RELEASE OF ANTIMICROBIAL COMPOUNDS FROM GLASS-IONOMER DENTAL CEMENTS

By

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DECLARATION

"I certify that this work has not been accepted in substance for any degree, and is not concurrently being submitted for any purpose, other than that of the PhD thesis being studied at the University of Greenwich. I also declare that this work is the result of my own investigations except where otherwise identified by references and that I have not plagiarised another's work"

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ABSTRACT

This thesis reports a study of the possibility of using conventional glass-ionomer cements (GICs) as matrices for release of antimicrobial compounds. Sodium fusidate, cetyl pyridinium chloride (CPC), benzalkonium chloride (BACH), triclosan and triclosan/zinc citrate at concentrations ranging from 1% to 5% by weight were added into Fuji IX and Chemflex cements. Disc-diffusion studies showed antimicrobial effect against *Streptococcus mutan*s. Inhibition zones were proportional to the amount of added bactericide, CPC and BACH showed highest antibacterial activity. The release of the bactericides into water was studied for time intervals up to seven weeks. The amount of additive released varied from 0.61% to 5.00% of total bactericide added and samples containing more antimicrobial agent gave higher release into the surrounding water. The release was shown to be diffusion based for the first 2-4 weeks. Compressive strength and surface hardness of reformulated materials decreased in comparison with the control specimens. Addition of bactericides also decreased the amount of fluoride released. ^{27}Al MAS-NMR showed that aluminium switches its coordination number from four, Al (IV), in the glass phase to six, Al (VI), in the cement matrix and addition of antimicrobial agents reduced the rate of this change. Incorporation of additives also prolonged the working time. By contrast, water loss properties were not affected by additives. The overall conclusion is that the presence of additives affects the setting and maturation reactions of GICs. These results can be interpreted as showing that the additives having an effect on the conformation of the poly (acrylic acid) (PAA) component in solution. Changes in the conformation of the PAA also influence the release of key ions from the glass (Al^{3+}, Ca^{2+}, F^- and Na^+). Alteration in the balance of these ions, especially Al^{3+}, would result in slower cross-linking processes and lower cross-link density matrix. Additionally, adsorption properties of surfactants to GI aluminosilicate glass particles can also lead to reduction in the number of available active sites on the glass which can react with PAA. The reduction in available active sites on the glass will result in a lower bonding density and thus a weaker matrix. All above will leads to the observed changes in mechanical properties, working kinetics, F^- release and kinetics of conversion of Al (IV) to Al (VI).

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Dla moich rodziców…

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ABBREVIATIONS

Symbol	*Description*
ASTM	*American Society for Testing and Material*
^{27}Al	*Aluminium-27*
BACH	*Benzalkonium chloride*
CHX	*Chlorhexidine*
CHF	*Chemflex*
Cfu	*Colony forming units*
Conc	*Concentration*
CPC	*Cetyl pyridinium chloride*
CS	*Compressive strength*
^{o}C	*Degrees Celsius*
D	*Diffusion coefficient*
F^-	*Fluoride anion*
FDA	*Food and Drug Administration*
FIX	*Fuji IX*
FTIR	*Fourier Transform Infrared Spectroscopy*
GICs	*Glass-ionomer cements*
g	*Gram*
h	*Hour*
HPLC	*High Performance Liquid Chromatography*
ICP-OES	*Inductively Coupled Plasma-Optical Emission Spectrometry*
ISE	*Ion Selective Electrode*
ISO	*International Organisation for Standardisations*
L	*Liter*
LC	*Liquid chromatography*

m	*Meter*
MAS-NMR	*Magic Angle Spinning-Nuclear Magnetic Resonance Spectroscopy*
MS	*Mass Spectrometry*
MPa	*Mega Pascal*
M_t	*Mass at time t*
M_∞	*Mass at time infinitive*
mm	*Millimeters*
min	*Minute*
ml	*Milliliter*
nm	*Nanometer*
PPA	*Poly (acrylic acid)*
PBS	*Phosphate buffered saline medium*
ppm	*Parts per million*
RMGICs	*Resin modified glass-ionomer cements*
s	*Second*
SEM	*Scanning Electron Microscopy*
SF	*Sodium fusidate*
SIR	*Single Ion Recording*
T	*Triclosan*
T*	*Triclosan with addition of zinc citrate*
UV	*Ultraviolet*
w/w	*Weight by weight*
VHN	*Vicker's hardness number*
XRD	*X-ray diffraction*
ZC	*Zinc citrate*

LITERATURE REVIEW

1.1 General Introduction

Dental caries, postoperative sensitivity and gum diseases are still the most common dental disorders. Although not life threatening, they can cause discomfort, pain or even be responsible for loss of teeth. Dental diseases are caused by bacteria that are normally present in the mouth by either facilitating production of acids that dissolve mineral content of the tooth or by formation of dental plaque that when not treated might cause irritation or infections of the gums. Dental plaque begins to build up on teeth within 20 minutes after eating. If this plaque is not removed thoroughly and routinely, tooth decay will begin. A long-term dental challenge is to prevent bacterial colonisation and thus dental caries by other means than common technologies such as mechanical brushing or usage of mouth washings.

Restorative dentistry very often makes use of glass-ionomer cements (GICs) either as filling materials or bonding agents for crown and bridges [1, 2]. The great advantage of GICs over other restorative materials is that they can be placed into tooth cavities without an additional bonding agent [3, 4]. They also possess a fluoride-releasing property [5, 6] and are relatively biocompatible with the pulp [7, 8]. However, studies have shown that its fluoride-releasing activity is insufficient for effective antibacterial protection [9]. Thus, various attempts have been made to improve the antibacterial property of GICs by the inclusion of specific antimicrobial compounds [10, 11, 12 and 13]. The presence of bactericidal agents in GICs could prove to be of practical benefit in preventing caries, including secondary caries and periodontal disease in patients, and to ensure high standards of hygiene throughout the oral cavity.

The primary objective of this project is to evaluate the potential of GICs to act as a slow release device for the delivery of antimicrobial agents. Release properties (profile, diffusion coefficients and antimicrobial effects) will be studied, and also the effect on GICs of adding the active compounds on properties such as working time, water loss, fluoride release, compressive strength and Vicker's hardness will be determined. The effect of additives on maturation kinetics, using sold state MAS-NMR, will be investigated.

1.2 History and development of dental restoration

Although the idea of dental preservation can be traced to 11th century, the first conservative dental procedure, with the use of gold and amalgam can be tracked down to second half of 19th century [14]. At the same time (1855) the first acid-base cement zinc-oxychloride was discovered by Sorel [15]. The zinc-oxychloride discovered by Sorel was not found to be very successful as it caused irritation of pulp tissues. However, this material provided the foundation for development of acid-base cements, which due to their unique properties proved to be of the utmost value to dentistry.

Acid-base materials were the first dental materials that adhered to tooth substrates. They set quicker, were stronger and were more resistant to erosion than for example Portland cement [1, 16]. Sorel's discovery triggered the development of acid-base materials, starting with the development of magnesium chloride-oxide eugenol cement followed by zinc-oxide eugenol [15, 17 and 18]. Since then zinc-oxide eugenol had been continuously employed as a temporary filling, lining and luting material [17, 18].

Silicate cements were introduced in the 1870s. These cements were based on aluminofluorosilicate glass and phosphoric acid. The matrix phase of these cements constituted an aluminum-phosphate salt formed from the partial dissolution of the glass by the acid and a dispersed phase composed of residual glass particles. Silicate cements were the first tooth colored materials; however, they were brittle and required mechanical retention. At the same time (1892) zinc phosphate was discovered by Ames. Silicate cements and zinc phosphate remained the principle anterior restoratives for over 50 years [14, 19].

In addition to cements, in the 1940s the development of acrylic and polymer materials had been studied. The poly (methyl-methacrylate) based composites were developed in the mid-1940s. These materials however, lack adhesion to the tooth. They showed high polymerisation shrinkage, a large coefficient of thermal expansion and poor colour stability [17]. Zinc polycarboxylate cements were introduced to the market in 1969 [20, 21].

The early dental restoration described above only focused on the mechanical properties of materials and there was little appreciation of the material properties, and their biological consequences. This attitude might be explained by the quality of early dental materials. They were weak, susceptible to erosion and had poor dimensional stability. So concern with their physical and mechanical properties was paramount.

The late 1960s was the most creative period for development of dental materials and it was triggered by the changes in the outlook of the dental profession on dental restoration. During this period increasing attention was paid to problems of biocompatibility between the restoration of the tooth, adhesion and aesthetics. The change in attitude has led to a revolution in thinking and change of researchers approach to the development of dental materials [1, 19].

At that time Wilson and Kent started research on dental silicates where phosphoric acid was replaced by organic chelating acids, including poly (acrylic acid). This discovery resulted in the development of the first GICs. Early GICs however lacked workability and set slowly [7, 22 and 23]. In 1972 Wilson and Crisp discovered that when tartaric acid is added to the acid mixture, it improved the manipulation of these materials by sharpening the setting reaction [24]. This discovery triggered a number of improvements in GICs to meet the needs of contemporary applications of these materials [14].

1.3 An introduction to GICs

GICs belong to the class of cements known as acid–base cements. Acid-base cements involve the cement formation by both acid-base and hydration reaction [26]. In GICs, the reaction between the proton accepting glass powder and proton donating acid liquid is generally considered to be the mechanism involved in the cement formation. The product of the reaction is salt-like hydrogel which binds the unreacted powder particles into a cement mass [26] and the general reaction may be simplified as:

MO	+	**H_2A**	→	**MA**	+	**H_2O**
basic oxide		acid liquid		salt hydrogel		water

On the basis of their chemistry, GICs can be classified into five types namely: conventional GICs, resin modified glass-ionomer cements (RMGICs), hybrid ionomer cements dual-cured, tri-cured glass-ionomer cements and metal-reinforced glass-ionomer cements [1, 2].

RMGICs are conventional GI with addition of a photopolymerisable monomer, typically 2-hydroxyethylmethacrylate (HEMA), plus a photoinitiator which react to harden the material when a visible light beam is applied. Once the resin is cured the GI maturation reaction continues protected by the cured resin enclosure from moisture and drying out [27]. The addition of the resin component decreases the initial setting time as the lightcuring process only takes ~ 40 seconds. The resin also reduces handling difficulties and substantially increases the wear resistance and physical strength of the cement which makes it a very appealing material to use in the dental industry [1, 2, 28 and 29].

Hybrid ionomer cements dual-cured and tri-cured both combine an acid-base reaction of the traditional GI with a self-cure amine-peroxide polymerisation reaction [1, 2]. This type of modification improves the fracture toughness of GIC [30]. Metal-reinforced glass-ionomer cements are made by the addition of silver-amalgam alloy powder to conventional GI. Addition of metal increases the physical strength of the cement [30].

GICs are widely used in clinical dentistry as luting and bonding materials, restorative materials, and cavity liners and bases [25]. The classifications of GICs cements were adopted from the original Wilson and McLean model and are widely accepted.

Type I: Luting and bonding materials

GICs can be used for cementation of crowns, bridges, inlays and orthodontic appliances as well as for the bonding of composite resins and amalgam. An example of the use of GICs for the attachment of crowns is shown in Figure 1.1.

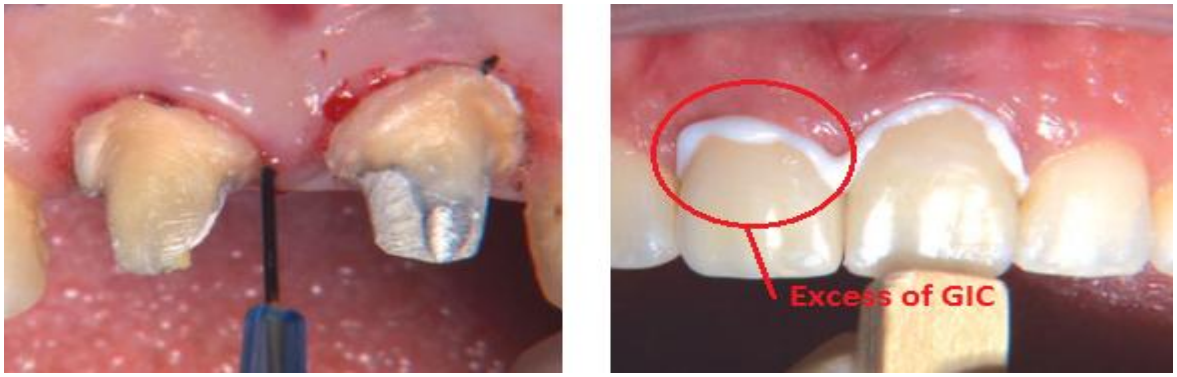

Figure 1.1: The upper incisor teeth prepared for crowns (on the left). Permanent crowns with a resin-modified GICs (RelyX Luting Plus Automix, 3M ESPE) (on the right) (photographs obtained from *Glidewell Laboratories* [31])

GICs to be used as luting and bonding materials require the following specifications:

- Powder/liquid ratio to be 1.5/1 up to 3.8/1 depending on the type of liquid used.
- Ultimate film thickness of about 20 microns maximum.
- Quick setting with early resistance to water uptake.

Type II

Type II.1: For restorations – aesthetic materials

GICs can be used for any application requiring minimal cavity preparation and minimal occlusal load which includes restoration of carious primary teeth. Use of GIC for restoration purposes is shown in Figure 1.2.

Requirements of use of GICs as restorative aesthetic materials are:

- Powder/liquid ratio to be 3/1 but can reach up to 6.8/1 if the polyacid is dehydrated and incorporated into the powder.
- Excellent shade range and translucency.
- Auto-cure cements, due to prolonged setting time, require immediate protection from moisture to avoid water uptake and loss.

Type II.2: Restorative materials

GICs are use where aesthetic considerations are not important, but rapid set and high physical properties are required.

Requirements of use of GICs as Type II.2 restorative materials are:

- Powder/liquid ratio to be 3/1 to 4/1.
- Fast set with early resistance to water uptake. The material can be trimmed and polished immediately after the initial set however it remains susceptible to dehydration for two weeks after placement.

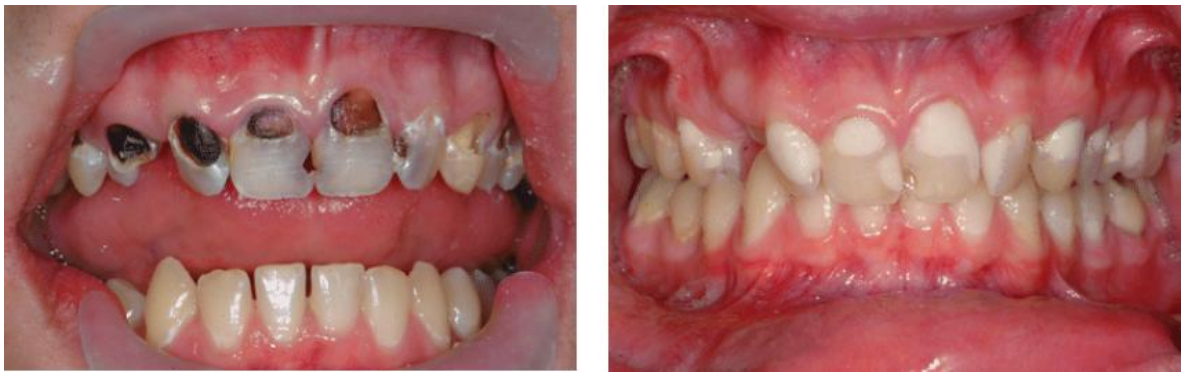

Figure 1.2: Front view of high caries activity (on the left). Front view of transitional restorations using GI restorative material (Fuji IX, GC) (on the right) (photographs obtained from *Journal of Dental Education* [32])

Type III: Liners and bases

GICs can be used either as a lining or as a base depending upon the powder/liquid ratio. A powder/liquid ratio of about 1.5/1 is used as a lining material under other restorative materials. A powder/liquid ratio of 3/1 up to 6.8/1 is used as a base or dentine substitute in a lamination technique with another restorative material. They can be used beneath both a composite resin and an amalgam [7, 23]. An example of the use of GI as a liner is shown in Figure 1.3.

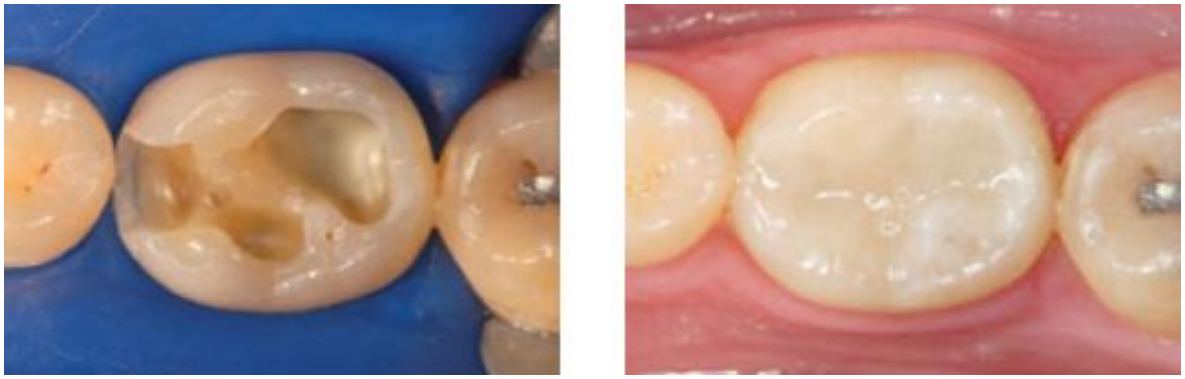

Figure 1.3: A resin-modified GIC liner (RelyX Luting Plus Automix, 3M ESPE) in tooth cavity (on the left). Finished restoration with dental composite-resin (Tetric EvoCeram, Ivoclar Vivadent) (on the right) (photographs obtained from *Dentistry Today* [33])

Outside dentistry these materials have been studied for possible application as artificial ear ossicles and bone substitute plates for craniofacial reconstruction [34]. Additionally the experimental studies have explored the possibility of using these materials for fixation of cochlear implants and sealing defects in the skull [35, 36].

1.3.1 Composition of GICc

The composition of a GIC is very complicated and varies with each material. Nevertheless, there are some features that are common for most of them. The two main constituents for all GI are: calcium or strontium aluminosilicate glass and a poly (alkenoic acid). The compositional range for useful glasses is wide and will depend on the intended application of the cement. The poly (alkenoic acid)s are homopolymers or copolymers of unsaturated carboxylic acids. The most common acid used is poly (acrylic acid) [25].

GICs are therefore unusual materials in that they are hybrid materials containing both organic and inorganic phases. In terms of an engineering classification they might be classified as composite materials but ones wherein the filler (glass) takes part in the setting reaction.

1.3.2 Glasses

The powders used in the GICs formulation are prepared from special ion-leachable glasses [37]. Although there are a number of potential glasses types used as GI powders and some of them are extremely complex, all of them contain silica, alumina and alkaline earth or rare earth oxide or fluoride [25]. The two essential glass types are: oxide glasses and fluoride glasses [25]. The examples of oxide and fluoride glasses are shown below in Figure 1.4.

SiO_2-Al_2O_3-CaO oxide glass

SiO_2-Al_2O_3-CaF_2 fluoride glass

Figure 1.4: Structure of oxide and fluoride glass [25]

The derivatives of these basic glass types are made by doping with sodium carbonate, calcium orthophosphate and in the case of fluoride glasses, aluminum fluoride is added [25]. In addition to fluoride glasses it has been reported that calcium can be replaced by strontium or lanthanum [25, 38]. The examples of oxide and fluoride glasses derivatives are shown below in Figure 1.5.

oxide glasses	**fluoride glasses**
SiO_2-Al_2O_3-CaO	**SiO_2-Al_2O_3-CaF_2**
SiO_2-Al_2O_3-CaO-P_2O_5	**SiO_2-Al_2O_3-CaO-CaF_2-$AlPO_4$**
SiO_2-Al_2O_3-CaO-Na_2O	**SiO_2-Al_2O_3-CaO-CaF_2-$AlPO_4$-Na_3AlF_6-AlF_3**

Figure 1.5: Structures of derivatives of oxide and fluoride glasses [25]

Glasses are prepared by fusion of the appropriate components in the temperature range of 1200^{o}C-1550^{o}C [39]. After fusion, molten glass is then shock-cooled by pouring it onto a metal plate or into water. The glass is then ground into a fine powder using a ball mill (4 μm for filling materials and 15 μm for luting cements) [2]. The composition of the fusion mixture is important. The ratio of alumina to quartz directly affects the reactivity of the prepared glasses towards poly (acrylic acid) liquids [37].

1.3.2.1 Glass structure

The distribution of the components within the glass is not uniform and it appears to form two distinctive phases. The presence of two phases is believed to result from a partial phase-separation – *via* spinodal decomposition [40]. Early studies of the composition of the G-200 GI conducted by Barry et al. (1972) showed that these glasses contained phase-separated droplets of complex structure as well as massive inclusion of fluoride [41]. Hill and Wilson (1986) reported that other opal glasses also contained phase-separated droplets similar to G-200. These phase-separated droplets contained large quantities of calcium with crystalline calcium and fluoride [7].

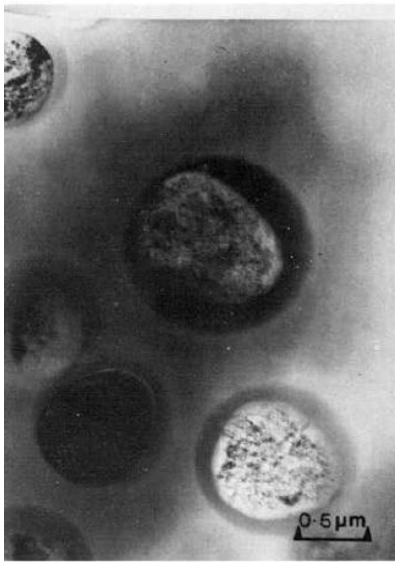

Figure 1.6: Micrograph of an opal aluminosilicate glass showing phase-separated droplets [41]

Phase separation has an effect on glass reactivity. Within GICs, acid attack occurs selectively at the phase-separated droplets which are rich in calcium and fluoride. As the main phase is depleted in calcium and fluoride it becomes less reactive towards the acid [7]. Phase-separated glasses produce stronger cements than clear glasses. The strongest cements produced from a clear glass have a compressive strength of 130 MPa and a flexural strength of 20 MPa, whereas phase separated glasses produce cements with compressive strength exceeding 200 MPa and flexural strengths exceeding 35 MPa [25].

1.3.2.2 Glass structure - cement forming ability

The formation of the cement is dependent on the ability of the glass to release cations to the acid solutions during the acid base reaction. This ability can be achieved by creating a basic site on the glass. Basicity of GI can be explained using the random network model of Zacheriasen [42].

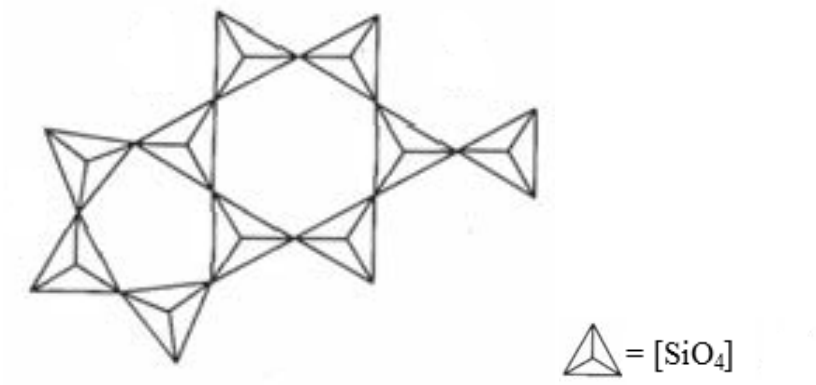

Figure 1.7: Schematic of silica tetrahedral network

In this model the glass is considered as a random assembly of SiO_4 tetrahedral units which link together to form a chain. The simplest glass of this type consists of a straight silica tetrahedral chain [42]. A schematic of a silica tetrahedral network is presented in Figure 1.7. This infinite three-dimensional network is electrically neutral and resistant to the acid attack. The cement forming glass must however acquire reactive sites that will be

susceptible to the acid attack during the acid-base reaction. Addition of network-modifying cations leads to the breakage of the network Si-O-Si and exposure of reactive non-bridging oxygen [27].

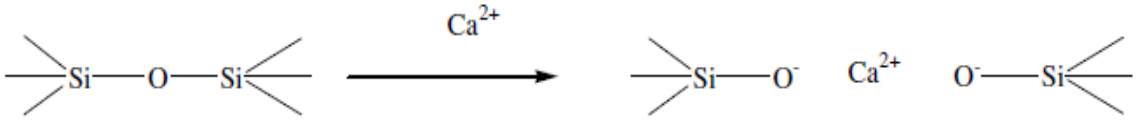

Figure 1.8: Schematic of the cation integration effect on the silica network

Aluminium plays a crucial role in cement formation ability. It not only acts as a network modifier in its six-coordinate form, it can also serve as a network former when four-coordinate. This property arises because Al^{3+} has a similar ionic radius to Si^{4+} and is able to replace Si^{4+} in the glass network. In the presence of sufficient amount of silica, alumina is forced to adopt the tetrahedral geometry of SiO_4. Replacement creates negatively charged sides that are reactive towards hydroxonium ion from the acid. The matrix must of course maintain its neutrality and therefore the negative charge is balanced by network modifying cations (Na^+, Ca^{2+}) [43].

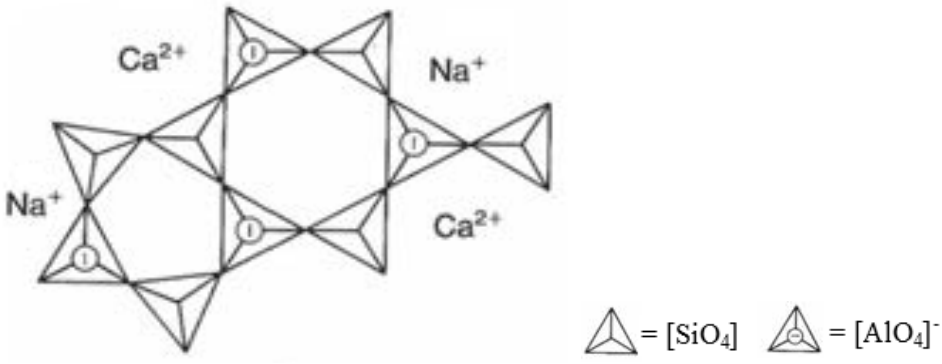

Figure 1.9: Schematic of aluminosilicate network

The cement-forming ability of the glass appears to be dependent on the chemical composition of the glass. The ratio of alumina to silica is critical and must exceed 1/2 by mass for the glass to be capable of forming cement. This condition is necessary to promote sufficient replacement of silicon by aluminium to ensure that the glass is basic [44, 45].

1.3.3 Poly (alkenoic acid)s

There are a number of poly (alkenoic acid)s that can be used in the GIC formulation [25]. In general they are homopolymers of acrylic acid and its copolymers with maleic acid [25, 46, 47 and 48]. The poly (acrylic acid) is not always contained in the liquid. Sometimes the dry acid is blended with glass powder and is activated by mixing with water or tartaric acid [49, 50]. A schematic of poly (acrylic acid) is presented in Figure 1.10.

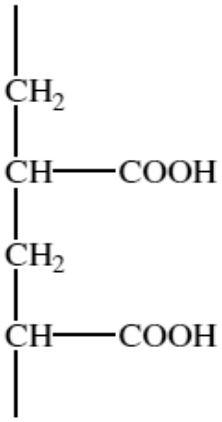

Figure 1.10: Schematic of poly (acrylic acid)

The use of homopolymers is very important in achieving the necessary handling properties of the glass and the resulting final properties of the cement [49, 50]. Maleic acid is a stronger acid than poly (acrylic acid) and contains more carboxyl groups. Additional carboxyl groups facilitate more rapid polycarboxylate cross-linking resulting in decreasing setting time. This property allows more conventional, less reactive glasses to be used which results in more aesthetic final set cement [49, 50].

Tartaric acid is an extremely important ingredient of GICs. It may be regarded as an essential constituent and is included in glass polyalkenoate cements as a reaction-controlling additive [51, 52, 53 and 54]. Tartaric acid increases the hardening rate, without decreasing and even sometimes increasing working time. Tartaric acid also strengthens and hardens the cement. Moreover, studies conducted by Crisp et al. (1980) showed that the addition of (+)-tartaric acid conferred the setting property on a glass G-288 that otherwise did not form a cement [55]. The action of the tartaric acid is associated with its ability to complex with ions released from the glass. Complex formation prevents ions from cross-linking the polymer chain until the chains become more linear and cross-linking can occur more readily [56].

Acids are prepared by free radical polymerisation in aqueous solution using ammonium persulfate as the initiator and propan-2-ol as the transfer agent [25, 57]. After polymerisation the solution is concentrated to 40-50% for use [22].

There are a number of requirements that cement-forming liquids have to meet which includes:

- sufficient acidity in order to decompose the basic powder and liberate cement-forming cations,
- contain an acid anion which forms stable complexes with released cations [22].

The concentration of the poly (acid) has a direct effect on the strength of the GICt. The increase in concentration of the poly (acid) increases solution viscosity, quite sharply above 45% by mass [58]. The strength of glass-polyalkenoate cements also increases, almost linearly, with poly (acid) concentration. This is achieved at the cost of producing over-thick cement pastes and loss of working time. Strength, fracture toughness and resistance to erosion are also improved when the molecular weight of poly (acid) increases [59, 60]. However, the increase of the molecular weight will accelerate the setting time processes and therefore working time will be lost [25].

1.4 Setting chemistry

The setting chemistry of GICs has been extensively studied by various techniques which include Fourier Transform Infrared (FTIR) spectroscopy [61], ^{13}C NMR (Carbon-13 Nuclear Magnetic Resonance) spectroscopy [62] and pH changes [63, 64]. All of these techniques indicate that the setting chemistry of these materials are based on an acid-base reaction that involves the neutralisation of poly (acid) by a glass powder (base) and formation of a metal polyacrylate hydrogel [65]. Water is the reaction medium and is also required to hydrate the metal polyalkanoate matrix. GICs set and harden by transfer of metal ions from the glass to the poly (acid) [65]. The stages of cement-forming reaction are described below.

The first stage of reaction is ionisation of a carboxyl group on the poly (acrylic acid) to COO^- and H^+ (i.e. hydrated proton, H_3O^+). H^+ ions from the acid penetrate the glass particles' surface, breaking down the aluminosilicate network, liberating the metal ions (aluminium and calcium), fluoride (if present) and silicic acid [66].

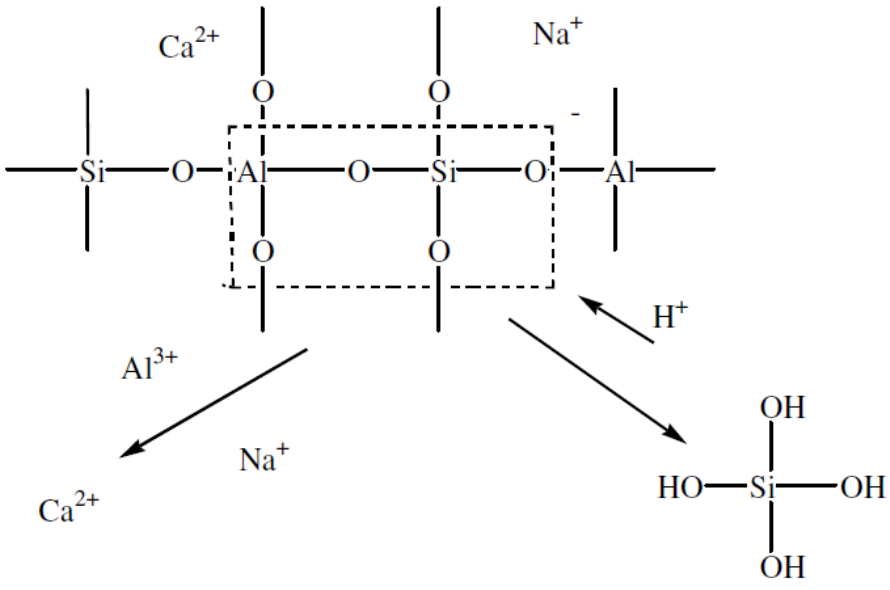

Figure 1.11: Decomposition of the aluminosilicate by acids [1]

As the reaction precedes further, the pH of the aqueous phase rises, the poly (alkenoic acid) ionises and most probably creates the electrostatic field which facilitates the migration of liberated metal ions into the aqueous phase [2]. Next stage involves gelation of the poly (acid) with calcium and aluminum metal ions to form matrix [25]. The formation of matrix units is sequential and involves formation of calcium acrylate followed by aluminium acrylate units [64, 65].

1.4.1 Hardening of GICs - formation of organic network

As stated above, the formation of salt units during the setting reactions of GICs is sequential [64, 65]. The occurrence of this process was assumed following Infrared spectroscopic studies conducted by Crisp et al. (1976) [67]. The studies showed order in which bands due to the respective metal carboxylates were appearing in the spectra. Later Nicholson et al. (1988) used a much more powerful FTIR technique to study the setting of GICs. The authors confirmed the sequential appearance of calcium and aluminium carboxylate observed by Crisp et al. (Table 1.1) [61].

Table 1.1: Time of first appearance of metal polyacrylate infrared spectroscopic bands in an experimental GIC [61]

Metal polyacrylate	**Time to first appearance (s)**
Ca-PAA	45
Al-PAA	540

Despite these results, there was doubt about the sequence of ion release. Cook et al. (1983) examined the composition of freshly prepared cements by dissolving them in 3% potassium hydroxide solution and analysing the resultant solutions using atomic absorption spectroscopy. The results showed that aluminium was present in the setting matrix early in the cure process indicating that aluminium and calcium were involved in the initial setting reaction [68]. These findings opened the question of why aluminium, whilst it is released from the glass matrix at the same time as calcium forms polyacrylates later. Wasson and Nicholson (1990) used Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) to look at the effect of washing on cement-forming glasses with dilute solutions of acetic acid. They examined washing chemical composition as a function of time. Results showed that the aluminium/calcium ratios in the washings were consistently smaller than the ratio in the glass. From these findings they concluded that aluminium is released from the glass in a form of condensed species and the delayed formation of aluminium polyacrylate is due to the slow extraction of the free Al^{3+} ions from this condensed form [69].

Formation of calcium polyacrylates is associated with initial setting reactions where an increase in viscosity of glass-ionomers is observed. Formation of aluminium acrylates is responsible for further hardening of the cement. This process lasts up to 24 hours and is accompanied by an increase in compressive strength of GIs [25].

1.4.2 Maturation reaction - formation of inorganic network

There are number of indications that the formation of calcium and aluminium polyacrylate cross-links are not the only type of reaction occurring within the cement matrix and that the cement matrix undergoes further reactions as it ages. The indications of these reactions are changes in physical and chemical properties of GI such as increase in translucency of the cement. The cements also become more resistant to desiccation. Probably the most difficult to explain is the continuous increase in strength up to one year based on the cross-linking of poly (carboxylic acid) by metal cations [70].

A number of studies have tried to explain this phenomenon. Wasson and Nicholson (1991) using ICP-OES to examine the effect of washing on cement-forming glasses with dilute solutions of acetic acid found that a variety of inorganic species (silicon and phosphorus) can be released from the glass. The existence of silicon and phosphorous within the matrix led to the suggestion that these elements formed a possible inorganic phase [71]. This hypothesis was supported by Hatton and Brook (1992). Hatton and Brook studied a section of set GICs using the electron microscope, and their studies confirmed the presence of silicon and phosphorus throughout the matrix [72].

This discovery was further tested by Wasson and Nicholson (1993). They prepared cement using no polymer, but based on acetic acid. Their results showed that the compressive strength of the prepared cement increased with time even though the acetic acid did not form insoluble salts with calcium and aluminum. These findings proved that the maturation of the organic component was unlikely to take any part in cement stabilisation and that the silica component leached from the powder contributed to the hardening of the cement [69, 73].

1.4.3 Cement structure

Set GIC can be characterised as a complex composite in which there is a matrix, consisting of calcium or strontium and aluminium (polyacrylates) (organic network) and glass particles, which act as filler, embedded within the matrix.

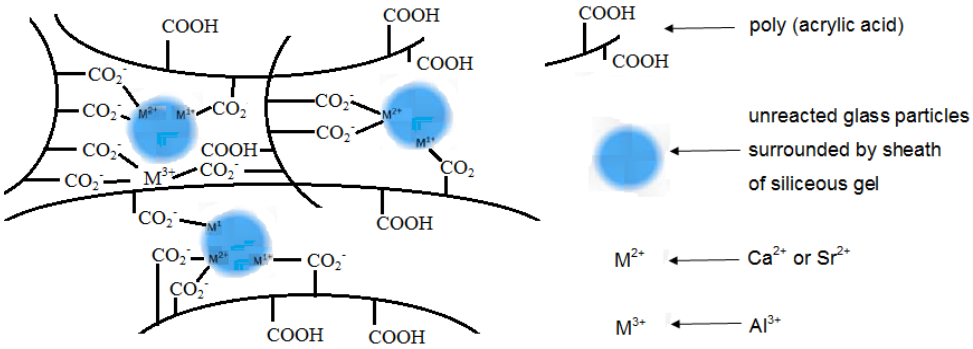

Figure 1.12: Schematic representation of set GICs structure

The glass particles comprise an unreacted core and are surrounded by a sheath of siliceous gel. The structure is held together by a combination of ionic cross-links, hydrogen bridges and chain entanglements [72]. A schema of the cement structure is presented in Figure 1.12.

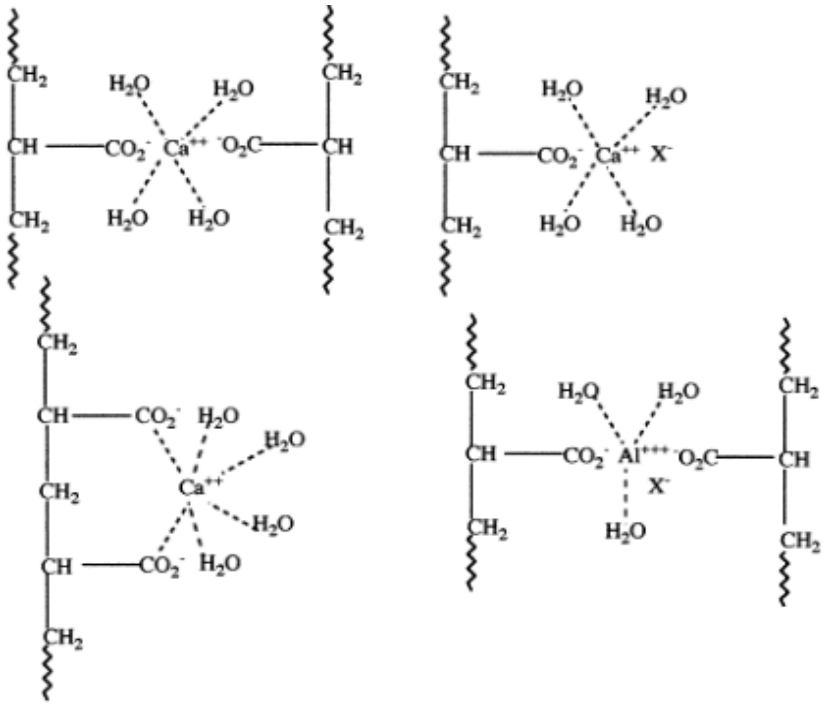

Figure 1.13: Possible inter- and intramolecular calcium and aluminium carboxylates in cured GIC (X represents H^- or F^- anions) [65]

Water that is used as a solvent remains in the cement and it can occupy various locations, for example coordination sites around metal cations or hydration regions around the polyanion chain. The principle of water configurations during cement maturation can be seen in Figure 1.13.

Taking into an account the role of water, a glass-ionomer can also be regarded as a glass filled poly (alkenoic acid), which is cross-linked by cations, via salt-bridges, and plasticised by water [65].

1.4.4 Hydration of polyanions

Water is a very important constituent of GICs. It acts both, as a solvent and as a component in the formation of the cement. It is also one of the reaction products formed during the acid-base reaction. In GIC water may serve to coordinate to certain sites around metal ions. It also hydrates the siliceous hydrogel that is formed from the glass after acid attack has liberated the various metal ions [1, 7].

Water occurs in the GIC in at least two different states [7]. These states have been classified as evaporable and non-evaporable water. This classification depends on whether the water can be removed by desiccation or remains bound firmly bound in the cement when subjected to such treatment [74]. In the glass-poly (acrylic acid) system the evaporable water is up to 5% by weight of the total cement, while the bound water is 18-28% [75]. This amount of tightly bound water is equivalent to five or six molecules of water for each acid group and associated metal cation. Hence at least ten molecules of water are involved in the hydration of each coordinated metal ion at a carboxylate site [1].

The bound, non-evaporable water is associated with intrinsic water spheres around the carboxylate anion-metal cation units, whereas evaporable water is associated with a secondary sheet around the polyacrylate chain. The ratio of non-evaporable to evaporable water is an important parameter which controls the mechanical properties of dental cements. The most highly hydrated cements are the strongest, have the greatest modulus and show the least deformation at fracture [26]. As the cement ages, the ratio of bound to non-bound water increases. These changes are associated with changes in strength modulus of GI [76].

1.4.5 Setting and maturation reactions - changes in ^{27}Al coordination number

The setting reactions of GICs had been studied using MAS-NMR [77, 78]. The advantages of this technique are it can probe the structure of amorphous glasses and give information on the local environment of selected species and their next nearest neighbors [78].

^{27}Al NMR studies conducted by Stamboulis et al. (2004) have shown that aluminum exhibits three distinct sites at 45-60, 20 and 0 ppm which are attributed to four, five and six-coordinate aluminium ions respectively [78]. As the cement sets, these peaks change in relative intensity, though all three may remain apparent in cements aged for one year [79]. Changes in the relative intensity show that the proportion of Al (IV) decreases on setting and maturation, whilst the proportion of Al (VI) increases. This is consistent with a setting reaction based partly on the elution of aluminum ions from the aluminate tetrahedron within the glass and the formation of 6-coordinate aluminium ions in an octahedral geometry within the matrix. The six-fold coordination sites may be occupied by oxygen atoms in the polyacrylate carboxylate group, fluoride ions and/or water molecules. Four-coordinate aluminium ions remain in the set cement or are formed in the surface layer due to modification [43, 80]. A schematic of four-, five-, and six-coordinate aluminosilicate clusters can be seen in Figure 1.14.

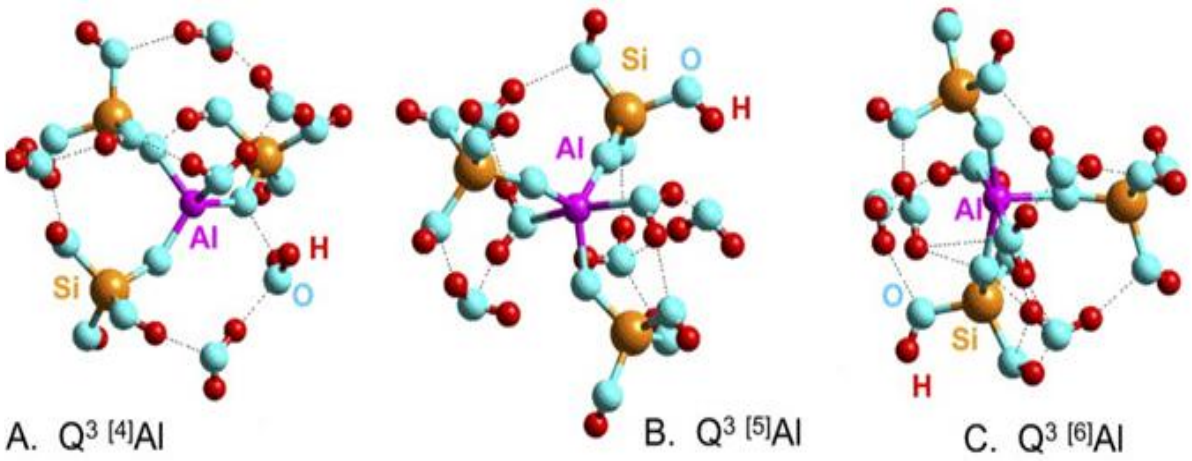

Figure 1.14: A schematic of four-, five-, and six-coordinate aluminosilicate clusters. Each cluster consists of a Q^3 Al surrounded by three Q^1Si and six water molecules. A. $Q^{3[4]}$Al covalently bonded to one H_2O molecule. B. $Q^{3[5]}$Al covalently bonded to two H_2O molecules. C. $Q^{3[6]}$Al covalently bonded to thee H_2O molecules [81]

1.5 Properties of GICs

GICs are dental materials, which are recognised for their unique properties which include: direct adhesion to the tooth structure [23, 81 and 82], biological compatibility [8], low coefficient of thermal expansion and thermal shrinkage [2, 83]. They can be translucent or tooth coloured. Translucency makes them a favored material both for the restoration of front teeth and to cement translucent porcelain teeth to metal posts [1]. They also have anticariogenic properties due to the release of fluoride [1]. A number of studies have also shown that these materials have the ability to release other ions and species with which they are doped [23, 83]. The least attractive property of GICs is their brittleness which narrows the range of possible applications of these materials [23, 2]. Typical fracture toughness values are 0.5-0.6 MPa $m^{1/2}$ which is low compared with composite resins and amalgam filling materials [1, 84].

1.5.1 Biocompatibility

Biocompatibility is defined as the ability of a material to perform with an appropriate host response in specific applications [85]. The biocompatibility therefore is not a property of a material but rather the ability to initiate an appropriate response from its host and vice versa. Whether this response is appropriate will depend on the side of the body it been inserted [7].

For dentistry use, GICs are formulated to meet the need of their specific applications, where they can be used as liners, bases, luting and direct restoration materials. The applications of GICs require them to be in direct contact with enamel, dentine, pulp and gums. The biocompatibility of GICs is therefore a subject of some importance [7]. Since the discovery of the GICs the biocompatibility of GICs has been intensively studied [7, 8]. In general, studies have showed that GICs have good biocompatibility [7, 8]. Pulp response showed slight reaction, which resolved with time if bacterial penetration was avoided [86]. Also, the cytotoxicity of fully set GICs was shown to be minimal [87]. Early studies show that these cements might cause some mild irritation when used as liners [88, 89], but there are no such reports in the more recent literature [87].

1.5.2 Adhesion to mineralised tissue

One of the most important characteristics of the GIC is its ability to adhere to untreated enamel and dentine as well as to bone and base metal [3]. This property allows a conservative approach to restoration, as it provides perfect mechanical attachment without the need of undercutting a healthy tooth. Adhesion of the GIC to the tooth surface also provides a perfect seal, protecting the pulp, eliminating secondary caries and protecting from bacterial microleackage between tooth and cement margin [4].

The full 80% of ultimate bond strength of GI to tooth structure develops within the first 15 minutes following placement [90]. The adhesive nature of these materials is not fully elucidated and it is thought to be a result of two processes [91, 92 and 93].

Initial adhesion, when the cement is applied to a tooth mineral as a fluid, is based upon hydrogen bonding, provided by the free carboxyl groups present in the fresh paste. As the cement sets the hydrogen bonds are replaced by ionic bonds involving ions coming from the cement or the hydroxyapatite phase of the tooth [91, 92 and 93]. Studies carried out by Wilson et al. (1983) showed that during adsorption, polyacrylate carboxyl groups enter the outer layer of the hydroxyapatite, displacing phosphate from the hydroxyapatite surface. Calcium ions are displaced equally with the phosphate ions so as to maintain electrical neutrality. This leads to the development of an ion-enriched layer of cement that is firmly attached to the tooth [94].

The mode of adhesion to dentine is a debatable, as the mechanism postulated above is based principally on adhesion to the apatite. Dentine contains weight fraction of collagen of around 20% w/w. Some have postulated that the absence of adhesion to collagen will explain the overall weaker bonding to dentine [95]. Wilson (1974) considered the bonding to collagen as well as to apatite as it contains both amino and carboxylic acid groups [93], however studies carried out by Jackson et al. (1986) suggested that polyacrylates are not adsorbed on collagen [7].

As the bonding to apatite is thought to be a principal mode of adhesion, the bond strength of the GI to the enamel is better than to dentine. The tensile bond strength of conventional GICs to untreated enamel ranges from 2.6-9.6 MPa. Tensile bond strength to dentine is about half that of enamel and ranges from 1.1-4.5 MPa [85]. These values are relatively low. However, this bond strength is more a measure of the tensile strength of the cement itself, since fractures are usually cohesive within the cement, leaving glass residues attached to the tooth [1].

1.5.3 Physical properties of GICs

The ability of dental material to withstand mechanical forces and perform effectively in the oral environment is probably one of the most important properties that determine its physical excellence. Brittleness is the most unfavorable characteristic of GIs and the resulting susceptibility to fracture and low wear resistance limit GI use in areas subjected to high mastication forces [1].

The testing of the mechanical properties of glass-ionomers and thus their applicability for particular applications are specified by International Organisation for Standardisation (ISO) 9917-1:2003 [96]. Three of the main tests for GICs guided by ISO are (i) working time (ii) setting time and (iii) compressive strength.

1.5.3.1 Compressive strength (CS) of GICs

CS is the resistance of a material to compressive force. CS testing can be used to evaluate the susceptibility of the materials to fracture. The test is prone to high degree of scatter as it is affected by imperfections in the specimen material such as cracks and an uneven surface. Change in CS can be used to approximate the molecular structure of the material [97]. Measuring CS at different time intervals can be used to study the maturation processes in GICs. The CS value required for commercial dental cements applications are specified by ISO 9917 and shown in Table 1.2 below [97].

Table 1.2: Showing compressive strength and Vicker's hardness of Fuji IX and Chemflex cement for different dental applications

Applications	Required CS (MPa)	Typical VHN
Luting	70	40
Restorative	130	60

1.5.3.2 Hardness of GICs

Hardness is defined as the resistance of a material to indentation. A hardness test can be used to evaluate the resistance of material to wear and plastic deformation by penetration [97, 98]. Change in hardness reflects the cure state of a material and the extent of the setting reactions [99, 100].

Various tests exist to measure the hardness of dental materials. The most common are: Knoop, Vicker's, Brinell and Rockwell. Some of the tests have advantages over one another and their suitability is determined by the mechanical properties of the tested material [101, 102 and 103].

The Vicker's and Knoop test are the most suitable for studying GICs and these techniques are the most often quoted in the literature [101, 102 and 103]. The Vicker's hardness of GICs is shown in Table 1.2. Note that the hardness test is not required by ISO in testing GICs so the values shown in the table are typical values quoted in the literature [104, 105 and 106].

1.5.4 Working time

Working time is the time after mixing at which it is still possible to manipulate a dental material without an adverse effect on its properties. Working time represents the time available to the clinician for the facile manipulation of the material, before placing it into tooth cavity [7]. There are two methods described by ISO for determining of working time: the oscillating rheometer and Gillmore needle indention test [96]. Indentation working times for GICs are shown in Table 1.3 below.

Table 1.3: Net working time (s) for Fuji IX and Chemflex cements for different dental applications measured at 23^{o}C

Applications	**Fuji IX**	**Chemflex**
Luting	120	120
Restorative	140	165

1.5.5 Fluoride (F^-) release

Studies show that a number of ions, both complex and simple, are derived from GICs when immersed in an aqueous medium. These species are native constituents of the cement glass that are released when exposed to the acid attack during the setting reaction [107].

F^- release is probably the most widely studied. Its release follows a very distinctive pattern and it involves at least two stages. The initial stage lasts up to 24 hours. It is non-linear with time and is characterised by a rapid burst of fluoride ion release. The initial high release is

likely due to F^- liberated from the glass particles during the setting reactions that entered the cement matrix [5].

Bell et al. (1999) studied the release of F^- from GICs in deionised water for a period of 60 days. They observed that the amount of F^- released during the 24 hours ranged from 15.3-155.2 ppm [6]. In vitro studies confirmed those findings and showed that the amount of F^- released during 24-48 hours and varied between 5-155 ppm [5, 6, 108, 109, 110 and 111].

After the initial stage, the F^- release slows down. The studies conducted by Creanor et al. (1994) showed that the concentration of F^- leached was reduced from 15-155 ppm at day one to about 0.9-4 ppm at day 60 [112]. Forsten (1977) studied F^- release for a period of five years, exposing cement samples to running water for most of this time and periodically determining F^- release over very short time intervals. These studies showed that the F^- release decreases with time and is proportional to the square root of time ($\sqrt{t}$) [113]. The $\sqrt{t}$ linear relationships indicates that the latter phase is diffusion-controlled and follows Fick's law of diffusion [114, 115]. The equation for second Fick's law of diffusion is shown below [113].

$$\mathbf{M_t/M_\infty = 2(Dt/\pi l^2)^{1/2}} \qquad (1.1)$$

Where:

M_t = mass uptake/loss at time t (s)

2l = thickness if the specimen (m)

M_∞ = equilibrium mass uptake/loss (g)

D = diffusion coefficient ($m^2\ s^{-1}$)

1.5.5.1 Biological aspects of F^- release

The antimicrobial effects of F^- on oral bacteria and plaque formation are well documented [117, 118 and 119]. It had been shown that a millimolar concentration of F^- in water can affect a variety of activities in several types of cells [116, 118 and 119].

F^- antimicrobial activity is based on the inhibition of the glycolytic enzyme enolase that takes part in the glycolic pathway. It is thought that F^- deactivates the enzyme by forming a complex with Mg^{2+} that forms part of the enolase molecule thereby inhibiting its activity [9, 120, 121 and 122]. Furthermore, it also has an effect on the bacterial biochemistry by inhibition of phosphatase which is also Mg^{2+} dependent [121]. There are also some indications that F^- generally has an adverse effect on bacterial growth [122].

Forss et al. (1991) have been investigating the F^- effect on *Streptococcus mutans* in plaque grown on GI and composite. Their studies showed evidently the decrease in a day-old *Streptococcus mutans* plaque grown adjacent to GI and composite as the concentration of F^- released from these materials into dental plaque increased [122].

Alongside antimicrobial properties, F^- has an effect on the demineralisation of tooth surfaces. This process is complicated and is based on the ability of F^- to reduce solubility of the apatite phase and to facilitate crystallisation of the mineralisation process [123, 124 and 125]. The fact that such dental restorative materials are able to release F^-, suggests that they may also have caries prevention potential [124, 125, 126 and 127].

1.6 Dental applications of GICs

1.6.1 Tooth structure

From the morphological point of view, the tooth can be divided into 4 main types of tissues which include: tooth enamel, dentine, dental pulp and cementum [128]. Enamel is composed of around 96% inorganic salt, mainly hydroxyapetaite (Ca_{10} $(PO_4)_6(OH)_2$) (with less than 1% w/w organic matter protein and lipids and 4-5% w/w water) however some evidence for the presence of octacalcium phosphate Ca_8H_2 $(PO_4)_6.5H_2O$ at the dentino-enamel junction has been found. Octacalcium phosphate is less stable than hydroxyapatite and can hydrolyse to hydroxyapetite. During this process, one unit cell of octacalcium phosphate is converted into two unit cells of hydroxyapatite. The enamel thickness varies within different parts of the crown, being smallest at the junction between enamel and dentine and highest at the central surface of the crown [129].

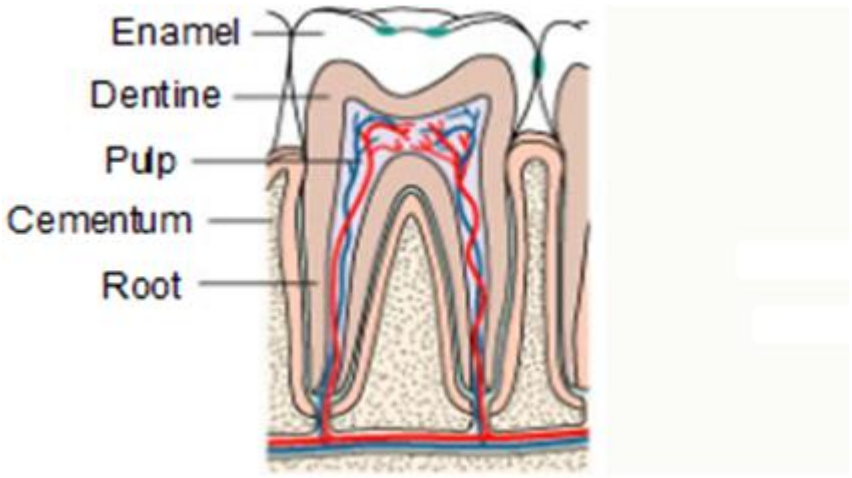

Figure 1.15: Schematic of tooth structure [130]

Dentine is situated below enamel in the crown section and underneath cementum on the root. It provides the bulk of the tooth. By weight, dentine consists of 70% w/w of mineral hydroxyapatite, 20% w/w percent is organic material- hydrated collagen [131] and 10% w/w percent is water [132]. The main characteristics of the dentine morphology are

"microscopic channels", dentinal tubules with diameters of the order of 1-5 μm which radiate outward through the dentine from the pulp to the exterior cementum or enamel border [133]. Tubules play an important role in the development of dentine and also are important for the physiology of dentine where they serve as transport channels for ions and molecules (calcium phospate and matrix gel proteins). These tubules contain fluid and cellular structures [134]. As a result, dentine is permeable which can increase the sensation of pain and the rate of tooth decay [135]. Dental pulp provides the internal structure of the tooth and consists of connective tissues containing nerves and blood vessels. Cementum is the part of the tooth which covers dentine outside the root and consists of a thin mineral layer [128, 131].

1.6.2 Enamel reminaneralisation and demineralisation

The presence of electrolytes (Na^+, K^+, Ca^{2+}, Mg^{2+}, PO_4^{3-}, HCO^{3-}) in the saliva provide a buffering system for acidic food and drinks, whereas thocyanate, peroxides, immunoglobin A, proteins and enzymes protect against antimicrobial attack. Probably the major role of saliva in relation to oral health is its ability to remineralise enamel structure. The driving forces for remineralisation is supersaturation of saliva by calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ions and it occurs when those ions precipitate and form crystals on the enamel.

As previously stated, 96% of the tooth enamel and 60% of dentine is composed of the inorganic salt- hydroxyapatite. This mineral content is highly susceptible to changes in pH in the oral environment. It is has been shown that a decrease of pH to 5.5 is sufficient to initiate the demineralisation processes. The first sign of presence of demineralised tissue is the so called "white spot lesion" [136]. The creation of a spot indicates the loss of mineral content and results in a decrease in surface micro hardness which if not treated might develop into tooth decay. If the buffering and remineralisation processes involving saliva are sufficiently rapid, it might possibly reverse cavity formation. The development of a lesion is therefore dependent of the rate of demineralisation and remineralisation. If remineralisation is not sufficient enough it will lead to caries lesion and if not treated early might effect in loss of a tooth [136].

1.6.3 Dental infections and their causes

Dental infections such as tooth decay and periodontal disease are one of the most common forms of bacterial infection in humans. In developed countries 60% to 90% of school children and the majority of adults are affected by these diseases [137, 138]. In developing countries such as Asia and South America the disease is widespread [139]. There are a number of factors that might contribute to susceptibility to dental caries and its development rate. It may be based upon genetic and socioeconomic factors, dietary habits, and dental hygiene [139, 140]. But the two main factors that cause dental caries are fermentable carbohydrates and caries-causing bacteria [140].

The primary ethological factor for dental caries is dental plaque [141]. Dental plaque in general terms is a diverse microbial community, mainly bacteria situated on the tooth surface, embedded in the matrix of polymers of bacterial and salivary origin [142]. The formation of the plaque on the tooth surface is characterised by progression from a limited number of pioneering microbial species to the complex form of mature dental plaque where bacteria live in very organised form [143]. Plaque formation is initiated by early coloniser bacteria. Early colonisers of the tooth are mainly *Nesseira* and *Streptococci*. These utilise sucrose to synthesise water-insoluble glucan, a sticky matrix, which provides the base for bacterial adhesion [141, 142, 143 and 144]. Bacteria within the biofilm produce organic acids, mainly lactic acid, *via* a fermentation process of carbohydrates. The fermentation of carbohydrates by *Streptococcus mutans* is the principal source of energy production for the organism however lactic acid released during this process significantly decreases the pH of oral environment [143].

As mentioned previously the tooth surface is mainly inorganic mineral. The mineral content of the tooth is very sensitive to changes in pH and a decrease to a pH of 5.5 can substantially dissolve the mineral content of the tooth. If salivary buffering is not sufficient, exposure of the tooth to so low a pH might develop a lesion and if not treated tooth decay [145].

Secondary caries is defined as a lesion at the margin of an existing restoration. It is the primary caries at the margin of an existing filling. Diseases occur in areas of plaque stagnation. For this reason the cervical margins of restorations are commonly affected [146]. Secondary caries are the most commonly diagnosed failure that results in replacement of any type of restoration. For directly placed restorations (resin-based composites and amalgam) the diagnosis of secondary caries accounts for about half of all restoration replacements. The consequence of secondary carries is an additional trauma to the tooth and tooth death [147].

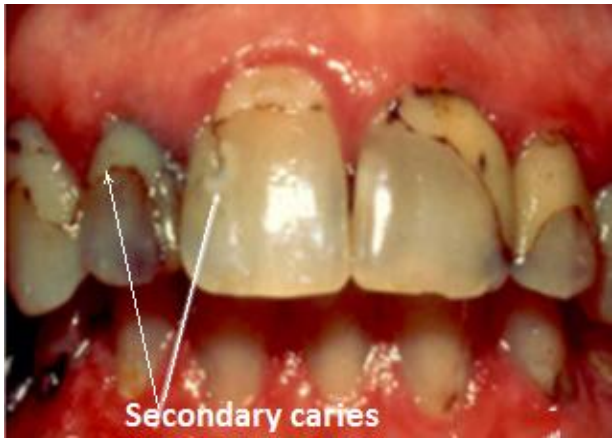

Figure 1.16: Showing secondary caries (photograph obtained from *The Internet Journal of Dental Science* [148])

1.6.4 Postoperative hypersensitivity

Postoperative sensitivity is not uncommon condition. It has been reported that a quarter of all patients, with dental amalgam restoration, experience some degree of postoperative irritation on the second day following restoration [149].

One of the most accepted proposals that accounts for tooth sensitivity is the hydronomic theory and according to it, tooth sensitivity can be triggered by fluid movement within dental tabulates. The main factors that are responsible for that process are dentine drying, heat generated during cavity preparation and bacterial penetration [149, 150]. The latter

might occur when a condition in the pulp is not well diagnosed before preparation of the cavity, especially when replacing an old leaking filling or the removal of secondary carries. Bacteria may be present deep down in the dentine, or in the local necrotic area of a pulp. The lack of any symptoms before a dental procedure might be due to good drainage of the inflammatory exudates thought the open caries lesion or loose filling. The permanent cementing of the inlay will block off the outward drainage causing accumulation of noxious substances in the pulp and will result in discomfort or pain [149, 150 and 151].

1.7 GICs as a reservoir for active species

The ability of GICs to release ions is an indication that these materials may be used as a reservoir for the slow release of organic species. This type of "hybrid GI" cement can be formed by addition of antimicrobial agents to the powder and/or liquid during material production. Such a material may acquire antimicrobial properties that could prevent local bacterial plaque accumulation.

The antimicrobial effect of the "hybrid GI" can occur in two different parts of the tooth:

1) On the outer surface of the tooth at the margin between tooth and restoration. Continuous exposure to oral fluids might facilitate the antimicrobial agent's release. This type of antimicrobial release can be primarily beneficial in the inhibition of secondary caries.

2) The inner surface of the restoration, isolated from the oral environment. The continuous flow of dentinal fluid creates a wet environment, which is conducive to the release of the antimicrobial agents. This type of antimicrobial release can be primarily beneficial in inhibition of secondary caries and postoperative sensitivity.

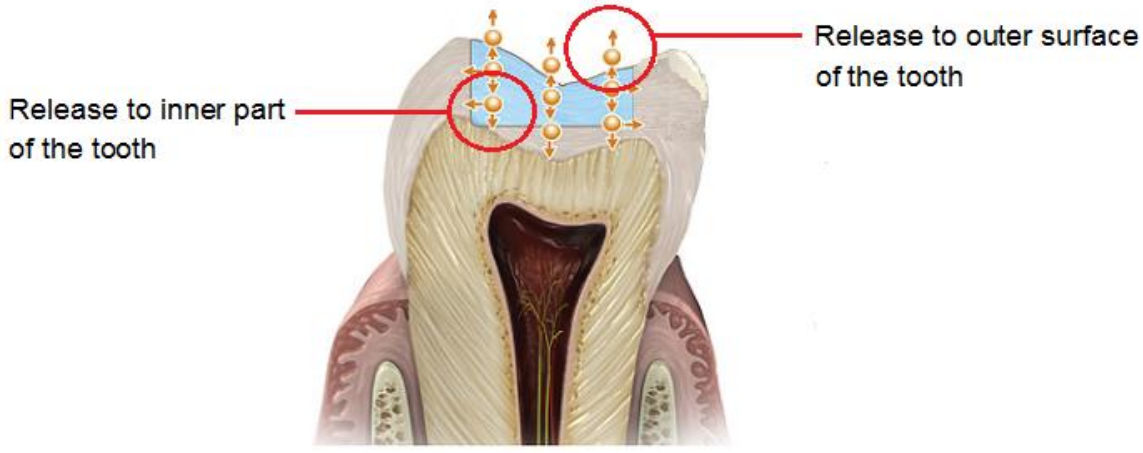

Figure 1.17: “Hybrid GI” (marked in blue) acting as a reservoir to release antimicrobial agent [152]

The ability of a restoration to act as an antimicrobial agent reservoir is mainly dependent on the kind and permeability of the filling material, the type and concentration of the antimicrobial agent and the mechanism for its release. However the effect of such an additive on the mechanical properties must be elucidated in order to be able evaluate whether the material is able to function in the oral environment.

Materials with a therapeutic effect have been subject to major scrutiny recently. Several attempts have been made to exploit these properties by incorporating antimicrobial agents into the cement matrix [10, 11, 12 and 124].

A number of studies have been performed to examine the inclusion and release of organic antimicrobial species [11, 13, 104, 152, 153, 154 and 155]. The use of chlorhexidine (CHX) is probably the most widely studied. Results show that only a small amount of this additive leaches out and its release is controlled by diffusion process [153]. It has also been established that the addition of CHX to GICs affects the mechanical properties of these materials [11, 12, 104, 154 and 156]. The summary of available literature in this topic is shown below in sections 1.7.1 – 1.7.6.3.

In this project two types of commercially available GICs namely: Fuji IX and Chemflex will be tested. Four antimicrobial agents (cetyl pyridinium chloride, benzalkonium chloride, sodium fusidate, triclosan/zinc citrate will be added at different proportions to the tested materials (comprehensive information on additives used is presented in sections 1.8 – 1.8.4.1).

Systematic analysis will be performed to determine the consequence of doping on mechanical properties of these materials. The release kinetics of the antimicrobial species and their effect on leaching properties of other species will be studied. Further the setting and maturation reactions and antimicrobial properties of the antimicrobial agent modified GICs will be tested. A detailed aim and objectives for the current study are presented in section 1.9.

1.7.1 Microbiology studies

Several studies have looked at the antimicrobial efficacy of GICs doped with various bactericides. Türkün et al. (2008) had studied the long term antimicrobial activity of CHX dieacetate and CHX digluconate modified GIC at different weight fractions (0.5, 1.25 and 2.5% w/w) against *Streptococcus mutans*, *Lactobacillus acidophilus* and *Candida albicans* using an agar diffusion plate. These studies showed that all the formulations were effective against the bacterial strains investigated and the inhibition zone was proportional to the fraction of added bactericides. Most of samples showed antimicrobial activity up to 50 days after mixing. The 2.5% group of CHX diacetate showed antibacterial activity up to 90 days [104].

Jedrychowski et al. (1983) looked at the antibacterial activity of GIC combined with CHX gluconate or CHX dihydrochloride at concentrations of 0%, 1%, 2%, 3%, 5% and 10% against *Streptococcus viridans, Streptococcus pyogenes, Streptococcus mutans, Lactobacillus acidophilus and Escherichia coli* using diffusion plate method. These studies showed that the addition of CHX gluconate or CHX dihydrochloride increased the antibacterial activity of GI restorative material [12].

Similar studies were performed by Botelho (2004). He examined the effect of CHX hydrochloride, cetyl pyridinium chloride, cetrimide and benzalkonium chloride added to Fuji IX GIC against *Streptococcus, Lactobacillus and Actinomyces*. The area of inhibition was taken after 24 hours and then was repeated for period of 11 weeks. All antimicrobial doped specimens showed significant bacterial inhibition which decreased at different rates over the test period [154].

Wren et al. (2008) investigated the antimicrobial behaviour of tri-sodium citrate modified GICs at weight fraction of 5%, 10% and 15% w/w. The disc-diffusion method was used against *Escherichia coli*, *Bactericides fragilis* and *Streptococcus epidermidis.* All the tri-sodium citrate modified cements were found to exhibit large inhibition zones against all the bacterial strains, especially the cement containing 15% w/w of tri-sodium citrate against *Escherichia coli* [13].

1.7.2 Mechanical studies

The studies cited above also looked at the effect of additives on the mechanical properties of GIC. These properties included surface hardness and CS. Türkün et al. (2008) studied surface hardness and CS of GIC doped with CHX diacetate and CHX digluconate at different weight fractions. The samples were stored in water for 24 hours and 10 days.

The Vicker's hardness test data showed statistically significant increases in hardness after 24 hours and 10 days. The fraction of dopant also had a significant effect upon the initial 24 hours hardness of the tested specimens. However, at day 10 almost all the tested groups demonstrated hardness values comparable with the control samples. Values for 24 hour conditioned samples varied between 23.55-35.93 VHN for CHX digluconate, 50.46-60.43 VHN for CHX diacetate and 57.23 VHN for the control sample. The 10 day conditioned samples exhibited hardness values of 57.46-63.83 VHN for digluconate, 62.75-63.8 VHN for CHX diacetate and 63.86 VHN for the control.

The CS data for 24 hour conditioned samples showed that most of the doped samples were comparable with control samples with the exception of 1.25% and 5% w/w CHX diacetate where the CS was significantly lower. The CS values ranged between 207.59-219.84 MPa for CHX digluconate, 177.91-213.55 MPa for CHX diacatate and 221.1 MPa for the control samples [104].

Palmer et al. (2004) also looked at the CS of GIC samples doped with CHX acetate a various weight fractions ranging from 0.5% to 13% w/w at one hour and 24 hours of maturation. The results showed that the additives decreased the CS of the samples. The one hour CS of the control sample had a mean value of 226.8 MPa, whereas the sample doped with 11.28% of CHX acetate exhibited CS values of 136.3 MPa. The control sample and samples doped with low concentration showed an increase in CS on maturation (269.9 MPa value for a 24 hour old control sample), whereas the CS of samples doped with higher fractions of dopant decreased with maturation (125.5 MPa for the 24 hours old - 11.28% w/w doped sample) [153].

1.7.3 Release studies

In the studies cited above, Palmer et al. (2004) looked also at the release of CHX acetate using High Performance Liquid Chromatography (HPLC). The pattern of release showed an initial rapid elution of material that leveled off to a constant value. Initial release was functionally linear with square root of time ($\sqrt{t}$) indicating a diffusion process. Within this linear region, the rate of release was comparable. All the measurable CHX was released within 22 days. After 240 days the release was equal to 3% to 5% of the incorporated CHX acetate and was concentration dependent [153].

1.8 Quaternary ammonium salts - general characteristics

Benzalkonium chloride and cetyl pyridinium chloride used in this study are classified as cationic quaternary ammonium compounds. Both of them are well known as effective antimicrobial agents [157]. Due to their low toxicity benzalkoniumm chloride and cetyl pyridinium chloride are commonly used as the antiseptic agents in many pharmaceutical, toiletry and oral hygiene products [158].

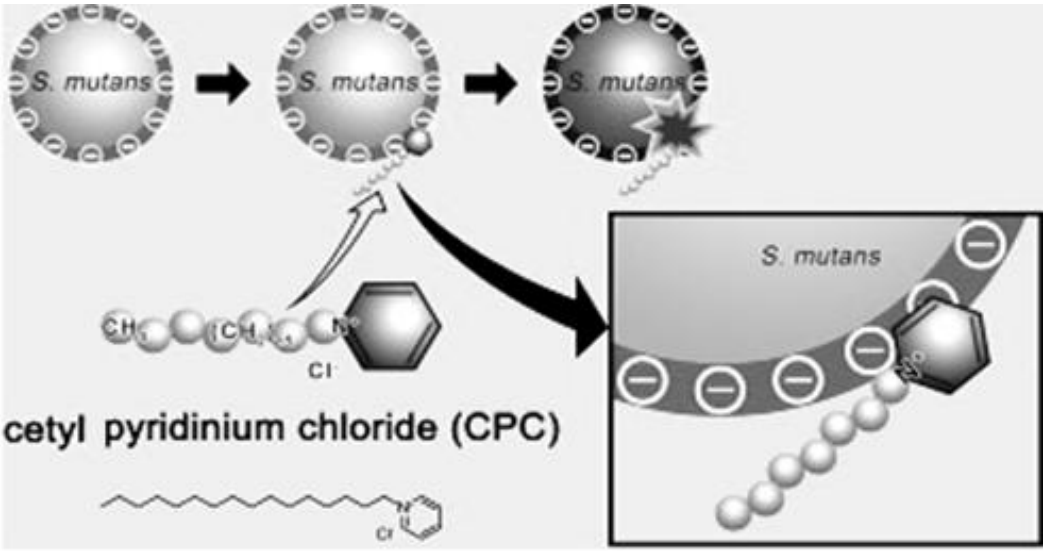

Figure 1.18: The antibacterial action mechanism of cetyl pyridinium chloride [159]

The antimicrobial property of these quaternary ammonium salts arise from the action of the ammonium or pyridinium group. The positively charged nitrogen group is attracted to the negatively charged cell membrane of the bacteria, the consequence of which is that the cell membrane loses its electrical balance and bursts under its own osmotic pressure (Figure 1.18) [159].

1.8.1 Benzalkonium chloride

The formula of benzalkonium chloride is $[C_6H_5CH_2N(CH_3)_2R]Cl$. Its molar mass varies as it is a mixture of alkylbenzyl dimethylammonium chlorides with various alkyl chain lengths from C_8H_{17} to $C_{18}H_{37}$ [17], with C_{12} and C_{14} predominating in pharmaceutical products [160].

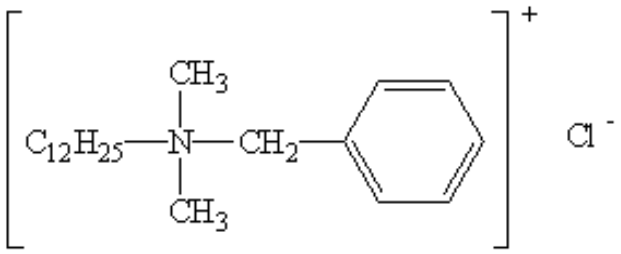

Figure 1.19: The structure of benzalkonium chloride

Benzalkonium chloride has been used for clinical purposes since 1935 as an antimicrobial additive [161]. Its applications include disinfecting instruments and preserving drugs in low concentration. It is also used in hygienic towels and wet wipes as well as to maintain the sterility of a variety of prescribed and over-the-counter products, such as cosmetics, infant care products, and pharmaceutical nasal sprays, ophthalmic solutions, etc. [162]. The Cosmetic Ingredient Review panel concluded that benzalkonium chloride can be safely used as an antimicrobial agent at concentrations up to 0.1% [163].

1.8.2 Cetyl pyridinium chloride

Cetyl pyridinium chloride has the formula $C_{21}H_{38}ClN$ with a molar mass of 358.01 g mol^{-1}. The structure of cetyl pyridinium chloride is shown below in Figure 1.20. Cetyl pyridinium chloride is a broad-spectrum antimicrobial agent with a long history of use to promote gingival health [164, 165].

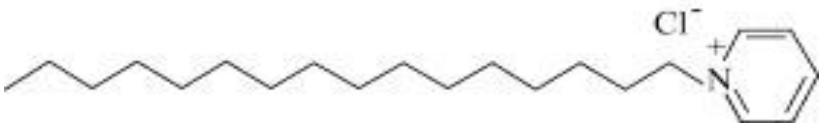

Figure 1.20: The structure of cetyl pyridinum chloride

The US Food and Drug Administration (FDA) Plaque Committee concluded that cetyl pyridinium chloride rinses must be formulated at concentrations of 0.045% to 0.1% cetyl pyridinium chloride with at least 72% to 77% chemically available cetyl pyridinium chloride to be considered safe and effective in an anti-gingivitis/anti-plaque rinse [166]. Cetyl pyridinium chloride applications may include mouthwashes, toothpastes, breath sprays as well as nasal sprays, anti-sore throat sprays, etc. [164, 165, 167 and 168].

1.8.3 Sodium fusidate

The formula of sodium fusidate is [$C_{31}H_{47}O_6Na$] with a molar mass of 538.69 g mol^{-1}. Sodium fusidate is an anionic sodium salt with a steroid-like structure. This antibacterial agent has unique structural features (Figure 1.21) including a tetracyclic ring system with an unusual chair–boat–chair conformation and a carboxylic acid bearing side chain attached by a double bond [169, 170].

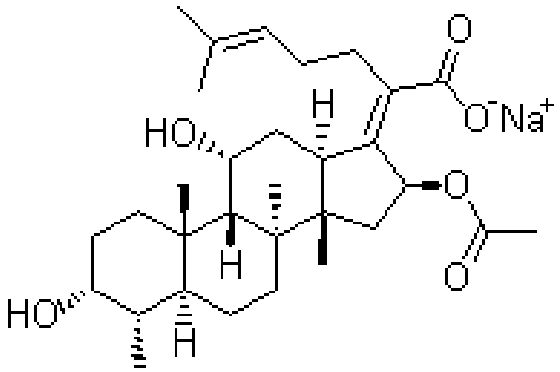

Figure 1.21: The structure of sodium fusidate

This steroid-like structure is responsible for the high penetration of sodium fusidate in tissues and for the absence of cross-resistance and cross-allergy with other clinically used antibiotics, which have made fusidate drugs a highly valuable antibiotic, especially for skin and eye infections [169, 170].

Sodium fusidate is a protein synthesis inhibitor. The antibacterial mode of action of sodium fusidate is through interference with the translocation enzyme and inhibition of the binding of the aminoacyl transfer ribonucleic acid to ribosome [171].

1.8.4 Triclosan

Triclosan is a non-ionic phenolic antiplaque agent. It is used in many skin products, toothpaste and liquid soaps [172]. It has beneficial effects on atopic dermatitis and it reduces eczema [173, 174]. At low concentrations triclosan acts as a bacteriostat, interfering with bacterial fatty acid synthesis. At higher concentrations it is bactericidal as it disrupts the plasma membrane, resulting in leakage of cellular components [175]. Triclosan has the formula $C_{12}H_7Cl_3O_2$ with molar mass of 289.54 g mol^{-1}. The structure of triclosan is shown below in Figure 1.22.

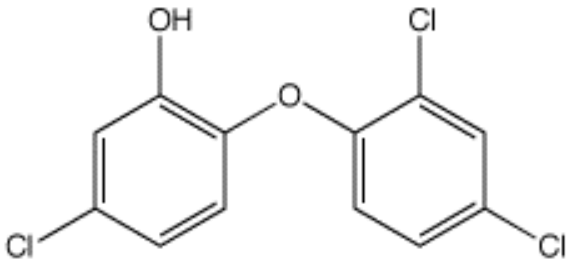

Figure 1.22: The structure of triclosan

Studies have shown that toothpastes containing 0.3% triclosan/copolymer have a moderate effect on inhibiting plaque accumulation [176] and that daily use may improve healing

following non-surgical treatment for advanced periodontal disease [177]. The use of triclosan is regulated by both the U.S. Food and Drug Administration and by the European Union. Triclosan's concentration used in toothpastes and other toiletry products is 0.3%, whereas mouthwashes 0.15% [178].

1.8.4.1 Triclosan and zinc citrate

Although triclosan is extensively used in cosmetic and household chemicals its poor solubility limits its applications. Zinc salts have also been shown to have a moderate inhibitory effect against plaque and gingivitis [179, 180 and 181]. Studies show that toothpastes containing triclosan combined with a zinc salt, inhibit plaque and supragingival calculus formation and have an effect in controlling development of the gingivitis [180, 181 and 182]. The combination of triclosan with zinc salt used in mouthwashes shows reductions in plaque formation and gingivitis control [183, 184 and 185]. Additionally dentifrice based on zinc citrate and triclosan inhibited plaque accumulation significantly more than either agent alone [185]. Traditionally the formulation of mouthwashes containing triclosan and zinc salts included alcohol as a solvent [184], however studies carried out by Schaeken et al. (1994) showed that mouthwashes based on other solvent systems then alcohol i.e. hydroalcoholic or aqueous has a similar preventive effect on gingivitis and supragingival plaque like the one based on alcohol [186].

In this project the combination of formulations of triclosan/zinc citrate were prepared to determine if the formulations containing zinc citrate in fact increases the antimicrobial action of the triclosan/zinc citrate formulation.

1.9 Aims and Objectives

The ability of GICs to release fluoride and its anti-cariogenic properties led to the conclusion that these materials can potentially be used as a slow release delivery system of antimicrobial agents. Several attempts have been made to develop GICs with antibacterial properties by the addition of bactericides such as chlorhexidine [10, 120] and tri-sodium citrate [13]. Studies showed that the addition of these products results in changes in setting reactions and mechanical properties of GICs [12, 152]. To date, simple tests such as Gillmore needle or mechanical testing have been used to investigate setting reactions [104]. Recently, Magic Angle Spinning-Nuclear Magnetic Resonance (MAS-NMR) was used to study the setting and maturation processes of GICs. Studies show that ^{27}Al coordination shifts from predominantly four (in the glass) to predominantly six (in the matrix) on maturation [77, 79].

The aim of the current study was to evaluate the effect of four antimicrobial agents, benzalkonium chloride, cetyl pyridinium chloride, sodium fusidate and triclosan/zinc citrate at different weight fractions of doping on setting and maturation processes of two commercially available GICs. A number of tests which included compressive strength and surface hardness were performed to determine the consequence of doping on mechanical properties of these materials. Also the effects of antimicrobial inclusion on maturation kinetics were evaluated using MAS-NMR. Gillmore needle testing was employed to investigate the setting reactions and the effect of the additives on these processes, also the effect of additives on water processes was evaluated using a desiccating system. The influence of additives on fluoride release was studied using a fluoride ion selective electrode. In parallel with this work the release kinetics of all additives was determined using UV, HPLC-UV and LC-MS. Finally, agar-disc diffusion tests were performed to determine antimicrobial properties of the bactericide's modified samples.

The aim of the current study was to develop further understanding of the setting and maturation processes occurring within GI dental materials when antimicrobial compounds are added as well as to evaluate the leaching processes of these bactericides. The findings of the current work might be useful to consider when GICs will be extended into bactericide modified cements.

1.9 References

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MATERIALS AND METHODS

2.1 Materials

The following Materials and Methods Chapter contains all the materials and techniques used during this project. There is a common experimental procedure section for all experiments. For experimental determination procedures that differed, additional procedures were specified.

Materials

Fuji IX: A strontium based tooth coloured glass-ionomer restorative material consisting of fluoro-aluminosilicate glass powder and 5% poly (acrylic acid) powder. The powder is mixed with poly (acrylic acid) 40-45% m/v (liquid) to produce a material which adheres to dentine and enamel producing tightly sealed cementations.
Dosage- 3.6 g powder/1.0 g liquid
The same powder/liquid ratio proposed is for testing purposes according to International Organisation for Standardisation (ISO) 9917:1991
Manufacturer-GC Co., Tokyo, Japan

Chemflex: A strontium based tooth coloured glass-ionomer luting material consisting of fluoro aluminosilicate glass powder (powder).
Dosage- 3.8 g powder/1.0 g liquid
The same powder/liquid ratio proposed is for testing purposes according to ISO 9917:1991
Manufacturer- Dentsply Ltd, Konstanz, Germany

Cetyl pyridinium chloride: A white powder, MW of 358.01 g mol^{-1}, > 99% of purity, melting point of 80-83^{o}C.
Manufacturer-Sigma, Dorset, UK

Benzalkonium chloride: A white powder, MW of ~ 390.00 g mol^{-1}, > 98% of purity, melting point of 34-37^{o}C.
Manufacturer- Sigma, Dorset, UK

Sodium fusidate: A white powder, MW of 538.69 g mol^{-1}, ≥ 98% HPLC grade.
Manufacturer- Sigma, Dorset, UK

Triclosan: A white powder, MW of 289.54 g mol^{-1} , ≥ 97% HPLC grade, melting point of 56-58^{o}C.
Manufacturer- Sigma, Dorset, UK

Zinc citrate: A white powder, MW of 610.40 g mol^{-1}, > 97% HPLC, melting point of 334^{o}C.
Manufacturer- Sigma, Dorset, UK

Solvent

HPLC grade water: MW of 18.01 g mol^{-1}, traces of ionic impurities <= 10 ppb, optical absorbance (au): 210-240 <= 0.01, 240-280 <= 0.005.
Manufacturer- Fisher Scientific, Loughborough, UK

Broth

Brain Heart Infusion broth: Manufacturer-Oxoid, Unipath Ltd. Basingstock, England

Bacterial medium

Streptococcus mutans*:* A strain ATCC 35664 (culti-loops).
Manufacturer- Oxoid, Unipath Ltd. Basingstock, England

Reagents

Sulphuric acid: A clear viscous liquid, MW of 98.08 g mol^{-1}, > 98 % H_2SO_4.
Manufacturer- Spectosol, Leicestershire, England

Acetonitrile: A clear liquid, MW of 41.05 g mol^{-1}, > 99.9% H_2SO_4.
Manufacturer- Fisher Scientific, Loughborough, UK

Methanol: A clear liquid, MW of 32.02 g mol^{-1}, > 95.0% CH_3OH.
Manufacturer- Fisher Scientific, Loughborough, UK

Orthophosphoric acid: A clear liquid, MW of 98.0 g mol^{-1}, > 85.0% H_3PO_4.
Manufacturer- Fisher Scientific, Loughborough, UK

2.2 Methods

The methods of the cement preparation for analysis are given it this section. Two groups of cement were formed, Fuji IX and Chemflex. In each of the groups, control samples and samples with 1%, 2%, 3% and 5% w/w of additives were prepared. In all cases additives were incorporated on weight fraction (w/w) bases so that for Fuji IX, 1% corresponded to 0.0101 g in 0.101 g of overall weight of powder and liquid, for 2% it corresponded to 0.0202 g in 0.101 g of overall weight of powder and liquid, for 3% it was 0.0304 g and for 5%, 0.0506 g. For Chemflex, 1% corresponded to 0.0096 g in 0.0960 g of overall weight of powder and liquid, for 2% it was 0.0192 g, for 3%, 0.0288 g and for 5%, 0.0480 g. To simplify w/w will be omitted. The methods of their preparation are described below.

Method for preparation of Fuji IX control samples

0.7920 g of Fuji IX cement powder was weighed on Dentsply paper pad using an analytical balance. Balance of weighed powder was returned to zero and 0.2200 g of Fuji IX cement liquid was place on it. Excess powder was removed using a stainless steel spatula. Dentsply paper with weighed powder and liquid was then placed on the glass slab and then mixed through for about 30 seconds until a uniform mass was obtained and no free powder remained. The freshly mixed cement paste was then transferred into 5 separate stainless steel moulds (4 mm diameter (± 0.1 mm) and 6 mm depth (± 0.1 mm)). The cements then were clamped between two stainless steel slides and allowed to cure in an incubator at 37°C for one hour. After one hour, the prepared specimens were unclamped and removed from the stainless steel moulds. Further specimen treatments differ for each determination and will be described in the corresponding sections.

Method for preparation of Chemflex control samples

The method of preparation was identical to the method explained above for Fuji IX material, except that 0.7600 g of Chemflex cement powder and 0.2000 g of liquid was used.

Method for preparation of doped cement samples

For the Fuji IX specimens containing 1%, 0.0101 g of cetyl pyridinium chloride was weighed, as for the glass powder, and incorporated into the cement paste at the mixing stage. For 2%, 0.0202 g of cetyl pyridinium chloride was used, for 3%, 0.0304 g and for 5%, 0.0506 g was used. For the Chemflex specimens, the masses of additive were 0.0096 g, 0.0192 g, 0.0288 g and 0.0480 g for 1%, 2%, 3% and 5 % levels of addition respectively. The detailed descriptions of each prepared formulation are given in Table 2.1.

Table 2.1: Composition of Fuji IX and Chemflex specimens for each of the formulations

Material	% of additives	Mass of cement powder/g	Mass of additive/g	Mass of zinc citrate/g	Mass of liquid/g
Fuji IX	0	0.7920	0.0000	-	0.2200
	1	0.7920	0.0101	-	0.2200
	1	0.7920	0.0101**	0.0101	0.2200
	2	0.7920	0.0202*	-	0.2200
	3	0.7920	0.0304	-	0.2200
	3	0.7920	0.0304**	0.0304	0.2200
	5	0.7920	0.0506*	-	0.2200
Chemflex	0	0.7600	0.0000	-	0.2000
	1	0.7600	0.0096	-	0.2000
	1	0.7600	0.0096**	0.0096	0.2000
	2	0.7600	0.0192*	-	0.2000
	3	0.7600	0.0288	-	0.2000
	3	0.7600	0.0288**	0.0288	0.2000
	5	0.7600	0.0480*	-	0.2000

* applicable to cetyl pyridinium chloride, benzalkonium chloride and sodium fusidate only

** applicable to triclosan only

2.3 Working time determination

The test employed in these studies was Gillmore needle indentation test. The Gillmore apparatus consists of an indenter needle (28 g) assembled into a rotary standard. The needle is lowered vertically to the surface of a specimen and left for 5 seconds. A test is performed every 15 seconds until needle fails to make complete circular indentation in the cement [1, 2].

2.3.1 Sample testing

Prior to mixing the glass powder and liquid, a stop watch was set up. After around one minute the specimen was unclamped and one of the microscope slides was removed from one side of the specimen. The specimen was placed centrally under the Gillmore needle with slide free side facing the needle. Next, the needle was slowly positioned on the surface of tested samples. The sample was tested every 15 seconds until needle failed to leave an impression on the surface of the specimen. The tests were performed three times for each formulation. Each sample was tested separately. The temperature of the room was 21^{o}C (± 1^{o}C) and was monitored using standard lab thermometer.

Figure 2.1: Image of Gillmore needle used for this work

2.3.2 Statistical analysis

Average working time for each formulation and its standard deviation was calculated at each interval and it was expressed in seconds. Student's t-test was performed to determine levels of significance between working times of control and doped specimens ($p < 0.05$).

2.4 Mechanical testing instrumentation

Compressive strength (CS)

CS is the most routinely used method of determination of strength of dental cements [3, 4]. The test is performed on the Universal Testing Machine. This consists of a compressive plate coupled to the load cell. The analysis is performed by applying an axial force to the flat ends of a cylindrical specimen at a constant rate. The force applied at moment of material failure is measured and recorded by an attached computer. CS is calculated by dividing the maximum load at failure by the original cross-sectional area of a specimen in a compression test and that can be calculated from equation below [1].

$$\sigma = F/A \qquad (2.1)$$

Where:

σ = stress

F = load applied (N)

A = area (m^2)

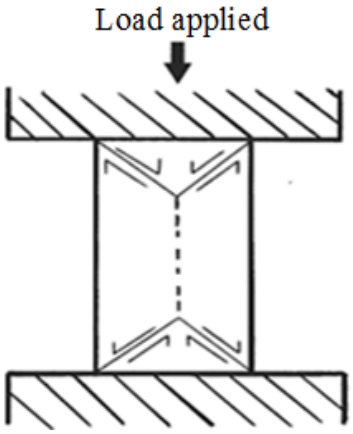

Figure 2.2: Schematic representation of compressive strength test [5]

Surface hardness

Surface hardness is not specified by ISO in testing glass-ionomers. However, surface properties of material are also determinant factors of material performance in the oral environment as it can influence the material polishing ability, scratching occurrence and materials resistance to load. The usual procedure involves the measurement of the depth or area of an indentation left by an indenter [6, 7].

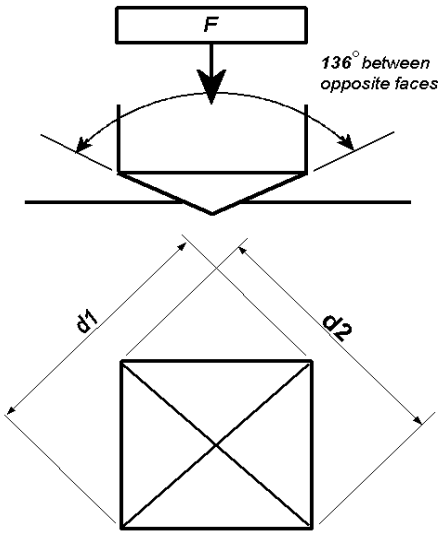

Figure 2.3: Showing Vicker's hardness diamond indenter [9]

In the study Vicker's hardness test was employed. Vicker's tester is equipped with a 136 degree pyramid-shape, square base diamond indenter. The test is performed by applying a constant load of 0.98 to 980 N to the material being tested. The load is normally applied for 10 to 15 seconds and the lengths of two diagonals of the indentation left in the surface of the material, after removal of the load, are measured using a microscope (Figure 2.3). The Vicker's hardness number is determined from the equation below [1, 8].

$$\mathbf{VHN = 1.854(F/d^2)} \qquad (2.2)$$

Where:

F = load (N)

d = arithmetic mean of the two diagonals, *d1* and *d2* (mm)

VHN = Vicker's hardness number

2.4.1 Preparation of the specimens for mechanical testing

For mechanical studies, all formulations of specimens described in section 2.2 were prepared. After one hour in the oven, the prepared specimens were unclamped, removed from the stainless steel moulds and placed into separate plastic containers containing 5 ml HPLC grade water. The specimens were stored in solutions for appropriate conditioning times. The conditioning period for each determination are given in Table 2.2. After conditioning period, the specimens were removed from the storage medium prior to the test and dried with tissue. The surface hardness test followed by the compressive test was carried out on each of the specimen.

Table 2.2: Conditioning period for particular dental material formulations

Additive	Material	Conditioning period				
		24 hours	1 week	3 weeks	5 weeks	7 weeks
Fuji IX	Cetyl pyridinium chloride	x	x	x	x	x
	Benzalkonium chloride	x	x	x	x	x
	Sodium fusidate	x	-	-	-	x
	Triclosan + (zinc citrate)*	x	-	-	-	x
Chemflex	Cetyl pyridinium chloride	x	x	x	x	x
	Benzalkonium chloride	x	x	x	x	x
	Sodium fusidate	x	-	-	-	x
	Triclosan + (zinc citrate)*	x	-	-	-	x

* valid for triclosan formulations containing additional zinc citrate

2.4.2 CS determination

CS was evaluated according to ISO 9917 [10]. The tensile strength tester (Hounsfield, H50kM1614 and the 5000 N load cell Hounsfield 308524, software QMat, Tinius Olsen) was used. Specimen was placed with a flat end up between the plates of the Universal Testing Machine. A compressive load along the long axis was applied at a crosshead speed of 1 mm min^{-1}. The maximum load applied before failure was determined and recorded in MPa.

2.4.3 Surface hardness determination

For the surface hardness measurements a micro hardness tester (Buehler, high quality hardness tester, 1600-6125) was used. The test specimen was positioned centrally below the indenter, a 0.98 N load was applied through the indenter with a dwell time of 10 seconds. Four indentations were made, two on each flat side of specimen at different location, away from the edge, and well away from each other. The size of the diamond pyramid indenter diagonals was measured using a microscope fitted with a graticule and the data were converted to Vicker's hardness numbers (VHN).

2.4.4 Data analysis

The average CS and surface hardness of each formulation and its standard deviation was calculated at each time interval, and expressed in MPa and VHN. Results were statistically analysed by Student's t-test at a significance level of $\alpha = 0.05$ and df = 4-8.

2.5 Water loss studies

Water loss studies were performed using a combination of analytical techniques. An analytical balance was used to determine water loss of specimens. The analytical balance used, consisted of a high precision (0.0001 g ± 100 μg) measuring pan enclosed within glass cabin with doors.

The desiccating conditions were established in a sealed glass desiccators containing concentrated sulphuric acid (H_2SO_4). The desiccator contained a metal mesh that provided a surface for samples and protected samples from direct contact with desiccating agent. The desiccating agent used in the studies was concentrated H_2SO_4. In a sealed desiccator, this gives at atmosphere of approximately 1% relative humidity.

Figure 2.4: Desiccating chamber

2.5.1 Preparation of specimens for water loss testing

For water loss studies, formulations containing 5% w/w of additive were prepared. The method of sample preparation is described in section 2.2. Fresh samples were then exposed to further treatments described in section 2.5.2

2.5.2 Water loss testing

Immediately after weighing, samples were transferred onto plastic weighting boats and placed in the desiccator. The water loss testing was performed by removing samples from the storage medium and weighing them using an analytical balance at 30 minutes, 1, 2, 3, 4, 5 hours and 24 hours. Testing was carried out weekly, until equilibrium was achieved.

2.5.3 Data analysis

The average mass for each formulation and its standard deviation was calculated at each time intervals and expressed in grams.

Percentage water loss

Percentage mass loss was calculated using equation below.

$$\textbf{Mass loss} = (M_\infty/M_t)*100 \qquad (2.3)$$

Where:

M_∞= mass lost at $_\infty$ time

M_t = mass at t time

2.5.4 Percentage water loss - statistical data analysis

Percentage water loss of water results were statistically analysed by Student's t-test at a significance level of $\alpha = 0.05$ and df = 4-8.

2.5.5 Diffusion graphs

Diffusion graphs for mass of water lost were plotted in terms of M_t/M_∞ against time $\sqrt{t}$ (s).

Where:

M_t = mass of analyte at time t (g)

M_∞ = mass of analyte at time infinity (g)

$\sqrt{t}$ = square root of time in (s)

Straight line graph was fitted into plotted data and straight line equations with their correlation coefficients were determined.

2.5.6 Diffusion coefficient

The diffusion coefficient of water was determined from the linear portion of the graphs, taking the slope and substituting into the equation below:

$$\mathbf{D = s^2\, \pi l^2/4} \qquad (2.4)$$

Where:

D = diffusion coefficient ($m^2\ s^{-1}$)

S^2 = square of diffusion graph slope

π = constant of a value of 3.15

l^2 =square of half thickness of the specimen (m^2)

2.6 MAS-NMR - determination of additives effect on ^{27}Al coordination

MAS-NMR – general characteristics

Magic Angle Spinning-Nuclear Magnetic Resonance (MAS-NMR) spectroscopy is an analytical technique used to elucidate information on the topology and three-dimensional structure of molecules [11]. A number of studies report use of MAS-NMR to elucidate the structure of dental materials [12, 13 and 14]. MAS-NMR can provide information on the structural changes of the particular element and its local environment in the material. Due to its specificity the technique has been employed to investigate the setting reactions of glass-ionomer cements [14].

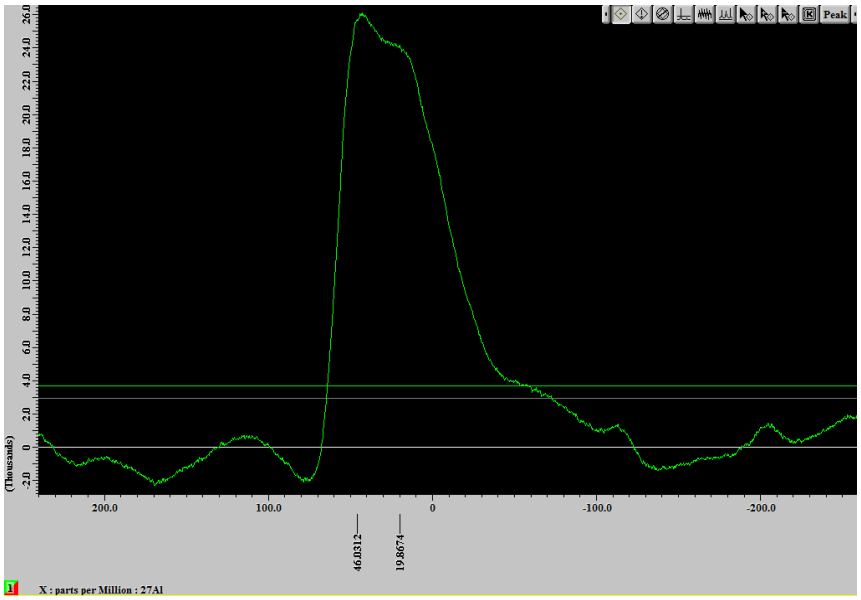

Figure 2.5: Solid state ^{27}Al MAS-NMR spectrum generated during this work for Fuji IX with no additive

2.6.1 Preparation of samples for ^{27}Al MAS-NMR studies

For ^{27}Al MAS-NMR studies control samples and samples doped with 5% weight fraction of cetyl pyridinium chloride (CPC) and benzalkonium chloride (BACH) were prepared. The method of the sample preparation for this determination differs from the method presented in section 2.2 in terms that samples were not cured in the oven. Instead, they were placed into individual plastic containers for 15 minutes, 1 hour, 5 hours, 24 hours, 1 week, 1 month and 4 months. Samples were stored at room temperature and ambient humidity. After the end of each storage time the specimens were removed from the storage plastic containers, placed into 25 ml glass container and approximately 15 ml of liquid nitrogen was poured into it. Once bubbling had stopped, samples were covered with cling film, pierced and placed into freeze-drier vacuum at -20^{o}C for 48 hours. After 24 hours samples were removed from freeze-drying and placed into desiccators under dinitrogen pentoxide (N_2O_5) environment for testing. Prior to testing, the samples were ground into a fine powder using pestle and mortar.

2.6.2 Sample testing

Samples were analysed using Jeol Eclipse, 300 MHz FT NMR spectrometer, incorporating SH30T6/HS solid state probe. Samples were packed into 6 mm diameter zirconium oxide rotors and spun at 78 MHz at the so-called magic-angle. The relaxation delay was set to 0.2 seconds, the number of spectral accumulations was 2000 and the 90^{o} pulse width was 4 μs. Samples were referenced with respect to a solid state spectrum of aluminium chloride hexahydrate ($AlCl_3.6H_20$). Obtained spectrums were processed using Delta software.

2.6.3 Assignment of chemical shift

^{27}Al chemical shift was determined from the highest point on the spectrum peak. The position of the peak was determined at the highest point in the spectrum, and quoted relative to $AlCl_3.6H_20$. Assignments were made using previously determined values as quoted in the literature [11].

2.6.4 Kinetics determination

The ratios of conversion of Al (IV) into Al (VI) were determined for studied aging times using equation below. The calculated ratios for control specimens were compared with ratios obtained for doped samples.

$$\textbf{Ratio = height of Al (VI) peak)/height of Al (IV) peak} \qquad (2.5)$$

2.7 Electrochemical determination of fluoride release

The fluoride electrode used in this experiment consisted of a membrane of a single lanthanum fluoride crystal, bonded into a glass of epoxy body. It is 100% selective for fluoride ions and is only interfered with by OH^- ions. The reproducibility of electrode measurements is ± 2% and it is concentration independent. Due to fluoride ion ability to form complexes with H^+ as well as other multivalent cations, it is advised to use Total Ionic Strength Acid Buffer (TISAB 3) decomplexing agent during fluoride analysis.

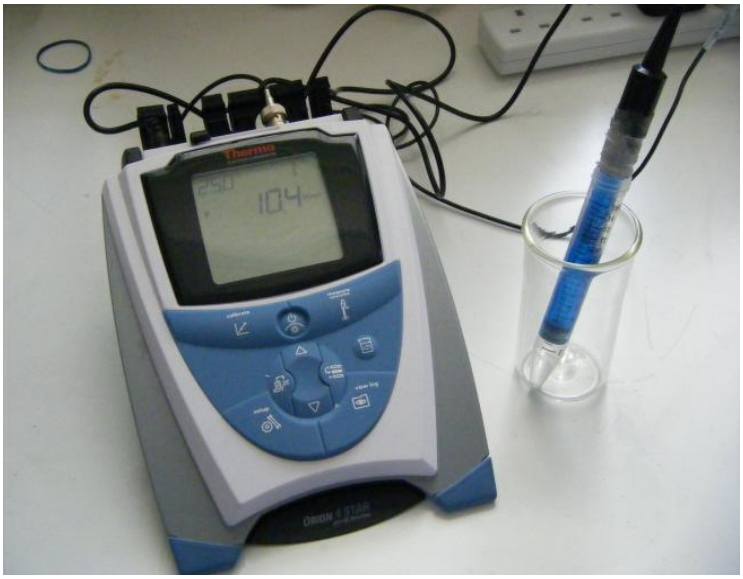

Figure 2.6: The pH/mv meter and an ion selective electrode used for this work

This decomplexes any associated fluoride complexes, e.g with Al^{3+} or H^+ [15, 16]. A potential is created the between the reference electrode and the ion selective electrode. The external electrode is immersed in the test solution, whereas the internal reference electrode is in a specific solution that donates ions of opposite charge to the tested ions. Both electrodes are connected to an external wire. Build up of ions on the membrane is compensated by opposite charged ions on the reference electrode becoming neutralised by reaction with electrode wire and electrons are forced through the external wire to the voltmeter [15, 16]. Concentration of unknown is determined from a slope of a graph.

Calibration graph is constructed from the response of a series of known solutions. Typical graph is plotted as the signal (potential) versus the log concentration of the analyte. Typical calibration curve of an ion selective electrode is shown below in Figure 2.7.

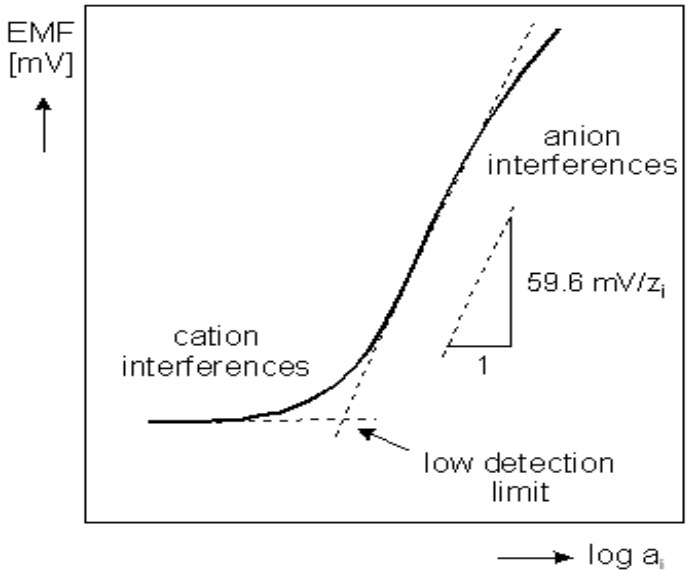

Figure 2.7: Calibration curve of the ion selective electrode [17]

2.7.1 Preparation of fluoride standards for calibration

Standard sodium fluoride (NaF) solutions of concentration of 1000 ppm, 100 ppm, 10 ppm, 1 ppm and 0.1 ppm were prepared using standard dilution method. For initial 1000 ppm of fluoride, 0.2210 g of NaF powder was weighed on the analytical balance, transferred into a 100 ml volumetric flask and filled up to the mark with HPLC grade water.

2.7.2 Calibration of fluoride meter

The meter was calibrated by placing fluoride electrode into lowest NaF standard until reading stabilised. Procedure was repeated with other standards following an ascending sequence. Note that automated meter was used for these studies which generated and stored slope. As the effect all obtained measurements were quoted directly in ppm.

2.7.3 Preparation of the specimens

For fluoride release study all formulations of specimens described in section 2.2 were prepared. Fresh samples were placed into separate plastic containers containing 5 ml HPLC grade water. Samples were placed into solution prior testing.

2.7.4 Testing of fluoride release

The release of fluoride was determined using Cole-Parmer fluoride ion selective electrode with pH/mv Orion 4 star meter (Thermo Electronic Corporation). The release of fluoride was measured every 15 minutes for an hour and then at 1, 2, 3, 4, 5 and 24 hours, and at 1, 3, 5 and 7 weeks. Determination was done by immersing the electrode into the test solution medium for an appropriate time, until the reading stabilised.

2.7.5 Data analysis

Prior analysis the response of meter was measured against standards and all results were corrected against obtained slope. Average of fluoride release for each formulation and its standard deviation was calculated at each time intervals and expressed in ppm.

2.8 Spectroscopic/spectrometric instrumentation - release of active species

In this section, the techniques used to determine release of active species will be explained. Release of sodium fusidate (SF) was tested by High Performance Liquid Chromatography-Ultraviolet (HPLC-UV) spectroscopy, whereas triclosan (T) release was determined by Liquid Chromatrograpy-Mass Spectrometry (LC-MS). Release of CPC and BACH evaluated using Ultraviolet (UV) spectroscopy. Techniques used were verified during preliminary tests to achieve appropriate detection of the studied material.

Most of sample the cells are small rectangular glass or quartz containers. The sample is dissolved in solvent while reference cell contains only solvent. Solvent type is chosen so that it does not absorb any significant amount of light in the wavelength range of interest (200-800 nm) [18, 19 and 20]. The intensities of the light that leaves the sample and reference cells are detected, subtracted and absorption calculated [20]. Performance of a quantitative analysis requires the preparation of a calibration curve. Concentration of analyte is calculated from calibration curve.

2.8.1 Preparation of standard solutions

SF standard solutions of concentration between $1x10^{-3}$- $8x10^{-6}$ mol L^{-1} were prepared using standard dilution method. For initial $1x10^{-3}$ mol L^{-1} of SF 0.539 g of powder was weight on the analytical balance, transferred into a 1000 ml volumetric flask and filled up to the mark with HPLC grade water. Preparation of standards solutions of T, CPC and BACH involved similar procedures, as for SF, however 0.289 g of T, 0.358 g of CPC and 0.390 g BACH was taken for preparation of initial solutions.

2.8.2 Preparation of the specimens

For the bactericide release studies, all formulations of the specimens described in section 2.2 were prepared. Fresh samples were placed into separate plastic containers containing 5 ml of HPLC grade water. Samples were placed into solution prior to testing.

2.8.3 Testing of release of active species

Release of SF

SF release studies were performed using HPLC-UV spectrophotometer (Agilent technologies 1100 series) with software (Acol). C_{18} chromatographic column 7.5 mm x 4.6 mm was used (Phenomenex 347700) with particle size of 5 μm. Mobile phase used was a mixture of acetonitrile, methanol and 0.01 M orthophosphoric acid with ratio of 5/2/3 relatively. Samples were analysed isocratically with mobile phase flow rate of 1 ml min^{-1} and injection volume of 10 μL.

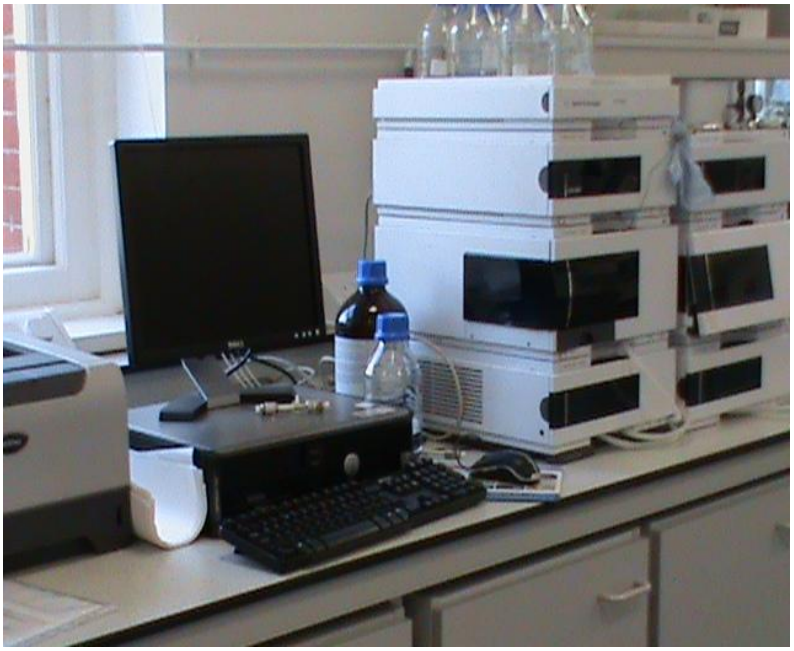

Figure 2.8: HPLC-UV spectrophotometer used for this work

20 μL of each studied sample was taken from sample container using 20 μL pipette. Samples were placed into 50 μL glass vials, sealed with rubber lead and placed into HPLC tray cell. The instrument was calibrated using SF standards prior to the experiment. Samples were analysed at 24 hours and seven weeks. The release of SF was determined at 235 nm.

Release of T

T release studies were performed using LC-MS spectrometer (Agilent technologies) with software (X-Calibur). 5.0 x 3.0 mm C_{18} chromatographic column was used (Phenomenex 129183-2) with particle size of 5 μm. Mobile phase used was a mixture of acetonitrile and water with ratio of 3/1.

Samples were analysed isocratically with mobile phase flow rate of 0.2 ml min^{-1} and injection volume of 100 μL. 200 μL of each studied sample was taken from sample container using 500 μL pipettes. Samples were placed into 500 μL glass vials, sealed with rubber lead and placed into LC-MS cell tray. The instrument was calibrated using T standards prior and the end of the experiment. Samples were analysed at 24 hours and seven weeks. Selected Ion Recording (SIR) method was used with analysis set into monitoring negative ion of 286.9 m/z.

Release of CPC and BACH

The release of CPC and BACH active species was determined at appropriate time intervals using a UV spectrophotometer (Hewlett Packard 8453) with software (Agilent Technologies). The instrument was calibrated using CPC and BACH standards prior the experiment. 1 ml of each studied sample was taken from the sample container using 5 ml syringe. Samples were poured into quartz vials and placed into tray cell. Samples were analysed every 15 minutes for the first hour and then at 1, 2, 3, 4, 5 and 24 hours, and at 1, 2, 3, 4, 5, 6 and 7 weeks. The release of cetyl pyridinium chloride and benzalkonium chloride was determined at 259 nm and 214 nm relatively.

2.8.4 Determination of bactericide concentration

The concentration of bactericide was determined from the slope of the calibration graph. Average mass for each formulation and its standard deviation was calculated at each time interval and expressed in mol L^{-1}.

2.8.5 Determination of bactericide mass

Released mass (M_{rel}) of particular bactericide was calculated from equation below and expressed in g.

$$\mathbf{M_{rel} = Conc*Rmm} \quad (2.6)$$

M_{rel} = mass released (g)
Conc = average concentration measured at time ∞ of each sample set (mol L^{-1})
Rmm = relative molar mass of particular analyte (g mol^{-1})

2.8.6 Determination of expected and released mass bactericide

From calculated averages of mass, the expected mass (M_{exp}) was calculated using equation below and expressed in g.

$$\mathbf{M_{exp} = (\Sigma_{mass}/5)x\%} \quad (2.7)$$

Where:
M_{exp} = mass expected (g)
(Σmass/5) = average mass of each sample set (g)
% = percentage of doping (%)

2.8.7 Determination of bactericide's recovery

Percentage recovery was calculated using equation below:

$$\mathbf{Recovery = (M_{exp}/M_r)*100} \quad (2.8)$$

Where:

M_{exp} = mass expected (g)

M_r = mass released (g)

2.8.8 Diffusion graphs

Diffusion graphs for bactericide release were plotted in terms of M_t/M_∞ against time $\sqrt{t}$ (s).

Where:

M_t = mass of analyte at time t (g)

M_∞ = mass of analyte at infinitive time (g)

$\sqrt{t}$ = square root of time in (s)

Straight line graph was fitted into plotted data and straight line equations with their correlation coefficients were determined.

2.8.9 Diffusion coefficient

The diffusion coefficient of bactericide was determined from the linear portion of the graphs, taking the slope and substituting into the equation 2.4.

2.9 Disc-diffusion test

Disc-diffusion test is a routinely used method to investigate the antimicrobial properties of dental materials [4, 21]. This method involves placing the tested material on an agar plate inoculated with oral bacteria. By this test method, an inhibition zone around the material is produced. To produce the zone of inhibition, the material needs to be able to leach a soluble antimicrobial agent. If the elution is not of adequate amounts of antimicrobial agent the zone of inhibition will not be produced [22]. In general, larger zones correlate with

concentration and/or potency of tested bactericide as well as susceptible of tested bacteria to specific antimicrobial agent [23]. The size of inhibition is measured using graduated ruler or sliding callipers [22]. Typical diffusion disc test is shown in Figure 2.9.

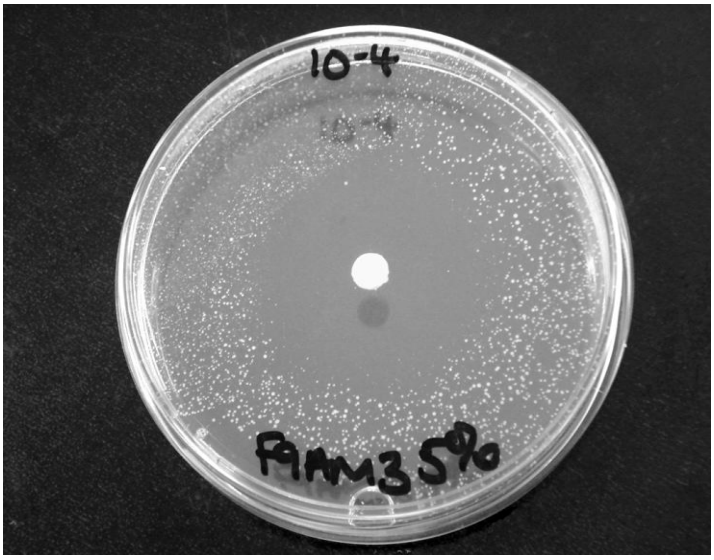

Figure 2.9: Disc-diffusion test obtained for this work

2.9.1 Preparation of specimens for microbial testing

For microbial testing all formulations described in section 2.2 were prepared, except of the samples with 2% weight fraction of antimicrobial agent. Method of the sample preparation for this determination differs from the method presented in section 2.2 in a fact that rubber moulds (4 mm diameter (± 0.1 mm) and 1 mm depth (± 0.1 mm)) were used instead. Samples were stored in a plastic container at room temperature and ambient humidity before the experiment.

2.9.2 Bacterial strain and culture conditions

Streptococcus mutans stock cultures were stored in 37^{o}C. This particular bacterial strain was chosen because this species is the main one that is associated with dental caries [24, 25

and 26]. The organisms were incubated at 37^{o}C aerobically overnight in a Brain Heart Infusion broth prior to use. Used media were chosen from Brain Columbian Blood agar.

2.9.3 Preparation of standard concentration of bacterial suspensions

Standard concentrations of bacterial suspensions of concentrations of $1x10^{-1}$ cfu ml^{-1} to $1x10^{-8}$ cfu ml^{-1} were prepared using standard dilution methods. All procedures were performed on the open bench over a gas flame under standard sterile conditions.

2.9.4 Preparation of standard microbial plates

Solutions were then used for preparation of standard microbial plates. 1 ml of each concentration of bacteria suspension was taken using a 1 ml automatic pipette, spread onto agar plate and seeded evenly on the agar using a storyline spreader. Also microbial plates with original stock solution were prepared. The bottle lids were sterilised in a gas flame, attached to the bottle and then shaken carefully. Petri dishes containing Brain Heart Infusion agar were used. All procedures were performed on the open bench over the gas flame under standard sterile conditions.

2.9.5 Preparation of microbial plates of investigated materials

Preparation of agar disc-diffusion involved applying 1 ml of appropriate concentration of culture bacteria using automatic pipette and seeding it evenly on the Heart Brain Fusion agar using of a sterilised spreaders.

Stock solution (prepared in section 2.9.2) and suspension of $1x10^{-4}$ cfu ml^{-1} concentration (prepared in section 2.9.3) were used. Two extreme concentrations were chosen with diluting factor differing by $1x10^{-4}$ to avoid bacterial overgrowth or undergrowth as initial concentration of suspension was not known at this point of the experiment. Glass-ionomer specimens with known concentration of additives were placed in the middle of each plate. Three replicates were prepared for each percentage and the plates were cultivated

aerobically for 48 hours at 37^{o}C. All procedures were performed on the open bench over the gas flame under standard sterile conditions.

2.9.6 Determination of the concentrations of standard microbial plates

The concentrations were calculated for plates seeded with original concentration and 1x10^{-4} cfu ml^{-1} concentration suspension by counting each colony. The plate for the higher concentration was divided into four equal sections and concentrations (cfu ml^{-1}) were counted from that section and then the amount multiplied by four to achieve the total concentration.

2.9.7 Determination of inhibition zone

The inhibition zone radius was measured for each disc at four different points around specimen using a graduated 0.5 mm ruler. The area of inhibition was calculated by adopting two different equations. For samples with regular circular inhibition zone the area was measured by equation 2.9 below:

$$\textbf{Inhibition zone} = 2\pi\,[(r1+r2+r3+r4)/4]^2 \qquad (2.9)$$

Where:

r = radius of the zone of inhibition

For samples with irregular inhibition zone the area was measured by equation 2.10 below:

$$\textbf{Inhibition zone} = (d1*d2)/2+(d2*d3)/2+(d3*d4)/2+(d4*d2)/2 \qquad (2.10)$$

Where:
d = diagonal (measured from the edge of the specimen to the peripheries of the inhibition zone)

All cements were analysed in triplicate and mean zone size and standard deviations calculated.

2.9.8 Statistical analysis

The inhibitory effect of individual bactericide and its concentration was determined using Student's t test. The inhibition zone sizes at different levels of doping for each bactericide were compared using Mann-Whitney U test.

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RESULTS

3.1 Setting kinetics studies - working time determination

In this section we report the effect of additives on the kinetics of working time of glass-ionomer cements (GICs). Additives such as cetyl pyridinium chloride (CPC), benzalkonium chloride (BACH), sodium fusidate (SF) at weight fraction of 5% (w/w) were added into Fuji IX and Chemflex. Also specimens doped with triclosan (T) and triclosan/zinc citrate (T/ZC) at a proportion of 3% were formulated. The specimens were prepared by the method described in Chapter 2.2. Working times were measured using the Gillmore needle. The resulting working times were compared with control (additive-free) specimens and the results are summarised in Table 3.1. Three measurements were taken for each group and the data was analysed using Student's t- test with significance set at $p < 0.05$. The results demonstrate that additives increase the working time of both Fuji IX and Chemflex. The increase in working time for Fuji IX doped specimen in comparison to control samples specimens varied between 1 second and 21 seconds. For Chemflex specimens the increase varied between 11 seconds and 32 seconds. Differences in the working time of the control against doped specimens were not statistically significant.

Table 3.1: Mean working time (s), SD in parenthesis for Fuji IX and Chemflex formulations doped with 5% w/w of additive

Material	**Control**	**CPC**	**BACH**	**SF**	**T**	**T***
Fuji IX	134 (19)	155 (22)	151 (20)	155 (17)	136 (5)	135 (5)
Chemflex	172 (22)	183 (5)	204 (5)	199 (2)	189 (0)	189 (0)

3.2 Mechanical characterisation

Compressive strength (CS) is an important property of dental materials, particularly in the processes of mastication when strong forces per unit area are applied. The ability of dental material to withstand these forces will determine its ultimate performance in its particular application. To fulfil these needs, the International Organisation for Standarisation and the British Institution set standard requirements for mechanical properties of dental materials for their particular application. The accepted CS of GICs for luting applications is 70 MPa and for restorative is 130 MPa [1]. Together as a criterion for clinical excellence, CS measurements can be used to provide information on the processes occurring during setting and maturation of these materials as this property reflects the extent of a material chemical structure [2, 3 and 4].

The current work investigated the effect of bactericides on the CS of reformulated materials. Additionally, the influence of bactericide on surface hardness (Vicker's hardness number, VHN) of reformulated cements was determined. Surface hardness is defined as the resistance of a material to indentation. Surface hardness testing can be used to evaluate the materials resistance to wear and plastic deformation by penetration [5, 6]. Change in surface hardness reflects the cure state of a material and the extent of the setting reactions. Surface hardness can also be used to give information of processes occurring on the surface of studied materials [7, 8].

Results in section 3.1 showed that addition of additives gives rise to elongation of working time. This part of the study examines whether the addition of antimicrobial additives to GICs affects the mechanical properties of the reformulated cement. CPC, BACH and SF at weight fractions of 1%, 2%, 3% and 5 % w/w were added into Fuji IX and Chemflex. Also formulations containing 1% and 3% w/w of T and T/ZC were prepared. Five cylindrical shape specimens of dimensions of 4 mm diameter (± 0.1 mm) and 6 mm depth (± 0.1 mm) were fabricated and placed into separate plastic cylinders (containing HPLC grade water) for an appropriate aging period of 24 hours, 1-7 weeks for CPC and BACH, and for 24 hours and 7 weeks for SF, T and T/ZC reformulated samples. The CS and surface hardness of each formulation were determined at each time interval from which the average CS and surface

hardness and its standard deviation was calculated, and expressed in MPa and VHN. Results were statistically analysed by Student's t-test at significance levels of $\alpha = 0.05$ and df = 4-8.

3.2.1 Compressive strength

The CS data for various cement formulations and ageing periods are shown in Tables 3.2 to 3.5. All statistical results are presented in Table A1 in Appendices.

Table 3.2: Mean compressive strength (MPa) for Fuji IX formulations, SD in parenthesis

	Cetyl pyridinium chloride					**Benzalkonium chloride**			
Time	**Control**	**1%**	**2%**	**3%**	**5%**	**1%**	**2%**	**3%**	**5%**
24 hours	152.9 (28.1)	131.9 (24.6)	101.5 (16.7)	90.0 (10.9)	65.6 (22.2)	125.1 (16.2)	108.7 (15.6)	108.0 (14.6)	82.9 (11.4)
1 week	133.2 (14.1)	123.6 (30.7)	102.2 (11.9)	60.9 (4.8)	47.4 (38.6)	94.5 (15.5)	84.8 (14.7)	74.0 (19.8)	71.0 (12.9)
3 weeks	133.1 (15.8)	110.0 (10.32)	83.8 (32.0)	75.2 (10.9)	67.6 (8.4)	105.8 (33.0)	102.6 (22.5)	89.4 (16.1)	87.3 (30.7)
5 weeks	152.4 (48.2)	107.8 (15.3)	102.9 (15.7)	85.4 (15.9)	81.9 (15.7)	131.0 (39.8)	117.7 (21.7)	110.8 (27.4)	88.6 (16.6)
7 weeks	185.0 (33.7)	106.7 (15.1)	102.1 (12.8)	66.1 (8.1)	42.7 (21.1)	125.6 (30.0)	115.4 (39.5)	100.8 (23.3)	89.0 (13.4)

All samples showed reduction in the CS on doping. The decrease was observed at all studied time intervals. The 24 hours data analysed by Students t-test (set at significant level of 0.05) showed that for Fuji IX, CPC and BACH had no significant effect on CS at levels of 1%. However, at 2%, 3% and 5% for CPC and BACH, there was a reduction in CS (significant to at least $p < 0.04$).

Similarly, lower levels of SF did not have any significant effect on CS. For SF, the reduction was significant (to at least $p < 0.04$) at 3% and 5% of additions. However, no considerable reduction in CS was observed for T and T/ZC formulations.

Table 3.3: Mean compressive strength (MPa) for Fuji IX formulations in, SD in parenthesis

	Sodium fusidate					**Triclosan**			
Time	**Control**	**1%**	**2%**	**3%**	**5%**	**1%**	**1%***	**3%**	**3%***
24 hours	152.9 (28.1)	151.3 (36.4)	137.2 (10.1)	106.7 (40.5)	90.1 (13.6)	150.7 (11.2)	141.5 (7.4)	128.4 (14.4)	126.1 (18.7)
7 weeks	185.0 (33.7)	133.8 (29.4)	133.5 (40.3)	116.6 (14.8)	81.3 (20.7)	155.8 (31.6)	148.7 (36.5)	139.7 (12.0)	125.3 (15.8)

**Triclosan samples additionally doped with zinc citrate*

For Chemflex, CPC caused statistically significant reductions in CS at addition of 2% and above ($p < 0.05$), whereas BACH caused statistically significant reductions in CS at all levels of addition. The decrease in CS caused by SF was significant at 5% ($p < 0.04$). No significant differences between the control and doped samples were calculated for T and T/ZC samples. Overall, Chemflex had lower CS values in comparison to Fuji IX.

Table 3.4: Mean compressive strength (MPa) for Chemflex formulations, SD in parenthesis

	Cetyl pyridinium chloride					Benzalkonium chloride			
Time	**Control**	**1%**	**2%**	**3%**	**5%**	**1%**	**2%**	**3%**	**5%**
24 hours	136.4 (27.8)	113.4 (20.5)	114.4 (7.8)	93.7 (23.9)	78.3 (9.0)	96.1 (16.3)	91.6 (13.6)	89.2 (18.0)	82.6 (3.4)
1 week	106.2 (12.5)	81.3 (6.4)	77.7 (8.5)	58.4 (6.9)	52.9 (7.0)	108.8 (6.9)	102.2 (7.4)	98.5 (30.8)	77.9 (30.9)
3 weeks	130.0 (6.9)	112.3 (31.6)	107.3 (19.0)	88.2 (22.7)	84.1 (19.0)	110.3 (30.0)	104.6 (7.9)	96.3 (28.7)	97.7 (12.8)
5 weeks	157.6 (14.0)	109.8 (13.5)	91.9 (14.0)	85.0 (7.8)	73.9 (8.2)	99.2 (19.7)	94.1 (20.0)	85.5 (25.2)	74.5 (19.1)
7 weeks	177.0 (31.9)	99.2 (29.2)	81.0 (11.6)	71.9 (9.9)	70.3 (20.1)	96.6 (11.7)	82.7 (16.5)	76.9 (17.6)	63.0 (17.6)

The increase in CS between 24 hours and seven weeks was observed for the control sample sets of both Fuji IX and Chemflex materials. However, the observed changes were no statistically significant. In contrast, when additives were present, a decrease in CS was observed, with the greatest reduction exhibit for specimens doped with BACH and CPC. However, no significant differences ($p < 0.05$) were observed for any of those sample sets.

The increase in CS was recorded for some samples doped with SF and T, but none of them were significant. Interestingly however, drop in CS of control specimens between 24 hours and one week were observed for both materials which become less pronounced at week three to achieve strength comparable to 24 hours data at week five.

Table 3.5: Mean compressive strength (MPa) for Chemflex formulations, SD in parenthesis

	Sodium fusidate					**Triclosan**			
Time	**Control**	**1%**	**2%**	**3%**	**5%**	**1%**	**1%***	**3%**	**3%***
24 hours	136.4 (27.8)	126.1 (24.2)	111.3 (22.6)	101.2 (36.6)	93.4 (12.5)	130.1 (7.4)	123.2 (13.0)	119.7 (4.9)	117.8 (12.6)
7 weeks	177.0 (31.9)	157.4 (36.3)	155.2 (21.1)	144.6 (19.3)	138.0 (23.5)	148.4 (12.8)	140.6 (16.7)	130.5 (10.8)	93.6 (19.3)

**Triclosan samples additionally doped with zinc citrate*

3.2.2 Surface hardness

The surface hardness data for various cement formulations and differing ageing periods are shown in Tables 3.6 to 3.9. All statistical results are presented in Table A2 in Appendices.

Table 3.6: Mean surface hardness (VHN) for Fuji IX formulations, SD in parenthesis

	Cetyl pyridinium chloride					**Benzalkonium chloride**			
Time	**Control**	**1%**	**2%**	**3%**	**5%**	**1%**	**2%**	**3%**	**5%**
24 hours	57.2 (5.9)	56.5 (8.1)	51.4 (6.2)	49.1 (8.5)	42.4 (5.4)	49.5 (5.5)	45.6 (4.1)	42.3 (7.0)	34.2 (5.7)
1 week	65.8 (7.0)	53.7 (6.2)	50.0 (10.2)	49.3 (11.4)	42.8 (8.0)	52.4 (6.2)	44.2 (8.4)	38.4 (7.4)	30.0 (4.9)
3 weeks	70.8 (11.1)	58.1 (13.4)	58.3 (9.3)	52.2 (3.8)	47.5 (9.6)	58.1 (13.4)	45.3 (6.9)	45.3 (7.0)	39.9 (10.3)
5 weeks	67.5 (4.2)	54.7 (7.1)	53.0 (5.4)	51.7 (13.7)	49.3 (8.4)	50.7 (7.3)	47.7 (6.7)	46.6 (4.4)	35.9 (6.6)
7 weeks	66.4 (8.2)	52.6 (5.1)	53.1 (10.8)	51.7 (5.2)	44.4 (7.0)	54.5 (10.2)	50.0 (5.3)	45.2 (4.7)	42.1 (3.0)

The decrease was observed at all studied time intervals. Lower concentrations of additives, i.e. 1% or 2% did not lead to statistically significant differences in surface hardness. Higher

concentrations, i.e. 3% or 5% generally did lead to reductions in surface hardness that were significant to at least ($p < 0.03$).

Table 3.7: Mean surface hardness (VHN) for Fuji IX formulations, SD in parenthesis

	Sodium fusidate					**Triclosan**			
Time	**Control**	**1%**	**2%**	**3%**	**5%**	**1%**	**1%***	**3%**	**3%***
24 hours	57.0 (5.8)	54.3 (7.9)	54.6 (7.4)	53.1 (11.5)	36.3 (7.9)	51.9 (8.9)	55.8 (15.1)	56.6 (9.7)	52.9 (6.4)
7 weeks	63.3 (7.1)	52.9 (8.2)	50.2 (5.0)	46.3 (7.4)	39.6 (4.1)	50.4 (4.6)	47.6 (3.1)	47.8 (4.3)	48.9 (4.4)

**Triclosan samples additionally doped with zinc citrate*

The 24 hours data showed that for Fuji IX, CPC had no significant effect on surface hardness at levels of 1%, 2% and 3%. However, at 5% there was a reduction in surface hardness (significant to $p < 0.02$). BACH reduced surface hardness to significant level at 2% and above ($p < 0.03$). SF reduced surface hardness to significant level only at 5% ($p < 0.01$). Similarly, for Chemflex, lower levels of CPC did not have any significant effect on surface hardness, whereas for BACH doped samples the reduction was significant (to at least $p < 0.03$) at 2%, 3% and 5% of additions. SF reduced surface hardness to significant level only at 5% ($p < 0.01$). No significant reduction in surface hardness was observed for Fuji IX and Chemflex doped with T and T/ZC. The increase in surface hardness between 24 hours and seven weeks was observed for the control sample sets of both Fuji IX and Chemflex. However, the observed changes were statistically significant only for Chemflex.

Table 3.8: Mean surface hardness (VHN) for Chemflex formulations, SD in parenthesis

	Cetyl pyridinium chloride					Benzalkonium chloride			
Time	**Control**	**1%**	**2%**	**3%**	**5%**	**1%**	**2%**	**3%**	**5%**
24 hours	45.0 (8.0)	40.1 (13.8)	43.2 (4.0)	37.9 (6.4)	34.7 (7.0)	38.5 (3.4)	31.2 (3.9)	30.6 (2.1)	23.5 (2.4)
1 week	65.8 (7.0)	47.1 (9.8)	45.1 (5.7)	42.6 (5.1)	37.6 (5.3)	44.0 (2.2)	43.6 (6.8)	43.0 (4.1)	38.0 (3.4)
3 weeks	70.8 (11.1)	51.9 (4.3)	46.2 (4.5)	44.5 (4.2)	47.5 (13.9)	49.6 (4.0)	42.8 (4.4)	39.6 (3.7)	36.3 (4.8)
5 weeks	67.5 (4.2)	55.8 (6.6)	50.7 (5.3)	48.6 (10.2)	47.3 (7.3)	48.1 (4.9)	50.0 (7.1)	40.6 (3.2)	38.2 (8.7)
7 weeks	58.5 (7.5)	54.6 (7.7)	56.8 (4.3)	53.3 (6.0)	52.8 (6.7)	49.0 (5.6)	50.4 (6.2)	43.2 (4.5)	32.4 (2.5)

The presence of additives changed this behaviour. Fuji IX and Chemflex specimens showed that the additives BACH and CPC led to increases in the surface hardness and in a case of Chemflex samples the increase in surface hardness was significant at 2% and above ($p < 0.03$). By contrast, the SF, T and T/ZC combinations in general caused a decrease in surface hardness with time, showing a significant decrease at 3% (to $p < 0.01$).

Table 3.9: Mean surface hardness (VHN) for Chemflex formulations, SD in parenthesis

	Sodium fusidate					Triclosan			
Time	**Control**	**1%**	**2%**	**3%**	**5%**	**1%**	**1%***	**3%**	**3%***
24 hours	49.2 (7.9)	44.2 (5.5)	36.6 (4.7)	35.5 (4.3)	33.7 (4.9)	53.9 (9.1)	50.2 (8.3)	42.4 (9.1)	46.3 (5.4)
7 weeks	51.9 (7.6)	47.5 (7.6)	35.2 (3.9)	31.3 (4.2)	28.0 (5.7)	41.1 (0.1)	42.0 (2.7)	37.2 (4.0)	34.5 (1.6)

**Triclosan samples additionally doped with zinc citrate*

3.3 Water loss studies

Results in section 3.2 showed that the addition of additives decreases both strength and hardness of materials and that decrease is proportional to percentage of doping. This part of the study examines whether the addition of antimicrobial additives to GICs affects the rate of loss of water from the cement matrix. CPC, BACH and SF at a weight fraction of 5% w/w were added into Fuji IX and Chemflex. Also specimens doped with 3% w/w of T and T/ZC were formulated. The specimens were prepared by the method described in Chapter 2.2. Samples were stored in desiccating chamber under concentrated sulphuric acid conditions. Water loss was measured using an analytical balance at 30 minutes, 1, 2, 3, 4, 5 and 24 hours. Testing was carried out weekly until equilibrium was achieved. Results for percentage mass loss under desiccating conditions are given in Table 3.10. Additionally, equilibrium mass losses for both Fuji IX and Chemflex for different formulations are presented in Figure 3.1.

Table 3.10: Mass loss data (%)

	Fuji IX						Chemflex					
Time	**C**	**CPC**	**BACH**	**SF**	**T**	**T***	**C**	**CPC**	**BACH**	**SF**	**T**	**T***
30 min	0.84	0.98	0.80	0.81	0.76	0.71	1.43	1.38	0.85	1.79	0.59	1.20
1 hours	1.14	1.33	1.14	1.28	0.96	0.90	2.01	1.65	1.35	2.67	0.94	1.69
2 hours	1.80	1.91	1.74	2.12	1.50	1.45	2.75	2.29	1.98	4.18	1.53	2.54
3 hours	2.17	2.38	2.15	2.78	1.85	1.80	3.42	2.87	2.55	4.99	2.01	3.08
4 hours	2.49	2.73	2.42	3.15	1.61	2.03	3.79	3.26	2.90	5.58	1.94	3.57
5 hours	2.89	2.99	2.69	3.54	2.21	2.08	4.21	3.54	3.31	5.97	2.48	4.00
24 hours	3.79	4.33	3.96	5.89	4.73	4.46	5.70	5.02	4.79	8.68	4.87	6.39
1 week	5.20	5.71	5.47	7.92	6.82	6.70	7.50	6.61	6.50	10.89	7.24	8.15
2 weeks	6.22	6.02	5.97	8.44	6.99	5.74	8.15	7.17	7.12	11.30	8.44	8.55
3 week	6.35	5.78	5.94	8.54	7.39	7.08	8.40	6.93	7.24	11.47	8.17	9.49

**Triclosan samples additionally doped with zinc citrate*

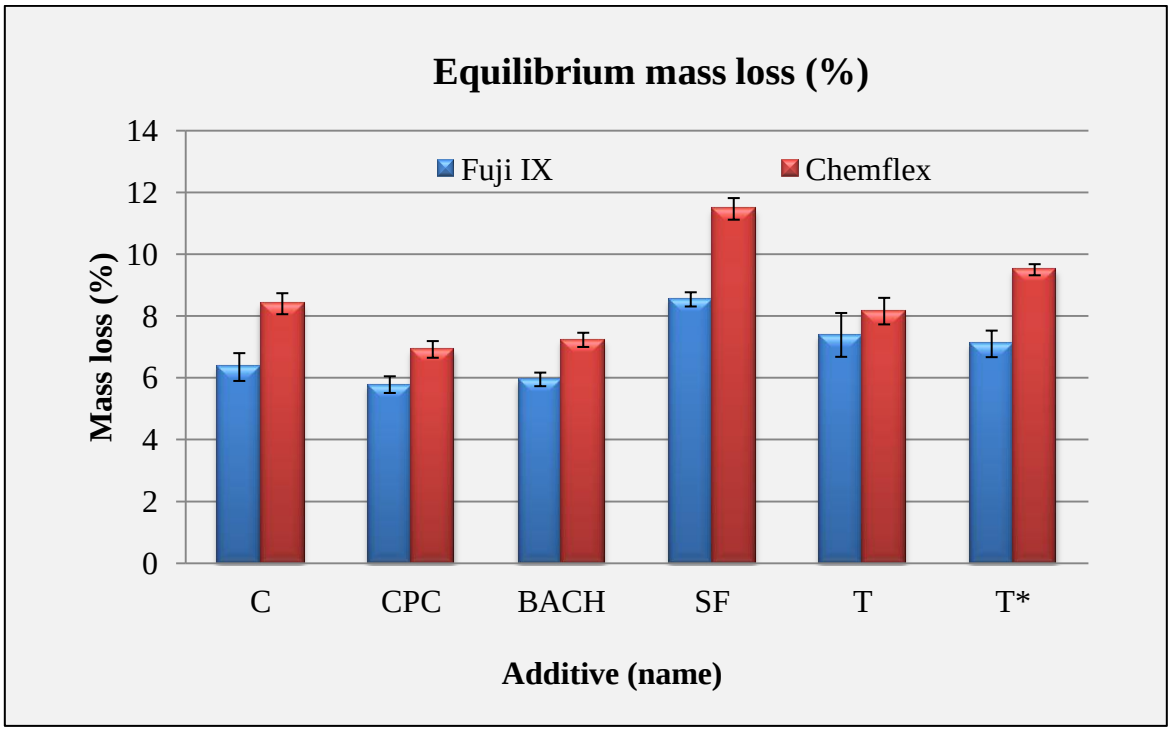

Figure 3.1: Equilibrium mass loss, SD as error bars

All the investigated samples exhibited a reduction in mass under desiccation conditions. The difference in relative fractional water loss from the control specimen as compared to the doped specimens was found not to be statistically significant.

Clear differences in the percentage water loss at equilibrium between Chemflex and Fuji IX material were observed. The percentage of water loss from the Chemflex samples was significant (to at least $p < 0.01$) in comparison with their initial masses. For Fuji IX, none of the differences were significant. This suggests that Chemflex is more susceptible to desiccation.

The effects of converting this data to the form required by Fick's law, i.e. M_t/M_∞ against $\sqrt{t}$, are shown in Figures 3.2 to 3.5. Straight lines were fitted through data, and the coefficients, r, for the correlation lines calculated for each formulation are shown in Table 3.11.

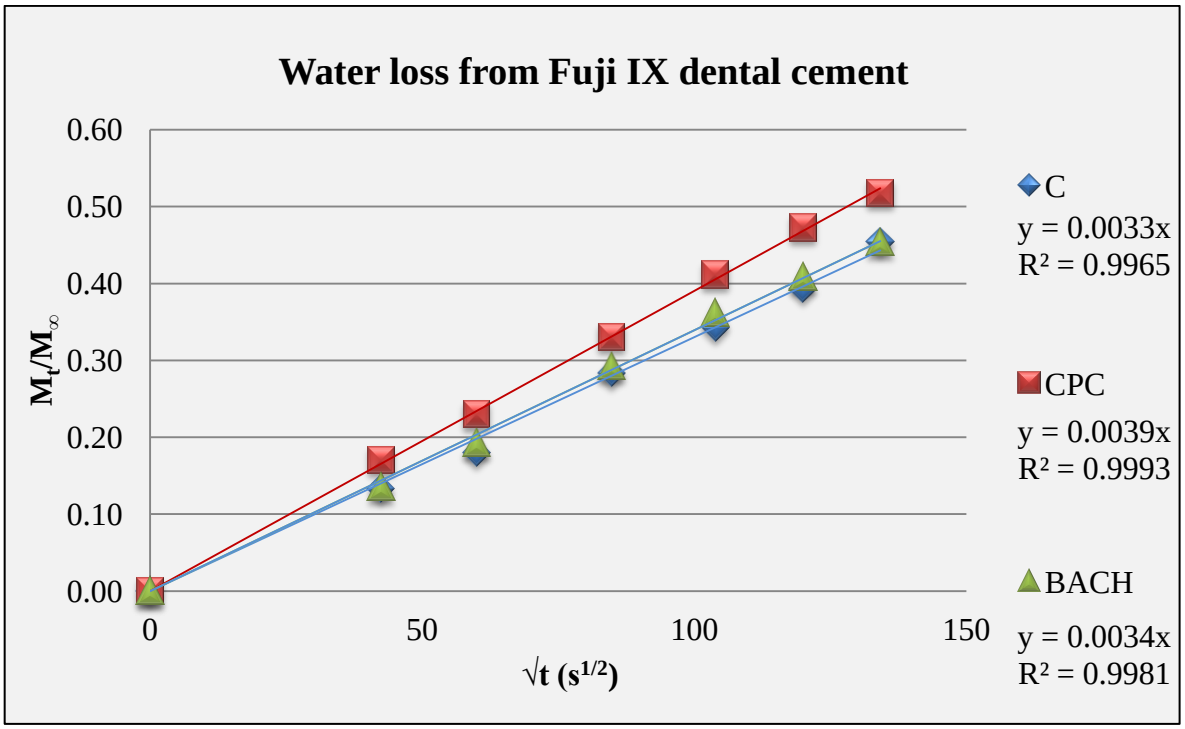

Figure 3.2: M_t/M_∞ vs √t for Fuji IX

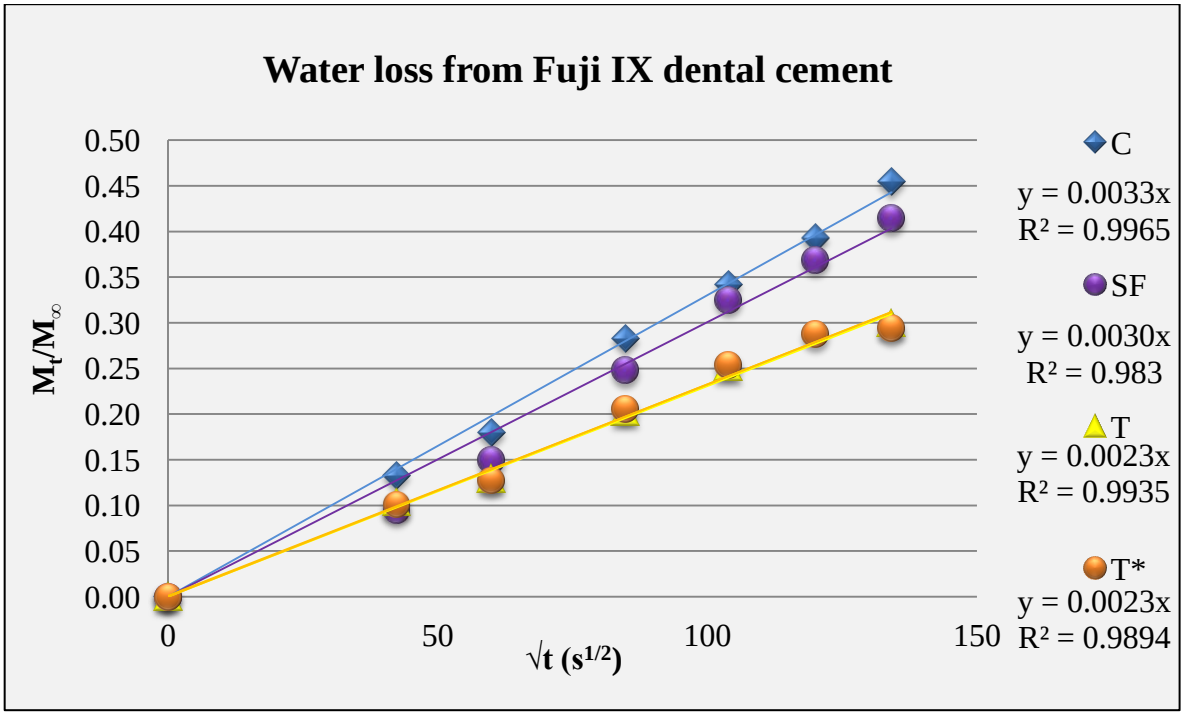

Figure 3.3: M_t/M_∞ vs √t for Fuji IX

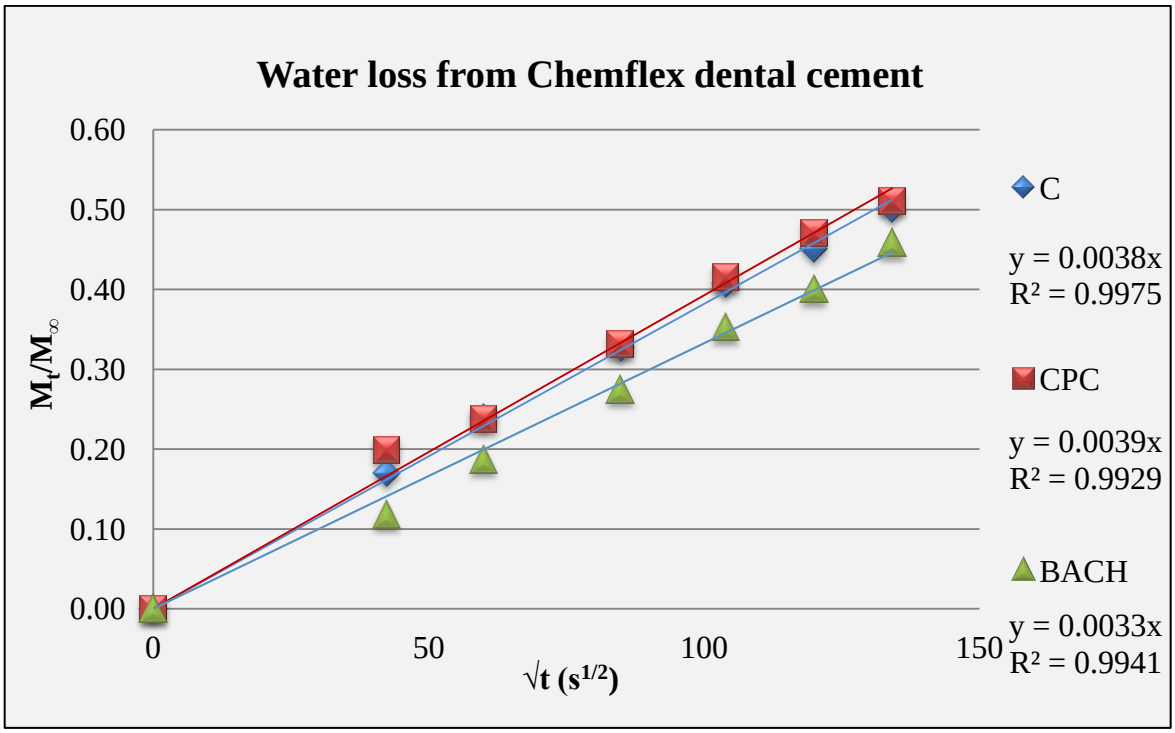

Figure 3.4: M_t/M_∞ vs $\sqrt{t}$ for Chemflex

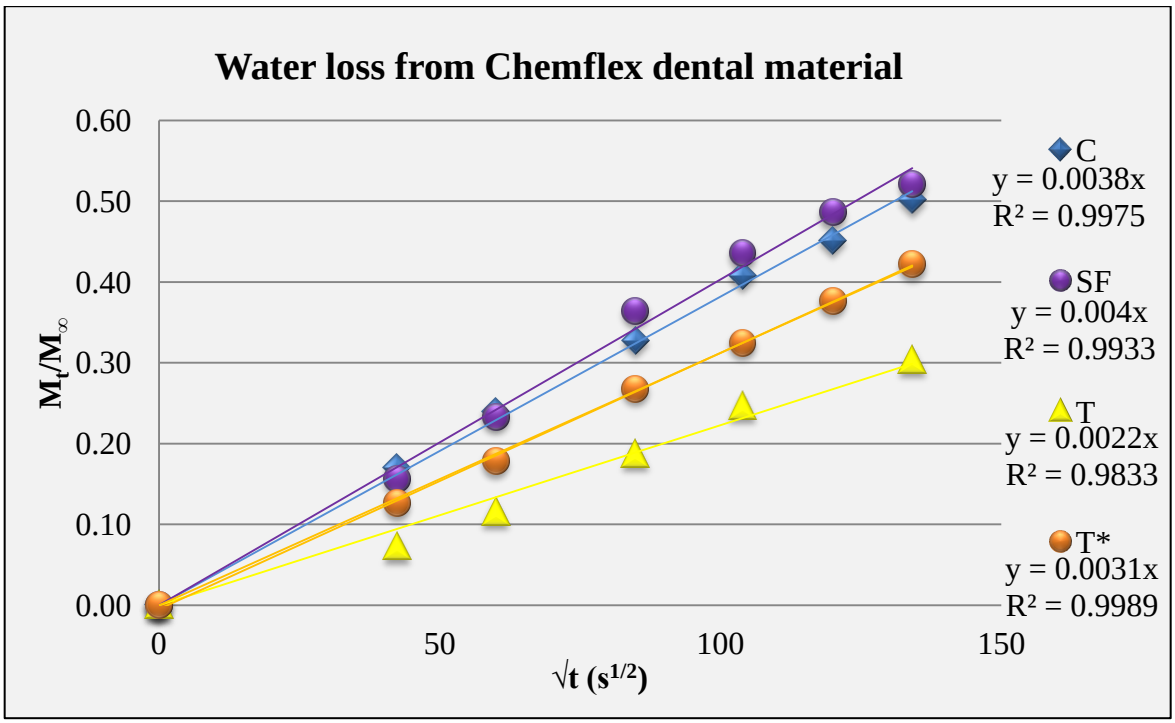

Figure 3.5: M_t/M_∞ vs $\sqrt{t}$ for Chemflex

Table 3.11: Linear regression equations and correlation coefficients for water loss plot (M_t/M_∞ vs $\sqrt{t}$)

Cement/amount and additive type	**Equation**	**Correlation Coefficient, r**
Fuji IX/		
Control	y=0.0033x	0.9965
Cetyl pyridinium chloride	y=0.0039x	0.9930
Benzalkonium chloride	y=0.0034x	0.9981
Sodium fusidate	y=0.0030x	0.9830
Triclosan	y=0.0023x	0.9935
Triclosan*	y=0.0023x	0.9900
Chemflex/		
Control	y=0.0038x	0.9975
Cetyl pyridinium chloride	y=0.0039x	0.9929
Benzalkonium chloride	y=0.0033x	0.9941
Sodium fusidate	y=0.0040x	0.9933
Triclosan	y=0.0022x	0.9833
Triclosan*	y=0.0031x	0.9989

The diffusion coefficients were determined from the linear portion of the graphs, taking the slope and substituting into the equation $D = s^2 \pi l^2/4$ and these are shown in Table 3.12.

An evaluation of the kinetics of water loss from the samples showed that water loss from both Fuji IX and Chemflex control specimens and doped specimens was based on

diffusion. The diffusion of water was observed for at least first five hours and can be satisfactorily described by the mathematical form of Fick's law in all cases (r-values of at least 0.9800). Diffusion coefficient values of water for the control and doped samples are comparable in all cases, varying between $3.42x10^{-11}$ m^2 s^{-1} to $5.93x10^{-11}$ m^2 s^{-1}. The slight differences show no trend with increasing amount of additive.

Table 3.12: Diffusion coefficients of water of investigated glasses and additive variations

Cement/amount and additive type	**Diffusion coefficient m^2 s^{-1}**
Fuji IX/	
Control	$3.42x10^{-11}$
Cetyl pyridinium chloride	$4.78x10^{-11}$
Benzalkonium chloride	$3.63x10^{-11}$
Sodium fusidate	$3.11x10^{-11}$
Triclosan	$1.66x10^{-11}$
Triclosan*	$1.66x10^{-11}$
Chemflex/	
Control	$4.54x10^{-11}$
Cetyl pyridinium chloride	$4.78x10^{-11}$
Benzalkonium chloride	$3.42x10^{-11}$
Sodium fusidate	$5.03x10^{-11}$
Triclosan	$1.59x10^{-11}$
Triclosan*	$3.02x10^{-11}$

3.4 ^{27}Al MAS-NMR

In section 3.2 the effect of antimicrobial additives on the mechanical properties of GICs has been examined. Additionally in section 3.1 the influence of additives on the setting kinetics was studied. The purpose of this section is to investigate how the kinetics of conversion of aluminium four-coordinate, Al (IV), to aluminium six-coordinate, Al (VI), during setting and maturation of GICs is altered by the presence of additives and to correlate the findings to the changes in mechanical and working times observed in section 3.1 and 3.2.

The additives CPC and BACH at a weight fraction of 5 % w/w were added into Fuji IX and Chemflex. Also control samples were prepared. Five specimens of each formulation were prepared by the method described in Chapter 2.2. However samples were not cured in the oven, instead they were stored in individual plastic containers for 15 minutes, 1 hour, 5 hours, 24 hours, 1 week, 1 month and 4 months at room temperature and ambient humidity. After the end of each storage time the specimens were removed from the plastic containers, placed into 25 ml glass container and approximately 15 ml of liquid nitrogen was poured into it. Once bubbling had stopped, samples were covered with cling-film, pierced and placed into freeze-drier vacuum at -20^{o}C for 48 hours. After 48 hours samples were removed from freeze-drying and placed into desiccators under dinitrogen pentoxide (N_2O_5) environment for testing. Prior to testing, the samples were grounded into a fine powder using pestle and mortar. Samples were analysed at appropriate time intervals using ^{27}Al MAS-NMR. ^{27}Al MAS-NMR spectra of Fuji IX and Chemflex glass powder, the control and doped specimens of cement at various stages of ageing are shown in Figures 3.8 to 3.13. In addition, spectra of the glass are presented in Figures 3.6 and 3.7.

As it can be seen, two main signals are found in both Fuji IX and Chemflex glass. The peak at the higher chemical shift was assigned to four-coordinate aluminium, Al (IV), and the lower chemical shift was attributed to six-coordinate, Al (VI). The spectrum of Fuji IX glass has a peak at 46.0 ppm and shoulder at 19.9-10.0 ppm. There was also a small peak at 5.0 ppm. Chemflex original glass showed large asymmetrical peak at 45.0 ppm- due to Al (VI) and smaller one at -2.0 ppm- due to Al (IV).

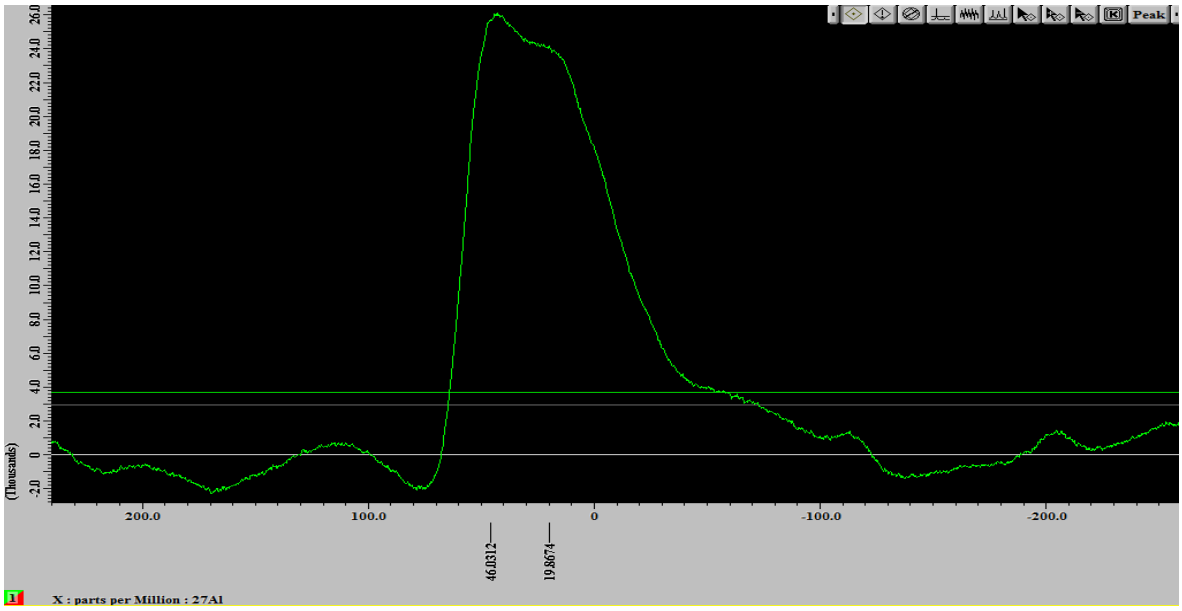

Figure 3.6: ^{27}Al MAS-NMR of Fuji IX glass

The spectra of cements showed similar features to the glass, with two main peaks observed. As before, the peak at higher chemical shift was assigned to Al (IV) and the one at the lower chemical shift to Al (VI). For Fuji IX control specimens there were also two substantial peaks found at around 45.5 ppm and around at -1.0 ppm.

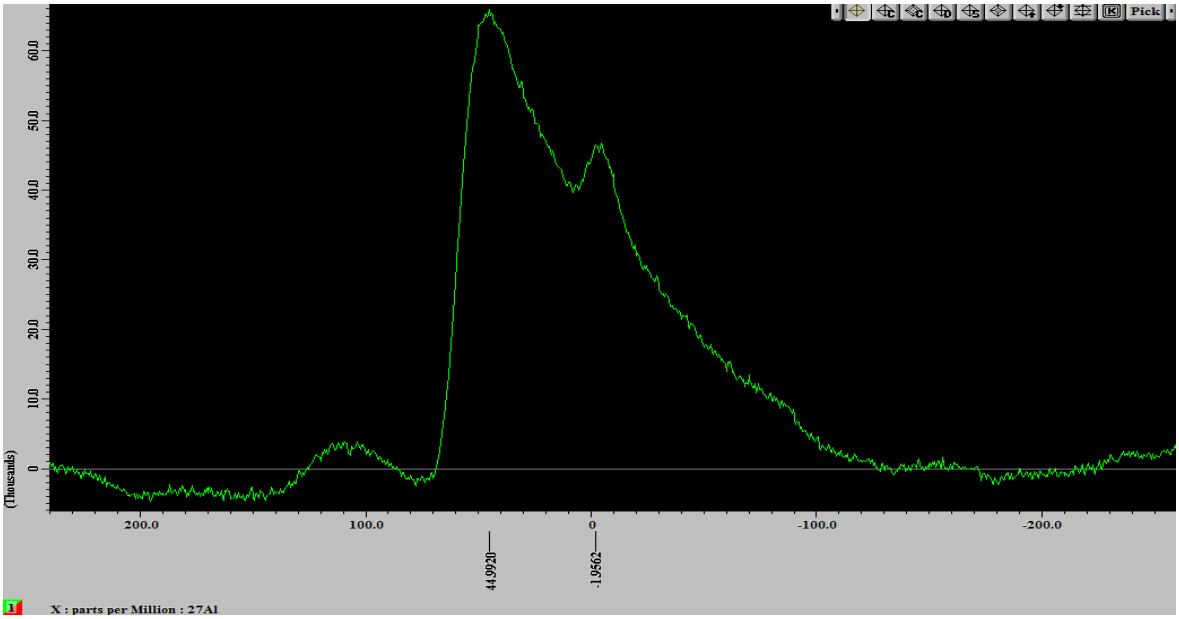

Figure 3.7: ^{27} Al MAS-NMR of Chemflex glass

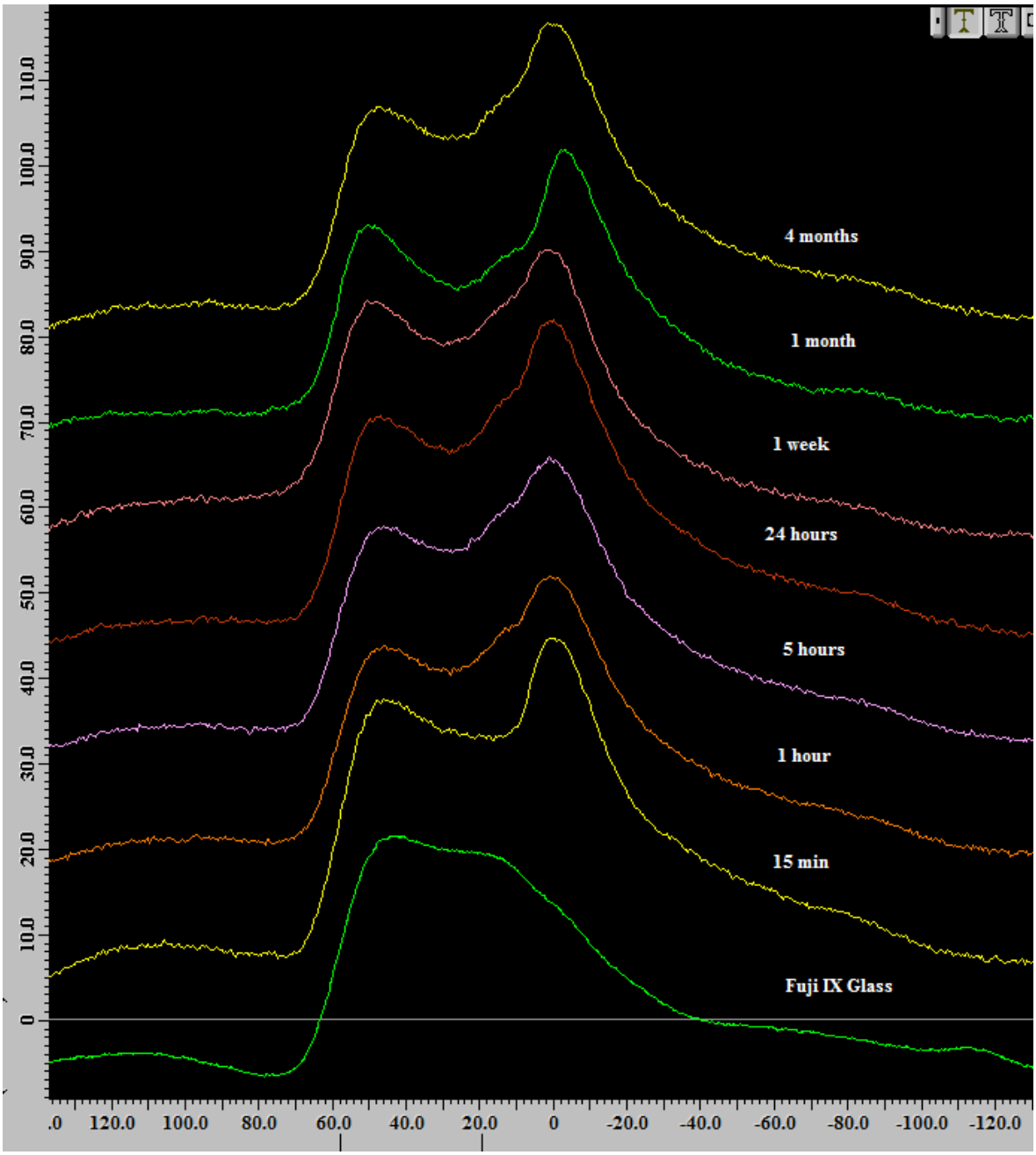

Figure 3.8: ^{27}Al MAS-NMR spectrum of Fuji IX dental cement – control samples

A clear change in intensity of these two peaks was observed as the cements set. For Fuji IX the peak at 45.5 ppm became smaller, whereas the intensity of the peak -1.0 ppm increased, and was greater than the peak at 45.5 ppm. The change in relative intensity between peaks were observed just after 15 minutes after cement mixing and they became more pronounced as the cement aged. The cement spectra also showed a shoulder between 30-15 ppm.

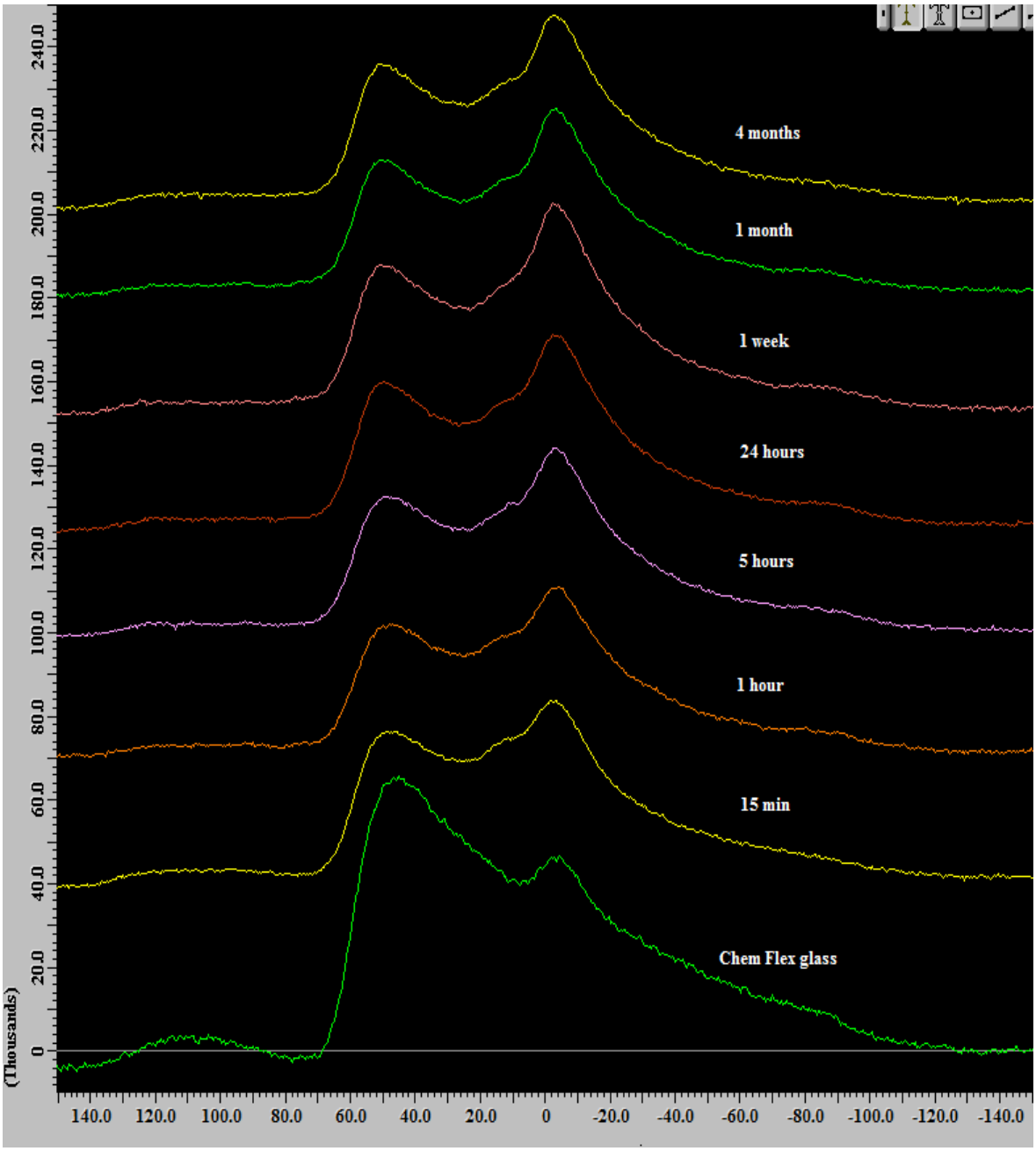

Figure 3.9: ^{27}Al MAS-NMR spectrum of Chemflex dental cement – control samples

For Chemflex two peaks were found, one around 48.4 ppm and second one at -2.0 ppm, and they were assigned to Al (IV) and Al (VI). The intensity of the peak at 48.4 ppm decreased and it became smaller at 15 minutes than the intensity of the peak at -2.0 ppm. Similar to Fuji IX, there was a gradual change in relative intensity observed, with Al (IV) decreasing relative to Al (VI) as the cements aged.

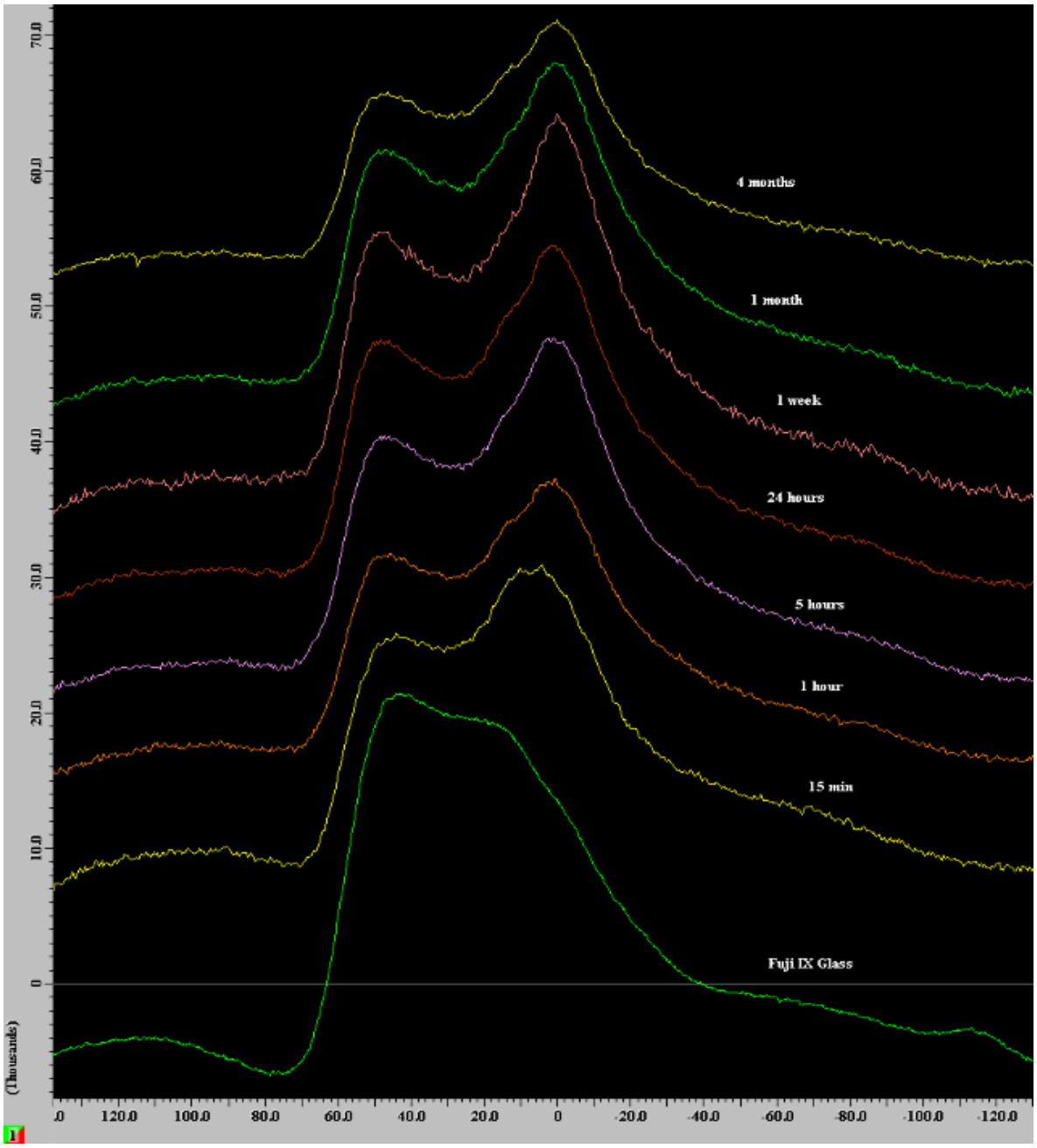

Figure 3.10: ^{27}Al MAS-NMR spectrum of Fuji IX dental cement – cetyl pyridinium chloride doped specimens

Similarly, two significant peaks at around 44.0 ppm and around at 4.4 ppm were observed for the Fuji IX sample doped with CPC. A small peak at around 44.0 ppm was due to Al (IV) and a larger one around at 4.4 ppm was assigned to Al (VI).

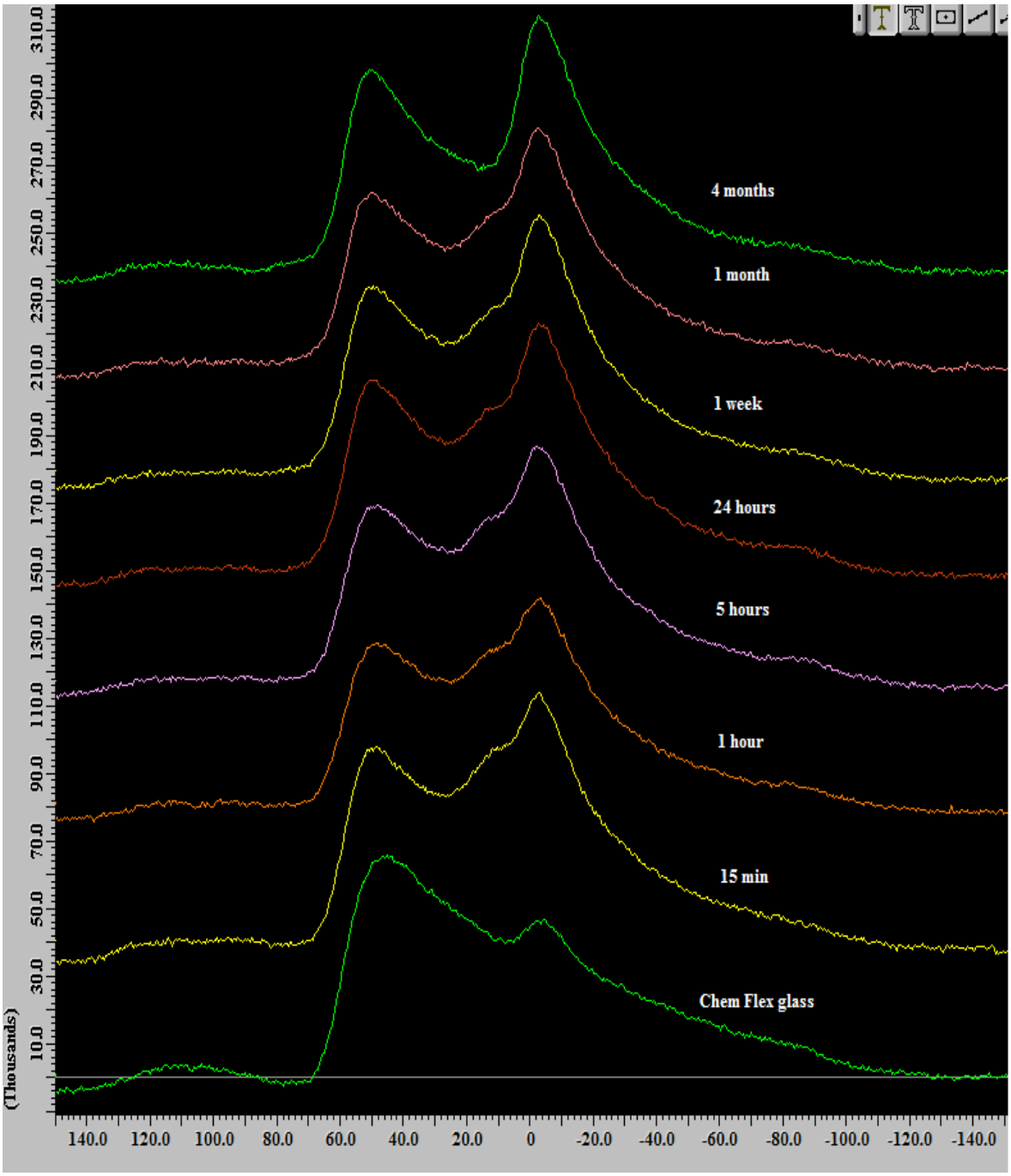

Figure 3.11: ^{27}Al MAS-NMR spectrum of Chemflex dental cement – cetyl pyridinium chloride doped specimens

The peaks for Chemflex cement doped with CPC were observed at around 48.4.0 ppm and around at -2.9 ppm. Fuji IX cement doped with BACH showed small peaks at around 46.0 ppm and larger ones around at 4.9 ppm.

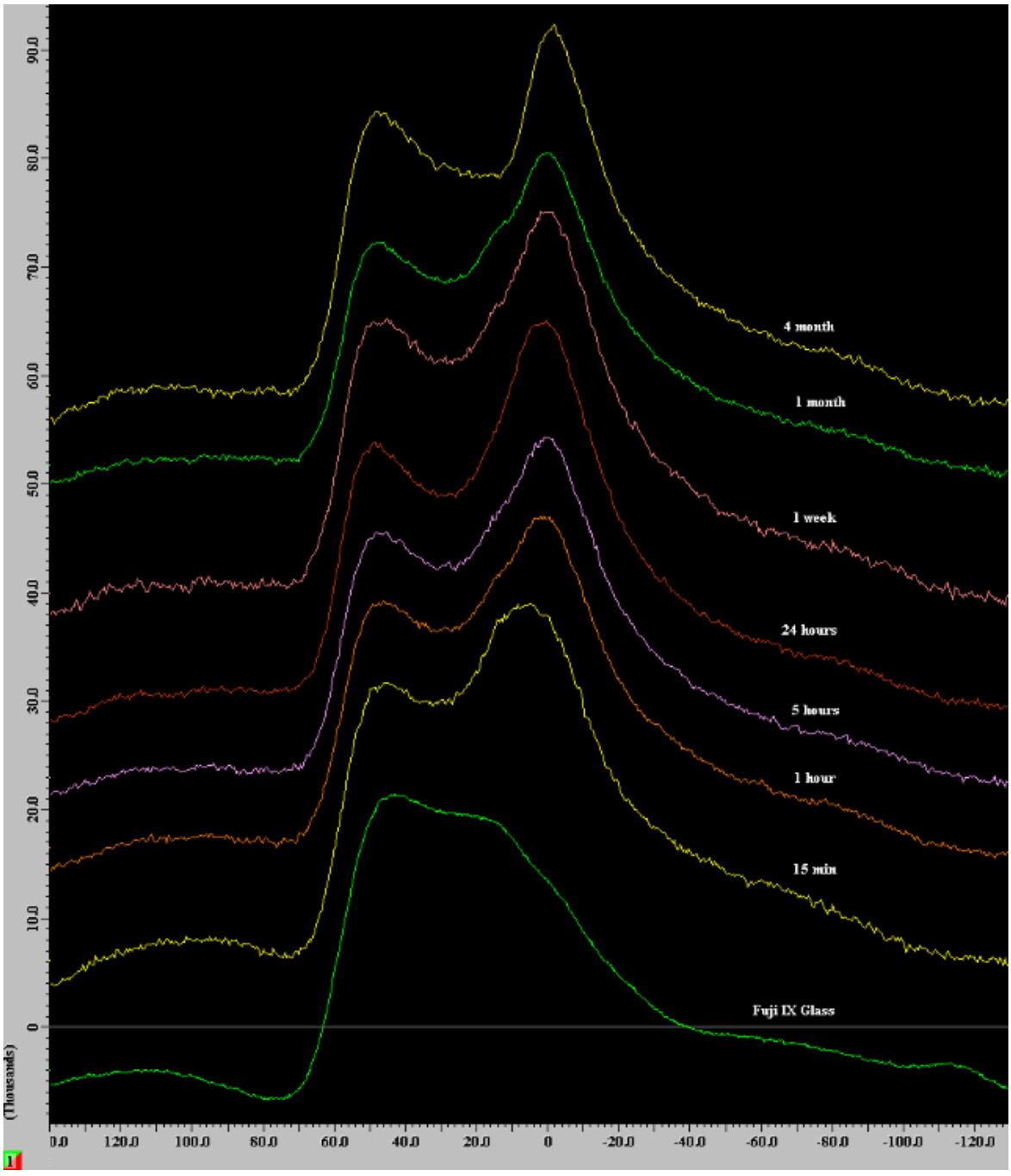

Figure 3.12: ^{27}Al MAS-NMR spectrum of Fuji IX dental cement – benzalkonium chloride doped specimens

Similarly, two significant peaks at around 49.9 ppm and around at -2.9 ppm were observed for the Chemflex samples doped with BACH and they were assigned to Al (IV) and Al (VI) respectively.

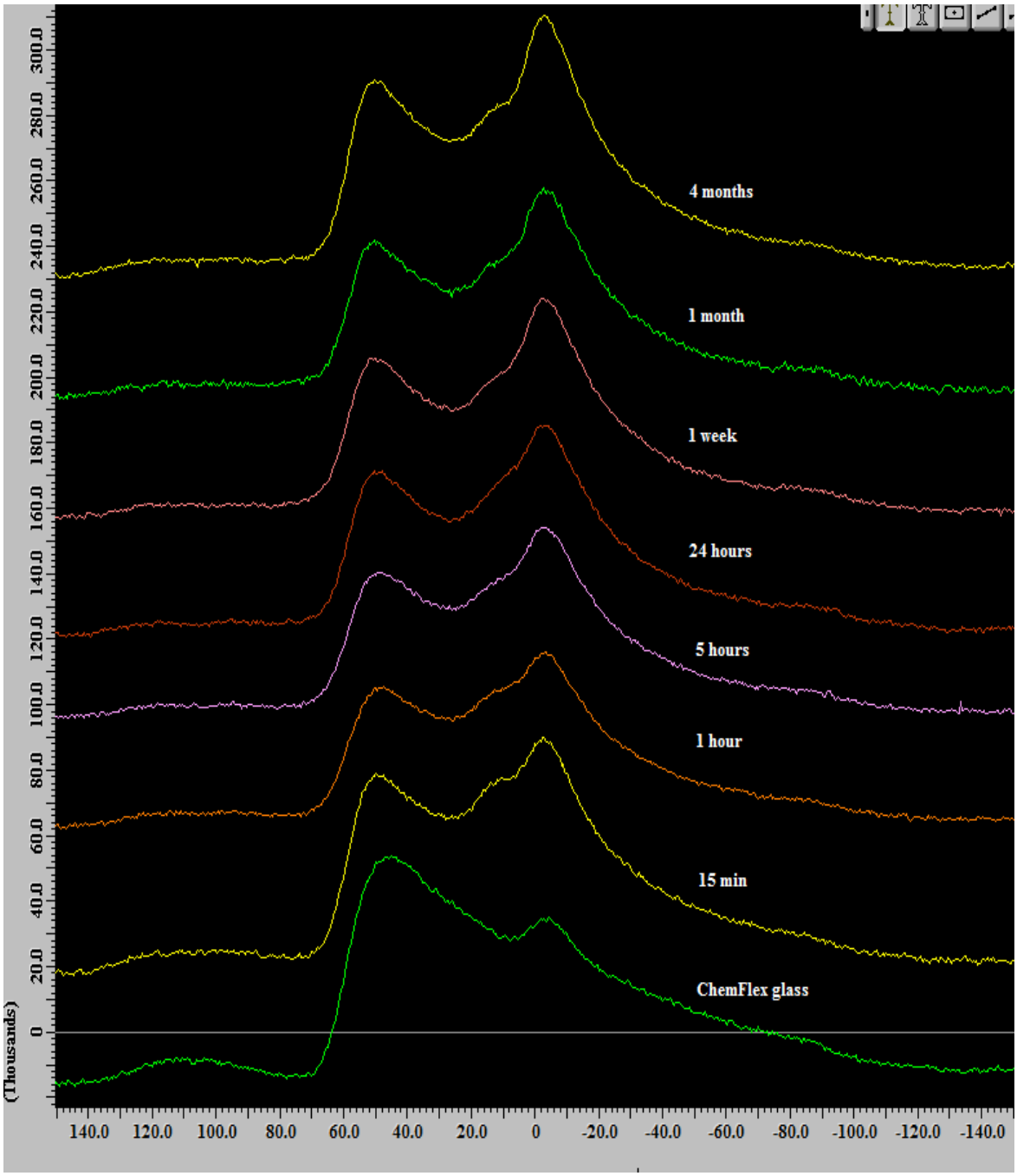

Figure 3.13: ^{27}Al MAS-NMR spectrum of Chemflex dental cement – benzalkonium chloride doped specimens

3.4.1 ^{27}Al MAS-NMR spectra and reaction kinetics

^{27}Al MAS-NMR data were used to determine the kinetics of conversion of Al (IV) into Al (VI). Heights of both Al (IV) and Al (VI) peaks were determined (Figure 3.14), their ratios were calculated and the results are presented in Table 3.13.

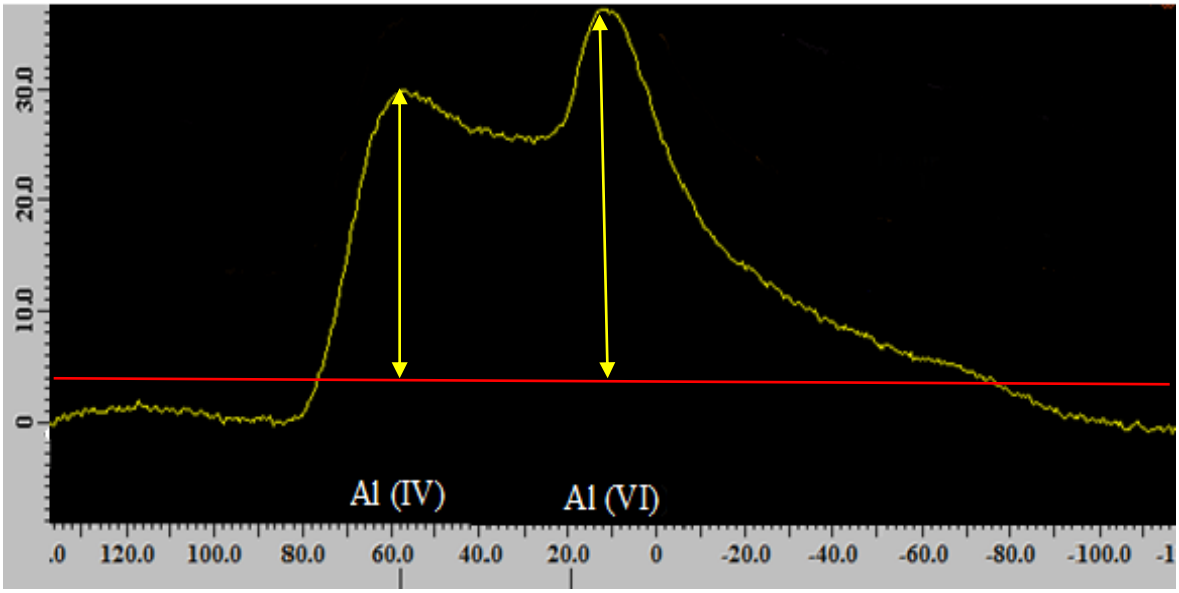

Figure 3.14: Showing measurement used for determination of height of Al (VI) peak and Al (IV) peak

Analysis of the data in Table 3.13 shows the changes of ratio of Al (VI) to Al (IV) during cement maturation. A clear increase in the ratios of Al (VI)/Al (IV) was observed for all cement formulations with time. For Fuji IX, the control specimens the Al (VI)/Al (IV) ratio increased from 1.22 at 15 minutes to 1.34 at 4 months. Doped Fuji IX samples showed ratios of 1.27 at 15 minutes and 1.38 at 4 months for CPC, and 1.23 at 15 minutes and 1.29 at 4 months for BACH. At the same aging periods, Chemflex showed ratios of 1.21 and 1.36 for the control specimens, 1.07 and 1.26 for CPC and 1.19 and 1.32 for BACH. In general, the control specimens exhibited slightly higher Al (VI)/Al (IV) ratios than doped ones, but the trend was less clear for Fuji IX. The ratios for Fuji IX were generally higher than for Chemflex.

Table 3.13: Ratios of Al (VI)/Al (IV)

	Fuji IX			**Chemflex**		
Time	**Control**	**CPC**	**BACH**	**Control**	**CPC**	**BACH**
Glass	0.93	-	-	0.74	-	-
15 min	1.22	1.27	1.23	1.21	1.07	1.19
1 hour	1.34	1.30	1.33	1.27	1.25	1.27
5 hours	1.32	1.35	1.36	1.30	1.28	1.30
24 hours	1.43	1.37	1.43	1.29	1.26	1.28
1 week	1.22	1.39	1.37	1.42	1.37	1.37
1 month	1.37	1.33	1.36	1.38	1.34	1.33
4 months	1.34	1.38	1.29	1.36	1.26	1.32

3.5 Fluoride release

Fluoride (F^-) release from GICs is clinically important as studies show that F^- released from GICs can have anticariogenic effect. A variety of mechanisms are involved in the anticariogenic effect of the F^-, which include the formation of fluoroapatite that has lower solubility than original carbonate apatite, the enhancement of the mineralisation and the inhibition of the microbial growth and metabolism [9, 10, 11, 12 and 13].

The release of F^- from GICs materials is well documented and is one of the most recognisable properties of these materials. This ability arises, because F^- is added to the flux (usually in a form of calcium fluoride (CaF_2) and/ or cryolite ($Na_3Al_2F_6$)) during GICs powders manufacturing for improvement of handling properties.

F^- is released from the glass powder during the GICs setting reaction when glass powder is mixed with poly (acid). During that reaction a variety of inorganic ions, including F^- are released from the glass. The di-and tri-valent metal cations released form ionic cross-links between the polymer chains. Mono-valent ions such as F^- are also released from the glass, but do not become bound within the resulting matrix, so can be released from the cement into surrounding liquids [14, 15].

The balance between the rate of release of ions from the glass into the matrix and the formation of cross-links during setting reactions is crucial in determining the properties of the final cement [15, 16 and 17]. In section 3.2 the effect of antimicrobial additives on mechanical properties of GICs has been examined. These findings clearly indicate that the addition of antimicrobial additives affect the mechanical properties of GICs and these changes are influenced by alternation of kinetics of setting and maturation processes by additives (section 3.1 and section 3.4).

As stated above release of F^- is associated with setting processes. Therefore the study of kinetics of release of F^- can gives us an inside on setting and subsequent maturation of

GICs. It can also provide information on additives influences on setting reaction of GI materials.

The purpose of this section is to examine the release of F^- from antimicrobial reformulated GICs and to determine if the observed changes in mechanical properties of reformulated materials might be related to the changes in kinetics of setting and maturations of GICs. CPC, BACH and SF at weight fractions of 1%, 2% 3% and 5% w/w were added into Fuji IX and Chemflex. Also specimens doped with 1% and 3% w/w of T and T/ZC were formulated. The specimens were prepared by the method described in Chapter 2.2. Five cylindrical shape specimens were fabricated and placed into separate plastic cylinders (containing HPLC grade water). F^- release was measured using F^- ion selective electrode at 15, 30, 45 minutes, 1, 2, 3, 4, 5, 24 hours, 1, 3, 5 and 7 weeks for CPC and BACH, and at 24 hours and 7 weeks for SF, T and T/ZC samples. Average of F^- release for each formulation and its standard deviation was calculated at each time interval and expressed in ppm. Results for cumulative F^- release for studied formulations at different times are given in Figures 3.15 to 3.22

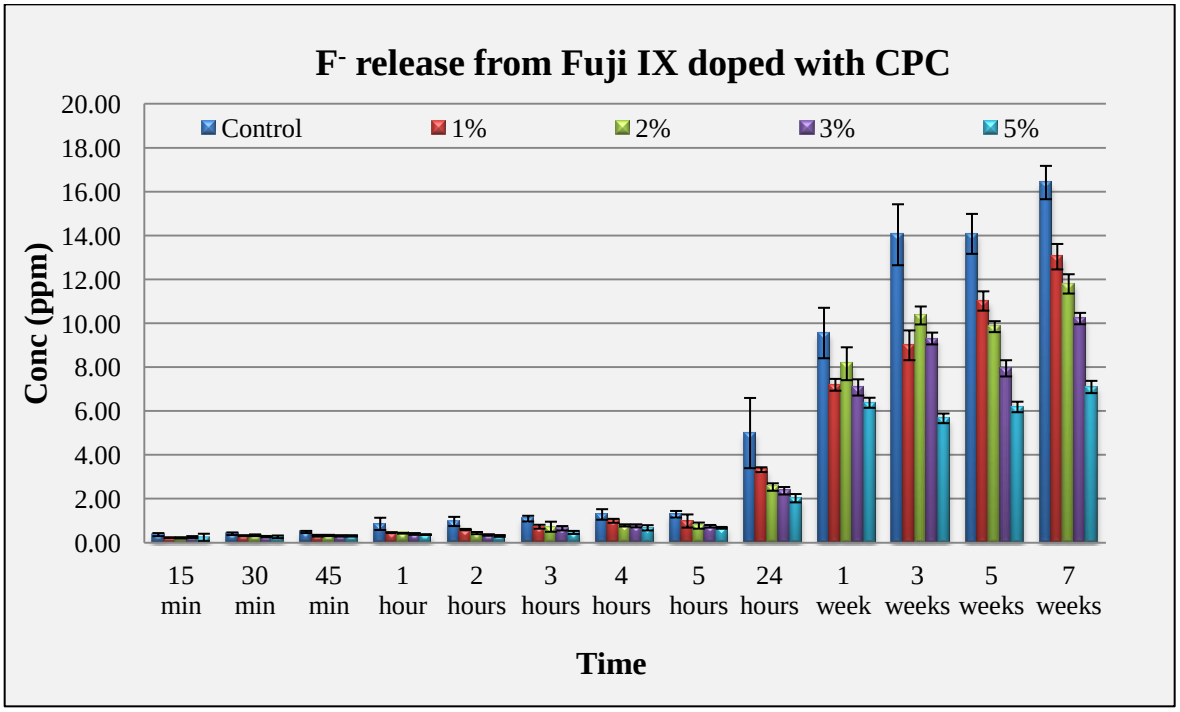

Figure 3.15: F^- release (ppm) from Fuji IX, SD presented as error bars

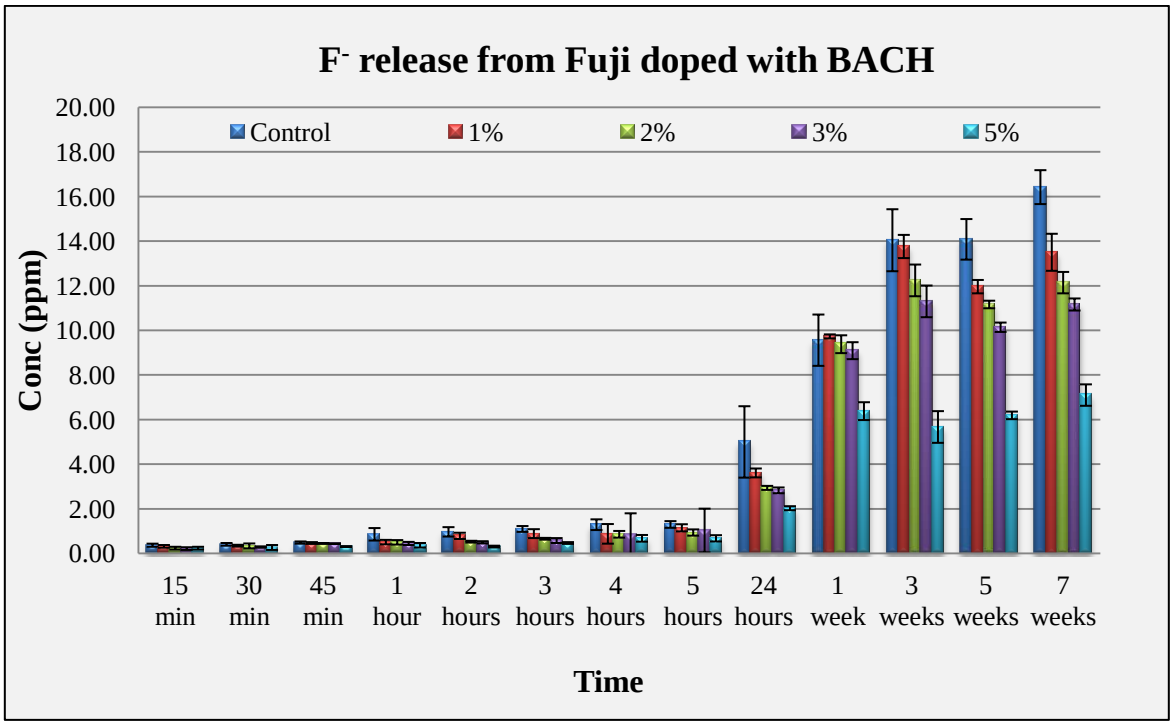

Figure 3.16: F^- release (ppm) from Fuji IX, SD presented as error bars

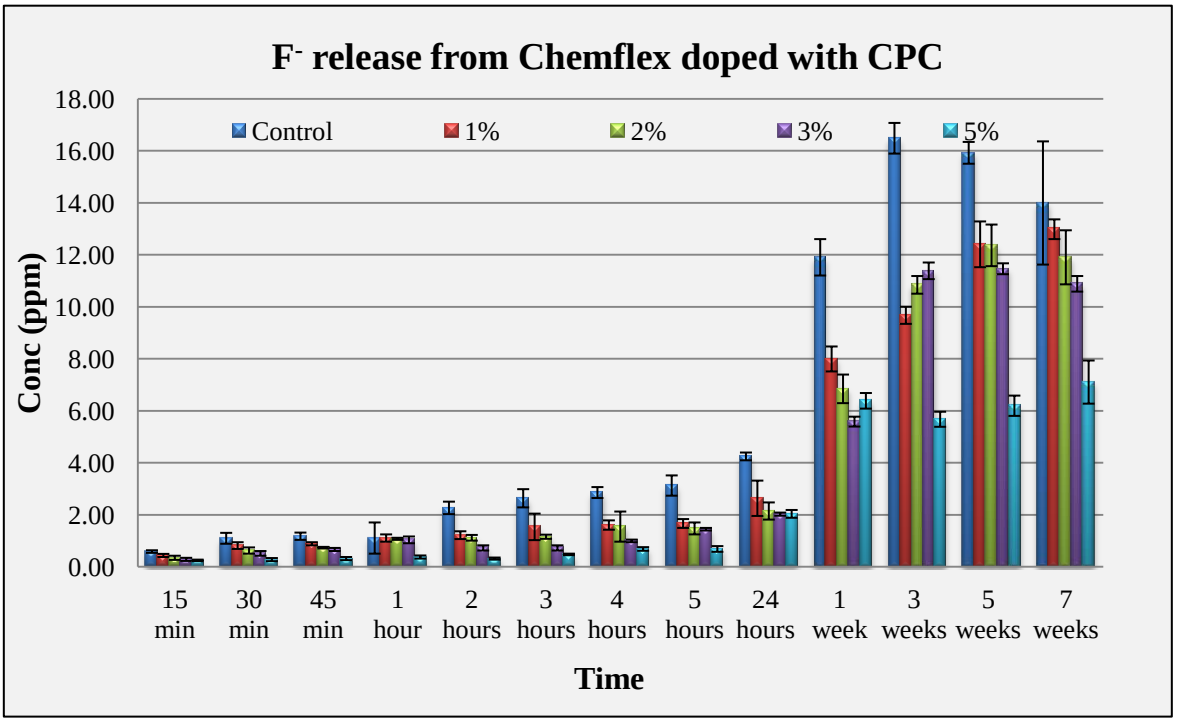

Figure 3.17: F^- release (ppm) from Chemflex, SD presented as error bars

Present findings showed that tested GICs cements continue to release F^- into solution. In general, the amount of F^- released was greater from control specimens than for doped specimens and this tendency was observed for most formulations and time periods studied. The reduction in F^- elution was significant for most of CPC and BACH samples and the significance increased with increasing percentage of doping ($p < 0.05$).

The seven weeks cumulative release for Fuji IX control samples was 16.42 ppm (± 0.76), whereas for Chemflex it was 13.99 ppm (± 2.37). Release from CPC doped samples was between 7.10 ppm (± 0.28) to 13.50 ppm (± 0.83) and for BACH doped samples between 10.56 ppm (± 0.83) to 13.98 ppm (± 0.67).

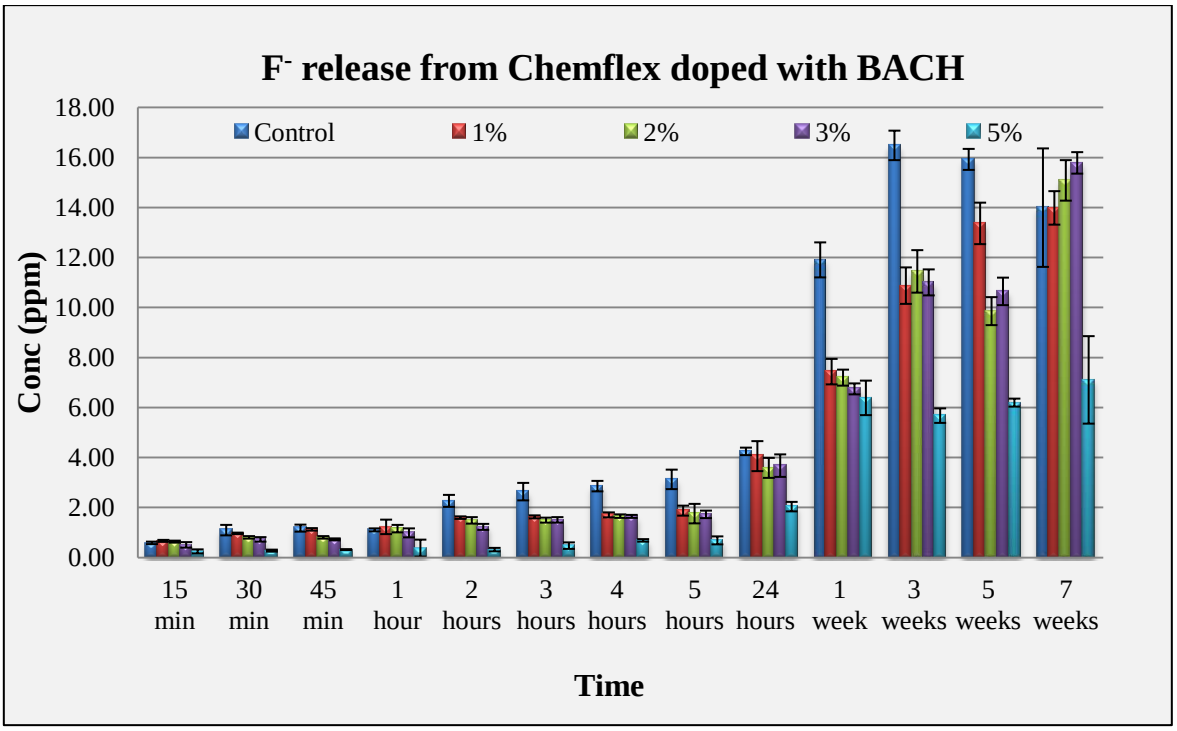

Figure 3.18: F^- release (ppm) from Chemflex, SD presented as error bars

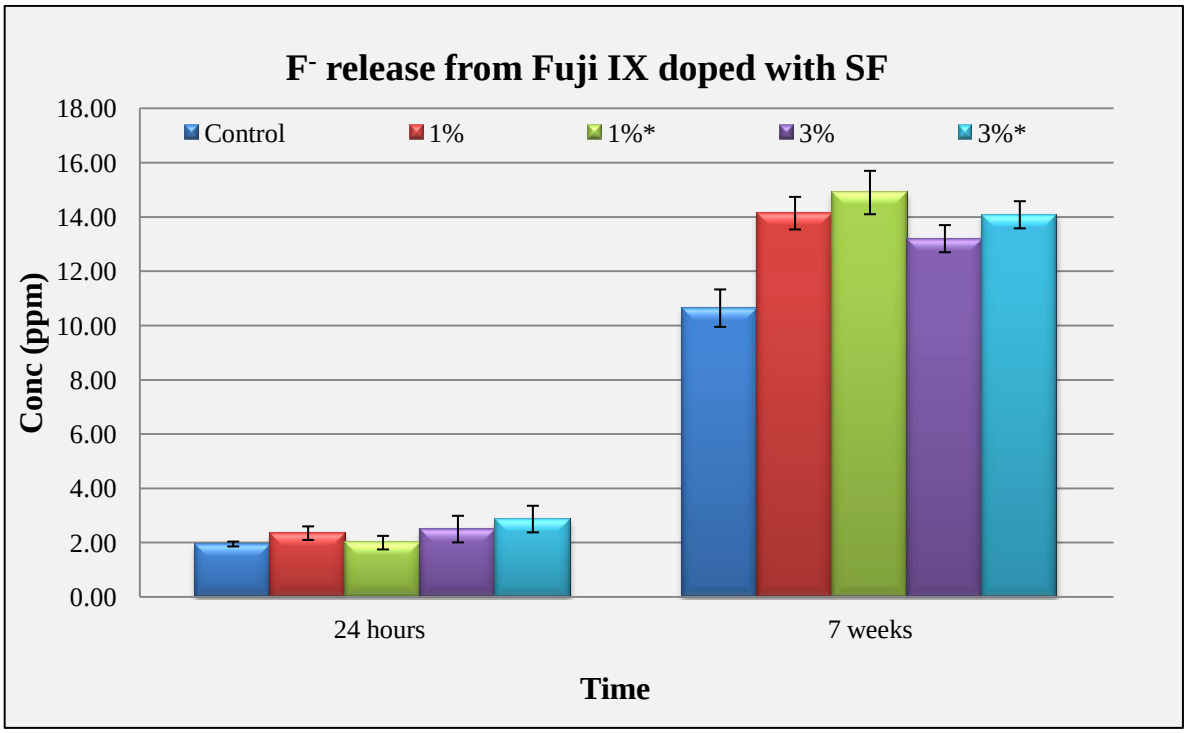

Figure 3.19: F^- release (ppm) from Fuji IX, SD presented as error bars

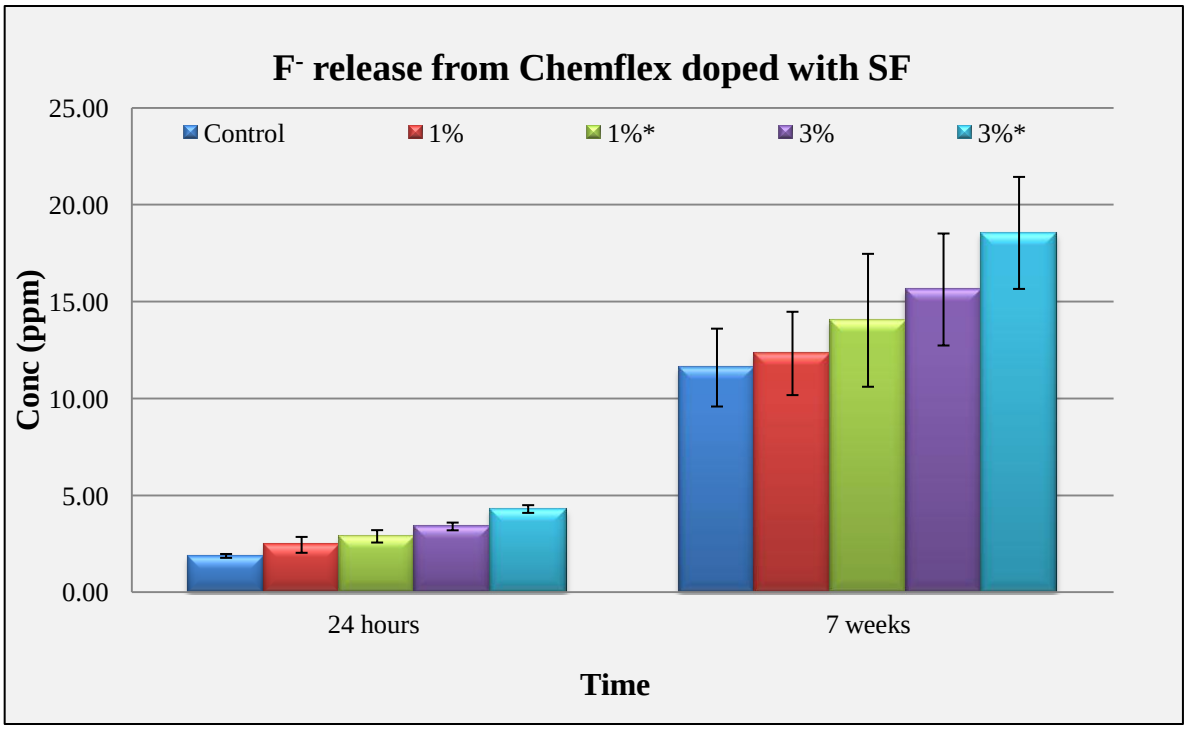

Figure 3.20: F^- release (ppm) from Chemflex, SD presented as error bars

Release of F^- from SF doped samples showed a different pattern, with a significant increase in F^- release with SF additive. The cumulative release varied between 10.64 ppm (± 0.49) to 18.55 ppm (± 2.89) and it was greatest for the highest level of addition.

Release of F^- from T doped samples ranged between 9.17 ppm (± 0.32) to 10.64 ppm (± 0.49) and for T/ZC between 9.02 ppm (± 0.97) to 10.64 ppm (± 0.49). Similarly to CPC and BACH formulations, T control samples show greater cumulative release in comparison with doped samples. The release tends to decrease with increasing percentage of doping, however this trend was not followed by Fuji IX samples. Additional doping of T samples with ZC did not influence leaching processes of F^-.

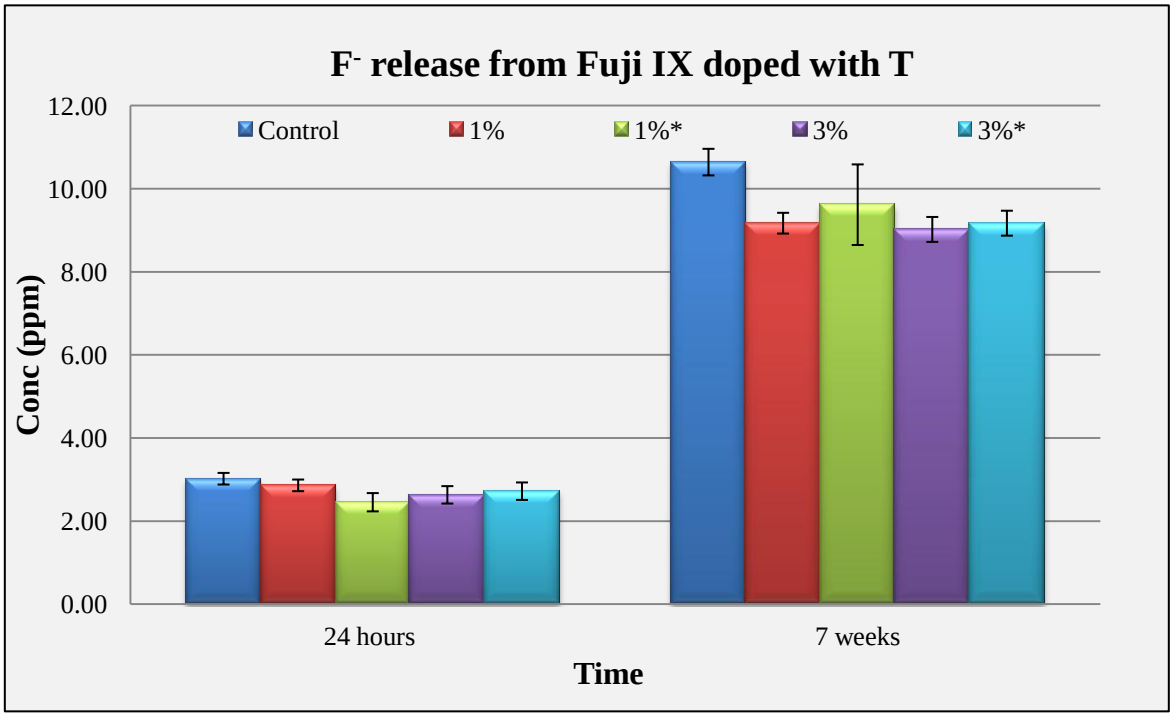

Figure 3.21: F^- release (ppm) from Fuji IX, SD presented as error bars

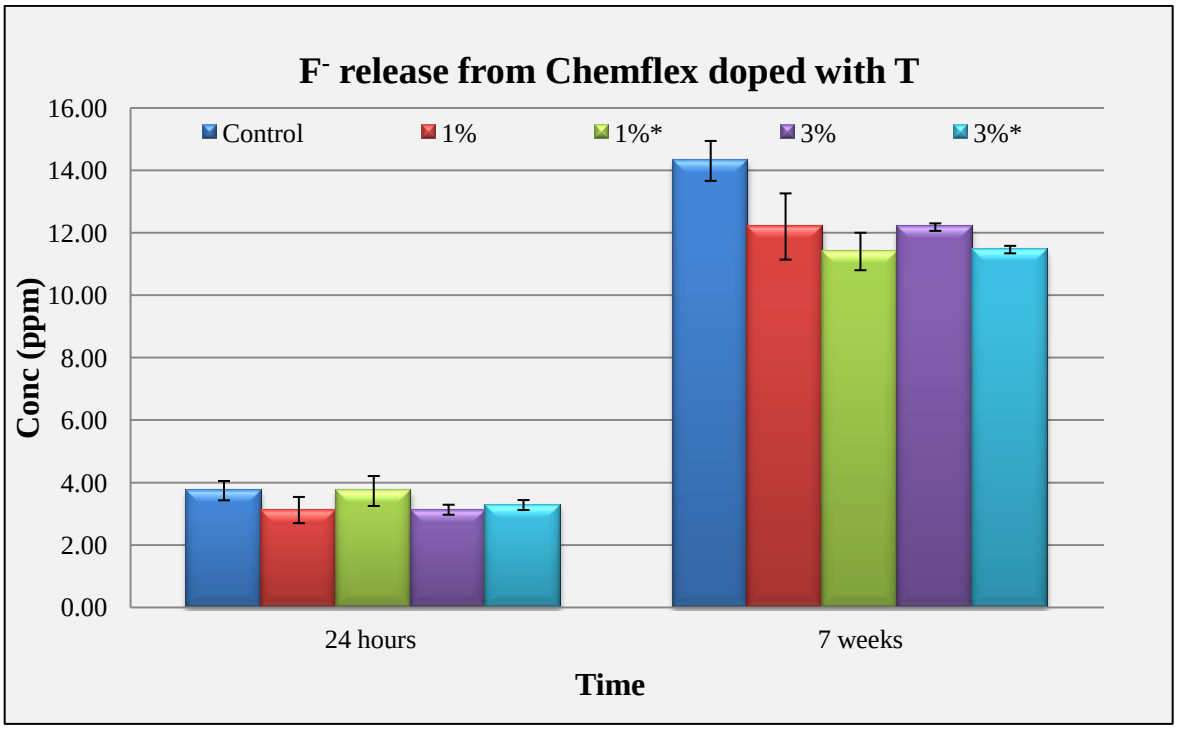

Figure 3.22: F^- release (ppm) from Chemflex, SD presented as error bars

The greatest cumulative release occurred in the first week, which ranged between 9.56 ppm for Fuji IX to 11.90 ppm for Chemflex, after which the release diminished until it became fairly constant.

Also percentage of F^- release between week one and week seven samples for CPC and BACH formulations were calculated. The percentages were calculated by dividing average release at week one by total release at week seven and multiplying by 100. Results showed that percentage F^- release at week one for Fuji IX material was lowest in comparison with samples doped with 5% of additives and they were 58%-72% for control and 77%-90% for 5% w/w of additions. Chemflex samples showed opposite trend. Control specimens released in percentage largest amount of F^- in comparison with doped specimens and they varied between 53%-72% for control samples and between 42%-43% for 5% w/w for doped specimens.

3.6 Antimicrobial additive release

The ability of GICs to release ions can potentially be beneficial in dentistry as it gives an indication that these materials can be used as a reservoir material for slow release of other species, such as antimicrobial compounds.

Antimicrobial dental materials could be used to inhibit recurrent caries and other bacterially induced dental diseases. However, in order to utilise this idea it is important to establish if reformulated cements by inclusion of bactericides are capable of releasing useful amounts of substance. It is also useful to know what the kinetics of release are. Studies on drug release kinetics might also provide important information into the function of material systems. The elucidation of the detailed transport mechanism and the structure-function relationship of a material system are critical to bridge the gap between the macroscopic data and the transport behaviour at the molecular level. Therefore, understanding the structure-function relationship of the material system is the key to the successful design of a delivery system for a particular application. Furthermore, determination of the mathematical model of release is a mean to elucidate solute transport mechanisms.

There have been several previous studies of the effect of incorporating antimicrobial agents into GICs [18, 19, 20, 21 and 22]. Addition of chlorhexidine is probably the most widely studied. Results show that only small amounts of this additive leach out and that an early stage of its release is controlled by diffusion process [18, 20 and 22].

In this section, the data for release of antimicrobial additives from cements obtained in the current project are reported. CPC, BACH and SF at weight fractions of 1%, 2% 3% and 5% w/w were added into Fuji IX and Chemflex. Also specimens doped with 1% and 3% w/w of T and T/ZC were formulated. The specimens were prepared by the method described in Chapter 2.2. Five cylindrical shape specimens were fabricated and placed into separate plastic cylinders (containing HPLC grade water). Several spectroscopic instrumentations were used in order to determination the release of additives. The release of additives was measured at 15 minutes, 30 minutes, 45 minutes, 1, 2, 3, 4 and 5 hours, 24 hours, 1 week, 3

weeks, 5 weeks and 7 weeks for CPC and BACH, and at 24 hours and 7 weeks for SF, T and T/ZC samples. The additives concentrations were calculated from calibration curves obtained from each additive. The release was studied at appropriate time intervals. The average bactericide release of each formulation and its standard deviation was calculated at each time interval, and expressed in mol L^{-1}. Results were statistically analysed by Student's t-test at levels of significance of $\alpha = 0.05$ and df = 4-8. Results of release of antimicrobial additives are presented in Figures 3.23 to 3.30. Additionally, the release equilibriums for additives from both Fuji IX and Chemflex are presented in Figure 3.31 and 3.32. The data was converted to the form required by Fick's law, i.e. M_t/M_∞ against $\sqrt{t}$. Straight lines were fitted through data using least squares regression and the coefficients, r, for the correlation calculated for each formulation. These are shown in Table 3.14. The diffusion coefficients were determined from the linear portion of the graphs, taking the slopes and substituting into the equation $D = s^2 \pi l^2/4$ and these are shown in Table 3.15.

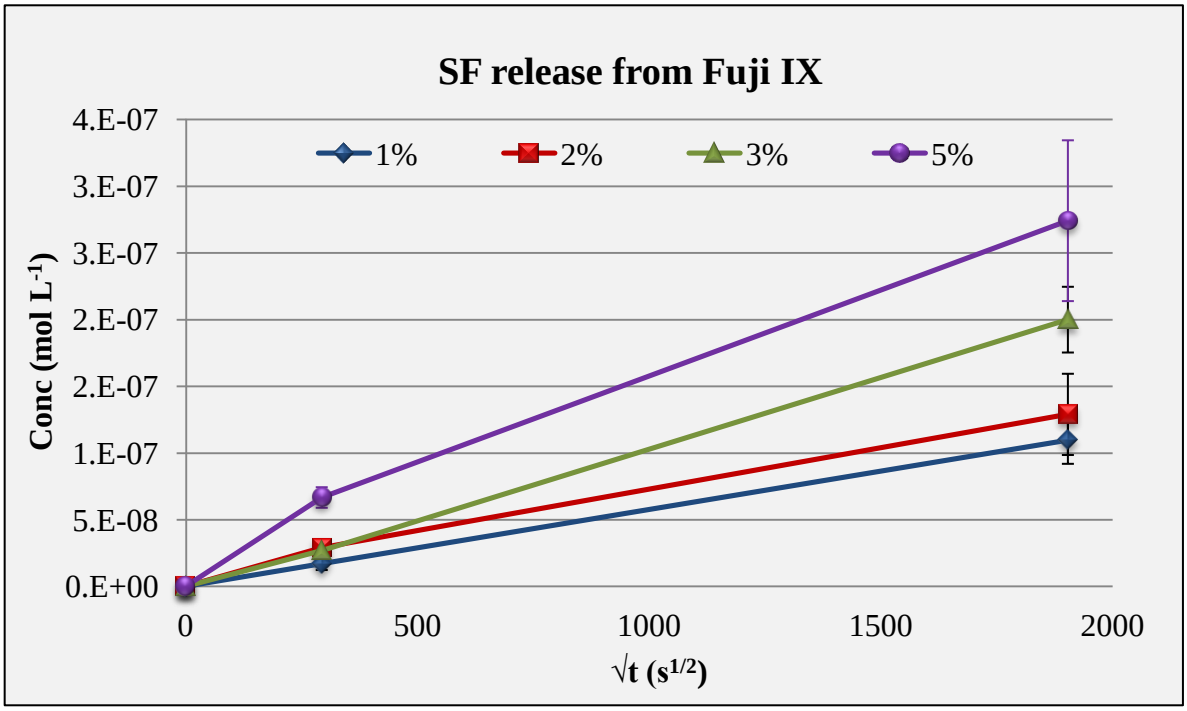

Figure 3.23: Sodium fusidate release from Fuji IX - HPLC-UV, SD as error bars

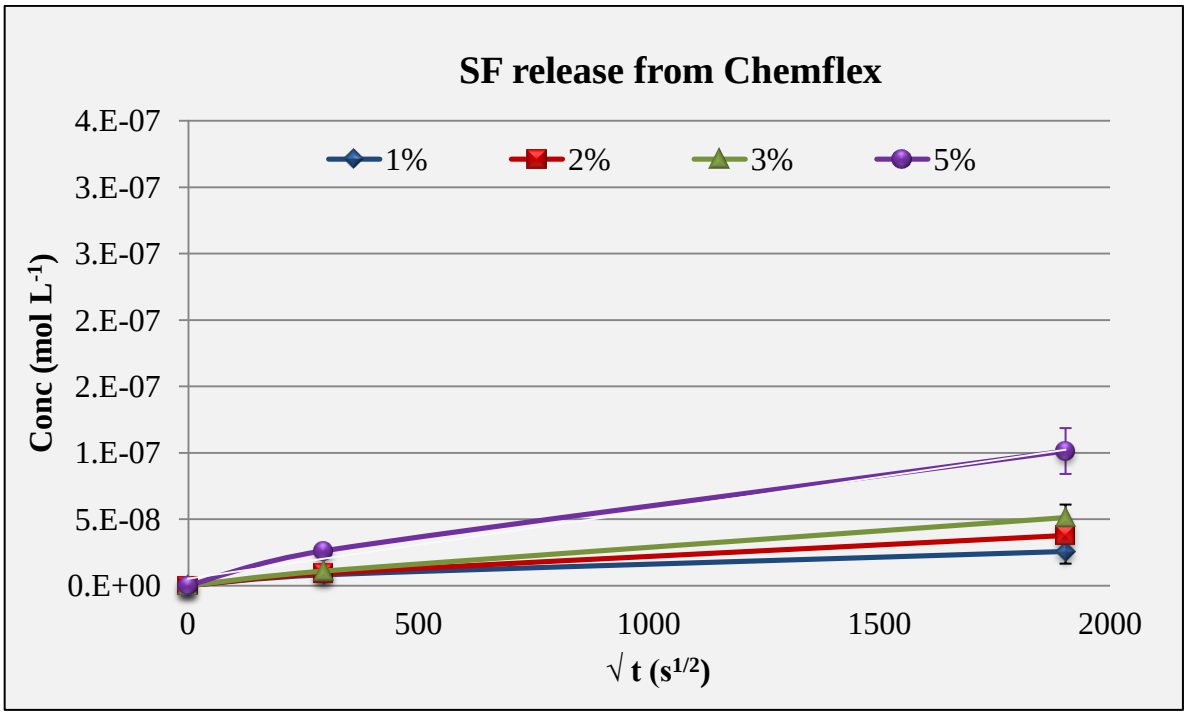

Figure 3.24: Sodium fusidate release from Chemflex - HPLC-UV, SD as error bars

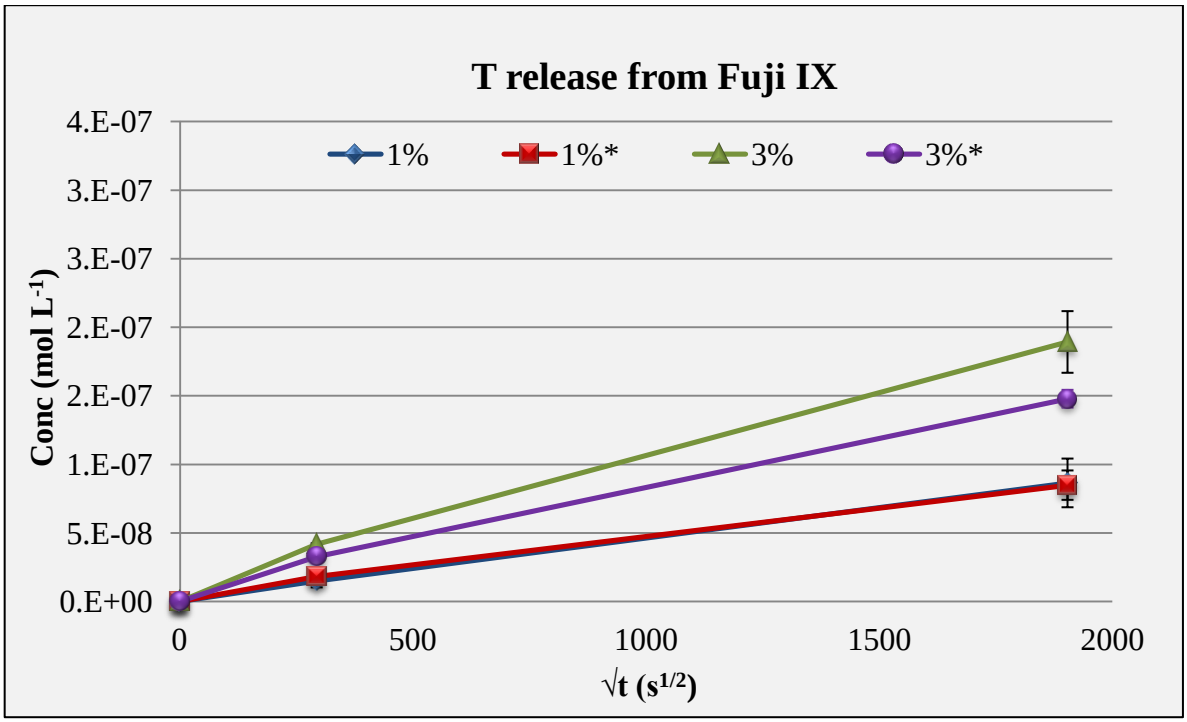

Figure 3.25: Triclosan release from Fuji IX - LC-MS, SD as error bars

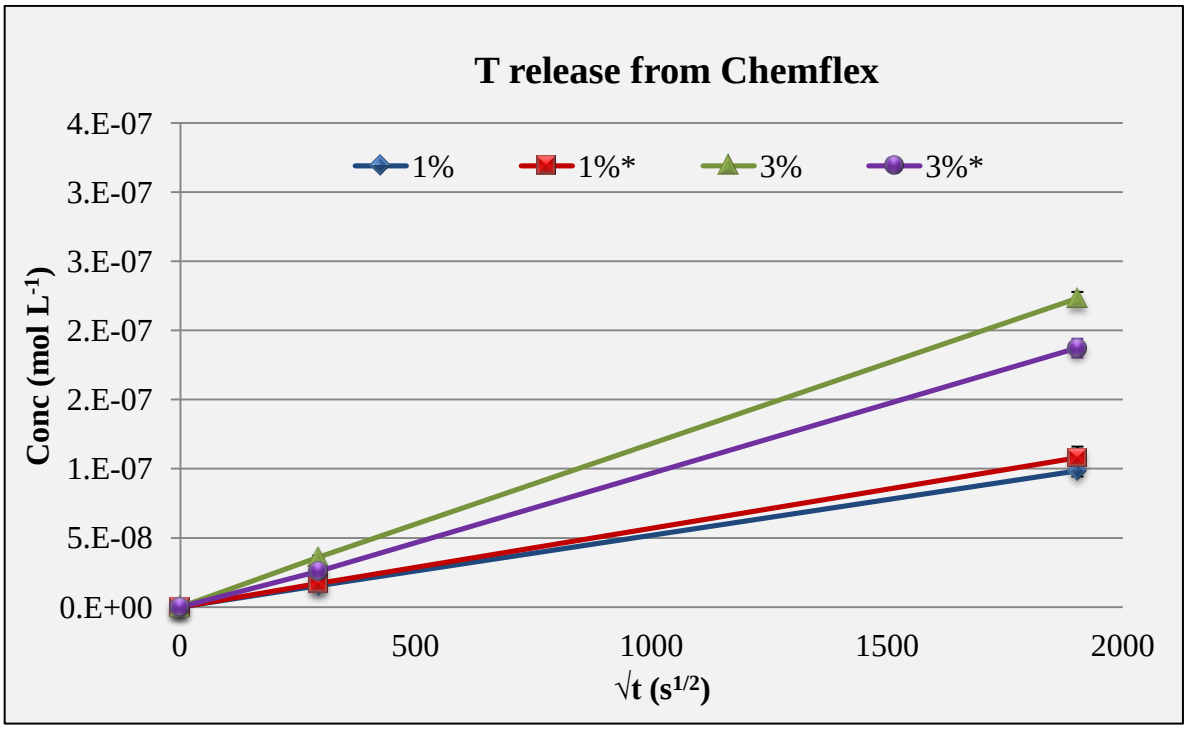

Figure 3.26: Triclosan release from Chemflex - LC-MS, SD as error bars

The results showed that both brands of GICs were able to release antimicrobial agents and the amount released varied with percentages (w/w) of doping. Fuji IX samples doped with SF released between $1.10x10^{-7}$ mol L^{-1} (± $1.78x10^{-8}$) to $2.74x10^{-7}$ mol L^{-1} (± $6.3x10^{-8}$). Chemflex released between $2.58x10^{-8}$ mol L^{-1} (± $9.20x10^{-9}$) to $1.10x10^{-7}$ mol L^{-1} (± $1.73x10^{-8}$) (Figures 3.23 and 3.24).

Fuji IX samples doped with T released between $9.48x10^{-8}$ mol L^{-1} (± $4.01x10^{-9}$) and $1.87x10^{-7}$ mol L^{-1} (± $6.8x10^{-9}$). Chemflex released between $8.64x10^{-8}$ mol L^{-1} (± $8.13x10^{-9}$) to $1.48x10^{-7}$ mol L^{-1} (± $8.81x10^{-9}$) (Figures 3.25 and 3.26).

Fuji IX samples doped with CPC and BACH released between $1.96x10^{-7}$ mol L^{-1} (± $5.71x10^{-9}$) and $7.52x10^{-7}$ mol L^{-1} (± $7.41x10^{-8}$) and between $1.90x10^{-7}$ mol L^{-1}(± $6.28x10^{-9}$) to $4.08x10^{-7}$ mol L^{-1} (± $6.30x10^{-8}$) (Figures 3.27 and 3.28).

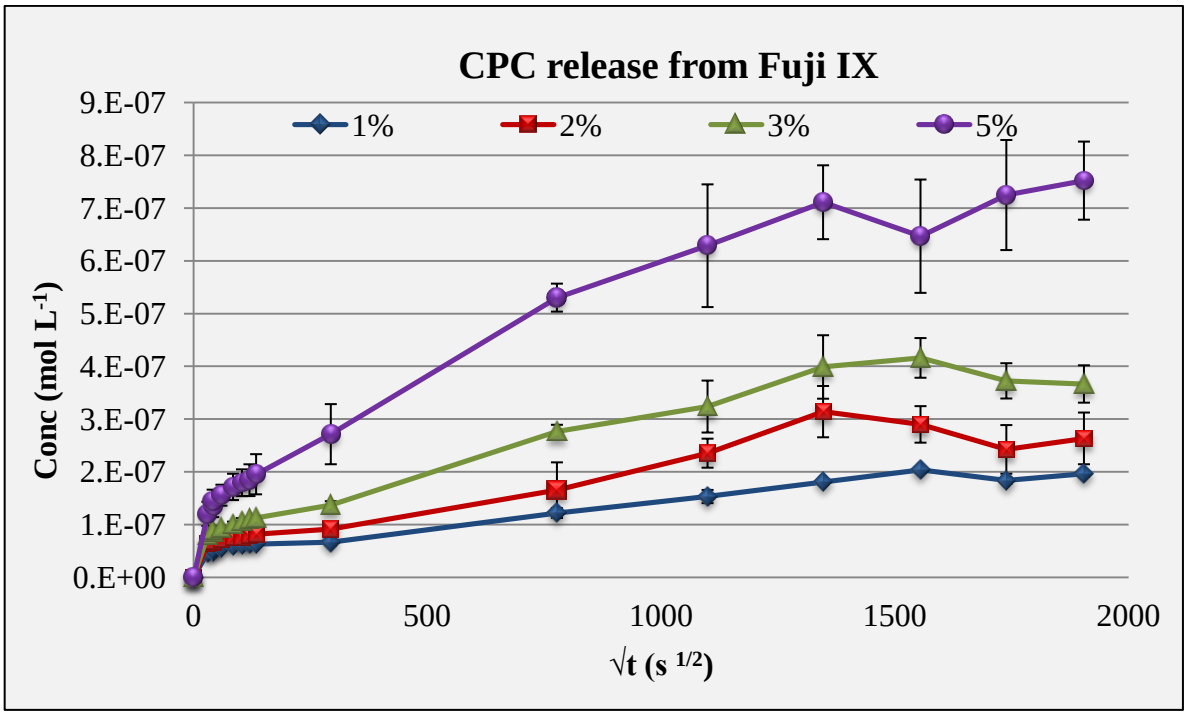

Figure 3.27: Cetyl pyridinium chloride release from Fuji IX-UV, SD as error bars

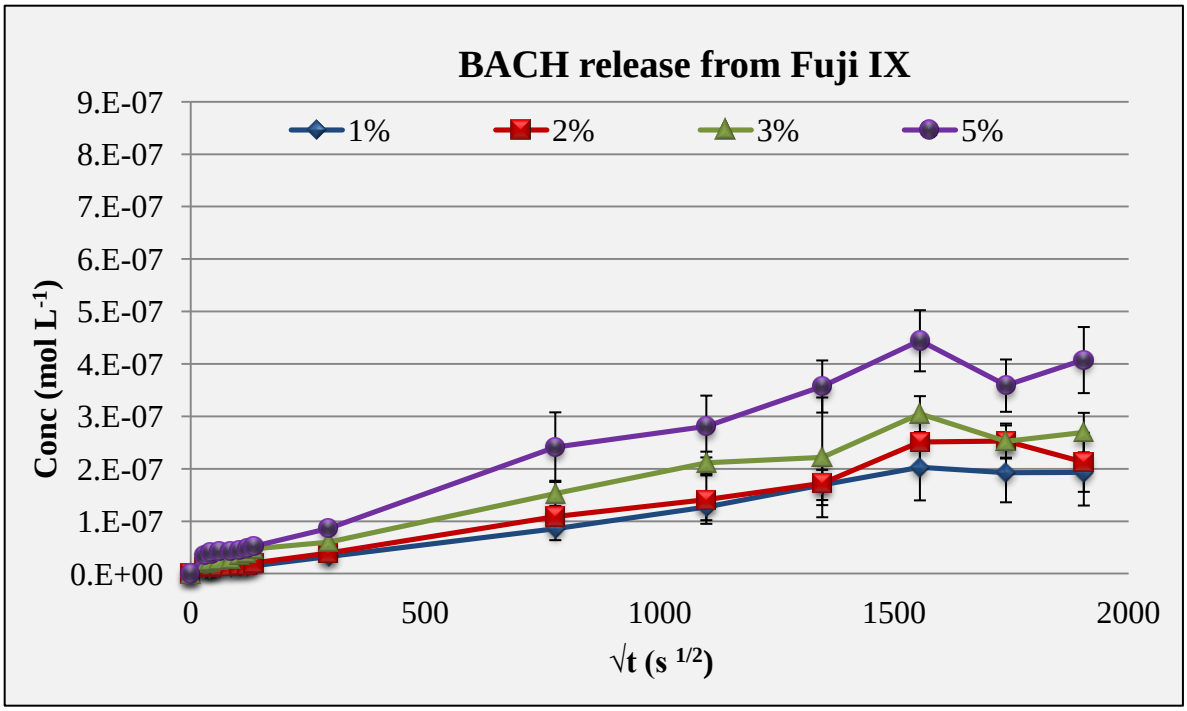

Figure 3.28: Benzalkonium chloride release from Fuji IX-UV, SD as error bars

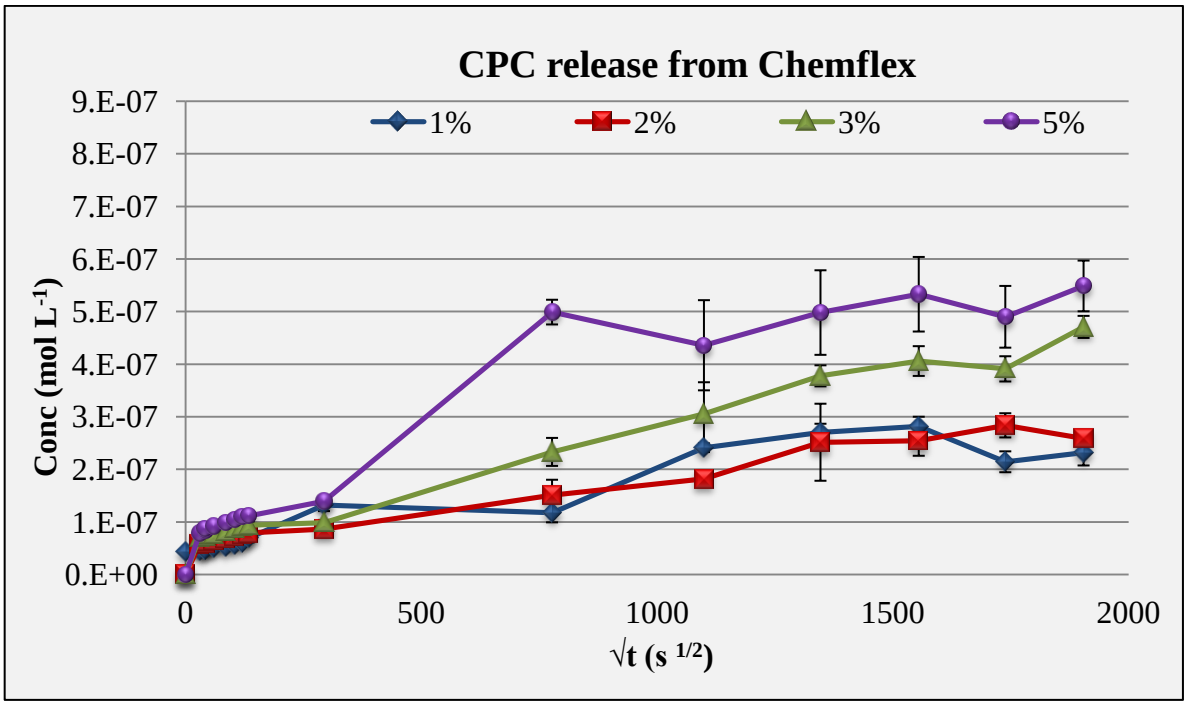

Figure 3.29: Cetyl pyridinium chloride release from Chemflex-UV, SD as error bars

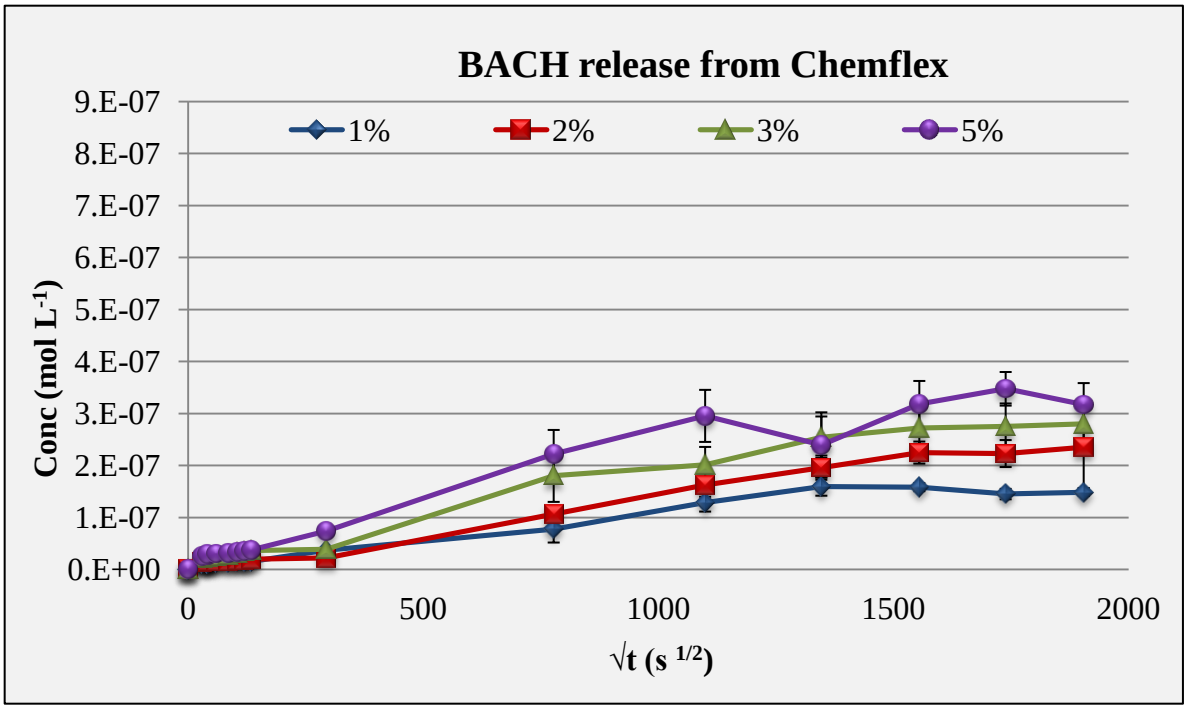

Figure 3.30: Benzalkonium chloride release from Chemflex-UV, SD as error bars

Chemflex samples containing CPC and BACH released between $2.14x10^{-7}$mol L^{-1} (± $2.44x10^{-8}$) to $5.49x10^{-7}$mol L^{-1} (± $4.82x10^{-8}$) and between $1.49x10^{-7}$mol L^{-1} (± $7.28x10^{-9}$) to $3.17x10^{-7}$mol L^{-1} (± $4.16x10^{-8}$) (Figures 3.29 and 3.30).

Calculated equilibrium recovery showed that only small amount of bactericide was released from the sample in each case. For example, for Fuji IX percentage of SF recovered varied between 2.14% (± 0.64) to 3.58% (± 0.62) of original amount of bactericide added, whereas Chemflex samples released between 0.61% (± 0.34) to 0.93% (± 0.34) of SF (Figures 3.31 and 3.32).

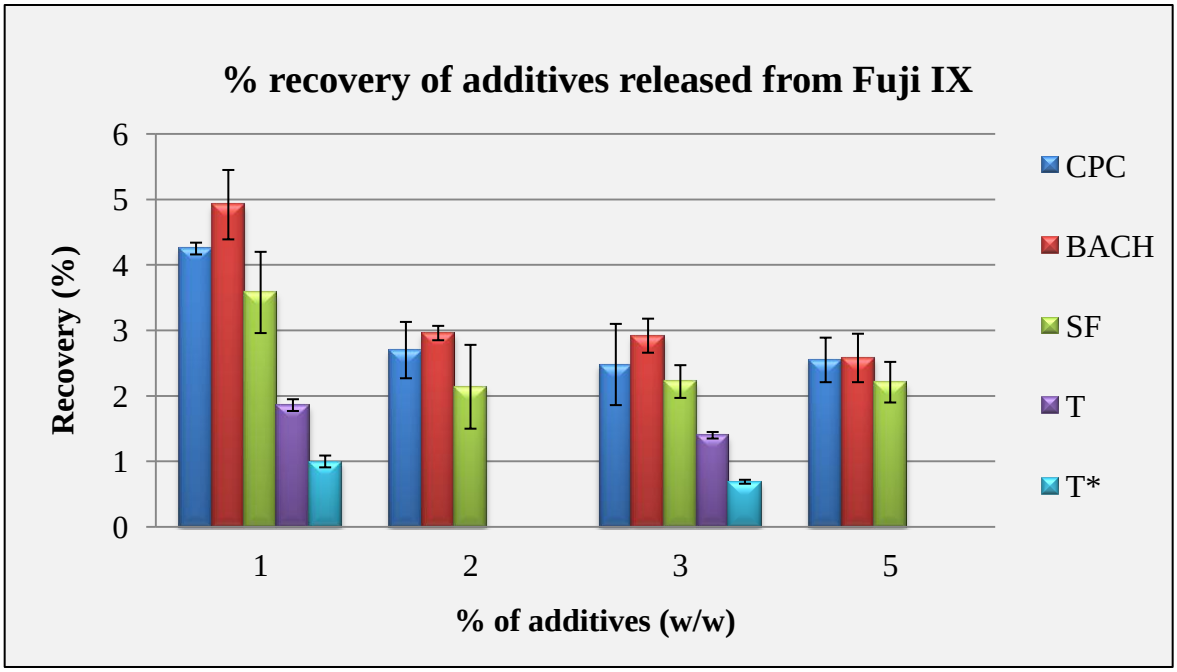

Figure 3.31: Equilibrium recovery of antimicrobial species, SD as error bars

Similarly small amounts were released for other additive. Total release of CPC and BACH was between 2.48 (± 0.62) - 4.92 (± 0.53) % for CPC and between 1.70 (± 0.23) - 5.00 (± 0.60) % for BACH and occurred within first week of release. The percentage of T recovered varied between 1.09% (± 0.09) to 1.86% (± 0.09), whereas the total percentage release of T from T/ZC reformulated samples varied between 0.51% (± 0.04) to 1.00 (± 0.09).

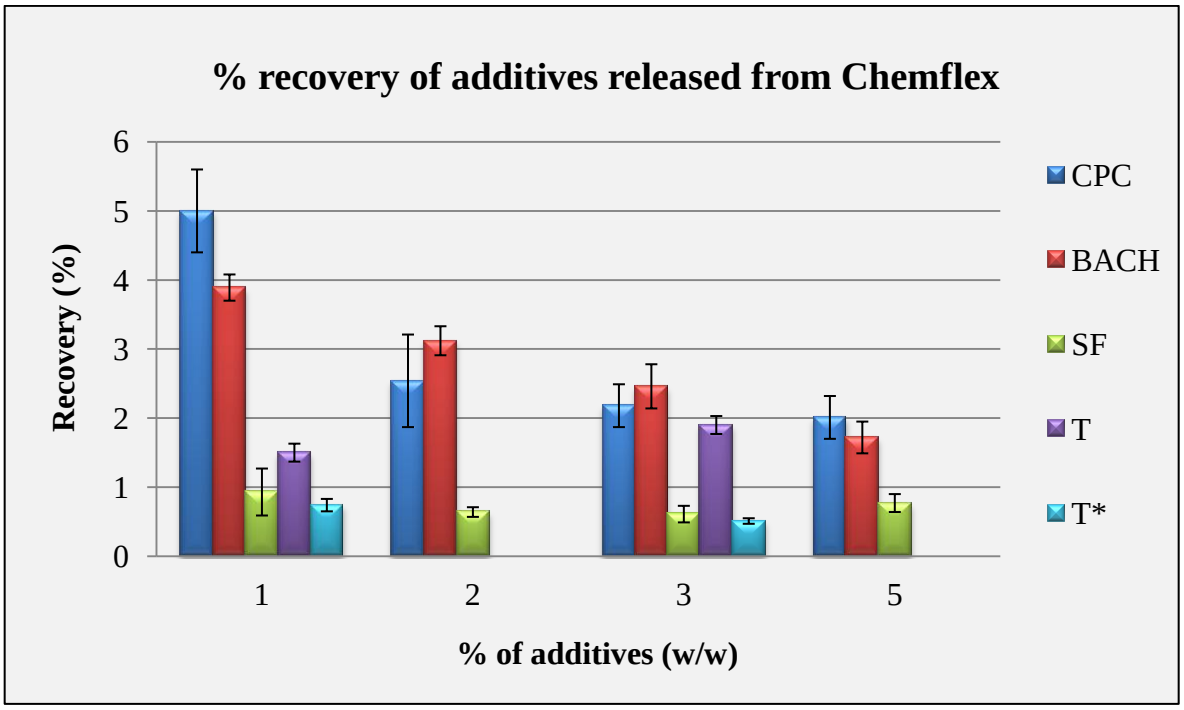

Figure 3.32: Equilibrium recovery of antimicrobial species, SD as error bars

In general, samples doped with CPC and BACH doped samples exhibited the greatest release followed by SF, T and T/ZC. In general, the percentage recovery of samples doped with lower percentages was greater than samples with higher percentages. The results also showed that Fuji IX was able to release statistically significantly greater amount of additive than Chemflex ($p < 0.001$) in all cases.

Current study also determined the kinetics of release of the antimicrobial. The result showed that the release of these active species clearly exhibits the region where the release rate is proportional to $\sqrt{t}$, hence occurs by diffusion. Correlation coefficients for this linear region were generally above 0.9900 and even in the worse cases, exceeded 0.9800, so diffusion could be assumed in all cases. In general, this linear region lasted for 2-4 weeks after exposure to water. The results for these studies are shown in Table 3.14, with the associated diffusion coefficients data in Table 3.15.

Table 3.14: Linear regression equations and correlation coefficients for release of cetyl pyridinium chloride and benzalkonium chloride

Cement/amount and additive type	**Equation**	**Correlation Coefficient, r**
Fuji IX/		
1% Cetyl pyridinium chloride	y =0.0005x+0.2417	0.9945
2% Cetyl pyridinium chloride	y=0.0006x+0.2249	0.9863
3% Cetyl pyridinium chloride	y=0.0006x+0.2155	0.9954
5% Cetyl pyridinium chloride	y=0.0006x+0.1835	0.9869
1% Benzalkonium chloride	y=0.0006x+0.0054	0.9934
2% Benzalkonium chloride	y=0.0006x+0.0274	0.9947
3% Benzalkonium chloride	y=0.0006x+0.0608	0.9899
5% Benzalkonium chloride	y=0.0006x+0.0599	0.9922
Chemflex/		
1% Cetyl pyridinium chloride	y=0.0005x+0.1915	0.9909
2% Cetyl pyridinium chloride	y=0.0004x+0.2283	0.9929
3% Cetyl pyridinium chloride	y=0.0005x+0.1292	0.9937
5% Cetyl pyridinium chloride	y=0.0006x+0.1314	0.9911
1% Benzalkonium chloride	y=0.0006x+0.0478	0.9941
2% Benzalkonium chloride	y=0.0006x+0.0092	0.9905
3% Benzalkonium chloride	y=0.0005x+0.0666	0.9905
5% Benzalkonium chloride	y=0.0005x+0.0612	0.9985

The diffusion coefficient between additives percentages and types were very comparable and they varied between $1.13x10^{-12}$ m^2 s^{-1} to $7.85x10^{-13}$ m^2 s^{-1}. In general, Chemflex exhibited lower diffusion coefficient value in comparison with Fuji IX.

Table 3.15: Diffusion coefficients of investigated glass-ionomer cements and additive variations

Cement/amount and additive type	**Diffusion coefficient $m^2 s^{-1}$**
Fuji IX/	
1% Cetyl pyridinium chloride	$7.85x10^{-13}$
2% Cetyl pyridinium chloride	$1.13x10^{-12}$
3% Cetyl pyridinium chloride	$1.13x10^{-12}$
5% Cetyl pyridinium chloride	$1.13x10^{-12}$
1% Benzalkonium chloride	$1.13x10^{-12}$
2% Benzalkonium chloride	$1.13x10^{-12}$
3% Benzalkonium chloride	$1.13x10^{-12}$
5% Benzalkonium chloride	$1.13x10^{-12}$
Chemflex/	
1% Cetyl pyridinium chloride	$7.85x10^{-13}$
2% Cetyl pyridinium chloride	$5.03x10^{-13}$
3% Cetyl pyridinium chloride	$7.85x10^{-13}$
5% Cetyl pyridinium chloride	$1.13x10^{-12}$
1% Benzalkonium chloride	$1.13x10^{-12}$
2% Benzalkonium chloride	$1.13x10^{-12}$
3% Benzalkonium chloride	$7.85x10^{-13}$
5% Benzalkonium chloride	$7.85x10^{-13}$

3.7 Antimicrobial studies

Materials with antimicrobial properties can be beneficial in dentistry. Such dental materials can be used to inhibit recurrent caries and other bacterially induced dental diseases. The concept can be utilised mainly in children and mentally and physically disabled people that are not able or/and have reduced ability to ensure appropriate levels of their oral hygiene.

In this section, the agar diffusion method was employed to study the antimicrobial properties of the bactericides reformulated GICs. CPC, BACH and SF at weight fractions of 1% 3% and 5% were added into Fuji IX and Chemflex. Also specimens doped with 1% and 3% of T and T/ZC were formulated. Detailed sample preparation and methods used for this experiment can be found in Chapter 2.9. The tests were performed against *Streptococcus mutans.* This particular bacterial strain was chosen because this species is the main one that is associated with caries formation [23, 24 and 25]. Plates were seeded with two different concentrations of bacterial suspensions and were inspected after 48 hours of incubation. These extreme conditions were chosen with dilution factor differing by $1x10^{-4}$ to avoid undergrowth or overgrowth as initial concentration of suspensions was not known at this point of the experiment. Due to the irregular inhibition zone of some of the samples the area of inhibition was determined by adopting two different methods. For samples with regular circular inhibition zones equation 3.1 was used.

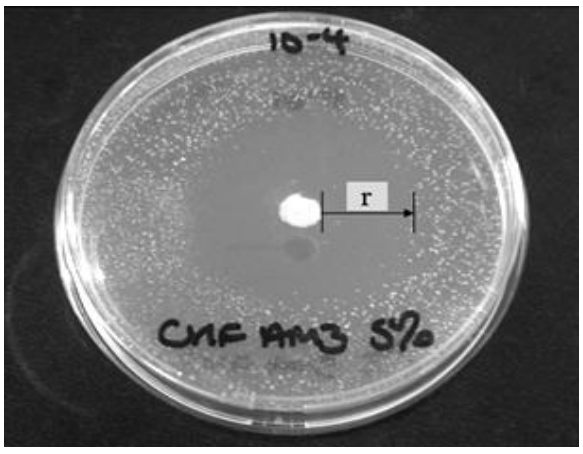

Figure 3.33: Measurement for radius used for samples with circular shape inhibition zone

$$\textbf{Inhibition zone} = 2\pi\,[(r1+r2+r3+r4)/4]^2 \quad (3.1)$$

Where r = radius of zone of inhibition

For samples with irregular inhibition zone the area was measured by equation 3.2.

$$\textbf{Inhibition zone} = (d1*d2)/2+(d2*d3)/2+(d3*d4)/2+(d4*d2)/2 \quad (3.2)$$

Where d = diagonal (measured from the edge of the specimen to the peripheries of the inhibition zone)

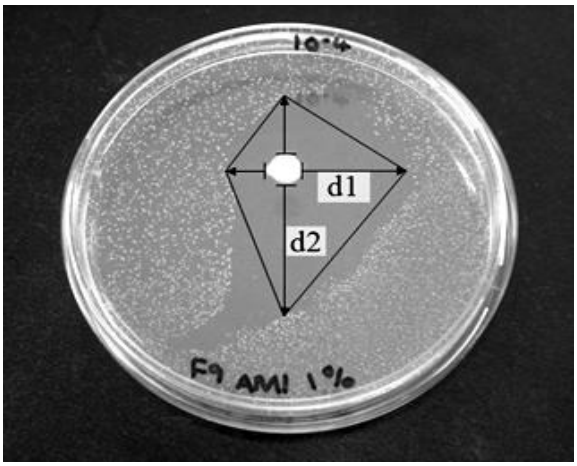

Figure 3.34: Measurement for diagonals used for samples with irregular shape inhibition zone

The results for agar-diffusion tests are shown in Figures 3.35 to 3.38. Additionally, the results of Mann-Whitney U test for both concentrations are presented in Table A.3 in Appendices. No apparent bacterial inhibition was observed for the control specimens. By

contrast, all bactericide's reformulated cements exhibited an inhibitory effect. Furthermore, statistical significances between each concentration of additive (1% vs 3%, 3% vs 5%) were found for both materials (to at least $p < 0.03$) and in general that was true for most compared groups (Table A.3 in Appendices).

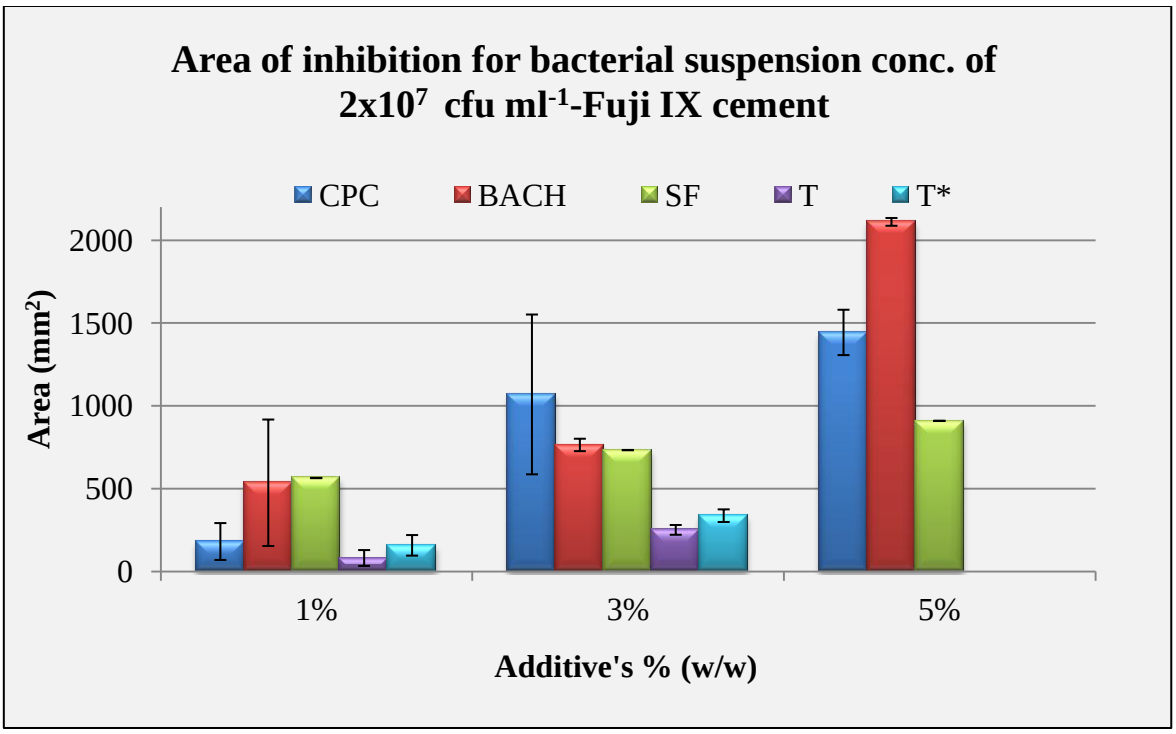

Figure 3.35: Area of inhibition (mm^2) of Fuji IX, SD depicted as error bars

No statistically significant differences were observed between specific additive loadings and these were: For Fuji IX: between 3-5% for samples doped with CPC, between 1-3% for BACH and between control and 1% for samples doped with T/ZC- plates seeded with $2x10^7$ cfu ml^{-1} concentration; for plates seeded with 1676 cfu ml^{-1} concentration they were between 3-5% for samples doped with CPC, and between 1-3% for BACH, and between 1-3% and 3-5% for SF.

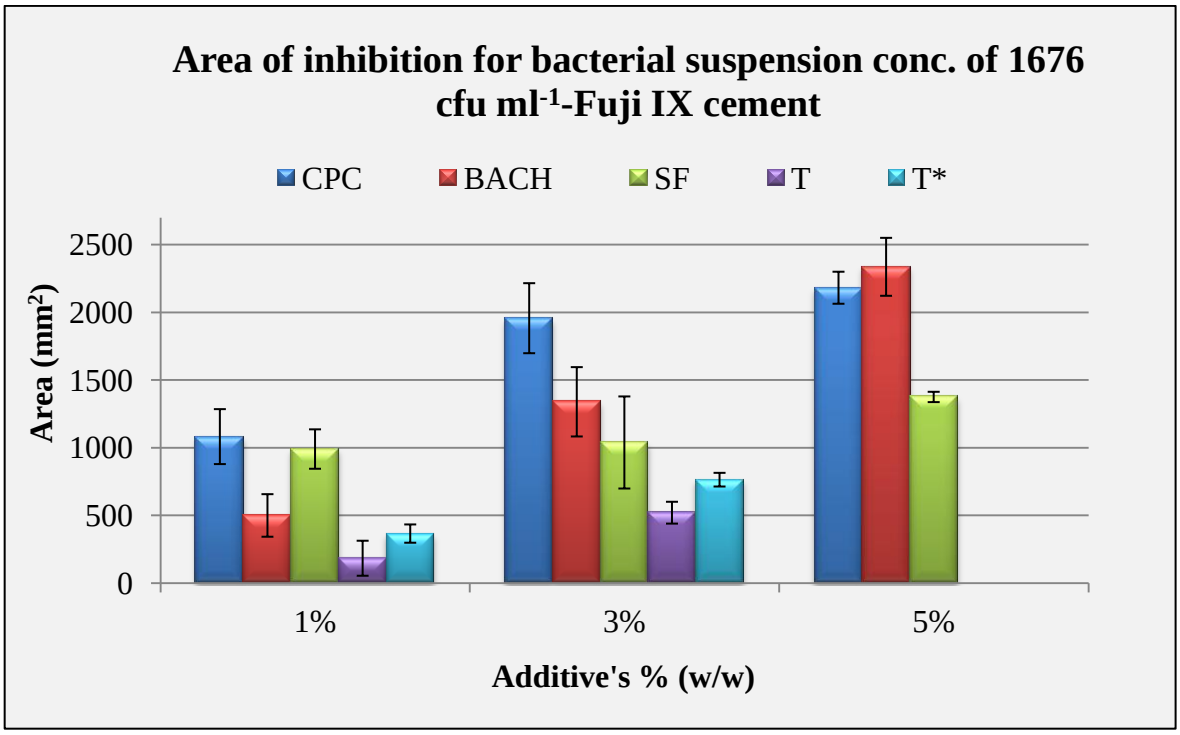

Figure 3.36: Area of inhibition (mm^2) of Fuji IX, SD depicted as error bars

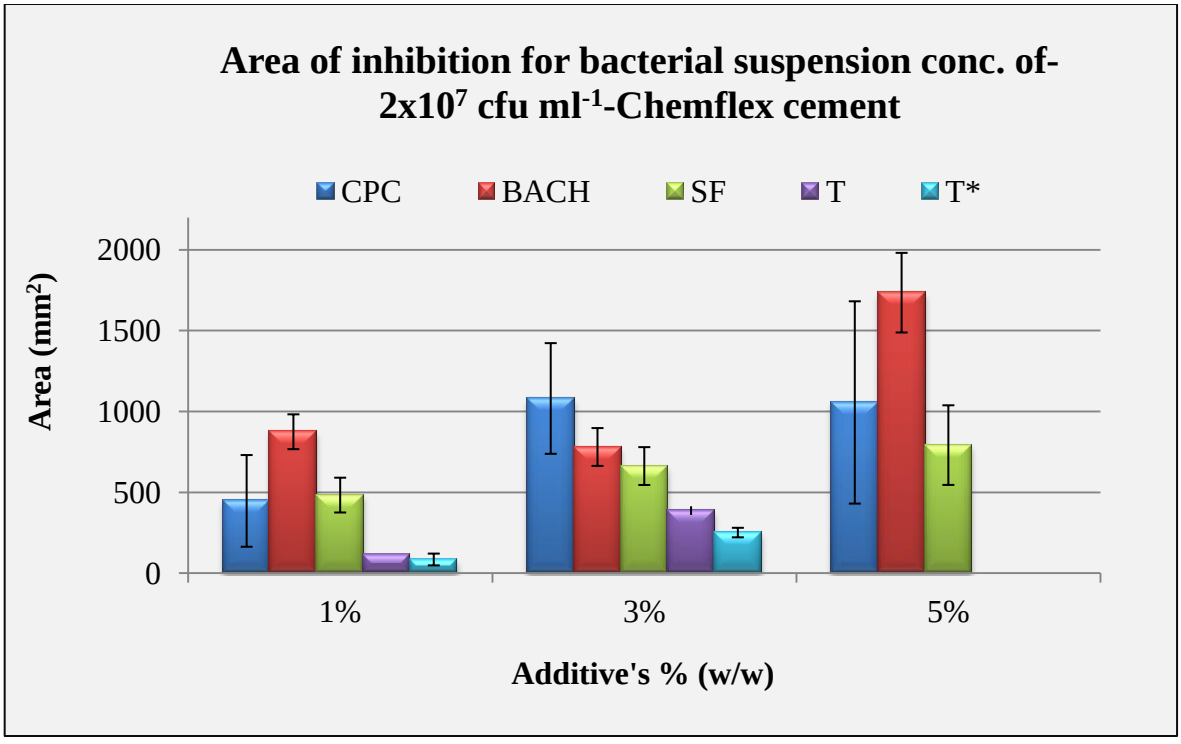

Figure 3.37: Area of inhibition (mm^2) of Chemflex, SD depicted as error bars

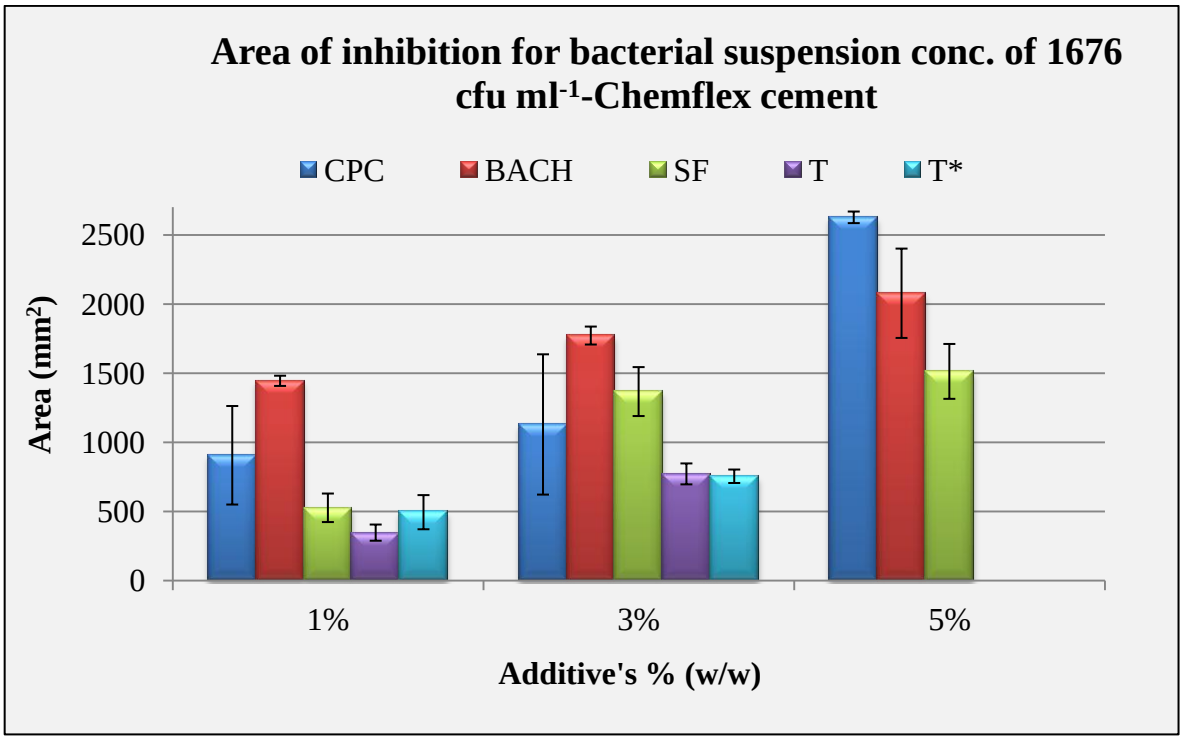

Figure 3.38: Area of inhibition (mm^2) of Chemflex, SD depicted as error bars

For Chemflex: between 3-5% for CPC, 1-3% for BACH and 1-3% and 3-5% for SF- plates seeded with $2x10^7$ cfu ml^{-1} concentration; and between 1-3% for CPC, 1-3% and 3-5% for BACH, and between 3-5% for SF- plates seed with 1676 cfu ml^{-1} concentration. There was no measurable antimicrobial action for both Fuji IX and Chemflex control specimens.

Results show that the antimicrobial activity of the tested materials is dependent upon the concentration of the disinfectant added. There was no measurable antimicrobial action for both Fuji IX and Chemflex control samples.

3.8 References

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DISCUSSIONS

Conventional glass-ionomer cements (GICs) have been used in dentistry for over 40 years [1]. The attractiveness of these materials is due to their intrinsic properties that make them useful as restorative and adhesive materials. These properties include:

- adhesion to moist tooth structures and base metals;
- anticariogenic properties due to the release of fluoride;
- thermal compatibility with tooth enamel;
- biocompatibility;
- and low cytotoxicity [2].

Although studies indicate that GICs might have anticarogenic capabilities due to the action of fluoride, recurrent dental decay and postoperative sensitivity are nowadays the main reasons of restorative failure [2, 3]. The ability of GICs to release fluoride ions however, indicates that these materials have the potential to act as ion release devices.

A study of the recent literature shows that dental materials modified with bactericides is the focus of strong research interest worldwide due to the possibilities of enhancing the materials' anticariogenic properties [4, 5, 6 and 7]. However, no commercial applications of materials formulated with bactericides had been reported to date. This is due to incomplete knowledge of the complex experimental factors and the interactions that might control the development of these antimicrobial devices. Therefore, significant research is still required to develop further understanding of the structure-property relationships in GI systems reformulated antimicrobial agents.

The objectives of current study was to advance on the fundamental understanding of the effect of antimicrobial onto setting and maturation reactions of GICs, the additives release mechanism and antimicrobial properties of reformulated materials. The understanding of the effect of the addition of bactericides on the physical and chemical properties of final cement is the base for developing a potential product. In the next section such interactions are discussed, and these are particularly important in advancing the fundamental

understanding of the effect of antimicrobial agents onto setting and maturation reactions in GICs, the additives release mechanism and antimicrobial properties of reformulated materials.

4.1 Setting kinetics studies - working time determination

The objective of this series of experiments was to evaluate the impact that the addition of antimicrobial agents may have upon working time. Working time is the time period measured after mixing at during which it is still possible to manipulate a dental material without an adverse effect on its properties. Working time is an important property, especially for dental clinicians, as it indicates the time that the material can be manipulated before being placed into the tooth cavity. Working time testing can be also be used to evaluate the setting reaction kinetics of GICs' [8].

The working time investigation showed that additives increase the working time of both Fuji IX and Chemflex. The increase in working time for Fuji IX specimens doped with CPC, BACH and SF varied between 1 second and 21 seconds with the lowest value obtained for T and T/ZC doped specimens. For Chemflex specimens the increase in working time varied between 11 seconds and 32 seconds. Differences in the working time of the control against doped specimens were found not to be statistically significant.

The working time characteristics of GICs are affected by a number of factors. These include the composition of the powder; the concentration, molecular mass and type of poly (alkenoic acid)s; the powder/liquid ratio; and the presence or absence of metal fluorides [9]. Since all of the tested formulations had similar powder compositions, levels of fluoride content and molecular mass and type of poly (alkenoic acid)s, all of these can be discounted as factors that influenced the working kinetics observed in the current study.

The mixing powder/liquid ratio is an important contributor which affects working times. Fleming et al. (2003) investigated the influence of powder/liquid ratio on cement

performance of ChemFil Superior. The authors identified that the variation between mixing ratios influenced the working times of resulted materials. The range of powder/liquid mixing ratios that were used included the manufacturers' recommended ratio (7.4/1.0 g ml^{-1}) and further mixing ratios containing 90%, 80% and 50% of the manufacturers' recommended powder content. Fleming et al. observed that the use of hand-mixed cements prepared to consistencies below that recommended by the manufacturers extended the working time available to the operator [10]. In the current study the extension of working time was observed despite the fact that the powder/liquid ratio was increased by the addition of antimicrobial compounds. The findings clearly demonstrate that a change in the equivalent powder/liquid ratio does not contribute to elongation of working times.

The working time of GICs is related with setting reaction and in particular with gelation processes where cations, aluminium (Al^{3+}) and calcium (Ca^{2+}), bind the carboxylic groups (COO^-) of the poly (acrylic acid) (PAA) to form initial matrix [11, 12]. The increase in working times therefore indicates that the presence of organic compounds added to the GICs destabilise the setting processes, by suppressing ion release, resulting in an elongation of working time. These results are in agreement with those of Türkün et al. (2008) who investigated the effect of chlorhexidine (CHX) diacetate and (CHX) digluconate at concentrations of 0.5%, 1.25% and 2.5% w/w in ChemFil GIC. This study confirms that organic compounds, when added to GICs, increase their working time [5]. A slight lengthening of working time may be an advantage, giving more time for a dentist to manipulate the dental material.

4.2 Mechanical characterisation

In Chapter 1, recent work on the use of GICs as antimicrobial slow release devices were reviewed [4, 5, 6 and 7]. However, in order to utilise the idea of using GICs as antimicrobial devices there is a need to understand the influence of these materials on chemical structures of the final cement.

The microstructure of GICs is formed as a result of the acid-base reactions between the proton donating acid liquid and proton accepting basic powder. During this process, the outer layer of the glass is attacked by the acid, with unreacted the glass particles bonding the COO^- of the PAA chains through ionic salt bridges with Al^{3+} and Ca^{2+} cations, both leached from the glass. The unreacted glass cores act as fillers within the resultant GI matrix [11, 12]. The quality of the microstructure formed will therefore depend upon the extent of the setting processes and it will be reflected in mechanical behaviour of obtained material [1]. Additionally, the mechanical properties of GICs are influenced by several other factors such as: the strength of the matrix phase, the volume fraction and mechanical properties of the dispersed particles, particle size, particle shape, density, and the bonding between the particle and the matrix [13, 14].

The most common tests used for the determination of the mechanical behaviour of materials modified by bactericides are compressive strength (CS), diametral tensile strength (DTS), flexural strength (FS), surface hardness (Vicker's hardness number, VHN, or Knoop) and wear rate [15, 16, 17 and 19].

Since most mastication forces are compressive in nature, it is important to investigate materials under this condition. The ability of a dental material to withstand these forces will determine its ultimate performance in its particular application. To fulfil these needs, the International Organisation for Standardisation and the British Standards Institution set standard requirements for mechanical properties of dental materials for their particular application [20].

In the current work, the CS and surface hardness of two GIC systems (Fuji IX and Chemflex) were analysed before and after their reformulation with bactericides, and the results are described in section 3.2. The 24 hours CS data showed that all doped specimens exhibited a lower CS (range 65.5- 151.3 MPa) compared to the control specimens (136.4-152.9 MPa). The extent of reduction depended on the weight fraction (w/w) and type of additive used. For cetyl pyridinium chloride (CPC), a significant decrease in CS was observed at 2% and above (to at least $p < 0.04$). For benzalkonium chloride (BACH), the

reduction was significant at all levels ($p < 0.05$). For sodium fusidate (SF) samples, significant differences were found at 3% and 5% ($p < 0.04$). No significant differences were observed between the control (additive-free) and triclosan's (T) reformulated specimens. Similarly, no significant differences were found between the control and triclosan/zinc citrate (T/ZC) samples.

In general, the CS of the samples with the highest concentrations of additives was significantly smaller then that of the control, but no influence on mechanical strength was observed for the incorporation of 1% weight fraction of antimicrobial agent. This is in good agreement with the literature [4, 22]. Palmer at al. (2004) looked at the 24 hour CS for fluoro-alumino silicate glass reformulated with CHX acetate at various concentrations. The findings of this study indicated that the addition of additives had a detrimental effect on the CS of doped samples and a decrease in CS was proportional to the percentage of doping [4]. Jedrychowski et al. (1983) reported that low concentrations of CHX dihydrohloride or CHX digluconate incorporated into Fuji Type II did not alter CS significantly [22].

Seven week CS data showed that control specimens become stronger after storage in water (177.0-185.0 MPa). However, no statistically significant differences were observed when the data was statistically analysed using Student's t-test ($p < 0.05$).

Interestingly, however, slight drops in the CS of the control specimens at 24 hours and one week were observed for both materials. This became less pronounced at week three to achieve a CS comparable to the 24 hours data obtained in week five. These findings are quite surprising. Cattani-Lorente et al. (1993) investigated the mechanical properties of commercial GICs stored in water for a period of one year [23]. The evolution of mechanical properties showed four distinctive patterns of change over time. These were characterised by:

- increase in strength or
- increase in strength over a period of six months, followed by its decrease,
- continuous decrease in strength, and
- an invariable strength with aging time.

The study showed that the aging mechanism of GICs is complex and can neither be characterised by a continuous increase nor a continuous decrease in strength. The authors concluded that the strengthening of GICs probably results from additional cross-linking and build-up of silica phase, whereas weakening might result from erosion and the plasticising effect of water [23]. Although these observations are reasonable, it must be remembered that the setting reactions of the GICs are strongly related to their composition. Therefore, the setting reactions of some of the materials will be completed after one day whilst in the other cases they will continue for over a year. In the case of Fuji IX and Chemflex it is expected that the setting processes will continue with time and that the related mechanical properties will improve, which is what has been observed for both materials. However, there is a decrease in CS at week one and two, and this can be attributed to the swelling effect of water observed by Cattani-Lorente et al.

In contrast to the control specimens, most of the doped specimens showed a decrease in CS during the storage period. The greatest reduction in CS was obtained for specimens doped with CPC and BACH. However, no significant differences ($p < 0.05$) were observed for any of those sample sets. The increase in CS was recorded for some samples doped with SF and T, but none of them were significant.

The increase in the CS of the control specimens can be easily analysed by the setting reactions of GICs. The calcium polycarboxylate is formed in the first 5-7 minutes after mixing. The aluminium polycarboxylate, which is more stable and improves mechanical properties, is formed between 24 hours to one year [24]. Thus, the observed increase in the CS of the control specimens between 24 hours and seven weeks can be attributed to the formation of aluminium carboxylate. The decrease in CS of doped specimens is more difficult to explain. It is well known that inhomogeneous dispersion of particles forms defects within the structure of the GICs, as it acts as a stress concentrator, and will reduce the materials CS [16]. The fact that the observed reduction in CS is downwards suggests that the additives are well dispersed within the cement matrix and that the lack of homogeneous dispersion within cement matrix can be discounted as a factor that reduces CS.

It had been also established that the release of agents from restoratives affects physical properties [4]. However, differences in CS observed in this study were not related to the release characteristics of the agent since the release data showed no significant differences in the amount of eluted bactericide.

The mixing powder/liquid ratio also affects mechanical properties [4, 10, 21 and 25]. The influence of changes in the powder/liquid ratio on the mechanical properties of dental materials has been identified by Billington et al. (1990) [21]. The authors noticed that in clinical practice, cements are mixed to produce a wide range of powder/liquid ratios and the range of mixing ratios did not include the manufacturers' recommended ratio for luting purposes. The authors determined that a decrease in the powder/liquid ratio for commercial cements (ChemFil II) from the manufacturer's recommended ratio of 6.8/1.0 to 5.0/1.0 decreases the CS by one-half. It was concluded that this restorative was often mixed in practice at much lower powder/liquid ratios than that recommended by the manufacturer and that this would impair the cement's mechanical properties [21].

Fleming et al. (2003) further investigated the effect of mixing on the mechanical properties of a GIC (ChemFil Superior) [10]. The authors observed that the reduction in relative powder/liquid ratio reduces the CS of the material. Fleming et al. concluded that the compressive fracture strength of the cement arises from the reinforcing glass filler particles. The reduction in the volume of reinforcing glass particles decreases the GIC's ability to resist the compressive forces which are manifested as failure at lower compressive loads [10].

In the current study the reduction in CS of the samples with the highest amounts of additives included was between 87%-43%. Therefore, one might conclude that the addition of antimicrobial agents affects mechanical properties as additional bactericide will change the powder/liquid ratio of the cement. While this is reasonable to accept it is important to point out that the addition of bactericides will increase the powder/liquid ratio. Therefore, considering the reasoning of Billington et al. and Fleming et al. it would be expected for the CS to improve, and this modification would result in shorter working times [9, 10].

Findings presented in section 3.1 indicate that the addition of antimicrobial compounds extend the working time. This clearly demonstrates that the reduction in CS is not due to a change in the equivalent powder/liquid ratio but is controlled by setting mechanism and its kinetics.

The interference in setting reactions by antimicrobial additives can occur in number of ways. Nicholson (1995, 1998) investigated the influence of metal halides (NaCl, KCl, KBr and KI) and sodium salts (NaF, Na_2SO_4 and $NaNO_3$) on the mechanical properties of GI and zinc polycarboxylate dental cements [26, 27]. Alkali metals halides have well-documented effects on polyelectrolytes in aqueous solutions. Specifically they are known to screen electrostatic interactions leading to a preference for conformations with high charged density, generally helical structures, and which allow the polyelectrolyte to develop increased ionisation [28, 29]. The main effect anticipated by Nicholson from this preference for highly charged conformation by metal halides and sodium salts was to increase ease of neutralisation, thus increasing in the setting rate. This behaviour was shown by zinc polycarboxylate and this change in rate of setting had no influence on mechanical properties of zinc polycarboxylate. The anticipated behaviour was not exhibited by GI. The results for GI batch showed that addition of ionic additives has no influence on its setting kinetics but led to significant reductions in its CS. Nicholson concluded that the observed behaviour of the investigated materials was related to the ability of ionic species such as metal halides and sodium salts to stabilise high-charge density polyacrylate molecule. As this high charged conformation eases the neutralisation of PAA, it leads to increases in the rate of the setting reactions, as it was observed in the case of zinc polycarboxylate [26, 27]. For GI the stabilisation of the more highly charged polyelectrolyte conformation did take place, but it did not result in an increase in settings. Nicholson suggested that if the neutralisation were to occur more rapidly, the resulting stiff structure would inhibit the formation of the inorganic network. The imbalance between the neutralisation of the polymer and formation of the inorganic network would be therefore responsible for the reduction in CS of GI [26]. Although Nicholson's finding could be used to explain the reduction in CS caused by additives observed in the current studies, this conjecture however does not fully explain the behaviour of the surfactants.

The interactions of surfactants with polyelectrolytes have been the subject of some recent studies [30, 31, 32 and 33]. These interactions are especially strong when the polyelectrolyte and an ionic surfactant are oppositely charge, as is the case with CPC and BACH. The opposite charge interactions result in cooperative binding of the surfactant to a polyanion. Binding begins at critical aggregation concentration (CAC) which often begins several orders of magnitude lower, than the critical micelle concentration (CMC) of the surfactant. These attractions happen due to the large electrostatic potential that exists in the vicinity of the polyanion. As a result the concentration of counterions in these regions is very high. The accumulation of counterions in these regions will result in a shielding of the carboxylate groups on the PAA. As a consequence the opposite effect on the PAA neutralisation to that proposed by Nicholson will be observed. It would be expected that neutralisation will be inhibited and consequently the release of ion and ion binding will be inhibited. Slower neutralisation of PAA slows down the working time. The shortening of the working time results in elongation and poor strength development. Such an interpretation is consistent with the observed relatively long working times presented in section 3.1 and reductions in the CS of the doped samples.

Additionally, surfactants can also be adsorbed to GI aluminosilicate glass particles. The adsorption of surfactants to silica surfaces is well documented in the literature [34, 35]. This phenomenon occurs because silica has a surface charge. The surface of silica is positively charged at low pH. Above the isoelectric point at pH range 2.0–3.52 the concentration of negative charge increases. At high pH values it is anionic and therefore, cationic surfactants will be adsorbed by negatively charged silica surface. This sort of behaviour will be expected for both CPC and BACH. Adsorption of surfactants can leads to reduction in the number of available active sites on the glass which can react with PAA, thus the leaching of ions from the glass and PAA neutralisation, results in a lower bonding density and thus a weaker matrix [28]. Furthermore, as all additives used are large molecules with molecular weights ranging between 290 and 535 g mol^{-1}, their interference with setting reactions could occur *via* steric hindrance of the active site on PAA and/or aluminosilicate glass, preventing the PAA and aluminosilicate glass reacting with each other.

The extent of reduction of CS depended upon the weight fraction (w/w) and type of additive used. CPC and BACH showed the greatest reductions in CS 24 hours after mixing in comparison with SF and T. This is probably induced by changes in surfactants adsorption conformation at the GI aluminosilicate glass particles. The conformation of surfactants during adsorption can be explained by adsorption isotherm. Somasundaran and Fuerstenau (1966) proposed a four-region model for the interpretation of the surfactant adsorption isotherm. In region I of the isotherm, surfactant monomers are electrostatically adsorbed to the substrate. In the region II, as the concentration of surfactant increases, aggregates are formed. Somasundaran and Fuerstenau have shown that surfactants are adsorbed to polar surfaces with the head-groups in contact with the surface. Region III shows an increase in the amount of the structure formed that was formed in region II to finally form bilayer in region IV [35, 36].

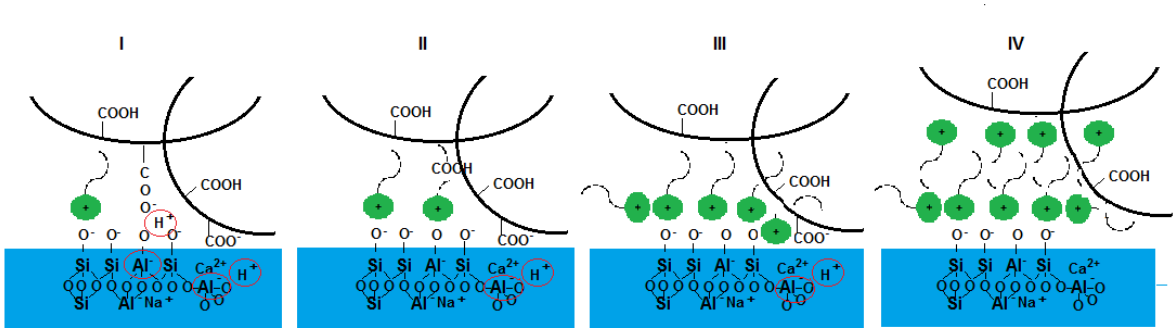

Figure 4.1: The proposed model of adsorption of cationic surfactants to aluminosilicate glass and its influence on the reaction of aluminosilicate glass with PAA. (I), (II), (III) and (IV) show the structures formed with increasing surfactant concentration. As the surfactant concentration increases from I to IV the amount of aluminosilicate glass surface area available for reaction with PAA is reduced

Figure 4.1 proposes in cartoon form a model of setting reaction inhibition in GI systems in the presence of surfactants based on the adsorption of surfactants molecules to the aluminosilicate glass surface. In region (I) minimal interference with the setting reaction occurs since this system has the lowest weight fraction of cationic additives. At low weight fractions of added surfactants no significant effects on CS are observed. In regions (II), (III)

the substrate surface charge is assumed to be neutralised. However, the solution activity of surfactants is not sufficient to lead to significant changes in CS. In region (IV) the solution concentration of surfactant is sufficient to form aggregates and saturation levels of surface coverage. This is observed at the highest weight fraction of surfactant used leading to significant reductions in CS.

Apart from the bulk mechanical properties of cement, the surface properties of the doped specimens were also evaluated. This property is important, especially considering that the main application of these materials is use in an oral environment. Surface hardness is a commonly used technique to determine the dental material's resistance to indentation. Surface hardness testing can be used to evaluate the materials resistance to wear and plastic deformation by penetration [15, 37]. Changes in surface hardness reflect the cure state of a material and the extent of reaction of the setting process. Surface hardness can also be used to give information on the processes occurring on the surface of studied materials [17, 38].

The surface hardness results were similar to those for CS. The reduction in the surface hardness of doped specimens was observed in comparison with the control specimens, and the extent of reduction was proportional to the weight fraction of added bactericide. The decrease was observed at all studied time intervals. Lower concentrations of additives at 1% or 2% did not lead to statistically significant differences in surface hardness. Higher concentrations, at 3% or 5%, led to reductions in surface hardness values that were significant (at $p < 0.03$). The 24 hour surface hardness data showed that all doped specimens exhibited lower values (range 23.5-56.6 VHN) in comparison with control specimens (57.0-57.2 VHN). The extent of reduction depended on the level and type of used additive. For CPC, a significant decrease in surface hardness was observed only at 5% (to at least $p < 0.02$). For samples doped with BACH, a significant decrease in surface hardness was observed at 2%, 3% and 5% (to at least $p < 0.03$). For SF, a significant decrease in surface hardness was observed only at 5% ($p < 0.05$). No significant reduction in surface hardness was observed for Fuji IX and Chemflex doped with T and T/ZC. These results correspond with the findings of Türkün et al. (2008) where CHX diacetate and CHX digluconate were incorporated at various concentrations into GICs. The findings of this

study also indicated that the addition of the additive CHX diacetate and CHX digluconate had a detrimental effect on the surface hardness of doped samples and the decrease in CS was proportional to the percentage of doping [5].

The increase in surface hardness between 24 hours and seven weeks was observed for the control sample sets of both Fuji IX and Chemflex. However, the changes observed were statistically significant only for Chemflex. These findings indicate that the hardening phase of the setting reactions of Chemflex was still taking place at week seven after the setting reactions. This observation is surprising taking in account that the powder/liquid ratio of Chemflex is greater than the Fuji IX; and it would be expected to be complete before the Fuji IX material [39, 40]. Therefore, one possible reason might be related to the smaller particle size of Fuji IX, resulting in a greater surface area available for reaction of polymeric acid with glass. The findings are in agreement with those observed by Silva et al. (2007) and Yap et al. (2002) where Fuji IX control samples showed no significant increase in surface hardness while other GI under the investigation (Ketac Molar, Vidron R, Vitramolar, Z 250 and Miracle Mix) exhibited consistent increase within the period of time under study [17, 38].

The results of seven weeks maturation of Fuji IX and Chemflex doped specimens showed that the additives CPC and BACH led to increases in the surface hardness over time to highly significant levels at weight fractions of 2 % and above ($p < 0.03$). By contrast, the T and T/ZC combinations caused a decrease in surface hardness with time, showing a significant decrease at weight fraction of 3% ($p < 0.01$).

It had been previously demonstrated that the variation in mechanical properties of GICs are the result of its maturation of a material and its setting kinetics [1]. The observations discussed above clearly show that the antibacterial agents added to the formulations reduce both the CS and surface hardness of GICs and that this reduction becomes more pronounced as higher amounts of additives are added. This indicates that the addition of antimicrobial compounds affects the setting processes of GICs and their maturation.

4.3 Water loss studies

Water is a very important constituent of GICs. It acts both as a solvent and a component in the formation of the cement and is also one of the products formed during the acid-base reaction. Water occurs in the GICs in at least two different states, which have been classified as evaporable and non-evaporable water [8]. The classification depends on whether the water can be removed by desiccation or remains bound within the cement [41]. As the cement ages, the ratio of bound to un-bound water increases. These changes are associated with changes in the strength modulus of GICs [42]. Water loss during the early stages of setting and maturation results in the formation of crazing, cracking, a loss of translucency and incomplete maturation [43]. Early desiccation also affects mechanical properties because the hardening and maturation processes are not completed. In effect, the matrix that is created is weaker and the material tends to release water faster [8].

All the investigated samples exhibited a reduction in mass under desiccation conditions. The difference in relative fractional water loss from the control specimen as compared to the doped specimens was found not to be statistically significant. Clear differences in the percentage water loss at equilibrium between Chemflex and Fuji IX material were observed. The percentage of water loss from the Chemflex samples was significant (to at least $p < 0.01$) in comparison with their initial masses. For Fuji IX, none of the differences were significant. This suggests that Chemflex is more susceptible to desiccation.

The hydration of GICs, defined as the ratios of non-evaporable water to evaporable water, is an important parameter, which controls the mechanical properties of these materials. The most highly hydrated cements have superior mechanical properties, including CS [12]. In this study Chemflex showed lower water loss in comparison to Fuji IX. This observation is rather surprising taking into account that the powder/liquid ratio of Chemflex is higher than Fuji IX and it would be expected that Chemflex contains less “free” water available for release [39, 40]. One possible reason for this phenomenon might be related to the bigger particle size of Chemflex, resulting in a lower surface area available for reaction with the PAA. Consequently, the resultant matrix will acquire a lower cross-link density and will be

more susceptible to desiccation. These findings are supported by the mechanical findings presented in section 3.2 where Chemflex exhibited a lower CS in comparison to Fuji IX.

An evaluation of the kinetics of water loss from the tested samples showed that water loss from both Fuji IX and Chemflex control specimens and doped specimens is based on diffusion. The diffusion coefficient of water was observed for at least the first five hours and can be satisfactorily described by the mathematical form of Fick's law described by equation below:

$$\mathbf{M_t/M_\infty = 2(Dt/\pi l^2)^{1/2}}$$

Where:

M_t = mass uptake/loss at time t (s)

2l = thickness if the specimen (m)

M_∞ = equilibrium mass uptake/loss (g)

D = diffusion coefficient ($m^2\ s^{-1}$)

If Fick's Law is obeyed M_t/M_∞ against $\sqrt{t}$ (s) plot slope needs to give straight line. The diffusion coefficient (D) is calculated from the line slope (s), where $s = 2(Dt/\pi l^2)^{1/2}$, from which $D = s^2\pi l^{\ 2}/4$ [56]. In all cases the regression coefficient values were greater than 0.9800. The findings are in agreement with the results of studies carried out on polymeric materials used in dentistry [45, 46, 47 and 48] where water transport in each of them has been shown to follow, in the early stages at least, Fick's law of diffusion.

It is difficult to relate findings of these studies with mechanical data shown in section 3.2. The absence of an obvious trend may indicate that the decrease in mechanical properties of the doped samples is more complex than the consequences of desiccation related imperfection, i.e. cracking of material. Also, the changes in the matrix structure are not easy to test by water loss studies.

4.4 ^{27}Al MAS-NMR studies

In this investigation aluminium-27 Magic Angle Spinning-Nuclear Magnetic Resonance (^{27}Al MAS-NMR) was used to investigate the effect of additives on the kinetics of the conversion of four-coordinate aluminium, Al (IV), to six-coordinate aluminium, Al (VI), during the setting and maturation reactions of GICs. An important aspect was to determine if there is any correlation between the kinetics of conversion of Al (IV) to Al (VI) with the observed changes in physical and chemical properties of the doped materials.

The setting processes of GICs have been investigated previously using ^{27}Al MAS-NMR [49, 50, 51, 52 and 53]. Studies conducted by Stamboulis et al. (2004) showed that aluminum (Al) exhibits three distinct regions at 45-60, 20 and 0 ppm which are attributed to Al (IV), Al (V) and Al (VI) [46]. In a glass, Al exists predominantly in four-coordination, forced into this state through the influence of tetrahedral silica. In cements, Al adopts its preferred six-coordinate state, so it changes its coordination from Al (IV) to Al (VI) as the setting reaction proceeds. This conversion is slow and is associated with changes in the relative intensity of Al (VI) to Al (IV) in the NMR spectrum. The change in ratio of Al (VI)/Al (IV) was observed for a period of one year. In addition to the peaks due to Al (IV) and Al (VI) a small peak attributed to Al (V) was also present during this time period [48]. The precise details of the six-coordinate state are not clear, but it is thought that it includes species such as carboxylate units, fluoride ions and water molecules occupying the increased number of coordination sites around the aluminium [53].

The findings of the current study are in agreement with these previous results reported in the literature. Fuji IX glass had a peak at 46.0 ppm, a shoulder at 19.9-10.0 ppm and a small peak at 5.0 ppm, which was assigned to Al (IV), Al (V) and Al (VI) respectively. Chemflex original glass showed a large asymmetric peak at 45.0 ppm and a smaller one at -2.0 ppm. The asymmetric peak at 45.0 ppm was assigned to Al (IV) and the presence of small proportions of Al (V). The peak at -2.0 ppm was due to Al (VI). The position of the peaks gave some structural information. In particular, the fact that the peak assigned to Al (IV) was at relatively low values (44-46 ppm) compared to about 60 ppm previously observed in

model glasses based $2SiO_2Al_2O_3CaOCaF_2$ [38] suggests that there is a higher incidence of Al–O–P bonds in these materials than in the models [50, 51].

The relative intensity of Al (VI) to Al (IV) at various time intervals was observed for both types of cement, both with and without additives. The results are shown in Table 3.13. Because of the quadrupolar nature of the ^{27}Al nucleus, these spectra are not truly quantitative and the values of relative intensity do not give true indications of the ratio of the two species present in the cement. However, the fact that the Al (VI) peak becomes larger relative to the Al (IV) is an indication that the relative amount of Al (VI) is growing within the cement, and that the changes observed in relative intensities give an indication of what is happening as the cements set and mature.

All cement samples showed an increase in Al (VI)/Al (IV) ratio as they aged, a result that confirms those previously reported [50, 51, 52 and 53]. This is consistent with the movement of Al from the glass to the cement matrix, with corresponding interactions with labile coordinating species. The presence of additives lowers this Al (VI)/Al (IV) ratio slightly at most time intervals, though this is not entirely true for Fuji IX doped with BACH.

These findings are consistent with the results of the mechanical properties of GICs presented in section 3.2. As shown, the CS and surface hardness of control specimens was improved during the seven week testing period. The materials also become less susceptible to desiccation (section 3.3).

The increase in CS of the control specimens is related to changes in the chemical structure within the cement. The first identifiable reaction product is calcium polyacrylate, and this can be shown to form 5-7 minutes after mixing. The aluminium polyacrylate takes longer to form and generally improves the mechanical properties [37]. Thus, the observed increase in CS of the control specimens between 24 hours and seven weeks in the current study can be attributed to the formation of aluminium polyacrylate. As matrix cross-linking increased, the cement became less susceptible to desiccation. The addition of antimicrobial

compounds changed this. Not only were these physical properties adversely affected at 24 hours, there was in general little or no improvement with time up to seven weeks, especially for those containing the highest level of antimicrobial component. Also, as presented in section 3.1, the working time of doped materials was extended, demonstrating clearly that the additives slowed down the setting reaction.

The results presented in this section clearly show that additives affect the kinetics of the conversion of Al (IV) to Al (VI). This indicates a possible correlation between the observed changes in the physical and mechanical properties of the doped materials and the kinetics of the conversion of the Al (IV) to Al (VI). The most likely explanation is that the additives may interfere with the setting reaction of GICs, causing less Al^{3+} to be released from the glass. This implies that less Al^{3+} is available to take part in the construction of the matrix during maturation which in turn alters the microstructure, and hence the properties of the matrix.

This interference with the setting process by the addition of antimicrobial additives may occur through a number of mechanisms. One possible mechanism is that the additives may hamper the reaction of PAA and aluminosilicate glass either by steric hindrance of active side-groups on PAA and/or the glass or by adsorbing to PAA and/or the active sites on the glass. Moreover, it is possible that additives might alter the conformation of the polymer and the ease with which it undergoes ionic cross-linking reactions. All of these possibilities are discussed in detail in section 4.2.

4.5 Fluoride release

The interest in the clinical use of GICs is mainly due to their behaviour as adhesive bioactive materials and their therapeutic action [8]. The latter arises from the ability of GICs to release fluoride (F^-) over an extended period of time. F^- release from GICs has attracted significant research attention and there are numerous studies on the amount and rate data for F^- release have been published [55, 56, 57 and 58]. Studies of the kinetics of F^-

release from GICs have shown that its release follows distinctive patterns and involves at least two stages. The initial stage lasts up to 24 hours, it is non-linear with time, and is characterised by a rapid release of F^- ions [55]. The second stage is linearly proportional to the square root of time ($\sqrt{t}$), indicating a diffusion-controlled process and this release is reported for a period of up to five years [41].

In section 3.5, the effect of addition of the antimicrobial compounds on F^- elution from two branded GICs into water has been investigated. The results show that all reformulated samples released F^- over the time period studied. In general, the amount of F^- released was greater for control specimens than for doped specimens, and this tendency was observed for most formulations and measured times.

Statistically significant reductions in F^- release were observed for samples doped with CPC and BACH at most levels of additives and time intervals ($p < 0.05$). The seven week cumulative release for Fuji IX control samples was 16.42 ppm (± 0.76), whereas for Chemflex the measurable F^- release was 13.99 ppm (± 2.37).

Release of F^- from SF doped samples showed a different pattern, with a significant increase in F^- release with SF additive. This increase varied with the amount of SF added and was greatest for the highest level. The cumulative release varied between 10.64 ppm (± 0.49) to 18.55 ppm (± 2.89).

The T and T/ZC samples showed similar behaviour to CPC and BACH where T cumulative F^- release ranged between 9.17 ppm (± 0.32) to 10.64 ppm (± 0.49) and for T/ZC 9.02 ppm (± 0.97) to 10.64 ppm (± 0.49). The presence of these additives reduced the level of F^- released, with larger amounts causing greater reductions. No statistically significant differences were found between Fuji IX and Chemflex materials. Additional doping of T samples with ZC did not influence the leaching processes of F^-.

F^- is released from the glass powder during the GIC setting reaction when the glass powder is mixed with PAA from where it is transported to the matrix [54]. As F^- remains unbound

it is therefore available for release. A variety of factors can influence the amount of F^- released, including the nature of the glass, specimen size and shape, and storage solutions.

The powder/liquid ratio is an important factor that influences the leaching of ions from the glass. Wilson and McLean (1988) have suggested that the decrease in the powder/liquid ratio results in an increased attack of the glass phase and, hence, in enhancement of F^- liberated from the glass [54]. In the current study a decrease in the F^- release is observed, which could be related to the presence of the additives in the glass powder. The additives may hamper the acid attack on the glass, resulting in a reduction of F^- liberated from this phase. This suggestion is supported by the observed increase in the working time shown in section 3.1. In addition, it is possible that equilibrium may have been established between F^- and additives, forcing F^- to reside in the cement.

The greatest cumulative release occurred in the first week, and ranged between 9.56 ppm for Fuji IX to 11.90 ppm for Chemflex, after which the release diminished until it became fairly constant. These findings are in agreement with a number of other *in vitro* studies, where it has been frequently shown that maximum cumulative F^- release occurs during first 24–48 hours and varies between 5-155 ppm, depending on the brand of GICs used and the geometry of the specimens [41, 55, 56, 57, 58, 59 and 60].

Water is another important impact factor which may affect the F^- release from the cement. Water in the cement allows ions interchange to occur with the surrounding liquid [61]. Therefore, it is possible that the additives will use up the available water, leaving less water to be available for the ions to move to the surrounding medium. This reasoning can explain the difference in the observed behaviour between Fuji IX and Chemflex. Chemflex has a higher powder/liquid ratio than Fuji IX [39, 40]. Therefore, there is less water available for acid-base reactions and, subsequently, for the solvation of ions, and so the delay in ion release observed for Chemflex material can be associated with the time that the water medium takes to penetration the cement. However, the results of water studies presented in section 3.3 clearly show that there is no difference in water properties between control and

doped specimens. This suggests that water balance in not influenced by doping and can be discounted as a factor that affects the rate of F^- release of Chemflex specimens.

F^- release is related to the setting reactions, as F^- is known to be transported from the glass to the matrix during the setting process [54]. The presence of the additives was generally found to slow the initial setting reaction, and to inhibit maturation, so it can be assumed that it reduced the amount of F^- transported into the matrix from the glass. As a result there is less F^- available for release from the cement. However, this reasoning does not apply to SF, as it increased the amount of F^- released. This suggests that SF enhances movement of F^- from the glass to the matrix, but at this stage it is difficult to speculate as to why this might be. One possibility for the origin of this phenomenon is the presence of sodium (Na^+) in SF. Na^+ is also present in the GI powder. During the setting reaction Na^+ is released from the glass to charge balance the polysalt matrix. It is well known that F^- forms complexes with Al^{3+} where three F^- ions are associated with Al^{3+}. It is therefore possible that F^- preferentially binds Na^+ in the matrix, leaving two "free" F^- ions which can then leach out into the solution. This assumption can be also supported by the findings of mechanical studies presented in section 3.2 where a slight increase in CS for SF samples was shown. That can be due to an increase in aluminium polycarboxylate cross-linking as Al^{3+} ions are freed by Na^+.

4.6 Antimicrobial additives release

"Drug release" refers to the process in which drug solutes migrate from the initial position in the material to the material's outer surface and then to the release medium [62]. The process is affected by multiple complex factors such as the physicochemical properties of the solutes, the structural characteristics of the material system, release environment, and the possible interactions between these factors [63].

In general, the study of drug release kinetics involves mathematical modelling. This provides the basis for the study of mass transport mechanisms that are involved in the drug

release [64]. Furthermore, mathematical modelling provides an insight into the function of material systems. Understanding the structure-function relationship of the material system is therefore the key to the successful design of a delivery system for a particular application [63].

In section 3.6, the release data of antimicrobial additives from cements obtained in the current study were reported. The results showed that both brands of GIC were able to release antimicrobial agents and the amount released varied with the weight fraction of bactericide added. The calculated equilibrium recovery showed that only a small amount of bactericide was released from the sample in each case. For example, for Fuji IX the fraction of SF recovered varied between 2.14% (± 0.64) to 3.58% (± 0.62) of the original amount of bactericide added; whereas Chemflex samples released between 0.61% (± 0.34) to 0.93% (± 0.34) of the total amount of SF added. The total release of CPC and BACH was between 2.48 (± 0.62) - 4.92 (± 0.53) % for CPC and between 1.70% (± 0.23) - 5.00 (± 0.60) % for BACH and occurred within the first week of release. The fraction of T recovered varied between 1.09% (± 0.09) to 1.86% (± 0.09), whereas the fraction of T released from the samples reformulated additionally with ZC varied between 0.51% (± 0.04) to 1.00% (± 0.09) of T. In general, the samples doped with CPC and BACH exhibited the greatest release followed by SF, T and T/ZC. The results also showed that Fuji IX was able to release statistically significantly greater amounts of additives than Chemflex ($p < 0.001$) in all cases. These results coincide with findings reported by Palmer at al. (2004) where CHX acetate was incorporated at various concentrations into fluoro-alumino silicate glass. The pattern of release showed an initial rapid elution of material that leveled off to a constant value. All measurable CHX acetate was released within 22 days. After 240 days the release was equal to 3-5% w/w of the incorporated CHX acetate and was concentration dependent [4].

The composition and microstructure of the materials has a great influence on the release of the drugs from it. Factors such as adsorption properties (interactions between antimicrobial agent and matrix), pore size, pore connectivity, pore geometry and matrix reactions with surrounding media (dissolution properties) are just a few of the factors that determine the

rate of the leaching of drugs into the surrounding media [62]. The current study showed that only a small portion of the incorporated bactericide was released into the water medium. The findings suggest that most of the bactericide remains either chemically or physically bound to cement.

As stated above, the drug ionic charge and interactions with the matrix, as well as its solubility and stability, are important factors that will determine the amount of the drug that can be leached out from the system [63, 64]. In addition the additives may be chemically bound to the cement substrates. The mode of interactions of PAA with additives and aluminosilicate glass with additives was discussed in details in section 4.2. Furthermore, it is possible that equilibrium may have been established between the employed additives and the ions mentioned above, forcing the additives to reside in the cement. Also, the low release obtained may be due to saturation of the solution in which the cement was immersed. However, this is unlikely, as the highest concentrations obtained for all formulations were well below the solubility limits of the additives used.

Another important factor that has a great influence on drug release is the material's composition and microstructure [64]. All the additives used are large molecules with molecular weights ranging from 290 - 535 g mol^{-1}. A low release profile for these materials could therefore be attributed to the size of the molecule, indicating the possibility that these molecules are physically embedded in the GIC matrix. Furthermore, the lower release of additives from Chemflex can be explained by the microstructure of the cement. Chemflex is a more viscous GIC then Fuji IX and so the cement matrix that was produced has a greater cross-linking density, causing the additive molecules to remain in the cement [39, 40]. These findings indicate that the structure of Fuji IX differs significantly from that of Chemflex and it was less likely to lock up additives (of all chemical types) than Fuji IX. Despite the fact that only a small proportion of the bactericides was released, the findings presented in section 3.7 clearly show that the substances are effective and are able to improve the antimicrobial character of the cements.

The current study also investigated the kinetics of drug release. The study of kinetics is necessary to elucidate the transport mechanism. The mechanism by which ions are transported from the GIC is commonly described by Fick's law of diffusion [56, 57]. Studies have shown that the release of parent ions is mainly controlled by diffusion and the cumulative release is linear with respect to √t [61]. A description of the detailed mechanism of F^- release can be found in Chapter 1.

The results obtained in this study show that the rate of release of these active species clearly exhibits a region where the release rate is proportional to √t, which is leads to the reasonable conclusion that release is diffusion controlled. The correlation coefficients obtained for the linear region were generally greater than 0.9900 and, even in the worst cases, exceeded 0.9800, indicating that diffusion control could be assumed in all cases. In general, this linear region lasted for 2-4 weeks after exposure to water. The results of the study coincide with study conducted by Palmer et al. (2004) in which it was observed that the release of CHX acetate was linear to √t, indicating a diffusion process [4].

The diffusion coefficient for molecular transport can be calculated from the slope of the linear graph [65]. The current study also attempted to determine the diffusion coefficient of studied formulations. The diffusion coefficients obtained for the various additives at different concentrations were comparable and they varied between $1.13x10^{-12}$ m^2 s^{-1} to $7.85x10^{-13}$ m^2 s^{-1}. In general, Chemflex exhibited a lower diffusion coefficient value in comparison with Fuji IX.

Diffusion is the process responsible for the movement of mass from one part of a system to another. Diffusion in GICs is complex and depends on the concentration gradient, cross-linking network density, and diffusant size [65]. The network cross-link density is an important factor in the determination of the kinetic of release of materials from GICs structure [63]. If the network is too dense it will slowdown and inhibit molecular movement, reducing additive release rates. If the network is less dense molecular transport is less inhibited. This suggests that the presence of additives inhibits the network formation and the observed reduction in mechanical properties in GICs may be associated with this

phenomenon. While this is a reasonable explanation, changes in network density would also be reflected in the release kinetics of these materials. However, the current study clearly shows that the release kinetics is not dependent on the amount of additives used; indicating that changes in mechanical properties must be influenced by other factors. The network density, however, has an effect upon kinetics of Chemflex release. As was observed, Chemflex in general exhibited a lower diffusion coefficient value in comparison with Fuji IX. The reason for this is that Chemflex is a more viscous GIC then Fuji IX and the cement matrix that resulted will have a greater cross-linking density, causing the molecules of the additives to remain in the cement [39, 40].

In addition to crosslink density, water uptake is another important impact factor that may affect the solute transport within the network. It is important to emphasise that the release of ions from GICs occurs because the cement itself contain a proportion of water, typically 8%-20%, which allows interchange to occur with the surrounding liquid [61]. Nicholson and Czarnecka (2008) studied the water uptake and loss kinetics of GICs. The analysis demonstrated that water uptake and water loss was linear with $\sqrt{t}$, which indicated a diffusion controlled process [66]. Thus, there seems to be a correlation between water uptake and release kinetics for the GICs matrices.

4.7 Antimicrobial studies

The antimicrobial studies presented in section 3.7 demonstrated that CPC, BACH, SF, T, T/ZC, when added to Fuji IX and Chemflex GICs, led to increased antimicrobial properties when compared to the cement alone. The antimicrobial activity of the reformulated materials was dependent upon the weight fraction (w/w) of the antimicrobial agent added. The inhibition zones became significantly larger as the fraction of anti-microbial compound added increased.

There was no measurable antimicrobial action observed for either the Fuji IX or the Chemflex control samples. The absence of an antimicrobial response in the control is in

agreement with the results of Botelho et al. (2003) and Yep et al. (1999). Both studies have reported that conventional GICs exhibited no antimicrobial effects in the agar diffusion test [68, 69]. This is despite the fact that these cements release F^- and also have a low pH at the start of setting, both of which might have an antimicrobial effect [69, 70, 71 and 72]. However, these effects are not sufficient to impart antimicrobial properties, and the addition of specific compounds, such as those used in the current study, is necessary in order to provide such properties.

An evaluation of the antimicrobial potency of the additives studied showed that CPC, BACH and SF have a similar antimicrobial response to *Streptococcus mutans* and was significantly lower than T and T/ZC. Also, the antimicrobial strength of T was significantly lower than T/ZC.

The susceptibility of the pathogens to the particular bactericides has a greater effect on the observed zone of inhibition [73]. In the current study, cationic disinfectants represented by CPC and BACH, anionic bactericide such as SF and neutral antimicrobial agent T was used. The antimicrobial activity of CPC and BACH is thought to arise from the absorption of the positively charged head group onto the negatively charged cell surfaces of the bacteria. This process is thought to be responsible for an increase in cell permeability and may disrupt the cell membranes. Although the detailed mechanism of the antibacterial effect of these materials has not been determined, it was suggested that the quaternary ammonium compounds, represented by CPC and BACH, cause lysis of the bacterial cells by binding to the cell wall components, causing leakage of the cytoplasmatic material [74, 75, 76 and 77]. The mode of action of SF is related to the inhibition of protein synthesis of the bacteria [78, 79]. The antimicrobial activity T is thought to occur via several mechanisms. At low concentrations it is bacteriostatic, it interferes with bacterial fatty acid synthesis. At high concentrations it is a bactericidal as it interferes with the permeability of plasma membranes, allowing leakage of intracellular content [79, 81]. All of the tested bactericides are potentially able to inhibit bacterial growth. CPC and BACH showed the greatest inhibition. The reason for this is that CPC and BACH are the most potent in the inhibition of *Streptococcus mutans* in comparison to the other additives tested. However,

as T is bacteriostatic at low concentrations, it is likely that the amount of additive leached out from the specimen was only potent enough to reduce the bacterial activity, rather than to cause it to cease completely. Samples additionally reformulated with ZC showed an improvement in antibacterial effect, though the sizes of the inhibition zones were smaller than those of CPC and BACH.

Another factor that can influence the size of the zone of inhibition is the rate of diffusion of the additive through agar. It is well established that the rate of diffusion of an antibiotic through agar is not always the same as it is dependent on the antibiotic, its molecular weight, its binding with agar and its solubility properties [82, 83]. Larger molecules will diffuse at a slower rate than lower molecular weight compounds. These factors, in combination, result in each antimicrobial having a unique breakpoint zone size, indicating susceptibility to that antimicrobial compound. While all the additives used have a similar molecular mass, the differences in the sizes of the zone of inhibition observed were therefore not due to the antibiotic size and can be discounted.

An additional reason could be related to the ability of the antibiotics to bind to agar. In the current study, the agar used was slightly negatively charged due to the presence of charged agaropectins [84]. Due to agar charge, the cationic compounds will move through it more slowly than anionic or neutral compounds such as SF and T. CPC and BACH are cationic additives and if the agar charge influences the rate of movement of additives, CPC and BACH would diffuse through the agar at the slowest rate.

Another important factor is the solubility of the additive. CPC, BACH and SF are all salts and are all soluble in water. T, on the other hand, is known to be slightly soluble in water [85]. Therefore, the reasons for the small zones of inhibitions of T doped samples obtained could be related to its low solubility and its low antimicrobial potency towards *Streptococcus mutans*. These suggestions are supported by the fact that the addition of ZC to the T formulation increased the antimicrobial activity. These findings are in agreement with the study performed by Cummins (2005), which showed that dentifrices containing combinations of ZC and T exhibited a greater plaque inhibition than either agent alone [86].

4.8 References

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CONCLUSIONS AND FUTURE WORK

5.1 Conclusions

The work carried out and presented in this thesis provides an extensive and systematic overview of the impact that anti-bactericidal agents have upon the curing kinetics and resultant mechanical properties of glass ionomer cements. The work reported has been diverse encompassing a number of disciplines ranging from materials chemistry to assessment of bactericidal efficacy. However, the major and initial motivation for the work remained the same throughout the project namely to make a contribution to our fundamental understanding of the effect of antimicrobial agents upon:

- the setting and maturation reactions in glass-ionomer cement (GIC) systems;
- the mechanisms of additive release;
- and the antimicrobial properties of the reformulated materials.

The GIC working time as determined using the Gillmore needle test showed that the addition of antimicrobial compounds to the GIC formula prolongs the working time as compared to control samples. The extension in working time for the doped specimens varied between 1 second to 31 seconds. In general, Fuji IX exhibited lower working times than Chemflex.

Water loss properties in the cements were not affected by the presence of additives. Water loss processes controlled by diffusion and it was observed over a period of three weeks. The diffusion of water from both the control and doped samples was comparable, which did not show and discernible trend and varied between 3.42×10^{-11} m^2 s^{-1} to 5.93×10^{-11} m^2 s^{-1}.

A range of mechanical properties, which included compressive strength (CS) and Vicker's hardness number (VHN), were measured for GIC specimens that were stored in HPLC grade water for various aging times of 24 hours, 1 week, 3 weeks, 5 weeks and 7 weeks. In general, the addition of antimicrobial compounds was found to reduce the CS when compared with the additive free samples. The CS of the doped specimens ranged from 65.6

MPa to 151.3 MPa and the control specimens from 136.4 MPa to 152.9 MPa. The extent of reduction depended upon the weight fraction (w/w) and type of additive used. It was observed that the 24 hour CS of those GIC samples containing the highest concentrations of additives were significantly lower than that of the control samples. Cetyl pyridinum chloride (CPC) and benzalkonium chloride (BACH) as opposed to the other samples had a greater influence on CS. The reduction in CS for CPC was significant at 2% and above. For BACH, the reduction was significant at all levels. By contrast, for the sodium fusidate (SF) samples the differences as compared with the control samples were only significant at 3% and 5% loading. No significant differences were observed between the controls and triclosan (T) containing specimens and the addition of zinc citrate (ZC) to the samples gave no differences at any level of significance. The findings indicate that the type of ionic charge can influence the kinetics of the setting and maturation reactions in slightly different ways. In general, the CS of the control specimens became higher after storage. For example the CS of Fuji IX control specimens increased from 152.0 MPa (± 28.1) at 24 hours to 185.0 MPa (± 33.7) at seven weeks. This increase in CS was not observed when additives were present.

The additives also reduced the surface hardness (Vicker's hardness number, VHN) of the doped materials. The reduction in surface hardness, like the reduction in CS, depended on the amount added, and decreased when increasing the amount of additive. The 24 hour surface hardness values for the control specimens were 57.2 VHN (± 5.9) for Fuji IX and 45.0 VHN (± 8.0) for Chemflex. The surface hardness of the doped specimens varied between 56.6 VHN (± 9.7) and 23.5 VHN (± 2.4). The addition of antimicrobial compounds did not affect the long-term storage properties in a way similar to that observed for CS. Surface hardness was found to increase for both control and doped specimens within 24 hour and seven weeks of study and in some cases the increase in surface hardness was statistically significant for doped specimens.

Finally, the release of fluoride (F^-) from the GICs was reduced in the presence of additives, and the extent of the reduction varied with the amount of additive present in the cement. In general, this was observed in most of the formulations, except those containing SF.

The kinetics of setting and maturation of GICs were determined using Aluminium-27 Magic Angle Spinning-Nuclear Magnetic Resonance (^{27}Al MAS-NMR) spectrometry. Results showed that aluminium switches its coordination number from four, Al (IV), in the glass to six, Al (VI), in the cement matrix. The addition of antimicrobial agents reduced the rate of these changes, since the calculated ratio of Al (VI)/Al (IV) was higher for the control specimens than the doped samples for all time intervals. This demonstrates that the presence of additives affect the setting and maturation reactions in the cement systems, a finding that is consistent with the reductions observed in mechanical properties.

It was concluded that the presence of additives affect the setting reactions, as indicated by working time measurements using the Gillmore needle. The prolongation of the setting reactions has a negative influence upon the mechanical properties of the final cement, both at 24 hours and at longer time intervals. These results can be interpreted as showing that the additives having an effect on the conformation of the poly (acrylic acid) (PAA) component in solution, which in turn should influence its degree of dissociation and hence its effective acidity. Changes in the conformation of the PAA also influence the released of key ions from the glass (Al^{3+}, Ca^{2+}, F^- and Na^+). Alteration in the balance of these ions, especially Al^{3+}, would result in slower cross-linking processes and lower cross-link density matrix. Additionally, adsorption properties of surfactants to GI aluminosilicate glass particles can also lead to reduction in the number of available active sites on the glass which can react with PAA. The reduction in available active sites on the glass will results in a lower bonding density and thus a weaker matrix. All above will leads to the observed changes in mechanical properties, working kinetics, F^- release and kinetics of conversion of Al (IV) to Al (VI). However, water transport behaviour appeared to be unaffected, with both the rate of water loss and the amount lost at equilibrium being the same for both the doped and control; cement samples, within statistical limits.

A release study was performed for each of the additives to determine the mechanisms that control the leaching processes. The amount of additive released depended upon the concentration fraction and type of additive. The greatest release was observed for CPC and BACH. The amount released into a fixed volume of aqueous solution varied from $1.49 \times 10^-$

^{7} mol L^{-1} (± $7.28x10^{-9}$) to $7.52x10^{-7}$ mol L^{-1} (± $7.41x10^{-8}$). The lowest amount released under the same conditions was observed for T samples and it varied between $8.64x10^{-8}$ mol L^{-1} (± $8.13x10^{-9}$) to $1.48x10^{-7}$ mol L^{-1} (± $8.81x10^{-9}$). These findings indicate that the ionic charge may have an influence upon the leaching processes. It was shown that the additives were released from the cement samples by a diffusion process, with diffusion coefficients varying between $1.13x10^{-12}$ m^2 s^{-1} to $7.85x10^{-13}$ m^2 s^{-1}. The release was independent of additive concentration. Although only small amounts were released between 0.51% (± 0.04) to 5.00% (± 0.60), the agar diffusion testing using cultures of *Streptococcus mutans* showed that the additives were released at sufficiently effective levels, causing inhibition of growth around the cement discs. The size of the inhibition zones varied with the amount and type of the antimicrobial compound present in the cement. The greatest inhibition zones were obtained for CPC and BACH. The sizes of the inhibition zones for CPC and BACH varied from 180.17 mm^2 (± 111.28) to 2110.17 mm^2 (± 23.47) for bacterial suspensions of $2x10^7$ cfu ml^{-1} and from 499.25 mm^2 (± 157.25) to 2627 mm^2 (± 41.94) for bacterial suspensions of 1676 cfu ml^{-1}. The smallest areal inhibition zones were obtained for T samples which varied between 80.74 mm^2 (± 47.88) to 386.22 mm^2 (± 26.74) for bacterial suspensions of $2x10^7$ cfu ml^{-1} and from 183.13 mm^2 (± 129.32) to 772.31 mm^2 (± 75.76) for bacterial suspensions of 1676 cfu ml^{-1}. This indicates that the additive's solubility and potency against *Streptococcus mutans* influences the inhibition zone size.

Overall, it can be concluded that GICs can be used as a delivery matrix for antimicrobial compounds. This ability is potentially clinically useful as the addition of antimicrobial additives enhances anticariogenic properties of these materials. The improvement of anticariogenic properties of dental materials dental materials is desirable, because it can potentially reduce the likelihood of reinfection of the restored tooth cavity. It should also prevent the occurrence of tooth decay and secondary carries formation.

5.2 Future work

On the basis of the above findings, further work could be carried out in order to advance the fundamental understanding of how the setting and maturation processes within GICs are altered when antimicrobial compounds are added. This would involve further study using ^{27}Al MAS-NMR, to identify the peak around 12 ppm, and to determine the role of the species causing it in the setting. Changes in the rate of neutralisation of the poly (acid) could also be determined by Fourier Transform Infrared (FTIR) spectroscopy, in order to correlate them with rate of change in aluminium coordination. Also, it would be useful to reformulate bactericidal GIC with optimum properties, taking into account the fact that the setting reaction is slower in the presence of antimicrobial compounds and the resulting physical properties are inferior to additive-free cements. Increasing the powder/liquid ratio slightly is likely to be beneficial, because this is known to increase the speed of setting and to increase the compressive strength of the set cement. Other useful items of further work are:

1. Determine wear properties of the reformulated materials. The wear test is not a standardised test but it can predict the clinical wear of materials. Suggested methods to be employed are included in references [1, 2].

2. The net setting time is the time measured from the start of mixing until the material has set. The setting time is an important property especially for dental clinicians, as it indicates the time needed for material to achieve a fully hardened state when the clinician can finish the restoration. It is therefore advised to determine the setting time of the materials doped with the additive, using methods described by International Organisation for Standardisation (ISO) [3].

3. The bioactivity of the material is an important property that can determine the possibility of the occurrence of adverse tissue effect in vivo of the reformulated

materials. The determination of bioactivity must be evaluated according to ISO standards [4, 5].

4. It is recommended that the microstructure of the doped samples be examined in order to understand how the bactericides are distributed in the cement matrix. Studies can be performed before and after water exposure. It is advised to test the samples at various leaching times up to equilibrium. These studies would employ two techniques; Energy-dispersive X-ray (EDX) spectroscopy can be employed to study the surface of the samples and Inductively Coupled Plasma-Optical Emission (ICP) to analyse the ion content of solutions.

5. The antimicrobial activity of the doped materials is probably one of the most important properties of these reformulated cements. The determination of microbial activity of the cements exposed to water is necessary in order to evaluate whether the treated cements still possess the desired antimicrobial properties. It is also advised to determine the antimicrobial activity of aged cements. The disc diffusion method used in the current study would be suitable for these determinations.

6. Although, the effect of additives upon the conversion kinetics of Al (IV) to Al (VI) was successfully determined by ^{27}Al MAS-NMR, an investigation of the un-identified peak in the ^{27}Al MAS-NMR spectrum at 12 ppm would be useful if only too understand what role if any it plays in the setting process in the GIC systems. High field MAS-NMR should be employed as it can mitigate the quadrupolar broadening effect of ^{27}Al thereby achieving better resolution of the peak. This study can be performed using the experimental method outlined in reference [6]. This study has the potential to be extended to include cements containing additives at varying states of maturation as some work indicates that the reduction in magnitude of this peak is time dependent [7, 8].

These investigations can be enhanced by the application of density functional theory in order to facilitate the calculation of likely NMR assignments. A neutron diffraction experiments can be used to obtain further structural information. These techniques together would be beneficial in extending our understanding of these materials and the effect of additives on GICs.

5.3 References

[1] Kunzelmann K. H. (1996) Glass-ionomer cements, cermet cements, "hybrid"-glass-ionomers and compomers-laboratory trials-wear resistance. *Transaction Academy of Dental Materials*, **9**:89–104.

[2] De Gee A. J., Van Duinen R. N. B., Werner A. and Davidson C. L. (1996) Early and Long-term wear of conventional and resin-modified glass-ionomers. *Journal of Dental Research,* **75**:1613.

[3] ISO Standard Proposal ISO/7489.

[4] ISO Standard Proposal ISO/TC 150/SC 1 Part 1 and 2.

[5] Lööf J., Svahn F., Jarmar T., Engqvist H. and Pameijer C. H. (2008) A comparative study of the bioactivity of three materials for dental applications. *Dental Materials*, **24**:(5):653-659.

[6] Zainuddin N., Karpukhina N., Hill R. G. and Law R. V. (2009) A Long-term Study on the Setting Reaction of Glass-Ionomer Cements by ^{27}Al MAS-NMR spectroscopy. *Dental Materials*, **25**:290–295.

[7] Pires R., Nunes T. G., Abrahams I., Hawkes G. E., Morais C. M. and Fernandez C. (2004) Stray-field imaging and multinuclear magnetic resonance spectroscopy studies on the setting of a commercial glass-ionomer cement. *Journal of Material Science-Material in Medicine,* **15**:201–208.

[8] Munhoz T., Karpukhina N., Hill R. G., Law R. V. and De Almaida L. H. (2010) A Setting of Commercial Glass-Ionomer Cements Fuji IX by ^{27}Al and ^{19}F MAS-NMR. *Journal of Dentistry,* **38**:325-330.

APPENDICES

APPENDIX 1

Compressive strength

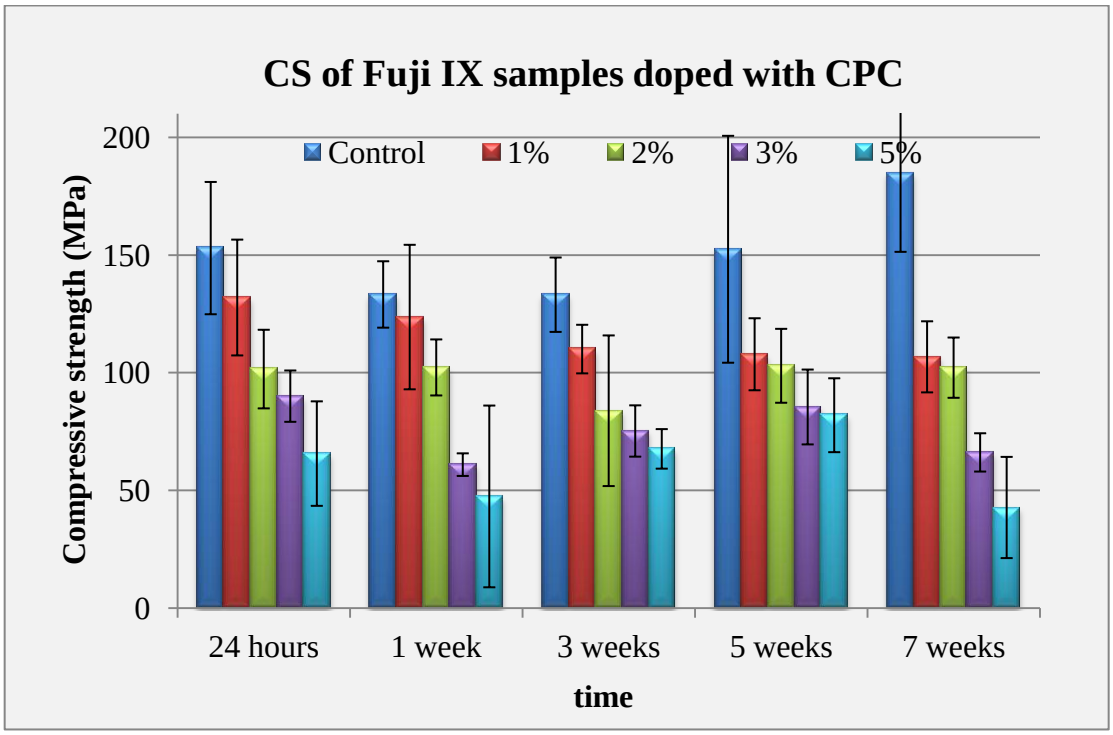

Figure A.1: Compressive strength (MPa) for Fuji IX doped with CPC, SD as error bars

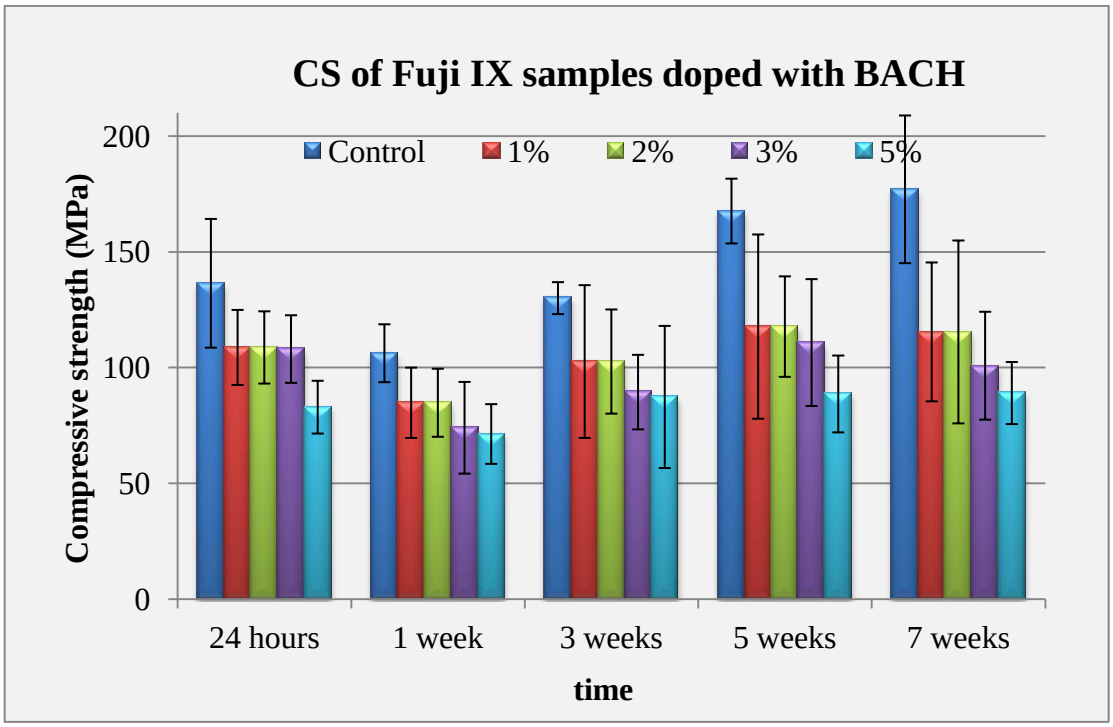

Figure A.2: Compressive strength (MPa) for Fuji IX doped with BACH, SD as error bars

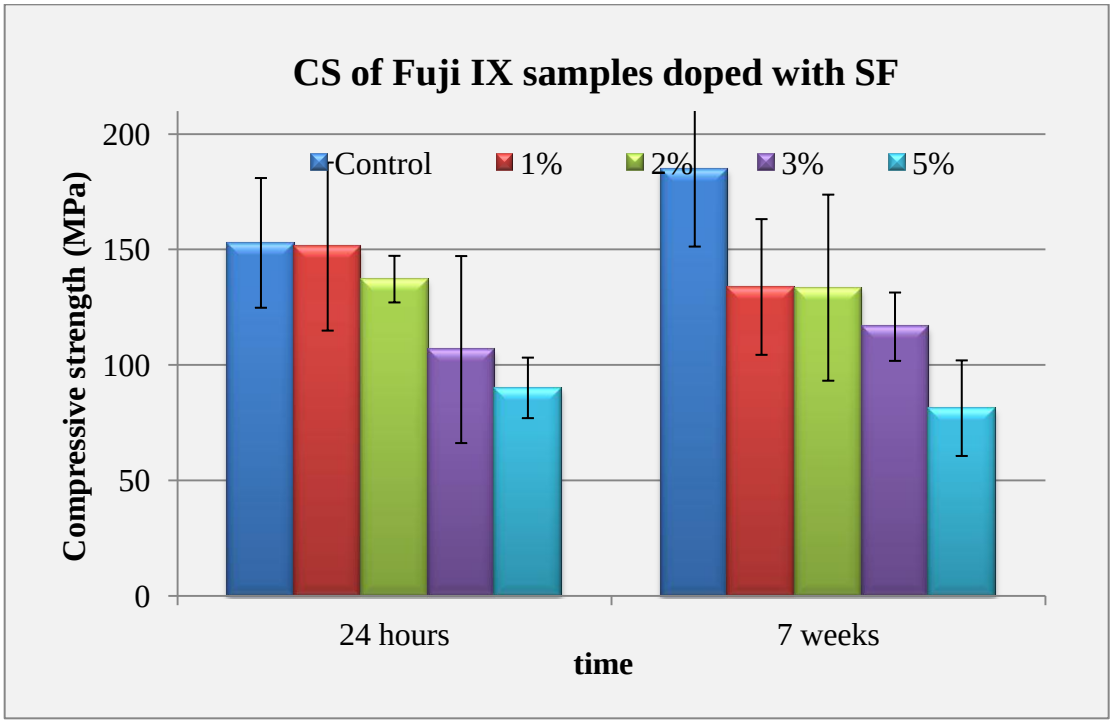

Figure A.3: Compressive strength (MPa) for Fuji IX doped with SF, SD as error bars

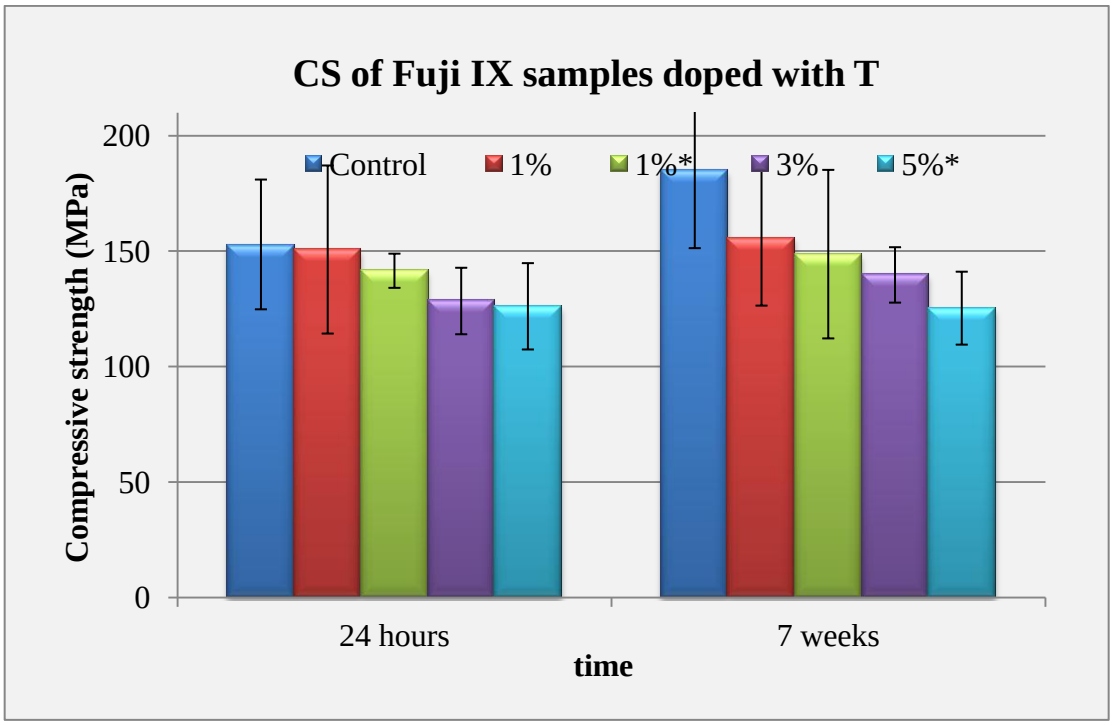

Figure A.4: Compressive strength (MPa) for Fuji IX doped with T, SD as error bars

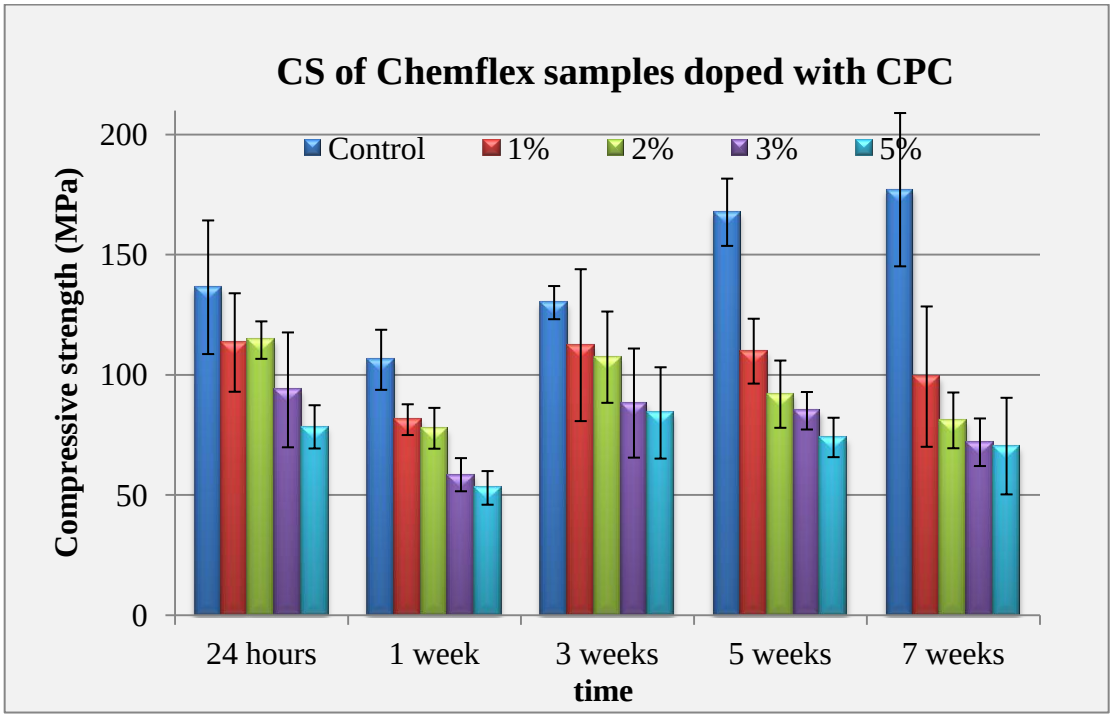

Figure A.5: Compressive strength (MPa) for Chemflex doped with CPC, SD as error bar

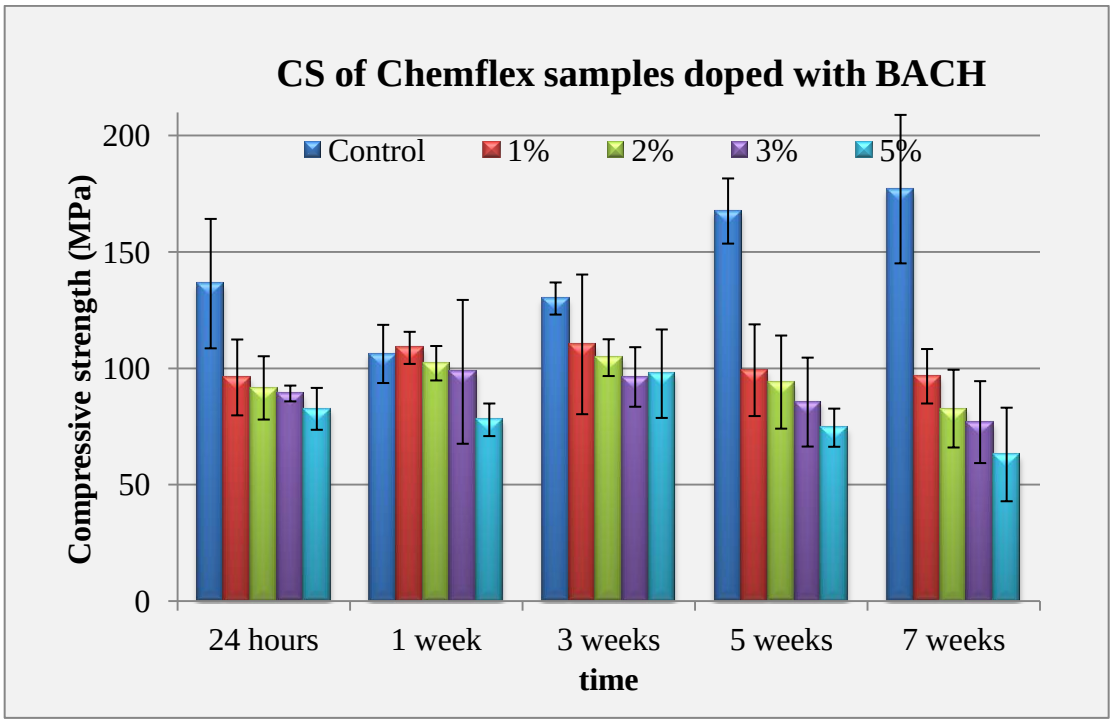

Figure A.6: Compressive strength (MPa) for Chemflex doped with BACH, SD as error bars

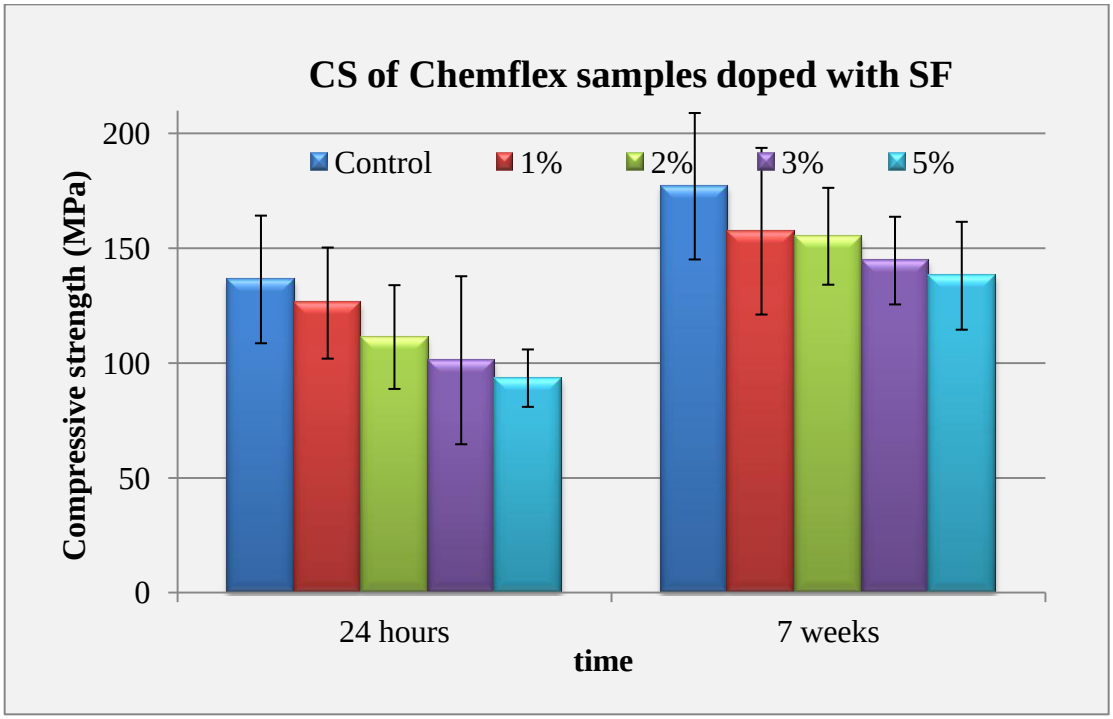

Figure A.7: Compressive strength (MPa) for Chemflex doped with SF, SD as error bars

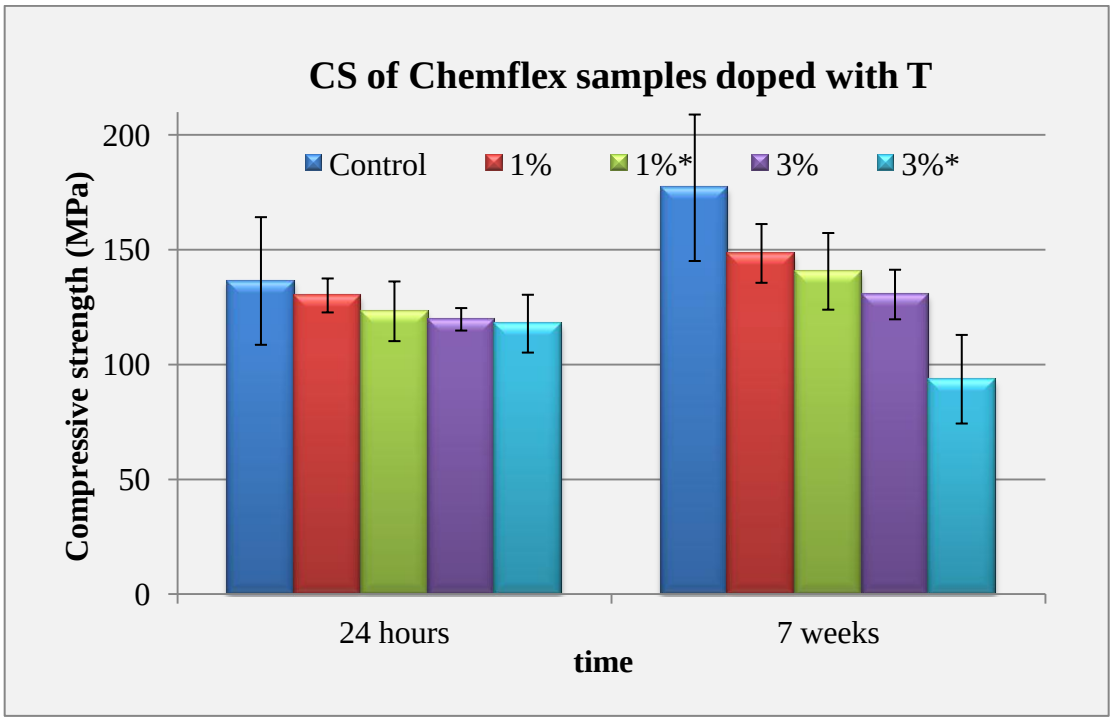

Figure A.8: Compressive strength (MPa) for Chemflex doped with T, SD as error bars

APPENDIX 2

Surface hardness

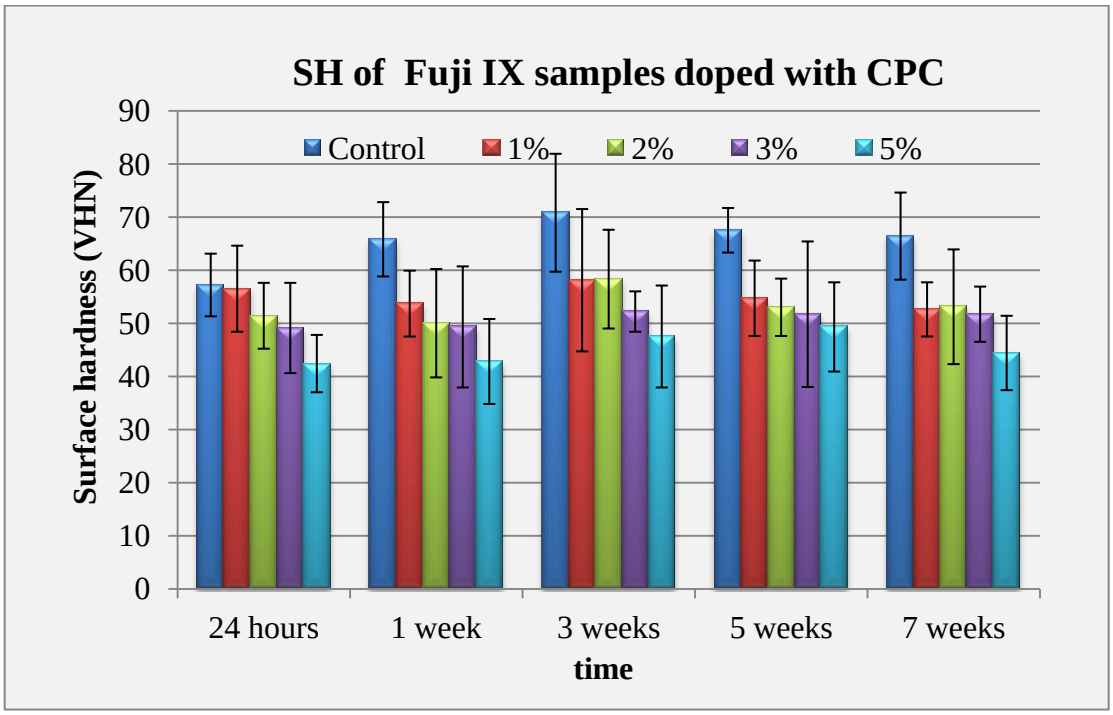

Figure A.9: Surface hardness (VHN) for Fuji IX doped with CPC, SD as error bars

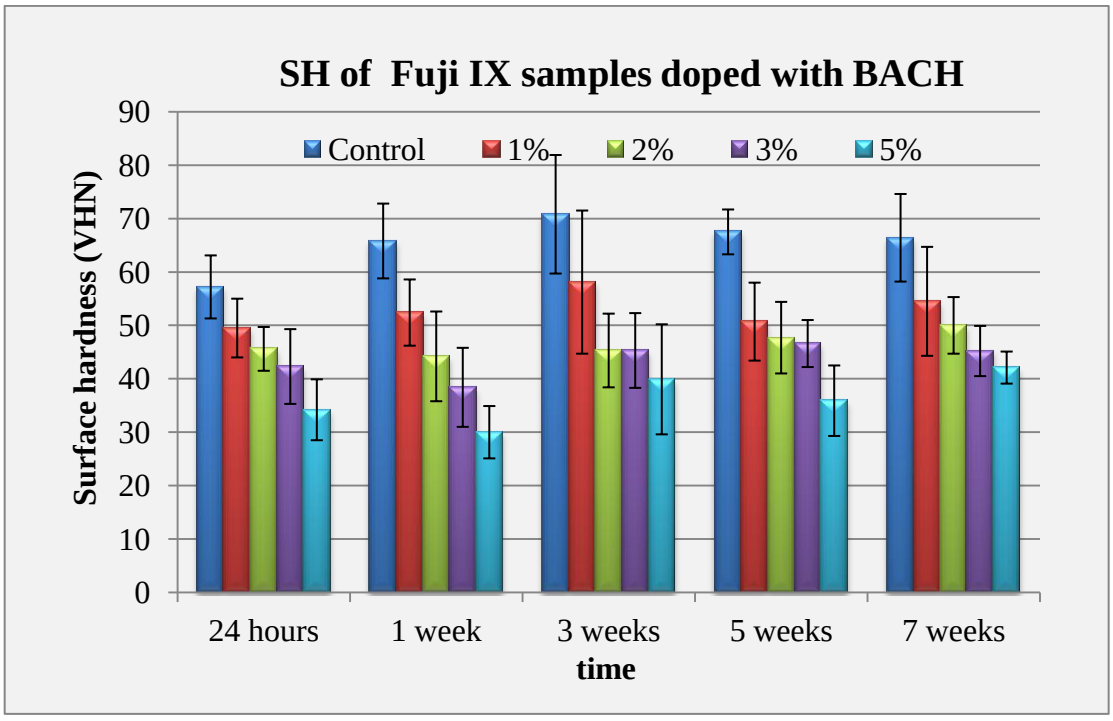

Figure A.10: Surface hardness (VHN) for Fuji IX doped with BACH, SD as error bars

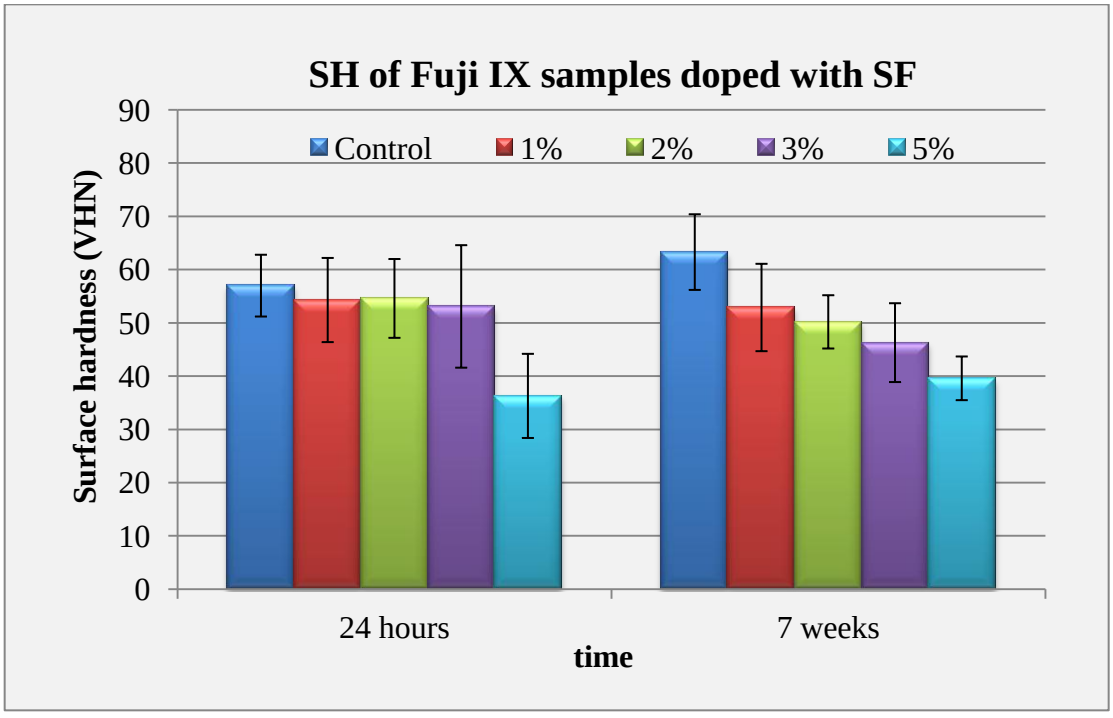

Figure A.11: Surface hardness (VHN) for Fuji IX doped with SF, SD as error bars

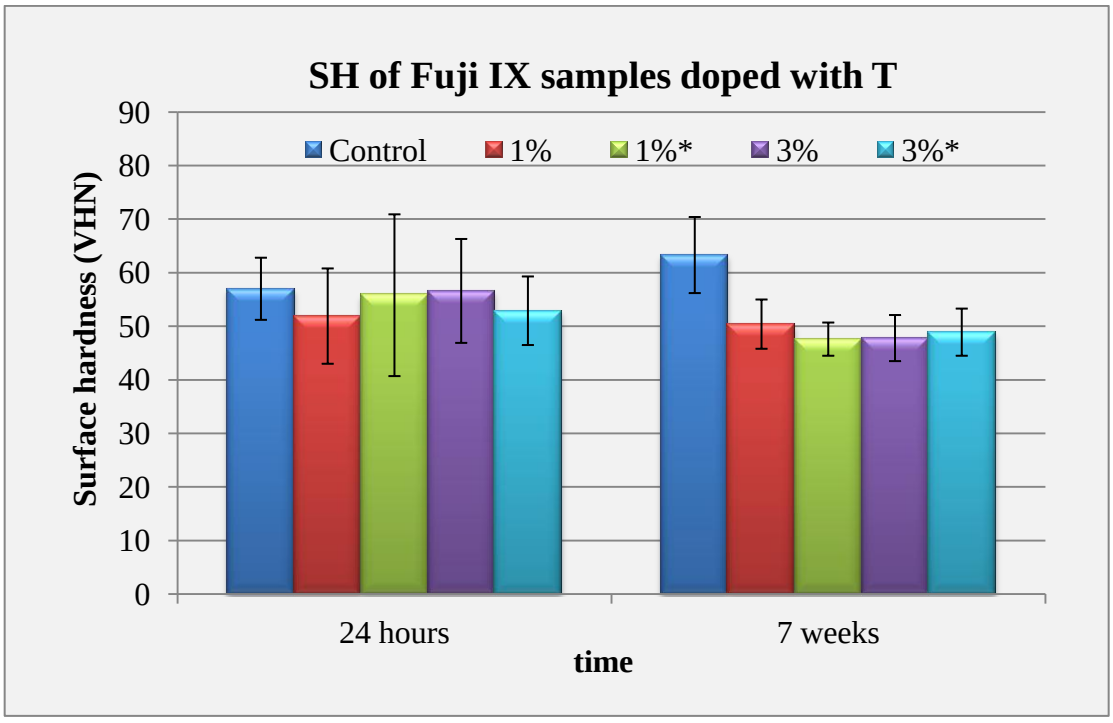

Figure A.12: Surface hardness (VHN) for Fuji IX doped with T, SD as error bars

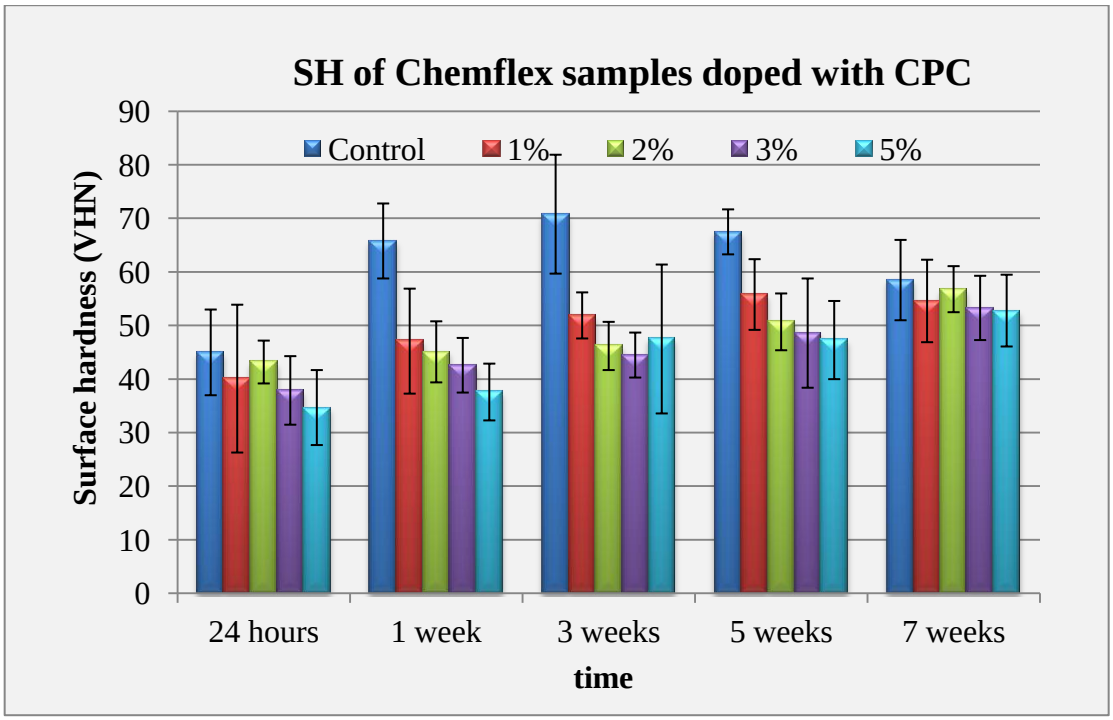

Figure A.13: Surface hardness (VHN) for Chemflex doped with CPC, SD as error bars

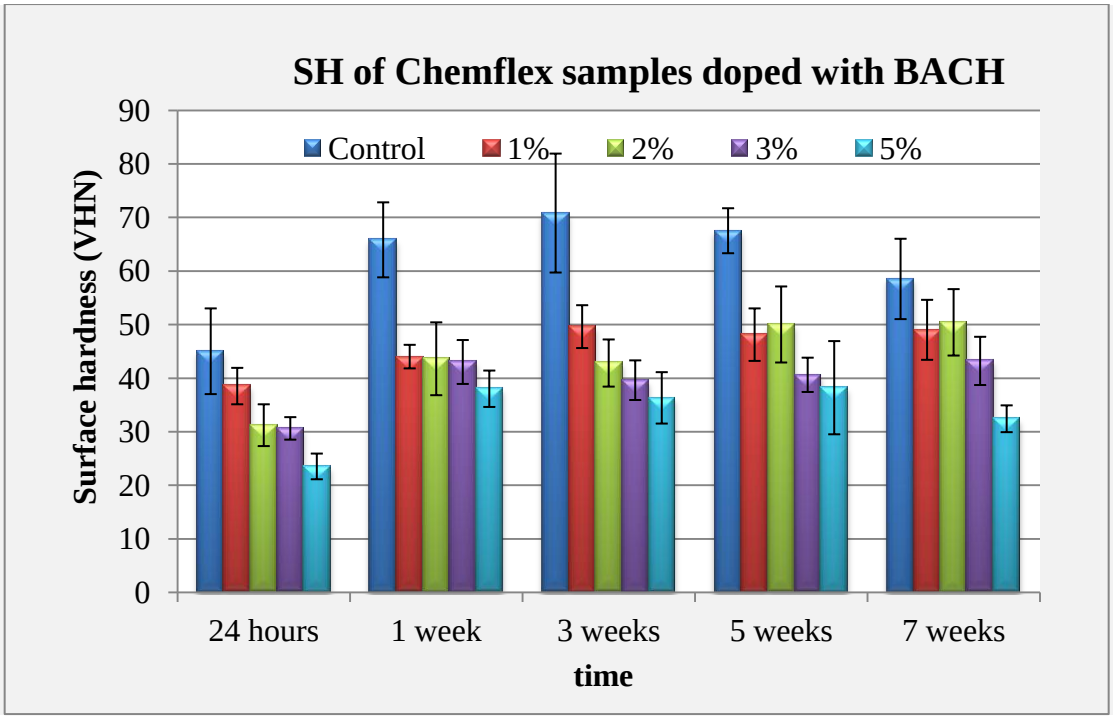

Figure A.14: Surface hardness (VHN) for Chemflex doped with BACH, SD as error bars

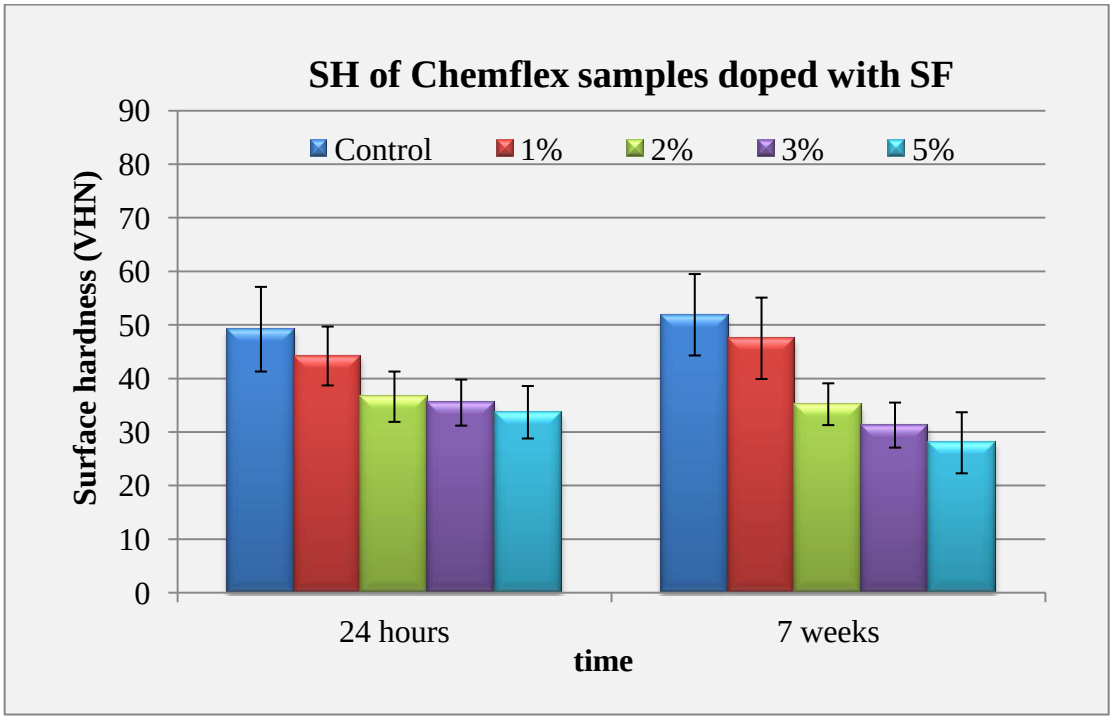

Figure A.15: Surface hardness (VHN) for Chemflex doped with SF, SD as error bars

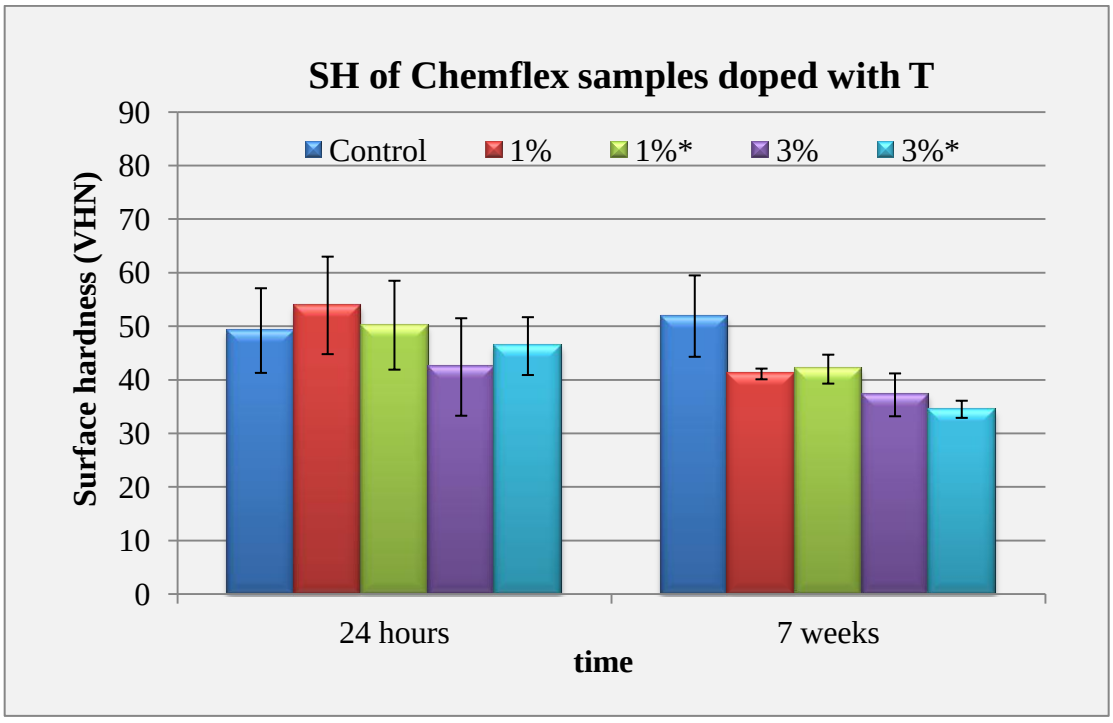

Figure A.16: Surface hardness (VHN) for Chemflex doped with T, SD as error bars

Table A.1: Student's t-test results for surface hardness of Fuji IX and Chemflex, data compares 24-hour compressive strength of control against doped samples

		Fuji IX		Chemflex	
Additives	%	t-value	p	t-value	p
CPC	1%	1.26	NS	1.49	NS
	2%	3.51	p<0.04	2.48	p<0.05
	3%	4.67	p<0.001	2.60	p<0.05
	5%	5.46	p<0.001	4.50	p<0.01
BACH	1%	1.92	NS	2.80	p<0.05
	2%	3.08	p<0.04	3.24	p<0.04
	3%	3.17	p<0.04	3.19	p<0.05
	5%	5.16	p<0.01	4.15	p<0.02
SF	1%	0.08	NS	0.65	NS
	2%	1.18	NS	1.44	NS
	3%	3.27	p<0.04	1.72	NS
	5%	4.50	p<0.02	3.15	p<0.04
T	1%	0.03	NS	0.49	NS
	3%	0.42	NS	1.32	NS
T*	1%	0.18	NS	0.97	NS
	3%	0.46	NS	1.36	NS

**Triclosan samples additionally doped with zinc citrate*

Table A.2: Student's t-test results for surface hardness of Fuji IX and Chemflex, data compares 24-hour surface hardness of control against doped samples

		Fuji IX		Chemflex	
Additives	%	t-value	p	t-value	p
CPC	1%	0.16	NS	0.69	NS
	2%	1.52	NS	0.45	NS
	3%	1.75	NS	1.55	NS
	5%	4.14	p<0.02	2.17	NS
BACH	1%	2.13	NS	1.67	NS
	2%	3.61	p<0.03	3.47	p<0.03
	3%	3.64	p<0.03	3.90	p<0.02
	5%	6.27	p<0.01	5.76	p<0.01
SF	1%	0.62	NS	0.18	NS
	2%	0.58	NS	2.02	NS
	3%	0.68	NS	2.34	NS
	5%	4.70	p<0.01	2.69	p<0.05
T	1%	1.07	NS	1.64	NS
	3%	1.17	NS	0.48	NS
T*	1%	0.08	NS	1.00	NS
	3%	1.06	NS	0.30	NS

**Triclosan samples additionally doped with zinc citrate*

APPENDIX 3

Antimicrobial additives release

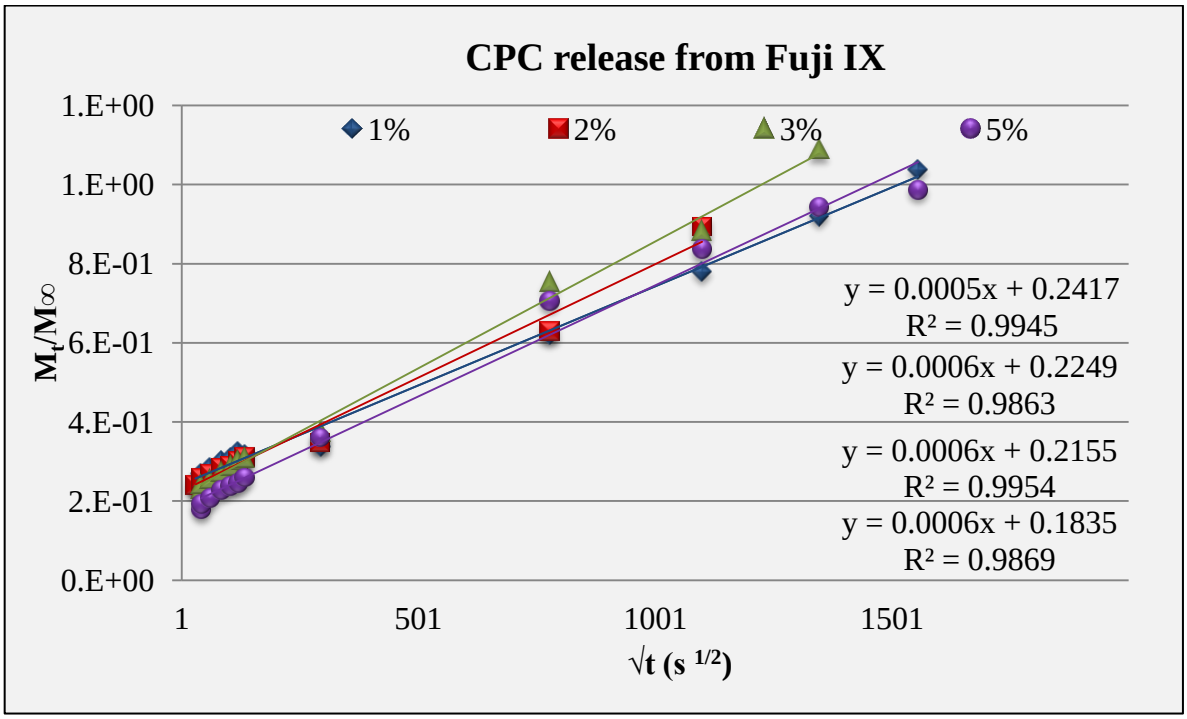

Figure A.17: M_t/M_∞ vs $\sqrt{t}$ for Fuji IX

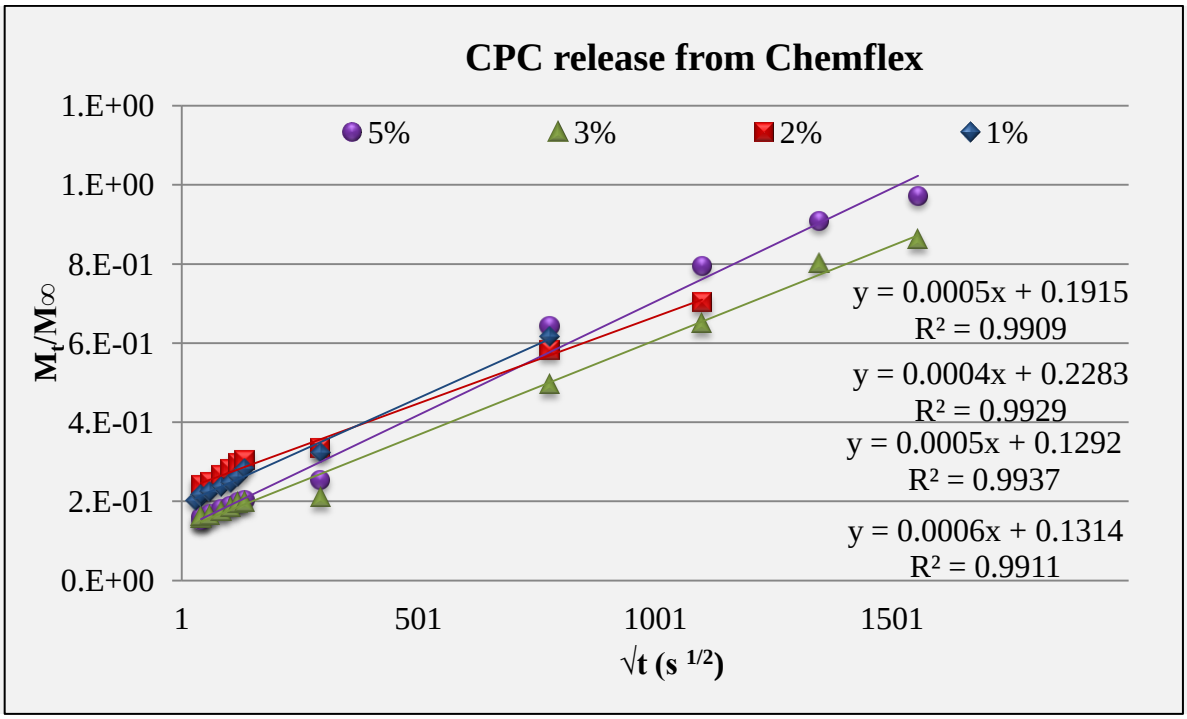

Figure A.18: M_t/M_∞ vs $\sqrt{t}$ for Chemflex

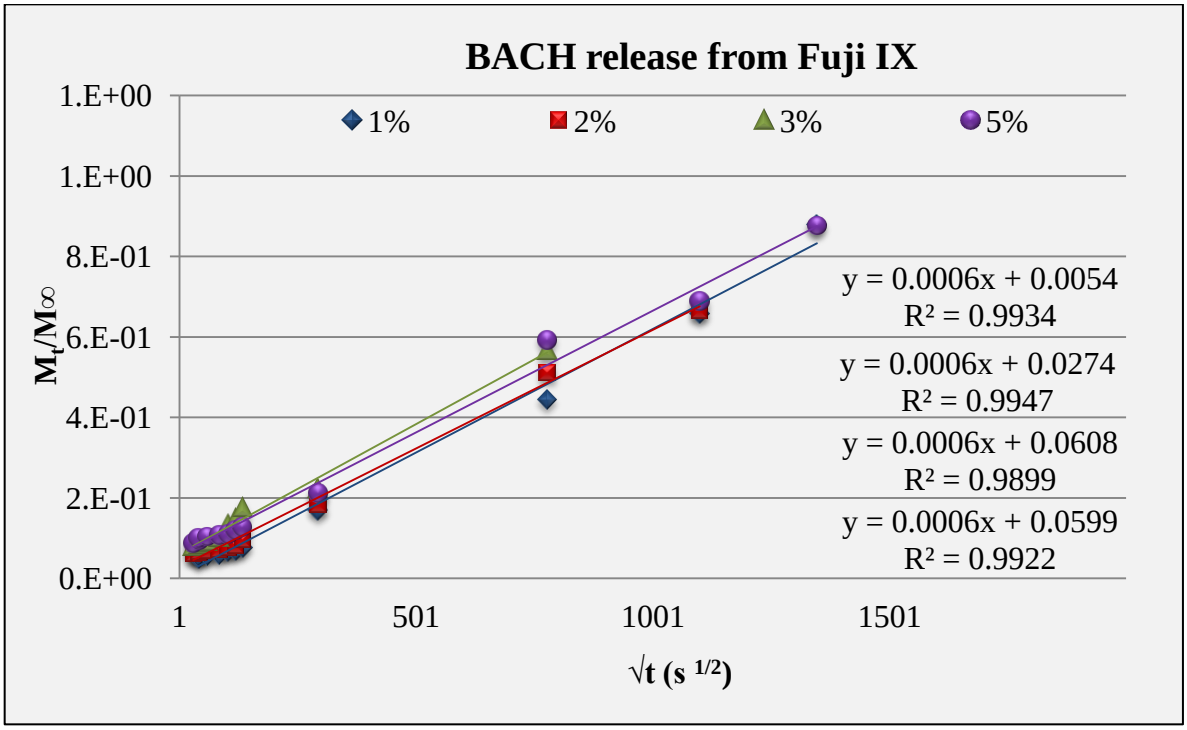

Figure A.19: M_t/M_∞ vs $\sqrt{t}$ for Fuji IX

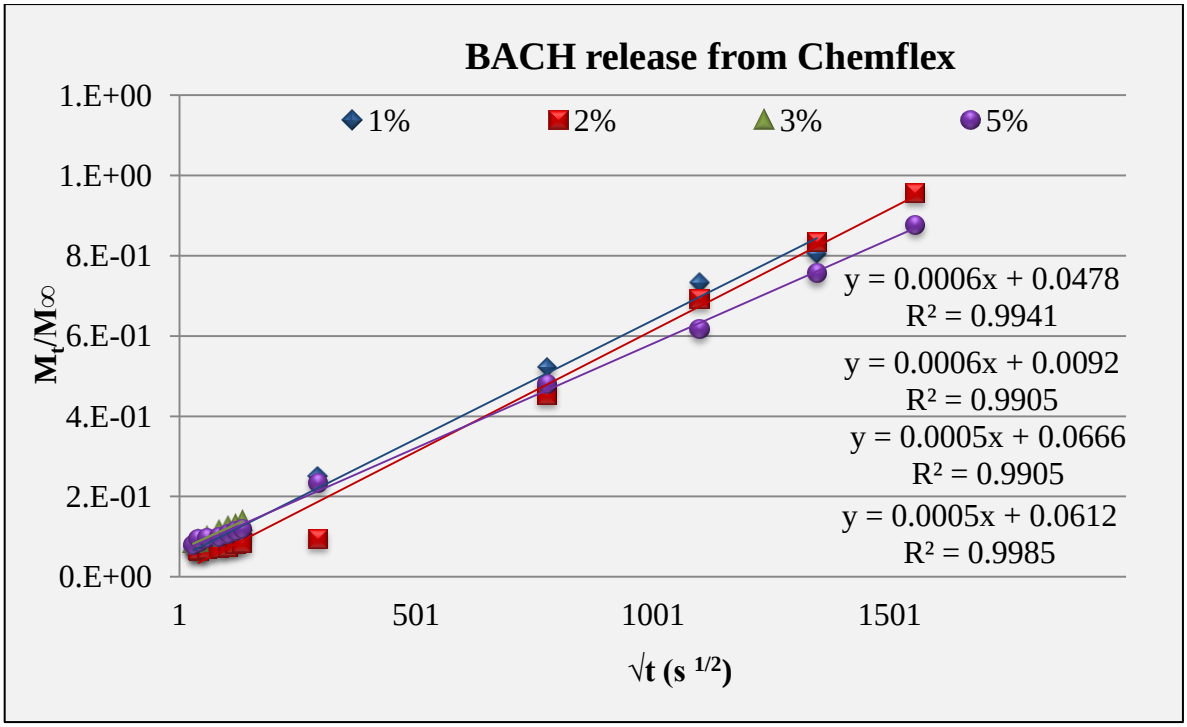

Figure A.20: M_t/M_∞ vs $\sqrt{t}$ for Chemflex

APPENDIX 4

Antimicrobial studies

Table A.3: Mann-Whitney U test results for Fuji IX and Chemflex, bacterial suspension at concentration of $2x10^7$ cfu ml^{-1} and 1673cfu ml^{-1}

		$2x10^7$ cfu ml^{-1}				1673 cfu ml^{-1}			
		Fuji IX		Chemflex		Fuji IX		Chemflex	
Additive	Compared (%)	Z	p	Z	p	Z	p	Z	p
CPC	**0-1**	2.12	p<0.03	2.12	p<0.03	2.12	p<0.03	2.12	p<0.03
	1-3	2.12	p<0.03	2.12	p<0.03	2.12	p<0.03	0.71	NS
	3-5	1.18	p<0.25	0.71	NS	0.71	NS	2.12	p<0.03
BACH	**0-1**	2.12	p<0.03	2.12	p<0.03	2.12	p<0.03	2.12	p<0.03
	1-3	1.18	p<0.25	1.65	p<0.10	2.12	p<0.03	0.23	NS
	3-5	2.12	p<0.03	2.12	p<0.03	2.12	p<0.03	1.18	p<0.25
SF	**0-1**	2.12	p<0.03	2.12	p<0.03	2.12	p<0.03	2.12	p<0.03
	1-3	2.12	p<0.03	1.65	p<0.10	0.71	NS	2.12	p<0.03
	3-5	2.12	p<0.03	0.47	NS	2.12	p<0.03	0.47	NS
T	**0-1**	2.12	p<0.03	2.12	p<0.03	2.12	p<0.03	2.12	p<0.03
	1-3	2.12	p<0.03	2.12	p<0.03	2.12	p<0.03	2.12	p<0.03
T*	**0-1**	0	NS	2.12	p<0.03	2.12	p<0.03	2.12	p<0.03
	1-3	2.53	p<0.01	2.12	p<0.03	2.53	p<0.01	2.12	p<0.03

**Triclosan samples additionally doped with zinc citrate*