Effect of acidic solution viscosity on enamel erosion

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Abstract: The objective of this in vitro study was to investigate the effects of viscosity changes of different acidic solutions on dental erosion. Bovine enamel samples (n = 240, Ø = 3 mm) were embedded in acrylic resin and were allocated to 30 groups (n = 8). Citric acid (CA) and phosphoric acid (PA) solutions at pH 2.5, 3, and 3.5 were prepared in de-ionized water (titratable acidity to pH 5.5: 31 ± 0.6 mmol OH(−)/l). The kinetic viscosities of the acidic solutions were adjusted to 1.5, 3, 6, 12, and 24 mm(2)/sec by the addition of hydroxypropyl cellulose (HPC) at different concentrations. Solutions were pumped over the enamel surface from a reservoir with a drop rate of 1 mL/min. Each specimen was eroded for 10 min at 20 °C. Erosion of enamel surfaces was measured by profilometry. Data were analyzed by analyses of variance and logarithmic regression analyses (p < 0.05). Enamel loss was dependent on viscosity, pH, and the kind of acid. The regression analyses showed that higher viscosity caused lower enamel erosion for both acids and all pH levels. Dental erosion is dependent not only on chemical factors of the acid, like pH and acid type, but also on acid viscosity.

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The impact of the viscosity variations of acidic solutions on enamel erosion

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ABSTRACT

**Aim:** The objective of this *in vitro* study was to investigate the effect of viscosity changes of different acidic solutions on dental erosion. **Material and methods:** Bovine enamel samples (n=240, φ=3 mm) were embedded in acrylic resin and were allocated to 30 groups (n=8). Citric acid (CA) and phosphoric acid (PA) solutions at pH 2.5, 3 and 3.5 were prepared in deionized water (titratable acidity to pH 5.5: 31 ± 0.6 mmol OH⁻/l). The kinetic viscosities of the acidic solutions were adjusted to 1.5, 3, 6, 12 and 24 mm²/s by adding hydroxypropyl cellulose (HPC) at different concentrations. Solutions were pumped over the enamel surface from a reservoir with a drop rate of 1ml/min. Each specimen was eroded for 10 min at 20°C. Erosion of enamel surfaces was measured using profilometry. Data were analysed using variance analyses and logarithmic regression analyses (p<0.05). **Results:** Enamel loss was dependent on viscosity, pH and kind of acid. The regression analyses showed that higher viscosity caused lower enamel erosion for both acids and all pH levels. **Conclusion:** Dental erosion is not only dependent on chemical factors of the acid, like pH and acid type, but also on acid viscosity.
INTRODUCTION

Dental erosion, is defined as the pathologic, chronic, localized loss of dental hard tissues that is chemically etched away from the tooth surface by acid and/or chelation without bacterial involvement (ten Cate and Imfeld, 1996). Numerous chemical factors, like pH, titratable acidity, degree of saturation, kind of acid and chelating properties, have been identified to influence the erosive potential of acids (West et al., 2000; Eisenburger and Addy 2003; Lussi et al., 2004; Attin et al., 2005; Lussi et al., 2012). Thereby, the frequency and duration of acid contact might determine the development and progression of erosive lesions. However, compared to the chemical factors, limited information about the effect of the physical or physico-chemical aspects of the acid on dental erosion is available yet. It is known that enamel and dentin erosion increased with increasing velocity of the acid flow, due to a faster ion exchange and clearance of dissolution products (Eisenburger and Addy 2003; Shellis et al., 2005; Wiegand et al., 2007; Attin et al., 2012). Moreover, the thermodynamic properties of acidic solutions might influence their adhesiveness to and displacement of tooth surfaces (Ireland et al., 1995), which in turn might also affect their erosive capability.

To the authors’ best of knowledge, there is no information available so far about a relation between the viscosity of acidic solutions and their erosive potential. Recent studies suggest that the erosion-inhibiting effect of polymers added to acidic solutions might not only be related to the formation of an erosion-protective layer on the surface, but also to a modification of the viscosity of the drink (Hughes et al., 2002; West et al., 2004; Barbour et al., 2005).
Therefore, the aim of this in vitro study was to investigate the effect of viscosity changes of different acidic solutions on dental erosion. The null hypothesis was that the erosivity of citric acid and phosphoric acid at different pH-values is not affected by their viscosity.

MATERIALS METHODS

Specimen preparation

Specimens were gained from intact bovine incisors of 2-3 years old cattles. The crowns were separated from roots and stored in 0.5% thymol solution for a maximum of 6 months at 5°C until used. A total of 240 cylindrical enamel specimens (diameter: 3 mm) were prepared using a water-cooled trephine bur and embedded in acrylic resin blocks (diameter: 6 mm, Paladur, Heraeus Kulzer, Germany). The enamel surfaces were then ground and fine ground with water-cooled discs (1200, 2400 and 4000 grit, Water Proof Silicon carbide Paper, Struers, Erkrath, Germany). The specimens were randomly allocated to 30 groups of each n = 8 specimens.

Preparation of acidic solutions

Citric acid (CA) and phosphoric acid (PA) solutions at pH levels of 2.5, 3.0 and 3.5 were used. CA solutions at pH 2.5, 3.0 and 3.5 were obtained by adding 2.81, 3.3 and 4.17 g/l (Fluka, Buchs, Switzerland) to deionized water, respectively. PA solutions at the respective pH-values were obtained by adding 30.5, 109.3 and 110.3 ml/l, respectively from a 3 mol/l stock solution (Merck, Darmstadt, Germany) to deionized water. In order to compare the respective acidic solutions under the same conditions, the titratable acidity (TA) of 31
mmol OH⁻/l to pH 5.5 was chosen which was dictated by pH 2.5 CA solution having a possible minimum titratable acidity of 31 mmol OH⁻/l to pH 5.5. The titratable acidities were measured with a titrator (Metrohm, Herisau, Switzerland).

The kinetic viscosities of the acidic solutions were adjusted to 1.5, 3, 6, 12 and 24 mm²/s, respectively by adding hydroxypropyl cellulose (HPC, Grade LM, HPC, Nippon Soda Company, Japan) at different concentrations. The viscosities were checked with a viscometer (Becker Research Equipment, Göttingen, Germany), which is described in detail in a previous study (Seeliger et al., 2010).

By adding 35.8 g HPC to a 1000 ml of the respective acid solution, a viscosity of 34 ± 1 mm²/s was obtained. These solutions were then diluted with the respective acid until the targeted viscosity was reached. After adjusting the viscosities the titratable acidities and the pH levels of the respective acidic solutions were then again measured and if necessary drops of NaOH or HCl were added for final adjustment.

The viscosities were chosen regarding the results of a preliminary test, which aimed to determine the viscosities of commercially available potentially erosive drinks. Twenty-five potentially erosive beverages marketed in Switzerland were chosen, and the viscosities of the beverages were measured 5 times for each beverage by means of the above-mentioned viscometer. The viscosities of the drinks ranged from 0.91 to 58.65 mm²/s, with only few drinks exhibiting a very high viscosity (≥ 20 mm²/s, Table 1).

**Experimental procedure**

From each specimen five baseline profiles were recorded with a stylus
profilometer (Perthometer S2; Mahr, Göttingen, Germany) with a distance of 50 µm between each profile. To ensure an exact repositioning of the samples during and after the experimental procedure, the profilometer and the samples were equipped with a custom-made jig (Wiegand et al., 2009).

All experiments were performed at a controlled room temperature of 20°C. The temperatures of the respective acidic solutions were also adjusted to 20-21°C as the viscosity is highly depended on the temperature of a liquid (Bourne, 2002). The respective acidic solutions were dropped from a reservoir into an inclined (45°) channel which was made from poly vinyl chloride with 8 mm in length of a flow rate of 1 ml/min and which directed the acidic solutions over the surface of an enamel specimen (Figure 1). The specimens were eroded for 10 min at a constant room temperature (20°C), this means that a total amount of 10 ml flow on each sample. Then, the specimens were rinsed with distilled water, and profilometry was performed again. Enamel wear was calculated by a custom-made software (4D Client, custom designed software; University Zurich, Zurich, Switzerland), comparing the baseline profiles with the respective profiles after erosion.

**Data analysis**

Descriptive statistics (mean, SD) were computed, and normality of the data was checked with Kolmogorov-Smirnov and Shapiro-Wilk’s tests. As normal distribution was found in all groups (p>0.05), data were analyzed by three-way ANOVA, factors being acid type, pH and viscosity. As significant interactions between the experimental factors were observed, the data were split according to the acid and analyzed further by using two times two-way ANOVAs. Again
significant interactions were found, so that the data were split according to pH, and six times one way-ANOVA followed by Scheffé’s post hoc tests was applied. Additionally a regression model was used to study the relation between viscosity and enamel erosion. The level of significance was set at $\alpha = 0.05$.

RESULTS

Generally, PA caused higher erosion than CA. Mean enamel loss by erosion with CA and PA is presented in Figure 2 a-b. All one-way ANOVAs showed significant differences in enamel loss. Generally, Scheffe’s post-hoc tests revealed highest loss for the acid with the lowest viscosity and lowest loss for the acid with the highest viscosity (2 a-b).

The logarithmic regression analysis revealed that enamel erosion decreased with increased viscosity (Figure 3). Thereby, at pH 2.5, enamel losses decreased from $6.8 \pm 0.6 \, \mu \text{m} \text{ (CA)}$ and $14.7 \pm 2.0 \, \mu \text{m} \text{ (PA)}$ at viscosity $1.5 \, \text{m}^2/\text{s}$ to $5.0 \pm 0.5 \, \mu \text{m} \text{ (CA)}$ and $10.4 \pm 1.2 \, \mu \text{m} \text{ (PA)}$ at viscosity $24 \, \text{mm}^2/\text{s}$. At pH 3, enamel losses decreased from $4.6 \pm 0.6 \, \mu \text{m} \text{ (CA)}$ and $7.9 \pm 1.4 \, \mu \text{m} \text{ (PA)}$ at viscosity $1.5 \, \text{mm}^2/\text{s}$ to $2.7 \pm 0.3 \, \mu \text{m} \text{ (CA)}$ and $5.6 \pm 0.5 \, \mu \text{m} \text{ (PA)}$ at viscosity $24 \, \text{mm}^2/\text{s}$. At pH 3.5, enamel losses decreased from $2.6 \pm 0.3 \, \mu \text{m} \text{ (CA)}$ and $2.5 \pm 0.5 \, \mu \text{m} \text{ (PA)}$ at viscosity $1.5 \, \text{mm}^2/\text{s}$ to $2.0 \pm 0.3 \, \mu \text{m} \text{ (CA)}$ and $1.5 \pm 0.2 \, \mu \text{m} \text{ (PA)}$ at viscosity $24 \, \text{mm}^2/\text{s}$.

DISCUSSION

To the first time it was shown that the erosive potential of acids is not only dependent on various chemical properties, such as pH, but also on the viscosity of the acidic solution. Therefore, the null hypothesis is rejected.
In order to isolate the effect of viscosity only, the tested acids (CA and PA) were adjusted to the same pH-values and the same titratable acidity. Titratable acidity is an important parameter to characterize the erosive potential of different acidic solutions for the same pH levels. In the present study, the titratable acidity was chosen according to the native titratable acidity of citric acid at pH 2.5. It was decided the measure the amount of base needed to raise the pH to 5.5, as a pH equal to or less than 5.5 is traditionally considered to be critical for enamel dissolution, although mineral loss may even begin at higher pH (Birkhed, 1984).

The acids and the ranges of pH-values were chosen according to our preliminary study and the composition of typically erosive beverages (Lussi et al., 2012). The tested increments of viscosities were determined according to the results of a preliminary test, which provided us the data that viscosities of potentially erosive beverages range from 0.91 to 58.65 mm²/s, with only few drinks exhibiting a very high viscosity ≥ 20 mm²/s. Therefore, it was decided to use the viscosities of 1.5, 3, 6, 12 and 24 mm²/s.

The viscosities were adjusted by adding hydroxypropyl cellulose (HPC), which is a derivative of polysaccharide cellulose and widely used in food, drug and cosmetics areas (Heitfeld et al., 2008) and is a non-ionic, water-soluble and pH-insensitive cellulose ether (Kadajji and Betaferi, 2011).

The set up of the present study allowed for a free flow of the acid drops over the surface of the enamel specimens, so that the viscosity of the liquid rather than any pump system determined the flow rate of the acid over the surface. Due to the fact that the viscosity is directly related to the temperature of fluids (Kestin et al., 1978), not only the experiments were undertaken at a standardized
temperature of 20ºC but also the temperature of the tested solutions was adjusted to 20ºC.

As the erosive enamel loss decreased with increasing viscosity, it can be assumed that the relative stickiness of the acid solutions with higher viscosity decreased the ion exchange and clearance of dissolution products. The clearance of dissolution products from the enamel surface is determined by the dissolution through a static liquid layer, which becomes partly saturated with respect to mineral dissolved from the underlying enamel (Shellis et al., 2005; Wiegand et al., 2007). At higher viscosity, this static layer might be thicker and less undersaturated, so that enamel erosion decreased. However, it is not known so far if there is also any interaction between the HPC polymer and the enamel surface, which might influence the erosive potential of the HPC-modified acids. In previous studies it was shown that polymer-modified citric acid solutions with a viscosity > 50 mPa s were less erosive than the unmodified solution under stirring conditions, probably due to the formation of a polymer layer on the surface (Beyer et al., 2010; Beyer et al., 2012). In this regard it has also to be discussed that the presence of the acquired salivary pellicle was not considered in the present study. The salivary pellicle is known to reduce erosive demineralization (Wiegand et al., 2008; Hannig et al., 2009), but might also affect the ability of liquids to adhere to enamel (Ireland et al., 1995). However, to minimize the variables in this study and to allow focusing on the viscosity, a salivary pellicle was not formed on the specimens’ surface.

Xanthan gum, pectins, chitosan derivatives, dextran, carrageenan, guar gum and cellulose ethers (e.g. hydroxypropylmethyl cellulose (HPMC), hydroxypropyl cellulose (HPC), hydroxyethyl cellulose (HEC), sodium carboxy methyl cellulose
(Na-CMC)) as well as albumin, starch or starch based derivatives are natural water soluble polymers and have been reported as inhibitors of dental erosion (West et al., 2004; Barbour et al., 2005; Hooper et al., 2007; Beyer et al., 2010; Gracia et al., 2010). Thereby, the erosion reduction mechanism was described as not only related to the formation of the gel-like polymer layer on enamel surfaces but also to an increased viscosity resulting in a reduced ion mobility and slower dissolution kinetics of polymer modified citric acid solutions (Beyer et al., 2010). The authors also hypothesized that the enamel protecting polymer layer was formed by the interactions between negatively charged carboxyl groups of polymers and calcium ions of the enamel surface and between negatively charged carboxyl groups of different polymer molecules in presence of positively charged calcium ions by forming a chelate complex (Beyer et al., 2010). HPC used in the present study is known to be non-ionic, so a binding to the surface seems relatively unlikely (see appendix).

In the present study, phosphoric acid generally caused higher enamel erosion than citric acid. This observation is in contrast to the results of previous studies, usually showing a higher erosive potential of citric acid, probably also due to the chelating properties of citric acid (Wiegand et al., 2007, Wiegand et al., 2008). It might be speculated that the free-flow mechanism of the respective acidic solutions did not provide enough time for the chelating ability of citric acid to become relevant. It must also be considered that different experimental conditions make a comparison between studies difficult, not least as the erosive potential of acids is influenced by temperature, concentration, exposure time and/or exposure type. To best of our knowledge there is no study regarding the comparison of erosive potential of CA and PA at same pH levels and same
titratable acidities which might be responsible for greater erosion for PA. It has also to be considered that bovine instead of human enamel was used in this study. In this regard it has to be mentioned that bovine enamel presents a higher susceptibility to erosion than human enamel (Attin et al. 2007), probably due to differences in morphological, chemical and physical properties. Although the differences between the two substrates must be considered when interpreting results obtained from any experiment using bovine tooth substrate (Yassen et al., 2011), the use of bovine specimens seems to be appropriate when relative rather than absolute differences are of interest (Wiegand and Attin, 2011).

Although the chemical factors (pH and kind of acid) had a higher influence on erosion than the physical aspect (viscosity), the present study demonstrated that modeling of erosive conditions is very complex and not only dependent on chemical aspects, like pH or titratable acidity, which are usually considered as erosion-determining factors.

Further studies have to show whether adding HPC to commonly consumed acidic beverages might influence their erosive potential.

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Figure Legends

**Figure 1.** Flow incline allowing for rinsing 8 specimens in parallel.  
**a:** Plastic tubes attached to the pump (Internal diameter: 3 mm)  
**b:** Acid drops  
**c:** Inclined U-form channel (Depth: 0.68 mm, Width: 2.00 mm)  
**d:** Enamel sample

**Figure 2a.** Enamel loss (µm, mean ± standard deviation) caused by CA at pH 2.5, 3.0 and 3.5 and different viscosities ranging from 1.5 to 24 mm²/s. Within each pH, significant differences between the viscosities were marked by different letters.

**Figure 2b.** Enamel loss (µm, mean ± standard deviation) caused by PA at pH 2.5, 3.0 and 3.5 and different viscosities ranging from 1.5 to 24 mm²/s. Within each pH, significant differences between the viscosities were marked by different letters.

**Figure 3.** Logarithmic regressions of viscosity of CA and PA at pH levels of 2.5, 3.0 and 3.5. (R Sq Linear = 0.579, 0.480, 0.338 for CA pH 2.5, 3.0 and 3.5, respectively; R Sq Linear = 0.689, 0.596, 0.263 for PA pH 2.5, 3.0 and 3.5, respectively)
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The impact of the viscosity variations of acidic solutions on enamel erosion

APPENDIX

INTRODUCTION

In previous studies it was shown that polymer-modified citric acid solutions were less erosive than the unmodified solution, probably due to the formation of a polymer layer on the surface (Beyer et al., 2010; Beyer et al., 2012). The authors hypothesized that a protecting polymer layer was formed by interactions between the polymers and the enamel surface (Beyer et al., 2010). To ensure that the reduction of enamel erosion by acidic solutions with higher viscosities was related to a decreased ion exchange and clearance of dissolution products rather than to a protection of enamel dissolution by any interaction between HPC and the enamel surface, the following additional experiment was performed. This experiment intended to analyse whether the pretreatment of enamel with HPC-containing acid solutions reduced further erosion by hydrochloric acid at pH 2.6 due to any kind of mechanical protection of the enamel crystals.

MATERIALS and METHODS

Cylindrical enamel discs (n = 92) were embedded in acrylic resin, and enamel surfaces were ground flat with water-cooled carborundum discs (1200, 2400 and 4000 grit, Water Proof Silicon carbide Paper, Struers, Erkrath, Germany).
**Calcium analysis**

Eighty-four specimens were randomly allocated to 14 groups (n = 6). They were pre-treated by immersion in citric or phosphoric acid (pH 2.5, 3 or 3.5) or water with or without addition of HPC (viscosity: 24mm$^2$/s obtained in the same way as in the present study) for 3 min.

Following each pre-treatment, the specimens were rinsed with deionized water for 30 seconds. Then, the specimens were fixed in a superfusion chamber and were eroded for 2 minutes HCl acid (pH 2.6) at 60 µl/min. The set-up was published in a previous study (Wiegand et al., 2008). Enamel loss was determined in every 15 s fraction by colorimetric analysis of calcium release.

The data were analysed with two-way ANOVA separately for both acids.

**SEM**

To further analyze any possible effect of the polymer itself on enamel erosion, SEM analysis was performed of enamel surfaces. Eight of the enamel specimens were eroded with citric and phosphoric acid or with the respective solutions with a viscosity of 24 mm$^2$/s (pH 3.0, each n = 2).

Before treatment with the test solutions, one half of the enamel surface was covered with PVC tape (Scotch, 3M, Germany) to obtain an untreated area (UT) which was protected against solution exposure. Then, the specimens were eroded for 10 min with the same construction used in the main study. After rinsing with distilled water for 30 s the tape was removed and the specimens were desiccated for 2 weeks in blue silica gel in a vacuum evaporator.
The specimens were mounted on aluminum stubs and sputter-coated with gold, and examined using a Supra 50 VP Scanning Electron Microscope (Carl Zeiss NTS, Oberkochen, Germany) with an acceleration voltage of 2 kV.

RESULTS

Calcium analysis

Calcium loss of enamel specimens pretreated with water with or without the addition of HPC amounted to $4.40 \pm 0.41$ and $4.36 \pm 0.26$ mmol/l, thus, was not different. The statistical analysis revealed that neither the pH nor the presence of HPC in the acid pre-treatment influenced further erosive calcium loss significantly (Appendix Figure 1 and 2).

SEM

The SEM images (Appendix Figure 3 and 4) revealed no differences between the surfaces eroded with HPC-containing acid and the surfaces, which were pretreated with the respective acid without HPC. No layer or precipitation was detected on the surfaces, indicating that no chemical binding between the polymer and enamel took place. This observation is especially true, when comparing these pictures with SEM images in a previous study (Beyer et al., 2010), which had presented distinctly visible adsorption of polymers (other than HPC) on enamel surfaces on SEM images.
Summarizing these results, it seems unlikely that calcium release and enamel dissolution, respectively, is significantly blocked by a chemical binding of HPC to the enamel surface.
APPENDIX REFERENCE


Appendix Figure Legends

Appendix Figure 1. Calcium loss (mmol/l, mean ± standard deviation) in enamel samples eroded with hydrochloric acid after the immersion in the respective citric acidic solutions with or without the addition of HPC.

Appendix Figure 2. Calcium loss (mmol/l, mean ± standard deviation) in enamel samples eroded with hydrochloric acid after the immersion in the respective phosphoric acidic solutions with or without the addition of HPC.

Appendix Figure 3. SEM images of enamel surfaces treated (10 min, pH 3.0) with CA (top) or HPC-containing CA (viscosity 24 mm2/s, bottom) with 2000x, 10000x and 50000x magnifications. UT: untreated area.

Appendix Figure 4. SEM images of enamel surfaces treated (10 min, pH 3.0) with PA (top) o- HPC containing PA (viscosity 24 mm²/s, bottom) with 2000x, 10000x and 50000x magnifications. UT: untreated area.
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