available at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/jden

A clinical study in situ to assess the effect of a food approved polymer on the erosion potential of drinks

S. Hooper [a], J. Hughes [a], D. Parker [b], M. Finke [b], R.G. Newcombe [c], M. Addy [a], N. West [a,*]

[a] Division of Restorative Dentistry, Bristol Dental School, Lower Maudlin Street, Bristol BS1 2LY, UK
[b] GlaxoSmithKline, Coleford, UK
[c] Department of Medical Statistics and Computing, University of Wales, College of Medicine, Cardiff, UK

ARTICLE INFO

Article history:
Received 16 October 2006
Received in revised form
5 March 2007
Accepted 11 March 2007

Keywords:
Tooth wear
Dental erosion
Drinks
Study in situ
Food polymers

ABSTRACT

Objectives: The consumption of acidic soft drinks continues to rise as do concerns regarding effects of frequent intake. The aim of the study was to determine the effect of acidic soft drinks containing food approved polymers, on dental enamel.

Methods: The study design was a single blind, randomised, five-treatment crossover clinical trial involving 15 healthy dentate subjects. Subjects wore an upper removable acrylic appliance retaining two enamel specimens and consumed 250 ml of beverage four times/day for 10 days. The following beverages were tested: (1) unmodified acidic soft drink, (2) experimental acidic soft drink with 0.02% polyphosphate, (3) experimental acidic soft drink with 0.02% polyphosphate and 0.01% calcium, (4) experimental acidic soft drink with 0.02% polyphosphate and 0.03% xanthan gum, and (5) mineral water. Tissue loss was measured at days 5 and 10 of each study leg using a profilometer.

Results: The order of erosion from most to least at day 10 was unmodified acidic soft drink > experimental acidic soft drink with polyphosphate > experimental acidic soft drink with polyphosphate + gum > experimental acidic soft drink with polyphosphate + calcium > mineral water. At day 10 the unmodified acidic soft drink was significantly ($p = 0.001$) more erosive than all other drinks.

Conclusions: Unmodified acidic soft drink with the addition of polyphosphate alone or combined with calcium or xanthan gum are all effective at reducing erosion of enamel compared with the unmodified soft acidic drink.

1. Introduction

The potential of acidic soft drinks to cause dental erosion has been established through a combination of epidemiological data[1,2] and studies *in vitro* and *in situ*.[3–13] The consumption of acidic soft drinks particularly by the young in many countries including the UK[14] is already high and has been increasing for years. It would therefore seem reasonable to expect soft drink manufacturers to try and improve their drinks properties from a dental perspective. To this end, a reduction in the erosive potential of some acidic soft drinks has been achieved through the careful manipulation of pH, titratable acidity and the addition of calcium.[4] It was assumed that calcium in the drink may inhibit the release of calcium from the enamel surface during the erosive demineralisation process. A further modification to the same drinks led to the addition

* *Corresponding author*. Tel.: +44 117 9284505; fax: +44 117 9284100.
E-mail address: N.X.West@bristol.ac.uk (N. West).

0300-5712/$ – see front matter
doi:10.1016/j.jdent.2007.03.001

of gum, resulting in reduction in erosion in excess of 90% in studies *in situ*.[15] Various other organic compounds, including polymers, are known to be adsorbed onto hydroxyapatite or enamel to reduce the rate of acidic dissolution.[16] Indeed, Schaad et al. (1994) found chemical groups with the ability to bind calcium ions, and phosphates were particularly effective.[17] Research in the dental field regarding these compounds is very limited. However, it is conceivable that on the same principle, the addition of polyphosphates to acidic soft drinks may reduce the rate of acidic dissolution of these minerals. Hence the aim of the present study was to determine the effects on enamel erosion of the addition of a food approved polymer/polyphosphate to such drinks, and a combination of this polyphosphate with other compounds known to reduce erosion of dental hard tissues, namely calcium[4] and xanthan gum.[15]

2. Method and materials

The study was a single blind, randomised, five-treatment crossover design balanced for residual effects[16] and involving 15 healthy dentate subjects. Inclusion and exclusion criteria were the same as in previous studies with subjects aged 18 years or older with no medical or pharmacotherapy histories which might influence the conduct or outcome of the study (e.g., acid reflux), no evidence of excessive tooth wear and with no fixed or removable orthodontic appliances or removable prostheses. The study was designed, conducted, monitored and reported according to the Guidelines for Good Clinical Practice. Approval for the study was obtained from the United Bristol Healthcare Trust Ethics Committee and subjects received verbal and written information on the study, and gave signed and witnessed consent to participate. Removable acrylic upper palatal appliances were prepared for each subject in which 2 enamel specimens, 1 anterior and 1 posterior, were placed during each of the five treatment periods.

The study used enamel specimens derived from surgically removed human third molars from 18 to 35 years old of either gender. Sterilisation of the teeth was achieved by soaking in 20,000 ppm hypochlorite for 24 h. Specimens were embedded in epoxy resin and polished to produce a flattened window of enamel with a surface profile of $\pm 0.1\ \mu m$ measured using a profilometer. Specimen dimensions were $8\ mm \times 5\ mm \times 2\ mm$. The surface profilometer (SF200 surfometer, Planer Products Ltd., Windmill Road, Sunbury-On-Thames, Middlesex TW16 7HD), measured the surface profiles of the enamel samples pre- and post-treatment. The head unit traversed the specimen at a constant velocity from left to right at a speed of 10 mm/min. The measuring head was fitted with a diamond stylus of 10 μm tip radius, the force of the stylus on the surface varying linearly with deflection at the rate of 8 mg force per micron deflection, the maximum force at 100 μm being 1.0 g. Prior to placing specimens in the appliances, baseline profiles were taken across the area delineated to be treated. Specimens were then taped with PVC tape to leave an exposed zone of enamel approximately 2 mm wide. Specimens were exposed to one of the drinks and the surface profile again recorded twice across the treated area, thus the amount of tissue loss in μm could be calculated for each drink by subtraction of baseline from the values after treatment. A detailed description of specimen preparation can be found in previous publications.[5]

At the beginning and end of each experimental day, appliances with the contained specimens, were soaked in a 0.2% chlorhexidine mouthwash[1] for 2 min. Overnight the appliances were placed in sealed containers on moist cotton wool. The beverage consumption was similar to all previous studies.[4,15] Appliances were in place from approximately 9 a.m. to 5 p.m. ($\pm$30 min) and were removed for 1 h between 12 and 1 p.m. to allow for consumption of lunch. Two hundred and fifty microliters of the allocated drink were imbibed at 9 a.m., 11 a.m., 1 p.m. and 3 p.m. Each volume of drink was sipped over a 10 min period and under supervision.

The five beverages in the study were (Details of drinks in Appendix A and Table 1).

1. Unmodified acidic soft drink.
2. Experimental acidic soft drink with 0.02% polyphosphate.
3. Experimental acidic soft drink with 0.02% polyphosphate and 0.01% calcium.
4. Experimental acidic soft drink with 0.02% polyphosphate and 0.03% xanthan gum.
5. Mineral water.

No other food or drink was to be consumed whilst the appliance was in place. All participants were asked to use a standard conventional fluoride toothpaste and conventional manual toothbrush throughout the duration of the study, provided by the sponsor. Specimens were re-measured twice on the profilometer across the treated area obtaining two surface profile tracings, after removal of the masking tape on days 5 and 10. To avoid cross-contamination, samples measured at day 5 were further disinfected by placing them in a 0.5% chlorhexidine in 70% spirit solution for at least 30 min. After retaping they were returned to the appliance for the completion of the study leg. At the end of each study period a rest period (washout) of at least 2 days was allowed. At the commencement of each study period, new specimens were placed in the appliances. If, at the 5 days measurement point, any specimens approached or exceeded 20 μm loss the subject was withdrawn from that leg of the study, in order to prevent potential unacceptable erosion to the participant.

3. Statistical methods

The mean of the two readings taken from each specimen was averaged at each of the three time measuring points (baseline, 5 and 10 days) for each subject for all five treatments. The average from the two specimens for each subject for each time point and treatment was then calculated. The change from baseline was used as the main outcome measure. As with other studies there was marked heterogeneity of variation and

[1] Corsodyl®, GlaxoSmithKline UK 980 Great West Road, Brentford, Middlesex, TW8 9GS UK.

Table 1 – Chemical properties of the drink formulations

	Drink				
	1	2	3	4	5
PH	3.4	3.4	3.4	3.4	7.2
Titratable acidity (w/w%)[a]	0.4	0.4	0.4	0.4	0.0
Calcium (mg/l)[b]	25	25	100	25	9.9
Sodium polyphosphate (mg/l)	0.0	200	200	200	0.0
Xanthan gum (mg/l)	0.0	0.0	0.0	300	0.0

[a] Titratable acidity (w/w%) expressed as citric acid monohydrate.
[b] Drinks 1, 2 and 4 contain 25 mg/l calcium as the water used to make up the product contained this level of calcium. Only the calcium polyphosphate drink had calcium specifically added.

comparisons between pairs of treatments were by non-parametric (Wilcoxon) tests.

4. Results

All of the subjects completed the study. The mean enamel loss (μm) relative to baseline with each treatment at days 5 and 10 is shown in Table 2. Observationally, the order of erosion from most to least was the unmodified acidic soft drink > experimental acidic soft drink + polyphosphate > experimental acidic soft drink + polyphosphate + gum > experimental acidic soft drink + polyphosphate + calcium > water for day 10. The non-parametric analysis of treatments for days 5 and 10 are shown in Tables 3 and 4. At days 5 and 10 the unmodified acidic soft drink was significantly more erosive than all other drinks. The experimental acidic soft drink + polyphosphate and the experimental acidic soft drink + polyphosphate + gum drinks were significantly ($P < 0.01$) more erosive than mineral water, albeit mean differences were small. The experimental acidic soft drink + polyphosphate + calcium did not cause significantly greater erosion than water at day 10. At day 10 the overall pattern for differences was similar to day 5 but with a less clear difference between the three polyphosphate containing drinks.

5. Discussion

This same *in situ* method has been used many times to study erosion of enamel and dentine by acidic soft drinks and the benefits of modifying drinks to reduce erosion.[3–5,15] Previous studies have shown that the addition of calcium alone[4] or together with xanthan gum[15] markedly reduces the erosive potential of these types of acidic soft drink. The mechanism for the effect of calcium must in part relate to the dynamics of the acid dissolution effect. As enamel is eroded by acid, calcium is released into the acid solution close to the enamel surface. As calcium is already present in solution, the rate of reaction is slowed, the levels of erosion decreasing as the ratio of calcium to acid is increased. It is postulated that the gum facilitates lower pH with raised titratable acidity of the test drink, whilst crucially, maintaining the low erosive properties of the original drink. Such products have been available to the general public for a few years to date.

Novel approaches or refinements to current soft drinks formulations are always being sort to reduce the erosive potential to dental hard tissues still further. Indeed the ultimate acidic soft drink would facilitate or stimulate remineralisation whilst maintaining the acidic taste so popular with the populous. The possible action of the polyphosphates has been hypothesised in the introduction to this study. Polymers that can be adsorbed onto the enamel surface induce a decrease in the rate of dissolution which strongly depends on the chemical nature of the polymer. Chemical groups with the ability to bind calcium ions such as phosphates are particularly efficient, playing potentially a powerful role in inhibiting enamel dissolution.[17] Indeed Schaad et al. (1994)[17] showed that ionic polymers of high molecular weight and of selected structures may be powerful agents for preventing the dissolution of enamel. One might further hypothesise that the addition of polyphosphate to a citric acid based drink would have an anti-erosion action similar to calcium; phosphate levels in the drink at the tooth interface, inhibiting the dissolution of phosphates from enamel hydroxyapatite. As calcium and gum addition have resulted in great success in lowering the erosive potential of drinks in the previous studies cited, these ingredients were also combined in the experimental design of the drinks for comparison.

The results demonstrated that the addition of polyphosphate had some effect at reducing erosion although this was minimal. The addition of polyphosphate with calcium or gum to the acidic soft drink, faired better. It is difficult to determine whether it was calcium or gum alone that resulted in this improvement or the combination of polyphosphate and calcium or gum as unfortunately a calcium or gum addition alone arm was not included in the study. At this juncture it is worth taking into account the magnitude of the effect already reported for calcium alone,[6] although of course the characteristics of each type of drink were different. It would also have been theoretically possible for the presence of calcium in the polyphosphate drink to render some of these polyphosphate groups ineffective and therefore reduce the protective capacity of polyphosphate in the calcium containing solution. The reason for the outcome is not clear and further research needs to be undertaken in this area. An additional arm to the study of an experimental soft drink and calcium only, would have been most useful in interpreting the results, although not the prime aim of the study.

Table 2 – Mean loss of enamel (μm)on days 5 and 10 produced by the five drink regimens

Regimen	Loss of enamel at day 5 (μm)	Loss of enamel at day 10 (μm)
Unmodified acidic soft drink		
N	13	14
Mean	2.92	6.04
S.D.	3.67	6.32
Minimum	−0.11	0.07
Maximum	13.22	22.06
Experimental acidic soft drink + polyphosphate + calcium		
N	15	15
Mean	0.06[2]	0.14
S.D.	0.13	0.31
Minimum	−0.05	−0.05
Maximum	0.36	1.14
Experimental acidic soft drink + polyphosphate		
N	14	14
Mean	0.18	1.62
S.D.	0.19	1.56
Minimum	−0.05	−0.04
Maximum	0.67	5.99
Experimental acidic soft drink + polyphosphate xanthan gum		
N	14	14
Mean	0.22	1.44
S.D.	0.48	0.82
Minimum	−0.04	−0.04
Maximum	1.84	2.50
Mineral water		
N	15	15
Mean	−0.03	0
S.D.	0.05	0.06
Minimum	−0.15	−0.07
Maximum	0.06	0.18

There was large variation in the enamel loss in the five treatment groups. This is due to the biological variation between then subjects and has been shown in previously documented erosion studies by this group.[3,4,6] The variation could be derived from for example saliva, pellicle, movements of the oral musculature. Variation in erosion is also shown between the anterior and posterior samples in a palatal appliance with the anterior samples showing greater surfaces changes however this is not significant (unpublished data from this research group). The samples are protected from the influences of the tongue to a large degree by the design of the appliance and the palatal wires, however the tongue has been shown to have a strong effect of removing dental hard tissue following as erosive challenge.[18]

Although extremely good results have been achieved at reducing the erosive properties of acidic soft drinks by addition of calcium and gum, but to achieve these ends the pH remained relatively high for a commercially available acidic soft drink, namely pH of 3.7–4.0. Ideally it would be advantageous to reduce the pH of a low erosive drink for two reasons. Firstly a low pH achieves a better fruity flavour for the drinks, and secondly improved microbiological stability is obtained. In this study the pH was lower (pH 3.4) to determine if a drink could be developed with the same low erosive properties already provided by the calcium technology,[3,4,6] but at a more common pH for soft drink manufacture. Hence these modified drinks were at a much lower pH to those cited in other low erosive studies.[4,15] The challenge to achieve a reduction in erosiveness was therefore much greater.

Perhaps the more important take home message from the data was that, despite causing significantly more erosion than water, the numerical differences or actual erosion levels were very small and, compared to the positive control drink without any additives, proportional reductions in erosion ranged from >89% to >97%.

In summary, polyphosphates alone appear to confer benefit to citric acid based drinks with regards to inhibition of erosion. However, when polyphosphates are added to a calcium or gum based citric acid drink, superior characteristics are achieved in respect to reduced erosion of enamel. These properties need to be explored in future clinical trials.

Table 3 – Comparisons of the loss of enamel, at 5 days between pairs of drink regimens

Contrast	Difference	95% confidence interval	Wilcoxon *p*-value
At 5 days			
Unmodified acidic soft drink vs. experimental acidic soft drink with polyphosphate	+2.75	+0.62 to +4.88	0.004
Unmodified acidic soft drink vs. experimental acidic soft drink with polyphosphate and xanthan gum	+2.69	+0.74 to +4.65	0.003
Unmodified acidic soft drink vs. experimental acidic soft drink with polyphosphate and calcium	+2.85	+0.70 to +5.00	0.002
Unmodified acidic soft drink vs. mineral water	+2.95	+0.74 to +5.15	0.002
Experimental acidic soft drink with polyphosphate vs. experimental acidic soft drink with polyphosphate and xanthan gum	−0.04	−0.25 to +0.17	0.51
Experimental acidic soft drink with polyphosphate vs. experimental acidic soft drink with polyphosphate and calcium	+0.12	+0.01 to +0.22	0.028
Experimental acidic soft drink with polyphosphate vs. mineral water	+0.21	+0.12 to +0.31	0.001
Experimental acidic soft drink with polyphosphate and xanthan gum vs. experimental acidic soft drink with polyphosphate and calcium	+0.16	−0.08 to +0.40	0.035
Experimental acidic soft drink with polyphosphate and xanthan gum vs. mineral water	+0.25	−0.02 to +0.52	0.002
Experimental acidic soft drink with polyphosphate and calcium vs. mineral water	+0.09	+0.01 to +0.17	0.017

Table 4 – Comparisons of the loss of enamel, at 10 days between pairs of drink regimens

Contrast	Difference	95% confidence interval	Wilcoxon p-value
At 10 days			
Unmodified acidic soft drink vs. experimental acidic soft drink with polyphosphate	+5.42	+2.39 to +8.45	0.001
Unmodified acidic soft drink vs. experimental acidic soft drink with polyphosphate and xanthan gum	+5.60	+2.23 to +8.98	0.001
Unmodified acidic soft drink vs. experimental acidic soft drink with polyphosphate and calcium	+5.88	+2.36 to +9.41	0.001
Unmodified acidic soft drink vs. mineral water	+6.06	+2.41 to +9.70	0.001
Experimental acidic soft drink with polyphosphate vs. experimental acidic soft drink with polyphosphate and xanthan gum	+0.18	−0.46 to +0.82	0.51
Experimental acidic soft drink with polyphosphate vs. experimental acidic soft drink with polyphosphate and calcium	+0.46	−0.40 to +1.33	0.17
Experimental acidic soft drink with polyphosphate vs. mineral water	+0.63	−0.26 to +1.53	0.002
Experimental acidic soft drink with polyphosphate and xanthan gum vs. experimental acidic soft drink with polyphosphate and calcium	+0.28	−0.17 to +0.73	0.026
Experimental acidic soft drink with polyphosphate and xanthan gum vs. mineral water	+0.46	−0.02 to +0.93	0.002
Experimental acidic soft drink with polyphosphate and calcium vs. mineral water	+0.15	−0.03 to +0.32	0.029

Appendix A. Drink formulations

All acidic soft drinks were based on the unmodified acidic soft drink. Drinks 2–4 had additional ingredients as documented below.

1. Unmodified acidic soft drink (ingredients in order of their concentration, starting with the greatest: water, citric acid, sodium citrate, potassium sorbate, sodium benzoate, aspartame, acesulfame K, flavouring).
2. Experimental acidic soft drink with added sodium polyphosphate (average degree of polymerisation: $n = 25$; Rhodia Consumer Specialities Limited; Earle Road Widnes, Cheshire, UK). The polyphosphate we used was a food grade polyphosphate (sodium hexamethaphosphate 696) with the general formula Na $(n + 2)$ PnO $(3n + 1)$ and $n = 25$. $N = 25$ therefore refers to the number of phosphate units that are joined together to make up the polyphosphate. The chain lengths of polyphosphates is difficult to characterise once a chain contains more than four phosphate units. The industry standard is therefore to classify polyphosphates by their average chain length, which is in the paper).
3. Experimental acidic soft drink with added sodium polyphosphate (as above) and with added calcium chloride (Hays Chemical Distribution, Swansea, UK).
4. Experimental acidic soft drink with added sodium polyphosphate (as above) and with added xanthan gum (CP Kelco UK Ltd., Surrey, UK).
5. Mineral water (Volvic, Danone Group, London, UK).

REFERENCES

1. Millward A, Shaw L, Smith AJ, Rippin JW, Harrington E. The distribution and severity of tooth wear and the relationship between erosion and dietary constituents in a group of children. *International Journal of Paediatric Dentistry* 1994;**4**:151–7.
2. Al-Dlaigan YH, Shaw L, Smith A. Dental erosion in a group of British 14-year-old school children. Part II. Influence of dietary intake. *British Dental Journal* 2001;**190**:258–61.
3. Hughes J, West NX, Parker D, Newcombe RG, Addy M. Development and evaluation of a low erosive blackcurrant drink in vitro and in situ. 1. Comparison with orange juice. *Journal of Dentistry* 1999;**27**:285–9.
4. West NX, Hughes J, Parker D, Newcombe RG, Addy M. Development and evaluation of a low erosive blackcurrant drink 2. Comparison with a conventional blackcurrant drink and orange juice. *Journal of Dentistry* 1999;**27**:341–4.
5. West NX, Maxwell A, Addy M, Parker D, Jackson RJ. A method to measure clinical erosion: the effect of orange juice consumption on erosion of enamel. *Journal of Dentistry* 1998;**4**:329–35.
6. Hughes J, West NX, Parker D, Newcombe RG, Addy M. Development and evaluation of a low erosive blackcurrant drink 3. Final drink and concentrate formulae comparisons in situ and overview of the concept. *Journal of Dentistry* 1999;**27**:345–50.
7. Hughes J, West NX, Parker D, Addy M. Effects of pH and concentration of citric, malic and lactic acids on enamel in vitro. *Journal of Dentistry* 1999;**28**:147–52.
8. West NX, Hughes J, Addy M. The erosion of dentine and enamel in vitro by dietary acids: the effect of temperature, acid character and concentration. *Journal of Oral Rehabilitation* 2000;**27**:875–80.
9. Eisenburger M, Hughes J, West NX, Jandt KD, Addy M. Ultrasonication as a method to study enamel demineralisation during acid erosion. *Caries Research* 2000;**34**:289–94.
10. Eisenburger M, Hughes J, West NX, Jandt KD, Addy M. The use of ultrasonication to study remineralisation of eroded enamel. *Caries Research* 2001;**35**:61–6.
11. Hunter ML, West NX, Hughes JA, Newcombe RG, Addy M. Relative susceptibility of deciduous and permanent dental hard tissues to erosion by a low pH fruit drink in vitro. *Journal of Dentistry* 2000;**28**:265–70.
12. Hunter ML, West NX, Hughes JA, Newcombe RG, Addy M. Erosion of deciduous and permanent dental hard tissue in the oral environment. *Journal of Dentistry* 2000;**28**:257–63.

13. West NX, Hughes J, Addy M. The effect of pH on the erosion of dentine and enamel by dietary acids in vitro. *Journal of Oral Rehabilitation* 2001;**28**:860–4.
14. The 1997 Sucralose Drinks Report. Tate & Lyle Speciality Sweeteners, Whiteknights, Reading RG6 6BX.
15. West NX, Hughes JA, Parker D, Weaver LJ, Moohan M, De'Ath J, Addy M. Modification of soft acidic drinks with xanthan gum to minimise erosion: a study in situ. *British Dental Journal* 2004;**196**:478–81.
16. Newcombe RG. Crossover trials comparing several treatments. *Journal of Clinical Periodontology* 1992;**19**:785–7.
17. Schaad P, Thomann J-M, Voegel J-C, Gramain P. Inhibition of dissolution of hydroxyapatite powder by adsorbed anionic polymers. *Colloids and Surfaces A Physicochemical and Engineering Aspects* 1994;**83**:285–92.
18. Gregg T, Mace S, West NX, Addy M. A study in vitro of the abrasive effect of the tongue on enamel and dentine softened by acid erosion. *Caries Research* 2004;**38**:557–60.