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Effects of Buffering Properties and Undissociated Acid Concentration on Dissolution of Dental Enamel in Relation to pH and Acid Type

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Key Words

Buffering · Citric acid · Dental erosion · Enamel · Lactic acid · Malic acid · pH-stat

Abstract

To quantify the relationships between buffering properties and acid erosion and hence improve models of erosive potential of acidic drinks, a pH-stat was used to measure the rate of enamel dissolution in solutions of citric, malic and lactic acids, with pH 2.4–3.6 and with acid concentrations adjusted to give buffer capacities (β) of 2–40 ($\text{mmol} \cdot \text{l}^{-1}$) · pH^{-1} for each pH. The corresponding undissociated acid concentrations, [HA], and titratable acidity to pH 5.5 ($\text{TA}_{5.5}$) were calculated. In relation to β , the dissolution rate and the strength of response to β varied with acid type (lactic > malic \geq citric) and decreased as pH increased. The patterns of variation of the dissolution rate with $\text{TA}_{5.5}$ were qualitatively similar to those for β , except that increasing pH above 2.8 had less effect on dissolution in citric and malic acids and none on dissolution in lactic acid. Variations of the dissolution rate with [HA] showed no systematic dependence on acid type but some dependence on pH. The results suggest that [HA], rather than buffering per se, is a major rate-controlling factor, probably owing to the importance of undissociated acid

as a readily diffusible source of H^+ ions in maintaining near-surface dissolution within the softened layer of enamel. $\text{TA}_{5.5}$ was more closely correlated with [HA] than was β , and seems to be the preferred practical measure of buffering. The relationship between [HA] and $\text{TA}_{5.5}$ differs between mono- and polybasic acids, so a separate analysis of products according to predominant acid type could improve multivariate models of erosive potential.

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A major cause of dental erosion is the consumption of acidic products, especially drinks but also acidic foods and even vitamin preparations [O'Sullivan and Curzon, 2000; Nunn et al., 2003]. Such products (except for a few, such as yoghurt) generally contain little calcium and phosphate, so are highly undersaturated with respect to the mineral phase of dental tissues. All studies agree that pH is a major factor determining how erosive such products are likely to be. With respect to the importance of buffering, small-scale studies using simple bivariate correlation have come to conflicting conclusions [Larsen and Nyvad, 1999; Mahoney et al., 2003; Jensdottir et al., 2005, 2006; Hemingway et al., 2006]. However, studies involving many products and multivariate regression

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methods have produced convincing evidence that erosion potential with respect to enamel is positively correlated with buffering capacity [Lussi et al., 1993, 1995, 2012]. Buffering properties are likely to be important in two aspects of erosion. In vivo, the strength of buffering will influence the rate of neutralization by saliva and hence the duration of an erosive challenge. Buffering will also influence the intrinsic capacity of the product to erode dental tissues (the erosive potential).

Further work on the relationship between buffering and the process of erosion is needed to address a number of questions. First, statistical analyses assume linear relationships between erosion and chemical properties. It is important to establish the form of the relationship between erosion and buffering, as nonlinear relationships with pH and possibly other properties such as degree of saturation already point to a need to refine current statistical models [Barbour et al., 2011]. Secondly, there is evidence that the effect of buffering on erosion varies with pH [Shellis et al., 2010; Barbour et al., 2011], so there is a case for studying buffering in relation to pH and also acid type, since pK_a influences the relationship between pH and buffering. Finally, as the strength of buffering can be measured in different ways, it is important to evaluate which is most suitable for use in erosion research, as discussed by Barbour et al. [2011]. The buffer capacity (β) is a measure of resistance to change in pH from the native value. Most published studies on erosion have measured the titratable acidity, which is the amount of base required to titrate the pH of a product from its native value to some predetermined value such as 7.0 or 5.5. These quantities are clearly related but measure somewhat different properties of a solution. A further quantity related to buffering is the concentration of undissociated acid, here abbreviated to [HA]. It has been suggested that the undissociated (molecular) form of a weak acid might be important as a mobile source of buffering in subsurface dissolution, as it should diffuse into the pores of a dental tissue more readily than the ionized form [Gray, 1962]. It has been shown that [HA] is an important factor in enamel caries [Featherstone and Rodgers, 1981] but not in root surface caries [Shellis, 2010]. A previous study on erosion by various acids suggested that early enamel softening had a more consistent relationship to buffer capacity than to [HA] [Barbour and Shellis, 2007].

Problems of this kind are best investigated by in vitro experiments on defined solutions. Some data of this type are available [Gray, 1962; Hughes et al., 2000; Barbour and Shellis, 2007; Shellis et al., 2010] but there exist disagreements, possibly because of different measurement

techniques. Therefore, the aim of this study was to explore the interrelationships between buffering and mineral dissolution rate of dental enamel, using several appropriate acids and a pH range typical of erosive products. Enamel was chosen as the substrate because it is significantly influenced by buffering capacity, whereas dentine is not [Shellis et al., 2010].

Materials and Methods

Reagents were purchased from Sigma-Aldrich (Poole, UK).

Enamel Specimens

Dental enamel was obtained from extracted human molars, which had been disinfected by exposure to a solution of sodium dichloroisocyanurate (HazTabs; Guest Medical, Aylesford, UK), containing $20,000 \text{ mg} \cdot \text{l}^{-1}$ of available chlorine for 24 h and then stored in 70% ethanol. The teeth were anonymized and their use for this purpose had been approved by the ethical committee of the University Hospitals Bristol Trust. Specimens were prepared as described by Shellis et al. [2010]. Briefly, planoparallel slices of enamel supported by a layer of dentine were cut from the lateral aspects of the cusps and the cut outer surfaces of the enamel lightly polished using 1,200-mesh silicon carbide in water. After coating the natural surfaces with nail varnish, images of the polished enamel specimens were obtained using a scanner (CanoScan 4400F; Canon Electronics) along with a scale marked in millimeters, and the surface areas of the unvarnished, polished portions were determined with the aid of a digitizing tablet (Graphire; Wacom, Vancouver, Wash., USA) and ImageJ software (<http://rsbweb.nih.gov/ij/>).

For use in the pH-stat, enamel specimens were attached with sticky wax to a glass tube fitted with a 14/23 cone, and nail varnish was applied to all surfaces except the test surface.

A total of 36 enamel specimens derived from 16 molars were used in these experiments.

Solutions

Three acids, all found in acidic dietary products, were used: citric acid (tribasic: found in citrus fruits and drinks), malic acid (dibasic: found in apples and wine) and lactic acid (monobasic: found in milk-derived products, wine and sauerkraut). The composition of the solutions, including pH, acid concentration, buffer capacity, [HA] and titratable acidity to pH 5.5, can be found in the supplementary online table (for online suppl. material, see www.karger.com/doi/10.1159/000351641). The experimental solutions were designed to provide buffer capacities (β) of 2, 5, 10, 20 and 40 ($\text{mmol} \cdot \text{l}^{-1} \cdot \text{pH}^{-1}$) at pH 2.4, 2.8, 3.2 and 3.6 at 37°C. The necessary calculations were performed by iteration using an ion speciation program [Shellis, 1988]. The pK_a values were as follows: lactic acid 3.86, malic acid 3.44 and 5.11, and citric acid 3.10, 4.71 and 6.42 (all adjusted for 37°C) [Martell and Smith, 1977]. For a given pH value, there is a minimum value for β (β_L), which represents the contribution due to the H^+ and OH^- ions in the solution [Butler, 1998]. At the lower pH values, it was not possible to obtain β as low as 2 ($\text{mmol} \cdot \text{l}^{-1} \cdot \text{pH}^{-1}$) (pH 2.8) or 5 ($\text{mmol} \cdot \text{l}^{-1} \cdot \text{pH}^{-1}$) (pH 2.4) because these values of β were less than β_L .

Since each solution is completely defined by the acid concentration and pH, for each value of β there is a unique value of [HA] and $TA_{5.5}$. [HA] was available from the usual output of the program. The titratable acid was calculated from the electroneutrality imbalance, U_{\pm} , which is also part of the program output. In a synthetic solution this quantity represents the concentration of acid (U_{\pm} negative) or base (U_{\pm} positive) necessary to achieve the measured pH [Brown and Chow, 1976; Shellis, 1988]. Therefore, the titratable acidity is the difference between the values of U_{\pm} at the initial pH and the final pH. A final pH of 5.5 was selected, for reasons given by Barbour et al. [2011]. U_{\pm} at this pH was determined using the total acid concentration obtained in the initial calculations of β and titratable acidity ($TA_{5.5}$) was determined as the difference in U_{\pm} between pH 5.5 and the initial pH.

Stock solutions of $2 \text{ mol} \cdot \text{l}^{-1}$ citric and malic acids were prepared from solids. Concentrated lactic acid (nominally 90% w/v) was diluted to approximately $2 \text{ mol} \cdot \text{l}^{-1}$ and the solution left for 2 weeks to allow hydrolysis of lactides. The true concentration ($1.80 \pm 0.01 \text{ mol} \cdot \text{l}^{-1}$) was then determined by titration against $1 \text{ mol} \cdot \text{l}^{-1}$ NaOH solution. Immediately before each experiment, the required solutions were prepared by dilution of these stock solutions with deionized water, using freshly calibrated digital pipettes (accuracy $\geq 98\%$).

Determination of Dissolution Rate

Dissolution was measured using a pH-stat (718 STAT Titrimo; Metrohm UK Ltd., Runcorn, UK) with a 50-ml double-walled glass reaction vessel fitted with a multiport lid. Water was pumped by a circulating bath (type GD120; Grant Instruments, Cambridge, UK) through the water jacket to maintain the reaction temperature at 37°C . In each experiment, 15 ml acid solution was introduced into the reaction vessel and the pH electrode and burette tip were fitted. The reaction solution was stirred at a constant rate using a disc-shaped magnetic follower (type LL; Labsales, Overton, UK) designed to minimize vortexing. After the system had reached equilibrium, the pH was initially adjusted by adding small quantities ($< 50 \mu\text{l}$) of concentrated KOH or HCl and finally adjusted using the pH-stat. The reaction was initiated by introducing the specimen on its holder and dissolution was allowed to proceed for 30 min. The addition of titrant ($50 \text{ mmol} \cdot \text{l}^{-1}$ HCl) during the course of the reaction was recorded electronically.

For each acid/pH combination, three determinations of dissolution rate were made for each value of β . Multiple measurements are possible for enamel if the specimen has not been exposed to surface-active agents [Barbour et al., 2005]. Therefore, three specimens were first randomized to each acid/pH combination. If more than one specimen from the same molar occurred for the same combination, one was exchanged for a specimen from a different combination. The sequence in which measurements on each specimen were made for the different values of β was then determined by a further randomization step. All randomization procedures were performed with the assistance of a random sequence generator (www.random.org).

Data Analysis

The rate of dissolution (V) was calculated as the slope of the linear portion of the plot of volume of acid versus time ($\text{ml} \cdot \text{s}^{-1}$). This was converted to micromoles of hydroxyapatite (HAp) $\cdot \text{s}^{-1} \cdot \text{m}^{-2}$ using the measured area of the enamel specimen, together with a pH- and acid-dependent factor converting micromoles of acid to micromoles of hydroxyapatite [Shellis et al., 2010].

Because most plots suggested a nonlinear relationship between V and β , $TA_{5.5}$ and [HA], data were fitted to power curves using Origin software, v. 6.1. For β and $TA_{5.5}$, the equation was: $V = aX^b$, where $X = \beta$ or $TA_{5.5}$. This curve predicts that $V = 0$ when $X = 0$, which seemed a reasonable assumption for these X variables. However, at [HA] = 0, the solution would still retain buffer capacity (β_L ; see above) and this implies that V would then not be zero but would have a limiting value (V_L) which would be that supported by β_L . Therefore, β_L was calculated for each pH and the value of V_L was calculated for each acid at that pH as $V_L = a\beta_L^b$, where a and b are the coefficients estimated from the curve of V fitted to β . It was then possible to fit the [HA] data sets to modified power curves: $V = V_L + a[\text{HA}]^b$.

To assess the suitability of the data for predicting erosive potential, linear regressions of dissolution rate against β and $TA_{5.5}$ were calculated. The data were then subjected to multiple regression, with pH and either β or $TA_{5.5}$ as independent variables and dissolution rate as the dependent variable, according to the model: $V = a + b_1(\text{pH}) + b_2(\beta \text{ or } TA_{5.5})$.

To assess the strength of correlation between the buffering variables, Pearson's r was calculated. For reasons explained in the Discussion, least-squares linear regressions were calculated for [HA] on $TA_{5.5}$, not only for the acids used in the study but also for tartaric acid (pK_a 3.01 and 4.36) and acetic acid (pK_a 4.76).

Results

In graphs showing the relationship between the dissolution rate and β (fig. 1), uniform axes are used to demonstrate variations with pH as well as with acid type and to give a true picture of the errors in the dissolution rate. Values of [HA] and $TA_{5.5}$ were numerically greater than corresponding values of β , the numerical increase being greatest for pH 2.4 and progressively less for pH 2.8, 3.2 and 3.6. As a result, on plots against [HA] and $TA_{5.5}$, data points tended to be densely crowded near the origin at the higher pH values if uniform x-axes were used. Therefore, axes with different scales are used for figures 2–5 to demonstrate clearly the relationship between the dissolution rate and $TA_{5.5}$ or [HA]. Also in the interests of clarity, error bars for the dissolution rate (± 1 SD) are provided only in figure 1 and the two data points for the highest dissolution rates are omitted from figure 6.

The coefficient of determination (R^2) for the fitted curves was 0.94–0.99. β_L and V_L decreased as pH increased and the dissolution rates observed at the lowest values of β (which were close to the respective β_L ; fig. 1) were consistent with the calculated V_L .

The rate of dissolution increased with increasing values of all three buffering variables, but the strength of the response (the overall steepness of the curve) varied with acid type and pH. Moreover, the relative importance of acid type and pH differed between the buffering variables.

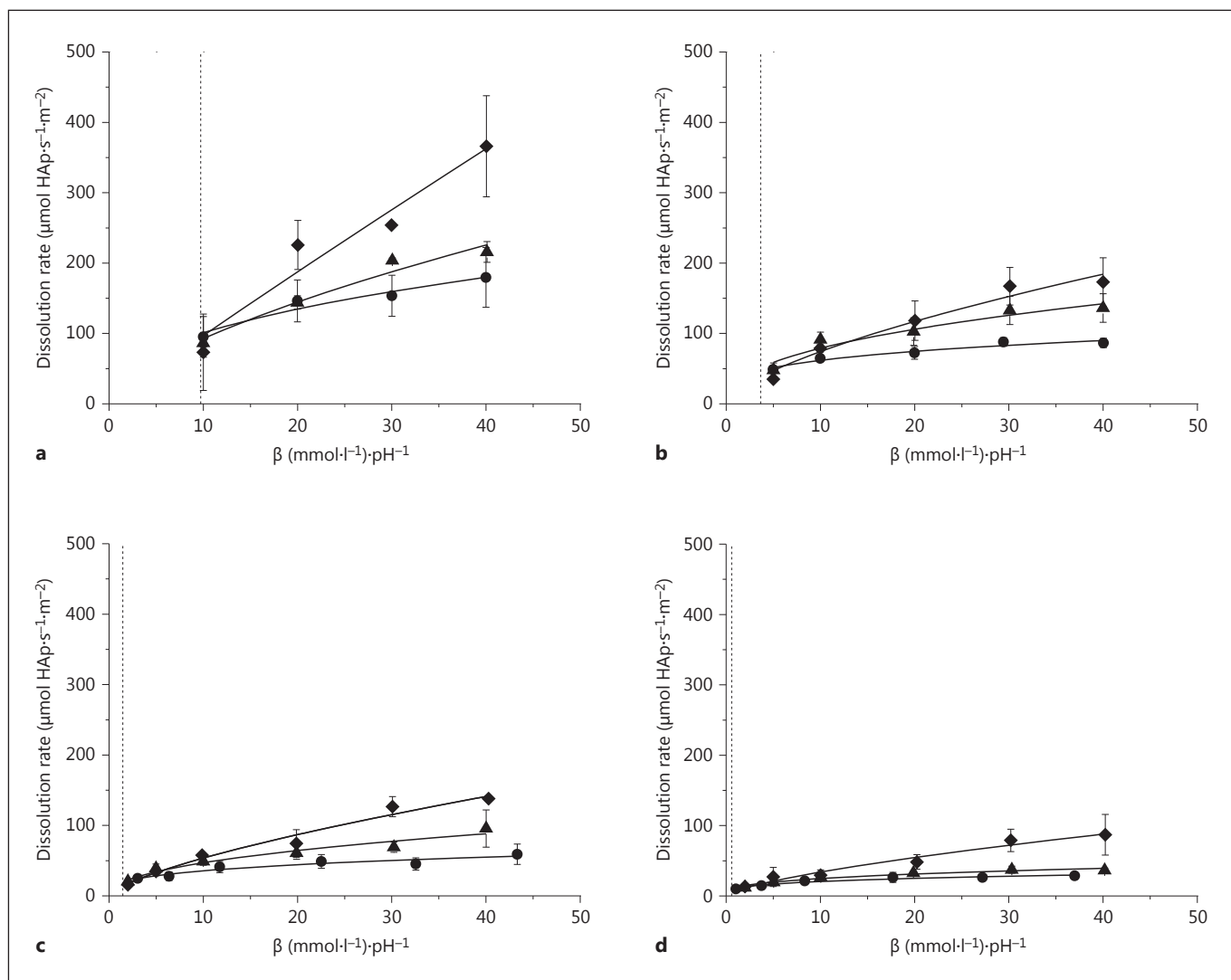


Fig. 1. Dissolution rate plotted against buffer capacity (β) to show variations with acid type. **a** pH 2.4. **b** pH 2.8. **c** pH 3.2. **d** pH 3.6. Diamonds = Lactic acid; triangles = malic acid; circles = citric acid. Vertical dashed lines indicate the values of β_L .

For any given pH, the strength of response to β decreased in the order lactic acid > malic acid > citric acid (fig. 1). Thus, although dissolution rates for the three acids were similar at low β , the curves diverged as β increased. Increasing pH was associated with reduction in both dissolution rate and the response to β . One result was that differences between the acids became smaller with increasing pH. In particular, at pH 3.2 and 3.6 there was very little difference between malic and citric acids and both acids showed very little increase in dissolution rate at $\beta > 10$ ($\text{mmol}\cdot\text{l}^{-1}\cdot\text{pH}^{-1}$) (fig. 1c, d). For citric and malic acids, the increase in pH from 2.4 to 2.8 resulted in

a larger fall in dissolution rate and response to β than further increases above 2.8.

With respect to variations with acid type, a qualitatively similar pattern was observed for $\text{TA}_{5.5}$ as for β (fig. 2), and the same convergence of malic and citric acids occurred at the higher pH values. For citric and malic acids the response to $\text{TA}_{5.5}$ decreased progressively with increasing pH, as for β (data for citric acid shown in fig. 3a). However, while the dissolution rate in lactic acid decreased between 2.4 and 2.8 there was no further change at higher pH, so that data points for pH 2.8–3.6 were superimposed (fig. 3b). Moreover, there was little reduction

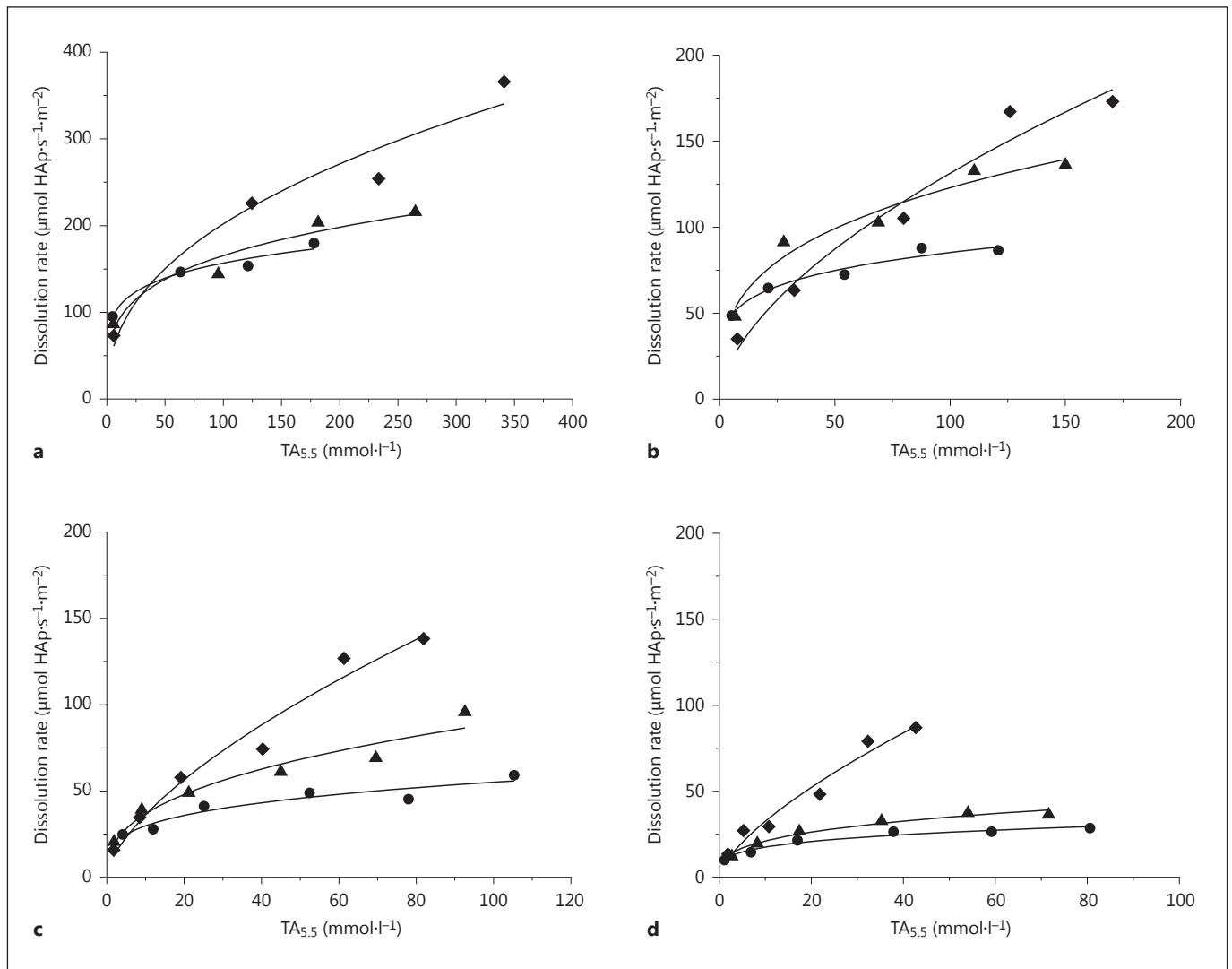


Fig. 2. Dissolution rate plotted against titratable acidity ($TA_{5.5}$) to show variations with acid type. **a** pH 2.4. **b** pH 2.8. **c** pH 3.2. **d** pH 3.6. Diamonds = Lactic acid; triangles = malic acid; circles = citric acid. Note that the axes vary between the four graphs.

in response to $TA_{5.5}$ at these values compared with that at pH 2.4.

On plots of dissolution rate against [HA], the data points were much closer together than on plots against β and $TA_{5.5}$, and the curves for the different acids were, within experimental error, superimposed (fig. 4). In the case of citric and malic acids, the dissolution rate decreased by about 50% between pH 2.4 and pH 2.8 and then by successively smaller increments with a further rise in pH (data for citric acid shown in fig. 5a). The corresponding plot for lactic acid was almost identical with that for $TA_{5.5}$, with a large decrease from pH 2.4 to pH 2.8

and no change between 2.8 and 3.6 (fig. 5b). In a plot of dissolution rate in all three acids against [HA], there was partial segregation of the data points according to pH, with the dissolution rate increasing from pH 3.6 to pH 2.4 (fig. 6).

The data of Hughes et al. [2000] on depth of erosion in citric, malic and lactic acids were plotted against [HA]. As for the dissolution rate (fig. 6), erosion depth was closely correlated with [HA] (fig. 7), again with partial segregation of data points according to pH.

Although power curves described the data more accurately, it was possible to fit linear regressions to the data

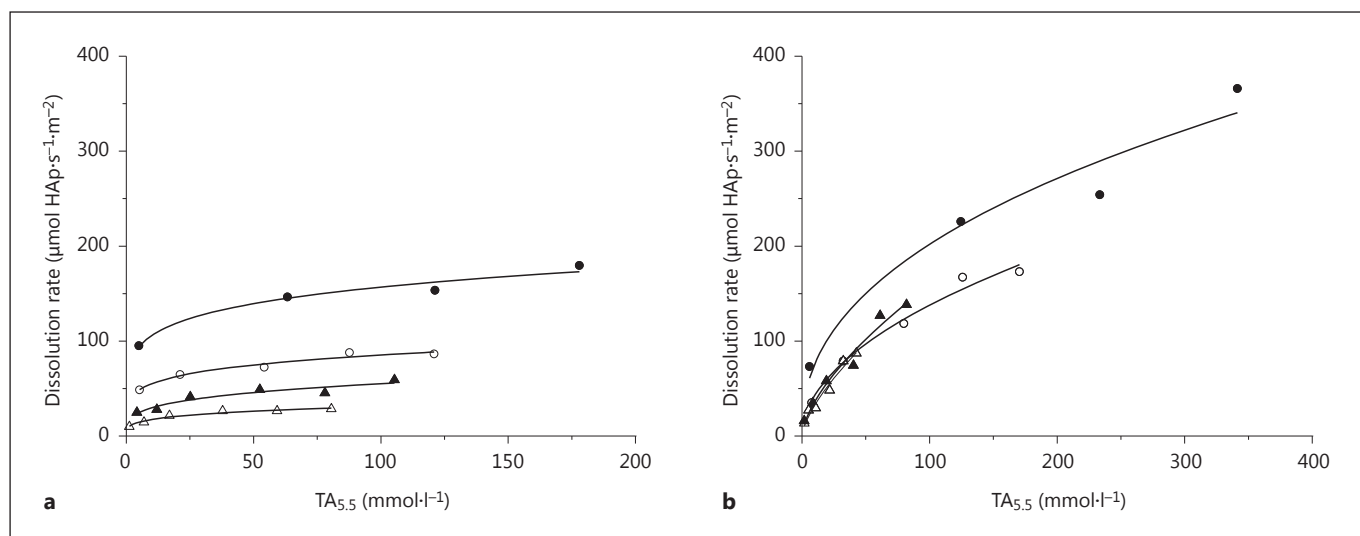


Fig. 3. Dissolution rate plotted against titratable acidity, to show variations with pH. Dissolution in citric acid (a) and lactic acid (b). Filled circles = pH 2.4; open circles = pH 2.8; filled triangles = pH 3.2; open triangles = pH 3.6. Note that the axes vary between the two graphs.

Table 1. Statistical parameters for multiple regressions, with rate of dissolution (V) as dependent variable and pH plus buffer capacity (β) or titratable acidity ($TA_{5.5}$) as independent variables

Acid	Coefficients (β)				Coefficients ($TA_{5.5}$)			
	a (constant)	b_1 (pH)	b_2 (β)	adjusted R^2	a (constant)	b_1 (pH)	b_2 ($TA_{5.5}$)	adjusted R^2
Citric	314.1	-88.7	1.01	0.859	281.2	-78.3	0.39	0.917
Malic	345.8	-99.5	1.91	0.875	245.5	-65.5	0.54	0.969
Lactic	379.8	-115.5	3.94	0.788	115.0	-24.3*	0.92	0.940
All acids	345.1	-100.7	2.29	0.708	209.2	-55.8	0.70	0.877
Citric + malic	329.3	-93.8	1.46	0.835	261.1	-71.6	0.49	0.934

Model: $V = a + b_1(\text{pH}) + b_2(\beta \text{ or } TA_{5.5})$. * $p = 0.106$, coefficient not significant.

for β and $TA_{5.5}$ ($r^2 = 0.79$ – 0.91 for citric acid, 0.82 – 0.95 for malic acid, 0.93 – 0.97 for lactic acid). In multiple regressions of dissolution on pH and β or $TA_{5.5}$ (table 1), up to 86% of variance in the dissolution rate was accounted for using β and up to 97% using $TA_{5.5}$. Models using the combined citric and malic acid data gave results intermediate between those using the separate data. The lowest values of R^2 were associated with regression of the combined data for the three acids. The only regression where pH was not a significant explanatory variable was for dissolution in lactic acid in relation to $TA_{5.5}$.

For each pH, $[HA]$ was linearly related to both β and $TA_{5.5}$. The regression lines for $[HA]$ on $TA_{5.5}$ (fig. 8) all

passed close to the origin. In the case of citric and malic acids, the slope decreased with increasing pH from 0.38 to 0.13 and from 0.51 to 0.30, respectively. The slopes were higher for lactic acid and almost constant (1.02–1.03). Linear regressions for the combined data for each acid showed high coefficients of determination (table 2), especially for lactic acid ($r^2 = 1.0$, approx.). The relationships between $[HA]$ and $TA_{5.5}$ were explored for two additional acids: acetic (pK_a 4.76) and tartaric ($pK_{5.5}$ 3.01, 4.36). Acetic acid gave similar results to lactic acid, the slopes for different pH being 1.07–1.13, and tartaric acid gave similar results for citric and malic acids, with slopes decreasing from 0.44 at pH 2.4 to 0.15 at pH 3.6. The coefficients of determina-

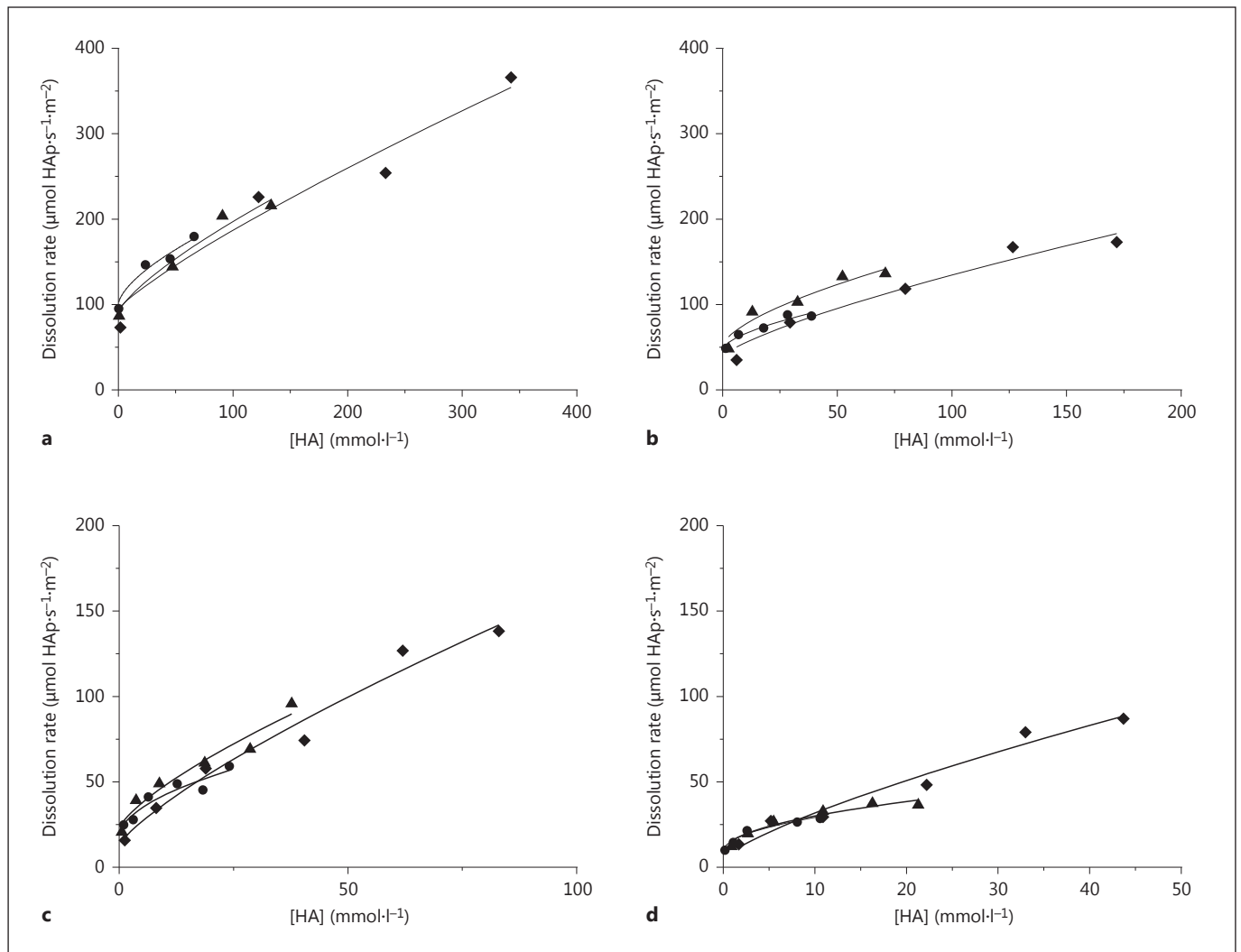


Fig. 4. Dissolution rate plotted against undissociated acid concentration, [HA], to show variations with acid type. **a** pH 2.4. **b** pH 2.8. **c** pH 3.2. **d** pH 3.6. Diamonds = Lactic acid; triangles = malic acid; circles = citric acid. Note that the axes vary between the four graphs.

tion for the overall regressions were also high (table 2). The lines relating [HA] to β showed large increases in slope as pH decreased and large negative intercepts at low pH 9 (not shown). Consequently, the correlation coefficients for the overall data were lower than for $TA_{5.5}$ (table 2). $TA_{5.5}$ showed a highly variable correlation with β (table 2).

Discussion

Regulations on the use of human tissues in research required the teeth to be treated for elimination of prions. Available evidence indicates that properties of enamel,

such as hardness and susceptibility to demineralization, are not affected by treatment with NaOCl [Shellis et al., 2011]. Moreover, as the source of chlorine used here is a less aggressive agent than NaOCl, and as specimen preparation involved removal of the superficial enamel directly exposed to the reagent, it seems unlikely that the disinfection procedure would have affected the results of this study. It is known that the solubility of enamel increases from the outer to the inner surfaces [Theuns et al., 1986]. The specimen preparation method ensures that the maximum depth of the test surface is the same in all specimens. Consequently, all specimens should initially have similar average solubilities. During each exposure to acid,

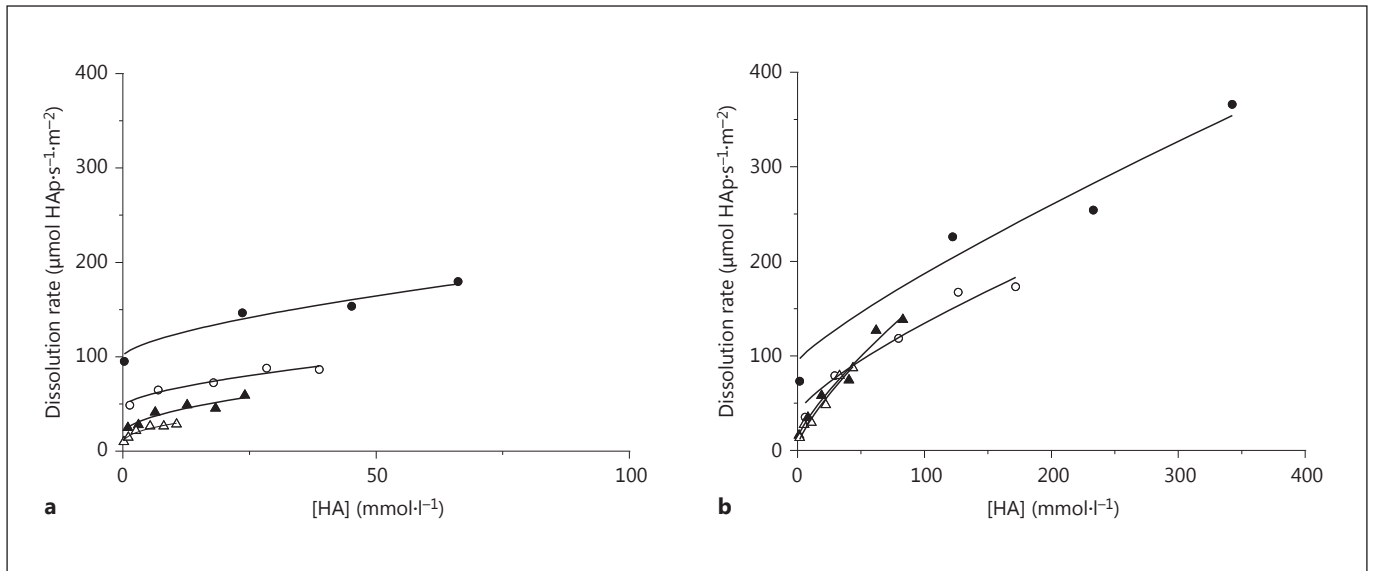


Fig. 5. Dissolution rate plotted against [HA] to show variations with pH. Dissolution in citric acid (a) and lactic acid (b). Filled circles = pH 2.4; open circles = pH 2.8; filled triangles = pH 3.2; open diamonds = pH 3.6. Note that the axes vary between the two graphs.

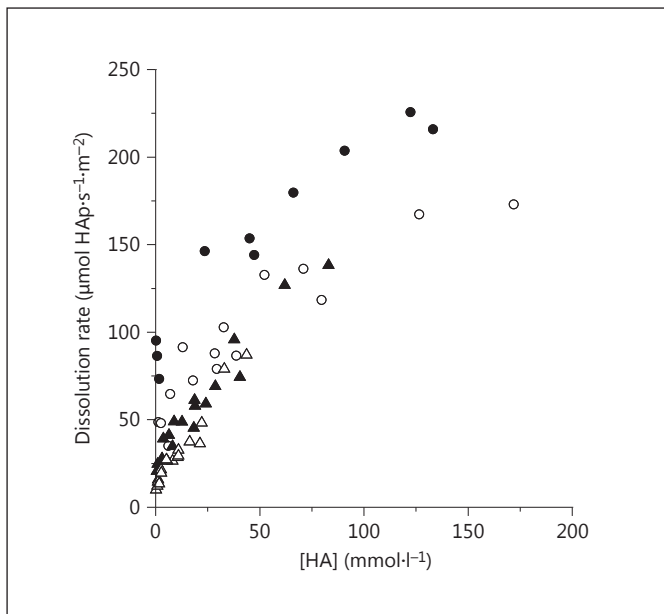


Fig. 6. All data for dissolution rate plotted against undissociated acid concentration to show variations with pH. Filled circles = pH 2.4; open circles = pH 2.8; filled triangles = pH 3.2; open triangles = pH 3.6. The data points for the two highest dissolution rates are omitted to improve clarity (see fig. 4).

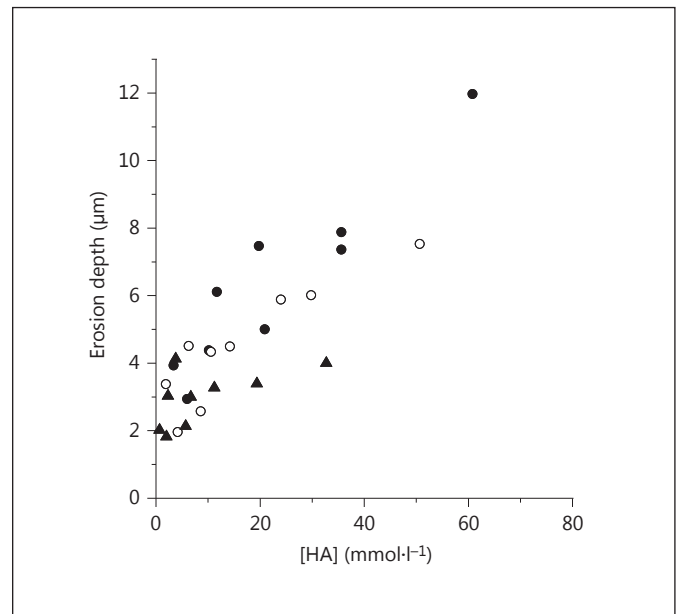


Fig. 7. Depth of erosion after 3×10 min exposure to citric, malic and lactic acids, plotted against concentration of undissociated acid. Erosion depth data from Hughes et al. [2000]; [HA] calculated from pH and acid concentration. Filled circles = pH 2.8; open circles = pH 3.3; filled triangles = pH 3.8.

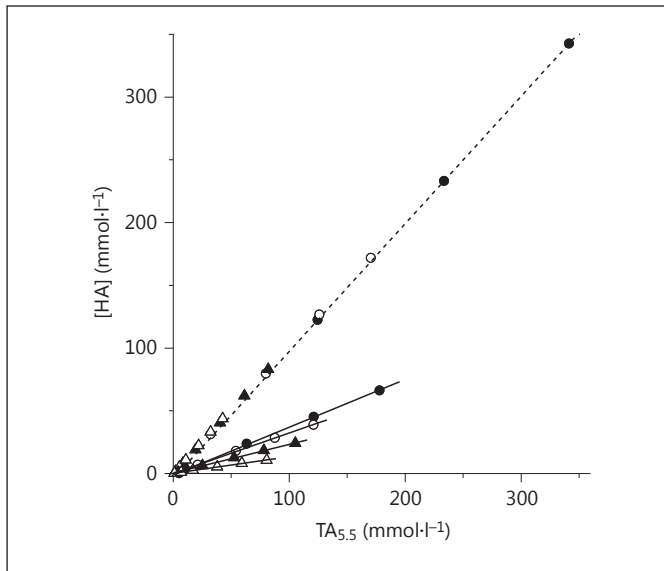


Fig. 8. Graphs of [HA] vs. $TA_{5.5}$. The upper (dashed) line is linear regression for lactic acid pH 2.4, with data points for pH 2.4–3.6 (regressions for pH 2.8–3.6 omitted for clarity). The lower set of four unbroken lines are linear regressions for citric acid, pH 2.4, 2.8, 3.2 and 3.6. Filled circles = pH 2.4; open circles = pH 2.8; filled triangles = pH 3.2; open triangles = pH 3.6.

Table 2. Correlations (Pearson's r) of [HA] and $TA_{5.5}$ with β for the acids used in this study, together with parameters of least-square regressions of [HA] on $TA_{5.5}$

Acid	β, r	[HA]		
		a	b	r^2
Citric				
[HA]	0.759			
$TA_{5.5}$	0.918	-3.07	0.34	0.949
Malic				
[HA]	0.715			
$TA_{5.5}$	0.787	-3.52	0.50	0.993
Tartaric				
$TA_{5.5}$		-4.92	0.44	0.987
Lactic				
[HA]	0.679			
$TA_{5.5}$	0.674	-0.64	1.01	1.000
Acetic				
$TA_{5.5}$		-0.09	1.12	1.000

Model: $[HA] = a + bTA_{5.5}$. Regression parameters for tartaric and acetic acids also included.

the dissolution rate will change as the softened layer forms. However, the rate is measured only after this process is complete and a steady state has been established [Shellis et al., 2010]. As each acid exposure results in loss of a layer of enamel, the depth of the test surface increases with successive exposures and this could lead to increasing solubility and dissolution rate. For this reason, it was essential to randomize the sequence of acid exposures to avoid systematic bias. The increase in depth of the test surface during a sequence of acid exposures would be more pronounced as pH decreased and this seems to be associated with a greater variance of dissolution rate at lower pH (fig. 1). We chose to conduct the experiments at 37°C, as in previous work [Barbour et al., 2007; Shellis et al., 2010]. Products relevant to this study may be consumed at various temperatures, between a few degrees above 0°C and intraoral temperature, and it is known that the rate of erosion increases with temperature [Barbour et al., 2006]. There seems no reason to believe that choice of a different temperature would affect our overall conclusions, but at low temperatures the lower rate of dissolution would probably reduce discrimination between treatments at higher pH.

The results confirm that buffering properties, expressed in terms of either β or TA, influence the erosive potential of acidic solutions. However, it is clear that the response of the dissolution rate to buffering is complex and varies over a wide range according to both pH and acid type.

The power curves fitted to the data were intended only to illustrate the nonlinear pattern of variation of the dissolution rate with buffering. The good statistical fit does not imply that this curve describes the kinetics of the dissolution rate in relation to buffering.

Barbour and Shellis [2007] suggested that β is a better predictor than [HA] of the extent of early erosion because the reduction in hardness and mechanical strength after exposure to different acids appeared to follow a common curve with β , whereas the response to [HA] was more variable. That study was conducted at pH 3.3 and the ranges of β and [HA] were 0–20 (mmol·l⁻¹)·pH⁻¹ and 0–20 (mmol·l⁻¹)·pH⁻¹, respectively. At similar pH (3.2, 3.6) we observed similar phenomena in this study. There was little difference between the three acids in the response to β (fig. 1c, d), as also seen in an analysis [Barbour et al., 2011] and in data of Hughes et al. [2000]. There was more scatter in relation to [HA]. For instance, the dissolution rate in lactic acid was lower than in malic or citric acids at low [HA] but became higher as [HA] increased. However, in the context of a wide pH range

[Hughes et al., 2000; Barbour et al., 2011; this study], it is clear that, contrary to the conclusion of Barbour and Shellis [2007], the dissolution rate varies with acid type and it is the relationship with [HA] that is most independent of acid type.

The importance of the undissociated (molecular) form of organic acids in caries development was stressed by Gray [1961] and Featherstone and Rodgers [1981], who argued that, because of its lack of charge, it would diffuse faster through the subsurface pores of the enamel than the dissociated (anionic) form and hence act as a mobile carrier of H^+ to the sites of demineralization, thereby maintaining the dissolution rate. This argument is also applicable to erosion, where dissolution occurs not only at the interface between solution and enamel, but also within the thin, partly demineralized 'softened' layer. The latter process can be described as 'near-surface demineralization'¹. We thus conclude that near-surface demineralization depends primarily on the concentration of the molecular form of acid(s) within the enamel pores and that it is less directly related to the buffering properties of the bulk solution. Figures 5 and 6 suggest that the dissolution rate is also partly controlled by pH. This could be due to proton-promoted dissolution, in which adsorption of H^+ to a solid weakens important bonds, facilitating the release of ions from the surface [Stumm, 1992].

The greater dependence on acid type of the relationships between the dissolution rate and β or $TA_{5.5}$ can to a large extent be explained by the difference in the contribution made by [HA] to these variables. All of the titratable acidity of lactic acid is accounted for by [HA] because it is a monobasic acid with a pK_a greater than all experimental pH. This property is correlated with the dissolution rate in lactic acid, being consistently greater than in the other acids, and with the nearly identical response of dissolution rate to pH in relation to both β and $TA_{5.5}$ (fig. 3b, 5b). [HA] accounted for only part of the titratable acidities of citric and malic acids because they are polybasic acids, with pK_{a2} greater than the experimental pH, so that a proportion of the titratable acidity is due to the singly ionized anion. The proportion of titratable acidity due to [HA] can be estimated approximately from the slopes

of the regressions of [HA] on $TA_{5.5}$ as follows: 38–51% at pH 2.4, 32–48% at pH 2.8, 23–41% at pH 3.2 and 13–30% at pH 3.6 (values for malic acid being always higher). Thus, [HA] contributes less to titratable acidity as pH increases. As the contribution to the titratable acidity of lactic acid was always 100% there was clearly a strong correlation between the relative response of dissolution to β or $TA_{5.5}$ (lactic > malic \geq citric) and these values.

From the point of view of predicting erosive potential, [HA] is not practically useful because it requires the concentrations of individual acids, as well as pH, to be determined. On the other hand, both $TA_{5.5}$ and β can be determined by titration. The results in table 1 suggest that either variable could be used to predict the dissolution rate when combined with pH in a multiple regression. However, as independent variables in multivariate models should not be correlated, only one buffering variable must be used and $TA_{5.5}$ seems to be preferable as it yielded higher values of R^2 . The nonsignificant coefficient for pH in the regression of the dissolution in lactic acid on $TA_{5.5}$ seems to be related to the restricted effect of pH seen in figure 3b. The b_2 coefficient associated with $TA_{5.5}$ for lactic acid was much higher than those for citric or malic acids (table 1). If this is due to the differences in the relationship between [HA] and $TA_{5.5}$ discussed above, models of erosive potential could be improved by treating products containing different acids separately. This is supported by the results for multiple regressions including individual acids, citric + malic acids or all three acids (table 1). Our calculations for acetic and tartaric acids support the hypothesis that the relationship between [HA] and $TA_{5.5}$ differs between monobasic acids on the one hand and di- or tribasic acids on the other (fig. 8). Therefore, we suggest that one way of improving models of erosive potential would be to perform separate analyses for products in which one of these two acid types are predominant. For example, pickles and lactic acid drinks (acetic and lactic acids) would not be included in the same model as wines, ciders, citrus fruits and drinks (citric, malic and tartaric acids).

Multiple regression analysis rests on certain assumptions. Among these are: (1) that there is a linear relationship between the dependent and each independent variable and (2) and that there is no correlation between independent variables. The relationship between the dissolution rate and β or $TA_{5.5}$ is nonlinear, and the effect of this on the accuracy of statistical models therefore needs to be assessed. It might be possible to improve precision by, for instance, excluding products with low buffering and hence avoiding the region where the curvature is

¹ Although demineralization within the softened layer takes place beneath the surface, the term 'subsurface demineralization' is best avoided in this context, as this term is firmly associated with caries, where demineralization can extend hundreds of micrometers beneath the surface. We suggest that the term 'near-surface demineralization' is appropriate to describe the softening process in erosion, as it emphasizes the limited extent of the softened layer (a few micrometers), while conveying the fact that it is not a process occurring at the surface itself.

most pronounced (see fig. 1, 2). As noted above, the correlation between β and $TA_{5.5}$ is a reason for not including both in multivariate models. The pH of many products is determined by the concentration of the acid-containing ingredient, e.g. fruit juices, so it would be worth assessing the correlation between pH and buffering as a preliminary step in constructing multivariate models of erosion prediction.

In conclusion, the present study has shown that the rate of dissolution of enamel is related to buffer capacity and titratable acidity, but the relationship to both variables is dependent on acid type and pH. The closer rela-

tionship with the concentration of undissociated acid is much less influenced by acid type and pH, probably because this entity acts as a readily diffusible source of H^+ ions because of its lack of charge. Because $[HA]$ is not easy to determine in dietary products, it cannot be used routinely to predict erosive potential. Titratable acidity to pH 5.5 is probably the preferred practical alternative method for measuring the contribution of buffering to erosive potential. However, the nonlinear relationships of the dissolution rate to pH and to buffering properties need to be taken into account when multivariate models of erosive potential are constructed.

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