

## BLOW OFF CARIES WITH BUBBLE GUM

Kishna Chaithanya G\*, Prabhakar AR, Saraswathi V., Naik, Shivani B

\*Post graduate student, Department of Pedodontics and Preventive Dentistry, Bapuji Dental Collage and Hospital, Davangere, India.

Head of the department, Professor, Department of Pedodontics and Preventive Dentistry, Bapuji Dental College and Hospital, Davangere, India.

Reader, Department of Pedodontics and Preventive Dentistry, Bapuji Dental College and Hospital, Davangere, India.

Lecturer, Department of Pedodontics and Preventive Dentistry, Bapuji Dental College and Hospital, Davangere, India.

### Abstract

#### Keywords:

*Casein phosphopeptide – amorphous calcium phosphate(CPP-ACP), Calcium concentration, phosphorus concentration, photometer, salivary pH and buffer capacity*

**Background:** Casein phosphopeptide – amorphous calcium phosphate (CPP-ACP) nanocomplexes incorporated into sugar-free chewing gums have shown to remineralize enamel subsurface lesions in situ. So this study was conducted to evaluate and estimate the salivary concentration of calcium and phosphorous, salivary pH and buffering capacity before and after chewing sugar free chewing gums containing CPP-ACP (Recaldent™).

**Material and methods:** Unstimulated saliva from each 18 selected subjects (aged between 8–14 years) was collected. Then each subject was given two pellets of chewing gum containing CPP-ACP and asked to chew for a period of 20 mins. Then the stimulated saliva was collected at intervals of 20, 40, 60, 90 min from each individual. They were assessed for calcium and phosphorous concentration using affiliated reagent kits and photometer while salivary pH and buffering capacity were assessed using Saliva Check kit.

**Results:** There was significant increase in salivary calcium level over a period of 20,40,60 mins than the resting salivary calcium level. The salivary phosphate level over a period of 20 mins was lower than at resting salivary phosphate level. The salivary pH and buffering capacity remained higher for the entire 90 mins period.

**Conclusions:** CPP-ACP containing chewing gum increases the level of calcium, salivary pH and buffer capacity, thereby may result in the remineralisation of tooth.

### Introduction

Saliva maintains the tooth integrity by demineralization and remineralization process. Salivary components like salivary calcium, inorganic phosphorous, buffering capacity, and pH etc plays a major role in initiation and progression of dental caries.<sup>1</sup>

Casein phosphopeptide amorphous calcium phosphate complexes (CPP-ACP), have been shown to have anticariogenic potential in laboratory, animal, and human insitu caries models. The CPP have remarkable ability to stabilize calcium phosphate in solution and substantially increase the level of calcium in dental plaque.<sup>2</sup>

Chewing gums are considered to be potential anticariogenic agents, because they can induce an increase in salivary pH. This higher pH can increase tooth mineral saturation during acid challenge and thus decrease demineralization. Increased calcium and phosphate concentrations in the oral environment can also increase tooth mineral saturation in oral fluids. Therefore, an increased anticaries effect might be anticipated from the release of these ions during chewing of gums fortified with appropriate calcium phosphate minerals.<sup>3</sup>

Studies have been conducted in the past to evaluate the buffering capacity of saliva and salivary pH after chewing gums containing xylitol, sorbitol and bicarbonate gum but the same properties are yet to be evaluated for chewing gums containing CPP-ACP.

Hence the aim of present study was to evaluate and estimate the salivary concentration of calcium and phosphorous, salivary pH and buffering capacity before and after chewing commercially available sugar free chewing gums containing CPP-ACP (Recaldent™).

## Materials and method

### Sample size determination

Based on previous study<sup>4</sup>, a sample size of 18 children (10 boys and 8 girls) was taken under 5% alpha error and 85% power of the test.

### Inclusion criteria

- Healthy, cooperative children aged between 8–14 years with a mean age  $10.72 \pm 1.34$ .

### Exclusion criteria

- Children who are allergic to gum ingredients.
- Children with psychological disorders, motor disorders and/or unidentified syndromes.<sup>5</sup>

**Study design:** This is an experimental, one group pretest post test design in vivo, study.

### Methods of saliva collection

Collection of sample was in the morning hours and from children who had breakfast two hours before and had not taken food or drink in between this period. Unstimulated and stimulated saliva was collected.

The research study was conducted on patients reporting to the Bapuji Dental college & hospital, Davangere.

Approval from the ethical committee of the institution was obtained.

### Procedure

The subjects were comfortably seated on the dental chair and were asked to rinse the mouth with distilled water. Two minutes later the subjects were asked to spit saliva into saliva collecting glass beaker till 1.5 ml of saliva was collected. Then the sample was labeled as baseline sample. Then the subject was given two pellets of CPP-ACP containing chewing gum {Figure 1} and was asked to chew the gum for 20 min following which the subject was asked to spit the chewing gum. Then the stimulated saliva was collected at intervals of 20, 40, 60, 90 min. {Figure 2} Then the samples was labeled after sample – 1,2,3, and 4 respectively and stored. The subjects was allowed to chew at their own natural chewing rate. During non-collection periods, subjects was asked to swallow their saliva.<sup>4</sup>

### Measurement of salivary Calcium and Phosphate

The samples was taken immediately to the laboratory and assessed for their calcium and phosphorus concentration using serum calcium and serum phosphorus reagent kits (ACCUCARE™ Lab-care Diagnostics Pvt. India) and photometer (Trans Asia Pvt Ltd.,)<sup>4</sup>{Figure 3}

### Measurement of salivary Buffering capacity

Buffering capacity of salivary samples was measured by using buffer strip (GC Asia Dental Pvt. Ltd.,). The buffer strip was removed from foil package and placed on to an absorbent tissue with the test side up. Using pipette, sufficient saliva was drawn from the collection cup and dispense one drop on to each of three test pads. Immediately the strip was turned around to 90 degree to soak up excess saliva on absorbent tissue. The test pads begin to change the colour immediately and after 2 mins the final result was calculated by adding the points according to final color of each pad. {Figure 4}

**Measurement of salivary pH**

The pH of the saliva samples was measured using a GC Saliva Check kit (GC Asia Dental Pvt. Ltd.). For pH evaluation, a pH test strip was immersed in the saliva for 10 seconds and compared for color change with a testing chart.<sup>6</sup>{Figure 5}



*Figure 1: Pellets of chewing gum given to the subject*



*Figure 2: Collection of saliva after chewing CPP-ACP containing chewing gum*



*Figure 3: Photometer*



Figure 4: Measurement of salivary buffer capacity by using GC Saliva Check kit (GC Asia Dental Pvt. Ltd.)



Figure 5: Measurement of salivary pH by using GC Saliva Check kit (GC Asia Dental Pvt. Ltd.)

## Results

The data collected was statistically analyzed using SPSS software (version 20.0). Repeated measures analysis was employed to find the differences between parameters at different time intervals.

### Salivary Calcium

Table 1 and Graph 1 shows the salivary calcium level (mean± sd.) obtained at baseline and after chewing two pellets of CPP-ACP containing chewing gum for 20 min. The mean salivary calcium level at baseline was  $1.71 \pm 0.84$  mg/dl and peak salivary calcium level ( $3.72 \pm 0.85$ ) mg/dl which occurred at 20mins post collection period and gradually decreased over a period of 90 mins. The salivary calcium level over a period of 20,40,60 mins was significantly higher ( $P < 0.0001^{**}$ ) than the resting salivary calcium level. The mean salivary calcium level at 90 mins post collection was  $1.71 \pm 0.70$  mg/dl which is not statistically significant when compared to resting salivary calcium level.

### Salivary Phosphate:

Table 2 shows and Graph 2 the salivary phosphate level (mean± s.d.) obtained at baseline and after chewing two pellets of CPP-ACP containing chewing gum for 20 min. The mean salivary phosphate level at base line was  $0.86 \pm 0.13$  mg/dl and at 20 mins collection period was ( $0.681 \pm 0.13$ ) mg/dl which was statistically significant ( $P < 0.0001^{**}$ ). The salivary phosphate level over a period of 40,60,90 mins post collection was not statistically significant.

### Salivary Buffer capacity

Table 3 and Graph 3 shows the salivary buffering capacity (mean± s.d.) obtained at rest and after chewing two pellets of CPP-ACP containing chewing gum for 20 min. The mean salivary buffering capacity at baseline was ( $7.22 \pm 1.39$ ) units and the peak buffering capacity ( $11.88 \pm 0.31$ ) occurred at 20 mins post collection period. The salivary buffering capacity remained significantly higher ( $P < 0.0001^{**}$ ) than the resting buffering capacity, for the entire 90-mins.

### Salivary pH

Table 4 and Graph 4 shows the salivary pH (mean± s.d.) obtained at baseline and after chewing two pellets of CPP-ACP containing chewing gum for 20 min. The mean salivary pH was  $7.07 \pm 0.21$  units at baseline and the peak pH

( $7.65 \pm 0.13$ ) occurred at 20 mins post collection period. The salivary pH remained significantly higher ( $P < 0.0001^{**}$ ) than the resting pH, for the entire 90-mins.

### Tables

**Table 1: Mean and Standard deviation of salivary calcium level at baseline and after chewing two pellets of CPP-ACP containing chewing gum for 20 min and over a period of 40,60 and 90 mins**

Parameter	Mean $\pm$ SD					F value	Significant (p value)
	Before chewing CPP-ACP containing gum	After chewing CPP-ACP containing gum					
		20mins	40mins	60mins	90mins		
Calcium (mg/dl)	1.71 $\pm$ 0.84	3.72 $\pm$ 0.88	2.78 $\pm$ 0.89	2.06 $\pm$ 0.74	1.71 $\pm$ 0.72	52.921	<0.0001**

**Table 2: Mean and Standard deviation of salivary phosphate level at baseline and after chewing two pellets of CPP-ACP containing chewing gum for 20 min and over a period of 40,60 and 90 mins**

Parameter	Mean $\pm$ SD					F value	Significant (p value)
	Before chewing CPP-ACP containing gum	After chewing CPP-ACP containing gum					
		20mins	40mins	60mins	90mins		
Phosphate (mg/dl)	0.86 $\pm$ 0.13	0.61 $\pm$ 0.13	0.78 $\pm$ 0.08	0.78 $\pm$ 0.15	0.79 $\pm$ 0.07	51.987	<0.0001**

**Table 3: Mean and Standard deviation of salivary buffer capacity level at baseline and after chewing two pellets of CPP-ACP containing chewing gum for 20 min and over a period of 40,60 and 90 mins**

Parameter	Mean $\pm$ SD					F value	Significant (p value)
	Before chewing CPP-ACP containing gum	After chewing CPP-ACP containing gum					
		20mins	40mins	60mins	90mins		
Buffer capacity	7.22 $\pm$ 1.39	11.88 $\pm$ 0.31	11.55 $\pm$ 0.49	11 $\pm$ 0.57	10.00 $\pm$ 0.79	141.79	<0.0001**

**Table 4: Mean and Standard deviation of salivary pH level at baseline and after chewing two pellets of CPP-ACP containing chewing gum for 20 min and over a period of 40,60 and 90 mins**

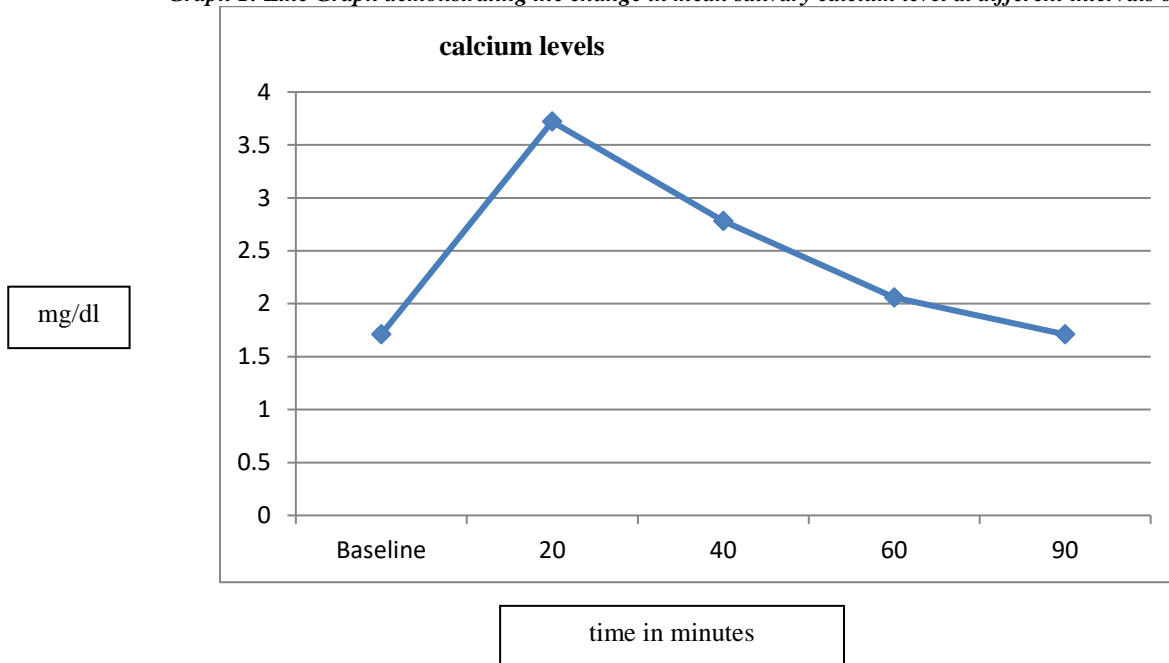
Parameter	Mean ± SD				F value	Significant (P value)	
	Before chewing CPP-ACP containing gum	After chewing CPP-ACP containing gum					
		20mins	40mins	60mins	90mins		
pH	7.07±0.21	7.65±0.13	7.45±0.17	7.33±0.2	7.27±0.21	3.76	<0.0001**

\*\*Statistically highly significant at p<0.01

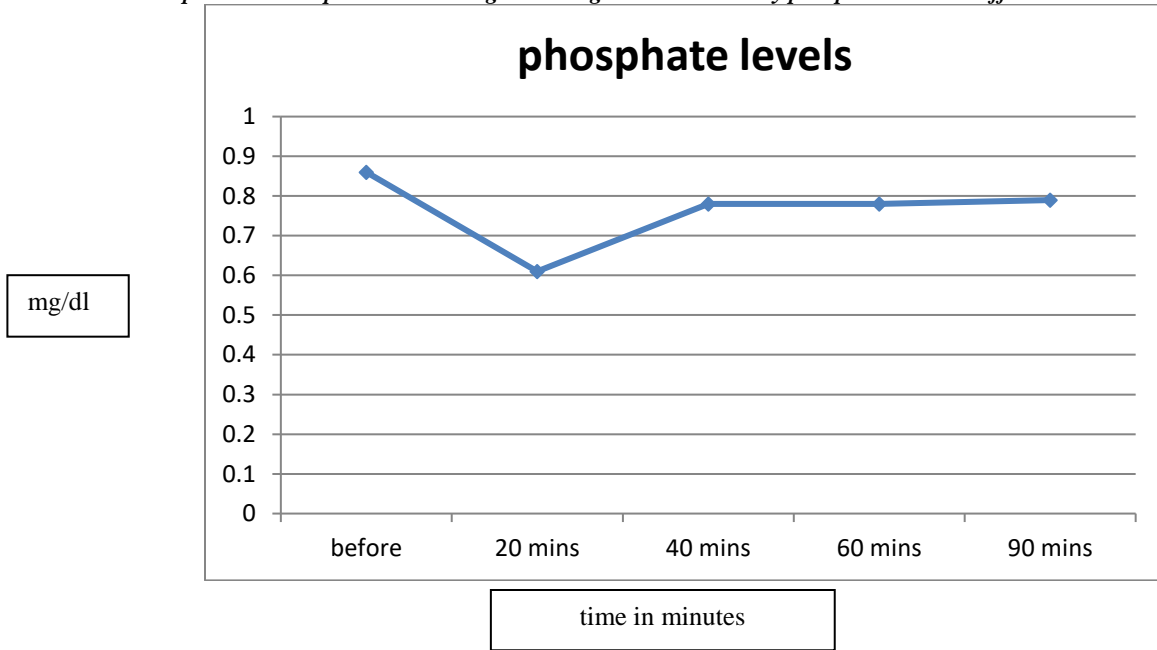
\*Statistically significant at p<0.05

**Graphs**

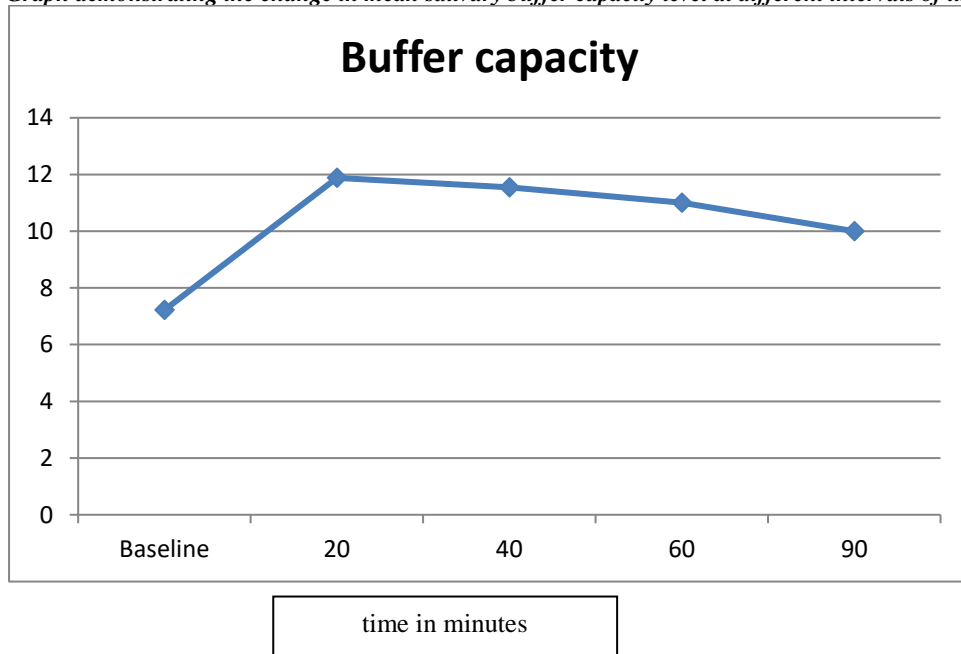
**Graph 1: Line Graph demonstrating the change in mean salivary calcium level at different intervals of time**

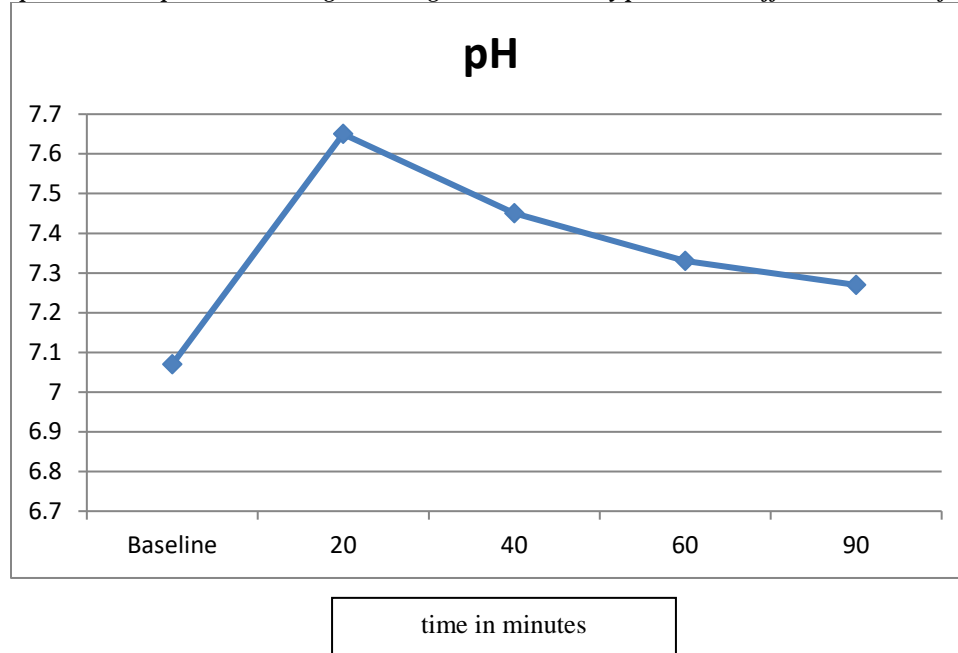


Graph2: Line Graph demonstrating the change in mean salivary phosphate level at different intervals of time



Graph3: Line Graph demonstrating the change in mean salivary buffer capacity level at different intervals of time



**Graph4: Line Graph demonstrating the change in mean salivary pH level at different intervals of time**

## Discussion

Demineralization and remineralisation equilibrium depends on the ionic concentration of calcium and phosphate in saliva and change in calcium and phosphate levels leads to initiation and progression of caries. It is clear that the presence of elevated levels of calcium and phosphorus in the saliva of children is protective against dental caries.<sup>7</sup>

Casein phosphopeptide amorphous calcium phosphate complexes (CPP-ACP), have remarkable ability to stabilize calcium phosphate in solution and substantially increase the level of calcium in dental plaque. Through their multiple phosphoryl residues the CPP bind to form clusters of Amorphous Phosphate (ACP) in metastable solution preventing the growth to the critical size required for nucleation and precipitation. Thus it buffers the free calcium and phosphate ion activities, thereby helping to maintain a state of super saturation with respect to tooth depressing demineralisation and enhancing remineralisation.<sup>2</sup>

CPP-ACP can be delivered via tooth mousse, chewing gum (chewing gum increases the salivary stimulation and the benefits of CPP-ACP are also present), mouth rinses and toothpastes and CPP-ACP helps in the reduction of tooth sensitivity when it is present in tooth pastes.<sup>8</sup>

The use of sugar-free chewing gum has been increasingly accepted as one adjunct to oral hygiene procedures. It has become a part of caries prevention program,<sup>9</sup> because, as a consequence of their effect on salivary flow, they can induce increase in plaque and salivary pH. This higher pH can increase tooth mineral saturation during an acid challenge and thus decrease demineralization. Increased calcium and phosphate concentrations in the oral environment can also increase tooth mineral saturation in oral fluids. Therefore, an increased anticaries effect might be anticipated from the release of these ions during chewing of gums fortified with appropriate calcium phosphate minerals.<sup>3</sup>

Furthermore, gums have advantages like they serve as calcium phosphate delivery vehicles such as

- (1) They can be made to contain a large amount of mineral ions, with a single stick of gum potentially holding nearly as much calcium as a litre of a typical remineralizing solution or saliva.
- (2) They can be chewed over a relatively extended period, while the practical application time for a remineralizing rinse is about 1 min.<sup>4</sup>



According to a study done by Dawes, the concentration of ions in the saliva keeps changing as the time progresses. Hence, in this study, salivary samples were taken at five different time intervals to record the variations in their salivary calcium and phosphorous, buffering capacity and pH.<sup>10</sup>

In this study Recaldent sugar free chewing gum containing CPP-ACP was used. The results showed the mean value of calcium of saliva before chewing CPP-ACP containing gum and over 90mins are 1.71,3.72,2.78,2.06 and 1.71 respectively mg/dL. In our study, we found that increase in mean value of calcium within the first 20 mins after chewing gum is in accordance with a study conducted by Santhosh BP et al who studied effect of casein phosphopeptide-amorphous calcium phosphate containing chewing gum on salivary concentration of calcium and phosphorus and concluded that CPP-ACP containing chewing gum would increase the level of calcium concentration of saliva, thereby supplying calcium to the whole dentition for a prolonged period after chewing the gum for 20 min.<sup>4</sup>

The mean value of phosphorus concentration was 0.86,0.61,0.78, 0.77 and 0.79 mg/dL respectively. In our study, we found that decrease in mean value of phosphorus after 20 mins of chewing gum is in accordance with a study conducted by Chow LC et al; who concluded that unstimulated saliva is well saturated with phosphorus and the concentration of phosphorus falls as the flow rate increases.<sup>11</sup>

Therefore, immediate decrease in the salivary concentration of phosphorus after chewing CPP-ACP containing chewing gum observed could be attributed to the increase in the salivary flow. This observation of the present study is supported by Vogel et al.<sup>3</sup>

Buffering capacity is promoted by carbonate-bicarbonate, phosphate, and proteins systems. The bicarbonate system is responsible for approximately 85% of salivary BC in the pH range from 7.2 to 6.8 in stimulated saliva. The concentration of these ions is higher in saliva collected after mechanical stimulation.<sup>12</sup> In our study, we found that increase in mean value of buffering capacity before and over 90 mins period is in accordance with a study conducted by Machiulskiene V et al. who studied Caries preventive effect of sugar-substituted chewing gum and concluded that buffering capacity can be increased with use of chewing gum.<sup>13</sup>

In our study, we found an increase in mean value of pH before and over 90 minutes period. This is in accordance with a study conducted by Polland et al who concluded that after 90-min gum chewing, the salivary flow was slightly increased and salivary pH was markedly increased above resting levels.<sup>14</sup> The results of this study are in agreement with the previous reports by Kumar S et al. who concluded that consumption of sugar-free chewing gums (xylitol), over a period of time, leads to an increase in the alkaline pH of saliva and plaque from an acidic pH and thereby prevent decay to the teeth.<sup>15</sup>

## Conclusion

CPP-ACP containing chewing gum would increase the level of calcium, salivary pH and buffer capacity, thereby supplying calcium and the phosphorus to the whole dentition for a prolonged period thus increases the remineralisation of tooth surface because of increased degree of super saturation.

## References

1. Humphrey SP, Williamson RT. A review of saliva: Normal composition, flow, and function. *J Prosthet Dent.* 2001 Feb;85(2):162-9.
2. Shen P, Cai F, Nowicki A, Vincent J, Reynolds EC. Remineralization of enamel subsurface lesions by sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. *J Dent Res* 2001;80:2066-70.
3. Vogel GL, Zhang Z, Carey CM, Ly A, Chow LC, Proskin HM. Composition of plaque and saliva following sucrose challenge and use of an  $\alpha$ -tricalcium phosphate containing chewing gum. *J Dent Res* 1998;77:518-24.
4. Santhosh BP, Jethmalani P, Shashibhushan KK, Subba Reddy VV. Effect of casein phosphopeptide-

- amorphous calcium phosphate containing chewing gum on salivary concentration of calcium and phosphorus: an in-vivo study. *J Indian Soc Pedod Prev Dent*. 2012 Apr-Jun;30(2):146-50.
5. Ribelles Llop M, Guinot Jimeno F, Mayne Acien R, Bellet Dalmau LJ. Effects of xylitol chewing gum on salivary flow rate, pH, buffering capacity and presence of *Streptococcus mutans* in saliva. *Eur J Paediatr Dent*. 2010 Mar;11(1):9-14.
  6. Bardow A, Moe D, Nyvad B, Nauntofte B. The buffer capacity and buffer systems of human whole saliva measured without loss of CO<sub>2</sub>. *Arch Oral Biol* 2000; 45:1-12
  7. Gandhi M, Damle SG. Relation of salivary inorganic phosphorus and alkaline phosphatase to the dental caries status in children. *J Indian Soc Pedod Prev Dent*. 2003 Dec;21(4):135-8.
  8. Farooq I, Moheet I, Imran Z, Farooq U. A review of novel dental caries preventive material: Casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) complex. *King saud university journal of dental sciences*.2013;4(2):47-51
  9. Itthagarun A, Wei SH. Chewing gum and saliva in oral health. *J Clin Dent*.1997;8(6):159-62.
  10. Dawes C. The effects of flow rate and duration of stimulation on the concentrations of protein and the main electrolytes in human parotid saliva. *Arch Oral Biol* 1969;14:277-94.
  11. Chow LC, Takagi S, Shern RJ, Chow TH, Takagi KK, Sieck BA. Effects on whole saliva of chewing gums containing calcium phosphates. *J Dent Res*. 1994 Jan;73(1):26-32.
  12. Antunes D P, Marinho RM, Garakis MC , Bresciani E .Buffer Capacity of Saliva as a Function of Time after Consumption of Sugary, Sugar-Free and Probiotic Chewing Gums. *Brazilian Research in Pediatric Dentistry and Integrated Clinic* 2015;15(1):153-161.
  13. Machiulskiene V, Nyvad B, Baelum V. Caries preventive effect of sugar-substituted chewing gum. *Community Dent Oral Epidemiol*. 2001;29(4):278-88.
  14. Polland K E, Higgins F, Orchardson R. Salivary flow rate and pH during prolonged gum chewing in humans. *Journal of Oral Rehabilitation* 2003;30: 861–865.
  15. Kumar S, SogiS S, Indushekar KR. Comparative evaluation of the effects of xylitol and sugar-free chewing gums on salivary and dental plaque pH in children *J Indian Soc Pedod Prev Dent*. 2013;31(4):240-244.