



# A randomised oral fluoride retention study comparing intra-oral kinetics of fluoride-containing dentifrices before and after dietary acid exposure

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## ABSTRACT

**Objective:** This exploratory, randomised, single-blind, crossover, study evaluated fluoride and calcium ion concentrations and pH following use of one of two 1450 ppm fluoride (NaF), 5% w/w KNO<sub>3</sub> dentifrices: (1) test dentifrice (with cocamidopropyl betaine) with an orange juice (OJ) rinse; (2) test dentifrice with a deionized (DI) water rinse or (3) comparator dentifrice (with sodium lauryl sulphate and tetrasodium pyrophosphate) with an OJ rinse.

**Design:** Eighteen participants used their assigned dentifrice, rinsed with DI water, then expectorate was collected. Sixty min post-brushing, participants rinsed with OJ or DI water then expectorate was collected. Saliva samples were collected pre-brushing and at 1, 5, 10, 15, 30 and 60 min post-brushing and following the 60 min OJ/DI water rinse. The pH of samples was taken.

**Results:** Significant differences ( $p < 0.05$ ) were found in salivary fluoride ion concentrations between test and comparator dentifrices at 30 and 60 min and following the 60 min OJ rinse, favouring the former. Significant differences were also found between test and comparator dentifrices for salivary calcium ion concentration at 1, 5 and 10 min ( $p < 0.0001$ ), favouring the former, and between test or comparator + OJ rinse and test + water rinse ( $p < 0.005$ ), favouring the latter. No pH differences were shown prior to OJ/water rinse. Products were generally well-tolerated.

**Conclusions:** Results confirmed that acid-labile fluoride is released from the oral cavity following a dietary acid challenge and showed that formulation excipients may impact on retention of such.

## 1. Introduction

Erosive tooth wear, the cumulative loss of hard dental tissue by a chemical-mechanical process not involving bacteria, can lead to changes in contour and surface morphology of teeth (Carvalho et al., 2015). Such erosion can compromise dental function, result in dentine hypersensitivity, and have negative aesthetic consequences (Barbour & Rees, 2006). The primary cause of erosive tooth wear is frequent exposure to acid via gastric hydrochloric acid (Moazzez & Bartlett, 2014) or dietary citric, phosphoric, or acetic acid (Barbour & Lussi, 2014). The early

stages of enamel erosion are thought to involve the release of minerals from enamel following diffusion of acids through the acquired salivary pellicle releasing minerals from the enamel (Lussi, Schlueter, Rakhmatullin, & Ganss, 2011). It is generally agreed that the condition can be reversed at this stage as a mineral scaffold exists for mineral deposition by calcium and phosphate ions naturally present in saliva (Amaechi & Higham, 2001). However, if the softened layer of enamel is not re-hardened and the acidic environment continues, progressive softening and dissolution of enamel can lead to permanent loss of volume with a softened layer superficial to the remaining sound tissue (Lussi

**Abbreviations:** AE, adverse events; ANCOVA, analysis of covariance; ANOVA, analysis of variance; CaF<sub>2</sub>, calcium fluoride; CI, confidence interval; DI, de-ionised; ITT, intent-to-treat; KNO<sub>3</sub>, potassium nitrate; NaF, sodium fluoride; OJ, orange juice; OST, oral soft tissue; PP, per protocol; ppm, parts per million; SLS, sodium lauryl sulphate; TISAB, total ionic strength adjustment buffer; w/w, weight/weight.

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et al., 2011) that is susceptible to wear from abrasive toothbrushing and other physical insults (Barbour & Rees, 2006).

Fluoride has been shown to protect hard dental tissue against acid-mediated dissolution and promote remineralisation by adsorbing to the surface of tooth enamel and reacting with calcium and phosphate ions found in the oral environment to form a mineral lower in solubility than the original enamel (ten Cate, 1999). Continuous exposure to slightly elevated fluoride concentrations is required to exploit the full remineralisation benefits of fluoride (Page, 1991). Clearance studies performed with fluoride-containing dentifrices have shown that following tooth brushing, a small concentration of fluoride remains in the saliva or is taken up by oral tissues (Duckworth, 2013; Duckworth & Morgan, 1991); this is believed to be stored in the form of calcium fluoride or a calcium fluoride-like material (Vogel, 2011). It is hypothesised that fluoride ions are slowly released from this reservoir, providing elevated levels of fluoride in saliva that can be sustained for several hours (Duckworth & Morgan, 1991).

It is understood that formulation excipients in fluoride-containing dentifrices may affect the availability, delivery and performance of the active ingredients (Barlow, Sufi, & Mason, 2009). It has also been reported that the surfactant sodium lauryl sulphate (SLS) may reduce retention of fluoride in the oral reservoir (Barkvoll, Rølla, & Lagerlöf, 1988; Rølla & Saxegaard, 1990; Vogel, 2011; Pessan et al., 2006; Vogel, Shim et al., 2006), meaning that while fluoride may be initially bioavailable at the point of application, less fluoride may be retained in the oral cavity leading to less fluoride being available for release into saliva to maintain an elevated fluoride concentration.

Other ingredients, such as condensed polyphosphates (e.g., sodium hexametaphosphate, sodium pyrophosphate and sodium trimetaphosphate), are known to be strong sequestrants of calcium (Irani & Clayton, 1962) and thus these materials would be expected to negatively impact salivary calcium ion activity. Further, condensed polyphosphates have been demonstrated to prevent formation of calculus through inhibiting the formation of calcium-phosphate like species and it may therefore similarly impede the formation of the calcium fluoride-like deposits seen within plaque (ten Cate, 1997) particularly since the amount of fluoride taken up by plaque is directly linked to the plaque calcium concentration (Whitford, Wasdin, Schafer, & Adair, 2002). Thus, these excipients that interact with salivary calcium ions may result in a decrease in the formation of calcium fluoride-like deposits, the primary source of bioavailable fluoride in the oral environment (Barkvoll et al., 1988; Vogel, 2011).

This randomised, crossover, salivary clearance study evaluated the changes in fluoride and calcium ion concentrations and pH values in saliva and expectorate samples after a single brushing with a test dentifrice containing 1450 ppm fluoride as sodium fluoride (NaF) and 5% w/w potassium nitrate ( $\text{KNO}_3$ ) with cocamidopropyl betaine as a surfactant. It also investigated these concentrations following an acid challenge (OJ) or control rinse (de-ionised [DI] water) 60 min after brushing. A dentifrice with identical NaF and  $\text{KNO}_3$  concentrations but containing the excipients SLS and sodium pyrophosphate was used as the comparator. The majority of other ingredients were identical (e.g., titanium dioxide, silica, polyethylene glycol, xanthan gum).

## 2. Materials and methods

This was an exploratory, randomised, analyst-blinded, crossover, intra-oral kinetics study with three experimental periods, performed at a single centre in a UK region where the supplied tap water was not fluoridated and the natural fluoride concentration was below 0.49 mg/mL (Drinking Water Inspectorate, 2019). The protocol was approved by an independent ethics committee (Manchester Consumer Healthcare Research Ethics Committee) and the study was conducted in accordance with the protocol, local regulations, and the Declaration of Helsinki. All enrolled participants provided written informed consent before undergoing study procedures. There was one amendment to the protocol, an

administrative change that did not affect study flow or outcomes. Anonymised individual participant data and study documents can be requested for further research from [www.clinicalstudydatarequest.com](http://www.clinicalstudydatarequest.com).

### 2.1. Participants

Eligible participants were aged between 18 and 65 years, with good general and oral health. Participants were required to have a minimum of 20 permanent natural teeth, a stimulated whole saliva flow rate  $\geq 0.8$  mL/min and an unstimulated whole saliva flow rate  $\geq 0.5$  mL/min at the screening visit. Exclusion criteria included: pregnancy; breast feeding; presence of a chronic debilitating disease; a xerostomia-causing condition; diabetes; known or suspected intolerance or hypersensitivity to the study materials; currently taking antibiotics or have taken them within 2 weeks of the screening visit; any medication that could affect salivary flow or cause xerostomia; use of multivitamins, and/or calcium or fluoride supplements within 7 days of the treatment phase. Dental exclusions included: evidence of untreated caries; gross periodontal disease; tongue or lip piercing or presence of dental implants; professional tooth cleaning or dental treatment during study; oral surgery or extraction within 6 weeks of the screening visit; oral symptoms including lesions, sores, or inflammation.

### 2.2. Study design and treatment

A unique screening number, assigned in ascending numerical order as each participant signed their consent form, identified each participant screened for study participation. Participants who met all inclusion and exclusion criteria were randomised to one of the treatments according to a randomisation schedule provided by the Biostatistics Department of the study sponsor. Randomisation numbers were assigned in ascending numerical order as each participant was determined to be fully eligible. The laboratory analysts, study statistician, data management staff, and other employees of the sponsor who may have influenced study outcomes were blinded to product allocation.

At screening (Visit 1), participants who met eligibility criteria were supplied with a non-fluoride, 5%  $\text{KNO}_3$  dentifrice to use twice daily during the 7 d ( $\pm 1$  d) washout period prior to each treatment visit. Participants presented to the study site at Visits 2, 3, and 4 having abstained from brushing their teeth on the morning of each appointment. Compliance with study restrictions and wash-out dentifrice use was checked. Participants were also required to abstain from drinking tea (due to the presence of fluoride) for 12 h prior to a treatment visit; from using antacids (due to the presence of calcium) and eating and drinking from 11 pm the night before a treatment visit; and from smoking within 2 h of a treatment visit. At each study visit a full oral soft tissue (OST) examination was conducted and eligibility to continue was assessed.

Participants were randomised to one of the following groups:

- **Test dentifrice plus an orange juice (OJ) rinse 60 min after brushing**
  - o Dentifrice containing 1450 ppm fluoride as NaF and 5% w/w  $\text{KNO}_3$  plus cocamidopropyl betaine (Sensodyne® Pronamel®; UK-marketed product; GSK Consumer Healthcare, Weybridge, Surrey, UK);
- **Test dentifrice plus a DI water rinse 60 min after brushing;**
- **Comparator dentifrice plus OJ rinse 60 min after brushing**
  - o Dentifrice containing 1450 ppm fluoride as NaF and 5% w/w  $\text{KNO}_3$  plus SLS and tetrasodium pyrophosphate (Colgate® Sensitive Enamel Protect; formerly UK marketed; Colgate-Palmolive [UK] Ltd., Guildford, UK).

Participants used their assigned treatment once under supervision at the study site (one treatment per visit). They brushed, in their usual manner, with 1.5 g ( $\pm 0.05$  g) of the allocated dentifrice (measured by

study staff and applied to a dry toothbrush [as supplied: Aquafresh® Clean Control – Everyday Clean, medium stiffness bristles] for 1 min then immediately expectorated into a labelled container. This was followed by an immediate oral rinse with 10 mL of DI water for 5 s, where participants vigorously swished the water around their mouth, then again expectorated into a labelled container. At 60 min post-brushing, participants swished their mouths vigorously with 10 mL of OJ or DI water (depending on their treatment allocation) for 30 s, which was expectorated into a labelled container. After a 7 d ( $\pm 1$  d) wash-out period, participants crossed over to an alternative treatment (Visit 3) and then to the final treatment (Visit 4). The procedures at Visits 2, 3, and 4 were the same.

The OJ pH was tested prior to study commencement and found to be 3.8. The pH of the deionised water was not determined but since this was kept sealed when not being used and new supplies were used regularly, we would expect the pH to be near 7.0. These were given to participants at room temperature.

### 2.3. Study assessments

At each test visit, a saliva sample was collected pre-brushing with further saliva samples taken at 1, 5, 10, 15, 30, and 60 min post-brushing and 1 min following the OJ/DI water rinse at 60 min. The sampling tolerance was  $\pm 20$  s for the 1 min timepoint and  $\pm 1$  min up to the 60 min timepoint. Also collected were the expectorant immediately after supervised brushing, the expectorant following the DI water rinse immediately after brushing and the expectorant following the 60 min OJ/DI water rinse (Fig. 1).

#### 2.3.1. Saliva collection

For the unstimulated collection, participants sat with their head tilted down for 1 min and swallowed any pooled saliva. They then sat for 2 min (3 min for pre-brushing sample) with their head tilted down, pooled saliva in their mouth then emptied it into a collection tube. For the stimulated saliva collection (for eligibility only), participants chewed on a piece of unflavoured gum for 1 min, swallowed any pooled saliva, chewed the gum for a further 2 min, then emptied pooled saliva into a collection tube. Samples were prepared for analysis and stored in the fridge until fluoride and calcium ion concentrations and pH measurements could be determined. Samples were analysed at ambient

temperature. Analysis was fully completed within 24 h of collection.

#### 2.3.2. Sample analysis

**2.3.2.1. Fluoride concentration.** For analysis of saliva, a 0.5 g aliquot was taken following homogenisation of the bulk saliva sample using a vortex mixer for 30 s. For expectorate analysis, a 0.5 g aliquot of homogenised expectorate was taken directly from the 60 min OJ or DI water rinse expectorates, or from the supernatant following centrifugation at 4200 rpm for 20 min. for the post-brushing and post-brushing rinse expectorate. For analysis of post OJ rinse expectorate, and of the OJ itself, sodium acetate buffer was added to the 0.5 g sample (50 g of OJ) to adjust the pH to 5–5.5. For each sample (saliva, expectorate supernatant or OJ), a 1:1 ratio of TISAB II was added. Concentration of fluoride ions was then measured using two calibrated Orion™ Ionplus fluoride electrodes, according to the direct calibration method detailed in the manufacturer's instructions (Thermo Fisher Scientific Inc., Waltham, MA, USA).

**2.3.2.2. Calcium concentration.** Calcium analysis took place if sufficient sample was left following fluoride analysis. For analysis of saliva, 0.5–1.0 g was taken following homogenisation (amount taken was dependent on amount left following fluoride analysis). For analysis of immediate post-brushing and post-DI-rinse (immediate and 60 min) expectorate, 1.0 g of supernatant was taken following centrifugation. For analysis of post OJ rinse expectorate, a 5.0 g sample was taken. The direct calibration method was used to determine the concentration of calcium ions of all samples by comparison to a series of standards. The OJ rinse expectorates, and the OJ itself, had calcium ion concentration determined by the known addition method. Ionic strength adjuster was added to all calcium standards and samples in the ratio 2–2.5% of the sample volume and mV readings were determined after 5 min. Concentration of free calcium ions was measured using two calibrated Orion™ Ionplus calcium electrodes, according to the manufacturer's instructions (Thermo Fisher Scientific Inc., Waltham, MA, USA), each covering a specific concentration range. The ppm calcium value for each sample was determined from the mV.

**2.3.2.3. Salivary pH.** Analysis of pH took place if sufficient sample was left following fluoride and calcium analysis. The pH of the unstimulated

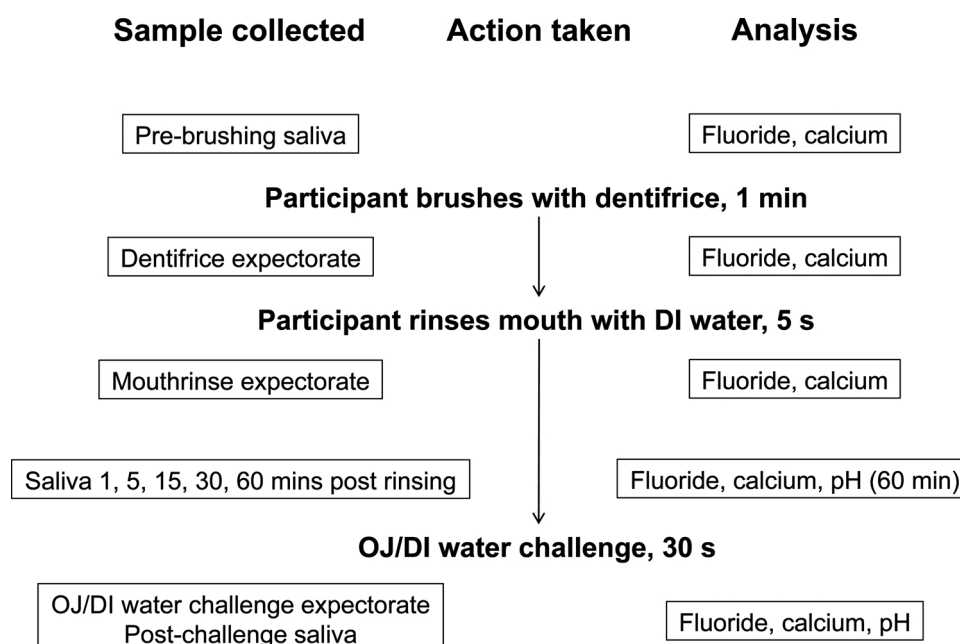


Fig. 1. Study procedures.

saliva samples just before and 1 min after the 60 min DI/OJ rinse and of the expectorate samples immediately post the 60 min DI/OJ rinse were measured using a calibrated semi-micro pH electrode (Thermo Fisher Scientific Inc., Waltham, MA, USA). The pH electrode was calibrated according to manufacturer's instructions using the pH standards 2, 4, 7, and 10.

## 2.4. Safety

Adverse events (AEs) were recorded from the start of the investigational period until 5 d following last administration of the investigational product. AEs were graded as mild, moderate or severe and assessed as to whether or not they were related to the study treatment by a trained examiner.

## 2.5. Evaluation criteria

The primary evaluation criterion for this exploratory study was the difference in fluoride and calcium ion concentrations following the OJ rinse compared with a DI water rinse 60 min after a single brushing with the test dentifrice. Secondary criteria were the differences in fluoride and calcium ion concentrations in saliva and expectorate samples following each treatment; differences in the pH of saliva and expectorates 60 min post-brushing, prior to and following the OJ or DI water rinse with each dentifrice, and change in fluoride and calcium ion concentrations and pH 60 min post-brushing before and following the OJ or DI water rinse.

## 2.6. Statistical analysis

This was an exploratory study, not powered for formal efficacy comparisons. It was planned to randomise 20 participants to ensure at least 16 completed the study. From a previous study with a similar design (data on file), this sample size was considered sufficient to assess the calcium and fluoride concentrations in saliva samples.

The safety population included all participants who fulfilled the study entry criteria and received at least one dose of study treatment. The intent-to-treat (ITT) population included all participants in the safety population who had at least one post-baseline outcome evaluation. The per protocol (PP) population was a subset of the ITT population. Participants with a protocol violation deemed to affect outcome assessments in all study periods were excluded from the PP population. Participants with a protocol violation deemed to affect outcome assessments in some (but not all) study periods were included in the PP population, but their data was excluded from the period(s) in which the protocol violation occurred. The primary analysis was performed on the PP population because it was deemed to provide better estimates for the oral fluoride retention model.

For outcome variables measured in all samples post-brushing and prior to the OJ or DI water rinse at 60 min, data from both test dentifrice arms (plus OJ or plus DI water rinse) were combined and compared with the comparator dentifrice.

Fluoride and calcium ion concentrations in saliva samples, and changes in fluoride and calcium ion concentrations and pH values from pre- to post-rinsing were analysed using analysis of covariance (ANCOVA), with treatment and period as fixed factors, participant as a random effect, and two baseline covariates (i) participant-level baseline concentration calculated as the mean baseline concentration across all periods within a participant and (ii) period-level baseline concentration minus participant-level baseline concentration. Fluoride and calcium ion concentrations in expectorate samples and pH values were analysed using analysis of variance (ANOVA), with treatment and period as fixed factors and participant as a random effect.

For all the analysis stated above, treatment differences and 95% confidence intervals are presented. All tests were two-sided and at the 5% level of significance. No adjustments for multiplicity were made. The

assumption of normality and homogeneity of variance were investigated and considered to be satisfied for all variables.

## 3. Results

The first participant was enrolled on March 24, 2014 with the last participant completing the study on 25 April 2014. A total of 53 participants were screened of whom 18 were randomised to a treatment group (Fig. 2). All 18 randomised participants completed the study and were included in the safety analysis and ITT population. One participant was excluded from the oral kinetics analysis (PP population) due to hypersalivation at a post-screening visit. Included participants were predominantly female ( $n = 13$ , 72.2%), with a mean age of 34.2 years (standard deviation 12.17; range 18–62). Treatment groups were well balanced for fluoride and calcium ion concentrations in saliva at baseline.

### 3.1. Fluoride and calcium ion concentrations in saliva

#### 3.1.1. Fluoride (Fig. 3, Table 1)

There was a sharp increase in fluoride ion concentration in saliva immediately post-brushing with both dentifrices, which decreased over time. Fluoride concentrations in the test dentifrice arms were numerically higher than in the comparator dentifrice arm at all timepoints, with a statistically significant difference observed at 30 and 60 min post-brushing ( $p < 0.05$ ). Following the OJ or DI water rinse at 60 min post-brushing, there were statistically significantly higher concentrations of salivary fluoride ions in the test + OJ rinse group compared with the comparator + OJ rinse group ( $p = 0.0436$ ). There were no other between-group significant differences.

#### 3.1.2. Calcium (Fig. 4, Table 1)

A depletion in calcium ion concentration in saliva was seen immediately after brushing and after the OJ rinse with both the test and comparator dentifrices. Statistically significant differences were observed in favour of the test dentifrice compared with the comparator dentifrice at 1, 5, and 10 min post-brushing ( $p < 0.0001$ ) and for both the test ( $p = 0.0003$ ) and comparator ( $p < 0.0001$ ) dentifrices following the OJ rinse at 60 min post-brushing compared with the test treatment after the DI water rinse at 60 min.

### 3.2. Fluoride and calcium ion concentrations in expectorates (Tables 2 and 3)

Statistically significantly higher fluoride ( $p = 0.0020$ ) and calcium ( $p < 0.0001$ ) ion concentrations were observed in the immediate post-brushing slurry expectorates for the test dentifrice compared with the comparator dentifrice. In the expectorate following the post-brushing DI water rinse, statistically significantly higher calcium ion concentrations were observed with the test dentifrice expectorate compared with the comparator dentifrice expectorate ( $p = 0.0007$ ).

Following the OJ/DI water rinse at 60 min post-brushing, statistically significantly higher fluoride and calcium ion concentrations were reported in the test ( $p < 0.0001$ ) or comparator ( $p < 0.0001$ ) dentifrice post-OJ expectorates compared with the test dentifrice DI water expectorate. Statistically significantly more fluoride ions were released with the test than the comparator dentifrice following the OJ rinse ( $p = 0.0047$ ) with no differences shown in calcium ion concentrations.

### 3.3. Saliva and expectorates pH values (Table 4)

Prior to the OJ or DI water rinse at 60 min post-brushing, no statistically significant differences between dentifrices were observed for salivary pH values. Following the OJ rinse with either the test ( $p = 0.0016$ ,  $p < 0.0001$  respectively) or comparator dentifrices ( $p = 0.0052$ ,  $p < 0.0001$  respectively), pH levels in saliva and expectorate were



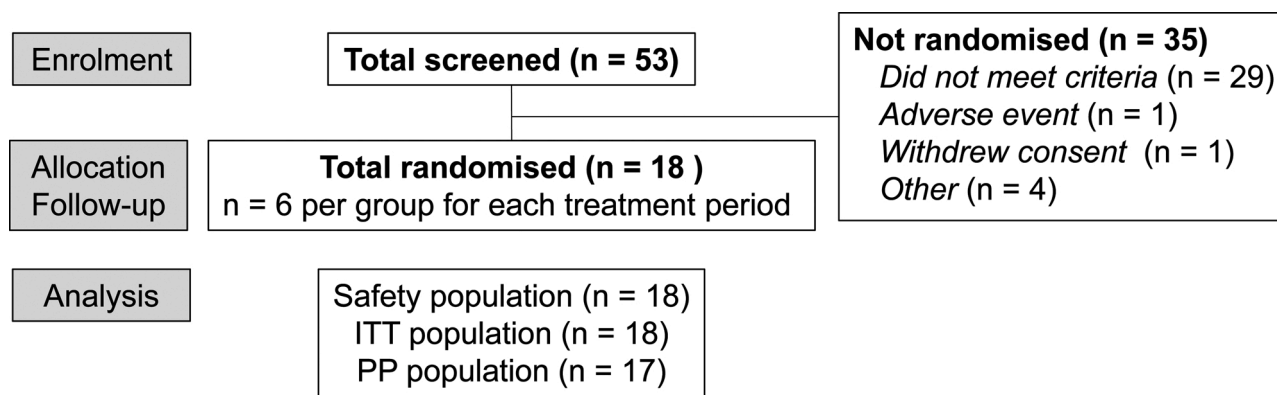
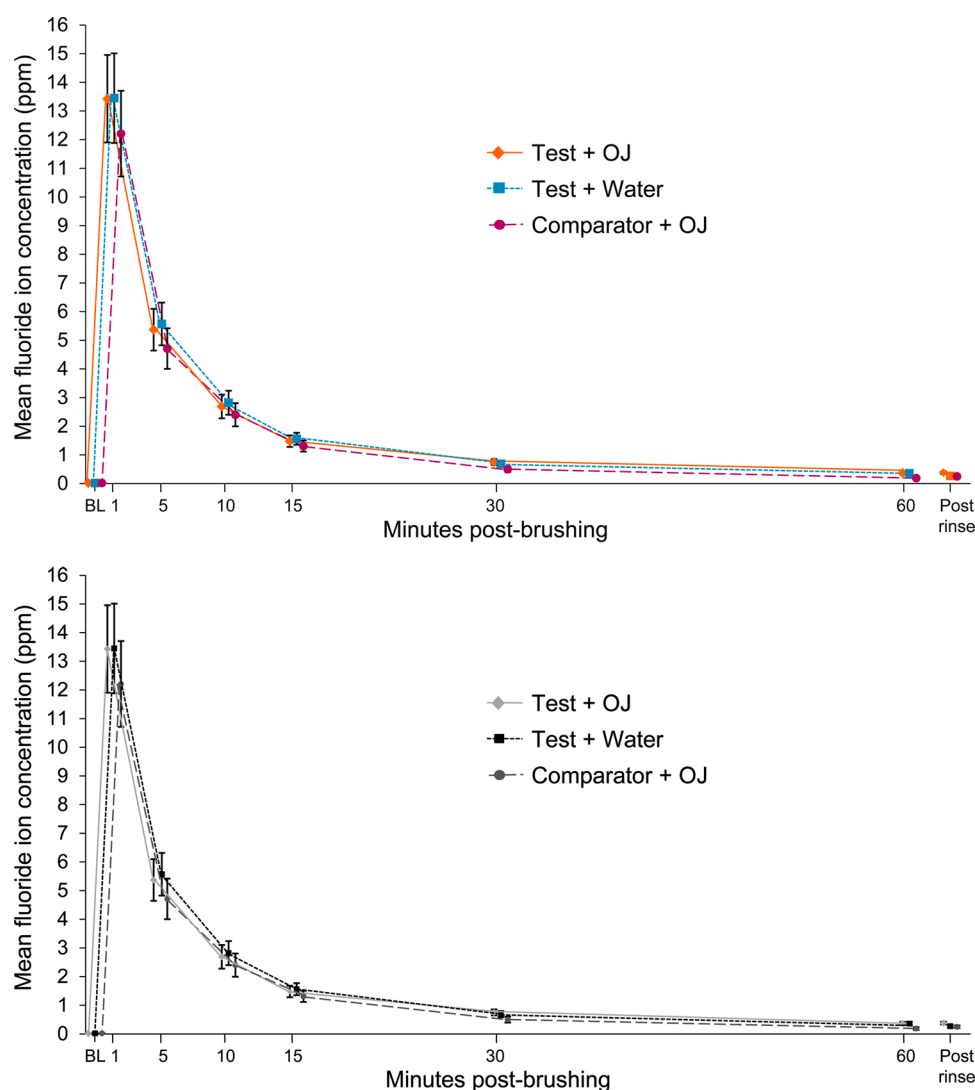


Fig. 2. Study flow.



significantly lower than the test dentifrice + DI water rinse, with no differences between them.

### 3.4. Change in salivary fluoride and calcium ion concentrations and pH from test dentifrice pre- to post-OJ or DI water rinse

At the 60 min post-brushing timepoint, no statistically significant

change from pre- to post-rinsing in salivary fluoride ion concentration was observed for the test dentifrice + OJ rinse compared with the test dentifrice + DI water rinse (difference: 0.11 [95% CI -0.01, 0.23],  $p = 0.0655$ ). A statistically significant change in pre- to post-rinse salivary calcium ion concentration (difference -7.40 [-11.7, -3.12],  $p = 0.0016$ ) and pH (difference -0.85 [-1.33, -0.37],  $p = 0.0015$ ) was observed in favour of the test dentifrice + DI water rinse compared with the test

**Table 1**

Treatment comparison for fluoride and calcium ion concentrations in saliva (PP population).

Time-point	Treatment comparison <sup>a</sup>	Fluoride ion conc (ppm)		Calcium ion conc (ppm)	
		Diff <sup>b,c</sup> (95% CI)	P-value <sup>c</sup>	Diff <sup>b,c</sup> (95% CI)	P-value <sup>c</sup>
1 min	Test vs. Comp	1.23 (−0.79, 3.25)	0.2209	10.89 (8.71, 13.07)	<0.0001
5 min	Test vs. Comp	0.76 (−0.26, 1.77)	0.1380	7.60 (5.73, 9.46)	<0.0001
10 min	Test vs. Comp	0.36 (−0.16, 0.88)	0.1675	6.14 (3.64, 8.65)	<0.0001
15 min	Test vs. Comp	0.21 (−0.03, 0.45)	0.0785	2.13 (−0.25, 4.51)	0.0761
30 min	Test vs. Comp	0.21 (0.07, 0.36)	0.0059	1.97 (−0.88, 4.82)	0.1639
60 min	Test vs. Comp	0.17 (0.08, 0.26)	0.0008	0.59 (−0.51, 1.69)	0.2707
60 min post rinse	Test/OJ vs. Comp/OJ	0.13 (0.00, 0.25)	0.0436	1.44 (−2.16, 5.03)	0.4157
	Test/OJ vs. Test/DI	0.11 (−0.01, 0.24)	0.0798	−8.08 (−12.0, −4.14)	0.0003
	Comp/OJ vs. Test/D	−0.01 (−0.13, 0.11)	0.8214	−9.52 (−13.1, −5.89)	<0.0001

CI: confidence interval; Comp: comparator OJ: orange juice rinse; DI: de-ionised water rinse; conc: concentration.

<sup>a</sup> Data from the two Test dentifrice arms (with OJ and DI water rinses) were combined for this treatment comparison.<sup>b</sup> Diff: Difference is first-named treatment minus second-named treatment such that a positive difference favours the first-named treatment.<sup>c</sup> From ANCOVA model with treatment and period as fixed factors, participant as a random effect, and two baseline covariates (i) participant-level baseline concentration calculated as the mean baseline concentration across all periods within a participant (ii) period level baseline concentration minus participant level baseline concentration.

dentifrice + OJ rinse.

### 3.5. Safety

A total of 12 treatment-emergent AEs, of which 11 were oral AEs, were reported by nine participants. None of the AEs were deemed to be related to treatment and no serious AEs were reported.

## 4. Discussion

Erosive tooth wear is increasingly common, particularly among young adults (Bartlett et al., 2013; Jaeggi & Lussi, 2014; Salas, Nascimento, Huysmans, & Demarco, 2015; Van't Spijker et al., 2009). For example, a recent European study of more than 3000 individuals aged between 18 and 35 demonstrated that 29% who attended a dental practice had erosive tooth wear (Bartlett et al., 2013). Erosion can have a serious impact on dental function and aesthetics (Barbour & Rees, 2006), therefore preventative management should be a priority to reduce or stop progression of lesions.

Using a fluoride-containing dentifrice has been shown to result in an initial sharp increase of salivary fluoride in the first few minutes after brushing, with a slower decrease over the following hour and levels above baseline for some hours (Duckworth & Morgan, 1991). This is due in part to the retention of fluoride in oral reservoirs, which is released into saliva as salivary fluoride concentrations decrease. This is reflected

in the results observed here, where, despite the shorter time-scale of this study, are similar to the biphasic decay reported previously (Duckworth & Morgan, 1991). The fluoride concentration decay has been rationalised in terms of a two-compartment model where salivary fluoride concentrations shortly after brushing are dominated by loosely adhered paste/paste slurry, which is rapidly cleared from the oral cavity through swallowing, with desorption from fluoride bound to the oral tissues accounting for the presence of longer term salivary fluoride (Duckworth & Morgan, 1991). Whilst the data presented here has not been mathematically fitted in the same way as previous publications (since this was not part of the statistical plan defined prior to the study start), the shape of the clearance data is very similar to that presented therein (Duckworth & Morgan, 1991; Duckworth, Jones, Nicholson, Jacobson, & Chestnutt, 1994).

In this study, for both treatment groups, slight, but still elevated, concentrations of salivary fluoride compared to baseline were observed at the 60 min timepoint (0.36/0.37 ppm for the test dentifrice groups, 0.19 for the comparator dentifrice). It has been demonstrated in *in vitro* caries models that a cariostatic concentration of fluoride in saliva is required to protect against lesions (Vogel, 2011). Assuming parallels can be drawn between the protective effects conferred by fluoride in *in vitro* models, even low concentrations could be expected to modify enamel demineralisation. In pH cycling models, fluoride concentrations as low as 0.014 ppm can reduce enamel demineralisation, though these models do not include an acidic challenge (Jacobson, Strang, & Stephen, 1991; Page, 1991).

The resistance of fluoride-treated enamel to acid-mediated dissolution has long been attributed to the formation of less soluble fluorinated hydroxyapatite or fluorapatite in or on the surface of enamel (Knappwost, 1956). The mechanism by which low concentrations of fluoride inhibit enamel dissolution is not fully understood; however, long term exposure to elevated concentrations of fluoride would favour enamel surface fluoridation and substitution of a proportion of the hydroxyl groups of enamel crystallites with fluoride ions (Tanizawa, Yoshiaki, Sawamura, & Suzuki, 1991). *In silico* (De Leeuw, 2004) and time resolved atomic force microscopy studies have demonstrated the expected reductions in enamel surface dissolution at the ultra-structural level conferred by fluoride (Parkinson, Shahzad, & Rees, 2010).

In this study, higher fluoride concentrations were observed in saliva samples after brushing with the test dentifrice compared to the comparator dentifrice at all timepoints, reaching statistical significance at 30 and 60 min. Interestingly, significantly higher fluoride concentrations were also observed in the test dentifrice expectorate immediately post-brushing, suggesting more fluoride is initially lost than with the comparator dentifrice. As hypothesised, the OJ challenge resulted in the release of significantly more fluoride in saliva and expectorate than the DI water challenge with both dentifrices. However, there were higher salivary and expectorate post-OJ challenge fluoride ion concentrations with the test dentifrice than with the comparator dentifrice. These findings suggest that even though initial fluoride in expectorate is greater, the oral fluoride reservoir is increased with the test dentifrice relative to the comparator dentifrice resulting in greater fluoride release in the event of a dietary acidic challenge.

While there was some decrease in salivary calcium ions with the test dentifrice after brushing, this was much more pronounced, and statistically significantly lower, with the comparator dentifrice. Additionally, the oral environment required at least 10 min to recover from the significant reduction of available calcium in saliva induced by application of the comparator product. The importance of calcium on fluoride retention in the oral cavity has been demonstrated in a series of studies where a pre-rinse of calcium ions prior to topical application of fluoride increased oral fluoride retention (Pessan et al., 2006; Vogel, Shim et al., 2006; Vogel, Chow, Carey, Schumacher, & Takagi, 2006). Whitford et al. (2002) also demonstrated that fluoride deposition in plaque was positively related to plaque calcium concentration. A conclusion of these studies was that saturating oral tissues with calcium prior to exposure to

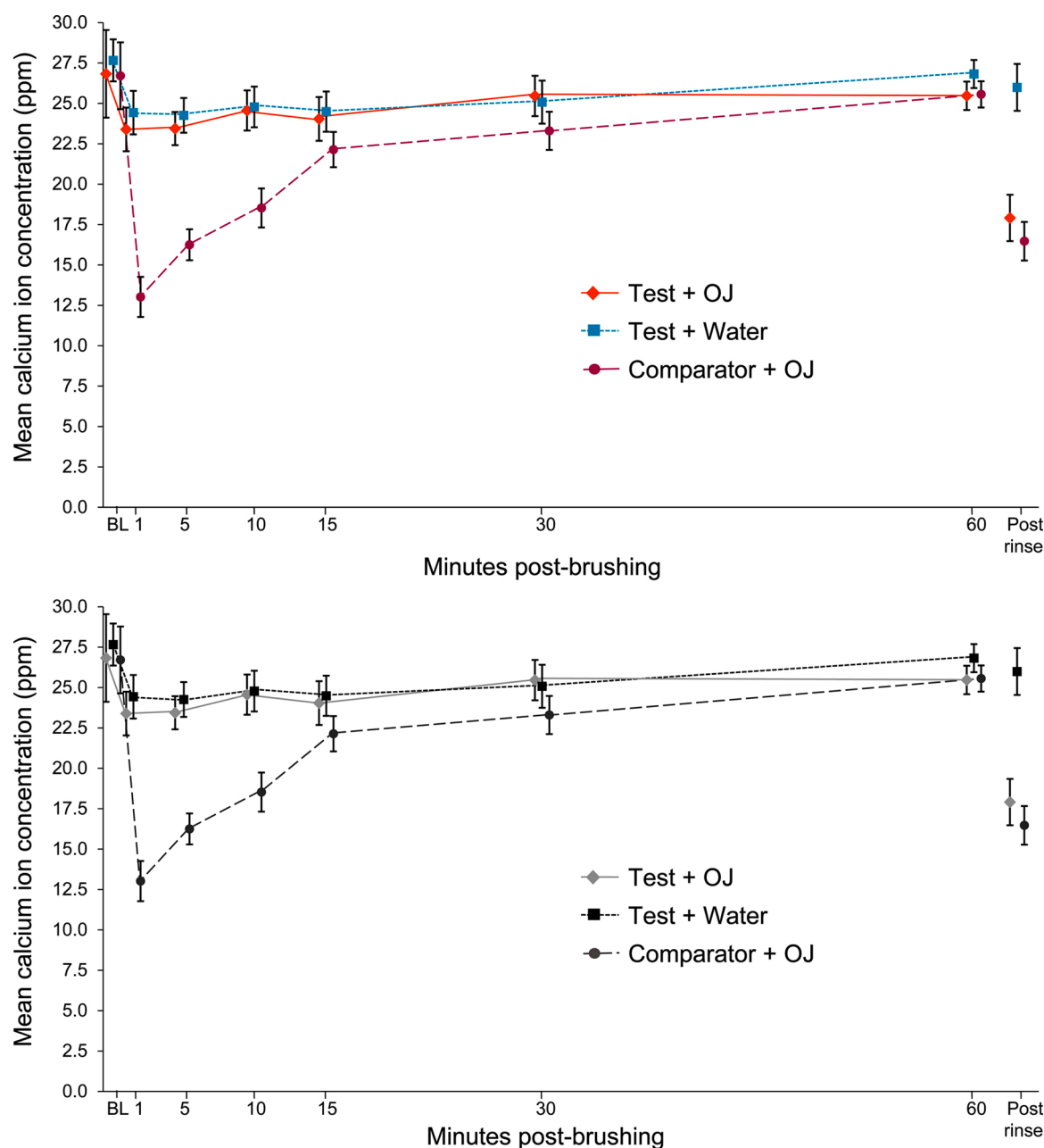


Fig. 4. Mean (SE) concentrations of calcium ions in saliva over time (PP population).

Data points are offset for clarity. Error bars represent between-participant variability. For baseline, mean  $\pm$  SE are presented. For all other timepoints, Adjusted Means  $\pm$  SE are presented. Adjusted Means and SE are from ANCOVA model with treatment and period as fixed factors, participant as a random effect, and two baseline covariates (i) participant-level baseline concentration calculated as the mean baseline concentration across all periods within a participant (ii) period level baseline concentration minus participant level baseline concentration.

fluoride greatly increased fluoride uptake through increased precipitation of calcium fluoride-like deposits. As such, the greater availability of calcium ions with the test dentifrice post-brushing may account for increased oral reservoir uptake.

Despite matching declared NaF and KNO<sub>3</sub> concentrations (and the majority of other ingredients), using *in vitro* models, the test dentifrice used here has been shown in previous studies to be statistically significantly superior to the comparator dentifrice with regard to erosive lesion re-hardening, surface softening inhibition and enamel fluoride uptake (Barlow et al., 2009; Fowler et al., 2009). This has led to the hypothesis that formulation excipients, such as surfactants, gums, and ion-sequestrants, play a role in fluoride bioavailability (Barlow et al., 2009; Fowler et al., 2009). The significant differences between the test

and comparator dentifrices may be linked to the presence of SLS and pyrophosphates in the latter. SLS is anionic and known to bind to calcium ions (Piacentini, Martinet, Beninati, & Folk, 1988). The presence of SLS in one study was shown to reduce deposition of fluoride (as CaF<sub>2</sub>) (Barkvoll et al., 1988). However, in a separate study, Vogel and colleagues (Vogel, Shim et al., 2006) demonstrated that the presence of SLS in a fluoridated mouthrinse did not adversely affect fluoride concentration in saliva 1 h after rinsing. Pyrophosphates are also well-known sequestrants of calcium ions (Irani & Clayton, 1962), and have been shown to interfere with fluoride-associated remineralisation (Zero, Cavaretta Siegel, Fu, & Li, 2000) as they bind to the enamel surface (Shellis, Addy, & Rees, 2005). As such, it may be the presence of these excipients in the comparator dentifrice that could be leading to lower

**Table 2**

Expectorate fluoride and calcium ion concentrations (PP population).

	Immediately post-brushing	DI water rinse post-brushing	OJ/DI water rinse, 60 min post-brushing
Fluoride concentration (ppm) (adjusted mean [standard error])			
Test + OJ	301.1 (17.24)	12.6 (1.55)	0.16 (0.013)
Test + Water	291.4 (17.61)	12.5 (1.61)	0.04 (0.013)
Comp + OJ	255.1 (16.65)	12.2 (1.45)	0.11 (0.011)
Calcium ion concentration (ppm) (adjusted mean [standard error])			
Test + OJ	10.37 (0.55)	4.9 (0.78)	91.9 (1.932)
Test + Water	11.38 (0.56)	5.9 (0.81)	4.7 (1.942)
Comp + OJ	2.49 (0.49)	2.9 (0.75)	88.3 (1.733)

From ANOVA model with treatment and period as fixed factors, participant as a random effect.

Comp: comparator OJ: orange juice rinse; Water: de-ionised water rinse.

**Table 3**

Treatment comparison of fluoride and calcium ions in expectorate (PP population).

Timepoint	Treatment comparison	Fluoride		Calcium	
		Diff <sup>a</sup> (95% CI)	P-value <sup>c</sup>	Diff <sup>a</sup> (95% CI)	P-value <sup>c</sup>
Post-brushing	Test vs. Comp <sup>a</sup>	41.19 (16.50, 65.89)	0.0020	8.39 (7.26, 9.52)	<0.0001
DI water rinse, immediately post-brushing	Test vs. Comp <sup>a</sup>	0.27 (-2.94, 3.49)	0.8618	2.55 (1.19, 3.92)	0.0007
60 min OJ/ Water challenge rinse expectorate	Test + OJ vs. Comp + OJ	0.05 (0.02, 0.08)	0.0047	3.56 (-1.35, 8.46)	0.1480
	Test + OJ vs. Test + Water	0.12 (0.09, 0.15)	<0.0001	87.19: (82.05, 92.34)	<0.0001
	Test + Water vs. Comp + OJ	0.07 (0.04, 0.10)	<0.0001	83.64: (78.69, 88.58)	<0.0001

From ANOVA model with treatment and period as fixed factors, participant as a random effect.

CI: confidence interval; Comp: comparator OJ: orange juice rinse; Water: de-ionised water rinse.

<sup>a</sup> Data from the two Test arms (with orange juice and water rinses) were combined for this treatment comparison.

<sup>b</sup> Diff: Difference is first-named treatment minus second-named such that a positive difference favours the first-named treatment.

<sup>c</sup> From ANOVA model with treatment and period as fixed factors, and participant as a random effect.

fluoride ion concentrations. Further studies are needed to investigate the impact of individual excipients.

This study also evaluated pH values but did not find a difference prior to or after rinsing with OJ 60 min post-brushing. As expected, differences were found depending on whether the rinse was OJ or DI water.

A limitation of this study is that it explored only one fluoride source (NaF) and concentration (1450 ppm); it may also be informative to explore fluoride kinetics in combination with established intra-oral models of enamel erosion. Additionally, while the dentifrices were chosen to be as similar as possible, it is accepted that there could have been minor formulation differences that also contributed to the results. It would also be of interest to examine how the Comparator dentifrice behaved after both an OJ and a water rinse. Finally, it is of note that the methodology employed in this study, where the acidic drink is applied only once but for a duration that exceeds a typical sipping occasion, is

**Table 4**

pH values and treatment comparisons of saliva and expectorate at 60 min pre- and post-OJ/DI water rinse (PP population).

	Pre 60 min OJ/ DI water rinse	Diff <sup>a</sup> (95% CI) p-value <sup>c</sup>	Post 60 min OJ/ DI water rinse	Diff <sup>a</sup> (95% CI) p-value <sup>c</sup>
Saliva pH (adjusted mean [standard error] <sup>a</sup> )				
Test + OJ	7.2 (0.08)		6.6 (0.17)	
Test + Water	7.1 (0.09)		7.4 (0.18)	
Comparator + OJ	7.2 (0.08)		6.8 (0.15)	
Treatment comparison	Test vs Comp <sup>a</sup>	-0.07 (-0.24, 0.10) 0.4132	Test + OJ vs. Comp + OJ	-0.13 (-0.54, 0.29) 0.5432
			Test + OJ vs. Test + Water	-0.76 (-1.21, -0.32) 0.0016
			Test + Water vs. Comp + OJ	-0.64 (-1.07, -0.21) 0.0052
Expectorate pH (adjusted mean [standard error] <sup>a</sup> )				
			4.0 (0.04)	
			6.6 (0.04)	
			4.0 (0.15)	
			Test + OJ vs. Comp + OJ	-0.01 (-0.11, 0.09) 0.8049
			Test + OJ vs. Test + Water	-2.65 (-2.75, -2.55)
			Water	<0.0001
			Test + Water vs. Comp + OJ	-2.64 (-2.74, -2.54)
				<0.0001

CI: confidence interval; Comp: comparator OJ: orange juice rinse; Water: de-ionised water rinse.

<sup>a</sup> Data from the two Test arms (with orange juice and water rinses) were combined for this treatment comparison.

<sup>b</sup> Diff: Difference is first-named treatment minus second-named such that a positive difference favours the first-named treatment.

<sup>c</sup> From ANOVA model with treatment and period as fixed factors, and participant as a random effect.

clearly a simplification of the complexities of real-life exposure to acidic drinks where shorter duration sips may be expected but with multiple sipping occasions. It would be interesting to understand how the pattern of sipping behaviour impacts fluoride availability in the oral cavity, particularly to understand if the oral fluoride reservoir is completely depleted after extensive acidic drink consumption and whether different drinking patterns (e.g., frequent small sips compared to fewer larger sips) affects the salivary fluoride concentration.

## 5. Conclusions

This exploratory study confirms that acid labile fluoride is retained in the oral cavity following a single brushing and is released into saliva in the event of a typical dietary acid challenge. The results also suggest that dentifrice formulations with identical declared fluoride concentrations (1450 ppm fluoride as NaF) may not deliver fluoride in the same way. Dentifrice excipients may play a significant role in the retention of fluoride in the oral environment.

## Authorship contributions

CP, MN, GB, and RK contributed to the conception and design of the study and analysis of the data. PH, GVT, MN, and TB contributed to the running of the study and data acquisition. All authors contributed to the drafting of the manuscript and read and approved the final version.



## Declaration of Competing Interest

The authors report no declarations of interest.

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