Fluoride is a key dentifrice ingredient for mitigating dental erosion and promoting remineralisation in tooth enamel. A dentifrice formulation (NaF/CL - where C = Copolymer, L = Lactate), optimised for fluoride delivery, containing sodium fluoride, polyvinylmethylether–maleic anhydride (PVM/MA) copolymer and lactate ion at controlled pH of 6.2 is compared with six commercial dentifrices from European and US regulatory regions. The in vitro study utilised white light interferometry (WLI) and dynamic secondary ion mass spectrometry (DSIMS) to assess dental erosion resistance and remineralisation potential of the dentifrices. For WLI, polished enamel samples were immersed in dentifrice slurry (1:3 wt./wt. in artificial saliva, 2 min), brushed, washed in deionised water, acid challenged (1% citric acid, pH 3.8, 5 min), washed and air dried. Surface roughness and bulk tissue loss were measured. DSIMS samples for fluoride uptake were acid challenged (1% citric acid, pH 3.8, 5 min), rinsed, immersed in dentifrice slurry (1:3 wt./wt. in artificial saliva, 2 min), washed and air dried. DSIMS samples for $^{44}$Ca uptake were prepared similarly but with $^{44}$Ca-doped artificial saliva. The NaF/CL dentifrice provided highest protection against dental erosion, with highest fluoride and $^{44}$Ca uptake in all treatment groups ($n = 5$ per group). Dentifrices containing other fluoride salts and/or ingredients known to inhibit fluoride uptake (e.g., polyphosphates, sodium lauryl sulfate), performed significantly worse.

**KEYWORDS**

demineralisation, dental erosion, dynamic secondary ion mass spectrometry, enamel, remineralisation, white light interferometry

1 | **INTRODUCTION**

The loss of human tooth enamel through wear can occur by three defined processes, that is, dental erosion, dental attrition and dental abrasion. Dental erosion involves the chemical loss of mineralised tooth substance caused by exposure to acids not derived from oral bacteria, that is, from stomach acids or food and drink. This results in softening and removal of enamel across the whole tooth surface. The process typically extends from $\sim$0.1 μm depth in the early stages of erosion to beyond $\sim$100 μm after prolonged exposure.

In dental attrition the physical loss of mineralised tooth material is caused by tooth-to-tooth contact, for example, tooth grinding (known as bruxism), whereas dental abrasion is caused by contact with objects other than teeth. Typical examples of dental abrasion are the effects of chewing certain foods or tooth brushing.
Concern over the increasing prevalence of dental erosion has been growing for more than 20 years,\textsuperscript{7} and it has recently been described as ‘an increasingly relevant problem’.\textsuperscript{8} Dental erosion is distinct from dental caries, which has a tendency to form in localised and relatively inaccessible areas of the teeth when food containing sugars or starch is converted to acids by dental plaque bacteria present on the tooth surface. Fluoride has been shown to be effective at inhibiting dietary acid-mediated erosion of dental hard tissues as well as promoting repair of demineralised incipient erosive lesions, that is, ‘remineralisation’.\textsuperscript{9–11} In vitro studies, primarily aimed at investigating caries prevention, have also shown that adequate availability of calcium, phosphate and fluoride ions can produce significant remineralisation of lesions in enamel.\textsuperscript{12,13} The challenge, therefore, is to design delivery systems which provide sufficient quantities of these active ions to optimise remineralisation.

Fluoride is clearly a key ingredient in dentifrices for mitigation of dental erosion and promotion of remineralisation. However, dentifrices are complex vehicles for therapeutic agents, and several ingredients commonly found in formulations can interfere with fluoride action. This may occur by direct precipitation of fluoride ion via certain polyvalent metal ions; reduction of fluoride binding to enamel surfaces by ionic surfactants, for example, sodium lauryl sulfate;\textsuperscript{14} or interference with fluoride-promoted remineralisation processes by polyvalent metal ions and polyphosphates.\textsuperscript{15,16} It is not possible to simply add more fluoride to formulations to offset these losses because national and international regulatory bodies set limits to dentifrice fluoride content in order to avoid harmful over-exposure from ingested product. Hence, the avoidance of fluoride bioactivity loss is critical.

One of the dentifrice formulations used in this study, Pronamel, was developed over a decade ago to avoid the use of the interfering ingredients described above.\textsuperscript{16} In this study the established formulation technology has been further developed to include agents which have been found to be able to promote fluoride’s action on enamel. Specifically, the combination of polyvinylmethylether–maleic anhydride (PVM/MA) copolymer and lactate ion (as sodium lactate), with the formulation pH controlled to 6.2 (designated as NaF/CL in this study), has been found to increase fluoride uptake and enhance fluoride-mediated acid resistance.\textsuperscript{17} This modified formulation has the commercial name Pronamel Intensive Enamel Repair. In this study the two formulations were initially compared using standard US Federal Drug Administration (FDA) assays, that is, enamel fluoride uptake (EFU)\textsuperscript{18} and enamel solubility reduction (ESR),\textsuperscript{19} in order to assess the potential benefits.

With this background of varying dentifrice formulations and constant iterative improvements there is an associated analytical challenge to apply methods which adequately monitor the effects of erosion, the ingress of any beneficial agents into enamel surfaces and the progress of remineralisation on the tooth surface. The initial prerequisite for such experiments is to obtain a set of human tooth enamel samples which are effectively identical in terms of physical uniformity and composition. Hence, all enamel samples are subjected to an identical cleaning and polishing procedure designed to remove surface debris and any fluoride in the enamel from water fluoridation and/or toothpaste use.

Over the past few decades a number of analytical techniques have been developed and utilised for the assessment of the chemical composition and physical structure of tooth enamel lesions. These techniques have been reviewed extensively\textsuperscript{20–22} and include standard radiography,\textsuperscript{23} transverse micro-radiography (TMR),\textsuperscript{24} secondary ion mass spectrometry (SIMS),\textsuperscript{25} electron probe micro-analysis (EPMA),\textsuperscript{26} surface microhardness (SMH) and cross-sectional microhardness (CSMH). For erosion studies, SMH has been consistently used to characterise mineralisation status and surface strength, with profilometry-based techniques also providing further physical information on topography and erosion wear depth.\textsuperscript{27–29} In a series of studies the present authors have used a combination of white light interferometry (WLI) to measure bulk tissue loss through erosive challenges and dynamic secondary ion mass spectrometry (DSIMS) to compare uptake of fluoride, from various dentifrice treatments, into lesioned enamel.\textsuperscript{30,31} WLI provides measurement of surface roughness and tissue loss in the z plane (depth) on the nanometer to tens of microns scale, whereas DSIMS allows acquisition of chemical images from cross sections of treated enamel with sub-micron spatial resolution and detection sensitivities on the ppb–ppm scale for, for example, fluoride. Retrospective line-scan analysis from DSIMS allows reconstruction of in-depth relative concentration profiles, for example, for assessment of relative fluoride uptake.

A significant challenge arises when the purpose of the investigation is to monitor chemical compositional changes of remineralised lesions, because the remineralising agents (i.e., calcium and phosphate) are chemically indistinguishable from the enamel substrate. In order to overcome this challenge, \textsuperscript{44}Ca-labelling of calcium phosphate in a dentifrice has been used to successfully demonstrate uptake of ‘new’ calcium into demineralised enamel.\textsuperscript{32} In more recent remineralisation studies, the present authors have used isotopically enhanced calcium chloride containing \textasciitilde97\% \textsuperscript{44}Ca in the remineralising artificial saliva medium.\textsuperscript{33} Measurement of the cross-sectional distribution of calcium isotopes (e.g., \textsuperscript{42}Ca, \textsuperscript{44}Ca) by DSIMS imaging, following treatment of enamel with \textsuperscript{44}Ca-labelled artificial saliva, provides clear distinction between ‘original’ calcium and ‘new’ calcium. It is clear, therefore, that WLI and DSIMS enable the quantification and visualisation of the effects of different toothpaste formulations on fluoride uptake, remineralisation and protection against demineralisation.

The main aim of this study was to assess the effects of different dentifrice formulations, marketed in European and US regulatory regions, by measuring relative fluoride uptake, surface roughening due to demineralisation, bulk tissue loss after brushing and uptake of new calcium—that is, remineralisation—using artificial erosive lesions in human enamel in vitro. A range of commercial dentifrices were placed into three groups, that is, two groups from the European regulatory region (EU1 and EU2, where the upper limit on fluoride content is 1500 ppm F) and one group from the US regulatory region (US, where the standard fluoride level is 1100–1150 ppm F). Each group included a fluoride-free control. The ability of the dentifrices to suppress enamel surface roughening due to demineralisation and bulk tissue loss (after brushing) was assessed using WLI. DSIMS was used to measure the relative uptake of fluoride and calcium, in separate experiments.
2 | MATERIALS AND METHODS

2.1 | Pronamel formulations

A summary of the dentifrices used in the initial assessment of the two Pronamel formulations is shown in Table 1.

2.2 | EFU and ESR assays

These two assays were initially employed to compare Pronamel Intensive Enamel Repair and Pronamel in relation to the ability of the formulations to deliver fluoride to enamel and protect against erosive acid challenge. Industry-standard fluoride performance measures were employed: the EFU (FDA method #40) and the ESR (FDA method #33). Briefly, the EFU method measures the amount of fluoride taken up by cut, polished and pre-demineralised enamel surfaces after standardised treatment with a toothpaste slurry. After treatment, fluoride delivered to the enamel surface is solubilised by exposure of the surface to 1 M perchloric acid and quantified by fluoride ion-specific electrode. Low levels of fluoride are generally found in placebo-treated samples due to naturally occurring fluoride in the teeth. In contrast, the ESR method measures the protection of enamel surfaces from mineral loss due to fluoride delivered to those surfaces by a toothpaste. It measures the amount of mineral lost from cut and polished enamel surfaces (pre-demineralised via a standardised acid challenge), after the surfaces have been treated with a toothpaste slurry and compares this with the amount lost from an equivalent acid exposure without the toothpaste slurry treatment. Mineral loss is quantified as the amount of phosphate dissolved from the enamel surface, measured using photoelectric colorimetry. The reduction in mineral loss due to treatment is calculated as a percentage (i.e., [mineral loss post-treatment/mineral loss pre-treatment] x 100). Hence, the EFU is a measure of the ability of the product to deliver the active ingredient (fluoride) to enamel surfaces, and the ESR is a measure of the ability of the delivered active ingredient to protect those surfaces from acid exposure.

2.3 | Commercial dentifrices

The commercial dentifrices used in the WLI and DSIMS studies are shown in Table 2 below.

2.4 | Enamel sample preparation

Human tooth enamel samples were randomly selected and embedded in ~5 mm thick acrylic resin blocks. All enamel samples were polished with progressively finer silicon carbide paper before a final polishing stage with P1200 and P2400 grit silicon carbide paper. This procedure is designed to provide a smooth, flat, uniform surface on which to perform studies and to remove surface debris and any fluoride already present in the enamel from water fluoridation and/or prior toothpaste use.

2.5 | Artificial saliva

The composition of the artificial saliva employed for enamel tissue loss and fluoride uptake experiments was as follows: magnesium chloride (0.2 mM), calcium chloride dihydrate (1.0 mM), potassium dihydrogen orthophosphate (4.0 mM), N-2-hydroxyethylpiperazine-N'-ethanesulphonic acid [HEPES] (20.0 mM), potassium chloride (16.0 mM) and ammonium chloride (4.5 mM). The pH was adjusted to 7.0 using potassium hydroxide (1.0 M). All ingredients in the artificial saliva were obtained from Sigma-Aldrich (Dorset, UK). For the calcium uptake experiments, calcium chloride dihydrate was replaced by calcium chloride dihydrate where the 44Ca content was ~97%. The 44Ca isotopically enriched calcium chloride was also obtained from Sigma-Aldrich UK.

2.6 | WLI

Prior to any treatment regime, an erosion window was created on each resin-mounted human enamel specimen by placing acid-resistant adhesive tape across the enamel surface, leaving approximately 50% of the enamel surface area protected. For each of the three studies, specimens were divided into four treatment groups (n = 5) and immersed into one of the dentifrice slurries (1:3 wt/wt in artificial saliva, 2 min) followed by controlled manual brushing for 2 min before washing for 1 min with deionised water. All specimens were then subjected to an acid challenge by suspension in 1% citric acid, pH 3.8 for 5 min, without agitation. Finally, specimens were washed with deionised water and air dried.

Prior to WLI analysis, the position of the protective tape was marked on each sample and then the tape carefully removed to expose a non-eroded reference region, immediately adjacent to the

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### TABLE 1 Dentifrices used in the EFU and ESR assay of Pronamel formulations

| Dentifrice                        | Identity code            | Fluoride content (source) | Additional formulants          |
|----------------------------------|--------------------------|---------------------------|--------------------------------|
| F-free placebo of Pronamel Intensive Enamel Repair | Fluoride-free control | None                       | PVM/MA copolymer, sodium lactate |
| Pronamel                         | NaF                      | 1150 ppm F (NaF)          | None                           |
| Pronamel Intensive Enamel Repair | NaF/CL                   | 1150 ppm F (NaF)          | PVM/MA copolymer, sodium lactate |
void pixels were removed and outlier spikes filtered. Data to produce images and surface roughness parameters. In each area, eroded area. The samples were wiped with an isopropanol-soaked lint-free cloth to remove any tape adhesive residue.

A MicroXAM (ADE phase shift) white light interferometer was used to acquire topographic data. Each enamel sample was initially scanned over three areas of \(687 \times 511 \mu m\) containing exposed/treated and tape-protected regions, to determine the step heights. This was followed by scanning three areas of \(687 \times 511 \mu m\) within the exposed/treated region for surface roughness determination. A \(Z\) (height) range of up to 300 \(\mu m\) was used for the measurements. Proprietary image analysis software (SPIP™—Image Metrology A/S) was then used to process the raw data to produce images and surface roughness parameters. In each area, void pixels were removed and outlier spikes filtered.

For the roughness analysis areas, a \(2^\circ\) polynomial plane correction was applied to remove gross form (curvature) and allow accurate roughness measurements.

For the step height analysis areas, the manual tilt function was used to maximise the flatness of the reference area. An averaged line scan was used across the interface between the treated and untreated regions to determine the step height.

### 2.7 | DSIMS

#### 2.7.1 | Fluoride uptake studies

In each of the three fluoride uptake studies, 20 polished enamel samples were initially subjected to an acid challenge by suspending in 1% citric acid, pH 3.8 for 5 min, without agitation. After washing with deionised water, specimens were then divided into four treatment groups (\(n = 5\)) and immersed into one of the dentifrice slurries (1:3 wt % in artificial saliva) for 2 min before washing for 1 min with deionised water, followed by air drying.

The central \(\sim 10 mm \times 10 mm\) section of each resin embedded specimen, containing the treated enamel, was removed using a junior hacksaw. For each \(n = 5\) study group the resultant sections were stacked and embedded in acrylic resin (Struers Epofix resin and hardener mixed in the ratio 15:2 vol) within a 25 mm diameter silicone holder. After curing for 24 h the resin blocks were removed and mechanically ground with 80 grit silicon carbide paper to expose cross-sections of the treated enamel. Progressively finer grit silicon carbide papers (P280, P800, P2400 Buehler Carbimet™) were used to gradually flatten and polish the enamel sections before finely polishing with 6 and 1 \(\mu m\) diamond pastes (Kemet International Ltd). The polished sections were then sputter coated with a thin gold layer, prior to DSIMS analysis, to prevent excessive sample charging.

DSIMS analysis was carried out using a Cameca ims 4f instrument. A 15 keV \(O_2^+\) primary ion beam was used with analysis beam current of \(\sim 100pA\) and image data (256 \(\times\) 256 pixel format) acquired in negative secondary ion detection mode, monitoring fluoride and representative enamel substrate species from areas of typically 100 \(\mu m \times 100 \mu m\) containing the original treated enamel surface region. All areas were ion beam etched with a primary beam current of typically 500 nA for several minutes, in order to remove polishing artefacts. For analysis, the sample extraction voltage was \(\sim 4.5\) keV, and a normal incidence electron beam, at the same nominal accelerating potential, was used to supply low energy electrons for charge compensation. Retrospective line scans were subsequently obtained from the digitally stored image data which allowed calculation of the variation of \(F^-/O^-\) signal intensity ratio across the fluoride uptake region of the enamel sections.

#### 2.7.2 | \(^{44}Ca\) uptake studies

For each of the three \(^{44}Ca\) uptake studies, 20 polished enamel samples were initially subjected to an acid challenge by suspending in 1% citric acid, pH 3.8 for 5 min, without agitation. After washing with deionised water, specimens were then divided into four treatment
groups (n = 5) and immersed into one of the dentifrice slurries (1:3 wt/wt in artificial saliva) for 2 min before washing for 1 min with deionised water, followed by air drying.

Each specimen was subsequently placed into an artificial saliva solution for 24 h. For the dentifrice treatments in each of the three studies, this solution contained calcium significantly enriched with $^{44}\text{Ca}$ (i.e., $\sim$97% $^{44}\text{Ca}$ as calcium chloride). For the fluoride-free treatment group in each study, a standard artificial saliva solution was used as a control (identical to the artificial saliva used for dentifrice treatments but containing $^{40}\text{Ca}$ as calcium chloride). Specimens were then washed for 1 min with deionised water and air dried.

DSIMS analysis was carried out using a Cameca ims 4f instrument. A 15 keV O$_2^-$ primary ion beam was used with analysis beam current of $\sim$200pA and image data (256 $\times$ 256 pixel format) acquired in positive secondary ion detection mode, monitoring $^{40}\text{Ca}^+$, $^{42}\text{Ca}^+$ and $^{44}\text{Ca}^+$ species from areas of typically 100 $\mu$m $\times$ 100 $\mu$m containing the original treated enamel surface region. All areas were ion beam etched with a primary beam current of typically 500 nA for several minutes, in order to remove polishing artefacts. For analysis, the sample extraction voltage was +4.5 keV, and a normal incidence electron beam was used for charge compensation. Retrospective line scans were subsequently obtained from the digitally stored image data which allowed calculation of the variation of $^{44}\text{Ca}/^{42}\text{Ca}$ signal intensity ratio across the enamel sections, from surface to bulk regions.

### 2.8 Statistical analysis

For EFU and ESR data, statistical analyses were performed with a one-way analysis of variance (ANOVA) model using SigmaPlot 13.0 software. Because significant differences were indicated, the individual means were analysed by Student Newman–Keuls (SNK) pairwise analysis. Significance was determined at $p < 0.05$.

Each set of measurement data from WLI and DSIMS analysis were analysed with a Student T test (two tailed, unequal variance) to test statistical significance at greater than 95% confidence ($p < 0.05$) between treatment groups.

### 3 RESULTS

#### 3.1 EFU and ESR

The results of the comparison of EFU and ESR values for the NaF and NaF/CL formulations, compared to a fluoride-free placebo, are presented in Table 3.

> It is evident that both fluoride uptake and enamel protection are enhanced in NaF/CL. This was confirmed by statistical analysis to be significant at greater than 95% confidence in both cases.

> On the basis of the positive benefits offered by NaF/CL in the EFU and ESR data, this modified formulation was included in the three study groups for comparison with other commercial dentifrices using the WLI and DSIMS techniques.

#### 3.2 Surface roughness and bulk tissue loss by WLI

Mean surface roughness measurements of brushed/acid-challenged regions of each of the four treatment groups (n = 5) are presented in Figure 1 for the EU1, EU2 and US studies.

In the EU1 and EU2 studies, NaF/CL was shown to provide the highest level of protection against enamel surface roughening, with the fluoride-free control showing the least protection. For the EU1 study, the respective mean group Sa values are NaF/CL (0.13 μm); SnF$_2$/hexametaphosphate (HMP) (0.89 μm); sodium monofluorophosphate (SMFP)/calcium silicate (CS) (1.11 μm) and fluoride-free control (1.38 μm). Statistical analysis of the surface roughness data by Student T test (four treatment groups at n = 5 samples per group and three measurement areas per sample) indicates Sa differences between all treatment groups in the EU1 study are statistically significant at 95% confidence level except in the case of SnF$_2$/HMP versus SMFP/CS ($p$ value 0.07). For the EU2 study, respective mean group Sa values are NaF/CL (0.20 μm); NaF/tetra-sodium pyrophosphate (Pyr)/sodium lauryl sulfate (SLS) (0.76 μm); ZnHA (1.53 μm) and fluoride-free control (1.54 μm). Statistical analysis showed the Sa differences between all treatment groups in the study to be significant at 95% confidence level ($p$ values significantly less than 0.05), except for ZnHA versus fluoride-free control ($p$ value 0.22). Finally, for the US study, the mean Sa values are NaF/CL (0.10 μm); SnF$_2$/sodium lauryl sulfate (SLS)/Zn (0.58 μm); NaF/Pyr/SLS (US)(0.59 μm) and fluoride-free control (1.55 μm). For this study, statistical analysis indicated the difference in Sa values was significant between all groups at 95% confidence level except for SnF$_2$/SLS/Zn versus NaF/Pyr/SLS (US) ($p$ value 0.98). The mean values of bulk tissue loss (step height) for each of the four dentifrice treatment groups in the three studies are shown in Figure 2.

For both the EU1 and EU2 studies, the dentifrice NaF/CL clearly demonstrates the greatest protection against bulk tissue loss. In the EU1 study, the values for mean step heights are NaF/CL (0.83 μm); SnF$_2$/HMP (8.7 μm); SMFP/CS (15.0 μm) and fluoride-free control $\text{TABLE 3}$ Comparison of mean EFU and mean ESR values for the NaF and NaF/CL formulations, compared to a fluoride-free control

| Product               | Mean EFU value (μg/g) | Std. error | Mean ESR value (%) | Std. error |
|-----------------------|-----------------------|------------|-------------------|------------|
| Fluoride-free control | 52                    | 6          | –7.88             | 2.03       |
| NaF                   | 1800                  | 38         | 4.83              | 2.17       |
| NaF/CL                | 1978                  | 59         | 20.55             | 1.03       |

TABLE 3
(52.7 μm). The relative scale of bulk tissue loss in this study is illustrated in Figure 3 where representative 3D images from each of the four treatment groups are shown.

The 3D views include the tape-protected (untreated) regions and the treated (brushed and acid-challenged) areas.

For the EU2 study, mean step heights are NaF/CL (1.1 μm); NaF/Pyr/SLS (EU) (18.6 μm); ZnHA (83.2 μm) and fluoride-free control (181.8 μm). In the US study NaF/CL showed the highest protection against bulk tissue loss, followed by SnF₂/SLS/Zn and NaF/Pyr/SLS (US) respectively. The step height values for the US study are NaF/CL (0.88 μm); SnF₂/SLS/Zn (8.3 μm); NaF/Pyr/SLS (US) (12.0 μm) and fluoride-free control (43.4 μm).

The step height differences between all treatment groups in the EU1, EU2 and US studies are statistically significant at 95% confidence level with all p values for inter-group comparisons being significantly below 0.05.
Examination of the cross-sectional DSIMS images for fluoride from a minimum of two areas from each of the \( n = 5 \) samples within all treatment groups indicated that fluoride uptake occurred within a depth regime of up to 50 \( \mu \text{m} \) into the treated enamel surface, under the experimental conditions employed in this study. Hence, from the line scans generated from individual images, a fluoride uptake integral value was calculated by integrating the F/O DSIMS ratio values from each depth increment from the original enamel surface to 50 \( \mu \text{m} \) depth, for each of the 40 areas analysed within each of the three study groups. The mean fluoride uptake integrals for each treatment group are plotted in Figure 4.

For the EU1 and EU2 studies, the highest fluoride uptake is provided by NaF/CL. In the EU1 study, NaF/CL shows an approximately 2.8 times higher fluoride uptake c.f. SMFP/CS and ~9 times higher uptake than SnF\(_2\)/HMP within the upper 50 \( \mu \text{m} \) of the lesioned enamel surfaces. Statistical analysis shows \( p \) values of significantly less than 0.05 for the differences between fluoride uptake across all four treatment groups indicating a statistical significance at 95% confidence level.

In the EU2 study, NaF/CL provides a fluoride uptake ~2 times higher than NaF/Pyr/SLS (EU) and ~60 times greater than ZnHA.
(which does not contain added fluoride). The fluoride uptake differences between NaF/CL, NaF/Pyr/SLS (EU) and ZnHA have p values all significantly below 0.05, that is, statistically significant at the 95% confidence level. The difference between dentifrice ZnHA and the fluoride-free control is not significant at the 95% level (p value 0.95). For the US study, NaF/CL has the highest fluoride uptake within the four treatment groups, that is, ~5 times higher uptake compared to NaF/Pyr/SLS (US) and ~38 times higher F uptake c.f. SnF2/SLS/Zn. The inter-group differences in F uptake are all statistically significant at 95% confidence level with p values all less than 0.05.

A series of representative DSIMS image overlays are presented in Figure 5 for the EU1 study group.

In each image, fluoride uptake is clearly shown by the designation of red colouration to F with the tooth enamel substrate represented by O signal and shown in cyan.

### 3.4 | 44Calcium uptake by DSIMS

Examination of the cross-sectional DSIMS images for 44Ca from all sample areas within treatment groups in the three studies indicated that 44Ca uptake occurred within a depth of up to ~30 μm on the treated enamel surface. Based on this observation, a series of 44Ca uptake integrals were calculated from the line scans, for each sample area, by integrating the 44Ca/42Ca DSIMS ratio values from each depth increment, over the 30 μm depth range. This process was carried out for each of the 40 analysed sample areas in each of the three study groups. The mean 44Ca uptake integrals for each treatment group within the three studies are plotted in Figure 6.

In the EU1 and EU2 studies, NaF/CL consistently exhibited the highest level of 44calcium uptake. For the EU1 study, NaF/CL showed an approximately twofold higher uptake of 44Ca compared to SMFP/CS and ~2.4 times higher than SnF2/HMP. Statistical analysis by Student T test shows the uptake integral differences between all treatment groups are significant at the 95% confidence level except for SnF2/HMP versus SMFP/CS (p value 0.28). In the EU2 study, NaF/CL demonstrates a ~2.9 times higher 44Ca uptake than NaF/Pyr/SLS (EU) and ~5 times higher than ZnHA. The differences between all four treatment groups are statistically significant at the 95% confidence level with p values <0.05 for all group comparisons. For the US study, NaF/CL has a 44Ca uptake integral which is 2.7 times higher than NaF/Pyr/SLS (US) and 6.3 times higher than SnF2/SLS/Zn. The statistical differences between all treatment groups are again significant at the 95% confidence level.

**FIGURE 5** Representative DSIMS image overlays (F, red; O, cyan) showing relative fluoride uptake and penetration into the enamel surface, EU1 study

**FIGURE 6** Comparison of mean relative 44Ca uptake for treatment groups in the EU1, EU2 and US studies, measured by DSIMS
A series of representative $^{44}\text{Ca}$ DSIMS image overlays are presented in Figure 7 for the EU2 study.

In each of the images $^{44}\text{Ca}^+$ signal is represented by blue colour with $^{42}\text{Ca}^+$ signal shown in yellow. For the NaF/CL overlay image there is a clear excess of blue colouration in the near surface region, indicating uptake of $^{44}\text{Ca}$. The images for dentifrices NaF/Pyr/SLS (EU) and ZnHA show progressively lower relative uptake.

4 | DISCUSSION

It has already been established that fluoride is a key ingredient in dentifrices for mitigation of dental erosion by inhibition of demineralisation and promotion of remineralisation. The delivery of fluoride to the tooth surface from a dentifrice is optimal when ingredients which interfere with fluoride delivery are removed from formulation and agents which can increase uptake of fluoride are present.

In this study two key aspects of dentifrice development have been addressed, that is, iterative formulation improvement and a comparison of a range of competitive formulations which have been designed to provide improved resistance to dental erosion through enhanced fluoride uptake and repair demineralised enamel through remineralisation.

A recent clinical study, in which 'in situ' enamel specimens were mounted in an intra-oral appliance, used surface microhardness measurements to compare a sodium fluoride-based dentifrice, containing PVM/MA copolymer and sodium lactate with the formulation pH controlled to 6.2 (NaF/CL in this study), with a control formulation containing stannous fluoride and zinc citrate. The NaF/CL formulation was found to increase fluoride uptake, remineralisation and overall fluoride-mediated protection against dietary acid compared to the control. In this study the NaF/CL formulation has been further characterised by comparing EFU and ESR values against an established sodium fluoride-containing formulation (NaF). Data from these FDA standard assays confirmed the potential benefit of the NaF/CL formulation for improving resistance to dental erosion and promoting remineralisation.

The second part of the study compared the relative effectiveness of the NaF/CL formulation against other commercial dentifrices from European and US regulatory regions, which contain different sources of fluoride and/or different formulation excipients. Dentifrices were divided into two European and one US group, with multiple treated specimens of human tooth enamel within each group subjected to surface roughness and bulk tissue loss measurement by WLI along with separate studies of fluoride and calcium uptake by DSIMS, respectively. The combined use of WLI and DSIMS provides a useful analytical approach whereby the physical effects of dentifrice treatment cycles can be measured and directly compared with any concomitant chemical compositional changes to the enamel structure.

In order to assess comparative resistance to dental erosion provided by the dentifrices, enamel specimens were subjected to a dentifrice treatment/brushing/acid challenge cycle, followed by measurement of surface roughening and bulk tissue loss within treated areas. The ADE phase shift white light interferometer used in the present study has a notably high (nm scale) resolution in the z plane, with Sa values (mean surface roughness) therefore providing a measure of dental erosion, even in the early stages of attack by dietary acids. Measurement of step heights also provides an assessment of the dentifrices' ability to suppress bulk tissue loss after the dentifrice treatment/brushing/acid challenge cycle. In all three studies, the level of surface roughening and bulk tissue loss was lowest following treatment with NaF/CL and highest in the fluoride-free controls. Across the three study groups, the formulations containing sodium fluoride or stannous fluoride generally provided lower surface roughening and better suppression of bulk tissue loss than those containing SMFP or no fluoride.

The WLI results described above provide an indirect measurement endpoint for assessment of the efficacy of dentifrice treatments containing fluoride. However, the use of DSIMS analysis provides a direct semi-quantitative comparison of fluoride uptake into enamel specimens. The combination of high spatial resolution, elementally specific chemical imaging of treated enamel cross-sections along with retrospective line-scan acquisitions from the digitally stored data provides a clear and unequivocal means of studying fluoride uptake from the enamel surface through to the bulk region. In the three studies, fluoride uptake occurred to a depth of up to ~50 μm and was highest in all cases for the formulation NaF/CL with fluoride-free controls showing the expected lowest uptake. For both the EU2 and US studies, NaF/Pyr/SLS provided the second highest fluoride uptake, that is, having relative uptakes of ~0.5 and ~0.2 respectively, compared to NaF/CL. The trends noted for fluoride uptake were also broadly exhibited in the DSIMS $^{44}\text{Ca}$ uptake studies, with NaF/CL showing the highest uptake in all three study groups. Notably,
NaF/Pyr/SLS again provided the second highest $^{44}\text{Ca}$ uptake in both EU2 and US groups, whereas SMFP/CS yielded the second highest uptake in EU1 group. The observed broad correlational trends between fluoride uptake and $^{44}\text{Ca}$ uptake confirm the role of free fluoride ion in promoting remineralisation, as reported previously by the present authors.\(^{53}\)

From the results within the two parts of this study, it is clear that formulation ingredients can have a significant impact on dentifrice performance in terms of protection against dental erosion by fluoride uptake, promotion of remineralisation and protection against demineralisation. In the first part of the study, one aspect of the potential improvement of the NaF/CL formulation, c.f. NaF, was the control of pH. It has been known for many years that a limited lowering of the pH of fluoride treatments\(^ {24}\) increases fluoride uptake to enamel without leading to demineralisation.\(^ {55}\) It has also been suggested to have the potential to enhance caries protection from toothpastes.\(^ {36,37}\) A further formulation change was the inclusion of the carboxylic acid copolymer PVM/MA. Such materials have potential for multi-point attachment of the polymer chains to cationic sites on enamel surfaces and have been shown by in vitro studies to enhance resistance to demineralisation, presumably by a surface-stabilising mechanism.\(^ {38,39}\) In the NaF/CL formulation it is apparent that the protective effect of the polymer system has been achieved without compromising fluoride uptake or remineralisation.

All of the dentifrices tested within each of the WLI and DSIMS study groups, that is, EU1, EU2 and US groups, were formulated with the same or very similar fluoride concentrations. It is therefore reasonable to assume that the measured differences in fluoride uptake and any ensuing benefits must be related to the presence of ingredients which either restrict or promote F uptake or to mechanistic differences in the delivery of free fluoride ion from solution to the enamel surface. With the exception of NaF/CL, all other commercial dentifrice formulations contained ingredients which have been found to either limit fluoride uptake, remineralisation or both, that is, sodium lauryl sulfate – SLS\(^ {14}\), polyphosphates\(^ {40-42}\) and polyvalent metal ions.\(^ {43,44}\) The inhibitory effects displayed by these agents are believed to be related to their affinity for enamel surfaces. Sodium lauryl sulfate has been shown to inhibit uptake of fluoride into enamel from alkali-soluble sources such as sodium fluoride.\(^ {14}\) Polyvalent metal ions have an affinity for surface-bound phosphate groups and polyphosphates for surface-bound calcium groups.\(^ {45,46}\) Such surface interactions may inhibit the integration of fluoride into the hydroxyapatite (HA) crystal structure as well as interfering directly with HA crystal growth during remineralisation. It must also be noted, however, that polyphosphates and polyvalent metal ions can have positive effects on enamel protection. They have been shown to reduce demineralisation of enamel subjected to acid challenge, most likely due to their enamel surface-binding and stabilising properties.\(^ {29,47,48}\) The balance of these positive and negative effects will determine the overall protective effect of the individual formulation. Finally, the form of fluoride used is also important to its protective effect against dental erosion. It would be reasonable to assume that formulations containing sodium fluoride and stannous fluoride will have essentially all fluoride available as free fluoride ion in solution under the experimental conditions used in this study. However, it is notable that the stannous fluoride-containing formulations, SnF$_2$/HMP in the EU1 study and SnF$_2$/SLS/Zn in the US study, were ranked below NaF/CL in the mean surface roughness/bulk tissue loss measurements by WLI but performed relatively poorly in terms of fluoride uptake (and consequently $^{44}\text{Ca}$ uptake) measured by DSIMS. It is known that stannous fluoride treatment results in formation of a protective barrier layer on the enamel surface but the exact nature of that layer is not yet clear.\(^ {49,50}\) It has also been suggested that stannous fluoride treatments may result in the formation of metal-rich precipitates on the enamel surface, for example Ca(SnF$_3$)$_2$, SnOHPO$_4$ or Sn$_3$F$_3$PO$_4$.\(^ {51}\) From the relatively low fluoride uptake demonstrated in this study it seems likely that treatment with these stannous fluoride formulations results in formation of Sn-based compounds which do not contain significant fluoride levels. In effect the dominant effect would appear to be formation of a Sn-based compound at the enamel surface with fluoride uptake restricted by a possible combination of that process and the known limiting effects of sodium lauryl sulfate, polyphosphates or polyvalent metal ions (i.e., Zn in SnF$_2$/SLS/Zn).

The dentifrice containing SMFP (SMFP/CS in the EU1 study) showed significantly lower performance in surface roughness, bulk tissue loss, fluoride uptake and $^{44}\text{Ca}$ uptake compared to NaF/CL. The release of free fluoride ion from SMFP will depend on cleavage of the covalent bond between phosphate and fluoride by enzymatic hydrolysis during treatment. In the mouth, this hydrolysis occurs mainly by the action of plaque bacteria.\(^ {52}\) Although this mechanism is relevant to demineralisation due to caries which occurs under plaque-coated surfaces, it is of little relevance to dental erosion which occurs principally on exposed surfaces with little or no plaque.\(^ {53}\) It is also known that the presence of sodium lauryl sulfate may inhibit this hydrolysis process when present in SMFP formulations.\(^ {54}\) Hence, it is not surprising that release of fluoride ion-mediated effects were significantly lower in SMFP/CS compared to the NaF/CL formulation.

## 5 | Conclusions

This study has shown the significant improvement in EFU and reduction of enamel solubility achieved by the addition of PVM/MA copolymer and sodium lactate at pH 6.2 (NaF/CL), to an established sodium fluoride-based dentifrice formulation (NaF). Comparisons of the NaF/CL formulation were undertaken with a range of commercial dentifrices from the European and US regulatory regions, which contained different fluoride sources and formulation ingredients, measuring (i) surface roughness and bulk tissue loss by WLI after a treatment/brushing/acid challenge cycle and (ii) fluoride uptake and $^{44}\text{Ca}$ uptake after acid challenge/dentifrice treatment cycles by DSIMS.

These in vitro studies showed that the NaF/CL formulation provided superior performance in promoting remineralisation and protecting against dental erosion.
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