An Investigation of Oily Formulations for the Management of Xerostomia

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Abstract

The physicochemical properties of natural saliva are responsible for many of its functions, yet an understanding about how the physicochemical properties of a saliva substitute influence clinical efficacy is limited. The aim of this thesis was to investigate oily formulations as potential saliva substitutes for individuals with xerostomia. Physicochemical properties of different compositions were investigated with an emphasis on rheology, as viscoelasticity was hypothesised to be important in developing a retentive saliva substitute that could lubricate the dynamic environment of the oral cavity. Further, a pilot study was conducted on healthy participants to determine the taste acceptability and retention of a selected oily formulation.

An initial investigation into the rheological properties of natural saliva confirmed that unstimulated saliva was shear thinning whereas stimulated saliva was a Newtonian fluid. In addition, natural whole saliva had a tan $\delta$ that was greater than one at low frequencies, indicating a higher contribution of viscous behaviour compared to elastic behaviour. At oscillatory frequencies greater than 5.2 Hz, tan $\delta$ was less than one, indicating that elastic behaviour was dominant. This was not observed in the commercially available saliva substitute (OralBalance®), which had a tan $\delta$ less than one, independent of frequency. Although this commercial saliva substitute was shear thinning, apparent viscosity was significantly higher at any shear rate ($p < 0.01$).

Emulsions, which contain both oil and water, were hypothesised to offer advantages over current saliva substitutes by combining the lubricating properties of oil with the acceptability of water. Compositions were prepared with food- and pharmaceutical-grade excipients and characterised using pseudo-ternary phase diagrams and polarised light microscopy. These investigations confirmed that the physical features of the formulations depended on excipient selection as well as the composition of each component. Compositions of rice bran oil, water and a surfactant mix of soy lecithin and propylene glycol at a weight ratio of 1:1 w/w, which formed coarse emulsions that were o/w, w/o or pseudo-bicontinuous depending upon their composition, were selected for further characterisation. Viscoelastic properties were analysed using both flow and oscillatory rheology and were found to be influenced by composition, frequency and shear stress.
This research demonstrated that the rheologically structured compositions exhibited shear thinning flow behaviour, although apparent viscosity was higher than for natural saliva at any shear rate. Interestingly, threshold frequencies were determined for some compositions where a peak in tan δ was observed, coinciding with a reduction in the storage modulus and increase in loss modulus. Compositions were able to maintain their rheological structure in the presence of up to 50% w/w water. Those with a rice bran oil, surfactant mix (lecithin and propylene glycol 1:1 w/w) and water ratio of 2:3:5 and 2:2:6 (w/w), exhibited frequency-dependent behaviour similar to that of natural saliva, where tan δ was greater than one at low frequencies and less than one at higher frequencies. These compositions were of particular interest. Viscous behaviour at low frequencies was hypothesised to offer lubrication in the oral cavity at rest whereas elastic behaviour at higher frequencies was thought to promote retention during higher shear tasks such as speaking and eating. Finally, the clinical application of a selected composition was trialled in a clinical study and showed that the retention of the emulsion immediately after rinsing was 8.34% higher than water (p = 0.003) and 4.57% higher than a 1% w/v methylcellulose substitute (p = 0.06). Information gained from this study will greatly facilitate the future development of successful saliva substitutes, which in turn will alleviate the terrible burden of morbidity suffered by xerostomia patients.
For Dad.
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<th>Description</th>
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<tbody>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>CMC</td>
<td>carboxymethylcellulose</td>
</tr>
<tr>
<td>CV</td>
<td>coefficient of variation</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared</td>
</tr>
<tr>
<td>G’</td>
<td>storage modulus</td>
</tr>
<tr>
<td>G”</td>
<td>loss modulus</td>
</tr>
<tr>
<td>GRAS</td>
<td>Generally regarded as safe</td>
</tr>
<tr>
<td>HEC</td>
<td>hydroxyethylcellulose</td>
</tr>
<tr>
<td>Ig</td>
<td>immunoglobulin</td>
</tr>
<tr>
<td>IPM</td>
<td>isopropyl myristate</td>
</tr>
<tr>
<td>LVR</td>
<td>linear viscoelastic region</td>
</tr>
<tr>
<td>MANOVA</td>
<td>multivariate analysis of variance</td>
</tr>
<tr>
<td>OGT</td>
<td>oxygenated glycerol triester</td>
</tr>
<tr>
<td>PEG</td>
<td>polyethylene glycol</td>
</tr>
<tr>
<td>PG</td>
<td>propylene glycol</td>
</tr>
<tr>
<td>RBO</td>
<td>rice bran oil</td>
</tr>
<tr>
<td>SALS</td>
<td>small-angle light scattering</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SE</td>
<td>standard error</td>
</tr>
<tr>
<td>SM</td>
<td>surfactant mix</td>
</tr>
<tr>
<td>tan δ</td>
<td>loss tangent</td>
</tr>
</tbody>
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Chapter one

General introduction
1 General introduction

1.1 Saliva and the acquired pellicle

Saliva is a complex fluid that surrounds all tissues in the oral cavity. It is predominantly secreted from three major paired glands as well as numerous minor glands that line the oral cavity, with very small contributions from non-glandular sources including crevicular fluid, oral microorganisms, host-derived cells and cellular constituents and dietary components (Leone & Oppenheim, 2001). Whole saliva consists of water, electrolytes, proteins and antimicrobial factors that contribute to its numerous functions.

1.1.1 Secretion of saliva

In healthy individuals an average of 0.5 to 1.5 L of whole saliva is secreted daily, with a pH between approximately 6.5 and 7.4 (Bardow et al., 2000; Pedersen et al., 2002; Edgar et al., 2004). The flow rate of saliva varies depending upon the degree of stimulation; the composition of its different constituents varies according to the flow rate and nature of the stimulus. The unstimulated flow rate is approximately 0.3 - 0.4 mL/min and this rate increases to 1.5 - 2.0 mL/min upon stimulation (Edgar et al., 2004; de Almeida et al., 2008). Saliva is secreted from three major (parotid, submandibular and sublingual) and minor (labial, lingual, buccal and palatal) salivary glands. The major glands include the parotid, located under and ventral to the ears; the submandibular, located at the midpoint of the mandible; and the sublingual, located under the tongue on the floor of the mouth (Figure 1.1). The composition and amount of saliva secreted varies from each gland.
Salivary glands primarily consist of two epithelial cell types, acinar cells and ductal cells, connected by tight junctions. Acinar cells are responsible for the secretion of salivary fluid as well as the majority of salivary proteins. There are two types of salivary acinar cells – serous and mucous. Serous cells produce a watery secretion, rich in enzymes and ions, whereas mucous cells produce a viscous secretion, rich in glycoproteins (Dodds et al., 2005; Mese & Matsuo, 2007). Striated duct cells modify the ionic composition of saliva, reabsorbing sodium and chloride and adding potassium and bicarbonate. Thereafter, the saliva passes through the collecting ducts. As the striated ductal cells are poorly permeable to water, the fluid entering the mouth is hypotonic (Turner & Sugiya, 2002; Catalan et al., 2009). The composition of saliva changes depending on flow rate, because at a slower basal rate saliva moves more slowly through the ducts, allowing more time for the striated ductal cells to modify the composition. Conversely, when saliva is stimulated the flow rate increases so that saliva passes rapidly through the ducts with little modification (Edgar et al., 2004). The composition of salivary fluid secreted is also dependent on the properties of the acinar cells. Parotid glands have serous acinar cells and produce a serous, watery secretion. Submandibular glands consist of both serous and mucous acini and produce a mixed secretion, whereas the mucous acinar cells found in sublingual and minor glands result in a secretion that is predominantly mucous in character (Figure 1.2).
Figure 1.2 Histology of the three major glands, showing the submandibular gland with serous cells grouped around the periphery of acinar cells and mucous cells in direct contact with the ductal system; the serous acinar cells of the parotid gland; the larger more irregular mucous acinar cells of the sublingual gland. Adapted from Martinez-Matrigal et al. (2007).

Salivary secretion is regulated by the parasympathetic and sympathetic pathways of the autonomic nervous system. In the absence of stimulation, saliva is secreted at a basal or unstimulated flow rate. Input from taste (gustatory), mastication (physical), and to a lesser extent psychogenic, namely smell, sight and thought, results in the release of neurotransmitters that stimulate secretion and increase the flow rate, known as the stimulated flow rate (Sreebny et al., 1992). In the parasympathetic system, fluid secretion is primarily activated by the binding of acetylcholine to muscarinic M3 receptors at the surface of salivary acinar cells. This results in a cascade of events leading to an increase in intracellular $\text{Ca}^{2+}$ from transporters on salivary acinar cells (including $\alpha$-adrenergic, substance P and M1, M2, M4 and M5 transporter subtypes) (Melvin et al., 2005). An osmotic gradient is created across the lumen, ultimately leading to salivary secretion. As osmosis requires an electrolyte gradient for transport, no net movement of water across the membrane occurs once equilibrium is reached. The striated duct then modifies the fluid so that the secretion is hypotonic when it enters the mouth (Edgar et al., 2004). This pathway is primarily responsible for the secretion of water and electrolytes. The sympathetic pathway controls macromolecule secretion by releasing noradrenaline, which binds to $\beta$-adrenergic receptors and leads to a sequence of events that results in salivary protein exocytosis from the salivary glands (Turner & Sugiya, 2002; Edgar et al., 2004; Melvin et al., 2005; Mese & Matsuo, 2007).

When the saliva has entered the mouth from the salivary glands some is lost by swallowing, evaporation or absorption through the oral mucosa. In healthy individuals the fluid input is greater than that lost by evaporation or absorption through the oral...
mucosa so that the remainder is swallowed. The most widely used model of salivary clearance is that of Dawes (1983), which mimics the action of an incomplete siphon. The minimal retained salivary volume is the residual volume in the mouth after swallowing, which then increases until a maximal volume is reached and swallowing is initiated. Lagerlöf and Dawes (1984) determined the mean maximal volume in healthy individuals before swallowing and residual volume after swallowing to be 1.07 mL (range 0.52 to 2.14 mL) and 0.77 mL (range 0.38 to 1.73 mL) respectively. Further, they established a correlation whereby as flow of fluid into the mouth increased, both the maximal volume swallowed and frequency of swallowing increased. This model has been widely accepted and shows a wide range, thus accounting for interindividual variability (Bergdahl, 2000; Dawes, 2004).

1.1.2 Composition of saliva

Saliva consists of about 99% water, with the remainder consisting of organic molecules (including proteins, glycoproteins lipids, glucose and urea) and electrolytes (Sreebny et al., 1992), as outlined in Table 1.1.

<table>
<thead>
<tr>
<th>Inorganic components</th>
<th>Organic components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>Mucins (MUC7 and MUC5B)</td>
</tr>
<tr>
<td>Potassium</td>
<td>Proline-rich proteins</td>
</tr>
<tr>
<td>Calcium</td>
<td>Statherin</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>Cystatins</td>
</tr>
<tr>
<td>Chloride</td>
<td>Histadines</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Immunoglobulin A (IgA)</td>
</tr>
<tr>
<td>Phosphate</td>
<td>Amino acids</td>
</tr>
<tr>
<td>Thiocyanate</td>
<td>Serum albumin</td>
</tr>
<tr>
<td>Iodide</td>
<td>Lactoferrin</td>
</tr>
<tr>
<td>Fluoride</td>
<td>Fibronectin</td>
</tr>
<tr>
<td>Ammonia</td>
<td></td>
</tr>
</tbody>
</table>
Many of the proteins found in saliva contain high levels (up to 40%) of the amino acid proline, and as such are termed proline-rich proteins. These proteins are further divided into acidic, basic or glycosylated proline-rich proteins depending on their charge and degree of glycosylation.

Saliva appears to be the source of components of the acquired pellicle. This is the biofilm that forms over tooth surfaces (Hannig & Hannig, 2009). The acquired enamel pellicle is a biofilm, formed by the selective adsorption of salivary proteins, serum proteins and microbial products with a high affinity for enamel and hydroxyapatite surfaces at the enamel-saliva interface. It forms on a variety of surfaces with different characteristics in the oral cavity including enamel, mucosa and various oral reconstructive materials such as alloy, porcelain, composites and polymers (Vassilakos et al., 1992; Ranc et al., 2006). Many of the glycoproteins present in saliva are also found in the pellicle. These include mucins, amylase, lysozyme, peroxidases, IgA and proline-rich proteins, which suggests a dynamic interface exists in the oral cavity between saliva and tooth surfaces. The pellicle has an important role in oral homeostasis as it acts as a diffusion barrier, reducing both the rate of loss of calcium and phosphate ions and protecting enamel apatite from the acidic environment. In addition, it has a role in lubrication of the tooth surfaces and facilitates the selective adhesion of certain types of bacteria, thus modulating the bacterial environment. The acquisition of oral flora begins within the first few hours of life, when pioneer species are introduced and alter the oral environment to facilitate colonisation by other bacteria until a highly dynamic equilibrium is reached (Hannig & Joiner, 2006; Samaranayake, 2006). The microbial composition changes as the oral environment is altered, for example the introduction of teeth provides a medium for the growth of organisms that prefer hard-tissue enamel colonisation as well as an anaerobic environment in the gingival crevice. The introduction of prosthetic appliances also alters the microbial composition (Samaranayake, 2006).

1.1.3 Functions of saliva

Saliva has a diverse range of functions, all of which ultimately serve to maintain homeostasis within the oral cavity (Mandel, 1989). These functions reflect the properties of saliva and include cleansing, lubrication, mucosal integrity, microbial
balance, buffering, remineralisation and taste (Table 1.2) (Sreebny et al., 1992; Ferguson & Barker, 1994; Ferguson, 2002).

Table 1.2 Key functions of saliva and the salivary components responsible for them. Adapted from Sreebny et al. (1992).

<table>
<thead>
<tr>
<th>Function</th>
<th>Salivary components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cleansing</td>
<td>Water, mucins</td>
</tr>
<tr>
<td>Lubrication</td>
<td>Mucins, proline-rich proteins, water</td>
</tr>
<tr>
<td>Mucosal integrity</td>
<td>Mucins, electrolytes, water, growth factors</td>
</tr>
<tr>
<td>Microbial balance</td>
<td>Lysozyme, lactoferrin, lactoperoxidase, mucins, cystatins, histatins, secretory IgA, proline-rich proteins</td>
</tr>
<tr>
<td>Buffering</td>
<td>Bicarbonate, phosphate ions</td>
</tr>
<tr>
<td>Remineralisation</td>
<td>Calcium, phosphate, statherin, anionic proline-rich proteins</td>
</tr>
<tr>
<td>Taste</td>
<td>Water, gustin</td>
</tr>
<tr>
<td>Digestion</td>
<td>Amylases, lipase, ribonuclease, proteases, water, mucins</td>
</tr>
</tbody>
</table>

1.1.3.1 Cleansing properties of saliva

The cleansing function of saliva can be attributed to both its fluidity and the mucous coating that covers all oral surfaces. While the mucous coating prevents the lodging of particles in the mouth, the water content allows surfaces to be continually washed. Muscle activity works synergistically with saliva to assist in shifting particles from teeth and soft tissues towards the back of the mouth and subsequent swallowing (Ferguson & Barker, 1994).

1.1.3.2 Lubricating properties of saliva

The lubricating function of saliva protects the oral surfaces from damage and allows free movement of both food and oral surfaces within the mouth, which is important for mastication, deglutition and speech (Ferguson, 1993; Ferguson & Barker, 1994; Bongaerts et al., 2007). The mucinous glycoproteins and proline-rich proteins in saliva combined with the water content provide the unique viscoelastic charged biofilm that covers the surfaces of the oral cavity. Remarkably, the mucin component of saliva is able to adsorb onto a wide variety of surface structures in the mouth (Yakubov et al.,
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2009). This includes the hydrophilic apatite surfaces of teeth as well as the lipophilic mucosal surfaces (Ranc et al., 2006).

1.1.3.3 Role of saliva in maintaining mucosal integrity

The protective biofilm covering the surfaces of the oral cavity further acts as a barrier to irritants and harmful substances and allows maintenance of a hydrated state within the mucosa (Ferguson & Barker, 1994). Epidermal and transforming growth factors in saliva further contribute to mucosal integrity by promoting tissue growth, differentiation and wound healing (Morris-Wiman et al., 2000; Mese & Matsuo, 2007).

1.1.3.4 Antimicrobial components of saliva

Several natural antimicrobial systems present in saliva modulate the growth of bacteria, fungi and viruses in order to maintain a healthy commensal microenvironment (Lagerlöf & Oliveby, 1994). The major antimicrobial proteins present in human whole saliva include lactoferrin, lysozyme, peroxidases, α-amylase, proline-rich proteins, histatin, cystatins and salivary IgA.

**Lactoferrin**

Lactoferrin is secreted by the major and minor salivary glands as well as oral leukocytes. It has a high affinity for iron, which is required for bacterial growth, resulting in a bacteriostatic effect through iron sequestration. It is also present in an iron-free state known as apo-lactoferrin and this molecule is able to bind directly and irreversibly to bacteria, resulting in a bactericidal effect. In addition, antimicrobial domains within the lactoferrin molecule may be released by host or microbial proteases (Edgar et al., 2004).

**Lysozyme**

Lysozyme is secreted into saliva from the major and minor salivary glands as well as gingival crevicular fluid and salivary leukocytes (Edgar et al., 2004). It is a strongly cationic protein that targets gram-positive bacteria and candida yeasts by hydrolysing the bacterial cell wall and activating bacterial autolysins that destroy bacterial cell contents (Rölla et al., 1983; Hannig et al., 2005; de Almeida et al., 2008). Lysozyme is the primary bactericidal protein in the pellicle and is able to bind to specific bacteria,
inhibiting or facilitating both aggregation and adherence (Hannig et al., 2005). The concentration of lysozyme does not appear to be related to the incidence or prevalence of caries and it has been demonstrated to be present in equal levels in newborn babies and adults, indicating that its antimicrobial effect begins before teeth eruption (Edgar et al., 2004).

**Peroxidases**

There are two types of peroxidase present in saliva. Salivary peroxidase (also known as sialoperoxidase) is secreted from the parotid and submandibular glands, whereas myeloperoxidase is secreted primarily through gingival crevices. Both enzymes catalyse the oxidation of salivary thiocyanate (SCN\(^-\)) to hypohionate (OSCN\(^-\)) and subsequently hypohiocyanous acid (HOCSN) (**Figure 1.3**). An increase in the concentration of OSCN\(^-\) and HOCSN reduces acid production by dental plaque following stimulation by sugars (Tenovuo et al., 1982). Peroxidases function both to provide antimicrobial activity against a variety of microbes and to protect host proteins and cells from hydrogen peroxide (H\(_2\)O\(_2\)), which is constantly produced by aerobic bacteria and is toxic to mucosal and gingival cells (Tenovuo, 2002; Edgar et al., 2004). They have also been shown to protect biologically active proteins from destruction by radicals of oxygen (Ericson & Bratt, 1987). In addition, peroxidase is able to bind to some bacteria and has an agonistic effect with lysozyme in the control of bacterial adhesion to the hydroxyapatite surface (Roger et al., 1994; Hannig et al., 2005).

\[
H_2O_2 + SCN^- \rightarrow OSCN^- + H_2O \\
H^+ + OSCN^- \rightarrow HOSCN
\]

**Figure 1.3** Oxidation of thiocyanate (SCN\(^-\)) and subsequent degradation of hydrogen peroxide (H\(_2\)O\(_2\)).

**α-Amylase**

α-Amylase is the most abundant salivary enzyme, contributing up to 50% of total proteins produced by the salivary glands (Edgar et al., 2004). The majority of α-
amylase is secreted by the parotid glands (80%), with the remainder originating from the submandibular glands. Although α-amylase is predominantly associated with the digestive function of saliva it also interacts with specific bacteria to modulate their surface attachment to the pellicle.

**Proline-rich proteins**

Proline-rich proteins comprise a complex group of proteins with a variety of functions. They have a fundamental role in the formation of the salivary pellicle and control the microbial environment by selectively promoting and inhibiting bacterial adhesion to the tooth surface (Bennick, 1982; Hannig & Joiner, 2006).

**Cystatins**

Cysteine-containing phosphoproteins, or cystatins, are multifunctional proteins mainly secreted in submandibular and sublingual saliva. The release of proteolytic enzymes from bacteria leads to a cascade of events, whereby proteolytic enzymes and lysosomal cysteine proteases from the host cells are activated leading to tissue degradation (Baron et al., 1999). Cystatins assist in the protection against periodontal tissue destruction by selectively inhibiting proteases from bacteria and leukocytes, thus preventing any untoward proteolysis (Edgar et al., 2004).

**Histatins**

Histatins are a group of peptides with significant antifungal action as well as some antibacterial activity. They target the mitochondrion, inducing the release of adenosine triphosphate (ATP) into the cytoplasm and subsequently causing fungal cell lysis. They are particularly effective against *Candida albicans* (Kavanagh & Dowd, 2004).

**Immunoglobulins**

Igs (mainly secretory IgA from salivary glands, as well as some IgG from crevicular fluid) produced by the common mucosal immune system are present in saliva, often from the first weeks of life. Salivary IgA antibodies are dimeric molecules that also comprise a secretory component, a small glycoprotein that makes the molecule more resistant to proteases in the oral environment (Edgar et al., 2004). They act by limiting microbial adherence and preventing the penetration of foreign antigens to oral surfaces.
(McNabb & Tomasi, 1981; Lenander-Lumikari & Loimaranta, 2000), although there is conflicting evidence regarding any significant relationship between salivary IgA and dental caries (Marcotte & Lavoie, 1998).

1.1.3.5  pH and buffering capacity of saliva

In healthy adults, the pH of unstimulated human whole saliva is generally in the range of 6.5 to 7.4 (Bardow et al., 2000; Pedersen et al., 2002). Saliva contains bicarbonate and phosphate as well as proteins that assist in the regulation of pH in the oral cavity; the physiological pH of saliva increases as salivary flow rate increases (Farsi, 2007). This is important because when pH falls below 5.7, which is common following ingestion of even modest amounts of carbohydrate as well as acidic foods, enamel begins to lose surface calcium and phosphate, resulting in demineralisation of hydroxyapatite (Figure 1.4). The buffering capacity of saliva results in an increase in pH (Ericsson, 1959), which helps drive the equation to the left, and as Ca$^{2+}$ and PO$_4^{3-}$ become supersaturated, remineralisation of hydroxyapatite is promoted.

\[
\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \xleftrightarrow{\text{Acid}} \xrightarrow{\text{Neutral}} 10\text{Ca}^{2+} + 6\text{PO}_4^{3-} + 2\text{OH}^- + \text{6H}^+ + 2\text{H}^+ + \text{6HPO}_4^{2-} + 2\text{H}_2\text{O}
\]

Figure 1.4  A simplified equation showing the effect of pH on dissolution and precipitation of hydroxyapatite (Ca$_{10}$(PO$_4$)$_6$(OH)$_2$).

1.1.3.6  Saliva and tooth remineralisation

Saliva is supersaturated with calcium and phosphate relative to tooth minerals. The mineral phase of teeth consists of hydroxyapatite, which freely incorporates foreign ions into its crystalline lattice. The enamel pellicle regulates dissolution and precipitation of hydroxyapatite. Solubility is highly dependent on the pH of the
environment and acidic ions, which increase solubility, thus promoting hydroxyapatite demineralisation (Figure 1.4). Fluoride, on the other hand, decreases solubility resulting in reduced demineralisation. In addition, proline-rich proteins and statherin bind calcium, maintaining supersaturation by inhibiting the spontaneous precipitation of calcium phosphate salts (Edgar et al., 2004; Proctor et al., 2005).

1.1.3.7 Taste perception and saliva

In order for foods to be tasted, components must be in aqueous solution to allow interactions with taste buds of the tongue. Saliva provides the aqueous medium required for the sense of taste to be experienced. Taste receptors are adapted to the taste of saliva, therefore, the levels of glucose, sodium, urea, chloride and glutamate in saliva must be lower than that of the gustatory substance in order to perceive the taste of sweet, salty, bitter, sour and umami, respectively (Dodds et al., 2005; de Almeida et al., 2008). The umami, or savoury, taste is a basic one, recognised by salivary glutamate and rich in foods such as chicken broth, meat extracts and cheese (Mese & Matsuo, 2007).

1.1.3.8 Role of saliva in digestion

Enzymes present in saliva, in particular amylase, assist in the breakdown of retained food particles and digestion of carbohydrates by hydrolysing the \( \alpha \)-1,4-glucosidic linkages in dietary starch (Hannig et al., 2005). However, this is thought to be of little significance in the general process of digestion (Ferguson & Barker, 1994).

1.2 Salivary hypofunction and xerostomia

By virtue of its composition and range of functions, saliva has a fundamental role in maintaining homeostasis in the oral cavity. Any alteration in the composition or flow rate of saliva will interrupt this equilibrium as the flow rate, clearance, calcium phosphate levels, pH and buffer capacity of saliva influence bacterial metabolism, adsorption to oral tissues and elimination as well as maintenance of tooth mineralisation (Dodds et al., 2005). Salivary gland hypofunction is defined as an objective reduction in flow rate or altered composition of stimulated or unstimulated saliva (Fox et al., 1985; Nederfors, 2000). Clinical effects include the sensation of a dry mouth, a burning sensation, difficulty speaking, chewing, swallowing and sleeping.
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(Ferguson, 1989; Chambers et al., 2007). Additionally, salivary gland hypofunction is associated with an increased incidence of dental caries and periodontal disease (Ferguson, 1989; Greenspan, 1996; Dodds et al., 2005). Xerostomia is defined as the subjective complaint of oral dryness and is associated with either permanent or transient salivary hypofunction (Sreebny & Valdini, 1988; Davies, 1997). The most common symptom in patients with salivary gland hypofunction is a persistent dry mouth (Ferguson, 1993, 2002). Researchers have endeavoured to quantify this subjective complaint; the general consensus is that individuals begin to experience symptoms of oral dryness when salivary flow rate is reduced by approximately 50% of their normal rate (Dawes, 1987; Sreebny et al., 1992; Suh et al., 2007). Salivary flow rate is affected by a number of factors, including level of hydration, body posture, circadian rhythms, medication and stimulation and as such there is a large variation within, as well as between, individuals. During the day the average unstimulated whole flow rate in healthy adults is between 0.3 and 0.4 mL/min, rising to up to 7.0 mL/min upon stimulation (Edgar et al., 2004; Preetha & Banerjee, 2005). During sleep, the flow rate drops to as low as 0.1 mL/min (Porter et al., 2004). Some researchers have suggested arbitrary salivary flow rates of less than 0.1 mL/min for unstimulated saliva or 0.5 mL/min for stimulated saliva to be indicative of salivary hypofunction (Lee et al., 2002; Edgar et al., 2004; de Almeida et al., 2008). In practice, salivary hypofunction may also be diagnosed if the composition and therefore, quality, of saliva is altered as well as in a diminished quantity of saliva so individuals with a diagnostically “normal” flow rate may still experience salivary hypofunction.

1.2.1 Complications of xerostomia

Patients with salivary hypofunction often have trouble eating, as a lack of lubrication makes mastication and deglutition difficult. Speech may also become a problem (Ferguson, 1993). Dysgeusia, or alteration in taste function, sometimes occurs as saliva provides the aqueous medium required for food to be tasted (Ferguson, 1993; de Almeida et al., 2008). Furthermore, the risk of dental caries and infections of the oral mucosa are considerably increased due to disruption of normal oral homeostasis (Dodds et al., 2005). Persistent salivary hypofunction impedes the ability of the salivary system to restore neutral pH levels following the ingestion of food and beverages, resulting in an acidic environment that not only promotes tooth demineralisation, but
also provides microorganisms with a modified setting for colonisation (Edgar et al., 2004). This effect is compounded by a reduced ability of saliva to form a protective barrier over the mucosa. In particular, the colonisation of Candida on the mucosa becomes more common (Ferguson, 1993). The reduction in salivary flow reduces the clearance of microorganisms from the oral environment, further promoting colonisation and accumulation, and enhancing the acidity of the environment by retaining carbohydrate particles within the oral cavity (Mandel, 1989; Sreebny et al., 1992). Plaque products from retained carbohydrates can promote gingivitis which, over a period of time, may develop into periodontal disease, particularly in the absence of sufficient oral hygiene measures (Ferguson, 1993; Edgar et al., 2004). Additionally, in the absence of sufficient enamel remineralisation, patients are more susceptible to further loss of enamel as a result of chemical dental erosion and physical abrasion, eventually resulting in a need for dental prostheses (Mandel, 1989; Edgar et al., 2004). Full acrylic dental prostheses require a thin salivary film over the mucosa to provide adequate adhesion. When the quantity or quality of saliva is altered such that this film is compromised, dental use is impaired. Titanium implants are better tolerated than mucosal-borne dentures, as they are fixed to the bone. With mucosal-borne dentures, adhesion is not sufficient for retention in patients with salivary hypofunction and the tissues are exposed to abrasion and trauma from the prostheses, which may further increase the risk of microbial infection (Edgar et al., 2004).

Ultimately, the complications associated with salivary hypofunction may significantly impair the health-related quality of life. Difficulty chewing, swallowing and tasting transforms to a lack of enjoyment from eating and may result in compromised nutritional status. This, along with speech difficulties and poor sleep, may also impair social interactions. Friable tissues in the oral mucosa are likely to develop traumatic lesions, particularly in patients with dentures, causing pain. Infections within the oral mucosa may lead to systemic disease, especially in patients who are already medically compromised (Edgar et al., 2004). An association between periodontal disease and cardiovascular disease has been established, however, it is unclear whether this relationship is causal or if oral health is simply an indicator of general hygiene and health care practices (DeStefano et al., 1993; Hujoel et al., 2000).
1.2.2 Prevalence of xerostomia

There is a large disparity in the prevalence of xerostomia in the general adult population, with reports that vary between 13% and 57% (Sreebny, 2010). Xerostomia is more prevalent in females than males and age is a known risk factor, with prevalence increasing to between 42% and 63% in individuals over the age of 65 years (Orellana et al., 2006; Sreebny, 2010). It is unknown whether this is a natural consequence of age-related salivary gland degeneration, or due to the increasing prevalence of systemic diseases and consequent higher intake of medication that occurs with age (Sreebny, 2010).

1.2.3 Aetiology of xerostomia

The feeling of oral dryness is sensed when salivary flow is reduced to approximately half of a patient’s normal flow rate and in order for such a reduction, more than one gland must be affected (Dawes, 1987; Sreebny et al., 1992; Nederfors, 2000; Suh et al., 2007). Xerostomia occurs as a consequence of functional or structural disorders that result in an alteration in the quantity or quality of saliva (Ferguson, 1989). Medication is the most common cause of functional salivary hypofunction and is reversible on cessation of the offending drug (Sreebny et al., 1989). Individuals suffering anxiety neurosis and depression are at increased risk of xerostomia, however, this is likely to be compounded by the medication used for treatment (Ferguson, 1993).

Structural disorders of the salivary glands occur when the acinar function is compromised as a result of atrophy and destruction of salivary acinar cells. This is common following radiation therapy to the head and neck region as well as chemotherapy, and in disorders such as inflammatory exocrinopathy (Sjögren’s syndrome), sarcoidosis and human immunodeficiency virus (HIV) (Ferguson & Barker, 1994; Porter et al., 2004; Napeñas et al., 2009). As salivary glands age, structural changes take place as acinar cells are replaced with adipose and fibrotic tissues, although it has been demonstrated that this does not necessarily translate to any age-related changes in salivary flow (Ship et al., 1995). Salivary glands in older individuals are, however, more likely to be affected by medication and as the intake of medication increases with age, it is probable that ageing is a contributing factor towards salivary hypofunction (Sreebny et al., 1992; Ship et al., 1995; Edgar et al., 2004).
**Medication**

Xerostomia is a side effect of many medications, some of which are in common use (Table 1.3). This adverse effect is increased by the additive effect of taking multiple medications, such as is common practice in elderly patients (Porter et al., 2004). Although xerostomia is a side effect of many medications, few have been tested for any objective change in salivary flow (Napeñas et al., 2009). Any medication that inhibits the binding of neurotransmitters to acinar cells or interferes with ion transport may reduce the quantity and quality of salivary secretion; therefore, drugs such as tricyclic antidepressants, atropine and clonidine that inhibit binding to cholinergic or α-adrenergic receptors commonly reported to cause salivary hyposalivation (Croog et al., 1986; Atkinson et al., 1989; Edgar et al., 2004; Nederfors et al., 2004; Gómez-Moreno et al., 2013). At normal dose ranges this effect is generally reversible upon discontinuation of the drug (Sreebny et al., 1992) and where appropriate, changing to a different medication for the same condition may reduce the complaint.

**Table 1.3** Medication that commonly causes xerostomia or salivary hypofunction.

<table>
<thead>
<tr>
<th>Medication group</th>
<th>Examples</th>
</tr>
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<tbody>
<tr>
<td>Anticholinergics/antispasmodics</td>
<td>Atropine</td>
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<tr>
<td></td>
<td>Hyoscyamine</td>
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<td></td>
<td>Oxybutynin</td>
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<tr>
<td>Tricyclic antidepressants</td>
<td>Amitriptyline</td>
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<td></td>
<td>Nortriptyline</td>
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<td>Antipsychotics</td>
<td>Trifluoperazine</td>
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<td></td>
<td>Chlorpromazine</td>
</tr>
<tr>
<td>α-adrenergic agonists/antagonists</td>
<td>Methyldopa</td>
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<tr>
<td></td>
<td>Clonidine</td>
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<tr>
<td></td>
<td>Prazosin</td>
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<tr>
<td>Retinoids</td>
<td>Isotretinoin</td>
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<tr>
<td>Antihistamines</td>
<td>Diphenhydramine</td>
</tr>
<tr>
<td></td>
<td>Brompheniramine</td>
</tr>
<tr>
<td></td>
<td>Promethazine</td>
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<tr>
<td></td>
<td>Dexchlorpheniramine</td>
</tr>
</tbody>
</table>
Radiation

Radiation therapy for the treatment of head and neck cancer often involves direct exposure to the salivary glands, causing a reduction in the production and alteration in the composition of saliva that is dependent on time, dose and radiation field (Dreizen et al., 1977; Valdez et al., 1993). The parotid gland is most often implicated because serous acinar cells are the most sensitive to radiation-induced apoptosis, causing intermitotic and interphase cell death (Stephens et al., 1991; Henson et al., 1999). This results in an increase in the viscosity of saliva within the first weeks following radiation therapy. Eventually, mucous cells are also affected and the quantity of saliva produced is further reduced (Henson et al., 1999). A radiation dose threshold of 23-25 Gy has been established above which salivary gland destruction is permanent and below which a degree of salivary gland function may return upon completion of radiation therapy (Eisbruch et al., 1999). Techniques have been developed in an attempt to preserve salivary gland function, such as giving the lowest effective dose and altering beam geometry to limit radiation exposure to salivary glands. Using a unilateral radiation field, for example, can preserve contralateral parotid gland function, causing less xerostomia compared to bilateral radiation fields (Eneroth et al., 1972; Robertson et al., 1986).

Systemic diseases

Inflammatory exocrinopathy, or Sjögren’s syndrome, is an autoimmune disease characterised by an inflammatory infiltration of the exocrine glands that results in a progressive loss of function. Although salivary and lachrymal glands are predominantly affected, other exocrine glands may also be involved. Inflammatory exocrinopathy may exist alongside connective tissue disorders including rheumatoid arthritis, systemic lupus erythematosus, progressive systemic sclerosis and polymyositis. Sarcoidosis is another chronic inflammatory disease that causes the formation of granulomata, resulting in salivary hypofunction if the salivary glands are involved (Sreebny et al., 1992). Salivary gland involvement occurs in some individuals infected with HIV and is characterised by recurrent or persistent major salivary gland enlargement and xerostomia.
1.3 The management of xerostomia

The management of patients with xerostomia requires a broad, multifaceted approach. Standard oral hygiene practices are essential to maintain the oral cavity and prevent progression of teeth caries. Patients with adequate residual salivary gland function may benefit from the use of sialogogues to stimulate secretion, however, a number of patients with severe salivary hypofunction have inadequate functional tissue to respond to sialogogues and a salivary substitute is the only alternative (Ferguson & Barker, 1994; Ferguson, 2002).

1.3.1 Sialogogues

Sialogogues stimulate salivary gland secretion. Sugar-free chewing gum is an ideal gustatory sialogogue as it stimulates saliva over at least 30 minutes with continued flavour release as well as the masticating action of the jaws (Ferguson, 1993; Frost, 2008). Evidence indicates that chewing gum increases the secretion of saliva in those with residual salivary gland function (Olsson et al., 1991; Furness et al., 2011). Acidic substances may also be used to stimulate salivation, but these must be calcium-buffered against erosion, as the stimulated saliva may not be sufficient to maintain plaque pH. If chewing gum provides insufficient stimulation, use of a pharmacological sialogogue such as pilocarpine or cevimeline may be appropriate as a second level of treatment (Ferguson, 1993; Ferguson & Barker, 1994; Petrone et al., 2002; Chainani-Wu et al., 2006). These are cholinergic agonists that act predominantly on the muscarinic receptors, and have demonstrated efficacy in individuals with residual salivary gland function (Ferguson, 1993; Porter et al., 2004; Jensen et al., 2010).

1.3.2 Saliva substitutes

The goal of a saliva substitute, as the name implies, is to replace saliva. As the composition and secretion of saliva is complex, most substitutes aim, at best, to relieve the sensation of a dry mouth, lubricate the oral cavity and protect the teeth from decay (Hahnel et al., 2009). The most common saliva substitute is water, however, this provides very temporary relief as it is rapidly washed away and fails to lubricate oral surfaces sufficiently. Small sips frequently, day and night, are required (Ferguson, 2002; Frost, 2008).
In an attempt to improve lubricating properties, solutions containing glycerol have been developed. In the past, acidic components, such as lemon juice or citric acid, have been included to encourage any residual gland function. However, patients who require a substitute often do not respond to sialogogues and repeated application could further damage the already compromised oral cavity by reducing pH, thereby increasing the risk of dental caries (Ferguson & Barker, 1994). In addition, some oral bacteria are able to ferment glycerol, resulting in the local production of acids. Rinses have been trialled containing saline, magnesium hydroxide or sodium bicarbonate in an attempt to raise plaque pH. Although they reportedly improved subjective symptoms of oral dryness, this was comparable only to water as no significant lasting effect was observed. The pH of the substitute is important, however, as continued exposure to an acidic substance will increase the risk of enamel demineralisation (Meyer-Lueckel et al., 2002). Research has demonstrated that fluoride stabilises demineralisation, reducing the sensitivity of enamel to an acidic environment (Meyer-Lueckel et al., 2002; Zandim et al., 2010). Mouthwashes containing chlorhexidine may also be beneficial in controlling plaque. However, individuals may find them to be astringent and long-term use has the propensity to stain teeth brown (Fardal & Turnbull, 1986). Alcohol-containing mouthwashes cause drying of the mouth and damage to the already compromised mucosa, so should be avoided.

In order to obtain a lasting effect, more complex substitutes have been developed to increase viscosity. In 1972, Matzker and Schreiber introduced sodium carboxymethylcellulose (CMC) into a phosphate-buffered saline solution, with the aim of improving viscosity and therefore, retention (Matzker & Schreiber, 1972). Over the years electrolytes and fluoride have been included to assist in tooth remineralisation, resulting in products such as VA-OraLube® (Shannon et al., 1977; Shannon et al., 1978). However, Klestov et al. (1981) compared VA-OraLube® spray with a glycerin mouth rinse and found that while the CMC in VA-OraLube® increased viscosity, it did not provide the same lubricating properties or protective effects as natural saliva. Preetha and Banerjee (2005) compared the viscosity and surface tension of natural saliva and substitutes containing CMC or xanthan gum and found that the substitutes did not exhibit the same physicochemical properties as natural saliva. Although the lubricating properties of CMC may improve some symptoms of xerostomia, they have minimal effect on gustatory function (Temmel et al., 2005). In addition, CMC-based
saliva substitutes do not have the same non-Newtonian properties as natural saliva (Marks & Roberts, 1983; Vissink et al., 1983; Epstein & Stevenson-Moore, 1992). Hydroxyethylcellulose (HEC), a polymer that forms a non-Newtonian pseudoplastic gel with water (Jones et al., 2002) is available as a saliva substitute (OralBalance®, Biotène, GlaxoSmithKline, Middlesex, UK) and has shown some benefit over CMC-containing products (Epstein et al., 1999). This suggests that the flow properties of a saliva substitute are important markers of efficacy.

With the rationale of better assimilating natural saliva, animal mucins, derived from bovine or porcine salivary glands, have been used, as it is these glycoproteins in natural saliva that largely contribute to its unique viscoelasticity (Adikwu, 2006). However, these mucin-based substitutes are unacceptable for vegetarians as well as in certain religious groups, which may limit their widespread use (Ferguson, 2002). There is conflicting evidence regarding the rheological properties of animal mucins compared to human whole saliva. Although some research has confirmed comparable rheological properties between animal mucins and natural saliva in vitro (Vissink et al., 1984; Park et al., 2007; Yakubov et al., 2009), in vivo studies have been inconclusive, with many comparative studies failing to find a significant difference in performance compared to placebo (Sweeney et al., 1997). Olsson and Axéll (1991) compared the subjective and objective efficacy of a CMC-based substitute with a mucin-based one. Both substitutes showed similar effects objectively and subjectively, but within about 15 minutes the residual saliva had returned to the basal value for each patient.

Linseed oil-based saliva substitutes, such as Salinum®, have also shown some promise. Christersson et al. (2000) compared the film-forming properties of natural saliva with that of substitutes based on porcine mucin, CMC or linseed extract, and found that while natural whole saliva had the lowest viscosity and linseed oil substitute had the highest viscosity, the linseed substitute had an initial surface tension closest to that of the saliva. The mucin substitute had a viscosity approximately twice that of natural whole saliva and was able to form a film on hydrophobic substrates.

Lubricants have been incorporated into saliva substitute preparations for the purpose of reducing moisture loss from tissue in the oral cavity. Oxygenated glycerol triester (OGT) spray forms a lipid film over the oral mucosa and has been shown to provide superior subjective and objective relief of xerostomia in older patients (Mouly et al.,
2007b) as well as in patients with xerostomia resulting from psychotropic medication (Mouly et al., 2007a).

Although many saliva substitutes are available commercially and have been proven to provide some subjective and objective relief, this appears to be limited and many patients still resort to frequent small sips of water for symptom relief (Ferguson, 2002; Momm et al., 2005). Further, using in vitro models, some commercially available saliva substitutes (Glandosane® and OralBalance®) have been shown to cause demineralisation (Smith et al., 2001; Meyer-Lueckel et al., 2002; Tschoppe et al., 2008). This outcome is not acceptable as patients with salivary hypofunction already have impaired tooth remineralisation capabilities due to the absence of natural saliva (Dodds et al., 2005). Smith et al. (2001) found little correlation between mineral dissolution and pH of saliva substitutes, suggesting that other factors such as the presence of inorganic ions and titratable acidity play a significant role in the mineral dissolution process.

1.3.3 Treatment of xerostomia in New Zealand

In New Zealand, there are two funded treatment options for patients with dry mouth. Pilocarpine may be prescribed for individuals with residual salivary gland function. As commercial pilocarpine lozenges are not available, an oral liquid is extemporaneously compounded in a pharmacy by diluting pilocarpine 4% eye drops (Fawcett et al., 1994; Wilson et al., 2012). Alternatively, a saliva substitute consisting of 1% w/v methylcellulose in water may be compounded based on the standard formula available in the New Zealand Pharmaceutical Schedule (Wilson et al., 2012).

1.3.4 Summary of treatment options

Although there are a variety of saliva substitutes commercially available, it is evident from the literature that there is ambiguity regarding their efficacy. Despite the severe impact that salivary gland hypofunction and xerostomia can have on the quality of life of sufferers, current saliva substitutes fail to provide adequate long-term relief, particularly in patients with little or no salivary gland function (Sweeney et al., 1997; Ferguson, 2002; Frost, 2008; Furness et al., 2011). Moreover, some commercially available formulations may be detrimental by promoting tooth decay in individuals with an already compromised oral cavity. The physicochemical properties of saliva are
essential in maintaining homeostasis in the oral cavity and are responsible for many of its properties. These properties depend upon a myriad of internal and external factors such as time of day, salivary gland of origin, nature of stimulation and individual differences in composition (Rantonen & Meurman, 1998). Therefore, the physicochemical properties of a saliva substitute are important in determining efficacy. While a need to resemble the physicochemical properties of natural saliva as closely as possible has been identified (Hahnel et al., 2009), it is hypothesised that this is not true for all properties. Saliva is continually secreted, whereas the goal of a saliva substitute is to be retained in the oral cavity for prolonged periods of time. Formulations that lubricate the oral cavity, such as OGT spray have demonstrated some promise, indicating that lubrication may be important in oral efficacy.

1.4 Oils and emulsions as saliva substitutes

Oils lubricate mucosal surfaces. As such, they are often used in cosmetic products for their lubricating and moisturising properties (Lerma-García et al., 2009). As an OGT spray has shown some promise in lubricating the oral cavity (Mouly et al., 2007a; Mouly et al., 2007b), it is hypothesised that oily formulations may be beneficial over the more traditional polymer- and mucin- based systems. It has been reported that xerostomia patients tend to sip water frequently (Ferguson, 2002; Frost, 2008), therefore, it follows that a saliva substitute may be optimised by incorporating a lubricating and moisturising oil phase with the palatable aqueous phase of water in an emulsion.

An emulsion is defined as a thermodynamically unstable dispersion of two immiscible liquids, such as oil and water, where one phase is dispersed as liquid droplets in a continuous medium. Depending upon the composition of the two immiscible liquids an oil-in-water (o/w) or water-in-oil (w/o) emulsion will result. The formation of an emulsion requires a large amount of energy, as the interfacial area of the emulsion is much larger than that of the individual bulk phases. To reduce the tendency for an emulsion to revert to its most thermodynamically stable form, surfactants are generally incorporated into the system, which adsorb at the interface between the two phases, forming an elastic film that reduces interfacial tension, which in turn lowers the energy required for the process of emulsification (Figure 1.5) (Walstra, 2003). When droplets are insufficiently stabilised, they collide with each other and reversible processes such
as creaming and aggregation occur, which can lead to rupture of the interfacial film surrounding the droplets, causing them to irreversibly coalesce into larger droplets with a smaller surface area.

![Surfactant molecule](image)

**Figure 1.5** Schematic representation of a surfactant molecule showing the hydrophilic head and lipophilic tail, as well as how the surfactant orients at the oil-water interface of an oil droplet for an o/w emulsion.

The physical properties of emulsions are highly variable. Alterations in the composition of the continuous and dispersed phases as well as the type of surfactant used can significantly impact mean droplet size ($\bar{x}$), volume fraction and droplet polydispersity. The volume fraction ($\phi$) is defined as the volume of the dispersed phase ($V_D$) as a function of the total volume of emulsion ($V_E$) (Equation 1.1). Polydispersity describes the droplet size distribution, an index (PDI) that can be obtained by dividing the standard deviation (SD) of droplet size ($d$) by $\bar{x}$ (Equation 1.2). The PDI can also be expressed as a percentage to define the coefficient of variation (CV) of the droplet size distribution.

$$\phi = \frac{V_D}{V_E} \quad \text{Equation 1.1}$$

$$PDI = \frac{SD_d}{\bar{x}} \quad \text{Equation 1.2}$$

These properties influence the feeling and ease of application of emulsions, which could be important considerations in an emulsion for spreading and retention in the
mouth. At low volume fractions, emulsions tend to be simple viscous liquids, whereas at high volume fractions are elastic solids, with a high shear modulus (Mason, 1999).

A microemulsion is defined as an emulsion of oil, water and surfactant mix (SM) that is optically isotropic and thermodynamically stable (Danielsson & Lindman, 1981). This is due to the small droplet diameter of the dispersed phase, which is usually around 3 to 30 nm (Gradzielski, 2008). Isotropy means uniformity in all directions, therefore, when viewed through cross-polarised light, the vibration direction of light will not change, and no image will be observed. Microemulsions form a variety of structures and phases depending upon the composition of oil, SM and water. They have a low interfacial energy and are able to form spontaneously (Moulik & Paul, 1998).

It was hypothesised that emulsions could offer advantages over current saliva substitutes by combining the lubricating and moisturising properties of oil with the palatability of water. In particular, o/w emulsions are often used for topical administration due to their water miscibility, low irritation and spreadability onto the site of application (Becher, 1985).

### 1.4.1 Choice of excipients

The most important feature of any formulation designed for delivery to the oral cavity is pharmaceutical acceptability. Taste, however, is also important, especially as the goal of a saliva substitute is to remain in the oral cavity for prolonged periods, therefore, will have contact with taste receptors for an extended time. Individuals with salivary hypofunction have an altered sense of taste (Mese & Matsuo, 2007) and this must also be taken into consideration. There are a variety of pharmaceutical and food-grade oils available that are suitable for oral ingestion. In a study investigating the efficacy of food-grade edible oils for the relief of xerostomia, rice bran oil (RBO) was found to provide a higher level of both relief and taste tolerance compared to extra virgin olive oil (Lawn, 2007). RBO is a bland tasting oil with a slightly nutty, earthly flavour and a fatty acid composition consisting primarily of palmitic, oleic and linoleic acids (Orthoefer & Eastman, 2005). It has numerous uses in foods, soaps, feed formulations and as a specialty ingredient in cosmetics (Lerma-García et al., 2009).

Generally, a combination of two surfactants is more effective in emulsification compared to a single surfactant in order to reduce the interfacial tension and improve
stability. Toxicity is an important consideration in the selection of surfactants. Non-ionic surfactants exhibit minimal toxicity and are often used. Examples include polyoxyethylene sorbitan monooleate (Tween 80) and sorbitan monooleate (Span 80). Although they tend to have a bitter taste (ChemicalBook, 2010), they are inexpensive, well characterised and exhibit minimal toxicity (Yaghmur et al., 2002; Cho et al., 2008). Lecithin consists of phosphatidylcholine (PC) and free fatty acids at different concentrations depending on purity. It originates from soy or egg and is used as an emulsifying agent in many cosmetic, food and pharmaceutical products (Hoepfner et al., 2007). Soy lecithin is an integral part of cell membranes, rendering it biocompatible and generally regarded as safe (GRAS) by the Food and Drug Administration (FDA, 2006). It has a taste similar to soybean oil (Hoepfner et al., 2007). Lecithin contains hydrated zwitterionic head groups, however it is strongly lipophilic due to its long hydrocarbon chains. To form balanced microemulsions, a polar co-solvent is required to increase the hydrophilic-lipophilic balance (Shinoda et al., 1991). Typically a short- or medium-chain alcohol is used, however, use is limited in pharmaceutical preparations by irritancy and toxicity issues (Cilek et al., 2006). In addition, the drying effect of alcohol may render it inappropriate for incorporation into formulations designed to provide xerostomia.

1.4.2 Emulsions in the oral cavity

Much work has been done on the oral processing of emulsions. Lubrication of the oral surfaces depends on the stability of the emulsion (McClements, 2007; Dresselhuis et al., 2008a). In turn, both physical and chemical aspects influence emulsion stability. The droplets themselves may be altered by hydrolysis and oxidation due to chemical instability, or physical instability may occur whereby creaming, flocculation or coalescence alters the spatial distribution of the droplets (McClements, 2010). When emulsions mix with saliva, flocculation occurs. This depends on the net charge of emulsion droplets and charged molecules present in saliva, and is influenced by depletion interactions, van der Waal’s forces and electrostatic interactions between salivary proteins and emulsion droplets (Malone et al., 2003; Silletti et al., 2007b; Sarkar et al., 2009).

Vingerhoeds and co-workers (2005) investigated the stability of emulsions stabilised by weakly negatively charged proteins (whey protein isolate, β-casein, sodium caseinate
and β-casein) or neutral surfactants (Tween 20) in the presence of mucin, which is negatively charged at neutral pH (Vingerhoeds et al., 2005; Silletti et al., 2007a, 2007b). Using porcine gastric mucin as a model mucin and whole saliva, they found that an average porcine gastric mucin concentration of 0.4% w/w was required in order for flocculation to occur in these emulsions. However, the average concentration of mucin required for flocculation of the emulsions in saliva samples was much lower (0.02% wt.), indicating that other salivary components also contribute to this phenomenon (Vingerhoeds et al., 2005). In a similar study, no flocculation was observed in highly negatively charged emulsions with a zeta potential of -90 mV (stabilised by sodium dodecylsulfate) or -75 mV (stabilised with diacetyl tartaric acid ester of monoglyceride) (Silletti et al., 2007b). In the absence of mucin, electrolytes in saliva have also been shown to aggregate oil droplets stabilised by lactoferrin at neutral pH. This has been termed the ‘salt effect’, with flocculation attributed to screening of the positively charged lactoferrin molecules on the droplet surface with the negatively charged electrolytes (phosphates, citrates and chlorides) from saliva (Sarkar et al., 2009). From these studies it appears that reversible flocculation occurs in weakly negatively charged and neutral emulsions via depletion interactions of mucins and other salivary components. These salivary components, which are not adsorbed at the oil-water interface, are pushed out of the area between emulsion droplets, creating an osmotic pressure gradient within the continuous phase that promotes flocculation of emulsion droplets (Smith & Williams, 1995; McClements, 2000). This process is reversible on dilution with water. In emulsions with higher negative charge, electrostatic repulsion has been proposed to prevent the droplets from coming into contact with one another, minimising flocculation. A bridging mechanism has been proposed for positively charged emulsions where electrostatic attraction causes the salivary proteins to bind with emulsion droplets (Silletti et al., 2007b).

Saliva will affect the sensory perception of an emulsion in the oral cavity via several mechanisms. In individuals with functioning salivary gland tissue, saliva will mix with the emulsion. Dresselhuis et al. (2008b) investigated the lipid retention of emulsions in five subjects, where each subject processed 10 mL emulsion around their mouth for 15 seconds before expectorating at set intervals following rinsing with water. The fluid expectorated was measured and the amount of lipid was determined gravimetrically using the Röse Gottlieb method. In this method, diacetyl ether and light petroleum are
added to the emulsion. The lipid is extracted into the ether layer, which is then removed from the bulk of the emulsion, dried and weighed (IDF, 1987). Participants were instructed to process the emulsion at a rate of 1 Hz, although it was not mentioned how this was achieved. They found that more lipid remained for emulsions that were sensitive to coalescence and that this was more difficult to remove by rinsing with water. Increasing droplet size also enhanced retention whereas increasing the fat content increased the absolute amount retained but reduced the relative fat retention. (Dresselhuis et al., 2008b). This suggests that coarse emulsions, which are less stable than microemulsions and have a larger droplet size, may provide better retention in the oral cavity compared to microemulsion systems.

1.4.3 Retention of liquids in the oral cavity

The amount of saliva available in the mouth is important in the ability to speak, efficiency of mastication and swallowing, and controlling the development of caries (Dirix et al., 2008). When saliva is secreted from the salivary glands it may be lost by swallowing, evaporation or absorption through the oral mucosa. In healthy individuals the fluid input is greater than that lost by evaporation or absorption through the oral mucosa – the remainder is swallowed.

To determine retention of materials in the oral cavity, several methods have been investigated. Turbidity measurements have been used to analyse the presence and persistence of food on oral coatings following ingestion of custard-based desserts, where the mouth is subsequently rinsed with 20 mL water following ingestion and the turbidity of the rinse is measured (Prinz et al., 2006; de Wijk et al., 2009). Although the objective turbidity measurements were found to relate well to subjective mouth-feel, the application of this method was limited to substances that have sufficient initial turbidity. Phenol red has been identified as an effective non-absorbable marker for mucosal drug absorption (Tucker, 1988; Dawes, 2006). In one study where participants chewed gum containing 0.5 mg phenol red for ten minutes and expectorated all saliva produced during that time, 96.7% ± 6.4% was recovered from the saliva and gum combined (Dawes, 2006). Previous studies have shown that it is absorbed through damaged gastrointestinal mucosa (Alich et al., 1984), however the effect of inflamed or damaged oral mucosa has not yet been investigated. In addition, colour is pH-dependent, changing from yellow to red over a pH range of 6.8 to 8.2, so samples may
need to be pH-adjusted prior to analysis. Although it was shown that phenol red analysis was not affected by saliva in a single healthy individual (Tucker, 1988), this may not be true in conditions such as salivary hypofunction where there is an alteration in pH in the oral cavity (Jensen et al., 2003).

1.5 Rheology with respect to saliva

Rheology describes the deformation of a material under the influence of an external stress (Equation 1.3), where stress ($\sigma$) is the product of force ($F$) across an area ($A$) (Menard, 2008).

$$\sigma = \frac{F}{A}$$  \hspace{1cm} \text{Equation 1.3}

There are two types of stress, depending on the direction in which force is applied. Normal stress describes those forces acting perpendicular to a plane, whereas shear stress describes those forces acting tangential to a plane. The deformation of a solid material that occurs when stress is applied is defined as the strain. If the applied force is perpendicular to the direction of movement then elongational (or compressional) strain ($\varepsilon$) results, which is defined as the ratio of extension or compression of a material ($dl$) compared to its original length ($l$) (Equation 1.4):

$$\varepsilon = \frac{dl}{l}$$  \hspace{1cm} \text{Equation 1.4}

Liquids, on the other hand, will relieve a strain resulting from an imposed stress by flowing. When a liquid is subjected to a force, a velocity gradient exists between planes in the liquid, whereby the plane directly exposed to the force flows the most and the plane furthest away from the force remains stationary. This gradient, $\gamma$, is termed the shear rate (Equation 1.5) and the principle is illustrated in Figure 1.6.

$$\gamma = \frac{dv}{dr}$$  \hspace{1cm} \text{Equation 1.5}
Elasticity describes the deformation of a material in response to stress, and its ability to reform when the stress is removed. Therefore, it is related to energy stored during flow. The deformation of an ideal elastic or solid is described by Hooke’s Law, which states that when a material is subjected to an infinitely small deformation, the applied stress is proportional to the resultant strain ($\gamma'$) (Equation 1.6). This ratio is called Young’s modulus ($E$).

$$E = \frac{\sigma}{\gamma'}$$  \hspace{1cm} \textbf{Equation 1.6}

Viscosity is a measure of the resistance of a liquid to flow or movement and is related to the energy used during flow. A purely viscous liquid obeys Newton’s Law of Flow in that applied stress is directly proportional to the rate of strain but independent of strain itself (Stanley & Taylor, 1993). This proportionality constant is termed the Newtonian viscosity ($\eta$) and is defined by Equation 1.7.

$$\eta = \frac{\sigma}{\gamma'}$$  \hspace{1cm} \textbf{Equation 1.7}

Whole saliva is classified as a non-Newtonian material because it does not obey Newton’s Law of Flow that “shear stress is linearly proportional to shear rate” (Hatton et al., 1987; Preetha & Banerjee, 2005). Instead, flow properties are dependent on the magnitude of applied shear stress – as shear stress increases, the shear rate increases.
and apparent viscosity, which is maximal at rest, decreases. This shear thinning behaviour has physiological significance because it allows for coating of the entire cavity as well as mucosal surface lubrication at rest and flow during mastication. Early studies on saliva assumed it to be a simple Newtonian fluid but as more sophisticated techniques for measuring rheological properties were developed, the shear thinning and linear viscoelasticity of saliva were detected, as well as a yield stress, leading to the conclusion that saliva exhibited gel-like properties (Davis, 1971). This yield stress has since been disregarded as saliva, like all biological fluids, contains surface-active molecules that can adsorb at liquid interfaces at the rim of the rheometer, forming an elastic layer that results in the measurement of a yield stress and gel-like properties uncharacteristic of the rheology of bulk saliva (Waterman et al., 1988; Stokes & Davies, 2007). This adsorption can be minimised by applying a thin layer of surfactant solution around the rim of the rheometer plates (Stokes & Davies, 2007). In the oral cavity, shear rates ranging from 0.1 and 1.0 s⁻¹ at rest (Corcoran et al., 2006) and increasing to 60 and 160 s⁻¹ during swallowing and speaking, respectively (Vissink et al., 1984) have been reported.

The behaviour of viscous and elastic materials can be represented using mechanical models. A viscous Newtonian fluid may be represented by the movement of a piston inside a liquid-filled cylinder, or dashpot (Figure 1.7a), whereby the piston extends at a rate dependent upon applied stress and liquid viscosity. When the applied stress is removed there is no return to the starting position due to the disruption of weak intermolecular forces. An elastic Hookean solid may be represented by the movement of a spring (Figure 1.7b), whereby stretching of intermolecular forces occurs and the applied stress is stored in the spring such that when it is removed the spring returns to its equilibrium position. The behaviour of real materials, including saliva, is based on a combination of both viscous and elastic properties, hence the term viscoelasticity. In saliva, this relates to its ability to be drawn out into threads and hold water whilst simultaneously remaining in contact with the mucosa for a period of time (Marks & Roberts, 1983). Several models have been established to describe viscoelasticity, using combinations of the spring and dashpot. The spring and dashpot are combined in series to form a Maxwell element (Figure 1.7c) or in parallel to form a Voigt element (Figure 1.7d). The Maxwell model describes a system under a constant applied stress. This results in displacement of the spring due to the strain on the material, and the
movement of the piston in the dashpot due to viscous flow. When the stress is removed, the spring returns to its original state but the dashpot does not. In the Voigt model, on the other hand, the drag of the viscous fluid in the dashpot influences the extension and compression of the spring (representing the elastic behaviour). Therefore, the strain varies exponentially over time. This strain can be measured and is known as deformation, or compliance. The Maxwell and Voigt models may also be combined into a generalised model that accounts for the flow and deformation of a material (Harris, 1977; Sinko, 2011).

![Diagram](image)

**Figure 1.7** Schematic representation of a) a purely viscous material, b) a purely elastic material, and examples representing viscoelastic materials using c) a Maxwell element and d) a Voigt element.

Oscillatory rheology is a non-destructive technique whereby samples are exposed to an applied stress that varies sinusoidally at a frequency (f), which provides information about the interparticle and intermolecular forces in the material being measured. The complex modulus (G*) of the material describes the overall resistance to deformation and can be calculated by the ratio of applied stress (σ) to resultant measured strain (γ) (Equation 1.8), whereas the phase angle, δ, describes the difference between the applied stress and resulting strain.

\[
G^* = \frac{\sigma}{\gamma}
\]  

*Equation 1.8*
During this process the energy stored (reversible deformation) represents the elasticity of the sample and is termed the storage modulus (\(G'\), Equation 1.9), whereas the energy lost (irreversible deformation) represents the viscous behaviour of the sample and is termed the loss modulus (\(G''\), Equation 1.10).

\[
G' = G^* \cos \delta \quad \text{Equation 1.9}
\]

\[
G'' = G^* \sin \delta \quad \text{Equation 1.10}
\]

Before conducting an oscillatory test the linear viscoelastic region (LVR) of the material to be tested must be determined. The LVR is the region in which stress is directly proportional to strain. It is determined by conducting an amplitude sweep where the sample is subjected to an increasing stress and the resulting strain is measured. The transition to a non-linear relationship between stress and strain means that the torque response of the material to sinusoidal strain is no longer following a pure sinusoidal pattern. This results in a sharp decrease in \(G^*\) and indicates sample deformation. The overall complex viscosity (\(\eta^*\)) is a measure of the difference in resistance of a material to deformation (regardless of whether the deformation is elastic or viscous) as a function of frequency, where \(\omega\) is the angular frequency (Equation 1.11).

\[
\eta^* = \sqrt{\frac{(G')^2 + (G'')^2}{\omega}} \quad \text{Equation 1.11}
\]

The loss tangent (\(\tan \delta\)) is a measure of the relative contribution of viscous and elastic moduli of a material at a given frequency (Barnes et al., 1989) (Equation 1.12). A \(\tan \delta\) of greater than one indicates that viscous behaviour dominates over elastic behaviour whereas a \(\tan \delta\) less than one indicates that elastic behaviour dominates; \(\tan \delta = 0\) for purely elastic materials (Harris, 1977).

\[
\tan \delta = \frac{G''}{G'} \quad \text{Equation 1.12}
\]

The viscoelasticity of saliva has been shown to be dependent on flow rate, age and composition (Zussman et al., 2007). Mucins are large glycoproteins that are able to
adsorb onto a range of surfaces (Yakubov et al., 2009). The two types of mucins present in saliva, MUC5B and MUC7, are thought to influence its overall viscoelastic properties. Several studies have confirmed that saliva secreted from the parotid gland is less viscoelastic than that secreted from the submandibular or sublingual glands (van der Reijden et al., 1993; Stading et al., 2009). The difference in rheology of saliva from each gland is hypothesised to be due to mucin concentration as sublingual salivary secretions are more mucous than serous parotid salivary secretions (van der Reijden et al., 1993). In addition, unstimulated whole saliva is more viscous than stimulated whole saliva. Research by Davies and co-authors (2009) found that the ingestion of acidic beverages such as cola and ice tea significantly increased the flow rate and elasticity of whole saliva compared to water. The increase in elasticity was proposed to be a defence mechanism to protect the oral cavity from the acid environment. The mechanical action of chewing gum and mint flavour showed a similar increased flow rate to acidic beverages, however, no significant difference in elasticity compared to water was observed.

The discrepancies in rheological properties of saliva may reflect different roles from each gland. The high elasticity of sublingual saliva at low shear rates combined with adhesion to the oral mucosa may support a higher retention, while the high viscosity prevents dehydration of the oral mucosa. On the other hand, saliva secreted from the submandibular gland provides the lubrication necessary for effective speaking, swallowing and bolus formation (van der Reijden et al., 1993; Schipper et al., 2007; Zussman et al., 2007).

1.6 Conclusions

Xerostomia can significantly impair oral health and quality of life, yet current treatment options for individuals with little or no residual salivary gland function are limited. Saliva has a diverse range of functions and complex physicochemical properties that are able to adapt to the oral environment. Emulsions have potential as saliva substitutes due to the lubricating properties of oil combined with the palatability of water; however, further understanding of the properties of such formulations is required. The rheological properties of saliva are important in the lubrication and retention of the oral cavity. For this reason it is hypothesised that viscoelasticity plays a pivotal role in the efficacy of a saliva substitute.
1.7 Research hypothesis

Treatment options for xerostomia are limited, with current saliva substitutes offering minimal efficacy, particularly in individuals with severely restricted residual salivary gland function. The rheological properties of natural saliva are thought to be responsible for many of its functions, yet an understanding about how the physicochemical properties of a saliva substitute influence clinical efficacy is limited. While viscoelasticity is hypothesised to be an important indicator of efficacy of saliva substitutes, retention in the absence of continual secretion is also important. Therefore, the viscoelasticity of an effective saliva substitute is expected to differ from that of natural saliva. It is hypothesised that pleasant-tasting oils may provide an alternative approach for lubricating oral mucosal surfaces. Therefore, the physicochemical properties of different oil-based formulations, with a focus on rheology, were investigated with the aim of developing a retentive saliva substitute that could lubricate the dynamic environment of the oral cavity.

1.8 Thesis aims

- To identify and evaluate combinations of pharmaceutically acceptable surfactants using pseudo-ternary phase diagrams and polarised light microscopy;
- To gain an understanding of the rheology of natural saliva by comparing the flow and oscillatory rheology of submandibular saliva collected using a custom-fabricated device with whole saliva collected using the expectoration method, and compare these results to that of a commercially available polymer-based saliva substitute;
- To investigate the rheological properties and droplet characteristics of selected emulsions in order to identify compositions worthy of trialling clinically;
- To evaluate the clinical efficacy of a selected emulsion in a pilot study in individuals with normal salivary function, in order to develop markers to determine the taste acceptability and retention of a selected formulation.
Chapter two

Preliminary investigations
2 Preliminary investigations

This chapter describes preliminary results that developed methods for use in later chapters and is divided into two sections. The first reports preliminary investigations of a range of potential oil and water based systems, while the second examines the rheological properties of saliva. In Section 2.2, combinations of pharmaceutically acceptable surfactants and oils were examined using pseudo-ternary phase diagrams and optical microscopy, in order to select a combination for further investigation. In Section 2.3, the rheological properties of whole saliva and submandibular saliva were investigated and compared with a polymer-based commercial saliva substitute. As the rheology of whole saliva has previously been reported (Stokes & Davies, 2007), this served as an introduction to the methods required in the rheology of materials, a technique that will be applied to potential saliva substitutes in later chapters.

2.1 Chapter aims

• To identify and study pharmaceutical surfactants with potential application in saliva substitute formulations, using pseudo-phase diagrams and polarised light microscopy;
• To investigate the rheology of submandibular saliva collected using a custom-fabricated device compared with whole saliva collected using the expectoration method to determine whether the flow properties of submandibular saliva collected with the device are representative of whole saliva;
• To compare the rheological properties of saliva with the rheology of a commercially available saliva substitute.

2.2 Investigation of excipients

2.2.1 Introduction

The pharmaceutical acceptability of excipients is a key consideration of formulations for the oral cavity (Lawrence & Rees, 2000). In this chapter, an investigation into possible oils and surfactants was conducted. RBO was selected as the oil phase following a pilot study that subjectively investigated xerostomia relief and taste tolerance following the use of a variety of edible oils. Results showed that while none of the tested oils were sufficiently retentive, RBO provided the highest level of relief
and taste tolerance (Lawn, 2007). Non-ionic surfactants such as polyoxyethylene sorbitan monooleate (Tween 80) and sorbitan monooleate (Span 80) are inexpensive, well characterised and exhibit minimal toxicity (Yaghmur et al., 2002; Cho et al., 2008), and were investigated for their potential application in the present work. Lecithin was used in this study as it is a surfactant used as an emulsifying agent in many cosmetic, food and pharmaceutical products (Hoepfner et al., 2007). As an integral part of cell membranes, lecithin is biocompatible and GRAS according to the Food and Drug Administration (FDA, 2006). Typically a short- or medium-chain alcohol is added as a co-solvent for lecithin, however in pharmaceutical preparations use is limited by irritancy and toxicity issues (Cilek et al., 2006). In addition, the drying effect of alcohol renders it inappropriate for incorporation into formulations designed to provide xerostomia relief. As such, a preliminary investigation of alternative co-solvents for lecithin was conducted. These included glycerol, polyethylene glycol (PEG) 400 and propylene glycol (PG). Isopropyl myristate (IPM) was investigated as an alternative, pharmaceutical-grade oil. IPM is a non-toxic ester and was selected as a second oil as it is pharmaceutically acceptable and has good systemic and local tolerance (Hoepfner et al., 2007).

A number of techniques may be employed in the characterisation of emulsions, but this preliminary chapter focuses on pseudo-ternary phase diagrams and polarised light microscopy. Pseudo-ternary phase diagrams are useful in determining the relationship between the composition of a formulation and the physical phase behaviour (Lawrence & Rees, 2000). This is of particular interest in the present work because it is likely that formulations will be exposed to continual water uptake in the oral cavity due to the presence of residual saliva and the ingestion of liquids. Polarised light microscopy is often used in the characterisation of emulsions to investigate the microstructure and determine if the system is isotropic or anisotropic. Anisotropic materials have properties that differ depending on the direction of measurement. Anisotropy can be observed using polarised light microscopy when refractive index changes occur in the sample of interest depending on polarisation. This is characteristic of liquid crystalline structures, and is also known as birefringence. The Becke line test was used to determine the phase with the higher refractive index, thus confirming whether the formulations were water-in-oil (w/o) or oil-in-water (o/w) type emulsions. The Becke line is a bright line that exists at the boundary between two phases. When the focal
distance of the microscope is increased, the line moves towards the medium with the higher refractive index. The test can be used to determine the relative refractive index between two phases.

The aim of this section was to identify and investigate pharmaceutically acceptable surfactants and determine their phase behaviour in the presence of RBO and water. A surfactant combination was then selected for further investigation in Chapter 3 as a template emulsion for the relief of xerostomia.

2.2.2 Materials and methods

2.2.2.1 Materials

RBO was purchased from Bespoke Foods (London, UK). Soy lecithin (Lipoid-S100) was purchased from Lipoid GmBH (Ludwigshafen, Germany). Product specification data provided by the manufacturer stated that the lecithin consisted of 96.8% dry weight phosphatidylcholine and less than 0.05% free fatty acids. IPM, Tween 80, Span 80, glycerol, PEG 400 and PG were purchased from Sigma (St Louis, MO, USA) and 99.8% ethanol was obtained from Anchor Ethanol Ltd (Auckland, NZ). Distilled water was used throughout the experiments and all materials were used as received from the manufacturer without any further purification.

2.2.2.2 Tween 80 and Span 80 as surfactants for RBO-water compositions

Compositions were prepared by weighing appropriate amounts of RBO, Span 80 and Tween 80 such that the total weight of each sample was 300 mg. The weight of each component varied in increments of 10% w/w so that 66 different combinations were possible (Figure 2.1). These were mixed for three minutes in a vortex mixer (Chiltern MT19) and examined for visual homogeneity. In order to determine the volume of water (with a density of 1 g/mL) incorporated into the system before a coarse emulsion formed, distilled water was added drop-wise to each formulation and the weight of the mixture was recorded after addition of each drop. Compositions were then vortexed for a further 30 seconds and examined for visual clarity. This process was repeated until the mixture became turbid, indicating the formation of a coarse emulsion. Turbidity was determined visually as the transition from a transparent to opaque system. Mixtures were left to equilibrate for 24 hours at room temperature before being re-
examined for turbidity. If turbidity had disappeared during this time then the process was repeated. The total weight of water incorporated before the system became turbid was then calculated as a relative percentage along with the concentration of surfactants and oil, and plotted on a contour surface plot using Matlab R2013a (8.1.0.604, MathWorks Inc, Natick, MA, USA). From this data, an optimal ratio of Tween 80 and Span 80 of 9:1 w/w was determined and a further pseudo-ternary phase diagram was created by first mixing Tween 80 and Span 80 at this ratio, then adding water and RBO at various w/w ratios as outlined in Figure 2.1. Each composition was again vortexed for three minutes before being visually assessed for turbidity. Based on their physical appearance, compositions were classed as “semi solid-like” when they had the visual appearance of a gel-like system and did not flow on initial inversion, or “liquid like”, where they flowed freely on inversion. Data was then plotted using Origin Pro 8.5.1 (OriginLab Corporation, Northampton, MA, USA).

![Figure 2.1 Phase triangle set-up showing each of the 66 possible combinations in a three-component system when the concentration of each excipient (A, B and C) was varied in 10% w/w increments.](image)
2.2.2.3 Soy lecithin as a surfactant for RBO-water compositions

Soy lecithin was investigated as a potential surfactant in emulsions containing RBO and water. Initially, the solubility of lecithin was investigated by addition of co-solvents including ethanol, PG, PEG 400, RBO, glycerol or Tween 80 at a lecithin to co-solvent ratio of 1:2 or 1:3 w/w. Compositions were vortexed for three minutes and then magnetically mixed at room temperature for 24 hours before being examined for visual homogeneity. They were then left to stand at room temperature for a further seven days before being re-examined for homogeneity.

PG was selected for further investigation as a co-solvent for lecithin. To determine the solubility of soy lecithin in PG, Lecithin:PG combinations were prepared at weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 w/w and vortex-mixed for three minutes before being magnetically stirred at room temperature for 24 hours. Where the lecithin failed to completely dissolve, further mixtures were prepared with PG added in 1% w/w increments and the mixing process was repeated until the approximate solubility was determined.

RBO was added in 10% w/w increments to aliquots of the surfactant compositions, as demonstrated in Table 2.1. These were vortex-mixed for three minutes before being left to equilibrate for 24 hours and assessed for visual clarity. The solubility of lecithin in PG and RBO was thus determined and plotted onto a pseudo-ternary phase diagram using Origin Pro 8.5.1 (OriginLab Corporation, Northampton, MA, USA). Compositions were then selected to determine the effect of water addition. Distilled water was added drop-wise to these compositions and the weight of the mixture was recorded after addition of each drop. Compositions were then vortexed for a further 30 seconds and examined for visual clarity. This process was repeated until the mixture became turbid, indicating the formation of a coarse emulsion. Mixtures were left to equilibrate for 24 hours at room temperature before being re-examined for turbidity. If turbidity had disappeared during this time, then the process was repeated. The total weight of water incorporated before the system became turbid was then calculated as a relative percentage along with the concentration of surfactants and oil, and plotted onto pseudo-ternary phase diagrams as described above.
Pseudo-ternary phase diagrams were prepared with RBO, water and surfactant combinations of either lecithin and PG (1:1 w/w) or lecithin and ethanol (1:1 w/w). The SMs were prepared by adding equal amounts of lecithin to co-solvent (ethanol or PG) and magnetically mixing for 24 hours, after which time the lecithin had completely dissolved. SM, RBO and water were then combined at different weight ratios (Figure 2.1) and mixed by vortexing for three minutes.

Table 2.1 Compositions of RBO and a surfactant mixture of lecithin (Lec) and PG at weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1.

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<td>0.07</td>
<td>0.06</td>
<td>0.05</td>
<td>0.04</td>
<td>0.03</td>
<td>0.02</td>
<td>0.01</td>
</tr>
</tbody>
</table>

2.2.2.4 IPM as the oil phase for lecithin-PG combinations

To investigate the effect of using IPM as the oil phase, the surfactant was prepared as described in the previous section, using lecithin and PG at a weight ratio of 1:1 w/w. Pseudo-ternary phase diagrams were then prepared by combining IPM, SM and water at different weight ratios and vortexing for three minutes so that 66 different compositions were evaluated (Figure 2.1). Compositions were left to equilibrate for 24 hours at room temperature before being examined for turbidity and this data was plotted onto a pseudo-ternary phase diagram using Origin Pro 8.5.1 (OriginLab Corporation, Northampton, MA, USA).
2.2.2.5 Polarised light microscopy of compositions

Following a 24-hour equilibration period at room temperature, selected compositions were examined under both polarised and non-polarised light using a Motic BA300Pol light microscope (Motic Incorporation, Hong Kong) coupled with a Moticam 2300 digital camera (Motic Incorporation, Hong Kong). Compositions were selected and prepared as described above with RBO, water, and a SM of either Tween 80 and Span 80 (9:1 w/w), lecithin and PG (1:1 w/w), or lecithin and ethanol (1:1 w/w) at varying concentrations. The compositions consisting of IPM, a SM of lecithin and PG (1:1 w/w) and water were also evaluated. Formulations were examined that had been prepared by both vortex-mixing and magnetic stirring in order to determine any differences in droplet size as a result of mixing technique. A drop of each mixture was placed on a slide and observed using a 40x, 60x and 100x objective lens. Images were captured both with and without a polarising filter to determine whether optical birefringence existed in the mixture. The Becke line test was used to determine the relative refractive index of the dispersed and continuous phases. As the focal distance increases, the Becke line moves towards the medium with the higher refractive index and can be used to determine whether the formulations were o/w or w/o type emulsions.

2.2.3 Results

2.2.3.1 Tween 80 and Span 80 as surfactants for RBO-water compositions

The transition of compositions containing different weight ratios of RBO, Tween 80 and Span 80 as water content increased is shown schematically in the shaded contour plot in Figure 2.2. The area below the shaded contour indicates transparent compositions and the area above indicates turbid compositions.
This transition from transparent to turbid indicated the change from a microemulsion to a coarse emulsion. To determine the effect of adding water in 10% w/w increments, data were plotted in a series of pseudo-ternary diagrams (Figure 2.3). All compositions were turbid after addition of 40% w/w water (Figure 2.3d). Compositions that were able to accommodate a relative percentage water uptake of greater than 20% are listed in Table 2.2. The composition that consisted of a relative w/w percentage of 7% RBO, 6% Span 80, 55% Tween 80 and 32% water (equivalent to a Tween 80 to Span 80 ratio of approximately 9:1 w/w) was selected for further investigation, because it was able to incorporate the most water (32% w/w) before forming a turbid emulsion.

The pseudo-ternary phase diagram for compositions containing water, RBO and a SM of 90% w/w Tween 80 and 10% w/w Span 80 is shown in Figure 2.4. As the weight fraction of water in the system increased, there was a physical phase transition from transparent liquid-like microemulsion to a turbid solid-like coarse emulsion and then to a turbid liquid-like coarse emulsion.
Figure 2.3  Phase diagrams of different w/w compositions of Tween 80, Span 80 and RBO showing the transition from microemulsion (unshaded) to coarse emulsion (shaded) following the addition of a) 10% w/w water, b) 20% w/w water, c) 30% w/w water and d) 40% w/w water.

Table 2.2 The relative percentage of RBO, Tween 80, Span 80 and water at the point in which the system became turbid, in formulations with greater than 20% w/w water uptake.

<table>
<thead>
<tr>
<th>Relative fraction of each component (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBO</td>
</tr>
<tr>
<td>-----</td>
</tr>
<tr>
<td>7.0</td>
</tr>
<tr>
<td>15</td>
</tr>
<tr>
<td>8.0</td>
</tr>
</tbody>
</table>
Figure 2.4 Pseudo-ternary phase diagram showing the physical phase changes from microemulsion (striped), semi solid-like (shaded) and liquid-like (unshaded), for different compositions (w/w) of RBO, water and a SM of Tween 80 and Span 80 (9:1 w/w).
2.2.3.2 Soy lecithin as a surfactant for RBO-water compositions

A summary of the miscibility of soy lecithin in different co-solvents is presented in Table 2.3. Ethanol and PG were able to dissolve lecithin at a weight ratio of both 1:2 w/w and 1:3 w/w. Lecithin was immiscible in RBO, glycerol, PEG 400 and Tween 80 at both weight ratios tested.

Table 2.3 Summary of the visual features of soy lecithin formulations dissolved in a variety of co-solvents after a time period of three minutes (immediately following vortex mixing), 24 hours of magnetic stirring and one week of magnetic stirring. Formulations were classed as miscible (M), immiscible (IM) or turbid (T), where miscibility could not be determined.

<table>
<thead>
<tr>
<th>Co-solvent</th>
<th>Lecithin:co-solvent 1:2 w/w</th>
<th>Lecithin:co-solvent 1:3 w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 min 24 h 1 week</td>
<td>3 min 24 h 1 week</td>
</tr>
<tr>
<td>Ethanol</td>
<td>M M M</td>
<td>M M M</td>
</tr>
<tr>
<td>PG</td>
<td>T M M</td>
<td>T M M</td>
</tr>
<tr>
<td>PEG 400</td>
<td>IM IM IM</td>
<td>IM IM IM</td>
</tr>
<tr>
<td>RBO</td>
<td>IM IM IM</td>
<td>IM IM IM</td>
</tr>
<tr>
<td>Glycerol</td>
<td>IM IM IM</td>
<td>IM IM IM</td>
</tr>
<tr>
<td>Tween 80</td>
<td>IM IM IM</td>
<td>IM IM IM</td>
</tr>
</tbody>
</table>

The minimum amount of PG required to successfully dissolve the soy lecithin at room temperature within 24 hours was determined. Results indicated that although a visually homogenous SM formed with a lecithin to PG ratio of up to 6:4 w/w, these mixtures were viscous and had a semi-solid appearance. The minimum concentration required to dissolve lecithin within 24 hours was 48% w/w PG and 52% w/w lecithin (Table 2.4).

The effect of adding RBO to the lecithin/PG compositions is presented in Figure 2.5a. As the relative weight fraction of RBO was increased, the amount of PG required to dissolve lecithin decreased, with only 8% w/w PG required in the presence of 60% w/w RBO. From this solubility investigation, a region on the pseudo-ternary phase diagram was selected to determine the transition to a turbid emulsion as water was added to the system (Figure 2.5). The phase change to a turbid emulsion occurred rapidly, with many of the compositions becoming turbid after the addition of 5% w/w water (Figure 2.5b). Following the addition of 10% w/w water, only one composition, of 74% w/w RBO, 12.5% w/w PG and 13.5% w/w lecithin, remained transparent, with a maximum
water uptake of 10.9% w/w (Figure 2.5c). A lecithin to PG ratio of 1:1 w/w was therefore selected for further investigations in systems containing RBO and water.

When compositions containing RBO, water and SM (1:1 w/w lecithin and PG) were investigated, structural changes were observed in all compositions as the concentration of SM was reduced, with formulations appearing more liquid-like with decreasing SM concentration (Figure 2.6). Turbid emulsions formed in the presence of less than 80% w/w RBO and 10% w/w water; however, systems were able to maintain a viscous, semi-solid state in the presence of up to 50% w/w water depending on the composition of oil and SM.

Table 2.4 Summary of the visual features of different compositions of lecithin and PG, in order to determine the minimum amount of PG required to successfully dissolve lecithin.

<table>
<thead>
<tr>
<th>SM composition (% w/w)</th>
<th>Time to visual homogeneity</th>
<th>Physical appearance at 24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lecithin</td>
<td>PG</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>90</td>
<td>3 min</td>
</tr>
<tr>
<td>20</td>
<td>80</td>
<td>3 min</td>
</tr>
<tr>
<td>30</td>
<td>70</td>
<td>12 h</td>
</tr>
<tr>
<td>40</td>
<td>60</td>
<td>12 h</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>24 h</td>
</tr>
<tr>
<td>52</td>
<td>48</td>
<td>24 h</td>
</tr>
<tr>
<td>53</td>
<td>47</td>
<td>24 h</td>
</tr>
<tr>
<td>55</td>
<td>45</td>
<td>24 h</td>
</tr>
<tr>
<td>57</td>
<td>43</td>
<td>24 h</td>
</tr>
<tr>
<td>58</td>
<td>42</td>
<td>24 h</td>
</tr>
<tr>
<td>60</td>
<td>40</td>
<td>24 h</td>
</tr>
<tr>
<td>70</td>
<td>30</td>
<td>n/a</td>
</tr>
<tr>
<td>80</td>
<td>20</td>
<td>n/a</td>
</tr>
<tr>
<td>90</td>
<td>10</td>
<td>n/a</td>
</tr>
</tbody>
</table>
Figure 2.5 Pseudo-ternary phase diagram showing a) the RBO, lecithin and PG compositions in which lecithin dissolved (shaded region) with the area selected for investigated the effect of water addition (dashed line); and the appearance of a turbid emulsion (striped region) after adding b) 5% w/w water and c) 10% w/w water, with maximum water uptake observed in the composition marked •.
Figure 2.6 Pseudo-ternary phase diagram of transparent liquid (striped), turbid semi-solid (shaded) and turbid liquid (unshaded) compositions for RBO, SM (1:1 w/w lecithin and PG) and water compositions.
2.2.3.3 IPM as the oil phase for lecithin-PG combinations

In the pseudo-ternary phase diagram of IPM, SM (lecithin and PG, 1:1 w/w) and water, compositions were liquid-like or semi solid-like depending on their composition. Transparent emulsions formed in compositions containing between 30% and 70% (w/w) SM as well as between 10% and 60% (w/w) IPM (Figure 2.7). Within this region, compositions exhibited semi-solid or liquid like behaviour.

Figure 2.7 Pseudo-ternary phase diagram for IPM, 1:1 w/w lecithin and PG (SM) and water compositions showing semi-solid (shaded), liquid (unshaded) and transparent (striped) compositions.
2.2.3.4 Polarised light microscopy of compositions

Polarised light microscopy was used to identify microemulsions, which are optically isotropic and exhibit no birefringence under polarised light. The presence of liquid crystalline structures were also identified, which are anisotropic and can be seen under polarised light. When Tween 80 and Span 80 were included as surfactants, no liquid crystalline structures were observed. However, when lecithin was present, many of the formulations demonstrated birefringence, indicating the presence of liquid crystals. A summary of the different microstructures formed within the tested RBO compositions is given in Figure 2.8. In order to obtain the clearest images, compositions with a SM of Tween 80 and Span 80 were captured using non-polarised light microscopy whereas compositions of lecithin and ethanol or PG were captured using polarised light microscopy. Flocculation was evident in some compositions with lower SM concentrations (below 40% w/w), as seen at weight ratios of 10% RBO, 30% SM and 60% water in Figure 2.8.
### Figure 2.8
Selected microstructure observed in compositions of RBO, water (W) and a SM of either 9:1 w/w Tween 80 and Span 80 (T80 + S80), 1:1 w/w lecithin in ethanol (Lec + EtOH) or 1:1 w/w lecithin in PG (Lec + PG). Black scale bars indicate 10 µm.
When IPM was the oil phase, droplets were not visible in the compositions that appeared transparent at a macroscopic level (Figure 2.7). Under polarised light, however, some structures showed birefringence, suggesting that liquid crystalline structures were present. In Figure 2.9, a comparison between the polarised light microscopy images obtained using IPM or RBO as the oil phase is presented. For any given oil fraction, droplets appeared to be less densely packed for IPM compositions compared to when RBO was the oil phase.

<table>
<thead>
<tr>
<th>Composition (w/w)</th>
<th>Oil</th>
<th>SM</th>
<th>W</th>
<th>IPM</th>
<th>RBO</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 60 10</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 60 20</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 30 50</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 30 40</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 2.9 Microstructure of selected compositions containing a 1:1 (w/w) lecithin and PG (SM), distilled water (W) and either IPM or RBO as the oil phase. Black scale bars indicate 10 µm.
Light microscopy was used to measure droplet size and to determine whether the RBO systems were w/o or o/w using the Becke line Test. Formulations were classified as o/w, w/o or pseudo-bicontinuous, where structures were present but no continuous or dispersed phase was observed. This is illustrated in Figure 2.10 for compositions or RBO, water and a SM of lecithin and PG (1:1 w/w). Table 2.5 shows that all of the lecithin-based compositions were either pseudo-bicontinuous or o/w emulsions, indicating that the SM forms part of the aqueous phase. This was highlighted in the formulation containing 60% w/w RBO and 40% w/w SM, as dispersed droplets were observed within a continuous phase, in the absence of water.

Figure 2.10  Pseudo-ternary phase diagram for RBO, SM (1:1 w/w lecithin and PG) and water compositions based on the Becke Line Test, showing o/w (grey), pseudo-bicontinuous (striped) and w/o (unshaded) regions.
Table 2.5 Characteristics of different compositions based on the Becke line test to determine whether these were o/w, w/o, microemulsion (ME) or pseudo-bicontinuous (BC). SM was either 9:1 w/w Tween 80 and Span 80 (T80 + S80), 1:1 w/w lecithin and ethanol (Lec + EtOH) or 1:1 w/w lecithin and PG (Lec + PG).

<table>
<thead>
<tr>
<th>Composition (% w/w)</th>
<th>SM</th>
<th>Water</th>
<th>T80 + S80</th>
<th>Lec + EtOH</th>
<th>Lec + PG</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBO 20</td>
<td>60</td>
<td>20</td>
<td>ME</td>
<td>o/w</td>
<td>BC</td>
</tr>
<tr>
<td>30</td>
<td>60</td>
<td>10</td>
<td>ME</td>
<td>o/w</td>
<td>o/w</td>
</tr>
<tr>
<td>40</td>
<td>60</td>
<td>0</td>
<td>-</td>
<td>o/w</td>
<td>o/w</td>
</tr>
<tr>
<td>20</td>
<td>50</td>
<td>30</td>
<td>o/w</td>
<td>o/w</td>
<td>o/w</td>
</tr>
<tr>
<td>30</td>
<td>50</td>
<td>20</td>
<td>BC</td>
<td>o/w</td>
<td>o/w</td>
</tr>
<tr>
<td>40</td>
<td>50</td>
<td>10</td>
<td>o/w</td>
<td>o/w</td>
<td>o/w</td>
</tr>
<tr>
<td>20</td>
<td>40</td>
<td>40</td>
<td>o/w</td>
<td>o/w</td>
<td>o/w</td>
</tr>
<tr>
<td>30</td>
<td>40</td>
<td>30</td>
<td>o/w</td>
<td>o/w</td>
<td>o/w</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>60</td>
<td>o/w</td>
<td>o/w</td>
<td>o/w</td>
</tr>
<tr>
<td>20</td>
<td>30</td>
<td>50</td>
<td>o/w</td>
<td>o/w</td>
<td>o/w</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>70</td>
<td>o/w</td>
<td>o/w</td>
<td>o/w</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
<td>60</td>
<td>o/w</td>
<td>o/w</td>
<td>o/w</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>80</td>
<td>o/w</td>
<td>o/w</td>
<td>o/w</td>
</tr>
</tbody>
</table>

2.2.4 Discussion

In the formulation of a saliva substitute, it is essential that all excipients are pharmaceutically acceptable for oral administration (Lawrence & Rees, 2000). This preliminary work involved an investigation of Tween 80, Span 80 and lecithin as potential surfactants in RBO emulsions based on this fundamental criterion. It is known that a mixture of surfactants are advantageous in emulsions, resulting in smaller droplet sizes and decreased turbidity in comparison to a single surfactant (Cho et al., 2008).

In an initial investigation of the optimal ratio of Tween 80 and Span 80, compositions that were able to incorporate a relative percentage of greater than 20% water before becoming turbid were considered desirable. The composition with a relative weight ratio of 7% RBO, 6% Span 80, 55% Tween 80 and 32% water, corresponding with a
Tween 80 to Span 80 weight ratio of 9:1, was selected for further investigation because this system was able to incorporate the most water (32% w/w) before a turbid emulsion developed. This also corresponded with the findings of Cho and colleagues (2008), who studied emulsions with canola oil and various non-ionic surfactant mixtures and found that the clearest microemulsions were generally formed at a weight ratio of 10% Span 80 and 90% Tween 80. They found that this corresponded with a smaller droplet size and improved stability over two months. In the present study, the pseudo-ternary phase diagram of RBO, water and SM of Tween 80 and Span 80 at a 9:1 w/w ratio demonstrated the change from a microemulsion to a coarse emulsion when the water content was more than 10% w/w, or the SM was less than 50% w/w. In addition, a transition from a semi solid-like system to a liquid-like emulsion was observed with increasing water content. This is important when considering a formulation for the oral cavity, as the water content in the system is likely to increase over time due to residual saliva and the ingestion of other liquids such as water, with xerostomia patients known to require frequent sips of water (Ferguson, 2002).

Soy lecithin is one of few surfactants to be listed as GRAS by the US Food and Drug Administration (FDA, 2006; Xu et al., 2011). It is commonly used in both food and pharmaceutical products (Hoepfner et al., 2007). In addition, it is of natural origin and is readily available (Xu et al., 2011), making it an appealing surfactant choice for the present study. An initial screening of potential co-solvents revealed that of those tested, lecithin was soluble in ethanol and PG but not in Tween 80, PEG 400, RBO or glycerol. An investigation into the combination of lecithin and ethanol demonstrated a transition from semi-solid like to liquid-like emulsion occurred when the relative weight fraction of lecithin to ethanol was below 1:2 w/w. Ethanol was not pursued as a potential co-solvent as it was considered unsuitable for oral administration due to potential toxicity and irritancy issues (Cilek et al., 2006).

The soy lecithin used in this research (Lipoid S100) consisted of 96.8% dry weight phosphatidylcholine. Phosphatidylcholine is a zwitterionic phospholipid that contains positively charged choline groups and negatively charged phosphate and carbonyl groups (McClements, 2005). However, phosphatidylcholine alone has shown inferior emulsifying properties compared to soy lecithin (Yeadon et al., 1958; Rydhag & Wilton, 1981), which also contains small amounts of other phospholipids, including
phosphatidyl ethanolamine and phosphatidylinositol. The phosphatidylinositol phospholipid in commercial soy lecithin is responsible for its weak negative charge at physiological pH (Wang & Wang, 2008; Xu et al., 2011). The superior surface activity of soy lecithin compared to phosphatidylcholine alone has been attributed to the combination of surface-active components (Xu et al., 2011). When soy lecithin was used as the surfactant, different emulsion structures formed depending upon the SM concentration, which has also been demonstrated in previous studies (Moreno et al., 2003).

PG was investigated as a co-solvent for lecithin and the solubility point of lecithin in PG was determined to be 0.52:0.48 w/w. Therefore, a 1:1 w/w ratio of lecithin and PG was selected as the SM and characterised with RBO and water using a pseudo-ternary phase diagram. Turbid emulsions formed when the weight ratio of SM was under 74% w/w and water was under 10% w/w, but these turbid systems were able to maintain their viscous, semi-solid state in the presence of up to 50% w/w water, to form a lecithin organogel-like composition. Lecithin organogels were first described in 1988 (Scartazzini & Luisi, 1988) and since then have been researched extensively for their potential application in topical delivery, where their lipid-rich composition provides a moistening effect. Their amphiphilic nature also allows partitioning into the skin and enhanced permeability of incorporated drug molecules (Kumar & Katare, 2005). As prolonged hydration is a final goal in the present formulations, lecithin formulations were of particular interest. In addition, PG is found in many oral applications including toothpastes (LaKind et al., 1999).

When IPM was used as the oil phase, a large microemulsion region was observed in the presence of between 30% and 70% (w/w) SM and 10% to 60% (w/w) IPM. Within this region, compositions were classed as semi-solid or liquid like based on visual observation. Structures were different to those observed when RBO was the oil phase, suggesting that the choice of oil impacted the resulting emulsion. This may be because oil composition influences the curvature of the surfactant film. Short-chain fatty acids penetrate the lipophilic chain of the surfactant monolayer, resulting in an increase in negative curvature that may promote different structure formation. Short-chain fatty acids are also more effective at penetrating this film compared to bulkier, long-chain fatty acids (Flanagan & Singh, 2006), resulting in different microstructure formation.
The fatty acids in RBO are mainly unsaturated (oleic acid and linoleic acid) (Orthoefer & Eastman, 2005), whereas the fatty acids in IPM are mainly saturated (myristic acid) (Hoepfner et al., 2007). Both saturated and unsaturated fatty acids can enhance penetration of the surfactant monolayer, thus affect the microstructure of the emulsion by acting as co-surfactants (Lawrence & Rees, 2000; Talegaonkar et al., 2008).

Microstructure varied depending on the composition and SM used. In the presence of a high surfactant concentration, microemulsions formed in the Tween 80 and Span 80 systems. In the lecithin and RBO compositions, turbid emulsions formed when the water concentration was over 10% w/w. Turbidity indicated that droplets were greater than 0.1 µm in diameter (Mollet & Grubenmann, 2001), hence these compositions were defined as coarse emulsions. When viewed under polarised light, a number of compositions exhibited structures with no clear dispersed or continuous phase. In a previous study, Hickey and co-workers (2006) examined different compositions of IPM, lecithin, n-propanol and water and observed a similar phenomenon. They termed these structures pseudo-bicontinuous and hypothesised that this was due to flexible, disorganised interfaces that existed when similar amounts of aqueous and oil phases were present. This is relevant for the present compositions, as in the oral cavity an emulsion is likely to mix with residual saliva and other aqueous liquids, thus altering its composition. In the presence of natural saliva, flocculation may occur due to bridging of salivary proteins, in particular mucins, with emulsifiers present in the formulation (Vingerhoeds et al., 2005; Silletti et al., 2007b, 2007a). This is likely to be limited in patients with minimal residual salivary gland function; however, the ingestion of water and other aqueous liquids may cause a similar effect. In addition, this phenomenon is reversible in the presence of negatively charged or neutral surfactants, because there is no electrostatic attraction between these molecules and the negatively charged mucins. In this case, flocculation may occur due to depletion forces, whereby mucins and other salivary proteins present in the continuous phase push the emulsion droplets together to form flocs (Smith & Williams, 1995). This process is reversible upon dilution or shear (Vingerhoeds et al., 2005; Silletti et al., 2007b). As soy lecithin is a weakly negatively charged surfactant (Wang & Wang, 2008; Xu et al., 2011), any flocculation that may occur should be reversible. However, depending upon the droplet size, oil type, surfactant concentration and stability of the emulsion, shear has also been demonstrated to induce coalescence by forcing droplets together (Dresselhuis et al., 2008a). Due to
the complexity of the oral environment, it is hypothesised that characterising the rheological behaviour of these RBO, water and lecithin/PG systems will provide an insight into the viscous and elastic behaviour of these formulations at frequencies and shear rates similar to those in the oral cavity. The viscoelasticity of natural saliva is considered fundamental in its action within the oral cavity (Bongaerts et al., 2007; Haward et al., 2010). Therefore, these properties are thought to be instrumental in the development of an effective substitute.

2.2.5 Conclusions

Although the systems containing Tween 80 and Span 80 produced reasonably predictable results with a clear microemulsion region and no liquid crystalline structures, the ability of the lecithin and PG systems to retain their organogel-like structure in the presence of up to 50% w/w water was of particular interest. When IPM was the oil phase, a large variation in microstructure was observed depending on the concentration of each component. From these preliminary investigations it was decided that emulsion systems containing RBO, water and a SM of lecithin and PG (1:1 w/w) had the biggest potential for saliva substitutes in the treatment of xerostomia. Further investigation of these systems with an emphasis on rheology is required in order to better understand the behaviour of these compositions in controlled shear environments.

2.3 An investigation of natural saliva

2.3.1 Introduction

Human saliva is a complex fluid predominantly secreted by three paired glands, as well as numerous minor glands in the oral cavity. The composition and flow rate of saliva are sensitive to a number of internal and external factors including biological rhythms, level of hydration, body posture, biological rhythms and psychological stimuli (de Almeida et al., 2008). The flow rate and composition of saliva also vary significantly from each salivary gland. Parotid saliva is serous, whereas saliva produced by the sublingual and minor glands is predominantly mucous in character and submandibular saliva is a mixed secretion (Mese & Matsuo, 2007). Whereas saliva is continually secreted, a saliva substitute must remain in the oral cavity for a prolonged period of time in order to be effective. While it is important that some physicochemical properties of a potential saliva substitute match that of saliva, it was hypothesised that
other properties will need to be different in order to provide prolonged relief. As the rheological properties of emulsions have been identified as important in the consideration of an effective saliva substitute, the purpose of this investigation was to measure the rheological properties of whole saliva collected using the expectoration method (Stokes & Davies, 2007) and submandibular saliva collected using a custom-fabricated collecting device. Like natural whole saliva, the commercially available HEC polymer-based substitute, OralBalance®, exhibits pseudoplastic flow properties (Jones et al., 2002). Research suggests superior efficacy over other polymer-based substitutes, such as CMC, which do not exhibit the same non-Newtonian flow properties as natural saliva (Marks & Roberts, 1983; Vissink et al., 1983; Epstein & Stevenson-Moore, 1992). The rheological properties of a commercial saliva substitute (OralBalance® gel) were also evaluated to gauge the magnitude of any differences between a commercially available saliva substitute and natural saliva.

2.3.2 Materials and methods

2.3.2.1 Materials

Sodium dodecyl sulphate and citric acid were obtained from Sigma (St Louis, MO, USA). A HEC commercial saliva substitute (Biotène® OralBalance® gel, GlaxoSmithKline, Middlesex, UK) was purchased from a local pharmacy. The fabrication of the device used to collect submandibular saliva is described in detail in Appendix A.

2.3.2.2 Methods

Saliva obtained from one participant using the collecting device was compared with whole saliva collected from the same individual using the expectoration or ‘spit’ method, described by Stokes and Davies (2007). The donor refrained from eating or drinking for at least 90 minutes prior to collection. Saliva was collected over a five-minute period, either by allowing saliva to pool in the mouth before forcefully expectorating into a collection cup every 30 seconds (expectoration method) or by inserting the collection device into the mouth and directing the tubing such that saliva flowed freely from Wharton’s duct into the collection cup for a five-minute period (device method). In both cases, stimulated saliva was obtained by rubbing the dorsum of the tongue with 5% citric acid using a cotton tip every 60 seconds. The mass and pH
of the collected saliva were recorded immediately following collection using a mass balance (Mettler AT201, Greifensee, Switzerland) and handheld pH meter (pH Spear, Eutech Instruments Pte Ltd, Singapore). The pH meter was calibrated using standards of pH 4.00, 7.00 and 10.00 at 20°C (Merck Pty Ltd, Kilsyth, VIC, Australia) prior to use and rinsed thoroughly with tap water following each measurement. The density of saliva was assumed to be 1 g/mL (Kerr, 1961; Lentner, 1981).

Rheological measurements were performed on both saliva samples and the HEC saliva substitute using a Paar Physica MCR 301 rheometer (Anton Paar GmbH, Graz, Austria) with a direct strain oscillation (DSO) option, which allowed strain-controlled oscillatory tests. The temperature was controlled with a Peltier temperature control unit (PTD 200) and a Peltier control hood. The same measurement geometry as reported by Stokes and Davies (2007) was selected, with a 0.02 radian cone and 50 mm diameter plate. The gap was set to 0.207 mm and the minimum volume of sample required for each measurement was 1.86 mL. Saliva was poured onto the plate immediately following collection and any air bubbles or particulate matter were carefully removed using a stainless steel spatula. The cone was then lowered and a small amount of 0.1% sodium dodecyl sulphate surfactant solution was brushed around the rim of the plates to minimise adsorption of surface-active molecules at the interface. Flow curves were obtained by measuring shear stress as a function of shear rate in the range of 1 s\(^{-1}\) to 1000 s\(^{-1}\). A frequency sweep was performed in the frequency range 0.1 Hz to 15 Hz to determine the change in viscoelastic behaviour as a function of the rate of application of stress. \(G'\) (Equation 1.9), \(G''\) (Equation 1.10), Complex viscosity (\(\eta^*\), Equation 1.11) and \(\tan \delta\) (Equation 1.12) were then calculated. All measurements were performed in triplicate at 37°C using fresh saliva of HEC substitute.

Statistical analyses were performed by analysis of variance (ANOVA) using Stata 11.1 (Stata Corp, College Station, Texas, USA). For the flow curves (apparent viscosity) and frequency sweeps (complex viscosity and \(\tan \delta\)), a repeated measure ANOVA was used, with shear rate and frequency being the respective repeated-measure variables. Post-hoc Bonferroni tests were conducted to identify individual differences. In all cases, a p-value of less than 0.05 was considered significant.
2.3.3 Results

The volume of saliva secreted when the glands were stimulated with 5% citric acid was significantly higher than when saliva was unstimulated (p < 0.01) (Table 2.6). For both unstimulated and stimulated saliva, the volume obtained using the collecting device was approximately half of that collected using the expectoration method (p < 0.01). The pH was highest in stimulated saliva obtained using the collecting device and lowest in stimulated saliva obtained via the expectoration method (Table 2.6). An interaction between collection method and saliva type was found to be statistically significant for both pH and saliva flow (p < 0.01).

Both unstimulated and stimulated saliva collected with the device exhibited pseudoplastic behaviour, with apparent viscosity decreasing with increasing shear rate (p < 0.01) (Figure 2.11). In comparison, whole saliva collected by expectoration appeared Newtonian under stimulated conditions and pseudoplastic when unstimulated (Figure 2.11, inset). The apparent viscosity of saliva collected using the device (stimulated and unstimulated) was similar to that of unstimulated saliva collected by the expectoration method, while the apparent viscosity of stimulated saliva collected by expectoration was lower at all shear rates below 400 s⁻¹.

Like unstimulated whole saliva, the HEC saliva substitute was shear thinning (Figure 2.12), but apparent viscosity was considerably higher than for natural saliva at any given shear rate (p < 0.01), as shown in Table 2.7. The relationship between shear rate and shear stress was described by a second-order polynomial equation (R² = 0.999; Figure 2.12 inset).

### Table 2.6 Summary of the flow rate (mL/min ± SD) and pH (± SD) of unstimulated and stimulated saliva collected using the device compared to the expectoration method. Data was collected in triplicate from a single donor and is presented as mean (± SD).

<table>
<thead>
<tr>
<th></th>
<th>Saliva (mL/min)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unstimulated</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collecting device</td>
<td>0.31 (0.06)</td>
<td>7.06 (0.10)</td>
</tr>
<tr>
<td>Expectoration method</td>
<td>0.71 (0.11)</td>
<td>7.15 (0.05)</td>
</tr>
<tr>
<td><strong>Stimulated</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collecting device</td>
<td>1.72 (0.19)</td>
<td>7.51 (0.07)</td>
</tr>
<tr>
<td>Expectoration method</td>
<td>3.30 (0.33)</td>
<td>6.61 (0.23)</td>
</tr>
</tbody>
</table>
Figure 2.11 Steady shear rheology of stimulated (squares) and unstimulated (circles) saliva showing viscosity as a function of shear rate collected using the device (filled symbols) and expectoration method (empty symbols). The inset figure shows a model that fits shear stress as a function of shear rate for the expectoration method. Data is presented as mean (± SD), n = 3.
Figure 2.12 Steady shear rheology of the HEC saliva substitute. The inset figure shows a polynomial model fitted to the shear stress as a function of shear rate. Data is presented as mean (± SD), n = 3.
Table 2.7 Summary of flow properties at selected shear rates for unstimulated and stimulated saliva collected using the expectoration method (whole saliva), the collecting device (submandibular saliva) and the HEC saliva substitute. Data is presented as mean (± SD), n = 3.

<table>
<thead>
<tr>
<th>Shear rate (1/s)</th>
<th>Shear stress (Pa)</th>
<th>Apparent viscosity (Pa.s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Unstimulated</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>whole saliva</td>
<td>1</td>
<td>0.01 (0.002)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.05 (0.01)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.33 (0.05)</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>1.87 (0.06)</td>
</tr>
<tr>
<td>stimulated whole saliva</td>
<td>1</td>
<td>0.002 (0.001)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.02 (0.002)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.20 (0.001)</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>1.60 (0.02)</td>
</tr>
<tr>
<td><strong>Unstimulated</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>submandibular saliva</td>
<td>1</td>
<td>0.007 (0.004)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.06 (0.02)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.38 (0.08)</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>1.67 (0.02)</td>
</tr>
<tr>
<td>stimulated submandibular saliva</td>
<td>1</td>
<td>0.02 (0.01)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.04 (0.01)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.25 (0.04)</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>1.55 (0.19)</td>
</tr>
<tr>
<td><strong>HEC saliva substitute</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>254.5 (13.4)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>423.5 (12.0)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>869.0 (52.3)</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>2155.0 (49.5)</td>
</tr>
</tbody>
</table>
The G’ and G” of natural saliva were determined by performing dynamic frequency sweeps. A strain of 5% was selected based on preliminary amplitude sweep testing, because viscoelasticity was found to be linear at this strain (Figure 2.13). The G’ and G” were lower for saliva collected by the expectoration method compared to saliva collected using the device, although this difference diminished as frequency increased (Figure 2.14).

**Figure 2.13** Amplitude sweep showing the complex modulus (G’, open symbols and G”, closed symbols) of unstimulated whole saliva at representative oscillatory frequencies of 0.1 Hz (squares), 1.7 Hz (triangles) and 2.4 Hz (circles). LVR = linear viscoelastic region.
Figure 2.14 Dynamic rheological properties of both whole saliva collected using the expectoration method (open symbols) and submandibular saliva collected using the device (closed symbols), showing $G'$ as a function of frequency for a) unstimulated saliva and b) stimulated saliva and $G''$ as a function of frequency for c) unstimulated saliva and d) stimulated saliva. Data is presented as mean ($\pm$ SD), n = 3.
Figure 2.15a and Figure 2.15b show the complex viscosity of stimulated and unstimulated saliva, respectively. A statistically significant difference in complex viscosity of saliva collected by the two methods was observed ($p < 0.01$), while the effect of frequency on complex viscosity was not statistically significant ($p = 0.17$). In addition, large between-test variability in complex viscosity was observed for stimulated saliva collected using the device (Figure 2.15b). The $\tan \delta$ for both unstimulated and stimulated saliva collected using the device remained reasonably constant over a frequency range of 0.1 to 8.0 Hz (Figure 2.15c-d). In comparison, $\tan \delta$ at any frequency was greater for saliva collected by expectoration than saliva collected using the device. For unstimulated saliva collected by the expectoration method, $\tan \delta$ decreased with increasing frequency (Figure 2.15c) and was greater than one at frequencies below 5.2 Hz and less than one at higher frequencies. For stimulated saliva collected by expectoration, $\tan \delta$ was less than one at frequencies below 0.4 Hz and increased with increasing frequency up to approximately 1.6 Hz, before decreasing at higher frequencies and, like unstimulated saliva, was less than one at frequencies greater than 7.5 Hz. A repeated-measures ANOVA (frequency being the repeated measure) showed that the $\tan \delta$ of saliva was significantly affected by stimulation ($p < 0.01$), and the interaction between saliva type and collection method was also statistically significant ($p < 0.01$).
Figure 2.15 Dynamic rheological properties of both whole saliva collected using the expectoration method (open symbols) and submandibular saliva collected using the device (closed symbols). The overall dynamic complex viscosity is shown as a function of frequency for a) unstimulated saliva and b) stimulated saliva. Similarly, the tan δ is shown as a function of frequency for c) unstimulated saliva and d) saliva stimulated with 5% citric acid solution. Data is presented as mean (± SD), n = 3.

The HEC saliva substitute showed different rheological properties compared to natural saliva (Figure 2.16), with both G’ and G” up to four orders of magnitude higher than natural saliva at any given frequency. The complex viscosity of the HEC saliva substitute decreased with increasing frequency (Figure 2.16c) and was significantly higher than for unstimulated natural saliva collected using the expectoration method (p < 0.01). Similarly, the tan δ of the HEC saliva substitute was significantly lower compared to unstimulated saliva at any given frequency (p < 0.01).
2.3.4 Discussion

The objective of the work reported in this section was to gain an understanding of the rheological properties of saliva and to develop some methods for measuring rheology for later application to emulsions for potential saliva substitutes. Preliminary data comparing the rheological properties of submandibular saliva collected using a device, and whole saliva collected using the expectoration method, showed that saliva collected using the device resulted in a total yield of approximately half of that collected by the expectoration method. Saliva collected using the device exhibited flow behaviour that was similar to unstimulated saliva collected using the expectoration method. In
addition, a comparison between natural saliva and a commercial saliva substitute showed significantly different rheological properties.

Saliva was analysed from one donor only and while it would be worthwhile repeating the study with more people in order to determine the reproducibility of these findings, it was outside the scope of this research to do so. In addition, further analysis of the composition of saliva collected using the device may give some insight into flow-rate-related differences in composition of submandibular saliva.

The volume of saliva collected was lower when the collecting device was used (0.31 ± 0.06 mL/min unstimulated or 1.72 ± 0.19 mL/min stimulated) compared with the expectoration method (0.71 ± 0.11 mL/min unstimulated or 3.30 ± 0.33 mL/min stimulated). This was anticipated because the device collects saliva from one gland only, whereas the expectoration method collects whole saliva, which is secreted from all glands. When saliva is exposed to air, CO₂ is gradually released, causing an associated rise in pH, which is dependent upon exposure time and temperature (Edgar et al., 2004). For this reason, pH measurements are not often used in the characterisation of saliva ex situ. In the present study, however, pH comparisons were made for saliva collected using different methods, and assumed that the amount of CO₂ released was constant in each sample. A possible explanation for the higher pH of stimulated saliva when collected using the device (7.5 ± 0.07) compared to with the expectoration method (6.6 ± 0.23) is that the device occludes and collects saliva directly from Wharton’s duct, so that there is no contamination of the sample by the citric acid used to stimulate salivary flow.

Unstimulated saliva exhibited pseudoplastic or shear thinning behaviour, whereby the viscosity of saliva decreased as the shear rate was increased, as shown in Figure 2.11. This behaviour is of physiological significance because it allows for coating and lubrication of the entire oral cavity at rest, with adequate flow at the higher shear rates encountered during mastication (Hatton et al., 1987; Preetha & Banerjee, 2005). The HEC saliva substitute was also shear thinning, but apparent viscosity was significantly higher at any given shear rate (p < 0.0001). The viscosity of a saliva substitute is an important factor in patient acceptability along with taste, delivery method and severity of xerostomia (Epstein et al., 1999; Shahdad et al., 2005).
Unstimulated saliva was more viscous than stimulated saliva, most likely because saliva contains a high concentration of glycoproteins (such as mucins) that contribute to its weak gel structure; when saliva is stimulated, parotid gland secretion increases, resulting in a more serous or watery fluid. Similarly, both the apparent viscosity and complex viscosity of stimulated and unstimulated saliva collected using the device were significantly higher than for stimulated saliva collected using the expectoration method. Again, this is likely due to the higher mucin concentration in submandibular saliva (van der Reijden et al., 1993).

The tan δ is a measure of the relative contribution of the viscous and elastic moduli of a material (G”/G’) at a given frequency (Barnes et al., 1989). The changes that occurred in tan δ at higher frequencies may be a result of changes in the molecular motion of particles within saliva, and possibly an overall alteration in saliva’s structural properties. In general, saliva collected using the device was more elastic (lower tan δ) than that collected by expectoration. Unstimulated whole saliva had a tan δ that was greater than one at low frequencies (< 5.2 Hz) and less than one at frequencies over 5.2 Hz. The reason for this is likely to be functional, with viscous behaviour at low frequencies promoting lubrication of the oral cavity at rest and elastic properties at higher frequencies promoting lubrication during high-shear tasks such as mastication and deglutition. Interestingly, stimulated whole saliva had a tan δ of less than one up to 0.4 Hz, greater than one at frequencies up to 7.5 Hz and less than one at higher frequencies. Differences in rheological properties of saliva collected with the two methods and under stimulated and non-stimulated conditions may reflect the different roles of saliva derived from each gland. Stimulated whole saliva has a greater relative contribution from the parotid gland. This saliva is classified as mainly serous, providing mechanical cleansing of residues such as food debris (de Almeida et al., 2008) and providing a medium for taste and subsequent swallowing (Ferguson & Barker, 1994; Mese & Matsuo, 2007). Hence, it is less elastic than sublingual and submandibular gland saliva, which contains glycoproteins that are important in lubricating oral cavity surfaces (de Almeida et al., 2008). It is interesting to note that with the collecting device, there was little difference in viscoelastic properties between stimulated and unstimulated saliva, indicating that the composition of saliva secreted by the submandibular gland is not sensitive to stimulation.
The viscoelastic properties of the HEC saliva substitute were significantly different to those of natural saliva, and tan δ was less than one throughout the tested frequency range, indicating that elastic or solid-like properties dominated over viscous or liquid-like properties. Whether this is a desirable property remains to be seen, however, it is hypothesised that such a high elasticity will limit its efficacy during high-shear tasks such as speaking, mastication and deglutition. This may explain why many clinical studies on substitutes on similar saliva substitutes have shown limited efficacy (Klestov et al., 1981; Olsson & Axell, 1991; Momm et al., 2005).

2.3.5 Conclusions

The differences in rheology of both unstimulated and stimulated saliva observed in this research highlights the complexity of human saliva. The unique rheology of saliva has been reported to play an important role in the way that it functions in the oral cavity (Bongaerts et al., 2007; Stokes & Davies, 2007; Chen & Stokes, 2012). The non-Newtonian behaviour and frequency-dependent tan δ are hypothesised to be of particular importance in the function of both unstimulated saliva and an effective saliva substitute.
Chapter three

Physicochemical characterisation of emulsions as saliva substitutes
3 Physicochemical characterisation of emulsions as saliva substitutes

3.1 Introduction

The purpose of this chapter was to investigate the physicochemical properties of emulsions. Following the investigations conducted on different SMs in Chapter 2, a combination of lecithin and PG (1:1 w/w) was selected as the SM. RBO was used as the oil phase and IPM was investigated as an alternative oil to determine the importance of oil type on rheological structure.

In Chapter 1, the unique viscoelasticity of saliva was highlighted. The viscoelastic properties of saliva substitutes are considered to be important markers of efficacy (Vissink et al., 1984; Park et al., 2007). However, saliva has the ability to be secreted continually, whereas a saliva substitute needs to remain in the oral cavity for extended periods of time. It is hypothesised, therefore, that although the viscoelastic properties of a saliva substitute are important in determining efficacy, emulating the viscoelasticity of natural saliva may result in a saliva substitute that is insufficiently retentive in the oral cavity. This may explain why many studies have confirmed comparable rheological properties between mucin-based substitutes and natural saliva in vitro (Park et al., 2007; Yakubov et al., 2009), yet in vivo studies on the same substitutes have been inconclusive (Olsson & Axell, 1991; Sweeney et al., 1997) and ambiguous as far as longer term studies are concerned (Gómez-Moreno et al., 2013).

3.2 Chapter aims

- To investigate the viscoelastic properties of lecithin-based RBO emulsions;
- To determine the droplet characteristics of selected compositions;
- To investigate the effect on viscoelasticity when IPM is the oil phase.

3.3 Materials and methods

3.3.1 Materials

Soybean lecithin (Lipoid S-100) was purchased from Lipoid GmBH (Ludwigshafen, Germany). Product specification data provided by the manufacturer stated that the lecithin consisted of 96.8% w/w phosphatidylcholine and less than 0.05% w/w free fatty acids. PG and IPM were purchased from Sigma (St Louis, MO, USA) and RBO
was from Bespoke Foods (London, UK). All materials were used as received from the manufacturer without any further purification.

Two microparticle size standards based on polystyrene monodisperse with nominal particle diameters of 3.0 µm and 5.0 µm were from Fluka Analytical (Buchs, Switzerland). Intralipid® 20% w/v (a sterile, non-pyrogenic fat emulsion prepared for IV administration as a source of calories and essential fatty acids) was used as a standard emulsion for the light scattering measurements (Pharmatel Fresenius Kabi AB, Sweden).

### 3.3.2 Preparation of RBO compositions

The SM used in the present study consisted of soy lecithin dissolved in PG at a ratio of 1:1 w/w. PG was gradually added to the lecithin and magnetically stirred at room temperature until a transparent mixture was achieved. The prepared SM was stored at room temperature for at least 24 hours before further use. Compositions were then prepared by mixing predetermined weight ratios of SM, water and RBO in a vial. Compositions were magnetically stirred and left to settle for 12 hours prior to further investigation. The ratio of oil, SM and water varied by increments of 10% w/w such that 66 different combinations were prepared for each oil in order to establish the phase behaviour of different systems.

### 3.3.3 Flow rheology of RBO compositions

The flow properties of each composition were determined at 37°C within a shear rate range of 0 to 200 s⁻¹ using an AR 2000 rotational rheometer (T.A. Instruments, Surrey, England) in flow mode. A parallel plate was used with 40 mm solvent trap steel plate measurement geometry and the fixed gap between the two plates was pre-set to 1000 µm. Compositions were applied to the stationary plate and allowed to equilibrate for three minutes before analysis. Analyses were conducted in triplicate for each formulation using AR Instrument Control software (T.A. Instruments, Surrey, England) and results were analysed using the Power Law model (Equation 3.1), where $\sigma$ is the applied shearing stress (Pa), $k$ is the flow consistency (Pa.s), $\gamma$ is the shear rate (s⁻¹) and $n$ the behaviour index (dimensionless).
3.3.4 Oscillatory rheology of RBO compositions

Oscillatory (or dynamic) rheological analyses of formulations were performed at 37°C using an AR 2000 rotational rheometer (T.A. Instruments, Surrey, England). A 40 mm solvent trap steel parallel plate geometry was used with a fixed gap of 1000 µm. Initially, an amplitude sweep was conducted at different frequencies to identify the LVR, where $G'$ and $G''$ are independent of stress and strain and from this data a oscillation stress amplitude of 5 Pa was selected. Compositions were applied to the lower stationary plate of the rheometer and allowed to equilibrate for three minutes before a sweep was conducted within a frequency range of 0.1 Hz to 10 Hz. The oscillatory rheological properties were measured in triplicate for each formulation and the $G'$ (Equation 1.9), $G''$ (Equation 1.10) and tan δ (Equation 1.12) were calculated using AR Instrument Control software (T.A. Instruments, Surrey, England). In addition, frequency sweeps were conducted at temperatures ranging from 20°C to 37°C on selected formulations to assess whether the rheological properties were affected by temperatures varying from room temperature (20°C) up to the physiological temperature of the oral cavity (37°C).

The statistical significance ($p < 0.05$) of the oscillatory data was investigated by dividing RBO compositions into two groups based on whether they exhibited solid-like (tan δ < 1) or liquid-like (tan δ > 1) properties at low frequencies. The viscoelastic properties of these compositions ($G'$, $G''$ and tan δ) at a representative frequency of 5.1 Hz were compared using a one-way ANOVA (Stata 11.1, StataCorp, College Station, Texas, USA). Differences in individual compositions within each group were identified using Tukey’s HSD post hoc test. The effect of temperature on viscoelastic properties were analysed using the Kruskal-Wallis Rank Test. In all cases, the level of statistical significance was set to $p < 0.05$.

3.3.5 Droplet size analysis of RBO compositions

Selected RBO compositions (Table 3.1) were examined under a light microscope at rest and following exposure to oscillatory frequencies of 0 Hz, 1 Hz, 6 Hz and 10 Hz, in
order to determine whether structural changes in the droplets could be observed at different frequencies.

### Table 3.1 RBO compositions investigated using droplet sizing techniques.

<table>
<thead>
<tr>
<th>SM (% w/w)</th>
<th>RBO (% w/w)</th>
<th>W (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>10</td>
<td>30</td>
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<tr>
<td>40</td>
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<td>60</td>
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<td>30</td>
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<td>50</td>
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</tbody>
</table>

The droplet size distribution of compositions was investigated using a laser diffraction particle sizer (LA-950V2, Horiba Instruments Inc., Irvine, CA, USA), with distilled water as the dispersion media. Since the test emulsions were slightly yellow, they were expected to preferentially absorb the blue light of the particle sizer, resulting in a lower blue transmission than expected. Therefore, optimal red transmission was investigated. The resulting particle size distribution of compositions at different red transmissions (%) was determined and $\chi^2$ values were compared (Equation 3.2). $\theta_i$ is the SD of scattered light intensity at each channel of the detector, $y_i$ is the measured scattered light at each $i$ of the detector, and $y(x_i)$ is the calculated scattered light at each channel of the detector, based on the chosen refractive index and reported particle size distribution. From this preliminary work it was found that the most reliable results were obtained with a red transmission between 89.5% and 93%.

$$\chi^2 = \sum \left\{ \frac{1}{\theta_i^2} [y_i - y(x_i)]^2 \right\}$$  \hspace{1cm} \text{Equation 3.2}

The refractive index was then optimised using the inbuilt software (LA-950, Horiba Instruments Inc., Irvine, CA, USA), which optimises the calculation used to determine droplet distribution by calculating the $\chi^2$ of results obtained using the real refractive
index of RBO (1.47). From the initial range of imaginary refractive indices of 0.0001, 0.001, 0.01, 0.1 or 0.5, an imaginary refractive index of 0.01i was selected.

These parameters were applied to the test compositions and the resulting droplet diameter and droplet size distributions were compared with the droplet diameters estimated from the polarised light microscopy images in Chapter 2 (Section 2.2.3.4).

To ensure an even distribution of each composition within the dispersion media, 0.1 mL of the mixture was added to 0.9 mL water and gently mixed using a 360° rotary mixer. Approximately 300 µL of diluted emulsion was then added to the dispersion media in the particle sizer to achieve the desired red transmission between 92% and 89% (approximately 300 µL formulation). Each measurement was performed in triplicate. Volume mean diameter was calculated and the CV for each composition was determined by dividing the SD by the mean volume distribution of 10% (D_{10}), 50% (D_{50}; also the median) and 90% (D_{90}) of the droplets.

### 3.3.6 Small-angle light scattering of RBO compositions

Small-angle light scattering (SALS) was used to determine changes in the emulsion droplets as a function of frequency. Oscillatory rheological analyses were performed at 20°C, to reduce evaporation of the compositions during the testing period, using a DHR Series rotational rheometer (T.A. Instruments, Surrey, England). A SALS accessory (T.A. Instruments, Surrey, England) was attached to the top of the rheometer with a Class II 0.95 mW diode laser (wavelength 635 nm, 1.1 mm circular beam and beam divergence of 0.7 mrad). A 50 mm quartz parallel plate geometry was used with a fixed gap of 1000 µm and a stress amplitude of 5 Pa was selected, as in the oscillatory rheology analysis.

A 3 µm alignment suspension, consisting of 2% w/v polystyrene beads, was diluted by a factor of 200 to obtain a suspension with a microparticle concentration of 0.05% w/v. The scattering pattern was then determined by placing two to three drops of the microparticle suspension over the laser window on the Peltier plate. The geometry gap was closed to 1000 µm and the laser focused on the sample. The camera settings were then adjusted until a scattering pattern of concentric rings was obtained (Figure 3.1). Following this alignment procedure, the camera settings were not altered for the remainder of the experiment. Five images were taken using a LuCam Capture camera
(Lumenera Corporation, Nepean, ON, Canada) and later averaged using ImageJ 10.2 (Public Domain Java Image Processing Programme, National Institute of Health, USA). To obtain a background image, the process was repeated using water, which was subtracted from each averaged image.

Figure 3.1 Typical scattering pattern of concentric rings obtained using the 3 µm polystyrene beads, after alignment.

The SALS set-up was calibrated according to the T.A. Instruments operating manual (Surrey, England). Initially, the radial profile angle of the averaged image was determined and integrated to measure normalised integrated intensity as a function of distance from the centre point, in pixels. The predicted Mie scattering pattern of the 3 µm microparticles was then calculated using MiePlot 4.3 (Philip Laven, Geneva, Switzerland) by entering the required variables (Table 3.2) and plotting predicted relative intensity as a function of scattering angle (θ).

Table 3.2 Variables required to predict scattering angle using MiePlot.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrated particle diameter* (dₖ)</td>
<td>3.113 µm</td>
</tr>
<tr>
<td>CV*</td>
<td>1.97%</td>
</tr>
<tr>
<td>Refractive index of sphere*</td>
<td>1.5905</td>
</tr>
<tr>
<td>Refractive index of medium* (n)</td>
<td>1.332</td>
</tr>
<tr>
<td>Wavelength of laser light (λ₀)</td>
<td>0.6328 µm</td>
</tr>
</tbody>
</table>

*Obtained from product specification data
To determine correction factors for the observed data, the predicted scattering pattern was overlayed on the plot of observed scattering pattern (Figure 3.2). A correction factor ($F_C$) was calculated to adjust for the pixel position relative to the light source (Equation 3.3). Intensity was corrected using $F_C$ and a y-shift correction factor ($F_y$), which was manually adjusted until the first peak of the observed scattering pattern had the same magnitude as the first peak of the predicted scattering pattern (Equation 3.4). The measured radius in pixels ($r$) was converted to scattering angle ($\theta$, degrees) using Equation 3.5, where the optics factor ($F_O$) was adjusted until the first peak of the observed scattering pattern overlapped the first peak of the predicted pattern (Figure 3.2). These correction factors were then applied to the sample data.

\[
F_C = \left( \cos \frac{\theta \pi}{180} \right)^3
\]

Equation 3.3

\[
I_{corr} = \frac{(I * F_y)}{F_C}
\]

Equation 3.4

\[
\theta = \frac{180 \cdot \arctan (r_0)}{\pi}
\]

Equation 3.5

Figure 3.2 The predicted scattering pattern obtained from the Laven data (dashed line) compared to the measured scattering pattern (solid line) a) before and b) after calibration using correction factors $F_O$ and $F_y$. 

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Following the calibration step, an optimal dilution factor was selected. To do this, a 5 µm microparticle size standard suspension was diluted by a factor of 100, 200 and 300 with distilled water. As the initial suspension consisted of 10% w/v polystyrene beads, these dilution factors resulted in suspensions with a particle concentration of 0.1%, 0.05% and 0.03% (w/v), respectively. A step sweep was conducted on each composition within a frequency range of 0.1 to 1.0 Hz. Approximately 1.96 mL mixture was applied to the lower stationary plate of the rheometer and allowed to equilibrate for three minutes. Each composition was held for 30 seconds at pre-determined frequencies of 0.1, 2, 4, 6, 8 and 10 Hz so that five images could be captured using a LuCam Capture camera (Lumenera Corporation, Nepean, ON, Canada). These were averaged using ImageJ 10.2 (Public Domain Java Image Processing Programme, National Institute of Health, USA) and the averaged water background image was subtracted. Radial profile angle was determined and data was adjusted using the correction factors described above to gain adjusted intensity as a function of scattering angle. A scattering pattern with two defined peaks, similar to that shown in Figure 3.2, was considered optimal as it indicated sufficient dilution to avoid multiple scattering. This procedure was then repeated using Intralipid®, as a standard emulsion, diluted by a factor of 200, 500, 1000, 2000 and 4000 using distilled water. Intralipid® contains 20% w/v oil, so these dilution factors corresponded with an oil concentration (w/v) of 0.1%, 0.04%, 0.02%, 0.01% and 0.05%, respectively. From these preliminary tests, a target emulsion concentration between 0.02% and 0.04% (w/v) was selected.

The selected test compositions (Table 3.1) contained up to 30% w/w RBO and between 30% and 60% w/w water. To obtain an emulsion concentration within the desired range of 0.02% to 0.04% w/v, each composition was diluted by a factor of 2000 using distilled water and mixed gently using a 360° rotary mixer. Approximately 1.96 mL sample was then applied to the lower stationary plate of the rheometer and allowed to equilibrate for three minutes before a step sweep was conducted within a frequency range of 0.1 to 10 Hz. Each composition was held for 30 seconds at pre-determined frequencies of 0.1, 2, 4, 6, 8 and 10 Hz so that images could be captured. Five images were obtained at each frequency using a LuCam Capture camera (Lumenera Corporation, Nepean, ON, Canada). These were averaged using ImageJ 10.2 (Public Domain Java Image Processing Programme, National Institute of Health, USA) and the
averaged water background image was subtracted. Radial profile angle was determined and data was adjusted using the correction factors described above to gain adjusted intensity as a function of scattering angle.

3.3.7 Rheology of compositions with IPM as the oil phase

The effect of oil type on the rheology of compositions was investigated by substituting RBO with IPM. Sample preparation, flow rheology and oscillatory rheology measurements were conducted using the methods and parameters described in Sections 3.3.2 to 3.3.4 above.

3.3.8 Volume fraction of the oil phase

The density of RBO, IPM and a SM of lecithin and PG (1:1 w/w) were calculated by determining the mass of 1 mL formulation using a mass balance (Mettler AT201, Greifensee, Switzerland). Measurements were taken in triplicate and the mean density of each component were then used to convert the weight ratio of compositions into a volume ratio, to determine the volume fraction of the oil phase in each composition (Equation 1.1).

3.3.9 Cryo-SEM of RBO and IPM composition

Structural differences in compositions containing 50% w/w SM, 20% w/w water and 30% w/w RBO or IPM were compared using cryo-field emission scanning electron microscopy (cryo-SEM). Samples were loaded into copper rivets and plunge frozen in liquid propane using a Reichert KF 80 cryofixation system (Leica, Wetzlar, Germany) at a temperature of -180°C. Samples were stored in liquid nitrogen before being transferred into the cryo-stage (Gatan, Alto 2500, UK). Samples were fractured and viewed under the microscope (JEOL, JSM-6700F, Japan) at a temperature of -140°C and accelerating voltage of 5.0 kV.
3.4 Results

3.4.1 Flow rheology of RBO compositions

The tested RBO compositions illustrated pseudoplastic or shear thinning behaviour. Flow consistency \((k)\) and rate indices \((n)\), calculated using Equation 3.1, are shown in Table 3.3. In general, as SM concentration decreased, flow consistency decreased and rate index increased. Increasing RBO concentration at any given SM concentration resulted in an increase in flow consistency. For example, compositions containing 30% w/w SM had a flow consistency of 12.1 \(\pm\) 2.5 Pa.s with 40% w/w RBO compared to 5.2 \(\pm\) 0.2 Pa.s with 30% w/w RBO. Physical state did not always correspond with flow consistency. Although a liquid-like state was observed in the composition of 40% RBO, 30% SM and 30% water (w/w), the flow consistency index of 12.1 \(\pm\) 2.5 Pa.s was similar to that observed in the semi-solid like compositions containing RBO:SM:water weight ratios of 10:40:50% or 20:40:40% (9.5 \(\pm\) 0.3 Pa.s and 13.0 \(\pm\) 1.3 Pa.s, respectively).

Table 3.3 Application of the power law model to determine the consistency index \((k)\) and rate index \((n)\) of compositions containing varying amounts of SM, RBO and water (W). Data is presented as mean \((\pm\) SD), \(n = 3\).

<table>
<thead>
<tr>
<th>Conc. (% w/w)</th>
<th>Physical features</th>
<th>Flow rheology data</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBO</td>
<td>SM</td>
<td>W</td>
</tr>
<tr>
<td>10</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>40</td>
<td>50</td>
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<td>40</td>
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</tr>
</tbody>
</table>

BC = pseudo-bicontinuous
3.4.2 Oscillatory rheology of RBO compositions

Frequency sweeps were conducted in order to investigate the viscoelastic behaviour of compositions as a function of the rate of application of stress. An oscillation stress of 5 Pa was selected, as the $G'$ and $G''$ of all tested compositions were linear at this oscillation stress. Typical examples are shown in Figure 3.3, where the LVR was determined to be between 3.1 and 10 Pa.

![Figure 3.3](image_url)

**Figure 3.3** Amplitude sweep showing the complex modulus ($G'$, open symbols and $G''$, closed symbols) of a representative composition of 40% RBO, 30% SM and 30% water (w/w) at oscillatory frequencies of 4 Hz (circles), 6 Hz (squares) and 8 Hz (triangles). The dashed line indicates the LVR.
An overview of the relationship between RBO and SM concentration and viscoelasticity of compositions at selected frequencies are shown in Figure 3.4 and Figure 3.5. Compositions containing at least 40% w/w SM exhibited behaviour consistent with rheologically structured semi-solids (\(\tan \delta < 1\)), with \(G'\) greater than \(G''\) over the entire tested frequency range. A summary of the viscoelastic properties (\(G'\), \(G''\) and \(\tan \delta\)) of selected compositions at frequencies of 1.1 Hz, 5.1 Hz and 10 Hz are presented in Table 3.4. SM concentration had the largest impact on the rheological properties of the compositions. Interestingly, some of the formulations that appeared liquid-like visually had \(\tan \delta\) values that were greater than one at frequencies below 5 Hz but less than one at higher frequencies (Figure 3.6) suggesting the viscoelastic properties of these formulations were frequency-dependent. Viscous properties dominated at low frequencies but elastic properties dominated at higher frequencies. When compositions were divided into two groups based on whether \(\tan \delta\) was less than one at all frequencies or at frequencies over 5 Hz only, statistically significant differences in \(G'\), \(G''\) and \(\tan \delta\) were observed (Figure 3.7). As the RBO to water ratio increased up to 3:1 (w/w), \(G'\) and \(G''\) increased, suggesting the rheological structure was enhanced. At RBO to water ratios of greater than 3:1 (w/w) these moduli decreased, indicating the rheological structure was then reduced as the oil fraction increased.

As SM concentrations decreased, \(G'\) and \(G''\) tended to decrease whereas \(\tan \delta\) increased for any given RBO concentration over the tested frequency range. This is illustrated in Figure 3.7, using a representative frequency of 5.1 Hz. At a RBO concentration of 30% (w/w) and a frequency of 5.1 Hz, \(G'\) ranged from 383.4 ± 11.7 Pa at 60% w/w SM down to 100.2 ± 7.5 Pa at 40% w/w SM (\(p < 0.05\)), although \(\tan \delta\) was less than one throughout the tested frequency range for both compositions.
Figure 3.4 Summary of $G'$ and $G''$ for compositions containing varying concentrations (% w/w) of SM and RBO at frequencies of a) 1 Hz, b) 5 Hz and c) 10 Hz. Colour bars highlight the magnitude of $G'$ and $G''$. 
Figure 3.5  Summary of tan δ values for compositions containing varying concentrations (% w/w) of SM and RBO at frequencies of a) 1 Hz, b) 5 Hz and c) 10 Hz. The right-hand panel indicates whether tan δ > 1 or < 1 at each frequency, according to the colour bar.
Table 3.4 Summary of the viscoelastic properties (G’, G” and tan δ) of selected formulations containing varying concentrations (conc.) of SM, RBO and water (W) at representative frequencies of 1.1 Hz, 5.1 Hz and 10.0 Hz. Data is presented as mean (± SD), n = 3.

<table>
<thead>
<tr>
<th>Conc. (% w/w)</th>
<th>Frequency (Hz)</th>
<th>Oscillatory rheology data</th>
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<tbody>
<tr>
<td>SM</td>
<td>RBO</td>
<td>W</td>
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<tr>
<td>60</td>
<td>10</td>
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</table>
Figure 3.6 Phase diagram showing the transition from liquid-like ($\tan \delta > 1$, indicating a higher relative contribution of viscous behaviour) to solid-like ($\tan \delta < 1$, indicating a higher contribution of elastic behaviour) for systems containing different ratios of RBO, SM and water, at frequencies of 1 Hz (shaded), 5 Hz (striped) and 10 Hz (dotted line).
Figure 3.7 $G'$, $G''$ and tan $\delta$ at a representative frequency of 5.1 Hz for compositions where tan $\delta < 1$ at all frequencies (a-c) and those where tan $\delta < 1$ at higher frequencies only (d-f). Data is sorted by 60% SM (black), 50% SM (diagonal stripes), 40% SM (dark grey), 30% SM (white), 20% SM (horizontal lines) and 10% SM (light grey). Statistical significance ($p < 0.05$) was determined between compositions with the same SM concentration (*) or oil concentration (^). Data is presented as mean (± SD), $n = 3$; N indicates that the rheological structure of the composition was insufficient to measure.
Semi-solid compositions containing more than 30% w/w SM produced negative peaks in $G'$ that corresponded with positive peaks in $G''$ at the same frequencies (Table 3.5, Figure 3.8). The frequency at which the peaks occurred was dependent on the composition and corresponded with a reversible dip in $G'$ and peak in $G''$ and $\tan \delta$ (Figure 3.8). As the ratio of oil to water increased at any given SM concentration, the threshold frequency at which the peak occurred increased up to a ratio of 10% RBO to 50% water (w/w), after which the threshold frequency decreased. In addition, the peak frequency was proportionately higher in formulations with a higher peak $G'$ and $G''$. These were also more elastic formulations (with a higher absolute $G'$ and lower absolute $\tan \delta$). The magnitude of the peaks, on the other hand, were independent of the viscoelasticity of the compositions, with $\tan \delta$ ranging from 0.06 to 0.33. Figure 3.8 shows $G'$, $G''$ and $\tan \delta$ as a function of frequency for compositions containing 40% w/w SM, RBO and water. As the ratio of RBO to water decreased, both the $G'$ and $G''$ also decreased. An exception to this was the formulation containing an oil to water ratio of 5:1 w/w, which did not exhibit a peak in $G''$.

**Table 3.5** Magnitude of peak $G'$, $G''$ and $\tan \delta$ and the frequency at which this occurred for formulations containing varying amounts of RBO, SM and water. Absolute values indicate the absolute (abs.) peak $G'$, $G''$ or $\tan \delta$ and relative (rel.) values indicate the peak $G'$, $G''$ or $\tan \delta$ relative to the baseline value for that formulation. Negative values indicate the peak was below the baseline value.

<table>
<thead>
<tr>
<th>Conc. (% w/w)</th>
<th>Frequency (Hz)</th>
<th>Peak G' (Pa)</th>
<th>Peak G'' (Pa)</th>
<th>Peak Tan δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>SM</td>
<td>RBO</td>
<td>W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>10</td>
<td>30</td>
<td>6.04</td>
<td>140.2</td>
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<tr>
<td>60</td>
<td>20</td>
<td>20</td>
<td>7.69</td>
<td>217.4</td>
</tr>
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<td>50</td>
<td>30</td>
<td>20</td>
<td>8.68</td>
<td>292.6</td>
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<td>40</td>
<td>10</td>
<td>7.36</td>
<td>215.1</td>
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<td>10</td>
<td>50</td>
<td>2.41</td>
<td>22.2</td>
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<td>20</td>
<td>4.72</td>
<td>170.3</td>
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<tr>
<td>40</td>
<td>50</td>
<td>10</td>
<td>3.73</td>
<td>72.7</td>
</tr>
</tbody>
</table>
Figure 3.8  a) $G'$,  b) $G''$ and c) $\tan \delta$ as a function of frequency for compositions containing 40% SM and a RBO:water ratio (w/w) of 5:1 ($\times$), 4:2 (■), 3:3 (□), 2:4 (○) and 1:5 (○). $G'$ indicates the viscous component of a material whereas $G''$ indicates the elastic component. Data is presented as mean ($\pm$ SD), n = 3.
A temperature sweep was performed on selected formulations to determine whether the rheological properties were affected by conditions ranging from room temperature (20°C) to physiological temperature (37°C). The tan δ of a representative formulation consisting of 10% RBO, 60% SM and 30% water (w/w) is plotted in Figure 3.9. At low frequencies, tan δ increased with increasing temperature whereas at higher frequencies, tan δ decreased with increasing temperature. However, these differences were not significant (p > 0.05). A peak in tan δ was observed at 4.9 Hz regardless of temperature.

**Figure 3.9** Tan δ as a function of frequency for a representative formulation (10% RBO, 60% SM and 30% water w/w), at temperatures of 20°C (■), 24°C (■), 30°C (■), 34°C (■), and 37°C (□).
3.4.3 Droplet size analysis of RBO compositions

Light microscopy after compositions were exposed to frequencies of 1 Hz, 6 Hz and 10 Hz suggested that the droplet size of the dispersed phase increased when frequency increased above the peak threshold (Figure 3.10).

![Light microscopy images at 10x magnification](image)

**Figure 3.10** Polarised light microscopy images at 10x magnification of a formulation consisting of 10% RBO, 60% SM and 30% water (w/w), when the formulation was a) unsheared, b) sheared at 1 Hz (before the peak in tan δ), c) sheared at 6 Hz (at the peak in tan δ) and d) sheared at 10 Hz (after the peak in tan δ). Scale bars represent 10 µm.

Laser diffraction was used to investigate the droplet size of the dispersed phase in selected o/w compositions. The optimal %T for droplet size analysis was found to be between 89.5 % and 93% and red transmission, as this was the region where $\chi^2$ was lowest and $D_{50}$ most constant (Figure 3.11).
Figure 3.11 The effect of red transmission on $D_{50}$ (■) and $\chi^2$ (□) in a composition of 20% w/w RBO, 30% w/w SM and 50% w/w water. Data is presented as mean (± SD), n = 3. Dashed line indicates the region of optimal red transmission.

The droplet size of each composition was measured in triplicate. The coefficient of variation (CV) was less than 5% in all compositions except for that consisting of 10% RBO, 60% SM and 30% water (w/w), which had a CV of 22% at $D_{10}$ (Table 3.6). As these values are within the reproducibility limits indicated by the United States Pharmacopeia, values from each composition are reported based on data from one of the three replicates for the remainder of this section. The droplet size distribution correlated reasonably well with the images obtained from light microscopy measurements in Chapter 2 (Figure 3.12), although droplet diameters less than 2 μm were not detectable by light microscopy images.
**Table 3.6** Mean droplet diameters $D_{10}$, $D_{50}$ and $D_{90}$ (± SD), and CV of compositions of different RBO, SM and water (W) concentrations (conc.) (n = 3).

<table>
<thead>
<tr>
<th>Conc. (% w/w)</th>
<th>Droplet diameter (µm)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$D_{10}$</td>
<td>$D_{50}$</td>
</tr>
<tr>
<td>RBO SM W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 30 60</td>
<td>0.32 (0.00)</td>
<td>4.32 (0.03)</td>
</tr>
<tr>
<td>10 40 50</td>
<td>1.92 (0.04)</td>
<td>4.91 (0.01)</td>
</tr>
<tr>
<td>10 60 30</td>
<td>0.65 (0.14)</td>
<td>3.27 (0.04)</td>
</tr>
<tr>
<td>20 30 50</td>
<td>1.55 (0.02)</td>
<td>11.18 (0.08)</td>
</tr>
<tr>
<td>20 40 40</td>
<td>0.64 (0.02)</td>
<td>10.50 (0.09)</td>
</tr>
<tr>
<td>30 40 30</td>
<td>1.41 (0.01)</td>
<td>23.24 (0.05)</td>
</tr>
</tbody>
</table>

* Shown in Figure 3.12

**Figure 3.12** Typical droplet diameters obtained by **a)** light microscopy and **b)** laser diffractometry for a representative composition of 20% w/w RBO, 40% w/w SM and 40% w/w water. Scale bar represents 10 µm.
Emulsions exhibited bimodal or trimodal distributions, with droplet sizes that were dependent upon composition (Table 3.7). In compositions of 10% w/w RBO, droplet distribution was bimodal with 30% and 60% SM (w/w) (Figure 3.13a), whereas a trimodal distribution was observed with 40% w/w SM, where droplets were up to 175 µm in diameter. In compositions of 20% w/w RBO, droplet distribution was trimodal (Figure 3.13b). Figure 3.13c shows the droplet size distribution of different compositions with median droplet diameter increasing with increasing RBO to water ratio. Conversely, the peak diameter of compositions decreased as the RBO to water ratio increased (Table 3.7).

<table>
<thead>
<tr>
<th>Composition (% w/w)</th>
<th>Peak diameter (µm)</th>
<th>Mode size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>SM</td>
<td>RBO</td>
<td>W</td>
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<tr>
<td>30</td>
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</table>
Figure 3.13 Typical volume size distribution of droplets for compositions containing a) 10\% RBO or b) 20\% RBO, and SM of 30\% (solid line), 40\% (dashed line) or 60\% (dot-dashed line); and c) typical median volume of distribution ($D_{50}$, *) showing the range of 10\% ($D_{10}$) to 90\% ($D_{90}$), in compositions containing different weight ratios of RBO, SM and water (RBO:SM:W).
3.4.4 SALS of RBO compositions

The scattering patterns observed when the 5 \( \mu \)m microparticles were diluted to 0.1\%, 0.05\% and 0.03\% in distilled water (w/v) are shown in Figure 3.14a. Clear peaks were observed at particle concentrations of 0.05\% w/v and 0.03\% w/v. Similarly, peaks were clearest when Intralipid® was diluted to 0.02\% w/v and 0.04\% w/v (Figure 3.14b).

The test compositions (Table 3.1) had a water content ranging from 30\% w/w to 60\% w/w. Therefore, to obtain a droplet concentration within the range of 0.02\% w/v to 0.04\% w/v, a dilution factor of 2000 was used. A temperature of 20°C was selected, because in the absence of a solvent trap with the quartz plate, evaporation of the composition was observed at 37°C over the testing period. In addition, when investigating the effect of temperature in Section 3.4.2, viscoelastic peaks were still observed at a temperature of 20°C (Figure 3.9). No differences in scattering pattern were observed at different frequencies for any given composition (Figure 3.15) and these diluted mixtures had little rheological structure (Figure 3.16). However, different light scattering patterns were observed when different compositions were compared at any given frequency (Figure 3.17) where compositions with a higher SM concentration showed higher peak intensities.
Figure 3.14  Averaged images after water subtraction and resulting scattering patterns observed when diluting a) 5 µm particles and b) Intralipid® emulsion at different particle concentrations.
Figure 3.15 Typical scattering patterns at frequencies of 0.1 Hz, 2 Hz, 4 Hz, 6 Hz, 8 Hz and 10 Hz of compositions containing a weight ratio of a) 20% RBO, 40% SM, 40% water and b) 20% RBO, 30% SM, 50% water. Averaged images with water subtracted are shown at a representative frequency of 6 Hz.
Figure 3.16  Viscoelastic profile of a composition consisting of 30% w/w RBO, 40% SM and 30% water (w/w), showing typical $G'$ (○) and $G''$ (●). Values of compositions diluted by a factor of 2000 for SALS. Data is presented as mean (± SD), n = 5.

Figure 3.17  Typical SALS image and corresponding scattering pattern of compositions composed of different weight ratios (w/w) of RBO:SM:water, at a representative frequency of 8 Hz.
3.4.5 IPM as the oil phase

When IPM was the oil phase, the viscosity of compositions depended significantly upon their composition. These compositions were either shear thinning or Newtonian depending on the ratio of IPM to water (Figure 3.18).

![Figure 3.18](image_url) Apparent viscosity as a function of shear rate for a Newtonian composition with a weight ratio of 30% IPM, 40% SM and 30% water (■), and for a non-Newtonian, shear thinning composition with a weight ratio of 20% IPM, 40% SM and 40% water (○). Data is presented as mean (± SD), n = 3.

For any SM concentration greater than 30% w/w, compositions with an IPM to water ratio of less than one exhibited the behaviour of rheologically structured semi-solids (tan δ < 1), with G’ greater than G” over the entire tested frequency range (Table 3.8). This was also in accordance with visual examination as these compositions had the appearance of semi-solids. A summary of the viscoelastic properties in different compositions of IPM, SM and water is given in Figures 3.19 and 3.20.
Table 3.8 Summary of the viscoelastic properties (G’, G” and Tan δ) of selected formulations containing varying concentrations (conc.) of SM, IPM and water (W) at representative frequencies (freq.) of 1.1 Hz, 5.1 Hz and 10.0 Hz. Data is presented as mean (± SD), n = 3.

<table>
<thead>
<tr>
<th>Conc. (% w/w)</th>
<th>Freq. (Hz)</th>
<th>Oscillatory rheology data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>G' (Pa)</td>
</tr>
<tr>
<td>SM</td>
<td>IPM</td>
<td>W</td>
</tr>
<tr>
<td>60</td>
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</tbody>
</table>
Figure 3.19 Summary of G’ and G’’ for compositions containing varying concentrations (% w/w) of SM and IPM at frequencies of a) 1 Hz, b) 5 Hz and c) 10 Hz. Colour bars highlight the magnitude of G’ and G’’. 

Chapter 3 – Physicochemical characterisation
Figure 3.20 Summary of tan δ values for compositions containing varying concentrations (% w/w) of SM and IPM at oscillatory frequencies of a) 1 Hz, b) 5 Hz and c) 10 Hz. The right-hand panel indicates whether tan δ > 1 or < 1 at each frequency, according to the colour bar.
The concentration of IPM appeared to be the most important determinant of viscoelasticity. The most marked change occurring in the SM range of 40% to 60%, where $G'$ increased up to four orders of magnitude by varying IPM concentration. Altering the ratio of IPM to water not only altered viscoelasticity, but also the way the formulation responded to increasing frequency (Figure 3.21). The most viscoelastic formulation was obtained with 40% SM and 20% IPM, with a $\tan \delta$ of between 0.01 and 0.02 over the entire tested frequency range. Interestingly, peaks similar to those observed in the RBO compositions were also observed in IPM compositions that contained 10% w/w IPM and more than 40% w/w SM (Figure 3.22, Table 3.9).
Figure 3.21  a) $G'$, b) $G''$ and c) $\tan \delta$ as a function of frequency for compositions containing 40% w/w SM and an IPM:water ratio (w/w) of 3:3 (□), 2:4 (●) and 1:5 (○). Data is presented as mean (± SD), n = 3.
Figure 3.22  a) $G'$, b) $G''$ and c) $\tan \delta$ as a function of frequency for compositions containing 10% w/w IPM and a SM:water ratio (w/w) of 5:4 (●), 6:3 (■), and 7:2 (○). Data is presented as mean (± SD), $n = 3$. 

Chapter 3 – Physicochemical characterisation
Table 3.9 Magnitude of peak G’, G” and tan δ and the frequency at which this occurred for formulations containing varying amounts of IPM, SM and water. Absolute values indicate the absolute (abs.) peak G’, G” or tan δ and relative (rel.) values indicate the peak G’, G” or tan δ relative to the baseline value for that formulation. Negative values indicate the peak was below the baseline value.

<table>
<thead>
<tr>
<th>Conc. (% w/w)</th>
<th>Frequency (Hz)</th>
<th>Peak G’ (Pa)</th>
<th>Peak G” (Pa)</th>
<th>Peak Tan δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>70 10 20</td>
<td>9.01</td>
<td>316.80 -37.46</td>
<td>88.26 5.89</td>
<td>0.27 0.05</td>
</tr>
<tr>
<td>60 10 30</td>
<td>6.04</td>
<td>133.23 -18.51</td>
<td>37.75 14.62</td>
<td>0.28 0.12</td>
</tr>
<tr>
<td>50 10 40</td>
<td>8.68</td>
<td>276.00 -25.45</td>
<td>- -</td>
<td>0.27 0.04</td>
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</table>

3.4.6 Volume fraction of the oil phase

The density of RBO was calculated to be 0.92 ± 0.006 g/mL, whereas the density of IPM was 0.85 ± 0.002 g/mL. The density of the lecithin/PG (1/1 w/w) SM was 1.02 ± 0.001 g/mL and the density of water was 1.00 ± 0.000 g/mL. Using these values, the v/w concentration of each component was calculated and the volume fraction (v/v) of the oil phase in different weight ratios of oil, SM and water were determined using Equation 1.1 (Table 3.10). For any given weight ratio, the volume fraction of oil was between 1% and 2% v/v lower when IPM was the oil phase compared to when RBO was the oil phase.
Table 3.10 Summary of the volume of the weight ratio (v/w) of different compositions of oil (RBO or IPM), SM and water (W) and corresponding oil volume fraction (v/v) for RBO and IPM.

<table>
<thead>
<tr>
<th>Conc. (w/w)</th>
<th>Conc. (v/w)</th>
<th>Oil volume fraction (v/v)</th>
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<tr>
<td></td>
<td>Oil</td>
<td>SM</td>
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<tr>
<td>0.10</td>
<td>0.40</td>
<td>0.50</td>
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<td>0.60</td>
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3.4.7 Cryo-SEM of RBO and IPM compositions

When RBO was the oil phase, both a continuous and dispersed phase were identified using cryo-SEM (Figure 3.23a), whereas when IPM was the oil phase a more complex structure was observed (Figure 3.23b).

![Image](https://via.placeholder.com/150)

**Figure 3.23** Cryo-SEM images of compositions consisting of 50% w/w SM, 20% w/w water and a) 30% w/w RBO or b) 30% w/w IPM. Scale bars represent 10 µm.
3.5 Discussion

The present study investigated the rheology of emulsions for potential use as saliva substitutes in patients with severe xerostomia. Emulsions were hypothesised to provide prolonged relief by combining the lubricating properties of oil with the palatability of water. Results showed that the rheological properties of the formulations depended upon their composition as well as the shear rate and frequency to which they were exposed. Frequency-dependent compositions were identified in RBO compositions containing less than 40% w/w SM, where the G” dominated G’ at oscillatory frequencies below 5 Hz (tan δ > 1) and elastic behaviour dominated at higher oscillatory frequencies (tan δ < 1) in a similar pattern to that observed for natural saliva in Chapter 2. This was successfully plotted onto a ternary phase diagram by grouping formulations based on their tan δ at selected frequencies (Figure 3.9). Although Chapter 2 reported complex viscosity, this chapter focused on the G’, G” and tan δ, as these were the values thought to be most relevant to the functions of natural saliva.

Emulsions exhibit a wide variety of rheological characteristics depending on their structure, composition and droplet interactions (Walstra, 2003; McClements, 2005, 2010). In emulsions, viscosity tends to increase with increasing droplet concentration (Walstra, 2003; McClements, 2010). In flocculated emulsions, the floc structure deforms and breaks down with increasing shear stress, resulting in shear thinning behaviour (McClements, 2010). The oil type influences the emulsion characteristics in the way that it penetrates the lipophilic region of the surfactant monolayer. Short-chain triglycerides are more effective at penetrating this film compared to bulkier, long-chain triglycerides (Talegaonkar et al., 2008). In Chapter 2, the microstructure of compositions varied depending on the surfactant and oil type used, as well as the concentration of each component. Therefore, it follows that the physicochemical properties of emulsions would also depend on these factors.

The oral cavity is a complex, dynamic environment (Vissink et al., 1984; Corcoran et al., 2006) with different forces and stresses occurring simultaneously on different surfaces and in different functional entities within the mouth (Ranc et al., 2006; Yakubov et al., 2009). Saliva is continually secreted at a flow rate and composition dependent on a myriad of external factors. Although saliva substitutes are as yet unable to emulate natural saliva in this way, an understanding of the rheological properties...
should provide some insight into the way these compositions may behave within the oral cavity. However, while the rheological characterisation of formulations and comparison to natural saliva are important features in developing a saliva substitute, clinical studies will ultimately be required in order to truly understand how rheological properties correlate with prolonged xerostomia relief.

Rheologically structured RBO compositions exhibited shear thinning behaviour. Unstimulated natural saliva is classified as a non-Newtonian fluid as it is pseudoplastic, with viscosity decreasing with increasing shear rate (Stokes & Davies, 2007). This contributes to the ability of saliva to lubricate the oral cavity at rest and flow at higher shear rates during mastication and deglutition (Hatton et al., 1987; Preetha & Banerjee, 2005). Clearly, a non-Newtonian saliva substitute may be considered beneficial in this respect. Although the RBO compositions exhibited pseudoplastic properties, the apparent viscosity was higher than that reported for natural saliva at any given shear rate (Stokes & Davies, 2007). The flow consistency provides an indication of the viscosity of a fluid, so long as the two fluids have a similar rate index (Björn et al., 2012). It would be expected that a fluid with a higher flow consistency would be more viscous and therefore, visually more semi solid-like. This was not the case in the composition of 10% RBO, 40% SM and 50% water (w/w), which was classed as semi solid-like based on visual observation, but had a lower flow consistency than the composition of 40% RBO, 30% SM and 30% water (w/w), which was classed as liquid-like. While RBO compositions consisting of less than 40% w/w SM exhibited a lower apparent viscosity, these values were still two orders of magnitude higher than that of natural saliva for any given shear. Although a highly viscous formulation may assist in lubrication of the oral cavity, functional difficulties in mastication may arise when a saliva substitute is too viscous as it must be able to function both at rest as well as in an environment of high shear (Vissink et al., 1984).

Compositions investigated in this study were unique in that viscoelasticity remained on dilution with aqueous fluids, whereas normally, dilution lowers the elasticity, thus facilitating removal from the oral cavity. This is important, because in the oral cavity the formulations will be exposed to additional moisture, including residual saliva and the ingestion of liquid. Compared to the findings in Chapter 2, where the viscoelasticity of natural whole saliva was determined, the tested formulations had G’
and G” values that were higher than whole saliva, but lower than the HEC saliva substitute. However, the relative contribution of viscous to elastic behaviour (tan δ) for compositions containing 20:20:60 and 20:30:50 RBO:SM:W (% w/w) were analogous to whole saliva, with tan δ less than one at higher frequencies only. This frequency-dependent behaviour may be beneficial in a saliva substitute, because the viscous behaviour that dominates at low frequencies is hypothesised to improve lubrication of the oral cavity at rest, whereas the elasticity at higher frequencies may improve retention during mastication and deglutition. The elasticity of saliva has been suggested to have a protective function in the oral cavity, reducing tooth decay caused by the ingestion of food and beverages by lubricating the oral cavity (Hahn Berg et al., 2003; Stokes & Davies, 2007). Compared to natural whole saliva, the combination of a similar tan δ yet higher overall G’ and G” that was observed in the tested compositions may provide prolonged lubrication, and therefore, retention, in the absence of continual secretion.

In the present study, RBO compositions with a higher fraction of oil droplets exhibited a higher G’, which corresponded with the findings of a previous study (Pal, 2006). Results in Chapter 2 showed that different structures formed depending upon their composition; emulsions were identified as o/w up to approximately 50% w/w RBO, after which oil became the continuous phase. Importantly, an emulsion in the oral cavity is likely to mix with residual saliva and other aqueous liquids, thus altering its composition. In the oral processing of emulsion droplets in food, lubrication of the oral surfaces depends on the stability of the emulsion. The droplets themselves may be altered by hydrolysis and oxidation due to chemical instability, or physical instability may occur whereby creaming, flocculation or coalescence alters the spatial distribution of the droplets (McClements, 2010). Recent research has focussed on emulsion stability in the presence of saliva (Kileast & Clegg, 2002; Dresselhuis et al., 2008b; Silletti et al., 2008; Vingerhoeds et al., 2009; van Aken et al., 2011). It has been reported that when emulsions mix with saliva, flocculation occurs and this is thought to be caused primarily by mucin, with other salivary components also contributing to this phenomenon (Vingerhoeds et al., 2005). This is likely to be limited in patients with minimal residual salivary gland function; however, the ingestion of water and other aqueous liquids may cause a similar effect.
While limited data exists with respect to oscillatory frequencies in the oral cavity, an oscillatory frequency of 8 Hz has been shown to correlate with perceived thickness (Stanley & Taylor, 1993). Based on this value, the effect of compositions containing greater than 40% w/w SM may be influenced by chewing, with a sharp reduction in $G'$ and increase in $G''$ at this frequency. The viscosity of natural saliva is thought to prevent dehydration of the oral mucosa while the high relative elasticity is thought to offer protection and lubrication in the oral cavity (Stokes & Davies, 2007). Where a higher $G'$ has been reported to improve retention and mucoadhesion in the oral cavity (Jones et al., 1997; Jones et al., 2001), liquid is required for effective lubrication during mastication (Pedersen et al., 2002). The reversible peak in viscous behaviour and reduction in elastic behaviour at oscillatory frequencies similar to those experienced during mastication, may be beneficial in the process of mastication and deglutition in the absence of natural saliva. This concept has not been reported previously and further studies are warranted in order to further investigate the occurrence of these peaks and if a similar phenomenon is observed *in vivo*.

The visually solid-like emulsions exhibited a high $G'$ and low tan $\delta$, confirming the presence of rigid structures and strong intermolecular interactions. Peaks in $G'$ and $G''$ were observed in many of the formulations, with the critical frequency at which the peak occurred depending upon the relative composition of RBO, water and SM. A similar finding has been reported previously in the $G'$ of emulsions that consist of both a Newtonian dispersed and continuous phase. This has been associated with relaxation in the shape of the deformed droplets and is termed a relaxation shoulder (Erni et al., 2007; Erni et al., 2011). Light microscopy indicated that the size of the droplets in the dispersed phase might increase after the threshold frequency, suggesting that the peak occurs due to droplet aggregation. Oscillatory rheology is a non-destructive technique; therefore, the rheological structure of materials historically remains intact within the LVR. Another possible explanation for this phenomenon is that in the coarse emulsions investigated here, the droplet size of the dispersed phase was not homogenous. Droplets may be subject to Brownian motion (Deyrail et al., 2009) and are likely to aggregate when they collide as a result of vibration in different directions. The relative weight fraction of each phase influences the vibration amplitude of dispersed droplets in response to frequency. The frequency at which this occurs would depend on the relative concentration of each component, as the volume fraction of oil
droplets may affect the size and proximity of the droplets to one another (Mason, 1999). When investigating these peaks compared to the microstructure determined in Chapter 2, it was evident that the compositions that exhibited these frequency dependent peaks were o/w type emulsions, with the exception of the composition of 50% RBO, 40% SM and 10% water (w/w). This was a pseudo-bicontinuous system that exhibited a small drop in $G'$ at 3.7 Hz (-7.1 Pa), corresponding with a peak in $\tan \delta$ (0.16), but no change in $G''$. The behaviour of droplets in the dispersed phase of selected compositions was investigated using light microscopy, following exposure of compositions to different frequencies. Resulting images indicated that droplet microstructure changed in response to frequency. However, following exposure to the set frequency, compositions were transferred from the rheometer to a glass slide before being examined. It is possible that results would differ under continued oscillatory conditions.

SALS was investigated to determine the scattering pattern of droplets while the formulation was exposed to an oscillatory force. No differences in the scattering pattern of compositions were observed at different frequencies. However, the compositions had to be diluted by a factor of 2000 to avoid multiple light scattering, which is a known limiting factor to in the application of SALS to rheology (Alexander & Dalgleish, 2006). Dilution results in a larger spatial distance between droplets and is therefore expected to alter droplet characteristics. If some sort of aggregation were responsible for the peaks, this would not occur in these dilute conditions. Additionally, dilution resulted in a lack of rheological structure, as seen in Figure 3.16. If the peak in $G'$ and $G''$ was not observed in these diluted compositions, it follows that no change in microstructure would be observed. Different compositions had different scattering patterns, suggesting that this method was useful in differentiating between compositions. As the wavelength was fixed in the present study, the light intensity observed at any scattering angle was dependent on droplet diameter, as well as destructive or constructive interference between transmitted and reflected light (Olesik & Kinzer, 2006). In scattering measurements, the peak intensity of smaller particles occurs at larger angles. As particle diameter increases, this peak is observed at smaller angles (Deyrail et al., 2009). Therefore, a higher peak angle is associated with a smaller droplet size. In the present study, droplet size was inversely proportional to SM
concentration and indeed, peaks were observed at larger angles in compositions with a SM concentration of 40% w/w compared to 30% w/w (Figure 3.17).

In particle size analysis, the development of an appropriate dispersion technique is vital, with variations in scattering pattern occurring as a function of scattering angle, particle size and refractive index (Shekunov et al., 2007). Errors in the estimation of the imaginary and real components of refractive index have been shown to result in errors in the resulting particle size and particle size distribution (Beekman et al., 2005; Keck & Müller, 2008). In order to confirm reproducibility of light diffractometry measurements, the United States Pharmacopeia (2006) specifies a CV of less than 10% at $D_{50}$ and less than 15% at $D_{10}$ and $D_{90}$ for particle size measurements, based on at least three replicates. In the present study, after investigating the appropriate dispersion technique, this criterion was met for all compositions except the one consisting of a weight ratio of 10% RBO, 60% SM and 30% water, which had a CV of 22% at $D_{10}$. However, as the reported droplet diameters were less than 10 $\mu$m, the maximum CV may be doubled to 30% at $D_{10}$, so this CV was still considered to be within the acceptable range. The droplet distribution in the emulsions were identified as bimodal or trimodal depending on the composition. Interestingly, the low CV that was observed was despite the fact that homogenisation techniques were not used to control droplet size.

The effect of increasing temperature from 20°C to 37°C was investigated to determine whether it altered the observed peaks or the critical frequency at which they occurred. Although temperature did not affect the frequency at which the viscoelastic peak was observed, $\tan \delta$ increased with increasing temperature up until the peak. After the peak, $\tan \delta$ began to decrease from 20°C to 37°C. A possible explanation for this is that a small amount of evaporation occurred resulting in altered rheological properties (Zhang et al., 2008), despite the presence of the solvent trap steel plate on the rheometer. In reality however, evaporation is also likely to occur in the oral cavity. The effect of temperature is an important consideration in developing an appropriate delivery device. The higher $\tan \delta$ observed at room temperature with oscillatory frequencies over 5 Hz may promote initial spreadability in the delivery of a formulation to the oral cavity. In addition, the lower $\tan \delta$ at 37°C and frequencies greater than 3.5 Hz may promote lubrication and prolonged retention within the oral cavity.
When RBO was replaced with IPM in the compositions, rheological properties were considerably different. When IPM was used as the oil phase, emulsions were shear thinning or Newtonian depending upon their composition. At any given SM concentration, the viscoelasticity of IPM compositions varied depending on the concentration of IPM and water. The largest $G'$ and $G''$ values were observed at an IPM concentration of 20% w/w. In compositions containing more than 40% w/w SM, increasing the IPM concentration from 20% w/w to 30% w/w and reducing the water concentration by 10% w/w resulted in a large reduction in both $G'$ and $G''$, corresponding with an increase in $\tan \delta$. This may be explained by the microstructure of the IPM compositions. The compositions that exhibited the highest rheological structure were identified in Chapter 2 as possessing a liquid crystalline microstructure when observed under polarised light. The IPM compositions did not exhibit the frequency-dependent change from liquid-like to solid-like that was seen in some RBO compositions, and the large increase in $G'$ and $G''$ (up to four orders of magnitude compared to natural saliva) was observed with increasing water concentration, which may limit spreadability in the oral cavity. Therefore, RBO compositions were considered beneficial in this respect.

The measured density of $0.92 \pm 0.006 \text{ g/mL}$ and $0.85 \pm 0.002 \text{ g/mL}$ for RBO and IPM, respectively, corresponded with those reported in the material safety data sheets ($0.92 \text{ g/mL}$ for RBO and $0.85 \text{ g/mL}$ for IPM). This resulted in a slightly lower volume fraction of IPM compared to RBO, at any given weight ratio. The volume fraction of the oil phase influences the rheological properties of an emulsion (Sinko, 2011). Further, the oil type influences the emulsion characteristics in the way that it penetrates the lipophilic region of the surfactant monolayer.

The microstructure of the IPM composition observed under cryo-SEM was markedly different to that of RBO. The microstructure was similar to that observed in previous lecithin-based studies, where IPM was the oil phase (Graf et al., 2008). The reason for the differences was likely due to the chemical composition of the two oils. Whereas RBO consists of 38% monounsaturated, 37% polyunsaturated and 25% saturated fatty acids (Orthoefer & Eastman, 2005), IPM is an ester that consists of isopropyl alcohol esters and saturated high molecular-weight fatty acids, particularly myristic acid. RBO compositions primarily formed o/w emulsions and the differences in rheology are likely
due to interactions between the droplets at different frequencies. The IPM compositions, on the other hand, formed more complex liquid crystalline structures, which were more resistant to changing frequency. Marked changes in viscoelasticity, by up to four orders of magnitude for \( G' \), occurred when the IPM to water ratio was decreased from 1:1 to 1:2 (w/w).

3.6 Conclusions

The viscoelastic properties of the lecithin-based emulsions depended on the oil used, with marked differences observed when RBO was replaced with IPM. This was most likely due to a different microstructure that forms due to the difference in the fatty acid composition of the two oils. RBO compositions were shear thinning whereas IPM compositions were either shear thinning or Newtonian, depending upon composition. When RBO was the oil phase, a frequency-dependent region was identified using a pseudo-ternary phase diagram, where viscous behaviour dominated at frequencies below 5 Hz and elastic behaviour dominated at higher frequencies. The droplet size distribution of the tested RBO emulsions were found to be bimodal or trimodal, depending upon their composition.

An emulsion consisting of RBO, lecithin and water has potential application as a saliva substitute \textit{in vivo}. Viscoelastic properties of formulations remained in the presence of up to 50% w/w water, which is an important consideration as xerostomia patients are likely to sip water continuously throughout the day and may also have residual saliva function. These factors will contribute to an increased aqueous content in the oral cavity. Although the elasticity of these formulations was higher than that of natural saliva, it may contribute to an improved retention and mucoadhesion in the mouth in the absence of an ability to continually secrete saliva. Frequency-dependent formulations may offer sustained relief of xerostomia with viscous behaviour dominating at low frequencies, resulting in improved lubrication at rest and elastic behaviour at higher frequencies improving retention during high-shear tasks. Further clinical studies will allow for an investigation of this hypothesis.
Chapter four

Clinical application: A pilot study
Chapter 4 – Clinical application

4 Clinical application: A pilot study

4.1 Introduction

In Chapter 3, the physicochemical properties of different compositions of RBO, SM and water were investigated for their potential as saliva substitutes. Some of these compositions demonstrated frequency-dependent viscoelasticity. It was hypothesised that viscoelasticity may offer sustained relief of xerostomia, with liquid-like behaviour at low frequencies improving lubrication at rest and solid-like behaviour at high frequencies improving retention during high-shear tasks such as swallowing and speaking. However, in order to determine the clinical efficacy of these compositions in patients, clinical studies are required. The focus of this chapter was on establishing methods for testing the retention of liquids in the oral cavity, and applying these to investigate the efficacy of an emulsion containing RBO, SM and water in a pilot clinical trial using healthy volunteers.

4.1.1 Establishing markers of efficacy

Many studies have investigated the clinical efficacy of saliva substitutes using a range of subjective and objective methods (Olsson & Axell, 1991; Momm et al., 2005; Dirix et al., 2007; Mouly et al., 2007b). Objective measures often include dry mouth assessments. However, the present study was a preliminary investigation using participants with normal salivary function and therefore, such assessments were not considered useful at this stage. As highlighted in Chapter 1, there is a paucity of data with respect to the retention of liquids in the oral cavity after simple rinsing. While turbidity measurements have been used to determine the presence of custard-based desserts (Prinz et al., 2006; de Wijk et al., 2009), initial turbidity in the formulation is required. Although this method may be useful for determining retention of turbid emulsions, this approach is not suitable for polymer-based saliva substitutes or water, which were intended to act as controls in this study. Phenol red has been used as a marker for drug absorption from the oral mucosa (Tucker, 1988; Dawes, 2006). While phenol red analysis was unaffected by saliva in a single, presumably healthy individual (Tucker, 1988), the effect of an acidic oral environment, which is typical in individuals with salivary hypofunction, has not been elucidated. The development of a marker for
the retention of liquids in the oral cavity would be beneficial when examining the
efficacy of saliva substitutes.

Lithium may be a useful marker for the retention of liquids in the oral cavity because it is ubiquitous, non-radioactive and easily measured at low concentrations using a lithium-specific spectrophotometric assay (Rumbelow & Peake, 2001). In addition, the efficacy of lithium as a non-toxic therapeutic marker has been established in other applications. Lithium ions are an effective marker in the measurement of cardiac output, as only trace amounts are normally present in the blood and its pharmacokinetics have been well established (Linton et al., 1993; Birch, 1999; Cecconi et al., 2009). The concentration of lithium in serum is routinely monitored in patients taking therapeutic doses of lithium carbonate for the treatment of mania, bipolar disorder and recurrent unipolar depression. The daily dose range for lithium carbonate is 400 mg to 1200 mg (equivalent to between 80 mg and 225 mg lithium), and this is adjusted accordingly to achieve a serum lithium concentration of 0.4 to 1.0 mmol/L (Sweetman, 2007), which is routinely monitored in these patients. Therefore, most laboratories are equipped to measure lithium concentration at low doses, often using a spectrophotometric assay. The basis of this assay is that lithium ions, present in the sample, react with a substituted porphyrin compound under alkaline reaction conditions. Complexation of porphyrin with lithium ions results in a decrease in absorbance at approximately 510 nm, which is directly proportional to the lithium concentration in the sample (Rumbelow & Peake, 2001) (Figure 4.1). The concentration of lithium can thus be determined by the change in absorbance of the sample.
Figure 4.1 The reaction of lithium and porphyrin (HP⁵⁻) to form a lithium-porphyrin complex (LiP⁵⁻) and resulting LiP⁵⁻ structure that induces a decrease in absorbance.

The measuring range of lithium using the spectrophotometric assay is between 0.05 and 3.00 mmol/L (Cobas C®, Roche Diagnostics GmbH, Mannheim, Germany). The lithium intake data from different countries has been examined and a daily intake of 14.3 µg per kg bodyweight per day has been established, which equates to 1.0 mg per day for a 70 kg adult. The main dietary sources of lithium include grains, animal products, vegetables and drinking water (Schrauzer, 2002; Aral & Vecchio-Sadus, 2008). A lithium concentration of 1.0 mmol/L, which equates to 0.07 mg lithium, would be sufficient to measure using the spectrophotometric assay, and is significantly below the average daily intake. Lithium ions can therefore be considered a safe marker compound worthy of further investigation.

Lithium carbonate (Li₂CO₃) is soluble in water so in an emulsion, lithium would be associated with the aqueous phase. As emulsions have the potential to coalesce in the oral cavity, particularly in the presence of saliva (Kilcast & Clegg, 2002; Dresselhuis et
al., 2008b; Silletti et al., 2008; Vingerhoeds et al., 2009; van Aken et al., 2011), a measure of the aqueous retention is not necessarily indicative of oil retention in the oral cavity. Gravimetric analysis is often used to determine the oil content of emulsions and generally involves evaporation of the aqueous phase (Hong et al., 2003; Dresselhuis et al., 2008b). Some emulsions can be separated by the use of membrane filters (Lipp et al., 1988; Chakrabarty et al., 2008; de Morais Coutinho et al., 2009), and ultracentrifugation has been effective in the separation of mayonnaise, which is a typical food emulsion (Jacobsen et al., 1998). Alternatively, the presence of oil in samples can be detected using Fourier transform infrared (FTIR) spectroscopy (Che Man et al., 2011; Rohman & Man, 2012), which does not necessarily require separation of the two phases.

Sensory perception is important when considering patient acceptability (Momm et al., 2005). Studies of the sensory perception of foods in the oral cavity often use quantitative 100-mm continuous scales (Kilcast & Clegg, 2002; Ali et al., 2011; van Aken et al., 2011), with anchor points appropriate to the property being tested, such as ‘like extremely’ and ‘dislike extremely’ in order to determine overall taste acceptability. Emulsion droplets have been shown to influence textural sensory perception of liquid emulsions by incorporating into the coating in the oral mucosa, increasing the viscosity and spreading of oil at surfaces in the oral cavity (van Aken et al., 2011). The feeling of textural thickness has been demonstrated to be directly proportional to the viscous force between the tongue and the roof of the mouth (Kokini et al., 1977).

4.1.2 Compositions to evaluate in the pilot study

In Chapter 3, different compositions of RBO, SM and water were investigated as potential saliva substitutes. Some compositions exhibited frequency-dependent behaviour, and these were hypothesised to offer sustained relief of xerostomia, with viscous properties at low frequencies improving lubrication at rest and elastic behaviour at higher frequencies improving retention during high-shear tasks such as swallowing and speaking. The peaks in tan δ observed at certain frequencies in some formulations were also of interest. The composition containing 20% w/w RBO, 40% w/w SM and 40% w/w water was selected for the present study. This formulation was in the frequency-dependent region, with a peak in tan δ that occurred at 3.40 Hz. Similar
compositions with a higher water concentration had similar tan δ to natural saliva, therefore, it was thought that as the water content in the oral cavity increased, this composition could behave more like natural saliva.

Water is often the preferred treatment option for xerostomia (Epstein & Stevenson-Moore, 1992; Ferguson, 2002); however, it offers only temporary relief and fails to lubricate the oral surfaces (Ferguson, 2002; Frost, 2008). Although many commercial saliva substitutes are available that could be used as controls in this study, only one is Government funded in New Zealand. It is an extemporaneously compounded product, with a standard formula consisting of 1% w/v methylcellulose in water (Wilson et al., 2012). A preservative may be included if necessary, but the addition of any further excipients, such as a flavouring agent, are not funded. As flavour has not been tested in the emulsion compositions, the use of unflavoured formulations as controls were considered optimal as a control in the present study. Both of the above-mentioned treatment options are highly relevant, with the 1% w/v methylcellulose suspension being the only funded option in New Zealand and water being the most commonly used treatment option. Therefore, the three formulations tested in the present study were the emulsion, consisting of 20% w/w RBO, 40% w/w SM and 40% w/w water; 1% w/v methylcellulose suspension; and water.

4.2 Chapter aims

- To evaluate the use of lithium as a marker for measuring the retention of fluids in the oral cavity;
- To apply this method to determine the retention of an emulsion intended as a saliva substitute compared to two controls (water and 1% w/v methylcellulose);
- To investigate sensory perception of the emulsion.

4.3 Materials and methods

4.3.1 Materials

Lithium carbonate was purchased from Merck KGaA (Darmstadt, Germany). A 100 mmol/L, pH 7.4 sodium phosphate buffer was prepared using sodium dihydrogen phosphate and disodium hydrogen phosphate purchased from Sigma Aldrich (St Louis, MO, USA), and high-viscosity sodium CMC and PG were also purchased from Sigma
Aldrich (St Louis, MO, USA). Methylcellulose was purchased from ABM Pharma Ltd (North Shore City, New Zealand), RBO was from Bespoke Foods (UK) and soy lecithin (Lipoid S-100) was purchased from Lipoid GmBH (Ludwigshafen, Germany). Distilled water was used where required and all materials were used as received without any further purification. Lithium analysis was conducted at Southern Community Laboratories (Dunedin, New Zealand). Infinity Lithium Liquid Stable Reagent (Thermo Fisher Scientific, Middletown, USA) was used in the validation study and Cobas C (Roche Diagnostics, Mannheim, Germany) lithium reagent was used in the clinical study, due to differing availability of reagents during the validation and testing periods of the study. The lithium reagents were stored at 4°C in a capped, opaque bottle to limit degradation. Once opened, the reagent was stored in the analyser and tests were completed within 24 hours, which was within the recommended manufacturer guidelines.

4.3.2 Study A: Validation of lithium as a marker of retention in the oral cavity

4.3.2.1 Validation of lithium assay for saliva samples

The lithium reagent used in the present study was intended for the quantitative determination of lithium in human serum or EDTA plasma in vitro. In order to validate its reliability to determine lithium concentration in saliva, unstimulated whole saliva collected from a single donor using the expectoration method described in Chapter 2 was spiked with known concentrations of lithium (0, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.6 mmol/L) in vitro. To ensure that the potassium concentration present in saliva would not interfere with the measured lithium concentration, 50 mmol/L potassium was added to some of the lithium-spiked saliva samples. Standards containing a known amount of saliva and mouth rinse were also analysed. All samples were prepared in triplicate and centrifuged at 3220 x g at a temperature of 4°C for ten minutes before analysis. Lithium concentrations were determined in a clinical laboratory (Southern Community Laboratories, Dunedin, New Zealand) using a spectrophotometric assay after reacting the lithium with the Infinity® reagent, which contains a substituted porphyrin. These results were compared to the known concentration of lithium in each sample to determine the standard curve. A further dilution series of 0.2, 0.4, 0.6, 0.8 and 1.0 mmol/L lithium dissolved in pH 7.4 100 mmol/L sodium phosphate buffer were
prepared and tested using the Cobas C® reagent. These were then plotted on the standard curve to confirm the reliability of results using this alternative reagent.

4.3.2.2 Validation procedure for determining retention of liquids in the oral cavity

Two mouth rinses were prepared (Appendix B), each containing 1 mmol/L lithium dissolved in the 100 mmol/L sodium phosphate buffer. One mouth rinse also contained 1% w/v CMC.

Twenty healthy subjects between the ages of 20 and 60 years old gave informed consent (Appendix C) and participated in a cross-over study. The study was approved by the University of Otago Ethics Committee (reference code 10/195, Dunedin, New Zealand). Of the participants, ten were males aged 30.6 ± 7.8 years (median 26.5 years) and ten were females aged 32.7 ± 11.5 years (median 26 years). All were dentate individuals who were considered to be in general good health. Participants were asked to refrain from food or beverages (with the exception of water) for at least 30 minutes prior to the study and were instructed to cleanse their mouth by consuming 200 mL water before and after each mouth rinse. They were randomly assigned to either mouth rinse A (‘water’) or mouth rinse B (‘1% w/v CMC’) using a binary random number generator (Haahr & Haahr, 2012), and instructed to gently swill 10 mL of the prepared mouth rinse for 30 seconds before expectorating the contents of their mouth into a pre-weighed collection cup. The mass expectorated into the cup was measured immediately, using a mass balance (Mettler AT201, Greifensee, Switzerland). Following a ten-minute washout period, participants were instructed to follow the same procedure with the second rinse so that one sample of each rinse was obtained from all participants. Samples (4 mL) were transferred to Microsep centrifugal tubes with a 0.45 µm filter (Pall Corporation, Ann Arbor, MI, USA) and centrifuged (3220 x g) at a temperature of 4°C for ten minutes. This was to reduce the CMC concentration as most was retained by the filter and to remove other contaminants, such as food particles that may have been expectorated with the mouth rinse. The concentration of lithium in the filtrate was then analysed by spectrophotometric assay in a clinical laboratory using standard protocols for lithium measurement (Southern Community Laboratories, Dunedin, New Zealand). The concentration of lithium in the initial mouth rinse was analysed using the same
technique to determine whether filtration affected the measured lithium concentration. The amount of liquid retained in the mouth was then calculated using the initial ($i$) and expectorated ($exp$) concentration ($C$) and volume ($V$) values for each mouth rinse (Equation 4.1).

\[
\text{Liquid retained (\%)} = \left( \frac{(C_i V_i) - (C_{exp} V_{exp})}{(C_i V_i)} \right) \times 100
\]

Equation 4.1

Statistical analysis was performed using Stata 11.1 (Stata Corp, College Station, Texas, USA). The retention of water and 1% w/v CMC were compared using a paired Student’s t-test, following a variance-comparison test that confirmed the assumption of equal variance. The relationship between age, gender and liquid retained were investigated using the Spearman rank correlation coefficient.

4.3.3 Study B: An investigation of potential saliva substitutes in the oral cavity

4.3.3.1 Preparation of formulations as saliva substitutes

Formulations were prepared according to the manufacturing sheets in Appendix B. In brief, the aqueous rinse (‘water’) was prepared by spiking a 100 mmol/L sodium phosphate solution (pH 7.4) with 1 mmol/L lithium, as in the validation study. The standard saliva substitute formula (‘polymer’) listed in the New Zealand Pharmaceutical Schedule was prepared by suspending 1% w/v methylcellulose in distilled water that was spiked with 1 mmol/L lithium. The emulsion (‘emulsion’) was prepared in the same way as described in Chapter 3, using a composition of 20% w/w RBO, 40% w/w SM (soy lecithin and RBO at a weight ratio of 1:1) and 40% w/w water. The aqueous phase was spiked with lithium so that the total concentration in the emulsion was 1 mmol/L. Formulations were refrigerated and used within 48 hours of preparation.

4.3.3.2 Test procedure for determining the retention of formulations

The second trial involved thirty volunteers aged between 19 and 36 (mean age 27) and was approved by the University of Otago Ethics Committee (reference code 12/252, Dunedin, New Zealand). Volunteers were asked to read through an information sheet and sign a consent form prior to participation (Appendix D). All were dentate
individuals who considered themselves to be in good general health. They were asked to refrain from eating or drinking for 60 minutes prior to their session. Each participant was randomly assigned to a formulation (water, polymer or emulsion) using a three-digit random integer generator (Haahr & Haahr, 2012) so that there were ten participants assigned to each formulation. At the start of their session participants were asked to rinse their mouths by consuming 200 mL tap water. Basal salivary flow rate was then measured using the expectorating technique described in Chapter 2. Participants sat with their heads tilted forward to allow saliva to pool in the mouth before forcefully expectorating into a pre-weighed collection cup at 30-second intervals for a five-minute period. The mass of saliva expectorated was determined immediately using a mass balance (Mettler AT201, Greifensee, Switzerland) in order to determine a basal salivary flow rate (mL/min) for each participant, where the density of saliva was assumed to be 1 g/mL (Kerr, 1961; Lentner, 1981).

Each participant rinsed their mouth with 10 mL of their assigned formulation by gently swilling it for 30 seconds before expectorating into a cup. The mass expectorated was determined immediately, using a mass balance (Mettler AT201, Greifensee, Switzerland) and the samples were stored at a temperature of -20°C for up to four weeks before analysis, according to the 2009 Salimetrics® saliva collection and handling advice guidelines (State College, PA, USA). Following expectoration, participants were given an assessment form to complete (Appendix E) to determine the perceived taste, intensity and thickness of the formulations using a 100-mm continuous analogue scale labelled with anchors such as ‘like extremely’ and ‘dislike extremely’ for taste acceptability; ‘extremely intense’ and ‘not intense at all’ for intensity in the mouth; and ‘the thickness of water’ and ‘the thickness of yoghurt’ for perceived thickness.

Participants were then asked to remain seated for a further five minutes, during which time they were able to speak and swallow as normal. After five minutes they were given 10 mL distilled water to swill around their mouth for 30 seconds before expectorating into a pre-weighed collection cup. Mass expectorated was again measured using a mass balance (Mettler AT201, Greifensee, Switzerland) and another assessment form completed. This was further repeated after ten minutes. Samples were stored at a temperature of -20°C for up to four weeks, until required for analysis.
4.3.3.3 Retention of the aqueous component of formulations

Each expectorated sample was centrifuged using a Microsep centrifugal device with 0.45 µm filter (Pall Corporation, Ann Arbor, MI, USA) for ten minutes at 3220 x g and a temperature of 4°C. This was to remove contaminants from the aqueous phase and in the case of the emulsion group, separate the aqueous phase from the emulsion. The concentration of lithium in each initial formulation as well as in each sample was analysed in a clinical laboratory (Southern Community Laboratories, Dunedin, New Zealand) using the Cobas C® lithium reagent. The amount of liquid retained in the mouth was then calculated using Equation 4.1, where the initial volume was 10 mL and the initial concentration of lithium was taken as the measured initial concentration for each formulation.

4.3.3.4 FTIR spectroscopy of emulsions following oral rinsing

FTIR measurements were performed using a Varian 3100 Excalibur Series FTIR spectrometer (Varian Incorporated, California, USA) equipped with calcium fluoride (CaF₂) BioCell windows (pathlength 6 µm, Biotools Incorporated, Jupiter, FL, USA). Each measurement included 64 scans at a resolution of 4 cm⁻¹ and absorbance range of 1000 to 4000 cm⁻¹. Data collection was facilitated using Resolutions Pro software (Agilent Technologies, Santa Clara, CA, USA).

A background measurement of air was recorded immediately before sample measurement. The spectrum of water vapour was measured and later subtracted from each sample spectra. A sample volume of 15 µL was placed in the centre of one CaF₂ disc and covered with the second CaF₂ disc. The two discs were aligned in the same way for each measurement. Standards of RBO, SM, water and emulsion were measured to confirm the presence and wavenumber of peaks associated with each component. The emulsion was then diluted to 20%, 10%, 5% and 1% w/w in distilled water and measured using FTIR to determine the limit of detection of the emulsion components over the range of 1000 cm⁻¹ and 4000 cm⁻¹. FTIR spectra for each of the expectorated samples in the emulsion group (at time 0, 5 and 10 minutes) were then acquired and examined for the presence of emulsion components peaks. Water vapour was subtracted from each spectra using Origin Pro 8.5.1 (OriginLab Corporation, Northampton, MA, USA).
4.3.3.5 Gravimetric analysis of emulsions following oral rinsing

A preliminary investigation was conducted to determine whether the tested emulsion could be separated by ultracentrifugation (Beckman Coulter Incorporated, Palo Alto, CA., USA) at 197500 \( x \) g for 60 minutes, based on a previous study (Jacobsen et al., 1998). This failed to separate the aqueous phase of the emulsion. Instead, centrifugation with a 0.45 \( \mu \)m filter to separate the aqueous phase, was used (Hong et al., 2003). The liquid expectorated in the emulsion group from each participant at each time (0, 5 and 10 minutes) was transferred to a 0.45 \( \mu \)m filter separating Microsep centrifugal device (Pall Corporation, Ann Arbor, MI, USA). The mass of the filter before and after the liquid was added was recorded. Samples were then centrifuged for ten minutes at 3220 \( x \) g and a temperature of 4°C to separate any excess water from the emulsion. The filter was then re-weighed to determine the mass of emulsion collected on the filter.

4.3.3.6 Statistical analysis

A multivariate analysis of variance (MANOVA) was used to model basal salivary flow, volume of liquid expectorated, percentage of liquid retained and the participant assessment form responses (perceived taste, intensity and thickness) as a function of formulation (emulsion, polymer or water) immediately following rinsing \( (t = 0) \). The difference in perceived taste, intensity and thickness between 0 and five minutes after initial rinsing were also analysed. The percentage of liquid retained was log transformed in order to achieve sufficient normality and homogeneity in each formulation group. Post-hoc tests were conducted to determine the adjusted predicted values of the dependent variables for each formulation. Average marginal effects between the emulsion group and the control groups (polymer and water) were then compared. All analyses were performed using Stata 11.1 (StatCorp, Texas, USA), and statistical significance was set to \( p < 0.05 \).
4.4 Results

4.4.1 Study A: Validation of lithium as a marker of retention in the oral cavity

4.4.1.1 Validation of lithium assay for saliva samples

In the initial validation study, the predicted concentration of lithium was directly proportional to the measured concentration of lithium using the Infinity® reagent. The standard curve had a slope of 1.002 and standard error (SE) of 0.002 ($R^2 > 0.999$) over the range of 0.2 to 1.60 mmol/L (Figure 4.2). Lithium concentrations in the saliva standards were measured using the Cobas C® reagent and results were found to fit on the same standard curve (Figure 4.2). The standard curve of samples spiked with 50 mmol/L potassium had a slope of 1.004 ± SE 0.003 ($R^2 > 0.999$; Figure 4.2, inset). The reliability of the reagent for use in saliva samples was therefore verified using both the Infinity® and Cobas C® reagent within the range of 0.2 to 1.60 mmol/L ($R^2 > 0.999$). The CV was calculated to be 2.9% at 1.00 mmol/L, with a range from 1.0% at 1.5 mmol/L to 5.0% at 0.2 mmol/L.
Figure 4.2 Validation of procedure using standard addition by measuring the lithium concentration (measured [Li⁺]) in saliva samples spiked with known concentrations of lithium (predicted [Li⁺]). The standard curve was established using the Infinity® reagent (filled symbols) and the validity of the standard curve for the Cobas C® reagent was later determined (empty symbols). Potassium was included in samples at a concentration of 50 mmol/L to exclude the possibility of interference from potassium present in saliva (inset). Data is presented as mean (± SD), n = 3.

4.4.1.2 Validation procedure for determining retention of liquids in the oral cavity

The recovery of lithium from 1.00 mmol/L standard solutions with a known volume of saliva were calculated to be 101 ± 3.0% from the water solution and 97 ± 3.4% from the CMC solution, based on three replicates. The volume of liquid expectorated and lithium concentration in each group is summarised in Table 4.1. No significant correlation in the volume of liquid retained within the oral cavity was observed when participants were categorised according to gender or age (p > 0.05). The concentration of lithium expectorated was 0.91 ± 0.03 mmol/L for water and 0.81 ± 0.04 mmol/L for 1% w/v CMC. The percentage of liquid retained for each formulation was calculated
using Equation 4.1. A higher proportion of 1% w/v CMC was retained (15.3 ± 4.1%) compared to water (10.4 ± 4.7%), and the difference of 4.9% (95% CI: 2.4%, 7.6%) was statistically significant (p < 0.01). It should be noted that four out of the twenty participants retained more water than CMC (Figure 4.3), however, one such participant retained 22.5% water, (over 8% more than the next highest water retainer) and another such participant retained 5.4% CMC (5% less than the next lowest oil retainer) and these could be potential outliers.

Table 4.1 Volume of liquid expectorated (V$_{exp}$), measured lithium concentration in expectorated sample (C$_{exp}$) and calculated percentage of liquid retained following 30 seconds oral rinsing with mouth rinse A or B. Data is presented as mean (± SD) and separated into gender as well as age (< 30 or ≥ 30), where number of subjects = n.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age</th>
<th>n</th>
<th>V$_{exp}$ (mL)</th>
<th>C$_{exp}$ (mmol/L)</th>
<th>liquid retained (%)</th>
<th>V$_{exp}$ (mL)</th>
<th>C$_{exp}$ (mmol/L)</th>
<th>liquid retained (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>&lt; 30</td>
<td>5</td>
<td>10.2 (1.0)</td>
<td>0.92 (0.05)</td>
<td>7.4 (5.9)</td>
<td>9.7 (0.6)</td>
<td>0.82 (0.03)</td>
<td>15.5 (3.0)</td>
</tr>
<tr>
<td></td>
<td>≥ 30</td>
<td>5</td>
<td>10.0 (0.2)</td>
<td>0.91 (0.03)</td>
<td>10.2 (2.5)</td>
<td>10.0 (0.4)</td>
<td>0.81 (0.04)</td>
<td>14.0 (3.9)</td>
</tr>
<tr>
<td>Male</td>
<td>&lt; 30</td>
<td>5</td>
<td>9.6 (0.3)</td>
<td>0.93 (0.02)</td>
<td>12.3 (1.2)</td>
<td>9.5 (0.5)</td>
<td>0.85 (0.03)</td>
<td>13.7 (5.6)</td>
</tr>
<tr>
<td></td>
<td>≥ 30</td>
<td>5</td>
<td>10.1 (0.8)</td>
<td>0.89 (0.03)</td>
<td>11.5 (6.6)</td>
<td>10.1 (0.6)</td>
<td>0.77 (0.04)</td>
<td>18.1 (3.5)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>20</td>
<td>10.0 (0.7)</td>
<td>0.91 (0.03)</td>
<td>10.4 (4.7)</td>
<td>9.9 (0.5)</td>
<td>0.81 (0.04)</td>
<td>15.3 (4.2)</td>
</tr>
</tbody>
</table>
4.4.2 Study B: An investigation of potential saliva substitutes in the oral cavity

The baseline characteristics of participants in each formulation group are summarised in Table 4.2. The basal salivary flow rate was not significantly different in any of the three formulation groups (p > 0.05).

Table 4.2 Baseline characteristics of participants (n) assigned to each formulation (emulsion, polymer and water). Basal salivary flow rate is presented as mean (± SD).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>n</th>
<th>Median age</th>
<th>Basal salivary flow rate (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emulsion</td>
<td>10</td>
<td>27</td>
<td>0.56 (0.25)</td>
</tr>
<tr>
<td>Polymer</td>
<td>10</td>
<td>25.5</td>
<td>0.46 (0.20)</td>
</tr>
<tr>
<td>Water</td>
<td>10</td>
<td>29</td>
<td>0.51 (0.37)</td>
</tr>
<tr>
<td>Overall</td>
<td>30</td>
<td>27</td>
<td>0.51 (0.27)</td>
</tr>
</tbody>
</table>

Figure 4.3 Distribution of liquid retained (%) in the oral cavity for water compared to 1% w/v CMC suspension for each participant (n=20).
4.4.2.1 Perceived taste, thickness and intensity of formulations

Although there were ten participants in each formulation group, not all of them could feel the formulation in their mouth after five or ten minutes. Perceived taste, intensity and thickness only applied if the formulation could be felt; therefore, the number of observations changed for each formulation depending on whether the participants could feel the formulation after five and ten minutes, as demonstrated in Table 4.3. A summary of perceived taste, intensity and thickness of each formulation is given in Figure 4.4. Statistical significance between formulations was only determined at time 0, and the difference between taste, intensity and thickness at time 0 and 5 minutes. This was because the number of participants who could still feel the formulation after ten minutes was small (n = 5 for the emulsion group; n = 3 for the polymer and water groups, respectively). The error bars indicate a large degree of variability in responses between participants in each formulation group. At time 0, the emulsion group ranked lower with respect to taste (31.0 ± 15.7%) compared to the polymer group (48.7 ± 19.2%) or water group (44.5 ± 16.9%); however, there was no significant difference in taste between the three groups (p > 0.05). At time 0, thickness was significantly higher in the emulsion group (66.9 ± 29.8%) compared to both the polymer group (23.3 ± 14.9%, p < 0.001) and the water group (19.5 ± 9.6%, p < 0.001). The difference in perceived thickness between the polymer and water group was not significant (p = 0.68). Further, the reduction in perceived thickness from 0 to 5 minutes was 66 ± 17.1% in the emulsion group, which was a significant reduction compared to that of the polymer group (17.7 ± 10.9%) and water group (14.25 ± 10.2%) in the water group (p < 0.001; Figure 4.5). No significant differences in perceived intensity of the formulations were observed immediately following rinsing and although a larger reduction in perceived intensity from 0 to 5 minutes was observed in the emulsion group (31.8 ± 27.4%) compared to the polymer (18.5 ± 12.5%) or water (11.0 ± 13.6%), these differences were not significant (p = 0.26 and 0.06, respectively).

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>n (emulsion)</th>
<th>n (polymer)</th>
<th>n (water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 4.3 Number of participants (n) in each formulation group out of a possible 10 who reported that they could still feel the formulation in their mouth after 0, 5 and 10 minutes.
Figure 4.4 Summary of participant responses with respect to perceived a) taste, b) intensity and c) thickness after rinsing with emulsion (■), polymer (■) or water (□) after 0, 5 and 10 minutes. Anchor points from the questionnaire are labelled on the y-axis. The * indicates p < 0.05 compared to the other formulation groups at time 0. Data is presented as mean ± (SD).
4.4.2.2 Retention of the aqueous component of formulations

The initial lithium concentration of both the emulsion and polymer formulation was 1.02 mmol/L. This concentration was 2% higher than the target concentration of 1.00 mmol/L, but within the calculated CV of 2.9% (Section 4.4.1.1) for a 1.00 mmol/L lithium solution. Therefore, the presence of emulsion or polymer in the formulation did not appear to affect the measurement of lithium. The initial lithium concentration of the water solution was 1.05 mmol/L, which was 5% higher than the 1.00 mmol/L target concentration and greater than the 2.9% CV, most likely due to discrepancies in the preparation of the formulation. This was corrected for in Equation 4.1, since initial lithium concentration \( C_i \) represents the initial measured lithium concentration in the formulation. Following the filtration step, the recovery of 1.00 mmol/L lithium from three replicate standards of each solution mixed with saliva were calculated to be 101 ± 4.4% from the water solution, 98 ± 4.6% from the polymer solution and 98 ± 2.6% from the emulsion.

Immediately following rinsing with the formulations \((t = 0)\), lithium concentration in the expectorant was lowest in the emulsion group and highest in the water group. This corresponded with retention of 18.39 ± 4.89% in the emulsion group compared to 13.82 ± 7.64% in the polymer group and 10.05 ± 2.54 in the water group (Table 4.4). The difference in mean retention of the emulsion group compared to the water group
(8.34 ± SE 2.71%) was statistically significant (p = 0.003), but the difference in the emulsion group compared to the polymer group (4.57 ± SE 2.71%) was not (p = 0.06). No significant difference was observed in the polymer group compared to the water (p = 0.26). The distribution of liquid retained within each treatment group is shown in Figure 4.6. The largest variation and highest overall retention was observed in the polymer group, with values of over 26% observed in two participants. The lithium concentration in three of the emulsion participants was too viscous to measure using the lithium assay; therefore, the retention was only established in seven participants, instead of ten as in the polymer and water groups.

Table 4.4 Summary of formulations before and immediately after rinsing (time 0), n = number of subjects. \(C_i\) represents the lithium concentration in the initial formulation and \(C_{exp}\) represents the lithium concentration in the expectorated sample. These values, along with the measured final volume \((V_{exp})\) and 10 mL initial volume were used to determine the portion of liquid retained. Data is presented as mean (± SD).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>n</th>
<th>(C_i) (mmol/L)</th>
<th>(V_{exp}) (mL)</th>
<th>(C_{exp}) (mmol/L)</th>
<th>Liquid retained (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emulsion</td>
<td>7</td>
<td>1.02</td>
<td>10.13 (0.86)</td>
<td>0.79 (0.07)</td>
<td>18.4 (4.9)</td>
</tr>
<tr>
<td>Polymer</td>
<td>10</td>
<td>1.02</td>
<td>10.02 (0.71)</td>
<td>0.87 (0.05)</td>
<td>13.8 (7.6)</td>
</tr>
<tr>
<td>Water</td>
<td>10</td>
<td>1.05</td>
<td>10.40 (0.87)</td>
<td>0.91 (0.07)</td>
<td>10.1 (2.5)</td>
</tr>
</tbody>
</table>

After five and ten minutes, the lithium concentration in many expectorated samples were below the 0.05 mmol/L limit of detection. Interestingly, the number of expectorated samples with a detectable amount of lithium (≥ 0.05 mmol/L) after five and ten minutes was highest in the water formulation (four participants with \([\text{Li}^+]\) of 0.06 ± 0.005 mmol/L at five minutes and three participants with \([\text{Li}^+]\) of 0.06 ± 0.01 mmol/L at ten minutes) (Table 4.5).
Figure 4.6 Distribution of liquid retained (%) in the oral cavity for the emulsion, polymer and water groups. Three single data points (•) were identified as potential outliers but were included in the statistical analysis due to the small sample size (n=7 for the emulsion group and n=10 for the polymer and water groups).

Table 4.5 Summary of volume ($V_{exp}$) and lithium concentration ($C_{exp}$) of liquid expectorated after 5 and 10 minutes of oral rinsing, where n (total) = the total number of subjects. As the limit of detection of the lithium assay (LD) was 0.05 mmol/L, samples from subjects (n (LD)) with a measured $C_{exp}$ greater than 0.04 mmol/L were identified and an adjusted mean $C_{exp}$, $C_{exp}$ (adjusted), was determined using only these values. Data is presented as mean (± SD).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Formulation</th>
<th>n (total)</th>
<th>$V_{exp}$ (mL)</th>
<th>$C_{exp}$ (mmol/L)</th>
<th>n (LD)</th>
<th>$C_{exp}$ (adjusted) (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Emulsion</td>
<td>10</td>
<td>10.24 (0.82)</td>
<td>0.02 (0.02)</td>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td>5</td>
<td>Polymer</td>
<td>10</td>
<td>9.99 (0.63)</td>
<td>0.04 (0.02)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Water</td>
<td>10</td>
<td>9.88 (0.57)</td>
<td>0.02 (0.02)</td>
<td>4</td>
<td>0.06 (0.005)</td>
</tr>
<tr>
<td>10</td>
<td>Emulsion</td>
<td>10</td>
<td>10.08 (0.63)</td>
<td>0.02 (0.01)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Polymer</td>
<td>10</td>
<td>10.21 (0.62)</td>
<td>0.04 (0.02)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Water</td>
<td>10</td>
<td>9.92 (0.84)</td>
<td>0.02 (0.02)</td>
<td>3</td>
<td>0.06 (0.01)</td>
</tr>
</tbody>
</table>
4.4.2.3 FTIR spectroscopy of emulsions following oral rinsing

The spectra for 100% RBO, SM and water are shown in Figure 4.7. The observed peaks in each of these samples were expected to be visible in the emulsion samples, which were made up of these three components. Indeed, RBO and SM peaks at approximately 2926 cm\(^{-1}\), 2855 cm\(^{-1}\) and 1746 cm\(^{-1}\) were visible in the emulsion (Figure 4.8b). The presence of emulsion peaks, indicating RBO and SM in the diluted emulsion samples, are summarised in Table 4.6. Peaks were visible when the emulsion was diluted to 5% w/w in distilled water (Figure 4.8c-e), but at 1% w/w the peak at 1746 cm\(^{-1}\) was no longer visible (Figure 4.8f). FTIR spectroscopy was used in the clinical study to detect the presence of emulsion, indicated by peaks at approximately 2926 cm\(^{-1}\), 2855 cm\(^{-1}\) and 1746 cm\(^{-1}\), in expectorated samples. Since this method was able to detect RBO and SM in emulsions diluted to 5% w/w, and each emulsion consisted of 20% w/w RBO, a limit of detection of 1% RBO was established.
Figure 4.7 FTIR spectra for a) RBO, b) SM and c) water that make up the emulsion, displaying –CH₂ stretching at between 2855 and 2926 cm⁻¹ and -C=O stretching at approximately 1745 cm⁻¹, -OH stretching at approximately 3400 cm⁻¹ and -OH bending at approximately 1643 cm⁻¹.
Figure 4.8 FTIR spectra showing a) emulsion peaks at 2926 cm$^{-1}$, 2855 cm$^{-1}$ and 1746 cm$^{-1}$ compared with b) an emulsion consisting of 20% RBO, 40% SM and 40% water (w/w) and the effect of diluting this in distilled water to c) 20% emulsion, d) 10% emulsion, e) 5% emulsion and f) 1% emulsion. The water spectra is given in g) for reference.
Table 4.6  Presence (✓) or absence (-) of emulsion peaks of 100% emulsion, 20% emulsion, 10% emulsion, 5% emulsion and 1% emulsion in distilled water.

<table>
<thead>
<tr>
<th>Emulsion content (%)</th>
<th>-CH\textsubscript{2} stretch</th>
<th>-C=O stretch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2924-2926 cm\textsuperscript{-1}</td>
<td>2853-2855 cm\textsuperscript{-1}</td>
</tr>
<tr>
<td>100</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>20</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>10</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>5</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>1</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

Peaks were detected in the expectorated emulsion rinse at time 0 for all participants. After five minutes, peaks were only detected for three participants at 2926 cm\textsuperscript{-1}, suggesting that insufficient oil was expectorated after 30 seconds oral rinsing with water (Table 4.7). The FTIR spectra of expectorated samples from one representative participant after each time interval is displayed in Figure 4.9. The peak identified at five minutes is considerably smaller than that identified at immediately following rinsing.

Table 4.7  The presence (✓) or absence (-) of FTIR spectral peaks at 1740-1746, 2855 and 2926 cm\textsuperscript{-1} in the expectorated mouth rinses of each of the ten participants (n).

<table>
<thead>
<tr>
<th>n</th>
<th>CH\textsubscript{2} stretch</th>
<th>CH\textsubscript{2} stretch</th>
<th>C=O stretch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2926 cm\textsuperscript{-1}</td>
<td>2855 cm\textsuperscript{-1}</td>
<td>1740-1746 cm\textsuperscript{-1}</td>
</tr>
<tr>
<td>0 min</td>
<td>5 min</td>
<td>0 min</td>
<td>5 min</td>
</tr>
<tr>
<td>1</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
</tr>
<tr>
<td>2</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
</tr>
<tr>
<td>3</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>4</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>5</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
</tr>
<tr>
<td>6</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>7</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
</tr>
<tr>
<td>8</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
</tr>
<tr>
<td>9</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
</tr>
<tr>
<td>10</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
</tr>
</tbody>
</table>
Figure 4.9 FTIR spectra of expectorated samples from one representative participant at a) 0 minutes, b) 5 minutes and c) 10 minutes after oral rinsing with an emulsion. Peaks detected that indicate the presence of RBO are marked *.

4.4.2.4 Gravimetric analysis of emulsions following oral rinsing

The portion of expectorated sample unable to pass through the 0.45 μm filter, summarised as a percentage of sample originally in the filter since this was different for each sample, is shown in Table 4.8. Although large variability existed between
subjects at each time interval, a clear difference existed in the portion of sample unable to pass through the filter at each time interval, with $87.9 \pm 4.8\%$ at 0 minutes (following oral rinsing with the emulsion), compared to $14.0 \pm 8.8\%$ and $6.14 \pm 1.44\%$ following oral rinsing with water after five and ten minutes, respectively.

Table 4.8  The amount of expectorated emulsion sample remaining in the centrifugal device filter (% remaining) for each subject as well as overall mean ($\pm$ SD) immediately after rinsing with the emulsion (time 0), and after five and ten minutes.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Subject</th>
<th>0</th>
<th>5</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>80.5</td>
<td>20.0</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>89.1</td>
<td>8.7</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>87.4</td>
<td>7.1</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>93.4</td>
<td>10.1</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>90.6</td>
<td>24.3</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>87.0</td>
<td>32.7</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>83.2</td>
<td>9.3</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>82.6</td>
<td>6.5</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>89.6</td>
<td>7.9</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>90.3</td>
<td>13.9</td>
<td>3.6</td>
</tr>
<tr>
<td>Mean ($\pm$SD)</td>
<td></td>
<td>87.9 (4.8)</td>
<td>14.0 (8.8)</td>
<td>6.14 (1.44)</td>
</tr>
</tbody>
</table>

4.5  Discussion

The present study demonstrated that a standard laboratory assay was able to determine the concentration of lithium in saliva samples within the range of 0.05 to 1.60 mmol/L ($R^2 > 0.999$). After 30 seconds of oral rinsing with a lithium-tagged mouth rinse, the concentration of lithium in the expectorant was successfully determined and the amount remaining in the oral cavity was estimated. After 30 seconds of oral rinsing with a simple aqueous solution, $10.4 \pm 4.7\%$ was retained in the oral cavity. In addition, a relationship between increasing viscosity and a higher retention was confirmed, with $15.3 \pm 4.2\%$ of 1% w/v CMC suspension being retained following 30 seconds of oral rinsing. This corresponded with the expectation that the increased viscosity of the
CMC suspension would enhance retention. No difference was observed with respect to gender or age within the individuals tested.

The use of a water-soluble compound such as lithium ions as a marker for retention in the oral cavity required several assumptions. First, all liquid not expectorated was assumed to remain in the oral cavity. Lithium is a small monovalent ion (molecular weight 6.94 g/mol) and when ingested orally is absorbed from the gastrointestinal tract via sodium channels (Schrauzer, 2002). As such, it is plausible that a small amount of lithium may diffuse from the medium and be absorbed via sodium channels within the oral cavity. Although the rinsing period was only 30 seconds, it may result in an over-estimation of the volume of liquid retained in the oral cavity. Further, if lithium is absorbed when investigating the retention of formulations over extended time periods then the volume of formulation remaining in the oral cavity after five and ten minutes may be under-estimated. Many studies that involve the collection of saliva assume that all secreted saliva is expectorated during the collection period (Navazesh & Christensen, 1982; Lagerlöf & Dawes, 1984; Anderson et al., 2001; Beltzer et al., 2010). However, Dawes (2006) found that in a population of experienced saliva collectors, 30% inadvertently swallowed some of their saliva. This has implications in the present study; if participants inadvertently swallowed any of the formulation during the 30-second swilling period, the volume of liquid expectorated would be lower, resulting in an over-estimation of that remaining in the oral cavity. While the calculated percentage of liquid remaining in the oral cavity is an estimation of retention, these values can be used to compare differences in retention between different formulations. Recently, an ex vivo retention model has been developed to simulate retention in the oral cavity using porcine buccal mucosa (Madsen et al., 2013). The application of this method to the formulations tested in the present study may offer an extended understanding of their retention in the oral cavity, particularly over longer time periods.

Following 30 seconds of oral rinsing, aqueous retention of the initial formulation was highest in the emulsion group, indicating that the emulsion was retained to a greater extent than the water or polymer suspension. Although only the difference between the emulsion and water group was significant in the present study, the sample size tested was small (n=10 in each group) and between-subject variability high; therefore, it
would be worthwhile repeating this study using a larger sample size. Interestingly, the largest variability was observed in the polymer group, with estimated retention ranging from 7.5% up to 26.7% immediately after rinsing. Unfortunately, three of the expectorated samples in the emulsion group were too viscous to be drawn into the sampling probe. Therefore, lithium concentration could not be determined in these samples and retention data was only obtained from seven participants in this group. The mean basal salivary flow rate in the three participants who produced the viscous expectorated samples was 0.38 ± 0.05 mL/min, compared to 0.64 ± 0.26 mL/min in the participants where lithium concentration could be measured in their expectorant. The more viscous samples may be the result of simple dilution, whereby the expectorated samples of participants with a higher basal salivary flow rate are more dilute and therefore, less viscous, compared to participants with a lower basal salivary flow rate. However, this would not be expected to influence the viscosity of expectorated sample able to pass through the 0.45 µm filter. Previous research has demonstrated that when emulsions mix with saliva, the emulsion may become destabilised whereby creaming, flocculation or coalescence may alter the spatial distribution of the droplets (Kilcast & Clegg, 2002; Dresselhuis et al., 2008b; Silletti et al., 2008; Vingerhoeds et al., 2009; McClements, 2010; van Aken et al., 2011). The 0.45 µm filter was used to extract the lithium-containing aqueous phase from the bulk of the emulsion. It is possible that in these three participants, the lower amount of saliva present altered the composition of the emulsion so that more was able to pass through the filter, resulting in a more viscous filtrate. This has important implications for future research since the emulsion is targeted for clinical use in individuals with salivary hypofunction. Further research into the separation of a sufficiently aqueous filtrate to determine lithium concentration in individuals with minimal salivary gland secretion is required, to improve the measure of emulsion retention.

The data obtained from the questionnaires provided information about perceived taste, intensity and thickness. Responses were subjective; with participants marking the point on the scale that they thought best described the formulation, with no reference formulation for comparison. This is likely to explain the large variability that was observed between participants, who were untrained in sensory perception. However, in a clinical environment it is the arbitrary mouth-feel and taste perception that influences patient acceptability, therefore, large variability can be expected. Taste in particular is
an important consideration in patient acceptability (Momm et al., 2005). Individuals with salivary hypofunction often have altered taste sensitivity (Temmel et al., 2005). While the present study indicates that further development of flavour is required, this ought to be based on preferences of individuals with xerostomia (de Almeida et al., 2008). After ten minutes, 50% of participants in the emulsion group were still able to feel the emulsion in their mouth, compared to 30% in the polymer and water groups. This indicates that the emulsion may be retained in the mouth for longer than the two control groups. However, this finding was not reflected in the measurement of lithium expectorated, which at ten minutes was detectable only in 30% of the samples expectorated from the water group and in no samples expectorated from the emulsion or polymer groups. Interestingly, the three participants in the water group who reported that they could feel the formulation in their mouths after ten minutes were also the three subjects in whom lithium concentration was measurable at ten minutes. It is possible that the emulsion was indeed retained in the oral cavity of the participants who reported they could feel it after ten minutes, but that the process of rinsing with 10 mL water was insufficient to facilitate its removal from the oral cavity. Lithium was a measure of aqueous retention and was not reflective of the retention of oil. It is also possible that the presence of oil in the oral cavity was responsible for the overall sensation of retention, whereas the aqueous phase (along with lithium ions) may have been cleared. Rinsing with 10 mL water may have also diluted the remaining formulation such that the lithium concentration was below the 0.05 mmol/L limit of detection. This would explain the inability to detect lithium in the expectorant.

The absorption peaks of RBO obtained by FTIR spectroscopy in the present study corresponded with those observed previously (Che Man et al., 2011). Emulsion peaks were detectable to an emulsion concentration of 5% w/w. As the initial emulsion consisted of 20% w/w RBO, the detectable limit of RBO was considered to be 1% w/w. FTIR spectra of emulsion samples demonstrated absorption peaks in the sample expectorated immediately after 30 seconds of rinsing. This was expected since the emulsion consisted of 20% w/w RBO and was swirled around the mouth for 30 seconds before being expectorated directly into a collection cup. Hence, mouth rinses were diluted only with saliva produced over the 30-second rinsing period. After five minutes, small peaks were detectable in only 30% of the samples, despite 50% of participants in the emulsion group reporting they could still feel the formulation in their
moutches. This may be related to the inability of water to remove the emulsion from the oral cavity, or it may be due to the 10 mL water resulting in the initial formulation being diluted to below the calculated 1% level of detection for RBO. Assuming the aqueous retention reflects the amount of oil retained and no separation of the emulsion occurs, the expected oil concentration remaining in the mouth immediately after rinsing can be calculated by multiplying the concentration of RBO in the initial mouth rinse (20%, or 0.2 g/mL) by the quantity of emulsion retained, (18.39%, or 1.839 mL for a 10 mL sample), resulting in a value of 0.368 g RBO. Even if no clearance from the oral cavity took place after five minutes, dilution after swirling 10 mL of water would result in a RBO concentration of 3.68% w/v. The secretion of saliva would further dilute RBO concentration, but this was also swallowed throughout the five-minute interval.

FTIR spectroscopy was an effective tool for qualitatively determining the presence of oil in expectorated samples, but future research may benefit from obtaining swabs directly from the wall of the oral mucosa so that FTIR measurements are conducted on undiluted samples. As FTIR analysis requires only a 15 µL sample, it may be possible to determine the presence of oil using this method. In addition, further investigation into the relationship between the area of the oil peak in relation to oil content under strictly controlled conditions may allow for a more quantitative approach.

Simple gravimetric analysis was used as another potential measure of RBO retained following oral rinsing. This has been done successfully in previous studies by weighing the sample and subsequently evaporating the aqueous phase, using acetone to increase the solubility of surfactants into water and accelerate demulsification, then weighing the remaining oil (Hong et al., 2003). However, this was not possible in the present study as the aqueous phase was required for analysis of lithium concentration. Instead, each sample from the emulsion group was weighed before and after centrifugation using a 0.45 µm filter. Water easily passes through such a filter, but the stability of the emulsion means that some water is likely to remain in the filter. Therefore, the measured portion retained in the filter can be used to infer the presence of emulsion, oil and any other contaminants in the sample. More expectorated sample remained in the filter immediately following rinsing with the emulsion compared to five minutes after initial rinsing. This was expected since the initial expectorated sample consisted of the emulsion plus saliva, whereas the sample expectorated after ten
minutes consisted of residual emulsion, saliva and 10 mL water. The amount of sample remaining in the filter was lowest after ten minutes.

The emulsion investigated in this clinical trial, consisting of 20% w/w RBO, 40% w/w SM and 40% w/w water, was selected based on the findings from Chapter 3. This composition was in the frequency-dependent region, with viscous properties dominating at low frequencies and elastic properties dominating at higher frequencies. In addition, viscoelasticity remained with increasing water content. Participants in this study had normal basal salivary flow rates and therefore, the formulations would be expected to mix with saliva secreted during the 30-second rinsing period. While viscoelasticity of the emulsion was expected to remain during this process, it was not tested in the present study. The shear rate in the oral cavity plays a role in emulsion destabilisation (Dresselhuis et al., 2008a). Further, saliva has the ability to destabilise emulsions by flocculation caused by numerous mechanisms such as van der Waals’ forces and depletion or electrostatic interactions between salivary proteins and emulsion droplets (Silletti et al., 2007b, 2007a; Sarkar et al., 2009). This will affect the retention of emulsions in the oral cavity, with saliva facilitating the efficient removal of emulsion droplets from oral surfaces (Dresselhuis et al., 2008b). The retention of these formulations is hypothesised to differ in the absence of saliva; therefore, further research in individuals with salivary hypofunction is required to gauge efficacy within the target population. This will help determine the extent to which the retention of a saliva substitute in a healthy oral mucosa translates to that of an unhealthy mucosa.

In this chapter, the use of lithium as a marker for retention of fluids in the oral cavity was validated after 30 seconds of rinsing. This method showed that initial retention in the oral cavity of the emulsion consisting of a weight ratio of 20% oil, 40% SM and 40% water was higher than for 1% w/v methylcellulose or water. In the emulsion group, neither lithium concentration nor oil in the expectorant were measurable after five minutes, despite 50% of participants claiming they could still feel the formulation in their mouth. This may be because rinsing with 10 mL water was insufficient to remove residual formulation from the mucosal surfaces. It is more likely, however, to be explained by the dilution of water combined with secretion of saliva resulting in expectorated samples that had oil and lithium concentrations that were below the limit of detection.
4.6 Conclusions

The emulsion developed in this thesis, consisting of 20% w/w RBO, 40% w/w SM and 40% w/w water, showed potential as a saliva substitute, with superior retention immediately following rinsing compared to a 1% w/v methylcellulose suspension and water. Lithium was validated as a suitable marker for determining retention of the aqueous component of a saliva substitute, but further research into methods of determining retention over extended time periods are required. FTIR spectroscopy was effective at determining the presence of the emulsion in expectorated samples immediately after rinsing and investigations into the use of this method on samples swabbed directly from the oral cavity may allow this method to be applied over longer time periods. Further modification of the formulation, with the inclusion of a bioadhesive agent, may be beneficial in improving retention for extended time periods. The emulsion rated lower in taste acceptability compared to the water or methylcellulose suspension, indicating that the taste profile of the emulsion requires further investigation. In addition, while the present study successfully validated a protocol for investigating the efficacy of formulations in the oral cavity, further clinical studies in participants with salivary hypofunction are pertinent.
Chapter five

General discussion and future directions
5 General discussion and future directions

Oily formulations consisting of lecithin, RBO and water have shown exciting potential as saliva substitutes, both in vitro and in an in vivo pilot study on individuals with normal salivary function. Despite the severe impact that xerostomia can have on the quality of life of sufferers, current saliva substitutes fail to provide adequate long-term relief, particularly in patients with little or no salivary gland function (Sweeney et al., 1997; Ferguson, 2002; Frost, 2008). The fundamental issue in developing an effective substitute is that, in the absence of continual secretion, a saliva substitute must be retentive in the oral cavity. The present research contributed to addressing this issue in several ways. Firstly, oily systems were developed with the aim of improving lubrication and retention in the mouth. Secondly, the rheological properties of these systems were assessed in vitro under simulated oral conditions, as these properties were hypothesised to be important indicators of efficacy. Thirdly, these parameters were combined in an in vivo pilot study to determine retention and taste acceptability of a selected emulsion compared to current treatment options for xerostomia. Finally, a number of analytical techniques were developed, such as the use of lithium as a marker for retention in the oral cavity as well as the use of FTIR spectrometry to determine the presence of oil in expectorated samples. The present research is multidisciplinary and results are highly relevant to the fields of both pharmacy and dentistry.

Different compositions were characterised using pseudo-ternary phase diagrams, polarised light microscopy (Chapter 2), flow and oscillatory rheology, laser diffraction and SALS (Chapter 3) techniques. In doing this, several compositions, in particular formulations number 24, 31 and 31 (Table 5.1) from the phase diagram in Figure 1.1, were highlighted as worthy of further investigation.

Table 5.1 Compositions of RBO, SM (lecithin and PG 1:1 w/w) and water identified in this thesis as showing potential as saliva substitutes.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>RBO (% w/w)</th>
<th>SM (% w/w)</th>
<th>Water (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>20</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>31</td>
<td>20</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>39</td>
<td>20</td>
<td>20</td>
<td>60</td>
</tr>
</tbody>
</table>
All of these compositions were o/w emulsions that contained 20% w/w RBO. Formulation 24 appeared semi solid-like, whereas formulations 31 and 39 were liquid-like. The droplet distribution for both formulation 24 and 31 were trimodal, with similar peak volume diameters (0.39 µm, 2.98 µm and 22.80 µm for formulation 24 and of 0.39 µm, 3.41 µm and 19.90 µm for formulation 31). This follows since the same amount of RBO, which was the dispersed phase, was present in both compositions. SALS showed that peak intensity occurred at a larger angle for formulation 24 compared to formulation 31, indicating that the droplet size of formulation 31 was larger than 24, which corresponded with the D_{50} values of 11.18 ± 0.08 µm and 10.50 ± 0.09 µm for formulations 31 and 24, respectively. The droplet distribution for formulation 39 was not determined.

Oscillatory rheology showed that a negative peak in G’ and corresponding positive peak in G” and tan δ were observed at 3.40 Hz for formulation 24, whereas no peaks were observed in formulation 31 or 39. The relationship between oscillatory frequency and tan δ was similar between natural saliva (Chapter 2) and formulations 31 and 39 (Chapter 3). In natural saliva, the reason for this is thought to be functional, with viscous behaviour at low frequencies promoting lubrication of the oral cavity at rest and elastic properties at higher frequencies promoting lubrication during higher shear tasks such as speaking and swallowing (Stokes & Davies, 2007). Clearly, these features will also be beneficial in a saliva substitute.

The oscillatory rheology data for formulations 24, 31 and 39 are presented alongside that of natural unstimulated saliva and the HEC saliva substitute in Figure 5.1. The G’ and G” of the emulsion compositions were up to four-fold higher than those observed for natural saliva in the preliminary investigation in Chapter 2, but up to four-fold lower compared to the tested commercial saliva substitute (Figure 5.1a-b). However, the frequency-dependent tan δ values that were observed for formulations 31 and 39 were similar to that observed for natural saliva in that tan δ was less than one at high frequencies only (Figure 5.1c). The tan δ of the commercial saliva substitute (OralBalance®) was less than one, independent of frequency.
Figure 5.1 Comparison of a) $G'$, b) $G''$ and c) $\tan \delta$ of four compositions containing a RBO:SM:water weight ratio of 2:4:4 (●), 2:3:5 (□) and 2:2:6 (■) with natural saliva (×) and a commercial saliva substitute (Δ). Data is presented as mean (± SD), n = 3.
Formulation 24 was selected for the clinical pilot study in 24 for several reasons. The organogel-like structure was hypothesised to offer prolonged hydration in the oral cavity, based on previous research investigating lecithin organogels on the skin surface (Scartazzini & Luisi, 1988; Kumar & Katare, 2005). In addition, as this composition was diluted in the oral cavity by natural saliva and the consumption of liquids, the viscoelasticity of the system was expected to decrease. Based on the pseudo-ternary phase diagram (Figure 3.6), it was hypothesised that viscoelasticity would become more similar to that observed in formulations 31 and 39, thus, tan δ would be similar to natural saliva. Results from the clinical investigation were promising, showing that in individuals with normal salivary function, the initial retention in the oral cavity of the emulsion (formulation 24) was higher than for both water and 1% w/v methylcellulose. This indicates that the emulsion may be superior at coating oral surfaces and lubricating the mouth after rinsing. In addition, more participants reported that they could feel the emulsion in their mouths after five minutes compared to the water or 1% w/v methylcellulose, which suggests that the emulsion may also be retained in the oral cavity for longer. It would be worthwhile in future research to test formulation 31 and maybe even formulation 39, and investigate mouth-feel and retention over longer time periods. These findings also support previous studies that have found lubricating, oil-based systems to be superior over aqueous saliva substitutes (Mouly et al., 2007a; Mouly et al., 2007b; Ship et al., 2007).

The tested emulsion ranked lower in perceived taste compared to the methylcellulose or water mouth rinses. Flavour has not yet been considered beyond the general taste acceptability of the oil and surfactant used. Because saliva is an important factor in taste perception, it follows that taste is impaired in individuals with salivary hypofunction (Matsuo et al., 1997; Mese & Matsuo, 2007). Therefore, flavour and taste acceptability studies should be conducted on individuals with xerostomia, with the goal of achieving a flavour that is considered relatively neutral in this target population.

It is hypothesised that the retention of liquids in the oral cavity will differ in individuals with xerostomia compared to those with normal salivary function. The next phase in this research would be to test the formulation in individuals with xerostomia. Indeed, a study investigating the efficacy of formulations developed in this thesis in a population of individuals who have xerostomia as a consequence of radiotherapy to the head and
neck region is currently underway. Outcome measures include oral health-related quality of life, the ability to swallow and patient acceptability. Information obtained from this study will provide valuable feedback from individuals with salivary hypofunction. This will allow for further correlations to be made between physicochemical properties and clinical efficacy of these formulations as saliva substitutes, which can be modified to achieve optimal efficacy and patient acceptability.

The behaviour of a variety of different pharmaceutical surfactants was investigated in Chapter 2, using pseudo-ternary phase diagrams and polarised light microscopy. Compositions with a SM of lecithin and PG (1:1 w/w) that contained more than 10% RBO formed turbid emulsions, indicating a droplet size greater than 0.1 µm (Mollet & Grubenmann, 2001). Research has suggested that the larger droplet size of coarse emulsions leads to improved retention in the oral cavity compared to microemulsions, which have droplet sizes under 0.1 µm (Dresselhuis et al., 2008b). Additionally, these turbid systems were able to maintain their viscous, semi-solid state in the presence of up to 50% w/w water to form lecithin organogel-like structures. Lecithin organogels have shown promise in dermal delivery as their lipid-rich environment allows for the maintenance of skin hydration in topical applications (Scartazzini & Luisi, 1988; Kumar & Katare, 2005). Organogels also enhance skin penetration and molecule transport (Kumar & Katare, 2005); it would be of interest to investigate if this also occurs in the oral mucosa and to investigate these RBO/lecithin organogel-like compositions for their potential as drug delivery systems.

As highlighted in Chapter 1, saliva consists primarily of water. It is shear thinning and has a unique viscoelasticity (confirmed in Chapter 2), for which glycoproteins such as mucin are thought to be responsible (Yakubov et al., 2009). While researchers have identified the importance of rheological properties in the efficacy of potential saliva substitutes (Vissink et al., 1984), much of this work has focused on the use of mucin-based substitutes, as these have comparable rheological properties to natural saliva (Park et al., 2007; Yakubov et al., 2009). However, saliva is intrinsically regulated and continually secreted in response to a range of both internal and external factors (Sreebny et al., 1992; Edgar et al., 2004; Melvin et al., 2005; Catalan et al., 2009). Hence, a saliva substitute needs to remain in the oral cavity for extended periods of time. While rheological properties are thought to be important in developing effective
saliva substitutes, retention in the absence of continual secretion is a key consideration and therefore, the rheological profile of an effective substitute can be expected to differ from natural saliva. This might explain why many comparative studies fail to find any significant difference in the efficacy of mucin-based substitutes compared to placebo (Sweeney et al., 1997).

The rheological properties of different RBO, SM (1:1 w/w lecithin and PG) and water compositions were investigated in Chapter 3. The compositions tested were shear thinning, which has been identified as an important feature of both natural saliva and saliva substitutes, allowing for coating and lubrication of the mouth at rest and adequate flow during higher shear rates (Hatton et al., 1987; Preetha & Banerjee, 2005). The apparent viscosity of the emulsions at any shear rate was higher than for natural saliva but lower than for the HEC saliva substitute. The emulsion compositions were tested in a shear rate range from 0 to 200 s\(^{-1}\) using a parallel plate measurement geometry, whereas natural saliva and the HEC saliva substitute were tested in a shear rate range from 1 to 1000 s\(^{-1}\) using a cone and plate measurement geometry. This was because the preliminary investigation on natural saliva was modelled on a previous study by Stokes and Davies (2007), whereas the investigation of emulsions was based on a simulation of the known shear rates in the oral cavity, which range from 0.1 and 1.0 s\(^{-1}\) at rest (Corcoran et al., 2006) and up to 60 and 160 s\(^{-1}\) during swallowing and speaking, respectively (Vissink et al., 1984). In hindsight, using the same parameters in both investigations could have allowed for more robust comparisons to be drawn between natural saliva and the emulsion compositions.

Theoretically, rheological test results should be the same when using different measurement geometries, but in practice, different results are often obtained depending upon the measurement geometry selected (Motamed & Bahia, 2011). In order to determine the rheological properties of the emulsions developed in this thesis, a parallel plate measurement geometry was selected as this technique allowed for more accurate measurements of samples containing a variety of particle sizes (Mezger, 2006). Shear rate varies across a parallel plate measurement geometry, whereas it remains constant with a cone and plate geometry (Mezger, 2006; Mewis & Wagner, 2012). Because shear rates are also expected to vary across the oral cavity, the parallel plate measurement geometry was considered superior in simulating the oral environment.
Similarly, different measurement parameters were used when investigating the oscillatory rheology of emulsions in Chapter 3 and the oscillatory rheology of natural saliva and the HEC saliva substitute in Chapter 2. A parallel plate measurement geometry and oscillatory frequencies ranging from 0.1 to 10 Hz were selected for the emulsions and a cone and plate geometry and oscillatory frequencies ranging from 0.1 to 15 Hz were selected for the preliminary investigation on natural saliva, for the reasons explained above. However, the viscoelastic properties of natural saliva determined in this study were analogous to those described in previous studies using both a cone and plate geometry (Stokes & Davies, 2007) and a parallel plate geometry (Stading et al., 2009). This suggests that the measurement geometry is not an important factor when determining the viscoelasticity of natural saliva, and allows some comparisons to be drawn between the results in Chapter 2 and Chapter 3 of this thesis.

In the preliminary investigation of natural saliva, saliva was collected from one donor only, with large standard deviations that demonstrate the large intra-individual variability of natural saliva. Although these results were similar to those obtained in previous studies, these studies also had small sample sizes of n = 1 (Stokes & Davies, 2007) or n = 2 (Stading et al., 2009). It would be worthwhile conducting a larger study to investigate the magnitude of inter-individual variability in order to confirm if these results are representative of the population as a whole.

The frequency-dependent viscoelastic peaks observed in many of the RBO compositions are likely due to deformation or relaxation of droplets, a phenomenon that has been previously reported for systems with a Newtonian dispersed and continuous phase (Erni et al., 2007; Erni et al., 2011). Although the application of SALS to investigate this structural change was limited by the significant dilution required to avoid multiple scattering, it was successfully applied to investigate different scattering patterns in different compositions. An investigation into alternative methods of defining microstructural changes that occur at these peaks would be beneficial in future work. Modification of the rheometer to incorporate a light microscope, or the use of a high-speed camera to measure droplet deformation as the compositions are exposed to different forces may also provide further insight into how this affects the droplet characteristics. In addition, it would be worthwhile determining the rheological properties of homogenised emulsions with known droplet sizes to investigate whether the viscoelastic peaks are still observed in these compositions. By formulating
emulsions with a range of known droplet sizes, the effect of droplet diameter and the critical diameter at which these viscoelastic peaks are observed may be elucidated.

In the preliminary section of this thesis (Chapter 2), natural saliva was compared with OralBalance®, a commercially available HEC-based saliva substitute. The HEC saliva substitute was selected because it is a commercial product that is available to purchase from most New Zealand pharmacies as well as a variety of online stores. In the clinical study (Chapter 4), the efficacy of an emulsion was compared to two placebos, water and a 1% w/v methylcellulose suspension. Water was selected because it is often the only treatment available to individuals with salivary hypofunction (Epstein & Stevenson-Moore, 1992; Ferguson, 2002). The 1% w/v methylcellulose suspension was selected because it is the only Government-funded saliva substitute currently available in New Zealand (Wilson et al., 2012) and was therefore considered more clinically relevant compared to the HEC saliva substitute. Methylcellulose has been shown to lack elasticity (Marks & Roberts, 1983), which may explain why, in the clinical study, retention in the emulsion group was superior.

Emulsions consist of an oil phase and an aqueous phase, therefore, have potential as delivery systems for both lipid-soluble and water-soluble excipients. The present research has focused on investigating a platform in which other excipients may be added to further imitate saliva, thus improve efficacy and reduce long-term complications of xerostomia, such as dental caries and periodontal disease. Further research is required in order to investigate the bioadhesion and surface properties of the compositions, as these are also thought to reflect retention. In addition, incorporating other proteins, polymers and salts, based on the composition of natural saliva as well as the therapeutic potential of the excipient, may optimise the physicochemical properties of these formulations. An example is the addition of salts such as fluoride and calcium phosphate, or proteins such as casein, which have been shown to enhance remineralisation (Reynolds et al., 2003). Glycoproteins such as mucin are found in natural saliva, and although the use of mucin in aqueous solution has demonstrated limited clinical evidence as an effective saliva substitute (Olsson & Axell, 1991; Sweeney et al., 1997), its inclusion in a more complex system has yet to be characterised. The addition of the above-mentioned excipients will likely alter the physicochemical properties of the formulations. For example, a previous study has
shown that the addition of salts disrupt the oil droplets in an o/w emulsion (Whittinghill et al., 2000). Therefore, careful monitoring of the physicochemical properties when further excipients are added will be important in order to achieve a balance between desired excipients and optimal physicochemical properties.

The inclusion of a sialagogue into the formulation may be beneficial in stimulating any residual salivary gland function. Pilocarpine or cevimeline are often used as pharmacological sialagogues (Petrone et al., 2002; Chainani-Wu et al., 2006). Recently, it was found that a sialagogue spray of 1% malic acid effectively improved the feeling of dry mouth and increased both unstimulated and stimulated salivary flow rates in individuals with xerostomia relating to antidepressant use (Gómez-Moreno et al., 2013). An investigation into the most effective sialagogue for local delivery in the oral cavity, including drug release studies to determine the rate at which these agents absorb into the mucosa from the emulsion system will allow for optimal efficacy. Such saliva substitutes would then act by both replacing lost saliva and stimulating residual saliva, which may provide superior relief.

In future work, the delivery method for the formulations developed in this thesis needs to be considered. Current saliva substitutes are available in a variety of delivery systems including gels, mouth rinses and sprays (Hahnel et al., 2009; Furness et al., 2011). Sprays make intuitive sense as delivery systems for the oral cavity as they can be delivered in small, non-intrusive and easy-to-use systems. However, the ability of compositions to be delivered via a spray mechanism as well as the stability of the emulsion droplets requires further investigation. Stability studies are required before compositions may be used for prolonged periods of time. The inclusion of an appropriate preservative for an emulsion system requires careful consideration, as many, such as sodium benzoate, are only effective in acidic environments (Sznitowska et al., 2002; Han & Washington, 2005). In emulsions, preservatives can partition out of the aqueous phase or form complexes with other excipients in the emulsion, thus reducing efficacy (Mollet & Grubenmann, 2001). Therefore, it is pertinent that microbial studies be carried out on the final product. The surface charge and pH of different compositions also requires further investigation. Surface charge is known to affect emulsion stability (Wang & Wang, 2008; Sarkar et al., 2009) and is dependent on the composition of the emulsion (Xu et al., 2011). The pH of a saliva substitute is
important as individuals with xerostomia already tend to have a reduced ability to restore neutral pH levels following the consumption of food and beverages, which may promote tooth demineralisation and provide microorganisms with a modified setting for colonisation (Edgar et al., 2004).

As discussed in Chapter 1, natural saliva forms an elastic film at the tooth-air interface that is thought to offer enamel protection (Dawes et al., 1963; Hay, 1967; Lendenmann et al., 2000; Hannig & Joiner, 2006). Therefore, the film-forming capacity of saliva substitutes is thought to be important in clinical efficacy (Christersson et al., 2000). It would be worthwhile in future studies to investigate the surface rheology and film-forming properties of these emulsions and modify them by adding proteins and other excipients so that they behave in a similar manner to natural saliva.

Tribology is the study of the lubrication, friction and wear of surfaces relative to one another. The application of tribology in the oral cavity, where interactions exist between teeth, lips, food, the tongue and the palate, has only been realised in recent years. In the oral cavity, saliva exists as a thin, lubricating film, or pellicle, that coats all surfaces, as well as a mobile film that interacts with food (Stokes, 2012). In the absence of saliva, the ability of a substitute to form a similar film on all surfaces may be important in its efficacy. It is hypothesised that in the sensory perception of foods, the rheology of the bulk of the food is important in the early stages of oral processing whereas the tribology of thin films and interactions with saliva are important during the later stages (Chen & Stokes, 2012). Gaining an understanding of the lubricating effect and interfacial rheology of these formulations compared to saliva will be beneficial not only in the development of effective saliva substitutes, but may have application in the delivery of similar formulations to other mucoadhesive sites.

RBO is a bland tasting oil with a slightly nutty, earthly flavour and a fatty acid composition consisting primarily of palmitic, oleic and linoleic acids (Orthoefer & Eastman, 2005). In this research, RBO was selected as the oil due to its taste tolerance in individuals with xerostomia (Lawn, 2007). In Chapter 2 and Chapter 3 it was shown that the type of oil affected the type of emulsion that formed, which in turn affected rheological behaviour. It is hypothesised that the contact angle of different oils, which provides information regarding spreadability, is an important factor in the ability of a formulation to spread in the oral cavity. This could be investigated further by
comparing the clinical efficacy of emulsions composed of different oil types and may assist in further determining which physicochemical properties are important in a saliva substitute. PG was selected as a co-solvent as it was able to dissolve lecithin and is a GRAS substance according to the FDA (2006), however, when applied occlusively to the skin, irritation may occur (Wahlberg & Nilsson, 1984). The oral mucosa of individuals with xerostomia may be more sensitive to this irritant effect. This will be revealed after clinical trials in patients with xerostomia and it may be worthwhile investigating alternative lecithin co-solvents.

It would be interesting to conduct a larger clinical study, with bigger sample sizes and using a variety of different emulsion compositions, including the effect of using IPM as the oil phase. In theory, this would allow for more correlations to be drawn between the physicochemical properties of a potential saliva substitute and its clinical efficacy. However, this would be an extremely complex process and in order to be worthwhile, careful planning and systematic analysis of one physicochemical parameter while controlling the others would be required.

It is suggested that the oily formulations developed in this project will provide improved relief over current saliva substitutes, due to the moistening and lubricating properties of oil, combined with the palatability of water. Results from the pilot study described in Chapter 4 suggest that immediately after oral rinsing with the emulsion, retention was higher compared to the polymer or water rinses. While FTIR showed promising results in the detection of oil, future research may benefit by taking swabs directly from surfaces in the oral cavity, instead of by oral rinsing. In doing so, dilution would be avoided; therefore, oil should be detectable at lower in-mouth concentrations. In addition, establishing a formula based on the peak area obtained by FTIR would mean that an absolute value for the concentration of oil present might be determined. Measuring retention of the emulsions from swabs taken from the oral cavity would also reduce the number of assumptions required. In the present project, it was assumed that any liquid not expectorated immediately after rinsing was retained in the oral cavity, and that lithium remained in solution and was be absorbed by the oral mucosa. While these assumptions were considered valid due to the short, 30-second rinsing period, the intention of these formulations is to remain in the oral cavity for longer time periods.
Chapter 5 – General discussion

and future work will need to further develop methods for determining retention over extended time periods.

5.1 Conclusions

The oily formulations developed and characterised in this thesis have shown exciting promise as saliva substitutes. Of particular interest are compositions that are able to maintain their viscoelastic properties upon dilution with water, as this is likely to occur in the oral environment. Although the compositions evaluated here were more viscoelastic than natural saliva, they were less viscoelastic than the commercially used HEC saliva substitute. In addition, some compositions had tan δ values greater than one at low frequencies and less than one at higher frequencies, which is an important characteristic of natural saliva. A selected composition of 20% w/w RBO, 40% w/w SM and 40% w/w water showed some promise over both water and a saliva substitute currently prescribed in New Zealand in healthy volunteers. The compositions developed in this work will provide a platform emulsion, upon which the addition and modification of other excipients to enhance bioadhesion and promote enamel remineralisation may be investigated. Further clinical studies into the effect of emulsions with different viscoelasticity in individuals with salivary hypofunction will provide insight into how this affects retention, efficacy and acceptability within the target patient population.
References


References


Appendix A

Device for the collection of submandibular saliva

In order to create a saliva collection device that is non-invasive, accurate impressions detailing the entire floor-of-mouth were required. To obtain this level of accuracy an adaptation of the altered cast technique for lower Kennedy Class I impressions was employed (Bauman & DeBoer, 1982). Heavy body Impregum impression material (3M Espe Ltd, Minnesota) with a two-sheet plastic spacer was used to obtain an initial impression of the lower floor of mouth (Figure A.1).

![Figure A.1](image)

**Figure A.1**  a) The floor of the mouth before the impression was taken, showing Wharton’s duct; b) Heavy Body material was used for the initial impression; c) Light Body material was then syringed onto the initial impression d) the final impression had a high level of detail.

The plastic spacer was then removed and light body Impregum impression material (3M Espe Ltd, Minnesota) syringed onto the hard body. A model was poured in stone and a chrome cobalt framework designed (Figure A.2). The material for the
framework was chosen because of its strength and rigidity without causing discomfort to the participant.

![Figure A.2](image)

**Figure A.2**  
*a) Construction of wax pattern with chrome cobalt base based on the stone impression and b) the chrome cobalt framework before the addition of composite resin.*

The metal framework design capped the lower anterior teeth and was designed to allow for the insertion of stainless steel tubing to the floor of the mouth without causing tongue irritation. Retention mesh was added to the posterior occlusal area and the device was constructed with retention around the lower canine area. The device was then tested in the participant’s mouth and a bite taken with dental wax to ensure no interference with the tubing. The appliance was returned to the model and wax was placed onto the cast in the region of the floor of the mouth and connected to the appliance. Upon removal from the cast just prior to clinical use impression adhesive was applied to the wax and light body impression material was syringed onto the surface before being reinserted into the participant’s mouth to allow a highly accurate impression to be taken of the floor of the mouth on a stable framework. This was particularly important in the submandibular gland area. The original model was trimmed to allow the framework and new impression to be seated and new stone was poured into the impression to allow accurate reproduction of detail in the region of the submandibular gland opening. Wharton’s duct was located in the mouth and marked on the cast. A relieving well was placed over this specific region on the model and the tubing connected ([Figure A.3]).
Figure A.3  a) Wharton’s duct was relieved using wax; b) Composite resin was placed onto the occlusal surface (arrow shows occlusion at Wharton’s duct); c) Mouth fit designed for minimal interference from the collection tube or fitting surface.

A wax-up of the finished device was constructed with the posterior bite planes. ClearSplint composite resin (Astron Dental Ltd, Illinois) was used for the bite plane and lingual plate. The lingual plate serves to create a seal over the salivary gland, preventing contamination of the saliva. Resin was mixed according to the manufacturer’s instructions and cured. Composite resin was used to obtain an optimal fit because it self-adjusts in the mouth when pre-softened in warm water.
Appendix B

Manufacturing sheets for clinical trial

Calculations

For a 100 mL, 100 mmol/L lithium aliquot:

\[ n (\text{Li}^+) = c \times V = 0.1 \text{ L} \times 0.1 \text{ mol/L} = 0.01 \text{ mol Li}^+ \]

\[ m (\text{Li}^+) = n \times M = 0.01 \text{ mol} \times 6.941 \text{ g/mol} = 0.0694 \text{ g Li}^+ \]

2 mol Li\(^+\) = 1 mol Li\(_2\)CO\(_3\)

\[ m (\text{Li}^+)/g (\text{Li}_2\text{CO}_3) = (2 \times M (\text{Li}^+))/M (\text{Li}_2\text{CO}_3) = (2 \times 6.941 \text{ g/mol})/73.880 \text{ g/mol} = 0.1879 \text{ g Li}^+/g \text{ Li}_2\text{CO}_3 \]

\[ = 0.069g \text{ Li}^+/0.1879g \text{ Li}^+/g \text{ Li}_2\text{CO}_3 = 0.3694 \text{ g Li}^+ \text{ required} \]

For a 200 mL, 1 mmol/L lithium mouth rinse solution:

Aliquot contains 0.1 mol/L lithium

1 mL of a 100 mmol/L lithium solution = 1 mmol/L lithium in 100 mL

2.0 mL of a 100 mmol/L lithium solution = 1.0 mmol/L lithium in 200 mL

= 2.0 mL lithium aliquot in 200 mL solution

1. Lithium aliquot (100 mmol/L) batch sheet

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity required</th>
<th>Quantity measured</th>
<th>Batch number</th>
<th>Expiry date</th>
<th>Checked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li(_2)CO(_3)</td>
<td>0.3694 g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distilled water</td>
<td></td>
<td>To 100 mL</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Method

Weigh Li\(_2\)CO\(_3\) using a mass balance (Mettler AT201, Greifensee, Switzerland) and transfer to 100 mL measuring flask. Make up to 100 mL with distilled water, mix to dissolve.

Label appropriately.

*Final yield: 100 mL of a 100 mmol/L lithium solution*

Final Product (lithium aliquot):

<table>
<thead>
<tr>
<th>Batch Number</th>
<th>Storage</th>
<th>Keep refrigerated (4°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturing Date</td>
<td>Expiry Date</td>
<td>7 days</td>
</tr>
</tbody>
</table>
## Appendix B

### 2. Study A:

**Mouth rinse A - ‘Water’ batch sheet**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity in formula</th>
<th>Quantity required</th>
<th>Batch number</th>
<th>Expiry date</th>
<th>Checked</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mmol/L lithium aliquot</td>
<td>2.00 mL</td>
<td>2.00 mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium phosphate buffer (pH 7.4, 100mmol/L)</td>
<td>to 200 mL</td>
<td>to 200 mL</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Method**

Check sodium phosphate buffer has pH = 7.4. Measure 2.00 mL lithium aliquot with a pipette. Make up to volume with distilled water, mix well. Transfer to stock bottle, label appropriately. *Final yield: 200 mL of a 1 mmol/L lithium solution*

**Final Product (Study A, ‘water’):**

<table>
<thead>
<tr>
<th>Batch Number</th>
<th>Storage</th>
<th>Expiry Date</th>
<th>Check</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>7 days</td>
</tr>
</tbody>
</table>
3. **Study B, Test mouth rinse A: ‘Water’ batch sheet**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity in formula</th>
<th>Quantity required</th>
<th>Batch number</th>
<th>Expiry date</th>
<th>Checked</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mmol/L lithium aliquot</td>
<td>1%</td>
<td>2.00 mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium phosphate buffer (pH 7.4, 100mmol/L)</td>
<td>to 100%</td>
<td>to 200 mL</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Method**
Check sodium phosphate buffer has pH = 7.4.
Measure 2.00 mL lithium aliquot using a pipette.
Make up to volume, mix well.
Transfer to stock bottle, label appropriately.

*Final yield: 200 mL of a 1 mmol/L lithium solution*

**Final Product (Study B, water):**

<table>
<thead>
<tr>
<th>Batch Number</th>
<th>Storage</th>
<th>Keep refrigerated (4°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturing Date</td>
<td>Expiry Date</td>
<td>7 days</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Checked</th>
<th>-------</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Run</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Run</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4. Study B, test mouth rinse B: ‘Polymer’ batch sheet

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity in formula</th>
<th>Quantity required</th>
<th>Batch number</th>
<th>Expiry date</th>
<th>Checked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl cellulose</td>
<td>5g</td>
<td>2g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lithium aliquot</td>
<td>-</td>
<td>2.00mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distilled water</td>
<td>to 500mL</td>
<td>to 200mL</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Method**

(Saliva substitute - NZ Pharmaceutical Schedule Standard Formula)
Reduce particle size of methylcellulose in mortar and pestle. Add water while mixing with mortar to suspend particles and avoid clumps. Add some more water if required. Transfer to a 200mL measure, use some of the water to wash out mortar and pestle and transfer to final measure. Add lithium aliquot, stir and make up to final volume with distilled water. Transfer to stock bottle and label appropriately.

*Final yield: 200mL of a 1 mmol/L lithium, 1% w/v methylcellulose suspension.*

**Final Product (Study B, polymer):**

<table>
<thead>
<tr>
<th>Batch Number</th>
<th>Storage</th>
<th>Keep refrigerated (4°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturing Date</td>
<td>Expiry Date</td>
<td>7 days</td>
</tr>
</tbody>
</table>
5. Study B, test mouth rinse C: ‘Emulsion’ batch sheet

**Surfactant mix:**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity in formula</th>
<th>Quantity required</th>
<th>Batch number</th>
<th>Expiry date</th>
<th>Checked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lecithin (Lipoid S-100)</td>
<td>50%</td>
<td>60g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>50%</td>
<td>60g</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Method**

Weigh lecithin and propylene glycol; transfer into a stock bottle, cover and magnetically stir overnight (it takes about 24 hours for the lecithin to completely dissolve). This is the SM for the emulsion below.

*Final yield: 120g SM*

**Lithium-tagged aqueous phase:**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity in formula</th>
<th>Quantity required</th>
<th>Batch number</th>
<th>Expiry date</th>
<th>Checked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium aliquot</td>
<td>2.00 mL</td>
<td>2.00 mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distilled water</td>
<td>to 80 mL</td>
<td>to 80 mL</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Method**

Measure 2.00 mL lithium aliquot, make up to 80 mL with distilled water. Mix well. This is the aqueous phase for the emulsion below.

*Final yield: 80 mL water tagged with lithium (to get 1 mmol/L in the final emulsion below)*

**Emulsion (20% w/w RBO, 40% w/w SM, 40% w/w water):**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity in formula</th>
<th>Quantity required</th>
<th>Batch number</th>
<th>Expiry date</th>
<th>Checked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surfactant mix (above)</td>
<td>40%</td>
<td>80g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice bran oil</td>
<td>20%</td>
<td>40g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aqueous phase (above)</td>
<td>40%</td>
<td>80g</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Method**

Weigh the appropriate amount of SM, RBO and water into a stock bottle. Magnetically mix until homogenous. Transfer to a spray bottle and label appropriately.

*Final yield: 200g.*

*Water weighed - due to viscosity it is difficult to obtain a total volume easily*

**Final product (Study B, emulsion):**

<table>
<thead>
<tr>
<th>Batch Number</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Manufacturing Date</th>
<th>Expiry Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix C

Participant information sheet and consent form, study A:
Validation of the procedure for determining retention of liquids in the oral cavity

Reference Number 10/195
November 2010

RETENTION OF LIQUID IN THE MOUTH
INFORMATION SHEET FOR
PARTICIPANTS

Thank you for showing an interest in this project. Please read this information sheet carefully before deciding whether or not to participate. If you decide to participate we thank you. If you decide not to take part there will be no disadvantage to you of any kind and we thank you for considering our request.

What is the Aim of the Project?

The aim of this project is to determine how much of any liquid is retained in your oral cavity or swallowed after mouth rinsing. The study will establish a method for measuring retention of substances in your mouth so that we can use this to further the development of oral products for patients with impaired saliva production.

What Type of Participants are being sought?

We are currently inviting 17 healthy participants over 18 years old without any missing teeth, who are not currently taking any of the following medication:

- Lithium
- ACE inhibitors/angiotensin II antagonists (captopril, enalapril, lisinopril, candesartan, losartan, valsartan)
- Calcium-channel blockers (verapamil, diltiazem)
- Diuretics (bendrofluazide, furosemide, bumetanide)
- Antiepileptics (phenytoin, phenobarbital, carbamazepine)
- Antimicrobials (doxycycline, metronidazole, spectinomycin, tetracycline)
- Antimigraine medication (sumatriptan)
- Antineoplastics (cisplatin)
Appendix C

- Antipsychotics/anxiolytics (chlorpromazine, flupentixol decanoate, fluphenazine decanoate, haloperidol, clozapine, risperidone, diazepam)
- Non-steroidal anti-inflammatory agents (celecoxib, diclofenac, ibuprofen, indomethacin, mefenamic acid, naproxen, piroxicam)

**What Will Participants be Asked to Do?**

Should you agree to take part in this project, you will be asked to rinse your mouth with a tasteless mouth rinse for thirty seconds before spitting it into a cup. The rinse will be tagged with a small amount of lithium, to be used as a marker to determine how much of the mouth rinse is left behind in your mouth. As participants will be required to spit out the rinse after thirty seconds, the amount of lithium swallowed will be negligible. You will be asked to refrain from eating or drinking anything other than water for the thirty minutes prior to testing.

Please be aware that you may decide not to take part in the project without any disadvantage to yourself of any kind.

**What Data or Information will be Collected and What Use will be Made of it?**

Your age and gender will be recorded. Samples containing saliva will be collected and analysed to determine the amount of liquid that remains in the mouth. These will be destroyed following analysis of the lithium concentration.

The data collected will be securely stored in such a way that only those mentioned below will be able to gain access to it. At the end of the project any personal information will be destroyed immediately except that, as required by the University's research policy, any raw data on which the results of the project depend will be retained in secure storage for five years, after which it will be destroyed.

The results of the project may be published and will be available in the University of Otago Library (Dunedin, New Zealand) but every attempt will be made to preserve your anonymity.

You are most welcome to request a copy of the results of the project should you wish.

**Can Participants Change their Mind and Withdraw from the Project?**

You may withdraw from participation in the project at any time and without any disadvantage to yourself of any kind.

**What if Participants have any Questions?**

If you have any questions about our project, either now or in the future, please feel free to contact either:-

Sara Hanning (PhD student) or Professor Jules Kieser
School of Pharmacy or Faculty of Dentistry
Phone: 4795285 or Phone: 4797083

This study has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (ph 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.
RETENTION OF LIQUID IN THE MOUTH
CONSENT FORM FOR PARTICIPANTS

I have read the Information Sheet concerning this project and understand what it is about. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:-

1. My participation in the project is entirely voluntary;

2. I am free to withdraw from the project at any time without any disadvantage;

3. I am not expected to experience any discomfort and risk during this project, however if I have any concerns I can contact the researchers at any time;

4. The results of the project may be published and will be available in the University of Otago Library (Dunedin, New Zealand) but every attempt will be made to preserve my anonymity.

I agree to take part in this project.

............................................................................. ..................................................
(Signature of participant) (Date)

This study has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (ph 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.
Appendix D

Participant information sheet and consent form, study B:
An emulsion for potential use as a saliva substitute

Reference Number 12/252
September 2012

AN EMULSION FOR POTENTIAL USE AS A SALIVA SUBSTITUTE
INFORMATION SHEET FOR PARTICIPANTS

Thank you for showing an interest in this project. Please read this information sheet carefully before deciding whether or not to participate. If you decide to participate we thank you. If you decide not to take part there will be no disadvantage to you and we thank you for considering our request.

What is the Aim of the Project?

The aim of this project is to determine the taste acceptability of an oily formulation for potential use as a saliva substitute in the mouth, and also to determine how much is retained in the mouth following simple rinsing.

What Type of Participants are being sought?

We are currently inviting 30 healthy participants over the age of 18 years old without any missing teeth, who are not currently (or in the last month) taking any of the following medication:

- Lithium
- ACE inhibitors/angiotensin II antagonists (captopril, enalapril, lisinopril, candesartan, losartan, valsartan)
- Calcium-channel blockers (verapamil, diltiazem)
- Diuretics (bendrofluazide, furosemide, bumetanide)
- Antiepileptics (phenytoin, Phoanobarbital, carbamazepine)
- Antimicrobials (doxycycline, metronidazole, spectinomycin, tetracycline)
- Antimigraine medication (sumatriptan)
- Antineoplastics (cisplatin)
- Antipsychotics/anxiolytics (chlorpromazine, flupentixol decanoate, fluphenazine decanoate, haloperidol, clozpare, risperidone, diazepam)
What will Participants be Asked to Do?

Should you agree to take part in this project, you will be asked to take part in one session of approximately 30 minutes duration. You will be asked to refrain from eating or drinking anything other than water for 60 minutes prior to your session time. During the session you will be asked to spit into a cup for five minutes to determine your salivary flow rate before being assigned a mouth rinse to swill round your mouth before spitting into a cup. You will be asked to fill out some questions relating to the feel and taste of this mouth rinse while remaining seated. A similar process will be repeated after five and ten minutes. The rinses will be tagged with a small amount of lithium as a marker to determine how much of the mouth rinse is left behind in your mouth. The lithium concentration in the mouth rinse is less than that found in most food and as you will spit out the rinse after 30 seconds, the amount of lithium swallowed will be negligible.

Please be aware that you may decide not to take part in the project without any disadvantage to yourself of any kind.

What Data or Information will be Collected and What Use will be Made of it?

Your age and gender will be recorded. Samples containing saliva will be collected and analysed to determine the amount of liquid that remains in the mouth. These will be destroyed following analysis of the lithium concentration.

The data collected will be securely stored in such a way that only those mentioned below will be able to gain access to it. Data obtained as a result of the research will be retained for at least 5 years in secure storage. Any personal information held on the participants will be destroyed at the completion of the research even though the data derived from the research will, in most cases, be kept for much longer or possibly indefinitely.

The results of the project may be published and will be available in the University of Otago Library (Dunedin, New Zealand) but every attempt will be made to preserve your anonymity.

You are most welcome to request a copy of the results of the project should you wish.

Can Participants Change their Mind and Withdraw from the Project?

You may withdraw from participation in the project at any time and without any disadvantage to yourself of any kind.

What if Participants have any Questions?

If you have any questions about our project, either now or in the future, please feel free to contact either:-

Sara Hanning (PhD student) or Professor Jules Kieser
School of Pharmacy Faculty of Dentistry
Phone: 03 479 5285 Phone: 03 479 7083
Email: sara.hanning@otago.ac.nz Email: jules.kieser@otago.ac.nz

This study has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (ph 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.
AN EMULSION FOR POTENTIAL USE AS A SALIVA SUBSTITUTE

CONSENT FORM FOR PARTICIPANTS

I have read the Information Sheet concerning this project and understand what it is about. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:-

1. My participation in the project is entirely voluntary;

2. I am free to withdraw from the project at any time without any disadvantage;

3. I am not expected to experience any discomfort and risk during this project, however if I have any concerns I can contact the researchers at any time;

4. The results of the project may be published and will be available in the University of Otago Library (Dunedin, New Zealand) but every attempt will be made to preserve my anonymity.

I agree to take part in this project.

............................................................................
(Signature of participant) ...................................(Date)

This study has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (ph 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.
Appendix E

Participant questionnaire, study B:
An emulsion for potential use as a saliva substitute

Participant ID______________________________ Date_____________________

Formulation (circle)  1  2  3  Time (circle)  0  5  10

Patient Acceptability and Retention of an Emulsion for Potential Use as a Saliva Substitute

Participant Questionnaire

Please answer the following questions regarding the formulation by marking with an ‘X’ on the scale provided:

1. How would you rate the taste of the formulation?

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Like extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. How would you rate the intensity of the taste of the formulation?

<table>
<thead>
<tr>
<th>Not intense at all (i.e. no taste)</th>
<th>Extremely intense (i.e. taste explosion)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. Can you feel the formulation in your mouth? [circle one]

Yes  No

If you answered yes above:

4. How thick does the formulation feel in your mouth?

The thickness of water  The thickness of yoghurt

5. Please comment on any other features associated with the feel of the formulation in the mouth:

_____________________________________________________________________________________
___________________________________________________________________________________
_____________________________________________________________________________________
_____________________________________________________________________________________

Like extremely  Dislike extremely