Effects of Calcium and Phosphate on Dissolution of Enamel, Dentin and Hydroxyapatite in Citric Acid

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Abstract

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21   The aim was to evaluate the effect of dissolved calcium and phosphate on dissolution

22   rate of enamel, dentin and compressed hydroxyapatite (HA) in citric acid solution as a

23   function of pH. At pH 2.5, dissolution rate of enamel increased significantly by 6% in 20

24   mmol/L added calcium but, otherwise, dissolution rates of neither enamel, dentin nor HA

25   were significantly affected by 10 or 20 mmol/L calcium. However, enamel dissolution

26   rate was reduced by $\geq$ 50 mmol/L calcium. At pH 3.25 and 4.0, 10-20 mmol/L calcium

27   inhibited dissolution of enamel by 29-100% and HA by 65-75% but did not affect dentin

28   dissolution. Phosphate (10 or 20 mmol/L) did not inhibit dissolution of enamel, dentin or

29   HA at any pH, but there were increases in dissolution rate of all three substrates at pH

30   2.5 and, in one test with dentine (at 20 mmol/L phosphate), at pH 3.25. The results

31   suggest that calcium addition to soft drinks and other acidic products such as

32   medications may reduce erosivity against enamel, provided that pH is not too low; that

33   phosphate would not reduce erosivity against enamel; and that neither calcium nor

34   phosphate at these concentrations would reduce erosivity against dentin.

Introduction

35

36   Erosive tooth wear has a high prevalence in some populations, particularly among

37   children and young people (JAEGGI, 2006; SCHLUETER and LUKA, 2018; SHREESTA and

38   RAJBHANDARI, 2018; ENTEZAMI et al., 2021; YIP et al., 2022). The condition appears to be

39   influenced by several factors associated with socioeconomic status (ENTEZAMI et al.,

40   2021), with occupation and with medical conditions which involve regurgitation

41   (SCHLUETER and LUKA, 2018; YIP et al., 2022).

42   Soft drinks, and possibly fruit juices, have been identified as risk factors (LI et al., 2012;

43   SALAS et al., 2015; LI al., 2022) and there is interest in investigating the factors that

44   determine their erosive potential and in finding ways to make them less erosive. In vitro

45   tests show that pH, buffering properties and calcium concentration have a statistically

46   significant negative association with erosive potential of a variety of drinks and other

47   products in multiple regression analyses (HARA & ZERO, 2008; LUSSI ET AL., 2011, and

48   references therein). In addition, degree of saturation, phosphate concentration and

49   fluoride concentration show significant bivariate correlations with erosive potential.

50   In principle, the erosive potential can be reduced by adding calcium or phosphate

51   (GRENBY, 1996). In this context, 'phosphate' signifies orthophosphate. These

52   modifications would reduce the degree of undersaturation with respect to dental

53   mineral, through the common-ion effect. Many experiments have been conducted with

54   complex phosphates, but these are not relevant to the present discussion as any effect

55   they may have will be due to surface effects, not to their effect on the degree of

56   saturation. Addition of calcium to acid solutions, soft drinks or candies are known to

57   reduce *in vitro* dissolution of enamel (ATTIN ET AL., 2003, 2005; BARBOUR ET AL., 2003;

58   DAVIS ET AL., 2007; JENSDOTTIR ET AL., 2005; MAGALHÃES ET AL., 2009) or hydroxyapatite

59   (ANDON ET AL., 1992; BARBOUR ET AL., 2008; HEMINGWAY ET AL., 2008). Addition of about

60   10 mmol/L calcium to fruit-based drinks successfully reduced erosion in situ (WEST ET

61   AL., 1999; HOOPER ET AL., 2004).

62   Information about the effect of phosphate addition on erosion potential is, however, very

63   limited. MCDONALD & STOOKEY (1975) reported large reduction in erosion by soft drinks

64   or organic acid solutions supplemented by addition of phosphate. ATTIN ET AL. (2003)

65 reported that supplementation of citric acid solution with low concentrations of

66 phosphate significantly reduced softening and surface loss of enamel.

67 Dissolution of hydroxyapatite and dental tissues increases with decreasing pH, and

68 dissolution is progressively reduced by increasing calcium concentrations at pH 3.2-3.3

69 (BARBOUR ET AL., 2003, 2008; HEMINGWAY ET AL., 2008). However, there remain lacunae

70 in knowledge of interactions between pH and concentration of calcium or phosphate.

71 Moreover, there is little information in this respect for dentin (SHELLIS ET AL., 2010). The

72 aim of the present study was to examine systematically the effects of adding calcium

73 and phosphate to citric acid solutions on dissolution at defined pH, using, not only

74 enamel and dentin, but also compressed hydroxyapatite (HA): a useful model for dental

75 hard tissues in erosion studies (BARBOUR ET AL., 2005).

Materials and Methods

77 Specimens were prepared as described by SHELLIS ET AL. (2010). Slices, about 1.3 mm

78 thick, of which the outer cut surface was composed entirely of enamel, were prepared

79 from the thick enamel lateral to the cusps of human molars, by cutting parallel with the

80 surface tangent at depths of 1 and 2.5 mm. Dentin specimens were prepared by serially

81 sectioning human tooth roots parallel with the long axis, and selecting slices that were

82 tangential to the surface and did not include part of the pulp cavity. The outer cut

83 surfaces of both enamel and dentin specimens were polished using 1200 grade silicon

84 carbide powder in water, rinsed in water and dried. The natural surfaces of the

85 specimens, and areas of cementum on the dentin specimens, were coated with nail

86 varnish. The areas of the unvarnished surfaces of the specimens were determined by

87 digitally scanning the specimens alongside a millimetre scale, using a Canoscan 4400F

88   scanner (Canon UK, Reigate, U.K.) and by image analysis of the scanned images using

89   a Wacom digitising tablet and ScionImage software (www.scioncorp.com; Scioncorp,

90   Frederick, MD, USA). For each specimen, the mean of three area determinations was

91   calculated. For use in the pH-stat, specimens were attached with wax to the tip of a gas

92   inlet tube (Quickfit) which fitted the reaction vessel lid.

93   Discs of compressed HA (HiMed Inc, Old Bethpage, NY, USA), diameter 12 mm, were

94   conditioned by ultrasonication and pre-treatment in citric acid solution prior to use, to

95   ensure consistency of response (SHELLIS ET AL., 2010). After fixation to the specimen

96   holder, the back and edges were coated with nail varnish.

97   *pH-stat measurements*

98   A Metrohm Combistat system was used in conjunction with a Metrohm 713 digital pH

99   meter (Metrohm UK, Runcorn, U.K.) as described by SHELLIS ET AL. (2010), using 15 mL

100   citric acid solution, maintained at 36°C in a 50 mL double-walled reaction vessel and

101   magnetically stirred at a constant rate. The glass combination electrode was calibrated

102   using buffers with an accuracy of 0.01 and nominal pH values of 2.0, 4.0 and 7.0

103   (Sigma-Aldrich, Poole, Dorset, U.K.). After equilibration and pH adjustment, the reaction

104   was initiated by introducing the specimen on its holder and addition of titrant (50 mmol/L

105   HCl) was recorded for an appropriate time.

106   *Experimental details*

107   Dissolution in 14.3 mmol/L citric acid was studied at pH 2.5, 3.25 and 4.0. Dissolution of

108   enamel, HA and dentin was studied at 0, 10 and 20 mmol/L calcium or phosphate.

109   Calcium was added as $CaCl_2.2H_2O$ and phosphate as KH_2PO_4. Multiple measurements

110   were possible for enamel and hydroxyapatite, since preliminary experiments showed

111   that the effects of calcium or phosphate were not persistent, so four enamel and four HA

112   specimens were used in the experiments with calcium and another four of each in the

113   experiments with phosphate. In these experiments, the order of treatments was

114   determined by a random sequence generator (www.random.org). Exposure of dentin to

115   acid alters the surface by creating a superficial layer of demineralized collagen (SELVIG,

116   1968; KINNEY ET AL., 1995), so four separate dentin specimens were used for each

117   combination of pH and calcium or phosphate concentration.

118   The pH-stat system used for the bulk of the measurements (Series 1) failed before the

119   experiments on the effect of dentin had been completed: no data had been collected for

120   the experiments on 10 mmol/L phosphate. Therefore, all of the dentin/phosphate

121   experiments were repeated (Series 2) with a different pH-stat system (718 STAT Titrino:

122   Metrohm UK, Runcorn, U.K.). However, the same reaction vessel was used, and all

123   variables such as temperature, stirrer and stirring rate were the same.

124   The results of the experiments with the first pH-stat indicated that dissolution rate

125   sometimes increased following addition of calcium and, to a lesser extent, phosphate. A

126   possible explanation for this result is that the additions, by raising the ionic strength,

127   could reduce ion activity coefficients and hence the degree of saturation. It was

128   calculated that addition of calcium and phosphate salts raised ionic strength by 10.2-

129   59.5 mmol/L (Table 1). Therefore, in supplementary experiments the effects of ionic

130   strength on enamel and HA dissolution rate at pH 2.5 (4 specimens of each) were

131   investigated. Rates were measured, in random order, in 14.3 mmol/L citric acid

132   containing 0 (control), 10, 20, 30 or 60 mmol/L KCl.

133   *Data analysis*

134   After an initial period (typically < 60 s), the dissolution rate of HA or enamel was

135   constant and the slope of this linear part of the titrant addition recording was the

136   outcome variable. For dentin, the rate decreased with time and the outcome variable

137   was the initial velocity, estimated as the first derivative of a third-degree polynomial

138   fitted to the data (r^2 > 0.999). Units were converted to nmol HA/min/mm^2 by multiplying

139   the rate of acid consumption (mL acid/min), as obtained above, by 1000CF/A, where C

140   = the titrant concentration (50 mmol/L); A = exposed surface area of specimen (mm^2)

141   and F was a factor (= mol HA dissolved per mol H^+ consumed), calculated as described

142   by Shellis et al. (2010).

143   At each pH, enamel and HA dissolution rates were compared with the controls (0 Ca or

144   P) by paired t-test. Dentin dissolution rates in the presence of calcium or phosphate

145   were compared with that for the controls by unpaired t-test. Enamel and HA dissolution

146   rates at different KCl concentrations were compared by repeated-measures ANOVA.

147   Statistical calculations were performed using XLSTAT v. 7.5 (Addinsoft). Other

148   calculations were performed using ion speciation programs IONPRODUCT (Shellis,

149   1988) and CurTiPot (GUTZ, 1992-2021).

150   **Results**

151   *Effect of calcium (Figure 1)*

152   At pH 2.5, 10 mmol/L calcium had no significant effect on enamel dissolution rate, while

153   20 mmol/L calcium significantly increased the mean rate compared with the controls by

154   6.1% (*p*=0.007). At pH 3.25 and 4.0, 10 and 20 mmol/L calcium significantly reduced

155 dissolution rate, by 29% and 46% respectively ($p \leq 0.004$) At pH 4.0, 10 mmol/L calcium

156 reduced dissolution by 83% (0.003). In the presence of 20 mmol/L calcium, dissolution

157 was not detectable.

158 At pH 2.5, addition of 10 mmol/L calcium resulted in a slight and statistically non-

159 significant increase in HA dissolution rate ($p=0.053$) but 20 mmol/L calcium had no

160 effect. At pH 3.25, dissolution rate was reduced significantly in 10 mmol/L calcium by

161 66% at pH 3.25 and 76% in 20 mmol/L calcium ($p \leq 0.01$). The corresponding reductions

162 at pH 4.0 were 69% and 72% ($p \leq 0.013$).

163 The dissolution rate of dentin was not significantly affected by either 10 or 20 mmol/L

164 calcium at any pH (p-0.08-0.89)..

165 *Effect of phosphate (Figures 2 and 3)*

166 Despite efforts to use the same reaction conditions, the results obtained with the

167 replacement pH-stat (Series 2) were consistently higher than those of Series 1.

168 Therefore, the Series 2 data were analysed separately.

169 At pH 2.5, enamel dissolution rate increased significantly and substantially: by 14%

170 ($p=0.043$) and 24% in the presence of 10 or 20 mmol/L phosphate respectively

171 ($p=0.007$). At pH 3.25 and 4.0, neither phosphate concentration significantly affected

172 dissolution rate ($p \geq 0.208$).

173 At pH 2.5, addition of 10 mmol/L phosphate significantly increased HA dissolution rate

174 by 25% ($p=0.002$) but 20 mmol/L had no significant effect ($p=0.809$). At pH 3.25 and

175 4.0, phosphate did not affect dissolution rate.

176   In Series 1 (Figure 2), at pH 2.5, 20 mmol/L phosphate significantly increased dentin

177   dissolution rate by 25% (p=0.035), but, at pH 3.25 and pH 4.0, phosphate had no

178   significant effect.

179   In Series 2 (Figure 3), at pH 2.5, 10 mmol/L phosphate significantly increased

180   dissolution rate of dentin by 46% (p=0.024) and 20 mmol/L phosphate increased the

181   rate by 51% but this was not significant. At pH 3.25, dissolution rate was increased by

182   21% (p=0.08) at 10 mmol/L phosphate and 34% at 20 mmol/L phosphate (p=0.015). At

183   pH 4.0, dissolution rate increased by 9% and 12% in the presence of 10 and 20 mmol/L

184   phosphate (p=0.27 and 0.06 respectively).

185   *Effect of ionic strength (Figure 4)*

186   In the presence of 10-60 mM KCl there was a non-significant increase in dissolution rate

187   of enamel (p=0.077) and no change in that of HA (p=0.79).

Discussion

189   The pH range was chosen as representative of soft drinks (LUSSI ET AL., 2011). In

190   agreement with previous studies (e.g. DAVIS & WINTER, 1980; BARBOUR ET AL., 2003,

191   2008; HEMINGWAY ET AL., 2008), dissolution rate fell rapidly from pH 2.5 to pH 3.25 and

192   then more slowly to pH 4.0. The concentrations of added calcium and phosphate were

193   based on the fact that addition of 10 mmol/L calcium successfully reduced the erosivity

194   of a fruit-based drink in situ (WEST, 1999). Addition of calcium or phosphate carries with

195   it increased risk of calcium phosphate precipitation and of taste alteration. If added as

196   basic salts, there can also be a rise in pH which both affects taste adversely and

197   reduces stability against bacterial contamination and proliferation. Thus high calcium or

198   phosphate concentrations are not practical. We therefore confined our experiments to

199   two relatively low concentrations.

200   At pH 3.25 and 4.0, the enamel dissolution rate responded to calcium as predicted by

201   the common-ion effect. This agrees with previous studies which had been conducted at

202   pH 3.0-3.7 (BARBOUR ET AL., 2003; ATTIN, 2005; JENSDOTTIR ET AL., 2005; MAGALHÃES ET

203   AL., 2009). We found that at pH 2.5 enamel dissolution rate was not reduced by 10 or 20

204   mmol/L calcium and even increased. This effect was also observed in preliminary

205   experiments which showed that, at this pH, the rate was only reduced by 3% at 50

206   mmol/L then continued to fall up to 200 mmol/L calcium (data not shown).

207   However, our results do not agree with the study by ATTIN ET AL. (2003), who reported

208   that addition of only 0.5-1.5 mmol/L calcium reduced softening of enamel and surface

209   loss by 52 mmol/L citric acid, pH 2.21.

210   Despite the marked structural differences between the two materials (SHELLIS ET AL.,

211   2010), the dissolution rate of compressed HA responded to calcium and phosphate in a

212   very similar fashion to that of enamel. Although compressed HA is less influenced by

213   acid concentration (SHELLIS ET AL. 2010), the evidence supports the utility of this material

214   as a convenient substitute for enamel.

215   At pH 3.25 and 4.0, neither calcium nor phosphate reduced the rate of dentin

216   dissolution. Because the surface of eroding dentin is covered by demineralized

217   collagenous matrix, the advancing front of demineralization becomes isolated from the

218   bulk solution and exchange between the two will be slow and diffusion-controlled. The

219   demineralization process might then be relatively less responsive to changes in the

220   driving force in the bulk solution. Constant-composition dissolution of dentin is

221   insensitive to the degree of undersaturation (PASCHALIS ET AL., 1996). Thus, modification

222   of drinks which have a pH in the range 3.25-4.0 by addition of low concentrations of

223   calcium or phosphate is unlikely to reduce erosion of exposed root surfaces in adults.

224   The reason for the lack of effect of added phosphate seems to be the very low

225   concentration of PO_4^{3-} ions at these pH values (Table 3).

226   At pH 2.5, addition of phosphate tended to increase the rate of dissolution of both

227   enamel and dentin. There are two possible explanations for this phenomenon. First, the

228   increased ionic strength (Table 1), by reducing ion activities, could lower the degree of

229   saturation and hence increase dissolution rate. However, our experiments with enamel

230   and hydroxyapatite suggest that the rate, at least of these materials, is not increased by

231   the observed increase in ionic strength (see also BARBOUR ET AL., 2008). The second

232   possibility is that the increased buffer capacity in the calcium- and phosphate-containing

233   solutions (Table 1) is likely to cause an increase in the dissolution rate. Buffering is an

234   important factor in erosion in vitro (SHELLIS ET AL., 2010, 2013) and has been identified

235   as important in the erosive potential of soft drinks (LUSSI ET AL., 2011).

236   At first sight, the data of MCDONALD & STOOKEY (1973) suggest that phosphate could

237   reduce erosion potential if added at high enough concentration, since they observed

238   large reductions in calcium release from enamel when 83 mmol/L phosphate was added

239   to soft drinks or to three organic acid solutions. However, addition of NaH_2PO_4 will also

240   raise the pH of the solution. Using CurTiPot software we calculate that pH of the acid

241   solutions used by MCDONALD & STOOKEY (1973) would have increased from initial values

242   of 2.53, 2.68 and 2.58 by 0.54, 0.63 and 0.56 respectively. Figures 2 and 3 suggests

243   that such increases in pH would produce significant reductions in dissolution rate of

244   dentin.

245   The effect on pH of adding calcium or phosphate salts to acid solutions may have

246   influenced results of previous studies. Alterations of solution pH after supplementation

247   with calcium are seen in MAGALHÃES ET AL. (2009) and in JENSDOTTIR ET AL. (2005). In

248   the latter, pH fell after addition of calcium citrate. In the study of calcium-containing soft

249   drinks by DAVIS ET AL. (2007), an increase of calcium concentration by 8.4-36.9 mmol/L[1]

250   reduced erosion depth in enamel by 60-100% and in dentin by 7-36% after 25 h

251   exposure. However, in 3/4 comparisons, the pH of the high-calcium drink was 0.33-0.4

252   units higher than that of the low-calcium drink. In the remaining comparison (equal pH)

253   the difference in dissolution rates of both enamel and dentin was much lower than in the

254   other comparisons and it appears that the erosive potential of the drinks was

255   determined in part by pH. The reduction of in situ erosion by a drink with an original pH

256   of about 2.9 was not achieved solely by addition of calcium, since it was added as

257    $CaCO_3$, which raised the pH by nearly 1 unit (WEST ET AL., 1999). For each modification

258   of a drink by calcium addition, the reduced erosive potential has to be balanced against

259   possible changes in pH and hence palatability and shelf life.

260   In conclusion, the effects of calcium and phosphate on dissolution rate in the pH-stat

261   are highly pH- and tissue-dependent. The results suggest that, with respect to enamel,

262   calcium at the concentrations used here would not usefully reduce erosivity of highly

263   acidic drinks, that phosphate would not reduce erosivity at any pH $\leq$ 4.0 and that neither

264   would reduce erosivity against dentin, but that calcium would reduce erosion by

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[1] Our calculations, assuming a serving size of 236.6 mL (8 ounces) and a Daily Value for calcium of 1000 mg.

265   moderately acidic drinks (pH $\geq$ 3.25). Compressed HA responded to changes in calcium

266   and phosphate concentration in a very similar manner to enamel highlighting its utility as

267   a convenient and consistent alternative to enamel without the constraints of human

268   tissue legislation and the biological variability that enamel inevitably exhibits.

269   We recognise that our experiments were conducted under 'clean' conditions, so the

270   results may well not be transferable directly to clinical conditions. A particularly

271   important factor is the presence in vivo of salivary pellicle, which is well known to reduce

272   the rate of dissolution of dental enamel in acid (HANNIG AND HANNIG, 2014). However, in

273   vitro measurements of erosion of enamel specimens coated with a salivary pellicle

274   (LUSSI ET AL., this volume) support the results of the present study. The quantitative

275   response of dental hard tissues to modification of erosive conditions is likely to differ in

276   vivo. However, we suggest that addition of calcium or phosphate to erosive products

277   should not be assumed to simply reduce dissolution of dental minerals. The overall

278   effect, influenced by interactions between calcium and phosphate ion concentrations,

279   pH and buffering, may be difficult to predict.

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References

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285 ANDON M B, KANERVAS R L, ROTRUCK J T, SMITH K T: Method of preventing tooth enamel

286 erosion utilizing an acidic beverage containing calcium. US Patent 5,108,761 (1992)

287 ATTIN T, MEYER K, HELLWIG E, BUCHALLA W, LENNON Á M: Effect of mineral supplements

288 to citric acid on enamel erosion. Arch Oral Biol 48: 753-759 (2003)

289 ATTIN T, WEISS K, BECKER K, BUCHALLA W, WIEGAND A: Impact of modified acidic soft

290 drinks on enamel erosion. Oral Dis 11: 7-12 (2005)

291 BARBOUR M E, PARKER D M, ALLEN G C, JANDT K D: Enamel dissolution in citric acid as a

292 function of calcium and phosphate concentrations and degree of saturation with respect

293 to hydroxyapatite. Eur J Oral Sci 111: 428-433 (2003)

294 BARBOUR M E, SHELLIS R P, PARKER D M, ALLEN G C, ADDY M: Inhibition of

295 hydroxyapatite dissolution by whole casein: the effects of pH, protein concentration,

296 calcium, and ionic strength. Eur J Oral Sci 116: 473-478 (2008)

297 BROWN W E: Solubilities of phosphates and other sparingly soluble compounds. In:

298 GRIFFITH E J, BEETON A, SPENCER J M, MITCHELL D T (editors).Environmental phosphorus

299 handbook, Wiley, New York, pp 203-228 (1973)

300 DAVIS R E, MARSHALL T A, QIAN F, WARREN J J, WEFEL J S: In vitro protection against

301 dental erosion afforded by commercially available, calcium-fortified 100 percent juices. J

302 Am Dent Ass 138: 1593-1598 (2007)

303 DAVIS W B, WINTER P J: The effect of abrasion on enamel and dentin after exposure to

304 dietary acid. Br Dent J 148: 253-256 (2007)

305 ENTEZAMI S, PERES K G, LI H, ALBARKI Z, HIJAZI M, AHMED K E: Tooth wear and

306 socioeconomic status in childhood and adulthood: Findings from a systematic review

307 and meta-analysis of observational studies. J Dent doi.org/10.1016/j.jdent.2021.103827

308 (2021)

309 GRENBY T H: Lessening dental erosive potential by product modification. Eur J Oral Sci

310 104: 221-8 (1996)

311 GUTZ I G R CurTiPot software. http://www2.iq.usp.br/docente/gutz/Curtipot_.html (1992-

312 2021)

313 HANNIG M, HANNIG, C: The pellicle and erosion. In: LUSSI A, GANSS, C (Editors) Erosive

314 Tooth Wear. From Diagnosis to Therapy. Monogr Oral Sci 25, Karger, Basel, pp 206-

315 214 (2014)

316 HARA A T, ZERO D T: Analysis of the erosive potential of calcium-containing acidic

317 beverages. Eur J Oral Sci 116: 60-65 (2008)

318 HEMINGWAY C A, SHELLIS R P, PARKER D M, ADDY M, BARBOUR M E: Inhibition of

319 hydroxyapatite dissolution by ovalbumin as a function of pH, calcium concentration,

320 protein concentration and acid type. Caries Res 42: 348-353 (2008)

321 HOOPER S, WEST N X, SHARID N, SMITH S, NORTH M, DE'ATH J, PARKER D M, ROEDIG-

322 PENMAN A, ADDY M: A comparison of enamel erosion by a new sports drink compared to

323 two proprietary products: a controlled cross-over study in situ. J Dent 32: 541-545.

324 (2004)

325 JAEGGI T, LUSSI A: Prevalence, incidence and distribution of erosion In: LUSSI A (Editor)

326 Erosive Tooth Wear. From Diagnosis to Therapy. Monogr Oral Sci 25, Karger, Basel, pp

327 55-73 (2014)

328 JENSDOTTIR T, NAUNTOFTE B, BUCHWALD C, BARDOW A: Effects of calcium on the erosive

329 potential of acidic candies in saliva. Caries Res 41: 68-73 (2007)

330 KINNEY J H, BALOOCH M, HAUPT D L, MARSHALL S J, MARSHALL G W: Mineral distribution

331 and dimensional changes in human dentin during demineralization. J Dent Res 74:

332 1179-1184 (1995)

333 LI H, ZOU Y, DING G: Dietary factors associated with dental erosion: A meta-analysis.

334 PLoS ONE 7: e42626. doi:10.1371/journal.pone.0042626. (2012)

335 LUSSI A, MEGERT B, SHELLIS R P, WANG XA: Analysis of the erosive effect of different

336 dietary substances and medications. Br J Nutr doi:10.1017/S0007114511002820 (2011)

337 MAGALHÃES C A, MORAES C M, RIOS D, BUZALAF M A R: Effect of ion supplementation of

338 a commercial soft drink on tooth enamel erosion. Food Additives Contam 26: 152-156

339 (2009)

340 MCDONALD J L, STOOKEY G K: Laboratory studies concerning the effect of acid-

341 containing beverages on enamel dissolution and experimental dental caries. J Dent Res

342 52: 211-216 (1973)

343 PASCHALIS E P, TAN J, NANCOLLAS G H: Constant composition dissolution kinetics studies

344 of human dentin. J Dent Res 75: 1019-1026 (1996)

345 SALAS M M, NASCIMENTO G G, VARGAS-FERREIRA F, TARQUINIO S B, HUYSMANS M C,

346 DEMARCO F F: Diet influenced tooth erosion prevalence in children and adolescents:

347 Results of a meta-analysis and meta-regression. J Dent 43: 865-875 (2015)

348 SCHLUETER N, LUKA B: Erosive tooth wear-a review on global prevalence and on its

349 prevalence in risk groups. Br Dent J 224: 364-370 (2018)

350 SELVIG K A: Ultrastructural changes in human dentin exposed to a weak acid. Arch Oral

351 Biol 13: 719-34 (1968)

352 SHELLIS R P: A microcomputer program to evaluate the saturation of complex solutions

353 with respect to biominerals. Computer Appl Biosci 4: 373-379. (1988)

354 SHELLIS R P, BARBOUR M E, JONES S B, HUGHES J A, HEMINGWAY C A, ADDY M: Effects of

355 pH and acid concentration on dissolution of enamel, dentin, and compressed

356 hydroxyapatite. Eur J Oral Sci 118: 475–482 (2010)

357 SHRESTHA D, RAJBHANDARI P: Prevalence and associated risk factors of tooth wear. J

358 Nepal Med Assoc 56: 719-723 (2018)

359 WEST N X, HUGHES J A, PARKER D M, NEWCOMBE R G, ADDY M: Development and

360 evaluation of a low erosive blackcurrant juice drink 2.Comparison with a conventional

361 blackcurrant juice drink and orange juice. J Dent 27: 341-344 (1999)

362 YIP K, LAM P P Y, YIU C K: Prevalence and associated factors of erosive tooth wear

363 among preschool children - A systematic review and meta-analysis. Healthcare 10, 491.

364 https://doi.org/10.3390/healthcare10030491 (2022)

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367   **Table 1. Properties of test solutions**

pH	Added Ca mmol/L	Increase in ionic strength mmol/L	Buffer capacity β mmol/(L.pH)	Added P mmol/L	Increase in ionic strength mmol/L	Buffer capacity β mmol/(L.pH)	$[PO_4^{3-}]$ mmol/L
2.5	0		13.5	0		13.5	
	10	29.7	14.8	10	10.2	19.0	$7.24.10^{-12}$
	20	59.5	15.4	20	20.3	24.1	$1.81.10^{-13}$
3.25	0		10.6	0		10.6	
	10	28.1	10.8	10	9.5	12.3	$4.04.10^{-12}$
	20	56.8	11.0	20	19.0	13.9	$9.84.10^{-12}$
4.0	0		9.0	0		9.0	
	10	28.2	9.0	10	17.4	9.6	$1.67.10^{-10}$
	20	53.6	9.4	20	27.6	10.1	$3.92.10^{-10}$

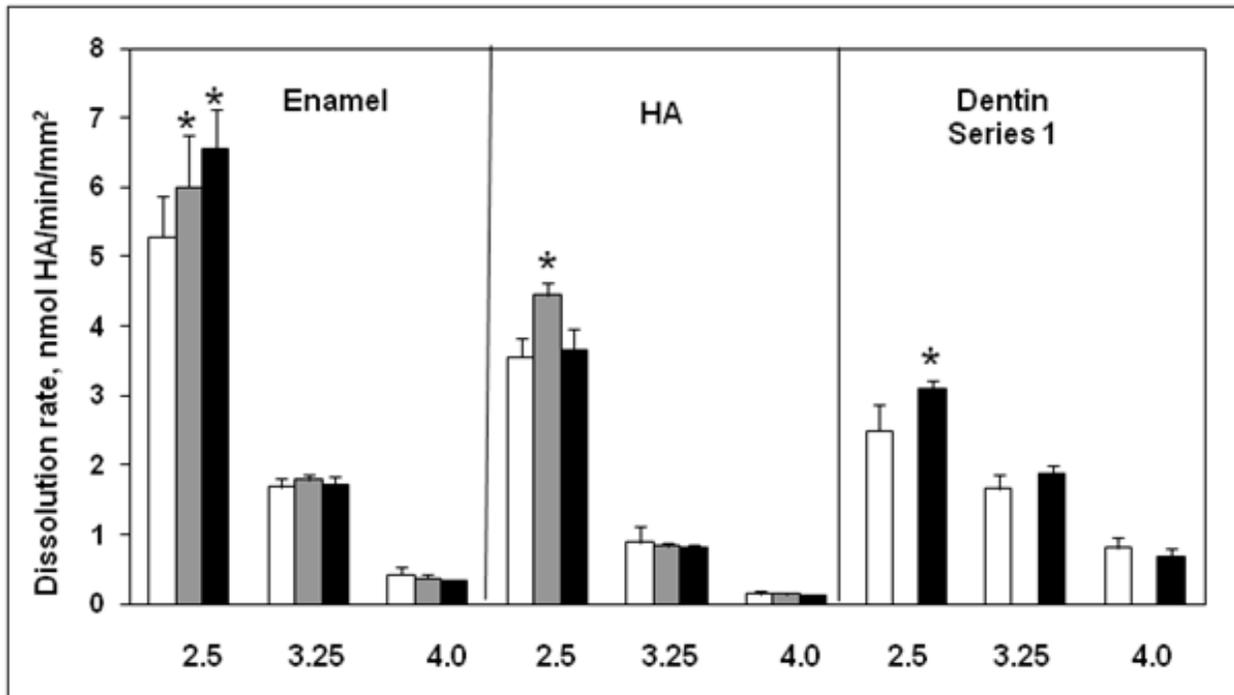368   β = buffer capacity; P = total phosphate

369
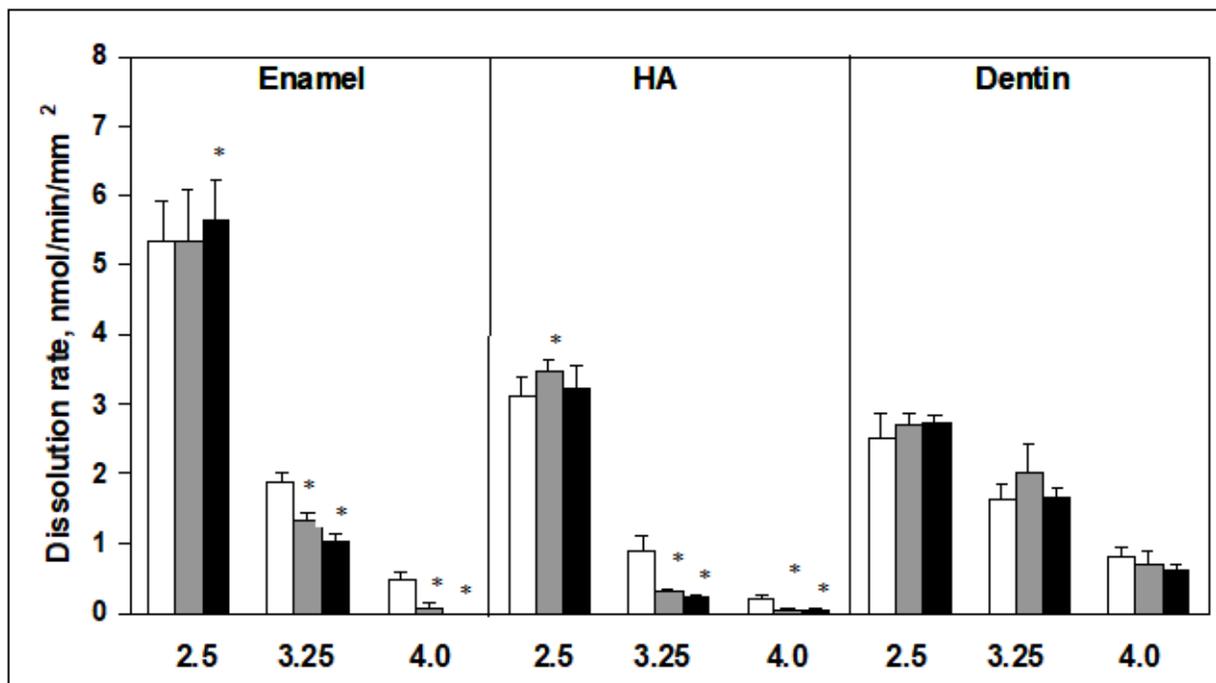
Figures

Figure 1. Effect of calcium on dissolution of enamel, compressed hydroxyapatite and dentin. White bars = control (no calcium); grey bars = 10 mmol/L calcium; black bars = 20 mmol/L calcium. Error bars = 1 SD. Asterisks indicate significant difference from control.
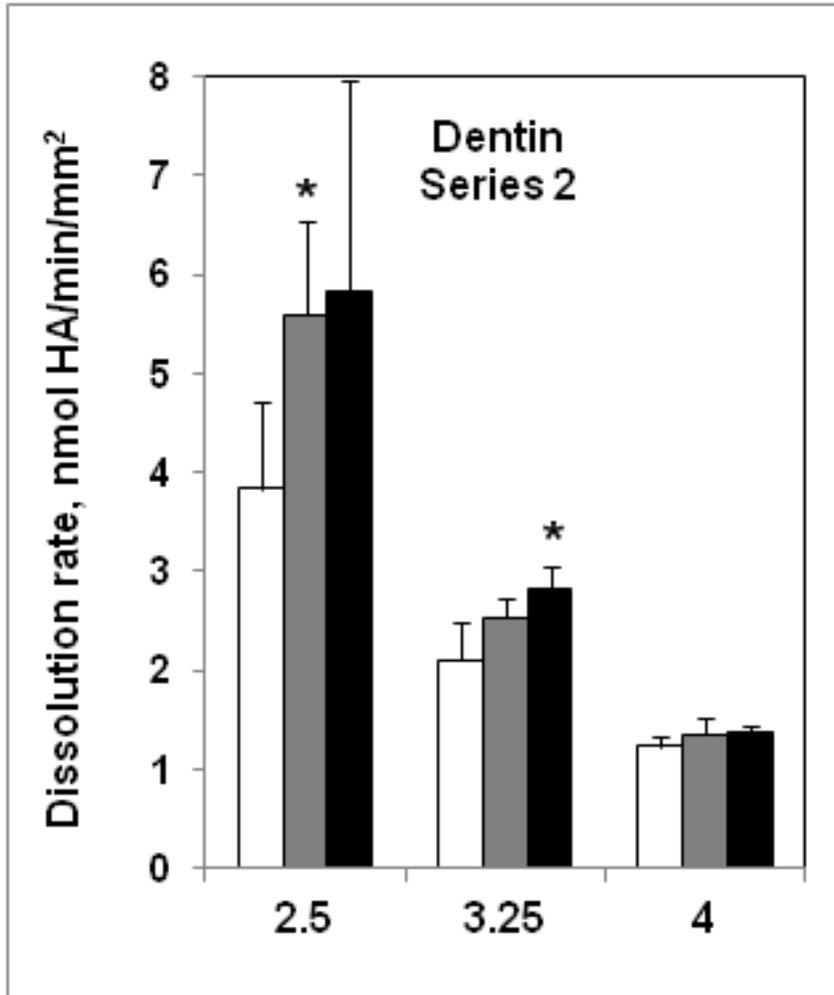
377

378 Figure 2. Effect of phosphate on dissolution of enamel, compressed hydroxyapatite and

379 dentin (Series 1). White bars = control (no phosphate); grey bars = 10 mmol/L

380 phosphate; black bars = 20 mmol/L phosphate. Error bars = 1 SD. Asterisks indicate
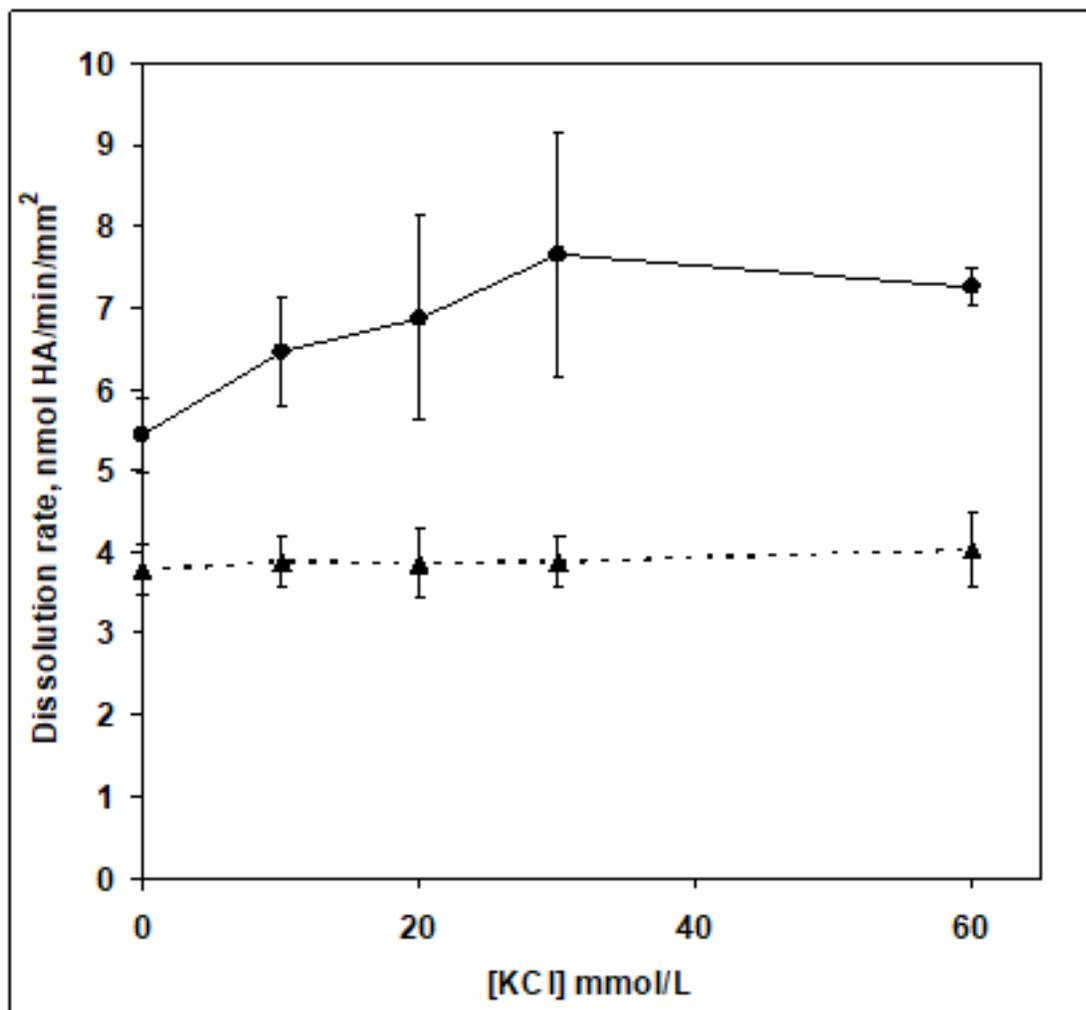
381 significant difference from control.

382

Figure 3. Effect of phosphate on dissolution of dentin (Series 2). White bars = control (no phosphate); grey bars = 10 mmol/L phosphate; black bars = 20 mmol/L phosphate. Error bars = 1 SD. Asterisks indicate significant difference from control.

388

389 Figure 4. Effect of KCl concentration on dissolution rate of enamel (solid line) and

390 compressed hydroxyapatite (broken line). Error bars = ± 1 SD.

391