1 (0% TCP), 12.41 ± 3.24 MPa dry and 9.21 ± 2.44 MPa wet) showed clinically acceptable bond strengths in aqueous solution, which was not statistically different to Aegis Ortho or Fugi Ortho LC.

The TCP containing experimental composites (experimental composite 2 (1% TCP), 8.84 ± 2.24 MPa dry, experimental composite 3 (5% TCP), 8.37 ± 2.14 MPa dry and experimental composite 4 (10% TCP), 8.07 ± 1.89 MPa dry) showed they have clinically acceptable bond strengths in dry storage conditions. However, in aqueous solution these composites

suffered significant hydrolytic degradation and produced clinically unacceptably low bond strengths (experimental composite 2 (1% TCP), 2.26 $\pm$ 0.57 MPa wet, experimental composite 3 (5% TCP), 1.93 $\pm$ 0.46 MPa wet and experimental composite 4 (10% TCP), 1.46 $\pm$ 0.54 MPa wet). As the percentage weight of calcium phosphate within the composite was increased the bond strength further deteriorated. The susceptibility to hydrolytic degradation of these composites resulted in a significantly greater number of cohesive bond failures.

However, the TCP containing experimental composite (experimental composite 4, containing 10% TCP) was capable of releasing a significantly greater concentration (up to eight times higher) of calcium ions ($89.2 \times 10^{-5} \pm 18.6 \times 10^{-5}$ M) than the ACP composite (Aegis Ortho) which contained 38% ACP ($11.9 \times 10^{-5} \pm 4.5 \times 10^{-5}$ M). This enhanced ability to leach ions comes at the cost of reducing the mechanical integrity of the composite which leads to greater cohesive bond failure and reduced bond strength.

Therefore, although producing lower bond strengths than the ACP composite (Aegis Ortho), the TCP experimental composites are likely to be capable of a much greater remineralistion effect.

Chapter Six

Future Work

Chapter Six: Future work

This study has shown that orthodontic adhesives suffer from hydrolytic degradation. The bond strength of composite, RMGIC, amorphous calcium phosphate (ACP) composite and tricalcium phosphate (TCP) composite adhesives are reduced when exposed to an aqueous environment.

This study has shown that TCP experimental composite with unhybridised calcium phosphate filler has a greater ability to leach compared to commercially available ACP composite (Aegis Ortho). However, the concentration of calcium and phosphate ions required to produce a clinically significant reduction in enamel demineralisation would require further investigation.

In this study bonding agents were applied to the enamel surface prior to application of the ACP composite (Aegis Ortho) according to the manufacturer's recommendation. It was also applied to the enamel surface prior to the application of the TCP experimental composites. The bonding agent acts as a barrier between the enamel surface and the calcium and phosphate ions leached from the composite. Therefore, although calcium phosphate can be leached into the local environment, it cannot directly leach into the enamel. Omitting the bonding agent or modification of the composite adhesive to include acidic monomers may allow direct adhesion of the adhesive to the enamel surface. Further investigation could be carried out to assess if the effect of omission of the bonding agent affected the bond strength of the adhesives and the effect on remineralisation of enamel if the bonding agent barrier was to be eliminated.

Chapter Seven

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Chapter Seven: References

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Chapter Eight

Appendices

Chapter Eight: Appendices

8.1 Appendix 1 - Raw data for bond strength tests

8.1.1 Group 1 Transbond XT (Dry)

Specimen	Newtons	Stress value (Mpa)	ARI
1	141.65	15.57	2
2	179.7	19.75	3
3	235.58	25.89	3
4	125.97	13.84	2
5	79.52	8.74	3
6	133.56	14.68	2
7	204.57	22.48	2
8	88.68	9.75	3
9	104.78	11.51	2
10	155.89	17.13	2
11	176.23	19.37	2
12	144.89	15.92	3
13	90.76	9.97	2
14	130.78	14.37	3
15	200.76	22.06	2
16	140.78	15.47	3
17	144.78	15.91	2
18	187.67	20.62	2
19	101.05	11.10	3
20	180.57	19.84	2
21	166.89	18.34	2
22	150.78	16.57	2
23	99.98	10.99	2
24	81.38	8.94	3
25	123.33	13.55	3
26	115.59	12.70	2
27	119.9	13.18	3
28	116.62	12.82	2
29	111.45	12.25	3
30	139	15.27	2
Sum	4173.09	458.58	
Mean	139.103	15.29	
S.D	39.13	4.30	

8.1.2 Group 2 Transbond XT (Wet)

Specimen	Newtons	Stress value (Mpa)	ARI
1	126.28	13.88	2
2	86.15	9.47	3
3	91.57	10.06	2
4	118.56	13.03	3
5	104.73	11.51	2
6	158.73	17.44	3
7	95.89	10.54	3
8	112.78	12.39	2
9	108.36	11.91	3
10	127.73	14.04	3
11	73.1	8.03	2
12	111.56	12.26	3
13	108.62	11.94	3
14	89.67	9.85	2
15	109.9	12.08	1
16	148.82	16.35	3
17	121.12	13.31	2
18	120.99	13.30	1
19	101.45	11.15	2
20	98.23	10.79	1
21	73.79	8.11	2
22	105.5	11.59	3
23	107.33	11.79	2
24	118.38	13.01	2
25	103.71	11.40	3
26	107.4	11.80	2
27	138.62	15.23	1
28	129.72	14.25	2
29	139.92	15.38	2
30	120.26	13.22	2
sum	3358.87	369.11	
mean	111.96	12.30	
S.D	19.88	2.18	

8.1.3 Group 3 Fugi Ortho LC (Dry)

Specimen	Newtons	Stress value (Mpa)	ARI
1	94.23	10.35	2
2	69.65	7.65	2
3	86.92	9.55	3
4	204.38	22.46	2
5	115.89	12.74	3
6	117.22	12.88	1
7	99.11	10.89	2
8	110.21	12.11	3
9	101.65	11.17	2
10	86.39	9.49	3
11	127.38	14.00	2
12	104.27	11.46	2
13	97.78	10.75	2
14	98.37	10.81	3
15	100.98	11.10	3
16	132.27	14.54	2
17	133.24	14.64	3
18	101.75	11.18	2
19	75.38	8.28	3
20	156.43	17.19	2
21	144.97	15.93	2
22	146.88	16.14	3
23	86.32	9.49	2
24	97.38	10.70	1
25	77.38	8.50	2
26	88.39	9.71	2
27	79.98	8.79	2
28	88.36	9.71	1
29	85.39	9.38	1
30	101.88	11.20	2
Sum	3210.43	352.79	
Mean	107.01	11.76	
S.D	28.72	3.16	

8.1.4 Group 4 Fugi Ortho LC (Wet)

Specimen	Newtons	Stress value (Mpa)	ARI
1	52.36	5.75	2
2	201.89	22.19	2
3	40.24	4.42	0
4	185.27	20.36	2
5	176.29	19.37	3
6	128.65	14.14	2
7	106.52	11.71	3
8	39.91	4.39	2
9	111.72	12.28	3
10	55.29	6.08	3
11	68.25	7.50	3
12	77.47	8.51	2
13	64.22	7.06	2
14	54.92	6.04	3
15	91.01	10.00	2
16	84.33	9.27	2
17	55.82	6.13	2
18	74.74	8.21	2
19	68.35	7.51	3
20	88.02	9.67	0
21	62.79	6.90	3
22	73.59	8.09	2
23	67.31	7.40	0
24	67.53	7.42	2
25	83.18	9.14	2
26	82.68	9.09	3
27	89.84	9.87	2
28	93.36	10.26	0
29	81.1	8.91	2
30	79.94	8.78	0
Sum	2606.59	286.44	
Mean	86.89	9.55	
S.D	39.54	4.35	

8.1.5 Group 5 Aegis Ortho (Dry)

Specimen	Newtons	Stress value (Mpa)	ARI
1	97.32	10.69	3
2	112.64	12.38	1
3	98.54	10.83	3
4	107.77	11.84	2
5	101.28	11.13	3
6	127.62	14.02	3
7	99.01	10.88	3
8	198.76	21.84	2
9	138.02	15.17	1
10	88.21	9.69	3
11	103.22	11.34	3
12	115.87	12.73	3
13	111.32	12.23	3
14	106.23	11.67	3
15	101.82	11.19	1
16	95.28	10.47	3
17	83.48	9.17	3
18	79.27	8.71	3
19	77.94	8.56	1
20	81.92	9.00	3
21	148.93	16.37	2
22	158.44	17.41	3
23	144.67	15.90	1
24	102.57	11.27	3
25	82.67	9.08	2
26	74.87	8.23	2
27	79.79	8.77	1
28	135.55	14.90	1
29	91.92	10.10	2
30	100.11	11.00	1
Sum	3245.04	356.60	
mean	108.17	11.89	
S.D	28.03	3.08	

8.1.6 Group 6 Aegis Ortho (Wet)

Specimen	Newtons	Stress value (Mpa)	ARI
1	53.33	5.86	3
2	112.54	12.37	3
3	162.8	17.89	2
4	50.6	5.56	2
5	125.38	13.78	2
6	35.9	3.95	3
7	36.9	4.05	2
8	113.65	12.49	3
9	105.65	11.61	2
10	83.79	9.21	2
11	97.21	10.68	2
12	93.28	10.25	2
13	154.38	16.96	3
14	73.28	8.05	3
15	84.55	9.29	3
16	90.28	9.92	2
17	68.95	7.58	3
18	71.78	7.89	3
19	45.98	5.05	2
20	82.47	9.06	2
21	72.35	7.95	3
22	168.39	18.50	2
23	81.66	8.97	3
24	74.47	8.18	2
25	55.39	6.09	2
26	131.44	14.44	2
27	152.48	16.76	3
28	49.2	5.41	3
29	103.73	11.40	2
30	120.23	13.21	3
Sum	2752.04	302.42	
mean	91.73	10.08	
S.D	37.18	4.09	

8.1.7 Group 7 Experimental composite 1 (0% TCP) (Dry)

Specimen	Newtons	Stress value (Mpa)	ARI
1	100.23	11.01	3
2	120.76	13.27	2
3	101	11.10	2
4	97.55	10.72	1
5	109.37	12.02	2
6	104.28	11.46	3
7	79.63	8.75	0
8	111.93	12.30	2
9	97.33	10.70	3
10	103.64	11.39	2
11	176.39	19.38	3
12	102.66	11.28	2
13	96.78	10.64	3
14	85.83	9.43	0
15	91.18	10.02	2
16	109.92	12.08	2
17	187.29	20.58	3
18	138.44	15.21	2
19	127.83	14.05	3
20	117.92	12.96	2
21	111.64	12.27	3
22	110.2	12.11	2
23	165.28	18.16	3
24	78.33	8.61	2
25	85.92	9.44	2
26	73.82	8.11	2
27	162.22	17.83	3
28	98.3	10.80	3
29	88.83	9.76	2
30	152.98	16.81	3
Sum	3387.48	372.25	
Mean	112.92	12.41	
S.D	29.48	3.24	

8.1.8 Group 8 Experimental composite 1 (0% TCP) (Wet)

Specimen	Newtons	Stress value (Mpa)	ARI
1	72.62	7.98	0
2	94.88	10.43	3
3	79.01	8.68	0
4	101.02	11.10	2
5	54.67	6.01	2
6	93.33	10.26	2
7	102.08	11.22	2
8	60.27	6.62	2
9	68.43	7.52	2
10	94.78	10.42	2
11	138.99	15.27	3
12	89.09	9.79	2
13	66.28	7.28	2
14	82.03	9.01	0
15	85.5	9.40	2
16	70.58	7.76	3
17	63.47	6.97	2
18	52.92	5.82	3
19	54.78	6.02	1
20	71.11	7.81	1
21	127.76	14.04	3
22	98.46	10.82	2
23	68.4	7.52	3
24	71.03	7.81	2
25	138.2	15.19	3
26	77.83	8.55	2
27	92.46	10.16	3
28	85.93	9.44	2
29	79.43	8.73	2
30	80.19	8.81	3
sum	2515.53	276.43	
mean	83.851	9.21	
S.D	22.23	2.44	

8.1.9 Group 9 Experimental composite 2 (1% TCP) (Dry)

Specimen	Newtons	Stress value (Mpa)	ARI
1	81.82	8.99	3
2	90.62	9.96	2
3	83.17	9.14	2
4	70.66	7.76	3
5	75.71	8.32	2
6	68.82	7.56	2
7	132.55	14.57	3
8	65.73	7.22	3
9	76.28	8.38	2
10	68.55	7.53	1
11	77.09	8.47	3
12	81.3	8.93	2
13	65.31	7.18	2
14	82.02	9.01	2
15	68.33	7.51	1
16	71.87	7.90	2
17	74.78	8.22	3
18	69.93	7.68	3
19	145.56	16.00	2
20	72.56	7.97	2
21	61.9	6.80	1
22	76.63	8.42	2
23	70.82	7.78	3
24	79.81	8.77	2
25	80.8	8.88	3
26	72.38	7.95	2
27	75.81	8.33	0
28	59.27	6.51	2
29	81.56	8.96	2
30	132.77	14.59	3
SUM	2414.41	265.32	
MEAN	80.480	8.84	
S.D	20.42	2.24	

8.1.10 Group 10 Experimental composite 2 (1% TCP) (Wet)

Specimen	Newtons	Stress value (Mpa)	ARI
1	10.82	1.19	2
2	18.83	2.07	2
3	21.52	2.36	2
4	21.67	2.38	2
5	26.36	2.90	2
6	27.01	2.97	2
7	26.12	2.87	2
8	16.28	1.79	2
9	21.11	2.32	2
10	28.66	3.15	2
11	24.87	2.73	1
12	23.61	2.59	1
13	19.02	2.09	1
14	22.59	2.48	2
15	16.18	1.78	2
16	29.01	3.19	1
17	14.82	1.63	2
18	9.2	1.01	1
19	17.35	1.91	2
20	22.81	2.51	2
21	24.72	2.72	2
22	18.04	1.98	2
23	30.81	3.39	1
24	17.7	1.95	1
25	19.23	2.11	2
26	17.54	1.93	1
27	17.33	1.90	2
28	20.17	2.22	2
29	17.58	1.93	2
30	15.38	1.69	2
SUM	616.34	67.73	
MEAN	20.54	2.26	
S.D	5.21	0.57	

8.1.11 Group 11 Experimental composite 3 (5% TCP) (Dry)

Specimen	Newtons	Stress value (Mpa)	ARI
1	61.71	6.78	3
2	85.19	9.36	2
3	128.39	14.11	2
4	70.29	7.72	1
5	67.82	7.45	3
6	70.75	7.77	2
7	63.7	7.00	2
8	81.03	8.90	3
9	71.16	7.82	2
10	65.55	7.20	3
11	71.73	7.88	2
12	66.51	7.31	3
13	70.69	7.77	3
14	64.26	7.06	2
15	71.15	7.82	2
16	75.78	8.33	2
17	69.52	7.64	3
18	73.71	8.10	2
19	146.1	16.05	3
20	67.88	7.46	2
21	68.05	7.48	3
22	61.72	6.78	3
23	69.74	7.66	2
24	115.72	12.72	3
25	64.58	7.10	3
26	79.33	8.72	1
27	75.25	8.27	2
28	71.06	7.81	3
29	62.69	6.89	2
30	74.46	8.18	2
SUM	2285.52	251.16	
MEAN	76.184	8.37	
S.D	19.50	2.14	

8.1.12 Group 12 Experimental composite 3 (5% TCP) (Wet)

Specimen	Newtons	Stress value (Mpa)	ARI
1	15.29	1.68	1
2	17.74	1.95	2
3	18.62	2.05	2
4	26.61	2.92	1
5	19.02	2.09	2
6	20.46	2.25	2
7	16.36	1.80	2
8	25.14	2.76	2
9	17.92	1.97	2
10	20.29	2.23	2
11	11.83	1.30	2
12	17.23	1.89	2
13	21.73	2.39	2
14	11.8	1.30	1
15	17.47	1.92	1
16	12.74	1.40	2
17	19.2	2.11	2
18	19.81	2.18	2
19	9.94	1.09	2
20	13.11	1.44	2
21	16.87	1.85	1
22	12.74	1.40	2
23	15.3	1.68	2
24	18.55	2.04	2
25	14.03	1.54	1
26	15.87	1.74	2
27	26.76	2.94	2
28	14.93	1.64	2
29	21.77	2.39	2
30	18.25	2.01	2
SUM	527.38	57.95	2
MEAN	17.57	1.93	
S.D	4.20	0.46	

8.1.13 Group 13 Experimental composite 4 (10% TCP) (Dry)

Specimen	Newtons	Stress value (Mpa)	ARI
1	70.48	7.75	3
2	87.29	9.59	2
3	52.78	5.80	2
4	69.01	7.58	2
5	91.29	10.03	2
6	65.63	7.21	2
7	53.72	5.90	2
8	112.78	12.39	3
9	68.44	7.52	2
10	64.08	7.04	3
11	75.87	8.34	1
12	61.1	6.71	2
13	62.88	6.91	1
14	77.72	8.54	2
15	110.78	12.17	1
16	58.82	6.46	2
17	71.55	7.86	3
18	70.61	7.76	2
19	76.94	8.45	3
20	58.17	6.39	2
21	67.89	7.46	3
22	58.36	6.41	2
23	66.99	7.36	2
24	69.78	7.67	3
25	63.83	7.01	2
26	124.67	13.70	3
27	68.84	7.56	0
28	86.9	9.55	2
29	72.18	7.93	3
30	63.99	7.03	3
sum	2203.37	242.13	
mean	73.44	8.07	
S.D	17.17	1.89	

8.1.14 Group 14 Experimental composite 4 (10% TCP) (Wet)

Specimen	Newtons	Stress value (Mpa)	ARI
1	10.89	1.20	1
2	14.07	1.55	1
3	14.66	1.61	2
4	18.92	2.08	2
5	25.19	2.77	1
6	18.43	2.03	2
7	10.78	1.18	1
8	6.87	0.75	2
9	9.72	1.07	1
10	24.98	2.75	1
11	16.27	1.79	1
12	17.2	1.89	2
13	13.24	1.45	1
14	4.11	0.45	2
15	14.82	1.63	1
16	13.86	1.52	2
17	15.39	1.69	2
18	9.29	1.02	1
19	11.39	1.25	2
20	8.89	0.98	1
21	12.62	1.39	2
22	15.26	1.68	2
23	5.16	0.57	2
24	12.92	1.42	1
25	8.28	0.91	2
26	11.83	1.30	2
27	16.46	1.81	2
28	11.82	1.30	1
29	17.43	1.92	2
30	9.02	0.99	1
SUM	399.77	43.93	
MEAN	13.326	1.46	
S.D	4.93	0.54	

8.2 Appendix 2 - Raw data for calcium ion release tests

8.2.1 ACP 1

SAMPLE	mV	log value	Conc. (antilog) (M)
1	210	-3.865	0.000136
2	190	-4.537	0.000029
3	204	-4.0666	0.000086
4	205	-4.033	0.000093
5	200	-8.402	0.000063
6	200	-4.201	0.000063
7	200	-4.201	0.000063
8	205	-4.033	0.000093
9	205	-4.033	0.000093
10	200	-4.201	0.000063
		Mean	0.000078
		S.D	0.000029
Calibration Solution			
0.1M	300		
0.01M	260		
0.001M	230		
0.0001M	214		

8.2.2 ACP 2

SAMPLE	mV	log value	Conc. (antilog) (M)
1	206	-4.0514	0.000089
2	205	-4.0845	0.000082
3	205	-4.0845	0.000082
4	200	-4.25	0.000056
5	210	-3.919	0.000121
6	200	-4.25	0.000056
7	205	-4.0845	0.000082
8	195	-4.4155	0.000038
9	205	-4.0845	0.000082
10	200	-4.25	0.000056
		Mean	0.000075
		S.D	0.000023
Calibration solution			
0.1M	300		
0.01M	265		
0.001M	235		
0.0001M	210		

8.2.3 ACP 3

SAMPLE	mV	log value	Conc. (antilog) (M)
1	200	-3.8559	0.000139
2	205	-3.7159	0.000192
3	200	-3.8559	0.000139
4	204	-3.7439	0.000180
5	210	-3.5759	0.000266
6	200	-3.8559	0.000139
7	205	-3.7159	0.000192
8	200	-3.8559	0.000139
9	205	-3.7159	0.000192
10	205	-3.7159	0.000192
		Mean	0.000177
		S.D	0.000040
Calibration solution			
0.1M	305		
0.01M	265		
0.001M	220		
0.0001M	205		

8.2.4 ACP 4

SAMPLE	mV	log value	Conc. (antilog) (M)
1	215	-3.706	0.000197
2	210	-3.873	0.000134
3	200	-4.207	0.000062
4	215	-3.706	0.000197
5	210	-3.873	0.000134
6	205	-4.04	0.000091
7	200	-4.207	0.000062
8	205	-4.04	0.000091
9	205	-4.04	0.000091
10	205	-4.04	0.000091
		Mean	0.000115
		S.D	0.000049
Calibration solution			
0.1M	300		
0.01M	260		
0.001M	235		
0.0001M	210		

8.2.5 ACP 5

SAMPLE	mV	log value	Conc. (antilog) (M)
1	205	-3.959	0.000110
2	210	-3.806	0.000156
3	210	-3.806	0.000156
4	215	-3.653	0.000222
5	215	-3.653	0.000222
6	205	-3.959	0.000110
7	210	-3.806	0.000156
8	205	-3.959	0.000110
9	205	-3.959	0.000110
10	210	-3.806	0.000156
		Mean	0.000151
		S.D	0.000044
Calibration solution			
0.1M	305		
0.01M	265		
0.001M	230		
0.0001M	210		

8.2.6 TCP 1

SAMPLE	mV	log value	Conc. (antilog) (M)
1	238	-2.9242	0.001191
2	230	-3.193	0.000641
3	240	-2.857	0.001390
4	235	-3.025	0.000944
5	244	-2.7226	0.001894
6	240	-2.857	0.001390
7	235	-3.025	0.000944
8	235	-3.025	0.000944
9	240	-2.857	0.001390
10	240	-2.857	0.001390
		Mean	0.001212
		S.D	0.000355
Calibration solution			
0.1M	300		
0.01M	260		
0.001M	230		
0.0001M	214		

8.2.7 TCP 2

SAMPLE	mV	log value	Conc. (antilog) (M)
1	215	-3.7535	0.000176
2	240	-2.926	0.001186
3	230	-3.257	0.000553
4	225	-3.4225	0.000378
5	238	-2.9922	0.001018
6	230	-3.257	0.000553
7	240	-2.926	0.001186
8	235	-3.0915	0.000810
9	240	-2.926	0.001186
10	235	-3.0915	0.000810
		Mean	0.000786
		S.D	0.000362
Calibration solution			
0.1M	300		
0.01M	265		
0.001M	235		
0.0001M	210		

8.2.8 TCP 3

SAMPLE	mV	log value	Conc. (antilog) (M)
1	215	-3.4359	0.000367
2	210	-3.5759	0.000266
3	215	-3.4359	0.000367
4	235	-2.8759	0.001331
5	220	-3.2959	0.000506
6	230	-3.0159	0.000964
7	235	-2.8759	0.001331
8	230	-3.0159	0.000964
9	235	-2.8759	0.001331
10	230	-3.0159	0.000964
		Mean	0.000839
		S.D	0.000429
Calibration solution			
0.1M	305		
0.01M	265		
0.001M	220		
0.0001M	205		

8.2.9 TCP 4

SAMPLE	mV	log value	Conc. (antilog) (M)
1	230	-3.205	0.000624
2	235	-3.038	0.000916
3	220	-3.539	0.000289
4	235	-3.038	0.000916
5	230	-3.205	0.000624
6	240	-2.871	0.001346
7	235	-3.038	0.000916
8	235	-3.038	0.000916
9	240	-2.871	0.001346
10	235	-3.038	0.000916
		Mean	0.000881
		S.D	0.000320
Calibration solution			
0.1M	300		
0.01M	260		
0.001M	235		
0.0001M	210		

8.2.10 TCP 5

SAMPLE	mV	log value	Conc. (antilog) (M)
1	220	-3.5	0.000316
2	225	-3.347	0.000450
3	235	-3.041	0.000910
4	225	-3.347	0.000450
5	230	-3.194	0.000640
6	235	-3.041	0.000910
7	230	-3.194	0.000640
8	240	-2.888	0.001294
9	235	-3.041	0.000910
10	235	-3.041	0.000910
		Mean	0.000743
		S.D	0.000296
Calibration solution			
0.1M	305		
0.01M	265		
0.001M	230		
0.0001M	210		

8.3 Appendix 3 - Statistical test results for bond strength tests, ARI and mechanism of bond failure

8.3.1 Dry bond strengths

Kruskal-Wallis Test: Bond Strength Dry versus Adhesive Dry

```
Kruskal-Wallis Test on Bond Strength Dry

Adhesive Dry     N  Median  Ave Rank      Z
Aegis Dry       30  11.159     132.0   2.58
CaP 0 Dry       30  11.424     138.9   3.26
CaP 1 Dry       30   8.325      69.1  -3.54
CaP 10 Dry      30   7.574      48.6  -5.54
CaP 5 Dry       30   7.771      53.4  -5.07
RMGIC Dry       30  10.994     129.1   2.29
Transbond Dry   30  14.976     167.4   6.02
Overall        210             105.5

H = 109.49  DF = 6  P = 0.000
```

Mann-Whitney Test and CI: Transbond Dry, RMGIC Dry

```
               N  Median
Transbond Dry  30  14.976
RMGIC Dry      30  10.994


Point estimate for ETA1-ETA2 is 3.453
95.2 Percent CI for ETA1-ETA2 is (1.507,5.212)
W = 1146.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0007
```

Mann-Whitney Test and CI: Transbond Dry, Aegis Dry

```
               N  Median
Transbond Dry  30  14.976
Aegis Dry      30  11.159


Point estimate for ETA1-ETA2 is 3.352
95.2 Percent CI for ETA1-ETA2 is (1.402,5.042)
W = 1139.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0010
```

Mann-Whitney Test and CI: Transbond Dry, CaP 0 Dry

```
                N  Median
Transbond Dry  30  14.976
CaP 0 Dry      30  11.424


Point estimate for ETA1-ETA2 is 2.810
95.2 Percent CI for ETA1-ETA2 is (0.859,4.640)
W = 1108.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0044
```

Mann-Whitney Test and CI: Transbond Dry, CaP 1 Dry

```
                N  Median
Transbond Dry  30  14.976
CaP 1 Dry      30   8.325


Point estimate for ETA1-ETA2 is 6.271
95.2 Percent CI for ETA1-ETA2 is (4.540,7.785)
W = 1303.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

Mann-Whitney Test and CI: Transbond Dry, CaP 5 Dry

```
                N  Median
Transbond Dry  30  14.976
CaP 5 Dry      30   7.771


Point estimate for ETA1-ETA2 is 6.648
95.2 Percent CI for ETA1-ETA2 is (5.006,8.226)
W = 1320.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

Mann-Whitney Test and CI: Transbond Dry, CaP 10 Dry

```
                N  Median
Transbond Dry  30  14.976
CaP 10 Dry     30   7.574


Point estimate for ETA1-ETA2 is 6.920
95.2 Percent CI for ETA1-ETA2 is (5.216,8.535)
W = 1330.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

Mann-Whitney Test and CI: RMGIC Dry, Aegis Dry

```
            N  Median
RMGIC Dry  30  10.994
Aegis Dry  30  11.159


Point estimate for ETA1-ETA2 is -0.145
95.2 Percent CI for ETA1-ETA2 is (-1.387,1.039)
W = 897.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.7958
```

Mann-Whitney Test and CI: RMGIC Dry, CaP 0 Dry

```
            N  Median
RMGIC Dry  30  10.994
CaP 0 Dry  30  11.424


Point estimate for ETA1-ETA2 is -0.636
95.2 Percent CI for ETA1-ETA2 is (-1.837,0.651)
W = 850.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.3403
```

Mann-Whitney Test and CI: RMGIC Dry, CaP 1 Dry

```
            N  Median
RMGIC Dry  30  10.994
CaP 1 Dry  30   8.325


Point estimate for ETA1-ETA2 is 2.535
95.2 Percent CI for ETA1-ETA2 is (1.738,3.534)
W = 1234.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

Mann-Whitney Test and CI: RMGIC Dry, CaP 5 Dry

```
            N  Median
RMGIC Dry  30  10.994
CaP 5 Dry  30   7.771


Point estimate for ETA1-ETA2 is 3.040
95.2 Percent CI for ETA1-ETA2 is (2.085,3.886)
W = 1269.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

Mann-Whitney Test and CI: RMGIC Dry, CaP 10 Dry

```
             N  Median
RMGIC Dry   30  10.994
CaP 10 Dry  30   7.574


Point estimate for ETA1-ETA2 is 3.298
95.2 Percent CI for ETA1-ETA2 is (2.350,4.260)
W = 1263.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

Mann-Whitney Test and CI: Aegis Dry, CaP 0 Dry

```
            N  Median
Aegis Dry  30  11.159
CaP 0 Dry  30  11.424


Point estimate for ETA1-ETA2 is -0.420
95.2 Percent CI for ETA1-ETA2 is (-1.765,0.714)
W = 864.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.4553
```

Mann-Whitney Test and CI: Aegis Dry, CaP 1 Dry

```
            N  Median
Aegis Dry  30  11.159
CaP 1 Dry  30   8.325


Point estimate for ETA1-ETA2 is 2.798
95.2 Percent CI for ETA1-ETA2 is (1.860,3.669)
W = 1246.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

Mann-Whitney Test and CI: Aegis Dry, CaP 5 Dry

```
            N  Median
Aegis Dry  30  11.159
CaP 5 Dry  30   7.771


Point estimate for ETA1-ETA2 is 3.308
95.2 Percent CI for ETA1-ETA2 is (2.283,4.069)
W = 1276.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

Mann-Whitney Test and CI: Aegis Dry, CaP 10 Dry

```
             N  Median
Aegis Dry   30  11.159
CaP 10 Dry  30   7.574


Point estimate for ETA1-ETA2 is 3.459
95.2 Percent CI for ETA1-ETA2 is (2.482,4.431)
W = 1275.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

Mann-Whitney Test and CI: CaP 0 Dry, CaP 1 Dry

```
            N  Median
CaP 0 Dry  30  11.424
CaP 1 Dry  30   8.325


Point estimate for ETA1-ETA2 is 3.181
95.2 Percent CI for ETA1-ETA2 is (2.299,4.126)
W = 1259.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

Mann-Whitney Test and CI: CaP 0 Dry, CaP 5 Dry

```
            N  Median
CaP 0 Dry  30  11.424
CaP 5 Dry  30   7.771


Point estimate for ETA1-ETA2 is 3.641
95.2 Percent CI for ETA1-ETA2 is (2.861,4.499)
W = 1284.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

Mann-Whitney Test and CI: CaP 0 Dry, CaP 10 Dry

```
             N  Median
CaP 0 Dry   30  11.424
CaP 10 Dry  30   7.574


Point estimate for ETA1-ETA2 is 3.852
95.2 Percent CI for ETA1-ETA2 is (2.961,4.867)
W = 1282.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

Mann-Whitney Test and CI: CaP 1 Dry, CaP 5 Dry

```
             N  Median
CaP 1 Dry   30   8.325
CaP 5 Dry   30   7.771


Point estimate for ETA1-ETA2 is 0.498
95.2 Percent CI for ETA1-ETA2 is (0.014,0.954)
W = 1051.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0451
```

Mann-Whitney Test and CI: CaP 1 Dry, CaP 10 Dry

```
             N  Median
CaP 1 Dry   30   8.325
CaP 10 Dry  30   7.574


Point estimate for ETA1-ETA2 is 0.704
95.2 Percent CI for ETA1-ETA2 is (0.100,1.294)
W = 1069.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0232
```

Mann-Whitney Test and CI: CaP 5 Dry, CaP 10 Dry

```
             N  Median
CaP 5 Dry   30   7.771
CaP 10 Dry  30   7.574


Point estimate for ETA1-ETA2 is 0.242
95.2 Percent CI for ETA1-ETA2 is (-0.264,0.749)
W = 977.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.3632
```

8.3.2 Wet bond strengths

Kruskal-Wallis Test: Bond Srength Wet versus Adhesive Wet

```
Kruskal-Wallis Test on Bond Srength Wet

Adhesive Wet     N  Median  Ave Rank      Z
Aegis Wet       30   9.249     147.9   4.13
CaP 0 Wet       30   8.770     139.6   3.32
CaP 1 Wet       30   2.165      61.2  -4.31
CaP 10 Wet      30   1.437      26.8  -7.66
CaP 5 wet       30   1.935      48.5  -5.55
RMGIC Wet       30   8.649     138.3   3.19
Transbond Wet   30  12.007     176.2   6.88
Overall        210              105.5

H = 166.06  DF = 6  P = 0.000
H = 166.06  DF = 6  P = 0.000  (adjusted for ties)
```

Mann-Whitney Test and CI: Transbond wet, RMGIC wet

```
                N  Median
Transbond wet  30  12.007
RMGIC wet      30   8.649


Point estimate for ETA1-ETA2 is 3.637
95.2 Percent CI for ETA1-ETA2 is (2.281,4.839)
W = 1191.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

Mann-Whitney Test and CI: Transbond wet, Aegis Wet

```
                N  Median
Transbond wet  30  12.007
Aegis Wet      30   9.249


Point estimate for ETA1-ETA2 is 2.627
95.2 Percent CI for ETA1-ETA2 is (0.771,4.189)
W = 1097.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0073
```

Mann-Whitney Test and CI: Transbond wet, CaP 0 Wet

```
                N  Median
Transbond wet  30  12.007
CaP 0 Wet      30   8.770


Point estimate for ETA1-ETA2 is 3.254
95.2 Percent CI for ETA1-ETA2 is (2.113,4.414)
W = 1228.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

Mann-Whitney Test and CI: Transbond wet, CaP 1 Wet

```
                N  Median
Transbond wet  30  12.007
CaP 1 Wet      30   2.165


Point estimate for ETA1-ETA2 is 9.907
95.2 Percent CI for ETA1-ETA2 is (9.244,10.709)
W = 1365.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

Mann-Whitney Test and CI: Transbond wet, CaP 5 Wet

```
                N  Median
Transbond wet  30  12.007
CaP 5 Wet      30   1.935


Point estimate for ETA1-ETA2 is 10.215
95.2 Percent CI for ETA1-ETA2 is (9.625,11.047)
W = 1365.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
The test is significant at 0.0000 (adjusted for ties)
```

Mann-Whitney Test and CI: Transbond wet, CaP 10 Wet

```
                N  Median
Transbond wet  30  12.007
CaP 10 Wet     30   1.437


Point estimate for ETA1-ETA2 is 10.684
95.2 Percent CI for ETA1-ETA2 is (10.099,11.487)
W = 1365.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

Mann-Whitney Test and CI: RMGIC wet, Aegis Wet

```
            N  Median
RMGIC wet  30   8.649
Aegis Wet  30   9.249


Point estimate for ETA1-ETA2 is -0.689
95.2 Percent CI for ETA1-ETA2 is (-2.740,1.082)
W = 861.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.4290
```

Mann-Whitney Test and CI: RMGIC wet, CaP 0 Wet

```
            N  Median
RMGIC wet  30   8.649
CaP 0 Wet  30   8.770


Point estimate for ETA1-ETA2 is -0.325
95.2 Percent CI for ETA1-ETA2 is (-1.585,1.037)
W = 878.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.5895
```

Mann-Whitney Test and CI: RMGIC wet, CaP 1 Wet

```
            N  Median
RMGIC wet  30   8.649
CaP 1 Wet  30   2.165


Point estimate for ETA1-ETA2 is 6.298
95.2 Percent CI for ETA1-ETA2 is (5.367,7.159)
W = 1365.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

Mann-Whitney Test and CI: RMGIC wet, CaP 5 Wet

```
            N  Median
RMGIC wet  30   8.649
CaP 5 Wet  30   1.935


Point estimate for ETA1-ETA2 is 6.673
95.2 Percent CI for ETA1-ETA2 is (5.657,7.484)
W = 1365.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
The test is significant at 0.0000 (adjusted for ties)
```

Mann-Whitney Test and CI: RMGIC wet, CaP 10 Wet

```
             N  Median
RMGIC wet   30   8.649
CaP 10 Wet  30   1.437


Point estimate for ETA1-ETA2 is 7.104
95.2 Percent CI for ETA1-ETA2 is (6.145,7.977)
W = 1365.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

Mann-Whitney Test and CI: Aegis Wet, CaP 0 Wet

```
            N  Median
Aegis Wet  30   9.249
CaP 0 Wet  30   8.770


Point estimate for ETA1-ETA2 is 0.509
95.2 Percent CI for ETA1-ETA2 is (-1.181,2.350)
W = 965.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.4643
```

Mann-Whitney Test and CI: Aegis Wet, CaP 1 Wet

```
            N  Median
Aegis Wet  30   9.249
CaP 1 Wet  30   2.165


Point estimate for ETA1-ETA2 is 7.175
95.2 Percent CI for ETA1-ETA2 is (5.963,8.981)
W = 1365.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

Mann-Whitney Test and CI: Aegis Wet, CaP 5 Wet

```
            N  Median
Aegis Wet  30   9.249
CaP 5 Wet  30   1.935


Point estimate for ETA1-ETA2 is 7.476
95.2 Percent CI for ETA1-ETA2 is (6.234,9.360)
W = 1365.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
The test is significant at 0.0000 (adjusted for ties)
```

Mann-Whitney Test and CI: Aegis Wet, CaP 10 Wet

```
             N  Median
Aegis Wet   30   9.249
CaP 10 Wet  30   1.437


Point estimate for ETA1-ETA2 is 7.954
95.2 Percent CI for ETA1-ETA2 is (6.700,9.723)
W = 1365.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

Mann-Whitney Test and CI: CaP 0 Wet, CaP 1 Wet

```
            N  Median
CaP 0 Wet  30   8.770
CaP 1 Wet  30   2.165


Point estimate for ETA1-ETA2 is 6.625
95.2 Percent CI for ETA1-ETA2 is (5.786,7.510)
W = 1365.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

Mann-Whitney Test and CI: CaP 0 Wet, CaP 5 Wet

```
            N  Median
CaP 0 Wet  30   8.770
CaP 5 Wet  30   1.935


Point estimate for ETA1-ETA2 is 6.890
95.2 Percent CI for ETA1-ETA2 is (6.075,7.840)
W = 1365.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
The test is significant at 0.0000 (adjusted for ties)
```

Mann-Whitney Test and CI: CaP 0 Wet, CaP 10 Wet

```
             N  Median
CaP 0 Wet   30   8.770
CaP 10 Wet  30   1.437


Point estimate for ETA1-ETA2 is 7.385
95.2 Percent CI for ETA1-ETA2 is (6.559,8.335)
W = 1365.0
```

```
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

Mann-Whitney Test and CI: CaP 1 Wet, CaP 5 Wet

```
            N  Median
CaP 1 Wet  30  2.1648
CaP 5 Wet  30  1.9346


Point estimate for ETA1-ETA2 is 0.3286
95.2 Percent CI for ETA1-ETA2 is (0.0395,0.6131)
W = 1066.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0256
The test is significant at 0.0256 (adjusted for ties)
```

Mann-Whitney Test and CI: CaP 1 Wet, CaP 10 Wet

```
             N  Median
CaP 1 Wet   30  2.1648
CaP 10 Wet  30  1.4374


Point estimate for ETA1-ETA2 is 0.7994
95.2 Percent CI for ETA1-ETA2 is (0.5076,1.0922)
W = 1234.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
The test is significant at 0.0000 (adjusted for ties)
```

Mann-Whitney Test and CI: CaP 5 Wet, CaP 10 Wet

```
             N  Median
CaP 5 Wet   30  1.9346
CaP 10 Wet  30  1.4374


Point estimate for ETA1-ETA2 is 0.4659
95.2 Percent CI for ETA1-ETA2 is (0.2197,0.7296)
W = 1155.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0004
The test is significant at 0.0004 (adjusted for ties)
```

8.3.3 Dry vs Wet bond strengths

Mann-Whitney Test and CI: Transbond Dry, Transbond wet

```
                N  Median
Transbond Dry  30  14.976
Transbond wet  30  12.007


Point estimate for ETA1-ETA2 is 2.606
95.2 Percent CI for ETA1-ETA2 is (0.833,4.413)
W = 1107.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0046
```

Mann-Whitney Test and CI: RMGIC Dry, RMGIC wet

```
             N  Median
RMGIC Dry   30  10.994
RMGIC wet   30   8.649


Point estimate for ETA1-ETA2 is 2.491
95.2 Percent CI for ETA1-ETA2 is (1.268,3.865)
W = 1151.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0005
```

Mann-Whitney Test and CI: Aegis Dry, Aegis Wet

```
             N  Median
Aegis Dry   30  11.159
Aegis Wet   30   9.249


Point estimate for ETA1-ETA2 is 1.968
95.2 Percent CI for ETA1-ETA2 is (0.028,3.613)
W = 1052.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0436
```

Mann-Whitney Test and CI: CaP 0 Dry, CaP 0 Wet

```
             N  Median
CaP 0 Dry   30  11.424
CaP 0 Wet   30   8.770


Point estimate for ETA1-ETA2 is 2.833
95.2 Percent CI for ETA1-ETA2 is (1.680,4.112)
W = 1203.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

Mann-Whitney Test and CI: CaP 1 Dry, CaP 1 Wet

```
             N  Median
CaP 1 Dry   30   8.325
CaP 1 Wet   30   2.165
```

```
Point estimate for ETA1-ETA2 is 6.020
95.2 Percent CI for ETA1-ETA2 is (5.618,6.480)
W = 1365.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

Mann-Whitney Test and CI: CaP 5 Dry, CaP 5 Wet

```
            N  Median
CaP 5 Dry  30   7.771
CaP 5 Wet  30   1.935


Point estimate for ETA1-ETA2 is 5.843
95.2 Percent CI for ETA1-ETA2 is (5.555,6.180)
W = 1365.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
The test is significant at 0.0000 (adjusted for ties)
```

Mann-Whitney Test and CI: CaP 10 Dry, CaP 10 Wet

```
             N  Median
CaP 10 Dry  30   7.574
CaP 10 Wet  30   1.437


Point estimate for ETA1-ETA2 is 6.144
95.2 Percent CI for ETA1-ETA2 is (5.733,6.611)
W = 1365.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
```

8.3.4 Dry ARI

Kruskal-Wallis Test: ARI Dry versus Adhesive Dry

```
Kruskal-Wallis Test on ARI Dry

Adhesive Dry     N  Median  Ave Rank      Z
Aegis Dry       30   3.000     109.8   0.42
CaP 0 Dry       30   2.000     107.0   0.14
CaP 1 Dry       30   2.000      99.2  -0.62
CaP 10 Dry      30   2.000      99.2  -0.62
CaP 5 Dry       30   2.000     113.0   0.73
RMGIC Dry       30   2.000      96.5  -0.88
Transbond Dry   30   2.000     114.0   0.83
Overall        210              105.5

H = 2.52  DF = 6  P = 0.866
H = 3.07  DF = 6  P = 0.800  (adjusted for ties)
```

Mann-Whitney Test and CI: ARI Trandbond Dry, ARI RMGIC Dry

```
                    N  Median
ARI Trandbond Dry  30  2.0000
ARI RMGIC Dry      30  2.0000


Point estimate for ETA1-ETA2 is -0.0000
95.2 Percent CI for ETA1-ETA2 is (0.0001,0.9999)
W = 996.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.2340
The test is significant at 0.1718 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI Trandbond Dry, ARI Aegis Dry

```
                    N  Median
ARI Trandbond Dry  30  2.0000
ARI Aegis Dry      30  3.0000


Point estimate for ETA1-ETA2 is -0.0000
95.2 Percent CI for ETA1-ETA2 is (-0.0000,-0.0001)
W = 927.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.8650
The test is significant at 0.8522 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI Trandbond Dry, ARI CaP 0 Dry

```
                    N  Median
ARI Trandbond Dry  30  2.0000
ARI CaP 0 Dry      30  2.0000


Point estimate for ETA1-ETA2 is -0.0000
95.2 Percent CI for ETA1-ETA2 is (-0.0001,0.0001)
W = 942.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.6952
The test is significant at 0.6552 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI Trandbond Dry, ARI CaP 1 Dry

```
                    N  Median
ARI Trandbond Dry  30  2.0000
ARI CaP 1 Dry      30  2.0000


Point estimate for ETA1-ETA2 is 0.0000
95.2 Percent CI for ETA1-ETA2 is (0.0001,0.0001)
W = 981.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.3329
The test is significant at 0.2694 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI Trandbond Dry, ARI CaP 5 Dry

```
                    N  Median
ARI Trandbond Dry  30  2.0000
ARI CaP 5 Dry      30  2.0000


Point estimate for ETA1-ETA2 is 0.0000
95.2 Percent CI for ETA1-ETA2 is (-0.0001,-0.0002)
W = 918.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.9705
The test is significant at 0.9662 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI Trandbond Dry, ARI CaP 10 dry

```
                    N  Median
ARI Trandbond Dry  30  2.0000
ARI CaP 10 dry     30  2.0000


Point estimate for ETA1-ETA2 is 0.0000
95.2 Percent CI for ETA1-ETA2 is (0.0001,0.0001)
W = 981.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.3329
The test is significant at 0.2694 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI RMGIC Dry, ARI Aegis Dry

```
                 N  Median
ARI RMGIC Dry   30  2.0000
ARI Aegis Dry   30  3.0000


Point estimate for ETA1-ETA2 is 0.0000
95.2 Percent CI for ETA1-ETA2 is (-1.0001,-0.0001)
W = 866.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.4733
The test is significant at 0.4403 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI RMGIC Dry, ARI CaP 0 Dry

```
                 N  Median
ARI RMGIC Dry   30  2.0000
ARI CaP 0 Dry   30  2.0000
```

```
Point estimate for ETA1-ETA2 is -0.0000
95.2 Percent CI for ETA1-ETA2 is (0.0000,-0.0000)
W = 869.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.5059
The test is significant at 0.4584 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI RMGIC Dry, ARI CaP 1 Dry

```
                N  Median
ARI RMGIC Dry  30  2.0000
ARI CaP 1 Dry  30  2.0000


Point estimate for ETA1-ETA2 is -0.0000
95.2 Percent CI for ETA1-ETA2 is (0.0001,0.0002)
W = 904.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.8766
The test is significant at 0.8623 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI RMGIC Dry, ARI CaP 5 Dry

```
                N  Median
ARI RMGIC Dry  30  2.0000
ARI CaP 5 Dry  30  2.0000


Point estimate for ETA1-ETA2 is 0.0000
95.2 Percent CI for ETA1-ETA2 is (-1.0001,-0.0001)
W = 842.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.2838
The test is significant at 0.2302 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI RMGIC Dry, ARI CaP 10 dry

```
                 N  Median
ARI RMGIC Dry   30  2.0000
ARI CaP 10 dry  30  2.0000


Point estimate for ETA1-ETA2 is -0.0000
95.2 Percent CI for ETA1-ETA2 is (0.0001,0.0002)
W = 904.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.8766
The test is significant at 0.8623 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI Aegis Dry, ARI CaP 0 Dry

```
                N  Median
ARI Aegis Dry  30  3.0000
ARI CaP 0 Dry  30  2.0000


Point estimate for ETA1-ETA2 is 0.0000
95.2 Percent CI for ETA1-ETA2 is (-0.0002,0.9999)
W = 932.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.8073
The test is significant at 0.7916 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI Aegis Dry, ARI CaP 1 Dry

```
                 N  Median
ARI Aegis Dry   30  3.0000
ARI CaP 1 Dry   30  2.0000


Point estimate for ETA1-ETA2 is -0.0000
95.2 Percent CI for ETA1-ETA2 is (0.0002,0.9999)
W = 957.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.5395
The test is significant at 0.5091 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI Aegis Dry, ARI CaP 5 Dry

```
                 N  Median
ARI Aegis Dry   30  3.0000
ARI CaP 5 Dry   30  2.0000


Point estimate for ETA1-ETA2 is 0.0000
95.2 Percent CI for ETA1-ETA2 is (0.0001,-0.0003)
W = 906.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.9000
The test is significant at 0.8909 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI Aegis Dry, ARI CaP 10 dry

```
                  N  Median
ARI Aegis Dry    30  3.0000
ARI CaP 10 dry   30  2.0000


Point estimate for ETA1-ETA2 is -0.0000
95.2 Percent CI for ETA1-ETA2 is (0.0002,0.9999)
W = 957.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.5395
The test is significant at 0.5091 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI CaP 0 Dry, ARI CaP 1 Dry

```
                 N  Median
ARI CaP 0 Dry   30  2.0000
ARI CaP 1 Dry   30  2.0000


Point estimate for ETA1-ETA2 is 0.0000
95.2 Percent CI for ETA1-ETA2 is (-0.0001,-0.0001)
W = 948.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.6256
The test is significant at 0.5883 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI CaP 0 Dry, ARI CaP 5 Dry

```
                 N  Median
ARI CaP 0 Dry   30  2.0000
ARI CaP 5 Dry   30  2.0000


Point estimate for ETA1-ETA2 is -0.0000
95.2 Percent CI for ETA1-ETA2 is (-0.0000,-0.0001)
W = 890.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.7227
```

```
The test is significant at 0.6921 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI CaP 0 Dry, ARI CaP 10 dry

```
                  N  Median
ARI CaP 0 Dry    30  2.0000
ARI CaP 10 dry   30  2.0000


Point estimate for ETA1-ETA2 is 0.0000
95.2 Percent CI for ETA1-ETA2 is (-0.0001,-0.0001)
W = 948.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.6256
The test is significant at 0.5883 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI CaP 1 Dry, ARI CaP 5 Dry

```
                 N  Median
ARI CaP 1 Dry   30  2.0000
ARI CaP 5 Dry   30  2.0000


Point estimate for ETA1-ETA2 is 0.0000
95.2 Percent CI for ETA1-ETA2 is (-1.0000,-0.0000)
W = 855.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.3790
The test is significant at 0.3270 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI CaP 1 Dry, ARI CaP 10 dry

```
                  N  Median
ARI CaP 1 Dry    30  2.0000
ARI CaP 10 dry   30  2.0000


Point estimate for ETA1-ETA2 is -0.0000
95.2 Percent CI for ETA1-ETA2 is (-0.0002,0.0002)
W = 915.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 1.0000
The test is significant at 1.0000 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI CaP 5 Dry, ARI CaP 10 dry

```
                  N  Median
ARI CaP 5 Dry    30  2.0000
ARI CaP 10 dry   30  2.0000


Point estimate for ETA1-ETA2 is -0.0000
95.2 Percent CI for ETA1-ETA2 is (0.0000,1.0000)
W = 975.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.3790
The test is significant at 0.3270 (adjusted for ties)
```

8.3.5 Wet ARI

Kruskal-Wallis Test: ARI wet versus Adhesive Wet

```
Kruskal-Wallis Test on ARI wet

Adhesive Wet     N  Median  Ave Rank      Z
Aegis Wet       30   2.000     144.2   3.77
CaP 0 Wet       30   2.000     114.8   0.91
CaP 1 Wet       30   2.000      83.8  -2.11
CaP 10 Wet      30   2.000      67.9  -3.66
CaP 5 wet       30   2.000      89.1  -1.60
RMGIC Wet       30   2.000     113.5   0.77
Transbond Wet   30   2.000     125.2   1.92
Overall        210             105.5

H = 34.04  DF = 6  P = 0.000
H = 43.86  DF = 6  P = 0.000  (adjusted for ties)
```

Mann-Whitney Test and CI: ARI Transbond Wet, ARI RMGIC Wet

```
                     N  Median
ARI Transbond Wet   30  2.0000
ARI RMGIC Wet       30  2.0000


Point estimate for ETA1-ETA2 is 0.0000
95.2 Percent CI for ETA1-ETA2 is (0.0000,0.9999)
W = 960.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.5059
The test is significant at 0.4637 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI Transbond Wet, ARI Aegis Wet

```
                     N  Median
ARI Transbond Wet   30  2.0000
ARI Aegis Wet       30  2.0000


Point estimate for ETA1-ETA2 is 0.0000
95.2 Percent CI for ETA1-ETA2 is (-1.0001,-0.0003)
W = 838.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.2581
The test is significant at 0.2031 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI Transbond Wet, ARI CaP 0 Wet

```
                     N  Median
ARI Transbond Wet   30  2.0000
ARI CaP 0 Wet       30  2.0000


Point estimate for ETA1-ETA2 is -0.0000
95.2 Percent CI for ETA1-ETA2 is (-0.0004,0.9999)
```

```
W = 956.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.5444
The test is significant at 0.5043 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI Transbond Wet, ARI CaP 1 Wet

```
                    N  Median
ARI Transbond Wet  30  2.0000
ARI CaP 1 Wet      30  2.0000


Point estimate for ETA1-ETA2 is 0.5000
95.2 Percent CI for ETA1-ETA2 is (0.0001,0.9999)
W = 1096.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0076
The test is significant at 0.0021 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI Transbond Wet, ARI CaP 5 Wet

```
                    N  Median
ARI Transbond Wet  30  2.0000
ARI CaP 5 Wet      30  2.0000


Point estimate for ETA1-ETA2 is -0.0000
95.2 Percent CI for ETA1-ETA2 is (0.0002,1.0001)
W = 1077.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0170
The test is significant at 0.0047 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI Transbond Wet, ARI CaP 10 Wet

```
                    N  Median
ARI Transbond Wet  30  2.0000
ARI CaP 10 Wet     30  2.0000


Point estimate for ETA1-ETA2 is 1.0000
95.2 Percent CI for ETA1-ETA2 is (0.0002,1.0000)
W = 1153.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0004
The test is significant at 0.0001 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI RMGIC Wet, ARI Aegis Wet

```
                N  Median
ARI RMGIC Wet  30  2.0000
ARI Aegis Wet  30  2.0000


Point estimate for ETA1-ETA2 is 0.0000
95.2 Percent CI for ETA1-ETA2 is (-1.0000,-0.0000)
W = 800.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0905
The test is significant at 0.0571 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI RMGIC Wet, ARI CaP 0 Wet

```
                N  Median
ARI RMGIC Wet  30  2.0000
```

```
ARI CaP 0 Wet  30  2.0000


Point estimate for ETA1-ETA2 is 0.0000
95.2 Percent CI for ETA1-ETA2 is (-0.0002,-0.0003)
W = 910.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.9470
The test is significant at 0.9414 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI RMGIC Wet, ARI CaP 1 Wet

```
                N  Median
ARI RMGIC Wet  30  2.0000
ARI CaP 1 Wet  30  2.0000


Point estimate for ETA1-ETA2 is -0.0000
95.2 Percent CI for ETA1-ETA2 is (-0.0001,1.0003)
W = 1039.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0679
The test is significant at 0.0338 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI RMGIC Wet, ARI CaP 5 Wet

```
                N  Median
ARI RMGIC Wet  30  2.0000
ARI CaP 5 Wet  30  2.0000


Point estimate for ETA1-ETA2 is 0.0000
95.2 Percent CI for ETA1-ETA2 is (0.0001,0.9998)
W = 1023.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.1120
The test is significant at 0.0573 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI RMGIC Wet, ARI CaP 10 Wet

```
                 N  Median
ARI RMGIC Wet   30  2.0000
ARI CaP 10 Wet  30  2.0000


Point estimate for ETA1-ETA2 is 1.0000
95.2 Percent CI for ETA1-ETA2 is (-0.0003,1.0000)
W = 1087.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0112
The test is significant at 0.0054 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI Aegis Wet, ARI CaP 0 Wet

```
                N  Median
ARI Aegis Wet  30  2.0000
ARI CaP 0 Wet  30  2.0000


Point estimate for ETA1-ETA2 is 0.0000
95.2 Percent CI for ETA1-ETA2 is (0.0000,1.0001)
W = 1030.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0905
The test is significant at 0.0572 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI Aegis Wet, ARI CaP 1 Wet

```
                N  Median
ARI Aegis Wet  30  2.0000
ARI CaP 1 Wet  30  2.0000


Point estimate for ETA1-ETA2 is 1.0000
95.2 Percent CI for ETA1-ETA2 is (-0.0001,1.0002)
W = 1189.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0001
The test is significant at 0.0000 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI Aegis Wet, ARI CaP 5 Wet

```
                N  Median
ARI Aegis Wet  30  2.0000
ARI CaP 5 Wet  30  2.0000


Point estimate for ETA1-ETA2 is 1.0000
95.2 Percent CI for ETA1-ETA2 is (0.0000,0.9999)
W = 1173.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0001
The test is significant at 0.0000 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI Aegis Wet, ARI CaP 10 Wet

```
                 N  Median
ARI Aegis Wet   30  2.0000
ARI CaP 10 Wet  30  2.0000


Point estimate for ETA1-ETA2 is 1.0000
95.2 Percent CI for ETA1-ETA2 is (1.0000,1.0000)
W = 1237.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
The test is significant at 0.0000 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI CaP 0 Wet, ARI CaP 1 Wet

```
                N  Median
ARI CaP 0 Wet  30  2.0000
ARI CaP 1 Wet  30  2.0000


Point estimate for ETA1-ETA2 is 0.0000
95.2 Percent CI for ETA1-ETA2 is (0.0002,1.0001)
W = 1047.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0519
The test is significant at 0.0236 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI CaP 0 Wet, ARI CaP 5 Wet

```
                N  Median
ARI CaP 0 Wet  30  2.0000
ARI CaP 5 Wet  30  2.0000


Point estimate for ETA1-ETA2 is -0.0000
```

```
95.2 Percent CI for ETA1-ETA2 is (0.0001,1.0002)
W = 1029.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0933
The test is significant at 0.0446 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI CaP 0 Wet, ARI CaP 10 Wet

```
                 N  Median
ARI CaP 0 Wet   30  2.0000
ARI CaP 10 Wet  30  2.0000


Point estimate for ETA1-ETA2 is 1.0000
95.2 Percent CI for ETA1-ETA2 is (0.0000,0.9998)
W = 1101.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0061
The test is significant at 0.0025 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI CaP 1 Wet, ARI CaP 5 Wet

```
                N  Median
ARI CaP 1 Wet  30  2.0000
ARI CaP 5 Wet  30  2.0000


Point estimate for ETA1-ETA2 is 0.0000
95.2 Percent CI for ETA1-ETA2 is (0.0001,0.0000)
W = 885.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.6627
The test is significant at 0.5517 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI CaP 1 Wet, ARI CaP 10 Wet

```
                 N  Median
ARI CaP 1 Wet   30  2.0000
ARI CaP 10 Wet  30  2.0000


Point estimate for ETA1-ETA2 is -0.0000
95.2 Percent CI for ETA1-ETA2 is (0.0002,0.0002)
W = 1005.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.1858
The test is significant at 0.1129 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI CaP 5 Wet, ARI CaP 10 Wet

```
                 N  Median
ARI CaP 5 Wet   30  2.0000
ARI CaP 10 Wet  30  2.0000


Point estimate for ETA1-ETA2 is 0.0000
95.2 Percent CI for ETA1-ETA2 is (0.0001,1.0001)
W = 1035.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0773
The test is significant at 0.0305 (adjusted for ties)
```

8.3.6 Dry vs Wet ARI

Mann-Whitney Test and CI: ARI Trandbond Dry, ARI Transbond Wet

```
                     N  Median
ARI Trandbond Dry   30  2.0000
ARI Transbond Wet   30  2.0000


Point estimate for ETA1-ETA2 is -0.0000
95.2 Percent CI for ETA1-ETA2 is (0.0000,-0.0002)
W = 966.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.4553
The test is significant at 0.3971 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI RMGIC Dry, ARI RMGIC Wet

```
                  N  Median
ARI RMGIC Dry    30  2.0000
ARI RMGIC Wet    30  2.0000


Point estimate for ETA1-ETA2 is -0.0000
95.2 Percent CI for ETA1-ETA2 is (0.0002,-0.0000)
W = 935.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.7675
The test is significant at 0.7419 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI Aegis Dry, ARI Aegis Wet

```
                  N  Median
ARI Aegis Dry    30  3.0000
ARI Aegis Wet    30  2.0000


Point estimate for ETA1-ETA2 is 0.0000
95.2 Percent CI for ETA1-ETA2 is (-1.0000,0.0001)
W = 881.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.6204
The test is significant at 0.5852 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI CaP 0 Dry, ARI CaP 0 Wet

```
                  N  Median
ARI CaP 0 Dry    30  2.0000
ARI CaP 0 Wet    30  2.0000


Point estimate for ETA1-ETA2 is 0.0000
95.2 Percent CI for ETA1-ETA2 is (0.0001,1.0002)
W = 973.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.3953
The test is significant at 0.3475 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI CaP 1 Dry, ARI CaP 1 Wet

```
                 N  Median
ARI CaP 1 Dry   30  2.0000
ARI CaP 1 Wet   30  2.0000


Point estimate for ETA1-ETA2 is 0.0000
95.2 Percent CI for ETA1-ETA2 is (0.0001,0.9999)
W = 1081.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0144
The test is significant at 0.0043 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI CaP 5 Dry, ARI CaP 5 Wet

```
                 N  Median
ARI CaP 5 Dry   30  2.0000
ARI CaP 5 Wet   30  2.0000


Point estimate for ETA1-ETA2 is 1.0000
95.2 Percent CI for ETA1-ETA2 is (-0.0001,0.9999)
W = 1131.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0014
The test is significant at 0.0002 (adjusted for ties)
```

Mann-Whitney Test and CI: ARI CaP 10 dry, ARI CaP 10 Wet

```
                  N  Median
ARI CaP 10 dry   30  2.0000
ARI CaP 10 Wet   30  2.0000


Point estimate for ETA1-ETA2 is 1.0000
95.2 Percent CI for ETA1-ETA2 is (-0.0002,1.0002)
W = 1138.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0010
The test is significant at 0.0003 (adjusted for ties)
```

8.3.7 Dry mechanism of bond failure

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

             adhesive  cohesive
             failure   failure  Total
    TB        12        18       30
              10.50     19.50
              0.214     0.115

    RMGIC     9         21       30
              10.50     19.50
              0.214     0.115

Total         21        39       60

Chi-Sq = 0.659, DF = 1, P-Value = 0.417
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

             adhesive  cohesive
             failure   failure  Total
    TB         12        18       30
               14.00     16.00
               0.286     0.250

    Aegis      16        14       30
               14.00     16.00
               0.286     0.250

Total          28        32       60

Chi-Sq = 1.071, DF = 1, P-Value = 0.301
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

             adhesive  cohesive
             failure   failure  Total
    TB        12        18       30
              13.00     17.00
              0.077     0.059

    CaP0      14        16       30
              13.00     17.00
              0.077     0.059

Total         26        34       60

Chi-Sq = 0.271, DF = 1, P-Value = 0.602
```

Chi-Square Test: adhesive failure, cohesive failure

Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

	adhesive failure	cohesive failure	Total
TB	12	18	30
	11.50	18.50	
	0.022	0.014	
CaP1	11	19	30
	11.50	18.50	
	0.022	0.014	
Total	23	37	60

Chi-Sq = 0.071, DF = 1, P-Value = 0.791

Chi-Square Test: adhesive failure, cohesive failure

Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

	adhesive failure	cohesive failure	Total
TB	12	18	30
	12.50	17.50	
	0.020	0.014	
Cap5	13	17	30
	12.50	17.50	
	0.020	0.014	
Total	25	35	60

Chi-Sq = 0.069, DF = 1, P-Value = 0.793

Chi-Square Test: adhesive failure, cohesive failure

Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

	adhesive failure	cohesive failure	Total
TB	12	18	30
	11.50	18.50	
	0.022	0.014	
CaP10	11	19	30
	11.50	18.50	
	0.022	0.014	
Total	23	37	60

Chi-Sq = 0.071, DF = 1, P-Value = 0.791

Chi-Square Test: adhesive failure, cohesive failure

Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

```
                adhesive  cohesive
                failure   failure  Total
    RMGIC         9         21       30
                  12.50     17.50
                  0.980     0.700

    Aegis         16        14       30
                  12.50     17.50
                  0.980     0.700

Total             25        35       60

Chi-Sq = 3.360, DF = 1, P-Value = 0.067
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

                adhesive   cohesive
                failure    failure     Total
    RMGIC         9         21          30
                  11.50     18.50
                  0.543     0.338

    CaP0          14        16          30
                  11.50     18.50
                  0.543     0.338

Total             23        37          60

Chi-Sq = 1.763, DF = 1, P-Value = 0.184
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

               adhesive  cohesive
               failure   failure    Total
    RMGIC       9         21         30
                10.00     20.00
                0.100     0.050

    CaP1        11        19         30
                10.00     20.00
                0.100     0.050

Total           20        40         60

Chi-Sq = 0.300, DF = 1, P-Value = 0.584
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

                adhesive  cohesive
                failure   failure      Total
    RMGIC         9         21          30
                  11.00     19.00
                  0.364     0.211
```

```
    CaP5          13         17          30
                  11.00      19.00
                  0.364      0.211

Total             22         38          60

Chi-Sq = 1.148, DF = 1, P-Value = 0.284
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

              adhesive  cohesive
              failure   failure      Total
    RMGIC       9         21          30
                10.00     20.00
                0.100     0.050

    CaP10       11        19          30
                10.00     20.00
                0.100     0.050

Total           20        40          60

Chi-Sq = 0.300, DF = 1, P-Value = 0.584
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

              adhesive  cohesive
              failure   failure      Total
    Aegis       16        14          30
                15.00     15.00
                0.067     0.067

    CaP0        14        16          30
                15.00     15.00
                0.067     0.067

Total           30        30          60

Chi-Sq = 0.267, DF = 1, P-Value = 0.606
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

              adhesive  cohesive
              failure   failure     Total
    Aegis       16        14         30
                13.50     16.50
```

```
                0.463     0.379

    CaP1        11        19        30
                13.50     16.50
                0.463     0.379

Total           27        33        60

Chi-Sq = 1.684, DF = 1, P-Value = 0.194
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

               adhesive  cohesive
               failure   failure     Total
    Aegis       16        14          30
                14.50     15.50
                0.155     0.145

    CaP5        13        17          30
                14.50     15.50
                0.155     0.145

Total           29        31          60

Chi-Sq = 0.601, DF = 1, P-Value = 0.438
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

               adhesive  cohesive
               failure   failure  Total
    Aegis       16        14        30
                13.50     16.50
                0.463     0.379

    CaP10       11        19        30
                13.50     16.50
                0.463     0.379

Total           27        33        60

Chi-Sq = 1.684, DF = 1, P-Value = 0.194
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

              adhesive  cohesive
              failure   failure  Total
    CaP 0       14        16       30
                12.50     17.50
                0.180     0.129

    CaP 1       11        19       30
                12.50     17.50
                0.180     0.129
```

```
Total            25        35       60

Chi-Sq = 0.617, DF = 1, P-Value = 0.432
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

               adhesive  cohesive
               failure   failure       Total
    CaP0        14        16            30
                13.50     16.50
                0.019     0.015

    CaP5        13        17            30
                13.50     16.50
                0.019     0.015

Total           27        33            60

Chi-Sq = 0.067, DF = 1, P-Value = 0.795
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

               adhesive  cohesive
               failure   failure  Total
    CaP0        14        16        30
                12.50     17.50
                0.180     0.129

    Cap10       11        19        30
                12.50     17.50
                0.180     0.129

Total           25        35        60

Chi-Sq = 0.617, DF = 1, P-Value = 0.432
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

               adhesive  cohesive
               failure   failure  Total
    CaP1        11        19       30
                12.00     18.00
                0.083     0.056

    CaP5        13        17       30
                12.00     18.00
                0.083     0.056

Total           24        36       60

Chi-Sq = 0.278, DF = 1, P-Value = 0.598
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

             adhesive  cohesive
             failure   failure  Total
    CaP1        11        19       30
                11.00     19.00
                0.000     0.000

    CaP 10      11        19       30
                11.00     19.00
                0.000     0.000

Total           22        38       60

Chi-Sq = 0.000, DF = 1, P-Value = 1.000
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

              adhesive  cohesive
              failure   failure  Total
    CaP5        13        17       30
                12.00     18.00
                0.083     0.056

    CaP10       11        19       30
                12.00     18.00
                0.083     0.056

Total           24        36       60

Chi-Sq = 0.278, DF = 1, P-Value = 0.598
```

8.3.8 Wet mechanism of bond failure

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

              adhesive  cohesive
              failure   failure  Total
    TB        11        19        30
              12.50     17.50
              0.180     0.129

    RMGIC     14        16        30
              12.50     17.50
              0.180     0.129

Total         25        35        60

Chi-Sq = 0.617, DF = 1, P-Value = 0.432
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

             adhesive  cohesive
             failure   failure  Total
    TB        11        19        30
              12.50     17.50
              0.180     0.129

    Aegis     14        16        30
              12.50     17.50
              0.180     0.129

Total         25        35        60

Chi-Sq = 0.617, DF = 1, P-Value = 0.432
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

              adhesive  cohesive
              failure   failure  Total
    TB        11        19         30
              11.50     18.50
              0.022     0.014

    CaP0      12        18         30
              11.50     18.50
```

```
              0.022     0.014

Total         23        37         60

Chi-Sq = 0.071, DF = 1, P-Value = 0.791
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

              adhesive  cohesive
              failure   failure  Total
    TB        11        19         30
              5.50     24.50
              5.500     1.235

    CaP1      0         30         30
              5.50     24.50
              5.500     1.235

Total         11        49         60

Chi-Sq = 13.469, DF = 1, P-Value = 0.000
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

             adhesive  cohesive
             failure   failure    Total
    TB        11         19         30
              5.50      24.50
              5.500     1.235

    CaP5      0         30         30
              5.50      24.50
              5.500     1.235

Total         11        49         60

Chi-Sq = 13.469, DF = 1, P-Value = 0.000
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

             adhesive  cohesive
             failure   failure  Total
    TB        11        19        30
              5.50      24.50
              5.500     1.235

    CaP10     0         30        30
              5.50     24.50
              5.500     1.235

Total         11        49       60

Chi-Sq = 13.469, DF = 1, P-Value = 0.000
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

                adhesive  cohesive
                failure   failure  Total
    RMGIC       14        16         30
                14.00     16.00
                0.000     0.000

    Aegis       14        16         30
                14.00     16.00
                0.000     0.000

Total           28        32         60

Chi-Sq = 0.000, DF = 1, P-Value = 1.000
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

               adhesive  cohesive
               failure   failure    Total
    RMGIC        14        16        30
                 13.00     17.00
                 0.077     0.059

    CaP0         12        18        30
                 13.00     17.00
                 0.077     0.059

Total            26        34        60

Chi-Sq = 0.271, DF = 1, P-Value = 0.602
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

               adhesive  cohesive
               failure   failure    Total
    RMGIC        14        16        30
                 7.00      23.00
                 7.000     2.130

    CaP1         0         30        30
                 7.00      23.00
                 7.000     2.130

Total            14        46        60

Chi-Sq = 18.261, DF = 1, P-Value = 0.000
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
```

```
Chi-Square contributions are printed below expected counts

               adhesive  cohesive
               failure   failure     Total
    RMGIC       14        16          30
                7.00     23.00
                7.000     2.130

    CaP5        0         30          30
                7.00     23.00
                7.000     2.130

Total           14        46          60

Chi-Sq = 18.261, DF = 1, P-Value = 0.000
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

               adhesive  cohesive
               failure   failure     Total
    RMGIC       14        16          30
                7.00     23.00
                7.000     2.130

    CaP10       0         30          30
                7.00     23.00
                7.000     2.130

Total           14        46          60

Chi-Sq = 18.261, DF = 1, P-Value = 0.000
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

               adhesive  cohesive
               failure   failure    Total
    Aegis       14        16         30
                13.00     17.00
                0.077     0.059

    CaP0        12        18         30
                13.00     17.00
                0.077     0.059

Total           26        34         60

Chi-Sq = 0.271, DF = 1, P-Value = 0.602
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

              adhesive  cohesive
              failure   failure    Total
    Aegis       14        16        30
                7.00      23.00
```

```
                7.000     2.130

    CaP1        0         30        30
                7.00      23.00
                7.000     2.130

Total           14        46        60

Chi-Sq = 18.261, DF = 1, P-Value = 0.000
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

                adhesive  cohesive
                failure   failure    Total
    Aegis        14        16         30
                 7.00      23.00
                 7.000     2.130

    CaP5         0        30          30
                 7.00     23.00
                 7.000     2.130

Total            14        46         60

Chi-Sq = 18.261, DF = 1, P-Value = 0.000
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

                adhesive  cohesive
                failure   failure    Total
    Aegis        14        16         30
                 7.00      23.00
                 7.000     2.130

    CaP10        0         30         30
                 7.00      23.00
                 7.000     2.130

Total            14        46         60

Chi-Sq = 18.261, DF = 1, P-Value = 0.000
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

                 adhesive  cohesive
                 failure   failure    Total
    CaP0          12        18         30
                  6.00      24.00
                  6.000     1.500

    CaP1          0         30         30
                  6.00     24.00
                  6.000     1.500
```

```
Total             12        48         60

Chi-Sq = 15.000, DF = 1, P-Value = 0.000
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

               adhesive  cohesive
               failure   failure       Total
    CaP0       12        18             30
               6.00      24.00
               6.000     1.500

    CaP5       0         30             30
               6.00      24.00
               6.000     1.500

Total          12        48             60

Chi-Sq = 15.000, DF = 1, P-Value = 0.000
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

              adhesive  cohesive
              failure   failure     Total
    CaP0       12        18          30
               6.00      24.00
               6.000     1.500

    CaP10      0         30          30
               6.00      24.00
               6.000     1.500

Total          12        48          60

Chi-Sq = 15.000, DF = 1, P-Value = 0.000
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

               adhesive  cohesive
               failure   failure      Total
    CaP1         0        30           30
                 0.00     30.00
                 0.000    0.000

    CaP5                  30           30
                 0.00     30.00
                 0.000    0.000

Total            0        60           60

Chi-Sq = 0.000, DF = 1, P-Value = 1.000
2 cells with expected counts less than 5.
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

               adhesive  cohesive
               failure   failure      Total
    CaP1         0        30           30
                 0.00     30.00
                 0.000    0.000

    CaP10        0        30           30
                 0.00     30.00
                 0.000     0.000

Total            0        60           60

Chi-Sq = 0.000, DF = 1, P-Value = 1.000
2 cells with expected counts less than 5.
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

              adhesive  cohesive
              failure   failure      Total
    CaP1         0         30           30
                 0.00      30.00
                 0.000     0.000

    CaP10        0         30           30
                 0.00      30.00
                 0.000     0.000

Total            0        60            60

Chi-Sq = 0.000, DF = 1, P-Value = 1.000
2 cells with expected counts less than 5.
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

               adhesive  cohesive
               failure   failure       Total
    CaP5         0        30             30
                 0.00     30.00
                 0.000    0.000

    CaP10        0        30             30
                 0.00     30.00
                 0.000    0.000

Total            0        60             60
```

```
Chi-Sq = 0.000, DF = 1, P-Value = 1.000
2 cells with expected counts less than 5.
```

8.3.9 Dry vs Wet mechanism of bond failure

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

               adhesive  cohesive
               failure   failure    Total
    TB dry       12        18        30
                 11.50     18.50
                 0.022     0.014

    TB wet       11        19        30
                 11.50     18.50
                 0.022     0.014

Total            23        37        60

Chi-Sq = 0.071, DF = 1, P-Value = 0.791
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

                    adhesive  cohesive
                    failure   failure     Total
    RMGIC dry         9         21         30
                      11.50     18.50
                      0.543     0.338

    RMGIC wet         14        16         30
                      11.50     18.50
                      0.543     0.338

Total                 23        37         60

Chi-Sq = 1.763, DF = 1, P-Value = 0.184
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

                    adhesive  cohesive
                    failure   failure     Total
    Aegis dry        16        14           30
                     15.00     15.00
                     0.067     0.067

    Aegis wet        14        16           30
                     15.00     15.00
                     0.067     0.067

Total                30        30           60

Chi-Sq = 0.267, DF = 1, P-Value = 0.606
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

                  adhesive  cohesive
                  failure   failure    Total
    CaP0 dry       14        16         30
                   13.00     17.00
                   0.077     0.059

    CaP0 wet       12        18         30
                   13.00     17.00
                   0.077     0.059

Total              26        34         60

Chi-Sq = 0.271, DF = 1, P-Value = 0.602
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

                   adhesive  cohesive
                   failure   failure    Total
    CaP1 dry        11        19         30
                    5.50      24.50
                    5.500     1.235

    CaP1 wet        0         30         30
                    5.50      24.50
                    5.500     1.235

Total               11        49         60

Chi-Sq = 13.469, DF = 1, P-Value = 0.000
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

                   adhesive  cohesive
                   failure   failure      Total
    CaP5 dry       13        17             30
                   6.50      23.50
                   6.500     1.798

    CaP5 wet       0         30             30
                   6.50      23.50
                   6.500     1.798

Total              13        47             60

Chi-Sq = 16.596, DF = 1, P-Value = 0.000
```

Chi-Square Test: adhesive failure, cohesive failure

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts
```

	adhesive failure	cohesive failure	Total
CaP10 dry	11	19	30
	5.50	24.50	
	5.500	1.235	
CaP10 wet	0	30	30
	5.50	24.50	
	5.500	1.235	
Total	11	49	60

Chi-Sq = 13.469, DF = 1, P-Value = 0.000

8.4 Appendix 4 - Statistical test results for calcium ion release test

Kruskal-Wallis Test: Calcium ion versus ACP/TCP

```
Kruskal-Wallis Test on Calcium ion

ACP/TCP    N     Median  Ave Rank      Z
ACP       50  0.0001099      25.7  -8.54
TCP       50  0.0009162      75.3   8.54
Overall  100                 50.5

H = 73.02  DF = 1  P = 0.000
H = 73.08  DF = 1  P = 0.000  (adjusted for ties)
```

Mann-Whitney Test and CI: Calcium ACP, Calcium TCP

```
              N   Median
Calcium ACP  50  0.00011
Calcium TCP  50  0.00092


Point estimate for ETA1-ETA2 is -0.00080
95.0 Percent CI for ETA1-ETA2 is (-0.00085,-0.00072)
W = 1285.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
The test is significant at 0.0000 (adjusted for ties)
```