Evaluation of fluoride release from teeth after topical application of NaF, SnF_2 and APF and antimicrobial activity on *mutans streptococci*

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The objectives of this study were to evaluate and compare the amount and pattern of fluoride release from teeth after topical application of 2% NaF, 8% SnF_2 and 1.23% APF at different time intervals. The growth inhibitory effects of this released fluoride ion was assessed on mutans streptococci (MS) and correlated with the fluoride release. Forty premolars divided into four groups were subjected to different topical fluoride treatments. All the teeth were immersed individually in deionized water and were transferred to containers at 1 hour, 1 day and 1 week time intervals. 240 samples in total were used for fluoride estimation by ion selective electrode method and the samples from the other sub-group were used for evaluation of antimicrobial activity on mutans streptococci (MS) by bacterial inhibition assay method. The results showed that the highest fluoride release (7.83±0.55ppm) was seen in SnF_2 treated specimens, as compared to that of NaF (3.71±0.60ppm) and APF (3.30±0.51ppm), the difference being statistically significant ($P<0.01$). This was observed immediately after 1 hour, followed by a drastic reduction thereafter. No zones of inhibition were observed at the released fluoride concentrations at different time intervals in the different groups. In conclusion: 8% SnF_2 is expected to have greater anticaries property from the high fluoride releasing property for prolonged period of time.

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INTRODUCTION

Despite the profusion of rhetoric to the contrary, dental caries is a critical concern even today. It places a large financial, health and time burden most frequently on those least able to bear it. The answer to this major public health problem is prevention, through the use of fluorides.[1]

The current focus of attention in fluoride research is the role of firmly and loosely bound fluoride in caries prevention. Firmly bound fluoride refers to fluoride incorporated in the crystalline lattice of hydroxyapatite, i.e. fluoridated hydroxyapatite (FAP), whereas loosely bound fluoride pertains to fluoride adsorbed to apatite and fluoride leaching from relatively soluble fluoride containing deposits, i.e. calcium fluoride (CaF_2). Fluoride in low concentration, i.e. ambient fluoride released from CaF_2 at enamel-plaque-saliva interface has greater caries preventive effects than firmly bound FAP. CaF_2 acts as a fluoride reservoir on the tooth surface and it is formed only during treatment with high concentration fluoride solutions.[2-5]

There is still debate, as to whether the antimicrobial effects of fluoride do contribute to caries prevention, since fluoride concentrations needed for antimicrobial effects surpass significantly the concentrations needed to reduce the solubility of apatite.[6]

Based on these observations and facts, the current study was undertaken to investigate the fluoride dissolution after topical application and to study the antimicrobial activity of this released fluoride on *mutans streptococci* (MS). The objectives of the present study were:

1. Comparative evaluation of the amount and pattern of enamel bound fluoride ion released after topical

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application of 2% NaF, 8% SnF_2 and 1.23% APF at 1 hour, 1 day and 1 week time intervals (at neutral pH).
2. To study and compare the growth inhibitory effects of this released fluoride ion by bacterial inhibition assay method on *mutans streptococci*.
3. To compare and correlate, the fluoride release and antimicrobial activity of these topical fluorides.

MATERIALS AND METHODS

The study was done in two parts. Analysis of fluoride release from teeth and evaluation of antimicrobial activity on *mutans streptococci*.

Fourty caries-free sound premolars, extracted for orthodontic purposes were washed thoroughly to remove blood, saliva and tissue debris. The anatomic roots of all 40 teeth were coated with nail varnish. The teeth were randomly divided into four groups of 10 each for respective topical fluoride application.

Group I: 2% NaF solution (10 teeth)
Group II: 8% SnF_2 solution (10 teeth)
Group III: 1.23% APF gel (10 teeth)
Group IV: Control/ Baseline – No fluoride application (10 teeth)

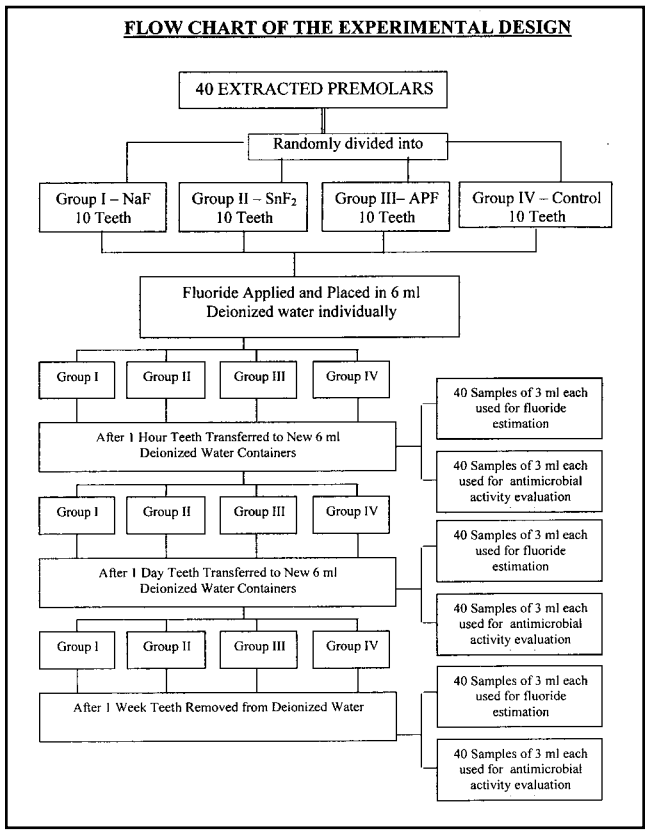
Estimation of fluoride release

After fluoride application, all 40 teeth were immersed individually separate in 40 tightly capped plastic containers containing 6 ml of deionized water in each container at neutral pH. After 1 hour each tooth was removed and transferred to new 6ml deionized water containers. After 1 day each tooth was removed and transferred to new 6ml water containers. After 1 week each tooth was removed from the container. These 120 samples of 6ml each, of 1 hour, 1 day and 1 week time intervals were divided into two subgroups of 3ml each. The 3ml sample of each of these specimens was used for fluoride estimation and remaining 3ml samples were used for evaluation of antimicrobial activity (Total 240 samples). (See flow chart of the experimental design).

Estimation of fluoride release was done using fluoride ion selective electrode method. The fluoride concentrations in all 120 samples were recorded (in ppm) and the values were subjected to statistical analysis.

Evaluation of antimicrobial activity

Mutans streptococci were isolated from saliva on Mitis-Salivarius agar plates. Antimicrobial activity of all 120 sample solutions of different time intervals was studied using bacterial growth inhibitory assay method. Assays of test organisms were done on Mitis-Salivarius agar by lawn culturing the organisms from the pure culture using sterile cotton swabs to obtain a carpet growth. Four wells were prepared on each agar plate using a standard bore. A sample from 4 different groups



of 100µl each was placed in 4 different wells for each group. These culture plates were incubated for 48 hours at 37ºC for the growth to become appreciable. The antimicrobial activity of the sample solutions was assessed by observing the zone of inhibition around each well. The values obtained were subjected to statistical analysis.

RESULTS

Fluoride release

The amount of fluoride ion released from each tooth specimen in ppm after topical fluoride application at 1 hour, 1 day and 1week time intervals is presented in Table 1. Group I: NaF exhibited maximum amount of fluoride release 3.71±0.60 at 1 hour time interval as compared to 0.50±0.05 at 1 day and 0.44±0.02 at 1 week time interval, the difference being statistically highly significant ($P<0.001$) (Table 2). Group II: SnF_2 showed highest amount of fluoride release 7.83 ± 0.55 at 1hour time interval as compared to 0.89 ± 0.09 at 1 day and 0.71±0.06 at 1 week time interval, the difference being statistically highly significant ($P<0.001$) (Table 3). Group III: APF showed maximum amount of fluoride release 3.30±0.51 at 1 hour time interval as compared to 0.72±0.04 at 1 day and 0.50±0.06 at 1 week time interval, the difference being statistically highly significant ($P<0.001$) (Table 4). Group IV: Control also showed a mean fluoride release of 0.50±0.03 at 1 hour time interval, which was greater than 0.44 0.02 at 1 day and 0.43±0.02 at 1 week time interval (Table 5).

TABLE 1. FLUORIDE RELEASE (ppm) FROM TEETH BY VARIOUS GROUPS AT DIFFERENT TIME INTERVALS

SP. NO.	GROUP-I NaF			GROUP-II SnF2			GROUP-III APF			GROUP-IV CONTROL		
	1 HOUR	1 DAY	1 WEEK	1 HOUR	1 DAY	1 WEEK	1 HOUR	1 DAY	1 WEEK	1 HOUR	1 DAY	1 WEEK
1	4.44	0.54	0.46	8.14	0.98	0.68	3.92	0.72	0.56	0.48	0.46	0.46
2	4.10	0.54	0.46	8.04	0.96	0.80	2.84	0.68	0.56	0.46	0.46	0.44
3	2.64	0.44	0.42	7.42	0.82	0.66	2.68	0.68	0.46	0.48	0.40	0.42
4	2.70	0.42	0.40	6.96	0.78	0.64	2.98	0.66	0.46	0.52	0.40	0.40
5	3.68	0.52	0.44	7.92	0.82	0.66	3.48	0.70	0.47	0.54	0.42	0.40
6	4.05	0.54	0.44	8.02	0.98	0.78	3.90	0.78	0.48	0.48	0.44	0.42
7	3.98	0.44	0.44	8.78	0.82	0.68	3.84	0.74	0.52	0.54	0.46	0.46
8	3.58	0.52	0.42	7.00	0.76	0.70	2.88	0.74	0.46	0.50	0.44	0.40
9	3.92	0.52	0.44	7.98	0.96	0.82	2.78	0.70	0.48	0.46	0.46	0.42
10	4.05	0.54	0.46	8.04	0.98	0.70	3.70	0.76	0.56	0.54	0.46	0.44

At 1hour time interval, the highest amount of fluoride release 7.83±0.55 was observed in Group II: SnF_2 as compared to all other groups, which was statistically significant (P<0.01). No statistically significant difference in fluoride release was observed between Group I: NaF (3.71±0.60) and Group III: APF (3.30±0.51). Fluoride release was greater in Group I: NaF and Group III: APF when compared to Group IV: Control, the difference being statistically significant (P<0.01). Least amount of fluoride release 0.50±0.03 was seen in Group IV: Control, as compared to all the other groups (P<0.01) (Table 6).

At 1 day time interval, maximum fluoride release 0.89±0.09 was seen in Group II: SnF_2 as compared to all other groups, the difference being statistically significant (P<0.01). The fluoride release by Group III: APF (0.72±0.04) was greater than Group I: NaF (0.50±0.05) and Group IV: Control (0.44±0.02), but less than Group II: SnF_2, the difference being statistically significant (P<0.01). No statistically significant difference in fluoride release was observed between Group I: NaF and Group IV: Control (Table 7).

At 1 week interval, there was no statistically significant difference when fluoride release was compared between Group I: NaF (0.44±0.02), Group III: APF (0.50±0.06) and Group IV: Control (0.43±0.02). But high amounts of fluoride release continued to occur in Group II: SnF_2 (0.71±0.06), which was statistically significant when compared to Group I: NaF and Group IV: Control (P<0.01) and Group III: APF (P<0.05) (Table 8).

Hence, fluoride release was highest within 1 hour for

Table 2. COMPARISON OF THE AMOUNT AND PATTERN OF FLUORIDE RELEASE (ppm) IN GROUP-I (NaF) AT DIFFERENT TIME INTERVALS

Time Intervals	Mean ± SD	Difference in Reduction	Percentage of Reduction	t-value*	p-value
1 Hour	3.71 ± 0.60	–	–	–	–
1 Day	0.50 ± 0.05	3.21 ± 0.56	86%	18.1	<0.001
1 Week	0.44 ± 0.02	3.27 ± 0.58	88%	17.9	<0.001

* Paired t-test
p<0.001, HS = **Highly significant**
p<0.01, S = **Significant**

Table 3. COMPARISON OF THE AMOUNT AND PATTERN OF FLUORIDE RELEASE (ppm) IN GROUP-II (SnF_2) AT DIFFERENT TIME INTERVALS

Time Intervals	Mean ± SD	Difference in Reduction	Percentage of Reduction	t-value*	p-value
1 Hour	7.83 ± 0.55	-	-	-	-
1 Day	0.89 ± 0.09	6.94 ± 0.51	87%	43.0	<0.001
1 Week	0.71 ± 0.06	7.12 ± 0.54	91%	41.8	<0.001

* Paired t-test
p<0.001, HS = **Highly significant**
p<0.01, S = **Significant**

Table 4. COMPARISON OF THE AMOUNT AND PATTERN OF FLUORIDE RELEASE (ppm) IN GROUP-III (APF) AT DIFFERENT TIME INTERVALS

Time Intervals	Mean ± SD	Difference in Reduction	Percentage of Reduction	t-value*	p-value
1 Hour	3.30 ± 0.51	-	-	-	-
1 Day	0.72 ± 0.04	2.58 ± 0.49	78%	16.8	<0.001
1 Week	0.50 ± 0.06	2.80 ± 0.50	85%	17.9	<0.001

* Paired t-test
p<0.001, HS = **Highly significant**
p<0.0, S = **Significant**

Table 5. COMPARISON OF THE AMOUNT AND PATTERN OF FLUORIDE RELEASE (ppm) IN GROUP-IV (Control) AT DIFFERENT TIME INTERVALS

Time Intervals	Mean ± SD	Difference in Reduction	Percentage of Reduction	t-value*	p-value
1 Hour	0.50 ± 0.03	-	-	-	-
1 Day	0.44 ± 0.02	0.06 ± 0.04	12%	4.3	<0.01
1 Week	0.43 ± 0.02	0.07 ± 0.04	14%	5.7	<0.001

* Paired t-test
p<0.001, HS = Highly significant
p<0.01, S = Significant

Table 6. INTER-GROUP COMPARISON OF THE FLUORIDE RELEASE (ppm) BETWEEN VARIOUS GROUPS AT 1 HOUR TIME INTERVAL

GROUPS	Mean ± SD	Range	DIFFERENCE BETWEEN GROUPS*			
			I	II	III	IV
I NaF	3.71 ± 0.60	2.64 – 4.44	–	<0.01 S	NS	<0.01 S
II SnF_2	7.83 ± 0.55	6.96 – 8.78	–	–	<0.01 S	<0.01 S
III APF	3.30 ± 0.51	2.68 – 3.92	–	–	–	<0.01 S
IV Control	0.50 ± 0.03	0.46 – 0.54	–	–	–	–
ANOVA-F	392.8	P<0.001				

* Newman-Keul's Range Test
S = Significant
NS = Not significant

Table 7. INTER-GROUP COMPARISON OF THE FLUORIDE RELEASE (ppm) BETWEEN VARIOUS GROUPS AT 1 DAY TIME INTERVAL

GROUPS	Mean ± SD	Range	DIFFERENCE BETWEEN GROUPS*			
			I	II	III	IV
I NaF	0.50 ± 0.05	0.42 – 0.54	–	<0.01 S	<0.01 S	NS
II SnF_2	0.89 ± 0.09	0.76 – 0.98	–	–	<0.01 S	<0.01 S
III APF	0.72 ± 0.04	0.66 – .078	–	–	–	<0.01 S
IV Control	0.44 ± 0.02	0.40 – 0.46	–	–	–	–
ANOVA-F	127.6	P<0.001				

* Newman-Keul's Range Test
S = Significant
NS = Not significant

Table 8. INTER-GROUP COMPARISON OF THE FLUORIDE RELEASE (ppm) BETWEEN VARIOUS GROUPS AT 1 WEEK TIME INTERVAL

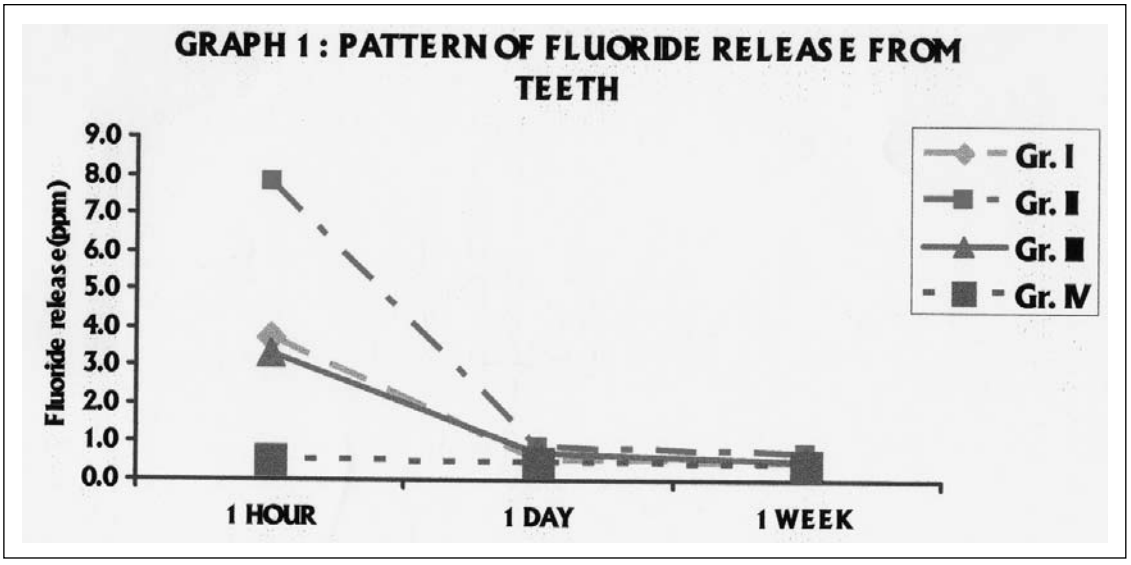
GROUPS	Mean ± SD	Range	DIFFERENCE BETWEEN GROUPS*			
			I	II	III	IV
I NaF	0.44 ± 0.02	0.40 – 0.46	–	<0.01 S	NS	NS
II SnF_2	0.71 ± 0.06	0.64 – 0.82	–	–	<0.05 S	<0.01 S
III APF	0.50 ± 0.06	0.46 – .056	–	–	–	NS
IV Control	0.43 ± 0.02	0.40 – 0.46	–	–	–	–
ANOVA-F	8.46	P<0.001				

* Newman-Keul's Range Test
S = Significant
NS = Not significant

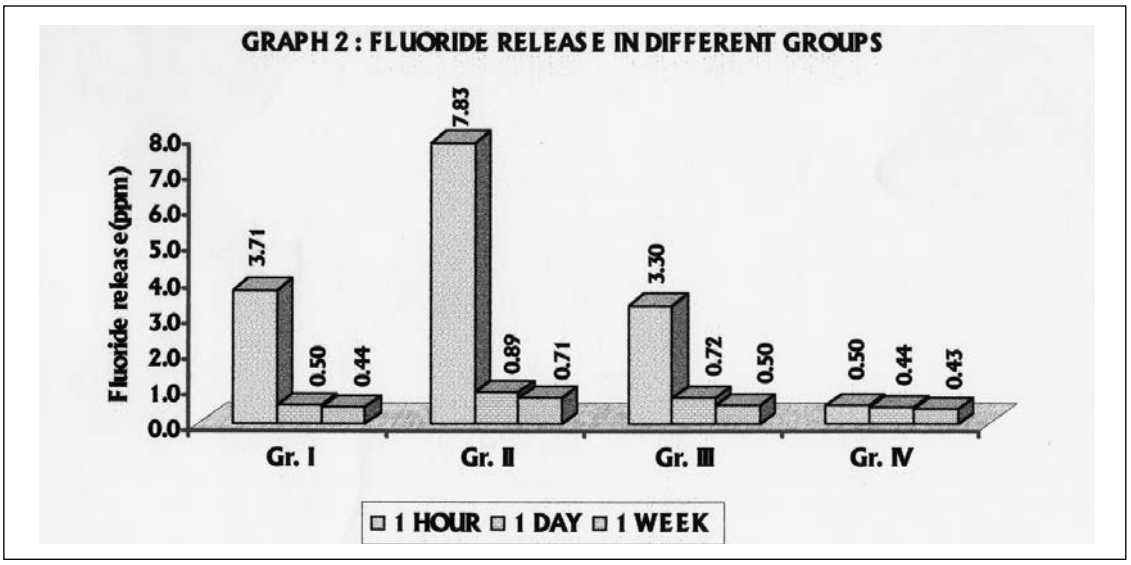
all the groups and reduced drastically on 1st day except for Group IV: Control. The release reduced gradually after 1 week (Graph 1). Highest fluoride release was observed in Group II: SnF_2 when compared to all other groups, at three different time intervals (Graph 2). For all the groups maximum amount of fluoride was released at 1 hour time interval and the release was reduced thereafter (Graph 3).

Antimicrobial activity

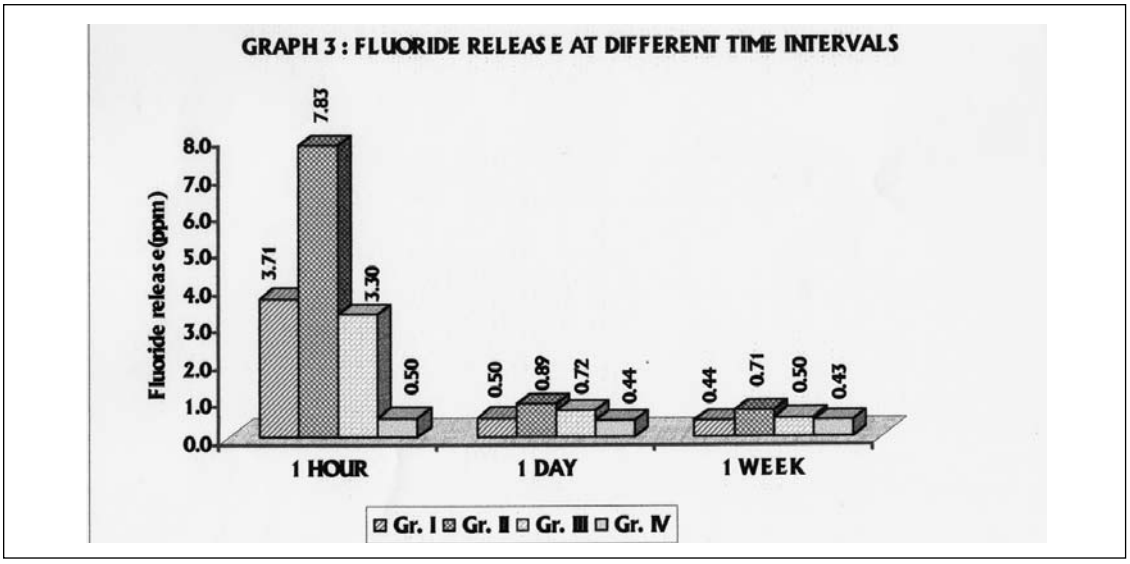
No circular zone of bacterial growth inhibition was observed for all 120 sample solutions of 4 different groups at 3 different time intervals. Hence no direct correlation was observed between fluoride release and the zone of inhibition in the present study (Table 9).



Graph 1. Pattern of fluoride release from teeth



Graph 2. Fluoride release in different groups



Graph 3. Fluoride release in different groups

Table 9. CORRELATION BETWEEN FLUORIDE RELEASE (ppm) AND ZONE OF INHIBITION (mm)

GROUPS	1 HOUR		1 DAY		1 WEEK	
	Fluoride Release (ppm)	Zone of Inhibition (mm)	Fluoride Release (ppm)	Zone of Inhibition (mm)	Fluoride Release (ppm)	Zone of Inhibition (mm)
I – NaF	3.71 ± 0.60	0.00 ± 0.00	0.50 ± 0.05	0.00 ± 0.00	0.44 ± 0.02	0.00 ± 0.00
II – SnF_2	7.83 ± 0.55	0.00 ± 0.00	0.89 ± 0.09	0.00 ± 0.00	0.71 ± 0.06	0.00 ± 0.00
III – APF	3.30 ± 0.51	0.00 ± 0.00	0.72 ± 0.04	0.00 ± 0.00	0.50 ±0.06	0.00 ± 0.00
IV – Control	0.50 ± 0.03	0.00 ±0.00	0.44 ± 0.02	0.00 ± 0.00	0.43 ± 0.02	0.00 ± 0.00

DISCUSSION

A dogma had existed for many decades that fluoride has to be ingested and acts mainly pre-eruptively, however recent studies conclude that caries preventive effects of fluoride are almost exclusively topical.[7] Retention of fluoride in saliva after topical application has become a major field of interest in caries research. The presence of trace quantities of fluoride released from enamel is critical, if the caries process is to be driven in the direction of remineralization. CaF_2, the undesirable reaction product of earlier attempts to incorporate fluoride into enamel in permanently bound state, has become a celebrated cariostatic moiety.[8] Therefore the clinical effects of fluoride are strictly dependent on the methods that deliver the fluoride ion to the surface of the tooth. Fluoride introduced into the oral cavity is cleared with passage of time, hence a continuous supply of fluoride is essential for anti-caries effect.

Fluoride release

The phenomenon of fluoride release is a complex interaction of many factors and involves several phases.[9] Fluoride release after topical fluoride application depends on pH, dose, concentration, duration and frequency of application.[4,5] The dissolution rate of CaF_2 is pH dependent, i.e. the release is increased as pH decreases.[10] This *in vitro* study gives an indication of the cumulative amount of fluoride release possible at neutral pH at different time intervals which is not possible to record *in vivo*, since fluoride is lost during eating and swallowing activities,. Constant pH cannot be maintained and the exposure from other sources of fluoride cannot be controlled. There is also a possible reuptake of released fluoride into the tooth during acid challenge.[9]

At 1 hour time interval, highest amount of fluoride release ranging from 6.96 to 8.78ppm was observed in Group II: SnF_2 which was significantly higher than NaF (2.64 to 4.44) and APF (2.68 to 3.92) treated specimens ($p<0.01$)(Table 6). This is because of the high fluoride concentration in 8% SnF_2 solution (19400 ppmF-).[11] These findings are similar to that of Skartveit *et al.*[12] where they have stated that higher release from SnF_2 treated specimens is due to high uptake of fluoride. At 1 day time interval, there was a drastic reduction in fluoride release in all the groups except in the control group. In the present study the reduction in fluoride release was 86%, 87% and 78% in NaF, SnF_2 and APF treated specimens respectively, whereas only 12% reduction occurred in the control group (Tables 2 to 5). At 1 week time interval, the fluoride release almost reached baseline values (except in Group II: SnF_2)(Table 8).

These values represent the fluoride release from each tooth specimen, thus fluoride release after topical application in oral cavity will be significantly higher. The release will be still greater during acid challenge.[10] The above observations clearly show that 8% Stannous fluoride had highest fluoride release when compared to 2% NaF and 1.23% APF gel at three different time intervals. Further, it is expected that the dynamics of release and re-uptake will continue to occur for prolonged period of time.

Antimicrobial activity

Antimicrobial activity of released fluoride by all the groups at three different time intervals was assessed on *mutans streptococci*, using diffusion technique, because the released fluoride can exert an antimicrobial activity by direct contact with the microorganisms through diffusion.

In the present study, there was no direct correlation between fluoride release and antimicrobial activity. Since no zone of inhibition was observed in all the groups at three different time intervals (Table.9). The lack of correlation between fluoride release and zone of inhibition can be attributed to the following reasons. a) Fluoride at the released concentrations, might have played a role only in inhibition of acid production and not in growth inhibition of *mutans streptococci*[6] b) The amount of fluoride release that occurred may not be sufficient to produce growth inhibition because various studies have shown that the minimum fluoride required for prevention of *mutans streptococci* growth range from 20 to 300ppm.[6,13,14] In this study, the highest fluoride release observed was 8.78ppm, which was very far from the minimum fluoride required to produce growth inhibition. c) Fluoride may be released as a complex salt and not in ionic form, which is required for growth inhibition. d) The normal constituents used for the preparation of bacterial culture media such as inorganic salts, amino acids and proteins may interact with fluoride and modify the antimicrobial property.

Similar findings were observed by Yap *et al.*[9] and Fischman and Tinanoff[15] where they have found no correlation between fluoride release and antimicrobial property. The zones of inhibition produced restorative materials may be due to combination of ZnO and fluoride, low pH and other chemicals liberated from these materials.[9,15,16]

According to Featherstone[3] fluoride works primarily via topical mechanisms by a) reducing demineralization in acidic conditions, b) enhancing remineralization and c) inhibiting bacterial metabolism. But it is not clear to what extent antimicrobial activity can contribute to caries prevention, as fluoride concentrations needed for the anti-microbial effects significantly surpass the concentration needed to reduce the solubility of apatite. There is no doubt that fluoride has a subtle antimicrobial effect as it reduces acid production. But much is yet to be known about the interaction of fluo-

ride with the plaque microflora. Not appreciating the importance of antimicrobial activity might be an underestimation, if overshadowed by the influence of fluoride on remineralization and demineralization.[2,6,8]

Fluoride in the next century…

Since a sizeable group of young children still suffer from high prevalence and incidence of dental caries, emphasis should now be placed on targeting these high risk individuals, who can receive maximum benefit from topical fluoride application, at an acceptable cost without significant harm.[11] Further research on the mechanism of fluoride release from teeth and assessment of the antimicrobial activity will help to gain an insight into the complete understanding of the critical role of fluoride in caries prevention.

CONCLUSIONS

The following conclusions were drawn from this study:

1. Highest amount of fluoride release occurred immediately within 1 hour in all the groups, except in control group, followed by drastic reduction at 1 day and gradual reduction thereafter at 1 week time interval.
2. Highest fluoride release was observed in SnF_2 treated specimens when compared to NaF and APF at all the three different time intervals. Thus, 8% SnF_2 is expected to have greater anticaries potential than 2% NaF and 1.23% APF gel, due to its high fluoride releasing property for prolonged period of time.
3. No zone of inhibition of bacterial growth was observed on the 120 sample solutions of 4 different groups at 3 different time intervals. Although fluoride has antimicrobial activity, no direct correlation was observed between fluoride release and zone of inhibition at less than 7.83±0.55 ppm in the present study.

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