COMPARATIVE FLUORIDE UPTAKE INTO ENAMEL FROM VARIOUS TOPICAL FLUORIDE AGENTS

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

SIGNED STATEMENT

This report contains no material which has been accepted for the award of any other degree in any university. To the best of my knowledge and belief, it contains no material previously published or written by another person, except where due reference is made in the text of the report.

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For Mum and Dad,

to whom I am indebted,

for their many sacrifices, support and inspiration

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SUMMARY

The incidence of dental caries in Australian children has been dramatically reduced over the last two decades. This has been achieved mainly through the wide spread use of systemic and topical fluorides, which have permitted such a result to be achieved, despite the maintenance of high levels of refined sugar intake per head of population over this time.

More recent evidence has focused on the high rate of effectiveness of topical fluorides in achieving this result, even in areas without water fluoridation. Fluoride dentifrices have been considered responsible for much of this result. However the availability of additional topical fluoride agents for patients with higher rate or risks of caries development, have made it possible for a high level of prevention to be achieved even in such cases.

At the same time, there is increasing evidence of mild fluorosis becoming a frequently detected problem in Australian children. This has led to the dental profession recognising the need to be more aware of the many sources of fluoride ion available in the community, and being far more knowledgable about the levels used in clinical practice. The need for a greater understanding of the most effective dose response results of topical fluoride use means that we need more information on the kinetics of fluoride action in preventing dental caries.

While we have a general understanding that fluoride works at the tooth surface mainly by inhibiting demineralisation and enhancing remineralisation during an acidic challenge, there is not a great deal of data available to indicate which methods of topical fluoride use or which agents are the most dose response effective.

It quickly becomes evident clinically that differing agents may be effective to varying degrees in patients with differing caries aetiological factors, eg. xerostomia, erosive caries, etc. Hence for the dental practitioner to be able to prescribe that type of fluoride and schedule of use which will be most beneficial for a particular patient and have least side effect, it is necessary to understand the kinetics of action of each one. It also involves being knowledgable about the multiplicity of types of topical fluoride vehicle, with their differing concentrations of fluoride ion, and recommended frequency and methods of application.

The critical requirement for basic effectiveness appears to be that a certain optimal concentration of fluoride ion be present at the site of acidic challenge through out each day. This concentrations will vary for each person according to the concentration of acid produced and duration of its activity, taking into account the potential for natural protection and repair present largely through salivary action in that patient. While this concentration may be delivered through frequent use of lower concentration topical fluorides, it may be enhanced significantly through the potential for fluoride ion storage in enamel. This inturn may result from pre-eruptive incorporation of increased concentrations of fluoride ion or through post eruptive incorporation through use of the more highly concentrated forms of topical fluoride.

One of the objectives in this project was to determine what the potential for uptake of fluoride ion into sound enamel was using a representative variety of those topical fluorides available commercially in Australia, and including analysis of some alternative forms not available commercially at present. To achieve this it was necessary to use a reliable method of analysis of fluoride uptake levels into enamel.

The method chosen to isolate fluoride ion was an acid biopsy system, based on that originally developed by Wetherell (1965) and later modified by Tyler and Comer (1985). This method was slightly modified to permit direct analysis of fluoride content of biopsy solutions using a fluoride selective electrode, attached to a high impedance pH meter. A second objective of the project was to determine how accurately this method isolated and measured fluoride uptake.

In general, the method was found to show great promise, though a few aspects of the biopsy system need further refinement. Hence the data obtained was viewed to be more in the nature of a pilot study, pending resolution of these few difficulties. The data obtained did indicate clearly that the acidulated fluoride gels provided the highest levels of storage of fluoride ion, above a number of products which contained even higher concentrations of fluoride ion. The alternative substances tested also showed great promise as providing high levels of storage and thus with further investigations may be subjected to comprehensive clinical trial, pending ethical clearance.

CHAPTER 1

1.0 INTRODUCTION.

There has been a significant reduction in caries incidence in western countries over the last 25 years. This has been achieved largely as a result of the widespread use of systemic and topical fluorides. The fact that the reductions in caries incidence have been comparable in communities with and without community water fluoridation programmes has led to the recognition that topical methods of fluoride use, in particular the use of fluoride containing dentifrices, can result in high levels of inhibition of caries. This was first pointed out by Fejerskov et al (1981).

Since that time, there has been increasing recognition that even greater protection against caries can be achieved at the individual or small group level, by the use of topical fluorides supplemental to dentifrices, eg. in mouth rinses, gels, varnishes, or strong solutions. Not only are these widely used in clinical practices for protection against caries development, but they can also be used to achieve control of the incipient carious lesion, or its arrestment at the advanced stage. Mouth rinses are widely used in school based caries protection programmes, and with gels in home based self care programmes.

The problem facing the dental clinician today is deciding which topical fluoride agent will be the most effective in preventing new caries development, or in controlling existing caries in patients where differing rates or types of caries development, or differing aetiological factors, are diagnosed. A factor making it difficult to determine the

relative benefit of one topical agent over another is our incomplete understanding of the way fluoride inhibits dental caries development. In the 1970's it was generally thought that the major benefits of fluoride were from its pre-eruptive incorporation into the developing tooth structure, with subsequent formation of crystals of apatite less soluble in oral acids. The current understanding is that its presence at the site of demineralisation, permits its major effect in promoting remineralisation of apatites and in inhibiting their demineralisation at the plaque/tooth interface, to be realised. This requires its presence at certain minimal concentrations at this site, when the daily round of ion exchange takes place. To achieve this concentration, frequent utilisation of low concentration topical fluorides has been recommended as being most effective. It is also realised that the presence of elevated levels of stored fluoride throughout tooth mineral, either from pre-eruptive incorporation in developing enamel and dentine, or by surface adsorption and storage, can enhance the continuous availability of fluoride ion at potential demineralisation sites for limited periods, and thus tooth protection. It is this latter factor, ie the potential for storage of fluoride within surface enamel provided by the differing topical fluoride agents available, that is the subject of this investigation.

While this factor is just one component of fluoride's role in inhibiting caries, there is increasing evidence that storage of high concentrations of fluoride in tooth mineral can provide enhanced protection against rampant caries and even to a certain extent against erosion by strong acids. It is essential therefore for there to be further investigations into new products or alternative methods of application which can increase this level of protection.

One of the factors which has slowed our progress in determining storage levels of fluoride ion has been the limitations of methods available to isolate it from the tooth structure. Chemical biopsy has been most widely used though it has some disadvantages. Frustration with chemical biopsy led to the development of a microabrasive technique requiring specialised equipment. However, this method also has significant disadvantages. The recently improved technological development of electron and proton probes will hopefully provide more easily attainable and accurate information of fluoride concentration profiles throughout teeth. These are extremely expensive and are not widely available as yet.

The objectives in this investigation are therefore:

(a) to determine whether a modification of a chemical biopsy system of fluoride extraction from enamel and its analysis provides an accurate basis for fluoride estimation.

(b) to determine the comparative intrinsic absorption rate of fluoride incorporation into enamel "in vitro" from representative commercially available topical fluoride agents, and following the use of some alternative methods of application, and materials.

It is hoped that the information gained may permit a more rational prescription of topical fluoride agents for particular requirements, in particular the provision of prolonged levels of protection to those with higher levels of caries risk.

CHAPTER 2:

REVIEW OF LITERATURE.

2.0 COMPARATIVE UPTAKE OF FLUORIDE ION INTO ENAMEL FROM TOPICAL FLUORIDE AGENTS.

2.1 THE ROLE OF FLUORIDE IN INHIBITING CARIES.

2.1.1 NATURE OF THE TOOTH AND ITS ORAL ENVIRONMENT.

2.1.1.1 ENAMEL COMPOSITION AND STRUCTURE.

Enamel is the hardest calcified tissue in the human body and forms a protective covering of variable thickness of up to 2.5 mm over the coronal dentine. It is made up of 96% inorganic and 4% organic substance by weight, and water. These occupy 86% and 2% of enamel by volume respectively. The inorganic constituent is similar to hydroxyapatite (HA): $Ca_{10}(PO_4)_6(OH)_2$. At eruption, the apatite contains a number of impurities which increase its solubility in oral acids. Carbonate and magnesium are the major contaminants. On dissolution carbonates can be replaced by hydroxyl or fluoride ions (ten Cate and Featherstone 1991).

Histologically enamel is made up of enamel prisms, rod sheaths and a cementing interprismatic substance. The prisms are described to be of a key hole shape measuring about 5μm in breath and 9μm in depth; and are oriented at right angles to the dentine surface of all permanent teeth with their diameter increasing from the DEJ to the enamel surface at a ratio of 1:2. The enamel crystals are densely packed and arranged in rods with inter rod substances which follow a wavy course from the dentine to the enamel

surface. The crystals are separated from each other by tiny intercrystalline substances filled with water and organic material, which inturn form a diffusion channel through which mineral and acid ions can travel in and out of the tooth.

2.1.1.2 NATURE OF THE NATURAL ORAL ENVIRONMENT OF THE TEETH.

2.1.1.2.1 SALIVA.

The most significant oral environmental protective factor is saliva. In daily use the word saliva describes the combined fluids present in the mouth. This fluid is made up of the secretions from the different secretary fluids, crevicular fluid plus a mixture of food debris, micro organisms and usually contains desquamated cells from the oral epithelium. A more accurate definition of saliva would be secretions from the discrete glands, the parotid, submandibular, sublingual and the large number of minor salivary glands spread over the mucosa of the parotid, cheeks and lips. However it is important to realise that the composition of saliva (ie. proteins and electrolytes) secreted from the various glands differ.

An adult person produces about 1- 1.5 litres of saliva / day. Saliva is present as a protinatious film covering all surfaces of the oral cavity. The thickness of the film is 1/10-1/100 of a mm. The velocity of formation of this film is the major factor responsible for distributing material within the oral cavity and for eliminating the various substances invading the cavity. Speed of movement will be strongly dependant on the amount and composition of saliva. The oral fluid is composed of an organic and inorganic component. The organic component comprises proteins, carbohydrates,

enzymes, etc. The inorganic component is made up of a range of electrolytes including sodium, potassium, calcium and phosphates which determine the saturation with respect to hydroxyapatite and fluorapatite. Each component of the saliva plays an important role in the oral environment.

2.1.1.2.2 FUNCTIONS OF SALIVA.

The presence of mucin in the saliva is important for keeping the mucosa moistened, and for creating a bolus which helps in swallowing and speech and lubrication of hard and soft tissues to reduce abrasion. Mucin may also interact with host cells to modulate wound healing (Heaney et al 1986). Saliva also influences the perception of taste. It dilutes substances introduced in the oral cavity and aids in oral clearance.

Saliva also contains immunoglobulins to act against micro organisms. A series of buffer systems, mainly carbonic acid/ bicarbonate system in the saliva protect the oral cavity against damaging pH changes.

Saliva contains calcium and phosphorus in a supersaturated state which is maintained by salivary proteins which hold calcium and phosphorous in a reversible state. This means that there is an excess of calcium and phosphorous to precipitate and remineralise.

2.1.1.2.3 PELLICLE.

Saliva is separated from the tooth by a thin acellular layer of adsorbed salivary proteins and other macromolecules which is closely adherent to the tooth, called pellicle, and which is 10 μm in thickness.

The surface of the tooth is negatively charged in a normal oral environment. The calcium and phosphate ions in the ratio of 10:1 in the saliva are attracted to the opposite charge and form a hydration layer. Because of the higher level of calcium ions the net charge of the enamel surface with the hydration layer is negative. The hydration layer now attracts negative charges. Negative charges on macromolecules are found in side chains ending with end groups of phosphate or sulphate and also in amino acids, apatate and glutanate.

2.1.2 NATURE AND MECHANISM OF ENAMEL CARIES.

2.1.2.1 THE CHEMICAL PROCESS OF DENTAL CARIES.

Dental caries is defined as the result of dissolution of the dental hard tissues caused by bacterial degradation products. The caries process is initiated by the bacterial fermentation of carbohydrates leading to the formation of several organic acids such as lactic, acetic and formic acids and a fall in pH results (Thylstrup and Fejerskov 1994).

This fall in pH results in the increased solubility of the enamel apatites. Thylstrup and Fejerskov (1994) report that a pH drop of 1 unit within the range of pH 4-7, gives rise to a ten fold rise in the solubility of enamel apatites.

The pH at which saliva is exactly saturated with respect to enamel apatites is defined as the critical pH and it ranges between 5.3 and 5.5. Below the critical pH enamel demineralisation occurs and above the critical pH enamel tends to remineralise. The value of the critical pH depends on the concentration of the calcium and phosphate in saliva and plaque fluid (Thylstrup and Fejerskov 1994).

At pH below the critical level the saliva is undersaturated with respect to enamel apatite and supersaturated with respect to fluorapatite, which in time leads to a carious lesion. However if the aqueous solution is undersaturated with respect to both hydroxy apatite and fluorapatite then both apatites dissolve resulting in a lesion resembling natural erosion or rampant caries.

During the first stage of carious development signs of dissolution of the outermost enamel may be visible as surface roughening and reduced translucency. This inturn opens up channels between the crystals and the prisms through which acid diffuses into the enamel. When the surrounding aqueous phase is undersaturated with respect to HA and supersaturated with respect to fluorapatite, the HA dissolves and fluorapatite or fluoride enriched apatite is formed. The resultant lesion is a carious lesion in which the dissolving HA originates from subsurface enamel and fluoride enriched apatite is formed in the surface enamel layer. When pH is above 6, the aqueous phase is supersaturated with respect to both HA and fluorapatite resulting in redeposition of minerals in surface enamel and, with higher pH (above 7.2) calculus formation (Thylstrup and Fejerskov 1994).

When the overall level of demineralisation is greater than the level of enamel remineralisation over a long period of time, caries will result, and a clinically detectible white spot lesion appears in enamel.

After the white spot lesion with a surface zone covering the subsurface lesion has been established, the diffusion of the acids and the protons from the plaque determine the fate

of the carious lesion. In chronic caries, the diffusion is so slow that although the pH change may be rapid in plaque covering enamel due to carbohydrate consumption, the pH within the lesion fluid remains relatively stable.

2.1.2.2 MICROSCOPIC FEATURES IN THE EARLY SURFACE LESION.

Silverstone (1973) has described four different regions in the surface enamel in early enamel lesion under the polarised microscope- Surface zone, lesion body, the dark (or + zone), and the translucent zone.

The surface and the dark zone are a result of predominantly remineralisation phenomenon, whereas the body of the lesion and the translucent zone are considered to be result of predominantly demineralisation (Silverstone et al 1988).

Frank (1990) demonstrated certain structural changes in enamel surface above the subsurface lesion under scanning electron microscopy and transmission electron microscopy, which included (a) enlarged prism junctions (b) diffuse mineral destruction in the prism cores (c) destruction of the interprismatic substance.

2.1.2.3 THE NATURE OF DEMINERALISATION REACTIONS AT THE ENAMEL SURFACE.

Elder (1993) suggests that the tooth enamel is degraded in the oral cavity by 3 main types of attack.

(a) surface softening or short term exposure to low pH such as enamel dissolution that occurs as a result of ingestion of food or beverage.

(b) subsurface demineralisation or lesion formation occurring due to the protecting properties of adsorbed macromolecules (pellicle and plaque) during long term exposure under mildly acidic conditions.

(c) surface etching following strong acid exposure for short periods of time.

Demineralisation reactions at the enamel surface leading to dental caries are usually due to the colonisation of bacteria and partial dissolution of individual crystal peripheries leading to uniform increase in the intercrystalline space. Bacterial growth on the dental hard tissues is seen after omitting tooth brushing for about 3 days. At this stage there is a loss of mineral of up to 20-100 μm from the outer surface which indicates a slight increase in the porosity.

After 14 days of undisturbed plaque accumulation the white spot lesion has been demonstrated with characteristic subsurface demineralisation. There is a further loss of mineral from enamel with further increase in porosity with preferential mineral loss from the subsurface enamel. In addition there is direct dissolution of the surface with microerosion involving the larger crystals (Elder 1993).

Following 3-4 weeks of undisturbed plaque accumulation the macroscopic changes are visible without air drying and classical zones of the white spot lesion can be observed under a polarised light microscope. The outer surface exhibits a dissolution of thin overlapping perikymata and developmental irregularities. Thylstrup and Fejerskov (1994) best describe the features of the clinical white spot lesion as follows- "*along the periphery of the facet irregular fissures and defects can be observed. In the opaque*

enamel surface cervical to the facet irregular holes are seen. These are deepened and contain more irregular Tomes processes which appear to merge forming areas of irregular cracks or fissures. Finally, the enamel exhibits distinct pattern of dissolution with widened intercrystalline spaces and minor fractures of the perikymata edge. The fracture may be such that microcavities may be formed. The honey comb pattern of the enamel rods is seen at the bottom of these microcavities. The surface enamel surrounding these microcavities exhibits abrasion with irregular scratches but in between rows of Tomes process pits and irregular deeper holes. The enamel surface in such areas are smooth in contrast to the enamel surface in macrocavities which appear granular indicative of merging ends of the individual crystals."

2.1.3 THE ROLE OF FLUORIDE IN INHIBITING CARIES.

2.1.3.1 CLINICAL EVIDENCE.

There is voluminous literature describing the marked inhibition of caries development in western countries over the last two decades. One of the most significant reductions has been experienced in South Australia, where mean new caries tooth (DMFT) scores in 12 year olds has declined from 7.8 in 1970 to 0.25 in 1993 (SADS Report, 1993).

Significant reductions have also been reported in some European and Northern American communities. This was initially thought to have been mainly due to the effect of systemic fluorides from water fluoridation, and where not available, the use of supplementary fluoride protection in the form of tablets or drops for ingestion. However, by the late 1970's it was becoming evident that large reductions in caries development were evident also in those areas without water fluoridation.

Fejerskov et al (1981) was amongst the first to recognise that the increasingly widespread use of fluoride containing dentifrices was resulting in a higher level of protection than dental scientists had predicted. Initial estimates for this level of protection provided by daily topical application of fluoride dentifrices were in the order of 20- 30 % reduction in prevalence. However, over a 20 year period in non- fluoridated areas of South Australia, reductions in new caries development of around 80% have been recorded (SADS Report, 1993).

Further evidence also demonstrated that, at the individual or small group level, additional use of other topical fluorides, such as mouth rinses, gels, strong solutions or varnishes could result in even higher levels of protection to 100 % levels (Thylstrup and Fejerskov 1991, Mc Intyre and Blackmore, 1993).

The efficacy of both systemic and topical fluorides has been clearly established and accepted, in enabling a high level of control of caries development to occur.

2.1.3.2 LABORATORY EVIDENCE OF THE ROLE OF FLUORIDE IN INHIBITING CARIES.

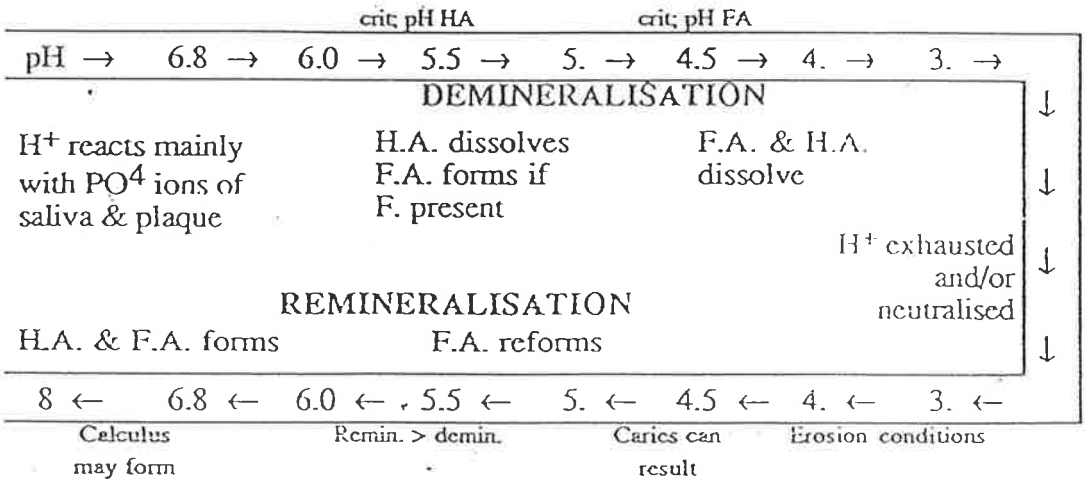
2.1.3.2.1 INHIBITION OF DEMINERALISATION.

The rate of progression of a carious lesion is mainly dependant on the rate at which the apatite crystals dissolve. When the pH level is lowered from 7 to about 5 the solubility of the enamel increases 50 times (Thylstrup and Fejerskov, 1994). A major factor that reduces enamel solubility is fluoride. It tends to decrease enamel solubility either by its presence in the enamel or by its presence in the aqueous phase around the crystals.

Fluoride because of its electronegative character and small ionic size has a strong affinity for the apatite crystal lattice thereby replacing the OH ions and forming fluorapatite or fluoride enriched apatite which is more stable and less soluble than HA.

The basic demineralisation reaction intraorally is as described previously by Fejerskov and Thylstrup (1994) (refer page 13). The role of fluoride in this process has been summarised by Mc Intyre (1992) with the following diagram:

Fig 1: Nature of a hypothetical pH cycle at the tooth surface.



In this hypothetically simplified model, acid ions begin to react increasingly with the surface enamel apatite as the plaque fluid saliva interface becomes undersaturated with calcium with respect to hydroxyapatite. However, as the dissolution of apatite proceeds with reducing pH, any fluoride at this location reacts with available calcium and phosphate to reform (remineralise) a fluoride enriched apatite (HAP). This reaction

occurs particularly at the enamel surface where diffusing ions are held by surface organic deposits, and their concentrations are highest.

If the pH drops below 4.5 the critical pH for fluorapatite to dissolve, then even the FA or HAF will begin to breakdown, providing conditions for rampant caries, or if maintained, erosion to occur.

This model is based on findings from a number of sources, in particular ten Cate (1983), Larsen (1978), Thylstrup and Fejerskov (1994) and Featherstone (1992).

An initial classical experiment done by ten Cate and Duijsters (1983) using demineralisation solutions with calcium and phosphorous concentrations up to 2.2 mMol/ litre and fluoride solution content of 0.2-2 ppm fluoride at pH 5.0-4.0 respectively, showed marked inhibition of dissolution of enamel.

Margolis et al (1986) found adequate protection against lesion formation at pH 4.3 using demineralisation solutions with calcium and phosphate concentrates of up to 11.7 mMol/ litre and 6.0 mMol/ litre respectively and fluoride content of 0.025 ppm. However when no calcium and phosphate ions were present in the start of demineralisation a higher fluoride concentration was essential to inhibit lesion formation.

Featherstone (1992) found in "in vitro/ in vivo" studies that concentrations of fluoride as low as 0.05 ppm inhibited lesion development. Thus it is now recognised that even very

low concentrations of fluoride ions, if maintained in contact with the tooth during the demineralisation process, can significantly inhibit the caries process. Maintenance of low concentrations of fluoride in the oral environment is possible by the use of topical fluorides, which reduce the susceptibility of the enamel to acid dissolution by their uptake on to the surface of apatite crystals, or by their retention in plaque or pellicle at the tooth surface.

Clarkson et al (1986) proposed that during lesion formation the fluoride content of enamel mineral increases even in the subsurface lesion where the fluoride content is low in intact enamel.

2.1.3.2.2 ENHANCEMENT OF REMINERALISATION.

Shellis and Duckworth (1994) describe remineralisation as a process by which partly dissolved crystals are induced to grow by precipitation of mineral ions from solution so that the process of mineral loss is to some extent reversed.

Both saliva and plaque fluid are supersaturated with hydroxyapatite at neutral pH and support remineralisation. The extent to which fluoride contributes to the remineralisation process has been demonstrated experimentally, though is still not fully understood.

Experiments by Varughese and Moreno (1981) also show an increase in the rate of crystal growth with fluoride concentrations as low as 0.05 ppm.

Nancollas and Tomazic (1974) have proposed that at normal pH and supersaturation levels found in resting plaque fluid, apatite growth proceeds by way of formation of intermediate solids especially Octacalciumphosphate (OCP) which then hydrolyses to hydroxy apatite. In the presence of fluoride it hydrolyses to fluorapatite or fluorhydroxyapatite. Thus by remineralising with fluoride not only is the lost mineral replaced but there is also an increased resistance to later demineralisation (Eanes, 1980).

"In vitro" studies by Koulourides et al (1981) have shown a 3 to 5 fold increase in the rate of hardness when acid treated enamel specimens were repeatedly immersed in remineralising solutions containing 1 ppm fluoride. In a similar experiment Feagin (1973) showed fluoride content up to 4 ppm in the surface-softened enamel.

The process of remineralising subsurface lesions is difficult because it is a diffusion controlled process, and the relative low porosity of the surface enamel may reduce the mineral uptake (Arends and ten Cate 1981, White et al 1988a). This can be better explained by the fact that fluoride enhances deposition primarily in the surface layer leading to blocking of the surface layer pores.

Christoffersen et al (1984) derived the optimal fluoride gradient to achieve remineralisation without inhibiting complete remineralisation. Greene and Newbrun (1986) concluded that lesion cannot be repaired by more than 80 % of the mineral originally removed due to the surface layer being fully mineralised.

In summary, there is a great deal of evidence to show that fluoride ion, even at concentrations as low as 0.05 ppm, can inhibit dental caries formation in enamel. The fluoride ion may be delivered in a number of ways, which may be classified as pre-eruptive or systemic and post-eruptive or largely topical. Irrespective of the means of delivery, it inhibits caries development largely by a process of inhibition of demineralisation and enhancement of remineralisation, at the site of a demineralisation reaction. This is usually at the tooth/ plaque/ saliva interface. Whether its origin is through pre-eruptive incorporation into apatite structures, or topical delivery, it is essential for the fluoride to be liberated in an ionised form at the site of demineralisation, for it to be effective.

2.2 INTRA ORAL MECHANISM AND KINETICS OF ACTION OF FLUORIDE.

2.2.1 RELATIVE CONTRIBUTIONS OF THE VARIOUS MODES OF ACTION OF FLUORIDE IN INHIBITING CARIES.

2.2.1.1 PRE VS POST ERUPTIVE EFFECTIVENESS OF FLUORIDE.

From the forgoing descriptions, it is clear that the fluoride ion is very effective in inhibiting the caries process. The precise way in which this is achieved intra- orally is still not fully understood. In the 1970's it was believed that systemic fluorides were the most effective in inhibiting caries. This was believed to be due to ingested fluoride being incorporated into mineralising apatite material as FA. This was known from chemical analysis to be less soluble in oral acids than HA. It was also believed this enlarged the apatite crystal size, also making such crystals more difficult to dissolve, and reducing pit and fissure depth in an altered crown morphology. However, subsequent inquiry has shown this concept not to be entirely accurate. Evidence has

been produced that teeth from a subject who drank fluoridated water through out life are still subject to dissolution in artificial carious systems, even though the rate may be reduced.

Levine (1991) pointed out that unless a continuous supply of fluoride is available to the enamel surface after eruption, then the fluoride incorporated within the tooth is lost by ionic exchange. Thus fluoride present in the tooth structure from pre eruptive uptake acts largely by its release in ionised form, to enable it to inhibit demineralisation and enhance remineralisation.

There is also evidence that higher concentrations of fluoride have a bacteriostatic effect on organisms present in plaque at the tooth surface. Loesche (1975) and others have demonstrated that concentrations of fluoride as low as 19 ppm can exert some degree of metabolic inhibition on bacteria (Marsh and Bradshaw, 1990).

In summary, fluoride is currently believed to exert its caries inhibitory effects by:

(i) inhibiting demineralisation

(ii) enhancing remineralisation

(iii) exerting some bacteriostatic effect

The bulk of scientific evidence does not support the concept of altered crown morphology, given the low human ingestion concentrations compared to laboratory animals where this has been demonstrated.

Both systemic and topical fluorides act by the above process and contribute to its effectiveness clinically. However, it has been demonstrated that its continuing effectiveness requires a continuous optimal concentration to be maintained at the tooth surface, whether dissolved from that resulting from systemic ingestion, or topically applied daily. The latter produces the most effective long term protection. However, the optimal concentration required to provide total protection depends on many factors which contribute to the overall cariogenicity of the oral environment, viz dietary sucrose frequency, salivary protection capacity, oral hygiene, etc. Even so, higher levels of cariogenic potential can be controlled if extra topical fluoride ion is provided on a frequent basis (Mc Intyre and Blackmore, 1993, Fung and Mc Intyre, 1994). This will be discussed later.

A further aspect of topical fluoride action which needs to be highlighted is that, as well as acting immediately at the tooth surface to inhibit the caries process in the enamel structure, it may be retained in saliva or adhered to soft tissues for some time after use. The longer the ion is retained, the greater inhibitory potential it has.

2.2.1.2 MECHANISM OF ACTION OF FLUORIDE IN PLAQUE AND SALIVA.

Fluoride is present in oral fluids and accumulates in plaque. The normal concentration of fluoride in resting saliva is 1 mMol/ litre (0.02 ppm) and this is significantly lower than the fluoride concentration in plaque and gingival fluids (Gron et al 1968). Fluoride concentration in saliva does not depend to a large extent on the flow rate although concentration is higher in unstimulated saliva.

Intake of systemic fluoride leads to a temporary increase in the plasma fluoride concentration which in turn leads to a corresponding increase in the salivary fluoride concentration; with high doses of systemic fluoride of up to 10 mg, saliva fluoride as high as 14 mMol/ litre can be achieved for short periods of time (Shannon and Edmonds 1972)

At low concentrations (< 4 mMol/ litre) in oral fluids, fluoride undergoes a slight reaction with the tooth surface to form fluorhydroxyapatite. However at higher concentrations calcium fluoride is formed at the tooth surface (Dawes and Weatherell 1990).

The sources of plaque fluoride include saliva, gingival fluid, diet, topically applied therapeutic agents and possibly from demineralised tooth mineral.

Plaque fluoride exists in three forms- as free fluoride ions, ionisable(loosely bound) and as strongly bound. The ionisable fluoride can be released by cold 0.5 Mol/ litre Perchloric acid and it constitutes less than 5% of the plaque fluoride. During sugar fermentation by plaque and the accompanying pH drop, more fluoride may be released as fluoride ions from the loosely bound fluoride (Thylstrup and Fejerskov 1986).

Methods are now available for the analysis of fluoride concentration in plaque from single tooth surfaces. Weatherell et al (1986, 1988) described a wide range of fluoride concentrations at different sites in the mouth after rinsing with fluoride solutions, showing the importance from discreet rather than pooled sites. However,

Carey et al (1989) showed no consistent site related fluoride concentration differences in overnight accumulated plaque fluid from upper, lower, approximal, buccal, anterior, posterior single tooth surfaces.

Wilson and Ashley (1988) reported that the total plaque fluoride was lower in pooled approximal plaque than in pooled smooth surface plaque. However, Gaugler and Bruton (1982) report a 5-6 fold higher fluoride values than did Wilson and Ashley (1988) in the interproximal plaque fluoride content.

The strongly bound fluoride contributes about half the total plaque fluoride and is detectable after digestion in hot concentrated acids. Thylstrup and Fejerskov (1986) propose that the living bacteria and mineralised parts of the microbial deposits may be the main binding substances for this fluoride fraction. Gron et al (1969) suggested that the bound fluoride was present as extracellular mineral similar to fluorapatite. However, Jenkins et al (1969) disputed this and suggested that plaque fluoride was mainly bound intracellularly by plaque bacteria, either in a completely dialyzable form (Singer et al 1970) or as only about 50% dialyzable fluoride, the remaining being non-dialyzable or tightly bound.

Thylstrup and Fejerskov (1986) suggest that the mechanism by which fluoride might interfere with initial bacterial adhesion to tooth surfaces other than by changing the composition of the pellicle remain purely speculative. However evidence suggests that unless fluoride concentration is raised massively, plaque bound or free fluoride has little or no inhibitory effect on acidogenicity (Tatevossian 1990). Fluoride concentration

higher than 40 ppm markedly retards the formation of extracellular polysaccharides, a substance thought to play a role in bacterial adhesion (Luoma et al 1975). However, studies by Kilian et al 1979, have not been able to demonstrate any changes which can be related to fluoride.

Geddes and Bowen (1990) proposed that fluoride enters micro organisms against a concentration gradient and accumulates intracellularly as the extracellular pH decreases. The transport of HF into cells results in its uptake as H^+ and F^- in the more alkaline intracellular fluid. Ionic fluoride then becomes more available for enzyme inhibition leading to a slower rate of acid production. Fluoride also increases the cell membrane permeability and can rapidly leak out of the bacterium as the cytoplasmic pH falls, contributing to the plaque matrix fluoride.

Several investigators have tried to relate the caries reducing effect of fluoride to changes in microbial composition of bacterial deposits on the tooth surface. There are no absolute consensus as to whether fluoride might change the microbial composition of plaque (Thylstrup and Fejerskov 1986). However, it is assumed that fluoride from plaque can be released to interact with the underlying hard tissue to suppress demineralisation and enhance remineralisation, although, experimental evidence to support this is lacking (Geddes and Bowen 1990).

Geddes and Bowen (1990) further describe the anticariogenic property of plaque fluoride as follows- *"high concentration will eliminate sensitive bacterial populations. Sublethal concentrations will alter carbohydrate metabolism in ways which include*

reduced acidogenicity and altered extracellular, insoluble polysaccharide production and possible adhesion. Also sublethal concentration of fluoride may alter acid tolerance in S. mutans and other microorganisms leading to a less acidogenic plaque flora." However, Myers and Handelman (1971) found no detectable changes in the proportions of the total number of streptococci, lactobacillus, acidogenic bacteria, extracellular polysaccharide producing streptococci which were represented by colonies similar to *S. mutans* or intracellular polysaccharide storing microorganisms.

2.2.1.3 NATURE OF FLUORIDE STORAGE IN ENAMEL AND DENTINE AND POSSIBLE DIFFERENCES IN KINETICS OF ACTION OF BOTH SOURCES OF FLUORIDE.

When fluoride reacts with enamel, two different products may result; When the fluoride concentration in the solution used is high, calcium fluoride is formed and at low concentrations fluoride is taken up in the apatite lattice (Spinelli et al 1971) resulting in the formation of flurapatite or fluoride enriched apatite (ten Cate 1991).

There has been considerable debate concerning the roles of calcium fluoride (loosely bound) and fluorapatite (firmly bound) fluoride for caries prevention. One school of thought views firmly bound fluoride as being most beneficial in caries prevention owing to its lower solubility and increased retention within dental tissues (Mellberg and Ripa 1983, Ericsson 1977). Another school of thought views loosely bound fluoride to act as a potential "reservoir" source of solution fluoride enhancing remineralisation and retarding demineralisation (Arends et al 1984, ten Cate 1984, Rolla and Saxegaard 1990).

CaF_2 is observable in the electron microscope as spherical globules in the enamel with a diameter ranging from 4-15 nm (Larsen and Fejerskov 1978). Rolla and Saxegaard (1990) pointed out that the size of the globules depend on the pH and the concentration of the fluoride solution, the period of exposure and the phosphate content of the solution.

Mc Cann (1968) had earlier proposed that CaF_2 is soluble in saliva (12-15 mg/ litre) and it would be rapidly lost in the oral cavity and that the clinical effect of fluoride was related to formation of firmly bound fluoride only.

However recent research has now shown that CaF_2 is stable in saliva at neutral pH owing to the adsorption of HPO_4 and proteins. Low pH causes loss of adsorbed HPO_4 and dissolution of CaF_2 (Rolla 1988). In addition Chander et al (1982) has shown that HPO_4 adsorbed to CaF_2 may cause formation of a surface layer of fluorapatite onto the CaF_2 crystals through a dissolution/ precipitation reaction. This layer is more stable and will release fluoride at low pH.

Studies by Ogaard (1988) showed that shark enamel containing 30,000 ppm of fluoride has a limited caries resistance which indicate that presence or formation of fluorapatite is not a major aspect of the cariostatic mechanism. Based on research on the beneficial effects of FA and CaF_2 it has been shown that FA is a reaction product of enamel/ apatite fluoridation with or without CaF_2 formation (White et al 1988b). Secondly, fluoride in solution has a greater influence on both remineralisation and demineralisation than in the formation of either bulk precipitate. Although both CaF_2

and FA can produce F in the solution phase, FA provides solution fluoride mainly under demineralisation conditions while CaF_2 elevated solution fluoride levels at both neutral and lower pH conditions (White et al 1990).

Caslavska et al (1991) have shown that APF treatment produce large amounts of CaF_2 especially from amine and ammonium based formulations. However, Chow (1990) concluded that APF does not appear to be highly effective in increasing the tooth bound fluoride content. Fluoride varnishes are believed to deposit CaF_2 and maintain increased fluoride concentrations as long as 24 weeks after application (Dijkmann et al 1983). In vitro studies by Laufer et al 1981 have shown significant increase in the fluoride content by immersing teeth in fluoride containing calcium phosphate solutions for several days.

Bruun and Givskov (1993) pointed out that use of fluoride dentifrices or mouth rinses with 0.2% NaF resulted in very little CaF_2 formation. Contrary to this in a study done by Saxegaard and Rolla in 1989 involving mouth rinses, a moderate amount of fluoride was acquired by sound enamel and more as CaF_2 than as FA. On etched enamel incorporation of firmly bound fluoride continued, which may indicate that CaF_2 was transformed into fluoridated apatite probably through remineralisation during pH cycling in plaque covering the etched enamel. It was also found that loss of CaF_2 was arrested after 1-2 days on sound enamel at 70 % and on etched enamel at 40 % of the original level. This study emphasised that frequent application of fluoride (ie. through rinsing or tooth brushing with fluoride containing products) will secure a constant CaF_2 depot on the tooth which may last long enough to be of clinical significance.

In general increased time of exposure, increased concentration, lowered pH and pretreatment with calcium have been shown to be effective means of increasing CaF_2 deposition in enamel "in vitro" (Saxegaard and Rolla 1988).

The thickness of CaF_2 formed on/in enamel after a topical application was estimated to be in the range of 0.02-0.2 μm (Dijkman et al 1982 a,b, Saxegaard and Rolla 1988).

Larsen et al (1981) pointed out that when an acidulated fluoride solution was used or the enamel was presoftened the loosely bound fluoride persisted for longer periods of time.

2.2.2 KINETICS OF FLUORIDE ACTION INTRAORALLY.

2.2.2.1 FLUORIDE DOSE RESPONSE FACTORS IN THE INHIBITION OF DEMINERALISATION OF ENAMEL AND DENTINE.

As stated previously, many investigators have shown that the most effective use of topical fluorides for the majority of the population is daily, low concentrations in a form readily available to the tooth surface. Concentrations as low as 0.05 ppm have been shown to inhibit mineral loss "in vitro", and the small amounts retained intra orally from brushing (of the order of 5 ppm in saliva immediately following brushing, diminishing to normal salivary background levels by 2 hours, Zero et al, 1988) have been shown by inference from Australian data (Carr 1988) to reduce new caries development in children 12 and under by 80-90 % including those in nonfluoridated water regions.

However, Mc Intyre and Blackmore (1993) have shown that even in cases of continuing high cariogenic diets, and Billings et al (1988) have shown in severely radiation xerostomic patients, almost all caries can be controlled by the use of higher concentrations of topical fluoride more frequently than traditionally used. Featherstone et al (1990) have pointed out that to achieve a halving of mineral loss in artificial caries systems in enamel, a log unit increase (ie 10x) in concentration of fluoride ion is needed at the tooth surface. However, this is achievable with the more concentrated topical fluoride agents currently available, though their retention rate and thus frequency of use are dependant on many factors, including salivary clearance rates (Featherstone et al 1992a), or storage capacity in enamel as CaF_2. Thus more information on these properties is needed before a schedule of treatment is prescribed.

2.2.2.2 CONCENTRATION OF FLUORIDE DELIVERED TO THE TOOTH SITE FROM VARIOUS TOPICAL FLUORIDE AGENTS.

ten Cate and Simons (1986) determined the optimal concentration of fluoride in a dentifrice without loss of its efficacy, it was concluded that 300 ppm of fluoride in a dentifrice should be sufficient to inhibit demineralisation. However in high caries risk patients a larger supply of fluoride is required (De Kloet et al 1986).

In another study by O'Reilly and Featherstone (1987), on teeth with orthodontic brackets in place, it was demonstrated that when NaF dentifrices containing 1100 ppm F, were applied, measurable demineralisation could occur adjacent to the brackets. However, fluoride dentifrice in conjunction with fluoride mouth rinse, was able to control demineralisation.

Billings et al (1988) investigated salivary clearance in xerostomic patients and demonstrated significant higher fluoride retention after different treatments (gels and mouth rinses) in xerostomic patients as compared to normal subjects.

Meyerowitz et al (1991) compared use of 0.05 % NaF mouth rinse used twice daily with 1.1 % NaF gel used once daily in xerostomic patients. Results indicate that fluoride concentration of 0.1 ppm was maintained constantly in saliva with use of twice daily NaF rinse.

Fluoride clearance in the normal mouth is relatively rapid and this effectively limits the duration of its effect (Lambrou et al 1981). Zero et al (1988) investigated the fluoride clearance in saliva for periods up to 24 hrs. Fluoride rinse with 225 ppm fluoride was retained at elevated levels for up to 2 hrs in the saliva of normal subjects, whereas use of fluoride dentifrices followed by subsequent water rinse decreased fluoride retention. These studies concluded that levels of 0.05 ppm and more can readily be achieved for periods of 1-2 hrs by the use of combination of fluoride dentifrices and fluoride mouth rinses.

Zero et al (1988) compared fluoride concentrations in unstimulated whole saliva in normal patients following use of fluoride dentifrices and fluoride rinses. These results suggest that fluoride rinses may be more effective than a fluoride dentifrice on the basis of fluoride retention.

2.2.2.3 FACTORS AFFECTING FLUORIDE CONCENTRATION AT THE TOOTH SITE FROM VARIOUS FLUORIDE AGENTS.

Method of fluoride delivery, fluoride concentration of the agent, and the time of application (day time vs night time) are important factors influencing fluoride levels in the mouth (Zero et al 1988).

The concentration of fluoride in the oral fluids is mainly influenced by the concentration of the fluoride product used, time since the last exposure (Bruun et al 1982), mode of delivery and a complex interaction of factors which influence fluoride clearance and retention (Zero et al 1988).

Zero et al (1988) compared the influence of various topical fluoride agents available such as fluoride dentifrices, fluoride rinses, fluoride gels. Fluoride retention was significantly higher from fluoride gels compared to other topical agents used with high levels for up to 2 hrs after treatment.

The retention rate from fluoride rinse and fluoride dentifrice were intermediate, with fluoride rinse being significantly greater than fluoride dentifrice. In the same study it was also found saliva fluoride concentration was significantly higher after night time application than after day time application.

Other factors which may influence increased concentration of fluoride at tooth surface include older patients where an increased fluoride concentration is attributed to enlarged interproximal spaces as a result of gingival recession (Zero et al 1988).

Mode of delivery of fluoride is also important in that use of a fluoride rinse (226 ppm) was shown to result in higher fluoride levels than fluoride dentifrice (1,100 ppm) (Zero et al 1988). This is because the act of rinsing with tap water after brushing reduces the fluoride retained in the mouth.

Studies by Zero et al (1988) also indicate that the method of delivery and the fluoride concentration of the topical agents are more important parameters than the dose. The concentration provides the driving force for fluoride to pass into the deeper plaque layers adjacent to the tooth surface and determines the initial concentration in oral retention sites.

Brudevold et al (1963) suggested use of fluoride in acid solution can increase fluoride uptake by hydroxyapatite and more effectively reduce its solubility. De Paula et al (1971) observed that fluoride uptake from APF was increased by etching the enamel surface with 50 % phosphoric acid.

Studies by Tyler and Poole (1984) confirmed that fluoride acquisition increased with decreasing pH and showed Ammonium bifluoride to be more effective as an "In vitro" fluoridating agent than APF.

2.2.2.4 EXAMPLES OF THE EFFECTIVENESS OF FLUORIDE IN INHIBITING DEMINERALISATION.

Radiation therapy in cancer patients causes iatrogenic hyposalivation, which inturn leads to changes in the oral microflora and rapid dental caries (Wescott et al 1975). In the absence of fluoride therapy there is rapid development of dental caries in radiation induced xerostomic subjects (Dreizen et al 1977).

The cariogenic challenge is further increased with the tendency of hyposalivation patients to have more soft, high carbohydrate containing foods (Brown et al 1975). In these situations the use of topical fluorides has been found to be very effective in caries control. Daly et al (1972) reported the use of 1.1% Neutral NaF gel (5000 ppm) applied daily in trays while Wescott et al (1975) investigated the use of 0.4 % Stannous fluoride gel applied by tooth brush. Johansen and Olsen (1979) described use of 1.1 % Neutral NaF gel (5000 ppm) or 1.23% APF (12,3000 ppm) twice daily for 2 weeks. and then once daily for 2 weeks followed by twice daily use of fluoride mouth rinse containing low concentration fluoride. Meyerowitz et al (1991) evaluated the effect of a twice daily topical application of 0.05 % NaF mouth rinse on caries in the oral cavity of subjects suffering from radiation induced hyposalivation. The results showed that twice daily oral rinse with 0.05 % NaF can prevent caries developing.

2.2.3 CURRENT EVIDENCE OF INSOLUBILITY OF CaF_2 AT NORMAL pH.

Recent clinical evidence indicates that CaF_2 can persist on the tooth surface for weeks or months after topical fluoride application and is quite insoluble in saliva at neutral pH (Caslavska et al 1991, Saxegaard and Rolla, 1989). This is due to the coating of pellicle

proteins and secondary phosphate on the enamel. However, when there is a drop in the pH (as seen during a carious attack) there is loss of pellicle coating resulting in an increased dissolution of CaF_2 (Chander et al 1982, Lagerlof et al 1988). The fluoride ions thus released may inhibit demineralisation or may increase rate of remineralisation (Arends and Christoffersen, 1990). Recent evidence also suggests that CaF_2 is a precursor of fluorapatite, which inturn is formed when pH drops in plaque and not during topical fluoride application (Rolla, 1988). The amount of CaF_2 in enamel depends on pH, exposure time and concentration of the fluoride solution (Saxegaard and Rolla, 1989). CaF_2 formation can be increased by lowering pH of preparation. Laboratory evidence by Rolla (1988) indicates that a continuous layer of CaF_2 covering the enamel surface completely, can protect the enamel to a higher degree than fluorapatite, because the solubility of CaF_2 is less pH dependent than that of fluorapatite.

2.2.4 USE OF HIGHER CONCENTRATION FLUORIDE FOR HIGH CARIES CASES.

Newbrun (1992 a,b) suggested an increased cariostatic effect with increasing fluoride content. In 1985, Hagan et al in a clinical study showed 0.6% APF to be less effective than 1.23% APF. The dose/response relationship to fluoride for concentrated preparations is controversial, and studies by Ogaard and Rolla (1992) showed no difference in the cariostatic effect following use of 0.05%, 0.2%, or 2% NaF solution daily, weekly or as a single application. Studies by Galagan and Knutson (1948) also showed no significant difference in cariostatic effect between neutral 2% NaF or 1% NaF solution. Recent studies by Jones et al (1994) have shown that prior application of

APF 1.23% gel to enamel can result in enhanced protection to a simulated gastric acid solution (0.06M HCl). Again the more frequent the application, the more protective was the topical fluoride in the strong acidic challenge situation. Hence the matter of a dose response effectiveness of topical fluoride is still controversial.

2.2.5 SAFETY ASPECTS OF TOPICAL FLUORIDE.

Ekstrand et al (1981) showed high plasma fluoride values in children following application of APF gel due to varying amounts retained in the mouth and swallowed. However, this was when no suction or expectoration of excess fluoride in saliva was permitted. Use of fluoride gels with foam lined trays and suction during treatment and one minute expectoration after treatment considerably reduced risk of side effects (Lecompte and Whitford 1982). Although thought to be more toxic than gels due to its high fluoride concentration of 2.3%, Duraphat is less toxic as a result of the slow release of the set varnish from the teeth (Ekstrand et al 1980). Plasma fluoride levels after use of Fluorprotector is lower than Duraphat due probably to its lower fluoride concentration.

The probable toxic dose of fluoride ion is considered now to be in the order of 5.0 mg/ kg of body weight (Whitford 1987). Thus the amount ingested during topical fluoride application, if applied with normal caution are well below this level. The main concern for toxicity is with infants swallowing excess supplemental systemic fluoride. A further concern with children is the risk of fluorosis resulting from swallowing significant amounts of topical fluoride. Hence it is advised for strong concentrations of topical fluoride not to be used for infants without close supervision. In general, when

fluoride ion is ingested in total amounts of 1 to 3 mg per day as in optimal fluoridated communities, it is considered safe from the toxicity aspect.

2.2.6 APPLICATION TECHNIQUE - PROPHYLAXIS AND DRYING.

Drying of teeth before application of fluoride solution and gels is recommended to avoid dilution of the agent and also to facilitate application (Ripa 1990). Prophylaxis however does not seem to make a difference in fluoride uptake and is no longer considered necessary before varnish or gel application. Pumice prophylaxis is time consuming and studies by Seppa (1983 a,b) indicate that fluoride uptake by enamel is not reduced by plaque or pellicle.

2.2.7 EVIDENCE ON INCREASED UPTAKE FOLLOWING ACIDULATION.

Laboratory evidence indicates that fluoride concentration in enamel were increased by lowering the pH of topical fluoride solutions. Brudevold et al in 1963 developed a acidulated preparation of sodium fluoride with phosphoric acid.

Nikiforuk, 1985, best describes the mode of action of acidulated fluorides as follows *"reaction of enamel hydroxyapatite with fluoride results in the formation of fluorapatite. However, acidic solutions that contain high fluoride concentrations lead to enamel demineralisation and CaF_2 formation, and phosphate release"*.

Commercial APF preparations are the most preferred topical fluoride agents. And have shown clinical evidence of caries reduction from 20 to 40% (Horowitz and Doyle 1971).

2.2.8 SUMMARY.

Fluoride ion exerts its inhibitory effect on dental caries mainly through its ability to inhibit demineralisation and enhance remineralisation at the site of acid-apatite interaction. While it also exerts an inhibitory effect on bacterial metabolism, this appears to be mainly with higher concentrations, which are retained for a period of time.

The ability of stored fluoride to provide long term protection has been well established. Calcium fluoride storage results from use of high concentration topical fluorides, and appears to be currently regarded as one of the most beneficial methods of storage.

The dose response effectiveness of topical fluoride protection is still controversial, with investigations showing contradictory results. This may suggest that differing types of topical fluoride used in different ways may provide variable responses, and that further study is necessary to determine more precisely all parameters relating to such investigations. Also, response is obviously determined largely by frequency of utilisation, method of application, severity of acidic challenge, and other host factors which are difficult to analyse.

2.3 METHODS FOR ASSAYING FLUORIDE UPTAKE IN ENAMEL.

2.3.1 INTRODUCTION.

There have been two aspects to the successful assaying of fluoride uptake. Firstly a method to biopsy definable amounts of enamel mineral was required and this should preferentially enable sampling at segmental depths. A further requirement was to be able to separate all fluoride, including that thoroughly bound as apatite form, for

analysis. The second objective was the need to be able to accurately analyse the concentrations of fluoride ion which have been separated. Some of the new technology methods of analysis, eg proton probe, do not require separation of fluoride and can be used directly. Traditional techniques for sampling enamel in order to determine the level of fluoride it contains can be broadly classified into groups; acid dissolution, mechanical abrasion, combination of both, and isotope uptake. SEM imaging, quantitative proton probe, secondary ion mass spectrophotometry and other methods are being developed and have just been considered sensitive enough to measure low concentrations of fluoride in teeth.

An important problem that arises with estimating the enamel fluoride uptake is that the absolute quantities of material being dealt with are minute, in some cases in the order of a nanogram, so loss of even a small amount may be significant. The other problem is the applicability of the different methods of assay in different situations. While purely abrasive methods are of little use in vivo, dissolution methods rely on assumption of nature of the material being sampled (density, porosity, and percentage of calcium present) and therefore have been claimed to be less useful for dental tissues other than in cases of white spot lesion (Williams 1992).

2.3.2 CHEMICAL BIOPSY.

As early as 1953, Jenkin and Spiers reported surface enamel to contain 4 to 5 times the fluoride present in deepened layers, obtained by dissolving 1 mg of enamel in acetic acid at pH 4.

Studies on pooled samples of ground enamel of different teeth by Brudevold et al (1956) reported surface enamel fluoride to increase with increasing age and that surface enamel contained 6-10 times the fluoride present in deeper layers.

Cooley (1961) used perchloric acid to dissolve 100 um thick layers from enamel blocks of known dimension. Muhlemann et al (1964) described use of 15 sec exposure to 2 M Perchloric acid to dissolve 12 μm thick layers of enamel. The thickness of the enamel dissolved was directly proportional to the duration of exposure, for a given concentration of acid.

Weatherell and Hargraves (1965) measured enamel fluoride loss by etching the enamel surface with 6N Perchloric acid with mild agitation for periods ranging from 5 to 50 seconds. Loss of enamel was measured by weighing before and after etching. Results indicated that the thickness of enamel removed from the tooth was directly proportional to the amount of enamel dissolved and duration of acid exposure. Weatherell and Hargreves (1965) also suggested that concentrated HCl might be a suitable etching agent, as perchloric acid is dangerous to human health.

Hotz et al (1970) reported an acid biopsy method using Perchloric acid. The enamel surface was coated with copal ether varnish except for a 3x3 mm window which was exposed to 10 ml of 2M Perchloric acid applied on a filter paper disk and then placed on the window. Following an exposure of 6-7 seconds enamel loss of up to 10 μm layer was reported.

2.3.3 MICROABRASION.

Brudevold et al (1968) developed an abrasion method of enamel biopsy which involved polishing off 0.2 mg of enamel from the tooth surface, using a midget filter cone impregnated with silicon carbide particles and coated with glycerine. The resultant slurry was then analysed for F^- and Ca_2^+ using a fluoride electrode and atomic absorption spectrometry. Fluoride concentrations of 400 to 2500 ppm were reported in the 2 μm depth of enamel removed. In 1973, Mellberg et al improved this abrasion technique by controlling the biopsy area, the polishing speed, and the pressure exerted and by using a standard time of abrasion. The enamel surface was covered with a flexible vinyl adhesive tape except for a circular window 4.7 mm in diameter. The polishing speed was 900 rpm and the pressure exerted was maintained at 200 gms. A similar study was done by Aasenden and Moreno (1971) where windows of 20.1 mm^2 and 15.9 mm^2 were used. Results indicated fluoride levels of 2100 ppm in biopsy depth of 1.1 -3.8 μm.

The abrasion method of enamel biopsy was further modified by Wei and Wefel (1975) by incorporating a electronic counter to monitor the number of revolutions made by the abrasive cone. Fluoride levels of 2258 ppm were obtained at a mean depth of 1.67 μm following a load of 200 gm at 300 revolutions.

Haberman et al (1980) utilised a specially constructed microdrill to sample enamel in white spot lesions.

Iyer et al (1983) combined the abrasion method with the chemical dissolution method of enamel biopsy. Windows of 0.20 cm^2 were exposed to 0.5 M Perchloric acid. The thickness of enamel removed was found to be 8-12 μm.

Another technique was developed by Weatherell et al (1985) which has an advantage of being applicable to tissues not amneable to acid etching. Weatherell et al utilised abrasion with simultaneous recording of the thickness of the adsorbed layer.

Reintsema et al (1986) compared Secondary Ion Mass Spectrophotometry (SIMS) with the microdrill technique "in vivo". Results obtained using the microdrill method compared well with those from SIMS although SIMS tended to relatively overestimate the fluoride concentration.

2.3.4 ANALYSIS.

Studies by various workers on fluoride analysis were carried out mainly by Calorimetric or Gravimetric methods, or by involving the hydrolysis of SiF_2, Titrimetric, Mass Spectrometric, Gas Chromatographic, Radiometric, Electroanalytical, and fluoride ion electrode methods.

Bowes and Murray (1935) evaluated some of the then available methods of fluoride analysis and concluded that colorimetric method utilising Zirconium alizarin was the most sensitive. They obtained values of 0.025 % (W/W) fluoride for human enamel (85 ppm, 4.4 mMol/ dm^3).

Singe and Armstrong (1965) reported a method of fluoride analysis which involved diffusion of hydrogen fluoride in a specially constructed cell. Jackson and Weidman (1959) estimated fluoride content of enamel and dentine by titrating the fluorine distillate using thorium nitrate with chromeazurol S as an indicator. The problem with this method was that several ions such as phosphate, arsenate and sulphate markedly interfered with the reaction. Leach and Griffiths (1963) modified the method used by Singe and Armstrong (1965) to increase sensitivity while dealing with smaller samples.

Frant and Ross (1966) developed the fluoride ion sensitive electrode, a more sensitive method of fluoride determination. This method has been used by many workers for determining fluoride ion activity (Mc Cann, 1968, Brudevold, Mc Cann and Gron 1968, Larsen, Kold and Von der Fehr 1972). The fluoride electrode has the advantage over other methods in that it is simple, rapid and reliable. The fluoride electrode is highly selective to fluoride and gives a Nernstian response over a wide range of fluoride concentrations.

Swift (1967) has used spark source mass spectrometry to analyse the trace elements of bovine tooth enamel. In this method it is possible to look for trace elements in addition to fluoride.

In 1970, Baud and Bang used electron probe and X-ray diffraction analysis to investigate enamel treated in vitro by fluoride solutions.

Weatherell et al (1973) used microlitre drop samples under oil to avoid evaporation, on the surface of a fluoride electrode to analyse fluoride. Of particular benefit was their finding that direct measurement of fluoride could be made using the fluoride selective electrode with a special high impedance pH metre, at low pH levels. At pH 2, the F ion was ionised as HF, and potential complexing molecules, eg. Ca remaining in ionised form, thus not interfering with the reading.

Other methods of fluoride analysis were developed. Munksgaard and Bruun (1973) used gas chromatography to convert fluoride to volatile trimethylfluosilane by treating the sample with trimethylchlorosilane reagent and is then passed through a gas chromatograph. In 1976 Hercules and Craig utilised X-ray photoelectron spectroscopy (ESCA), while Petersson et al (1978) utilised secondary ion mass spectrometry (SIMS).

Besides the above mentioned methods Diggens and Ross (1981) performed the electrochemical analysis of fluoride at a pH below the Pk of hydrofluoric acid, where HF was predominantly present, which inturn increased sensitivity of the fluoride electrode. This method was modified by Tyler and Comer (1985) to measure fluoride and calcium simultaneously in biopsy samples etched from enamel by 0.2 M Hydrochloric acid.

Other methods of fluoride analysis include the F MAS-NMR (Mass activation analysis-nuclear magnetic resonance -White et al 1988 b) to study reactions of fluoride with hydroxyapatite and enamel. Vogel et al (1990) described use of micropipette procedures for sample transference and dilution, miniature and microelectrode and adaptive

measures to allow the use of fluoride electrodes. In 1991, Caslavsca and Duschner reported effects of a surface active cation on fluoride/ enamel interactions in vitro using elecronspectroscopy (XPS).

2.3.5 SUMMARY.

Of the methods used for fluoride separation, there appears to be disadvantages with both chemical biopsy and microabrasion methods. With microabrasion, it is difficult to section to the same depth at the same site, and it needs specialised equipment to measure depth of sampling. After sampling, it is still necessary to measure fluoride content while it is still bound to apatite. With chemical biopsy, using HCl, the method developed initially by Wetherell (1965) and later modified by Tyler and Comer (1985) appeared to be the least complex, and possibly most reproducible. Of the methods used to analyse fluoride concentration, the fluoride specific electrode appeared to be that method most readily available, which was considered reliable. The advantage of the Tyler and Comer method was that it permitted direct reading of fluoride from biopsied enamel mineral.

CHAPTER 3:

3.0 MATERIALS AND METHOD.

3.1 Overview.

As stated in the introduction, the objective in this study was to determine:

(a) the accuracy and practicability of a modification of the chemical biopsy method for separation of fluoride from tooth enamel.

(b) the relative uptake of fluoride (ie. concentration) into whole enamel from a wide variety of topical fluoride agents currently available to the dental profession in Australia, together with some experimental agents not commercially available, and in some cases, following alternative usage methods to those traditionally recommended.

It was decided in this investigation, to test the suitability of a chemical biopsy system of fluoride separation from enamel developed initially by Weatherell (1965), and later modified by Tyler and Comer (1985) which allowed direct measurement of fluoride ion levels. This eliminated the need for specialised equipment needed for a microabrasion biopsy system, and used HCl instead of the more dangerous perchloric acid. This method also permitted direct analysis of concentrations of fluoride ion using a fluoride ion specific electrode, rather than require time consuming prior separation by acid hydrolysis and siloxane diffusion as with Taves technique (1968). Various aspects of its accuracy and reliability were confirmed prior to its use for the project.

Human enamel was used rather than bovine enamel, even though this introduced the problem of unknown background fluoride levels in individual teeth. A method was developed to take this factor into account.

Ten topical fluoride agents representative of those commonly available in Australia, were tested initially for fluoride uptake into enamel "in vitro" following application times used clinically.

Some were then tested using an "in vivo- in vitro" system, to determine whether prior coating `of enamel with pellicle and plaque made significant difference to uptake levels.

Finally, investigations into whether time of contact significantly altered uptake of fluoride from acidulated F gels, and tests of some alternative methods of application, and topical F agents not commercially available, were carried out. It is proposed that such knowledge will permit a more rational prescription of topical fluoride agents for specific requirements, in particular the provision of prolonged levels of protection to those with higher levels of caries risk.

3.2. DEVELOPMENT AND EVALUATION OF AN ACID ETCH BIOPSY SYSTEM FOR ENAMEL DISSOLUTION AND RELEASE OF FLUORIDE ION FOR ESTIMATION.

3.2.1 The method of biopsy chosen for this study was a variation of that developed by Tyler and Comer (1985). This involved the use of strong Hydrochloric Acid (HCl) for serial etching of enamel surfaces, and subsequent direct measurement of fluoride levels

in the etching solution using a combination fluoride specific electrode. The main difference between this and other chemical biopsy systems was the findings by these investigators that the Orion fluoride specific electrode could be used to measure concentrations of fluoride ion at low pH (approx. pH 2) if a high impedance pH meter was used, whereas the makers of the electrode had recommended it to be used between pH 5- 6.5 for optimal results. An exhaustive analysis by Tyler and Comer demonstrated that not only was it possibly to show a Nernstian- type response in fluoride ion measurement at low pH, but that the measurements detected much lower concentrations of ion. They reported no deterioration in the electrode (glass) through etching by the hydrofluoric acid (HF) being measured at this pH over a two year period, stating this was due to the fast response of the fluoride differential cell and the low concentrations of fluoride ion being analysed in this enamel etch system.

It was realised by Tyler and Comer that at this pH, it was mainly HF which was being measured. This had the advantage that potential binding agents of fluoride, eg. calcium, would not be firmly bound to the fluoride at this pH, making it more easily available for measurement. Following further personal discussion with Dr Tyler by Dr Mc Intyre (project supervisor), it was decided to use a 0.1 M HCl (pH: 1) solution in such an etching biopsy system.

It was considered necessary firstly to determine the consistency of the mv response using fluoride electrode analysis, at pH 1 in comparison to results at other pH levels.* A selection of fluoride solutions of differing concentration ions viz- 0.005, 0.01, 0.5, 0.1, 1.0, 10.0, and 100.0 ppm which had been prepared in HCl solutions at pH 6, 5, 4, 3,

* Experiment repeated twice with similar results.

2, 1, were tested for millivolt (mv) response using an Orion Combination Fluoride Selective Electrode (model no EA 940), in combination with an Orion 901 pH meter. As stated earlier, this pH meter has two high impedance inputs, which are required for accurate measurement at such low pH levels (Tyler and Comer, 1985). See Fig. 2 & 3.

The following graph (Fig. 4 and Table 1) shows the mv response against fluoride ion concentrations at differing pH. Clearly a typical Nernstian response has resulted for most of the higher concentration of fluoride ion at all pH levels, the mv readings increasing to reflect the increased concentration of acid ions. This confirmed the findings by Tyler and Comer (1985) and thus it was decided to choose an acid etch system using 0.1 M HCl for acid biopsy of enamel, and to permit direct measurement of fluoride ions from the etch solution. As even slight variations in pH of the etch solution result in significant differences in mv. reading at this low pH, it will be essential to consistently monitor the pH of etching solutions prior to, and following use.

FIG 2: pH METER.

FIG 3: GENERAL ARRANGEMENT OF EQUIPMENT.

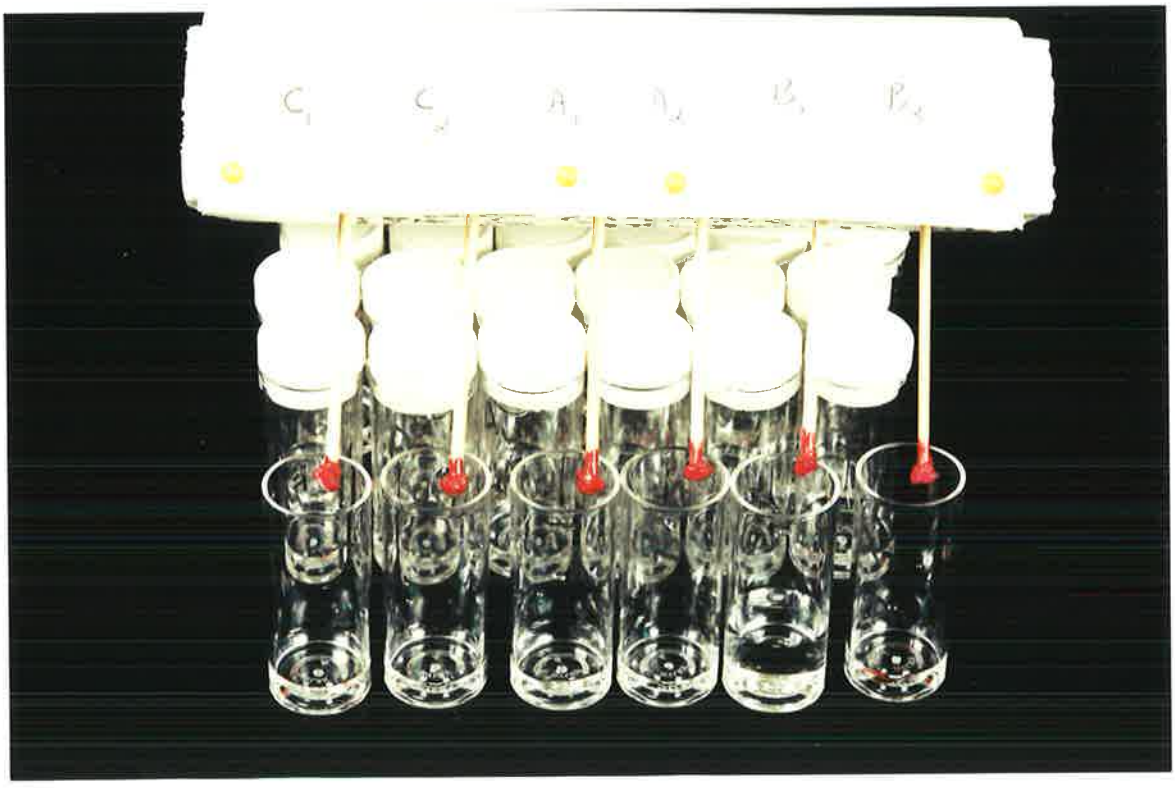


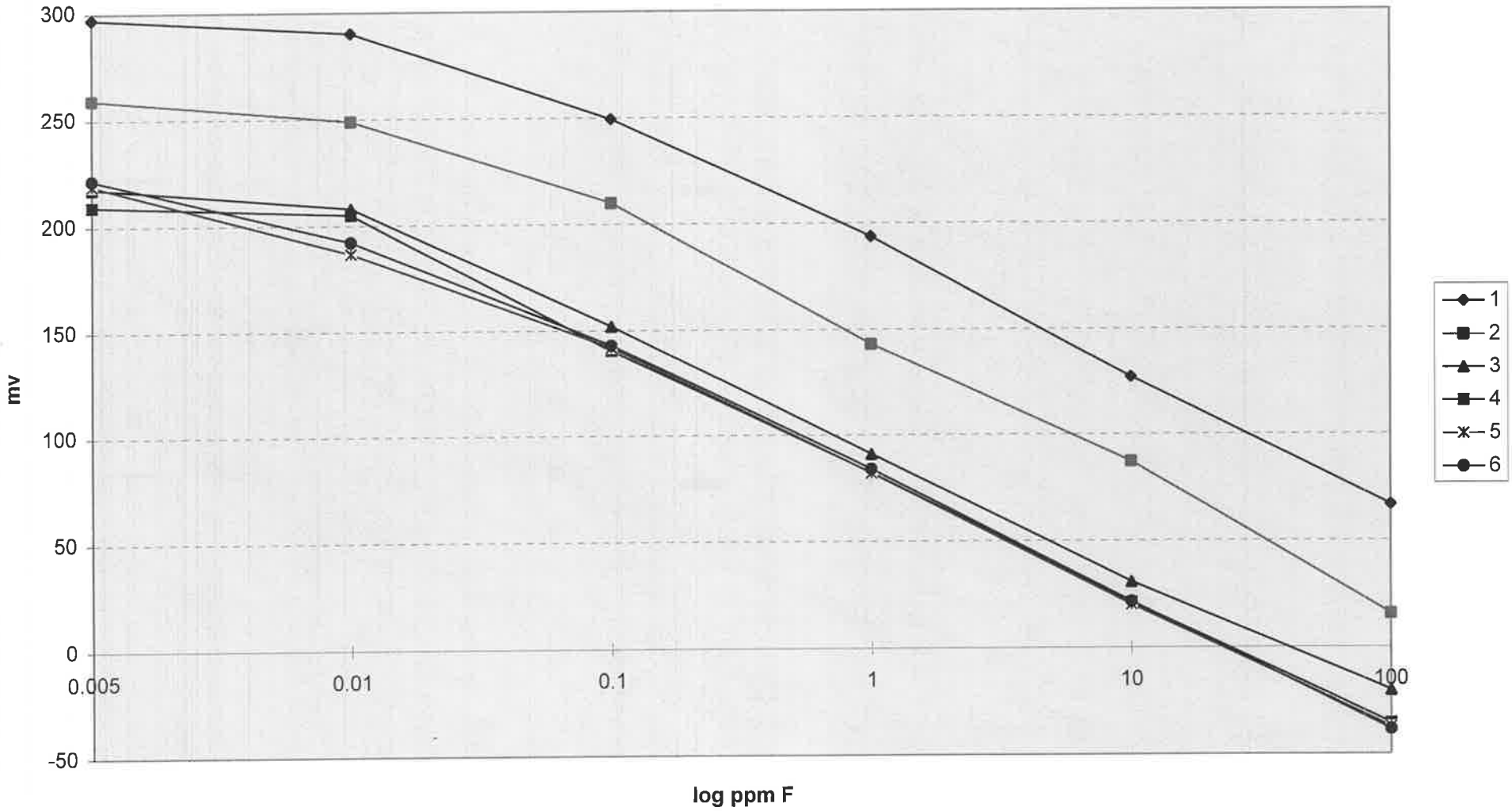
TABLE 1: mv RESPONSE FROM FLUORIDE ION CONCENTRATIONS AT DIFFERENT pH.

F Con (ppm)

pH	0.005	0.01	0.1	1	10	100
1	297.1	290.3	249.4	193.8	127.1	66.6
2	259.2	248.9	210	143.2	87.1	15.8
3	217.7	208.1	152	91	30.9	-20.2
4	209.3	205	140.4	82.1	21.3	-35.5
5	219	186.8	142	82	20.2	-37.2
6	221.5	192.3	143	83.9	21.6	-38.3

mv

FIGURE 4: mv RESPONSE FROM FLUORIDE ION CONCENTRATIONS AT DIFFERENT pH



3.3 SELECTION AND PREPARATION OF TOOTH ENAMEL SAMPLES.

3.3.1 It was decided to use extracted human teeth for these studies. The teeth used were first and second molars of unknown origin, which had been stored at 4°C in thymol water. Plaque and any calcified deposits on the enamel* were removed using scalers taking care not to scratch the enamel surface. Specimens showing surface defects such as cracks, abrasions, extensive staining, or decalcified areas were discarded and all surfaces analysed were free from caries

Preliminary testing of background fluoride levels from similar fixed areas of enamel on different teeth, showed marked differences in background levels between teeth. Hence as all the teeth were from unknown origin and thus with unknown previous fluoride contact, it was considered necessary to test fluoride uptake from each topical fluoride agent on samples of enamel from the same tooth, thus permitting measurement of uptake against known background levels of fluoride ion. This measure was considered necessary to eliminate this source of error on a comparative basis between teeth. Thus, six enamel slabs were cut from the approximal surfaces** of each tooth by using a diamond saw. An effort was made to cut as perpendicular and parallel to the surface as possible. After cutting all slabs were sterilised in 1% Ethylene Oxide vapour to destroy any adsorbed viral or bacterial contaminants. The enamel sections from one tooth were stored together in plastic containers containing thymol DDW until use.

The surfaces were then painted with acid and water resistant varnish with an exception of a window measuring 2x4 mm ensuring equal surface area contact with topical fluoride through out the procedure. Accuracy of surface window area was ensured by

* Sections were checked under stereomicroscope.
** buccal and lingual surfaces.

measurement with a flexible ruler. The slabs were then mounted on wooden sticks with the help of white sticky wax. In each experiment two of these six slates were untreated and were kept as control samples to provide base line levels from which increase in fluoride levels after topical fluoride applications could be determined for each group. The remaining four slates were coated with topical fluoride agents in a manner closely approximating that used clinically, for the specified time, following which they were rinsed thoroughly with DDW, and then dried. The topical fluoride materials used, and their method of application will be described in future sections.

3.2.2 METHOD FOR INCREMENTAL BIOPSY OF ENAMEL.

In order to take samples of mineral at sequential depths, it was decided to test variations in exposure time to the 0.1M HCl biopsy solution, to determine the depth of mineral sampled.* The sequence of exposure times chosen were 1,2,2,5,5,5 minute exposure times, resulting in a 20 minute overall exposure time. The depths of sequential enamel loss were consistently found to be as in Table 2.

Table 2: Depth of enamel biopsy with accumulative minutes of acid exposure.

Minutes	Depth in um
1	0*
3	12
5	25
10	36.6
15	45.6
20	54

* surface etch only

* Separate vials.

Some dissolution of mineral occurred lateral to the varnish protection lines. As these were similar in all cases this was not considered to constitute a problem, except if precise volumes of enamel were being measured. A further test investigated whether prior treatment with concentrated fluorides, eg. APF 1.23% gel, reduced the depth of biopsy with time. It was found not to affect the rate or depth of biopsy. This would be because of the low pH (pH:1), which would rapidly dissolve any apatite, whether it had F stored as FA or CaF_2.

3.3.3 COMPARATIVE BACKGROUND FLUORIDE LEVELS ON SLATES OF ENAMEL FROM DIFFERING SURFACES OF THE SAME TOOTH.

Weatherell et al (1986) and others have demonstrated that differing regions of the mouth have varying concentrations of fluoride ion in contact with them. This would most likely result in differing surfaces of the same tooth having different concentrations of fluoride ion adsorbed to the surface. To test the level of such differences background fluoride concentrations at different biopsy depths from all six slates taken from the same tooth, were measured for 3 teeth. Table 3 shows the differing concentrations present. As these ranged from little difference to approximately 2.5 times difference between lowest and highest background level, in some teeth, a further test was carried out to determine whether there were consistent differences between known surfaces. While there were some differences in background levels from differing surfaces, there emerged no consistent pattern of elevated levels from specific surfaces. Again, the differences between different teeth were significant. These results confirmed the belief that it was necessary to test uptake for specific categories of products or application methods on enamel from the same teeth, and that despite the differences in background

concentrations between surfaces, if two slates were used for each test category, the means should provide data that were within the confines of biological variability.

TABLE 3: COMPARISON OF BACKGROUND FLUORIDE LEVELS IN 3 TEETH IN PPM AT DIFFERING DEPTHS OF BIOPSY (CUMULATIVE DATA, A-F= DIFFERENT SURFACES).

	A1	A3	A5	A10	A15	A20
A	0.027	0.051	0.07	0.103	0.132	0.153
B	0.021	0.042	0.061	0.096	0.122	0.141
C	0.035	0.087	0.119	0.157	0.181	0.199
D	0.032	0.064	0.087	0.127	0.157	0.178
E	0.025	0.048	0.067	0.104	0.134	0.155
F	0.023	0.049	0.07	0.112	0.144	0.17
	B1	B3	B5	B10	B15	B20
A	0.08	0.162	0.197	0.317	0.387	0.441
B	0.033	0.083	0.106	0.188	0.238	0.273
C	0.054	0.124	0.15	0.236	0.288	0.325
D	0.058	0.144	0.177	0.287	0.349	0.393
E	0.025	0.062	0.08	0.138	0.175	0.205
F	0.03	0.076	0.097	0.177	0.223	0.261
	C1	C3	C5	C10	C15	C20
A	0.015	0.038	0.057	0.09	0.116	0.139
B	0.026	0.07	0.102	0.164	0.206	0.231
C	0.03	0.072	0.105	0.163	0.203	0.228
D	0.056	0.128	0.182	0.264	0.322	0.354
E	0.03	0.082	0.119	0.183	0.235	0.264
F	0.027	0.069	0.102	0.158	0.202	0.228

CUMULATIVE TOTAL FOR WHOLE TOOTH (A, B, AND C) AT THE END OF 20 MINUTES.

A20	0.996
B20	1.898
C20	1.444

TABLE 4: COMPARISON OF FLUORIDE LEVELS IN 6 SURFACES- MESIAL (M), DISTAL (D), DISTOBUCCAL (DB), MESIOBUCCAL (MB), DISTOLINGUAL (DL), MESIOLINGUAL (ML) ON 4 TEETH IN PPM.

This is a 20 minute biopsy result and does not measure incremental depth.

	M	D	DB	MB	DL	ML
1	0.12	0.16	0.21	0.23	0.16	0.19
2	0.24	0.21	0.21	0.21	0.07	0.38
3	0.38	0.41	0.35	0.5	0.33	0.35
4	0.25	0.2	0.23	0.19	0.17	0.16

3.4 TOPICAL FLUORIDE PRODUCTS TESTED.

3.4.1 Representative selection of commercially available, commonly used topical fluoride products tested, including labelled concentrations.

1. Colgate *Thixofluor* gel, 4 minute APF gel, 1.23%, 12,300 ppm F. (Colgate Oral Care).

2. Oral B *Minute* gel, 1 minute APF gel, 1.23%, 12,300 ppm F. (OB Labs, Pty Ltd, Sydney, Australia).

3. Johnson and Johnson "Neupro Neutral", NaF gel 2%, 0.9% F ion, 9000 ppm F. (Dentsply International Inc, U.S.A).

4. Oral B *Fluorinse* mouthrinse, NaF, 0.2%, 1000 ppm F. (OB Labs, Pty Ltd, Sydney, Australia).

5. Colgate *Dentamint* mouthrinse, NaF, 0.02 %, 100 ppm F. (Colgate Oral Care).

6. *Colgate Total* toothpaste, NaF, 0.22%, 1000 ppm F. (Colgate Oral Care).

7. *Colgate Fluoriguard* toothpaste, MFP, 0.76%, 1000 ppm F. (Colgate Oral Care).

8. *Colgate Junior* toothpaste, NaMFP, 0.304%, 400 ppm F. (Colgate Oral Care).

9. *Colgate Sparkle* toothpaste, NaMFP, 0.76%, 1000 ppm F. (Colgate Oral Care).

10. *Pepsodent* toothpaste. (Hindustan Lever Dental Research, India).

11. *Duraphat* Varnish, 25,000 ppm F. (Rhone Poulenc Rorer, Germany).

12. Stannous Fluoride (SnF_2) solution, 48,500 ppm F. (Floran, Creighton Pharmaceuticals).

13. Silver Fluoride (AgF) solution, 60,000 ppm F. (Creighton Pharmaceuticals).

3.4.2 Special products tested, not available commercially.

(a) Titanium Tetrafluoride (TiF_4), a solution made to 30,400 ppm F. (Aldrich Chemical Company Inc. Milwaukee, Wis, U.S.A).

(b) Ammonium bifluoride (NH_4F_2), a solution made to 38,000 ppm F. (Aldrich Chemical Company Inc. Milwaukee, Wis, U.S.A).

(c) Tartaric acid gel (TAF), containing 11.3% Tartaric acid, F ion at 11,038 ppm, pH 3.5, was specially prepared by Colgate Oral Care Australia for experimental "in vitro" use only. It was intended for testing as an alternative to APF gels currently available commercially, which contain HF and H_3PO_4 in high enough concentrations to damage glass based restorative products. While this gel is currently being tested comprehensively for its effectiveness, and effect on enamel and restorative materials, the initial requirement was to determine whether fluoride ion uptake was as high as from the APF gels currently available.

3.4.3 METHOD OF USE OF EACH PRODUCT.

Fluoride gels (APF, NaF, TAF gel):

Of the acidulated phosphate containing gels, there are two available which recommend different periods of application, 4 minutes in one case and 1 minute in the other. However, whether there is any difference in the uptake over comparable times for these gels is not known. For this reason, it was decided to use these gels both as recommended by the manufacturers (ie. Thixofluor, 4 min: Minute gel, 1 min) and also at different times for specific investigations. Both NaF and TaF gels were applied for 4 minutes, with TAF being applied for 20 minutes in one test.

In the initial experiments using acidulated gels, the Thixofluor or Minute gels were applied in excess to an exposed window of dried enamel, and left for 4 minutes and 1 minute respectively. At the end of 4 minutes and 1 minute, the gels were washed away with a jet of DDW for 10 seconds and then dried with a jet of air. In a later investigation of the uptake rate following different lengths of exposure, the experiment was repeated with other samples at 1,2,3,4,5,10,20, and 40 minutes of contact of APF gel with enamel windows. Tooth samples were stored in a humid environment while waiting biopsy.

Toothpaste:

Tooth brushing times varies for different people, with an increasing tendency in Paedodontics for children to leave paste in contact with the tooth for as long as possible before washing it or in some cases without washing it at all. Also following active brushing, there is residual concentration of fluoride in saliva coating tooth surface. This remains for some time following use. For this reason it was decided to leave the tooth paste on in every case for 5 minutes to simulate total contact time. Tooth paste was applied in excess to an exposed window of dried enamel, and left for 5 minutes. At the end of 5 minutes, the paste was washed away with a jet of DDW for 10 seconds and then dried with a jet of air.

Mouth rinse:

Normally, patients are instructed to rinse with Mouth rinse for 1 minute followed by spitting out of bulk material and residual material until comfortable. Saliva would dilute the remaining material. For this reason a time span of 1 minute contact between the tooth and the mouth rinse was chosen to simulate clinical use. Slates were placed in a

* Customary in Paediatric dentistry in S.Australia.

mouthrinse solution for 1 minute. At the end of 1 minute slates were withdrawn and washed with a jet of DDW for 10 seconds and dried with a jet of air.

Varnish:

Varnish is specifically designed to set on the teeth, and provide contact with the tooth for up to 24 hrs. However, it is frequently removed soon after application in children by the tongue and lip movement. Hence in this project, varnish was applied in excess on to exposed windows of dried enamel for 4 minutes or 24 hrs. It was then gently removed by a cotton applicator, followed by washing with a jet of DDW, and then dried with a jet of air.

SnF_2 (20%), AgF_2 (40%):

Slates were placed in the concentrated form of these agents, as supplied commercially for 5 minutes, then washed with a jet of DDW for 10 seconds and dried with a jet of air.

Variations in method of use, time of application or products used:

It was attempted to use each product in a way simulating clinical use and contact periods as closely as possible. Variations on some of the methods and periods of contact with enamel were used to determine whether these altered levels of uptake of fluoride ion. The major variations in method of application were

(1) Increased length of exposure to gels etc.

ie. uptake was measured following application of two gels (Minute gel and Thixofluor gel) at 1, 2, 3, 4, 5, 10, 15, 20, and 40 minutes.

(2) Acid etch before local application.

Enamel windows were etched with 37% phosphoric acid for 30 seconds, rinsed with DDW and then dried with a jet of air prior to toothpaste application and results obtained were compared with those slates that were not etched prior to tooth paste application. Tooth paste was applied in excess to exposed window of dry enamel, and left for 5 minutes. At the end of 5 minutes, paste was washed away with a jet of DDW for 10 seconds and then dried with a jet of air.

(3) Non Commercially available materials.

TiF_4 (30,400 ppm) and NH_4F_2 (38,000 ppm).

Solutions of these materials were made up to the stated concentrations which were similar to those present in SnF_2 and AgF. Slates were placed in the concentrated solution as supplied for 5 minutes. At the end of 5 minutes slates were washed with a jet of DDW for 5 minutes and then dried with a jet of air. Experiment was also repeated using diluted TiF_4 (30 ppm) and Ammonium bifluoride (38 ppm).

3.4.4 CONFIRMATION OF CONCENTRATIONS OF FLUORIDE ION IN SOME `COMMERCIAL PRODUCTS TESTED.

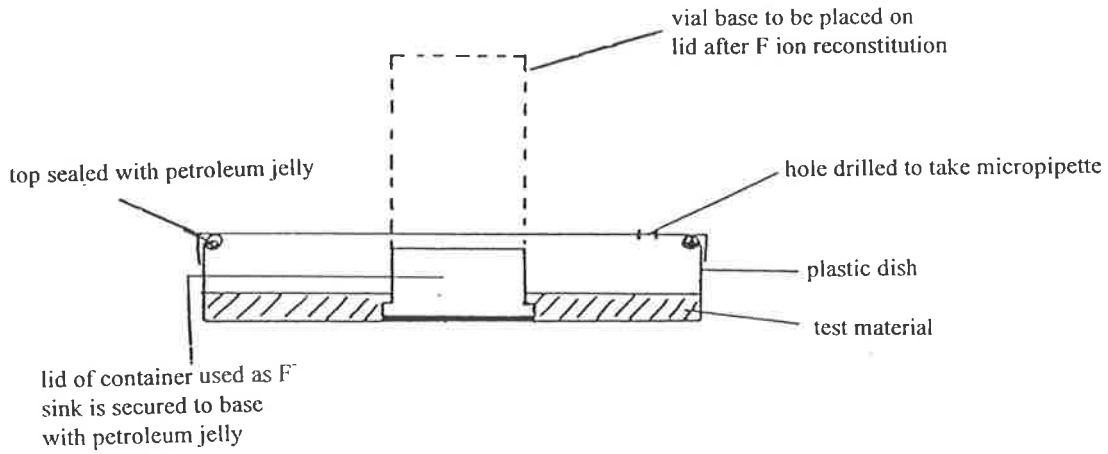
This was achieved using the TAVES (1968) method of fluoride ion separation described below, followed by reconstitution of separated fluoride ion and measurement with a Fluoride Selective Electrode (See table 5). The Taves method of separation of fluoride ion is achieved by the hydrolysis of the product with 6 M HCl, which has been saturated with Hexamethyldisiloxane (HMDS). The HF formed by acid hydrolysis then forms a

silane which is highly volatile. Within the closed system, this volatile Trimethylfluorosilane finds its way into a fluoride sink, containing NaOH. This neutralises the trimethylfluorosilane and retains the fluoride in the sink. Taves reported that 98% of fluoride ion is separated within the first hour after hydrolysis commences.

The Taves separation procedure is carried out as follows:

1. Assemble base plastic dish with fluoride sink as in Figure 5.

FIGURE 5



2. Place .5 ml of 1.65M NaOH into fluoride sink

3. Place material to be tested into outer are, if solid crush and weigh, if liquid measure 3 mls into outer area. If less than 3 mls add DDW to make 3 mls noting factor of dilution

4. Seal top to base. Mark each clearly as to test agent. Use standards: 1x100 ppm F, 1x10 ppm F, 1x1 ppm F, 2x.1 ppm F, 2x DDW

5. Syringe in through hole in lid 1 ml 6M HCl saturated in HMDS, seal hole in lid immediately with parafilm and petroleum jelly

6. Shake at least 8 hrs at minimum rate ensuring all containers are held firmly

7. Stop shaker, remove tops from bases individually and remove fluoride sink wiping jelly from base. Place onto marked trays (for identification). Place onto drying oven until all moisture is lost

8. Reconstitute fluoride with 1 ml 1.34 M Acetic Acid. Place bases on caps and shake on mixer for minimum of 8 hrs.*

9. Measure mv reading for each category and read concentrations from standard graph

The following results were obtained for five of the test products (Table 5). As will be seen, the results obtained showed accurate recordings for two of the products, the remainder being not too widely divergent. These small variations were not of great significance to the overall results.

Table 5: Confirmation of concentration of fluoride ion in commercial products tested by using the TAVES technique.

PRODUCT USED	RESULTS FROM TAVES IN PPM
TOTAL TP- 1000 PPM	1000
JUNIOR TP- 400 PPM	370
FLUORINSE- 1000 PPM	1000
THIXO GEL-12,300 PPM	10,300
DURAPHAT- 25,000 PPM	20,900

* As recommended by Taves to allow time for hydrolysis of proteins, etc, to reach F crystals.

* 3 samples.

3.5 THE EXPERIMENTAL "IN VITRO" UPTAKE SYSTEM.

3.5.1 General method used.

As previously stated, in general, two slates from a tooth were used for background levels of fluoride ions to be determined (often referred to as controls). Two of the remaining slates were used for each agent or method of application being tested. This permitted fluoride uptake levels to be estimated against known background levels of fluoride ion in the enamel, thus providing a reasonably accurate method of estimation. The six slates of enamel from each tooth were mounted on sticks, and these sticks were placed into Polystyrene blocks such that the tooth samples can be simultaneously lowered into biopsy vials described later. The experimental slates then were coated with topical fluoride agents such as described for prescribed periods, washed and dried. These were now ready for biopsy.

3.6 THE BIOPSY METHOD.

3.6.1 PREPARATIONS OF BIOPSY SOLUTIONS.

Several hundred vials, 2 cms in diameter (10 ml capacity), had 2 mls of 0.1M HCl placed in them and were then capped. These were numbered according to the test categories being analysed and placed into biopsy boxes as described below.

Boxes 12 cms wide and 12 cms long were prepared so as to take 6 vials length wise and breath wise. The six slates of enamel were lowered into these vials (see Figure 3), such that the exposed surface was beneath the surface of the biopsy acid. Checks were made to ensure each biopsy tube was identified correctly. Biopsies were made at room

temperature, for prescribed periods of time, as described previously- 1,2,2,5,5,5 minutes (a total of 20 minutes).

3.6.2 Regular sampling checks on pH of the etching solution were made following exposure to treated enamel. No significant variations to pH were observed following biopsy.

3.6.3 It was realised that, despite through rinsing prior to biopsy, some F ion may remain bound to the protective varnish. A brief test was made to determine whether this was so. To achieve this, two sample slates were totally painted with varnish, and a pencilled window of similar dimensions drawn on. These slates were coated over a similar area as test teeth with APF 1.23% gel, before biopsy. Two slates were used as controls, and two as test slates. Results (Table 6) showed that there was no incidence of residual fluoride on the varnish for these slates which were entirely covered with varnish.

Table 6: Test to confirm that insignificant amounts of fluoride ion were retained on slates totally covered in varnish (Individual and cumulative results). *

	1	2	3	5	5	5
Control	0.06	0.058	0.08	0.045	0.02	0.75
Covered	0.05	0.017	0.02	0.009	0.005	0.005
APF	0.5	0.34	0.34	0.48	0.18	0.13
	1	3	5	10	15	20
Control	0.06	0.118	0.198	0.243	0.263	1.013
Covered	0.05	0.067	0.087	0.096	0.101	0.106
APF	0.5	0.84	1.18	1.66	1.84	1.97

* Controls not varnished though from the same tooth.

3.7 ANALYSIS OF FLUORIDE LEVELS.

3.7.1 USE OF FLUORIDE SELECTIVE ELECTRODE AND STANDARD SOLUTIONS

As stated previously, fluoride concentrations in biopsy solutions were read directly using an Orion Combined Fluoride Specific Electrode (Model 94-09) with an Orion 109 pH metre. The pH metre was used manually, rather than automatically, because the fluoride levels were frequently low, and at this concentration the relationship of log concentration of fluoride to millivolt reading were not strictly linear.

Fluoride standards of 0.005, 0.01, 0.05, 0.1, 1, 10, 100 and at pH 1.0 (equal to 0.1 M HCl) were used to prepare standard graphs. Unknown sample concentrations were estimated from these graphs.

3.7.2 ESTIMATION OF UPTAKE

As stated previously, two slates of each six were used to determine background levels of fluoride ion at sequential depths, and two each were used for a particular product, to determine the uptake of fluoride ion into the sound enamel from that product. To achieve this, the mean background levels in ppm (BG.) were subtracted from the mean uptake and background levels in ppm from products A and B (Total.Up A, Total. Up B). This permited the mean uptake above background levels to be determined as follows:

Mean uptake (ppm) = Total. Up A - BG. (ppm)

or Total. Up B - BG. (ppm).

This is purely a comparative study and the intent is not to measure absolute amounts of fluoride ions. Window size was the same in every case which means that the relationship between results in ppm would be exactly the same as expressed in microgram F/ volume of enamel. These can be easily transposed into nanogram quantities as follows:

1 ppm = 1 μg/ gm of enamel

or 1 nanogram/ mg of enamel

X ppm = X ng/ mg of enamel

As all biopsy values were the same, a ready comparison between uptake in each case can be made by comparing the ppm values directly.

Tables of incremental and cumulative concentrations of fluoride ion released were prepared, and further tables of comparative uptake levels between products, with differing methods of application tested.

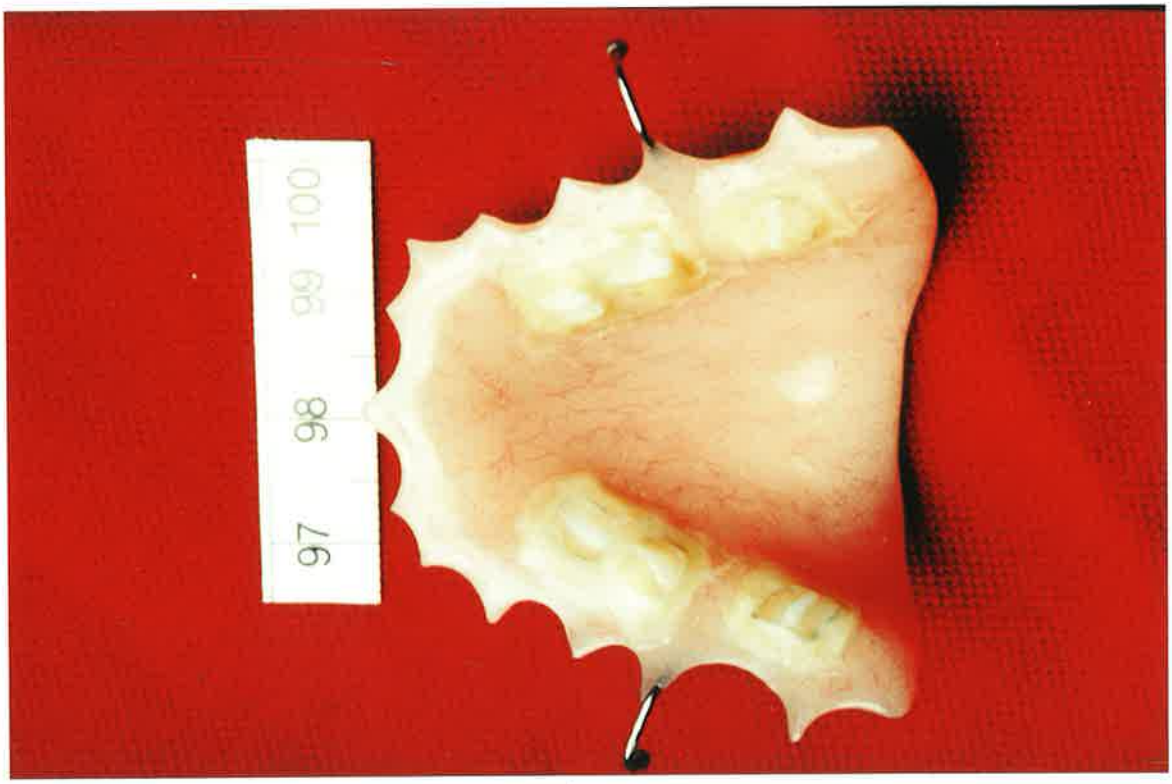
3.7.3 STATISTICAL ANALYSIS

It was originally intended to look for statistical levels of significance between product fluoride uptake values, using simple "t" test methods. However, as will be seen, the results did not lend themselves to useful statistical analysis at this stage. This will be discussed further in the discussion section.

3.8. FLUORIDE UPTAKE USING AN "IN VITRO/ INVIVO" TEST SYSTEM.

While the objective initially has been to measure intrinsic potential for fluoride ion uptake into enamel "in vitro", it is realised that intraorally, there are other factors which might significantly affect uptake levels. These include primary pellicle, and to a lesser extent plaque. Hence it was considered essential to measure uptake under intra oral conditions using an "in vitro-in vivo" test system. This involved use of an oral appliance onto which test slates of sterilised enamel for the one tooth could be placed. Human teeth of unknown origin were sectioned and sterilised as described above, and painted with varnish to leave windows exposed. Six slabs were mounted in specially prepared grooves of individually designed oral appliance by means of inlay dental wax. This wax is rigid at room temperature, and significantly adhesive at mouth temperature to retain the slates in place. Particular care was taken to leave the enamel windows uncovered with wax and to place the slab surface flush with, or slightly below the oral surface of the appliance. The last two slabs placed on either side was used as controls (for background levels to be recorded). See Fig 6.

FIG 6: APPLIANCE WITH 6 SLATES OF ENAMEL MOUNTED.



The appliance was worn by a volunteer. The participant had 28 teeth, was in good general health, had no active caries or periodontal disease, lived in fluoridated water area, had a salivary flow rate within the normal range, and was not on antibiotics or medications with hyposalivatory side-effects. The participant used the appliance for 3 nights consequently, for adequate plaque and pellicle build up prior to testing a particular product. During this period the appliance was cleaned with a tooth brush under tap water with no tooth paste, however, the enamel slabs were not brushed . The appliance was placed in DDW so as to ensure 100% humidity when not in the mouth. A non fluoridated tooth paste was used and the participant refrained from all kinds of

additional fluoride supply during the experimental period. The local drinking water contained 0.7 ppm F.

Products were tested for F uptake into enamel using the method described below:
Each product was tested with two enamel slates and the last two slates in the appliance were used as controls. Products were used to simulate clinical application for the specified time. For gels, this was generally 4 minutes, since the product would be placed in contact with the tooth surface for this time (eg. gel on trays, etc). Following this time of contact outside the mouth, two control slate and two test slate was removed, washed and dried thoroughly. The plate was then placed for 60 minutes in the mouth (where gel would have been cleared naturally), following which the remaining slates were washed thoroughly with DDW to ensure that no fluoride remnants were on the surfaces .They were then air dried and removed from the appliance. All slates from the plate were then mounted on wooden sticks for chemical biopsy. Ethical approval for this "in vitro-in vivo" method of testing had been previously obtained from the committee for the Ethics of Human Experimentation, The University of Adelaide.

CHAPTER 4

4.0 RESULTS.

This chapter provides the results for the following investigations. The results are presented in bulk initially in tabular form with raw data, and secondly in histogram form, for each experiment.

4.1 Uptake of fluoride ion into enamel from 10 differing commonly used topical F agents, indicating uptake following set cumulative times of biopsy.

Table and Fig 4.1.1 Cumulative uptake of fluoride from Thixofluor APF gel (1.23%) following 4 minute application

Table and Fig 4.1.2 Cumulative uptake of fluoride from NaF gel (2%) following 4 minute application

Table and Fig 4.1.3 Cumulative uptake of fluoride from *Minute* APF 1.23% gel following 1 minute application

Table and Fig 4.1.4 Cumulative uptake of fluoride from *Dentamint* mouthrinse following 4 minute application

Table and Fig 4.1.5 Cumulative uptake of fluoride from *Fluorinse* mouthrinse following 2 minute application

Table and Fig 4.1.6 Cumulative uptake of fluoride from *Colgate Total* (NaF 0.22%) toothpaste following 5 minute application

Table and Fig 4.1.7 Cumulative uptake of fluoride from *Colgate Fluoriguard* (MFP 0.76%) toothpaste following 5 minute application

4.2 Uptake into enamel of Fluoride ion from 2 different APF 1.23% gels at 1, 2, 3, 4, 5, 10, 20 and 40 minutes.

4.3 Variation in method of application and use of products not commercially available.

4.4 "In vivo" test indicating uptake of fluoride ion into enamel from commonly used topical F agents, following set cumulative times of biopsy.

TABULAR FORM OF RAW DATA

4.1.1 Individual uptake of fluoride in ppm from APF gel (1.23%) following 4 min application

	1 MIN	2 MIN	2 MIN	5 MIN	5 MIN	5 MIN
APF GEL	1.4	0.58	0.4	0.68	0.48	0.37
Control	0.14	0.067	0.05	0.076	0.055	0.048

Cumulative uptake of fluoride in ppm from APF gel (1.23%) following 4 min application

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
APF GEL	1.4	1.98	2.38	3.06	3.54	3.91
Control	0.14	0.207	0.257	0.333	0.388	0.436

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
APF-C	1.26	1.773	2.123	2.727	3.152	3.474

4.1.2 Individual uptake of fluoride in ppm from NaF gel (2%) following 4 min application

	1 MIN	2 MIN	2 MIN	5 MIN	5 MIN	5 MIN
NaF GEL	0.33	0.12	0.072	0.13	0.082	0.062
NaF GEL	0.8	1.7	0.33	0.25	0.12	0.082
C	0.066	0.078	0.082	0.15	0.1	0.176
C	0.066	0.074	0.07	0.13	0.084	0.06

Cumulative uptake of fluoride in ppm from NaF gel (2%) following 4 min application

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
NaF GEL	0.33	0.45	0.522	0.652	0.734	0.796
NaF GEL	0.8	2.5	2.83	3.08	3.2	3.282
C	0.066	0.144	0.226	0.376	0.476	0.552
C	0.066	0.14	0.21	0.34	0.424	0.484

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
NaF GEL	0.565	1.475	1.676	1.866	1.967	2.039
C	0.066	0.142	0.218	0.358	0.45	0.568

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
NaF-C	0.499	1.33	1.458	1.508	1.517	1.521

4.1.3 Individual uptake of fluoride in ppm from Minute gel following 1 min application

	1 MIN	2 MIN	2 MIN	5 MIN	5 MIN	5 MIN
MIN GEL	0.23	0.14	0.082	0.16	0.045	0.037
MIN GEL	0.32	0.2	0.11	0.16	0.045	0.037
C	0.042	0.058	0.042	0.074	0.021	0.022
C	0.064	0.094	0.054	0.12	0.046	0.029

Cumulative uptake of fluoride in ppm from Minute gel following 1 min application

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
MIN GEL	0.23	0.37	0.452	0.612	0.657	0.694
MIN GEL	0.32	0.52	0.63	0.79	0.835	0.872
C	0.042	0.1	0.142	0.216	0.237	0.259
C	0.064	0.158	0.212	0.332	0.378	0.407

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
MIN GEL	0.275	0.445	0.541	0.701	0.746	0.783
C	0.053	0.129	0.177	0.274	0.3075	0.333

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
M-C	0.222	0.316	0.364	0.427	0.4385	0.45

4.1.4 Individual uptake of fluoride in ppm from Dentamint following 4 min application

	1 MIN	2 MIN	2 MIN	5 MIN	5 MIN	5 MIN
D	0.072	0.025	0.04	0.062	0.05	0.042
D	0.072	0.03	0.042	0.072	0.06	0.044
C	0.042	0.019	0.028	0.048	0.038	0.03
C	0.062	0.024	0.034	0.054	0.04	0.025

Cumulative uptake of fluoride in ppm from Dentamint following 4 min application

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
D	0.072	0.097	0.137	0.199	0.249	0.291
D	0.072	0.102	0.144	0.216	0.276	0.32
C	0.042	0.061	0.089	0.137	0.175	0.205
C	0.062	0.086	0.12	0.174	0.214	0.239

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
D	0.072	0.0995	0.1405	0.2075	0.2625	0.3055
C	0.052	0.0735	0.1045	0.1555	0.1945	0.222

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
D-C	0.02	0.026	0.036	0.052	0.068	0.0835

4.1.5 Individual uptake of fluoride in ppm from Fluorinse mouthrinse following 2 min application

	1 MIN	2 MIN	2 MIN	5 MIN	5 MIN	5 MIN
FR	0.13	0.047	0.062	0.094	0.074	0.056
FR	0.13	0.04	0.05	0.08	0.062	0.048
C	0.042	0.019	0.028	0.048	0.038	0.03
C	0.062	0.024	0.034	0.054	0.04	0.025

Cumulative uptake of fluoride in ppm from Fluorinse mouthrinse following 2 min application

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
FR	0.13	0.177	0.239	0.333	0.407	0.463
FR	0.13	0.17	0.22	0.3	0.362	0.41
C	0.042	0.061	0.089	0.137	0.175	0.205
C	0.062	0.086	0.12	0.174	0.214	0.239

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
FR	0.13	0.1735	0.2295	0.3165	0.3845	0.4365
C	0.052	0.0735	0.1045	0.1555	0.1945	0.222

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
FR-C	0.078	0.1	0.125	0.161	0.19	0.2145

4.1.6 Individual uptake of fluoride in ppm from Colgate Total toothpaste following 5 min application

	1 MIN	2 MIN	2 MIN	5 MIN	5 MIN	5 MIN
NaF	0.026	0.082	0.036	0.086	0.062	0.054
NaF	0.062	0.078	0.056	0.094	0.07	0.078
C	0.018	0.045	0.03	0.074	0.056	0.045
C	0.054	0.09	0.06	0.1	0.068	0.05

Cumulative uptake of fluoride in ppm from Colgate Total toothpaste following 5 min application

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
NaF	0.026	0.108	0.144	0.23	0.292	0.346
NaF	0.062	0.14	0.196	0.29	0.36	0.438
C	0.018	0.063	0.093	0.167	0.223	0.268
C	0.054	0.144	0.204	0.304	0.372	0.422

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
NaF	0.044	0.124	0.17	0.26	0.326	0.392
C	0.036	0.1035	0.1485	0.2355	0.2975	0.345

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
NaF-C	0.008	0.0205	0.0215	0.0245	0.0285	0.047

4.1.7 Individual uptake of fluoride in ppm from Colgate Fluoriguard toothpaste following 5 min application

	1 MIN	2 MIN	2 MIN	5 MIN	5 MIN	5 MIN
MFP	0.026	0.035	0.012	0.1	0.018	0.016
MFP	0.043	0.045	0.045	0.052	0.05	0.03
C	0.018	0.045	0.03	0.074	0.056	0.045
C	0.054	0.09	0.06	0.1	0.068	0.05

Cumulative uptake of fluoride in ppm from Colgate Fluoriguard toothpaste following 5 min application

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
MFP	0.026	0.061	0.073	0.173	0.191	0.207
MFP	0.043	0.088	0.133	0.185	0.235	0.265
C	0.018	0.063	0.093	0.167	0.223	0.268
C	0.054	0.144	0.204	0.304	0.372	0.422

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
MFP	0.0345	0.0745	0.02155	0.179	0.213	0.236
C	0.036	0.1035	0.1485	0.2355	0.2975	0.345

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
MFP-C	-0.0015	-0.029	-0.0455	-0.0565	-0.0845	-0.109

4.1.8 Individual uptake of fluoride in ppm from Duraphat following 24 hrs and 4 min application

	1 MIN	2 MIN	2 MIN	5 MIN	5 MIN	5 MIN
D24	0.052	0.037	0.024	0.041	0.037	0.017
D24	0.052	0.037	0.02	0.04	0.037	0.017
D4	0.037	0.041	0.027	0.046	0.037	0.017
D4	0.052	0.043	0.03	0.047	0.04	0.023
C	0.052	0.052	0.041	0.056	0.049	0.025
C	0.035	0.041	0.029	0.046	0.046	0.023

Cumulative uptake of fluoride in ppm from Duraphat following 24 hrs and 4 min application

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
D24	0.052	0.089	0.113	0.154	0.191	0.208
D24	0.052	0.089	0.109	0.149	0.186	0.203
D4	0.037	0.078	0.105	0.151	0.188	0.205
D4	0.052	0.095	0.125	0.172	0.212	0.235
C	0.0252	0.104	0.145	0.201	0.25	0.275
C	0.035	0.076	0.105	0.151	0.197	0.22

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
D 24	0.052	0.089	0.111	0.1515	0.1885	0.2055
D4	0.0445	0.0865	0.115	0.1615	0.2	0.22
C	0.0301	0.09	0.125	0.176	0.2235	0.2475

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
D24-C	0.0085	-0.001	-0.014	-0.0245	-0.035	-0.042
D4-C	0.001	-0.0035	-0.01	-0.0145	-0.0235	-0.0275

4.1.9 Individual uptake of fluoride in ppm from SnF (20%) following 5 min application

	1 MIN	2 MIN	2 MIN	5 MIN	5 MIN	5 MIN
SnF	0.076	0.052	0.05	0.096	0.07	0.056
SnF	0.094	0.05	0.064	0.11	0.086	0.062
C	0.04	0.036	0.036	0.066	0.048	0.04
C	0.075	0.044	0.036	0.064	0.05	0.027

Cumulative uptake of fluoride in ppm from SnF (20%) following 5 min application

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
SnF	0.076	0.128	0.178	0.274	0.344	0.4
SnF	0.094	0.144	0.208	0.318	0.404	0.466
C	0.04	0.076	0.112	0.178	0.226	0.266
C	0.075	0.119	0.155	0.219	0.269	0.296

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
SnF	0.085	0.136	0.193	0.296	0.374	0.433
C	0.0575	0.0975	0.1335	0.1985	0.2475	0.281

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
SnF-C	0.0275	0.0385	0.0595	0.0975	0.1265	0.152

4.1.10 Individual uptake of fluoride in ppm from AgF (40%) following 5 min application

	1 MIN	2 MIN	2 MIN	5 MIN	5 MIN	5 MIN
AgF	0.25	0.074	0.082	0.09	0.15	0.096
AgF	0.095	0.054	0.052	0.024	0.09	0.064
C	0.04	0.036	0.036	0.066	0.048	0.04
C	0.075	0.044	0.036	0.064	0.05	0.027

Cumulative uptake of fluoride in ppm from AgF (40%) following 5 min application

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
AgF	0.25	0.324	0.406	0.496	0.646	0.742
AgF	0.095	0.149	0.201	0.225	0.315	0.379
C	0.04	0.076	0.112	0.178	0.226	0.266
C	0.075	0.119	0.155	0.219	0.269	0.296

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
AgF	0.1725	0.2365	0.3155	0.3605	0.4805	0.5605
C	0.0575	0.0975	0.1135	0.1985	0.2475	0.281

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
AgF-C	0.115	0.139	0.17	0.198	0.233	0.2795

4.2.1.1 Individual uptake of fluoride in ppm from Minute gel and Thixofluor following application at 1 and 2 mins

	1 MIN	2 MIN	2 MIN	5 MIN	5 MIN	5 MIN
Min Gel-1	0.24	0.25	0.14	0.21	0.12	0.12
T Gel-1	0.3	0.3	0.2	0.35	0.26	0.17
Min G-2	0.42	0.43	0.3	0.52	0.38	0.27
T Gel-2	0.42	0.43	0.28	0.45	0.29	0.24
Control	0.078	0.12	0.09	0.13	0.078	0.056
Control	0.074	0.12	0.074	0.09	0.062	0.098

Cumulative uptake of fluoride in ppm from Minute gel and Thixoflour following application at 1 and 2mins

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
MG1	0.24	0.49	0.63	0.84	0.96	1.08
TG1	0.3	0.6	0.8	1.15	1.41	1.58
MG2	0.42	0.85	1.15	1.67	2.05	2.32
TG2	0.42	0.85	1.13	1.58	1.87	2.11
C	0.078	0.198	0.288	0.418	0.496	0.552
C	0.074	0.194	0.268	0.358	0.42	0.518

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
MG1	0.24	0.49	0.63	0.84	0.96	1.08
TG1	0.3	0.6	0.8	1.15	1.41	1.58
MG2	0.42	0.85	1.15	1.67	2.05	2.32
TG2	0.42	0.85	1.13	1.58	1.87	2.11
C	0.076	0.196	0.278	0.388	0.458	0.535

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
MG1-C	0.164	0.294	0.352	0.452	0.502	0.545
TG1-C	0.224	0.404	0.522	0.762	0.952	1.045
MG2-C	0.344	0.654	0.872	1.282	1.592	1.785
TG2-C	0.344	0.654	0.852	1.192	1.412	1.575

4.2.1.2 Individual uptake of fluoride in ppm from Minute gel and Thixofluor following application at 3 and 4 mins

	1 MIN	2 MIN	2 MIN	5 MIN	5 MIN	5 MIN
Min Gel-3	0.44	0.18	0.11	0.18	0.12	0.094
T Gel-3	0.96	0.37	0.27	0.44	0.3	0.22
Min G-4	0.54	0.18	0.12	0.19	0.14	0.11
T Gel-4	1.4	0.58	0.4	0.68	0.48	0.37
Control	0.13	0.064	0.05	0.076	0.054	0.05
Control	0.15	0.07	0.05	0.076	0.056	0.046

Cumulative uptake of fluoride in ppm from Minute gel and Thixoflour following application at 3 and 4 mins

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
Min Gel-3	0.44	0.62	0.73	0.91	1.03	1.124
T Gel-3	0.96	1.33	1.6	2.04	2.34	2.56
Min G-4	0.54	0.72	0.84	1.03	1.17	1.28
T Gel-4	1.4	1.98	2.38	3.06	3.54	3.91
Control	0.13	0.194	0.244	0.32	0.374	0.424
Control	0.15	0.22	0.27	0.346	0.402	0.448

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
Min Gel-3	0.44	0.62	0.73	0.91	1.03	1.124
T Gel-3	0.96	1.33	1.6	2.04	2.34	2.56
Min G-4	0.54	0.72	0.84	1.03	1.17	1.28
T Gel-4	1.4	1.98	2.38	3.06	3.54	3.91
C	0.14	0.207	0.257	0.333	0.388	0.436

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
MG3-C	0.3	0.413	0.473	0.577	0.642	0.688
TG3-C	0.82	1.123	1.343	1.707	1.952	2.124
MG4-C	0.4	0.513	0.583	0.697	0.782	0.844
TG4-C	1.26	1.773	2.123	2.727	3.152	3.474

4.2.1.3 Individual uptake of fluoride in ppm from Minute gel and Thixofluor following application at 5 and 10 mins

	1 MIN	2 MIN	2 MIN	5 MIN	5 MIN	5 MIN
Min Gel-5	0.53	0.34	0.13	0.18	0.12	0.12
T Gel-5	0.54	0.3	0.17	0.22	0.14	0.11
Min G-10	0.3	0.23	0.14	0.2	0.13	0.11
T Gel-10	0.4	0.33	0.19	0.28	0.18	0.15
Control	0.09	0.08	0.066	0.11	0.09	0.084
Control	0.07	0.058	0.046	0.08	0.07	0.062

Cumulative uptake of fluoride in ppm from Minute gel and Thixoflour following application at 5 and 10 mins

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
MG5	0.53	0.87	1	1.18	1.3	1.42
TG5	0.54	0.84	1.01	1.23	1.37	1.48
MG10	0.3	0.53	0.67	0.87	1	1.11
TG10	0.4	0.73	0.92	1.2	1.38	1.53
C	0.09	0.17	0.236	0.346	0.436	0.52
C	0.07	0.128	0.174	0.254	0.324	0.386

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
MG5	0.53	0.87	1	1.18	1.3	1.42
TG5	0.54	0.84	1.01	1.23	1.37	1.48
MG10	0.3	0.53	0.67	0.87	1	1.11
TG10	0.4	0.73	0.92	1.2	1.38	1.53
C	0.08	0.149	0.205	0.3	0.38	0.453

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
MG1-C	0.45	0.721	0.795	0.88	0.92	0.067
TG1-C	0.46	0.691	0.805	0.93	0.99	1.027
MG2-C	0.22	0.381	0.465	0.57	0.62	0.657
TG2-C	0.32	0.581	0.751	0.9	1	1.077

4.2.1.4 Individual uptake of fluoride in ppm from Minute gel and Thixofluor following application at 20 and 40 mins

	1 MIN	2 MIN	2 MIN	5 MIN	5 MIN	5 MIN
Min G-20	0.15	0.14	0.08	0.1	0.076	0.09
T Gel-20	0.22	0.26	0.21	0.56	0.38	0.25
Min G-40	0.74	0.44	0.25	0.28	0.18	0.12
T Gel-40	0.48	0.54	0.46	0.96	0.58	0.33
Control	0.098	0.078	0.064	0.08	0.072	0.054
Control	0.058	0.034	0.023	0.032	0.03	0.024

Cumulative uptake of fluoride from Minute gel and Thixoflour following application at 20 and 40mins

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
M G 20	0.15	0.29	0.37	0.47	0.546	0.636
T G 20	0.22	0.48	0.69	1.25	1.63	1.88
M G 40	0.74	1.18	1.43	1.71	1.89	2.01
T G 40	0.48	1.02	1.48	2.44	3.02	3.35
C	0.098	0.176	0.24	0.32	0.392	0.446
C	0.058	0.092	0.115	0.147	0.177	0.201

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
M G 20	0.15	0.29	0.37	0.47	0.546	0.636
T G 20	0.22	0.48	0.69	1.25	1.63	1.88
M G 40	0.74	1.18	1.43	1.71	1.89	2.01
T G 40	0.48	1.02	1.48	2.44	3.02	3.35
C	0.078	0.134	0.1775	0.2335	0.2845	0.3235

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
M G 20-C	0.072	0.156	0.1925	0.2365	0.2615	0.3125
T G 20-C	0.142	0.346	0.5125	1.0165	1.3455	1.5565
M G 40-C	0.662	1.046	1.2525	1.4765	1.6055	1.6865
T G 40-C	0.402	0.886	1.3025	2.2065	2.7355	3.0265

4.3.1 Individual results in ppm comparing difference in fluoride uptake from tooth paste (Tartar control) with and without prior etch

	20 MIN
ETCH	0.44
ETCH	0.6
NO ETCH	0.42
NO ETCH	0.46
C	0.33
C	0.4

Results in ppm comparing difference in fluoride uptake from tooth paste (Tartar control) with and without prior etch

	20 MIN
ETCH	0.52
NO ETCH	0.44
C	0.365

	20 MIN
ETCH-C	0.155
N E-C	0.075

4.3.2 Individual uptake of fluoride in ppm from Perioclean, Colgate sparkle, Pepsodent, tooth pastes following 5 min application after prior etch with 37% phosphoric acid for 30 secs

	1 MIN	2 MIN	2 MIN	5 MIN	5 MIN	5 MIN
P C	0.045	0.043	0.043	0.051	0.038	0.045
C S	0.036	0.056	0.044	0.051	0.045	0.061
P D	0.016	0.029	0.027	0.061	0.04	0.035
C	0.018	0.027	0.027	0.041	0.035	0.035
C	0.017	0.027	0.027	0.044	0.038	0.033

Cumulative uptake of fluoride in ppm from Tartar control, Perioclean, Colgate sparkle, Pepsodent, tooth pastes following 5 min application after prior etch with 37% phosphoric acid for 30 secs

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
T C	0.024	0.059	0.086	0.13	0.175	0.216
P C	0.045	0.088	0.131	0.182	0.22	0.265
C S	0.036	0.092	0.136	0.187	0.232	0.293
P D	0.016	0.045	0.072	0.133	0.173	0.208
C	0.018	0.045	0.072	0.113	0.148	0.183
C	0.017	0.044	0.071	0.115	0.153	0.186

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
T C	0.024	0.059	0.086	0.13	0.175	0.216
P C	0.045	0.088	0.131	0.182	0.22	0.265
C S	0.036	0.092	0.136	0.187	0.232	0.293
P D	0.016	0.045	0.072	0.133	0.173	0.208
C	0.0175	0.0445	0.0715	0.114	0.1505	0.1845

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
TC-C	0.0065	0.0145	0.0145	0.016	0.0245	0.0315
PC-C	0.0275	0.0435	0.0595	0.068	0.0695	0.0805
CS-C	0.0185	0.0475	0.0645	0.073	0.0815	0.1085
PD-C	-0.0015	0.0005	0.0005	0.019	0.0225	0.0235

4.3.3 Individual results in ppm of the comparative uptake of fluoride from TiF4 using concentrated (30,400 ppm) and diluted (30 ppm) solutions.

	1 MIN	2 MIN	2 MIN	5 MIN	5 MIN	5 MIN
TiF4 Con	2.4	1.1	1	1.5	0.88	0.78
TiF4 Con	2.3	1.3	1.9	1.1	0.64	0.68
TiF4 Dil	1.2	0.62	0.6	0.7	0.68	0.62
TiF4 Dil	1.1	0.48	0.7	0.64	0.74	0.62
C	1.2	0.9	0.62	0.62	0.74	0.6
C	1.1	0.78	0.92	0.76	0.74	0.6

Cumulative results in ppm of the comparative uptake of fluoride from TiF4 using concentrated (30,400 ppm) and diluted (30 ppm) solutions.

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
TiF4 Con	2.4	3.5	4.5	6	6.88	7.66
TiF4 Con	2.3	3.6	5.5	6.6	7.24	7.92
TiF4 Dil	1.2	1.82	2.42	3.12	3.8	4.42
TiF4 Dil	1.1	1.58	2.28	2.92	3.66	4.28
C	1.2	2.1	2.72	3.34	4.08	4.68
C	1.1	1.88	2.8	3.56	4.3	4.9

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
TiF4 Con	2.35	3.5	5	6.3	7.06	7.79
TiF4 Dil	1.15	1.7	2.35	3.02	3.73	4.35
C	1.15	1.99	2.76	3.45	4.19	4.79

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
TiF4 C-C	1.2	1.51	2.24	2.85	2.87	3
TiF4 D-C	0	-0.29	-0.41	-0.43	-0.46	-0.44

4.3.4 Individual results in ppm of the comparative uptake of fluoride from Ammonium Bifluoride using concentrated (38,000 ppm) and diluted (38 ppm) solutions

	1 MIN	2 MIN	2 MIN	5 MIN	5 MIN	5 MIN
NH4F2 C	1	1.9	0.74	0.82	0.66	0.8
NH4F2 C	1	0.78	0.7	0.66	0.66	0.66
NH4F2 D	0.8	0.7	0.64	0.64	0.62	0.66
NH4F2 D	0.82	0.86	0.74	0.82	0.78	0.68
C	0.64	0.62	0.6	0.56	0.58	0.58
C	0.58	0.6	0.56	0.62	0.6	0.58

Cumulative results in ppm of the comparative uptake of fluoride from Ammonium Bifluoride using concentrated (38,000 ppm) and diluted (38 ppm) solutions

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
NH4F2 C	1	2.9	3.64	4.46	5.12	5.92
NH4F2 C	1	1.78	2.48	3.14	3.8	4.46
NH4F2 D	0.8	1.5	2.14	2.78	3.4	4.06
NH4F2 D	0.82	1.68	2.42	3.24	4.02	4.7
C	0.64	1.26	1.86	2.42	3	3.58
C	0.58	1.18	1.74	2.36	2.96	3.54

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
NH4F2 C	1	2.34	3.06	3.8	4.46	5.19
NH4F2 D	0.81	1.59	2.28	3.01	3.71	4.38
C	0.61	1.22	1.8	2.39	2.98	3.56

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
NC-C	0.39	1.12	1.26	1.41	1.48	1.63
ND-C	0.2	0.37	0.48	0.62	0.73	0.82

4.3.5 Individual results in ppm comparing uptake of fluoride from TaF gel following 4 minute application

	1 MIN	2 MIN	2 MIN	5 MIN	5 MIN	5 MIN
C	0.11	0.12	0.1	0.15	0.12	0.074
C	0.1	0.1	0.094	0.15	0.1	0.074
C	0.052	0.04	0.041	0.064	0.054	0.058
TaF	0.23	0.18	0.15	0.25	0.17	0.12
TaF	0.34	0.21	0.19	0.27	0.2	0.11
TaF	0.23	0.18	0.13	0.22	0.14	0.096

Cumulative results in ppm comparing uptake of fluoride from TaF gel following 4 minute application

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
C	0.11	0.23	0.33	0.48	0.6	0.674
C	0.1	0.2	0.294	0.444	0.544	0.618
C	0.052	0.092	0.133	0.197	0.251	0.309
TaF	0.23	0.41	0.56	0.81	0.98	1.1
TaF	0.34	0.55	0.74	1.01	1.21	1.32
TaF	0.23	0.41	0.54	0.76	0.9	0.996

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
C	0.087333	0.174	0.252333	0.373667	0.465	0.533667
TaF	0.266667	0.456667	0.613333	0.86	1.03	1.138667

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
TaF-C	0.179333	0.282667	0.361	0.486333	0.565	0.605

4.3.6 Individual results in ppm comparing uptake of fluoride from TaF gel following 20 minute application

	1 MIN	2 MIN	2 MIN	5 MIN	5 MIN	5 MIN
C	0.047	0.056	0.052	0.06	0.054	0.045
C	0.086	0.12	0.11	0.17	0.086	0.058
C	0.088	0.12	0.088	0.13	0.088	0.074
TaF	0.68	0.58	0.47	0.8	0.66	0.6
TaF	0.45	0.29	0.21	0.34	0.25	0.24
TaF	0.64	0.56	0.44	0.74	0.5	0.47

Cumulative results in ppm comparing uptake of fluoride from TaF gel following 20 minute application

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
C	0.047	0.103	0.155	0.215	0.269	0.314
C	0.086	0.206	0.316	0.486	0.572	0.63
C	0.088	0.208	0.296	0.426	0.514	0.588
TaF	0.68	1.26	1.73	2.53	3.19	3.79
TaF	0.45	0.74	0.95	1.29	1.54	1.78
TaF	0.64	1.2	1.64	2.38	2.88	3.35

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
C	0.073667	0.172333	0.255667	0.375667	0.451667	0.451667
TaF	0.59	1.066667	1.44	2.066667	2.536667	2.973333

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
TaF-C	0.516333	0.894333	1.184333	1.691	2.085	2.521667

4.4.1 Individual results in ppm comparing uptake of fluoride from APF (1.23%) gel following 4 minute application outside the mouth and 60 minutes in the mouth

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
APF 4	0.38	0.35	0.31	0.82	0.62	0.62
APF 4	0.52	0.42	0.36	0.96	0.76	0.68
APF 60	0.46	0.42	0.38	1	0.82	0.52
APF 60	0.29	0.32	0.25	0.62	0.5	0.38
C	0.09	0.058	0.07	0.092	0.05	0.025
C	0.03	0.079	0.06	0.084	0.054	0.046

Cumulative results in ppm comparing uptake of fluoride from APF (1.23%) gel following 4 minute application outside the mouth and 60 minutes in the mouth

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
APF 4	0.38	0.73	1.04	1.86	2.48	3.1
APF 4	0.52	0.94	1.3	2.26	3.02	3.7
APF 60	0.46	0.88	1.26	2.26	3.08	3.6
APF 60	0.29	0.61	0.86	1.48	1.98	2.36
C	0.09	0.148	0.218	0.31	0.36	0.385
C	0.03	0.109	0.169	0.253	0.307	0.353

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
APF 4	0.45	0.835	1.17	2.06	2.75	3.4
APF 60	0.375	0.745	1.06	1.87	2.53	2.98
C	0.06	0.1285	0.1935	0.2815	0.3335	0.369

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
APF4-C	0.39	0.7065	0.9765	1.7785	2.4165	3.031
APF60-C	0.315	0.6165	0.8665	1.5885	2.1965	2.611

4.4.2 Individual results in ppm comparing uptake of fluoride from NaF (2%) gel following 4 minute application outside the mouth and 60 minutes in the mouth

	1 MIN	2 MIN	2 MIN	5 MIN	5 MIN	5 MIN
NaF 4	0.039	0.13	0.088	0.16	0.064	0.062
NaF 4	0.22	0.13	0.08	0.18	0.062	0.044
NaF 60	0.076	0.07	0.054	0.14	0.06	0.036
NaF 60	0.1	0.084	0.066	0.17	0.06	0.07
C	0.044	0.084	0.056	0.088	0.054	0.045
C	0.066	0.11	0.094	0.24	0.1	0.064

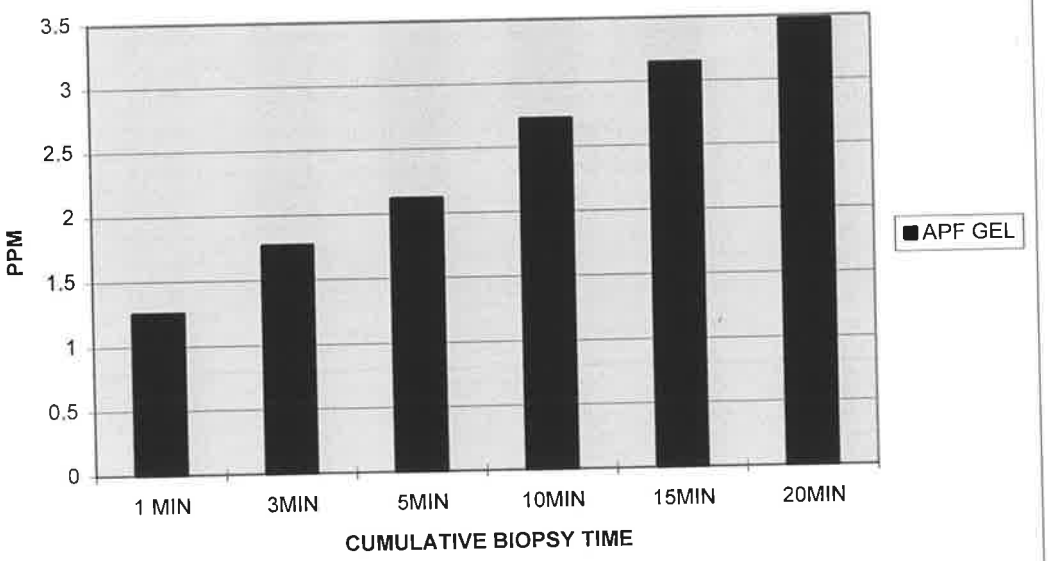
Cumulative results in ppm comparing uptake of fluoride from NaF (2%) gel following 4 minute application outside the mouth and 60 minutes in the mouth

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
NaF 4	0.039	0.169	0.257	0.417	0.481	0.543
NaF 4	0.22	0.35	0.43	0.61	0.672	0.716
NaF 60	0.076	0.146	0.2	0.34	0.4	0.436
NaF 60	0.1	0.184	0.25	0.42	0.48	0.55
C	0.044	0.128	0.184	0.272	0.326	0.371
C	0.066	0.176	0.27	0.51	0.61	0.674

	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
NaF 4	0.1295	0.2595	0.3435	0.5135	0.5765	0.6295
NaF 60	0.088	0.165	0.225	0.38	0.44	0.493
C	0.055	0.152	0.227	0.391	0.468	0.5225

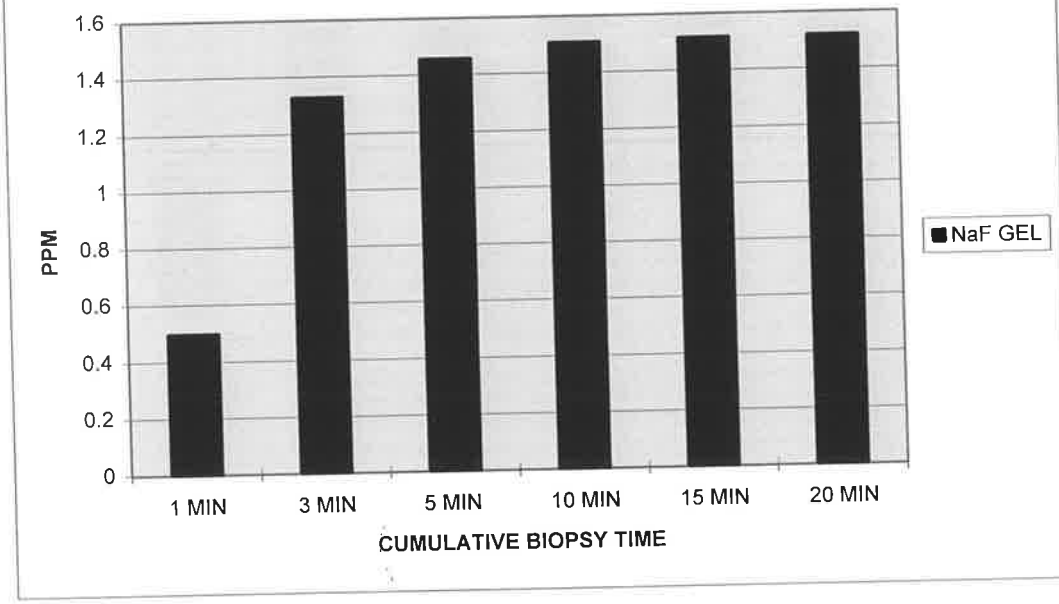
	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
NaF 4-C	0.0745	0.1075	0.1165	0.1225	0.1085	0.107
NaF 60-C	0.033	0.013	-0.002	-0.011	-0.028	-0.0295

4.1.1 CUMULATIVE UPTAKE OF FLUORIDE FROM APF GEL (1.23%) FOLLOWING 4 MIN APPLICATION



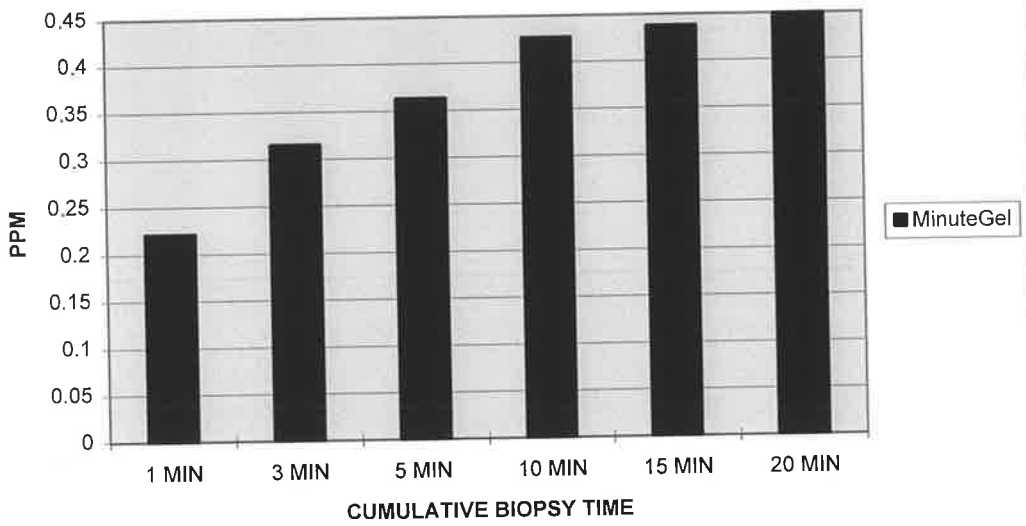
	1 MIN	3MIN	5MIN	10MIN	15MIN	20MIN
APF GEL	1.26	1.773	2.123	2.727	3.152	3.474

4.1.2 CUMULATIVE UPTAKE OF FLUORIDE FROM NaF GEL (2%) FOLLOWING 4 MINUTE APPLICATION



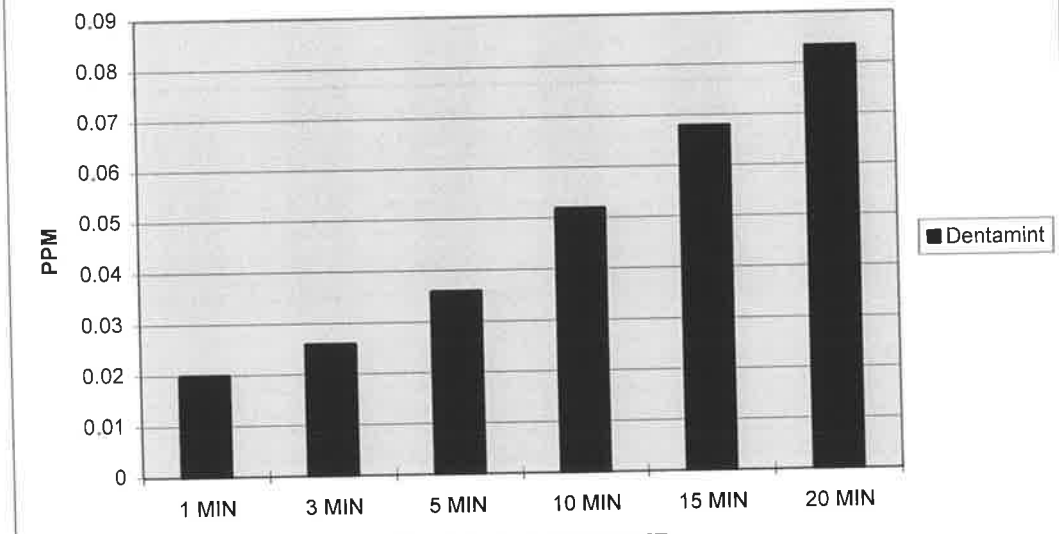
	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
NaF GEL	0.499	1.33	1.458	1.508	1.517	1.521

4.1.3 CUMULATIVE UPTAKE OF FLUORIDE FROM MINUTE GEL FOLLOWING ONE MINUTE APPLICATION



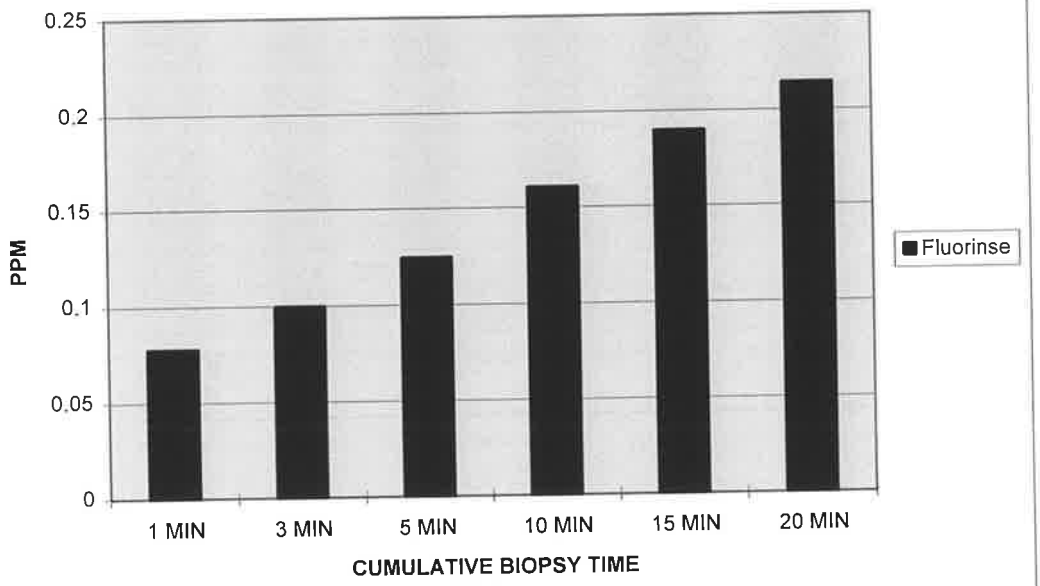
	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
MinuteGel	0.222	0.316	0.364	0.427	0.4385	0.45

4.1.4 CUMULATIVE UPTAKE OF FLUORIDE FROM DENTAMINT FOLLOWING 4 MIN APPLICATION



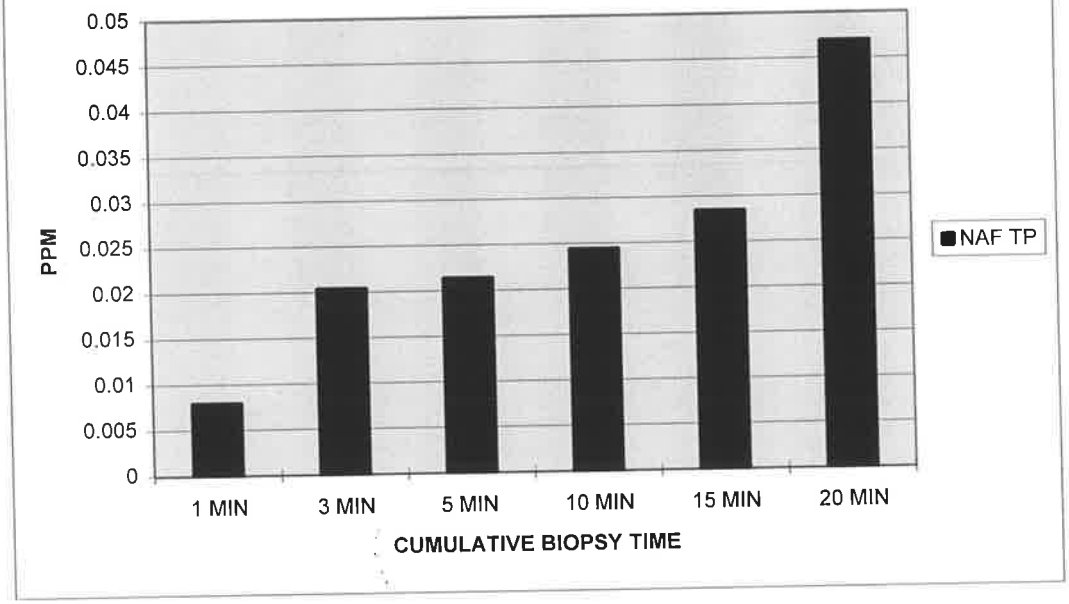
	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
Dentamint	0.02	0.026	0.036	0.052	0.068	0.0835

4.1.5 CUMULATIVE UPTAKE OF FLUORIDE FROM FLUORINSE MOUTHRINSE FOLLOWING 2 MINUTE APPLICATION



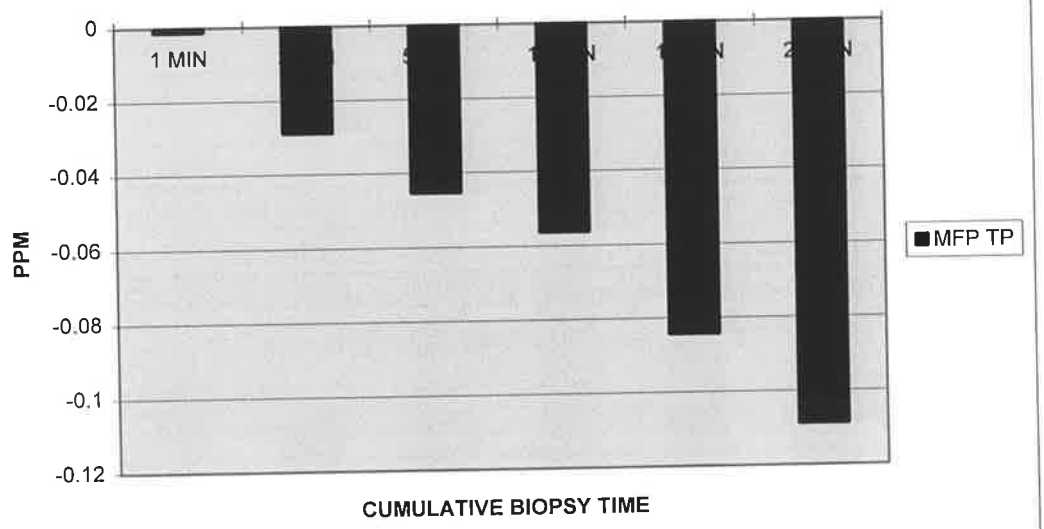
	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
Fluorinse	0.078	0.1	0.125	0.161	0.19	0.2145

4.1.6 CUMULATIVE UPTAKE OF FLUORIDE FROM COLGATE TOTAL TOOTHPASTE FOLLOWING 5 MINUTE APPLICATION



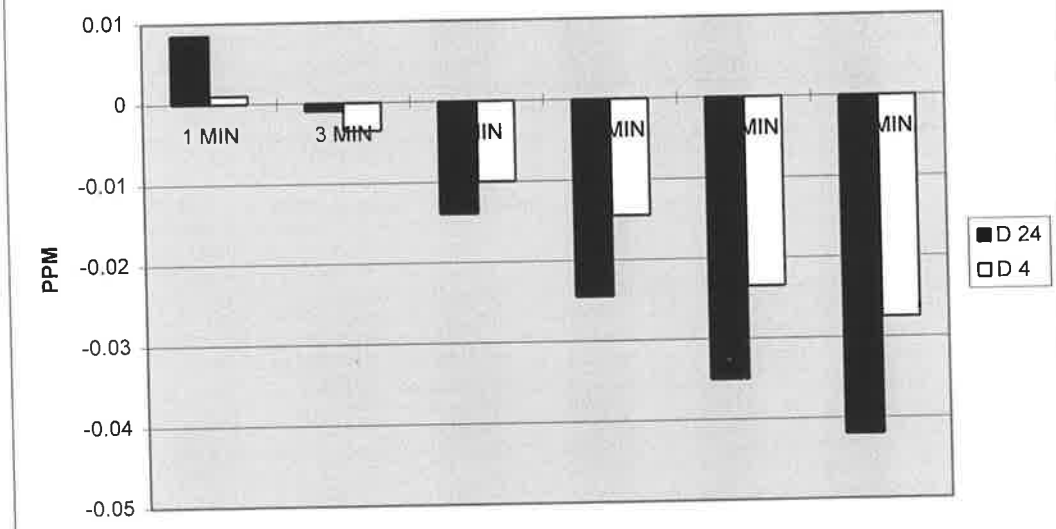
	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
NAF TP	0.008	0.0205	0.0215	0.0245	0.0285	0.047

4.1.7 CUMULATIVE UPTAKE OF FLUORIDE FROM COLGATE FLUORIGUARD TOOTHPASTE FOLLOWING 5 MIN APPLICATION



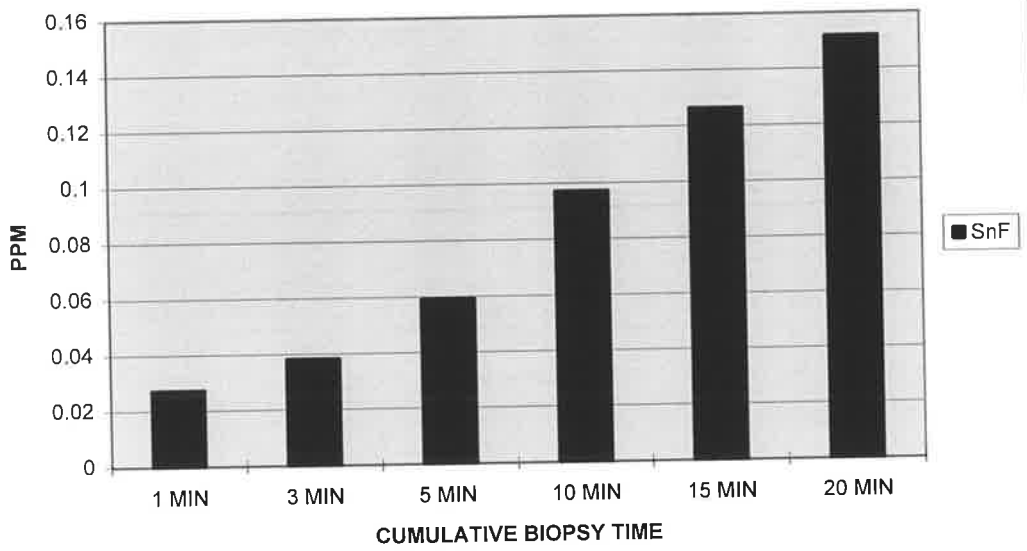
	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
MFP TP	-0.0015	-0.029	-0.0455	-0.0565	-0.0845	-0.109

4.1.8 COMPARATIVE UPTAKE OF FLUORIDE FROM DURAPHAT FOLLOWING 24 HRS AND 4 MIN APPLICATION



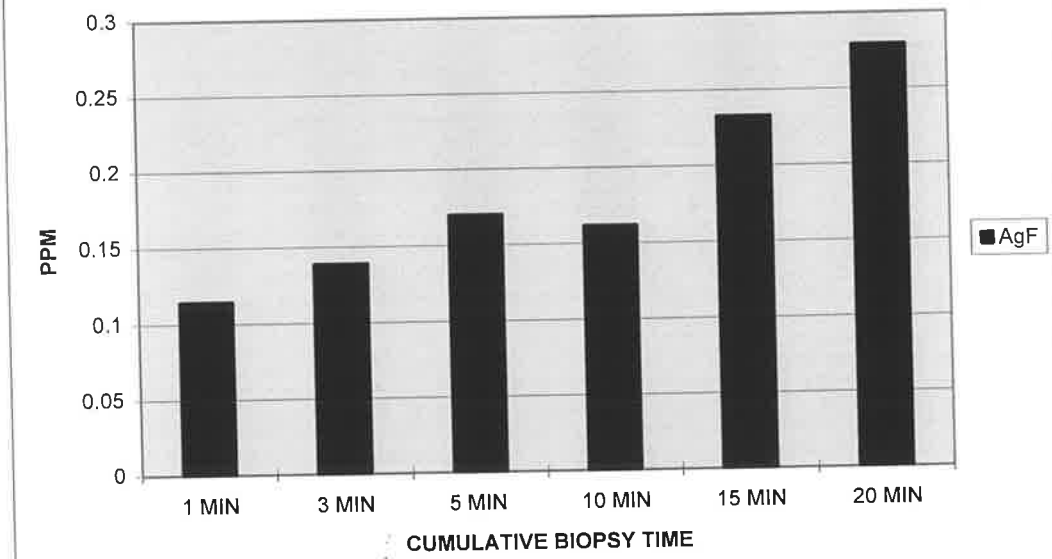
	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
D 24	0.0085	-0.001	-0.014	-0.0245	-0.035	-0.042
D 4	0.001	-0.0035	-0.01	-0.0145	-0.0235	-0.0275

4.1.9 CUMULATIVE UPTAKE OF FLUORIDE FROM SnF2 (20%) FOLLOWING 5 MIN APPLICATION



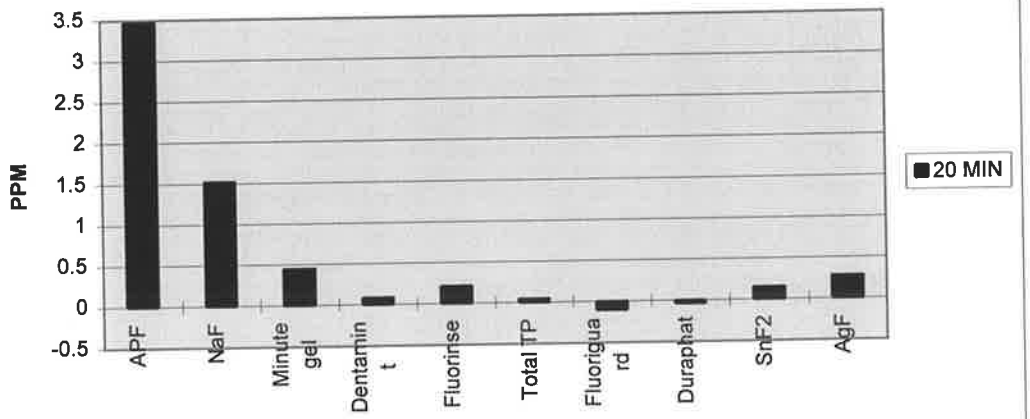
	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
SnF	0.0275	0.0385	0.0595	0.0975	0.1265	0.152

4.1.10 CUMULATIVE UPTAKE OF FLUORIDE FROM AgF (40%) FOLLOWING 5 MIN APPLICATION



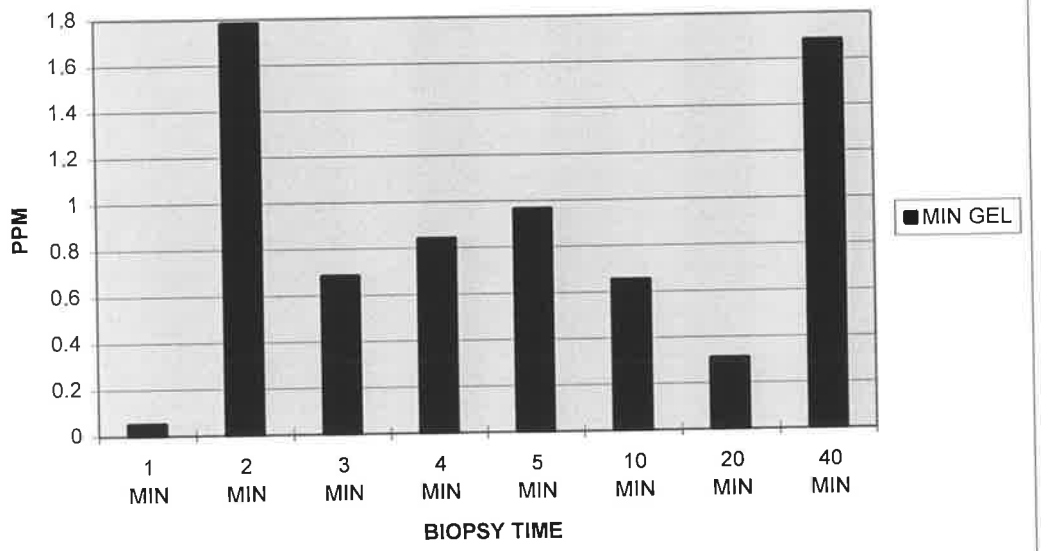
	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
AgF	0.115	0.139	0.17	0.162	0.233	0.2795

4.1.11 Summary graph comparing fluoride uptake from APF gel (4 min) and NaF gel (4 min), Minute gel (1 min), Dentamint (4 min), Fluorinse (2 min), Colgate Total (5 min), Colgate Fluoriguard (5 min), Duraphat (4 min), SnF2 (5 min), AgF (5 min).



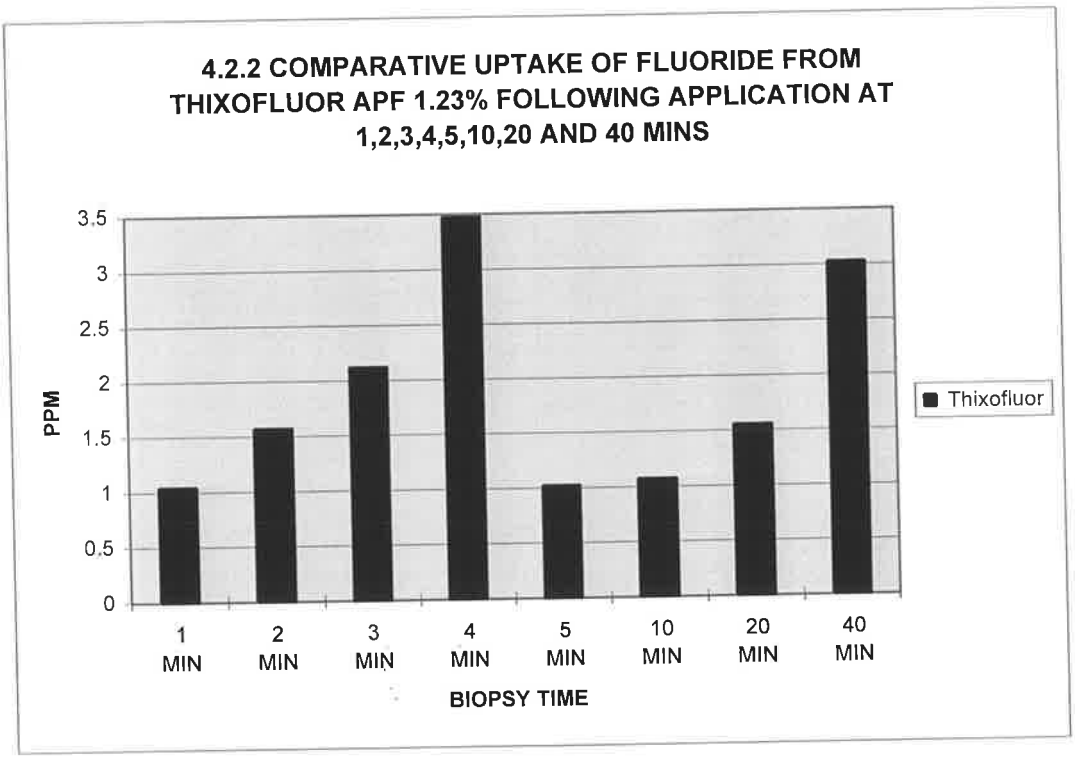
	20 MIN
APF	3.474
NaF	1.521
Minute gel	0.45
Dentamint	0.0835
Fluorinse	0.2145
Total TP	0.0475
Fluoriguard	-0.109
Duraphat	-0.042
SnF2	0.152
AgF	0.2795

4.2.1 COMPARATIVE UPTAKE OF FLUORIDE FROM MINUTE APF 1.23% GEL FOLLOWING APPLICATION AT 1,2,3,4,5,10,20 AND 40 MINS



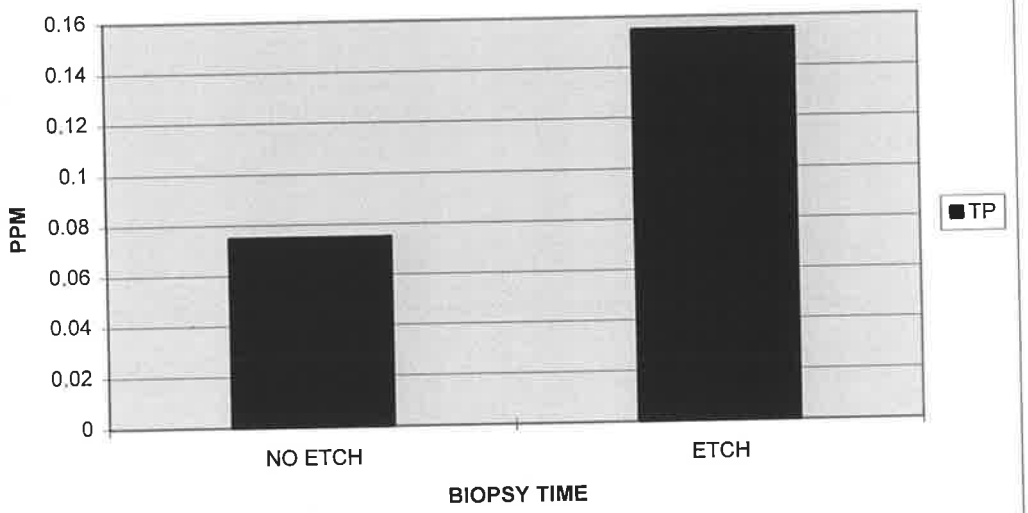
	1 MIN	2 MIN	3 MIN	4 MIN	5 MIN	10 MIN	20 MIN	40 MIN
MIN GEL	0.0545	1.785	0.688	0.844	0.967	0.657	0.3125	1.6865

4.2.2 COMPARATIVE UPTAKE OF FLUORIDE FROM THIXOFLUOR APF 1.23% FOLLOWING APPLICATION AT 1,2,3,4,5,10,20 AND 40 MINS



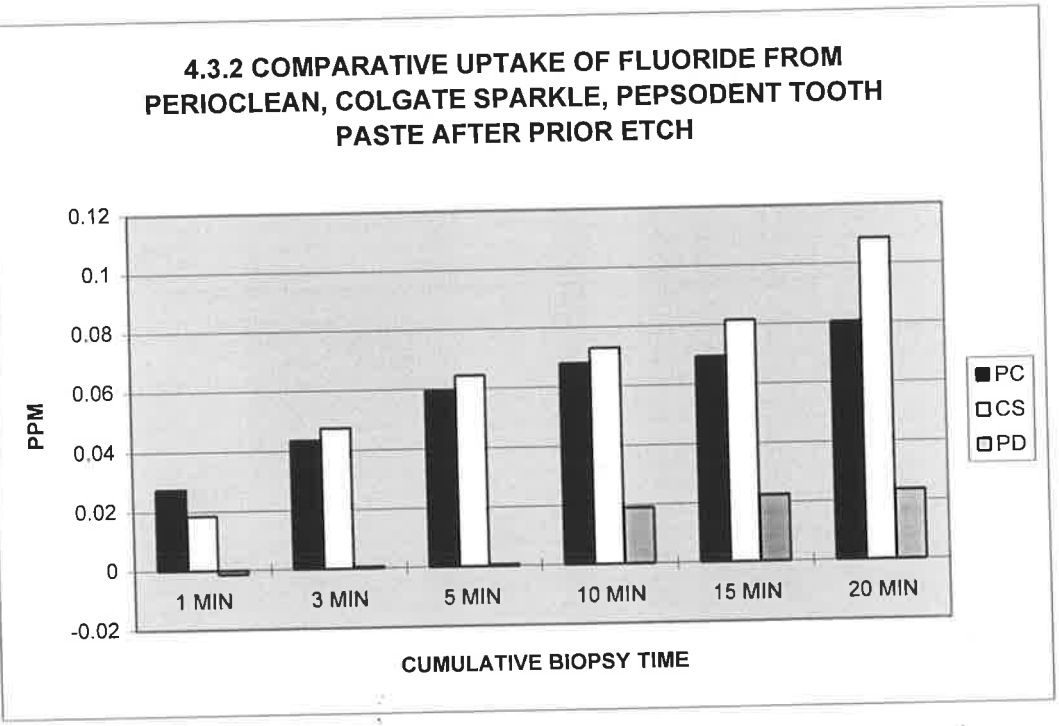
	1 MIN	2 MIN	3 MIN	4 MIN	5 MIN	10 MIN	20 MIN	40 MIN
Thixofluor	1.045	1.575	2.124	3.474	1.027	1.077	1.5565	3.0265

4.3.1 COMPARATIVE DIFFERENCE IN FLUORIDE UPTAKE FROM TOOTH PASTE (TARTAR CONTROL) WITH AND WITHOUT PRIOR ETCH



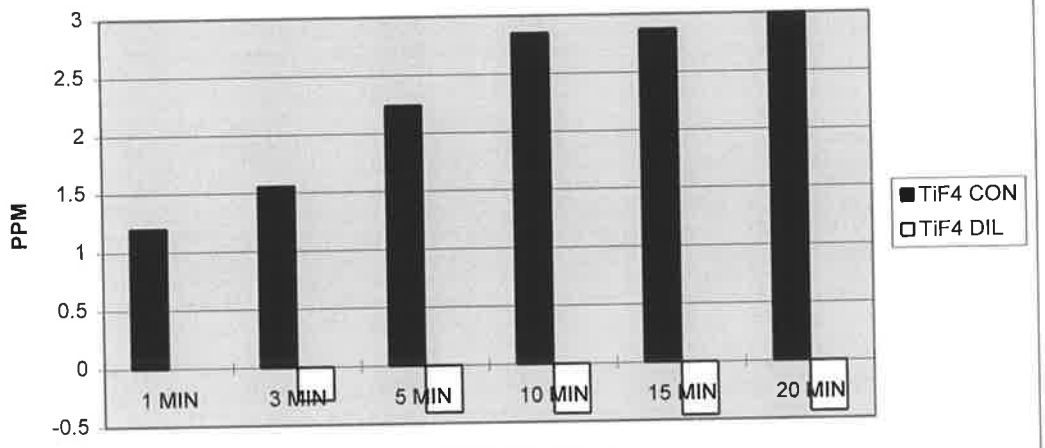
	TP
NO ETCH	0.075
ETCH	0.155

4.3.2 COMPARATIVE UPTAKE OF FLUORIDE FROM PERIOCLEAN, COLGATE SPARKLE, PEPSODENT TOOTH PASTE AFTER PRIOR ETCH



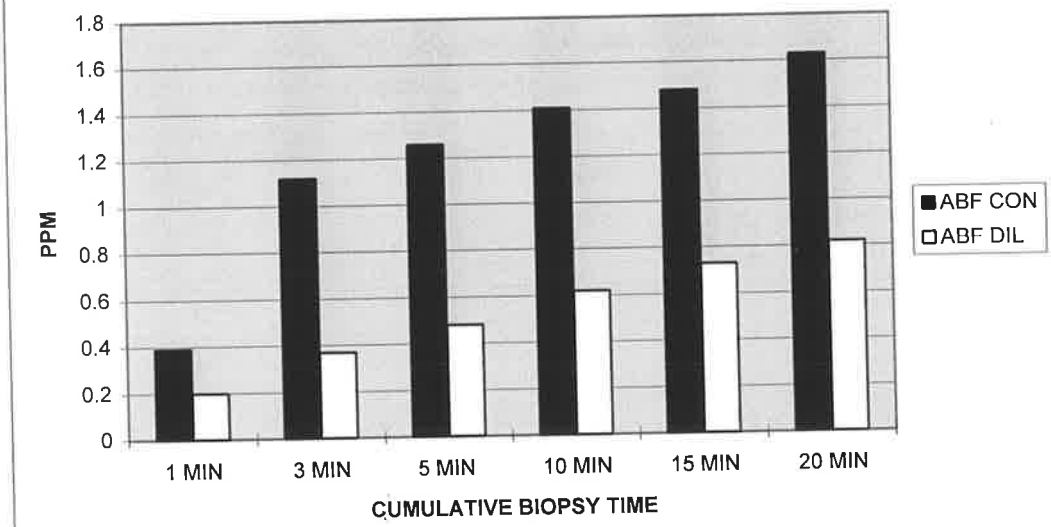
	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
PC	0.0275	0.0435	0.0595	0.068	0.0695	0.0805
CS	0.0185	0.0475	0.0645	0.073	0.0815	0.1085
PD	-0.0015	0.0005	0.0005	0.019	0.0225	0.0235

4.3.3 COMPARATIVE UPTAKE OF FLUORIDE FROM TIF4 USING CONCENTRATED (30,400 PPM) AND DILUTED (30 PPM) SOLUTIONS

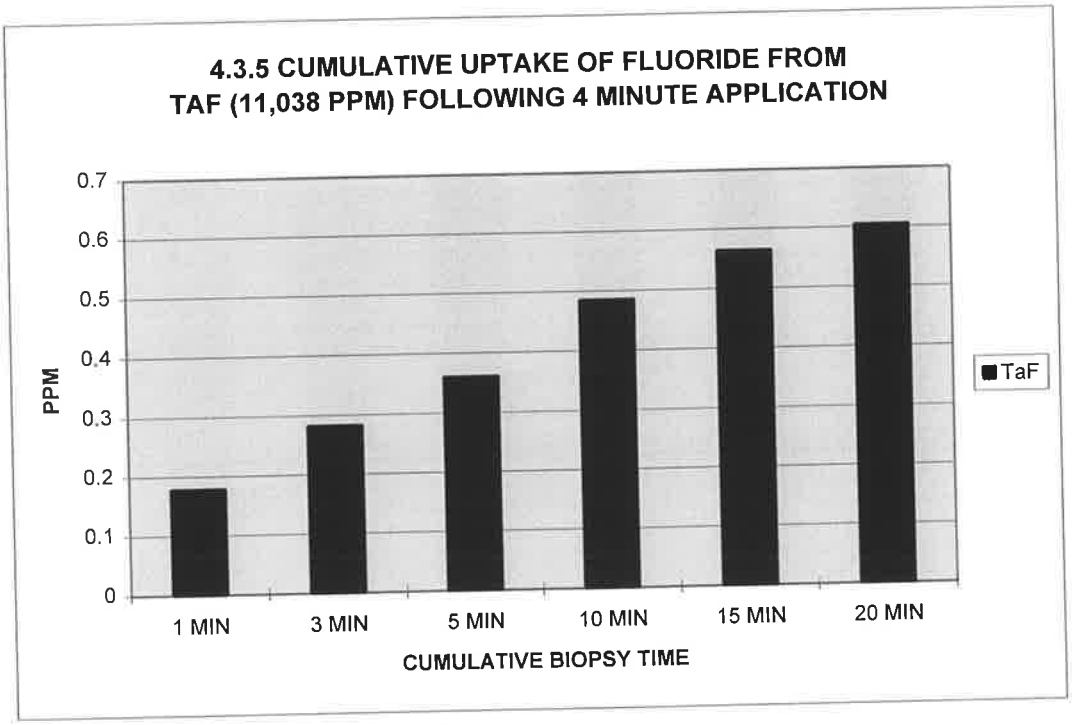


	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
TiF4 CON	1.2	1.56	2.24	2.85	2.87	3
TiF4 DIL	0	-0.29	-0.41	-0.43	-0.46	-0.44

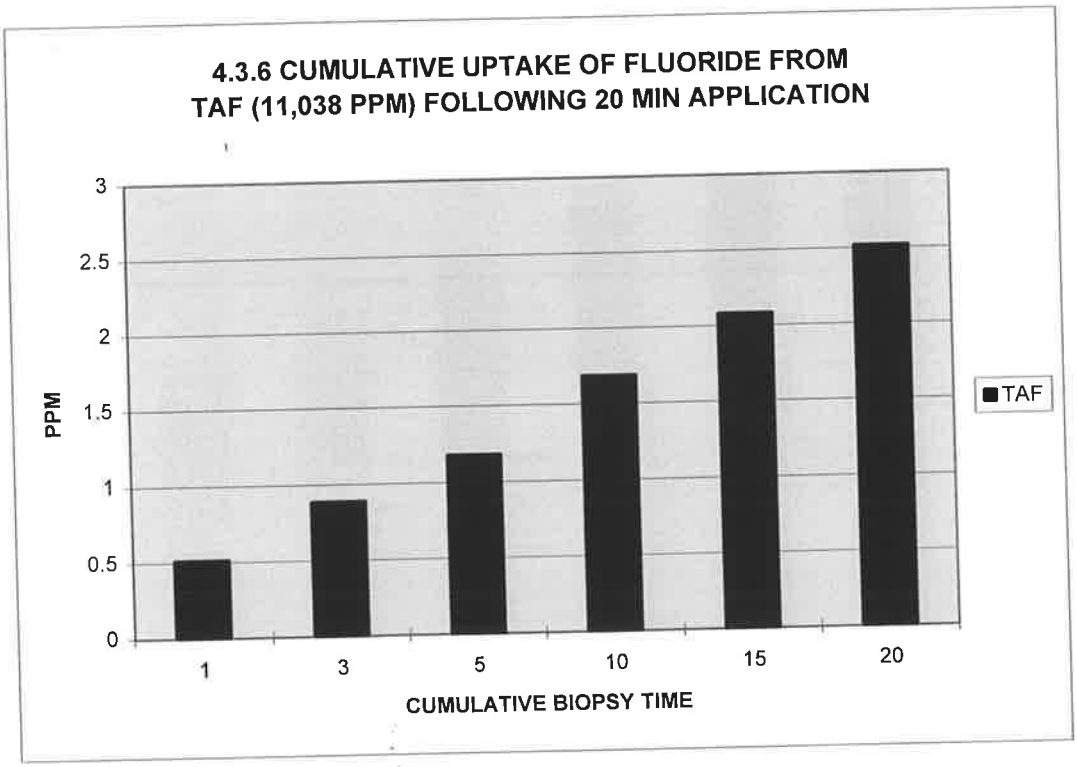
4.3.4 COMPARATIVE UPTAKE OF FLUORIDE FROM AMMONIUM BIFLUORIDE, USING CONCENTRATED (38,000 PPM) AND DILUTED (38 PPM) SOLUTIONS



	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
ABF CON	0.39	1.12	1.26	1.41	1.48	1.63
ABF DIL	0.2	0.37	0.48	0.62	0.73	0.82

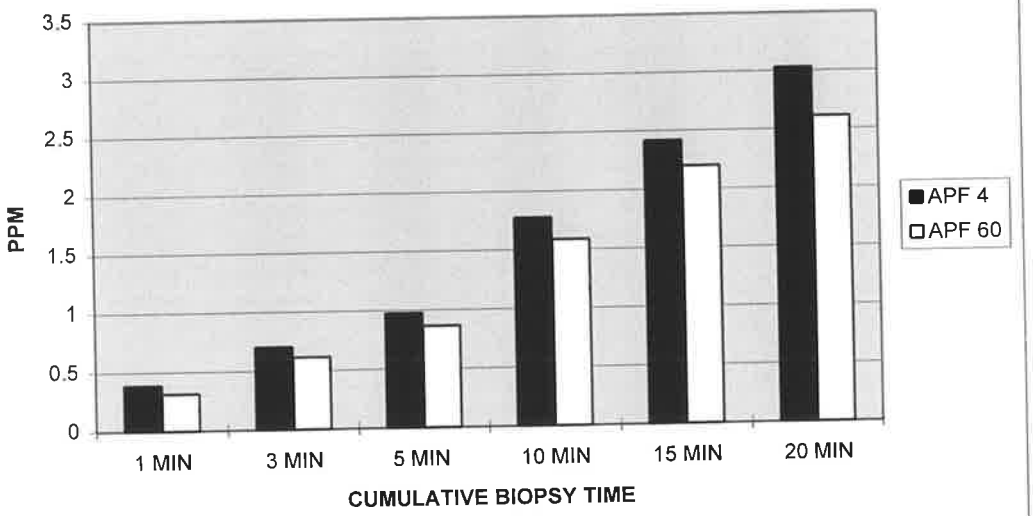


	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
TaF	0.179	0.283	0.361	0.486	0.565	0.605



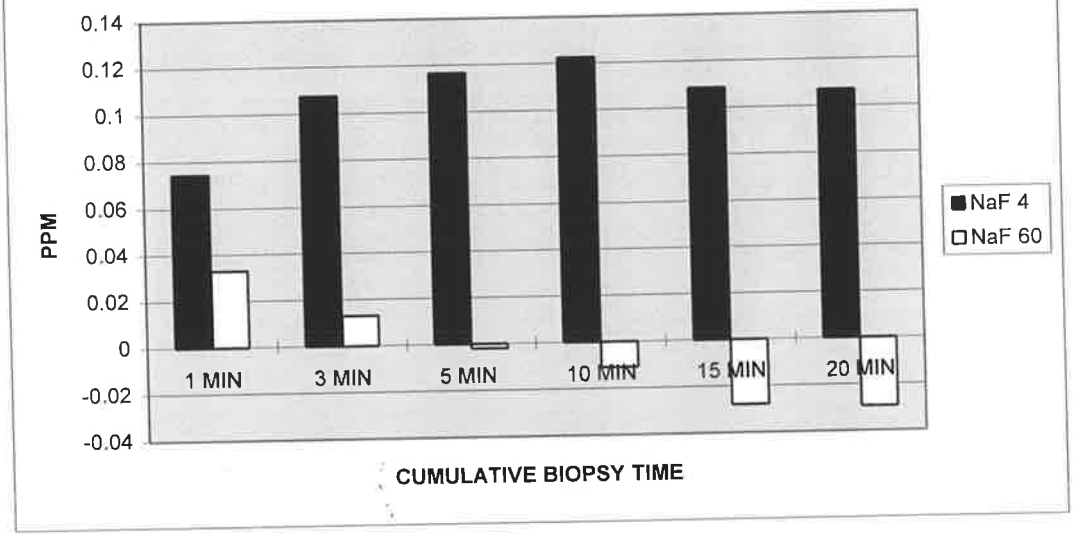
	1	3	5	10	15	20
TAF	0.516	0.894	1.184	1.691	2.0854	2.522

4.4.1 COMPARATIVE UPTAKE OF FLUORIDE FROM APF (1.23%) GEL FOLLOWING 4 MIN APPLICATION OUTSIDE THE MOUTH FOLLOWED BY PLACEMENT FOR 60 MIN IN THE MOUTH



	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
APF 4	0.39	0.7065	0.9765	1.7785	2.4165	3.031
APF 60	0.315	0.6165	0.8665	1.5885	2.1965	2.611

4.4.2 COMPARATIVE UPTAKE OF FLUORIDE FROM NAF (2%) GEL FOLLOWING 4 MIN APPLICATION OUTSIDE THE MOUTH FOLLOWED BY PLACEMENT FOR 60 MIN IN THE MOUTH



	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN
NaF 4	0.0745	0.1075	0.1165	0.1225	0.1085	0.107
NaF 60	0.033	0.013	-0.002	-0.011	-0.028	-0.0295

CHAPTER 5:

5.0 DISCUSSION.

The objectives in this study were:

(A) to determine whether a modification of a chemical biopsy system of fluoride extraction from enamel and its analysis provides an accurate basis for fluoride estimation.

(B) to determine the relative uptake of fluoride ion into whole enamel from a variety of topical fluoride agents.

These objectives have been partly met, time available limiting further investigations of some of the questions arising from some of these studies. Hence the project has become more of a pilot study, with further investigations needed to

(a) improve reliability of the sampling methods, and

(b) gain a greater bulk of data on fluoride ion uptake.

Because of the nature of the results, and some of the difficulties evident in the biopsy and sampling process, it was decided that there would be little point in subjecting the data to statistical analysis at this stage. This should be reserved until some of these difficulties have been overcome, and an increased sample size is used.

(A) Evaluation of effectiveness and reliability of the biopsy system.

The following questions need to be considered in relation to achievements of the first objective:

(i.) was the basic method of using six samples of enamel, cut from one tooth, beneficial in providing reasonable consistency in results?

(ii.) was the method of application of agents, and their removal, adequate?

(iii.) was the chemical biopsy method the best?

(iv.) was the method of fluoride analysis effective?

Commencing from the last question (iv), this method of direct analysis of biopsy solutions appeared to provide consistent and reliable results. The pH of biopsy solutions was frequently monitored before and after biopsy, and found to remain unaltered. This is reasonable given the very strong acidic conditions reacting with a small window of exposed enamel. It would have been preferable to present duplicated F ion measurements obtained at different times. However, this method has been used so widely in this laboratory, and duplicated results found to be consistent, this was not carried out on this occasion.

The method thus provides a considerable advantage irrespective of whether acid biopsy or microabrasion biopsy is used. In the latter, the fluoride ion has still to be chemically released and isolated from the abraded apatite, and this has been most frequently achieved using a strong acid.

A weakness in this system, and indeed all methods of F analysis currently used is their lack of sensitivity at low concentrations, even though at pH 1, there is evidence of a 10x increased sensitivity of this method compared with that at pH 5-6 (Tyler and Comer,

1985). Once the concentration goes below 0.01 ppm, the sensitivity decreases considerably.

A further problem, the occasional appearance of negative uptake, is considered to be one relating to sampling and will be discussed in the next few sections.

(iii) A number of problems became apparent in terms of depth of biopsy. Preliminary tests showed consistent depths of biopsy on a number of different teeth, irrespective of whether strong fluoride ion had been previously placed or not. However, it was later recognised that following use of a number of low fluoride concentration products, there still seemed to be relatively high levels of fluoride ion being detected between 15 and 20 minute biopsy times, even though the fluoride ion from these agents had been unlikely to have penetrated to this depth. Why this is so was not understood. It was realised that despite there being varnish protection at the margin of the windows, each increment of biopsy time resulted in some degree of lateral penetration of biopsy acid under the varnish undermining the marginal protection. It is known that the highest concentration of background fluoride ion are present at the surface of enamel. However, this should have been so for controls and test specimens. It must be considered also that fluoride from topical agents may have penetrated laterally into adjacent enamel. This would have been most likely in cases where acidulated gels or very high concentrations solutions were used. Hence, these residual levels of fluoride ion recorded at greater depth, may have resulted from lateral penetration of biopsy acids under the protective varnish.

In some cases, eg. for the strongly concentrated products, like APF 1.23% gels, it may also have been that, penetration may have exceeded the 54 μ metres of biopsy depth obtained after 20 minutes, and in such cases, the differences in concentrations from baseline levels were significantly higher than background levels to indicate that this was the case.

Dealing with the former problem, ie. lateral penetration of biopsy acids, may be difficult. It should be realised that the same conditions should have applied in all cases, and shown up consistently, as it did with most low concentration products. Hence it will be the low concentration products where this may have distorted the results in all cases. To deal with insufficient depth of biopsy for the stronger biopsy products, it may have been wiser to extend biopsy time to 30 minutes. Even so, the 20 minutes is greatly in excess of that time used by Tyler and Poole (1989) using a less concentrated biopsy acid. They stated that they kept this shorter time, as at increased time there was too much lateral etching evident, and they were looking for quantifiable volumes. It is evident that the matter of biopsy solution concentration and time of exposure need to be investigated much more fully, to provide more accurate results, particularly when dealing with low fluoride concentrations.

The major benefit of incremental biopsy was that it helped to indicate whether all additional fluoride ion had been reached and removed. This is seen particularly in Fig 4.1.2 where maximum fluoride uptake was reached by 10 mm of biopsy. (ie about 37 μm depth), with further biopsy not finding increased amounts.

A further benefit was to see the depth of penetration of fluoride ion from various sources, particularly with the high concentration products. As stated earlier, the evidence of penetration of fluoride ion to 50 μm in many low concentration neutral products is doubtful, and is more likely a result of continuing lateral etching of background fluoride in the surface levels of enamel. The data on incremental depth patterns of uptake has not been further explored in this report, due to the variability of some of the results, and difficulties evident in sampling biopsy method.

The problem of a few negative results from a few tests is very puzzling. For the lower concentration neutral fluoride materials, eg. tooth pastes, this might be explained in terms of an unusually high control (background) reading on the samples selected as controls, where the low level of uptake may not have made up for this high background reading.

However, in the case of *Duraphat* F varnish, this can hardly be an explanation. Such uptake results have not been reported by others, though this does not mean they were not obtained. However, Cruz et al (1992) showed that no firmly bound fluoride was present immediately after treatment, elevated levels being detected only one week after application. Hence this result may be accurate. This result needs further investigation, as it throws some doubt on the validity of the method until resolved.

(ii) It was difficult to determine which method of application "in vitro" most simulated clinical use. The intention was to select a method of application "in vitro" for each material, which accounted for the contact time of each agent with enamel when used "in

vivo", thus enabling a measure of intrinsic uptake potential by enamel of fluoride from that substance. However, in the low concentration materials, this resulted in concentrations within the lower reaches of sensitivity of the instruments, and thus these were probably less accurate. To have left these for longer periods of contact may have resulted in increased levels, but not provided comparisons of outcomes of routine clinical use. A further factor which may have reduced uptake was the lack of electrolyte containing moisture at the tooth surface. The teeth were kept moist in DDW until time for application of topical fluoride, and were lightly "blow dried" immediately before application. It may have been preferable to keep the teeth in an artificial saliva type of fluid and not dry the surface strongly before application of agents. Even so, it is difficult to see how the level of moisture may have been kept comparatively similar in each case.

It was decided that the topical fluoride agents should be totally washed off after the periods of time allocated, even though many of these linger in the saliva in contact with the tooth surface for some time after clinical or home self application. As it was impossible to simulate this "in vitro", the "in vitro- in vivo" test was introduced. This was intended to show whether, after topical fluoride had been left in place for one hour, there was any significant alteration in final uptake levels. The results obtained (Table and Fig 4.4) suggest that there is a slight reduction in concentration present in enamel following an extra 60 minutes retention intra orally in the case of APF gel, approximately one sixth of the initial concentration being lost over this time. Why this may happen is difficult to understand. However, it does suggest that the "in vitro" results obtained by the current methods are a useful indication of those which might be obtained "in vivo". The loss over time with the more neutral fluoride agents, however

seems to be quite marked, even though initial uptake rates were not severely reduced by the presence of pellicle. Hence, even though pellicle did not appear to significantly alter uptake in the short term, further problem of reduction in uptake levels with time was posed.*

(i.) Enamel slate sampling system.

It is evident that whatever method of biopsy is used, the background levels of fluoride ion present prior to any uptake test must be known. There must also be many other factors which may influence uptake rates, including surface deposits, and age of enamel. In this study, the samples were carefully cleaned of surface deposits before use and only those which showed no signs of staining, marked wear or cracks, ie. indicators of age, were used, to minimise such influences.

The decision to use slates of enamel from one tooth was important in minimising the considerable differences in background fluoride level between teeth. However, the differences between background levels of fluoride on slates from the same tooth were also significantly high, though it was considered that the use of two slates as control in each test would result in a mean value which was representative of the mean of all six samples. In retrospect,, to obtain a more reliable result, the test should have been triplicated, ie. have six specimens for each agent rather than just two as was used. Alternatively, it may have been better to use 3 slates as control for each tooth, with 3 for each agent being tested, using slates from two teeth for each test (ie. also 6 specimens). Despite these comments, the majority of results appeared to provide a credible profile of accumulation of fluoride levels at increasing depths within enamel and thus the results

* Small amounts of plaque might have been present.

obtained of fluoride uptake from different products were considered to provide useful pilot study level data to enable an initial evaluation of comparative intrinsic uptake potential to be made.

(B) Comparison of intrinsic fluoride ion uptake from a selection of commercially available products.

B(i). Comparison between 10 products

As the individual Y axis scales of graphs are different, this can be best seen in the summary graph (Fig 4.1.11) of 20 minute cumulative scores from each of these tests. As stated previously, the results from *Duraphat* F varnish are quite anomalous and need further investigation. Generally, the results are reasonably predictable on the basis of original concentration, except in terms of the gels. One form of acidulated gel obviously results in a far greater uptake level to greater depth than similar concentration of fluoride ion in a neutral form. Even so, close inspection of neutral gel fluoride uptake data (Table 4.1.2) shows an enormous difference in results between the two slates to which the gel was applied. This again indicates the need for many more slates to be used for each agent. Period of contact is obviously important, as will be seen more clearly from later tests. The Oral B *"Minute"* APF Gel however, resulted in a significantly reduced F ion uptake than the Colgate *"Thixofluor"* APF gel, even though both had basically similar constituents. The *"Thixofluor"* gel is claimed to be thixotropic, such that under pressure, more F ion is liberated. However, no pressure was used in these teeth. This matter also needs further investigation.

With very low concentrations, the profiles become more erratic due to reduced sensitivity of the test system at these concentrations of fluoride ion.

The uptake from AgF was unexpectedly low, particularly since this contained such high concentrations of fluoride (60,000 ppm). However, this confirms a previous finding by Alfonso and Gotjamanos (1993) that the fluoride ion in AgF did not penetrate deeply into enamel, when tested using ion exchange electrodes in a histochemical test. This was thought to be due to the binding of the heavy metallic silver ion at the surface of enamel forming a dense barrier which would not permit the fluoride ion to penetrate more deeply. A similar explanation may exist with SnF_2 where uptake was also unexpectedly low.

B(ii) Time uptake trials for the APF gels.

These were carried out, firstly to determine whether prolonged contact of APF gels with the enamel resulted in increased F ion uptake, or conversely whether shorter times of contact resulted in similar uptake. They were also carried out to check the relative uptake rates between the Colgate *Thixofluor* (4 min) gel, and the Oral B *Minute* (1 min) gel.

It is difficult to attempt to hypothesise why there should be such a difference in uptake levels between the two forms of gel, as stated earlier and this needs to be investigated further.

It is also difficult to attempt to explain why there should be a high level of uptake of *Thixofluor* gel at 4 mins, which one minute later is greatly reduced, only to return again some time later.

Whether this represents the culmination of a period of initial vigorous ion exchange activity, where rapid increase in fluoride ion concentration causes marked inhibition of continued acid ion penetration, to be followed by a period when the acid ions regain penetrating potential is not known. The reduction in fluoride ion may be attributed to an accumulation of acid ions dissolving the stored CaF_2 or FA formed, resulting in some of it diffusing to the surface, while some penetrates deeper into the tooth structure. It may simply be that the depth of biopsy was insufficient to detect more deeply penetrating fluoride ions in those tests with prolonged uptake time. A further study might follow this uptake on a minute by minute basis beyond 5 minutes, to see whether a series of pulses of F ion appear to penetrate into the enamel. It must be remembered that the nature of both the demineralisation and remineralisation reaction in enamel is of a moving pulse of ion exchange at the lesion front behaving as a diffusion controlled process of ion movement, which ebbs and flows according to the equilibrium of reaction of ions interacting at individual places within the lesion. The presence of strong acids within APF gel may bring about a complex system of pulsing of ion exchange throughout the enamel, with prolonged contact resulting in increased loss from surface diffusion.

Considering the clinical complications of prolonged retention, it is clear from these results that there is little benefit from increasing time of contact (except for *Minute* gel).

Also, in the case of *Thixofluor* a greatly reduced uptake results from shortening contact time.

It is important to restate that either pre or post eruptive storage is only one aspect of how fluorides work, and that low uptake rates do not mean reduced effectiveness. A good example is the effectiveness of tooth paste in caries prevention, even though little appears to be absorbed into enamel. However, where there is prolonged strong acidic challenge, as in rampant caries, there is much evidence to show that prior storage of high levels of fluoride ion in enamel enhances prolonged protection, and this is where the acidic gels, and other high concentration products provide some advantage.

B(iii) Effect of prior acid etch on uptake

The demonstration that fluoride uptake is greater in demineralised (White spot, carious lesions) than in intact, normal enamel led to the pretreatment of enamel by an etching agent to enhance fluoride uptake (Nikiforuk 1985). In this study enamel surface was etched with 37% phosphoric acid for 30 secs, rinsed and dried prior to fluoride application. These results confirmed those by De Paula et al (1971) in that there was a two fold increase in the fluoride uptake at the end of a 20 minute application period following etch as compared to direct application. However, how this finding can be applied clinically is difficult to consider. Etching of enamel should not be undertaken lightly. It may be best to consider these results as confirming that demineralised enamel has a higher uptake of fluoride than sound enamel.

B(iv) Use of alternative products not available commercially

(a) TiF_4 and NH_4F_2 results (Fig 4.3.3) confirm findings by others (Williams, 1992) that the high fluoride content salts TiF_4 and NH_4F_2 provide a very high level of uptake. Their toxicity levels are higher than currently used salts, and hence it is probably preferable to retain use of the less toxic products. One advantage they have, is that they are unlikely to result in staining, as SnF_2 has been shown to do. It would be valuable to look at intermediate concentrations of these products, as dilute NH_4F_2 appears to be well retained in enamel. These products appear very promising, and need further investigation.

(b) TAF

Evidence that fluoride concentrations in enamel were increased by lowering the pH of the topical fluoride solutions led to the development of acidulated phosphate fluoride (APF 1.23%, pH 3.2). To date APF gels are considered to be one of the more effective cariostatic reagent. However, frequent use results in corrosion of glass based restorative materials which may be due to the HF or H_3PO_4 components of the gel. Thus, alternative methods of acidulation are being investigated. As an alternative, Tartaric acid was chosen amongst other organic acids. It had the lowest pKa, and it had a history of wide use in foods, and hence a safe toxicity profile. The "in vitro" uptake of fluoride into enamel from TAF gel was less than that from APF gel after 4 minutes contact, though was much closer after 20 minutes. Initial pilot studies have not revealed any detrimental effects on enamel or cementum/ dentine after 6 months continuous contact. The long term effect on GIC's or Composite Resins has not yet been determined. It is not known to be corrosive of glass, though has a chelating potential. If this product

proves to be unreactive with tooth and restorative materials, its uptake rate into enamel makes it look promising as an APF alternative.

Overall, the evidence provided indicates that the higher concentration topical fluoride products do result in increased levels of storage in sound enamel. As stated repeatedly, this is only one factor relating to their effectiveness. Even so, it would suggest a more likely advantage in providing protection both against prolonged acidic challenge, or for a longer duration against milder challenge. Certain products appear to provide advantages over others, though further investigation is needed to clarify this point, due to many of the questions remaining unanswered.

CHAPTER 6

CONCLUSIONS.

As stated with the previous chapter, the objectives of the project were partly fulfilled, though the results must be viewed as those of a pilot study.

Quite a deal of valuable information has resulted. The basic biopsy and analytical method looks very promising, with not a great deal of work still being necessary to clear up some of the problems evident in the method. When this is achieved it will be necessary to gather a much greater quantity of data to average out the variable results obtained from different slates, even though these were from the same tooth.

Hence in retrospect it would have been preferable to spend more time collecting more data on only a few topical fluoride agents, and at the same time modifying the method through more detailed testing of its outcomes.

Despite these comments, the following have been achieved:

(a) It has been demonstrated that use of a 0.1 M HCl biopsy solution is feasible, and provides the advantage of permitting direct analysis of fluoride ion content if a high impedance pH meter is available. This provides a considerable time saving over previous methods.

(b) The use of slates of enamel from a single tooth eliminates yet another source of variability, ie. the differences in background fluoride levels between teeth. This should

permit fewer samples to be needed to provide an accurate mean value, ie. with minimal standard deviation.

(c) The comparative measurements of fluoride uptake have perhaps opened up more interesting questions than they have resolved. These include:

(i) why Thixofluor, APF 1.23% gel should demonstrate differing uptake rates for fluoride than Minute gel (APF 1.23%).

(ii) the mechanism which results in an increasing then decreasing level of uptake from acidulated fluoride gels over a period of time

(iii) the potential high levels of uptake from some of the alternative products, eg. TiF_4, NH_4F_2, and Tartaric Acid Acidulated F gel. These products may provide advantages over those currently available, and deserve further investigation.

(d) The "in vitro-in vivo" tests proved to be very useful, though themselves opened up some further questions needing further investigations. Those aspects of the investigation which require further analysis are :

(a) the time of acid biopsy, in particular the degree of lateral penetration of the acid, and the depth in relation to some of the more deeply penetrating materials.

(b) the method of application of the agents to the tooth surface, in particular the need for electrolyte solutions, and in general the search for methods which might simulate the "in vivo" situation more clearly.

It may be preferable to restrict the tests to the "in vitro-in vivo" test situation, though this needs to be achieved without risking safety to the subject.

(c) The uptake from the low concentration fluoride materials is so low, the results fall at the lower end of sensitivity of the analytical method, and hence may be less reliable. Hence there may be little point in pursuing these further. However, those from the more concentrated products need wider investigation. The particular questions to be followed, not covered in the previous sections are:

(i) the comparative uptake between the acidulated and neutral products

(ii) the effect of time contact on uptake levels

(iii) the effect of dilution on uptake rate of a number of the products, eg TiF_4, NH_4F_2.

(d) As stated previously, the number of specimens needed to provide an acceptable level of standard deviation for each category of test, needs to be investigated further. This would enable a more scientific evaluation of uptake levels, involving statistical analysis, to be carried out.

A general objective of the project was to begin to accumulate data which made it easier for the clinical dentist to decide which topical fluoride might be most appropriate for particular caries risk situations, in particular the high risk case. It was acknowledged that caries inhibition was achieved by fluoride over a number of modes of action. Primarily, there is the need for a certain optimal level of fluoride to be present on each occasion that an acidic challenge occurs at the tooth plaque or tooth/ saliva interface, ie. a number of times each day. This may be achieved by fluoride ions loosely bound to the plaque in the plaque fluid, which do not become incorporated to any significant level in the tooth surface. This is achieved largely by fluoride in dentifrice used twice each day. However, when the acidic challenge exceeds the potential for natural inhibition and

repair, via saliva and this low concentration additional fluoride ion, then greater concentrations of fluoride are needed over a prolonged period of time. This may be achieved by the use of more concentrated solutions from which fluoride ion may be retained in saliva and plaque for much longer periods of time. In some cases, the accompanying heavy metal ion gives an additional bacteriostatic effect. Also, some of this fluoride ion is adsorbed into the tooth surface and stored as CaF2 or FA. or in other forms.

The results from the comparative studies in the project give some initial data which demonstrate the relative uptake rates from various materials. Clearly, the acidulated gels provide the highest storage levels of all products tested, even though further analysis is needed to clarify the relative uptake from other concentrated projects. At the same time caution is needed as to when they can be safely used, and it is hoped that the alternative acidulated gels tested in this project may prove to be able to be used without detrimental effects on glass based restorative materials.

It is important to bear in mind that there may be other ways of optimising the benefits of fluoride by investigating new products or alternative methods of application such as prolonged contact, using other acidulated solutions, or pre etching the surface prior to topical fluoride application. This has been one of the aims of the present study. Some of these new materials show promise of improved protection.

The decision as to which topical agent is best to use has to be modified by other clinical judgements. For example relative uptake of fluoride ion from fluoride mouth washes

such as *Fluorinse* was twice that from dentifrices even though they had the same fluoride concentrations. However, use of mouth rinses need to be restricted for specific purposes. The rationale for this is that the individual who is brushing the teeth once or twice daily will be better protected against caries than an individual who rinses the mouth with a fluoride solution (Thylstrup and Fejerskov 1994). Also tooth brushing aims to eliminate plaque in addition to providing fluoride to the tooth surface, unlike mouth rinsing. Because of the importance of tooth brushing in total prevention required for the patient, mouth rinsing is usually used as an adjunct when increased protection is needed. A further factor in recommending use of mouth rises is the age of the patient, and potential to control the swallowing reflex. Otherwise ingestion of amounts likely to result in fluorosis may occur. This applies to all concentrated fluoride products for young children, they need to be strictly controlled.

This study demonstrates that there is still a great deal of work to be done in understanding how topical fluorides contribute to caries protection in enamel. However, the recent introduction of electron and proton probes, which can now detect concentrations of fluoride ion "in situ" in reactions of enamel, may hopefully make this process of analysis less complicated. At present, these new instruments are being evaluated, and can still only detect levels approximately 10-100 fold less than those that can be detected in the present biopsy and analysis system. It is expected that their sensitivity will be greatly enhanced in the years ahead. Hopefully the costs of the equipment will also make them more accessible. It is proposed, in the mean time, that the acid biopsy/ fluoride selective electrode system used here shows great promise in further studies of uptake of fluoride ion into enamel.

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ERRATA

p. xvi, 3rd parag. : **knowledgeable**
p. xvii, : **knowledgeable**
p. xvii, 3rd parag. : This **concentration** will vary
p. xviii, 2nd parag. : **Weatherell**
p. xvii, 3rd parag. : This **in turn**
p. 5 : 2nd parag. : different **secretory** fluids
p. 5 : 2nd parag. : mucosa of the **palate**, cheeks
p. 5 : 3rd parag. : **proteinatious**
p. 5 : 3rd parag. : **dependent**
p. 7 : 1st parag. : **aspartate** and **glutamate**
p. 8 : line 1 : At **pHs** below
p. 9 : 3rd parag. : **phenomena**
p. 12 : first line, 1st parag. : Fejerskov et al. (1981) **were**
p. 12 : last parag. : **dependent**
p. 22 : 2nd parag. : fluoride enters **micro-organisms**
p. 22 : 3rd parag. : There **is** no absolute
p. 23 : 1st parag. : Streptococci, **lactobacilli**, ...
p. 23 : 2nd parag. : formation of **fluorapatite**
p. 27 : 2nd parag. : **efficacy**;
p. 29 : 4th parag. : The retention **rates**
p. 29 : 3rd parag. : fluoride rinses **and** fluoride gels
p. 29 : 4th parag. : The retention **rates**
p. 30 : 3rd parag. : **DePaola** et al (1975)
p. 33 : Thus the amounts
p. 33 : Fluorprotector **are** lower
p. 34 : 3rd parag. : fluoride **concentrations** in
p. 37 : 2nd parag., 1st line : Weatherell and **Hargreaves** (1965)
p. 37 : 2nd parag., 6th line : Weatherell and **Hargreaves** (1965)
p. 39 : 2nd parag. : **amenable**
p. 40 : 1st line : **Singer**
p. 41 : 3rd parag. : **pK**
p. 42 : line 1 : **Caslavska**
p. 42 : **Weatherell** (1965)
p. 45 : top parag. : not only was it **possible** to show
p. 50 : 2nd parag. : **DDW**- Double distilled deionised water
p. 56 : Fluorinse- **0.22%** NaF
p. 56 : Duraphat- **22600** ppm F
p. 61 : 3. outer **area**
p. 62 : 7. Place **into** drying
p. 63 : last parag. : 6 vials in **length and breadth**
p. 64 : 3rd parag. : **thorough** rinsing prior to
pages 80, 81, 82, and 83 Headings : **Thixofluor**
p. 104 : 2nd parag. : the highest **concentrations** of
p. 105 : 2nd parag. : 10 **mins** biopsy
p. 110 : 2nd parag. : **Afonso** and Gotjamanos (1993)
p. 110 : 2nd parag. : was used in **Dentine**

p. 112 : last parag. : **DePaola** et al. (1975)
p. 113 : 2nd parag. : one of the more effective cariostatic **agents**
p. 120 : 2nd ref. : **Afonso**, FM., and Gotjamanos, T. (1993)
p. 123 : first ref. : Clarkson, BH., **Wefel**, JS.
p. 123 : last ref. : **DePaola** et al (1975)
p. 137 : Weatherall, JA and **Hargreaves**, JA (1965)