Role of mucoadhesive polymers in retention of toothpaste in the oral cavity

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Abstract

Retention of active ingredients of toothpastes in the mouth following brushing determines the efficiency of these oral care formulations. In this study, new *in vitro* methodologies for the observation and measurement of toothpaste retention in the oral cavity were developed and used to evaluate the efficiency of formulations containing different mucoadhesive hydrophilic polymers. The findings suggest that using Carbopol ETD 2020 and Carbopol Ultrez 10 as binders in toothpaste prolongs the retention time of these formulations in the oral cavity. The *in vitro* methodologies tested, coupled with texture analysis, were able to accurately characterise the behaviour of the toothpaste and produce detailed images showing how it is retained in the oral cavity. This study has not only produced a new method for studying the behaviour of toothpaste and other formulations in the oral cavity but is also the first to investigate how different types of mucoadhesive binders can be used to improve toothpaste retention.

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1. Introduction

Toothpaste formulations have continued to improve over the last century, from simple powders which contained very few active ingredients and simple flavours to the complex formulations which we have today [1]. Modern formulations are capable of cleaning, repairing, and protecting teeth whilst also leaving the user with a clean and pleasant feeling which in turn boosts their confidence. Although the quality of oral care products has greatly improved, dental caries and oral diseases are on the rise due to the increased availability of sugary foods and drinks and an increase in acidic drinks [2-4]. As a result, there is a need to further increase the effectiveness of oral care products to improve and maintain global oral health [5]. Flavouring agents included in these formulations contribute significantly to the overall experience of the product and leave a lasting impression on the consumer [6]. The pleasant aftertaste and mouthfeel left after brushing however is short-lived, with many flavour components and active ingredients being rapidly washed off the oral mucosal surfaces. Active ingredients, flavour compounds and any residual ingredients of the formulations are quickly removed by swallowing, the use of mouthwash post brushing, dissolution in saliva and removal from the oral cavity, or the mastication and consumption of food. As a result, some of the ingredients do not have the necessary time required at their active sites and their beneficial effects are soon lost [7]. For example, fluoride compounds need to be retained in the salivary reservoir in sufficient quantity to remineralise enamel. Work by Duckworth et al [8] however found that salivary fluoride concentration post brushing depletes rapidly. To overcome these issues and provide greater effectiveness and cleaning experience, the retention of these ingredients in the oral cavity needs to be improved.

All toothpastes use hydrophilic polymers as binding agents which hold the product together and thicken the formulation; these polymers are a major contributor to the formulations retention and control the release of active ingredients in the oral cavity [9-10]. Both natural polymers and synthetic polymers are used as binding agents in the oral care industry with carboxymethyl cellulose, xanthan gum, carrageenan, and poly(acrylic acid)-based polymers such as Carbopol being the most common. All these polymers are well documented mucoadhesives and their binding properties have been thoroughly investigated [11-14]. Mucoadhesion is the adhesion between one material and a mucosal surface of an organism which provides temporary retention [15-16]. By retaining the toothpaste for longer in the oral cavity, the release and clearance of active ingredients can be reduced, prolonging their residency. By studying the retention of these polymers in the oral cavity when incorporated into toothpaste, new formulations with enhanced retention of active ingredients can be developed to combat the rising number of dental caries worldwide [17-19].

When looking at the retention of active ingredients in a toothpaste, a suitable marker is needed which can mimic the ingredients and provide an accurate way of measuring their movement/retention in the oral cavity. As the active ingredients and flavour compounds used in formulations are not fluorescent, a fluorescent marker (fluorescein sodium) was used as it allows for the tracking and measurement of the toothpaste whilst providing an image of where the toothpaste is retained in the oral cavity, giving a more detailed view at how it adheres to the enamel and mucosal surfaces.

Currently, very little research has been carried out on how mucoadhesive toothpaste formulations are retained and how they adhere to mucosal surfaces in the mouth, with few papers published on mucoadhesive polymers enhancing toothpaste formulations [20]. In addition to this, we have been unable to find a standardised *in vitro* method for measuring retention of toothpaste. Therefore, novel methods designed specifically to observe and measure the retention and interaction of toothpaste in the oral cavity were developed in this study. Sheep oral mucosa with similar properties and teeth size to that of humans was used to develop the *in vitro* method [21]. These methods are designed to track and observe toothpaste and oral care formulations and to see how it interacts with different parts of the oral cavity, improving our understanding of where these formulations adhere potentially allowing future formulations to be tailored to specific conditions or to target specific areas in the mouth.

This study is the first to design specific methodologies for measuring toothpaste retention in the oral cavity and observing how mucoadhesive polymers can improve their retentive properties. Our hypothesis is that toothpaste formulations which incorporate compounds that exhibit strong mucoadhesive properties may persist in the oral cavity for longer, retaining the flavour compounds and active ingredient in the oral cavity for longer, extending the residence time, delaying release and prolonging their benefits. The results from this study will expand our knowledge on how oral care products work in the oral cavity, allowing the development of better oral care formulations which could improve the oral health of millions of people.

2. Experimental Section

2.1 Materials

Described in Supporting information.

2.2 Formulation of toothpaste using natural polymers

Sorbitol (200 g) and sodium benzoate (4 g) were added to water (250 mL) and stirred using an overhead mixer. Sodium hydroxide (1.2 g) was added during mixing and mixed until fully dissolved. Either xanthan gum (9.6 g), sodium carboxymethyl cellulose (9.6 g), Chitosan (9.6 g) or xanthan gum (6 g) with carrageenan (2 g) were added to glycerol (80 g) and were premixed before adding to the sorbitol solution and stirred for 1 h. TC15 silica (28 g), AC77 (92 g) and titanium dioxide (4 g) were slowly added to the hydrated polymer gel and mixed with a Silverson Industrial High Shear Mixer (UK) at a rate of 2000 rpm until a paste formed. Sodium laureth sulfate (SLS) (12 g) was added to water (80 mL) and mixed at 50 °C in a water bath until dissolved except in the case of the chitosan formulation. SLS solution and cocamidopropyl betaine (11.8 g) was added to the paste and mixed. The pH of the completed formulations was between 6-7. Rheological properties of toothpaste was compared to commercial formulations.

2.3 Formulation of toothpaste using synthetic polymers

Sorbitol (200 g) and sodium benzoate (4 g) were added to water (250 mL) and stirred using an overhead mixer. Either Carbopol 971P NF (9.6 g), Carbopol 974P NF (9.6 g), Carbopol ETD 2020 (9.6g) Carbopol Ultrez (9.6 g) or Gantrez S97 (9.6 g) was added to sorbitol solution and mixed for 1.5 h. TC15 silica (28 g), AC77 (92 g) and titanium dioxide (4 g) were mixed with polymer gel using a Silverson shear mixer at 2000 rpm. Then glycerol (80 g) was added. Sodium laureth sulfate (SLS) (12 g) was added to water (80 mL) and mixed at 50 °C in a water bath until dissolved. SLS solution and cocamidopropyl betaine (11.8 g) was added to the paste and mixed. Sodium hydroxide (1M) was used to adjust the pH of the formulation to pH 6-7. Rheological properties of toothpaste was compared to commercial formulations.

2.4 Rheology

Rheological properties of toothpaste samples were analysed using an Anton Paar MCR 302 rheometer fitted with a 40 mm diameter rotating plate adjusted to 25 °C. Samples were allowed equilibrate before being warmed to 25 °C in a water bath prior to being placed on the lower plate surface that was also at 25 °C and equilibrated for 3 min. All tests were run in triplicate. A total of 24 data points were generated during shearing with the shear rate measured over a rate of 0.01-100 s^{-1}.

2.5 Preparation of fluorescently labelled toothpaste

All samples were prepared by adding sodium fluorescein (0.1 % w/w) to toothpaste (1 g) on a glass disc and mixed until a homogenous paste was formed. The toothpaste was checked under the fluorescence microscope to ensure even distributed throughout the toothpaste. Increasing amounts of these formulations were dispersed in 3.5 L water to prepare standards for calibration. Aliquots of these standard dispersions (3 mL) were taken and their fluorescence intensity measured to establish a correlation between fluorescence intensity and the weight of toothpaste remaining on the jaw after washing (Figure S1).

2.6 *In vitro* brushing experiment

Fresh lamb jaws were obtained from Newman's Abattoir, Farnborough (UK). The specimens were immersed in a water bath set to 37 °C for 30 min prior to the experiment to simulate the oral cavity temperature. The jaw was taken out of the bath and images, using the fluorescence microscope, were taken to provide a background for comparison with later images. Fluorescent images were captured using a Leica MZ 10F fluorescent microscope (Germany) with the microscope settings standardised throughout all experiments: exposure time 57 ms, gain 10x, gamma 1, pseudocolor 527 nm and intensity 3. Sample fluorescence was measured using a Jasco FP-6200 spectrofluorometer (Japan) at λ_{ex} 460 nm and λ_{em} 512 nm. Pictures of the front teeth, the tongue and the molars were taken (Figure S2, Supporting information). An electrical toothbrush (Oral B®) was used to brush teeth and the tongue. Fluorescently labelled toothpaste (1 g) was placed on the toothbrush head and applied on the lamb model following the American Dental Association (ADA®) guidelines: the outer, the chewing and inside surfaces of all teeth, and the upper side of the tongue were brushed by gently moving the toothbrush back and forth for 2 min with the electric toothbrush held at a 45° angle to the gums. Images of the jaw were taken after toothpaste application. The jaw was then placed upside down in the water bath with a magnetic stirring bar creating a flow inside the bath. After 30 s, the jaw was removed from the water and images of the four areas were taken. An aliquot of water (3 mL) was taken after each wash for analysis by a Jasco FP 6200 fluorescence spectrometer with a peak excitation at 494 nm and peak emission at 512 nm. Images were taken after the jaw was immersed in water for 30, 60, 120, 300 and 600 s using a fluorescent microscope. The experiments were all conducted in triplicate. Fluorescent images were analysed using ImageJ software (NIH).

2.7 Wash off_{50} test

Wash off_{50} (WO50) test measuring how much saliva is required to reduce fluorescence observed by half, were performed to verify the findings of the *in vitro* brushing experiment (Figure S3). Background photos were taken with a fluorescent microscope before toothpaste application. Fluorescently labelled toothpaste (0.1 g) was brushed onto a sheep's tongue cut into 3 cm^2 squares. The tongue was photographed and artificial saliva (KCl (10 mM), $CaCl_2$ (4 mM), $NaHCO_3$ (2 mM), KH_2PO_4 (6.7 mM) and NaCl (7 mM) prepared in deionised water as described in [16]) was dripped at 1 mL/min onto the tongue to mimic the natural flow of stimulated saliva [22]. The tongue was then photographed and measurements were taken at 1, 3, 5, 7 and 10 mL. The WO50 values were calculated according to the method reported in [22].

2.8 Texture analysis

Adhesion experiments were carried out using a custom made mucoadhesive probe on a TA-XT plus, Stable Micro Systems, (UK). The contact time between the probe and the tissue was 30 s with 500 g of force before pulling apart with a removal speed of 1 $mm \cdot s^{-1}$. Sheep's tongues were used in the probe and on a platform as the surface to which the toothpaste (1 g) was applied (Figure S4). Each area of the tongue in the probe and platform was cut into 3 cm^2 sections and secured on the top container and bottom platform of the TA. Before each experiment, the tongue tissue section was conditioned with 100 µL of AS and incubated at 37 °C. During testing, artificial saliva (1 mL) was added to the bottom tissue and surrounded by water at 37 °C to maintain the tissue temperature during the test.

2.9 Statistical analysis

Described in Supporting information

3. Results

3.1 Formulation and rheology of toothpastes

All toothpastes were made following either the natural polymer or synthetic polymer recipes to provide a fair and accurate comparison between formulations and to eliminate any advantages that may be gained by adding further ingredients. The rheological properties of the toothpastes were measured as any that deviated significantly from the physical characteristics of a standard toothpaste would not be suitable for commercial use. The rheological behaviour of toothpaste is identified as that of a yield-stress, thixotropic material with a time-dependent behaviour. The application of shear stress to the toothpaste causes its viscosity to decrease allowing it to flow. The rheological properties of toothpastes are generally controlled by thickeners, abrasives and structure builders. By matching the rheological properties of the toothpaste as close to three commercial toothpaste, a better comparison can be drawn as toothpaste is renowned for having a characteristic rheological profile [23]. If the formulation was too thick then its retentive properties may be due to its rheological properties as opposed to its mucoadhesive behaviour. Too thin or watery formulations will not be retained on mucosal surfaces or the toothbrush when being applied. The rheological profiles of all toothpastes showed that as the shear rate increased and more stress was applied, the viscosity decreased. Figure 1 shows the rheological profiles of the novel and commercial formulations.

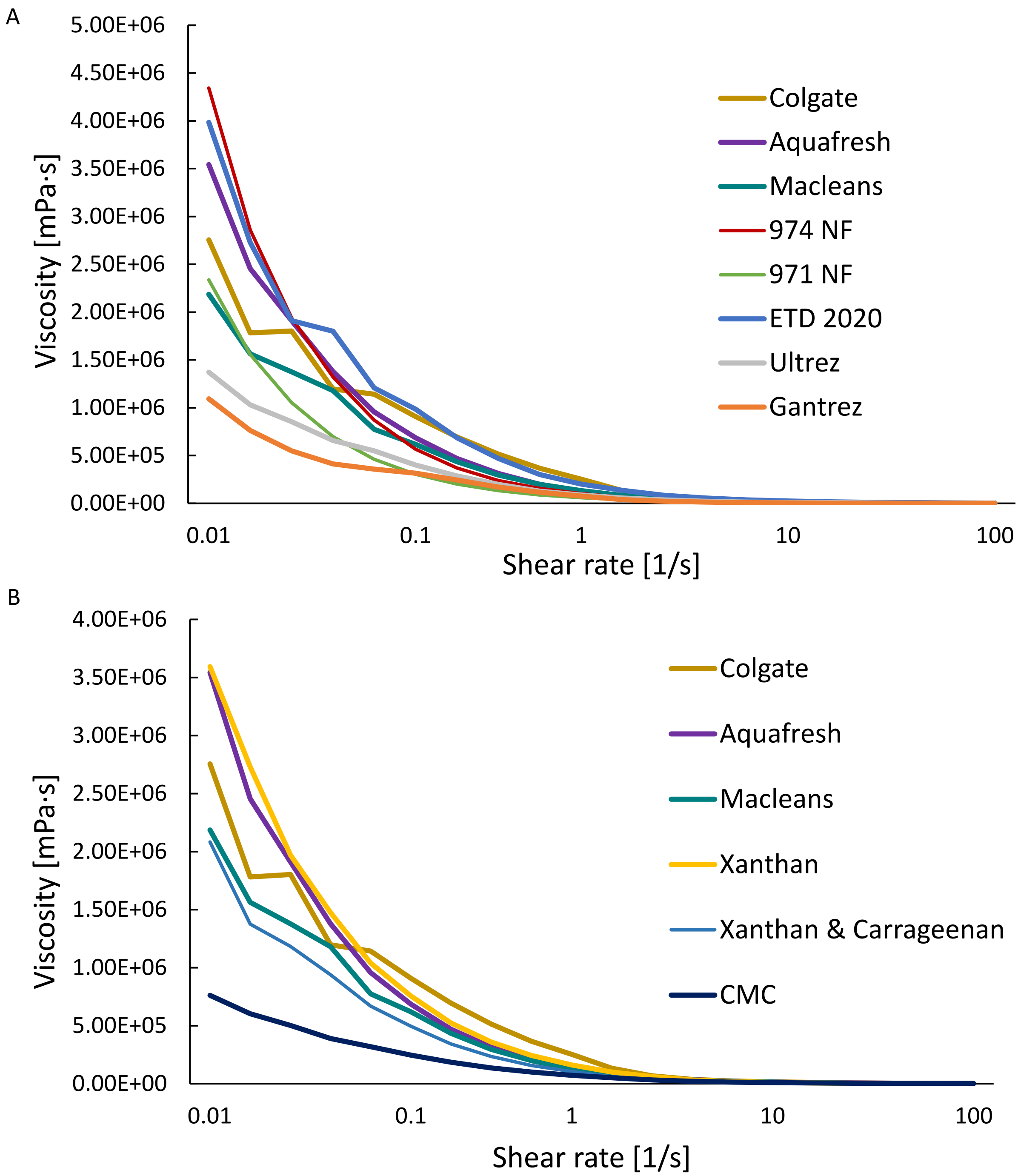

Figure 1 Rheological profiles of (A) synthetic polymer formulations and (B) natural polymer formulations compared to commercially available toothpastes

The initial viscosities of the commercial toothpaste varied although similar thickening agents are used in each formulation. The difference in viscosity could be down to different manufacturing techniques or different ingredients used in the formulation. The change in toothpaste viscosity from a semi-solid paste with a high viscosity to a free flowing one as shear rate increases is characteristic of Bingham plastics [11]. The novel toothpaste viscosity varied depending on polymer used with the Carbopol based toothpaste generally having higher initial viscosities with the exception of Carbopol Ultrez 10. This is unusual as Carbopol Ultrez 10 is known to have a very high viscosity in respect to some of the other polymers tested. However, by 18 s^{-1}, all toothpaste viscosities had reached near zero. The Carbopol polymers generally have a higher viscosity than the natural and commercial toothpaste due to their weakly-crosslinked microparticulate nature; at higher pHs these microparticles swell and may form a gel network [24]. Poly(acrylic acid)-based polymers are known to exhibit strong mucoadhesive properties as they are able to bind to mucins through hydrogen bonding [25]. The pH range for all the toothpastes tested were between 6 – 7; in order to keep them in line with the 3 commercial pastes which had a pH range of 6.3 - 6.7 and to mitigate any additional benefits from having a higher or lower pH. A lower pH in Carbopol formulations could increase the number of free carboxylic acid groups leading to an increase in hydrogen bonding and improved mucoadhesive properties of the formulation.

Although the toothpaste made with Ultrez 10 & Gantrez S97 had low initial viscosities compared to the commercial formulations, the retention studies and texture analysis results showed they have high retentive properties indicating that a low viscosity of the formulation does not necessarily mean it will have a low retention time in the oral cavity.

3.2 *In vitro* brushing test

The *in vitro* brushing test was developed to provide an accurate image of how toothpaste behaves and interacts inside the oral cavity with the focus on the teeth which were grouped into the front, the molars and the tongue as these are the most brushed areas of the mouth. The ability to track the toothpaste and observe where it is retained will improve the way formulations are developed, made and tested (Figure 2). The method allows a comparison between different toothpaste and oral care formulations. The retention profiles of the toothpaste (Figure 3) show that by varying the binding agent used in the formulations, the retention time of the toothpaste in the oral cavity changes. All formulations showed a decrease in fluorescence observed during the washing process indicating there was a loss of toothpaste from the mucosal and enamel surfaces.

Retaining toothpaste on the front teeth is considerably harder than on the molars as the front teeth have smooth surfaces which make it difficult for toothpaste to adhere. This means there is minimal physical protection for the toothpastes resulting in their quick removal. The paste once washed from the teeth, is either removed from the oral cavity or accumulates around the gum line where most of its action takes place. For this reason, the retention of toothpaste on the front teeth was measured on and around the tooth's surface.

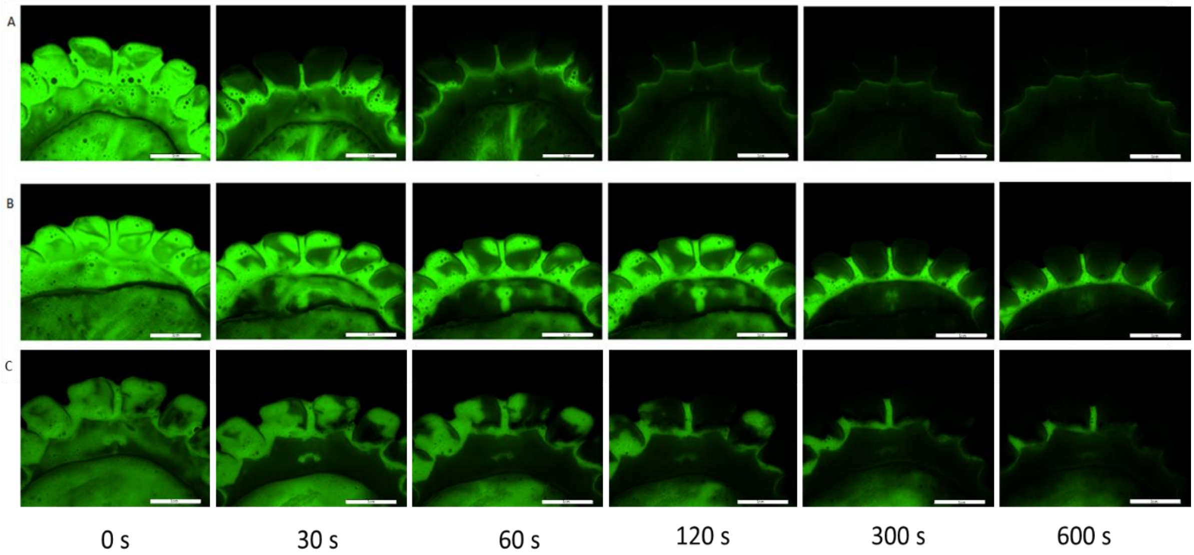

Figure 2 Fluorescence images of the front teeth after being brushed with fluorescently labelled (A) Colgate; (B) Carbopol Ultrez 10; (C) Carbopol ETD 2020 NF. Scale bar 1 cm

The Carbopol Ultrez and Carbopol ETD 2020 NF formulations were found to retain on the front teeth and the gum line for longer than the commercial brand over the course of the experiment, with other novel toothpaste formulations being retained longer at various points throughout the test. After 30 s of washing, the Colgate toothpaste was removed from the teeth whilst the formulations made from Carbopol ETD 2020 NF and Carbopol Ultrez 10 were still present on the teeth at 120 s. Retention on the teeth up to 120 s suggests that the toothpaste is able to form strong bonds with the mucus coating the teeth, allowing it to remain for longer than the other formulations. This is beneficial as the fluoride compounds are retained at their active site for longer improving the toothpastes performance. All the toothpastes exhibited the same pattern of washing off the enamel surface and gathering on the gum line and around the teeth, albeit at different rates, with the intensity of fluorescence observed reducing as time increases. The fluorescence observed in Figure 2 around the gum line and between the teeth is still intense after 600 s of washing indicating that a reasonable amount of toothpaste is still being retained. Unsurprisingly, the CMC novel formulation that uses the same binding agent as the Colgate toothpaste had similar fluorescence levels observed over time (Figure 3), suggesting the two toothpastes behave similarly although manufactured in different conditions with a more basic recipe indicating it is only the binder that plays a role in the retention of these formulations.

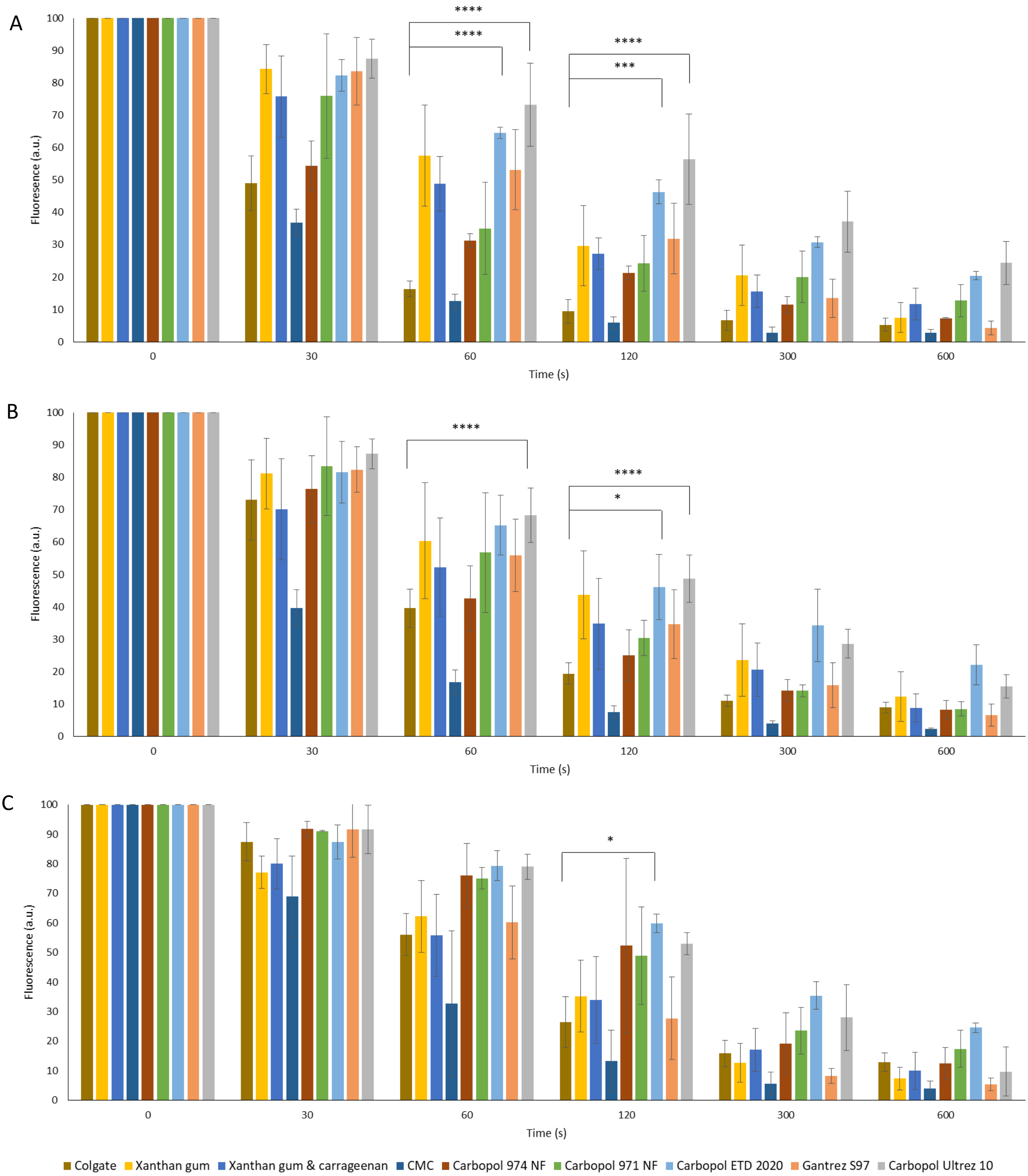

Figure 3 Fluorescence levels of all toothpaste formulations deposited on the (A) front teeth, (B) molars and (C) tongue tested using the developed in vitro method. All toothpastes were measured after being applied to the front teeth (0 s) and during the rinsing step at 30, 60, 120, 300 and 600s. Error bars represent standard deviation.

Generally, the novel formulations tested showed higher levels of fluorescence observed when compared to the Colgate toothpaste indicating it had a higher retention of the fluorescein. Overall, the retention of most toothpastes on the front teeth was low due to the smooth nature of the enamel surface when comparing Figure 2 with S5 & S6 with most of the toothpaste retaining around the gum line. Of the toothpastes tested, Carbopol ETD 2020 and Carbopol Ultrez 10 had the highest observable fluorescence at 600 s indicating they had the highest retention of fluorescein during the tests.

The morphology and gaps in between the molars provide protection for the toothpaste, slowing the rate at which it is washed away as shown in Figure S5. Once applied, the toothpaste covers the sides and fills the pits and grooves on the enamel surface. These spaces provide a certain level of protection for the formulations leading to an increase in their retention time. The Carbopol Ultrez and 2020 formulation can be seen between and around the teeth throughout the washing. Because of this added protection, the decrease in fluorescence observed for the toothpastes was slower than compared to the front teeth.

Once again, the CMC formulation showed poor retention throughout the test losing over 50% of observed fluorescence after 30 s of washing. The fluorescence observed from the Colgate toothpaste at 120 s, had decreased by ~ 80% with the Carbopol 2020 & Ultrez formulations only decreasing by ~ 50%. This decrease in fluorescence levels can be seen in Figure S5, where the toothpaste has been washed from the enamel and between the teeth with only residue around the gum line still visible whereas the Carbopol 2020 and Ultrez formulations however still had visible toothpaste around the molars at 120 s and even up to 600 s. These results again suggest that the Carbopol formulations are more mucoadhesive and retain longer than the commercial toothpaste on the molars.

The tongue, although not always brushed by the consumer, is still coated in toothpaste during brushing and contains the taste buds which will detect the cooling sensation from the menthol used in formulations. The large mucosal surface area and rough morphology of the tongue presents an ideal surface for the toothpaste to adhere to as the binders used are mucoadhesive and their presence will improve the overall retention of the formulations. All tests were performed on the front half of the tongue (Figure S6) as this is where the tongue would be brushed as the pharyngeal reflex would prevent or discourage brushing the back of the tongue. The gradual decrease of the fluorescence observed indicates that although toothpaste is being lost from the tongues surface, the more gradual decrease in fluorescence indicates that the toothpaste is retained on the surface better than it was on the front teeth. This would be due to it being a large mucosal surface and the binding agents used in these oral care formulations are all known mucoadhesives.

The results from the tongue retention showed that all the fluorescence levels observed decreased at a more gradual rate than for the front teeth and the molars as there are no enamel surfaces that the formulations can be removed from quickly when they are washed. This increased retention benefits the flavour oils and sweeteners in oral care formulations as a longer retention at their active sites, the longer lasting the taste.

After 30 s of washing, all the toothpastes showed a similar level of fluorescence intensity which indicated minimal toothpaste had been washed off the tongue. By 120 s the fluorescence levels observed for the Colgate toothpaste were roughly half of what was measured for the Carbopol 2020 & Ultrez formulations as more has been washed off the tongue. This can be seen in Figure S6 where toothpaste can still be seen in a sizeable quantity on the tongue for the Carbopol formulations, but only a residue for the Colgate paste. By 600 s, the vast majority of toothpaste has been washed off the tongue as seen in the images and fluorescence data with the Carbopol ETD 2020 and Ultrez formulation having higher final fluorescence levels than the other toothpaste.

The results from these tests show that the retention of a toothpaste is related to the binding agent used in the formulation. By using a polymer which exhibits strong mucoadhesive properties such as Carbopol, the amount of toothpaste lost from the oral cavity during rinsing can be reduced. The loss of a specific toothpaste can be attributed to its ability to form strong physical bonds with the mucosal surfaces of the oral cavity as toothpaste as a whole does not bind with the smooth enamel surfaces but can bind with the mucin coating them. Longer retention of formulations in the oral cavity will allow the increased retention of flavour oils, sweeteners and other anti-gingival actives on the tongue and mucosal surfaces as well as retaining fluoride compounds on or around the enamel surfaces. The *in vitro* results indicate that poly(acrylic acid)-based formulations containing Carbopol ETD 2020 & Carbopol Ultrez 10 have the longest retention on all the surfaces that were tested. This is down to the presence of carboxyl groups and their ability to form hydrogen bonds with the mucin oligosaccharide side chains and secondly, the long chains of the Carbopol ETD 2020 and Ultrez polymers increasing the number of hydrogen bonds that are able to form, and in turn increasing the mucoadhesion of the formulation [26]. Overall, the methodology developed was able to measure the retention of toothpaste in the oral cavity and provide high quality images showing where the toothpaste is retained, allowing for a more detailed and accurate model for the development of toothpaste and other oral care formulations.

From this test, it was decided that additional retention studies would be done on Carbopol 2020 and Ultrez 10 as these were the formulations that were retained the longest, and Gantrez S97 as although it had average performance in the test, it has known enhancement solubility of water insoluble compounds such as flavour oils in oral care formulations.

3.4 Toothpaste weight loss test

In addition to measuring the observed fluorescence, samples of the water used in section 3.3 were analysed using a fluorescence spectrometer as the more fluorescently labelled toothpaste that is washed off the jaw and dispersed into the water, the higher the fluorescein concentration in the water will be. Using the data obtained and creating calibration curves for each labelled toothpaste (Figure S1), the amount of toothpaste washed off the jaws could be calculated (Figure 4).

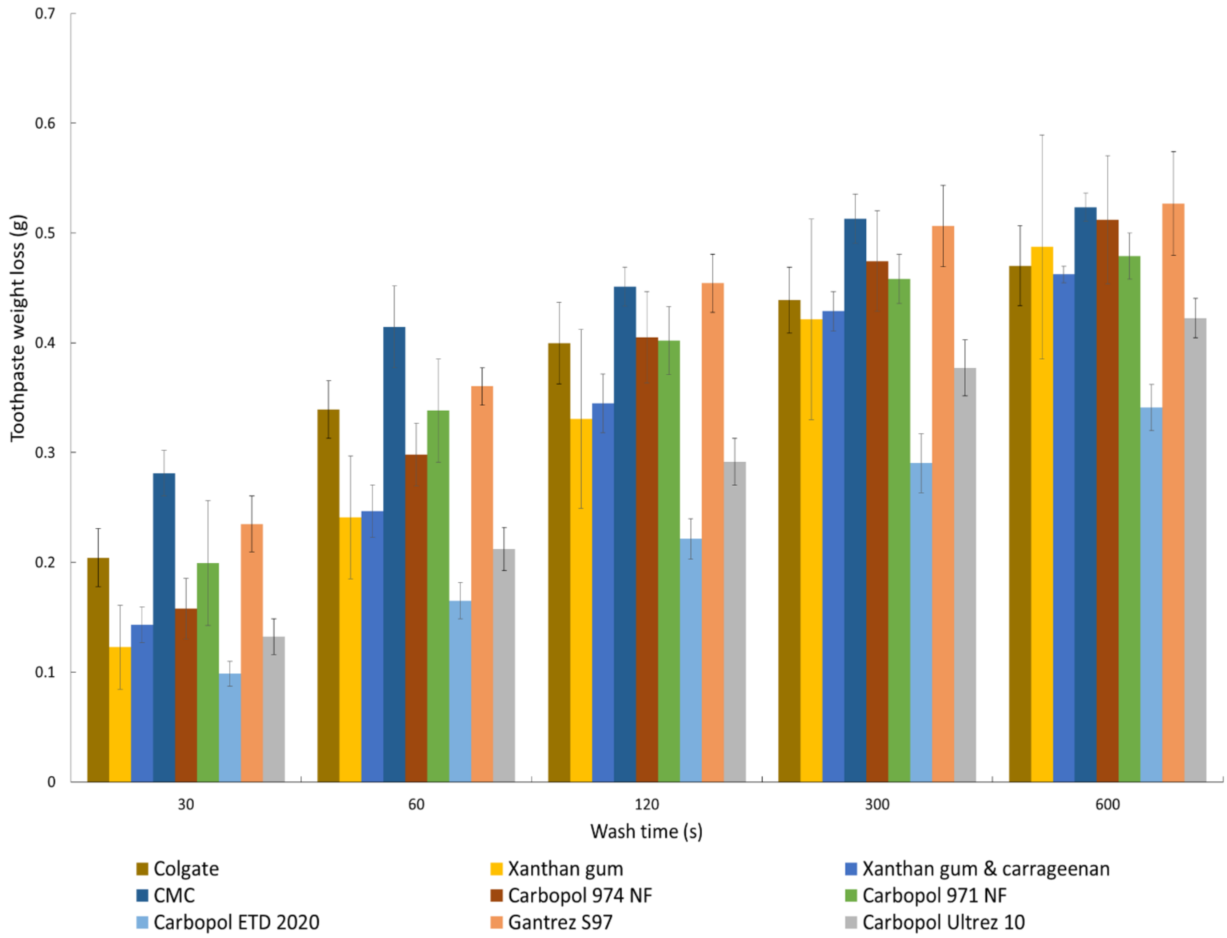

Figure 4 Toothpaste weight loss of all toothpastes tested calculated using fluoresce spectroscopy and calibration curves with regards to the length of time they are washed for. All test were repeated in triplicate and mean values are shown. Error bars represent standard deviation.

The Carbopol ETD 2020 and Ultrez 10 toothpaste lost the least amount of weight during the test which is consistent with the higher observed fluorescence over time in the previous test. This indicates that these formulations are retained the longest on the mucosal surfaces in the oral cavity.

3.5 Wash off$_{50}$ test.

To further verify the findings of the developed *in vitro* method discussed in 3.2, a standard wash off test for measuring retention was adapted to calculate the WO50 values for the formulations on oral mucosal surfaces [16, 27]. For this, three novel and one commercial toothpastes were applied to the tongue and washed with artificial saliva over a period of 10 min (Figure S7). The results from this test (Figure 5) showed that the novel formulations required more washing with AS to remove 50 % of the fluorescence observed than the commercial toothpaste. All toothpastes showed a decrease in fluorescence observed as the formulation was washed with more artificial saliva, with the Carbopol 2020 toothpaste requiring more AS to reduce the fluorescence levels by half.

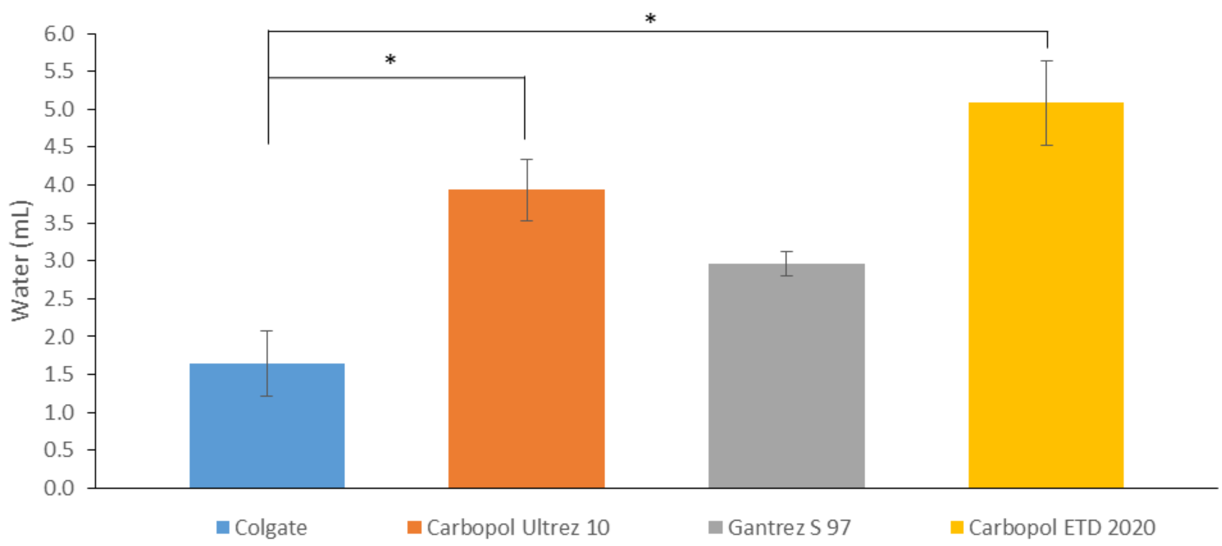

Figure 5 Calculated WO50 results for the commercial and novel formulations. Error bars represent standard deviation.

The results from this test support the findings of the developed method and show it is a feasible option for testing and developing toothpastes, and indicated that the three novel formulations tested have a higher retention in the oral cavity than the commercial toothpaste due to their improved mucoadhesive properties.

3.6 Texture analysis

Texture analysis was performed to find the maximal force of detachment required to separate the probe from the toothpaste and the total work of adhesion that was calculated from the area under the curve. A chitosan formulation was made to test its adhesive properties as it is commonly used as a positive control in tensile strength test of mucoadhesion. However, the formulation dried out quickly once brushed making it not commercially viable so it was not tested further than the texture analysis. The impractical nature of the chitosan formulation meant it could not be used in the other tests conducted. Of the toothpaste tested, the maximal force of attachment values for the chitosan was the highest (Figure 6). Chitosan is known to have some of the strongest mucoadhesive properties of any polymer which explains why it requires the highest amount of force to detach [28]. Further development of the chitosan toothpaste could lead to a longer retaining formulation.

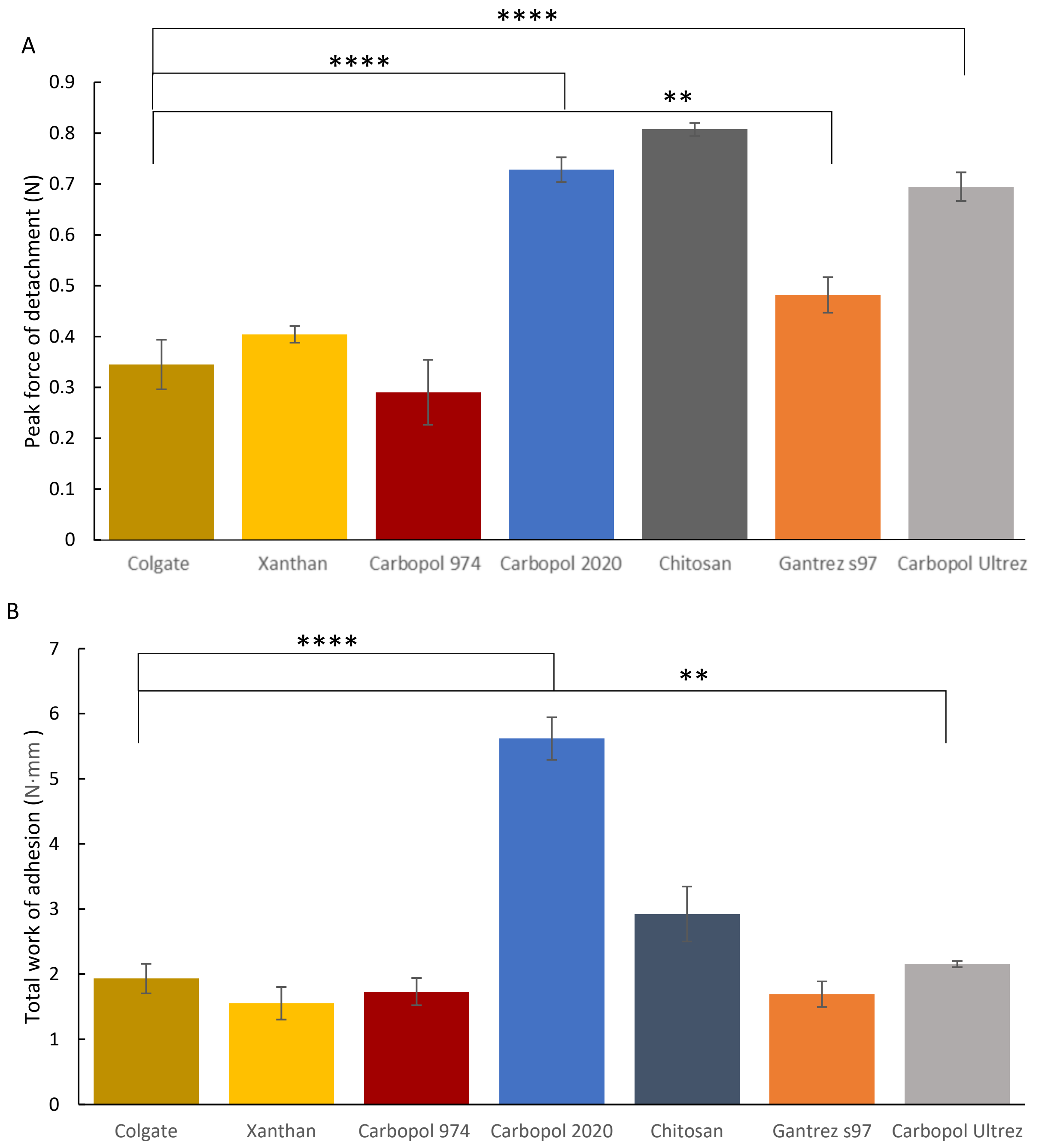

Figure 6 (A) Maximal force of detachment and B) total work of adhesion for novel and commercial toothpaste formulations determined by texture analysis. All samples were repeated in triplicate. Error bars represent standard deviation.

Carbopol ETD 2020 toothpaste had a high peak force and highest total work of adhesion which is consistent with the results from the in vitro and wash off tests. Most of the toothpastes tested had very similar maximal force of detachment and were grouped around 0.3 – 0.6 N and total work of adhesion of between 1 and 2 N·mm indicating that these formulations have very similar mucoadhesive properties. Although the Carbopol Ultrez formulation had a low viscosity in the rheological testing, it still had a high maximal force of detachment and total work indicating that the viscosity of the formulation is not necessary linked to its retention, but rather the polymer used and its mucoadhesive properties play a more important role.

4. Conclusion

The results from this study show that thickening agents used in toothpaste formulations affect their retention on mucosal surfaces in the oral cavity and, by using polymers with strong mucoadhesive properties, the retention time of the formulation can be prolonged. Formulations that can retain for longer will provide the most benefit to the users as it potentially means active ingredients can be held at their active sites for longer. Formulations containing Carbopol Ultrez 10 and Carbopol ETD 2020 NF were shown to be able to prolong the retention of the formulation in the oral cavity and adhere not only to mucosal surfaces, but also to enamel. The *in vitro* methodologies developed for this study provide a new way to measure the retention of oral care formulations and observe how they interact with teeth and mucosal surface in the mouth. These easy to use and inexpensive models provide a new and accessible means of testing toothpastes and oral care formulations in an *in vitro* environment. The findings of this study have the potential to change the way oral care formulations are made and tested with more knowledge on how different polymers affect retention and how these formulations behave in the oral cavity.

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