FLUORIDE RELEASE FROM LIGHT-CURED ORTHODONTIC BONDING MATERIALS

BY

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A Thesis
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1.0 ABSTRACT

The purpose of this study was to compare the rate of fluoride release with time of one non-fluoridated and three fluoride-containing orthodontic bonding materials in distilled water and artificial saliva. Materials tested were: Assure (Reliance, Itasca, IL), Fuji Ortho LC (GC America Inc., Alsip, IL), Python (TP Orthodontics Inc., LaPorte, IN), and Transbond XT (3M, St Paul, MN). Twenty specimens of each material were polymerized and placed in polyethylene tubes. Half the specimens were stored in 1 mL of distilled water and half in 1 mL of unstimulated artificial saliva, at 37°C and 100% relative humidity. Fluoride release was measured with an ion-specific electrode. Readings were taken at 1, 2, 3, 5, 7 and 9 days from time of immersion, then weekly for three weeks and monthly for 5 months. To prevent cumulative measurements, storage solutions were changed 24 h prior to the weekly and monthly readings.

Results showed Assure to release the most fluoride, followed by Fuji Ortho LC, Python, and Transbond. The fluoride release rates were greatest during the first days of testing, declining to low but stable levels. The type of storage medium did not dramatically affect fluoride release. Throughout the study, daily fluoride release rates of all three fluoride-containing materials were within the therapeutic range for the reduction of enamel demineralization.

The second part of the study tested the twenty samples of Assure for a further two-week period, after exposure to running and still distilled water. Although fluoride release rates declined with time, they were again within the therapeutic range. Release rates were similar in running and still water at all time points.

4.0 INTRODUCTION

Decalcification of the enamel surface adjacent to orthodontic brackets is one of a number of iatrogenic complications of orthodontic treatment (Gorelick *et al.*, 1982). Other potential complications include external root resorption, relapse following the completion of orthodontic treatment, gingivitis, and adverse pulpal reaction (Thilander, 1992). Enamel decalcification was chosen as the principal focus of this study, since there are claims that the slow release of fluoride from recently developed adhesive materials may either eliminate or reduce such lesions.

The importance of anticariogenicity dovetails with the importance of the attachment and microbial composition of biofilms in the oral environment in current research (Burne, 1998). Whereas advances in our understanding of biofilms may well allow inhibition of microbial attachment to the surfaces of both brackets and the enamel in future, this is not presently feasible. This study will focus on evaluation of the rate of release of fluoride from three light-cured fluoride-containing orthodontic bonding materials to provide a better understanding of this phenomenon.

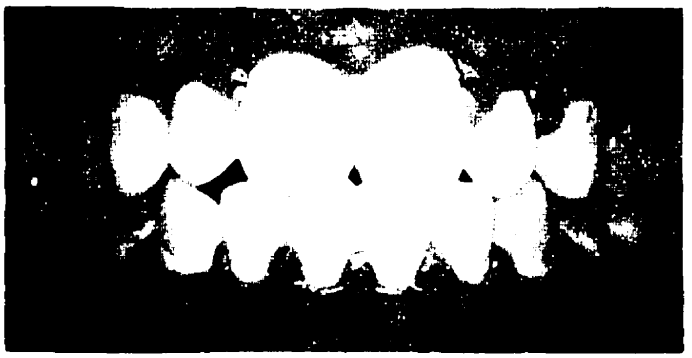
This introduction comprises eight sections. The first provides a brief review of decalcification in relation to orthodontics, followed by a description of topical fluoride, its benefits and mechanisms of action. This is followed by a history of fluoride inclusion into dental materials and a description of different types of materials. Measurement of fluoride release, a summary, and the hypotheses comprise the last three sections of the introduction.

4.1 Enamel decalcification in orthodontics

Decalcification of enamel adjacent to orthodontic brackets is prevalent in orthodontics (Gorelick *et al.*, 1982). Patients often have difficulty maintaining adequate oral hygiene with orthodontic appliances attached directly to the teeth. Plaque, which therefore accumulates readily on the surfaces of bands, brackets, and adhesive margins (Thilander, 1992), results in decalcification if the person's diet is conducive to caries formation, i.e. high in fermentable carbohydrate (Bowen, 1976). Bacterial acid production from such plaque micro-organisms as *Streptococcus mutans* and *Lactobacillus acidophilus* (Thilander, 1992) causes a drop in the pH of the oral environment and leads to diffusion of calcium and phosphate ions out of enamel (Millett *et al.*, 1999). The resulting crescent-shaped chalky white spot located near the gingival margin is made visible by subsurface tissue loss (Gorelick *et al.*, 1982).

Figure 4.1.

White spot lesions in a 16-year-old male patient after 2 years of fixed orthodontic treatment. Photo was taken at the time of debanding in 1998.



The white spot has only a slightly roughened surface initially, since the outermost enamel layer remains relatively intact (Millett *et al.*, 1999). Prolonged plaque accumulation results in the lesion gradually becoming excavated as the enamel surface is undermined (Shafer *et al.*, 1983) by further mineral loss. The majority of white-spot lesions do not progress to cavitation. For instance, Geiger *et al.* (1988) found that only one of 101 teeth with white spots exhibited cavitation. However, these unesthetic lesions may persist for years after the completion of orthodontic treatment (Øgaard, 1989).

Estimates of the presence of one or more white-spot lesions per patient in the orthodontically treated population range from 12.6% (Sonis and Snell, 1989), to 50% (Gorelick *et al.*, 1982) to 96% (Øgaard, 1989). The range of prevalence may be explained by the use of different visual analogue scales to define the differential prevalence of white spots. One scale had categories such as 1= no white spot, 2= slight, 3= severe, and 4= excessive or cavitated (Geiger *et al.*, 1988), while Øgaard, in 1989, used the size of the white spot (more or less than 1/3 of crown) as the defining factor of the visual scale. Sonis and Snell (1989) gave a score of 0 (no decalcification) to white spots < 1 mm in diameter, while a score of 3 meant the spot must cover at least 2/3 of the tooth's crown. Methodology of tooth examination differs as well, with both direct examination (Geiger *et al.*, 1988; Øgaard, 1989) and color photographs (Sonis and Snell, 1989; Millett *et al.*, 1999) being used.

Gorelick *et al.* (1982) found that 10.8% of 2211 orthodontically treated teeth (from a sample group of 49 males and 72 females, all under 18 years of age) had white spots. Only 3.6% of teeth in the control population (50 randomly selected children from the practices of two of the authors, examined prior to the placement of any brackets or

bands) exhibited white spots. There was no difference in incidence of white spots according to age and/or sex. No studies have been reported which correlate the prevalence of white spots in orthodontic patients with ethnic group or socioeconomic status. It has been suggested that acid etching of teeth prior to cementation of appliances may enhance decalcification (Haydar *et al.*, 1999). Interestingly, however, decalcification was not observed adjacent to any of the acid-etched and bonded lingual retainers of 60 patients (Gorelick *et al.*, 1982), even though calculus and/or plaque was frequently found. This finding suggests that acid etching does not, in itself, cause subsequent enamel decalcification, and may point to the protective role of saliva in preventing demineralization. Saliva may be particularly effective in the lower lingual region of the mouth due to a much higher salivary film velocity lingually than buccally. Assuming that the thickness of the salivary film is uniform (about 0.1 mm) throughout the mouth, Dawes *et al.* (1989) estimated salivary film velocity to be 7.6 mm/min in the lower-anterior lingual area of the mouth, and only 0.8 mm/min in the upper-anterior buccal region. These measurements were for unstimulated salivary flow rates. The authors postulated that the slow movement of the salivary film over, for example, the cervical areas of maxillary incisors would prolong the clearance time of metabolic products such as acid from the plaque.

In the maxilla, lateral incisors are the teeth most frequently affected by decalcification; in the mandible, premolars and first molars are at highest risk (Geiger *et al.*, 1988). Bands and brackets are placed closer to the gingival margin of these particular teeth because of their anatomic shape, making plaque removal more difficult to accomplish (Øgaard, 1989). Simplification of appliance design (i.e. fewer hooks, smaller

brackets) will facilitate plaque removal (Øgaard, 1989), although the crown height of these teeth may leave the operator no choice but to place brackets in close proximity to the gingival margin.

According to Croll (1990), existing white spots may be treated by micro-abrasion with a water-soluble acid/abrasive compound such as Prema (ESPE/Premier, Germany). There was a 50-75% success rate in the removal of white lesions through this method, with success depending chiefly on how deeply the lesion extended into the enamel. The technique does involve removal of surface enamel, however, and therefore prevention is the preferred approach to the problem of decalcification (Kindelan, 1996).

4.2 Topical fluoride - mechanisms of action

Topical fluoride application, for example in the form of a twice-daily application of 0.4% SnF_2 brush-on gel (Strateman and Shannon, 1974) or a once-daily 0.05% NaF rinse (Boyd, 1993), has been shown to reduce the risk of decalcification in adolescent orthodontic patients, compared with brushing with fluoridated toothpaste alone. Although the mechanism of action of fluoride is as yet incompletely understood, several mechanisms have been proposed to explain the effectiveness of topical fluoride agents:

1. Fluoride-containing agents such as rinses, gels and dentifrices may inhibit bacterial activity, thereby reducing acid production (Clarkson, 1991) if the concentration is sufficiently high, i.e. 12,000 ppm (Margolis and Moreno, 1990). Environmental pH and the presence of counter ions significantly affect the capacity of fluoride to inhibit bacterial growth (Hamilton and Bowden, 1996). These authors state that it is unrealistic to expect a dramatic decrease in salivary *S. mutans* levels following the

recommended use of topical fluorides. The scientific consensus is that the main mechanism of action of topical fluoride does not appear to be through inhibition of micro-organisms (Shafer *et al.*, 1983).

2. It is theorized that exposure of enamel to topical fluoride may allow incorporation of fluoride ions into the crystal lattice structure of enamel, forming a tightly bound complex such as fluorhydroxyapatite (FHA) within the enamel (White and Nancollas, 1990). The relatively small F^- ion either substitutes for the OH^- ion or occupies the hydroxyl vacancies in the hydroxyapatite lattice, ostensibly rendering the enamel less acid-soluble (Clarkson, 1991). Nuclear magnetic resonance imaging has identified FHA formation within surface enamel when exposed to low (1.5-10 ppm) concentrations of fluoride in solution in saliva or systemically (White and Nancollas, 1990). FHA was found in a laboratory study to be less soluble in lactic acid solution (pH =5.0) than hydroxyapatite (Margolis and Moreno, 1990). Clarkson (1991) argues, however, that unless the enamel is newly erupted or subsurface (i.e. with the outer portion ground down or removed, such as is often the method of preparation in *in vitro* trials), it will probably not form FHA due to topical exposure only of fluoride ions.
3. A more widely accepted theory is that the fluoride from topical sources forms globules of soluble calcium fluoride on the surfaces of teeth. As it dissolves in saliva or plaque fluid, the calcium fluoride is available to supply low concentrations of ambient fluoride to reduce demineralization and aid in remineralization of hard tissue (Rølla, 1988). Topical treatment of enamel with 1100 ppm NaF dentifrices, followed by immersion in demineralizing (acidic) and remineralizing (neutral) solutions,

resulted in increased remineralization and decreased demineralization rates compared with no fluoride exposure (White and Nancollas, 1990). According to the work of Clarkson *et al.* (1988), after topical fluoride applications (1.23% acidulated phosphate F^- and 8% SnF) to natural enamel surfaces, fluoride concentrations in enamel were elevated. They returned to pretreatment levels, however, after a wash in KOH, which would remove alkali-soluble reaction products, including calcium fluoride. From the results of this study, it appears unlikely that elevated FHA levels will be produced on an enamel surface already saturated with fluoride from topical applications.

Controversy exists as to whether the available fluoride is most effective when found in or on the enamel, within plaque, or dissolved in saliva. Fluoride from mouthrinses or pastes containing from 200-1000 ppm F^- can diffuse into plaque fluid, increasing the driving force for mineral deposition and resulting in remineralization of lesions (Margolis and Moreno, 1990). Ripa (1984) stated that fluoride is incorporated into the outer layers of enamel (as measured by proton activation analysis and abrasive enamel biopsy), whether or not a layer of plaque or pellicle is present. From a clinical standpoint, the most important consideration may be that ionic fluoride is present at the site of the developing lesion (Clarkson, 1991). Clarkson *et al.* (1988) showed an inverse relationship between the size of enamel lesions and the fluoride concentrations within the lesion.

Topical fluoride applications are effective when administered frequently at low concentrations, such as daily rinsing with a 0.05% NaF rinse and daily brushing with a standard fluoride toothpaste (Geiger *et al.*, 1988). Less frequent application of fluoride at higher concentrations, such as 8% stannous fluoride applied professionally every six

months, has also been proven effective (Margolis and Moreno, 1990). Dentifrice, gels, and rinses have all been investigated as methods of delivering fluoride to the patient in full-banded treatment. A once-daily 0.05% NaF rinse reduced white spot formation by 13.5% in a group of 26 adolescent orthodontic patients compared with that in 32 control subjects using fluoridated (1100 ppm F) toothpaste alone. Twice-daily applications of 0.4% SnF_2 gel reduced white spots by 22% compared with those in the controls (Boyd, 1993). Patients who complied (based on the amount of rinse they used per day) with a 0.05% sodium fluoride-rinsing regimen had 28% fewer white spots than those who did not use the rinse, even if their oral hygiene was consistently poor (Geiger *et al.*, 1992). Oral hygiene status was not clearly defined in the study, however. Presence of plaque does not impede the action of topical fluoride because fluoride accumulates in higher concentrations in plaque than in saliva, and therefore is able to aid in remineralization at the area of greatest need (Haydar *et al.*, 1999).

Self-administered topical fluoride treatments are highly dependent on patient cooperation. The 1988 Geiger *et al.* study of the effectiveness of home fluoride-rinse programs found that poor compliers (evaluated by questionnaire and interview of patient and parent) formed 52.5% of 101 subjects, even though materials were provided free of charge and detailed instructions were given. Stratemann and Shannon (1974) found that only 51 of 99 patients used a 0.4% stannous fluoride gel daily as directed. Those who followed instructions (as determined by questionnaire) experienced a 2% incidence of decalcification, compared with 58% in a control group of 110 adolescent patients. Topical fluorides may be administered in the dental office, such as during a professional

prophylaxis, but they tend to be time-consuming for the operator (Trimpeneers *et al.*, 1998).

4.3 Fluorides in dental cements

The potential advantages of a bracket-bonding material with sustained release of fluoride are that:

- a continuous release of fluoride would be possible adjacent to the bracket, the area at greatest risk of decalcification (Rawls, 1995). If release of fluoride were truly sustained, then a uniform dosage might be delivered over time (Rawls, 1991).
- direct involvement of health professionals is minimal, and the fluoride is continuously present over extended periods (Rawls, 1995). Fluoride must be released throughout the entire period of orthodontic treatment in order to protect against demineralization (Dunne *et al.*, 1996).
- the need for patient compliance is less than with self-administered delivery of fluoride (Rawls, 1995).

Slow release of fluoride over time is an effective means of caries inhibition, as observed from a study of lesion depth from photomicrographs of tooth sections subjected first to a fluoride rinse, then to a caries challenge (Clarkson *et al.*, 1988). Even very low concentrations of fluoride adjacent to restorative materials seem to impart protection against caries. Using the silicate cements as the historical "gold standard" for a caries-inhibiting material (although most of the evidence for the success of silicates as caries inhibitors is anecdotal), it was determined that a range of 6-10 $\mu g/F^-/day$ was sufficient to

inhibit caries formation in sound enamel (Rawls, 1995). However, this number is highly dependent on local factors such as diet, pH of surrounding fluid, salivary clearance, host resistance, and cariogenic bacterial levels (Clarkson, 1991). Since a cement's properties such as bond strength (Haydar *et al.*, 1999) and rate of degradation (Rawls, 1991) may suffer when fluoride is added, it must be ensured that the material is safe, is retained, and continues to function acceptably for its primary purpose (Rawls, 1995).

The first restorative material to contain fluoride was silicate cement, which had up to 15% fluoride salts (up to 13,000 ppm) added to it as a flux (Clarkson, 1991) to aid in its manufacture by lowering the fusion temperature. Thus, fluoride was being added to this type of cement long before its beneficial effect on caries resistance was recognized. Despite the gross marginal leakage that occurred with this material, recurrent decay rates were anecdotally reported as lower than with dental amalgam (Ripa, 1991). The fluoride present in the cement was thought to be released throughout the life of the restoration (White and Nancollas, 1990), being incorporated into the marginal enamel and making it more resistant to acid dissolution. Silicates have been found to release fluoride at an average daily rate of about 6 μg F^-/g (White and Nancollas, 1990). Although no longer used due to its poor mechanical properties (Ripa, 1991), silicate cement demonstrated the effectiveness of incorporating fluoride compounds into cements. Glass ionomer cements, developed for use in the early 1970's by McLean and Wilson (McLean *et al.*, 1994), were initially intended to replace the silicate cements as an esthetic restorative material. However, they have come to be used in a wider variety of circumstances, including orthodontic banding and bonding cements (Mount, 1998).

4.4 Properties of glass ionomer cements

The glass ionomer (GI) cements are prepared from a powder and a liquid. The powder contains silica, alumina, calcium fluoride, sodium fluoride, and/or aluminum phosphate. These materials are heated at temperatures of up to 1500°C to form fluoroaluminosilicate glass. The fluoride compounds, with their considerably lower melting temperatures, act as a flux at this stage to help form a cohesive liquefication. The cooled glass is then ground into fine (20-50 μm) particles (Phillips, 1982).

The liquid component is usually an aqueous solution (about 50% by weight) of polyacrylic acid. As the components are mixed, an acid-base reaction begins. H^+ is liberated from the polyacrylic acid and attacks the glass particles, releasing calcium and aluminum ions and fluoride complexes. A cross-linked metallic salt eventually precipitates out as more of these ions diffuse into the liquid (Millett and McCabe, 1996). These salts hydrate to form a gel matrix surrounding the remaining unreacted glass particles. The polyacrylic acid liquid tends to be quite viscous (Phillips, 1982); therefore, newer formulations freeze-dry the acid component and add it to the glass powder. In these cases the liquid used consists of water and tartaric acid, a chelating agent to control the rate of the acid-base reaction (Burgess *et al.*, 1994). The setting reaction continues for 24 hours and slowly for much longer, as cross-linkages continue to form. The relatively long setting time explains why GI cements should be protected from heavy loading (Sidhu and Watson, 1995), moisture contamination and dehydration for the first 24 h, in order to avoid a reduction in their physical properties (Momoi and McCabe, 1993). Other characteristic properties of the GI's include brittleness, adhesion to tooth structure, and fluoride release (McCabe, 1998).

The mechanism of fluoride release in GI's is not yet completely clear. Initially all fluoride present is contained within the glass, but as the glass particles are attacked by the acid, fluoride ions are released and become trapped in the hardening gel matrix (Trimpeneers *et al.*, 1998). Water present within the GI cement formulation acts as the medium of the setting reaction. After initial setting, but prior to contact with external sources of water, fluoride is present in three phases: 1) in the as-yet unreacted glass particles, 2) in the aqueous pore liquid, and 3) attached to the polysalt matrix gel via formation of complexes, for example with aluminum (Cranfield *et al.*, 1982).

Since the fluoride present in the liquid is loosely bound, it easily diffuses out of the freshly mixed cement into the aqueous environment, as evidenced by a significantly higher rate of fluoride release in water when a 2:1 powder-liquid ratio was used, compared with a 3:1 ratio (Cooley *et al.*, 1989). This mechanism of diffusion from the liquid may help explain the initial high levels (25-200 $\mu g/cm^2$/day) of fluoride released from many glass ionomers (Cooley *et al.*, 1989; McCabe, 1998).

A second proposed mechanism of fluoride release from GI's is a dissolution/diffusion process, described by Rawls (1991). As water from the oral environment diffuses into the matrix material, fluoride within the salt complexes dispersed in the matrix dissolves in the water. Driven by the concentration gradient, the fluoride diffuses through the matrix material into the surrounding environment. Both internal water from the formulation, and external sources from the aqueous oral environment, allow for diffusion of ionic species towards the surface of the cement (Millet and McCabe, 1996). The rate of diffusion probably depends on such factors as how tightly bound the fluoride is within the matrix, and how hydrophilic is the matrix

(Rawls, 1991). This mechanism is proposed as being responsible for the continued fluoride release of GI cements even months after initial setting (Rawls, 1995). However, as time passes, the fluoride must be extracted from deeper within the matrix, and the rate of diffusion will slow (Rawls, 1991). The rate-determining step will be the rate of diffusion of fluoride ions from the bulk cement to the outer surface. When dissolution occurs, loss of fluoride from the material may be accompanied by loss of other ions, such as calcium (Rawls, 1991). A lining cement material, XR-Ionomer (Sybron/Kerr, Romulus MI), has been shown to undergo visible dissolution with concomitant calcium release into water during a 4-week test period (Tam *et al.*, 1991). Over time, the structure of the cement may be weakened (potentially resulting in more bond failures, according to Tam *et al.*, 1991) and total available surface area of the cement is lessened, if dissolution with degradation is the major mechanism of fluoride release (Rawls, 1991).

4.5 Properties of composite resin and hybrid materials

Conventional (i.e. non-fluoride) composite resin materials are widely used for the bonding of brackets to acid-etched enamel. Favorable properties include high strength, low solubility, and ease of handling. However, they do not release fluoride or form a chemical bond to tooth structure. The bond strengths of GI cements tend to be lower than those of the conventional composite resins (CR's); i.e. 1-4 MPa compared with 8 MPa for a highly filled composite resin (Powers *et al.*, 1997). A GI showed a clinical failure rate of 51%, compared with 8% for a composite resin, when 112 brackets for each type of cement were evaluated over a 3-year period (Miguel *et al.*, 1995). In an attempt to improve on the shortcomings of GI cements while retaining their desirable properties,

"hybrid" materials were developed. These products fit somewhere along a continuum of materials; some whose properties, such as setting in the absence of light, are like those of the GI's (McLean *et al.*, 1994), and others which more closely resemble composite resins, with a high (60 MPa) flexural strength (Tam *et al.*, 1991). Experimental work on creating a resin-modified glass ionomer cement (by mixing the liquid from a commercial GI restorative with the liquid resin component of a light-activated CR) suggested that moisture sensitivity was reduced, although compressive strength, with a reduction of 13% from the CR, was not clinically acceptable (Mathis and Ferracane, 1989). Since the early experimental work, many commercial varieties of hybrid materials have been developed, as briefly summarized below:

4.5a Resin-modified glass ionomers

At one end of the hybrid continuum lie the resin-modified glass ionomer cements. Resin components like HEMA or Bis-GMA, as well as photo-initiators, are added to liquid polyacrylic acid or to the water-acid mixture to form these materials (Powers *et al.*, 1997). They have a dual setting reaction. Visible light or a chemical initiator will initiate the polymerization of vinyl groups like HEMA, as with conventional CR's. They will also undergo the classic GI acid-base setting reaction, measurable by a pH change as the reaction progresses. The pH is very low (2-3) immediately after mixing but rises significantly on setting (McCabe, 1998). Therefore, the resin-modified GI's will eventually set in the absence of light, which is a requirement for a material to be called a "glass ionomer" under the proposed nomenclature (McLean *et al.*, 1994). The name "resin-modified GI" suggests that the materials retain characteristics of the GI's, which are modified by the addition of resin. Common brand names include Fuji Ortho (GC,

Tokyo), Photac-Fil (ESPE, Seefeld, Germany), Vitrabond (3M, St Paul, MN), and Vitremer (3M). Many, such as Fuji Ortho and Fuji Ortho LC (GC, Tokyo), are recommended for use with unetched enamel (Powers *et al.*, 1997), although the effect of etching on bond strength has varied depending on the conditioning agent and type of cement (Millett *et al.*, 1996). Some materials within this group, such as Fuji LC (GC, Tokyo), have shown a large degree of volumetric change (33 μm protrusion from a cavity after 1 month water storage) due to water sorption (McCabe, 1998). The presence of hydrophilic poly-HEMA chains appears to be a contributory factor, causing the material to behave as a hydrogel (Meyer *et al.*, 1998). The significance of this finding may not be as great in orthodontic uses as in restorative applications (McCabe, 1998). However, absorbed water may act as a plasticizer, possibly reducing hardness and bond strength (Meyer *et al.*, 1998)

4.5b Modified composites

Toward the opposite end of the continuum lie the fluoride-containing composite resins, which resemble conventional CR's more closely than glass ionomers. Modified polyacids with unsaturated groups (Meyer *et al.*, 1998) and/or ion-leachable ground glass containing fluoride are added to conventional CR formulations (Sidhu and Watson, 1995) to form these materials. They do not depend on an acid-base reaction for hardening; instead, polymerization is the setting reaction. They are available as one paste (light-cured) or two-paste (chemically cured) systems. Most current market research is in this area. Brand names include Assure (Reliance, Itasca IL), Band-Lok (Reliance), Direct (Orthocare, Yorkshire UK), Fluorobond (Ormco, Glendora CA), Enlight (Ormco), Light-bond (Reliance), Rely-a-bond (Reliance), Python (TP Orthodontics, LaPorte IN), and

FluorEver OBA (Macro Chem, Billerica MA). Some of these brands, e.g. Python and Enlight, are available in both fluoridated and non-fluoridated formulations.

Overall, bond strengths for these materials of 7-15 MPa (Powers *et al.*, 1997) have compared favorably with those of conventional CR's. Another property shared by many materials in this group is water sorption, even though an initial volumetric shrinkage occurs as a result of polymerization (Meyer *et al.*, 1998). Weight gain and water sorption tend to be less than for the resin-modified GI materials. Variglass (Dentsply, Germany), a restorative material in this class, showed water sorption of 91 $\mu g/mm^3$, compared with 257 $\mu g/mm^3$ for Photac-Bond (ESPE, Germany), a resin-modified GI cement (McCabe, 1998).

A different mode of fluoride release from that of GI's, that of ion-exchange diffusion, has been proposed by Rawls (1991) for this class of materials. Fluoride is chemically bound to the polymer matrix and only very small amounts are released from any one site. Fluoride, found (e.g. as amine-HF groups) within a highly cross-linked three-dimensional network, is released when a F^- ion exchanges for another ion (e.g. Cl^-) in the oral environment. This occurs in response to a reactant entering the matrix and causing a chemical change. Because the ions are exchanged, no degradation of the material occurs as a result of fluoride release (Rawls, 1995). The re-organized polymer matrix therefore maintains structural integrity.

It is too simplistic, however, to assume that all resin-modified and conventional GI's release fluoride via diffusion, whereas all modified composites undergo ion-exchange. These models are meant to aid in our comprehension of the as-yet incompletely understood process of fluoride release.

4.c Compomers

Compomers, or polyacid-modified composite resins, are single pastes, which contain all the major ingredients of GI's and composites except for water. The exclusion of water ensures that polymerization will take precedence over the acid-base reaction, since there is no water to liberate cations from the glass particles. Later, when in contact with the oral fluids, a limited acid-base reaction may occur as water molecules penetrate the covalent polymeric network. The glass particles in a compomer are partially silanized (treated with a silane coupling agent) to allow them to bond directly to the resin matrix, which is composed of modified methacrylates such as Bis-GMA (Meyer *et al.*, 1998). Some brands are Compoglass (Vivadent, Schaan, Liechtenstein), Dyract (Dentsply/DeTrey, Kostanz, Germany), and Ana Compomer (Nordiska Dental, Helsingborg, Sweden).

These materials are commonly marketed as restorative materials (El-Kalla and Garcia-Godoy, 1999), although Compoglass has been tested in a recent bracket bonding study (Haydar *et al.*, 1999). Rates of fluoride release from this class of materials tend to be lower than those of the resin-modified GI's, such as 7.8 $\mu g/cm^2$ for Dyract after 1 week, compared with 25.9 $\mu g/cm^2$ for Fuji II LC (McCabe, 1998). Compomers do not contain water in their formulations. Therefore, in order for diffusion to occur, water must first diffuse from the aqueous environment into the material before diffusion of fluoride ions into the environment can commence. In short, of all the hybrid materials, the compomers seem to behave most like CR's and least like the glass ionomer cements.

4.5d Summary: advantages of including fluoride in an orthodontic cement

In summary, there are several advantages of including fluoride in a bracket-bonding adhesive.

1. The fluoride is supplied directly to the area where it is most needed, while minimizing unnecessary systemic dosage, i.e. >10 μmol/L plasma fluoride levels, according to Clarkson (1991). This is important, given that decalcification has been shown to occur as soon as four weeks after bonding. For instance, the premolars (slated for extraction) of patients who brushed with 1100 ppm NaF toothpaste had 14% more mineral loss at the cervical margin when the bracketed teeth were extracted one month later and tested for microhardness, compared with those who brushed and applied APF gel weekly (O'Reilly and Featherstone, 1987).
2. In theory, the fluoride can be delivered at a continuous low dose, over a long treatment time (for at least six months, according to Ripa, 1991).
3. The need for extra chair time and patient compliance are minimal (Rawls, 1991). It is interesting to note, however, that manufacturers are reluctant to claim caries inhibition or decalcification prevention related to the release of fluoride by their product. The reason is that government agencies such as the Food and Drug Administration might then classify the materials as drugs rather than devices, requiring more costly and stringent testing procedures (Rawls, 1991).

4.6 Measurement of fluoride release

4.6a *In vivo* studies

Clinical trials and *in vivo* studies are the benchmark by which orthodontic materials should ultimately be judged. However, *in vitro* studies provide the opportunity to create a more controlled environment for the testing of materials, especially new products with no previous database of knowledge from which to draw. Potential problems with split-mouth testing (where two quadrants of subjects' teeth have brackets bonded with one cement, the other two have a different type) is that as fluoride dissolves in the saliva, it may cross over to the "nonfluoride" segment of the mouth (Millett *et al.*, 1999). To overcome this problem, independent test and control subjects would be needed. However, variability of such factors as diet and caries risk of the subjects would then be increased (Millett *et al.*, 1999). When fluoride concentration was measured in plaque adjacent to brackets, it was found that different sites in the same mouth had different fluoride levels. Some studies test plaque concentrations of fluoride adjacent to cemented brackets (Chung *et al.*, 1998); however, lower incisors had higher concentrations of fluoride in the plaque than upper incisors (30 vs 5 ppm) within the same mouth (Arneberg *et al.*, 1997). A more detailed discussion of various testing methodologies for *in vivo* studies will be found in the Discussion section of this paper.

No one number can be chosen as the "optimum" or even "minimum" concentration of fluoride that must be maintained in the vicinity of an orthodontic bracket to provide protection against decalcification (Rawls, 1991). It is conditional upon the caries risk of the individual, oral hygiene, diet (Millett *et al.*, 1999), plaque microbiology, and exposure to other sources of fluoride (Clarkson *et al.*, 1988), to name several factors.

There has been very little standardization of the protocol for studies of fluoride released from dental cements. Measurement is very much dependent on methodology, which varies widely in the literature (Monteith *et al.*, 1999). Table 4.1 indicates some of the variations of different experimental details in a review of the literature.

Table 4.1

In vitro studies on fluoride release from orthodontic bonding agents.

(from Monteith et al., 1999)

Study	Materials	No. of test specimens per material	Volume and type of medium	Specimen type and size	Observation time	Units of fluoride release
Creekey et al. [11]	A	5	10 ml distilled water	Discs 15 mm dia. × 1 mm thick	3 months	$\mu g/cm^2$
	B					
	C					
	D					
Fox [4]	A	10	10 ml deionised water	Slabs 10 mm × 1 mm	20 weeks	µg
	B					
	E					
Chan et al. [12]	A	20 teeth	4 ml deionised water	Molar teeth with bonded brackets	5 weeks	mg
	F	5 discs A				
		2 discs F				
Rinhare et al. [13]	A	20 teeth	4 ml deionised water	Teeth with bonded brackets	5 weeks	mg
	F					
Øgaard et al. [14]	F	12	10 ml human saliva, 10 ml water	Discs 8.5 mm dia. × 3.5 mm thick	6 months	mg
Chani et al. [4]	E	12	5 ml demineralising solution	Premolar teeth with bonded brackets	8 days	µg
	C					
	G					
Newman and Rudolph [15]	A	ng	30 cm^3 distilled water	Discs 20 mm dia., 1.5 mm thick	1.5 year	ng/mm^2
	2 types 1% NaF 10% dentinsulfon					
Wilshire and Jones van Rensburg [9]	F	10	1 ml distilled water	Discs, vol. 56.57 mm^3 surface area 0.9 cm^2	85 weeks	$\mu g/cm^2$
	G					
Chadwick and Gordon [16]	H	10 teeth	1 ml distilled water	Premolar teeth with bonded brackets	1 month	µg
	I					

While no ISO standards exist for the best method of measurement, it should be kept in mind that the goal of *in vitro* studies is to simulate as closely as possible the *in vivo* situation, to provide the most clinically relevant data.

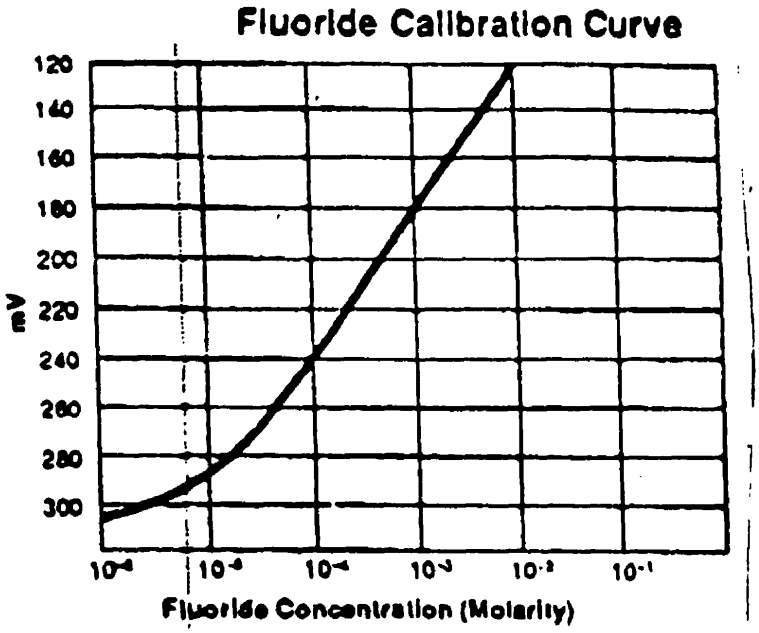
4.6b Detection of fluoride ion and units of fluoride release

Early studies used complicated techniques to estimate the amount of fluoride ion released. One, for example, distilled fluorine gas from sulphuric acid and estimated the amount with spectrophotometry (Tviet and Gjerdet, 1981). The standard procedure in the more recent literature, however, is to use an ion-sensitive electrode to detect fluoride ion in solution, as described by Fox (1990). The technique of ion-selective fluoride analysis has been described as accurate, sensitive and specific (Nakajima *et al.*, 1997). The sensor of the ion-specific electrode is a single crystal of lanthanum fluoride. A potential, the strength of which depends of the amount of fluoride ion present in solution, develops when the sensor contacts a solution. This potential is measured on a millivoltmeter, using a standard technique (Fox, 1990).

Before each use and regularly throughout the testing session, the fluoride electrode must be calibrated using a series of standard fluoride solutions containing known concentrations of fluoride ion, e.g. 1, 10, 100, and 1000 ppm. The solutions should bracket the F^- concentration of interest. A calibration curve of ppm vs Log[conc] of F^- ion may be plotted on semi-logarithmic paper (Fox, 1990) using the Nernst equation. At room temperature (approximately 20°C), the slope of the line will be constant at 58 mV per ten-fold concentration difference, except at very low concentrations of F^- ion (Rix, 1999).

Figure 4.2.

Example of a fluoride calibration curve, from Orion specifications for ion-sensitive electrodes.



The ppm reading for a particular solution can therefore be determined by plotting its millivolt reading on such a graph as shown in Figure 4.2. By thus measuring the ppm fluoride in a known volume of solution, the total amount of fluoride ion released per mL of solution can be calculated (Fox, 1990). Many studies commonly report fluoride released simply by mass; i.e. in μg (Monteith *et al.*, 1999). This is calculated by multiplying the ppm (1 ppm = 1 μg/mL) by the volume of the water sample in mL. Alternatively, fluoride release has been reported as a function of mass of the material e.g. ng/g (Rawls, 1995). However, according to the review of the literature by Monteith *et al.* (1999) it is not a commonly used unit. Despite the variety of units mentioned in the literature, there have been no studies discussing the accuracy of various fluoride release units.

4.6c Size and surface area of specimen

Many studies take the surface area of the sample into account by reporting fluoride release in units of $\mu g\ F^-/cm^2$ of sample area (Rix, 1999). This is calculated by dividing the total amount of fluoride released by the area of the sample disc in cm^2. A flat disc of cement will expose more surface area to the aqueous environment than a cube of equal mass. The volume of the sample of cement material has been shown to have a nearly linear effect on fluoride ion release (Newmann and Rudolph, 1994), i.e. a sample with twice the volume will release twice as much fluoride under the same conditions. An experiment that kept the volume of two groups of samples constant but varied the surface area available for fluoride release (by coating part of some specimens with Max Factor Diamond Hard nail varnish) found that the specimens with twice as much surface area released 1.3 times as much fluoride (Monteith *et al.*, 1999). Therefore, the relationship between surface area and fluoride release may not be linear, perhaps lending credence to the theory that diffusion of fluoride from the matrix to the surface of the material is the rate-limiting step (Rawls, 1995). Discs of up to 20 mm in diameter and 1.5 mm in thickness (Cranfield *et al.*, 1982) have been used to evaluate fluoride release. In orthodontics, however, a comparatively small amount of cement (estimated by Rix in 1999 to be 0.1 mm width adhesive at all perimeters of the bracket base) will be exposed to the oral environment.

4.6d Single-point vs. multi-day measurements

When fluoride is released by diffusion, the rate of release over time decays gradually, roughly as the square root of time. The reason for the rate decay is that as time progresses, the agent must be leached and extracted from deeper within the matrix (Rawls, 1991). When several such decay curves are compared, they may cross over each other, making any measurement at a single time point quite meaningless (Cranfield *et al.*, 1982). Therefore, a number of time points are plotted on a curve to determine the pattern of fluoride release over time. Although useful as an overview, a statistical disadvantage of this technique is that a large amount of data must be analyzed if comparisons are to be made at every time point. Therefore, it may be more meaningful to make statistical comparisons only at selected time points (Hassard, 1999).

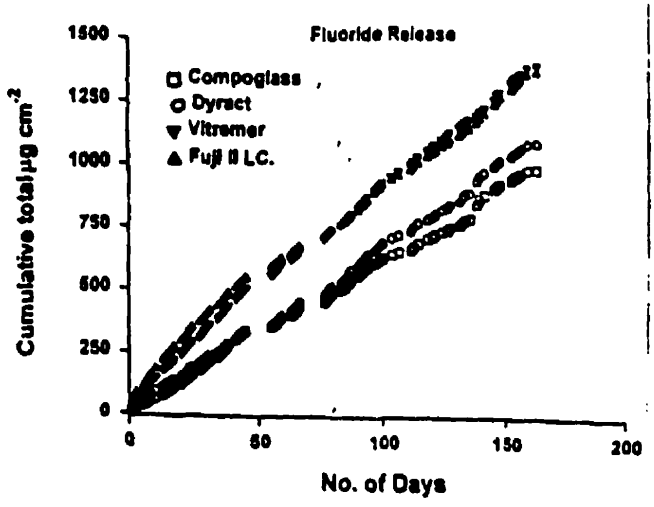
4.6e Cumulative vs. interval measurements

Cumulative measures record the total amount of fluoride released over time. Figure 4.3 shows cumulative release, measured with a fluoride-sensitive electrode, for 4 materials when storage water was replaced daily.

Figure 4.3.

Cumulative fluoride release over 6 months

(from McCabe, 1998)



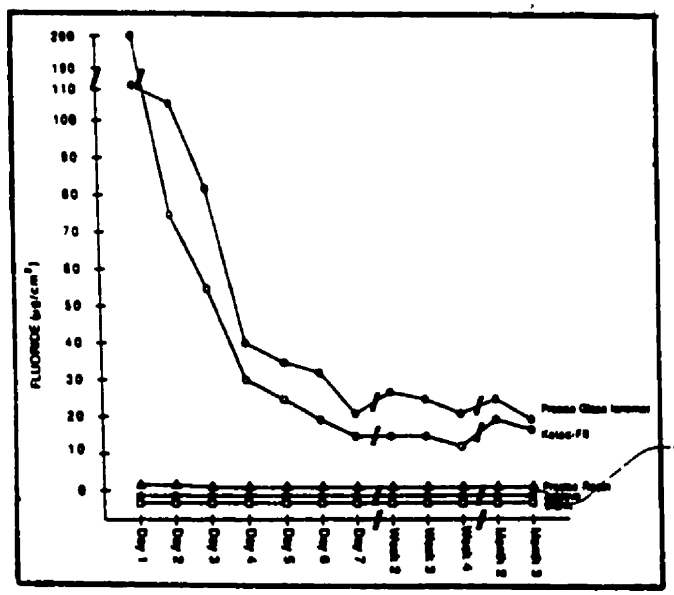
Cumulative fluoride release over 6 months.

The curve will show whether one material consistently releases more fluoride than another. Interval measurements involve changing the storage solution 24 hours prior to measurement. More frequent water changes become practically difficult, according to McCabe (1998). When readings are taken later, they will only show the fluoride released since the last water change. In this way, the amount of fluoride present at the site of action can be estimated more accurately (Cooley *et al.*, 1989). Whereas cumulative graphs show an increasing concentration of fluoride ion gradually reaching a plateau, interval graphs typically show a sudden burst of fluoride release (corresponding to the time period when the material is freshly set), followed by exponential decay (Wiltshire and Janse van Rensberg, 1995) to low levels after several weeks. Figure 4.4 shows fluoride released by discs of a GI and a CR, 15 mm diameter x 1 mm thick, placed in 10

mL distilled water for a total time period of 3 months. Measurements were taken daily for the first 7 days, weekly for the next 3 weeks, then monthly for 2 months. Solutions were changed 24 hours prior to sample analysis (Cooley *et al.*, 1989).

Figure 4.4.

Interval fluoride release of Ketac-Fil and Precise (from Cooley *et al.*, 1989)



The glass ionomers Ketac-Fil and Precise exhibited a "burst effect" of fluoride release and then diminished dramatically. The Precise composite resin released only minimal levels of fluoride for three days and then was unable to be recorded.

4.6f Type and volume of storage solution

Deionized distilled water is the most common solution used, although saline and methylcellulose (a component of some saliva substitutes) have been mentioned by Momoi and McCabe (1993). Carvahlo and Cury (1999) immersed specimens in a pH-cycling system (6 h in a demineralizing solution of calcium phosphate buffered to pH 4.3, then 18 h in a remineralizing solution of artificial saliva at pH 7.0). The gold standard is fresh natural saliva. Although Øgaard *et al.* (1997) have conducted some research using human saliva, experimental problems include different rates of salivary fluoride clearance

depending on the intraoral site, potential inconsistency in salivary F^- concentration due to dietary or topical fluoride sources (Dawes and Weatherell, 1990), and instability when used in long-term controlled studies (Leung and Darvell, 1997). Artificial saliva is a buffered solution containing Na, K, Mg, Cl, inorganic P, and small concentrations of other ionic species, depending on the exact formulation. It more closely resembles the complex chemistry of the oral environment than does deionized distilled water (El-Mallakh and Sarkar, 1990).

Use of artificial saliva as the storage medium has resulted in lower rates of total fluoride release (e.g.1.26 vs 7.62 $\mu g/cm^2$ over 15 days for Chelon-Fil) compared with deionized water (Carvahlo and Cury, 1999), but has also resulted in comparable F^- release from Photac-Fil compared with a self-cure GI (Ketac-Fil); when in distilled water the self-cure material released 6 times as much fluoride (Wandera *et al.*, 1996).

The greater the volume of water in which the samples are stored, the more dilute will be the fluoride. If results are given in units of ppm or μg/mL, then the amount of fluoride per mL of storage solution has already been calculated. If fluoride release rate is reported in different units, such as μg alone, caution should be exercised when comparing results from different studies.

4.6g Temperature of solution

Fluoride release in distilled water for some GI materials is significantly higher when measured at 37°C (mouth temperature) compared with 21°C (room temperature). For example, after 24 h, Fuji II released 800 ng/g/day at the higher temp, compared with 350 ng/g/day at room temperature. This pattern may indicate a greater diffusion rate of fluoride into solution as temperature increases (Jones *et al.*, 1987).

4.6h Total period for fluoride measurement

Since the average time of orthodontic brackets in the mouth is two years or more, and interval testing demonstrates that there is a rapid decay rate of fluoride release after the first week of testing, it is important to determine long-term rates of fluoride release. Many studies testing orthodontic cements are surprisingly short-term. The total number of days of fluoride release reported in the literature included eight (Ghani *et al.*, 1994), 30 (Chadwick and Gordon, 1995), and 43 (Chan *et al.*, 1990). However, according to the review of the literature by Monteith *et al.* (1999), more common time spans for testing are between 20 and 52 weeks. Few researchers have longer-term data, although Forsten (1998) has tested restorative materials for up to 8 years.

4.6i Use of buffer solutions

Most of the recent literature acknowledges the need to hold constant the pH of samples being tested at between pH 5 and 5.5, as differences in pH will result in inaccurate readings from the electrode (Monteith *et al.*, 1999). A commercial buffering solution, Total Ionic Strength Adjustment Buffer (TISAB), is specifically designed to accomplish this as well as freeing F^- bound to hydrogen ions and eliminating interference from other ionic species such as hydroxyl and aluminum. Aluminum and fluoride ions readily form complexes in the presence of excess fluoride ions (Meyer *et al.*, 1998). The ionanalyzer, which can only respond to an ionic form of fluoride, would not recognize these complexes as containing fluoride (Nakajima *et al.*, 1997). TISAB is an aqueous mixture of acetic acid, NaCl, CDTA, and sodium hydroxide (Fox, 1990). Addition of a specified amount of TISAB to every sample being tested, waiting for several minutes for

the pH to stabilize, and then testing the sample on the ionanalyzer has become the standard protocol for fluoride measurement (Chan *et al.*, 1990; Ghani *et al.*, 1994).

4.6j Cement disc model vs. tooth-bracket model

Most studies tested fluoride release from a disc of cement suspended in distilled water (Fox, 1990; Wiltshire and Janse van Rensberg, 1995; Trimpeneers *et al.*, 1998). However, even when smaller (0.94 cm^2 surface area) size discs are used (Wiltshire and Janse van Rensberg, 1995), it is likely that the amount of cement exposed to the environment exceeds that which would be available clinically. Several researchers have bonded brackets to extracted teeth and measured the fluoride release of the tooth-bracket model (Ashcraft *et al.*, 1997; Monteith *et al.*, 1999; Rix, 1999). A potential disadvantage of the tooth-bracket model is the possibility of fluoride on the tooth surface (in the form of CaF_2) diffusing into the solution during testing (Monteith *et al.*, 1999). When direct comparisons were made between fluoride release (in ppm) of the discs and the tooth/bracket models, Monteith *et al.* (1999) found that the tooth model resulted in generally lower rates of fluoride release than when discs were used. However, this study may be criticized since the relative surface areas were not taken into account in calculation of the units of fluoride release. Rix (1999) found that teeth bracketed with Fuji Ortho LC released more fluoride per unit area than Assure (i.e. 2.23 vs. 1.06 µg/tooth/day at day 1). Conversely, he showed that discs of the same two materials showed the opposite trend, i.e. more fluoride release per unit area in Assure (69.75 vs. 57 $\mu g/cm^2$/day at day 1). As long as unit area is accounted for in the units, there is no definitive evidence in the literature that the tooth-bracket model provides more accurate information on fluoride release than the disc model.

4.6k Frequency of storage solution change

This is perhaps the most controversial area of the methodology, because of the potential great differences in reported fluoride release when it is varied. Rix (1999), who compared changing water 24 h prior to testing to calculating daily release by dividing the cumulative release for one month by 30, found that 24 h fluoride release values were from 3 to 13 times greater than the "month-based" daily average. Some (but by no means all) reported methodologies for timing of water changes and fluoride measurements are:

1. Accumulation of fluoride over the whole observation period (Cranfield *et al.*, 1982), with periodic fluoride measurements but no water changes (cumulative measurements).
2. Daily water changes for a week, then weekly changes for a month, then monthly changes for several months, with no water changes 24 hours prior to taking measurements (measures are cumulative from the point of the previous water change). This method was used by Tviet and Gjerdet (1981).
3. Daily, weekly and monthly changes similar to 2 above, but with the added step of changing the water 24 hours prior to measurement to avoid cumulative values, similar to the present study and also used by Monteith *et al.* (1999).
4. Continuously flowing water over the samples with periodic testing by immersing samples in still, de-ionized water, then measuring fluoride release (Forsten, 1990).

In the mouth, a constant flow of saliva is washing away the free fluoride ion, but clearance rates are higher in the lower anterior segment than the upper anterior segment. Fluoride levels in saliva are subject to change depending on exposure to dentifrice,

fluoride-containing foods, and rinses, for example (Cranfield *et al.*, 1982). Hence, none of the *in vitro* models are able to simulate completely the *in vivo* situation.

4.7 Summary

In vitro studies to determine fluoride release into water or artificial saliva have been performed on orthodontic materials in the past. They can be considered a useful alternative to *in vivo* research because of the difficulty in standardizing factors in human subjects such as clearance rates of saliva in different intraoral segments, intraoral fluoride intake differences among subjects (Dawes and Weatherell, 1990), and potential crossover of fluoride in split-mouth trials (Millett *et al.*, 1999).

An *in vitro* trial can approach the best compromise of control and simulation of intraoral conditions, by maintaining samples at mouth temperature, frequent changing of solutions or immersing samples in running water, and testing fluoride release for a long enough time period to be meaningful for orthodontic purposes. The data gleaned from such an investigation would be useful in comparing fluoride release rates among materials, hence providing a benchmark for further tests of clinical performance.

4.8 Purpose

The purpose of the current study was to compare the rates of fluoride release over time from two new polyacid-modified CR materials (Assure[a] and Python[b]) with those from a resin-modified glass ionomer (Fuji Ortho LC[c]), and a non-fluoride composite resin control (Transbond XT[d]). All materials were light-cured materials designed for the bonding of orthodontic brackets. Both Assure and Python have been available for purchase for less than 2 years. The only published research on either material is by Rix (1999), comparing Assure with Fuji Ortho LC and an experimental material.

Deionized distilled water and artificial saliva were tested to determine differences in fluoride release due to storage media. In addition, the material that released the most fluoride was re-tested under continuously running deionized distilled water to simulate *in vivo* conditions more closely.

4.9 Hypotheses

1. That the resin-modified glass ionomer, Fuji Ortho LC, will release more fluoride than the other materials.
2. That the bonding agents will release more fluoride in deionized distilled water than in artificial saliva.
3. That the highest-releasing material will release less fluoride after exposure to running water than after immersion in still water.

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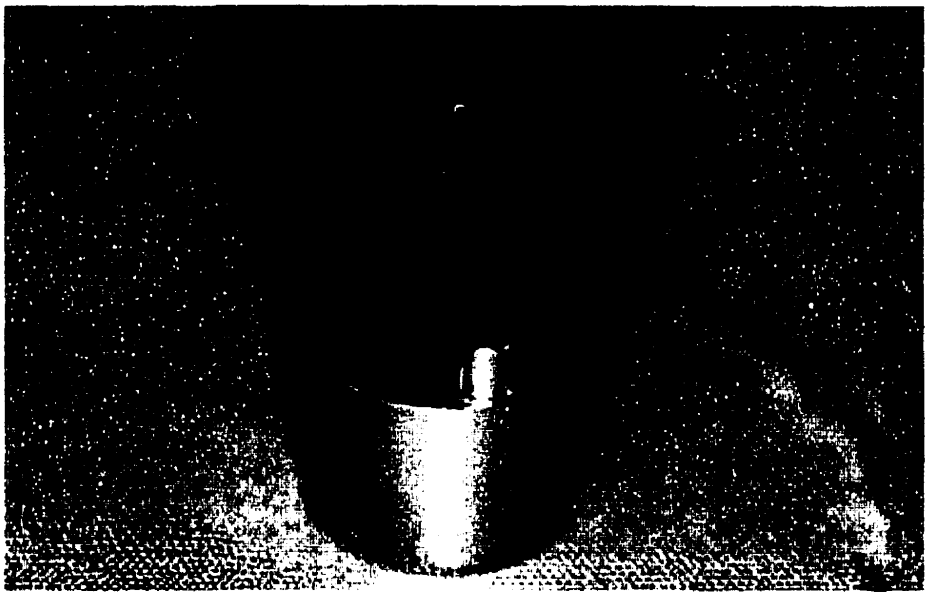
[a] Reliance Orthodontic Products, Inc., Itasca IL
[b] TP Orthodontics, LaPorte, IN
[c] GC Corp., Tokyo, Japan
[d] 3M Dental Products, Monrovia, CA

5.0 MATERIALS AND METHODS

5.1 Specimen fabrication

A steel and teflon split-mould was machined at The University of Pretoria, South Africa. It is capable of producing specimens of equal diameter (6 mm) and three different thicknesses. However, only the smallest thickness (2 mm) was used for this study. The surface area of the discs was calculated using the formula $[\pi\ d\ h] + [2\pi(r^2)]$, as stated by Monteith *et al.* (1999). The surface area of each specimen was found to be 0.9425 cm^2.

Figure 5.1. Split-mould



Twenty equal-sized (6 mm in diameter and 2 mm in thickness) specimens of each material were formed into disks using the mould. Names, manufacturers, lot numbers and a brief description of each material are found in Table 5.1.

Table 5.1 Materials used in the study

Material	Manufacturer	Lot Number	Description (as provided by the manufacturer)
Transbond XT	3M Dental Products, Monrovia, CA, USA	062697	Resin base: BIS-GMA and TEGMA (1:1ratio) with hybrid silica filler particles (average size 3 μm)
Python	TP Orthodontics, LaPorte, IN, USA	CE 0646 (no fault)	BisGMA system, containing a prepolymerized acrylic modified resin with an acrylic monomer and containing glass particles as an active filler
Assure	Reliance Orthodontic Products, Inc., Itasca IL, USA	039188	A fluoride-releasing paste.
Fuji Ortho LC	GC Corp., Tokyo, Japan	071267	Glass ionomer material with powder 100% alumino-silicate glass. Liquid is a mixture of distilled water, polyacrylic acid, HEMA, and initiator

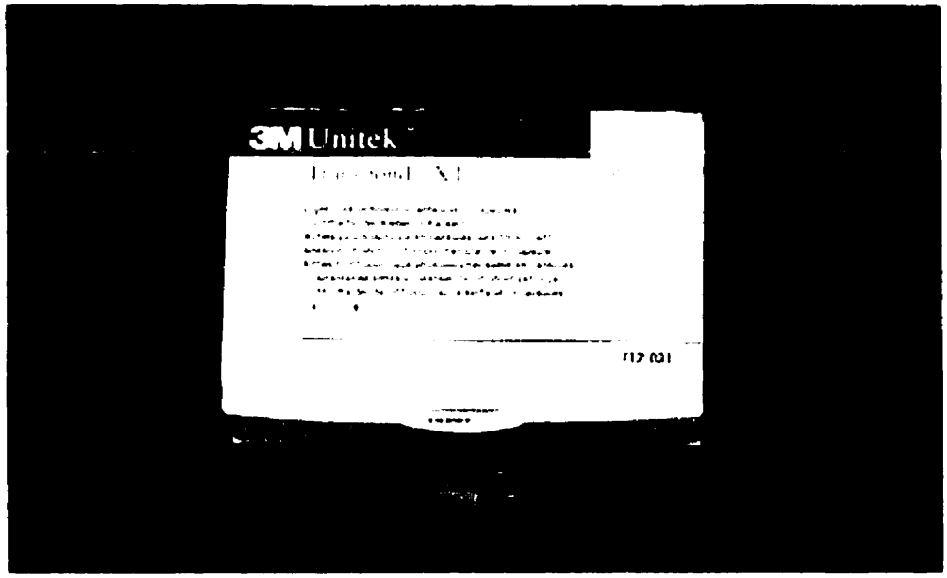
Materials were handled according to the following protocol:

5.1a Transbond XT

A capsule of Transbond XT adhesive paste was placed in the application gun supplied by the manufacturer and the paste was expressed into the mould, avoiding

inclusion of voids. The Transbond primer was not used. The mould was filled flush to the top with adhesive paste.

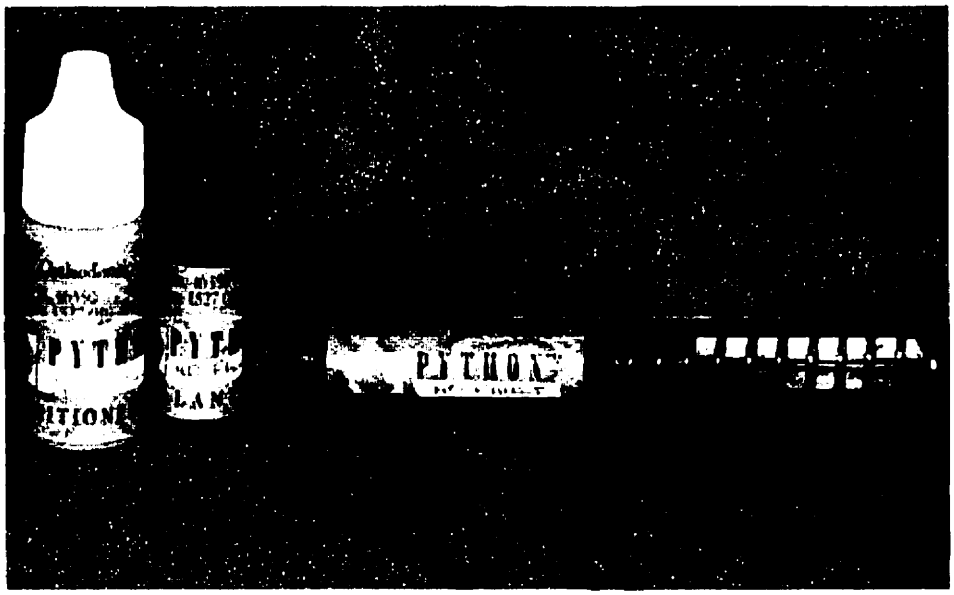
Figure 5.2 Transbond XT bonding resin



5.1b Python (fluoride-containing composite resin)

The syringe tip of Python adhesive paste was held just over the mould as the paste was expressed into the split-mould. The mould was filled completely. Neither Python conditioning liquid nor sealant was used.

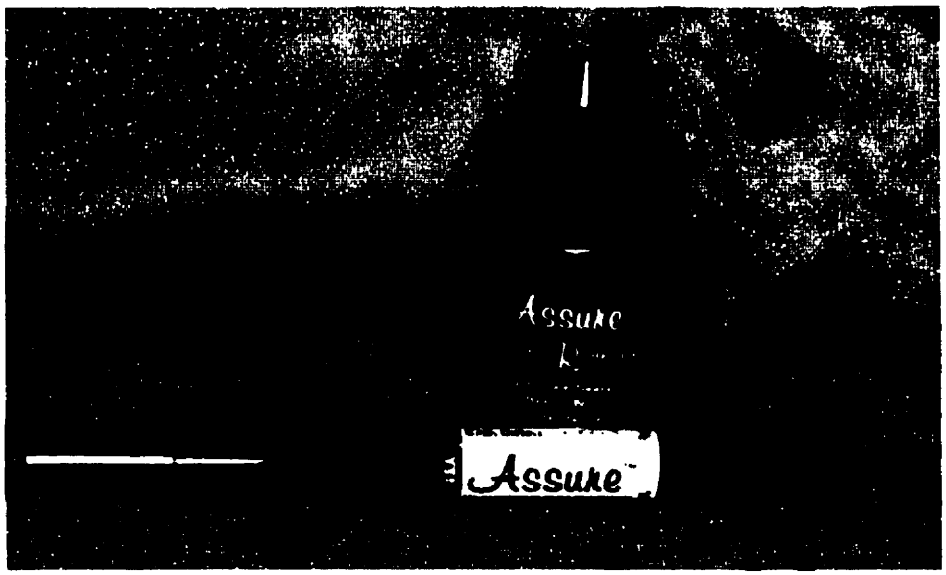
Figure 5.3 Python bonding resin



5.1c Assure (fluoride-containing composite resin)

Adhesive paste from the syringe was expressed into the split-mould in the same manner as described for the previous material. The light-cure sealant was not used.

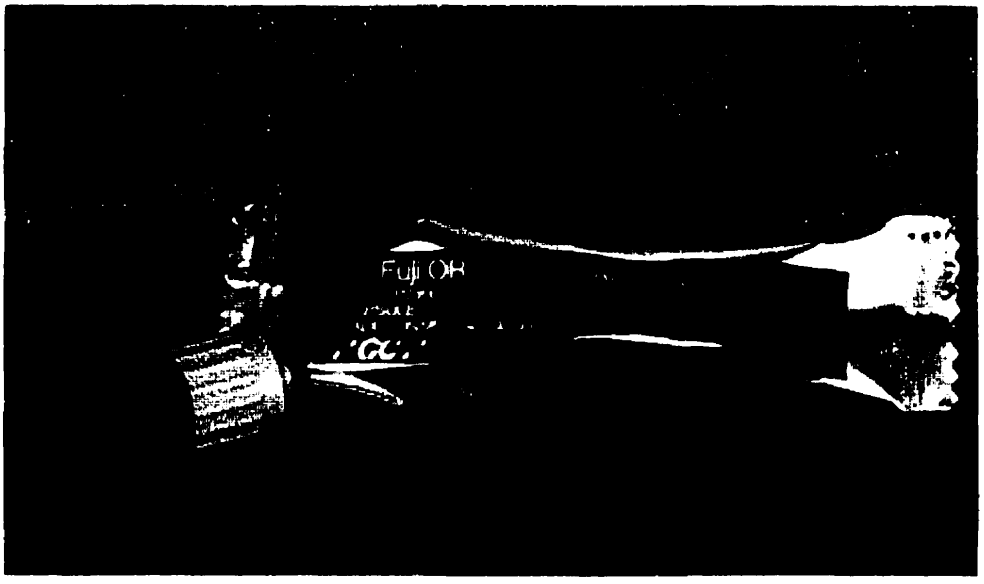
Figure 5.4 Assure bonding resin



5.1d Fuji Ortho LC (resin-modified glass ionomer cement – encapsulated)

The adhesive-filled capsule was squeezed together by hand to break the membrane separating powder and liquid. The capsule was then triturated for 10 seconds in a Vari-Mix III triturator (Caulk/Dentsply, Milford, DE) at approximately 4000 rpm. The capsule was then loaded into the application gun supplied by the manufacturer and the adhesive paste was squeezed into the mould.

Figure 5.5 Fuji Ortho LC bonding material

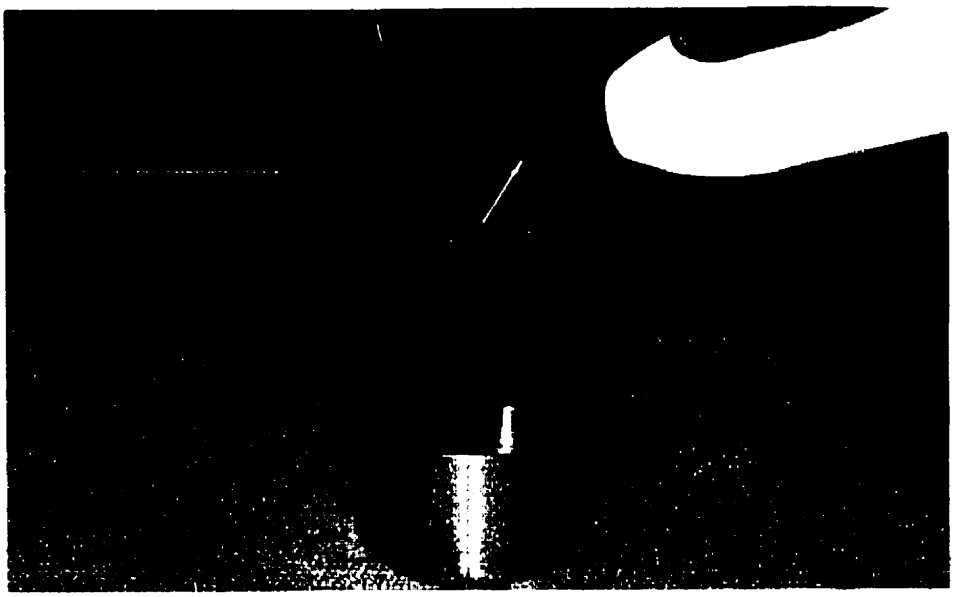


5.1 e Sample manufacturing technique

When the split-mould was filled flush to the top with a sample of each type of the uncured bonding material, a clear mylar strip (Palmero Health Care, Stratford, CT), was held with light finger pressure over the unset material, in contact with the superior surface of the mould. An Ortholux XT Visible Light Curing Unit (3M, St Paul, MN) was used to light-cure all materials. The light tip was held directly over, but not in contact

with, the sample. Curing time was 40 seconds per specimen for all material types. Once curing was completed, each specimen was released from the mould.

Figure 5.6 Filling of split-mould with Transbond XT



All discs were then weighed on a model 2001 MP2 electronic analytical balance (Sartorius, Gottingen, Germany) to the nearest 0.0001g. Each disc was then placed into an individual 10 mL polyethylene test tube (#14-956-3D, 12x75 mm with snap caps, Fisher Scientific, Pittsburgh PA), and labeled with the material type and specimen number. A total of 80 sample discs (20 of each material) were fabricated and light-cured in this manner. A time period of 24 hours elapsed between fabrication of the specimens and immersion in the sample solutions, during which time the samples were maintained in their test tubes in an incubator (Thelco Precision Scientific, model #18, Chicago, IL) at 37° C and 100% relative humidity.

5.2 Immersion solutions

5.2a Deionized distilled water

Deionized distilled water was used as the immersion solution for 10 samples of each material. Using an automatic pipette (Brinkman Dispensette 2 ml, Westbury NY), 1 mL of water was added to 10 test tubes for each material, for a total of 40 specimens.

5.2b Artificial saliva

Artificial unstimulated saliva was made up according to the following recipe, based on research by Dawes and Dong (1995).

Table 5.2 Components of unstimulated artificial saliva per litre of distilled water

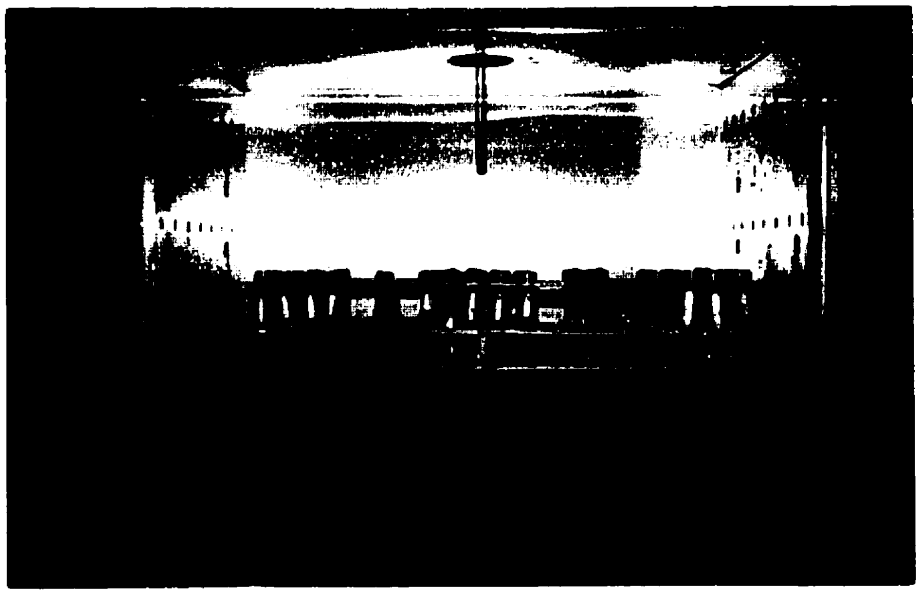
Component	Concentration (g/L)
KCl	1.0438
NaH_2PO_4	0.6805
$NaHCO_3$	0.4200
$CaCl_2$	0.0331
$MgCl_2$	0.0061

The pH of the solution was fixed at 6.95.

The remaining 40 specimens (10 of each material type) received 1 mL of the artificial saliva, measured with an automatic pipette as per the distilled water group. Two "blank" test tubes, one containing 1 mL of distilled water and one containing 1 mL of artificial saliva, but no specimens, were also included. All test tubes were then capped

with their polyethylene covers. The test tubes were placed in racks and stored in an incubator at 37°C and 100% relative humidity.

Figure 5.7 Storage of specimens in incubator



5.3 Fluoride electrode setup

A fluoride ion-specific combination electrode model 13-620-528 (Orion Research Inc. Beverly, MA) was connected to a pH meter (Radiometer pHM 82 standard pH meter, Copenhagen, Denmark). When not in use, the electrode was immersed in a standard solution of fluoride as per the manufacturer's instructions.

Figure 5.8 Fluoride electrode and pH meter

5.4 Fluoride electrode calibration

A series of standard solutions containing a known concentration of fluoride ion were made using serial dilutions of NaF in distilled water.

Table 5.3 Concentrations of fluoride ion in the standard NaF solutions

Parts per million (ppm)	g F^- / L
1000	1
100	0.1
10	0.01
1	0.001
0.6	0.0006
0.1	0.0003
0.1	0.0001

The operation of the electrode is based on the potential which develops across the electrode's membrane. The relationship between potential and fluoride ion activity (or concentration) is set forth by the Nernst equation:

$E = E° - S \log A$

Where:

E = measured electrode potential

E° = constant, sum of several system potentials

S = electrode slope

A = fluoride ion activity

At 20°C, the ideal Nernstian equation slope is –58 mV per decade increase in fluoride ion activity.

Calibration of the fluoride electrode system was performed using the full range of standard solutions prior to each test session and after every 20 readings.

5.5 Fluoride release measurement

Measurement of the solution fluoride levels and changing of the solutions was carried out daily for the first seven days, weekly for the next three weeks, and monthly for the next 5 months, for a total testing period of six months from time of first immersion in the solutions. Fresh batches of artificial saliva were made and used for all weekly and monthly testing sessions.

At the time of testing, each sample solution was collected in a 5 mL polyethylene test cup (Nalgene, Rochester NY). To each 1 mL of sample solution, an equal amount of

TISAB II (Orion Research Inc., Beverly MA) was added to the test cup. The TISAB II was added to obtain a constant background ionic strength and eliminate aluminum interference. The solutions in the test cups were allowed to reach room temperature prior to fluoride level testing, as per the instructions of the manufacturer of the electrode.

The electrode was removed from its standard solution, rinsed several times with distilled water and dried with a tissue, as per the manufacturer's instructions. The electrode tip was then immersed in the solution in the test cup. The reading (in mV) was allowed to stabilize for several minutes before being recorded. The electrode was rinsed and dried in between the recording of each sample solution. From the calibration curve determined by the standard solutions, the readings in mV recorded from the pH meter were converted by hand using semi-logarithmic graph paper to ppm (parts per million) values.

5.6 Running water testing

After the initial six months of fluoride release testing were complete, all samples were maintained in 1 mL of their respective solutions (either deionized distilled water or artificial saliva) in the capped test tubes at 37°C and 100% relative humidity.
Six months after the final test session, a trial test period under running distilled water was carried out on the Assure material only, for a total testing period of 2 weeks. This running water test was performed in order to simulate more closely salivary flow and to investigate the effect thereof on fluoride release.

The 10 sample discs of Assure in distilled water were placed in a 25-mL shallow plastic container (Nalgene, Rochester NY), on top of a test tube rack sitting in a large

basin. A digital flow controller (Masterflex Digistatic 7525-00, Cole-Parmer Instrument Co. Vernon Hills IL) was calibrated to provide a flow rate of deionized distilled water over the samples of 1 mL/min, which is roughly equal to the flow rate of saliva in the mouth in orthodontic patients (Forsberg *et al.*, 1992). The entire apparatus was kept in a walk-in incubator maintained at 37°C. A constant flow of distilled water thus ran over the samples, with overflow collected in, and periodically emptied from, the large basin below.

After 48 h of exposure to the continuous water flow, each sample was placed in 1 mL of deionized distilled still (not flowing) water for 24 h at 37°C. The resultant 10 x 1 mL samples of solution were each combined with 1 mL of TISAB II. The 10 water samples were then tested with the ion-sensitive electrode as previously described. The 10 discs of Assure were then placed back in the shallow dish under the flow of distilled water. The 24-hour soak in distilled water, followed by fluoride release testing, was repeated at 7 and 14 days from time of first exposure to running water.

Controls for the running water experiment consisted of 10 samples of the same material (Assure) which were the same age (12 months), had the same history of storage and handling, and had been tested for fluoride release the same number of times as the experimental samples. Each of the 10 control discs was placed in 1 mL of deionized distilled water in a capped test tube on Day 1 of the running water trial. Testing of these samples also occurred at 3, 7 and 14 days. Immediately after testing, the samples were replaced in their individual test tubes with 1 mL of fresh deionized distilled water and returned to the incubator until the next testing session. They were not exposed to continuously flowing water.

6.0 RESULTS

The Results segment of this thesis is divided into four sections. The first (6.1) presents the mean fluoride release values for all four materials in both distilled water and artificial saliva. Section 6.2 presents the statistical analyses of these values at days 1, 7, 91 and 183. Significant effects due to type of material and storage medium are examined in section 6.3, while the final section (6.4) presents the relative fluoride release values and statistical analyses from Assure in both running water and still water at days 1, 7, and 14.

6.1 Mean fluoride release in still water and artificial saliva

The daily fluoride release from the samples into distilled water and artificial saliva are summarized at all time points in Appendices I and II. The data were converted from mV to ppm using standard curves. They were subsequently transformed into $\mu g/cm^2/day$ by dividing each ppm value by the surface area of the discs (0.94 cm^2), with the unit $\mu g/cm^2/day$ selected to facilitate comparisons with the published literature. As previously discussed (section 4.6), the scientific community has yet to accept a fluoride release universal unit. However, $\mu g/cm^2/day$ is a common unit in the orthodontic literature.

The mean fluoride release rates in $\mu g/cm^2/day$ as a function of time, in distilled water and artificial saliva, for the materials evaluated in this study are illustrated in Figures 6.1 and 6.2.

Figure 6.1

Change in rate of fluoride release into distilled water with time (mean ± S.E.)

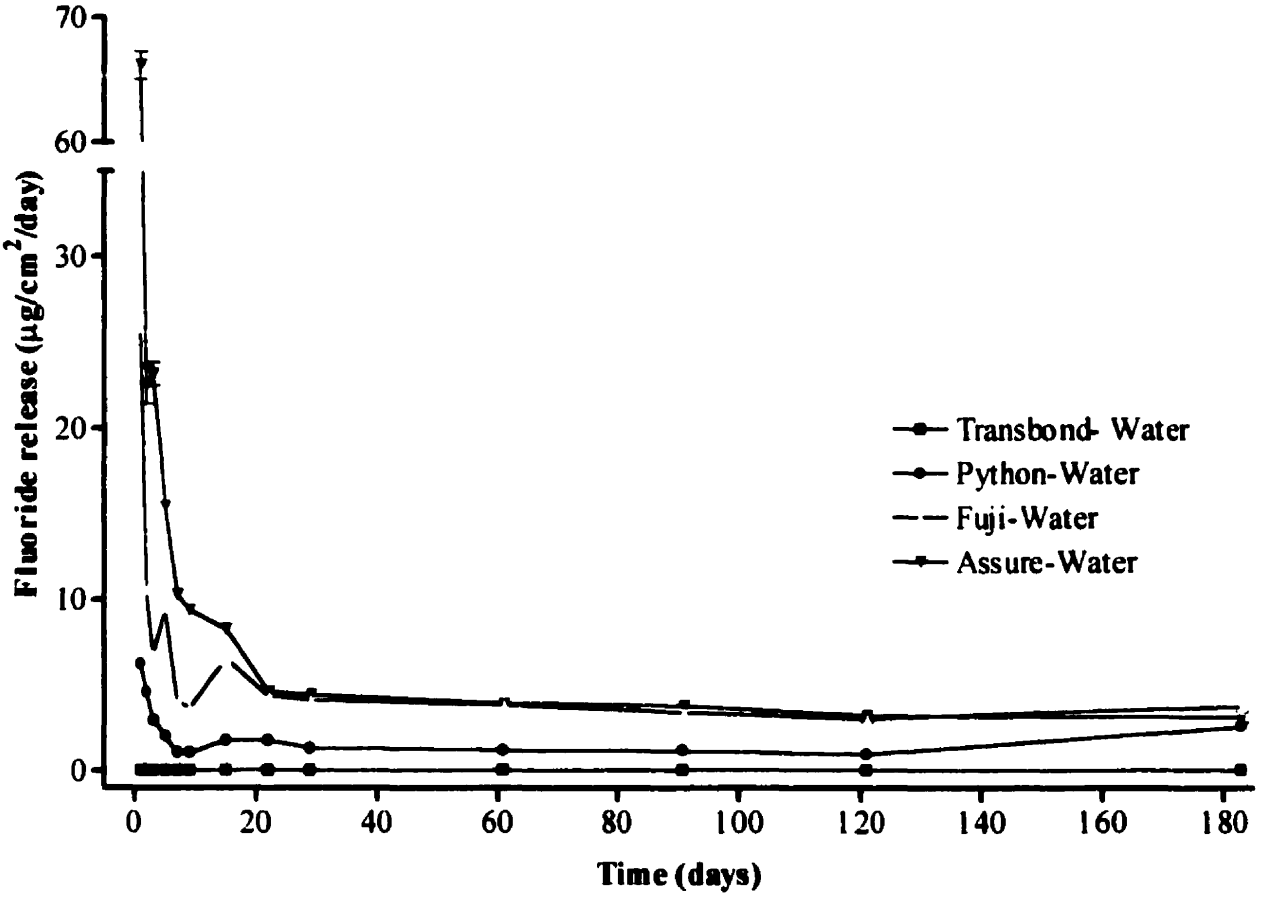


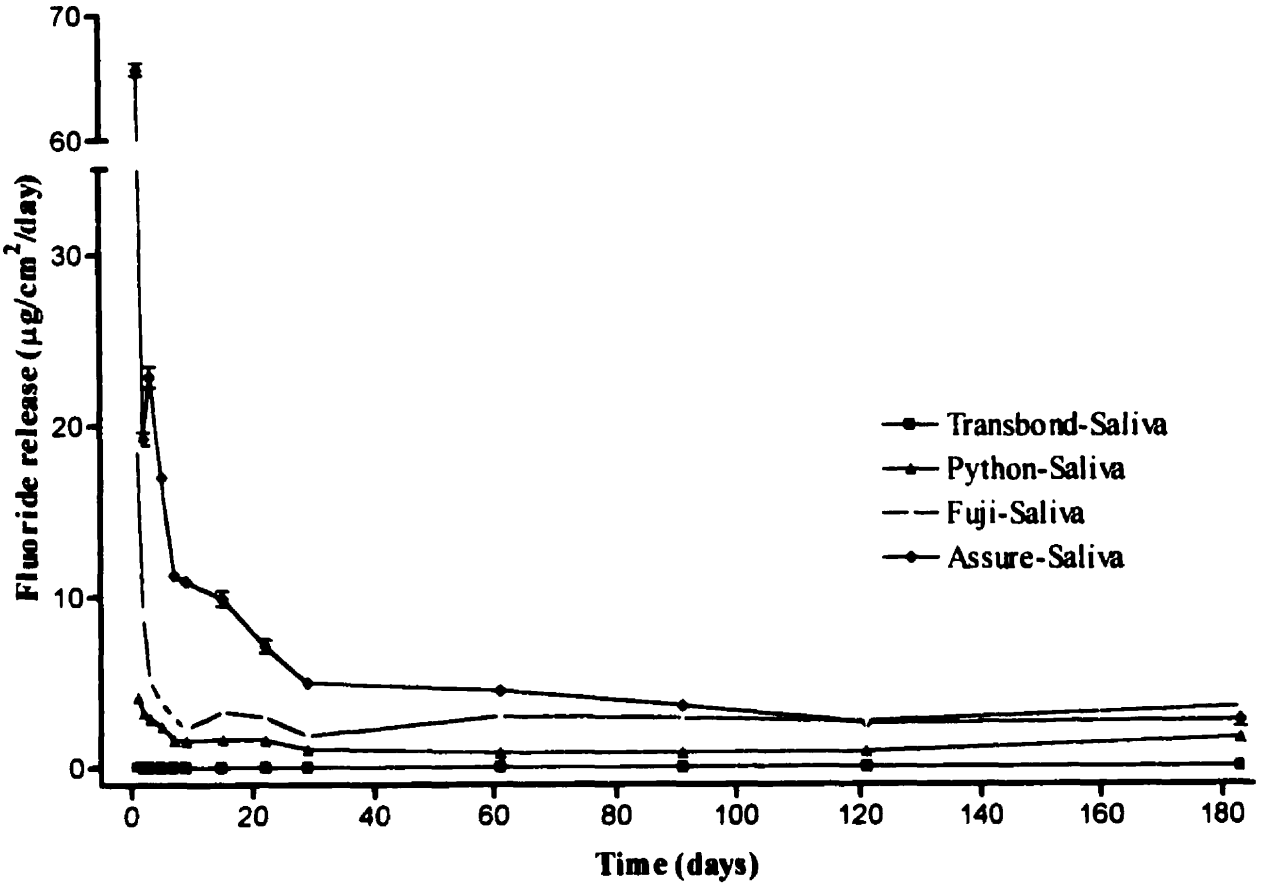
Figure 6.1 shows that the maximal fluoride release rates for each of the three fluoride-containing materials occurred during the first day of testing. For instance, Assure released 66.2 µg/cm^2 F$^-$/day at day 1, nearly 300% more fluoride than its closest competitor, Fuji Ortho LC, with 25.9 µg/cm^2/day. The fluoride release rate for Assure dropped to 22.9 µg/cm^2/day by day 3, and underwent a further drop of

23% to 17.0 $\mu g/cm^2$/day at day 5. The rate of release for Assure continued to decline to day 22, and subsequently remained relatively stable (between 3.1 and 4.7 $\mu g/cm^2$/day) until the end of the test period at 183 days. Assure also released more fluoride than any of the other tested materials until day 121, when it was surpassed by Fuji Ortho LC.

Fuji Ortho LC shared a similar pattern with Assure, with an initially high rate of fluoride release (25.9 $\mu g/cm^2$/day), declining to lower but relatively constant levels of between 3.8 and 4.4 $\mu g/cm^2$/day by day 15. Although Assure was found to release up to 320% more fluoride than Fuji Ortho LC during the first three days of testing, the differences in fluoride release between the two materials were less than 1 $\mu g/cm^2$/day from day 22 onward. Although Python had its highest rate of fluoride release (4.2 $\mu g/cm^2$/day) at day 1, Fuji Ortho LC at day 1 exceeded this amount by 410%. The fluoride released by Python declined to 1.7 $\mu g/cm^2$/day at day 15 and 1.0 $\mu g/cm^2$/day at day 91. The non-fluoride control material (Transbond) released less fluoride than all other materials at all time points, maintaining a constant rate of between 0.04 and 0.09 $\mu g/cm^2$ per day throughout the testing period, i.e. a 90% reduction compared with Python at day 1.

Figure 6.2

Change in rate of fluoride release into artificial saliva with time (mean ± S.E.)



In general, the patterns of fluoride release for the four materials in artificial saliva were very similar to those in distilled water (Fig. 6.2), with 0.04 to 0.09 µg/cm^2/day recorded for the Transbond material throughout the test period. Assure again released the highest amounts of fluoride until day 22. On the first day of testing, Assure released 350% more fluoride than Fuji Ortho LC, whereas Fuji released 450% more than Python. By day 2, the fluoride release rate had dropped by 66% (from 65.8 to 19.3 µg/cm^2/day) for Assure and by over 50% (from 18.8 to 8.9

$\mu g/cm^2/day$) for Fuji Ortho LC. After day 22, the difference in release rates between Assure and Fuji Ortho LC never exceeded 3.1 $\mu g/cm^2/day$ on any given day.

Python was again found to release less fluoride than any of the fluoride-containing materials, with its highest rate occurring at day 1 (6.3 $\mu g/cm^2/day$), and its lowest rate of 1.0 $\mu g/cm^2/day$ at day 121.

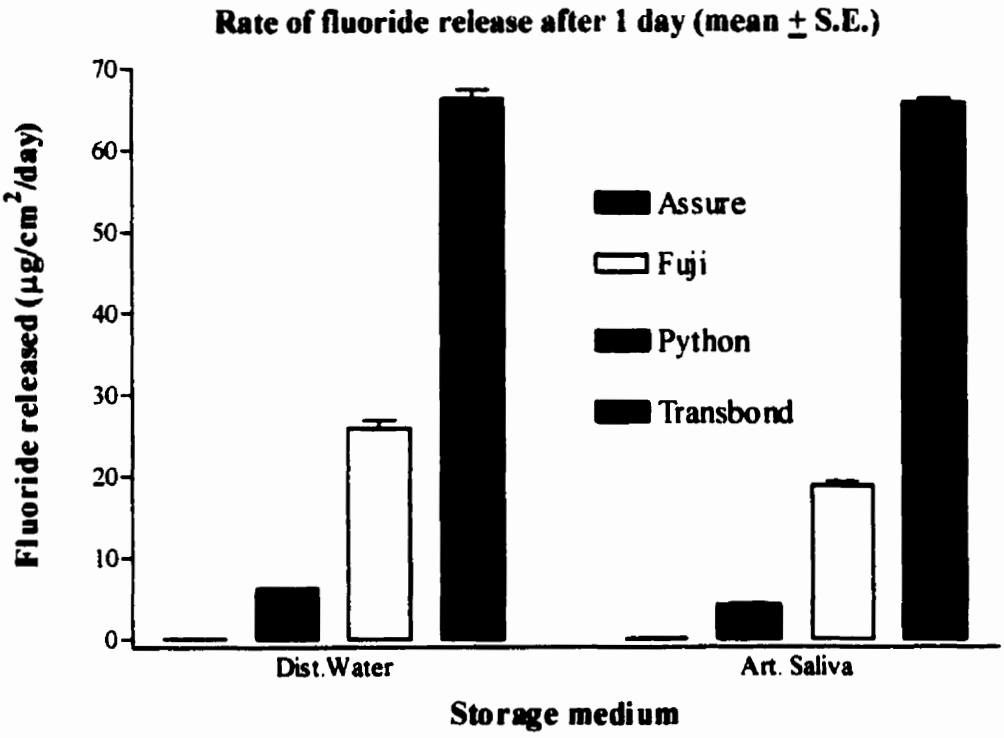
The "blank" test tubes of distilled water and artificial saliva recorded 0.03 ppm or less of fluoride at all of the time points, although these results were not included in the statistical analysis as they approached the detection limit of the fluoride-sensitive electrode.

6.2 Statistical analyses for the still water and artificial saliva tests

The mean amounts of fluoride released by the different sample discs were then evaluated by a two-way analysis of variance (ANOVA). The categorical variables in the two-way ANOVA were type of adhesive (Transbond, Python, Assure, or Fuji Ortho LC), and type of storage medium (distilled water or artificial saliva). Tukey's multiple comparison test was used to determine the statistical significance of the difference between pairs of material/medium combinations (e.g. Fuji-water and Fuji-saliva). The statistical tests were performed at the following time points: 1 day, 7 days, 91 days, and 183 days from time of first immersion of the discs in the storage media.

The rates of fluoride released by the four materials in each of the storage media at day 1 are illustrated in Figure 6.3, where the release for Transbond is so low that it is not visible on the graph.

Figure 6.3



Assure's rate of fluoride release exceeded those of the others in both media, releasing more fluoride in distilled water and in artificial saliva than the other three materials combined. Assure released similar amounts of fluoride in both media.

Fuji Ortho LC, the next highest fluoride-releasing material in this study, showed a 27.4% greater release of fluoride into distilled water than artificial saliva. Python, which released less than one-third the amount of fluoride of Fuji Ortho LC, had a 33.1% higher rate of release into distilled water than artificial saliva. The

difference in $\mu g/cm^2$/day between the Python-water and Python-saliva groups was only 2.1, however.

The two-way ANOVA performed for data from day 1 is summarized in Table 6.1.

Table 6.1 Two-way ANOVA for material, medium and interaction effects of fluoride release at day 1

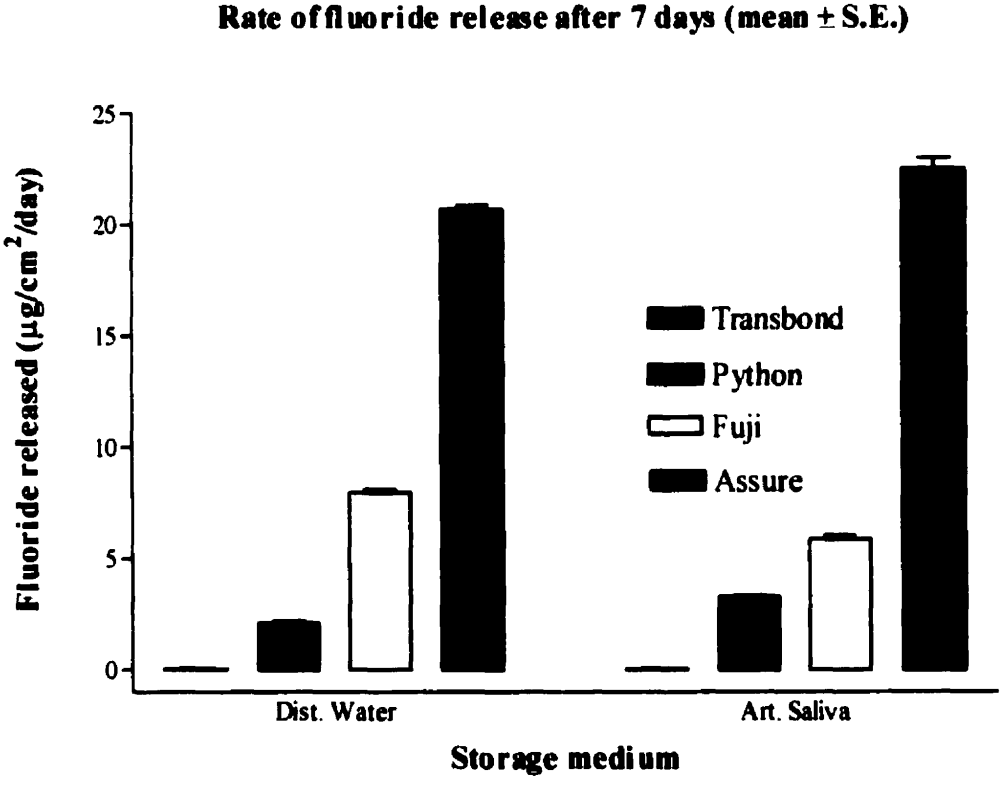
Source of variation	Interaction	Material	Storage Medium	Residual	Tukey's
% total variation	0.29	99.04	0.21		
p value	<0.0001 ***	<0.0001 ***	<0.0001 ***		
df	3	3	1	72	
Sum-of-squares	158.5	53770	115.7	248.4	
Mean square	52.83	17920	115.7		
F	15.31	5195	3.450		2.43

*** = significant at the $p < 0.0001$ level

Table 6.1 shows that at day 1 of testing, the rate of fluoride release was significantly different among the materials, although the differences varied with the type of storage medium. The interaction between the two factors was also significant. When pairs of material-media combinations were tested with Tukey's test, all materials were found to be significantly different from each other in rate of fluoride release, where the prioritized order of fluoride released was Assure > Fuji Ortho LC > Python > Transbond. Only Fuji Ortho LC, however, had a significantly different rate of release between the storage media, i.e. this release was 7.1 $\mu g/cm^2$/day higher in distilled water.

The rates of fluoride release for all four materials in distilled water and artificial saliva at day 7 of testing are illustrated in Figure 6.4. Note that although the scale on the y-axis was changed to represent a maximum of 30 $\mu g/cm^2$/day F^-, the rate of release from Transbond was so low as to be barely visible on the graph.

Figure 6.4



Assure was shown to release the most fluoride, followed by Fuji Ortho LC, Python, and lastly Transbond. Assure released over twice as much fluoride in each type of storage medium than did the closest competitor, Fuji Ortho LC. At day 7, the rate of fluoride release was 26.6% higher for Fuji Ortho LC in distilled water than in artificial saliva. The other two fluoride-containing materials showed the opposite trend, i.e. a greater fluoride release into artificial saliva than water. For Assure and

Python, respectively, the fluoride release rates were 8.1% and 35.8% higher in artificial saliva.

The results of the two-way Analysis of Variance performed for the data from day 7 of testing are summarized in Table 6.2.

Table 6.2 Two-way ANOVA for material, medium and interaction effects on rate of fluoride release at day 7

Source of variation	Interaction	Material	Storage Medium	Residual	Tukey's
% total variation	0.80	98.69	0.02		
p value	<0.0001 ***	<0.0001 ***	0.1156 ns		
df	3	3	1	72	
Sum-of-squares	44.95	5551	0.9769	27.72	
Mean square	14.98	1850	0.9769	0.3850	
F	38.92	4806	2.537		0.81

*** = significant at the $p < 0.0001$ level

ns = not significant

At day 7, all the materials were different from each other in rate of fluoride release, maintaining the same order as at day 1, with 98.69% of the total variation among the groups reflecting the type of material. The interaction between material and medium was also significant, in that three materials (Assure, Fuji Ortho LC and Python) released significantly different amounts of fluoride into distilled water than into artificial saliva. Assure released 1.8 $\mu g/cm^2$/day more fluoride and Python released 1.2 $\mu g/cm^2$/day more fluoride into artificial saliva than into distilled water.

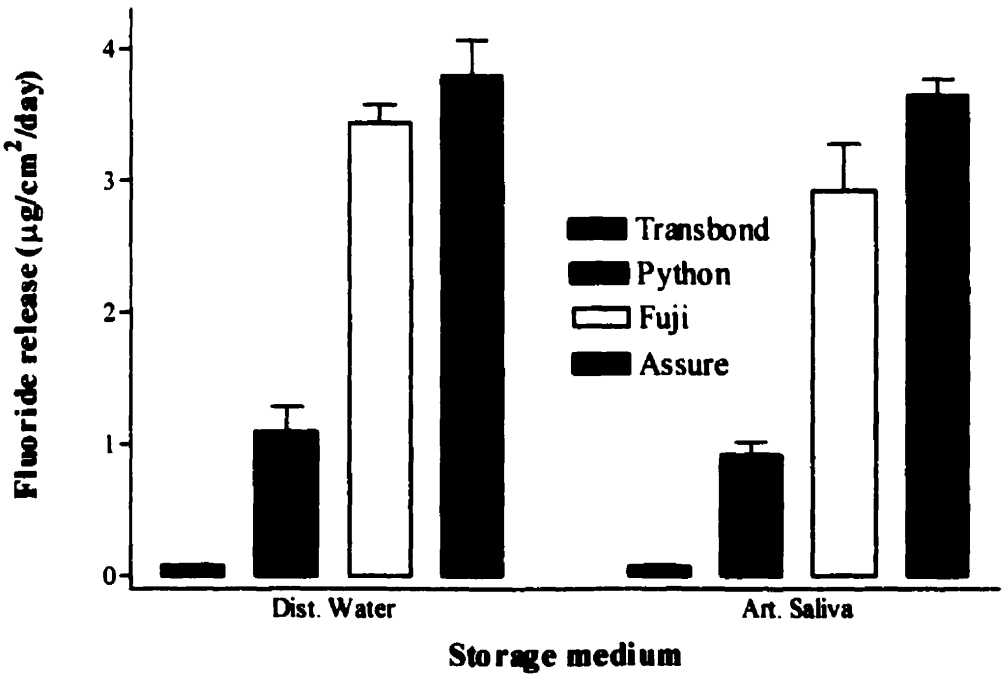
Although for Python this percentage difference was 35.8%, the actual difference in $\mu g/cm^2$/day being released into the artificial saliva vs. the water was quite small.

Fuji Ortho LC showed the opposite trend from Python and Assure, with 2.1 $\mu g/cm^2$/day higher F^- release into water than into artificial saliva. Transbond showed the same amount of fluoride released into both types of storage media.

The fluoride release rates at day 91 of the study are summarized in Figure 6.5. Because the fluoride release of the three fluoride-containing materials decreased considerably after day 7, with no group exceeding 4 $\mu g/cm^2$/day, the low rate of release for Transbond was clearly visible on this graph (Figure 6.5).

Figure 6.5

Rate of fluoride release after 91 days (mean + S.E.)



Unlike measurements from days 1 and 7, the rates of release between Fuji Ortho LC and Assure were similar. Python released less than half as much fluoride as either Fuji Ortho LC or Assure, with Transbond again releasing the least fluoride in both storage media. Table 6.3 details the results of the two-way ANOVA for data from day 91.

Table 6.3 Two-way ANOVA for material, media and interaction effects on rate of fluoride release at day 91

Source of variation	Interaction	Material	Storage Medium	Residual	Tukey's
% total variation	0.33	87.23	0.43		
p value	0.5846 ns	<0.0001 ***	0.1147 ns		
df	3	3	1	72	
Sum-of-squares	0.600	160	0.782	22.1	
Mean square	0.200	53.4	0.782	0.307	
F	0.652	174	2.55		0.72

*** = significant at the $p < 0.0001$ level

ns = not significant

At day 91, the only significant factor was the choice of material, which accounted for 87.23% of the total variation. When pairs of the same material but different media were compared with Tukey's test, no differences were apparent between types of media for any of the four materials, although Transbond released significantly less fluoride than all other adhesives.

There was also no difference in fluoride release between Fuji Ortho LC and Assure. Python exhibited significantly less fluoride release than both Fuji Ortho LC and Assure, in both distilled water and artificial saliva. The amount of fluoride released by Python was 2.3 $\mu g/cm^2$/day less than the release by Fuji Ortho LC in distilled water and 2.0 $\mu g/cm^2$/day less than the release by Fuji Ortho LC in artificial saliva.

The fluoride release at 183 days, the final day of testing, is illustrated in Figure 6.6. Again, all materials showed a mean daily fluoride release rate of less than 4 $\mu g/cm^2$ at this time point, though Transbond released consistently lower amounts than the three fluoride-containing materials.

Figure 6.6

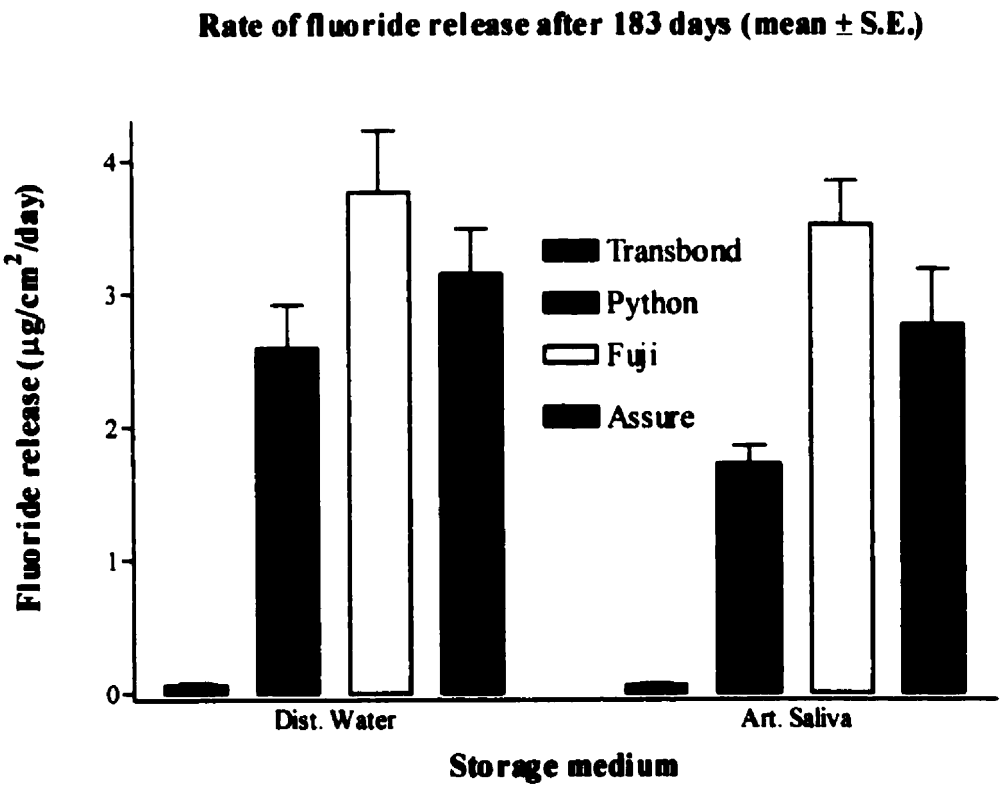


Table 6.4 Two-way ANOVA for material, medium and interaction effects on rate of fluoride release at day 183

Source of variation	Interaction	Material	Storage Medium	Residual	Tukey's
% total variation	0.93	67.07	1.32		
p value	0.5396 ns	<0.0001 ***	0.0831 ns		
df	3	3	1	72	
Sum-of-squares	1.77	128	2.51	58.4	
Mean square	0.589	42.6		0.812	
F	0.726	52.5	3.09		1.18

*** = significant at the $p < 0.0001$ level

ns = not significant

At day 183, the type of material proved once again the only significant variable, accounting for 67.07% of the total variation. There were no differences with storage medium for any of the four materials tested. Transbond released significantly less fluoride than all other materials. For example, Transbond-saliva released 25 times less fluoride than the next-lowest-releasing material, Python-saliva. While fluoride release showed no difference between Assure and Python or between Assure and Fuji Ortho LC, a significant difference was noted in the fluoride release in artificial saliva between Fuji Ortho LC and Python (1.8 $\mu g/cm^2/day$) at the final time period tested ($p < 0.0001$).

6.3 Summary of results of the distilled water vs. artificial saliva tests

The rates of fluoride release for all materials in distilled water and artificial saliva at days 1, 7, 91, and 183 are summarized in Table 6.5.

Table 6.5 Rate of fluoride release in $\mu g/cm^2$/day at days 1, 7, 91, and 183 in distilled water and in artificial saliva

Material	Day 1	Day 7	Day 91	Day 183
Transbond-water	0.1	0.1	0.1	0.1
Transbond-saliva	0.1	0.1	0.1	0.1
Python-water	6.3	2.1	1.2	2.6
Python-saliva	4.2	3.3	0.9	1.7
Fuji-water	25.9	8.0	3.4	3.8
Fuji-saliva	18.9	5.9	2.9	3.5
Assure-water	66.2	20.7	3.8	3.1
Assure-saliva	65.8	22.5	3.6	2.8

6.3 a. Materials effects

At day 1 and day 7, significantly different amounts of fluoride release were noted among all the materials, with the prioritized order being Assure > Fuji Ortho LC > Python > Transbond ($p < 0.0001$). Transbond, the non-fluoride control material, released significantly less fluoride than all other materials, both at day 91

and day 183 ($p < 0.0001$). At day 91, Python showed less fluoride release than both Assure and Fuji Ortho LC in both distilled water and artificial saliva ($p < 0.0001$). No differences in the rates of fluoride release were noted between Assure and Fuji Ortho LC ($p > 0.05$) at day 91. At day 183, a significant difference in fluoride release rates was noted between Fuji Ortho LC and Python ($p < 0.0001$), although no differences were apparent between Assure and Python, or between Assure and Fuji Ortho LC.

6.3 b. Storage medium effects

At day 1, Fuji Ortho LC released 7.1 $\mu g/cm^2$/day more fluoride in distilled water than in artificial saliva. None of the other materials showed different F^- release rates into the two storage media ($p > 0.05$). At day 7, three of the four materials (excluding Transbond) released significantly different amounts of fluoride according to storage medium. Assure released 1.82 $\mu g/cm^2$/day more fluoride ($p < 0.05$), whereas Python released 1.18 $\mu g/cm^2$/day more fluoride ($p < 0.05$) into artificial saliva than into distilled water. Fuji Ortho LC exhibited the opposite trend from Python and Assure, with a 2.12 $\mu g/cm^2$/day higher F^- release into water than artificial saliva ($p < 0.05$). No differences were, however, apparent between fluoride release in distilled water and artificial saliva for any of the four materials tested at day 91 and day 183 ($p > 0.05$).

6.4 Running water and still water tests on the Assure material

6.4a Results of the running water tested samples and still water tested controls

The 20 discs of Assure exposed to either still or running distilled water were tested for fluoride release at 2, 7, and 14 days from the beginning of the experiment, as summarized in Table 6.6.

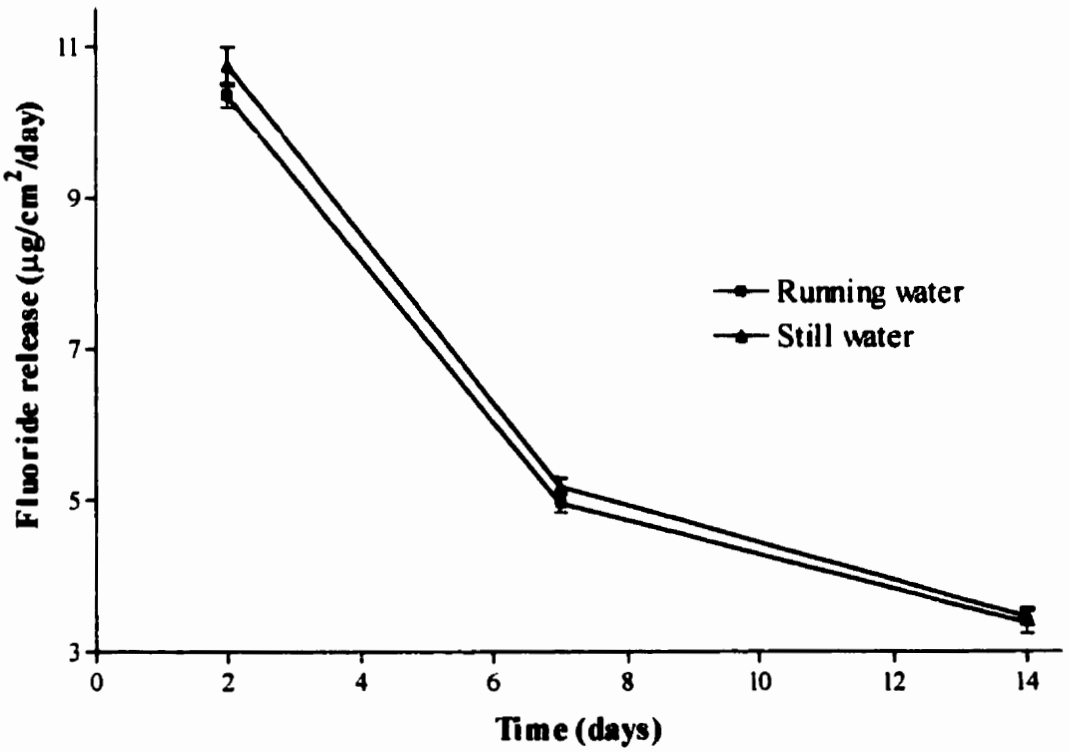
Table 6.6 Mean rates of fluoride release from Assure ($\mu g/cm^2/day$) in running and still water ($\pm$ S.D.) at days 2, 7, and 14

Day	Running water	Still water
2	10.3 $\pm$ 0.47	10.8 $\pm$ 0.77
7	5.0 $\pm$ 0.37	5.2 $\pm$ 0.35
14	3.4 $\pm$ 0.47	3.5 $\pm$ 0.35

The fluoride release data of Assure in running and in still distilled water over the course of the two-week testing period are displayed in Figure 6.7.

Figure 6.7

Rate of fluoride release from Assure in running and still water (± S.E.)



Similar patterns of declining fluoride release over time were apparent in both running and still water. From 0 to 7 days, both groups experienced a 52-53% decrease in fluoride release, with a further decline of 32-34% noted from days 7 to 14.

6.4b Statistical analysis for the running and still water samples

These data were then analyzed by means of a two-way ANOVA (Table 6.8) to determine differences due to time (n=3) and test conditions (n=2).

Table 6.8 Two-way ANOVA for time, test conditions and interaction effects on the rate of fluoride release in the running water vs. still water tested samples of Assure

Source of variation	Interaction	Test condition	Time	Residual
% total variation	0.05	0.15	97.59	
P value	0.5401 ns	0.0623 ns	p>0.0001 ***	
Df	2	1	2	54
Sum-of-squares	0.2912	0.8467	556.9	12.62
Mean square	0.1456	0.8467	278.5	0.2337
F	0.6231	3.623	1192	

*** = $p < 0.0001$

ns = not significant

Time was the only significant factor, with all three periods differing significantly from one another ($p < 0.0001$). The time period providing the greatest release of fluoride was day 2, followed by day 7 and then day 14. No differences were apparent in the fluoride release rates of Assure at any of the time points between running and still water test conditions ($p > 0.05$).

7.0 DISCUSSION

The first section (7.1) of this discussion compares the present results with those of other studies, specifically listing the effects from materials, type of storage medium (distilled water or artificial saliva), and running versus still water.

In the second section (7.2), the question of whether fluoride-releasing materials are effective at preventing decalcification is discussed. Special reference is made to research correlating fluoride release into water or saliva with measures of decalcification reduction for the same materials. The prime focus is to determine the accuracy of these investigative models in measuring protection against demineralization. Finally, the results of the present study are used to determine whether the tested materials are beneficial in reducing the prevalence of white spot lesions following the removal of orthodontic appliances.

7.1 Comparative evaluations

7.1a Materials effects

Throughout the present study, a general trend was noted for Assure to release more fluoride than Fuji Ortho LC, whereas the latter released more than Python. Transbond, the control adhesive, released significantly less fluoride than the other adhesives at all time periods. The most dramatic differences among the materials occurred during the initial two weeks of testing. All materials exhibited significant differences in rate of fluoride release at day 1 and day 7, with the order being Assure > Fuji Ortho LC > Python > Transbond. For example, Python released approximately 100 times more, Fuji Ortho LC released 400 times more, and Assure released 900 times more fluoride than Transbond at day 1. The few exceptions to this order of materials occurred in the final days of testing. For instance, the fluoride release in water from Fuji Ortho LC surpassed that of Assure by 0.62 $\mu g /cm^2/day$ at day 183, although this difference was not statistically significant.

With the exception of Transbond, a non-fluoride material with a consistently low fluoride release; i.e. 0.04 - 0.09 $\mu g/cm^2/day$ at all time periods, all materials released the most fluoride during the first day of testing. The material that released the most fluoride at day 1 was Assure in water, at 66.22 $\mu g/cm^2/day$. This "burst effect" of fluoride release has been noted in many previous studies. For instance, Wiltshire and Janse van Rensberg (1995) found that one orthodontic adhesive, FluorEver OBA (Macrochem Corp., Woburn MA), released 35 $\mu g\ F^-/cm^2/day$ on the first day of the study. The rate dropped approximately 75% to less than 10 $\mu g\ F^-/cm^2/day$ by Day 2 and remained below 5 $\mu g/cm^2/day$ for the remainder of the 36-day test period. Chan *et al.* (1990) found the

mean F^- release rate to be 181 $\mu g/cm^2$/day for the restorative resin version of FluorEver on day 1 of testing, decreasing 70% to 52.6 $\mu g/cm^2$/day on Day 3, then declining a further 80% to 10.5 $\mu g/cm^2$/day by day 43. Young *et al.* (1996) measured fluoride release from restorative GI's and found 15 $\mu g/cm^2$/day for Ketac-Fil (Espe/Premier, Seefeld, Germany) at day 1, which declined 66% to 5 $\mu g/cm^2$/day by Day 3, and continued to decrease until the end of the 33-day testing period, where the final rate of fluoride release of 2 $\mu g/cm^2$/day was noted as 13% that at day 1.

The trend for sharp declines in fluoride release after the first several days was also apparent in the present investigation. The fluoride release rates of all adhesives subsequently fell to lower levels (ranging from 2.1 $\mu g/cm^2$/day for Python to 18.9 $\mu g/cm^2$/day for Assure at day 9) after the first week of testing, and had reached nearly constant values by day 25.

Assure, a composite system, released more fluoride than all other materials at days 1 and 7. In a review of the literature, Erickson and Glasspoole (1995) stated that the 24-h fluoride release for a wide variety of conventional and hybrid GI materials varied between 40-100 $\mu g/cm^2$/day, while most fluoride-containing composite systems have been shown to release from 5-20% of that amount. Both systems showed a decrease in daily rates of fluoride release with time (Erickson and Glasspoole, 1995). Tam *et al.* (1991) found that two resin-modified glass ionomer lining materials, Vitrabond (3M, St Paul MN) and XR-Ionomer (Sybron/Kerr, Romulus MI), released 11.4 and 27.3 $\mu g/cm^2$/day, respectively, on day 1 of the study, whereas the fluoride-containing composite systems Timeline (Caulk/Dentsply, Milford DE) and Cavalite (Sybron/Kerr) released less than 5 $\mu g/cm^2$/day. Verbeeck *et al.* (1998) found that Ketac-fil, a

conventional GI, released approximately 125 $\mu g/cm^2$/day at day 1, compared with a release of approximately 5 $\mu g/cm^2$/day for Dyract, a compomer. Young *et al.* (1996) found that Ketac-fil released 54 times as much fluoride as Tetric (Vivadent, Liechtenstein), a fluoride-containing composite resin, after one day. In all three of the above studies, the materials most closely resembling conventional GI's released the most fluoride. Hence, the findings of the present study were unusual in that the material releasing fluoride at the highest rate was not the resin-modified GI, Fuji Ortho LC. As the manufacturers of Assure provided little detail on its proposed fluoride-releasing mechanism, the reasons for the higher rate of release than from Fuji Ortho LC are unclear. It is not marketed as a glass ionomer material, and does not set in the dark, which is one criterion for a material to be designated a GI according to McLean *et al.* (1994).

The only published results of fluoride release for Assure were by Rix (1999), who compared Assure with Fuji Ortho LC, Transbond, and an experimental cement. Rix found that Assure discs released a mean of 69.75 $\mu g\ F^-/cm^2$/day in distilled water at day 1, whereas a mean release rate of 66.22 $\mu g/cm^2$/day for Assure in distilled water was noted in the present study. The fluoride release from Transbond after one day was also similar between the two studies: Rix found a mean rate of 1.43 $\mu g/cm^2$/day (Rix, 1999), compared with a rate of 0.07 $\mu g/cm^2$/day in the present study. Less agreement was apparent between the two studies for the rate of fluoride release of Fuji Ortho LC after 1 day. For instance, Rix (1999) reported a rate of 57$\mu g/cm^2$/day, whereas only 25.87 $\mu g/cm^2$/day of fluoride (a difference of 55%) was released from Fuji Ortho LC into distilled water in the present study. McCabe (1998) found 25.9 $\mu g/cm^2$/day release for Fuji II LC (GC Corp, Tokyo, Japan), a material similar to Fuji Ortho LC, after 1 week of

storage with daily water changes. The present study found a release rate for Fuji Ortho LC at the same time period in distilled water of 7.98μg/cm^2/day, a difference of 69%. Although the reasons for these discrepancies remain obscure, they may result from chemical differences between the two materials, or differences in experimental design.

No published data for Python were available at this time. Despite some differences (such as those noted with Fuji Ortho LC), however, both the fluoride release patterns and the amounts of fluoride released showed a similar trend when compared with the results of other authors (Wiltshire and Janse van Rensberg, 1995; Young *et al.*, 1996; McCabe, 1998; and Rix, 1999).

7.1 b. Storage medium effects

The choice of storage medium was a significant variable at days 1 and 7. For instance, at day 1, Fuji Ortho LC was the only material that released significantly different amounts of fluoride in distilled water (25.87 μg/cm^2/day) and artificial saliva (18.78 μg/cm^2/day), a difference of 27.5%. At day 7, three of the four materials (Assure, Fuji Ortho LC and Python) released significantly different amounts of fluoride in the two solutions. With Fuji Ortho LC, 26.6% less fluoride was released in artificial saliva. In contrast, Python and Assure released 36% and 8% more F^-, respectively, in artificial saliva. However, variation in the amounts of fluoride released among the groups was markedly reduced after day 1. The greatest spread of values due to medium effect at day 7 was between Fuji-water (7.98 μg/cm^2/day) and Fuji-saliva (5.86 μg/cm^2/day), a 27% difference. Although statistically significant, the day 7 differences for Assure, Fuji, and Python of 2.12 μg/cm^2/day F^- or less are of doubtful clinical significance. At day 91 and

again at day 183, the four materials released similar amounts of fluoride in water and in artificial saliva. The reasons for the differences in fluoride release between distilled water and artificial saliva at early time points in the study clearly require further consideration.

Only a limited number of studies have compared fluoride release in water and saliva. For instance, Øgaard *et al.* (1992) compared fluoride release from disks of Orthodontic Cement VP 862 (Vivadent, Liechtenstein) in distilled water and human unstimulated saliva. After one hour, release was 4.8 times higher (a significant difference) into the water (0.96 ppm) as opposed to saliva (0.2 ppm). The experimental period was limited to 1 hour because a longer time *in vitro* with natural saliva was deemed unsuitable by the authors, due to the instability of salivary proteins. When the pH of the saliva was lowered to 4.0 by addition of hydrochloric acid, thereby simulating a severe caries challenge, the amount of F^- released increased to equal that measured in water. The authors stated that the pH-controlled mechanism of fluoride release in saliva was probably due to desorption of proteins and phosphate from the salivary protein-covered discs at lower pH, potentially increasing the rate of fluoride diffusion from the discs into the saliva. However, this has yet to be verified experimentally.

While synthetic saliva does not contain the proteins or enzymes found in human saliva, it has been used extensively in caries research (Macpherson and Dawes, 1994; Leung and Darvell, 1997), due to its extended shelf life compared with that of human saliva. The remainder of the studies discussed in this section tested cement samples in artificial saliva, rather than in natural saliva.

El-Mallakh and Sarkar (1990) studied four types of restorative GI cements in both de-ionized water and artificial saliva over a period of 60 days. For all materials, fluoride

release was consistently higher in water than in artificial saliva. For example, Fuji II (GC Corp. Tokyo, Japan), at day 3, released 20 ppm of fluoride in water and 3 ppm, or 15% as much, in artificial saliva. The authors felt that this pattern may have reflected the presence of various cations in the artificial saliva, although no examples were provided by the authors.

Wandera *et al.* (1996) compared fluoride release of three restorative GI cements in water and artificial saliva. All of the materials released significantly more fluoride in distilled water than in artificial saliva at 24 h. For example, Ketac-Fil released 102 ppm/mm^3 in water but only 13 ppm/mm^3, or 88% less, in artificial saliva. The samples in distilled water exhibited the usual pattern of a "burst" of fluoride release in the first several days of testing, followed by a gradual decrease over time. The samples stored in artificial saliva did not follow this pattern, in that an increase in the rate of F^- release was noted at 3-4 weeks and again at 8 weeks of the 9-week study. The authors suggested that these different fluoride release patterns might have been related to the absence of a well-defined concentration gradient in the artificial saliva. Possibly, chemical species such as sodium and phosphate may have had the potential to be adsorbed by the cement, perhaps acting as a barrier to reduce initial fluoride availability. Ions moving to the subsurface region under the adsorbed coating may have also been responsible for the later increase in fluoride release, although these suppositions remain untested.

When Karantakis *et al.* (2000) tested fluoride release from a variety of restorative materials in water, artificial saliva, and lactic acid, no significant differences were noted in the amounts of fluoride released in water vs. artificial saliva. Similar fluoride release patterns were noted in both these storage media, with the highest fluoride dissolution

during the first 24 hours. The authors explain the difference between their results and those of El-Mallakh and Sarkar (1990) by citing the more basic pH (7) of the artificial saliva formulation used in the Karantakis *et al.* (2000) study, as well as possible pH differences between double-distilled and deionized water.

In the present study, fluoride release from Fuji Ortho LC was 27.5% lower in artificial saliva than in distilled water at day 1, and 26.6% less at day 7, i.e. analagous with the results of El-Mallakh and Sarkar (1990) and Wandera *et al.* (1996). However, unlike the two cited studies, this trend did not last throughout the testing session. Fuji Ortho LC was the only material that released less fluoride into distilled water than into artificial saliva. Indeed, both Assure and Python released significantly more fluoride (8% more for Assure, and 36% more for Python) into artificial saliva at day 7. However, the clinical significance of these results for Assure and Python was questionable, because the actual differences in fluoride release rates between the artificial saliva and water groups at day 7 were only 1.82 $\mu g/cm^2$/day for Assure, and 1.18 $\mu g/cm^2$/day for Python. In addition, none of the materials had significantly different F^- release in water vs. artificial saliva by day 91 and day 183 of the present study. The isolated findings for Fuji Ortho LC early in the testing period may therefore have resulted from the particular formulation of artificial saliva used in this study. The slightly increased viscosity of the artificial saliva may have also acted as a physical barrier to the free diffusion of fluoride ions. However, one would then expect a decrease in fluoride release for all materials, which was not the case. In any event, the trend did not persist and, in general, the patterns of fluoride release for the four materials tested were very similar between distilled water and artificial saliva.

7.1 c. Running vs. still water effects

Rix (1999) noted that the fluoride release patterns of Assure and Fuji Ortho LC were markedly different depending on the timing of the water changes. In the first part of his study, daily water changes for one week were followed by weekly changes for several weeks, and subsequently by monthly changes. Fluoride measurements were taken immediately prior to water changes and were, therefore, cumulative from the previous water change. To estimate a rate of release per day for each of the materials, the cumulative measures from the weekly and monthly periods were divided by 7 and 28 days, respectively. This was based on the assumption that the very low levels of fluoride being released by this time (relative to the fluoride saturation levels) would have no effect on the fluoride released from the sample. To test this assumption, Rix (1999) performed a series of daily water changes on samples that had already been tested for fluoride release, as previously described, for 5 months. The fluoride release values from the samples receiving daily water changes were 3 to 13 times higher than the previous "month-based" daily average. For example, at day 140, Fuji Ortho LC released 1.56 $\mu g/cm^2$/day (based on cumulative release for 1 month divided by 28). The next day, 24 h after a water change, the rate of release was 21.35 $\mu g/cm^2$/day. After 10 daily water changes following the fifth month of observation, the daily fluoride release rate had increased by 310% for Fuji Ortho LC and 62% for Assure, when compared with the daily fluoride release rates based on the accumulated fluoride release during the fifth month. Rix concluded from this research that fluoride release depended on the timing of the water changes. He suggested that daily water changes or continuously flowing water would provide patterns of fluoride release that more closely resembled *in vivo* conditions.

The present investigation was similar to that of Rix (1999) in the overall length of time of measurement (183 days vs. 150 for Rix) and times of testing being daily for 7 days, followed by weekly and monthly. However, unlike Rix (1999), in the present investigation the storage solutions were changed 24 h prior to measurement to avoid cumulative readings. This storage pattern, while preferable to one with less frequent water changes, still represents a static equilibrium. Erickson and Glasspoole (1995) mentioned the need to replicate *in vivo* conditions more closely in order to give relevant information on clinical efficiency for fluoride-containing adhesives. In an attempt to simulate more closely the intraoral situation, where saliva is constantly clearing away the fluoride in solution (Dawes and Weatherell, 1990), part II of the present experiment was devised. The aged samples of Assure cement were tested under continuously flowing distilled water at a rate of 1 ml/min, similar to the flow rate of saliva in the mouths of orthodontic patients (Forsberg *et al.*, 1992). Although still not a true *in vivo* simulation, the model simulated the constant washing away of accumulated fluoride by saliva.

The only published literature on testing fluoride release under running water is by Forsten (1990, 1995,1998). In his review of the literature, Forsten (1998) stated that specimen storage for some weeks in water or different solutions does not occur *in vivo*, and hence his specimens were exposed to a continuous flow of running tap water. At certain time periods the specimens were transferred for a short time to a small amount of deionized water, to allow sufficient fluoride to accumulate in solution to facilitate its measurement.

For example, Forsten (1990) exposed 7 different glass ionomer materials to running tap water (at the speed of 1 L/min) for 2 years. The fluoride release from the

specimens was measured periodically after storage in distilled water. After 24 h, the fluoride release for Fuji II LC was on average 7.0 $\mu g/cm^2$/day, whereas this decreased to 1.4 $\mu g/cm^2$/day one month later. A similar study by Forsten (1995) tested different GI-based restorative materials for a total time of 2 years under running water with a flow rate of 0.5 L/min. Under these conditions, the fluoride release for Fuji II was 7.9 $\mu g/cm^2$/day after 24 h, and 1.13 $\mu g/cm^2$/day after 3 weeks, i.e. a 75.8% decrease. In both studies, all of the fluoride-releasing materials showed the classic pattern of a "burst effect" of fluoride release for the first several days, followed by a tapering off toward the end of testing. The fluoride release values tended to be lower than those found by researchers using a still water storage system. For example, Tam et al. (1991) found a 24-hour F^- release from Vitrabond of 11.4 $\mu g/cm^2$/day, and from XR Ionomer of 27.3 $\mu g/cm^2$/day.

Some GIC's, such as Fuji II, (Forsten, 1998), have demonstrated low but stable long-term fluoride release throughout the entire 8-year testing period. However, fluoride is probably not washed away as efficiently in the mouth as by this laboratory model. Films or layers on the fillings or in the overlying plaque could reduce the rate of release, or part of the released fluoride could accumulate in the layers covering the filling. Forsten felt that the running water model of fluoride release, while not perfect, was preferable to the still water testing so prevalent in the literature, e.g. Cranfield *et al.* (1982), Cooley *et al.* (1989) and Momoi and McCabe (1993).

In the present experiment, distilled water was used both for the running water and the short periods of immersion in still water prior to testing. The flow rate was 1 mL/min, felt to be an estimation of the salivary flow rate of orthodontic patients (Forsberg et al., 1992). This rate was 500-1000 % lower than the rates used in Forsten's research. A

control group of similarly aged and treated materials was used to determine any difference between the rates of fluoride release in running vs. still water.

The results showed no significant differences between the amounts of fluoride released in still or running water at 1, 7, or 14 days. The amount of fluoride released was, however, significantly lower at each successive test period, indicating a gradual decrease in fluoride eluted over time. It is interesting to note, however, that more fluoride was released since these samples of Assure were last tested in still water. Six months previously, at day 183, the 10 sample discs of Assure had a mean F^- release rate of 3.14 $\mu g/cm^2$/day into still water. Following this test time, each sample had been stored in 1 mL of water for 6 months until the running water tests began. Following 24 h of exposure to running water and a further 24-h soak in 1 mL of distilled water, the mean amount of fluoride released by the same 10 discs increased to 10.34 $\mu g/cm^2$/day, i.e. a 330% increase. This indicates that even after a total time period of 1 year, fluoride within the Assure discs was still available.

A possible explanation for the above increase in rate of fluoride release involves the kinetics of diffusion. One could assume that early in the testing period, there was a diffusion gradient from the center of the sample towards the edges, as fluoride from the outermost areas of the disc would pass into solution first. Once the samples had been exposed to the same small volume of water for six months, fluoride would diffuse from deeper within the composite matrix to the periphery, eventually rendering the concentration of fluoride constant throughout the disc. This would create a higher concentration at the periphery of the disc than that present at the end of the six-month test

period. This increased concentration could then increase the rate of fluoride release at the beginning of the new test period.

Rix (1999) also found an increase in fluoride release rates (310% for Fuji Ortho LC and 62% for Assure) when the frequency of water change was increased to daily from monthly. While no explanation for the increase was offered, it was stated that frequent water changes most closely approximate the clinical situation.

7.1 d. Longevity of fluoride release

Cranfield *et al.* (1982) stated that high release of fluoride from a glass ionomer cement, perhaps at a greater rate than it could be absorbed by the enamel, is of little value when it occurs only over a short period early in the lifespan of a restoration. However, in the orthodontic setting, an initial high burst of fluoride may be beneficial in remineralizing the etched enamel (Ghani *et al.*, 1994). In this investigation, Assure released up to 320% more fluoride than the next highest material, Fuji Ortho LC, during the first three days of testing. However, fluoride release from the three fluoride-containing adhesives had reached quite similar values by day 22. The samples in this study were tested for a total period of 183 days, although the 20 discs of Assure were retested in running and still distilled water after a further six months. The Assure discs released a mean fluoride amount of 10.5 $\mu g/cm^2$/day 12 months after their initial manufacture and immersion in solution. The results of this study seem to indicate that fluoride from Assure is still available for release into the surrounding environment at least 12 months after initial immersion.

To provide continuing protection against decalcification, materials must release meaningful amounts of fluoride throughout the entire intraoral period (Dunne *et al.*, 1996). Although few studies testing fluoride release of orthodontic bonding agents have persisted in testing for more than six months, long-term data of at least two years is preferred, since the average duration of fixed orthodontic treatment is 24 to 28 months (Thilander, 1992).

The present investigation consisted of 6 months of testing in distilled water and artificial saliva. The sample discs of Assure were tested again at 12 months under different conditions (running and still water). However, all samples were maintained in 1 mL of their respective storage solutions in an incubator. In a follow-up study, the long-term fluoride release of the aged samples will be investigated, increasing the total testing time to 24 months.

7.2 Summary

7.2 a. The level of fluoride necessary for protection against demineralization

How much fluoride is enough to protect against demineralization? In a restorative model, relatively high concentrations of fluoride in enamel are necessary to slow the progression of a caries lesion. For example, Clarkson *et al.* (1988) reported that when the fluoride concentration in the body of a caries lesion was > 400 ppm higher than that in sound enamel, the lesion progression was halted.

Much lower levels of ambient fluoride may be sufficient to protect sound enamel, e.g. adjacent to orthodontic brackets. For instance, Rawls (1987) found that decalcification was inhibited in sound enamel adjacent to a resin releasing fluoride at a

rate as low as 0.65 to 1.3 μg F^-/cm^2/day. This therapeutic range was determined by an experiment comparing *in vitro* and *in vivo* data on the same experimental materials. Fluoride release from sample discs into saline from an experimental fluoride-containing dental resin and two commercial silicate cements, Syntrex (Caulk, Milford DE), and MQ (SS White, Philadelphia PA) was measured and compared with enamel uptake of fluoride in acid-etch biopsies of enamel bonded with the same three materials.

Phase II (Reliance, Itasca IL), a fluoride-releasing adhesive, was found to release an average of 0.5-1.0 μg F^-/cm^2/day over a 30-day period. When tested *in vivo* on rats fed a cariogenic diet, with molars bonded with either the test or a control material (System I, Ormco Corp, Glendora CA), the fluoride-releasing material was found to reduce white-spot demineralization adjacent to the brackets by 31%, a highly significant difference (Dubroc *et al.*, 1994).

Rawls (1987) found the average fluoride release rate of an experimental self-cure bracket-bonding material to be 1.5 μg F^-/cm^2/day. In an independent study, Underwood *et al.* (1989) bonded the first premolars of patients, in whom these teeth were scheduled for extraction, with either the experimental material or a non-fluoride control. After 60 days in the mouth, the teeth were extracted and examined by polarized light microscopy for the presence of enamel lesions. There were 93% fewer dark-zone lesions (a histological feature found in natural early caries lesions) in the fluoride group than in the control group, a highly significant reduction in lesion progression.

The literature, therefore supports the model of a minimum mean release rate of 0.63-1.3 μg F^-/cm^2/day (Rawls, 1995). Three of the four materials tested, Assure, Fuji Ortho LC, and Python, sustained a fluoride release rate of greater than this amount once

fluoride release tapered off after the initial burst. At day 183, for example, the release rate for Assure in water was 3.14 μg F^-/cm^2/day, whereas Assure in artificial saliva released 2.76 μg F^-/cm^2/day. Fuji Ortho LC released 3.76 μg F^-/cm^2/day in water, and 3.52μg F^-/cm^2/day in artificial saliva. Python's release was 2.60 μg F^-/cm^2/day in water and 1.72 μg F^-/cm^2/day in artificial saliva. All three of the fluoride-releasing materials tested, therefore, released fluoride at rates above the minimum effective range proposed by Rawls (1995). Whether this release translates into increased protection against decalcification of the smooth surface enamel adjacent to the bracket can only be determined through further research.

Clinically, the initial burst of fluoride release may play a protective role by remineralizing the etched enamel (Wiltshire, 1996). Rølla and Saxegaard (1990) found that high rates of fluoride release (such as during the first 48 hours after adhesive placement on the teeth) forms calcium fluoride on the enamel surface. The calcium fluoride globules may act as a potential reservoir by releasing fluoride ions to remineralize the enamel surface (Erickson and Glasspoole, 1995). Basdra *et al.* (1996), studied fluoride release from discs of two fluoride systems into distilled water, and found high (110 μg/cm^2/day) release for Fluorobond/Concise and moderate (41.5 μg/cm^2/day) release for Rely-A-Bond after 48 hours. Although the release rates of the two materials were similar by day 14, the authors' concurrent evaluation of the extent of enamel demineralization showed a clear advantage for the Fluorobond/Concise system. These authors also suggested that even though the higher initial fluoride release for Fluorobond/ Concise was short-lived, it may have been responsible for greater deposition of calcium fluoride on the enamel surface. These globules could then have allowed slow release of

fluoride over an extended time, exerting a protective effect on the enamel. In the present study, Assure showed markedly higher rates of fluoride release than all other materials for the first two weeks. A clinical study of white-spot formation or an enamel biopsy study similar to that of Basdra *et al.* (1996) could help determine whether this early burst of fluoride release translates into long-term reduction in demineralization for Assure.

Therefore, the low but sustained fluoride release exhibited by Assure, Fuji Ortho LC and Python may lead to the formation of fluorapatite within the enamel (Wiltshire, 1996), protecting enamel in the medium to long-term range of orthodontic treatment, which could extend as long as 24-30 months clinically. In addition, the initial high burst of fluoride release shown by Assure may play a role in protection against enamel demineralization in the short-term, immediately after acid-etching of the enamel in preparation for bracket bonding.

7.2 b. *In vitro* vs. *in situ* studies

Considerable information exists from *in vitro* models to establish that fluoride-releasing materials can reduce demineralization of enamel (Erickson and Glasspoole, 1995). However, the evidence is inadequate to establish precisely how effective these materials are or under what conditions they might be effective. The authors suggest that *in situ* studies, which develop dose-response relationships for fluoride, could provide the necessary data to refine the interpretation of results from the *in vitro* models. However, for new materials without a database from which to draw, release of fluoride from discs into a liquid medium remains a useful and cost-effective way to gather baseline fluoride-release data in preparation for a clinical study. In this

way, a level of fluoride release may be determined to be effective at preventing demineralization of enamel adjacent to orthodontic brackets. In the light of results of Basdra *et al.* (1996), one suggested approach would be to bond brackets to premolar teeth (scheduled for extraction) *in situ* using these materials, extract the teeth after a period of time and examine the fluoride content in enamel. This would determine whether the high early fluoride release of Assure increased calcium fluoride concentration on the enamel surface. An enamel biopsy study would determine whether Assure's high initial fluoride release caused increased fluoride uptake by the enamel. In summary, data from fluoride release studies and clinical trials could be combined to determine better the clinical efficacy of orthodontic bonding adhesives.

8.0 RECOMMENDATIONS

8.1 *In vitro* research

Determination of fluoride release into liquid media is a common method for gathering baseline data on the rate of fluoride release from orthodontic bonding materials. All of the tested materials released fluoride at rates at or above the proposed therapeutic range of 0.63-1.3 μg F^-/cm^2/day (Rawls, 1995). Further *in vitro* research is warranted to determine whether these materials continue to release clinically useful levels of fluoride throughout the average orthodontic treatment period of 24-30 months. Further research could also compare several materials in running and still water over a longer time period to determine any differences in the long-term rate of fluoride release into running water, rather than in a static immersion solution.

8.2 In vivo research

Assuming that the results from *in vitro* research continue to show significant fluoride release, *in vivo* tests, such as decalcification studies and enamel biopsies, could be performed to provide a more complete database of information on these materials. When data from *in vitro* and *in vivo* studies are combined, the clinical efficacy of Assure, Fuji Ortho LC, and Python in reducing decalcification may be accurately assessed.

8.3 Recommendations for clinical use

At this point, there are no accessible published data on bond strength or bracket retention rates for the two newest materials in the study, Assure and Python. Independent clinical trials are necessary to determine whether these materials are indeed appropriate

for use in the bonding of orthodontic brackets. Transbond and Fuji Ortho LC, however, have been reported to have adequate mechanical properties for use in bracket bonding (Miguel *et al.*, 1995; Lippitz *et al.*, 1998; Gaworski *et al.*, 1999). Given the prevalence of decalcification among orthodontic patients, a fluoride-releasing material would be preferred over a non-fluoride adhesive, provided that the material's properties did not suffer as a result of the inclusion of fluoride. Based on the data from the present study, in combination with other independent published research, Fuji Ortho LC may be beneficial for clinical use where enamel decalcification is a concern for the clinician.

9.0 CONCLUSIONS

9.1 Distilled water vs. artificial saliva

Daily rates of fluoride release in distilled water and artificial saliva were calculated from a resin-modified glass ionomer cement (Fuji Ortho LC), two polyacid-modified composite resins (Assure and Python), and a control non-fluoride composite resin orthodontic bonding adhesive (Transbond XT) for 183 days of observation. Statistical comparisons between types of materials, types of storage media, and interactions were performed at 1, 7, 91, and 183 days from the time of first immersion of the discs. The findings were as follows:

1. At days 1 and 7, all materials were significantly different from one another in rate of fluoride release, with Assure having the highest rate, followed by Fuji Ortho LC, Python, and Transbond. Therefore, the first hypothesis, that Fuji Ortho LC would release the most fluoride, was rejected.
2. Transbond XT, the non-fluoride control material, displayed significantly lower rates of fluoride release than all other materials at all time points. Its fluoride release was below the therapeutic range proposed by Rawls (1995).
3. Although fluoride release rates decreased with time for all three fluoride-containing materials (Assure, Fuji Ortho LC and Python), these materials sustained rates of fluoride release throughout the study above the therapeutic range determined by Rawls (1995).
4. The type of storage medium created a significant difference in rate of fluoride release only at selected early time points in the study. At later time periods (91 and 183

days), no differences in fluoride release were noted for the materials in distilled water vs. artificial saliva. Hence the second hypothesis, that the materials would release more fluoride into distilled water than into artificial saliva, was rejected.

9.2 Running vs. still water

The 20 previously tested samples of Assure underwent a further immersion period under running or still distilled water. Ten discs were exposed to running water for a total period of two weeks, and were periodically removed and stored in 1 mL of distilled water for 24 hours to obtain fluoride release rates. The 10 control samples were stored in distilled water and tested at the same time points throughout the two-week period. Fluoride release rates in running and still water were compared at 2, 7, and 14, days from time of first immersion. The findings were as follows:

1. Fluoride release of Assure decreased with time after exposure to both running and still water. Fluoride release was within the "therapeutic range" (Rawls, 1995) at all time points.
2. There were no significant differences in the rates of fluoride release in running and still water at any of the time periods. Therefore, the third hypothesis, that Assure would release less fluoride after exposure to running water vs. still water, was rejected.

This research project has both answered questions and posed new questions. Further research, both *in vitro* and *in vivo*, is warranted.

9.0 APPENDICES

Appendix I Mean rate of fluoride release (μg/cm^2/day) of discs in distilled water (± S.D.)

Day	Transbond	Python	Fuji Ortho LC	Assure
1	0.1 ± 0.03	6.3 ± 0.09	25.9 ± 3.19	66.2 ± 3.48
2	0.1 ± 0.02	4.6 ± 0.81	10.6 ± 0.33	22.6 ± 3.50
3	0.0 ± 0.01	3.0 ± 0.32	6.8 ± 0.28	23.2 ± 2.12
5	0.0 ± 0.03	2.0 ± 0.34	9.45 ± 1.31	15.5 ± 1.37
7	0.1 ± 0.03	1.1 ± 0.15	4.0 ± 0.38	10.4 ± 0.53
9	0.1 ± 0.02	1.1 ± 0.18	3.9 ± 0.25	9.5 ± 1.48
15	0.1 ± 0.02	1.8 ± 0.15	6.6 ± 1.23	8.3 ± 0.41
22	0.1 ± 0.02	1.8 ± 0.25	4.4 ± 0.44	4.7 ± 0.25
29	0.1 ± 0.02	1.4 ± 0.41	4.2 ± 0.64	4.5 ± 0.41
61	0.1 ± 0.02	1.2 ± 0.53	3.9 ± 0.92	3.9 ± 0.62
91	0.1 ± 0.02	1.2 ± 0.49	3.4 ± 0.44	3.8 ± 0.84
121	0.1 ± 0.01	1.0 ± 0.47	3.1 ± 0.77	3.2 ± 0.96
183	0.1 ± 0.03	2.6 ± 1.02	3.8 ± 1.46	3.1 ± 1.01

Appendix II Mean rate of fluoride release (μg/cm^2/day) of discs in artificial saliva (± S.D.)

Day	Transbond	Python	Fuji Ortho LC	Assure
1	0.1 ± 0.01	4.2 ± 0.51	18.8 ± 1.61	65.8 ± 1.55
2	0.1 ± 0.03	3.3 ± 0.47	8.9 ± 1.57	19.3± 1.17
3	0.0 ± 0.01	2.9 ± 0.41	5.1 ± 0.98	22.9 ± 1.89
5	0.0 ± 0.02	2.5 ± 1.01	3.9 ± 1.30	17.0 ± 1.50
7	0.1 ± 0.02	1.7 ± 0.11	3.0 ± 0.53	10.9 ± 1.33
9	0.0 ± 0.01	1.6 ± 0.19	2.3 ± 1.01	10.9 ± 3.79
15	0.0 ± 0.01	1.7 ± 0.14	3.3 ± 0.44	15.7 ± 3.79
22	0.1 ± 0.02	1.7 ± 0.29	3.0 ± 0.73	7.1 ± 1.18
29	0.0 ± 0.0	1.1 ± 0.13	1.9 ± 0.37	5.0± 0.71
61	0.0 ± 0.00	0.9 ± 0.26	3.0 ± 1.31	4.5 ± 0.71
91	0.1 ± 0.02	0.9 ± 0.29	2.9 ± 1.12	3.6 ± 0.40
121	0.1 ± 0.02	1.0 ± 0.33	2.6 ± 1.34	2.6 ± 0.65
183	0.1 ± 0.08	1.7 ± 0.42	3.5 ± 1.03	2.8 ± 1.32

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