# COMPARATIVE EVALUATION OF AMINE FLUORIDE, CALCIUM SODIUM PHOSPHOSILICATE (NOVAMIN), NANO HYDROXY APATITE CONTAINING DENTRIFICES ON ENAMEL MICROHARDNESS: AN IN VITRO STUDY

Original Article

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## ABSTRACT

**Aim:** To evaluate and compare the microhardness of enamel surface after the application of fluoride, novamin, and hydroxyapatite containing dentifrices. **Materials and Methods:** Twenty freshly extracted premolars were collected and decoronation of all the teeth was done at cementoenamel junction. The crowns were sectioned mesiodistally into two halves with the help of diamond disc, and then the subsequent forty samples kept in 1% citric acid for the demineralization in an incubator at 350C temperature for 72 hours. The samples were randomly divided into four groups of 10 samples in each group, that is, Group A(Control Group), Group B (sodium fluoride dentifrice), Group C(Novamin dentifrices) and Group D(Hydroxyapatite dentifrices). Groups B, C & D were treated using prepared dentifrices slurries for 3 min daily, twice for 7 days at room temperature. The samples were preserved in artificial saliva in between treatment. The enamel surface microhardness was evaluated using Vickers hardness test at base level, after demineralization, as well as after remineralization. Statistical analysis of surface microhardness obtained at different stages was done by Student's t -test and $P < 0.05$ was considered statistically significant. **Result:** Group B C & D showed significant increase in microhardness as compare to Group A. **Conclusion:** nHAP based dentifrices showed greater increase in microhardness as compare to other groups.

**Keywords:** Amine fluoride, Demineralization, Dentifrices, Microhardness, Remineralization, Novamin, Nano hydroxyapatite, Artificial saliva, Citric acid.

**Received:** 02-05-2017; **Review Completed:** 05-09-2017; **Accepted:** 08-01-2018

## INTRODUCTION:

Regardless of the age, gender and ethnicity - dental caries, affects a major portion of the world's population. Dental caries is "an infectious microbiological disease of the teeth that results in localized dissolution and destruction of calcified tissues." It is caused due to physiological imbalance between oral microflora and pathological factors. The occurrence of caries is pH dependent. When pH drops below5.5, enamel dissolution starts, embarking demineralization.[1-2] Silverstone (1977) defined demineralization as the process of removing minerals, in the form of mineral ions, from dental enamel. Formation of bacterial acids lowers the pH to the point where the hydroxyapatite mineral of enamel dissolves and this mineral loss lead to cavitation in future. The immediate fluid environment involved in demineralization of a tooth is the fluid phase of plaque i.e. 'plaque fluid' and not the saliva. Neutralizing the oral pH opposes the process of demineralization. By definition, remineralization is the process whereby partially demineralized enamel is repaired through the recrystallization of tooth enamel mineral salts. Remineralization represents an important natural mechanism of the oral environment in its defense, against caries. From a clinical standpoint, remineralization has been associated with the arrest of developing caries and the reversal of clinically diagnosed incipient white spots. Carious lesion remineralization occurs when there is supersaturation of saliva with respect to calcium phosphate mineral phases.[26]

Numerous mechanisms are available for accelerated remineralization. It involves a delivery mechanism of ions to the affected area.[1] Various remineralizing agents like fluoride, potassium nitrate, Casein phosphopeptide stabilized amorphous calcium phosphate, unstabilized ACP, CPP stabilized amorphous calcium phosphate with fluoride are available.

The discovery of remineralizing agents such as fluorides is a boon for the field of dentistry. There are various types of INORGANIC FLUORIDE preparations available such as stannous fluoride, acidulated phosphate fluoride, sodium mono-fluorophosphate and sodium fluoride.1 Muhlemann in his study concluded that ORGANIC FLUORIDES such as amino fluoride(organic fluoride) have shown significant superior results than that of inorganic fluorides in decreasing the solubility of enamel.[3] The unique position of amine

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fluorides is based on their special molecular structure consisting of a hydrophilic (water soluble) and fluoride binding amine group attached to a hydrophobic (water insoluble) hydrocarbon chain.[4] Due to their surface activity, amine fluorides are rapidly dispersed in the oral cavity and wet all surfaces , thereby covering teeth with a homogeneous molecular layer and transporting fluoride ions to appropriate sites. [4]

Numerous other mechanisms are also available for accelerated remineralization of tooth structure besides fluoride such as bioactive glass, novamin, arginin, nano hydroxyapaptite, theobromine, self-assembling peptides, inotophoresis and sugar alcohol.

Recently, bioactive glass materials and nanohydroxyapatite have been introduced in many fields of dentistry. NovaMin (calcium sodium phosphosilicate bioactive glass.) is a known component made of bioactive glass particulates with a median size of less than 20 microns.[6-8]

**Nano-hydroxyapatite (n-HAp)** is considered one of the most biocompatible and bioactive materials, and has gained wide acceptance in medicine and dentistry in recent years. Synthetic nano-hydroxyapatite (n-HAP) has the same chemical-physical properties as the apatite structure within enamel. These products are envisaged to promote remineralization due to size-specific effects of the apatite nano-particles corresponding to the ultrastructure of the enamel.

- This study sought to test two hypotheses.

1. The first hypothesis was that each of the three dentifrices would **promote enamel remineralization** that is significantly greater than zero.

2. The second hypothesis was that the three dentifrices differ with respect to post-treatment remineralization. Of special interest was whether the **nHAP**-based dentifrices promote greater enamel remineralization and enamel microhardness relative to the fluoride dentifrice and novamin based.

Demineralization and remineralization have a crucial impact on the hardness and strength of tooth enamel. Thus, here we have done comparative evaluation of amine fluoride, calcium sodium phosphosilicate (novamin), nanohydroxy apatite containg dentrifices on enamel microhardness.

## MATERIALS AND METHOD

For this prospective study, 20 caries free, freshly extracted premolars were collected. Carious teeth were excluded from samples. The sample size was selected according to systematic random sampling method and ISO 24153:2009 standards.[9] Prophylaxis of all the samples were done using ultrasonic scalers and were polished with an abrasive paste (Smile and Shine, ICPA) and brush. Decoronation of all the teeth was done at cementoenamel junction, and the crowns were sectioned mesiodistally into two halves with the help of diamond disc. Then, the subsequent forty samples distributed in four groups by simple randomization, that is, Group A, Group B, Group C and Group D with 10 samples each.

- Group A (Control group)
- Group B (Sodium fluoride dentifrice)
- Group C (Calcium sodium phosphosilicate bioactiveglass dentifrices)
- Group D (Hydroxyapatite dentifrices).

The samples were placed in acrylic resin filled molds facing buccal surface upwards. A Vickers microhardness indenter (fuel instruments and engineer pvt ltd ) used to check the baseline microhardness under the load of 100 g, which was applied for 15 s at three different positions, each was 1 mm apart, and the mean was calculated.Samples were immersed in 1% citric acid (20 ml demineralizing solution) and stored in an incubator at 35°C temperature for 72 h for demineralization. After demineralization, once again, surface microhardness was calculated using the Vickers hardness indenter.

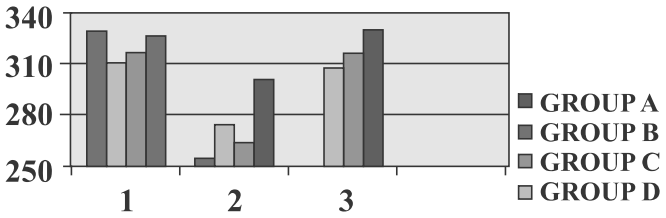
The samples of Group B, Group C and Group D were treated with prepared dentifrices slurries, i.e., Group B with sodium fluoride dentifrice slurry (Amflor™ toothpaste, Group pharmaceuticals) Group C with Calcium sodium phosphosilicate bioactiveglass dentifrices (Novamin) and Group D with Hydroxyapatite dentifrices( Aclaim) using same protocol, that is, 3 min, daily twice for 7 days. The samples were kept in artificial saliva (ICPA, Mumbai, Maharashtra, India) in between treatment. After the treatment, the enamel surface microhardness in all three groups evaluated with the help of Vickers hardness indenter and a comparative

analysis done. Statistical analysis of surface microhardness obtained at different stages done by Student's t-test and $P < 0.05$ was considered statistically significant.

## RESULTS:

| | | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Min | Max. | ANOVA P VALUE |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound | | | |
| Base line | CONTROL A | 10 | 328.30 | 36.11 | 11.419 | 302.47 | 354.13 | 287.00 | 387.00 | 0.383 |
| | B | 10 | 310.40 | 15.44 | 4.881 | 299.36 | 321.44 | 290.00 | 333.00 | |
| | C | 10 | 316.80 | 26.87 | 8.498 | 297.58 | 336.02 | 251.00 | 345.00 | |
| | D | 10 | 325.30 | 16.41 | 5.190 | 313.56 | 337.04 | 300.00 | 355.00 | |
| | Total | 40 | 320.20 | 25.22 | 3.987 | 312.14 | 328.26 | 251.00 | 387.00 | |
| After demineralization | CONTROL A | 10 | 253.70 | 67.84 | 21.453 | 205.17 | 302.23 | 158.00 | 351.00 | 0.069 |
| | B | 10 | 274.80 | 25.42 | 8.037 | 256.62 | 292.98 | 221.00 | 312.00 | |
| | C | 10 | 263.80 | 31.79 | 10.053 | 241.06 | 286.54 | 212.00 | 300.00 | |
| | D | 10 | 301.40 | 17.62 | 5.572 | 288.79 | 314.01 | 280.00 | 335.00 | |
| | Total | 40 | 273.43 | 42.90 | 6.784 | 259.70 | 287.15 | 158.00 | 351.00 | |
| After remineralization | CONTROL A | 0 | . | . | . | . | . | . | . | 0.022 |
| | B | 10 | 307.50 | 14.07 | 4.450 | 297.43 | 317.57 | 280.00 | 330.00 | |
| | C | 10 | 316.00 | 22.12 | 6.994 | 300.18 | 331.82 | 268.00 | 343.00 | |
| | D | 10 | 329.80 | 13.14 | 4.155 | 320.40 | 339.20 | 315.00 | 349.00 | |
| | Total | 30 | 317.77 | 18.82 | 3.436 | 310.74 | 324.79 | 268.00 | 349.00 | |

Vickers hardness values of the samples obtained at the baseline were in the range of 310.40- 328.30 VHN. After demineralization, mean microhardness in Group A decreased to 253.70 VHN, in Group B 274.80 VHN, in Group C 263.80 VHN and in Group D 301.40. After remineralization, mean microhardness in Group B increased to 307.50 VHN ,Group C increased to 316.00 VHN. And Group D increased to 329.80 VHN.




1. BASELINE
2. AFTER DEMINERALIZATION
3. AFTER REMINERALIZATION

**DISCUSSION** Early enamel caries can histologically be described as a subsurface carious lesion of enamel. Prominent feature being a subsurface demineralized zone with intact and unscathed enamel surface. Even though surface is intact, the mineral content is deficient. Hence, there is a lower microhardness of early enamel caries as opposed to sound enamel.[15] The battle to keep teeth strong and healthy is dependent upon the ratio between demineralization and remineralization. It is observed that the balance between demineralization and remineralization influences dental caries initiation and reversal.

This balance is dependent on factors such as presence of calcium, phosphate, and fluoride in saliva, as well as pH of saliva.[10]

During demineralization, Ca2+, OH−, PO42−, F−, CO3−, Na+ and Mg2+ get displaced from the enamel surface to the exterior. More the acidic environment, greater is the outward flow of ions. However, mineral content of surface is higher than the body of the lesion.[15] The demineralization process can be stopped by creating an environment conducive for remineralization by various remineralizing agents. The process of restoring lost mineral ions to the tooth structure and strengthening the lattice work is known as remineralization.[16] Remineralization of enamel occurs when the alkaline pH increases.[14]

Different fluoride combinations may show different results in prevention of caries. Dentifrices and mouthwashes are very popular in the world. The use of dentifrices as therapeutic agents is well accepted in dentistry.[17] Chaudhary *et al*. in their study observed that dentifrices can help enamel against erosion and caries by increasing the enamel microhardness with remineralizing effect.[21]

Here we have used amine fluoride which is organic fluoride which has showed promising results in studies done by Sh *et al*. who has evaluated effect of amine fluoride and sodium fluoride mouthwashes on enamel microhardness and they concluded that amine fluoride increased enamel microhardness more than sodium fluoride.[22] Galuscan *et al*. in their study observed that amine fluoride helps to promote the remineralization of initial lesions by releasing high quantities of fluoride molecules during the acid attack.[23]

Chronic exposure to low levels of fluoride can cause gastrointestinal, urogenital, and respiratory problems in normal individuals. On the other hand, the prevalence of dental fluorosis has increased noticeably in non-fluoridated areas and to a lesser extent in optimally fluoridated areas. Moreover, fluoride ions alone cannot completely remineralize carious lesions. Formation of each fluorapatite molecule requires calcium and phosphate in addition to fluoride ions. Thus, it is imperative to find an efficient, safe alternative to fluoride to completely prevent caries and remineralize the incipient enamel lesions.[27]

NovaMin comes in contact with saliva or any aqueous media, its active ingredient, inorganic chemical calcium sodium phosphor silicate, binds to the tooth surface in order to initiate the remineralization process on the tooth enamel. This is performed by providing silica, calcium, phosphorous and sodium ions to the tooth structure.[11-12]

Synthetic NHA has the same physicochemical properties as those of apatite in the enamel. It shows strong affinity to the tooth and can strongly adsorb on enamel surfaces. nanoHA has the potential to directly fill up defects and micropores on demineralized teeth. Once, nano-HA penetrates the enamel pores, nano-HA will act as a template in the precipitation process and will continuously attract a large amount of Ca2+ and PO3− 4 to the enamel surface to fill the vacant positions of the enamel calcium crystals. This in turn will promote crystal integrity and growth.

White et al. in their study evaluated different methods for enamel demineralization and remineralization such as direct methods and indirect methods. In the present study, indirect method, i.e., microhardness measurement method is used because it can measure changes in the physical parameters such as surface structural

strength, and also it is simple, fast and least destructive method to determine demineralization and remineralization.[18-19]

There are different types of microhardness tests which include Knoop, Vickers, and Brinnel. Darshan and Shashikiran in their study observed that even small changes can be detected easily, by the square shape indent obtained after Vickers hardness test. Hence, in the current study, Vickers hardness test was selected for investigation over Knoop and Brinnel test.[20]

The results of the current study showed significant increase in mean microhardness after Nano hydroxyapatite (Aclaim) remineralization than amine sodium fluoride (Amflor) remineralization and Calcium sodium phosphosilicate glass (Novamin) and it was statistically significant. These results are in accordance with study done in 2007 by K. OHTA et al who has observed that mineral density to demineralized surface can be increased by treatment with nano HAP. In 2013 Shreya S et al observed HAP showed definite potential as an effective remineralizing agent when compared to potassium nitrate and sodium monofluorophosphate.[25]

In these study significant increase in mean microhardness was greater in novamin group as compared to the fluoride group. These results are in accordance with study done in In 2012 M. Vahid et al in their study observed that NovaMin dentifrice appears to have a greater effect on remineralization of carious-like lesions when compared to that of fluoride containing dentifrice in permanent teeth.24 in 2010 sharma N et al has observed that Comparison of the potassium nitrate and stannous fluoride formulations, the dentifrice containing NovaMin provided more substantial and significant improvements in enamel microhardness.[28]

## CONCLUSION:

Demineralization of the tooth is a condition that is affected by diet, bacteria and limited use of protective agents found in fluoride, salivary buffers and antimicrobial agents. With clearer understanding of the implementation of these effective agents and new technologies accessible to dentists, we can create a more favorable relationship in which remineralization occurs more often than demineralization.

Under the limitation of this study we can conclude that NOVAMIN (calcium sodium phosphosilicate bioactive glass.) and aclaim tooth paste (Nano hydroxyapatite crystals) were more effective in restoring enamel microhardness than fluoride contaiong tooth paste (Amflor). And between NOVAMIN (calcium sodium phosphosilicate bioactive glass.) and aclaim tooth paste (Nano hydroxyapatite crystals) aclaims tootpaste shows more effctive results.

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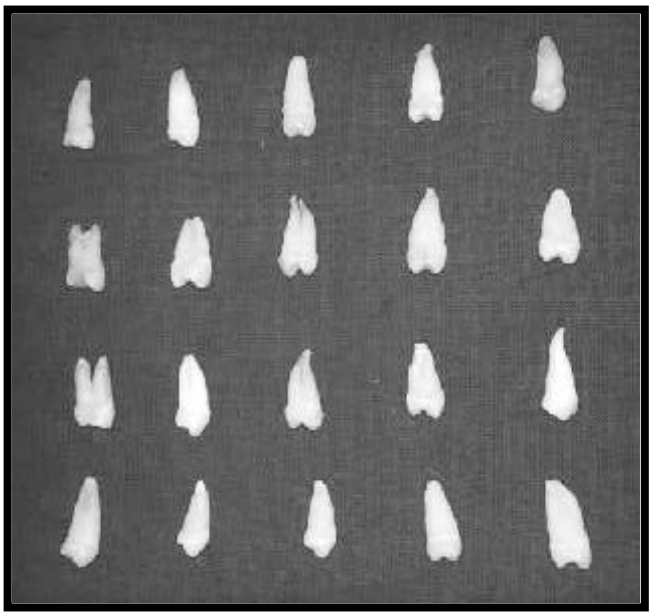
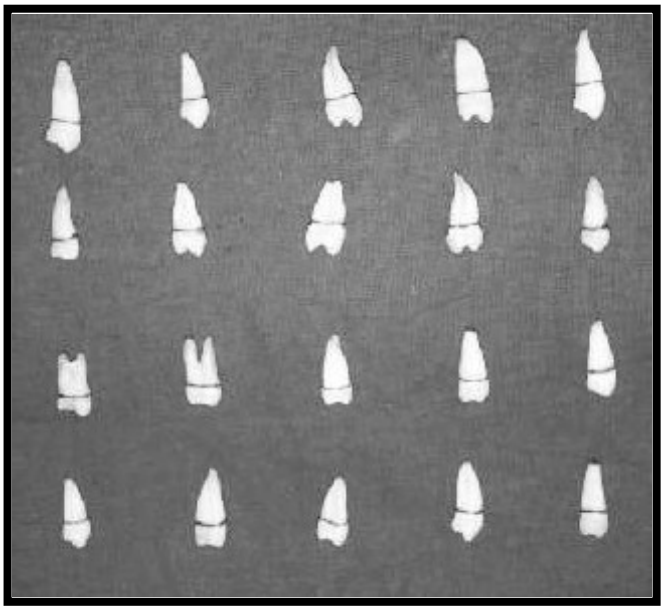
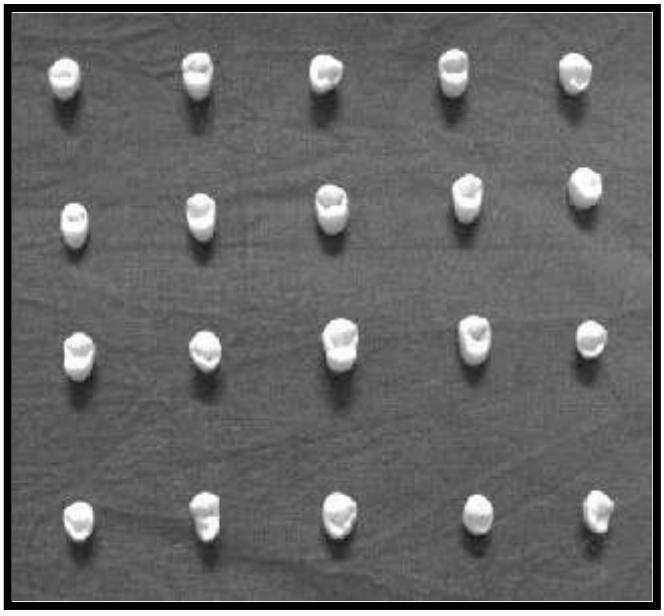


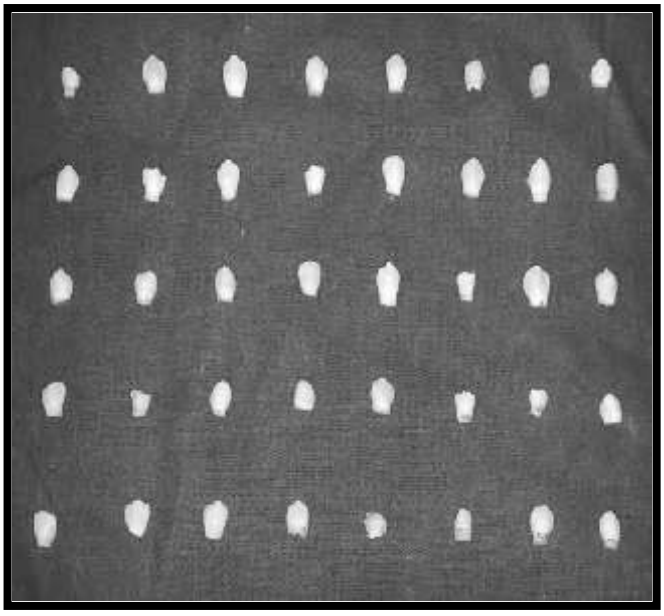
SINGLE ROOTED NATURAL PREMOLARS



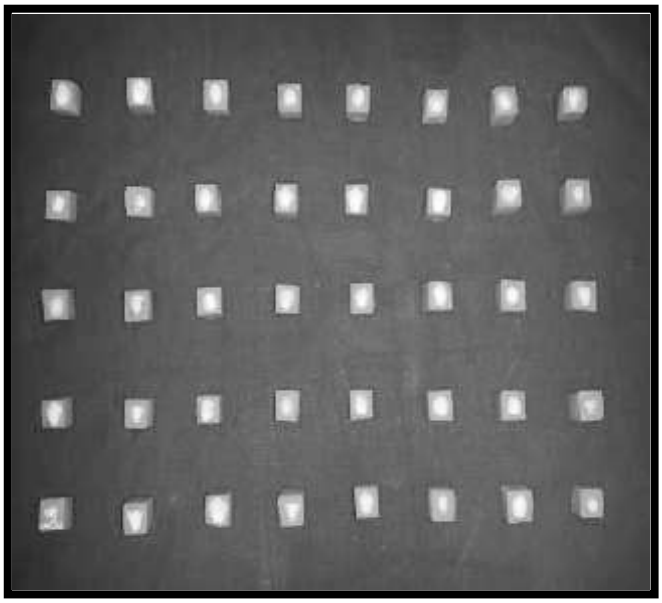
DEMARCATION LINE FOR DECORONATION



DECORONATED CROWNS



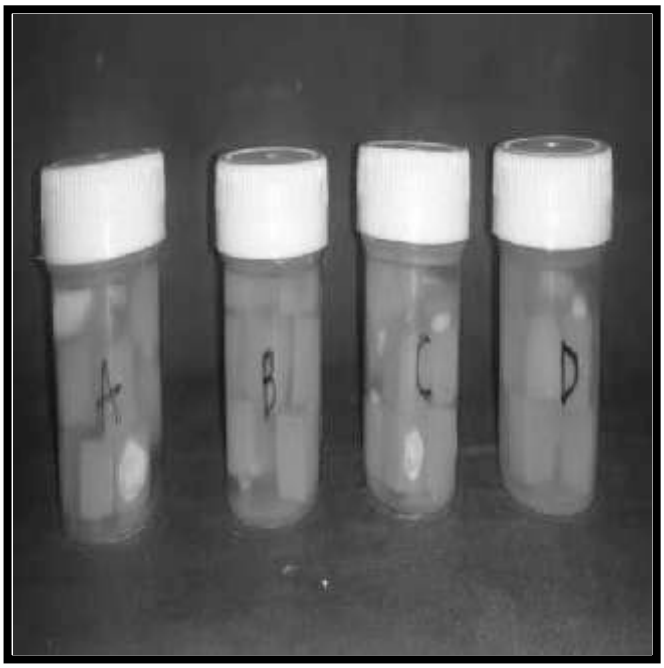
MESIO-DISTAL SECTION OF CROWN



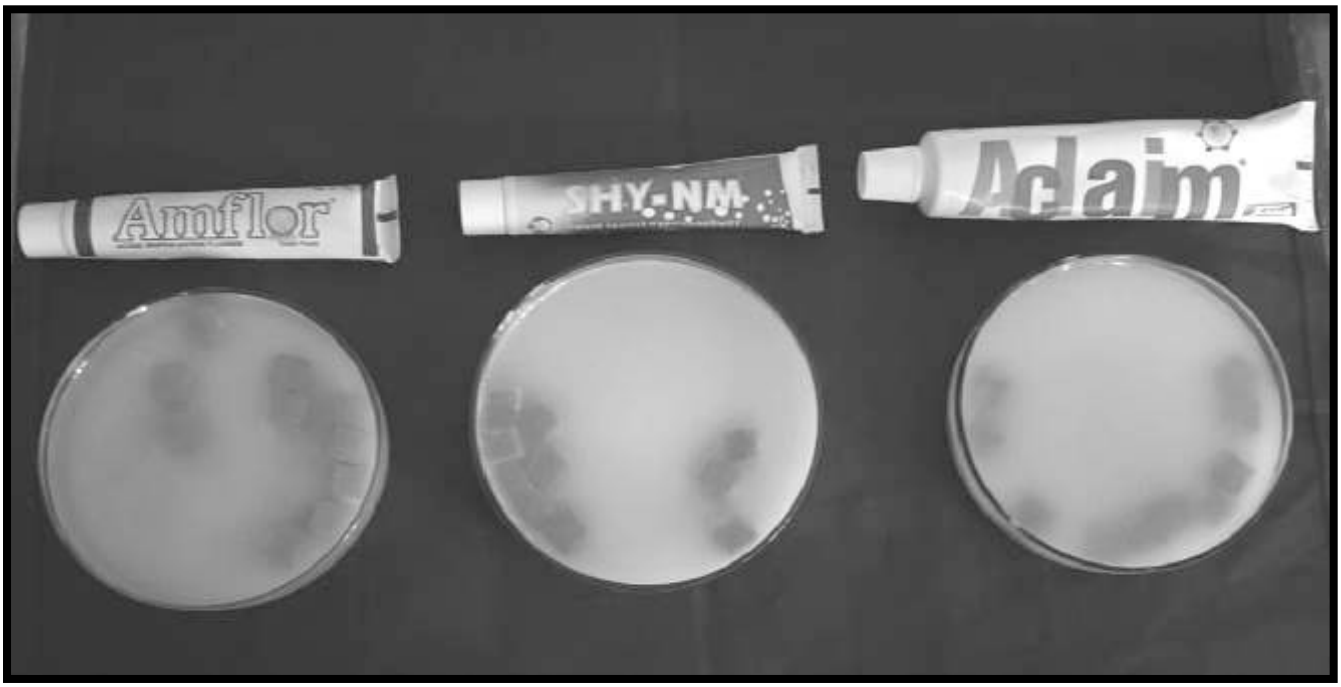
SAMPLE EMBEDDED ACRYLIC BLOCKS


SAMPLES IMMESED IN 1%CITRIC ACID

MICROHARDNESS TESTING




4 GROUPS




**SAMPLES EMBEDDED IN THE REMINERALIZING SOLUTION**


**ARTIFICIAL SALIVA**