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J DENT RES 2000 79: 983
DOI: 10.1177/00220345000790041501
The online version of this article can be found at:
http://jdr.sagepub.com/content/79/4/983
Effect of *in vitro* Acidification on Plaque Fluid Composition With and Without a NaF or a Controlled-release Fluoride Rinse

**INTRODUCTION**

Caries is strongly influenced by the concentrations of hydrogen, calcium, phosphate, and fluoride ions in the fluid environment of the teeth (Vogel *et al.*, 1990b, 1998; Margolis and Moreno, 1990). Although increases in calcium and phosphate ions can produce a cariostatic effect (Forward, 1994; Vogel *et al.*, 1998), a small increase in the fluid concentration of fluoride can have a profound anti-caries effect (ten Cate and Duijsters, 1983; Wefel and Harless, 1984; Margolis and Moreno, 1990). This observation has led to a heightened interest in labile fluoride reservoirs that could continue to release fluoride in the mouth after application. Although studies have indicated that absorbed fluoride (White *et al.*, 1994), perhaps mediated by calcium binding (Rose *et al.*, 1996), may be a significant fluoride source, most researchers have suggested that CaF$_2$ or “calcium-fluoride-like” deposits are the most important source of labile oral fluoride (Ogaard *et al.*, 1983; Arends and Christoffersen, 1990; Rølla and Saxegård, 1990). A two-component rinse has been shown to enhance the oral deposition of CaF$_2$ (Chow and Takagi, 1991; Chow *et al.*, 1992). These rinses are referred to as “controlled-release” (CR) rinses because the rate of hydrolysis of the SiF$_6^{2-}$ ion to free fluoride (contained in the “A” part of the CR rinse) is controlled by the pH, buffer capacity, and concentration of calcium (contained in the “B” part of the CR rinse). In theory, this control allows for greater penetration by rinse components into enamel or dentin pores, plaque, and mucosal tissue before the formation of CaF$_2$ deposits (Chow and Takagi, 1991; Chow *et al.*, 1992; Vogel *et al.*, 1992a, 1992b, 1997). In the case of a NaF rinse, deposition of CaF$_2$ during the period of rinse application is limited by the availability of calcium. Either 2 hrs (Vogel *et al.*, 1992a) or overnight (Vogel *et al.*, 1997) after these CR rinses are used, large increases in the plaque fluid, salivary, and especially whole plaque fluoride concentrations have been observed relative to a NaF rinse of the same total fluoride content.

Although these results suggest an increased remineralization potential for the controlled-release formulation, recent studies suggest that the release of fluoride during a cariogenic challenge at the surfaces of the teeth may be especially beneficial (reviewed by White *et al.*, 1994). There is, therefore, an interest in determining if these large controlled-release-rinse-induced fluoride deposits would, at an acid pH, release greater amounts of fluoride into plaque fluid than a conventional rinse. The purpose of this study was to compare the fluoride release from a CR rinse and a conventional NaF fluoride rinse under acidic conditions produced by the *in vitro* addition of a small volume of concentrated HCl. Because the release of calcium and phosphate ions at low pH may also provide cariostatic benefits that should be synergistic with those of fluoride, the concentrations of these ions were also examined. We chose an *in vitro* acid addition to avoid confounding interactions when a sucrose rinse is used to induce an *in vivo* or *in vitro* pH decrease. These include fluoride inhibition of acid production, salivary clearance patterns that maintain high concentrations of sucrose and fluoride

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**ABSTRACT**

Plaque fluid ion concentration changes, especially fluoride, in response to the pH decrease associated with a cariogenic episode are important components of the caries process. A “controlled-release” (CR) fluoride rinse, based on the controlled release of fluoride in the presence of calcium, has been shown to form large fluoride reservoirs in resting plaque. In this study, the *in vitro* acid-induced release of fluoride, and other ions, was examined in 48-hour-aged plaque fluid from subjects (*n* = 11) who received no rinse, or who used a 228-ppm CR or NaF fluoride rinse 1 hr before being sampled. After collection, the plaque was centrifuged to yield plaque fluid, acidified (0.1 µL of 0.5 mol/L HCl per milligram plaque), and then re-centrifuged before a second sample was obtained. Although previous studies indicated a higher plaque fluid fluoride after the new rinse relative to NaF, no statistically significant difference was observed here. Average fluoride release after acidification (average pH, 5.2) was statistically greater following the use of the CR rinse (153 µmol/L) compared with the NaF rinse (17 µmol/L). No fluoride release was seen in the no-rinse samples. The pH, free calcium, phosphate, acetate, propionate, and buffer capacity were not affected by the different amounts of fluoride deposited in the plaque. However, following acid addition, an increase in free calcium and phosphate was observed, which was also independent of the rinse. The large release of fluoride following acidification suggests that the new rinse may provide an improved cariostatic effect.

**KEY WORDS:** plaque fluid, rinses, fluoride, ions, acid.


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American Dental Association Health Foundation, Paffenbarger Research Center, Room A153, Bldg. 224, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA; 1Warner Lambert Company, 170 Tabor Road, Morris Plains, NJ 07950; and 2Faculty of Medicine & Dentistry, University of Western Ontario, London, ON, Canada N6A 5C1; *corresponding author, jvogel@nist.gov

Received March 1, 1999; Last revision September 7, 1999; Accepted September 23, 1999
that show that the water component of a sucrose rinse may induce the loss of plaque fluoride and other ions.

It is hypothesized that, after acidification of plaque to a cariogenic pH: (1) the difference in plaque fluid fluoride between the CR and NaF rinses will be increased due to the release of fluoride from the large plaque fluoride deposits induced by the CR rinse, (2) calcium and phosphate concentration will increase in plaque fluid following acidification, (3) neither rinse will influence the release of these ions, and (4) the buffer capacity will not be affected by differences in fluoride concentration induced by the rinses.

MATERIALS & METHODS

Subjects, Fluoride Administration, and Sample Sites

The procedures used in this experiment have been extensively described in recent publications (Vogel et al., 1990a, 1992a,b, 1997, 1998) and are only summarized here. Fluoride administration and plaque collection were done with the informed consent of the subjects following protocols reviewed and approved by the appropriate institutional review boards. Ten males and two females ranging in age from 27 to 56 years, and with no dentures or unfilled cavities, participated in this study. However, one subject failed to complete the CR rinse part of the protocol and was removed from the study. All subjects lived in a fluoridated water area and habitually used F-containing dentifrices. Subjects were issued an electric toothbrush (Oral B Laboratories, Redwood, CA, USA) to ensure good oral hygiene, and were frequently reminded to be diligent in brushing and flossing their teeth. Before each experiment, subjects accumulated plaque for 48 hrs and fasted overnight before morning plaque sampling. About 8:30 am, subjects either rinsed for one min with 20 mL of NaF or CR rinse, or they received no rinse. Plaque samples were then collected 1 hr later. The rinse concentration, 12 mmol/L (i.e., 228 ppm) fluoride, was chosen because it approximates current over-the-counter formulations and is near the maximum values observed in saliva samples obtained 30 sec after a one-minute brushing period with 1000 \( \mu g/g \) or 1500 \( \mu g/g \) (i.e., ppm) fluoride dentifrices (Brun et al., 1984). Separate upper and lower jaw pools of supragingival plaque samples were recovered from the easily accessible buccal-interproximal surfaces of the molar and premolar teeth (excluding the third molars). At least 1 wk separated the application of each of the rinses, which were administered in a random order.

In a few of the experiments, in which no rinse was administered, we obtained a second set of samples and used them to test for the effect of water addition on plaque composition. Eight samples were thus treated. Because preliminary experiments had indicated a release of anions when plaque samples were stored for several hours, 12 no-rinse samples were stored at room temperature for about 3 hrs and then re-analyzed for phosphate, propionate, and acetate.

Rinse Composition and Mechanism of Action

The NaF rinse consisted of 12 mmol/L sodium fluoride in distilled water. The CR rinse consisted of two parts mixed in equal volumes before application: Part A contained 4 mmol/L Na\textsubscript{2}SiF\textsubscript{6} (Aldrich, Milwaukee, WI, USA), while part B contained 20 mmol/L CaCl\textsubscript{2} and 50 mmol/L sodium acetate. It should be noted that previous CR formulations contained phosphate (Vogel et al., 1992a, 1997) and are often referred to in the literature as “two-component”, “two-solution” or A+B rinses.

Collection of Samples

Before plaque was sampled, plastic collection spatulas and mineral-oil-filled microrcentrifuge tubes were weighed together on a microbalance (Vogel et al., 1997). The oil in the centrifuge tube was water-saturated and equilibrated with a gas mixture having volume fractions of 5% CO\textsubscript{2} and 95% N\textsubscript{2} for the prevention of sample evaporation and pH changes associated with CO\textsubscript{2} loss (Vogel et al., 1990b). Next, each subject was asked to suck and then swallow to remove as much saliva as possible, after which the collection spatula, held with a hemostat, was used to collect the sample. The spatula and sample were then placed inside the oil-filled centrifuge tube that was re-weighed to determine the sample weight. The tube, spatula, and sample were then centrifuged (14,000 rpm and 2°C for 4 min) to expel the plaque from the spatula and to separate the fluid from the plaque solids. Next, the spatula was withdrawn, and small aliquots of the fluid phase of plaque (i.e., the plaque fluid) were recovered with oil-filled capillary micropipettes (Vogel et al., 1990b, 1992b). It should be noted that the removal of a small volume of plaque fluid does not significantly reduce the fluoride in the plaque residue (Vogel et al., 1997). Next, the tip of the centrifuge tube was cut, and the plaque distilled into oil-filled 200-µL PCR tubes (Rainin Instrument Co., Emeryville, CA, USA) containing about 0.1 µL of 0.5 mol/L HCl per milligram plaque. The volume of acid was delivered to the PCR tube by means of a calibrated miniature oil-filled Lange-Levy-type micropipette which was prepared by use of a pipette puller (Narishige, Seacliff, NY, USA). After 4 min, a time period sufficient for the acid to diffuse throughout the rather flat plaque residue, the sample was re-centrifuged for 2 min, and a sample of acidified plaque fluid was recovered (total exposure to acid, about 12 min). Sixty-six samples were thus collected and acidified. A similar procedure was used in the water addition experiments.

Several points should be noted with regard to this procedure. First, the volume and concentration of acid were chosen to: (1) obtain a pH between 4 and 5 based on the published buffer capacity of whole plaque (Shellis and Dibdin, 1988), (2) prevent excessive dilution of the sample [a dilution of about volume fraction 30% could be anticipated based on plaque being about a mass fraction of about 35% fluid (Tatevossian and Gould, 1976)], and (3) avoid acid strong enough to rupture cells before dilution into plaque fluid. The acid used here was 1/2 of the concentration used by others in the determination of plaque buffer capacity (Shellis and Dibdin, 1988). Second, the PCR tubes were used in this extraction because: (1) their conical aspect permitted the plaque and acid to form a thin rounded cake that would encourage a rapid diffusion of the acid throughout the cell mass, and (2) their shape was conical enough to permit an aliquot of fluid to be recovered after re-centrifugation. Third, samples were kept fairly large (average mass = 3.3 mg) so that adequate amounts of fluid could be recovered from these tubes. However, since the acid was added in whole volume increments, but the plaque weight was not controllable, the ratio of acid to plaque is only approximate. Thus, the magnitude of the pH values obtained is not of biological significance.

Analysis Methods

Aliquots of plaque fluid recovered before and after acid additions were split into two parts (A and B). Part A was deposited on the surface of an oil-covered, inverted fluoride electrode apparatus and
analyzed for calcium and pH by means of microelectrodes (Vogel et al., 1987, 1990b). However, one important improvement in these methods should be noted: A movable glass tube filled with water was pressed onto the calcium electrode body (Fig. 1) to aid in placement of the microelectrode in mineral oil without causing the loss of the water barrier in the tip of the electrode. This water barrier prevents contamination of the calcium ion exchange fluid by mineral oil or proteinaceous substances, which has proved to be the major problem in the use of calcium electrodes in oral fluids (Vogel et al., 1987). Aliquots of part A were then diluted, 9 parts sample with 1 part TISAB III (Orion, Cambridge, MA, USA), on the surface of the electrode for the determination of fluoride concentration (Vogel et al., 1992b, 1997). Part B was placed on the surface of a mineral-oil-filled 50-mm disposable Petri dish. Aliquots of these samples were then diluted for the determination of phosphate and organic acids by capillary electrophoresis. Because the capillary electrophoresis method used in this laboratory is described for the first time in this report, it is described in more detail.

The capillary electrophoresis instrument was a Waters Quanta 4000 (Waters Corp, Milford, MA, USA) with a 50-cm column of 375 μm o.d. x 75 μm inner diameter operating at 30 kV with the detector at positive polarity. Quantification was done by indirect detection at 254 nm, with 5 mmol/L sorbic acid (Sigma Chemical Co., St. Louis, MO, USA) as the displaced ion in the running buffer. This buffer was prepared daily with de-ionized, de-gassed water (from a 50 mmol/L sorbate stock solution that was neutralized to pH 5.7 with LiOH). Additionally, the diluted buffer contained 0.6 mmol/L tetradecltrimethylammonium bromide (TTAB) to reverse the electroosmotic flow (Sigma T-4762, prepared from a 300 mmol/L stock) and 3.0 mmol/L pH 5.7 MES [2-N-Morpholinoethanesulfonic acid] buffer to control the pH and hence the separation of relevant peaks (Sigma M-5287, prepared from a 750 mmol/L stock, neutralized to pH 5.7 with LiOH). Samples and standards were both diluted with the same capillary “to contain” micropipette (0.01 μL approximate volume) into 5 μL of diluent delivered to a PCR tube by a P10 Pipetman micropipette (Ramin, Woburn, MA, USA), thus obviating the need for calibration of the micropipette (Vogel et al., 1990b, 1997). The diluent was de-ionized water containing 0.1 mmol/L pentane sulfonic acid as a internal standard (used to compensate for small shifts in peak location, thereby ensuring proper peak identification). Sampling was done by means of a 30 s hydrostatic injection directly from the PCR tubes, which were Parafilm “M” (American National Can, Greenwich, CT, USA) to prevent evaporation. Prior to each injection, the capillary was given a six-second pre-wash with 0.1 mol/L HCl in isopropanol (to remove traces of mineral oil and to condition the column), followed by a two-minute wash with the running buffer. Peaks occurred between 2 min and 3 min from the application of voltage (current about 11 μA). The minimum detectable concentration is about 0.0025 mmol/L after dilution (equivalent to 1 mmol/L in the 0.01 μL of sample). It should be noted that several organic acids were detected by this system that are not reported here, notably lactate. However, in these fasted plaque samples, the concentrations of these ions were usually near the detection limit and were not quantified. Because of the large size of the samples, and hence higher accuracy, free calcium and pH measurements were performed only once. Because smaller aliquots were used for the anions (including fluoride), these analyses were performed in triplicate for increased accuracy. The estimated standard uncertainty of the replicates ranged from mass fraction 3% to 10%, depending on the type and concentration of the ion.

Calculation of the ion activity products (IAP) for dicalcium phosphate dihydrate (DCPD), hydroxyapatite (HAp), and fluorapatite (FAp) from these analytical data was performed as previously described (Vogel et al., 1997). The solubility products (Ksp) for these minerals at 25°C are \(-\log(Ksp)_{DCPD} = 6.58\) (Gregory et al., 1970), \(-\log(Ksp)_{HAp} = 58.5\) (McDowell et al., 1998), and \(-\log(Ksp)_{FAp} = 60.5\) (Moreno et al., 1977). Although
Table 1. Effect of Water Addition (0.1 μL/mg) on Baseline (i.e., no-rinse) Plaque Fluid Composition (n = 8 samples)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>Free Ca mmol/L</th>
<th>Phosphate mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before water</td>
<td>6.96 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.68 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.6 ± 4.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>After water</td>
<td>6.95 ± 0.26</td>
<td>0.73 ± 0.13</td>
<td>13.8 ± 3.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± standard deviation.

the solubility product of pure CaF<sub>2</sub> is -log(Ksp)<sub>CaF<sub>2</sub></sub> = 10.4 (McCann, 1968), it has been found that calcium fluoride formed in the presence of phosphate can exhibit ion activity products of about -log(Ksp)<sub>CaF<sub>2</sub></sub> = 10 (Christoffersen <em>et al.</em>, 1988, 1993). The solubility product for tooth enamel calculated, assuming that its stoichiometry is similar to that of HAp, has been found to be highly variable, ranging from -log(IAP)<sub>HAp</sub> = 52.1 to nearly as high as that of HAp after repeated extraction with acid (Patel and Brown, 1975). However, the single value of 54.3 has been frequently cited (Margolis <em>et al.</em>, 1988, 1993; Margolis and Moreno, 1992).

Statistical Methods

Descriptive statistics were calculated for all variables measured for each group and for upper and lower arches separately. Multiple regression analysis was used to determine the effects on plaque fluoride level of the independent variables which were measured both before and after the addition of acid. Interactions among variables were also assessed. A logarithmic transformation of the plaque fluoride level was used prior to an analysis of variance test of the null hypothesis that there is no difference in plaque fluoride levels between the two rinses. When the assumption of equal variances was violated, the Welch ANOVA test was used to determine statistically significant differences between the groups (Welch, 1951). All tests of the null hypothesis were two-sided and based on a level of significance (alpha) of 0.05. One no-rinse group sample was lost during the study, and thus 10 subjects were used in the statistical analysis. The data tables and figures, however, show the values for 11 subjects. Additionally, two values found in the CR rinse data (one fluoride and one calcium value from different samples) were very large and appear to be outliers (Grubbs and Beck, 1972). These data were not omitted from the analysis, but the effect of their omission on the average values is noted in the footnote to the tables. In the text and tables, ± refers to the standard deviation, which in this paper is used as a measure of the standard uncertainty.

RESULTS

The addition of 0.1 μL/mg distilled water to plaque (Table 1) had only a minimal effect on pH and calcium and phosphate concentrations in plaque fluid. However, the 12 samples that were stored for about 3 hrs had the following mean increases in the mass fraction of anion concentrations: 87% ± 38%, 15% ± 17%, and 173% ± 107% for acetate, phosphate, and propionate, respectively.

No significant differences in plaque fluid composition, except for the fluoride concentrations, were produced by the different fluoride rinses before acidification (Table 2). Both rinses produced plaque fluid fluoride concentrations that were statistically greater than those at the no-rinse baseline.

Although a larger mean plaque fluid fluoride level was observed with the CR rinse compared with the NaF rinse, the difference was not statistically significant. Except for phosphate, which was significantly higher at the upper molar sites, no other significant site-related concentration differences were found.

The mean plaque fluid fluoride values among the NaF, CR, and no-rinse groups (Table 2) after acidification were all found to be statistically different from each other. In terms of acid-induced fluoride release (Fig. 2), a large release is seen with the CR rinse plaque samples (average releases from upper and lower sites, 153 μmol/L), while only a small increase is seen with the NaF samples (average release, 17 μmol/L). Plaque acidification produced similar pH values (about 5.2) in each experimental group (Table 2) and resulted in a similar increase in the free calcium (61 of 66 samples) and phosphate (all samples). However, these increases were not correlated with the post-acidification pH. The number of micromoles of H<sup>+</sup> ions added per gram of plaque under observed pH curves (Fig. 3) was similar among all experimental groups. Because the initial pH values were similar (Table 2), and because most of the buffer capacity is below pH 6 (Shellis and Dibdin, 1988), the quantity plotted in Fig. 3 is essentially the integrated whole plaque buffer capacity required to reach the final pH.

All plaque samples were supersaturated with respect to enamel, HAp, FAp, and, except for one no-rinse sample, DCPD, before the acid addition (Table 2). After acidification, only 2 of the no-rinse samples, with very low pH values (3.69 and 3.81), became undersaturated with respect to FAp, so that, on average, all samples remained highly supersaturated with respect to this mineral. Most of the samples became undersaturated after the acid addition with respect to both enamel and DCPD (Table 2). Specifically, 57 out of the 66 acidified samples (those with pH < 5.4) had -log(IAP)<sub>enamel</sub> values > 54.3, and 53 of the samples had -log(IAP)<sub>DCPD</sub> > 6.6. All but one of the CR-rinse plaque-fluid samples were undersaturated with respect to CaF<sub>2</sub> before acidification. After acidification (Fig. 4), about half of the CR-rinse samples were either supersaturated or slightly undersaturated with respect to this mineral (i.e., in this Fig., are either above or below the dotted line, respectively). In contrast, all but one of the acidified NaF samples remained undersaturated with respect to calcium fluoride. With respect to the calcium fluoride ion activity products observed in the presence of phosphate described above (solid line, Fig. 4), two of the CR-rinse post-acidified samples appear to be slightly supersaturated. The CaF<sub>2</sub> saturation of the no-rinse samples was unaffected by acidification (Table 2) and, therefore, is not shown in Fig. 4.

DISCUSSION

The constancy of the plaque fluid pH, free calcium, and phosphate values (Table 1) after the addition of distilled water indicates that plaque buffer capacity and the mineral stores of these ions in plaque are sufficient to overcome an approximately 30% volume fraction dilution. The increases observed in acetate and propionate ion activity concentrations indicate that the three-hour storage of plaque samples are in agreement with a recent review describing the effect of plaque storage (Margolis and Moreno, 1994) and with observations made during studies of plaque buffer capacity (Shellis and Dibdin, 1988).
The no-rinse plaque fluid fluoride values (Table 2) are somewhat lower than previously obtained: 12.7 ± 2.8 μmol/L and 14.7 ± 4.9 μmol/L (Vogel et al., 1992a) and 83 ± 41 μmol/L (Vogel et al., 1997). However, the no-rinse free calcium and phosphate concentrations are similar both to our previous values (Carey et al., 1986; Vogel et al., 1998) and to the values obtained by others (Margolis and Moreno, 1994). Since there were no statistically significant differences in composition in the pre-acidiﬁed samples, except for ﬂuoride, neither the ﬂuoride in the rinses nor the calcium and acetate contained in the CR rinse altered the plaque ﬂuid composition.

Although the differences are not statistically signiﬁcant, the data (Table 2 with the outlier omitted) are in agreement with previous observations that ﬂuoride concentrations in the plaque ﬂuid are higher at lower molar sites compared with upper molar sites (Weatherell et al., 1988; Vogel et al., 1992b; Ekstrand, 1997). The opposite distribution for plaque ﬂuid phosphate agrees with the distribution of whole plaque phosphate noted by others (Ashley, 1975) and with recent studies in this laboratory (Vogel et al., unpublished data). Previous studies with these rinses (before acid values) found a signiﬁcantly lower concentration of plaque ﬂuid ﬂuoride one hour after rinsing with NaF compared with the CR rinse (Vogel et al., 1992a). However, the relative difference between the rinses in the current study was lessened, and the standard deviations were higher. Thus, no statistically signiﬁcant difference was found in plaque ﬂuid ﬂuoride following the two rinses.

Previous studies where a decrease in plaque ﬂuid pH was produced by the in vivo administration of sucrrose (Rankine et al., 1985, 1989; Margolis and Moreno, 1992; Margolis et al., 1993) exhibited an increase in total calcium, which is consistent with the free calcium values measured in the current experiments (Table 2). However, the increase in total phosphate observed here has been found in some studies (Rankine et al., 1985; Margolis and Moreno, 1992) but not in others (Rankine et al., 1989; Margolis et al., 1993). Although phosphate utilization of cells has been postulated to explain the lack of increase in these in vivo studies (Margolis and Moreno, 1992), this mechanism would not be expected to be of signiﬁcance in the fasted samples used in the current experiment, where the pH drop was induced by HCl addition rather than by sucrrose. Diffusion of phosphate into the aqueous phase of a sucrrose rinse may also be a factor, since it has been found (Vogel et al., unpublished data) that simply rinsing with water produced a decrease in plaque ﬂuid phosphate that was similar in magnitude to the increases in phosphate seen here. Recent studies (Vogel et al., 1998) have also found a larger decrease in plaque ﬂuid phosphate when salivary ﬂow, and hence diffusion into saliva, is increased during the chewing of sugarless gum. The post-acidiﬁcation release of either calcium or phosphate (Table 2) was also not affected by the ﬂuoride in the NaF rinse or by the presence of both calcium and fluoride in the CR rinse administered 1 hr before the acid addition. The integral buffer capacities (Fig. 3) are generally lower than those.

### Table 2. Plaque Fluid Composition, pH, and -log IAP Values (ion activity products) Before and After Addition of 0.5 mol/L HCl to Plaque (0.1 μL/mg)

<table>
<thead>
<tr>
<th>Rinse Type</th>
<th>pH</th>
<th>Fluoride μmol/L</th>
<th>Free Calcium mmol/L</th>
<th>Phosphate mmol/L</th>
<th>Acetate mmol/L</th>
<th>Propionate mmol/L</th>
<th>-log IAP DCPD</th>
<th>-log IAP enamel-HAp</th>
<th>-log IAP FAp</th>
<th>-log IAP CaF₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>No-rinse before</td>
<td>6.90 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.5 ± 2.1</td>
<td>0.76 ± 0.26</td>
<td>14.4 ± 4.4</td>
<td>15.8 ± 6.5</td>
<td>4.1 ± 2.8</td>
<td>6.26 ± 0.16</td>
<td>49.7 ± 1.4</td>
<td>47.9 ± 1.1</td>
<td>14.45 ± 0.44</td>
</tr>
<tr>
<td>No-rinse acid</td>
<td>5.15 ± 0.54</td>
<td>42 ± 20</td>
<td>2.6 ± 1.6</td>
<td>203 ± 5.2</td>
<td>16.5 ± 6.0</td>
<td>54 ± 3.8</td>
<td>7.02 ± 0.71</td>
<td>57.9 ± 4.4</td>
<td>54.6 ± 3.9</td>
<td>14.22 ± 0.50</td>
</tr>
<tr>
<td>NaF before</td>
<td>7.00 ± 0.27</td>
<td>54 ± 54</td>
<td>1.05 ± 0.50</td>
<td>147 ± 2.6</td>
<td>16.9 ± 7.9</td>
<td>48 ± 3.1</td>
<td>6.10 ± 0.25</td>
<td>48.6 ± 1.5</td>
<td>46.1 ± 1.4</td>
<td>12.65 ± 0.88</td>
</tr>
<tr>
<td>NaF acid</td>
<td>5.35 ± 0.45</td>
<td>94 ± 48</td>
<td>3.1 ± 1.5</td>
<td>19.3 ± 2.9</td>
<td>14.9 ± 3.9</td>
<td>53 ± 2.3</td>
<td>7.67 ± 0.48</td>
<td>56.2 ± 3.2</td>
<td>51.7 ± 2.8</td>
<td>11.47 ± 0.65</td>
</tr>
<tr>
<td>CR before</td>
<td>6.84 ± 0.24</td>
<td>124 ± 184</td>
<td>0.90 ± 0.54</td>
<td>140 ± 3.8</td>
<td>17.7 ± 5.7</td>
<td>43 ± 2.5</td>
<td>6.26 ± 0.19</td>
<td>49.9 ± 1.4</td>
<td>47.0 ± 0.9</td>
<td>12.3 ± 1.1</td>
</tr>
<tr>
<td>CR acid</td>
<td>5.22 ± 0.52</td>
<td>254 ± 174</td>
<td>3.6 ± 5.4</td>
<td>16.9 ± 4.5</td>
<td>17.3 ± 5.2</td>
<td>6.0 ± 3.8</td>
<td>7.03 ± 0.55</td>
<td>57.7 ± 3.5</td>
<td>52.7 ± 2.9</td>
<td>10.80 ± 0.83</td>
</tr>
</tbody>
</table>

<sup>a</sup> n = 11 subjects unless noted. The pH values (and hence the IAP values) depend on the ratio of acid added to plaque weight, which is only approximate.

<sup>b</sup> Samples with <log> IAP values > <log> Ksp are undersaturated. The <log> Ksp values for the relevant phases: dicalcium phosphate dihydrate (DCPD) = 6.6, hydroxyapatite (HAp) = 58.5, tooth enamel (calculated as HAp; see text) = 54.3, fluorapatite (FAp) = 60.5, and calcium fluoride (CaF₂) = 10.4. Average \(-\log IAP\) calculated as the average of the individual IAP values.

<sup>c</sup> ± = standard deviation. pH calculated as the average of the low log of the average hydrogen ion concentration (Margolis et al., 1988).

<sup>d</sup> One very high sample was in each of these upper molar CR rinse groups ("before" group fluoride = 638 μmol/L, "acid" group free calcium = 19.6 mmol/L). These were statistical outliers according to the criteria of Grubbs and Beck (1972). With these numbers omitted, the average values that were substantially changed were: ["before" group] fluoride = 72 ± 74; ["acid"] IAP(CaF₂) = 12.02 ± 0.78; ["acid"] free calcium = 1.97 ± 0.72. Exclusion of these values does not affect the conclusions.

<sup>e</sup> CR rinse statistically greater than NaF at p < 0.05.

<sup>f</sup> Upper site > lower site at p < 0.05.

<sup>g</sup> n = 10.
obtained by Shellis and Dibdin (1988) in a step-wise titration, but they are similar to those obtained by Strálfors (1948). The increase in whole plaque buffer capacity with decreasing pH is in agreement with these previous studies. The similarity of the pH response in this Fig. demonstrates that the various samples, in spite of their great differences in fluoride concentration, have a similar buffer capacity. This suggests that the influence of fluoride on buffer capacity must be small.

Although the release of calcium and phosphate ions at low pH (Table 2) would be consistent with mineral stores in plaque (Vogel et al., 1990b; Margolis and Moreno, 1992), it is probable that there are several processes involved. Specifically, more phosphate was released than calcium, a process inconsistent with the stoichiometry of calcium phosphate minerals likely to form in plaque at resting pH. Given the increase in calcium and phosphate in almost all the individual samples after acid addition, the lack of a pH correlation for these ions is certainly a reflection of the various kinds of calcium and phosphate stores and the different amounts of these stores in the samples. For example, pH-sensitive calcium binding has been demonstrated in dental plaque (Rose et al., 1994), and a very large standard deviation is often found in studies of the calcium and phosphate content of plaque (Ashley, 1975).

Nearly all of the samples became undersaturated with respect to enamel after acidification. But only the two no-rinse samples (with pH < 4 and fluoride concentrations of less than 3.2 μmol/L) became undersaturated with respect to FAp. This result is difficult to reconcile with studies demonstrating that shark enamel, which is a mass fraction of about 75% fluorapatite, can demineralize in an in vivo model (Ogaard et al., 1988). Nearly all the samples became undersaturated with respect to the nominal solubility of enamel - log (IAp)HAp = 54.3, indicating that sufficient acid was added for demineralization of tooth mineral. Although previously it has been suggested that plaque is in equilibrium with DCPD (Vogel et al., 1990b), most of the samples became undersaturated with respect to this mineral after acidification, which suggests that the amount of this mineral in two-day-old plaque is small.

Acidification of the no-rinse samples produced a small decrease in plaque fluid fluoride consistent with the dilution of the samples by the added acid. These results are in agreement with recent in vivo studies after sucrose administration (Tanaka and Margolis, 1999) and suggest that labile stores of fluoride in plaque samples must be low in samples collected 48 hrs following the use of a fluoride dentifrice. Although the CR rinse was devised to enhance deposition of CaF₂ in the oral environment, the fate of deposited CaF₂ after either rinse is the subject of some controversy, because the relatively high solubility of CaF₂ suggests that it would rapidly be lost by dissolution into saliva (McCann, 1968). One explanation for this observed persistence of CaF₂ is the presence of phosphate-containing calcium fluoride deposits (referred to as “calcium-fluoride-like”). The formation of these deposits has been found to diminish the rate of dissolution of pure CaF₂ dramatically when this mineral is exposed to phosphate-containing solutions (Christoffersen et al., 1988, 1995; Lagerlöf et al., 1988; Rolla and Saxegaard, 1990). Such deposits, which are very likely to form in saliva and more particularly in plaque fluid, given its very high phosphate content (Table 2), may explain how the NaF and CR rinse plaque samples could maintain a relatively high CaF₂ undersaturation in plaque fluid before acidification.

![Graph](image-url)
It has been suggested that the phosphate-containing “calcium-fluoride-like” material formed in vivo after these rinses loses phosphate and releases fluoride under acidic conditions (Rolla and Saxegaard, 1990). Consistent with this model, with the proposed mechanism of CaF$_2$ deposition after the use of a CR rinse, and with the very large in vivo and in vitro deposition of fluoride previously seen when this rinse is used (Chow and Takagi, 1991; Chow et al., 1992; Vogel et al., 1992a, 1997), acidification of the CR rinse plaque samples releases nearly 9 times more fluoride than acidification of the NaF samples (Fig. 2). The release of fluoride from these samples is negatively correlated with pH (correlation coefficient = 0.587, p < 0.05) if the point corresponding to the one fluoride outlier noted in Table 2 is removed from the dataset. No relationship between pH and fluoride release is seen in the NaF samples.

Recent studies found that, 1 hr after a NaF or CR rinse, whole plaque samples similar to those used here contain approximately 3.9 µg/g (NaF rinse) and 49 µg/g (CR rinse) extractable fluoride, measured by a procedure that extracts only loosely bound fluoride (Vogel et al., unpublished data). However, the difference in plaque fluid fluoride between these rinses (Table 2 “before” values) is much more modest, suggesting that the effect of phosphate “stabilization” of these deposits is difficult to predict. Specifically, phosphate, besides decreasing the rate of release of fluoride from pure CaF$_2$, can, as described above, increase the ion activity product (and thus makes this material more soluble) and can also act as a crystal growth poison (Christoffersen et al., 1988, 1995). The ion activity products with respect to CaF$_2$ (Fig. 4) after acidification of the CR rinse plaque samples are consistent with the former mechanism, since many of the samples became supersaturated with respect to pure CaF$_2$, but only two of these samples became slightly supersaturated with respect to the ion activity product of CaF$_2$ formed in the presence of phosphate. These latter two values may be explained by the variability of the ion activity products of “calcium-fluoride-like” deposits in the presence of very high plaque fluoride levels. The complex nature of the phosphate effect may explain why, in previous studies, where phosphate was present in the applied rinse, the CR rinse produced a relatively greater plaque fluid fluoride concentration, compared with the NaF rinse, than in the current study. Finally, it is important to note that, although the behavior of these samples, especially the CR samples, is consistent with the formation of “calcium-fluoride-like” deposits, it is also consistent with a recent bacterial fluoride binding model that suggests that fluoride is held by pH-sensitive calcium bridges (Rose et al., 1996).

Since, as noted above, the CR fluoride rinse deposits a much higher percentage of water-extractable fluoride in plaque, and since several studies have found that this rinse deposits up to 6 times more total fluoride than the NaF rinse (Vogel et al., 1992a, 1997), the moderate difference in plaque fluid fluoride concentration following these rinses suggests that a dramatic reduction in the rate of fluoride release must have been induced after the CR rinse. This high deposition and slow release at resting pH may be responsible for the maintenance of the high plaque fluid fluoride concentration found overnight after application of the CR rinse (Vogel et al., 1997). More significant, given the apparent importance of fluoride release during the caries process (Arends and Christoffersen, 1990; Margolis and Moreno, 1990; White et al., 1994), is the very large release of fluoride from plaque following use of a CR rinse at a low pH. These results suggest that a “controlled release” fluoride rinse may produce a superior cariostatic effect without increasing the amount of fluoride administered.

ACKNOWLEDGMENTS

This investigation was supported, in part, by USPHS Research Grants DE10840 and DE05534 to the American Dental Association Health Foundation from the National Institutes of Health-National Institute of Dental and Craniofacial Research and is part of the dental research program conducted by the National Institute of Standards and Technology in cooperation with the American Dental Association Health Foundation.

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