Novel Resin-composites for Minimally Invasive Restoration of Teeth

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Candidate declaration

I, Yousef Tariq Yousef Eshmawi, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signature:

Date: 13 July 2022
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Abstract

The use of resin restorative materials became predominant with the growing demand for tooth conservation procedures. The development of self-adhesive resin composite has simplified their application method. The addition of remineralising and antibacterial agents may further improve the longevity of these restorations by sealing gaps and cracks when applied on caries affected dentine layer. Therefore, it is essential to evaluate the effect of adding these agents on the stability of the experimental composites chemical, physical and mechanical properties.

Aims:

The aim was to investigate the effect of MCM and PLS on the polymerisation stability and paste shelf life of experimental composite formulations, and also evaluate the depth of cure of the experimental formulations and two commercially available restorative materials. In addition, the polymerisation shrinkage, subsequent water sorption-induced mass changes, expansion and hydrogen ion release from four experimental resin-composite formulations were evaluated. The simulated wear resistance of five experimental resin-composite formulations and two commercial restorative materials was also investigated. Furthermore, the aim was to assess the effects of the storage container, time of high-temperature paste aging, sample depth and curing on colour of four experimental resin-composite formulations.

Materials and methods:

Resin-composite formulations containing PLS (0, 3, 4, 6 or 8 wt%) & double
these levels of MCPM and three commercial self-adhesive restorative materials were evaluated. The polymerisation kinetics and paste shelf life were evaluated by using FTIR spectroscopy. Delay times, maximum reaction rate, 50% reaction time ($t_{0.5}$) and final degree of conversion ($\%DC$) were determined. Resin-composite pastes were stored at room temperature (7 months), 60°C (18 days or 9 months) and at 4°C (9 months). The aged formulations were compared to experimental formulations stored at 4°C and room temperature. Percentage mass and volume change upon water storage were determined gravimetrically regularly up to 11 weeks by using Archimedes Principle and the polymerisation shrinkage was measured using ISO 17304:2013. Storage solution acidity was evaluated at each time point and dried solid content was analysed after 3 months and 1 year using SEM, FTIR and EDX. The wear resistance was evaluated after 3 months and 1 year by using a dual axis-chewing simulator. L*a* b* values for pastes and composite discs were assessed using a spectrophotometer. Pastes samples were stored in sealed brown glass containers at 4°C, 60°C, or 80°C and studied before and after cure. Results were compared with previous data (Pitsillou, 2019) for paste stored in compules.

**Results:**

Experimental resin-composite formulations were only mildly affected by additive addition or high-temperature aging. Average experimental composite delay times were 4.9, 7.7 and 24.5s, at 1mm, 2mm and 4mm depths, respectively. The maximum rates of reaction were on average 5, 4 and 2%/s, at 1mm, 2mm and 4mm depth, respectively. The 50% reaction times were 12,
16 and 41s, at 1mm, 2mm and 4mm depth, respectively. At 1mm and 2 mm depth, %DC of 74% and 72% could be achieved with 20s light exposure. 40s light was required to gain an average of 64%DC at 4 mm depth. Experimental formulations delay times and rates of reaction showed major differences when compared to Activa (2, 1 and 11s and 3, 2 and 0.67%/s) and Vertise flow (2, 4 and 10s and 5, 4 and 1%/s) at 1mm, 2mm and 4mm depth, respectively. The polymerisation shrinkage ranged between 3.0 and 4.0%. PLS significantly influenced subsequent initial rate of mass change versus square root of time (p<0.05). After 11 weeks, mass changes were -0.9±0.2, 0.1±0.1, 1.1±0.1 and 1.2±0.1 wt% for F1, F2, F4 and F5, respectively. The final values of volume change were 3.7±0.1, 3.7±0.1, 3.9±0.3, and 2.6±0.1 vol.% for F1 (MCPM 16wt.% and 8wt.% PLS), F2 (MCPM 16wt.% and 4wt.% PLS), F4 (MCPM 8wt.% and 8wt.% PLS) and F5 (MCPM 8wt.% and 4wt.% PLS), respectively. Increasing both additives had a significant impact on volume change (p<0.05). H+ ion release was higher for composite formulations containing high levels of MCPM. The highest average H+ release by 11 weeks was 22±2 micromoles/sample and the lowest was 9±2 micromoles/sample for F1 and F5 respectively. SEM images showed signs of extensive crystallisation from the dried extracts. Elemental analysis from EDX of the dried extracts after 3 months showed P/Cl ratio was approximately 2, 4, 1, 2 as expected from the MCPM: PLS ratios in the evaluated formulations. For FTIR, The MCPM: PLS ratios in dried extracts are 3 times the MCPM: PLS wt.% ratio in the material for F1, F2 and F5. For F4, the ratio is 6 times that in the material. For the wear resistance, the highest surface volume loss after 3 months was 3.40±1.47
mm³ and the lowest was 2.21±0.06 mm³ for F2 and F4, respectively. After 1 year, the highest surface volume loss was 2.89±0.45 mm³ and the lowest was 1.72±0.40 mm³ for F7 (MCPM 8% small particle size and 4% PLS) and F4 respectively. Surface volume loss for commercial materials ranged between 1.20±0.46 to 1.72±0.26 mm³ with the highest average for Fuji II and the lowest for Activa. F7 showed higher surface volume loss and differed significantly to Activa (p=0.046), Fuji II and F4 (p<0.05). The b* values showed greater change than L* and a*. The average b* values for compule-stored, 1mm thick composite paste at 4°C, 25°C and 37°C, covered with acetate were 23 ±1 and 29 ±1.3 without acetate. Following 20s cure, they declined to 6 ±2 and 7.5 ±2 on top and bottom surfaces, respectively. For samples with 4 mm depth, the results ranged between 8.2 to 31.4 with an average of 11.7 ±2.4 for top surfaces and 28.3 ±3.8 for bottom surfaces. The bottom surfaces of the 4mm groups showed higher b* values than the top surfaces and the overall 1mm group, particularly regardless of the curing surfaces. The effect of the composition is evident after long time storage at high temperature as formulations with high PLS became yellower than those with lower PLS.

**Conclusion:**
The experimental formulations were stable upon high temperature aging. For experimental formulations and commercial materials, 20s light exposure is sufficient for good monomer conversion up to 2mm depth. Increased thickness causes an increase in delay time and a decrease in the rate of reaction. Polymerisation shrinkage was not significantly influenced by MCPM and PLS level. The increase of MCPM and PLS led to higher volume change and H⁺
release, while increasing PLS led to higher mass change. Reducing MCPM particle size led to lower wear resistance than commercial comparisons. Storage of resin-composite paste in compules instead of sealed containers caused greater colour instability. Sample thickness has a greater effect than high temperature storage conditions on colour stability. High PLS affected the colour stability upon long time storage at high temperature.
Impact Statement

In recent years, health care expenditures have been substantially increased around the world. Dental caries is one of the global health burdens that considerably affects low and middle-income countries (Petersen, 2004, Sharma et al., 2014). Untreated dental caries was found to be the most common condition among all global diseases with the highest productivity losses and expenditure found in Western Europe (Righolt et al., 2018, Kassebaum et al., 2017). The caries process is known to be interruptible by identifying active lesions and arresting these lesions at an early stage. If the carious lesion is not arrested, the caries process will progress to clinical cavitation of the tooth surface. This will require surgical removal of the infected carious lesion and the application of a biomimetic material.

Resin composite restorations and glass ionomer cements are the current main restorative materials after the Minamata Agreement in 2018 that banned the use of amalgam restorations (Fisher et al., 2018). Glass ionomer cements have lower flexural strength (up to 50 MPa) (Lohbauer, 2009) than resin composite (up to 145 MPa) (Jun et al., 2013) that are inadequate for bulk restoratives. However, composite failure is mostly attributed to water sorption, wear, bulk fracture and the formation of bacterial colonization and recurrent caries, resulting in subsequent need for restoration replacement (Sunnegardh-Gronberg et al., 2009, Rasines Alcaraz et al., 2014, Opdam et al., 2007, Bernardo et al., 2007, Bourbia et al., 2013). Therefore, the development of a novel self-adhering and therapeutic restorative material with bioactive properties is required to replace amalgam and to overcome problems heavily
associated with dental composites (Van Meerbeek and Frankenberger, 2019). This doctoral project evaluated restorative materials with self-adhesive and remineralising components. The smart restorative material was developed to enable single-step placement directly on caries affected dentine, saving treatment chair time and minimizing handling errors. The material entered the clinical trial phase, in January 2019, and preliminary reports and data gathered from the patients showed promising results (unpublished data). In this project, the stability of the smart restorative material was investigated with systematic variations in material components. The findings should allow better understanding of trends in material properties to further optimise the novel resin-composite formulations to be used for minimally invasive dentistry.
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<tr>
<td>4META</td>
<td>4-methacryloyloxyethy trimellitate anhydride</td>
</tr>
<tr>
<td>ATR</td>
<td>Attenuated total reflectance</td>
</tr>
<tr>
<td>ACP</td>
<td>Amorphous calcium phosphate</td>
</tr>
<tr>
<td>CaP</td>
<td>Calcium Phosphate</td>
</tr>
<tr>
<td>CQ</td>
<td>Camphorquinone</td>
</tr>
<tr>
<td>DC</td>
<td>Degree of conversion (%)</td>
</tr>
<tr>
<td>EDI</td>
<td>Eastman dental institute</td>
</tr>
<tr>
<td>EDX</td>
<td>Energy dispersive X-ray</td>
</tr>
<tr>
<td>FDA</td>
<td>US food and drug administration</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform spectroscopy</td>
</tr>
<tr>
<td>GIC</td>
<td>Glass ionomer cement</td>
</tr>
<tr>
<td>H⁺</td>
<td>Hydrogen ion</td>
</tr>
<tr>
<td>HEMA</td>
<td>Hydroxyethyl methacrylate</td>
</tr>
<tr>
<td>LED</td>
<td>Light-Emitting Diode</td>
</tr>
<tr>
<td>MCPM</td>
<td>Monocalcium Phosphate Monohydrate</td>
</tr>
<tr>
<td>N/A</td>
<td>Not available</td>
</tr>
<tr>
<td>PLR</td>
<td>Powder/Liquid Ratio</td>
</tr>
<tr>
<td>PLS</td>
<td>εPoly-L-Lysine</td>
</tr>
<tr>
<td>PPGDMA</td>
<td>Poly(propylene glycol) dimethacrylate</td>
</tr>
<tr>
<td>RMGI</td>
<td>Resin modified glass ionomer</td>
</tr>
<tr>
<td>RT</td>
<td>Room temperature</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Chemical Name</td>
</tr>
<tr>
<td>-------------</td>
<td>-----------------------------------</td>
</tr>
<tr>
<td>TEGDMA</td>
<td>Triethylene glycol dimethacrylate</td>
</tr>
<tr>
<td>UDMA</td>
<td>Urethane dimethacrylate</td>
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Figure 5.3 L* (A) a* (B) and b* (C) values versus time of paste storage (days) for uncured samples and set materials of F5 experimental formulation, stored at 4, 25 and 37°C (n=1) in compules by Pitsillou, 2019 and at 4°C in sealed containers (n=1). Abbreviations: Numbers: represent storage temperature in degree Celsius (°C). P: Uncured paste (red symbols). PA: Paste with acetate sheet (blue symbols). CT: Cured top (green symbols). CB: Cured bottom (black symbols) *: Paste stored in sealed containers. Diamond: 4°C. Triangle: 25°C. Square: 37°C Circle: Sealed glass container.

Figure 5.4 b* values for the uncured samples and set materials of F5 experimental formulation at 1mm depth light cured for 20s stored at 4°C and aged at 60°C for 112 days in compules by Pitsillou, 2019 and 609 days in sealed containers. Error bars are half the difference between samples. Paste (n=2) and set materials (60°C: n=1 and 4°C: n=2).

Figure 5.5 L*, a* and b* values versus time (days) for uncured samples of F5 experimental formulation, aged at 80 °C in compules (A) by Pitsillou, 2019 for 26 days and sealed containers for 28 days (n=1) (B).

Figure 5.6 b* values versus time (days) for uncured samples with (A) and without acetate sheet (B), and top and bottom surfaces of cured samples (C) of F5 experimental formulation, aged at 80°C in compules by Pitsillou,
2019 for 26 days and sealed containers for 28 days (n=1). Abbreviations:

Figure 5.7 Average b* values for the uncured samples of the experimental formulations at 1mm and 4mm depth aged at 80°C for 14 and 28 days. Error bars are half the difference between samples of each formulation (n=2).

Figure 5.8 b* values for the experimental formulations at 1mm depth cured for 20s and 4mm depth cured for 40s aged at 80°C for 14 days (A) and 28 days (B) (n=1).

Figure 5.9 Factorial analysis of the evaluated b* values: values and interactions of 2 variables (a1:MCPM, a2: PLS, a12: Interactions between the variables).

Figure 5.10 Factorial analysis of the evaluated b* values: values and interactions of 2 variables (a1:MCPM, a2: PLS, a12: Interactions between the variables).

Figure 5.11 Factorial analysis of the evaluated b* values: values and interactions of 2 variables (a1:MCPM, a2: PLS, a12: Interactions between the variables).

Figure 7.1 Hydrogen ion release versus SQRT (t/hr) for 11 weeks in deionised water. Error bars are 95% CI (n=3).
1. **Introduction**

1.1 **Dental Caries**

1.1.1 **Epidemiology and effect on quality of life**

Dental caries is one of the most common chronic infectious diseases and considered one of the major global public health challenges around the world, affecting up to 90% of children and adolescents (Petersen et al., 2005, Zaror et al., 2011). An estimated 2.5 billion people around the globe have untreated dental caries (Kassebaum et al., 2017). In the European region as a whole, it was reported that dental caries is the most common non-communicable disease affecting 20–94% of 6-year-old children (WHO, 2018).

The consequences of childhood caries is cumulative in the permanent dentition and involves higher risk of new carious lesions, higher rate of hospitalization and emergency room visits, increased treatment costs, loss of school days (Edelstein and Reisine, 2015) and increased days with restricted activity, diminished oral health-related quality of life and ability to learn (Blumenshine et al., 2008, Anil and Anand, 2017).

1.1.2 **Aetiology**

Dental caries is a dynamic process that results from a physiological imbalance in the mineral ions of the tooth structure and the dental plaque characterized
by demineralisation and remineralisation cycles (Gross et al., 2012, Featherstone, 2008). Dental plaque is an organic biofilm formed on tooth surfaces soon after cleaning. It frequently contains cariogenic bacteria such as *mutans* Streptococci and *Lactobacilli* species. These have been found to be the main aetiological agents responsible for dental caries (Bowen et al., 2018). The demineralisation phase starts with the formation of acids by plaque bacteria after exposure to a fermentable carbohydrate leading to the diffusion of calcium and phosphate ions of the enamel and dentine (Featherstone, 2008, Pitts et al., 2017, Anderson et al., 2018, Bowen et al., 2018). If mineral loss outweighs gain, over time, the caries process will progress to the clinical cavitation of the tooth surface (Featherstone, 2008).

### 1.2 Tooth hard tissue structure

#### 1.2.1 Enamel

Enamel is a highly mineralised outer layer of the crown portion of the tooth (Hsieh et al., 2013). It is hard (Knoop hardness: 343 kg/mm\(^2\)) and brittle serving as an important stiff and wear-resistant part of teeth (Sakaguchi and Powers, 2012). Enamel is the hardest substance in the human body and has a complex hierarchical structure composed of a high content of inorganic hydroxyapatite (HAp) crystals (25-30 nm thickness) (Sui et al., 2013). It consists of approximately 80-90 vol% inorganic content (around 96% by weight) (Bechtle et al., 2012) and low content of organic globular proteins (Ang et al., 2012, He and Swain, 2008). On the micro scale, enamel has notable features, in particular aligned long prisms or rods with a “keyhole”
shape with the top or head oriented toward the crown of the tooth and the bottom, or tail, oriented toward the root of the tooth (Figure 1.1) (Macho et al., 2003, Habelitz et al., 2001). An enamel rod is a tightly packed highly organised mass of hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ crystals measuring 4 $\mu$m wide and 8 $\mu$m high (Wefel and Dodds, 1999).

Figure 1.1 A cross section of the enamel microstructure that shows enamel rods (head and tail). The area around the enamel rod is known as interrod enamel. The figure was adapted from Habelitz et al., 2001.

### 1.2.2 Dentine

Dentine is a thick porous layer that forms the bulk of dental mineralised tissues beneath the enamel and in the root it is covered by cementum (Goldberg et al., 2011, Neves and Sharpe, 2018). Physically and chemically dentine closely resembles bone. It is less mineralised (55 vol% Inorganic) and has higher organic (mainly type I collagen) and water content than enamel. It acts as a second barrier to protect the soft pulp tissues from any infectious
agents. The pulp contains the odontoblasts that are responsible for dentine secretion throughout life (Goldberg et al., 2011). The dentine that is forming the initial shape of the tooth is called primary dentine. In permanent teeth, primary dentine is completed three years after tooth eruption. Subsequently, secondary dentine forms on the internal aspect of the pulp chamber and its formation continues for the rest of life (Zilberman and Smith, 2001). Reparative or tertiary dentine forms on the wall of the pulp cavity as a reaction to external stimulation such as attrition, abrasion, erosion, trauma, moderate rate caries lesions and operative procedures. The innermost layer of dentine is not fully mineralised and is called pre-dentine (it is the matrix secreted by odontoblasts).

The odontoblast cell bodies remain external to dentine but their processes exist within tubules in dentine. The tubules lengthen to form a tubular structure called the dentinal tubules (Goldberg et al., 2011). They radiate outward from the pulp through the dentine to the exterior cementum or enamel border (Ross, 2003). The tubules follow an S-shaped path from the outer surface of the dentine to the area nearest the pulp (Ten Cate, 1994). The diameter and density of the tubules are greatest near the pulp and lowest near the enamel and cementum (Figure 1.2) (Tjäderhane et al., 2009).

Dental caries is one of the most common challenging diseases to dentine. Dentine carious lesions consist of two distinct layers: the outer bacterially infected layer (infected dentine) and the inner layer (affected dentine) (Fusayama, 1979). The superficial layer is highly demineralized with irreversible degenerated collagen fibers, and physiologically unremineralizable
with disappearance of cross-linkages of collagen (Nakornchai et al., 2004). Conversely, the deeper layer is partially demineralized, physiologically remineralisable with expanded odontoblastic processes, sound collagen fibers, and apatite crystals bound to the fibers (Figure 1.3) (Terashima, 1970, Ohgushi and Fusayama, 1975, Kuboki et al., 1977). The cross-linking of collagen remains intact and can act as a scaffold for the remineralisation of the intertubular dentine. Therefore, caries affected dentine should be conserved during cavity preparation (Meraji et al., 2018, Perdigão, 2010).

Figure 1.2 Histogram showing the relationship between the number and radius of the dentinal tubules to the distance from the pulp. The figure was adapted from Tjäderhane et al., 2009.
1.2.3 Management of dental caries

1.2.3.1 Minimally Invasive Dentistry

The concept of minimally invasive dentistry is based on maximum preservation and conservation of healthy, natural tooth structure by detecting and treating dental caries on the microscopic level (Rainey, 2002, Wolff et al., 2007, Murdoch-Kinch and Mclean, 2003). The concept involves caries risk assessment, early detection of carious lesions, nonsurgical interventions and a modified restorative approach such as delayed restoration, smaller tooth preparations, adhesive dental materials and repair rather than replacement of failing restorations (Murdoch-Kinch and Mclean, 2003).

Figure 1.3 Caries lesion under the Field-emission scanning electron microscopy showing: (A) partially demineralised dentine in the transition to caries-affected dentine; (B) tubules are occluded with mineral deposits in the caries-affected area. The figure was adapted from Perdigão, 2010.
The caries process is known to be preventable and interruptible. The latter can be achieved by identifying demineralising (i.e., active) lesions and arresting or reversing these lesions at an early stage (Cury and Tenuta, 2009). Treatment of early carious lesions can be achieved by modifying the aetiologic factors through improving oral hygiene, control of plaque biofilm and cariogenic dietary habits, and increasing protective factors such as the application of fluoride, fissure sealants and ensuring adequate salivary flow (Featherstone, 2008). Due to lack of awareness and socioeconomic factors, primary prevention of the disease may fail and necessitate secondary or tertiary measures to stop the progression of the lesion (Longbottom et al., 2009). Cavitated lesions should be restored by surgical treatment to remove the carious lesion and to restore the tooth function and aesthetics. Several surgical intervention techniques can be used including minimally invasive operative intervention, atraumatic restorative treatment, crowns and prosthesis or extraction of the tooth.

1.2.3.2 Atraumatic restorative treatment (ART)

The Atraumatic Restorative Treatment (ART) is a minimally invasive approach to prevent dental caries and to stop its progression (Frencken et al., 1996). It was introduced almost 35 years ago, to manage cavitated dentine lesions in rural areas in which rotary-driven restorative care was not possible because of the lack of electricity, and piped water. At that time, dentists used hand instruments to enlarge small cavity openings or for the removal of soft carious dentine (Frencken, 2017). ART rarely requires local anesthesia and produces
less anxiety and pain to patients (Frencken, 2014). ART components can be divided into a preventive sealant and a restorative restoration. In ART sealants the material is placed by finger pressure over carious lesion and pits and fissures (Frencken, 2017). Hand instruments are only used to adjust the bite and to remove any excess material. The ART restorative procedure involves removing soft completely demineralized enamel-dentine tissues using only hand instruments and, restoring teeth with an adhesive restorative material. A restorative material that has the ability to bond to affected tooth substrates and exhibits a self-healing property to restore diseased tissues with sufficient mechanical properties was recommended (Frencken et al., 1996). High-viscosity glass ionomer cement (GIC) is currently used for ART (van’t Hof et al., 2006) due to its biocompatibility, chemical adhesion, fluoride release (Van Dijken and Pallesen, 2008) and handling properties (Casagrande et al., 2013, Ladewig et al., 2017).

1.3 Dental materials

1.3.1 Current direct restorative materials

The main conventional direct restorative materials currently available for restoring primary and permanent teeth include amalgam, conventional glass ionomer cement, resin-modified glass ionomer cement, high-viscosity glass ionomer cement, compomer, and resin-composite (Dhar et al., 2015).

Mercury-silver amalgam was the most commonly used and has been considered the gold standard in restorative materials (Mickenautesch et al.,
However, due to the Minamata agreement (Minamata convention on mercury, UN, 2013), its use in children was banned in July 2018 because of the potential toxicity of mercury to the environment (Fuks, 2015, Bakhurji et al., 2017, Federation, 2014).

Conventional glass ionomer has inferior compressive (87 MPa) and flexural strength (34.4 MPa) (Ilie and Hickel, 2007) and higher annual failure rate of 7.2% in posterior stress bearing cavities compared to amalgam (3%) and composite (2.2%) (de Lima Navarro et al., 2021, Manhart et al., 2004). Resin-modified glass ionomer cement was developed to improve the compressive (156.6 MPa) and flexural (83.1 MPa) strength and to overcome the brittle nature of the conventional glass ionomer cement (Croll and Nicholson, 2002, Espelid et al., 1999). However, due to their lower flexural strength, glass ionomer cements are not recommended bulk restoratives (Chadwick and Evans, 2007, Sidhu et al., 1997, Ellakuria et al., 2003, Heintze et al., 2022).

1.3.2 Resin-matrix composite

1.3.2.1 Composition

1.3.2.1.1 Resin matrix

Resin-matrix composite is composed of a plastic resin material that is known as the continuous phase (Anusavice, 2003). The light-activated polymerisable monomers binds to the reinforcing inorganic radiopaque glass or barium oxide filler particles/fibers that are dispersed in the matrix (Figure 1.5) (Miletic, 2018, Bayne et al., 2019). Resin composites commonly contain a blend of aromatic
and/or aliphatic dimethacrylate monomers such as bisphenol-A glycidyl dimethacrylate (Bis-GMA), urethane dimethacrylate (UDMA), triethylene glycol dimethacrylate (TEGDMA) (Figure 1.4) and non-acidic functionalized 2-hydroxyethyl methacrylate (HEMA) (Szczesio-Wlodarczyk et al., 2021). Bis-GMA was the first monomer to be used in dental resins. The large molecule with aromatic structure improves the mechanical properties of the composite (Anseth et al., 1995, Moszner and Hirt, 2012, Raptis et al., 1979). UDMA is an alternative matrix polymer that facilitates filler loading but increases polymerisation shrinkage. UDMA has lower viscosity than Bis-GMA and a highly flexible urethane linkage that may improve the monomer conversion and toughness of resin composites (Asmussen and Peutzfeldt, 1998, Stansbury and Dickens, 2001). Furthermore, UDMA does not contain hydroxyl groups, which results in lower water sorption than Bis-GMA contained composite (Barszczewska-Rybarek, 2019, Sideridou et al., 2003). Previous studies have revealed that UDMA has a lower residual monomers in the resulting polymer network and therefore less toxic than Bis-GMA (Issa et al., 2004). In addition, the UDMA molecule with its flexible aliphatic structure and urethane links is able to form a strong hydrogen bond that may further increase the toughness of the composite (Sideridou et al., 2002). Both Bis-GMA (1200 Pa·s) and UDMA (23.1 Pa·s) are viscous which makes them very difficult to blend and to be used as a restorative monomers alone and are usually thinned (Peutzfeldt, 1997, Ferracane, 2011, Sideridou et al., 2002). Diluent monomers such as TEGDMA are added as viscosity controllers to improve handling properties, cross-linking, flexibility and monomer conversion
of the resultant composite paste. Previous findings showed that UDMA was more reactive and had higher double bond conversion than Bis-GMA when used with similar TEGDMA concentrations. This could affect the polymerisation rate and final conversion of the polymerisation process (Dickens et al., 2003b). A functionalized hydrophilic monomer such as HEMA is used to improve adhesion and mixing of hydrophobic/hydrophilic parts in the same mixture (Moszner and Hirt, 2012). The addition of HEMA into UDMA/Bis-GMA resin matrix positively affected the flexural strength and modulus (Floyd and Dickens, 2006), which could be explained by the better mobility of Bis-GMA and UDMA in HEMA (Tauscher et al., 2017). Additionally, previous studies have found UDMA/HEMA showed higher monomer conversion than unblended UDMA matrix (Antonucci et al., 2006).
The soft mouldable resin paste requires an activator/initiator system to react and to turn into a hard restorative material. Visible photoinitiators such as camphorquinone/amine systems are the most commonly used in dental resin composites (Xu et al., 2017).

Monomers convert to polymers through a polymerisation reaction that follows a radical chain-growth sequence. The polymerisation reaction takes place in three stages (Fugolin and Pfeifer, 2017, Sakaguchi and Powers, 2012):

**MW**: Molecular weight; **η**: Viscosity

Figure 1.4 Commonly used dental monomers. "MW" and "η" indicate molecular weight and viscosity, respectively. The figure was adapted from Wang et al., 2020.
1. **Initiation**: Most dental polymers require light activation to initiate the reaction. The energy from the light decomposes the photoinitiator, which forms an active free radical species. These free radicals will interact with the monomers, by breaking the vinyl double bonds and converting them into single bonds.

2. **Propagation**: In this stage, rapid addition of other monomer molecules to the active center occurs, which co-polymerise via the carbon-carbon single bonds to provide the growing polymer chain.

3. **Termination**: At a certain point the polymer chain interrupts its growth, and the reaction terminates, with the radical centers being destroyed by a combination or a disproportionation reaction.

Effective polymerisation reaction forms a highly cross-linked polymer network that is able to achieve the desired functional properties (Fugolin and Pfeifer, 2017). During the polymerisation reaction, a double carbon bond reaction occurs. Monomers bind to other monomers forming a single carbon bond, first in a linear manner and later by cross-linking in between monomers. When the polymerisation reaction commences, the monomeric matrix is in the pre-gel stage, where monomers are not yet restricted in movements and can occupy positions in the matrix. As the polymerisation reaction advances, the material sets, and hardens as larger molecules are formed, reaching a point where movement is restricted—gel point (Milosevic, 2016).
Polymerisation shrinkage is one of the most common shortcomings of resin-composites. It is caused by the conversion of monomers into long, cross-linked polymers. Before monomer conversion the molecules are held together by van der Waals forces. After polymerisation, the van der Waals forces are replaced with short covalent bonds that result in substantial volumetric shrinkage of the composite (Münchow et al., 2018). The polymerisation shrinkage of flowable and non-flowable composites can range from 4.17 vol.% to 5.63 vol.% and 2 vol.% to 3.42 vol.%, respectively (Kleverlaan and Feilzer, 2005). The polymerisation shrinkage is directly proportional to the degree of conversion and the number of converted methacrylate groups in the monomer system (Braga and Ferracane, 2002). Shrinkage can also be determined by the bonding of composites to the tooth structure and by the free surfaces (Versluis et al., 1998). The configuration factor (C-factor) was introduced by Feilzer et al., 1987, which is defined as the ratio of the bonded to unbounded surfaces of the composite restoration (Feilzer et al., 1987). As the number of unbounded surfaces increases, there will be less stress generation. This is due to the fact that increased surface area will help to relieve the generated stresses. If the C-factor is high, then the shrinkage stress is high. These shrinkage stresses are not uniformly distributed at the tooth/composite interface (Braga et al., 2006). The consequences of shrinkage stresses are the formation of marginal gaps, leakage, cuspal deflection, and post operative sensitivity (Münchow et al., 2018). Therefore, polymerisation shrinkage can be reduced by incremental filling techniques to reduce shrinkage stresses and cuspal deflection (Park et al., 2008).
1.3.2.1.2 Filler particles
The incorporation of chemically inert filler particles into the resin matrix significantly reinforces the resin-matrix of composite materials. Increasing filler levels significantly improves several important properties such as dimensional stability, modulus of elasticity, strength, and hardness and wear resistance (Kundie et al., 2018). It also decreases thermal expansion, polymerisation shrinkage and water sorption of resin-composite (Chen, 2010). Crystalline quartz, and colloidal silica, non-crystalline silica glass with the incorporation of barium, strontium and zirconium are examples of fillers used in resin-composite (Anusavice, 2003).

Traditionally, restorative resins have been classified by filler size, type, loading and distribution. The classification of resin-composite according to filler size includes macrofilled composites (10 to 50 μm) that were strong but difficult to polish and retain surface smoothness and aesthetics, microfilled (40 to 50 nm) composites that were polishable but generally had lower flexural strength (50.5 MPa) and hybrid composites (10 to 50 μm and 40 nm) (Ferracane, 2011, Beun et al., 2007). The hybrid composite was further modified as midifill with average particle sizes slightly greater than 1 μm in addition to a portion of the 40 nm-sized fumed silica “microfillers” (Alzraikat et al., 2018). The modification in the filler particle size resulted in what is referred to as microhybrids (0.6 to 1 μm and 40 nm). Nanofilled (1 to 100 nm) and nanohybrid composites (a combination of microhybrid and nanofilled-size particles) were then introduced (Randolph et al., 2016). These materials are generally considered to be
universal composites as they can be used for most anterior and posterior applications based on their strength and polishability.

Another classification has emerged based on handling properties into packable and flowable composites. Conventional composites can be packed into cavities and proximal contacts. On the contrary, flowable composite has lower viscosity and can be made by decreasing the filler loading to 30-50 vol.%. Flowable composites can be used as a liner for packable composite restorations and as pits and fissure sealants. For flexural strength, conventional composites (117.4 MPa) are usually superior to flowables (102 MPa) (Attar et al., 2003).

1.3.2.1.3 Coupling agent
A silane coupling agent is used to promote adhesion between filler and resin matrix (Ferracane, 2011). Coupling agents are important as they form siloxane bonds to the filler and covalent bonds with the organic matrix to enhance mechanical properties of dental composites. They allow transfer of stresses from resin matrix to stiff filler particles. A commonly employed example is 3-methacryloyloxypropyl trimethoxysilane (MPTS) (Chen, 2010).

1.3.2.1.4 Optical modifiers
Resin-composites must have a visual appearance that mimics the corresponding optical properties of natural tooth structure. Optical modifiers are added to provide shading and varying degrees of opacity. Various pigments with minute amounts of metal oxide particles such as titanium
dioxide are used to achieve shading of resin-composites. The translucency and opacity can then be adjusted as needed to simulate enamel and dentine. Excessive translucency will result in too much light to pass through the restoration leading to less amount of light reflection to the observer. Therefore, the observer will perceive the restoration as too dark. This can be corrected by adding an opacifier such as titanium dioxide. However, adding too much opacifier might lead to excessive light reflection and the observer will perceive the restoration as too white (Anusavice, 2003).
Figure 1.5 Diagram showing the composition of resin-matrix composite
1.3.3 Adhesion to enamel and dentine

Tooth restoration traditionally relied on the surgical removal of sound tissue structure to provide mechanical retention in the prepared cavity. Direct adhesion to tissues was then introduced to prevent unnecessary removal of sound tissues. Buonocore (1955) found that acid etching could improve bonding to enamel. Fusayama, 1980 and Nakabayashi et al., 1991 have advocated the need of etching dentine to expose the dentinal collagen matrix to allow for adhesive infiltration to improve bonding to dentine (Fusayama, 1980, Fusayama, 1988, Nakabayashi et al., 1982, Nakabayashi et al., 1991). Due to the hydrophilic nature of the collagen matrix they suggested the use of monomers that have both hydrophilic and hydrophobic groups. The hydrophilic group helps to facilitate the permeation of monomers into collagen matrix to form a collagen resin hybridized layer, whereas the hydrophobic group facilitates bonding with the hydrophobic resin-matrix in the composite (Vaidyanathan and Vaidyanathan, 2009). The hybridization layer is the result of the infiltration of the primer into the exposed collagen matrix and the polymerisation of both the primer and adhesive to form an interdiffusion zone of both collagen and resin called the hybrid layer (Van Meerbeek et al., 1993).

1.3.4 Bonding to caries-affected dentine

Bonding to caries affected dentine is one of the essential approaches to preserve the remaining tooth structure to meet the contemporary minimally invasive philosophy. Previous studies found that bond strength to caries
affected dentine was lower than bonding to sound dentine (Nicoloso et al., 2017, Kucukyilmaz et al., 2017). This could be related to the chemical and morphological changes in caries-affected layer such as the decrease in the mineral content, alteration of the collagen structure and higher amount of water content (Nakajima et al., 2011, Isolan et al., 2018). Several studies have been conducted to improve adhesion to caries-affected dentine but a reduced performance in this substrate is still evident (Hass et al., 2019, Taniguchi et al., 2009, Grech et al., 2013, Isolan et al., 2018).

1.3.5 Recent advances in resin-matrix composite

1.3.5.1 Bulk fill resin-composites

Bulk fill resin-composite have been introduced to simplify the application method of the restorative material by avoiding the incremental layering technique (Van Ende et al., 2017, Chesterman et al., 2017). They are available as flowable and high viscosity bulk fill restoratives that can fill the cavity up to 5 mm (Ilie et al., 2013). With these it is necessary to control polymerisation shrinkage and depth of cure when larger increments are to be placed in the cavity. Manufacturers have attempted to increase the depth of cure by improving the material’s translucency, refractive index and the use of alternative photoinitiators. Reducing the filler content and increasing filler particle size decreases the amount of light scattering at the resin-filler interface (Ilie et al., 2013, Son et al., 2017, Miletic et al., 2017). The use of highly reactive photoinitiators such as Ivocerin in Tetric EvoCeram Bulk-Fill (Ivoclar Vivadent) allows larger increments to be polymerised when compared
to camphorquinone or lucrin (Vivadent). Additionally, light-curing methods should also be considered to improve the depth of cure. This includes the curing time, the distance between the light curing unit tip and the composite, and the amount of radiant exposure received by the composite (Bucuta and Ilie, 2014, Alrahlah et al., 2014). Previous studies identified that some of the available bulk-fill materials had significantly lower surface hardness as the depth increased from the surface with a bottom/top hardness ratio decreasing below 0.70 (Garcia et al., 2014, Issa et al., 2016). As composite polymerises, the material flows from the unbonded free margin to compensate for shrinkage. As polymerisation continues, the composite stiffens, and flow decreases. At the gel point, the composite is too rigid to flow, and the forces continuing to be generated by polymerisation shrinkage are transferred to the cavity wall, creating stress at the marginal interfaces. Only a portion of the total polymerisation shrinkage is translated into stress; the remainder is reduced by the composite flow. SureFil SDR is designed to reduce shrinkage stress by increasing flow with chemistry that slows the rate of polymerisation to reduce shrinkage stress (Burgess and Cakir, 2010). Although, flowable bulk fill composite has lower filler content, it is flexural strength (131.8 MPa) is comparable to conventional composites (131.2 MPa) (Ilie et al., 2013). High viscosity bulk fill composite provides superior handling properties than conventional resin composite but it still requires preceding application of a separate adhesive resin system as do all (Chesterman et al., 2017, Cieplik et al., 2021).
1.3.5.2 Self-adhesive resin-composites

Self-adhesive resin-composite has been developed to serve as a simple and easy approach to eliminate the need of using separate etching and adhesive system (Van Meerbeek and Frankenberger, 2019, Meerbeek et al., 2020). The material is based on the incorporation of acidic adhesive monomers such as glycerol phosphate dimethacrylate (GPDM) 2-methacryloyloxyethyl phenyl phosphate (Phenyl-P), 4-methacryloyloxyethy trimellitate (4-META) and 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP). These monomers are able to etch, infiltrate and bond to the substrate without the need of using the current acid etching and bonding steps (Yao et al., 2020). Functional monomers such as 4-META and 10-MDP are able to interact with the hydroxyapatite by creating ionic bonds between carboxylic or phosphate groups and calcium (Yoshioka et al., 2002). In addition to the chemical bonding, they provide shallower demineralisation and expose less collagen fibers, thus, creating a stable dentine interface that might prevent degradation of the collagen (Van Meerbeek and Yoshihara, 2014, Meerbeek et al., 2020).

Glass ionomer cements can be considered as self-adhesive materials, as they have the ability of forming ionic bond between calcium and the polyacid molecules in the material (Sidhu and Nicholson, 2016).

Self-adhesive composites have been investigated in several in vitro studies and the findings showed inferior shear bond strength and high interfacial defects compared to one-step self-etch or two-steps etch and rinse adhesives combined with flowable composites (Park et al., 2015, Mine et al., 2017, Brueckner et al., 2017, Peterson et al., 2018). Clinical trials have also
reflected their poor performance in comparison to conventional composites, limiting their clinical application (Çelik et al., 2015).

The current commercial self-adhesive composite materials as claimed by the manufacturer are Vertise Flow (Kerr, Orange, CA, USA) which contains the acidic monomer GPDM, Activa Bioactive (Pulpdent, Watertown, MA USA) that has polyacrylic acid is marketed as bioactive, Constic (DMG, Hanau, Germany) which contains 10-MDP and Surefil One (Dentsply, York, PA, USA). Due to the shortcomings of these restorative materials, they are indicated for small cavities and as a lining material. More research is still needed to improve these materials, as they are desired by clinicians.

1.3.5.3 Remineralising fillers
Remineralisation can be defined as the process of promoting ion deposition into the crystal voids of demineralised enamel and dentine by utilizing calcium (Ca) and phosphate (PO₄) ions from external sources to tooth structure (Cochrane et al., 2010). Calcium and phosphate ions provide the major components of hydroxyapatite [Ca₁₀(PO₄)₆(OH)₂] crystals and have a key role in influencing the tooth demineralisation and remineralisation processes (Li et al., 2014). The incorporation of calcium phosphate (CaP) fillers in dental resins could have the ability to release calcium/phosphate and substitute the mineral loss from enamel and dentine (Skrtic et al., 1996b, Dickens et al., 2003a). This may repair gaps and cracks in the affected areas of the hard tissue structures (Langhorst et al., 2009). In addition, at lower pH, Ca and PO₄ ions could be released in supersaturating levels, thus, providing a caries
inhibiting effect (Xu et al., 2009, Alkhouri, 2019). The ion release could be influenced by the size and surface treatment of CaP filler particles. Small sized filler particles (0.112 μm) have a higher surface area and faster release rate, while salinization treatment may increase the hydrophobicity of the particle surfaces resulting in lower water permeability and ion release (Xu et al., 2007b). The biaxial flexural strength of experimental resin composites was decreased upon the addition CaP fillers (Panpisut et al., 2020, Alania et al., 2016). This could be due to the absence of strong chemical and micromechanical interactions between the resin matrix and the CaP particles, which does not allow for an effective stress distribution between both phases (Braga, 2019). Another considerable concern is the incorporation of CaP fillers may increase water sorption leading to the degradation of the resin matrix. Therefore, it is crucial to optimise the total amount of CaP fillers (10 vol.% dicalcium phosphate dihydrate) in resin matrix to provide sufficient ion release without deterioration of the mechanical properties of the restorative material (Chiari et al., 2015, Vilela et al., 2020).

Several remineralising CaP fillers have been investigated including hydroxyapatite (HAp) \([\text{Ca}_{10} (\text{PO}_4)_{6} (\text{OH})_2]\), amorphous calcium phosphate (ACP, \(\text{Ca}_x\text{H}_y(\text{PO}_4)_{2n}\text{H}_2\text{O}\)) (Skrtic et al., 1996b, Skrtic et al., 1996a, Antonucci et al., 1996), tetracalcium phosphate (Ca\(_4\)(PO\(_4\))\(_2\)) (Xu et al., 2009), \(\beta\)-tricalciumphosphate (\(\beta\)-Ca\(_3\)(PO\(_4\))\(_2\)) (Karlinsey et al., 2010, Viana et al., 2020), dicalcium phosphate anhydrous (CaHPO\(_4\)) (Xu et al., 2006, Xu et al., 2007a, Xu et al., 2007b), dicalcium phosphate dihydrate (CaHPO\(_4\).2H\(_2\)O) (Chiari et al., 2015, Alania et al., 2016, Natale et al., 2018), and monocalcium
phosphate (MCPM) (Abou Neel et al., 2016).

Bioactive glasses (BGs) are another type of remineralising filler used in composites that are widely investigated to restore both hard and soft tissues (Miguez-Pacheco et al., 2015, Jang et al., 2018, Tezvergil-Mutluay et al., 2017). Unlike CaP fillers, bioactive glasses do not leave voids or pores when reacting with physiological fluids, which could improve the mechanical properties when used as orthodontic adhesives (Al-Eesa et al., 2019). Bioactive glasses are amorphous solids produced from a SiO$_2$–P$_2$O$_5$–CaO–Na$_2$O system that in contact with physiological fluids form an apatite-like layer on their surface (Tiskaya et al., 2021, Hench and Paschall, 1973).

1.3.5.4 Antibacterial agents

Although, numerous advancements have been made to improve caries prevention, caries prevalence remains high worldwide particularly among low socioeconomic populations (Pitts et al., 2017). Nowadays, tooth coloured resin-composites are the first choice for restoring carious lesions due to their excellent aesthetics and performance (Welch et al., 2010, Ferracane, 1995, Drummond, 2008). However, failure of composites is mostly attributed to the formation of caries lesions around the restoration (secondary caries), wear, fracture, water sorption, and degradation of the organic resin matrix, resulting in subsequent need for restoration replacement (Sunnegardh-Gronberg et al., 2009, Rasines Alcaraz et al., 2014, Opdam et al., 2007, Bernardo et al., 2007, Bourbia et al., 2013, Opdam et al., 2014, Demarco et al., 2017). Bacterial colonization and the formation of cariogenic biofilms on plastic resin
restoration is the major role for secondary caries (Zhang et al., 2016b, Bourbia and Finer, 2018). Therefore, the inhibition of biofilm formation and reduction of secondary caries should improve the longevity of the restorative material (Zhang et al., 2014). This could be achieved by the incorporation of bactericidal agents into resin composites to help suppression of residual infection and hence improve their longevity (Hojati et al., 2013, Ai et al., 2017).

1.3.5.4.1 Silver (Ag)

Silver (Ag) based nanoparticles are known to have antibacterial, antiviral and antifungal capabilities (Monteiro et al., 2009, Allaker, 2010). The antibacterial mechanism of Ag⁺ is thought to have three different ways to kill the bacteria; through damaging the cell wall, inhibiting DNA replication or denaturing cytoplasmic enzymes (Peng et al., 2012). The antibacterial property of Ag is mainly dependent on the size and surface area of Ag particles (Melo et al., 2013). Resin composite containing Ag nanoparticles with high surface area was found to leach more Ag⁺ after polymerisation (Cheng et al., 2011, Durner et al., 2011). However, excessive Ag may accumulate in skin, liver and kidneys raising biosafety concerns (Peng et al., 2012).

1.3.5.4.2 Quaternary ammonium compounds

The use of polymers with quaternary ammonium groups has been shown to have antibacterial effect and reduce microbial growth (Antonucci et al., 2012, Kourai et al., 2006). The antibacterial mechanism with these materials is thought to be contact killing by molecules penetrating the bacterial cell
membrane resulting in leakage of the cytoplasmic material, autolysis, and cell death of bacteria (Ahlström et al., 1999, Kawabata and Nishiguchi, 1988, Kenawy et al., 2002). An example of a quaternary ammonium monomer is 12-methacryloyloxydodecylpyridinium bromide (12-MDPB). The polymerisation of 12-MDPB with other monomers can form a polymer matrix with antimicrobial effect that has the ability to decrease the colonization of *Streptococcus mutans* and plaque accumulation (Thomé et al., 2009). The incorporation of nanoparticles into resin composite was found to have an effective antibacterial strategy compared to normal materials (Pietrokovski et al., 2016).

1.3.5.4.3 Zinc oxide (ZnO)

Zinc oxide (ZnO) powders have been used as opaque reinforcing fillers in resin composites (Bowen and Cleek, 1972). They are considered as broad-spectrum antibacterial agents that have the ability to kill several oral microbes known to contribute to caries (Fang et al., 2006, Kadiyala et al., 2018, Liu et al., 2014, Suresh et al., 2013). ZnO nanoparticles have been found to be more effective than larger particles on gram-negative and gram-positive bacteria (Adams et al., 2006, Jones et al., 2008). It has been found that resin composite containing ZnO nanoparticles has higher antibacterial effect on *Streptococcus mutans* than that of Ag (Kasraei et al., 2014). The antimicrobial mechanism of ZnO nanoparticles is by leaching Zn$^{2+}$ into the bacterial biofilm. Zinc ions have the ability to inhibit the transport and metabolism of sugar and disrupting enzyme systems of dental biofilm (Devulapalle and Mooser, 1994, Paunio, 1970, Aydin Sevinç and Hanley, 2010).
1.3.5.4.4 Chlorhexidine

Chlorhexidine (CHX) is an organic broad-spectrum antibacterial agent that is considered as positive control for evaluating antimicrobial materials (Genovesi et al., 2017, Supranoto et al., 2015). It has been commonly used, as an antiplaque agent due to its low toxicity to soft tissues (Wu et al., 2013). Its mechanism of action is by the attraction of the cationic CHX molecule to the negatively charged bacterial cell surface, with strong adsorption to phosphate-containing compounds. This alters the integrity of the bacterial cell membrane, and CHX is then attracted toward the inner cell membrane. In addition, CHX can bind to phospholipids in the inner membrane, increasing their permeability and resulting in the leakage of low molecular weight components, such as potassium ions. Furthermore, the positively charged dicationic molecule has the ability to bind to negatively charged surfaces including teeth and mucosa, which could act as reservoirs of CHX (Jones, 1997). CHX is effective against both Gram-positive and Gram-negative bacteria including aerobes and anaerobes (Davies et al., 1954, Hennessey, 1973, Emilson, 1977). It has also been shown that CHX can reduce the adherence of *Porphyromonas gingivalis* to epithelial cells (Grenier, 1996). CHX cannot be immobilised in a polymer matrix because it lacks a methacrylate double bond (Mittal et al., 2015). Two strategies have been used to incorporate CHX into resin composite including encapsulation or nanoparticulation. The nanoparticulation is by the synthesis of chlorhexidine-hexametaphosphate nanoparticles, which act as a slow-release device for soluble CHX (Barbour et al., 2013, Hook et al., 2014). Encapsulation is achieved by loading spherical particles of chlorhexidine
diacetate with CaCl$_2$ into carriers, which can provide sustained drug release (Luo et al., 2016).

1.3.5.4.5 PolyLysine

Polylysine is a polyamino acid that has been widely used in biomedical research and applications. The polyamino acid can be divided into α-polylysine and ε-polylysine. The α-polylysine is highly toxic and can be produced by artificial chemical synthesis (Shi et al., 2015). ε-Polylysine is a homopolymer of L-lysine, containing approximately 30 L-lysine subunits and can be synthesized from the bacterial fermentation by *Streptomyces albulus*. In addition, it has a broad-spectrum antibacterial effect and it is safe to be used as food preservative (Hiraki et al., 2003). The antibacterial mechanism of Polylysine is by binding to negatively charged phospholipid head groups in the lipid bilayer of bacterial membranes and destabilize them, thus increasing permeability. In addition, polylysine can destroy the lipopolysaccharide component of the outer membrane (Hyldgaard et al., 2014).

1.4 Statement of problem

Composite restorations, using materials with a resin matrix, have become predominant for many clinicians with the growing demand for aesthetics and tooth conservation techniques (Eltaahlah et al., 2018, Vetromilla et al., 2020). Treatment with composites demands moisture control and isolation, which in turn requires extra time and patient cooperation (Bücher et al., 2017).
The median longevity of posterior resin-composite restorations has been reported as 10 years, (Laske et al., 2016) with secondary caries (59%) and bulk fracture (38%) the most common causes of failure (Demarco et al., 2017, Eltahlah et al., 2018, Worthington et al., 2021). Among the contributing factors that affect the success rate is the tendency of resin-composite to accumulate biofilms (Bourbia et al., 2013) and the formation of micro-gaps at the tooth-restoration interface resulting in microleakage and subsequent bacterial invasion and secondary caries (Breschi et al., 2008, Spencer et al., 2010, Khvostenko et al., 2015).

Resin-composites have significantly evolved over the past years (Xu and Burgess, 2003, Samuel et al., 2009, Milward et al., 2011, Hosoya et al., 2011, Wei et al., 2011, Ferracane and Giannobile, 2014, Stansbury and Idacavage, 2016). The development of self-adhering flowable resin-composites eliminated the cardinal steps of etching, rinsing, priming, and bonding, which makes them more useful by saving treatment chair time and minimizing handling errors. However, they have been proposed as a restorative material for only a small-sized cavities and cavity lining (Goracci et al., 2013). The significant progresses that have been made on the development of resin-composite in the past years are still inefficient at arresting carious lesions (Xu et al., 2017, Ferracane, 2017). Most of the current restorative resin materials are considered inert and are not bioactive, with remineralisation properties, but the drive to develop such materials exists and is ongoing (Braga, 2019).
1.5 Solution

1.5.1 Previous work that led to the materials in this study

Several previous studies have been conducted at EDI research labs to improve the properties of composite formulations with antibacterial (CHX versus PLS) and remineralising calcium phosphates (mono with or without tricalcium or tristrontium phosphate). Various different diluent (TEGDMA, PPGDMA and HEMA) and acidic monomers (10-MDP and 4META) have been included with the base monomer UDMA. These are summarised in chronological order below with explanations for the gradual changes in formulation.

1.5.1.1 Addition of CHX into methacrylate polymers

In 2005, Leung et al. evaluated the monomer conversion, rate of reaction, mass and volume change of experimental composite formulations containing UDMA, TEGDMA and different levels of CHX and HEMA. The initial rates of polymerisation were not significantly affected by CHX level but increased linearly upon reducing the percentage of HEMA in the hydrophobic monomers UDMA and TEGDMA. Formulations containing 10% of the HEMA replaced by CHX had higher final monomer conversion than other formulations. The results suggest that CHX may stabilise the free radicals, slowing the termination stage. Furthermore, the addition of TEGDMA and UDMA leads to crosslinked structures that may reduce molecular diffusion rates, slowing in particular the termination step and enhancing the overall reaction rate (Young
and Lovell, 2011). Furthermore, raising crosslinking UDMA and TEGDMA levels could control water sorption-induced expansion that allows for control over CHX release rate and may compensate for polymerisation shrinkage (Leung et al., 2005). However, CHX is readily released only if considerable water sorption occurred or drug loading was excessively high.

1.5.1.2 Addition of reactive fillers

In 2009 Mehdawi et al. investigated composite formulations containing the same monomers (HEMA, TEGDMA and UDMA) with or without CHX. The study involved the addition of MCPM and β-tricalciumphosphate to encourage water sorption and to enhance the release of CHX and calcium phosphate content. The photo-activated composite was prepared by combining HEMA, TEGDMA, and UDMA in a ratio of 2:1:1 with 1 wt.% camphorquinone and 1 wt.% N, N-dimethyl-p-toluidine (DMPT). The filler phase contained equal masses of MCPM and β-tricalciumphosphate. The filler and monomers were mixed at a high or low powder/liquid ratio of 3:1 or 1:1, respectively. Chlorhexidine diacetate was also incorporated at a level of 0 or 5 wt.% of the liquid phase. Raman microscopy was used to quantify the level of monomer conversion. The resin composite samples showed 90% monomer conversion upon light exposure irrespective of the formulation. The findings also showed that increasing the PLR and CaP content promoted water sorption, encouraged expansion and enhanced calcium, phosphate and CHX release. Furthermore, addition of β-tricalciumphosphate encouraged brushite formation within the polymer. The results also revealed that the compressive
and biaxial flexural strength decreased significantly after 24hr of immersion of deionised water. The decrease in the mechanical properties was presumably related to the high solubility of MCPM that encouraged high water sorption which could plasticize the polymer matrix (Mehdawi et al., 2009).

1.5.1.3 Addition of non-reactive fillers

In 2013 Mehdawi et al. found that partial replacement of CaP with nanosilica–silicon carbide particles and a decrease in HEMA content significantly improved the compressive and biaxial flexural strength and provided better control of volumetric expansion of the composite. Due to the lack of refractive index matching, the formulations used in this study had to be cured by mixing chemical initiators with activator instead of light exposure (Mehdawi et al., 2013a).

Neither of these studies considered cure variation with depth and long-term strength reduction or modulus.

In 2015 Aljabo et al. studied the conversion versus depth, volumetric changes and mechanical properties of composite formulations containing the same monomers (UDMA, TEGDMA and HEMA) with varying levels of CHX and reactive fillers (MCPM and β-tricalciumphosphate). In addition, boro silicate glass fibers and barium boro alumina silicate glass fillers with close refractive index match to the monomer were added to the formulations. The authors found that the monomer conversion decreased with increasing CaP content and sample depth. Upon storage in deionised water, high CaP level resulted
in high water sorption induced mass and volume change and reduction in biaxial flexural strength and modulus. Water sorption can expand the polymerised resin matrix, or through enhancing lower density brushite formation, which increases the volume of the inorganic phase. The hydrophilic nature of the CaP fillers resulted in higher water sorption that may lead to the degradation of the organic resin matrix. Additionally, the absence of strong chemical and micromechanical interactions between the resin matrix and the CaP particles may also contribute to lower biaxial flexural strength and modulus (Aljabo et al., 2015). In addition it was found that hydroxyapatite precipitation mass was proportional to the CaP content (Aljabo et al., 2016).

1.5.1.4 PLS instead of CHX

Previous findings showed that the use of CHX could be toxic to odontoblast-like cells (Lessa et al., 2010). In addition, two incidents have been reported that CHX mouthwash has the potential of causing anaphylactic shock when used to wash tooth sockets following recent tooth extraction (Pemberton, 2016). PLS can serve as an alternative antimicrobial agent due to it is low toxicity to human cells and it is wide antimicrobial spectrum (Pemberton, 2016).

In 2016 Panpisut et al. evaluated composite formulations containing (UDMA, TEGDMA, DMPT, 4-META) and varying levels of MCPM, PLS and tristrontium phosphate (TSrP). The hygroscopic expansion, apatite formation and PLS release were found to be encouraged by the addition of these fillers. However,
the monomer conversion and mechanical properties of the composite formulation was decreased (Panpisut et al., 2016).

1.5.1.5 Change in monomer system

The use of the low molecular weight triethylene glycol dimethacrylate (TEGDMA) diluent monomer in previously described composite may result in high polymerisation shrinkage (Atai et al., 2005). Therefore, it would be beneficial to replace TEGDMA with alternative monomer system to improve shrinkage and conversion.

In 2016 Walters et al. evaluated the monomer conversion, strength and the cytocompatibility of resin composite formulations upon fully replacing Bis-GMA with UDMA and TEGDMA with PPGDMA. They found that composite containing UDMA has improved monomer conversion, cytocompatibility, depth of cure and biaxial flexural strength than those containing Bis-GMA. In addition, PPGDMA showed higher monomer conversion than TEGDMA and no detrimental increase in shrinkage when used with UDMA (Walters et al., 2016).

Another concern with previously described composites is the use of tertiary amine activator (DMPT) that was found to have both cyto- and geno-toxicity effects (Nomura et al., 2006, Chen et al., 2003). Walters et al. have showed that the replacement of TEGDMA with higher molecular weight PPGDMA can improve monomer conversion and may reduce the need for any amine activator (Walters et al., 2016).

Kangwankai et al. developed composite resin containing (UDMA, PPGDMA,
4-META) and different levels of MCPM, $\beta$-tricalciumphosphate, PLS and aluminosilicate glass. The monomer conversion, dimensional stability, flexural strength, modulus, surface roughness and apatite precipitation were investigated. The PPGDMA containing composites had higher monomer conversion than previous experimental composites (Aljabo et al., 2015, Panpisut et al., 2016). The increase of CaP and PLS led to higher water sorption, reduced strength, and increased mass and volume loss upon wear. In addition, active fillers enhanced hygroscopic expansion and surface apatite formation (Kangwankai et al., 2017).

Another study conducted in 2019 by Panpisut et al. compared TEGDMA/UDMA versus PPGDMA/UDMA-based bone composites with added (MCPM and TSrP) and (PLS). It was found that replacing TEGDMA with PPGDMA in the experimental composite increased the inhibition time, final monomer conversion and strontium release. PPGDMA also enhanced hygroscopic expansion and decreased polymerisation shrinkage with no detrimental effect on the mechanical properties of the composite (Panpisut et al., 2019).

1.5.1.6 Removal of TCP

$\beta$-tricalciumphosphate may not dissolve in a predictable fashion due to it is low solubility below pH 6.2 (Toya et al., 2001, Sakai et al., 2016). Therefore, $\beta$-tricalciumphosphate was removed from composite to avoid excessive water sorption and to improve it is mechanical properties.
Several recent studies have evaluated the properties of resin composite formulations containing UDMA, PPGDMA, 4-META and varying levels of MCPM and PLS with different powder/liquid ratios.

In 2018 Tzelepi found that formulations containing 10% MCPM and 2% PLS showed higher bond strength to dentine than those containing 5% MCPM and 5% PLS when no etching and bonding where used. Resin tag formation was also observed on SEM images for formulations with 8% MCPM and 4% PLS. This formulation (8% MCPM and 4% PLS) exhibited higher monomer conversion compared to commercial composite for samples with 2 and 4mm depth (Tzelepi, 2018).

1.5.1.7 Importance of antibacterial versus sealing action

Alkhouri evaluated resin composite formulations containing PLS (5% or 2%wt) and MCPM (8% or 4%wt) and found that all formulations were stable upon high temperature aging at 60°C for 6 months. The water sorption and solubility increased significantly with formulations containing high levels of MCPM and PLS. The author also observed resin tag formation in a caries like model that was created during the study in order to be used as a substrate to test the ability of the experimental formulations to form resin tags. Based on these results, formulations with 8% MCPM and 4% PLS were chosen as the final optimised formulation. This formulation showed better self-etching to enamel and adaptation to cavity walls than commercial comparators. Additionally, it formed resin tags in carious dentine with precipitation of minerals at the adhesion interface, which might be able to seal and stabilize carious dentine.
(Alkhouri, 2019). This could be related to the interaction of PLS with non-collagenous proteins that are highly concentrated around the dentinal tubules. This interaction might have encouraged the composite paste to be pulled into the dentinal tubules.

1.5.1.8 Acid etching by MPCM

MCPM can disproportionate in water to form dicalcium phosphate and phosphoric acid. The phosphoric acid may provide self-etch of hydroxyapatite in tooth structures forming more DCP (Xia et al., 2014).

Makrygiannaki investigated composite formulations containing 8% MPCM and 4% PLS with small versus large MPCM filler sizes. Makrygiannaki has evaluated the effect of MPCM filler particle size on mass, volume and acid release after immersion in deionised water. In addition, the surface roughness and subsequent volume loss after two-body wear test was also evaluated. The findings showed that formulations with small particle size did not have significant effect on mass and volume change. However, the acid release was lower than with formulations containing large particle size. Moreover, the experimental formulations showed lower surface roughness but high volume loss than commercial comparison (Makrygiannaki, 2018).

In 2019 Abdel-Hadi assessed the mass loss, pH and biaxial flexural strength of composite formulations with PLS (8% or 4%wt) and MPCM (16% or 8%wt) following immersion in deionised water for 1 week, 1 month and 1 year. All formulations provided the required remineralising and neutralising effect after 12 months. The pH was higher after 1 year compared with 1 month but similar
compared with 1 week and 1 year. Abdel-Hadi has concluded that although strength was similar after 12 months for all formulations, the higher mass loss in the high MCPM/PLS formulation could negatively affect the longevity of the restoration (Abdel-Hadi, 2019).

1.5.1.9 Colour stability of composites packaged in compules
Pitsillou investigated the colour stability of compule-packaged composite paste with 8% MCPM and 4% PLS at different storage time and temperature. The elevated temperatures significantly affected the colour stability and resulted in early discolouration of the composite samples. However, composite compules stored at 37°C were more colour stable than those stored at high temperature. Pitsillou also found that the colour stability of the experimental composite was lower than FUJI II LC, which could result in poorer aesthetics (Pitsillou, 2019).

1.5.1.10 PLS release and antibacterial action
Lygidakis et al. investigated the effect of incorporating (PLS) at different mass fractions (0.5, 1 and 2 wt%) on PLS release and Streptococcus mutans planktonic growth. The rate of PLS release was high in the first 24hr with 2 wt% in the filler. The final percentage release was not affected by increasing composite PLS level. In addition, the amount released into 1 mL of broth in 24hr was sufficient to reduce bacterial levels when the initial inoculum level was at $8 \times 10^5$ but their growth in number was only inhibited when the initial bacterial counts were raised 10-fold. With an initial inoculum of $5 \times 10^6$
bacteria, those detected on the composite surfaces after 72hr were mostly live with 1% or less PLS in the composite but dead with 2% PLS (Lygidakis et al., 2020).

Yaghmoor et al. evaluated the ability of increasing (PLS) and (MCPM) to prevent residual bacteria in cavities depositing and forming biofilms early on the composite surface. This was achieved by the incubation of composites with S. mutans and sucrose. The findings showed that increasing PLS resulted in linear decline in surface biofilm mass and thickness. In addition, rapid increase in dead bacteria within the biofilm was observed with PLS levels above 4%. The early PLS release was proportional to the square root of time. Release leveled below 100%, suggesting it was from surface layers, which increased in thickness with raising PLS level (Yaghmoor et al., 2020).

1.5.1.11 Replacement of 4-META with 10-MDP

In 2021 Delgado examined experimental composite containing PLS (8% or 4%wt) and MCPM (16% or 8%wt), four commercial bonding agents and three commercial self-adhesive restorative materials. The outcomes showed that bonding agents outperform self-adhesive composites in dentine interdiffusion due to their viscosity and chemical composition. Additionally, composite formulations were able to penetrate etched dentine and form long resin tags and good conversion levels. Delgado also created an optimised formulation by the replacement of 4-META with 10-MDP. The formulation showed comparable conversion and good mechanical properties to formulations containing 4-META. Delgado also suggested this could be a viable alternative
to enhance material properties and bonding (Delgado, 2021).

No previous studies have investigated the stability of experimental composite formulations containing high levels of MCPM (0, 6, 8, 12 or 16 wt%) and PLS (0, 3, 4, 6 or 8 wt%).

The aim of this study was to provide understanding of factors controlling new composite stability, component release kinetics, chemistries and their ability to resist abrasive wear.

The new experimental resin-composite formulations used in this thesis contains PolyLysine (PLS) and Monocalcium phosphate monohydrate (MCPM). The two novel agents were added to conventional composite fillers with different concentrations of PLS (0, 3, 4, 6 or 8 wt%) and MCPM (0, 6, 8, 12 or 16 wt%). The acidic adhesive monomer 4-methacryloyloxyethyl trimellitate (4-META) was mixed with PLS and MCPM and dissolved in a low shrinkage liquid dimethacrylate phase. Urethane dimethacrylate (UDMA) matrix monomer and a novel low shrinkage Polypropylene glycol dimethacrylate (PPGDMA) diluent monomer were incorporated with Camphorquinone (CQ) used as photoinitiator. More details on the formulations are described in the next chapter. PLS release into carious dentine may eradicate any residual bacteria remained in the cavity upon placement of the restorative resin restoration. In addition, the incorporation of MCPM could have the ability to release calcium and phosphate ions into the cavity and substitute the mineral loss resulting in hydroxyapatite formation within the
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tooth structure (Aljabo et al., 2016, Zhang et al., 2016a, Xie et al., 2017). This may seal and repair gaps in the affected areas resulting in better adhesion of the resin restoration to cavity walls, thus, preventing secondary caries. The functional adhesive monomer 4-META may have the ability to etch, infiltrate and provide effective bonding to the affected dentine. 4-META may react with water to form 4-MET with two carboxylic acid groups. These acid groups may interact with positive calcium ions in MCPP or the nitrogen groups in PLS. This may possibly provide an alternative to the silane-coupling agent, which is important for composite strength. The incorporation of the high molecular weight monomer PPGDMA may improve the polymerisation shrinkage of the experimental composite formulations.

1.6 Techniques used in this project

1.6.1 Attenuated total reflectance-fourier transform infrared spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) was used to evaluate polymerisation kinetics, paste shelf life, and dried extracts of set materials. Atoms in molecules are not static and display vibrations in well-defined patterns, called vibrational modes. FTIR uses infrared (IR) light, to determine energy differences between vibrational states of molecules in solid, liquid and gaseous phases. It is able to interact with matter, causing a net change in dipole moment during the vibration of the molecule. The concept of this technique is based on sample absorption to light at characteristic
wavenumbers, or frequencies in the mid-infrared region. These wavenumbers depend on the physicochemical properties of the corresponding molecule. The associated absorbance spectrum corresponds to the particular IR wavelengths absorbed by the sample, thus revealing details about its molecular structure (Rives, 2001, Pavia et al., 2014). An Attenuated-Total Reflectance (ATR) technique was used to obtain IR spectra. The technique involves the placement of the sample on a diamond crystal with a high refractive index. Then the IR beam is directed towards the crystal, and an internal reflectance occurs, where the wave extends beyond the crystal, into the sample. If the absorbance of energy happens in the IR region, the beam will be attenuated. The beam is again directed to the crystal and is picked up by a detector inside the equipment. This technique is non-invasive and sensitive.

1.6.2 Scanning electron microscopy and energy dispersive x-ray spectroscopy

Scanning Electron Microscopy (SEM) is a technique of electron microscope that is widely used in material science to study the surface microstructure and chemistry of materials. The concept of SEM is based on the emission of an electron beam that scans the surface of the material and the backscattered electrons are then picked up by a detector to produce high-resolution images. The image generated depends upon the energy of the beam and the nature of the sample (Kumar, 2013). In this study, SEM was used (Philips/FEI XL-30,
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Eindhoven, The Netherlands) to investigate the dried extracts of composite samples stored in deionised water. The beam energy was set at 5 kV and a current of approximately 200 pA. All of the samples were sputter-coated with gold/palladium for 90 seconds using a Polaron E5000 (Quorum, UK). Energy Dispersive X-ray Spectroscopy (EDX) was used to identify and quantify chemical elements in samples. EDX works through atom excitation by an electron beam, coupled with the SEM, which emits specific wavelength X-rays that are characteristic of the atomic structure of each element. The EDX detector is able to analyse the X-ray emissions and assign the elements (Scimeca et al., 2018). EDX was carried out with beam energy of 25 kV using INCA software X-sight 6650 detector (Oxford Instrument, UK).

1.6.3 Dual axis chewing simulator and surface profile measurement

Ideally, clinical trials are the best methods to test the wear characteristics of materials. However, in vivo studies are expensive, time-consuming and present confounding variables that could complicate the interpretation of the results (Hickel et al., 2007, Heintze, 2006). Dual axis-chewing simulators were developed to study the wear behaviour of dental restorative materials in-vitro and to overcome the difficulties in in-vivo methods. Chewing simulators have the ability to simulate the human chewing cycles in a laboratory using specific loads and frictional forces (Heintze et al., 2012). In the current study resin composite samples were subjected to a two-body wear test in a dual-axis chewing simulator (CS-4, SD Mechtronik GmbH, Feldkirchen-Westerham, Germany) with 120,000 loading cycles, against steatite balls (MUHLMEIER,
Baernau, Germany) with a diameter of 6mm. The masticatory cycle was achieved in a circular motion of 3mm in diameter and with a vertical force of 5kg.

The surface profile of the worn materials can be determined by using a profilometer (Fleming et al., 2016). Three-dimensional scanning of worn surfaces is the preferred method for measuring wear because it is quantitative and accurate. Scanners can be divided into contact and non-contact. Non-contact profilers are available in different types including point, line, area, and volume scanners. Contact profilers are able to evaluate irregular topology of occlusal surfaces by using spherical tipped styli. In contact profilers, resolution is limited by the size of the stylus tip with diameters of 0.1 mm or larger. In addition, they are accurate at relatively low cost and are not affected by the colour or transparency of the material. However, contact profilers are slow and require rigid surfaces. Both contact and non-contact point scanners are similar in the way they digitize surfaces. A non-contact laser scanner uses a light source or microscope that requires an opaque, diffuse and reflecting surface. Their resolution depends on the light source, which is typically less than 0.025 mm. Non-contact line profilers are using a straight line projected on the surface with a digital camera that captures images of the line as it moves across the surface. The known geometry of the system and triangulation enable calculating the surface points. Unlike line scanners, area scanners project a pattern over the surface and use triangulation, interferometry, phase shifting, or combinations of these to calculate surface points. Both line and area scanners have lower resolution than point scanners. Volume scanners
are CT-based, and their resolution depends on the voxel size, and ranges (DeLong, 2006). In this study, composite surface wear was analysed using a three-dimensional contact scanner (Renishaw Dental Scanner, Renishaw, Wotton-under-Edge, UK) with a 1mm diameter stylus. The three-dimensional image was then imported into the three-dimensional inspection software (Geomagic Control X v2014.0.0, 3D Systems Inc., Rock Hill, SC, USA) to determine the surface volume loss.

1.6.4 Colour measurement

Aesthetic dentistry relies on a proper colour match between the tooth structure and restorative materials (Trifkovic et al., 2018). Reliable methods for colour measurement are required for laboratory and clinical studies. Methods based on the colour match using shade guide such as Vita Classical and Vita-Zahnfabrik have been used in some clinical trials (Correa et al., 2016, Fernandes et al., 2017, Peixoto et al., 2019). The reliability of these shade guides depends on the experience, ability, and the lighting conditions (Gokce et al., 2010, Imbery et al., 2018, Corcodel et al., 2018). The use of spectrophotometers allows colour to be determined numerically and reduces subjective colour measurements. In addition, spectrophotometers can evaluate the direction of some colour differences (increased yellowness) (Igiel et al., 2017, Sullivan et al., 2019). The International Commission on Illumination (CIE) L*, a*, b* system is the most frequently used in studies of colour measurements in dentistry (Chu et al., 2010, Joiner and Luo, 2017). This system is based on the lightness (L*) and the chromaticity coordinates a*
(red-green axis) and b* (yellow-blue axis). The overall colour difference can be determined by calculating the change in all colour coordinates ($\Delta E$) (Gómez-Polo et al., 2016). In addition, the whiteness index (WI) can be used to determine the whiteness of the tooth and restorative materials (del Mar Pérez et al., 2016). The Lab coordinates can also be converted to other colour systems such as CIE Lch (lightness, chroma, and hue), CIE XYZ, and RGB (red-green-blue), for instance, allowing the yellowness index and $\Delta E$ to be calculated (Luo et al., 2001). The drawbacks of spectrometers are the high cost and lack of measuring specific areas of a specimen (Chu et al., 2010, Brook et al., 2007, Bhandari et al., 2019). Computer analysis of digital images was also reported to be a reliable method for tooth colour measurements (Bentley et al., 1999). In this method, the images produced via a digital camera are analysed using imaging software, enabling the collection of colour values from the images. This method is much cheaper than the use of spectrophotometers or colorimeters.
1.7 General aim

The general aim of this doctoral thesis was to evaluate the stability of novel resin composite formulations containing different levels of PLS (0, 3, 4, 6 or 8 wt%) & double these levels of MCPM to further optimise the formulations to be used for minimally invasive restoration of teeth and to overcome current material flaws.

1.7.1 Specific aims

1.7.1.1 Specific aim 1

The aim was to investigate the effect of MCPM and PLS on the polymerisation stability and paste shelf life of experimental composite formulations, and also evaluate the depth of cure of the experimental formulations and two commercially available restorative materials.

1.7.1.2 Objectives

The objectives were to evaluate the delay time, maximum rate of reaction, 50% reaction time ($t_{0.5}$), final degree of conversion (%DC) and the stability of experimental resin composite formulations with varying levels of PLS (0, 3, 4, 6 or 8 wt%) & MCPM (0, 6, 8, 12 or 16 wt%). Vertise Flow (Kerr) and ACTIVA kids (PULPDENT) were used as commercial comparisons. Resin composite pastes were stored at different temperatures and cured at different thicknesses and curing times.
1.7.1.3 Specific aim 2

The aim was to evaluate polymerisation shrinkage, subsequent water sorption-induced mass changes, expansion and hydrogen ion release from four experimental resin-composite formulations. The simulated wear resistance of five experimental resin-composite formulations and two commercial restorative materials was investigated.

1.7.1.4 Objectives

The project objectives were to evaluate the polymerisation shrinkage, mass and volume change of set materials in addition to their release of H⁺ ions and other components for four experimental resin-composite formulations containing PLS (8, 4wt%) & MCPM (16, 8wt%). Furthermore, simulated wear resistance of the four resin composite pastes in addition to a composite paste containing MCPM (8wt%) & PLS (4wt%) with a small MCPM particle size (10µm) was evaluated. ACTIVA kids (PULPDENT, Watertown, MA, USA) and Fuji II LC (GC corp., Tokyo, Japan) were used as commercial comparison.

1.7.1.5 Specific aim 3

The aim was to assess effects of storage container, time of high temperature paste aging, sample depth and curing on colour of four experimental resin-composite formulations.
1.7.1.6 Objectives

The project objectives were to assess effects on L* a* b* of storage container, sample depth and time of high temperature aging of four experimental resin-composite formulations containing PLS (8, 4wt%) & MCPM (16, 8wt%).
1.8 General null hypotheses

1. Varying the type of composite used (commercial versus experimental) has no significant effect on the polymerisation kinetics.

2. Varying the level of MCPP and PLS has no significant effect on the experimental composite polymerisation stability, shrinkage, mass or volume change following water-sorption, H⁺ release into water, components released versus time and wear resistance.

3. The storage container, high temperature storage, increasing components and sample thickness do will not have a significant effect on colour stability of experimental resin-composite pastes.
2. Materials and Methods

This chapter is a summary for the materials and methods used in this research project. The section comprises the following:

- Preparing solutions.
- Chemicals used in experimental formulations.
- Experimental composite paste preparation.
- Commercial comparators used.
- Sample preparation

2.1.1 Preparing solutions

2.1.1.1 Buoyancy medium preparation

The buoyancy medium was used along with the density kit (Mettler Toledo, AG204) for composite disc mass and volume change measurements to improve the wettability by reducing the surface tension of water. It was prepared in accordance with ISO 17304:2013 (BSI, 2013b) by topping up 5g of Sodium Lauryl sulphate with deionised water in a glass bottle until a final weight of 500ml. The bottle was then sealed and shaken until the solution was clear then stored at room temperature. The solution can be used for up to one month but was always prepared one day before using it.
2.1.2 Chemicals used in experimental formulations

The chemical agents used in the experimental composites are provided in Table 2.1. The FTIR spectra and peak assignments of the chemical agents are provided in Appendix 1: Table 7.1, Table 7.2 and Table 7.3.

Table 2.1 Chemicals used to prepare the experimental formulations

<table>
<thead>
<tr>
<th>Agent</th>
<th>Purity, company, Lot number and origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-methacryloyloxyethy trimellitate anhydride (4META) (Adhesive monomer)</td>
<td>Polysciences 697058</td>
</tr>
<tr>
<td>Camphorquinone (CQ) (Photoinitiator)</td>
<td>DMG 100134/90339</td>
</tr>
<tr>
<td>Hybrid aluminosilicate glass particles (Filler)</td>
<td>DMG 02110/688344, DMG 020684/680326</td>
</tr>
<tr>
<td>Mono-Calcium Phosphate Monohydrate (MCPM) (Remineralising agent)</td>
<td>MCP-369925, Himed, NY, USA</td>
</tr>
<tr>
<td>Poly(propylene glycol) dimethacrylate PPGDMA 400 (Diluent monomer)</td>
<td>Polysciences 626208</td>
</tr>
<tr>
<td>PolyLysine (PLS) (Antibacterial agent)</td>
<td>(4700g/mol, 20-50 µm particles size), Epolyly, 020120160203, Handary, Brussels, Belgium</td>
</tr>
<tr>
<td>Urethane dimethacrylate (UDMA) (Basic monomer)</td>
<td>DMG 100112/97406</td>
</tr>
<tr>
<td>Fumed silica (Filler)</td>
<td>(40 nm (10 wt.%) particles size), Aerosil OX50, Evonik industries, Germany153022145</td>
</tr>
</tbody>
</table>
2.1.3  Experimental composite paste preparation

Materials were prepared in bulk by Dr Wendy Xia with support from Synergy Devices Ltd. (Wycombe, UK) in April 2017. Formulations were stored in plastic black containers at RT or at 4°C until required. Resin-composite formulations were prepared by combining two phases; liquid and powder. The liquid phase was mixed by adding 4META 3 wt% (acidic monomer) and camphorquinone (CQ) 1 wt% (initiator) to poly (propylene glycol) dimethacrylate (PPGDM) 24 wt% (diluent monomer) and stirring for two hours at room temperature on a magnetic stirrer hot plate (Jeo Tech) until a clear liquid was achieved. Urethane dimethacrylate (UDMA) 72 wt% was then added as a base monomer and stirred for 24 hours until the liquid was again clear. The powder phase was made up of MCPM (16, 12, 8 or 6 wt.%), PLS (8, 6, 4 or 3 wt.%) and 3 different sizes of glass particles (7µm, 0.7µm silane treated aluminosilicate glasses and 40 nm silica particles at 60 wt%, 30 wt% and 10 wt% of total glass weight, respectively). To evaluate the effect of reducing MCPM particle size on the wear resistance of the experimental composites, smaller MCPM particle size (10µm) was used in composite containing 8wt% MCPM & 4wt% PLS. The total glass filler content varied between the formulations and was dependent on the total mass weight of MCPM and PLS in each formulation (Table 2.2). The powder (225g) was mixed without vacuum at 1000rpm for 30s using a speed mixer (DAC600.2 CM51, Synergy Devices Ltd). The monomers (75g) were added to the powder in plastic pots at powder/liquid ratio (PLR: 3:1) and mixed without vacuum at 2300rpm for 15s. Then, they were placed under vacuum for 3 minutes to reduce pressure to
around 20mbar. The formulations were mixed at 1800rpm for 30s with pressure of 17mbar.

The prepared formulations are summarised in Table 2.2.

Table 2.2 Experimental MCPM, PLS, glass filler and liquid content of the evaluated experimental resin-composite formulations used in this research with powder/liquid ratio of 3:1 (75wt.% powder). F6: composite formulation mixed without vacuum. F7: MCPM with small particle size

<table>
<thead>
<tr>
<th>Formulations</th>
<th>MCPM</th>
<th>PLS</th>
<th>Glass filler</th>
<th>Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(wt.% of filler)</td>
<td>(wt.% of monomer)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>16</td>
<td>8</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>16</td>
<td>4</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td>12</td>
<td>6</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>F4</td>
<td>8</td>
<td>8</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>F5</td>
<td>8</td>
<td>4</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>F6</td>
<td>8</td>
<td>4</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>F7</td>
<td>8</td>
<td>4</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>F8</td>
<td>6</td>
<td>3</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>F9 (Control)</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

3 wt% 4META
1 wt.% CQ
24 wt.% PPGDMA
72 wt.% UDMA
2.1.4 Commercial comparators used

The commercial restorative materials used in this study and their composition according to leaflets published on the manufacturer’s websites are summarized in Table 2.3. The materials were selected as they have relatively similar properties and application procedure as the experimental formulations.

<table>
<thead>
<tr>
<th>Material</th>
<th>Description</th>
<th>Manufacturer</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fuji II LC</td>
<td>Resin modified glass ionomer</td>
<td>GC America USA</td>
<td>HEMA 25-50%. Polycarboxylic acid, UDMA 1-5%, DMA 1-5%, Camphorquinone&lt;1%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Alumino-fluoro-silicate glass</td>
</tr>
<tr>
<td>ACTIVA™ KIDS</td>
<td>Resin modified glass ionomer</td>
<td>PULPDENT, Watertown, MA, USA</td>
<td>44.6% blend of UDMA and other methacrylates with modified polyacrylic acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sodium fluoride and silica (56 wt%)</td>
</tr>
<tr>
<td>Vertise™ Flow</td>
<td>Flowable resin composite</td>
<td>Kerr, Orange, CA, USA</td>
<td>5-10% HEMA, N/A% Bis-GMA, 5-10% UDMA and 1-5% GPDM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ytterbium fluoride and barium aluminosilicate (70 wt%)</td>
</tr>
</tbody>
</table>
2.1.5 Sample preparation

Cylindrical metal circlips (1 mm thickness × 10 mm diameter) were used to fabricate resin-composite samples. Moulds were placed on acetate sheet on top of a glass slab. Then, resin-composite was packed/dispensed into the mould and pressed between two acetate sheets. These were pressed to ensure consistent thickness, remove any excess materials and to avoid oxygen inhibition layer (Figure 2.1). Samples were light cured on top and bottom surfaces using a single emission peak light emitting diode (LED) light curing unit (LCU) (Demi Plus, Kerr, Orange, CA, USA) with power output from 1100 mW/cm$^2$ to peak of 1330 mW/cm$^2$ and spectral emission ranging from 450 through 470 nm. The LCU details were according to the manufacturer’s instructions. Each surface was cured in 4 overlapping irradiation cycles according to ISO:4049. Samples with 1 and 2 mm thickness were cured for 20s while samples with 4 mm thickness were cured for 40s. This method was always followed to prepare composite discs unless stated differently.
Figure 2.1 Example of disc-shape sample preparation
3. Polymerisation kinetics and paste shelf life

3.1 Introduction

The development of self-adhering flowable composites may eliminate the cardinal steps of etching, rinsing, priming, and bonding (Vichi et al., 2013). The introduction of this advancement holds great potential with respect to saving chair time and minimizing handling errors (Vichi et al., 2013). The incorporation of Mono-Calcium Phosphate Monohydrate (MCPM) and Polylysine (PLS) into resin-composite may also promote dentine sealing, self-repair and antimicrobial benefits (Gandolfi et al., 2011, Hyldgaard et al., 2014). These components or aging, however, may affect polymerisation kinetics. Adequate polymerisation of resin-composite is an essential requirement for long and predictable clinical performance (Shortall et al., 2013, Bayne, 2012, Leprince et al., 2013a, Shortall et al., 2015). Ferracane et al in 1985 found a significant positive correlation between increasing %DC and microhardness values of resin-composite samples (Ferracane, 1985). Likewise, in 2003, Palin et al. evaluated the %DC and flexural strength of several resin-composite materials and found that lower %DC values could significantly compromise the flexural strength properties of resin-composite (Palin et al., 2003). The application of resin-composites close to or beyond their expiry date may lead to significant clinical consequences that would result in functional failure (D'Alpino et al., 2015). The failure may result from premature reaction and consumption of inhibitors (Leprince et al., 2010).
Therefore, resin-composite products should be analysed to determine if they are susceptible to chemical degradation (D'Alpino et al., 2015).

3.2 Aims and objectives

The aim of this study was to evaluate the delay time, rate of reaction, $t_{0.5}$, \%DC and the stability of experimental resin composite formulations with varying levels of PLS and MCPM and Vertise Flow (Kerr) and ACTIVA kids (PULPDENT) used as commercial comparison.

3.3 Hypotheses

The null hypotheses are:

1. There is no significant difference between the proposed experimental formulations and commercial materials (Vertise Flow and ACTIVA kids) in terms of polymerisation kinetics.

2. Additive addition does not have a significant effect on the experimental composite polymerisation kinetics or paste stability.
3.4 Materials and methods

3.4.1 Materials and storage

The materials studied in this chapter are provided in Table 2.2. They have PLS ranging from 0 to 8wt% and MCPM from 0 to 16wt%. The mass weight ratio of MCPM/PLS ranges from 1:1 (with F4) to 4:1 (with F2). For all other formulations except the control with no PLS and MCPM this ratio is 2:1. All formulations (F1-F6, F8 and F9) were studied after storage at room temperature for 7 months following preparation. Additionally, samples were examined after storage at 4°C for 9 months or 60°C for 18 days or 9 months. Commercial comparison materials Activa and Vertise flow were stored at 4°C and used well within their expiry dates.
3.4.2 FTIR measurements

Samples (1 mm thickness × 10 mm diameter) were fabricated according to section 2.1.5. For the first set of experiments with room temperature stored experimental pastes and commercial materials, up to 4 metal circlip moulds were placed on acetate sheet on top of a glass slab to provide moulds of up to 4 mm depth. For all other higher temperature stored experimental pastes only 1mm thick samples were investigated, as thicker samples consumes a lot of materials. The mould with the acetate sheet was placed upside down on top of the Attenuated Total Reflectance (ATR) (Specac Ltd., UK) diamond crystal plate (Figure 3.1).

Figure 3.1 Resin-composite sample setup on the ATR diamond crystal plate inside a circlip of 1mm thickness and covered by acetate sheet
Fourier Transform Infrared (FTIR) (Perkin Elmer, UK) spectra were obtained from bottom surfaces of the samples (1mm, 2mm or 4mm thickness × 10 mm diameter). The spectra wavenumber range acquired was from 700 to 4000 cm⁻¹ at resolution of 4 cm⁻¹ for 20 minutes at 24°C (Young et al., 2004) before, during and after light exposure of the top surfaces. The light curing started 20s after beginning of scanning. All samples of 1 and 2 mm thickness were cured for 20s while samples of 4 mm thickness were cured for 40s. The curing times were chosen to compare the results with previous study (Aljabo et al., 2015) (n=3). Light cure was achieved using a single emission peak light emitting diode (LED) light curing unit (LCU) (Demi Plus, Kerr, Orange, CA, USA) with power output from 1100 mW/cm² to peak of 1330 mW/cm² and spectral emission ranging from 450 through 470 nm (Figure 3.2).
The degree of conversion was calculated using the following equation 3.1:

\[ DC(\%) = \frac{100(h_0 - h_t)}{h_0} \]  

3.1

Where \( h_0 \) and \( h_t \) represent methacrylate C=O peak absorbance at 1320 cm\(^{-1}\) initially and after 20 minutes using absorbance at 1335 cm\(^{-1}\) as background (Aljabo et al., 2015). An example of monomer conversion versus time is given in Figure 3.3.

![Figure 3.3 Representative FTIR spectrum profile plot of an experimental composite, showing the data points used to calculate delay time, rate of reaction and monomer conversion.](image)

To quantify the delay time and following rapid rate of reaction, straight line plots through degree of conversion data from the end of the delay to 50% conversion were used. The delay time, \( t_d \), was calculated from intercept with the time axis minus 20s (i.e. axis intercept – 20s) (Figure 3.3). The reaction
Chapter 3: Polymerisation kinetics and paste shelf life

rate, $R_{\text{max}}$, was calculated from the slope ($\%/s$) (Figure 3.3). Final maximum conversion, $\% \text{DC}_{\text{max}}$ was obtained from the y intercept of late time data plotted versus inverse time. Additionally, the time of light exposure required for 50\% conversion of this maximum level ($t_{0.5}$) was calculated using equation 3.2:

$$t_{0.5} = \frac{0.5 \% \text{DC}_{\text{max}}}{R_{\text{max}}} + t_d$$  \hspace{1cm} 3.2
3.5 Statistical analysis

Delay time, rate of reaction, $t_{0.5}$ and final %DC values were analyzed using the Statistical Package for the Social Sciences software (SPSS v.26.0 for Mac iOS (IBM Corporation, Armonk, NY, USA)). Shapiro-Wilk normality test was used to assess data distribution. For the comparison of means, parametric one-way ANOVA was utilized. Bartlett’s test of homogeneity was also employed. Non-parametric alternative Kruskal-Wallis H Test (distribution free) was used if a normal distribution assumption was violated. Post-hoc tests included Bonferroni for ANOVA, and Dunn’s test was used when non-parametric Kruskal-Wallis H test was employed. All analyses were carried out at a set significance level of 5%.
3.6 Results

3.6.1 Example spectra

3.6.1.1 Full experimental spectra reproducibility

3.6.1.1.1 Before cure

Figure 3.4 provides FTIR absorbance versus wavenumber (\text{/cm}) for 3 samples of F1, following storage at room temperature for 7 months in plastic pots and before light exposure. Generally initial spectra were highly reproducible. Noise around 1500 cm\(^{-1}\) (green spectrum in Figure 3.4) is caused by background variation. This variation has minimal effect around the 1320 cm\(^{-1}\) peak used for quantifying polymerisation.

![Figure 3.4 Initial FTIR spectra for F1 after storage at 24\textdegree{}C for 7 months and before cure.](image-url)
3.6.1.1.2 After cure

Figure 3.5 illustrates absorbance versus wavenumber (/cm) for the same F1 sample (1mm depth) at 20 minutes after 20s cure. With careful background control these spectra are highly reproducible.

Figure 3.5 Final FTIR spectra of 3 samples for F1 at 1mm depth cured for 20s and stored at 24°C.
3.6.1.1.3 Difference spectra

Figure 3.6 provides examples of difference spectra for F5 obtained by subtracting spectra before cure from those at 20 min following cure. Examples shown were stored at room temperature and cured for 20s (1mm or 2mm depth samples) or 40s (4mm deep sample). All show the same peaks and troughs as a consequence of the identical chemical processes but the level of change (and therefore of reaction) for the thicker sample is slightly reduced.

![Difference spectra diagram]

Figure 3.6 Difference between FTIR spectra at end versus beginning of scanning of F5 room temperature stored formulation. Samples of 1mm or 2mm depth were cured for 20s and those of 4mm depth for 40s. Spectra were obtained at 24°C.
3.6.1.1.4 Aging

Figure 3.7 provides FTIR absorbance versus wavenumber (\text{\textbar{cm}}) for 3 samples of F1. The formulation was stored at room temperature for 7 months in plastic pots, or aged at 60\textdegree C for 9 months in sealed glass containers, before light exposure. Peak assignments are provided in Table 3.1. The initial spectra before and after aging are consistent with good stability of main monomer components.

Figure 3.7 Initial FTIR spectra for F1 after storage at 24 \textdegree C for 7 months or aged at 60 \textdegree C for 9 months and before cure.
### Table 3.1 Peak assignment of the common methacrylate groups

<table>
<thead>
<tr>
<th>Wavenumber (cm⁻¹)</th>
<th>Peak Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3300</td>
<td>N–H stretch</td>
</tr>
<tr>
<td>2950–2850</td>
<td>C–H stretch</td>
</tr>
<tr>
<td>1700–1715</td>
<td>C=O stretch</td>
</tr>
<tr>
<td>1635–1640</td>
<td>C=C stretch</td>
</tr>
<tr>
<td>1520</td>
<td>N–H bend</td>
</tr>
<tr>
<td>1390, 1365</td>
<td>O–C–H bend</td>
</tr>
<tr>
<td>1320, 1300</td>
<td>C–O stretch</td>
</tr>
<tr>
<td>1000</td>
<td>Si–O stretch</td>
</tr>
</tbody>
</table>
Figure 3.8 illustrates absorbance versus wavenumber (\text{cm}^{-1}) for the same F1 sample (1mm depth) at 20 minutes after 20s cure. The final spectra before and after aging are comparable.

![Figure 3.8 Final FTIR spectra of 3 samples for F1 formulation at 1mm depth cured for 20s and stored at 24°C and 1 sample aged for 9 months at 60°C](image-url)
3.6.2 Polymerisation profile examples

Figure 3.9 shows examples of %DC versus time (s) for F5 formulation at 1mm and 2mm depth cured for 20s and 4mm depth cured for 40s at 24°C. With thin samples degree of conversion rose steeply soon after the light was turned on at 20s. Conversely with thicker samples there could be an extended delay time before any conversion.

Figure 3.9 %DC versus time (s) for F5 formulation at 1mm and 2mm depth cured for 20s and 4mm depth cured for 40s at 24°C. Light was turned on at 20s.
Figure 3.10 shows average %DC versus sample thickness for the same F5 samples. At 1mm and 2 mm depth, %DC of 71 and 74% could be achieved with 20s light exposure. At 4mm depth, %DC was significantly lower than 1mm depth ($p<0.05$). No other significant differences were found between groups.

![Figure 3.10 Mean ±SD of final %DC for F5 formulation at 1mm and 2mm depth cured for 20s and 4mm depth cured for 40s at 24°C (n=3).](image)
3.6.3 Average polymerisation kinetics parameters

For all formulations, major differences were seen between the commercial and experimental materials. Furthermore, large effects were caused by thickness and aging see Table 3.2 and Table 3.3.

3.6.3.1 Delay time

3.6.3.1.1 Thickness effect

Figure 3.11 shows delay times versus sample depth for all materials. All materials showed a thickness dependent delay followed by rapid polymerisation. Average experimental composite delay times were 4.9, 7.7 and 24.5s, at 1mm, 2mm and 4mm depth, respectively. At 1mm depth, experimental control (F9) had the highest delay time (6.8s ±1.6) and differed significantly to Activa ($p<0.001$) and VF ($p=0.002$), While F1 had higher delay time than Activa ($p=0.050$). For 2 mm depth, F1 had statistically higher delay time than Activa ($p=0.011$) and VF ($p=0.026$). For 4mm depth, VF had significantly lower delay time compared to F1 ($p=0.016$) and F5 ($p=0.049$). Comparing different thicknesses for each material, at 4mm depth, delay times were significantly higher than 1mm and 2mm for all materials ($p<0.001$). Furthermore, at 1mm depth, delay times were statistically lower than 2mm for all materials ($p<0.05$) except for experimental control, Activa and VF. No other significant differences were found between groups.
Table 3.2 Mean ±SD delay time, rate of reaction, final conversion, 50% reaction times of Activa kids and Vertise Flow at 1mm and 2mm depth light cured for 20s and 4mm depth light cured for 40s at room temperature (24°C) (n=3).

<table>
<thead>
<tr>
<th>Storage condition (°C)</th>
<th>Thickness (mm)</th>
<th>Delay time (s)</th>
<th>Rate of reaction (%DC/s)</th>
<th>50% reaction times (s)</th>
<th>Final conversion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ACTIVA kids</strong></td>
<td>1mm</td>
<td>2.0 ±1.1</td>
<td>3.0 ±0.6</td>
<td>13.5 ±1.6</td>
<td>65.7 ±0.7</td>
</tr>
<tr>
<td></td>
<td>2mm</td>
<td>1.7 ±2.6</td>
<td>2.1 ±0.5</td>
<td>16.8 ±0.8</td>
<td>61.5 ±0.6</td>
</tr>
<tr>
<td></td>
<td>4mm</td>
<td>11.6 ±0.2</td>
<td>0.7 ±0.1</td>
<td>52.5 ±3.4</td>
<td>55.0 ±0.9</td>
</tr>
<tr>
<td><strong>Vertise Flow</strong></td>
<td>1mm</td>
<td>2.6 ±1.1</td>
<td>4.9 ±0.7</td>
<td>10.1 ±0.0</td>
<td>72.6 ±0.6</td>
</tr>
<tr>
<td></td>
<td>2mm</td>
<td>4.5 ±0.6</td>
<td>4.1 ±0.1</td>
<td>13 ±0.3</td>
<td>71 ±0.0</td>
</tr>
<tr>
<td></td>
<td>4mm</td>
<td>10.6 ±0.8</td>
<td>1.8 ±0.1</td>
<td>28.5 ±0.8</td>
<td>65.6 ±2.0</td>
</tr>
</tbody>
</table>
### Chapter 3: Polymerisation kinetics and paste shelf life

Table 3.3 Mean ±SD delay time, rate of reaction, final conversion, 50% reaction times of experimental formulations at 1mm and 2mm depth light cured for 20s and 4mm depth light cured for 40s at room temperature (24°C) compared with formulations stored for 9 months at 60°C, (n=3).

<table>
<thead>
<tr>
<th>Storage condition (°C)</th>
<th>Thickness (mm)</th>
<th>Delay time (s)</th>
<th>Rate of reaction (%DC/s)</th>
<th>50% reaction times (s)</th>
<th>Final conversion (%)</th>
</tr>
</thead>
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<tr>
<td>16-8</td>
<td>60</td>
<td>3.2 ±0.1</td>
<td>6.3 ±0.3</td>
<td>9.2 ±0.5</td>
<td>74.8 ±1.0</td>
</tr>
<tr>
<td>16-4</td>
<td></td>
<td>3.1 ±0.4</td>
<td>6.8 ±0.6</td>
<td>8.8 ±0.3</td>
<td>77.1 ±1.8</td>
</tr>
<tr>
<td>12-6</td>
<td></td>
<td>2.9 ±0.0</td>
<td>6.1 ±0.2</td>
<td>8.6 ±0.4</td>
<td>69.3 ±1.5</td>
</tr>
<tr>
<td>8-8</td>
<td></td>
<td>2.4 ±0.0</td>
<td>6.2 ±0.2</td>
<td>8.5 ±0.6</td>
<td>75.9 ±1.7</td>
</tr>
<tr>
<td>8-4</td>
<td></td>
<td>3.0 ±0.4</td>
<td>7.1 ±1.1</td>
<td>8.2 ±0.8</td>
<td>71.5 ±3.2</td>
</tr>
<tr>
<td>6-3</td>
<td></td>
<td>2.4 ±0.7</td>
<td>6.6 ±0.4</td>
<td>7.9 ±1.1</td>
<td>72.8 ±4.5</td>
</tr>
<tr>
<td>Mean/std</td>
<td></td>
<td>2.9 ±0.4</td>
<td>6.5 ±0.4</td>
<td>8.5 ±0.4</td>
<td>73.6 ±2.9</td>
</tr>
<tr>
<td>16-8</td>
<td>24</td>
<td>5.1 ±1.0</td>
<td>5.5 ±0.4</td>
<td>12.0 ±0.6</td>
<td>76.5 ±1.9</td>
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<tr>
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<td>4.4 ±1.3</td>
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</tr>
<tr>
<td>12-6</td>
<td></td>
<td>4.6 ±0.3</td>
<td>4.4 ±0.4</td>
<td>13.0 ±0.9</td>
<td>73.3 ±0.8</td>
</tr>
<tr>
<td>8-8</td>
<td></td>
<td>4.6 ±0.5</td>
<td>5.4 ±1.3</td>
<td>11.9 ±1.3</td>
<td>74.8 ±0.5</td>
</tr>
<tr>
<td>8-4</td>
<td></td>
<td>4.8 ±0.9</td>
<td>4.8 ±1.3</td>
<td>12.5 ±1.1</td>
<td>70.9 ±0.5</td>
</tr>
<tr>
<td>8-4 NoVAC</td>
<td></td>
<td>4.3 ±0.1</td>
<td>5.3 ±0.1</td>
<td>11.7 ±0.1</td>
<td>78.7 ±2.3</td>
</tr>
<tr>
<td>6-3</td>
<td></td>
<td>4.5 ±0.9</td>
<td>4.9 ±0.2</td>
<td>12.5 ±0.6</td>
<td>79.4 ±2.2</td>
</tr>
<tr>
<td>0-0</td>
<td></td>
<td>6.8 ±1.6</td>
<td>4.6 ±0.7</td>
<td>15.2 ±2.4</td>
<td>75.4 ±3.6</td>
</tr>
<tr>
<td>Mean/std</td>
<td></td>
<td>4.9 ±0.8</td>
<td>5 ±0.4</td>
<td>12.6 ±1.2</td>
<td>75.6 ±2.8</td>
</tr>
<tr>
<td>16-8</td>
<td>24</td>
<td>8.9 ±0.4</td>
<td>3.7 ±0.4</td>
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<tr>
<td>16-4</td>
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<td>8.1 ±0.9</td>
<td>4.0 ±0.8</td>
<td>16.9 ±1.4</td>
<td>67.4 ±1.3</td>
</tr>
<tr>
<td>12-6</td>
<td></td>
<td>7.7 ±1.0</td>
<td>4.0 ±0.2</td>
<td>17.2 ±0.6</td>
<td>75.3 ±3.4</td>
</tr>
<tr>
<td>8-8</td>
<td></td>
<td>7.7 ±0.4</td>
<td>4.7 ±0.6</td>
<td>15.8 ±0.7</td>
<td>75.0 ±3.8</td>
</tr>
<tr>
<td>8-4</td>
<td></td>
<td>7.1 ±1.0</td>
<td>3.8 ±1.1</td>
<td>17.1 ±1.3</td>
<td>73.5 ±6.4</td>
</tr>
<tr>
<td>8-4 NoVAC</td>
<td></td>
<td>8.2 ±0.1</td>
<td>4.4 ±0.1</td>
<td>16.5 ±0.2</td>
<td>73.4 ±2.9</td>
</tr>
<tr>
<td>6-3</td>
<td></td>
<td>7.5 ±0.8</td>
<td>4.7 ±0.7</td>
<td>15.5 ±0.3</td>
<td>74.2 ±3.1</td>
</tr>
<tr>
<td>0-0</td>
<td></td>
<td>6.6 ±1.1</td>
<td>4.2 ±0.6</td>
<td>16.0 ±0.6</td>
<td>78.2 ±2.8</td>
</tr>
<tr>
<td>Mean/std</td>
<td></td>
<td>7.71 ±0.7</td>
<td>4.2 ±0.4</td>
<td>16.6 ±0.8</td>
<td>73 ±4</td>
</tr>
<tr>
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<td>1.8 ±0.5</td>
<td>44.9 ±3.5</td>
<td>56.6 ±1.7</td>
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<tr>
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<td>39.8 ±0.3</td>
<td>65.3 ±1.3</td>
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<tr>
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<td>64.6 ±0.3</td>
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<tr>
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<tr>
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<td>2.6 ±0.4</td>
<td>38.3 ±0.9</td>
<td>69.9 ±4.2</td>
</tr>
<tr>
<td>6-3</td>
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<td>23.4 ±0.0</td>
<td>2.0 ±0.3</td>
<td>39.1 ±1.9</td>
<td>63.6 ±0.6</td>
</tr>
<tr>
<td>0-0</td>
<td></td>
<td>20.7 ±2.5</td>
<td>2.8 ±0.2</td>
<td>35.1 ±1.4</td>
<td>79.9 ±1.3</td>
</tr>
<tr>
<td>Mean/std</td>
<td></td>
<td>24.5 ±2.4</td>
<td>2.1 ±0.5</td>
<td>41 ±4.6</td>
<td>64.5 ±8.9</td>
</tr>
</tbody>
</table>
Table 3.11 Mean ±SD of the delay time for the experimental formulations at 1mm and 2mm depth cured for 20s and 4mm depth cured for 40s stored at 24°C (n=3).

<table>
<thead>
<tr>
<th>Material</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F8</th>
<th>F9</th>
<th>Activa</th>
<th>VF</th>
<th>F6</th>
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<td>4</td>
<td>6</td>
<td>8</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCPM</td>
<td>16</td>
<td>16</td>
<td>12</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>0</td>
<td></td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3.11 Mean ±SD of the delay time for the experimental formulations at 1mm and 2mm depth cured for 20s and 4mm depth cured for 40s stored at 24°C (n=3).
3.6.3.1.2 Aging effects for 1 mm samples

Figure 3.12 shows delay times versus time of paste aging for formulations F1-F5 and F8. For the experimental formulations stored at room temperature, 18 days at 60°C and 9 months at 4°C or 60°C, the average delay times for 1mm thickness ranged between 2 to 5s. For formulations stored at 60°C for 18 days, F8 showed higher delay time than F3 ($p=0.008$) and F5 ($p=0.009$) and no other significant differences were found between groups.

![Figure 3.12 Mean ±SD of the delay time for the experimental formulations (1mm thickness, 20s light curing) stored at 4, 24 and 60°C (n=3).](image)
3.6.3.2 Rate of reaction

3.6.3.2.1 Thickness effect

Figure 3.13 shows reaction rates versus sample depth for all materials. For the experimental materials the maximum rates of reaction were on average 5, 4 and 2%/s, at 1, 2 and 4mm depth, respectively. At 1mm depth, Activa showed lower reaction rate than F1 ($p=0.026$), F2 ($p=0.047$), F6 ($p=0.010$). In addition, F4 had higher reaction rate than Activa at both 1mm ($p=0.048$) and 2mm ($p=0.049$) depth. For 4mm depth, the experimental control had significantly higher rates of reaction compared with Activa ($p=0.008$) and F2 ($p=0.036$). Also, Activa showed lower rate of reaction compared with F6 ($p=0.013$). For the differences between sample depths for each material, at 4mm depth, reaction rates were significantly lower than at 1mm and 2mm depths for all materials ($p<0.001$) except F5. At 1mm depth, reaction rates were higher than at 2mm depth for F1 and F6 ($p<0.05$). No other significant differences were found between groups.

Figure 3.13 Mean ±SD of the rate of reaction %DC/s for the experimental formulations at 1mm and 2mm depth cured for 20s and 4mm depth cured for 40s stored at 24°C (n=3).
3.6.3.2.2 Aging effects for 1 mm samples

Figure 3.14 shows reaction rates versus time of paste aging for formulations F1-F5 and F8. For the experimental formulations stored at room temperature, 18 days at 60°C and 9 months at 4°C or 60°C, their average rate of reaction at 1mm depth ranged between 4%/s to 6%/s. For formulations stored at 60°C for 18 days, F8 showed higher reaction rate than F3 ($p=0.002$) and F5 ($p=0.030$). No other significant differences were found between groups.

![Graph showing reaction rates vs time](image.png)

Figure 3.14 Mean ±SD of the rate of reaction %DC/s for the experimental formulations (1mm thickness, 20s light curing) stored at 4, 24 and 60°C (n=3).
3.6.3.3 50% reaction times

3.6.3.3.1 Thickness effect

Figure 3.15 provides times of 50% conversion versus sample depth for all materials. The 50% reaction times were 12, 16 and 41s, at 1mm, 2mm and 4mm depth, respectively. At 1mm depth, time of 50% conversion of experimental control (15.2 ±2.4) was higher than F2 (11.6s ±0.3) (p=0.043), F6 (11.7s ±0.1) (p=0.054) and VF (10.1s ±0.0) (p<0.001). In addition, VF (10.1s ±0.0) had lower 50% reaction times than F3 (13.0s ±0.9) (p=0.050) and Activa (13.5s ±1.6) (p=0.017). For 2mm depth, VF (13s ±0.3) had significantly lower time of 50% conversion than F1 (17.9s ±1.0) (p=0.013). For the comparison between different sample thicknesses for each material, at 4mm depth, times of 50% conversion were significantly higher than at 1mm and 2mm depths for all materials (p<0.001). At 1mm depth, times of 50% conversion were lower than at 2mm depth for all materials (p<0.05) except F9. No other significant differences were found between groups.
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Figure 3.15 Mean ±SD of the time at 50%DC for the experimental formulations at 1mm and 2mm depth cured for 20s and 4mm depth cured for 40s stored at 24°C (n=3).
3.6.3.3.2 Aging effects for 1 mm samples

Figure 3.16 shows 50% reaction times for each time of paste aging for formulations F1-F5 and F8. For the experimental formulations stored at room temperature, 18 days at 60°C and 9 months at 4°C or 60°C, the 50% reaction times ranged between 9s to 12s. No significant differences were found between experimental groups.

![Graph showing 50% reaction times for each time of paste aging](image)

**Figure 3.16** Mean ±SD of the time at 50%DC for the experimental formulations (1mm thickness, 20s light curing) stored at 4, 24 and 60°C (n=3).
3.6.3.4 Monomer conversion

3.6.3.4.1 Thickness effect

Figure 3.17 shows %DC versus sample depth for all materials. Experimental composite monomer polymerisation profiles for formulations stored at room temperature for 7 months were only mildly affected by additive addition. At 1mm and 2mm depth, final %DC of 74% and 72% could be achieved with 20s light exposure. 40s light was required to gain average of 64%DC at 4mm depth but this was decreased with higher MCPP levels. At 1mm depth, ACTIVA (65.7% ±0.7) had the lowest %DC out of all materials tested, significantly different to all (p<0.05) except F5. Furthermore, F5 (70.9% ±0.5) had lower %DC than F6 (78.7% ±2.3) (p=0.006) and F8 (79.4% ±2.2) (p=0.002), while VF (72.6% ±0.6) showed lower %DC than F8 (p=0.026). At 2mm depth, Activa (61.5% ±0.6) obtained the lowest %DC and differed significantly to all materials (p<0.05) except F1 (66.6% ±2.7), F2 (67.4% ±1.3) and VF (71% ±0.0). However, experimental control (78.2% ±2.8) showed higher %DC than F1 and F2. %DC was significantly lower at 4mm depth than at 1mm and 2mm depth for all materials (p<0.05) except experimental control (F9), F5 and F6. In addition, at 1mm depth %DC was higher than at 2mm for F1, F2, Activa and VF (p<0.05). No other significant differences were found between experimental groups.
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Figure 3.17 Mean ±SD of %DC for the experimental formulations at 1mm and 2mm depth cured for 20s and 4mm depth cured for 40s stored at 24°C (n=3).
3.6.3.4.2 Aging effects for 1 mm samples

Figure 3.18 shows %DC versus time of paste aging for formulations F1-F5 and F8. For the experimental formulations stored at room temperature, 18 days at 60°C and 9 months at 4°C or 60°C, the %DC ranged between 69% to 78%. At room temperature, F8 (79.4% ±2.2) had higher %DC compared to F5 (70.9% ±0.5) (p=0.003). For formulations stored at 60°C for 18 days, F8 obtained lower %DC than F1 (p=0.001) and F2 (p=0.0034), while F1 had higher %DC than F5 (p=0.022). For formulations stored at 60°C for 9 months, F2 showed higher %DC than F3 (p=0.044). No other significant differences were found between experimental groups.
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3.7 Discussion

The null hypothesis tested in the present study, which assumed that additive addition will not have a significant effect on the experimental composite polymerisation kinetics or paste stability could be rejected.

Accelerated aging protocol can be used to predict shelf-life stability and the long time performance of resin-composite in a shorter period of time (Hemmerich, 1998). According to the 10-degree rule, each 10°C increase above room temperature can double the reaction rate (Clark, 1991, ASTM, 2016). The accelerated aging protocol was evaluated according to Arrhenius model (ASTM, 2016) using the following equation 3.3:

\[
Real\ Time\ (RT) = Accelerated\ Aging\ Time\ (AAT) \times Q_{10}^{(t_{AA}-t_{RT}/10)} \quad 3.3
\]

Where:

\( t_{RT} \) is room temperature (22°C)
\( t_{AA} \) is the elevated temperature (60°C)
\( Q_{10} \) is the reaction rate coefficient (2)

Following equation 3.3, the accelerated aging time for formulations stored for 9 months at 60°C is approximately equivalent to 11 years of aging at room temperature (22°C), and formulations stored for 18 days at 60°C is approximately equivalent to 10 months of aging at room temperature (22°C) (Vismara et al., 2014). The chemical degradation could be a combination of effects arises from premature polymerisation, consumption of inhibitors, and
other factors that may be dependent on the environmental storage (Leprince et al., 2010). The results of this study showed that the experimental formulations were stable upon high temperature aging and had comparable rates and levels of conversion compared to formulations stored at room temperature and 4°C suggesting good shelf life. However, the results are not showing any trend and they seem to be time-dependent rather than the storage temperature. This could be related to a systematic error due to the timing of the test, as the study was conducted at different temperatures and humidity. In addition, the lack of cool-down protocol for the composites, after they were taken out of the incubators may also have affected the results.

The null hypothesis tested in the present study, which assumed that there is no significant difference between the proposed experimental formulations and commercial materials (Vertise Flow and ACTIVA kids) in terms of polymerisation kinetics has to be rejected.

ATR-FTIR detects conversion of monomers into polymers in the lower surface of the resin composite samples (in contact with the diamond). The delay time is the time between the start of light-curing and the actual start of monomers converting into polymers. It is a consequence of photobleaching being required to enable sufficient light penetration into deeper samples. The higher delay time with F9 than Activa andVF at 1mm depth could be related to the low viscosity of the flowable commercial composites that may improve the mobility of the monomer molecules and result in lower delay times. In addition, the bleaching shade used with Activa may improve light transmission through the sample and consequently result in lower delay time compared to
experimental control with relatively high CQ (1wt.% of the monomer) that makes the formulations more yellow.

The %DC has a significant impact on the performance of resin-composite restorations. It was found that %DC is positively correlated with increasing the amount of irradiance (mW/cm²) received by resin composite (Calheiros et al., 2008). Conversely, unreacted monomers may plasticize the polymer structure, degrading the mechanical properties of resin composite restorations (Arikawa et al., 2004, Lovell et al., 1999). The total radiant exposure (J/cm²) is the product of irradiance (mW/cm²) and exposure time (s). Comparable material properties may result from similar radiant exposure regardless of how it was obtained (amount of irradiance, exposure time). This principle is known as the "exposure reciprocity law" (Leprince et al., 2011). In this study, to standardize the amount of radiant exposure received by the resin-composite, the samples were cured with 0 mm distance between the LCU tip and the top surface of each sample and by using a single LED LCU. The light exposure times (20, 40s) were sufficient to obtain high %DC values on the bottom surfaces. However, it is important to note that %DC values were detected from the 4×4 mm ATR diamond crystal plate that doesn’t represent the %DC of the entire bottom surface.

Previously %DC has been found to be considerably influenced by the material composition, as increasing the monomer concentration, viscosity and opaque filler loading may decrease the %DC of the resin composite (Amirouche-Korichi et al., 2009, Gonçalves et al., 2009). Increasing the viscosity of the dimethacrylate monomer system could restrict the mobility of the molecules,
resulting in lower rate of reaction, more free radical entrapment, increased termination, and lower %DC values (Leprince et al., 2013b). Also, the type of photoinitiators could affect the %DC, as for one molecule of CQ only one active free radical is expected for polymerisation initiation (De Oliveira et al., 2015, Neumann et al., 2006). On the other hand the photoactivation process of alternative photoinitiator such as diphenyl (2,4,6-trimethylbenzoyl) phosphine oxide will lead to a cleavage reaction on the molecule resulting in more than one free radical to initiate the polymerisation reaction leading to higher %DC (De Oliveira et al., 2015).

Among the advantages of the new composite formulations is their ability to be placed in thicker layers than the maximum thickness (2 mm) often suggested for each resin-composite increment (Sakaguchi et al., 1992, Garoushi et al., 2016). In general, increasing the thickness of the samples (1, 2 and 4mm) resulted in lower %DC and rate of reaction. The results are consistent with a previous study that showed a linear decrease in %DC with increasing the thickness and CaP content (Aljabo et al., 2015). The photoinitiators can absorb the light and decrease the amount of light transmitted through the composite (Ogunyinka et al., 2007, Musanje and Darvell, 2006). Also, the mismatch in refractive indices of the filler particles (Silica=1.46, aluminosilicate glass=1.59) (Duminis et al., 2018) relative to the monomers (UDMA=1.48, PPGDMA=1.45) (Walters et al., 2016) can affect the light transmission to bottom surfaces by both scattering and refracting. In addition, the refractive index of the monomers change during polymerisation, which may result in change in light scattering (Odian, 2004, Howard et al., 2010, Shibayama et al.,
2005). It is also important to point out that the total glass filler content is increasing with decreasing the reactive fillers, which may reduce the refractive index mismatch with the polymerised matrix (1.6) caused by MCPM (1.51) and PLS (1.37) (Wang and Chang, 2003, Howard et al., 2010). This could explain the lower %DC of F1 and F2 when compared to experimental control with no additives and the lower %DC of F5 than F8. The lower %DC with Activa could be related to the higher amount of its dimethacrylate base monomer (50% UDMA), which may limit its immediate %DC (Delgado, 2021).

In addition, Activa has polyacid components of the glass ionomer family that undergo an acid-base reaction and has a dual-cure system (light and chemically activated polymerisation). Therefore, Activa has in fact three different setting mechanisms that tend to gradually improve its monomer conversion for up to 24 hours (Bandéca et al., 2009, Croll et al., 2015). The lower %DC with VF than F8 could be due to the refractive index mismatch caused by the prepolymerised fillers in VF. In addition, %DC measured with the FTIR may not be suitable in the presence of prepolymerised fillers. The measurement of the %DC might be taking into account parts of the sample that did not undergo polymerisation but were in fact prepolymerised (Aljabo et al., 2015).
3.8 Conclusion

Based on the findings of this study so far, the following can be concluded:

1) Experimental formulations were stable upon high temperature aging suggesting good shelf life.

2) For both experimental formulations and commercial materials, 20s light exposure is sufficient for good monomer conversion up to 2mm depth.

3) Increased thickness causes an increase in delay time and a decrease in rate of reaction.
4. Polymerisation shrinkage, mass and volume change, $H^+$ release, dried extracts and simulated wear resistance of set materials

4.1 Introduction

Polymerisation shrinkage is a consequence of the short covalent bond formation during the polymerisation reaction that leads to a reduction in distance between monomer molecules (Ferracane, 2008). Resin-composite restorations are subjected to fluids in the oral cavity and subsequent water sorption. Water uptake by set materials can change the mass and volume of resin-composite and could negatively affect their mechanical and physical properties (Aljabo et al., 2015, Panpisut et al., 2016, Suiter et al., 2016). Volumetric expansion due to water sorption of the composites may compensate the polymerisation shrinkage and relieve the residual shrinkage stress (Park and Ferracane, 2014).

Water uptake is also required to enable release of components such as MCPM ions and PLS in order to promote remineralising or bonding effects (Farrugia and Camilleri, 2015). Agent release, however, may lead to surface pores and subsequent low wear resistance and high surface roughness, which facilitates biofilm accumulation (Chen et al., 2009, Derchi et al., 2017). In composites, mono-calcium phosphate was found to react with water to form high porosity brushite crystals that expand the resin-matrix and releases
phosphoric acid (Aljabo et al., 2016, Panpisut et al., 2016, Abou Neel et al., 2016, Mehdawi et al., 2009, Aljabo et al., 2015). In water, MCPM (Ca(H₂PO₄)₂) can disproportionate to form dicalcium phosphate (DCP) (CaHPO₄) and phosphoric acid (H₃PO₄) via the following equilibrium 4.1:

\[
\text{Ca(H}_2\text{PO}_4\text{)}_2 \leftrightarrow \text{CaHPO}_4 + \text{H}_3\text{PO}_4
\]

In the presence of water, the phosphoric acid may provide self-etch of apatite (Ca₁₀ (PO₄)₆ (OH)₂) in tooth structures forming more DCP through the following equilibrium:

\[
\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 4\text{H}_3\text{PO}_4 \leftrightarrow 10\text{CaHPO}_4 + 2\text{H}_2\text{O}
\]

The DCP rapidly precipitates as brushite (dicalcium phosphate di-hydrate). This process requires water and produces crystals of lower density that occupy greater volume (Xia et al., 2014). This can compensate polymerisation shrinkage or fill gaps at the tooth restoration interface by expansion of the composite (Mehdawi et al., 2009). Furthermore, the reverse reaction upon buffering of the phosphoric acid by hydroxyl ions in dentinal fluid may support longer-term tooth remineralisation. Brushite is less soluble than hydroxyapatite below pH of 4 and therefore precipitates. At higher pH it becomes more soluble and so transforms back to hydroxyapatite (Table 4.1) (Chow, 2009).
Brushite may transform into calcium deficient hydroxyapatite upon neutralisation of phosphoric acid by oral fluids, whilst organic acids such as citric produce counter ions that can solubilise calcium. Phosphate ions may precipitate as other calcium phosphate species helping to reduce loss of calcium ions.

PLS has been found to be effective against a wide range of bacteria including dental pathogens such as \textit{S.mutans} and \textit{P.gingivalis} (Dima et al., 2020). It was reported that increasing PLS concentration above 250 ppm resulted in the reduction of survival rate, enzymatic activity, and adenosine triphosphate (ATP) levels of \textit{Staphylococcus aureus}. PLS has the ability to destroy the peptidoglycan of the cell wall and can affect the levels of several metabolites (Tan et al., 2019). It can also exert its activity by binding to negatively charged phospholipid head groups in the lipid bilayer of Gram-positive (\textit{Listeria innocua}) and Gram-negative (\textit{E. coli}) bacterial membranes and destabilized them, thus increasing permeability. In the case of \textit{E. coli}, PLS can destroy the lipopolysaccharide component of the outer membrane (Hyldgaard et al., 2014).
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PLS release would be restricted if the resin-composite is in direct contact with the dentine surface. Poor bonding technique, polymerisation shrinkage and stresses may damage the composite/dentine interface, leading to the formation of a water filled marginal gap. Rapid release of PLS into this gap could help to prevent biofilm formation and subsequent secondary caries. It was suggested that higher MCPM and PLS are required for biofilm destruction than planktonic bacteria (Yaghmoor et al., 2020).

However, excessive release of MCPM and PLS, may negatively affect surface roughness (14.7μm) upon wear (Kangwankai et al., 2017).

Wear of dental restorative materials can be influenced by many factors including contact with antagonist teeth (attrition), acid attack by certain beverages and fruits (erosion), chemical effect (corrosion) and the use of tooth paste and mastication of food (abrasion) (Tsujimoto et al., 2018, Tsujimoto et al., 2019).

Therefore, it is necessary to assess how properties vary with systematic variations in material components in order to both understand material properties and to further optimize the novel resin composite formulations to be used to restore children’s teeth. The aim of this study was to provide understanding of factors controlling new composite dimensional stability and component release kinetics and chemistries and their ability to resist abrasive wear.
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4.2 Objectives

The project objectives were to evaluate the polymerisation shrinkage, mass and volume change of set materials in addition to their release of $H^+$ ions and other components for four experimental resin-composite formulations containing PLS (8, 4wt%) & MCPM (16, 8 wt%). Furthermore, simulated wear resistance of the four resin composite pastes in addition to a composite paste containing MCPM (8 wt%) & PLS (4 wt%) with small MCPM particle size (10µm) was evaluated. ACTIVA kids (PULPDENT, Watertown, MA, USA) and Fuji II LC (GC corp., Tokyo, Japan) were used as commercial comparisons.

4.3 Hypotheses

The Null hypotheses are:

Increasing additive addition does not have a significant effect on the experimental composite:

- Dimensional stability and shrinkage.
- Mass or volume change following water-sorption.
- $H^+$ ion release into water.
- Components released versus time.
- Wear resistance.
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4.4 Materials and methods

4.4.1 Materials

The materials studied in this chapter were produced by Dr Wendy Xia (see section 2.1.3) and provided in Table 4.2. They have 8 or 4wt% PLS and 16 or 8wt% MCPM with inorganic filler content of 76wt%, 80wt%, 84wt% and 88wt% for F1, F2, F4 and F5, respectively. All formulations were studied after storage at room temperature for 7 months following preparation. Polymerisation shrinkage, percentage mass and volume change, storage solution acidity, dried solid content and wear resistance (F1, F2, F4 and F5) (n=3) were evaluated. In addition, wear of F7 was evaluated. Wear was assessed after 3 months (n=2) and 1 year (n=3) of immersion in deionised water. ACTIVA kids (PULPDENT, Watertown, MA, USA) and Fuji II LC (GC corp., Tokyo, Japan) were used as commercial comparisons in all studies.

Table 4.2 Experimental MCPM, PLS and glass filler content of the evaluated experimental resin-composite formulations used in this chapter. F7: MCPM with small particle size.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>MCPM (wt% of filler)</th>
<th>PLS (wt% of filler)</th>
<th>Glass filler (wt% of filler)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>16</td>
<td>8</td>
<td>76</td>
</tr>
<tr>
<td>F2</td>
<td>16</td>
<td>4</td>
<td>80</td>
</tr>
<tr>
<td>F4</td>
<td>8</td>
<td>8</td>
<td>84</td>
</tr>
<tr>
<td>F5 and F7</td>
<td>8</td>
<td>4</td>
<td>88</td>
</tr>
</tbody>
</table>
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4.4.2 Assessment of polymerisation shrinkage

4.4.2.1 Polymerisation shrinkage calculation

The polymerisation shrinkage was calculated as a percentage using Archimedes principal and ISO 17304: 2013. The densities of unpolymerised paste and polymerised discs were determined by measuring their mass in air and water and used in the following equations 4.3 and 4.4 to calculate polymerisation shrinkage (Rüttermann et al., 2007, Sun and Lin-Gibson, 2008):

\[
S(\%) = \frac{\rho_d - \rho_p}{\rho_d} \times 100
\]

\[
\rho_p = \frac{(M_{pa} - M_a) \times \rho_w}{M_{pa} - \left(\frac{\rho_w \times M_a}{\rho_a}\right) - M_{pw}}
\]

Where:

\( S \) is the polymerisation shrinkage (\%)

\( \rho_d \) is the density of the polymerised disc (g/cm\(^3\))

\( \rho_p \) is the density of the paste (g/cm\(^3\))

\( \rho_a \) is the density of the glass plate (g/cm\(^3\))

\( \rho_w \) is the density of water (g/cm\(^3\))

\( M_a \) is the mass of the glass plate in air (g)
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$M_{pa}$ is the mass of the paste plus glass in air (g)

$M_{pw}$ is the mass of the paste plus glass in water (g)

### 4.4.3 Solid sample preparation

Composite discs (2 mm thickness × 10 mm diameter) were prepared using 2*1 mm deep metal circlips placed on top of each other as a mould. All samples were light cured on top and bottom surfaces using a single emission peak LED LCU (Demi Plus, Kerr, Orange, CA, USA) with power output from 1100 mW/cm$^2$ to peak of 1330 mW/cm$^2$ and spectral emission ranging from 450 through 470 nm. Each surface was cured in 4 overlapping irradiation cycles according to ISO: 4049 (Figure 4.1). The curing time was 40s for each cycle. Total cure time was therefore 160s on each surface.

![Figure 4.1 Schematic diagram of overlapping irradiation zones for the preparation of the specimens](image-url)
4.4.4 Assessment of density

All samples were stored in 10 mL of deionised water in a sterilin tube and incubated at 37°C. The weight of each disc was recorded in air and water. Sodium lauryl sulphate (5g sodium lauryl sulfate in 500ml deionised water) was added to improve the wettability by reducing the surface tension of water. The weight and volume were determined by using a density kit (Mettler-Toledo AG, Greifensee, Switzerland) and balance equipped with accuracy to ±0.1mg. The weight measurements were recorded over time at 0, 2, 4, 6, 24, 48, 72 hours of storage and then every week up to 11 weeks. Percentage mass and volume change were calculated before and after aging in water over the 11 weeks (Mehdawi et al., 2013a).

4.4.4.1 Calculations of mass density and volume changes

Samples were weighed in air and water and then density (ρ), determined at different times using the following equation 4.5:

\[
\rho = \frac{A}{A - B}(\rho_0 - \rho_L) + \rho_L
\]  

4.5

The volume (V) of each sample was also calculated using wet and dry measurements following equation 4.6 below:

\[
V = \alpha \frac{A - B}{\rho_0 - \rho_L}
\]  

4.6
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Where:

$\rho$ is density of the sample

$A$ is weight of the sample in air

$B$ is weight of the sample in deionised water

$V$ is volume of the sample

$\rho_0$ is density of deionised water at the measured temperature

$\rho_L$ is density of air (0.0012 g/cm$^3$)

$\alpha$ is a weight correction factor (0.99985)

The changes in mass and volume were calculated as percentages following equation 4.7 for percentage increase in mass and equation 4.8 for percentage increase in volume.

$$\frac{\% \text{ Mass change}}{} = \frac{100(M_t - M_0)}{M_0} \quad 4.7$$

Where: $M_t$ is the mass at time $t$ after immersion, $M_0$ is the initial mass

$$\% \text{ Volume change} = \frac{100(V_t - V_0)}{V_0} \quad 4.8$$

Where: $V_t$ is the volume at time $t$ after immersion, $V_0$ is the initial volume
4.4.5 Assessment of H⁺ release of set materials

The water acidity was measured by using a pH meter and glass electrode (Orion star A111, Thermo Scientific). The pH meter was calibrated at room temperature (24°C) by using dilute buffers. The glass electrode was then rinsed with deionised water and placed in the sample storage water to obtain the pH from the meter. The acidity was recorded over time at 0, 2, 4, 6, 24, 48, 72 hours of storage and then every week up to 11 weeks without changing the storage water.

4.4.5.1 Calculation of H⁺ release

The H⁺ release overtime was calculated from the following equation 4.9:

\[
pH = -\log_{10}[H^+] \\
[H^+] = 10^{-pH}
\]

4.9

[H⁺] is the concentration of H⁺ ions in moles/L.

4.4.6 Assessment of dried extracts of set materials

Following the previous protocol for evaluating mass and volume change (section 4.4.4), all samples were removed from sterilin tubes. Tubes with the storage water were then placed uncovered under vacuum (Vacuutherm, Thermo scientific) of 1x10⁻² bar at 50°C for 7 days.

After water evaporation, the remaining dried extracts from one sterilin tube of each formulation were evaluated after 3 months and 1 year of storage in
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water. Scanning Electron Microscopy (SEM), Energy-dispersive X-ray spectroscopy (EDX) and Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy were used to evaluate the dried extracts. For SEM studies, the samples were sputter-coated under vacuum with gold/palladium for 90 seconds using a Polaron E5000 (Quorum, UK). The dried extracts were then scanned using an SEM (Philips/FEI XL-30, Eindhoven, The Netherlands) in the secondary electron imaging mode. This was performed with a current of 200pA and an energy beam of 5kV. For EDX studies, it was performed with a beam energy of 25 kV using INCA software X-sight 6650 detector (Oxford Instrument, UK). Nine EDX spectra were obtained from the sample extract. The surface of the dried extract was divided into 9 squares each 3x3mm. The acquisition time to map each square was 200s. To obtain the FTIR (Specac Ltd., UK, and Perkin Elmer, UK) spectra, the golden gate bridge of the ATR accessory was required to ensure good contact of the dried extracts with its diamond. All spectra were acquired from 700-4000 cm$^{-1}$ at a resolution of 4 cm$^{-1}$. Spectrum TimeBase v. 3.1.4 (Perkin-Elmer, MA, USA) was used for data processing.

4.4.7 Assessment of simulated wear resistance

The wear resistance of the four resin composites (F1, F2, F4 and F5) was evaluated after 3 months (n=2) and 1 year (n=3). In addition, composite paste containing MCPM (8wt%) & PLS (4wt%) with smaller MCPM particle size (F7), was evaluated after 1 year with ACTIVA kids (PULPDENT, Watertown, MA,
USA) and Fuji II LC (GC corp., Tokyo, Japan) used as commercial comparisons (n=3).

The wear testing was conducted by using a dual axis Chewing Simulator (CS-4, SD Mechtronik GmbH, Feldkirchen-Westerham, Germany) (Figure 4.2). The cylindrical samples were embedded in type IV die stone (Silky Rock die material violet, Whip Mix, Louisville, KY, USA) using the manufacturer sample holder (Figure 4.3). Steatite balls (MUHLMEIER, Baernau, Germany) with a diameter of 6mm were used as antagonist. The samples were then subjected to a two-body wear test in deionised water. The masticatory cycle was achieved in a circular motion of 3mm diameter and with a vertical force of 5 kg. Each sample was loaded at 4.28 Hz for a total of 120,000 chewing cycles. All other test parameters are summarized in Table 4.3 (Heintze, 2006, Heintze et al., 2012).
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Figure 4.2 Dual Axis Chewing Simulator

Figure 4.3 The cylindrical specimens embedded in type IV die stone using the manufacturer specimen holder.
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### Table 4.3 Wear test parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cycles</td>
<td>120,000</td>
</tr>
<tr>
<td>Force</td>
<td>5kg (49N)</td>
</tr>
<tr>
<td>Circle diameter</td>
<td>3mm</td>
</tr>
<tr>
<td>Intrusion depth</td>
<td>1mm</td>
</tr>
<tr>
<td>Speed</td>
<td>40mm/s</td>
</tr>
<tr>
<td>Frequency</td>
<td>4.28Hz</td>
</tr>
</tbody>
</table>
4.4.8 Calculation of the volume loss after simulated wear testing

Samples were subjected to a quantitative surface analysis using a CAD/CAM three-dimensional contact scanner (Renishaw Dental Scanner, Renishaw, Wotton-under-Edge, UK) (Figure 4.4). The scanner was calibrated before each use. An upper and lower point of the sample were determined to ensure the scan is within the working volume (X, Y and Z tolerances). Samples were then scanned using a 1mm diameter stylus. From each worn surface, a three-dimensional image was obtained (Figure 4.25). The three-dimensional image was then imported into the three-dimensional inspection software (Geomagic Control X v2014.0.0, 3D Systems Inc., Rock Hill, SC, USA). The worn area was selected and a reference plane for the depth measurement was defined to be parallel to and in the plane of the flat unworn surface surrounding the abraded area on the mesh. The volume between the reference plane and the worn surface was calculated and assumed as the volumetric loss (mm$^3$) (D'Arcangelo et al., 2014) (Matias1a et al., 2016).
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Figure 4.4 CAD/CAM three-dimensional contact scanner (Renishaw Dental Scanner)
4.5 Statistical analysis

Factorial analysis was used with two variables (MCPM and PLS) at high versus low levels, to assess their effects ($a_1$, $a_2$) and any interaction effect ($a_1a_2$) on the properties of composites (P) (Mehdawi et al., 2013b). The univariate general linear model in SPSS statistics version 25 for Windows (IBM, USA) was used to determine significant differences between groups ($\alpha=0.05$). The following equation 4.10 demonstrates the factorial expression employed:

$$\ln P = <\ln P> \pm a_1 \pm a_2 \pm a_1a_2$$  \hspace{1cm} 4.10

Where $<\ln P>$ is the average value of $\ln P$. The average effect of changing each variable ($a_i$) from low to high was calculated using the following equation 4.11:

$$a_i = 0.5\ln g_h/g_0$$  \hspace{1cm} 4.11

Where $g_h$ and $g_0$ are the geometric means of the property for samples sharing the same level of the variable (high versus low, respectively).

The following 4.12 equations were used to calculate the percentage increase or decrease in property (P) caused by a variable:

$$Increase\% = 100 \left( \exp^{2a_i} - 1 \right)$$

$$decrease\% = 100 \left( \frac{1}{\exp^{2a_i}} - 1 \right)$$  \hspace{1cm} 4.12
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4.6 Results

4.6.1 Densities and polymerisation shrinkage

Mean ±SD densities for paste and set materials are provided in Table 4.4. 
Measured polymerisation shrinkage results are illustrated in Figure 4.5. 
Results with higher PLS (F1 and F4) were slightly lower due to PLS lower 
density than filler particles. Polymerisation shrinkage ranged between 3.98% 
±0.04 and 2.96% ±0.02 for F2 and F1, respectively, which were significantly 
different (p<0.005). Results for F4 and F5 were in between, more variable and 
not significantly different from that of F2 (p>0.05).

Table 4.4 The calculated paste and disc densities for the evaluated formulations

<table>
<thead>
<tr>
<th></th>
<th>Paste density (g/cc)</th>
<th>Disc density (g/cc)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paste1</td>
<td>Paste2</td>
</tr>
<tr>
<td>F1</td>
<td>1.799</td>
<td>1.803</td>
</tr>
<tr>
<td>F2</td>
<td>1.838</td>
<td>1.853</td>
</tr>
<tr>
<td>F4</td>
<td>1.813</td>
<td>1.814</td>
</tr>
<tr>
<td>F5</td>
<td>1.855</td>
<td>1.865</td>
</tr>
</tbody>
</table>
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![Figure 4.5 Polymerisation shrinkage (%). Error bars are 95% CI (n=3).]
4.6.2 Assessment of mass and volume change

Figure 4.6 (A) shows plots of mass change versus square root of time. With all formulations, these increased linearly initially but began to level off or decline by 4 weeks.

Figure 4.6 (B) provides plots of volume change versus square root of time. These were linear until it began to level off and reached plateau values by 5 weeks.

Figure 4.6 Mean ±SD of mass (A) and volume (B) change in deionised water (over 11 weeks) as a function of square root of time (t/hr) of F1, F2, F4 and F5 (n=3).
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Figure 4.7 provides initial rate of mass and volume change versus square root of time. PLS significantly influenced initial rate of mass change ($p<0.05$). F2 (0.04%hr$^{-0.5}$ ±0.003) showed lower initial rate of mass change than F1 (0.06%hr$^{-0.5}$ ±0.004) ($p=0.015$) and F4 (0.06%hr$^{-0.5}$ ±0.003) ($p=0.003$). In addition, F4 had higher rate of mass change than F5 (0.05%hr$^{-0.5}$ ±0.008) ($p=0.046$). Results showed that composites with higher MCPM and PLS significantly increased initial rate of volume change ($p<0.05$). F1 (0.13%hr$^{-0.5}$ ±0.01) showed the highest initial rate of volume change and differed significantly to F2 (0.10%hr$^{-0.5}$ ±0.005), F4 (0.09%hr$^{-0.5}$ ±0.01) and F5 (0.07%hr$^{-0.5}$ ±0.004) ($p<0.001$). Furthermore, F5 showed lower volume change than F2 ($p<0.001$) and F4 ($p=0.002$).

![Initial rate of mass and volume change versus square root of time (%hr$^{-0.5}$). Error bars are 95% CI (n=3).]
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Figure 4.8 shows the final mass and volume change. After 11 weeks, F1 showed the lowest final mass change (-0.9 ±0.2) and differed significantly to F2 (0.1 ±0.1), F4 (1.1 ±0.1) and F5 (1.2 ±0.1) ($p<0.001$). In addition, F2 was significantly lower than F4 and F5 ($p<0.001$). The final values of volume change showed that F5 (2.6 ±0.1) was significantly lower than F1 (3.7 ±0.1), F2 (3.7 ±0.1) and F4 (3.9 ±0.3) ($p<0.001$).

![Figure 4.8 Mean ±SD of the final mass/volume change (11 weeks) in deionised water for different MCPM and PLS wt% of filler (n=3).](image-url)

<table>
<thead>
<tr>
<th>Material</th>
<th>PLS</th>
<th>MCPM</th>
<th>Final mass change (%)</th>
<th>Final volume change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>8</td>
<td>16</td>
<td>-0.9 ±0.2</td>
<td>3.7 ±0.1</td>
</tr>
<tr>
<td>F2</td>
<td>4</td>
<td>16</td>
<td>0.1 ±0.1</td>
<td>3.7 ±0.1</td>
</tr>
<tr>
<td>F4</td>
<td>8</td>
<td>8</td>
<td>1.1 ±0.1</td>
<td>3.9 ±0.3</td>
</tr>
<tr>
<td>F5</td>
<td>4</td>
<td>8</td>
<td>1.2 ±0.1</td>
<td>2.6 ±0.1</td>
</tr>
</tbody>
</table>
4.6.3 Assessment of H⁺ release

Unlike early mass and volume change, plotting H⁺ release versus SQRT gave no linear plots (see Appendix 2: Figure 7.1). Figure 4.9 and Figure 4.10 shows plots of H⁺ ion release versus time. H⁺ release was linear over time after 72hrs for all formulations but began to level off by 5 weeks for F1. Rates for H⁺ ion release were higher for composite formulations containing high levels of MCPM and PLS. These were 1.67, 0.96, 0.77 and 0.42 micromoles/(Lhr) for F1, F2, F4 and F5, respectively (R² > 0.95 in all cases). This followed burst release of 454, 319, 123 and 65 micromoles/L at 72hrs for F1, F2, F4 and F5, respectively. The final H⁺ ion release at 11 weeks was 2277 ±190, 2105 ±32, 1566 ±191 and 922 ±206 micromoles/L.

Figure 4.9 Hydrogen ion release versus time (hr) for 11 weeks in deionised water. Error bars are 95% CI (n=3).
Figure 4.10 Hydrogen ion release versus time (hr) after 72hrs in deionised water. Error bars are 95% CI (n=3).
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Figure 4.11 shows final H\(^+\) release (11 weeks) for all formulations. Both MCPM and PLS significantly influenced final H\(^+\) release \((p<0.05)\). F1 showed the highest H\(^+\) release and differed significantly to F4 \((p=0.002)\) and F5 \((p<0.001)\). In addition, F2 had higher H\(^+\) release than F4 \((p=0.009)\) and F5 \((p<0.001)\), while F4 showed higher H\(^+\) release than F5 \((p=0.003)\).

![Bar chart showing H\(^+\) release for all formulations](image)

Figure 4.11 11 weeks H\(^+\) release (micromoles/L) for all formulations. Error bars are 95% CI \((n=3)\).
4.6.4 Factorial analysis

Factorial analysis showed increasing PLS significantly increased mass change rate by 31% ($p<0.05$). Increasing either of the additives had significantly increased the initial volume change rate by 41% for MCPM and 31% for PLS ($p<0.05$) (Figure 4.12). Increasing MCPM and PLS resulted in a significant increase of final H$^+$ ion release by 135% and 59% ($p<0.05$), respectively. For the polymerisation shrinkage, SPSS general linear model indicated a significant interaction effect and showed that increasing both MCPM and PLS simultaneously led to lower polymerisation shrinkage by 15% ($p<0.05$). However, factorial analysis also showed that increasing either individually gave no significant effect as error bars for “a” values touch or overlap zero.

Figure 4.12 Factorial analysis of the evaluated properties: values and interactions of 2 variables (a1:MCPM, a2: PLS, a12: Interactions between the variables). Error bars are 95% CI ($n=3$).
4.6.5 Assessment of dried extracts from set materials

4.6.5.1 Scanning electron microscopy

Figure 4.13 shows SEM images of dried extracts from F1, F2, F4 and F5 samples immersed in deionised water for 3 months. Dried extracts from samples showed a relatively rough texture with different modifications to the topography depending on the area examined. After 3 months, dried extracts from formulations with high MCPM content (F1 and F2) show a smooth polymer area with MCPM platelets. It is noticeable that the volume fraction of the platelets increases with lowering PLS level to 4wt.% as with F2. Dried extracts from F4 show lots of polymer and bubbles, but with higher magnification, fibers like appearance are observed within the polymer surface. With F5 images, large aggregating spheres are noticed, on close inspection; spheres appear to have bundles of fibers.
Figure 4.13 SEM images of F1, F2, F4 and F5 dried extracts from samples immersed in deionised water for 3 months at 100-μm resolution and 200x magnification (Left) and 10-μm resolution and 2000x magnification (right).
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Figure 4.14 shows SEM images of dried extracts from F1, F2, F4 and F5 samples immersed in deionised water for 12 months. Dried extracts from formulations with high levels of MCPM show a polymer film that is approximately 10 micron thick with coarsened platelets structure. CaP platelets are approximately 3-micron diameter and <1 micron thick. F4 shows CaP fiber-like crystals dispersed within the polymer and creases that may have been formed upon water bubbles collapse. Dried extracts from F5 show coarsened structure with larger platelets and fibers.
Figure 4.14 SEM images of F1, F2, F4 and F5 dried extracts from samples immersed in deionised water for 12 months at 100-μm resolution and 200x magnification (Left) and 10-μm resolution and 2000x magnification (right).
4.6.5.2 FTIR spectra of the dried extracts

The FTIR spectra for the dried extracts were obtained after 12 months of immersion in deionised water (Figure 4.15). Peak assignments are provided in Table 3.1 and Appendix 1: Table 7.3. The MCPM peaks below 1300 cm\(^{-1}\) are 2.4 times the height obtained for the pure MCPM (compare Figure 4.15 and Figure 4.16) conversely the PLS peaks above 1300 cm\(^{-1}\) are 0.4 times the height for pure PLS for samples F1, F4 and F5 but approximately half this height for F2. Figure 4.17 shows the difference between spectra obtained for the extracts minus that expected from the pure components. The PLS broader peaks around 3200 cm\(^{-1}\) have partially shifted to lower wavenumbers while phosphate peak has been flattened/broadened.

Figure 4.15 FTIR spectra of the samples dried extracts for all the evaluated formulations after immersion in deionised water for 12 months.
Chapter 4: Polymerisation shrinkage, mass & volume change, $H^+$ release, dried extracts and wear resistance

Figure 4.16 MCPM and PLS FTIR spectra.

Figure 4.17 Difference FTIR spectra between actual spectra of the samples dried extracts for all the evaluated formulations after immersion in deionised water for 12 months and sums of relative levels of MCPM and PLS (F1= MCPM 2.4, PLS 0.4wt%, F2= MCPM 2.4, PLS 0.2wt%, F4= MCPM 2.28, PLS 0.35wt%, F5= MCPM 2.3, PLS 0.35wt%).
4.6.5.3 Energy dispersive x-ray spectroscopy- surface analysis of dried extracts

Elemental composition (wt.%) of the samples dried extracts after 3 and 12 months storage in deionised water are provided in Appendix 2: Table 7.4, Table 7.5 and Table 7.6.

Table 4.5 provides average molar ratios of the key elements in dried extracts from 3 and 12 months storage solutions.

Table 4.5 Mean ±SD of the molar ratios of the samples dried extracts key elements after 3 and 12 months storage in deionised water.

<table>
<thead>
<tr>
<th>3 months</th>
<th>F1</th>
<th>F2</th>
<th>F4</th>
<th>F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (±SD)</td>
<td>P/Ca</td>
<td>P/Cl</td>
<td>O/P</td>
<td></td>
</tr>
<tr>
<td>3.4 ±0.1</td>
<td>5.7 ±0.5</td>
<td>3 ±0.2</td>
<td>3.8 ±0.2</td>
<td></td>
</tr>
<tr>
<td>2.6 ±0.2</td>
<td>3.6 ±0.2</td>
<td>1.3 ±0.1</td>
<td>2.6 ±0.1</td>
<td></td>
</tr>
<tr>
<td>6.3 ±0.6</td>
<td>4.8 ±0.1</td>
<td>6.2 ±0.5</td>
<td>5.5 ±0.2</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>12 months</th>
<th>F1</th>
<th>F2</th>
<th>F4</th>
<th>F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (±SD)</td>
<td>P/Ca</td>
<td>P/Cl</td>
<td>O/P</td>
<td></td>
</tr>
<tr>
<td>2.8 ±0.1</td>
<td>2.7 ±0.2</td>
<td>2.5 ±0.1</td>
<td>2.7 ±0.1</td>
<td></td>
</tr>
<tr>
<td>1.4 ±0.2</td>
<td>6.6 ±1.6</td>
<td>2.1 ±0.2</td>
<td>2.3 ±0.2</td>
<td></td>
</tr>
<tr>
<td>6 ±0.7</td>
<td>5.9 ±0.4</td>
<td>6.6 ±0.4</td>
<td>5.6 ±0.2</td>
<td></td>
</tr>
</tbody>
</table>

The mass ratio of MCPM/PLS for F1, F2, F4 and F5 is 2, 4, 1 and 2. Molar ratios are obtained by multiplying these by 183/252=0.73
Figure 4.18 provides the P/Ca ratios from EDX spectra (n=9) of extracts in water after 3 months and 1 year sample storage.

At 3 months, the P/Ca ratio was higher than at 1 year due to initial high phosphoric acid release followed by slower release of Ca as dicalcium phosphate particularly for F2. Both MCPM and PLS significantly influenced P/Ca ratios ($p<0.05$). Results also showed that F2 (5.7 ±0.5) had the highest P/Ca ratio than F1 (3.4 ±0.1), F4 (3 ±0.2) and F5 (3.8 ±0.2) ($p<0.001$). Additionally, F4 had lower P/Ca ratio than F1 ($p=0.047$) and F5 ($p<0.001$).

At 1 year, F1 (2.8 ±0.1) had higher P/Ca ratio than F4 (2.5 ±0.1) ($p=0.001$).

<table>
<thead>
<tr>
<th>Material</th>
<th>3 month P/Ca</th>
<th>1 year P/Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLS</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>MCPM</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>F1</td>
<td>3.4 ±0.1</td>
<td>2.8 ±0.1</td>
</tr>
<tr>
<td>F2</td>
<td>5.7 ±0.5</td>
<td>3.3 ±0.2</td>
</tr>
<tr>
<td>F4</td>
<td>3 ±0.2</td>
<td>2.5 ±0.1</td>
</tr>
<tr>
<td>F5</td>
<td>3.8 ±0.2</td>
<td>3.1 ±0.1</td>
</tr>
</tbody>
</table>

Figure 4.18 Mean ±SD of the P/Ca (moles) of 9 EDX spectra after immersion in deionised water for 3 months and 1 year.
Figure 4.19 shows the P/Cl ratios from EDX spectra (n=9) of extracts in water after 3 months and 1 year sample storage.

At 3 months the ratio of P/Cl is approximately 2, 4, 1 and 2 as expected as the MCPM/PLS ratio is also 2, 4, 1 and 2 in F1, F2, F4 and F5, respectively. F2 (3.6 ±0.2) showed the highest P/Cl ratio than F1 (2.6 ±0.2), F4 (1.3 ±0.1) and F5 (2.6 ±0.1) (p<0.001). Furthermore, F4 had lower P/Cl ratio than F1 and F5 (p<0.001).

With F1, P/Cl decreases at 1 year consistent with phosphoric acid levelling off but some continuation of chloride possibly with PLS. The increase in P/Cl with F2 and F4 suggests some reduction in the rate of PLS vs phosphorus. F2 (6.6 ±1.6) showed the highest P/Cl ratio after 1 year and differed significantly to F1 (1.4 ±0.2), F4 (2.1 ±0.2) and F5 (2.3 ±0.2) (p<0.001).

Figure 4.19 Mean ±SD of the P/Cl (moles) of 9 EDX spectra after immersion in deionised water for 3 months and 1 year.
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Figure 4.20 shows plots of P/Ca and P/Cl versus MCPM/PLS molar ratio of EDX spectra (n=9) after immersion in deionised water for 3 months. The P/Ca and P/Cl ratio increases with increasing MCPM/PLS ratio.

Figure 4.20 Mean P/Ca and P/Cl (moles) versus MCPM/PLS HCl ratio (moles) of 9 EDX spectra after immersion in deionised water for 3 months. Error bars are 95% CI.
Figure 4.21 shows plots of P/Ca and P/Cl versus MCPM/PLS molar ratio of EDX spectra (n=9) after immersion in deionised water for 1 year. Increasing MCPM/PLS ratio has minimal effect on P/Ca and P/Cl ratio at 1 year for all formulations except F2, as it requires longer time to reach equilibrium.

Figure 4.21 Mean P/Ca and P/Cl (moles) versus MCPM/PLS HCL ratio (moles) from EDX spectra (n=9) after immersion in deionised water for 1 year. Error bars are 95% CI.
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For P/Ca, factorial analysis showed that increasing MCPM significantly increased the ratio by 30% after 3 months ($p<0.001$) and 5% after 1 year ($p=0.004$). Results also showed that increasing PLS led to lower P/Ca ratio by 45% after 3 months ($p<0.001$). In addition, increasing both MCPM and PLS simultaneously led to lower P/Ca ratio by 15% after 3 months ($p<0.001$) and higher ratio by 5% after 1 year ($p=0.01$).

For P/Cl, results showed that increasing MCPM significantly increased P/Cl ratio by 65% after 3 months and 33% after 1 year ($p<0.001$) (Figure 4.22). Increasing PLS resulted in a significant decrease of P/Cl ratio by 69% after 3 months and 131% after 1 year ($p<0.001$). SPSS general linear model indicated a significant interaction effect and showed that increasing both MCPM and PLS simultaneously led to higher P/Cl ratio by 21% after 3 months ($p=0.01$) and lower ratio by 115% after 1 year ($p<0.001$).

![Figure 4.22 Factorial analysis of the evaluated properties: values and interactions of 2 variables (a1: MCPM, a2: PLS, a12: Interactions between the variables). Error bars are 95% CI (n=9).](image-url)
4.6.6 Calculation of the volume loss after simulated wear testing

Figure 4.23 and Figure 4.24 illustrate the surface volume loss upon wear of samples that had been pre-stored in deionised water for 3 months and 1 year, respectively. After 3 months, the highest surface volume loss was 3.40 ±1.47 mm$^3$ and the lowest was 2.21 ±0.06 mm$^3$ for F2 and F4, respectively. No commercial comparisons were evaluated at 3 months. No statistical differences were found between groups.

For samples of the experimental composites stored for 1 year in deionised water, the highest surface volume loss was 2.89 ±0.45 mm$^3$ and the lowest was 1.72 ±0.40 mm$^3$ for F7 and F4 respectively. The surface volume loss for commercial materials ranged between 1.20 ±0.46 to 1.72 ±0.26 mm$^3$ with the highest average for Fuji II and the lowest for Activa but no statistical difference was found between them. In addition, F7 showed higher surface volume loss and differed significantly to Activa ($p=0.046$), Fuji II and F4 ($p<0.05$).

Figure 4.25 provides three-dimensional images of cylindrical samples after wear testing. All wear facets exhibited typical oval shapes resulting from abrasive wear by steatite antagonists. The worn surface on Fuji II sample appears to be longer than other samples. The likely cause is that samples had shifted in the initial stage of testing.
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dried extracts and wear resistance

Figure 4.23 Mean surface volume loss (mm$^3$) after simulated wear testing (3 months storage in 
deionised water). Error bars are half the difference between samples of each formulation (n=2).

Figure 4.24 Mean ±SD of the surface volume loss (mm$^3$) after simulated wear testing (1 year storage in 
deionised water) (n=3). Upper case letters represent statistical groups. Groups that do not share the 
same letter are significantly different (p<0.05).
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Figure 4.25 Three-dimensional images showing the wear facets
4.7 Discussion

4.7.1 Polymerisation shrinkage

The null hypothesis tested in the present study, which assumed increasing additive addition would not have a significant effect on the experimental composite dimensional stability and shrinkage, was accepted.

Theoretically, polymerisation shrinkage would be proportional to degree of conversion, monomer volume fraction, number of methacrylate groups per monomer and inverse average monomer molecular weight. In addition, filler load, size and shape can significantly influence the polymerisation shrinkage of the evaluated experimental composite formulations (Satterthwaite et al., 2009, Baroudi et al., 2007). Therefore, significant differences would be expected in polymerisation shrinkage between the formulations, as the inorganic filler content increases with decreasing the reactive fillers. Previous studies in our group showed that CaP had no significant effect while PLR had major effect on polymerisation shrinkage (Aljabo et al., 2015, Alkhouri, 2019). The lower polymerisation shrinkage with F1 could be related to the high internal porosity caused by MCPM. Furthermore, PLS might decrease shrinkage as it has lower density than the other fillers and so occupies a greater volume thereby reducing the volume fraction of the monomer phase.
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The replacement of TEGDMA with the high molecular weight PPGDMA may improve the polymerisation shrinkage for the experimental formulations (Kangwankai et al., 2017). The findings showed that MCPM and PLS had only minor effect on the measured shrinkage (F1: 2.9\%, F2: 3.9\%, F4: 3.7\% and F5: 3.6\%). The results are in agreement with previous investigations on the polymerisation shrinkage of commercial composites that ranged between 2 and 6 vol.\% (Kleverlaan and Feilzer, 2005). Also the findings showed relatively similar results when compared with previous studies on the polymerisation shrinkage in our research group that ranged between 2.5 to 3.5\% (Aljabo et al., 2015).

4.7.2 Assessment of mass and volume change and H\(^+\) release

The null hypothesis tested in the present study, which assumed increasing additive addition would not have a significant effect on the experimental composite mass or volume change following water-sorption and H\(^+\) ion release into water was rejected.

4.7.2.1 Mass change

The results of this study are in agreement with previous findings that showed early mass increase is proportional to square root of time for all experimental formulations (Kangwankai et al., 2017, Panpisut et al., 2016). The findings showed that increasing MCPM resulted in decreasing final mass change. The
water sorption would be expected to subsequently dissolve PLS and MCPM components resulting in their diffusion-controlled release. MCPM may then disproportionate into dicalcium phosphate (CaHPO$_4$) and phosphoric acid (H$_3$PO$_4$). The precipitation of dicalcium phosphate as lower solubility brushite could bind water within the composite. The final negative mass change of F1 might have been a consequence of greater mass loss compared with mass gain arising from water sorption. A possible explanation of the greater mass loss is the replacement of MCPM (2.22 g/cc) components with the lower density water molecules (1 g/cc). The final mass change results suggest that F4 and F5 with low MCPM have reached equilibrium at 11 weeks, while F1 and F2 continue to change. In this study, the 2mm sample thickness required longer time for the mass change (4 weeks) to level off or decline when compared to the 1mm sample thickness used in previous studies (Kangwankai et al., 2017, Panpisut et al., 2019). It is also important to point out that the monomer weight fraction is constant, but the volume fraction is not due to the lower PLS density. In addition, the total glass filler content is increasing with decreasing the reactive fillers. This could explain the variations in dimensional stability in this study as F5 (88wt.% glass filler) showed better dimensional stability upon water sorption than F1 (76wt.% glass filler).
4.7.2.2 Volume change

The water uptake is expected to expand the polymerised resin-matrix and PLS particles and result in subsequent volume increase of the resin composite samples (Mehdawi et al., 2013a). The volume expansion of the composites may compensate the polymerisation shrinkage and relieve the residual shrinkage stress (Park and Ferracane, 2014). However, imbalance between polymerisation shrinkage and volume expansion may result in marginal leakage and subsequent secondary caries. The findings showed that lowering either PLS or MCPM resulted in a lower volume change with lowest value presented when both were at low wt. % (F5: 2.5vol.%). The results also showed that increasing MCPM levels resulted in higher rate of volume change. The hydrophilic nature of PLS and MCPM may encourage water sorption and expands the surrounding polymer matrix. In addition, previous studies suggest this may be due to lower density brushite formation in the bulk of the materials forcing expansion of the surrounding resin matrix (Mehdawi et al., 2009, Mehdawi et al., 2013a). Similar effect of calcium phosphate (CaP) on mass and volume change was noticed in a previous study (Aljabo et al., 2015).

4.7.2.3 H⁺ release

The findings of this study showed that MCPM has markedly impacted H⁺ ion release. The considerable increase in H⁺ ions with F1 and F2 is related to the high levels of MCPM component that result in high phosphoric acid release. The
amount of phosphoric acid released can be assumed from the amount of MCPM released in water using the following equations:

\[
\text{Ca(H}_2\text{PO}_4)_2\cdot\text{H}_2\text{O} \rightarrow \text{CaHPO}_4 + \text{H}_3\text{PO}_4 + \text{H}_2\text{O}
\]

Therefore:

\[1 \text{ mol MCPM} \Rightarrow 1 \text{ mol } \text{H}_3\text{PO}_4\]

The number of moles of MCPM can be calculated from the following equation 4.13:

\[
(mol) \text{ MCPM} = \frac{A_{ave} \times W_i \times \text{MCPM}_i}{\text{MCPM}_{mw}}
\]

Where:

\[A_{ave}\] is the average weight of each specimen (0.3g)

\[W_i\] is the weight fractions of filler (0.75)

\[\text{MCPM}_i\] is the fraction of MCPM in the filler (0.16 or 0.08)

\[\text{MCPM}_{mw}\] is MCPM molecular weight (252g/mol)

For each sample, assuming its total mass is 0.3g, the percentage of maximum acid release from MCPM can be calculated (Table 4.6):
Chapter 4: Polymerisation shrinkage, mass & volume change, H+ release, dried extracts and wear resistance

Table 4.6 Mass/Moles of MCPM in each sample and H+ release at 11 weeks (%)

<table>
<thead>
<tr>
<th>Material</th>
<th>Mass of MCPM (g)</th>
<th>Mols of MCPM</th>
<th>H+ (mole/L)</th>
<th>H+ of MCPM at 11 weeks (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.036</td>
<td>0.00014</td>
<td>0.0023</td>
<td>16</td>
</tr>
<tr>
<td>F2</td>
<td>0.036</td>
<td>0.00014</td>
<td>0.0021</td>
<td>15</td>
</tr>
<tr>
<td>F4</td>
<td>0.018</td>
<td>7.14E-05</td>
<td>0.0016</td>
<td>22</td>
</tr>
<tr>
<td>F5</td>
<td>0.018</td>
<td>7.14E-05</td>
<td>0.0009</td>
<td>13</td>
</tr>
</tbody>
</table>

Moles released into the 10ml of storage solution is 100 times smaller than this figure

The thickness of each sample is 2mm (2000μm). The layer thickness of MCPM release from each sample if only from the top and bottom surfaces is then calculated to be 159, 147, 219 and 129μm for F1, F2, F4 and F5 respectively.

The amount of H+ released from each sample in 10ml of deionised water is 16% (0.000023mol/10ml) and 15% (0.000021mol/10ml) for F1 and F2, respectively. While, 22% (0.000016mol/10ml) and 13% (0.000009mol/10ml) of H+ was released with F4 and F5, respectively. The findings showed that increasing the amount of PLS resulted in higher percentage of phosphoric acid release. This could be related to the ability of PLS to enhance water sorption and the high aqueous solubility of MCPM in water.
4.7.3 Assessment of the dried extracts

The null hypothesis tested in the present study, which assumed increasing additive addition would not have a significant effect on components released versus time was rejected.

Component release may affect bacteria or cell compatibility due to antibacterial PLS and acid release vs time. Fast acid release could cause degradation of collagen. Slow acid attack results in dentinal tubules becoming blocked by crystals as a natural defence mechanism. Therefore, slow acid release may enable remineralisation and sealing of tubules. The material under trial has low MCPM and PLS and it would be beneficial to determine if increasing either or both components give valuable release that improves remineralisation process.

4.7.3.1 Scanning electron microscopy

For SEM images, a change in the morphology of the phases was noticeable. Early release of calcium and phosphate is required to supersaturate the storage water and initiate the precipitation process. After 3 months, high precipitation of CaP platelets would be expected due to high levels of MCPM in F1 and F2. In addition, the morphology of the aggregating spheres in F5 is similar to the morphology of hydroxyapatite crystals from a previous study on the characterisation of hydroxyapatite formation (Aljabo et al., 2015). After 12
months, as pH decreases below 4, brushite becomes less soluble and therefore precipitates to form a coarsened structure with larger platelets and fibers.

### 4.7.3.2 FTIR spectra of the dried extracts

When light interacts with matter, there is absorption of the incident radiation energy. The transmitted radiation is different, because of this energy absorbance. The amount of light absorbed by the sample is then expressed as the absorbance. This absorbance is directly proportional to the sample concentration. The Beer-Lambert Law describes the relationship between absorbance and concentration. This law explains how light and sample properties are correlated.

\[ A = \varepsilon c l \]  \hspace{1cm} 4.14

In order to semi-quantify MCPM and PLS levels in dried extracts, the spectra of raw MCPM and PLS components were obtained. A model was then created which allowed the prediction of individual contributions of each raw component, multiplying them by a constant, giving a final spectrum. This spectrum was then fitted to the spectrum of the dried extract. Increasing or decreasing the ratios of each component led to the determination of the closest fit. The relative sums and ratios of MCPM and PLS in dried extracts were as the following:

F1= MCPM (2.4wt.%), PLS (0.4wt.%), MCPM: PLS = 6:1
Chapter 4: Polymerisation shrinkage, mass & volume change, H+ release, dried extracts and wear resistance

F2 = MCPM (2.4 wt.%), PLS (0.2 wt.%), MCPM: PLS = 12:1

F4 = MCPM (2.28 wt.%), PLS (0.35 wt.%), MCPM: PLS = 6:1

F5 = MCPM (2.3 wt.%), PLS (0.35 wt.%), MCPM: PLS = 6:1

The MCPM: PLS ratios observed above are 3 times the MCPM: PLS wt.% ratio in the material for F1, F2 and F5. For F4, the ratio is 6 times that in the material. This may be a consequence of higher percentages of MCPM vs PLS release and/or the spectra of powders being very sensitive to the pressure applied with the FTIR anvil.

FTIR spectra showed that when there is more PLS some of the MCPM peaks disappear. This could be related to the presence of phosphoric acid or to the change in the crystallinity caused by PLS which might affect some of the peaks more than others or reducing water of crystalisation because of the effect on the peak $\sim 3300 \, \text{cm}^{-1}$.

4.7.3.3 Energy dispersive x-ray spectroscopy- surface analysis of dried extracts

Ideally, the P/Ca ratio was expected to be 2:1 as each MCPM molecule has two phosphorus and one calcium ion (Ca(H$_2$PO$_4$)$_2$). Higher ratios may suggest some phosphoric acid release. The P/Ca ratio at 3 months was higher than expected, which is due to the initial high phosphoric acid release and the lower rate of release of dicalcium phosphate. The high P/Ca ratio with F2 is due to high MCPM content that results in a high phosphoric acid release. Whilst lower PLS
content may reduce the amount of water sorption and results in a lower rate of calcium release.

The findings showed that P/Ca at 1 year is 2.67 suggesting the ratio on the surface of the material would be expected to have decreased from 2 to 1.33. For 2.67 need 1 MCPM + 0.67 H3PO4 (Chow, 2009). The most dominant ions were the oxygen, phosphorus, chloride and calcium. The breakdown of calcium phosphate resulted in O, P and Ca ions, while Cl is from PLS hydrochloride. The O/P ratio was expected to be 4:1 if only phosphate species were released as each MCPM molecule has 8 oxygen and 2 phosphorus ions. The results showed O/P ratios higher than expected which suggests PLS release. In addition, a lower amount of calcium and phosphate were released in distilled water than in simulated body fluids over time (Kangwankai et al., 2017).

### 4.7.4 Simulated wear testing

The null hypothesis tested in the present study, which assumed increasing additive addition would not have a significant effect on wear resistance was accepted.

Wear resistance is a property that needs to be carefully considered when choosing the appropriate restorative materials in clinical practice. It was found that wear is one of the significant modes of failure for posterior resin composite in
patients with bruxism and clenching habits (Ferracane, 2006). Therefore, investigating the wear resistance for materials development is of a vital importance. Loads, speed, friction mode, surface characteristics, and lubrication can influence the wear process. For resin composites, the wear process is mainly affected by the filler particle size and shape and the interfacial bonding strength. In this study, wear was evaluated by using quantitative methods to determine volume loss. The resin composite specimens were tested at 120,000 chewing cycles which represent six month of clinical wear (Heintze et al., 2012). Steatite balls were used as antagonists as they yield similar wear rates on composites as enamel (Shortall et al., 2002). The round shape of the antagonist provides a greater contact area compared to pointed antagonists. Therefore less fatigue wear is created (Heintze, 2006). The antagonists were not scanned before and after testing, which is considered one of the shortcomings of this study. The chewing forces were adjusted to 50 N which represents the mean physiological biting forces for patients without bruxism (Heintze et al., 2007). The findings showed that increasing MCPM resulted in higher surface volume loss as with F1 (2.2mm$^3$) and F2 (2.7mm$^3$). This could be related to high surface pores caused by MCPM release, which may result in lower wear resistance. In addition, F1 (76wt.%) and F2 (80wt.%) have lower glass filler content than other formulations (F4: 84wt.%, F5: 88wt.%), which may contribute to high surface volume loss. Furthermore, reducing MCPM particle size (F7: 10µm) may result in a higher release of CaP as the interface with water becomes larger (Xu et al., 2007b),
thus, higher surface pores and low wear resistance. The limitations of this study would be the lack of sample repetitions. In addition, operator uncertainty and reproducibility of the results were not evaluated. Furthermore, the actual unworn surface should have been scanned and superimposition software used following scanning of the wear cavity to have a true reading rather than an assumed reading. All the limitations of this study are due to the limited time and access to the EDI research lab, as we had to pack and move out of the lab. That was followed by the outbreak of COVID-19 and the lockdown.
4.8 Conclusion

Based on the findings of this study the following can be concluded:

1. Polymerisation shrinkage was not significantly influenced by MCPM and PLS.
2. The increase of MCPM and PLS led to higher volume change and H+ ion release, while increasing PLS led to higher mass change.
3. Reducing MCPM particle size led to lower wear resistance than commercial comparisons.
Chapter 5: Assessment of the colour stability of resin-composite pastes at different storage temperatures

5. Assessment of the colour stability of resin-composite pastes at different storage temperatures

5.1 Introduction

The increased demand for aesthetics in modern dentistry has greatly influenced the popularity of tooth-coloured restorative materials (Arregui et al., 2015). These dental materials are widely used due to their ability to match the colour with natural teeth (e Silva et al., 2014). Glass ionomers, dental ceramics and resin-composites are the common tooth-coloured restorative materials used in esthetic dentistry. The most common concerns with these materials are the colour stability and their long-term durability in the oral cavity. It was found that dental ceramics have the highest colour stability in the oral cavity followed by resin-composites and glass ionomers (Arregui et al., 2015). Resin-composites are more popular among tooth-coloured restorative materials due to their optimum strength, aesthetics and bonding to tooth structure (Bindal et al., 2015, Badra et al., 2005). Resin composites should have and maintain the same appearance as natural teeth to achieve the optimum esthetic results. However, resin-composites usually undergo colour change over time in the oral cavity (Choi et al., 2005, Correr et al., 2012, Bagheri et al., 2005).

Spectrophotometers are currently used to quantify the colour change (ΔE) of restorative materials by measuring the L*, a* and b* colour coordinates in the CIELAB color space (Zanetti et al., 2019). The L* coordinate represent the
brightness, the a* is the red or green component of the colour (positive a* indicates redness and negative a* represents greenness), whereas the b* coordinate is the yellow or blue component of the colour (positive b* represents yellowness and negative b* indicates blueness). For example, after composite polymerisation, a change in the b* coordinate toward the negative indicates that the composite has lost its yellowness. The discoloration of tooth-coloured restorative materials may occur due to internal or external factors. The internal factors include discoloration in the resin-matrix or at the matrix–filler interface (Yap and Wee, 2002). The external factors such as changes in temperature and humidity, staining due to exposure to solutions such as tea, coffee, coloured beverages or discoloration as a result of aging (Villalta et al., 2006).

5.2 Objectives
The project objectives were to assess effects on L* a* b* of storage container, sample depth and time of high temperature aging of four experimental resin-composite formulations containing PLS (8, 4wt%) & MCPM (16, 8wt%).

5.3 Hypotheses
The Null hypotheses are:

- Storage container.
- High temperature storage.
Chapter 5: Assessment of the colour stability of resin-composite pastes at different storage temperatures

- Increasing components.
- Sample thickness.

Will not have a significant effect on $L^* \ a^* \ b^*$ values of experimental resin-composite pastes.
5.4 Materials and Methods

5.4.1 Material preparation and storage

5.4.1.1 Paste stored in sealed glass containers

In this study, four experimental resin-composite pastes (PLS 8 or 4wt% and MCPM 16 or 8wt%) (F1, F2, F4 and F5) were evaluated. The glass filler content for each formulation is provided in Table 5.1. These were manufactured by Dr Wendy Xia and produced at Synergy Devices Ltd. (Wycombe, UK). The formulations were made on 12th of April 2017 and stored in plastic black containers at 4°C or in glass containers at 60°C for 20 months. All samples stored at 60°C were analyzed after this time whilst the F5 formulation stored at 4°C was used as a baseline for all studies. Additionally, some of each of the pastes stored at 4°C was subsequently transferred and stored at 80°C for a further 2 or 4 weeks before analysis.

Table 5.1 Experimental MCPM, PLS, glass filler, storage container, time and temperature of the evaluated experimental resin-composite formulations used in this chapter

<table>
<thead>
<tr>
<th>Formulations</th>
<th>MCPM (wt% of filler)</th>
<th>PLS (wt% of filler)</th>
<th>Glass filler (wt% of filler)</th>
<th>Storage container</th>
<th>Time and Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>16</td>
<td>8</td>
<td>76</td>
<td>Sealed glass container</td>
<td>60°C for 20 months.</td>
</tr>
<tr>
<td>F2</td>
<td>16</td>
<td>4</td>
<td>80</td>
<td></td>
<td>80°C for 2 or 4 weeks</td>
</tr>
<tr>
<td>F4</td>
<td>8</td>
<td>8</td>
<td>84</td>
<td></td>
<td>F5 stored at 4°C and used as a baseline.</td>
</tr>
<tr>
<td>F5</td>
<td>8</td>
<td>4</td>
<td>88</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.4.1.2 Paste stored in compules

Data produced earlier (Pitsillou, 2019) was reanalysed to enable comparison of this new data with the colour stability of the F5 resin composite formulation stored in compules. This formulation was manufactured by DMG on 11\textsuperscript{th} of December 2017 and placed into compules on the 3\textsuperscript{rd} of March 2018. Initial L* a* b* parameters were obtained using samples stored at 4°C. Paste in compules was analyzed after storage at 25°C for 11 months and 12 months or 37°C for 1, 3, 4 and 6 months. Additionally, at elevated temperatures of 60 and 80°C data for samples stored for 112 days or 26 days respectively was used (Table 5.2) (Pitsillou, 2019).

Table 5.2 MCPM, PLS, glass filler, time and temperature of F5 paste stored in compules by Pitsillou, 2019.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>MCPM</th>
<th>PLS</th>
<th>Glass filler</th>
<th>Storage container</th>
<th>Time and Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F5</td>
<td>8</td>
<td>4</td>
<td>88</td>
<td>Compules</td>
<td>- 25°C for 11 and 12 months.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- 37°C for 1, 3, 4 and 6 weeks.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- 60°C for 112 days.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- 80°C for 112 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- F5 stored at 4°C and used as a baseline.</td>
</tr>
</tbody>
</table>
5.4.2 Sample preparation

Composite discs (1 or 4mm thickness × 10 mm diameter) were prepared using cylindrical metal circlips (1 mm thickness × 10 mm diameter). The circlips were placed on acetate sheet on top of a glass slab to provide moulds of 1 or 4 mm thickness. Then, resin-composite was packed into the mould and a second piece of acetate sheet was placed on top of the circlip and compressed using a glass slab to achieve even thickness and prevent air inclusion. All samples were light cured at the center of the top surface of the disc (Figure 5.1) using a single emission peak light emitting diode (LED) light curing unit (LCU) (Demi Plus, Kerr, Orange, CA, USA) with power output from 1100 mW/cm² to peak of 1330 mW/cm² and spectral emission ranging from 450 through 470 nm. The LCU details were according to the manufacturer’s instructions. Samples with 1 mm thickness were cured for 20s. This curing time was chosen to enable comparison with the results of the previous study of 1mm thick samples of F5 packaged in compules (Pitsillou, 2019). To evaluate the effect of sample thickness on colour stability, new samples of 4 mm thickness were cured for 40s. For all formulations readings were taken from the top and bottom surfaces (n=1). For the pastes results were averaged giving n=2 in bar charts. For (Pitsillou, 2019) sample repetition number for pastes and cured samples was n=1.
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5.4.3 Spectroshade colour evaluation

The shade of the samples was recorded by using Spectroshade micro (MHT Optic Research, AG, Niederhasli, Switzerland). The spectrophotometer uses a combination of a digital camera and a light emitting diode. The device was calibrated using the white and green calibration tiles on the docking station provided by the manufacturer. The calibration was performed every time before an image of a sample was taken. Images of the new resin-composite pastes with acetate sheet left in place as well as of the top and bottom surfaces of the discs after polymerisation and acetate removed were obtained. The instrument was held directly against the composite sample and when a clear image was displayed on the screen the image was captured.

The International Commission on Illumination (CIE) L*, a*, b* coordinates of the colour were obtained for each image (Figure 5.2). The chromacity of the colour
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(b* values) were then imported, sorted and analysed in graphs in Microsoft Office Excel.

Figure 5.2 Shade analysis ($L^*$, $a^*$, $b^*$ coordinates).
5.5 Statistical analysis

Factorial analysis was used with two variables (MCPM and PLS) at high and low levels to assess the effect of variables \( (a_1, a_2) \) and their interactions \( (a_{1,2}) \) on the \( b^* \) parameter of composites \( (P) \) (Mehdawi et al., 2013a).

The following equation demonstrates the factorial expression employed:

\[
\ln P = <\ln P> \pm a_1 \pm a_2 \pm a_{1,2}
\]

Where \( <\ln P> \) is the average value of \( \ln P \). The average effect of changing each variable \( (a_i) \) from low to high was calculated using the following equation:

\[
a_i = 0.5 \ln \frac{g_h}{g_0}
\]

Where \( g_h \) and \( g_0 \) are the geometric means of the property of samples sharing the same level of the variable (high versus low, respectively).

The following equation 5.1 were used to calculate the percentage increase or decrease in property \( (P) \) caused by a variable \( (a_i) \):

\[
Increase\% = 100 \left( \exp^{2a_i} - 1 \right)
\]

\[
decrease\% = 100 \left( \frac{1}{\exp^{2a_i}} - 1 \right)
\]

5.1
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5.6 Results

5.6.1 Effect of Temperature

5.6.1.1 Baseline 4, 25 and 37 °C storage of F5, 1mm thick samples

Figure 5.3 illustrates baseline L*, a* and b* values of F5 resin-composite formulation stored in compules (Pitsillou, 2019) at 4, 25 and 37°C and sealed glass container at 4°C. The findings showed very low variability in L*, a* and b* at lower temperature storage irrespective of the storage container.

In general, removal of the acetate sheet from the paste raises b* and L* and lowers a* values. Following cure, a* and b* are closer to zero (i.e. reduction in green and yellow colour respectively due to photobleaching). Values on top surfaces were only marginally different from those on the bottom surfaces.

5.6.1.2 L* values

The average L* values for paste without acetate was 84.5 ±1 while the average value for paste with acetate was 78 ±1. After cure, the average value on top surfaces was 77 ±1.6 while bottom surfaces was 77 ±2.

5.6.1.3 a* values

The average a* values for paste without acetate was -7.1 ±0.4 while the average value for paste with acetate was -6.1 ±0.3. After cure, the average value on top surfaces was -2.1 ±0.3 while bottom surfaces was -2.4 ±0.5.
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5.6.1.4 $b^*$ values

The average $b^*$ values for paste without acetate was 29 ±1.3 while the average $b^*$ values for paste with acetate was 23 ±1. After cure, the average $b^*$ values on top surfaces was 6 ±2 while bottom surfaces was 7.5 ±2. $b^*$ values were used to evaluate colour stability in this study as they showed more significant change than $L^*$ and $a^*$ in the previous study (Pitsillou, 2019).
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Figure 5.3 L* (A) a* (B) and b* (C) values versus time of paste storage (days) for uncured samples and set materials of F5 experimental formulation, stored at 4, 25 and 37°C (n=1) in compules by Pitsillou, 2019 and at 4°C in sealed containers (n=1). Abbreviations: Numbers: represent storage temperature in degree Celsius (°C). P: Uncured paste (red symbols). PA: Paste with acetate sheet (blue symbols). CT: Cured top (green symbols). CB: Cured bottom (black symbols) *: Paste stored in sealed containers. Diamond: 4 °C. Triangle: 25 °C. Square: 37 °C Circle: Sealed glass container.
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5.6.2 Effect of 60°C storage on F5, 1mm thick samples

Figure 5.4 illustrates $b^*$ values for F5 resin-composite formulation stored in sealed glass container at 60°C or plastic container at 4°C both for 20 months versus compules (Pitsillou, 2019) at 60°C for 112 days. The colour was more stable for composite paste after long time storage (20 months) at 60°C in sealed glass container than paste stored in compules. Average $b^*$ values for paste stored in sealed container was 21 ±0.4 and 20 ±0.5 at 4°C and 60°C, respectively, whilst for paste stored in compule at 60°C was 34 ±0.3. Following cure, for all storage conditions, $b^*$ values on top surfaces for 1mm thick samples were only slightly lower than on bottom surfaces.

![Graph](image)

Figure 5.4 $b^*$ values for the uncured samples and set materials of F5 experimental formulation at 1mm depth light cured for 20s stored at 4°C and aged at 60°C for 112 days in compules by Pitsillou, 2019 and 609 days in sealed containers. Error bars are half the difference between samples. Paste ($n=2$) and set materials (60°C: $n=1$ and 4°C: $n=2$).
5.6.3 Effect of 80°C storage on F5, 1mm thick paste

Figure 5.5 illustrates plots of L*, a* and b* values versus time for F5 resin-composite paste following storage in compules (A) (Pitsillou, 2019) at 80°C for 0, 3, 7, 10, 14, 21 and 26 days or sealed glass container (B) for 0, 14 and 28 days.

5.6.3.1 L* values
Plots of L* values versus time slightly decreased linearly over time for paste stored in compules from 79 by -0.23/day. On the contrary L* values were more stable for paste stored in sealed containers and had an initial value of 77.

5.6.3.2 a* values
Plots of a* values versus time slightly increased linearly over time for paste stored in compules with initial value of -6.5 and rate of 0.15/day. a* values showed minor variation with time for paste stored in glass container and an initial value of -7.

5.6.3.3 b* values
b* values for paste stored in compules increased from 22 by 0.5/day. That in glass container showed minor variation and had an initial value of 20.5.
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Figure 5.5 L*, a* and b* values versus time (days) for uncured samples of F5 experimental formulation, aged at 80 °C in compules (A) by Pitsillou, 2019 for 26 days and sealed containers for 28 days (n=1) (B).
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Figure 5.6 illustrates plots of b* values versus time for uncured paste with (A) and without (B) acetate sheet, and cured samples (C) of F5 resin-composite formulation stored in compules (Pitsillou, 2019) at 80°C for 26 days or sealed glass container for 28 days.

Plots of b* values followed a linear trend for paste stored in compules and sealed glass containers. For uncured paste covered with acetate sheet, the values increased from 24.4 with rate of 0.49/day for paste stored in compules, conversely it showed minor variation with initial value of 21.3 and rate of -0.11/day for paste stored in sealed glass containers. For paste without acetate sheet, the values increased from 28.1 by 0.50/day for paste stored in compules, conversely it slightly decreased with time for paste stored in sealed glass containers with an initial value of 30.2 and rate of -0.31/day.

For paste stored in compules, b* values of cured samples increased over time with initial value of 5.3 by 1.08/day and 5.8 by 1.04/day on top and bottom surfaces respectively. Additionally, b* values on top (3.7 by 0.16/day) and bottom (5.3 by 0.10/day) surfaces of cured samples for paste stored in sealed glass containers were lower than those stored in compules.
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Figure 5.6 $b^*$ values versus time (days) for uncured samples with (A) and without acetate sheet (B), and top and bottom surfaces of cured samples (C) of F5 experimental formulation, aged at 80°C in compules by Pitsillou, 2019 for 26 days and sealed containers for 28 days (n=1). Abbreviations: Numbers: represent storage temperature in degree Celsius (°C). P: Uncured paste. PA: Paste with acetate sheet. CT: Cured top. CB: Cured bottom. *: Paste stored in sealed containers.
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5.6.4 Effect of thickness and composition

Figure 5.7 shows $b^*$ values of pastes of the four formulations of 1 and 4mm depth cured for 20 versus 40s respectively after storage at 80°C for 14 and 28 days in a glass container. These results show that all paste $b^*$ values at 4mm were higher than corresponding samples of 1mm. Average $b^*$ values for 1mm ranged between 18 ±0.3 and 26 ±0.6 and 4mm are 23.7 and 32 ±0.3. Pastes stored for 14 days showed higher $b^*$ values than equivalent samples at 28 days. The average $b^*$ values for pastes stored at 80°C for 14 days ranged between 31.9 ±0.1 to 24.2 ±0.2 with the highest value for the F4 4mm group and the lowest for F2 1mm group. The highest value for pastes stored at 80°C for 28 days was 29.25 ±0.35 and the lowest was 17.9 ±0.3 for F4 4mm and F5 1mm, respectively.

![Figure 5.7 Average b* values for the uncured samples of the experimental formulations at 1mm and 4mm depth aged at 80°C for 14 and 28 days. Error bars are half the difference between samples of each formulation (n=2).](image-url)
Figure 5.8 provides $b^*$ values for samples described in Figure 5.7 after polymerisation. All formulations showed a decrease in $b^*$ values after polymerisation. The bottom surfaces of the 4mm groups showed higher $b^*$ values than top surfaces and the overall 1mm group particularly regardless of the curing surfaces. For 1mm depth, $b^*$ values ranged between 7.2 to 14.2 with an average of 10 ±2 for top surfaces and 11 ±2.5 for bottom surfaces. For samples with 4 mm depth the results ranged between 8.2 to 31.4 with and average of 11.7 ±2.4 for top surfaces and 28.3 ±3.8 for bottom surfaces.

For a given thickness and time of high temperature storage, all formulations with higher PLS were more yellow than those with lower PLS. Average $b^*$ values for top surfaces of formulations stored for 14 days was 11 ±2 and bottom surfaces was 21 ±10. For formulations stored for 28 days, average $b^*$ values on top surfaces was 10.6 ±3 and bottom surfaces was 18.7 ±9.
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Figure 5.8 $b^*$ values for the experimental formulations at 1mm depth cured for 20s and 4mm depth cured for 40s aged at 80°C for 14 days (A) and 28 days (B) (n=1).
5.6.5 Factorial analysis

Figure 5.9 provides factorial analysis of effects of MCPM ($a_1$) and PLS ($a_2$) levels and their interactions ($a_1, a_2$) on $b^*$ values for pastes of 1 and 4mm depth after storage at 80°C for 14 and 28 days in a glass container.

From the $a$ values and using equation 5.1 the percentage changes can be shown as the following:

For pastes stored at 80°C for 28 days, increasing PLS led to higher $b^*$ values by 22% for the 1mm and 14% for the 4mm group. For paste stored for 14 days, increasing PLS led to higher $b^*$ values by 5% and 2% for the 1 and 4mm groups respectively.
Figure 5.10 illustrates factorial analysis of b* data for pastes of 1 and 4mm depth cured for 20 versus 40s respectively after storage at 80°C for 14 days in a glass container. For formulations stored at 80°C for 14 days, increasing PLS resulted in higher b* values by 50% and 39% on top and bottom surfaces for samples with 1mm depth, respectively, and 31% on top surfaces for samples with 4mm.

Figure 5.10 Factorial analysis of the evaluated b* values: values and interactions of 2 variables (a1: MCPM, a2: PLS, a12: Interactions between the variables)
Figure 5.11 illustrates factorial analysis of \( b^* \) values for pastes of 1 and 4mm depth cured for 20 versus 40s respectively after storage at 80°C for 28 days in a glass container. For the 1 mm discs, increasing PLS had increased the \( b^* \) values by 65% and 61% for the top and bottom surfaces, respectively. For the 4 mm discs, increasing PLS led to higher \( b^* \) values by 62% and 27% for the top and bottom surfaces, respectively.

![Factorial analysis of the evaluated \( b^* \) values: values and interactions of 2 variables (a1:MCPM, a2: PLS, a12: Interactions between the variables)](image-url)
5.7 Discussion

The CIE L*a*b* colour system was used as it is the most commonly used system to evaluate colour stability in dentistry. According to this system, L* represents lightness (grey scale) while a* is the red/green component of the colour and b* is the blue/yellow component of the colour (Sarafianou et al., 2012, Zanetti et al., 2019).

5.7.1 Effect of Temperature

5.7.1.1 Baseline 4, 25 and 37 °C storage on F5, 1mm thick samples

Baseline data in Figure 5.3 showed very low variability in b* values at lower temperature storage irrespective of the storage container, therefore it indicates good technique sensitivity that is highly reproducible. The small variability at time zero could be due to the compule storage at room temperature for several months before the start of the test. Also it could be related to the use of different batches, as the paste stored in compules was a DMG batch while paste stored in sealed glass container was made by Dr Wendy Xia and produced at Synergy.

5.7.1.2 Effect of 60°C storage on F5, 1mm thick samples

The first null hypothesis tested in the present study, that storage container would not have a significant effect on b* values of experimental resin-composite pastes was rejected.
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Upon light exposure paste yellow colour is rapidly photobleached. The level of bleaching is slightly higher on the upper surface. Significant yellowing of the sample in the compule upon 60°C storage for 16 weeks substantially reduced the ability of the sample to photobleach upon light exposure either on the top or lower surfaces. A possible reason could be due to light absorption by yellow dye components rather than the CQ. If the composite does not photobleach, CQ in lower layers remain yellow. Previous studies (Pitsillou, 2019) showed compule stored resin-composite at 60°C for 112 days showed higher paste b* values than baseline by 62%. The b* values increased linearly over the first 16 weeks up to a value of 33.4 with a change of 0.68/week (Pitsillou, 2019). Storage instead in a sealed brown glass container prevents paste yellowing at 60°C even with almost 6 times the level of time. The change in colour could be due to leaching of dye from the container or thermally induced degradation of CQ and monomer. This would be expected to then affect the rate of polymerisation. Long high temperature storage, does however, cause b* values to be higher after cure on both upper and lower surfaces due to additional colour more likely than photobleaching. However, storage times between groups are not systematic and it is difficult to draw comparisons.
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5.7.1.3 Effect of 80°C storage on F5, 1mm thick samples

The second null hypothesis tested in the present study, which assumed that high temperature storage would not have a significant effect on b* values of experimental resin-composite pastes was rejected.

Storage compules was evidently a contributing factor of colour changes observed with the tested resin-composite pastes and consequently of the cured discs stored at 60°C or 80°C. Plots of b* values for resin-composite formulation stored at extreme temperature of 80°C in compules showed very fast and linear alteration for both paste and discs up to 4 weeks (Figure 5.6). However, assumptions cannot be made on trend with only one sample repetition and no standard deviation. In addition, points do not lie fully on a line and therefore this relationship may not be real. The discolouration was identified visually to confirm the sensitivity of the method and to detect shade discrepancies (Pitsillou, 2019). These results were obtained from previous findings on colour stability (Pitsillou, 2019). Furthermore, yellowing of the samples could probably indicate low level leeching of dye from the red plastic tip of the compule. The discolouration might affect the amount of light transmitted through the sample, which could negatively affect the polymerisation process resulting in lower monomer conversion (Young, 2010). The light absorption by yellow dye components and CQ may affect the amount of light transmitted through the sample. This could result in higher delay times and lower rates of reaction, %DC and polymerisation shrinkage. Insufficient
monomer conversion would have deleterious impact on the mechanical properties as well as the biocompatibility of dental composites.

5.7.2 Effect of thickness and composition

The third null hypothesis tested in the present study, which assumed that increasing components would not have a significant effect on b* coordinate of experimental resin-composite pastes was rejected.

The overall effect of composition on colour stability for resin-composite pastes stored in sealed containers at 80°C was evident (Figure 5.8). Resin-composite pastes and discs with high PLS content led to higher b* values and darker samples after a long time storage at extremely high temperature of 80°C. These results are in agreement with previous study in our research group that showed PLS is affecting the polymerisation reaction of the tested resin-composite (Alkhouri, 2019). High PLS content may result in a higher amount of light scattering by increasing refractive index mismatch with the polymerised matrix. The thickness of the samples was obviously affecting the apparent colour and ability of light to penetrate to the lower surfaces of both pastes and cured discs. Samples with 4mm depth appeared more yellow than those of 1mm depth. This can be explained by the photoinitiators that can absorb the light and decrease the amount of light transmitted through the resin-composite sample (Ogunyinka et al., 2007, Musanje and Darvell, 2006). In addition, the translucency of
monomers change during polymerisation as a result of the refractive index rise (Odian, 2004, Howard et al., 2010) that resulting in light scattering associated with gelation and vitrification (Shibayama et al., 2005). Furthermore, it is important to point out that increasing sample thickness will increase the chroma of the colour. The results of this chapter confirm the effect of thickness, MCPM and PLS on the polymerisation kinetics described in chapter 3, as formulations containing high levels of MCPM and PLS resulted in a lower rate of reaction and %DC, particularly with increasing sample thickness. Therefore, the forth-null hypothesis tested in the present study was rejected. The hypothesis states that sample thickness would not have a significant effect on b* coordinate of experimental resin-composite pastes.

The limitations of this study would be the lack of sample repetitions and the baseline data for formulations with different levels of MCPM and PLS other than F5. This was due to the high risk of COVID-19 pandemic and the lockdown that result in limited access to the EDI research lab. The shortcoming of this study was also the lack of cool down protocol of the containers, after they were taken out of the incubators.
5.8 Conclusion

1. Resin-composite paste stored in compules had less colour stability than formulations stored in sealed containers.

2. Sample thickness has a greater effect than high temperature storage conditions on colour stability.

3. PLS is affecting the colour after long time storage at high temperature.
6. Summary and General Conclusion

Based on the work presented in each chapter and within the limitations of laboratory studies, the following can be concluded:

In Chapter 3 a comprehensive study was conducted to evaluate the effect of the additives on the polymerisation stability and paste shelf life of experimental composite formulations, and also evaluate the depth of cure of the experimental formulations and two commercially available restorative materials. The findings from this chapter proved that:

- Experimental formulations were stable upon high temperature aging suggesting good shelf life and competitive rates and levels of conversion to commercial materials.
- For both experimental formulations and commercial materials, 20s light exposure is sufficient for good monomer conversion up to 2mm depth.
- Increased thickness causes an increase in delay time and a decrease in rate of reaction.

In Chapter 4 the focus was put on understanding of factors controlling new composite stability, component release kinetics / chemistries and their ability to resist abrasive wear. It was found that:

- Polymerisation shrinkage was not significantly influenced by MCPM and PLS.
- The increase of MCPM and PLS led to higher volume change and H+ ion release, while increasing PLS led to higher mass change.
- Reducing MCPM particle size led to lower wear resistance than commercial comparisons.

The goal of Chapter 5 was to assess paste stability, photobleaching during cure and effect of sample depth on level of light penetration and lower surface photobleaching for formulations stored at high temperature for long time. The results from this chapter showed the following:

- Resin-composite paste stored in compules had less colour stability than formulations stored in sealed containers.

- Sample thickness has a greater effect than high temperature storage conditions on colour stability.

- PLS is affecting the colour after long time storage at high temperature.
7. Future work

- Studying paste shelf life of experimental composite formulations and commercial self-adhesive restorative materials using samples with 2 and 4mm depth.

- Examining water sorption of self-adhesive restorative materials after aging protocols with/without using simulated body fluids (SBF) to determine biodegradation of the monomer and filler phase. Quantifying leaching of monomers after aging protocols.

- Evaluating wear resistance and surface roughness of experimental formulations and self-adhesive restorative materials at different chewing cycles using SBF.

- Testing the colour change in water over time at 0, 2, 4, 6, 24, 48, 72 hours of storage and then every week for one month.

- Analysing surface nano hardness and cross-link density through ethanol softening test.

- Evaluate the fracture toughness with/without using SBF for self-repair (ISO 13586 Plastic determination of fracture toughness).

- Evaluate the flexural strength and analyse the fractured parts using scanning electron microscopy (SEM), electron dispersive X-ray analysis (EDX) system and raman spectroscopy.

- Investigate the component reactions at different powder/liquid ratio using raman spectroscopy.


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Bibliography


Table 7.1 FTIR spectra of chemicals used in the powder phase of the experimental composites

<table>
<thead>
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<th>Chemicals</th>
<th>FTIR spectra</th>
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</thead>
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<tr>
<td>Glass particles (7µm, 0.7µm)</td>
<td></td>
</tr>
<tr>
<td>Barium-alumino silicate</td>
<td></td>
</tr>
<tr>
<td>PLS</td>
<td></td>
</tr>
<tr>
<td>MCPM</td>
<td></td>
</tr>
</tbody>
</table>
Table 7.2 FTIR spectra of UDMA and PPGDMA used in the liquid phase of the experimental composites

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>FTIR spectra</th>
</tr>
</thead>
<tbody>
<tr>
<td>UDMA</td>
<td><img src="image" alt="FTIR spectrum of UDMA" /></td>
</tr>
<tr>
<td>PPGDMA</td>
<td><img src="image" alt="FTIR spectrum of PPGDMA" /></td>
</tr>
</tbody>
</table>
Table 7.3 Peak assignment of chemicals FTIR spectra

<table>
<thead>
<tr>
<th>Chemical agent</th>
<th>Wavenumber (cm⁻¹)</th>
<th>Peak Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass</td>
<td>1012</td>
<td>SiO stretch</td>
</tr>
<tr>
<td>PLS</td>
<td>1500</td>
<td>NH₃⁺</td>
</tr>
<tr>
<td></td>
<td>1560</td>
<td>Amide II N-H</td>
</tr>
<tr>
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Appendix 2

Figure 7.1 Hydrogen ion release versus SQRT (t/hr) for 11 weeks in deionised water. Error bars are 95% CI (n=3).
Table 7.4 Elemental composition (wt.%) of the samples dried extracts after 3 months storage in deionised water.

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Table 7.5 Elemental composition (wt.%) of the samples dried extracts for F1 and F2 after 12 months storage in deionised water.

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