Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 278 (2004) 391–406
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Bioadhesive, rheological, lubricant and other aspects of an oral gel formulation intended for the treatment of xerostomia

H.M. Kelly [a], P.B. Deasy [a,*], M. Busquet [b], A.A. Torrance [b]

[a] *School of Pharmacy, Trinity College, University of Dublin, Dublin, Ireland*
[b] *Department of Mechanical and Manufacturing Engineering, Trinity College, University of Dublin, Dublin, Ireland*

Received 22 January 2004; received in revised form 9 March 2004; accepted 18 March 2004

Available online 13 May 2004

Abstract

Xerostomia is commonly known as 'dry mouth' and is characterised by a reduction or loss in salivary production. A bioadhesive gel for its localised treatment was formulated to help enhance the residence time of the product, based on the polymer *Carbopol 974P*. The bioadhesion of various formulations was evaluated on different mucosal substrates, as simulations of the oral mucosa of xerostomic patients. Depending on the type of model substrate used, the mechanism of bioadhesion could alter. When the rheology of various formulations was examined, changes in bioadhesion were more easily interpreted, as the presence of other excipients caused an alteration in the rheological profile, with a change from a fully expanded and partially cross-linked system to an entangled system. Improving the lubricity of the product was considered important, with optimum incorporation of vegetable oil causing a desirable lowering of the observed friction of the product. The final complex formulation developed also contained salivary levels of electrolytes to help remineralisation of teeth, fluoride to prevent caries, zinc to enhance taste sensation, triclosan as the main anti-microbial/anti-inflammatory agent and non-cariogenic sweeteners with lemon flavour to increase the palatability of the product while stimulating any residual salivary function.

Keywords: Xerostomia; Saliva substitute; Bioadhesion; Rheology; Lubricity; *Carbopol 974P*; Excipients

1. Introduction

Xerostomia is a condition characterised by a reduction or loss in salivary flow, often with a concurrent change in the composition of the saliva, resulting in dryness of the oral cavity. This leads to changes in oral pH and microflora, causing an increased risk of infection, dental caries, mucositis, gingivitis and periodontitis (Plankhurst et al., 1996). There is also an increased risk of *Candida* infections and angular cheilitis (Chaushu et al., 2000). Xerostomia may lead to difficulties with some or all of the following, polydypsia, polyuria, sleeping, speaking, swallowing and mastication (Hamlet et al., 1997). There may be also changes in taste acuity (Mossman and Henkin, 1978).

There are a number of conditions that can result in the development of xerostomia. The most common are radiation treatment of the head and neck region, anticancer drug treatment and Sjögrens syndrome. However, other conditions have been reported to cause xerostomia including HIV (Schiødt et al., 1992), brain tumours and neurosurgical procedures (Ettinger, 1996).

* Corresponding author. Tel.: +353-1-6082784; fax: +353-1-6082783.
E-mail address: pdeasy@tcd.ie (P.B. Deasy).

0378-5173/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2004.03.022

Xerostomia while not considered a serious illness, can result in symptoms which patients find very distressing.

The treatment of xerostomia is dependent on a number of factors such as the severity of the condition, does the person have any residual salivary function remaining to be stimulated and is the xerostomia temporary or permanent. The side effect profile of the chosen treatment must also be considered, while the cost versus effectiveness of the chosen method needs to be evaluated. Treatments currently available vary considerably in effectiveness, cost, ease of use and side effect profile, and in many cases the results are highly subjective. Pilocarpine is the systemic drug of choice for use as a salivary stimulant. It is only of use in patients where there is still residual function left. The drug also has a poor side effect profile, including causing sweating, increased urinary frequency, flushing, dizziness and tachycardia, with all side effects being dose related (British National Formulary, 2002).

The most common option employed to treat xerostomia is the use of an artificial saliva or saliva substitute (oral lubricants). Saliva substitutes are used in those patients who have little or no residual salivary gland function. There are many saliva substitutes available on the market such as Luborant, Glandosane and Bioxtra, but none is ideal. This is due to the complex nature of saliva, making it difficult to produce a product that adequately mimics its properties. Common criticisms of currently available artificial salivas include little relief of symptoms, strange consistency, bad taste and short duration of action (Furomoto and Carter-Hanson, 1998). However, in their favour, they are a relatively cheap, safe and easy to use option with no problematic side effects. The ideal saliva substitute should be bioadhesive and lubricant, biocompatible and should also help protect against some of the many problems associated with xerostomia such as dental caries and *Candida* infections. It should also help relieve the distressing symptoms associated with xerostomia such as difficulty in talking, chewing and swallowing.

Commercial saliva substitutes contain either bioadhesive agents e.g. sodium carboxymethylcellulose (NaCMC), xanthan gum or animal mucin (bovine submaxillary mucin as in *Saliva Orthana*) (Levine et al., 1987). Mucin is a normal constituent of saliva and the mucin-based artificial saliva appears to be more effective and better tolerated than NaCMC-based ones (Sweeney et al., 1997). However, it would appear that many of the mucin-based products of bovine origin have now been discontinued, probably due to concerns regarding transmissible spongiform encephalopathy. The presence of NaCMC or xanthan gum increases the apparent viscosity of the product and provides adherence of the product in the oral cavity for a prolonged period of time. However, their duration of action is still considered to be too short, with a study by Olsson and Axéll (1991) showing changed friction values for only about 15 min, which was just twice as long as obtained using water. Artificial salivas should also contain electrolytes in concentrations similar to normal saliva and fluoride to help tooth remineralisation. Usually they contain an artificial, non-cariogenic sweetener like sorbitol and various flavourings.

Other than for the mucin-containing products, there have been few published studies regarding the benefits of artificial salivas. Anecdotal evidence refers to a product called Oralbalance gel being a preferred product (Ettinger, 1996); however, this has been replaced recently with a similar product called Bioxtra gel, which is hydroethylcellulose-based. There are some available results from clinical trials on saliva substitutes that show while many of the products contain a number of shortcomings, there is also a strong desire among most patients to continue using a saliva replacement. A NaCMC-based saliva substitute showed no significant advantage over a glycerin and lemon swab, although a significant number of patients preferred both of these options to distilled water (control) (Weisenfeld et al., 1983). A mucin containing spray (*S. Orthana*) showed no statistically significant difference between active spray (mucin containing) and placebo spray (no mucin), and neither spray had any major impact on oral microflora. However, the majority of patients in both treatment groups wished to continue using the spray (Sweeney et al., 1997). In another clinical trial of *S. Orthana*, significantly greater relief was offered from xerostomia compared with its base (no mucin) or water, and the *S. Orthana* was significantly better at relieving soreness than water. In terms of preference, *S. Orthana* was ranked significantly higher than either alternative (Duxbury et al., 1989).

Collectively these studies indicate that an ideal salivary substitute for the treatment of xerostomia has yet to be marketed, and this study describes the in vitro

development of hopefully an improved product, which will be tested subsequently in a panel of xerostomia patients to confirm clinical efficacy.

2. Materials and methods

2.1. Materials

Calcium chloride, sodium chloride, zinc sulphate (Merck), *Carbopol 974P* (BF Goodrich), cottonseed oil, ethylenediaminetetraacetic acid (EDTA), phenylmethylsulphonylfluoride (PMSF), porcine gastric mucin (type II—crude), potassium chloride, potassium phosphate (monobasic), sodium azide, sodium fluoride, sodium lauryl sulphate (SLS), sorbitol (Sigma), lemon flavouring (Dragoco), magnesium chloride, triethanolamine (BDH), olive oil (Don Carlos), sunflower oil (Tesco), triclosan 5000 (Kumar Organic Products), xylitol (Lancaster) and water (glass distilled) were used. All reagents were GPR unless otherwise indicated.

2.2. Method for preparation of formulations

Various formulations, some of which are detailed in Tables 1 and 2, were prepared as outlined in the following method. The relevant quantities of sodium fluoride, calcium chloride, potassium chloride, sodium chloride, magnesium chloride, potassium phosphate, zinc sulphate, xylitol, sorbitol and flavouring agent were added to 20 ml approximate of water and dissolved. The required quantity of *Carbopol 974P* was added with continuous stirring over 60 min and left overnight at room temperature to allow complete hydration of the polymer. In a separate beaker, SLS and triclosan were placed in 10 ml approximate of water, 1.0 ml of triethanolamine was added and the preparation was sonicated until the triclosan was fully solubilised. The solubilised triclosan solution was added to the hydrated polymer system, which was partially neutralised by the triethanolamine present in the triclosan solution. The pH was further adjusted to 6.75 ± 0.05 as a simulation of oral pH using triethanolamine and the product was adjusted to its final weight using water. If vegetable oil was present in the formulation, water was added to give the weight required before the addition of the oil. The oil was added and emulsification was carried out using an Ultra-Turrax T25 shear rate mixer at 7500 rpm for a period of 4–5 min. The product was then centrifuged at 2000 rpm for 10 min to remove any air bubbles trapped in the emulsion.

2.3. In vitro testing for bioadhesive strength

Bioadhesive strength of the lead formulations was determined in vitro using a texture analyser XT.RA

Table 1
Formulation of various products prepared for examination as saliva substitutes

Formulation	1	2	3	4	5	6	7	8	9	10
Sodium fluoride (mg)	0.42	0.42	0.42	0.42	0.42	0.42	0.42	–	–	0.42
Calcium chloride (g)	0.0166	0.0166	0.0166	0.0166	0.0166	0.0166	0.0166	–	–	0.0166
Potassium chloride (g)	0.12	0.12	0.12	0.12	0.12	0.12	0.12	–	–	0.12
Sodium chloride (g)	0.0844	0.0844	0.0844	0.0844	0.0844	0.0844	0.0844	–	–	0.0844
Magnesium chloride (g)	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	–	–	0.0058
Potassium phosphate (g)	0.0366	0.0366	0.0366	0.0366	0.0366	0.0366	0.0366	–	–	0.0366
Xylitol (g)	3.0	5.0	5.0	15.0	15.0	15.0	15.0	15.0	15.0	–
Sorbitol (g)	–	–	–	5.0	5.0	5.0	5.0	5.0	5.0	–
Zinc sulphate (g)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	–	–	0.05
Triclosan (g)	0.15	0.3	0.15	0.1	0.1	–	–	0.1	0.1	0.1
Na lauryl sulphate (g)	1.0	2.0	1.0	1.0	1.0	–	–	1.0	1.0	1.0
Lemon flavour (g)	0.25	0.25	0.25	0.3	0.4	0.4	0.400	0.4	0.4	0.4
Olive oil (g)				–	–	–	–	–	–	–
Carbopol 974P (g)	1.5	1.5	1.5	1.0	0.5	1.0	0.5	1.0	0.5	1.0
Water (g)	100	100	100	100	100	100	100	100	100	100

Table 2
Formulation of various products prepared for examination as saliva substitutes continued

Formulation	11	12	13	14	15	16	17	18	19	20
Sodium fluoride (mg)	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
Calcium chloride (g)	0.0166	0.0166	0.0166	0.0166	0.0166	0.0166	0.0166	0.0166	0.0166	0.0166
Potassium chloride (g)	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Sodium chloride (g)	0.0844	0.0844	0.0844	0.0844	0.0844	0.0844	0.0844	0.0844	0.0844	0.0844
Magnesium chloride (g)	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058
Potassium phosphate (g)	0.0366	0.0366	0.0366	0.0366	0.0366	0.0366	0.0366	0.0366	0.0366	0.0366
Xylitol (g)	–	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Sorbitol (g)	–	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Zinc sulphate (g)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Triclosan (g)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Na lauryl sulphate (g)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Lemon flavour (g)	0.4	0.5	0.5	0.5	1.0	1.0	1.0	1.0	1.0	1.0
Olive oil (g)	–	5.0	10.0	20.0	5.0	10.0	–	–	–	–
Sunflower oil (g)			–	–	–	–	5.0	10.0	15.0	20.0
Carbopol 974P (g)	0.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Water (g)	100	100	100	100	100	100	100	100	100	100

(Stable Micro Systems). The apparatus consisted of an upper metal probe (surface area 5.12 cm^2) to which the mucosa or mucus was attached. The sample was placed in a secure position on the lower platform. The probe was lowered at a fixed speed until it came in contact with the sample with a defined force, where it was held for a stated contact time. At the end of this time period, the probe retracted at a defined speed breaking the bioadhesive bond. This procedure was carried out under the adhesive mode. A profile of force versus time was obtained from which the maximum force of detachment F_{max} was measured in mN. The work of adhesion was determined from the area under the curve (N mm). Testing was performed on three different systems, *Carbopol 974* 1%, product 4 and product 15 using three different model substrates as simulations of the oral mucosa in xerostomic patients, dried porcine gastric mucin (DPGM), fresh porcine oral mucosa (FPOM) and fresh porcine gastric mucus (FPGM).

Dried porcine gastric mucin (20% (w/w)) was prepared by adding the required quantity of powder to distilled water and allowing it to hydrate overnight at 4 °C. Fresh porcine oral mucosa was obtained from freshly slaughtered pigs (within 1 h of slaughter). The top layer of tissue was excised from the lower connective tissue layers and cut into suitable sample sizes. The tissue was either used immediately or frozen at −20 °C, with greaseproof paper separating individual samples of tissue. The tissue was defrosted at 4 °C as required and used immediately.

Fresh porcine gastric mucus was extracted from recently slaughtered porcine stomach, using a method modified from Mortazavi et al., 1993. Batches of crude mucus were obtained by scraping porcine stomachs received just after slaughter. To the crude mucus was added an equal quantity of an isotonic solution containing PMSF (0.0175% (w/v)), sodium azide (0.02% (w/v)), EDTA (0.186% (w/v)) and sodium chloride (0.9% (w/v)). This mixture was homogenised by blending, with the resulting mixture centrifuged at 3000 g for 1.5 h at 4 °C using a temperature-controlled centrifuge (Sorvall, UK). The gel layers were removed from each centrifuge tube, pooled and exhaustively dialysed for 24 h at 4° C in a large volume of water and finally homogenised. If the mucus obtained was not used immediately, it was frozen at −20 °C until required and defrosted overnight at 4 °C before use.

When using DPGM or FPGM, the probe was covered with a thin layer of each, which was then dried using warm air (∼40 °C). For FPOM, a thin layer of the tissue was attached to the probe using an adhesive backing layer. The product gels were placed in a glass jar to a depth of 20 mm, with the container anchored to the platform. The probe was brought in contact with the test samples with a force of 0.1 N and held there for a specified period of time and then withdrawn at

a speed of 0.1 mm/s. Five replicates were performed for each sample.

2.4. Rheology

Rheological properties of various systems were evaluated using a Carri-Med rheometer CSL^{2}500 (TA Instruments, UK). All experiments used cone and plate test geometry (angle 4°). The linear viscoelastic region of the systems was first determined by torque sweep.

2.5. Rheological synergism with fresh porcine gastric mucus (FPGM)

Two grams samples of FPGM were mixed with an equal weight of the relevant system, and the pH was adjusted to 6.75 using 10% (w/v) triethanolamine. The final weight of the sample was adjusted to 4.5 g using water. Further mixtures containing 2 g of each system were adjusted to pH 6.75 and made up to 4.5 g using water. A sample containing mucus 2 g and distilled water 2.5 g was prepared also to act as a control. Oscillatory rheometry (triplicate determinations) was carried out on all of the samples listed in Table 3, using a frequency sweep of 0.1–10 Hz at 37 °C, with an oscillatory torque of 1350 μNm, which was determined to be within the linear viscoelastic range.

2.6. Friction testing of xerostomic products

Friction testing was performed on apparatus that had been constructed in-house. It consisted of a lower fixed plate onto which the test substrate and test substance could be placed, and an upper slider to which different geometries could be attached. The slider moved along the lower plate at varying speeds and differing distance, creating friction between the two surfaces that could be measured and analysed using in-house developed computer software.

A piece of *Perspex*® 10 cm in length was covered with a soft piece of leather. The leather was used to simulate the oral mucosa (Blanco-Fuente et al., 1996). The upper probe was also covered with the same leather. The sample was loaded onto the 10 cm plate and spread evenly over it. The instrument was put into run mode and the probe moved at a set speed up and down the fixed 10 cm plate for a defined period of time, measured in seconds. All samples were examined at the same speed and distance of travel. The friction was measured in terms of the friction coefficient, μ (force in the direction of motion divided by load applied to the slider). Testing was performed on *Carbopol 974P* 1% (w/w), product 4 and product 15.

Table 3
Carbopol 974P-based systems examined for rheological synergism

System	1	2	3	4	5	6	7
Porcine gastric mucus (g)	2.0	2.0	2.0	2.0			
Product 4 (g)	2.0				2.0		
Product 15 (g)		2.0				2.0	
Carbopol 974P 1% (g)			2.0				2.0
pH (adjusted to)	6.75	6.75	6.75		6.75	6.75	6.75
Water (g)	4.5	4.5	4.5	4.5	4.5	4.5	4.5

3. Results

3.1. Theory of formulation

Increased incidence of caries is a significant problem associated with xerostomia, as the excretion of minerals in saliva is affected and the pH of the oral cavity is reduced leading to demineralisation of teeth. The functions of the ions present in saliva are to contribute to the osmolarity of saliva and also to its buffering capacity. The buffering capacity of saliva is directly related to its flow, with the difference in resting and stimulated saliva related to the sodium and bicarbonate concentrations. Both sodium and potassium were included in representative formulations listed in Tables 1 and 2, as potassium is the predominant cation in the buffer systems of resting saliva, but sodium predominates in stimulated saliva. The quantities of minerals used in the formulations developed in this project were comparable to those levels of minerals found in natural whole saliva (Edgar, 1992) and are similar in quantity to those in Luborant. Fluoride is an important mineral in caries prevention and therefore is of particular relevance to xerostomic patients. Fluoride is not found in the diet to any large extent and only small quantities are secreted in the saliva. Therefore, fluoride supplementation is required to further improve the patient's chance of maintaining good oral health.

Saliva has a number of anti-microbial agents present as a defence mechanism against invasion by pathogenic bacteria e.g. lysozymes, histatins, peroxidase and lactoferrin (Whelton, 1996). Another effective antibacterial mechanism of saliva is its washing action i.e. swallowing results in clearance of large numbers of bacteria. A reduction in saliva production causes a rapid increase in the population and type of microorganisms present in the mouth (Brown et al., 1975). Therefore it was considered beneficial to include an anti-microbial agent in the formulation. Triclosan was the agent chosen, as it is already present in a number of proprietary products e.g. Colgate toothpaste, Sensodyne mouthwash (Rosling et al., 1997). Triclosan has been shown to be an effective anti-microbial agent for products used in the oral cavity with broad-spectrum activity against both gram-positive and gram-negative organisms, with the agent affecting gram-negative anaerobes to a greater extent than gram-positive groups (Bhargave and Leonar, 1996). It has been shown also to have a direct anti-inflammatory effect on the gingival tissues. Gaffar et al. (1995) found that triclosan inhibited several important mediators of gingival inflammation. That study also suggested that the necessary triclosan concentrations could probably be achieved in local tissues, such as the gingiva, from topical applications. In order to incorporate it into the formulation, SLS was chosen as a solubilising agent at a concentration of 1%. This is a solubilising agent of choice as it has been shown to have a synergistic action on the anti-microbial effect of triclosan (Waaler et al., 1993). SLS is already used in a number of pharmaceutical preparations such as a wetting agent in dentrifices, tablet lubricant/wetting agent and anionic emulsifier.

Zinc has been shown to exert a modest anti-plaque effect in simple solutions (Harrap et al., 1983), but when combined with triclosan the anti-plaque effect is greatly enhanced to the extent that a gingival health benefit can be achieved (Svatun et al., 1987). Zinc performs also another possible function in the formulation. A constant complaint of patients with xerostomia is the loss of taste sensation. When a zinc salt was included in a formulation in an uncontrolled study, there was some evidence that zinc therapy may have been useful in ameliorating taste impairment (Mossman and Henkin, 1978).

As xerostomia is usually a long-term condition, the flavour of the formulation is important, with a ‘fresh mouth’ feeling preferable, as the product may be used on a continual basis. Development of an acceptable flavour in the new products developed in this project was difficult mainly due to the presence of triclosan and SLS. Triclosan has a phenolic flavour, which if correctly masked confers a fresh mouth feeling, however, if concentrations are too high it gives an unacceptable bitter aftertaste. Xylitol and sorbitol were chosen as the sweetening agents because they are non-cariogenic, while a lemon flavour was selected due to its citrus, fresh taste and its ability to stimulate residual salivary function. Xylitol was the major sweetener used as it is fermented to acid more slowly than sorbitol, and therefore it does not depress plaque pH.

The optimum product developed in this project, unlike existing commercial products, contained sunflower oil as a stable o/w emulsion to help provide a lubricating effect similar to that of normal saliva, so facilitating ease of swallowing and speaking in xerostomic patients. A number of vegetable oils were initially screened at different concentrations e.g. olive oil 5, 10 and 20%, cottonseed oil, 5 and 10% and sunflower oil 5 and 10%. These oils were chosen due to their commercial availability and broad dietary use. A product containing olive oil 5% was originally the lead product, but the taste was not considered acceptable. Cottonseed oil also gave an unpleasant aftertaste, but sunflower oil left no appreciable aftertaste and also seemed to give a less oily texture to the product than was provided by olive oil. A concentration of sunflower oil 5% was chosen as it was considered the lowest level that would provide adequate lubricating effect and its long-term use might elevate essential fatty acid levels adequately in patients to be of additional benefit in the treatment of Sjögrens syndrome (Horrobin, 1984).

It is important that if the saliva substitute is to help in lubricating and protecting the oral cavity, it must be retained there for a prolonged period of time for its actions to be effective. The use of a bioadhesive polymer will help ensure the retention of the product in the oral cavity for an increased period over that achieved in its absence. *Carbopol 974P*, which is a lightly cross-linked polyacrylic acid, was chosen as the bioadhesive polymer of choice as this polymer type

has been shown to have good mucoadhesive properties (Jones et al., 1996) and is used in toothpaste and other orally administered products. Once *Carbopol 974P* is dispersed in water, the molecules begin to hydrate and partially uncoil. On conversion of the acidic polymer to a salt thickening occurs. *Carbopol 974P* polymer must be neutralised in order to achieve maximum apparent viscosity. Concentrations of neutralised *Carbopol 974P* (pH 6.75 ± 0.05) from 0.5 to 2.0% were initially examined in order to achieve the correct texture and consistency considered necessary for the final product, while still maintaining adequate bioadhesive ability. Both 2.0 and 1.5% were considered to be too viscous for ease of application when compared to existing commercial products, none of which available in Ireland contain this polymer, while *Carbopol 974P* 0.5% suffered from a lack of permanence. A concentration of 1.0% was chosen as suitable, as it showed good bioadhesion but was also of adequate consistency for use in the oral cavity.

Various concentrations of the bioadhesive polymer alone and in formulations (Tables 1 and 2) based on the selected excipients as justified above were evaluated for bioadhesive, rheological and lubricity properties considered important for its end-use. Some representative results of these studies, compared to aspects of commercial products available in Ireland, are presented below as an aid to the development to the final formulation. Preliminary studies on human saliva confirmed its lack of apparent viscosity, bioadhesion and lubricity compared to the optimum formulations developed in this project.

3.2. In vitro bioadhesion testing

Products 4 and 15 were examined for in vitro bioadhesion. Product 4 gave the largest peak detachment force readings at 60 s for bioadhesion with DPGM, followed by FPOM and FPGM (Fig. 1). However, the work of adhesion values at 60 s did not follow the same trend, with the greatest value being given by FPGM, followed by DPGM and FPOM. Over increasing contact time, the peak detachment force of product 4 using FPOM showed a continual increase, there was a rapid decline in bioadhesion after 60 s with FPGM, and DPGM showed little appreciable change over time (Fig. 2). The largest average peak detachment force is seen with DPGM. This mucin substrate showed an increase in bioadhesion for product 4 compared to *Carbopol 974P* 1%. The only possible explanation for this reverse trend is that a different method of bioadhesion is occurring with DPGM, perhaps mucin/polymer chain entanglement is becoming the predominant method due to the high concentration of mucin present. This is a possibility due to the increased space between the polymer chains as a result of the partial recoiling, leaving room for the mucin chains to penetrate.

Product 15, another complex formulation, showed a different pattern of bioadhesion at 60 s, with the largest bioadhesive force being given by FPGM, fol-

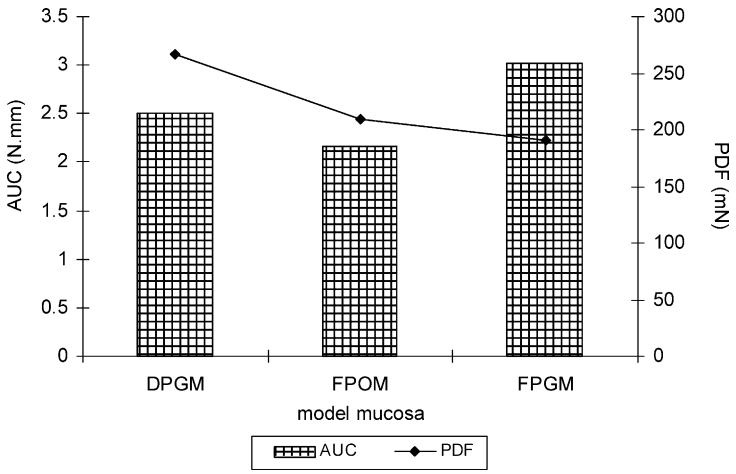
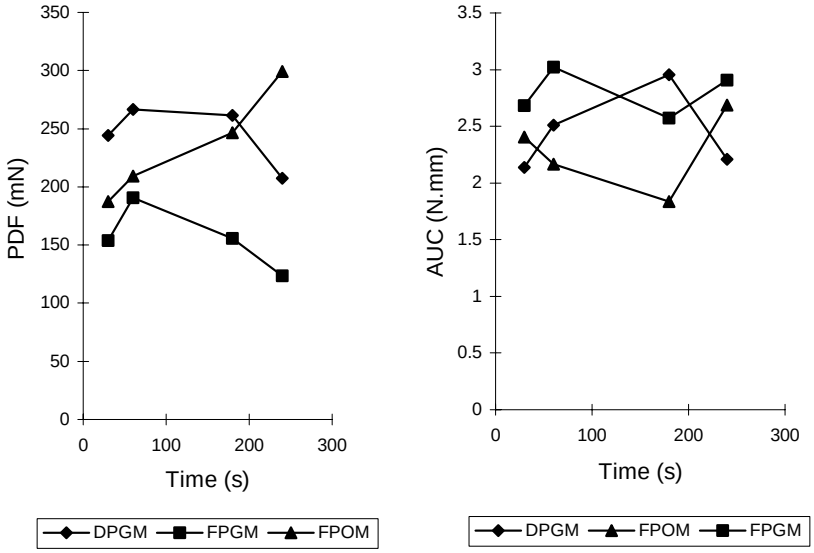


Fig. 1. Bioadhesion testing of product 4 at 60 s using three different substrates.



(a) PDF (b) Work of adhesion

Fig. 2. Bioadhesion of product 4 with contact time using three different substrates (a) PDF and (b) work of adhesion.

lowed by DPGM, with the weakest bioadhesive force being observed with FPOM, for both peak detachment force and work of adhesion (Fig. 3). The bioadhesion seen with product 15 is greater than that observed with product 4 for all substrates. When the peak detachment force of product 15 is examined with contact time, a decline in bioadhesion is seen with FPGM after 60 s, there is little change observed over time with DPGM and there is an increase in the value with FPOM over time, resulting in the FPOM showing the greatest peak

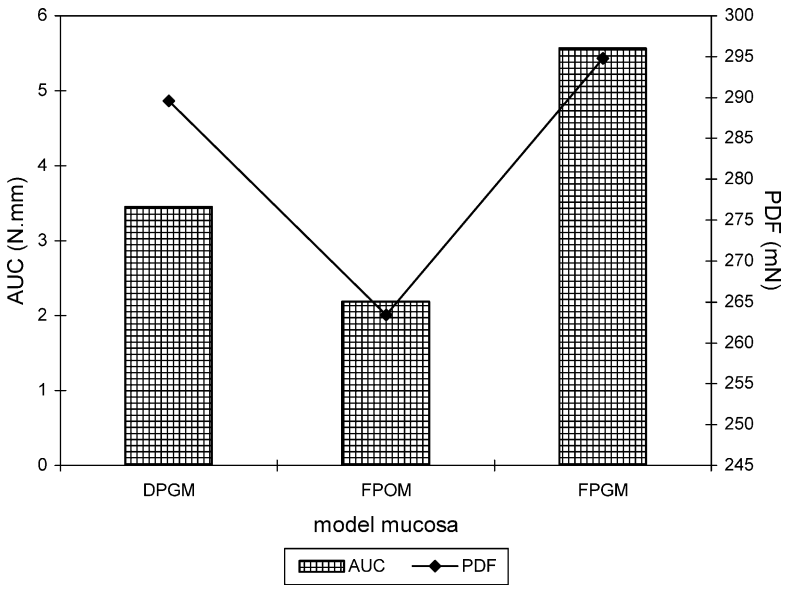
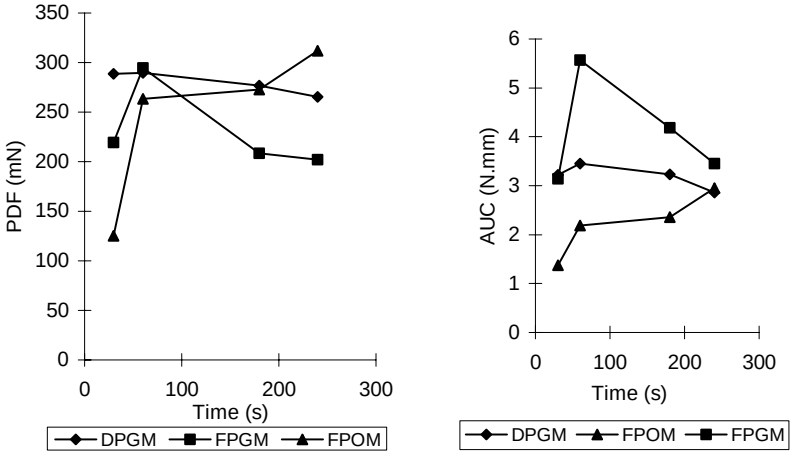


Fig. 3. Bioadhesion of product 15 at 60 s using three different substrates.



(a) PDF (b) Work of adhesion

Fig. 4. Bioadhesion testing of product 15 with contact time using three different substrates (a) PDF and (b) work of adhesion.

detachment force at 240 s (Fig. 4). The work of adhesion follows the same trend, however at 240 s all three substrates show approximately the same work of adhesion.

3.3. Rheology

Both *Carbopol 974* 1% and 0.5% show similar response over the frequency range, with a plateau response being achieved at 2 Hz approximately for 1% and 4 Hz approximately for 0.5%, continuing until the final result taken at 10 Hz (Fig. 5). The storage modulus G′ (indicative of the solid-like or elastic component of the material) of *Carbopol 974P* 0.5% is seen to be half the G′ for the 1% preparation throughout the frequency range, indicating that the G′ in this particular *Carbopol 974P* sample would appear to be concentration-dependent throughout the frequency range applied. The *Carbopol 974P* 0.5% shows almost equal storage and loss modulus (G″, indicative of the liquid-like component of the material) at plateau frequencies.

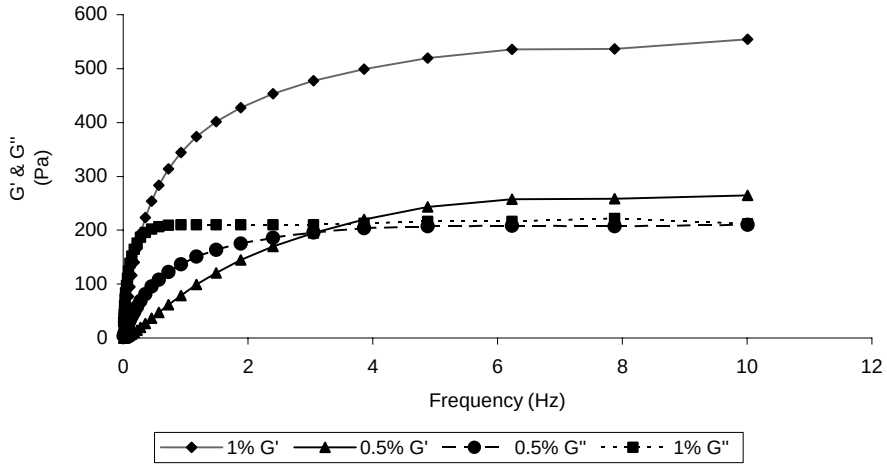


Fig. 5. The storage and loss modulus of *Carbopol 974P* 1% and 0.5%.

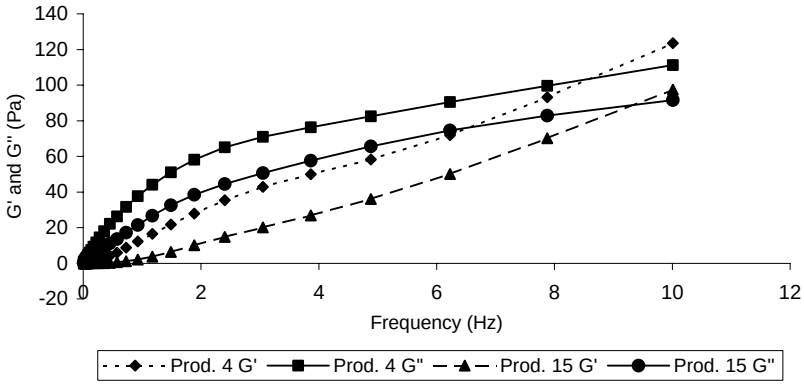


Fig. 6. The storage and loss modulus of product 4 and product 15.

Product 4 and product 15 contain a number of added excipients. The presence of these excipients cause a significant drop in the apparent viscosity of the system (Fig. 6) when compared to *Carbopol 974P* 1%. The excipients cause a significant change in the dynamic oscillatory rheology of the *Carbopol 974P* polymer system, with a large loss in the storage modulus and a change in the frequency dependence of the polymer. The storage modulus of product 4, which contains *Carbopol 974P* 1% shows a reduction to less than the storage modulus of *Carbopol 974P* 0.5%. The plateau effect seen with both *Carbopol 974P* 1% and 0.5% is not observed over the frequency range investigated with product 4 or product 15, with a gradual increase in the storage modulus being seen over the entire frequency range examined. The position of the intersection of G′ and G″ is also significantly changed, with an increase to much higher frequencies of between 8 and 9 Hz, depending on the particular formulation being examined. The loss modulus for product 4 is greater than the storage modulus over the frequency range up to 8.5 Hz, where a cross-over occurs between G′ and G″. Product 15 again shows a decrease in the storage modulus in comparison to product 4, with the difference being more significant at lower frequencies. Product 4 can be seen to have a slight up curve over the frequency range 0.1–4 Hz, with the curve flattening out above this point.

The presence or absence of the various excipients also has a pronounced effect on the intersection point of the storage and loss moduli (Fig. 7). The absence of the sweeteners xylitol and sorbitol, while causing a reduction in the storage modulus does not affect the cross-over point between G′ and G″, so they would not appear to have an impact on the polymer conformation. In the absence of triclosan, SLS and the salts, the intersection point shifts significantly downwards to 5 Hz approximately, with the storage and loss moduli almost equal in profile up to that point.

3.4. Correlation between oscillatory rheology and bioadhesion

It is evident from Figs. 6 and 7 that the addition of various excipients has a profound effect on the rheology of the *Carbopol 974P* polymer dispersion, with significant changes in the storage modulus, loss modulus and overall viscoelasticity being observed. The strong viscoelastic response seen with the *Carbopol 974P* 1% is far less evident in both product 4 and product 15, with the plateau effect observed in *Carbopol 974P* 1% from frequencies of 5 Hz and upwards not present in product 4 and product 15.

The relevance of these changes in oscillatory rheology is of considerable significance in relation to the trends observed during bioadhesion testing. It is clear from Fig. 8 that the lower the tan δ (ratio of G′ to G″), the greater the bioadhesive force achieved. This would point to elasticity being an important factor in determining bioadhesion, which has been previously noted in the literature (De Vries and Boddé, 1998).

3.5. Rheological synergism

Rheological synergism is widely used to examine the mucoadhesive properties of polymers. The

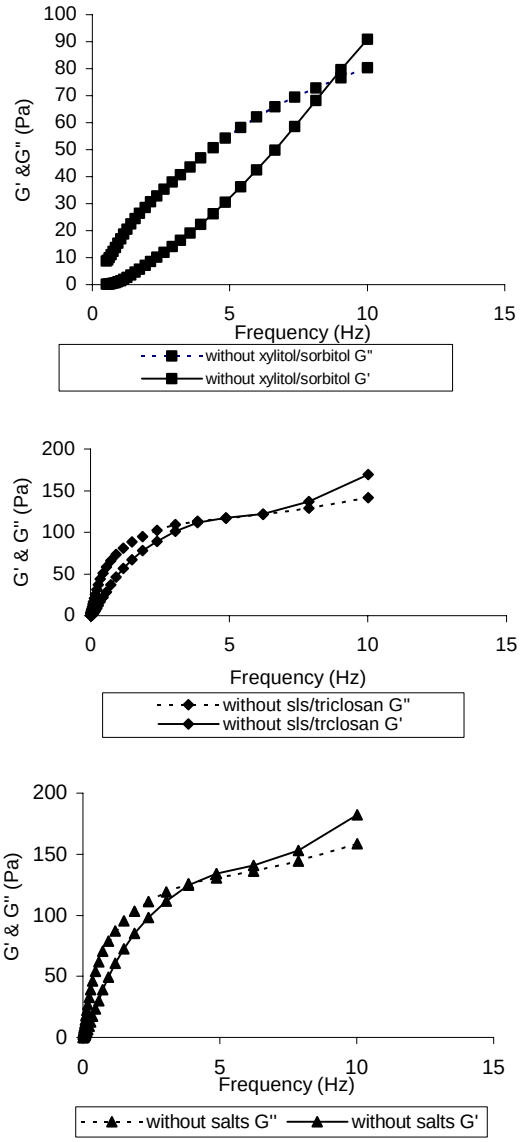


Fig. 7. The effect of excipients on the storage and loss moduli of *Carbopol 974P* 1%.

method simulates the interpenetration layer in the mucoadhesion process (Hägerström et al., 2000). This is a proposed mechanism of mucoadhesion, where the mucus and the mucoadhesive molecules become firstly entangled and secondly form non-covalent bonds (Mortazavi and Smart, 1994). The systems examined are outlined in Table 3. The *Carbopol 974P* 1%/mucus (system 3) at a representative frequency of 1 Hz showed no change in G′ and a reduction in G″ (Fig. 9). This resulted in a reduction in the tan δ, pointing to the system becoming more elastic in structure. It is evident that there is a significant drop in the G′ and G″ in system 3 compared to system 7. The frequency at which G′ and G″ intersect is lowered by the presence of the mucus, pointing to a change in the physical conformation of the system. A strong positive interaction was observed for product 4 and product 15 with a significant increase being seen in the storage modulus of both when mixed with mucus. This is considered to be due to interpenetration and secondary chemical bonding occurring between the system and the mucus. Product 15 shows a greater increase in its elasticity.

3.6. Friction testing

It is clear from Fig. 10(a) that the friction coefficient of *Carbopol 974P* 1% increases rapidly with time. It reaches 0.5 at 40 min. This is most likely due to dehydration of the polymer dispersion over time, resulting in the polymer dispersion becoming more concentrated, stickier and therefore having a greater friction coefficient. This increasing high friction coefficient would tend to give an unpleasant feeling and consistency in the oral cavity. Therefore, it is necessary to modify the polymer system to produce a lower friction coefficient. Product 4 has a low friction coefficeint for the first 6900 s (115 min), as seen in Fig. 10(b) and after that a gradual increase is seen to 9900 s (165 min), with a more rapid increase occurring thereafter. The friction coefficient of 0.5 is not reached until 15,000 s (250 min), which is a six-fold increase in time over the *Carbopol 974P* 1% polymer dispersion alone. Product 15 (Fig. 10c) has the lowest initial friction coefficient of the samples examined, with a value as low as 0.18 approximately, where it remains until 7,800 s (130 min). Thereafter, a gradual increase in the friction coefficient occurs with time, with it only reaching $\cong$ 0.3 by the end of the experiment at 18,000 s (300 min).

3.7. Comparison with commercial products

The main xerostomic products available on the Irish market are Luborant, Glandosane and Bioxtra gel. Luborant has very low apparent viscosity and gave no significant bioadhesion on testing, indicative of poor

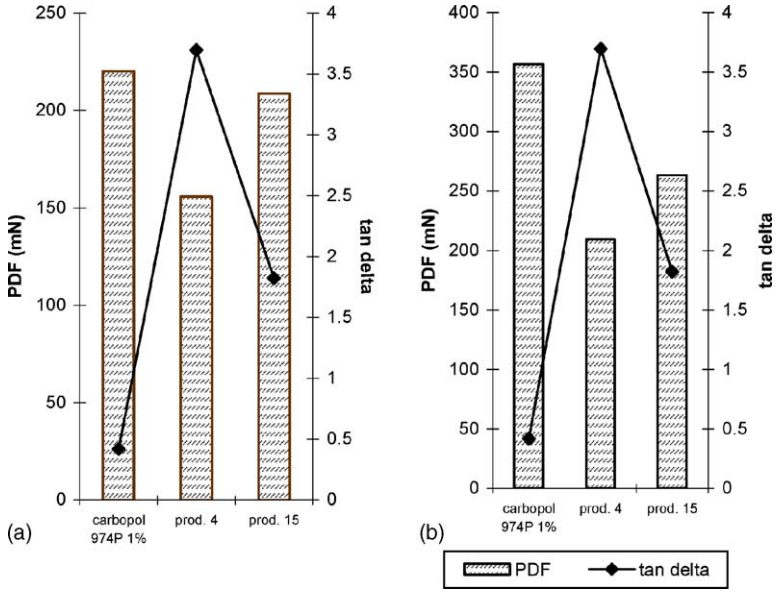


Fig. 8. Relationship between tan δ and PDF for (a) FPGM and (b) FPOM.

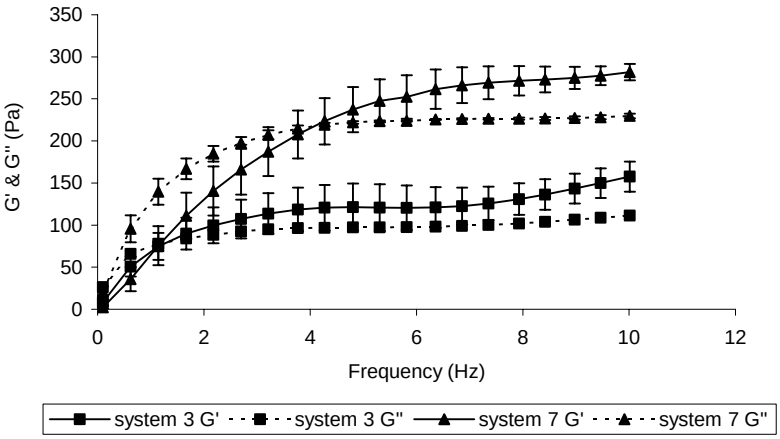


Fig. 9. Rheological synergism of *Carbopol 974P* 1%/mucus (system 3) compared to polymer alone (system 7).

residence time in the mouth. Glandosane has a low pH and is therefore suitable for use only in edentulous patients. Bioxtra gel shows an oscillatory profile similar to *Carbopol 974P* 1%, with the intersection between storage and loss moduli occurring at a lower frequency, indicating a gel structure with a higher level of cross-links than entanglements. The bioadhesive profile shows Bioxtra gel to have relatively good bioadhesion, similar to that seen with product 4 but significantly below that seen with products 15 or 17.

4. Discussion

The three model substrates give different patterns of bioadhesion dependent on the test substance used, and they also give different results as to which system has the strongest bioadhesion. Overall *Carbopol 974P* 1% and product 15 show the strongest bioadhesion, with their bioadhesion being almost identical with FPGM and DPGM. The bioadhesion of *Carbopol 974P* 1% was significantly greater than product 15 when using

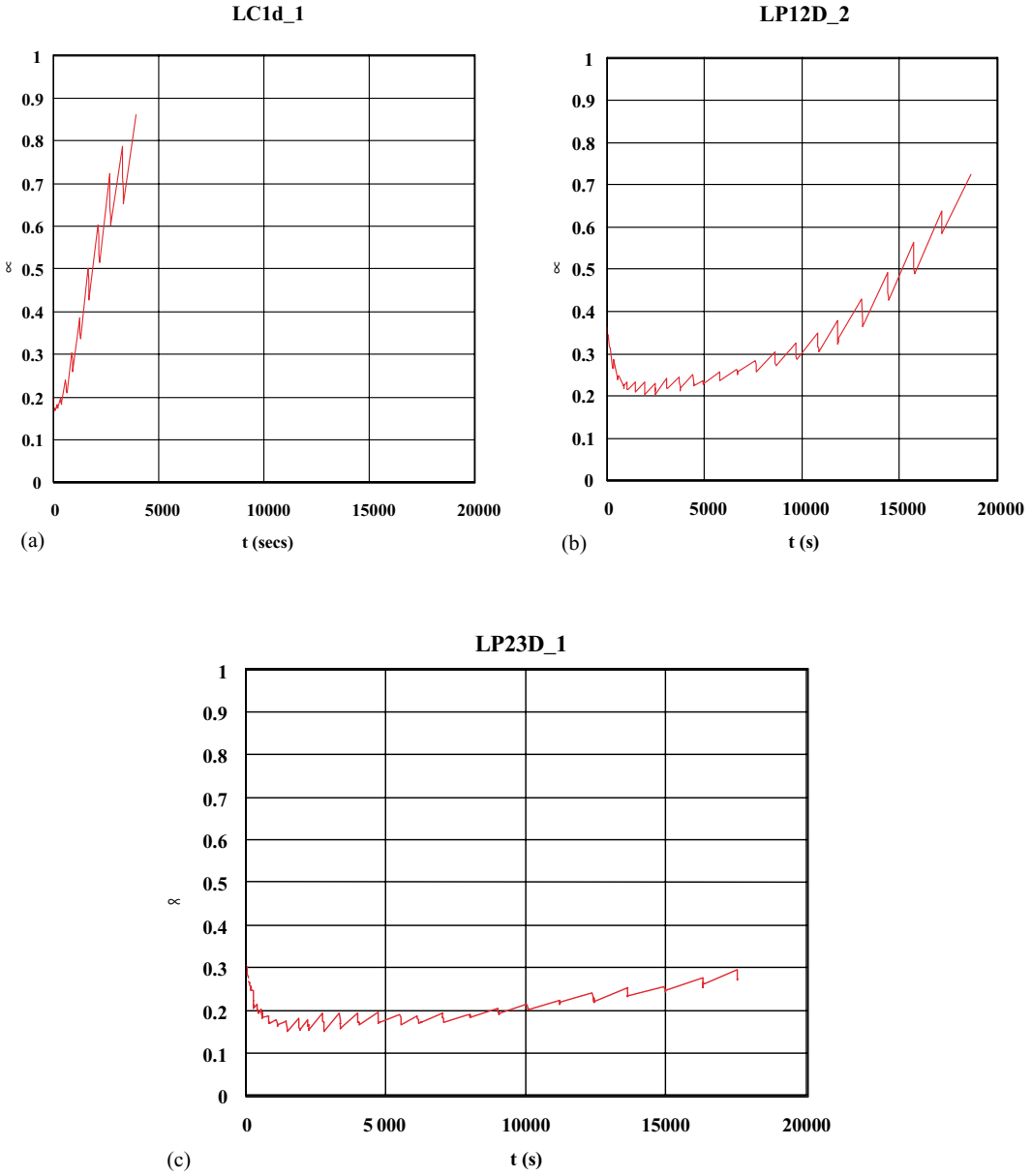


Fig. 10. Lubricity testing of (a) *Carbopol 974P* 1%, (b) product 4 and (c) product 15.

FPOM. Product 15 at nearly all times and with all substrates showed greater bioadhesion than product 4. The reduction in bioadhesion of product 4 and product 15 compared to *Carbopol 974P* 1% is most likely due to the presence of salts in the formulation of products 4 and 15. *Carbopol* polymers have been shown to be highly sensitive to ions, with electrolyte presence affecting bioadhesion to mucus. In composition, the only difference between product 4 and product 15 was the addition of 5% olive oil to the latter product. The presence of the oil appears to cause an increase in the bioadhesive force of the product, which could be due to a number of reasons. It could cause a change in the chemical conformation of the polymer resulting

in exposure of more binding sites. More likely the presence of the oil could help reduce overhydration at the mucus/polymer interface by acting as a barrier to penetration by water, resulting in a reduction in the rate at which hydration is able to occur. The presence of sunflower oil 5% (product 17) instead of the olive oil 5% caused only a slight drop in bioadhesion. Some increase in bioadhesion was seen when the sunflower oil was increased to 10% (product 18), however, it was still less than that observed with product 15. It is clear from these studies that the substrate used, the time allowed for bioadhesion to occur and the product tested all affect the results obtained.

The rheology of the products and *Carbopol 974P* was also examined. *Carbopol 974P* 1% gave comparatively flat curves, indicating that cross-links or entanglements within the gel prevent any substantial arrangement of the molecules, with the width of the plateau reflecting the degree of association within the gel. A plateau region that extends into the low frequency region reflects a highly cross-linked structure as was seen in the cross-over of G′ and G″ for 1% *Carbopol 974P*, which occurs at a much lower frequency than in the 0.5% *Carbopol 974P*, with the 1% plateau occurring before the 0.5%. It is obvious from the values determined that the *Carbopol 974P* 1% is predominantly elastic, with the storage modulus being of an order of three times greater than the loss modulus at plateau frequencies.

The presence of the salts in the gel formulation causes significant changes in the rheology of the gel, with their removal resulting in the largest increase in storage modulus, followed by the effect of removal of the triclosan and SLS (Fig. 7). The removal of the xylitol and sorbitol causes a reduction in the storage modulus, implying that the sweeteners contribute to the conformational structure of the polymer. This could be due to the large quantity of sweetener present, which acts to increase the structural integrity of the system. These results are to be expected, as the ionic nature of the salts will suppress electrostatic repulsion between molecules of the polymer resulting in it reverting to a more coiled form. This reduction in the expanded nature of the polymer will prevent the polymer chains coming into contact with one another and so will prevent contact occurring between polymer chains. This results in a change to the rheological profile with a lower storage modulus and a higher loss modulus, as the system will not have significant cross-linking but will have a much higher level of polymer chain entanglement.

These rheological results correlate well with bioadhesion results. *Carbopol 974P* 1% is shown to be a strongly cross-linked and entangled system, which would make it difficult for mucin chains to penetrate into the polymer (Fig. 5). Figs. 6 and 7 show the reduced level of cross-linking due to the presence of the excipients as they cause the polymer chains to revert to a partially coiled form. This will allow for inter-penetration and entanglement of polymer and mucus chains, which explains the increased bioadhesion seen with FPGM for product 4 and product 15, when compared to FPOM.

Friction testing was used to give an estimation of the lubricity of the product. There was a significant drop in the friction coefficient of product 4 compared to *Carbopol 974P* 1% which is probably in part due to the reduced apparent viscosity of the sample. However, the further reduction in the friction coefficient seen with product 15 cannot be simply explained by a reduction in apparent viscosity, as product 15 and product 4 have approximately the same apparent viscosity. The increase in the friction coeffecient over time for the *Carbopol 974P* is most likely due to dehydration of the sample, but this occurs more slowly in product 4 due to the reduced amount of water present and the increased number of excipients which help to retain the water. Product 15 shows lower friction coefficients than product 4, which is to be expected when an oil is added due to its lubricating nature. What is more interesting is the length of time that the friction coefficeint remains at this low level. The most likely reason for the lack of increase in the friction coefficient over time is that the oil forms a barrier to evaporation, preventing the sample from dehydrating to the same level as the two other samples.

Collectively these findings help in the selection of a preliminary product for testing in xerostomic patients and provide insight as to how the formulation of the product might be altered if in vivo testing warrants it.

5. Conclusions

As previously discussed, xerostomia patients can either suffer from prolonged total or partial loss of sali-

vary function. Where there is total loss there will be little mucus present in the oral cavity. It is therefore important that the product be able to adhere to the altered oral mucosal tissue. Product 15 showed good bioadhesion with FPOM, having a bioadhesion only 26% less than the *Carbopol 974P* 1% alone, suggesting that it should have prolonged activity in vivo. This product also showed good bioadhesion in the presence of FPGM as another mucosal substrate representative of oral conditions in xerostomic patients. This would suggest that this product would be suitable for use in patients suffering from partial or complete loss of salivary function. The problem of the tackiness of the polymer dispersion has been reduced with the addition of oil, providing good lubricity to product 15. Product 17 gave similar friction reduction and other properties to product 15, the change of lubricant to sunflower oil improving the taste and was considered to be the optimal formulation developed for in vivo testing. It was also considered likely to be more clinically effective than existing commercial products because of its ability to provide wider symptomatic relief in xerostomic patients.

Healthy human volunteers, while possibly useful for taste studies, are not suitable for modelling conditions in the oral cavity of patients requiring localised treatment with the intended product. Preliminary short-term studies in two patients suffering from xerostomia confirmed that product 17, has acceptable taste, mouth feel, lubricity and permanence, warranting its extensive long-term trial in such patients, where the pertinent correlation between the in vitro formulation development studies described in this project and successful clinical treatment needs to be established.

References

Blanco-Fuente, H., Villa-Dorrio, B., Anguiano-Igea, S., Otero-Espinar, F.J., Blanco-Mendez, J., 1996. Tanned leather: a good model for determining hydrogels bioadhesion. Int. J. Pharm. 138, 103–112.

Bhargave, H.N., Leonar, P.A., 1996. Triclosan—applications and safety. Am. J. Infect. Control 24, 209–218.

British National Formulary, 2002. The Pharmaceutical Press, London.

Brown, L.R., Dreizen, S., Handler, S., Johnston, D.A., 1975. Effect of radiation-induced xerostomia on human oral microflora. J. Dent. Res. 54, 740–750.

Chaushu, G., Bercovici, M., Dori, S., Waller, A., Taicher, S., Kronenburg, J., Talmi, Y., 2000. Salivary flow and its relation with oral symptoms in terminally ill patients. Cancer 88, 984–987.

De Vries, M.E., Boddé, H.E., 1998. Hydrogels for buccal drug delivery: properties relevant for mucoadhesion. J. Biomed. Mater. Res. 22, 1023–1032.

Duxbury, A.J., Thakker, N.S., Wastell, D.G., 1989. A double-blind cross-over trial of a mucin containing artificial saliva. Br. Dent. J. 166, 115–120.

Edgar, W.M., 1992. Saliva—its secretion, composition and functions. Br. Dent. J. 172, 305–312.

Ettinger, R.L., 1996. Review: xerostomia: a symptom which acts like a disease. Age Ageing 25, 409–412.

Furomoto, E.K., Carter-Hanson, C., 1998. Subjective and clinical evaluation of oral lubricants in xerostomic patients. Spec. Care Dent. 18, 113–118.

Gaffar, A., Scheri, D., Afflitto, J., Coleman, E.J., 1995. The effect of triclosan on mediators of gingival inflammation. J. Clin. Periodontol. 22, 480–484.

Hägerström, H., Paulsson, M., Edsman, K., 2000. Evaluation of mucoadhesion for two polyelectrolyte gels in simulated physiological conditions using a rheological method. Eur. J. Pharm. Sci. 9, 301–309.

Hamlet, S., Faull, J., Klein, B., Aref, A., Fontanesi, J., Stachler, R., Shamsa, F., Jones, L., Simpson, M., 1997. Mastication and swallowing in patients with postirradiation xerostomia. Int. J. Rad. Oncol. Biol. Phys. 37, 789–796.

Harrap, G.J., Saxton, C.A., Best, J.S., 1983. Inhibition of plaque growth by zinc salts. J. Periodontol. Res. 18, 634–642.

Horrobin, D.F., 1984. Essential fatty acid metabolism in diseases of connective tissue with special reference to scleroderma and to Sjögrens syndrome. Med. Hypotheses 14, 233–247.

Jones, D.S., Woolfson, A.D., Djokic, J., Coulter, W.A., 1996. Development and mechanical characterisation of bioadhesive semi-solid, polymeric systems containing tetracycline for the treatment of periodontal diseases. Pharm. Res. 13, 1734–1738.

Levine, M.J., Aguirre, A., Hatton, M.N., Tabak, L.A., 1987. Artificial salivas: present and future. J. Dent. Res. 66, 693–698.

Mortazavi, S.A., Carpenter, B.G., Smart, J.D., 1993. A comparative study on the role played by mucus glycoproteins in the rheological behaviour of the mucoadhesive/mucosal interface. Int. J. Pharm. 94, 195–201.

Mortazavi, S.A., Smart, J.D., 1994. Factors influencing gel strengthening at the mucoadhesive-mucus interface. J. Pharm. Pharmacol. 46, 86–90.

Mossman, K.L., Henkin, R.I., 1978. Radiation-induced changes in taste acuity in cancer patients. Int. J. Rad. Oncol. Biol. Phys. 4, 663–670.

Olsson, H., Axéll, T., 1991. Objective and subjective efficacy of saliva substitutes containing mucin and carboxymethylcellulose. Scand. J. Dent. Res. 99, 316–319.

Plankhurst, C.L., Dunne, S.M., Rogers, J.O., 1996. Restorative dentistry in the patient with dry mouth. Part 2. Problems and solutions. Oral Med. Dental Update.

Rosling, B., Dahlén, G., Volpe, A., Furuichi, Y., Ramberg, P., Lindhe, J., 1997. Effect of triclosan on the subgingival microbiota of periodontitis-susceptible subjects. J. Clin. Periodontol. 24, 881–887.

Schiødt, M., Dodd, C.C., Greenspan, D., Daniels, T.E., Chernoff, D., Hollander, H., Wara, D., Greenspan, J.S., 1992. Natural history of HIV associated salivary gland disease. Oral Surg. Oral Med. Oral Pathol. 74, 326–331.

Sweeney, M.P., Bagg, J., Baxter, W.P., Aitchison, T.C., 1997. Clinical trial of a mucin containing oral spray for the treatment of xerostomia in hospice patients. Palliative Med. 11, 225–232.

Svatun, B., Saxton, C.A., van der Ouderaa, F., Rolla, G., 1987. The influence of a dentrifice containing a zinc salt and a nonionic antimicrobial agent on the maintenance of gingival health. J. Clin. Periodontol. 14, 457–461.

Waaler, S.M., Rölla, G., Skjörland, K., Ögaard, B., 1993. Effects of oral rinsing with triclosan and sodium lauryl sulphate on plaque formation: a pilot study. Scand. J. Dent. Res. 101, 92–105.

Weisenfeld, D., Stewart, A.M., Mason, D.K., 1983. A critical assessment of oral lubricants in patients with xerostomia. Br. Dent. J. 10, 155–157.

Whelton, H., 1996. In: Edgar, W., O'Mullane, D.M. (Eds.), Saliva and Oral Health, second ed. British Dental Association. pp. 1–9 (Chapter 1).