



### EFFECT OF PH AND TIME ON CALCIUM RELEASE FROM SOUND ENAMEL: AN ATOMIC SPECTROMETRIC ANALYSIS

Shetty Shishir\*<sup>1</sup>, Hegde Mithra N<sup>2</sup>, Kumari Suchetha<sup>3</sup>, Shetty Smitha<sup>4</sup>, Bekal Mahesh<sup>5</sup>, Thimmaiah<sup>6</sup>

<sup>1</sup>Professor, Department of Conservative Dentistry and Endodontics, A.B. Shetty Memorial Institute of Dental Sciences, Nitte University, Mangalore, India

<sup>2</sup>Professor and Head of Department of Conservative Dentistry and Endodontics, A.B. Shetty Memorial Institute of Dental Sciences, Nitte University, Mangalore, India

<sup>3</sup>Head, Central Research Laboratory, Nitte University, Mangalore, India

<sup>4</sup>Senior lecturer, Department of Periodontics, A. B. Shetty Memorial Institute of Dental Sciences, Nitte University, Mangalore, India

<sup>5</sup>Research associate Central Research Laboratory, Nitte University, Mangalore, India

<sup>6</sup>Post Graduate, Department of Conservative Dentistry and Endodontics, A.B. Shetty Memorial Institute of Dental Sciences, Nitte University, Mangalore, India

\*Corresponding Author Email: shishirshetty15@gmail.com

Article Received on: 12/03/13 Revised on: 01/04/13 Approved for publication: 21/05/13

DOI: 10.7897/2230-8407.04654

IRJP is an official publication of Moksha Publishing House. Website: www.mokshaph.com

© All rights reserved.

#### ABSTRACT

Dental Caries is a disease of great concern which affects people of all races in all places, the exact etiology of caries though unknown; it is well established that the release of calcium and phosphate from hydroxyapatite crystal is due to decreased pH of saliva. However the relationship between calcium release, pH and time is not well documented.

The present study was designed to assess the calcium release from intact enamel when subjected to different pH at different time intervals. The enamel samples were subjected to acid challenge using Acetate buffer of pH of 3.5, 4, 4.5, 5, 5.5, 6 and 6.5 and buffer solution were analyzed for calcium release at specific time intervals for each pH using atomic absorption spectrometry. The results show that the rate of calcium release significantly increased from pH 5.0 to 3.5, however the calcium release was very little in pH 5.5 to 6.5. From the observations made from this study we can conclude that sound enamel is resistant to acid attack between pH of 5 to 5.5 and Time plays an important role in the reaction of enamel to acid.

**Keywords:** Caries, Sound enamel, Calcium, demineralization, pH, time.

#### INTRODUCTION

Dental Caries is a disease of great concern which affects people of all races in all places. History suggests that caries was not a disease with high prevalence until the 17<sup>th</sup> century. The reason can be attributed to the large consumption of fermentable carbohydrates in the form of sucrose for the last three centuries. The 20<sup>th</sup> century saw the establishment of Dentistry as a profession. Throughout the beginning of the century the profession mainly dealt with relieving pain to people by surgical intervention<sup>1</sup>.

It was only after Stephan 1942 demonstrated a sudden drop in plaque pH followed by a gradual increase, after consumption of a sucrose drink<sup>2</sup> and Dean in 1946 concluded that 1 PPM of fluoride was good for prevention of Caries<sup>3</sup> that a shift was observed in the management and understanding of Dental caries. The importance of plaque and salivary pH was realized and the research was shifted from treatment to prevention of dental caries.

The etiology of caries is still not fully understood. There have been many theories proposed and the etiologic factors vary from Microorganisms to saliva to diet. Whatever may be the reason ultimately, the calcium and Phosphate ions diffuse out of the Hydroxyapatite crystal resulting in caries. The solubility of Hydroxyapatite is greatly influenced by the pH of the solution in which it dissolving, unlike other salts like sodium chloride where the solubility is unaffected by the pH of the solution.

Research has proved that the rate of dissolution of Hydroxyapatite greatly increases below the pH 5.5, however the dissolution is not instantaneous and is dependent on the

amount of exposure of the Hydroxyapatite crystal to the acidic environment. Hence the present study was designed to assess the calcium release from intact enamel when subjected to different pH at different time intervals.

#### MATERIALS AND METHODS

Freshly extracted human Maxillary and Mandibular first molar teeth extracted on periodontal grounds from individuals below the age of 40. Teeth were selected based on randomized sampling method. Teeth with caries, hypoplastic lesions, stains, white spots, cracks, erosion, developmental anomaly or any other deformity were excluded.

The teeth were cleansed of visible blood and gross debris and were maintained in a hydrated state during storage. Extracted teeth were placed in sodium hypochlorite solution diluted with saline in a ratio of 1:10 in container with a secure lid to prevent leaking and labeled with the biohazard symbol.

Elimination of microbial growth was achieved by using an autoclave cycle for 40 minutes. Teeth that do not contain amalgam restorations were preferred because they can be safely autoclaved. However, Extracted teeth containing amalgam restorations, were immersed in 10% formalin solution for 2 weeks<sup>4</sup>.

Seven Enamel slabs of 2mm thickness were prepared from Buccal and Lingual surface of molars by using diamond discs under water cooling at a speed of 25,000 RPM using Micromotor with a contra angled hand piece. The surface of enamel on the buccal and lingual surface were first made flat and then polished to get a fine gloss using 3M Sof Lex polishing discs(J of American Science, 2010;6(11)). The

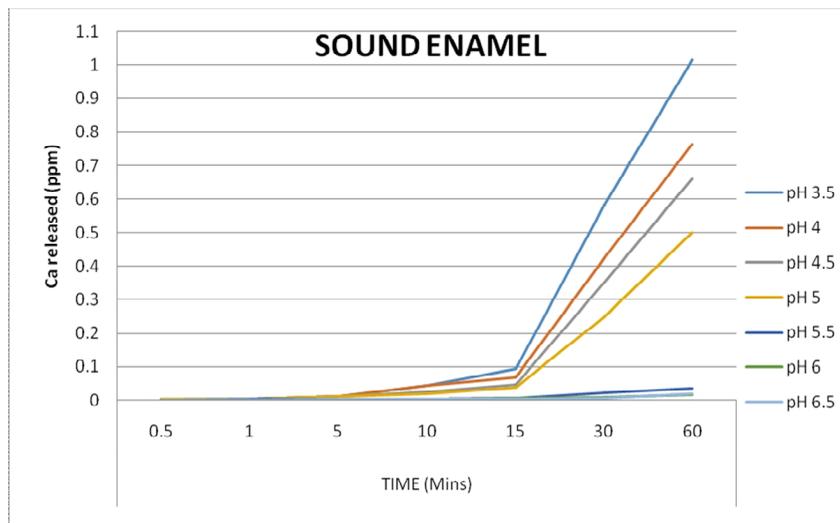
samples were prepared following all infection control protocols and barrier techniques. 0.2 M Acetate buffer was prepared for pH 3.5 to 6.5 at 0.5 intervals.

The samples of Intact Enamel were divided into seven subgroups each based on the pH of Acid buffer. Individual specimen of every subgroup was immersed in 50 ml of acetate buffer. The solution was continuously stirred using a magnetic stirrer at 100 RPM. 5 ml of the solution was poured to a sterile screw capped tubes at time intervals of 0,

0.5, 1, 5, 10, 15, 30 and 60 minutes using a micro pipette. All the centrifuge tubes were marked for future identification. The experiment was simultaneously conducted for all the 4 groups.

The acid buffers of all the groups for all time intervals were evaluated for calcium, using Atomic spectrometry (GBC 932 Plus atomic absorption spectrophotometer).

All the Experimental data were analyzed using one way ANOVA.



Graph 1: Y axis showing the amount of calcium released in ppm and X axis showing the time

## RESULTS

Enamel samples treated with Acetate Buffer showed increased amount of calcium release at all time intervals between pH 3.5-5.0, However calcium release was statistically not significant above pH 5.0 at all time intervals.

There was statistically significant amount of calcium release at time intervals of 15, 30 and 60 mins compared to time intervals below 15mins.

## DISCUSSION

Dental caries is a dynamic process involving periods of demineralization and remineralization. When demineralization is greater than remineralization the lesion progresses. The increased demineralization is attributed to decreased pH of oral fluids.

The present study was designed to assess the calcium release from intact enamel when subjected to different pH at different time intervals.

The rate of calcium release significantly increased from pH 5.0 to 3.5, however the calcium release was very little in pH 5.5 to 6.5. This may be because when pH is below 5.5 Hydroxyapatite dissolves in the saliva because the saliva is under saturated with hydroxyapatite, hence Sound Enamel is resistant to acid attack above Ph 5.5.<sup>1</sup>

Measurements of pH have made it possible to examine dental plaque as a metabolic unit and to identify the Stephan pH response as an important indicator of caries activity<sup>2</sup>. The results obtained indicates that along with the pH, This study showed that the calcium release proportionately increased with time in all pH groups, suggesting that the time also plays an important role in caries formation.

Sound enamel is resistant to acid attack between ph 5.0-5.5 but it showed vulnerability to lower pH. Time plays an

important role in the reaction of enamel to acid. However there is no pre-determined demarcation of time or amount of calcium released to distinguish the acid resistance of enamel.

## CONCLUSION

Sound enamel is resistant to acid attack between ph 5.0-5.5 but it showed vulnerability to lower pH.

Time plays an important role in the reaction of enamel to acid. However there is no pre-determined demarcation of time or amount of calcium released to distinguish the acid resistance of enamel.

## ACKNOWLEDGEMENT

We are grateful to the department of chemistry, National Institute of Technology, Suratkal, Mangalore and the Central research Laboratory for their enormous help and guidance in conducting the study.

## REFERENCES

- O. Fejerskov, E. A. M Kidd. Clinical cariology and operative dentistry in the 21st century. O. Fejerskov, E. A. M Kidd, editors, Dental caries and its clinical management, 2003: 3-6.
- Robert M. Stephan, Benjamin F. Miller. A Quantitative method for evaluating physical and chemical agents which modify production of acids in bacterial plaques on human teeth. J Dent Res 1943; 22: 45-51. <http://dx.doi.org/10.1177/00220345430220010601>
- O. Fejerskov. Changing Paradigms in concepts of dental caries: consequences for oral health care. Caries Res 2004; 38: 182-191. <http://dx.doi.org/10.1159/000077753> PMID: 15153687
- Pantera E. A, Schuster G.S. Sterilisation of extracted human teeth. J Dent Educ 1990; 54: 283-285. PMID: 2335661
- Kleinberg, G.N. Jenkins, R. Chatterjee and L. Wijeyeweera. The antimony pH electrode and its role in the assessment and interpretation of dental plaque pH. J Dent Res 61(10): 1139-1147, October 1982. <http://dx.doi.org/10.1177/00220345820610100601> PMID: 6749924
- B Nyvad, W. Crielaard, A Mira, N Takahashi, D. Beighton. "Dental Caries from a molecular microbiological perspective". Caries Research 2013; 47: 89-102 <http://dx.doi.org/10.1159/000345367> PMID: 23207320

7. J. M ten Cate, M. J. Larsen, E.I.F Pearce, O. Fejerskov. Chemical interactions between tooth and oral fluids, O. Fejerskov, E. A. M Kidd, editors, Dental Caries and its clinical management. 2003: 649-669.
8. Ferreira, Neto, Pereira, Boscolo: in vitro induction of enamel subsurface delineraization for evaluation of diagnostic imaging methods. Journal of applied oral science, sept/oct 2007. <http://dx.doi.org/10.1590/S1678-77572007000500004> PMID:19089167
9. Amir Azarpazhooh and hardy Limeback. Clinical efficacy of casein derivatives. JADA, vol. 139 July 2008, 916-924.
10. J. Neilands, L.G Peterson, D. Beighton, G Svensater: fluoride-supplemented milk inhibits acid tolerance in root caries biofilms. Caries res 2012;46:156-160. <http://dx.doi.org/10.1159/000337390> PMID:22488252
11. Junko Tanaka, Norio Mukai, Muto tanaka, Masahiro Tanaka. "Relationship between cariogenic bacteria and pH of dental plaque as margin of fixed prosthesis." International Journal of dentistry. Vol 2012
12. Axelsson P, Lindhe J and Nystorm B. "On the prevention of caries and periodontal disease." J ClinPeriodontol 1991;18:182-189. <http://dx.doi.org/10.1111/j.1600-051X.1991.tb01131.x> PMID:2061418
13. P.Anderson, M.P.Hector, M.A.Rampersad. "Critical pH in resting and stimulated whole saliva in groups of children and adults" International Journal of Paediatric Dentistry 2001;11:266-273 <http://dx.doi.org/10.1046/j.1365-263X.2001.00293.x> PMID:11570442
14. A.F.B Calvo, C.P.M Tabchoury, A.A Del BelCury, L.M.A Tenuta, W.J. da Silva, J.A.Cury. "Effect of Acidulated phosphate fluoride gel application time on enamel demineralization of deciduous and permanent teeth" .Caries res 2012;46:31-37. <http://dx.doi.org/10.1159/000335125> PMID:22237162
15. YoshaskamAgnihotri, Namratha Lakshmi Pragada, GauravPatri, PK Thajuraj. "The effect of CPP-ACP on remineralization of artificial caries like lesions: An invitro study". Indian journal of Multidisciplinary Dentistry, Vol 2, Issue 1, November 2011-January 2012.
16. I.Kleinber. "A mixed-bacteria ecological approach to understanding the role of the oral bacteria in dental caries causation: an alternative to Streptococcus Mutans and the specific plaque hypothesis" Crit Rev Oral Biol Med 2002 13(2);108-125. <http://dx.doi.org/10.1177/154411130201300202>

**Cite this article as:**

Shetty Shishir, Hegde Mithra N, Kumari Suchetha, Shetty Smitha, Bekal Mahesh, Thimmaiah. Effect of pH and time on calcium release from sound enamel: An atomic spectrometric analysis. Int. Res. J. Pharm. 2013; 4(6):240-242

Source of support: Nil, Conflict of interest: None Declared