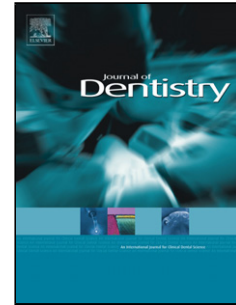


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Ion release and physical properties of CPP-ACP modified GIC in acid solutionsI. Zalizniak¹, J.E.A. Palamara¹, R.H.K. Wong¹, N. J. Cochrane¹, M.F. Burrow²,E.C. Reynolds^{1*}¹ Oral Health CRC, Melbourne Dental School, The University of Melbourne² Faculty of Dentistry, University of Hong Kong, Hong Kong.

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Short title: CPP-ACP in GIC**Keywords:** GIC, CPP-ACP, hardness, ion release

Ion release and physical properties of CPP-ACP modified GIC in acid solutions**Abstract**

A new glass-ionomer cement (GIC) (Fuji VIITM EP) includes 3% (w/w) casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) to enhance ion release.

Objectives: To assess this new GIC compared with a GIC without CPP-ACP (Fuji VIITM) with respect to ion release, changes in surface hardness and in mass under a variety of acidic and neutral conditions.

Methods: Eighty blocks of Fuji VIITM (F7) and Fuji VIITM EP (F7EP) were subjected to three acidic solutions (lactic and citric acids pH 5.0, hydrochloric acid pH 2.0) and water (pH 6.9) over a three day period. Ion release, surface hardness and weight measurements were carried out every 24 hours.

Results: Higher calcium ion release from F7EP was observed under all acidic conditions. Increased inorganic phosphate ion release was observed for F7EP in hydrochloric and citric acids. Fluoride ion release was similar between F7 and F7EP under all conditions but was significantly higher in acids compared with water. After three days there was no significant difference in surface hardness ($p > 0.05$) between the two materials under all conditions except hydrochloric acid. Minimal change in mass was observed for F7 and F7EP in water, lactic and hydrochloric acids, however citric acid caused significantly more mass loss compared with water ($p < 0.001$).

Conclusion: Incorporation of 3% w/w CPP-ACP into F7 enhanced calcium and phosphate ion release, with no significant change in fluoride ion release and no adverse effects on surface hardness or change in mass.

Clinical Significance Statement:

GICs have the potential to release fluoride ions particularly under acidic conditions associated with dental caries and erosion. A new GIC containing CPP-ACP and fluoride releases not only fluoride ions but also calcium and phosphate ions under acidic conditions which should help to inhibit demineralization associated with caries and erosion.

1. Introduction

Glass ionomer cements (GICs) are widely used for a variety of purposes such as intermediate restorations, caries stabilization, definitive restoration of micro-cavities or non-carious cervical lesions, adhering orthodontic brackets and bands, fissure sealing erupting molars and as a surface protecting material for high risk surfaces such as root surfaces.¹⁻² The main advantages of GICs are their strong ability to chemically bind to dentine and their ability to release fluoride ions. This fluoride ion release has been shown to slow the progression and aid the regression of early carious lesions.³ Tooth enamel will only demineralise when the fluid bathing the enamel crystals is undersaturated with respect to the enamel mineral (carbonated hydroxyapatite). Hence, calcium and phosphate ion activity is an important component influencing the level of enamel mineral saturation.

A number of investigators have explored the modification of dental materials in attempt to have them release calcium, phosphate and fluoride ions.⁴⁻⁶ Skrtic *et al.*⁶ explored modifying dental composites with bioactive glasses. Mazzaoui *et al.*⁵ and Al Zraikat *et al.*⁴ assessed the addition of casein phosphopeptide amorphous calcium phosphate (CPP-ACP) to GICs. The casein phosphopeptides stabilise calcium and phosphate ions in a bioavailable form that allow them to inhibit demineralisation and promote the remineralisation of early lesions.⁷ CPP-ACP is stable in the presence of fluoride and has been shown to work synergistically with fluoride.⁸ This makes CPP-ACP a promising additive to dental products and restorative materials. The study by Mazzaoui *et al.*⁵ was a proof of concept study that showed that CPP-ACP could be added to GIC. Al Zraikat *et al.*⁴ later explored the effect of CPP-ACP concentration on GIC mechanical properties. Additionally, both of these studies showed that the addition of CPP-ACP improved calcium and phosphate ion release in lactic acid.

The tooth surface can be exposed to a variety of demineralization challenges including: acids

formed from metabolic processes of oral bacteria (predominantly lactic acid); food acids such as citric and phosphoric acid that are commonly found in soft drinks;⁹ and hydrochloric acid from the regurgitation of stomach contents. If these acids overwhelm saliva's protective functions mineral may be lost from the teeth.¹⁰ Therefore, the overall aim of this study was to determine how GIC with CPP-ACP performed under a variety of acidic conditions compared with GIC without CPP-ACP in terms of ion release, change in hardness and change in mass. Fuji VII (F7) is a low viscosity strontium glass based ionomer cement and is commonly used as a surface protectant, providing effective wetting and intimate adhesion to tooth surfaces, as well as enhanced remineralization capabilities. It has higher fluoride release than most other GICs which makes it a good candidate to establish if there is an additional benefit through the incorporation of CPP-ACP as a source of calcium and phosphate ions together with the fluoride ions. This study will establish whether F7 plus CPP-ACP has the potential to further protect surrounding hard tissue and enhance the remineralization of demineralized tissue by additional ion release.

The research questions were: what effect on the GIC would the addition of CPP-ACP have on; (1) surface hardness; (2) change in mass under neutral or acidic environments and (3) calcium, phosphate and fluoride ion release under neutral or different acidic environments.

2. Materials and Methods

Fuji VII GIC (F7) and F7 with added 3% w/w CPP-ACP (F7EP) from the same batch were provided by GC Corporation (Japan) in capsule form. Polyvinyl siloxane impression material (eliteHD+ light body, Zhermack SpA, Badia Polesine, Italy) moulds were used to create standardised GIC blocks measuring 3mm x 6mm x 6mm (thickness x width x length). 40 blocks of each GIC were prepared by placing the materials in the mould with the top and

bottom surfaces covered by plastic strips, which was held between two glass slides. The glass slides were gently pressed together to extrude any excess material. The specimens were allowed to set inside the moulds for 24 hours in an incubator (37°C, ~100% relative humidity). After cooling to room temperature the blocks were removed from the moulds, and the two major parallel surfaces of the blocks were lapped with 600 grit paper (Norton Tufbak, Saint-Gobain Abrasives Ltd., Auckland, NZ).

Four different solutions were prepared to expose the blocks to a variety of acidic and neutral environments. The three acidic solutions were formulated to simulate a gastric erosive challenge (50mM NaCl adjusted to pH 2.0 with HCl), a dietary erosive challenge (50mM citric acid at pH 5.0) and a cariogenic acid challenge (50mM lactic acid at pH 5.0) (this concentration was selected based on values found previously in plaque fluid).¹¹ Ionic strength of all the acidic solutions was made up to 50 mM including the HCl solution which was modified with NaCl to approximate that found in stomach acid.¹²

The neutral solution was distilled deionised water at pH 6.9 (Millipore Corporation, Victoria, Australia).

Ten blocks of each type of GIC were exposed to 5mL of one of the four solutions. Solutions were changed every 24 hours and the samples were measured for change in mass, surface hardness and the solutions were analysed to determine ion release of calcium, phosphate and fluoride every 24 hours over 3 days.

The mass of each block was measured every 24 hours before surface hardness measurements were performed. Blocks were taken out of solution, and then weighed using a microbalance (Precisa XT 120A, Dietikon, Switzerland). Mass loss measurement was performed under the same conditions for each sample. Blocks were gently pat dried in the same manner and weighed immediately to ensure consistent treatment before testing. All mass loss measurements were obtained under ambient conditions of $60 \pm 5\%$ relative humidity and $23 \pm$

2° C. The percentage change in mass was a combination of water uptake (absorption) and dissolution (solubility) of the GIC.

Vickers microhardness measurements were determined from indentations on the lapped GIC surface using a Microhardness tester (MHT-10, Anton Paar GmbH, Graz, Austria) attached to a microscope (Leica DMPL, Leica Microsystems Wetzlar GmbH, Germany). Two indentations were made on each block (Force, 1.0 N; Dwell, 6 s; Rate, 0.99 N/min). The indentations were separated by a distance of at least three times the indentation size. Images of the indentations were acquired through a calibrated digital camera (Leica DFC320) mounted on the microscope (Leica DMPL, Leica Microsystems Wetzlar GmbH, Germany) and distance measurements made using Image Tool software (Version 3.0, UTHSC, San Antonio, TX) which were then converted into Vickers hardness values. Blocks were then placed into fresh batch of solution and returned to the incubator.

The ion release of calcium, phosphate and fluoride after each 24 hour period of storage was determined using atomic absorption spectroscopy, colorimetry and an ion-specific electrode respectively. To determine the calcium concentration sample solutions (1 mL) were acidified with 1M HCl (0.5 mL) and diluted with 2% lanthanum chloride (0.5 mL) and analysed on a Varian AA240 atomic absorption spectroscope (Varian Australia Pty. Ltd.) against a set of seven standards ranging from 0 to 250 μ M calcium. Inorganic phosphate ion concentrations were determined colorimetrically using a spectrophotometer (UV-visible spectrophotometer, Varian Australia, Pty. Ltd). The samples that were analysed were prepared by taking 100 μ L of solution, diluting with 500 μ L of 4.2% ammonium molybdate and adding 20 μ L 1.5% of Tween $\text{\textcircled{R}}$ 20 (Sigma-Aldrich, St. Louis, MO). The phosphate concentration was determined by comparing the spectrophotometer readings of the samples against a set of seven standards ranging in phosphate concentrations from 0 and 100 μ M. The concentration

of fluoride ions was determined using an ion-selective electrode (Radiometer analytical, ISE C301F, France) connected to an ion analyzer (Radiometer analytical, Ion Check 45, France). Sample solutions (1mL) were diluted with 1mL total ionic strength adjustment buffer (Merck Pty Ltd, Kilsyth, VIC, Australia) and measured against a set of eight fluoride standards ranging from 0 to 1000 μM .

The χ^2 test was used to test normality and data were found to be normally distributed. Single factor ANOVA was used to analyse the results using Bonferroni-Holm multiple comparison. Two-way ANOVA was used to determine the interaction between incorporation of CPP-ACP and exposure to different acids. Level of significance was set at $\alpha = 0.05$.

3. Results

Table 1 shows surface hardness of F7EP and F7 in three different acidic solutions and distilled deionised water measured over three days. A significant decrease in surface hardness was measured from day to day in all solutions except in the following cases. F7EP in citric and hydrochloric acids between day two and three did not register a significant change in surface hardness. F7 in water did not register a significant change between days one and two, and two and three. The surface hardness of F7EP in water was not significantly different between its initial hardness and day one as well as between days two and three. The initial hardness of F7EP was significantly ($p < 0.001$) lower than that of F7. However, after 24 hours of storage in lactic and citric acid F7EP had a similar surface hardness to F7 and was no longer significantly weaker for citric, lactic acids and water ($p > 0.05$), but it was still significantly ($p < 0.01$) weaker in hydrochloric acid. After three days of storage no significant difference in surface hardness could be measured between F7EP and F7 ($p > 0.05$) for citric, lactic acids and water, but F7EP still remained significantly ($p < 0.05$) weaker in hydrochloric acid.

Table 2 shows the mean relative mass change of F7 and F7EP blocks when stored in the four different solutions over three days. Each group is scaled according to its initial weight (100%). The greatest mass loss at all time points was from F7 and F7EP that were exposed to citric acid.

Figures 1a-c show the measured concentration of calcium, phosphate and fluoride ions released from F7 and F7EP when stored in the four different solutions over a three day period. The rate of calcium and phosphate ion release for F7EP was significantly greater than that of F7 for both citric and hydrochloric acids (Figures 1a and 1b), and there was also greater calcium ion release in lactic acid (Figure 1a). The fluoride ion release in all solutions was similar for both F7 and F7EP (Figure 1c).

Where a change in mass was detected for an exposure to an acidic solution over the three days (Table 2) the calcium and phosphate ion release was found to be greater for F7EP (Figures 1a and 1b). The difference in calcium and phosphate ion release as a function of change in mass for F7 and F7EP is shown in Figures 2a and 2b for citric and hydrochloric acids respectively (there was no measurable mass loss in lactic acid and water). These figures show that per unit of mass loss there was a greater release of calcium and phosphate ions from the GIC containing CPP-ACP.

4. Discussion

In all the tests conducted on F7 and F7EP, the materials behaved differently in water and acidic environments. The surface hardness of the GIC's significantly decreased in the acidic environments although this did not correlate to a change of mass. The release of ions was greater in acidic conditions compared with water. The release of calcium and phosphate ions from the material only under acidic conditions is ideal as this is when they would be needed to protect the adjacent tooth structure from demineralization. The higher release of calcium

and phosphate ions from F7EP under acidic conditions may saturate the fluid in the oral environment with appropriate ions thus acting as a "smart material" for the protection of at risk tooth surfaces. The 3 day continuous exposure *in vitro* would have been equivalent to a much longer term periodic acid challenge *in vivo*. However, the *in vitro* performance of these GIC materials should be confirmed clinically.

Previous studies have investigated ion release and surface hardness of various GICs in acidic and neutral environments.^{4-5, 13} Past results have shown ion release from F7 with incorporation of CPP-ACP when stored in neutral solution (water) or lactic acid.⁴ It has also been reported that lower pH environments negatively affect surface hardness of GICs.¹⁴ However, there have been no studies looking at ion release or surface hardness of GICs covering a wide variety of acids that can be encountered in the oral environment.

The results indicate that the degradation of surface hardness was pH dependent rather than solution dependent. Lactic and citric acids (both pH 5) produced a similar reduction in microhardness over three days, while HCl (pH 2) showed the greatest and water (pH 6.9) the least. Even though initially F7EP was slightly softer in surface hardness than F7, a converging pattern occurred across all solutions such that, except for HCl, the difference after three days was no longer significant. It is possible that F7EP has a greater buffering capacity than F7 alone as CPP-ACP has previously been shown to have buffering potential.¹⁵⁻¹⁶

As with surface hardness, there was an initial difference in the change in mass between these two GIC materials. This can be partly attributed to F7EP being slightly less dense than F7. Significant mass loss was observed for specimens placed in citric acid for both materials. While other solutions produced small changes from day to day the differences between them were not significant. The difference in the change in mass between F7EP and F7, in citric acid, was not significant when taking into account the difference in initial weights. Although surface hardness showed very similar trend in a decrease in hardness for

all solutions this was not repeated for change in mass. For citric and lactic acids the mass loss showed a large difference between the two acid solutions such that, in lactic acid a slight mass gain was observed for F7EP and a slight loss for F7. In citric acid a large mass loss was observed for both materials. A mass gain was observed in water. The chelating effect of the citric acid is likely to contribute to the large mass loss. Citric acid is a triprotic acid, which can chelate transition metals, in this case aluminium. Aluminium is an initial component of the GIC matrix in the form of aluminium phosphate and the aluminium ion is involved in the acid-base setting reaction. The cross-linking between the polyalkenoic acid chains is formed by a slow final maturation reaction involving aluminium ions, which increases the strength of the GIC.¹⁷ This hardening process may take days to complete. When exposed to citric acid Al^{3+} ions are complexed by citrate removing them from the GIC matrix leading to the greater mass loss of the material. Further evidence to support this theory can be seen in the phosphate ion release data shown in Figure 1b. The GIC without CPP-ACP released high levels of phosphate although it contained no CPP-ACP supporting the chelation of aluminium and the dissolution of the aluminium phosphate present within all GICs.

When subjected to aggressive environments such as citric, lactic and hydrochloric acids a substantial release of ions occurred from both GIC materials. Calcium release in the three acids ranged from 0.07 to 0.20 $\mu\text{mol}/\text{mm}^2$ for F7 but was higher for F7EP, ranging from 0.36 to 1.34 $\mu\text{mol}/\text{mm}^2$, equating to an increase of 564%, 405%, 462% for citric, lactic and hydrochloric acids respectively. This can be explained by the CPP-ACP acting as a source of bioavailable calcium ions. The CPP-ACP complexes will release calcium and phosphate ions in a pH dependent manner.^{16, 18} An increase in calcium ions would help to maintain the degree of saturation with respect to tooth mineral which would prevent demineralization. Phosphate ion release in lactic acid and water was not detectable for either GIC material. Daily phosphate ion release for citric acid ranged from 9.8 to 13.9 nmol/mm^2 for F7 and was

higher for F7EP, ranging from 14.5 to 17.6 nmol/mm², an average increase of 39%. Daily phosphate ion release for hydrochloric acid ranged from 0.42 to 0.51 nmol/mm² for F7 and was higher for F7EP, ranging from 0.7 to 1.07 nmol/mm², an average increase of 85%. This implies that some phosphate is being supplied by CPP-ACP, but unlike the calcium ion release a lot of the phosphate is also being supplied by the GIC matrix most likely from dissolution of AlPO₄ at the surface of the GIC. Dissolution of the AlPO₄ by citric and hydrochloric acids releases phosphate from the GIC matrix. Fluoride release from both materials was similar and highest in citric (103.53 and 108.46 μmol/mm²) and hydrochloric acids (107.40 and 109.38 μmol/mm²) for F7 and F7EP respectively (Figure 1b).

5. Conclusion

Although initial surface hardness of F7EP was slightly lower than F7, after three days of storage in three of the four types of solution there was no significant difference between the surface hardness values of the two GICs. Addition of CPP-ACP to F7 increased the release of calcium ions by an average of 477% when exposed to acidic solutions with no change in fluoride ion release. However, phosphate ion release ranged greatly depending on the type of acid exposure and whether CPP-ACP had been incorporated.

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Figure 1. Measured calcium (a), phosphate (b) and fluoride (c) ion release from F7 and F7EP when stored in the four different solutions over a three day period. Significant increase ($p < 0.05$) in calcium ions was measured for all groups (except water) between F7EP and F7. Significant increase ($p < 0.05$) in phosphate ion release was measured for citric and hydrochloric acids compared to water. Significantly more phosphate was released from F7EP compared to F7 in citric and hydrochloric acids. Fluoride ion release was significantly higher ($p < 0.005$) in all acids compared to water. Groups marked with same letter indicate no significant difference in fluoride ion release between F7 and F7EP when exposed to the same solution.

Figure 2. Difference in calcium and phosphate ion release as a function of change in mass between F7 and F7EP in citric acid (a) and hydrochloric acid (b).

Table 1. Mean surface hardness of F7 and F7EP in acidic and neutral solutions measured over three days

HV (kg/mm ²) n=10	F7 (Std. Dev.)				F7EP (Std. Dev.)			
	Water	Lactic acid	Hydrochloric acid	Citric acid	Water	Lactic acid	Hydrochloric acid	Citric acid
Initial	53.8 ^a (5.3)	51.8 ^b (5.5)	53.3 ^c (7.6)	53.2 ^d (7.1)	47.1 ^a (6.0)	46.2 ^b (6.2)	46.6 ^c (6.4)	46.7 ^d (7.0)
Day 1	48.5 ^e (6.8)	32.6 (5.1)	28.1 ^f (4.5)	39.5 (7.4)	45.6 ^e (4.3)	34.0 (5.9)	22.6 ^f (4.0)	38.1 (9.0)
Day 2	44.4 (4.2)	28.5 (3.1)	22.0 ^g (3.9)	32.9 ⁱ (5.8)	42.2 (3.3)	28.3 (4.8)	15.5 ^g (3.5)	28.5 ⁱ (6.3)
Day 3	42.0 (4.7)	24.8 (2.3)	15.4 ^h (2.7)	25.8 (5.6)	41.5 (3.2)	24.0 (3.0)	13.3 ^h (2.3)	25.6 (6.8)

a,b,d,c,e,f,g,h

Values marked with the same letter indicate a significant difference between GIC materials within the same solution for the same time period.

Table 2. Mean relative mass of F7 and F7EP when stored in the four different solutions over three days

(% relative to initial mass (100%) n=10)	F7 (Std. Dev.)				F7EP (Std. Dev.)			
	Water	Lactic acid	Hydrochloric acid	Citric acid	Water	Lactic acid	Hydrochloric acid	Citric acid
Day 1	100.3 ^a (5.7)	99.8 (3.8)	99.4 (4.5)	96.9 ^a (4.2)	101.5 ^d (5.1)	101.1 (5.5)	99.9 (4.3)	97.2 ^d (4.4)
Day 2	100.5 ^b (5.7)	99.7 (3.8)	98.6 (4.6)	92.8 ^b (4.3)	102.0 ^e (5.1)	101.3 (5.5)	99.5 (4.2)	93.2 ^e (4.5)
Day 3	100.6 ^c (5.6)	99.6 (3.8)	97.7 (4.6)	89.2 ^c (4.4)	102.2 ^f (5.2)	101.3 (5.4)	98.8 (4.4)	89.0 ^f (4.6)

^{a,b,c,d,e,f} Values marked indicate significant difference between different solutions within the same material for the same time period. Each group is scaled according to its initial weight (100%).

Figure 1 (a)

Calicum Ion Release from Fuji VII and Fuji VII EP

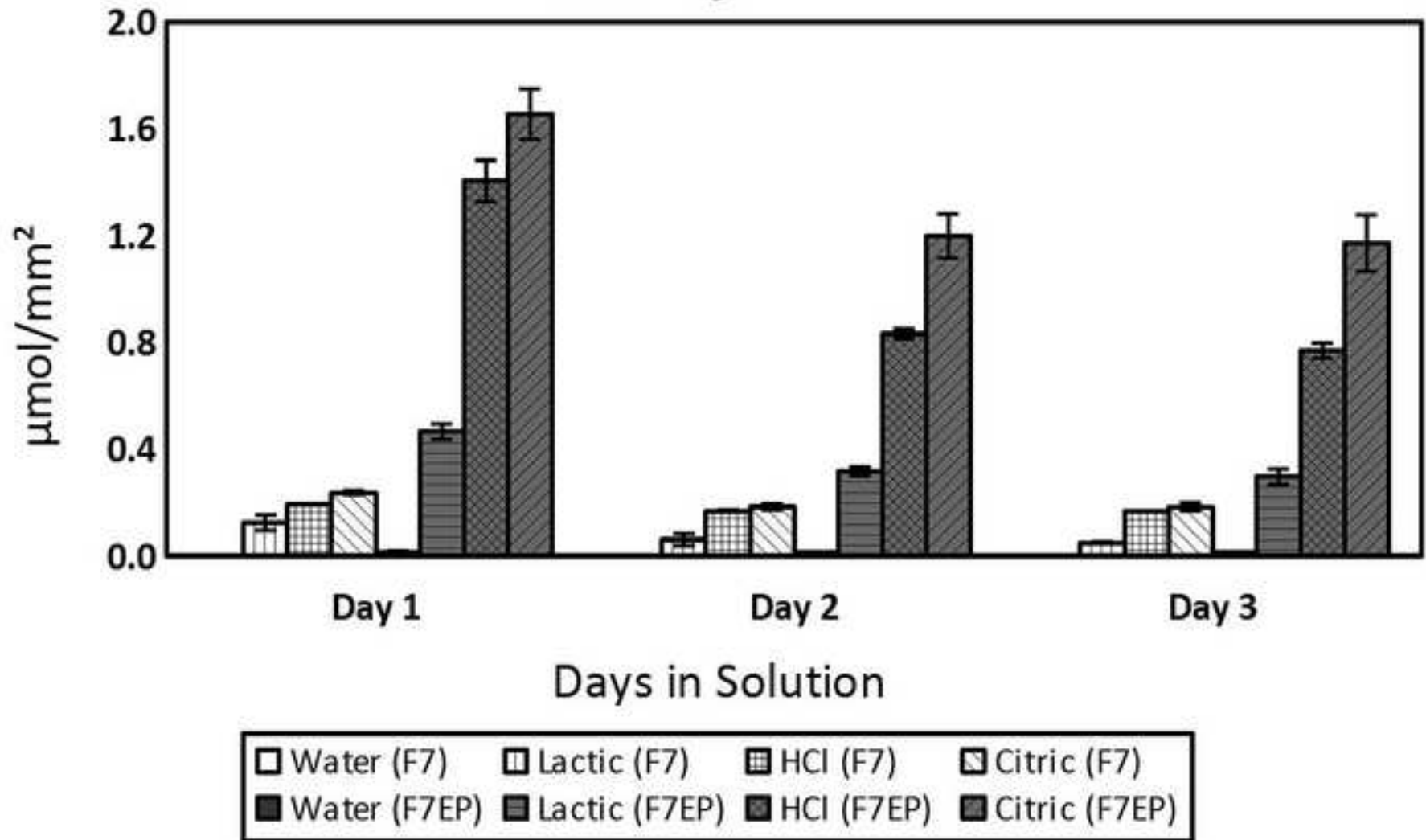


Figure 1 (b)

Phosphate Ion Release from Fuji VII and Fuji VII EP

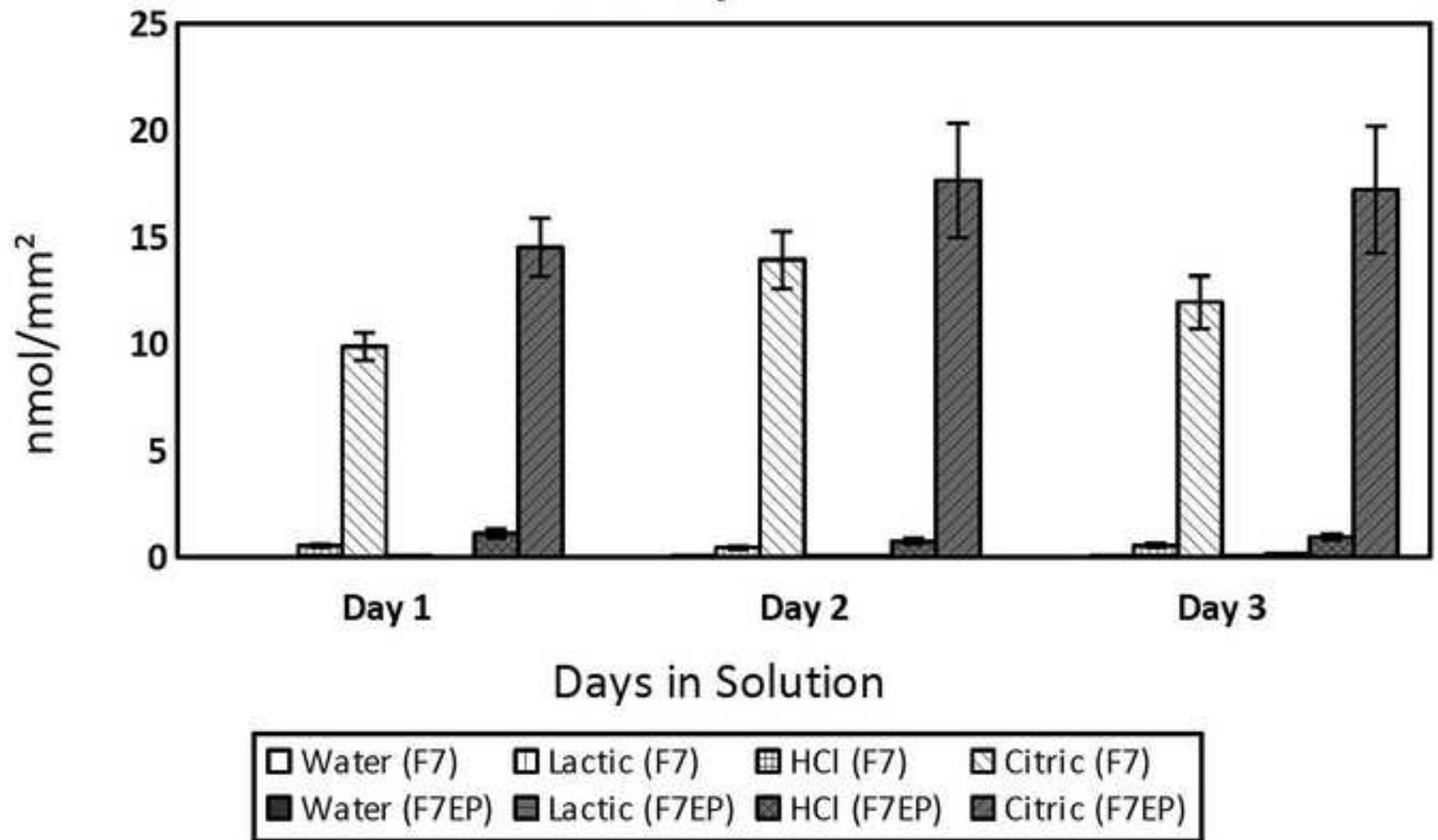


Figure 1 (c)

Fluoride Ion Release from Fuji VII and Fuji VII EP

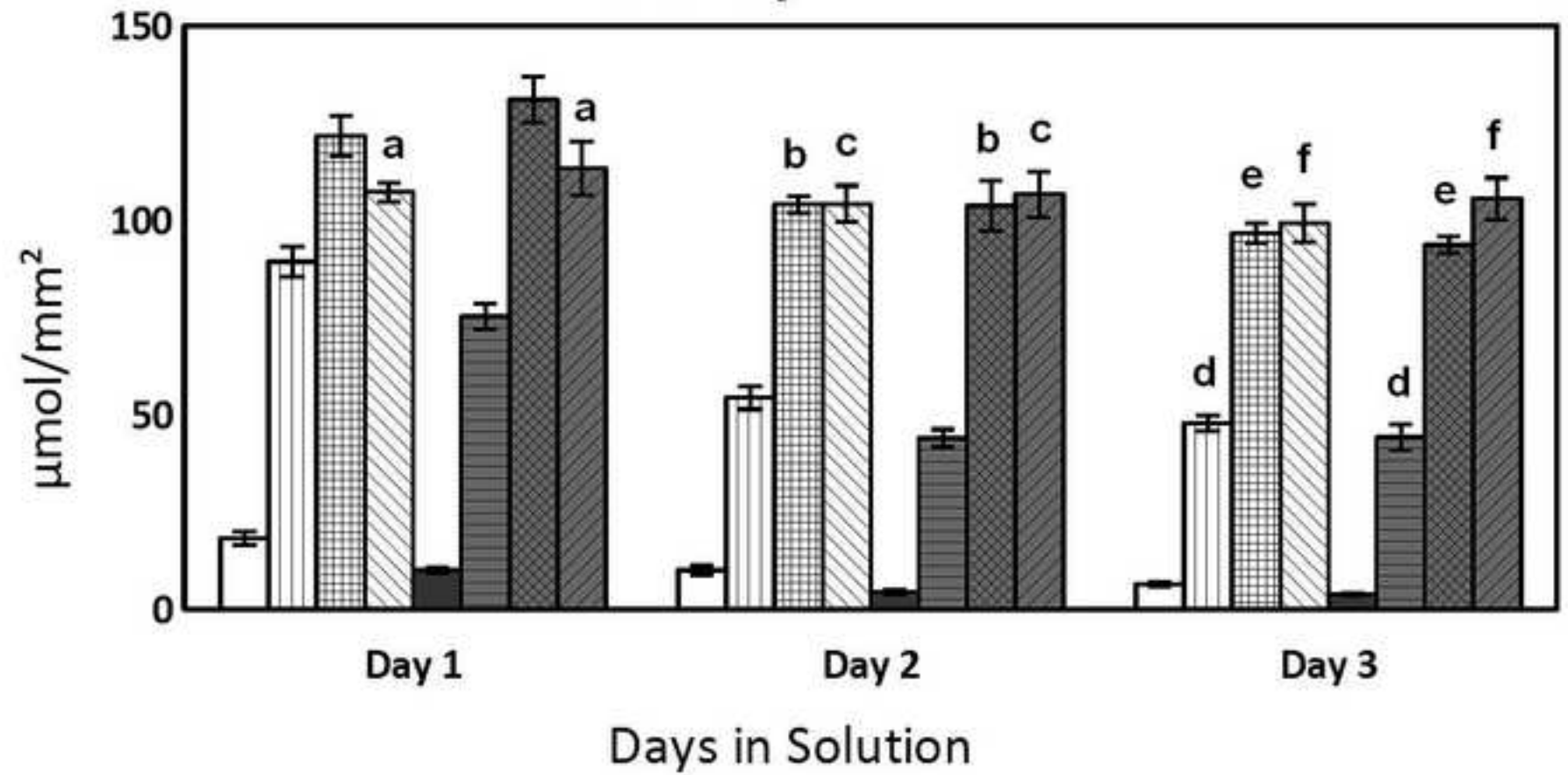


Figure 2 (a)

Citric Acid (pH 5.0)

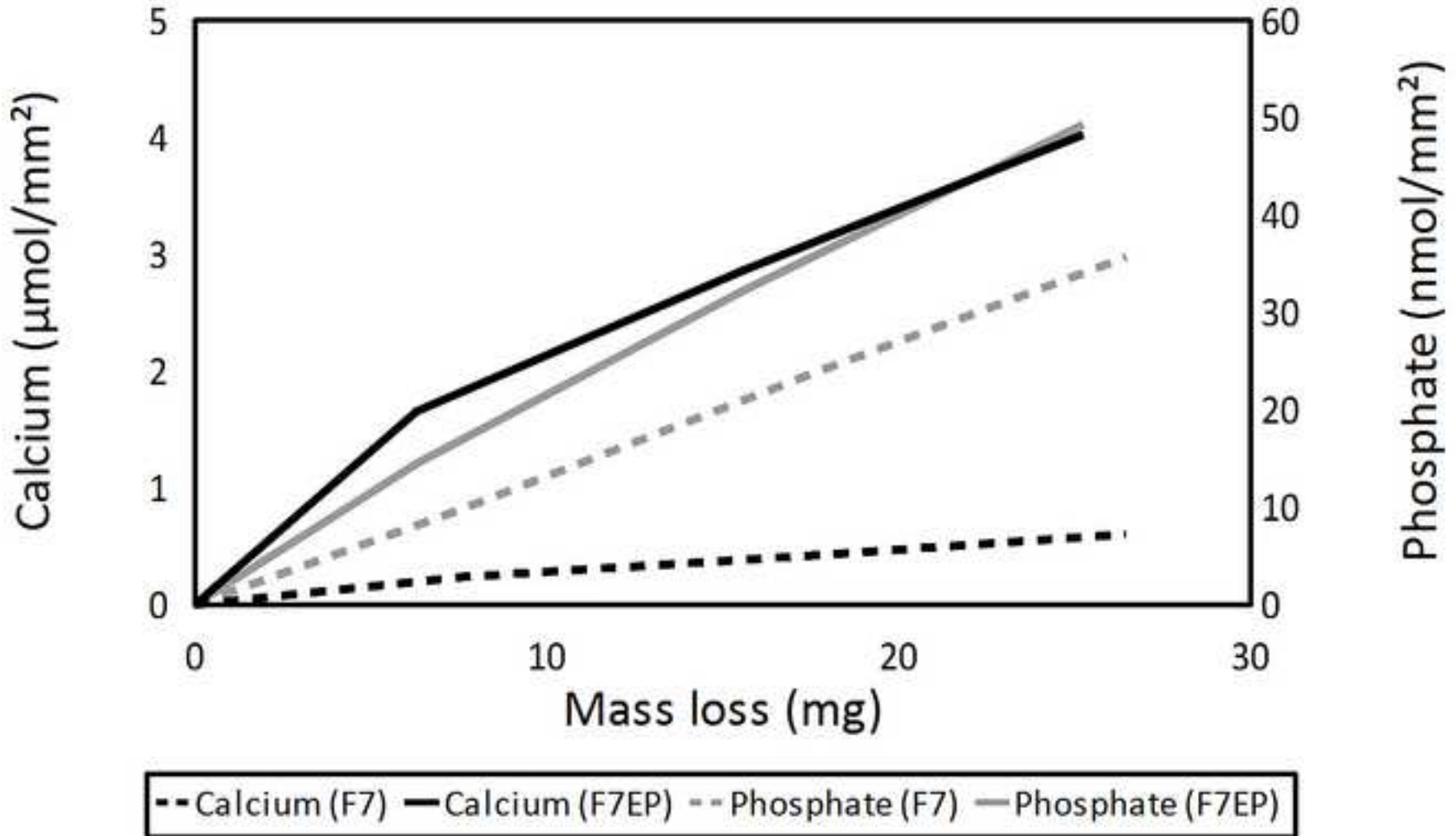
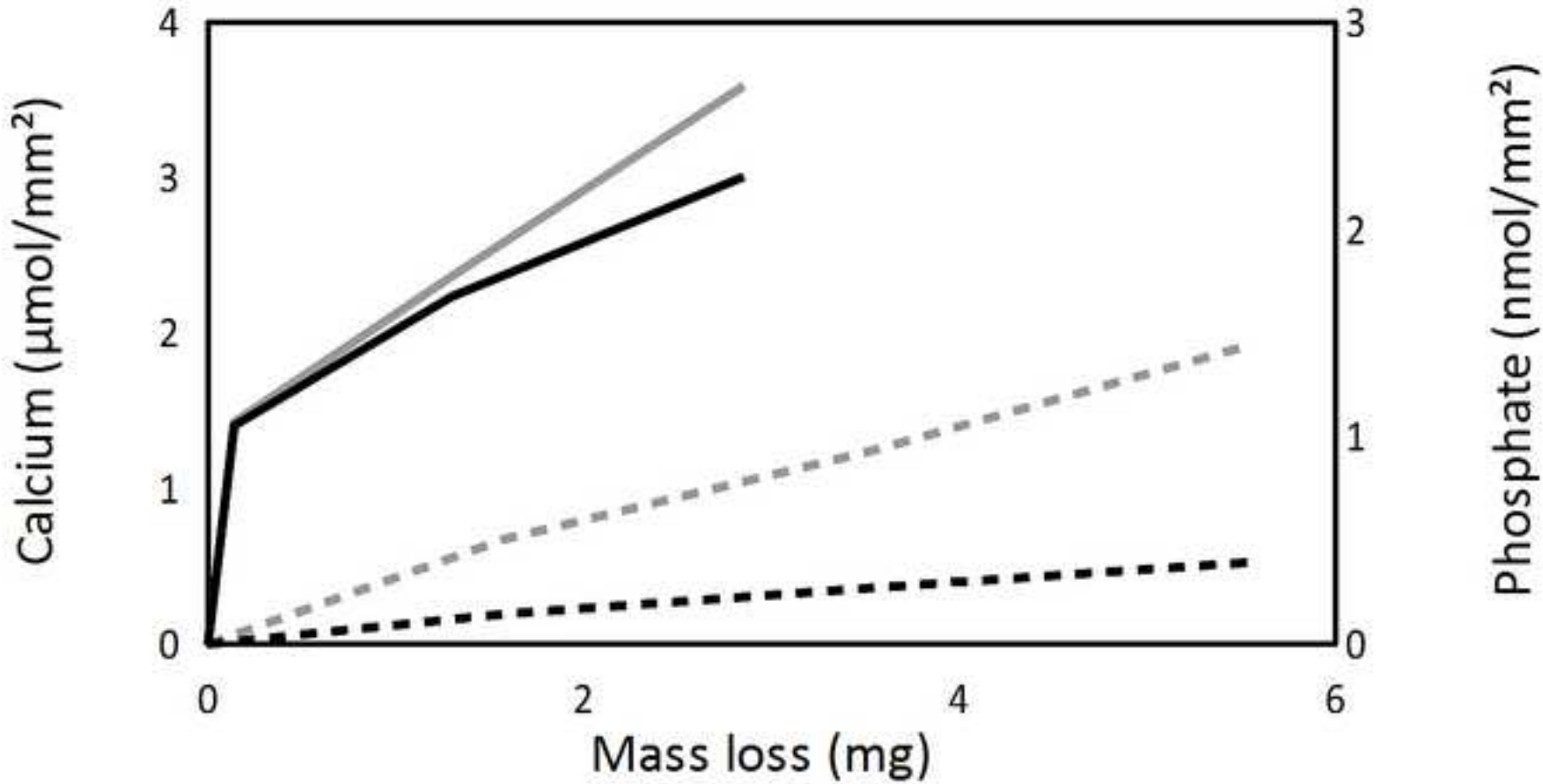


Figure 2 (b)

HCl (pH 2.0)



--- Calcium (F7) — Calcium (F7EP) --- Phosphate (F7) — Phosphate (F7EP)



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