# FLUORIDE-CONTAINING ANTI-CARIES pH-SENSITIVE RELEASE SYSTEM AND ITS EFFECT ON STREPTOCOCCUS MUTANS

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**ABSTRACT: The aim of this study was to evaluate the antimicrobial effects of a newly developed anti-caries pH-sensitive controlled release system against Streptococcus mutans. For this purpose 9, 30, and 45 mg of NaF were placed in multilayer polymeric structures consisting of three layers: poly-L-lactide (PLLA), 8% Eudragit E 100 + Eudragit RS 100, and Chitosan. The amount of fluoride ion (F) released was determined with an ionmeter. In order to assess the effect of this antimicrobial material releasing NaF in a controlled manner on *S. mutans*, the proliferation of this organism was recorded by preparing test discs with a standard surface area containing the active ingredient. In all the groups the F release began when the pH fell under 5.5, after 6 hr, and the number of *S.mutans* declined. In conclusion, the newly developed controlled release system can release intraorally the fluoride ion with its anti-caries properties when the pH falls below 5.5 and it may help significantly to improve caries prevention.**

Key words: Anti-caries effect of F; pH-sensitive intraoral F release; Streptococcus mutans.

## INTRODUCTION

In general, dental caries and periodontal diseases are included in the group of "dental plaque diseases." Although the implementation of common preventive approaches has decreased the incidence of dental caries, especially in the developed countries, this condition remains the most common infectious disease worldwide.[1-3]

Dental caries is a progressive disease caused by multiple factors that allow for the colonization of pathogenic microorganisms in the mouth, which is characterized by dental plaque accumulation. These factors include diet, the presence of a suitable dental substrate for the pathogens to colonize, and time.[3] Preventative measures and research focused on avoiding dental caries are all based on disrupting one or more of these mechanisms of caries formation. Understanding the process and dynamics of dental caries formation is critical to developing new preventative techniques.[2]

Dental plaque accumulation, the basic cause of dental caries, is a dynamic and complex process that begins when salivary proteins stick to the teeth, forming a pellicle. Plaque maturation then occurs as different combinations of about 300 types of microorganisms, an oral ecosystem, bind to pellicle proteins through adhesion and coadhesion. Thus, anti-caries methods focus on preventing the

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adhesion of microorganisms to teeth and disrupting their nutritional environment.[2,3]

As in all ecosystems, bacteria living in the dental plaque are influenced by the nutritional conditions and surrounding oral environment, and they develop various defense mechanisms that result in the dominance of certain organisms. One such organism, Streptococcus mutans, is identified as the primary source of caries formation and progression. This pathogen, which shows both acidogenic and aciduric characteristics, reduces plaque and salivary pH by fermenting dietary sucrose, causing demineralization of the inorganic components of the tooth.[2,3] In other words, a decrease in the oral pH contributes to both caries formation and the dominance of the pathogenic *S. mutans* group. Thus, intraoral placement of an anti-caries agent installed on a pH decrease-sensitive drug carrier system could aid in caries prevention.[3]

Fluoride has been the most widely studied anti-caries material since the 1930s due to its antimicrobial effect and ability to create protective changes on the tooth surface.[4,5] Nowadays, it is commonly found in over-the-counter toothpastes and mouthwashes, as well as topical professional preparations.[5] Controlled release fluoride tablets, such as 0.25 mg chewable tablets, have also been successfully used since the 1980s in children with high caries activity.[4,5] During the day, acid attacks occur in the mouth when pathogens ferment ingested sugary foods, resulting in the demineralization of tooth structure and the development of dental caries. The fluoride tablets continuously release fluoride, but are not ideal because the amount released does not depend on the environmental conditions.[6-8]

Although sustained release fluoride systems have been implemented as a successful preventative approach, the preparation of a pH-sensitive controlled release system (CRS) carrying traditional anti-caries agents like fluoride would help avoid the negative impact of certain oral environmental conditions. The system could adhere to the mucosa and release fluoride into a medium only when the pH is 5.5 or less.

Eudragit products represent one such potential biocompatible release system because they are pH-sensitive and known to be capable of pH-sensitive controlled drug release. Eudragit polymers are available with many different forms and characteristics. Eudragit E 100 (poly(butylmethacrylate-co-2-dimethylaminoethyl methacrylate-co-methyl methacrylate) 1:2:1) is a terpolymer which is positively charged at low pHs and is used as a pH responsive drug delivery system. It serves both to coat the active substances to protect them from light, humidity, and other environmental factors and also to release the contents of the tablet at low pHs where it is ionized. In 2007, Souza et al.[9] created microgranules by using Eudragit E100 as a drug delivery system. Another study[10] reported that Eudragit RS100 (poly(methacrylic acid-co-methyl methacrylate) 1:2), a pH responsive copolymer due to the negative charge it has at high pHs, has high mucoadhesive characteristics, making it a viable option for drug delivery in the mouth. The development of a preparation utilizing this material, which would release an anti-caries agent when the oral pH is below a critical value (pH 5.5), would be an important step in caries prevention.

The purpose of this study was to develop anti-caries preparations comprising fluoride microgranules in the mucoadhesive gel carrier using Eudragit E100 and Eudragit RS100 that could adhere to the mucosa. Additional objectives were as follows:

(i) At the 1st stage, to determine the release level of fluoride when the pH decreased and (ii) At the 2nd stage, to assess the antimicrobial effects of the developed preparations against *S. mutans* in a microbial culture medium.

## MATERIALS AND METHODS:

Sodium fluoride (NaF) was placed in multilayer polymeric structures consisting of three layers bound together through heat application. The system was designed as a multilayer polymeric structure in order to ensure the following: (i) the drug was applied directly to the target area, (ii) the drug concentration was retained at a constant level through its long term, slow, pH-controlled release, and (iii) the bioactive material was distributed homogeneously. The first layer was prepared using 4% poly-L-lactide (PLLA), the second layer was prepared using 8% Eudragit E 100 + Eudragit RS100 + the bioactive agent (NaF), and the last mucoadhesive layer was prepared using 1.5% (w/v) Chitosan (Figures 1 and 2).

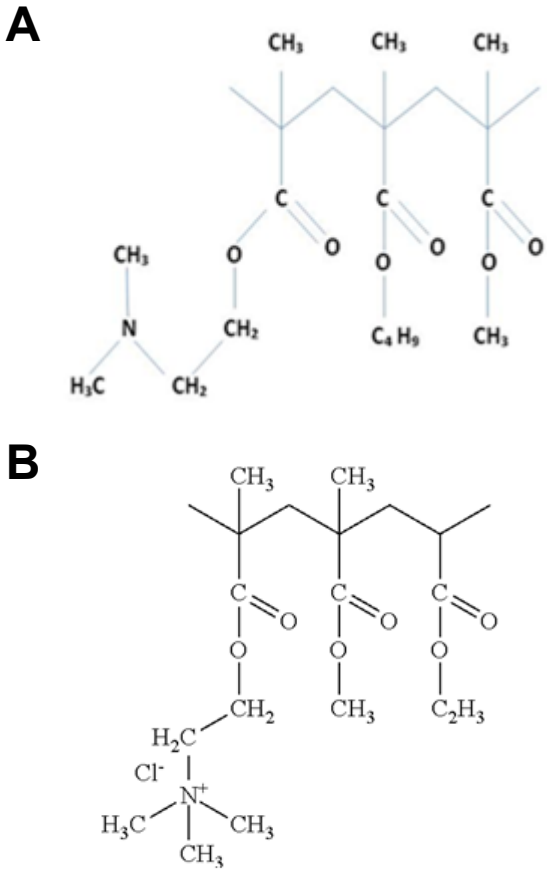


**Figure 1.** Chemical appearances of:
(A) Eudragit E100 and
(B) Eudragit RS100.

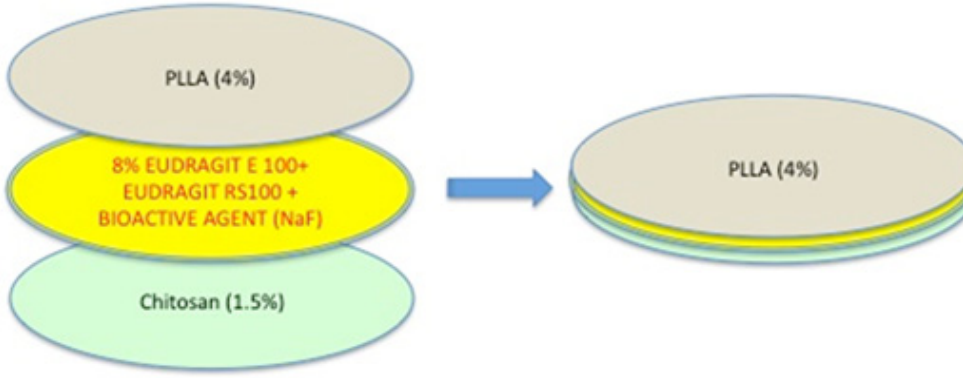


**Figure 2.** The structure of the 3-layer device with a first layer prepared using 4% poly-L-lactide (PLLA), a second layer prepared using 8% Eudragit E 100 + Eudragit RS100 + the bioactive agent (NaF), and the last mucoadhesive layer prepared using 1.5% (w/v) Chitosan.

The three layered film, 1.5 cm in diameter, was placed into the ultrafiltration cell system which was immersed in 10 mL of ultra pure water at room temperature. The medium was mixed with a magnetic stirrer and the top covered with parafilm to prevent evaporation. The pH of the medium was adjusted to 7 and then gradually reduced to 5.5 using 0.1 M HCl (0.25 hourly) for 6 hr. After 6 hr, when the pH fell below 5.5, the fluoride release increased rapidly. The fluoride ion concentration was determined with an ion meter (Orion, USA) specific for fluoride via direct potentiometry. Direct potentiometry is the most common analytical method for determining ion concentration. This method relies on the Nernst equation, which governs the potential difference across an ion specific membrane and a reference electrode.

In order to assess the effect of the developed antimicrobial controlled release system on *S. mutans*, the proliferation of this organism was recorded by preparing test discs of standard surface area containing the active ingredient. These test discs carrying 9, 30, and 45 mg of NaF were placed into 15 mL test tubes containing Yeast Extract Peptone Dextrose broth (Difco, USA) with 1% sucrose which was incorporated into the Yeast Extract Peptone Dextrose broth prior to use. A bacterial suspension containing $1.5 \times 10^8$ colony-forming units (CFU)/mL of the *S. mutans* strain was added into each tube. The optical density of the bacterial suspension spectrophotometrically at 625 nm (Shimadzu UV-1201V, Japan) was between 0.08 to 0.13 for the 0.5 McFarland standard equivalent to $1.5x10^8$ CFU/mL. To act as controls, a disc lacking the active substance was placed into one tube, and the bacterial suspension without a disc was placed into another. The test tubes were placed on a mini shaker (Shaker PSU-2T Boeco, Germany) with a gentle speed and then incubated in an anaerobic chamber (Bactron IV Shel Lab, USA) in an atmosphere containing 90–92% nitrogen, 3–5% hydrogen, and 5% carbon dioxide for 24 hr at 37ºC. At 0, 4, 8, 12, and 24 hr of incubation, the test tubes were vortexed smoothly, and media samples were taken from each tube and placed into a 24-well sterile cell culture plate in order to measure the pH values of

the medium by using indicator paper strips (Merck, Germany), and a digital pH meter (Hydrus 400, Fisher Brand, UK). Additionally, in order to assess the viable colonial counts in the media samples, 10-fold serial dilutions of the media samples were prepared, inoculated onto Mueller Hinton agar plates, and incubated in anaerobic conditions at 37ºC for 24 hr. At each period, media samples from each of the test tubes were inoculated onto three plates. The live bacterial colony counts and pH of the media samples from the test tubes, with and without the active ingredients on the discs, were statistically analyzed.

## RESULTS

When fluoride release was analyzed according to the change in pH, no significant fluoride release from the 3-layer film was observed within the first 6 hr (Figure 3). After 6 hr, as a result of the pH decrease (<5.5) in the medium, fluoride ion (F) release increased rapidly with 19.66 mg being released over 139 hr from the groups containing 30 mg and 45 mg NaF.

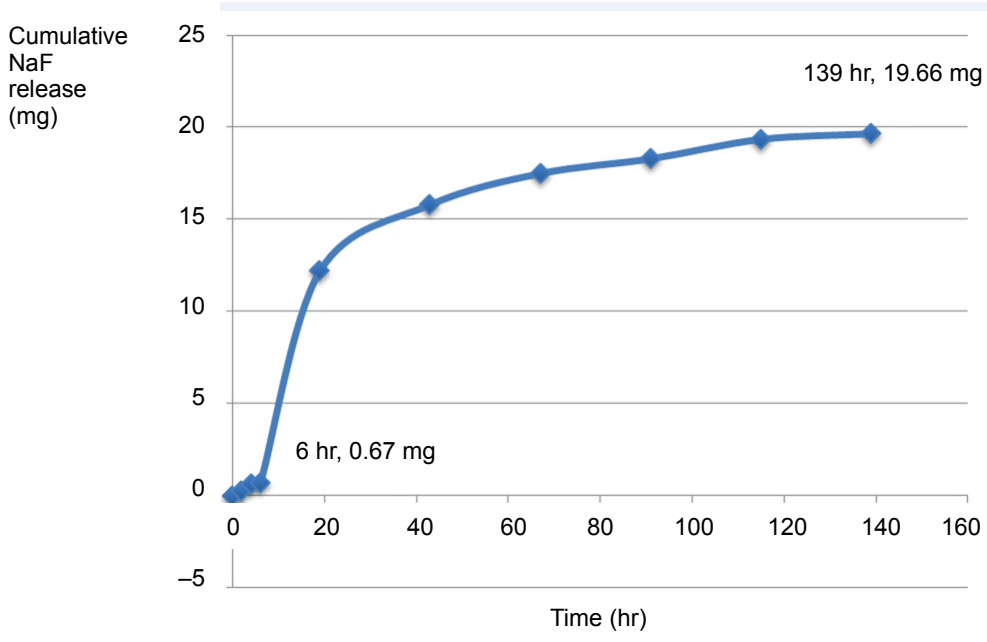


**Figure 3.** The release over time of NaF from a 3-layer film in the 30 and 45 mg NaF groups.

The media containing 9, 30, and 45 mg of NaF and the control medium all showed similar pH changes over time (Figure 4). While the pH of the 9 mg NaF sample was 6.17 at time 0 hr, it was 6.20 after 24 hr. Likewise, with the 30 mg NaF sample, while the pH was 6.19 at time 0 hr, it was 6.50 after 24 hr. With the 45 mg NaF sample, while the pH was 6.25 at time 0 hr, it was 6.74 after 24 hr. In the control medium the ph was 6.1 at 0 hr and 6.08 at 24 hr. The disc control and

growth control groups showed similar pH decreases. In the growth control group, the pH decreased from 5.97 at 0 hr to 4.47 at 24 hr. Similarly, the disc control group had a pH of 5.98 at 0 hr and of 4.5 at 24 hr.

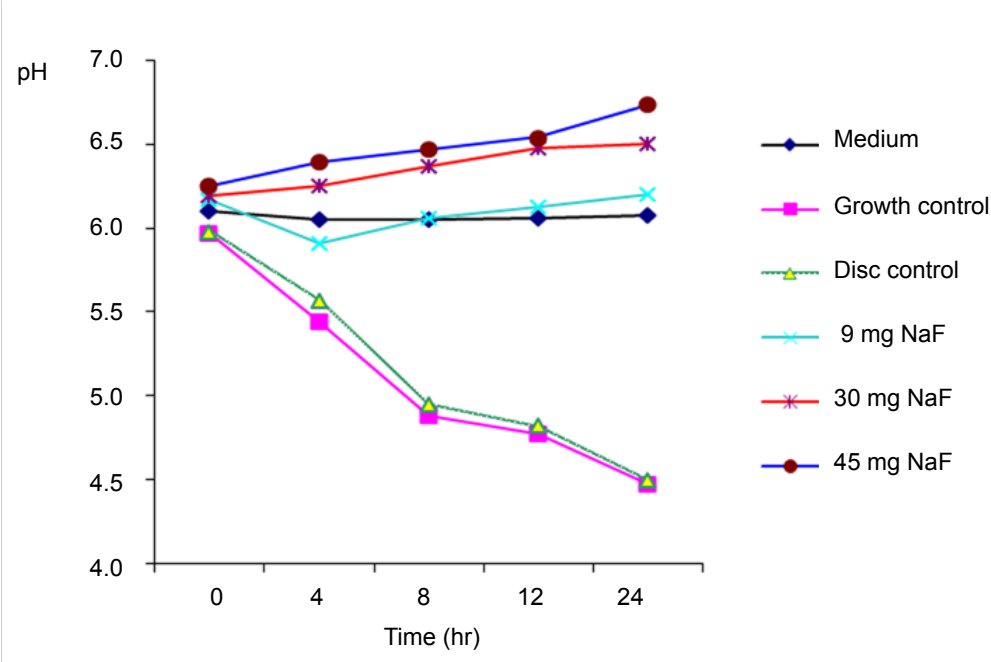


**Figure 4.** The variation of the pH of the medium with time in the medium, growth control, disc control, and 9, 30, and 45 mg NaF groups.

The 30 and 45 mg NaF carrying samples had the same effect on the number of colonies while the disc control and the 9 mg of NaF sample showed similar changes (Figure 5). With the 30 and 45 mg NaF samples, the bacterial count reached zero after 8 hr while in the disc control and the 9 mg NaF samples a similar level was reached after 24 hr. In the growth control group, the colony count increased at 4 hours and then returned to its initial value.

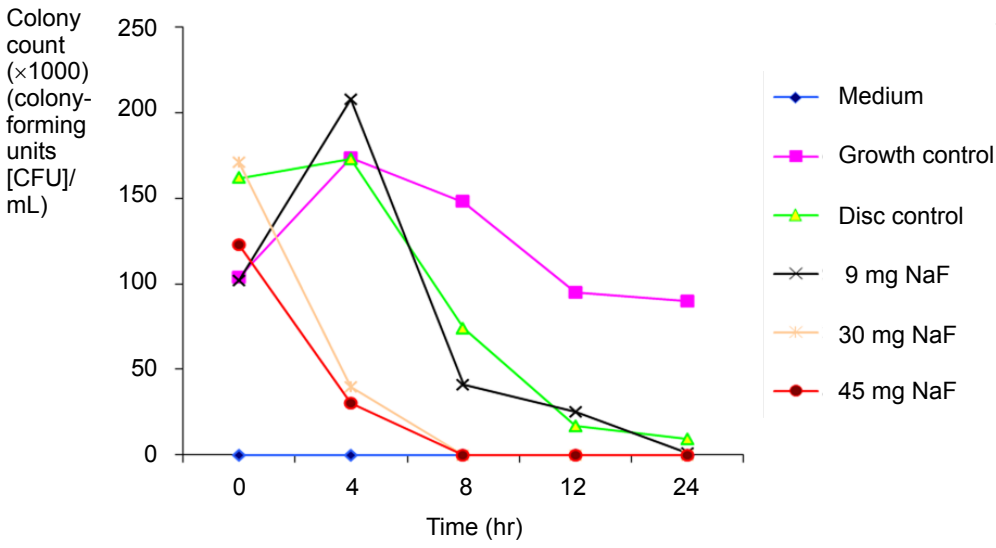


**Figure 5.** The influence of NaF released from the CRS device on the colony counts in the medium, growth control, disc control, and 9, 30, and 45 mg NaF groups.

## DISCUSSION

The major etiological factors for dental caries are supposed to be the high consumption of refined sugars and the presence of an acidogenic flora in the dental plaque.[11] Lactic acid is produced by the fermentation of refined sugars by streptococcus mutans. A major component of dental enamel is hydroxyapatite (HA), $Ca_{10}(PO_4)_6(OH)_2$, and this mineral content is sensitive to increases in acidity from the production of lactic acid. A tooth is in a constant state of back-and-forth demineralization and remineralization between the tooth and surrounding saliva. When the pH at the surface of the tooth drops below a critical level (pH 5.5), demineralization proceeds faster than remineralization and the formation of dental caries begins.[12]

The cariostatic effect of the fluoride ion is well supported by the literature.[13,14] Fluorides affect caries formation through a variety of mechanisms including reduction of demineralization, enhancement of remineralization, interference with pellicle and plaque formation, and inhibition of microbial growth and metabolism.[15] This preventative benefit is gained primarily by topical rather than systemic application, as reports have shown that the fluoride ion exerts its maximum cariostatic effect through its constant presence at the plaque-saliva-enamel interface.[16,17]

Fluoride has a direct inhibitory effect on the metabolic activity of cariogenic bacteria. Glycolysis is the metabolic breakdown of glucose and other sugars that releases energy in the form of ATP. Inhibition of glycolysis by fluoride is central to the concept that the anti-microbial effect of fluoride has a role in caries prevention.[18] Since the intraoral levels of F play a key role in the dynamics of

dental caries, it has been suggested that delivery systems can be used to control the dental caries incidence in high risk individuals. Numerous studies have investigated topical systems of slow and constant F release, which are mainly grouped into three types of devices: the polymeric membrane, the glass bead, and, more recently, a mixture of sodium fluoride (NaF) and hydroxyapatite.[8,19-21] The system that Cowsar et al.[19] developed in 1976 releases fluoride in the oral environment at constant, pre-determined linear rates of 0.02 to 1.0 mg/day for at least six months. With glass devices, the fluoride ion content changed in the range of 11.94 to 18.01 mg. The system of NaF and hydroxyapatite contained 18 mg of NaF and was intended to release 0.15 mg F/day. With our system, containing 45 mg F, all of the bacteria were eliminated in 8 hr.

In all these systems, a small amount of F is continuously released, and numerous studies in the literature have investigated whether this continuous release of fluoride increases the amount of fluoride in the systemic circulation. Mirth et al.[22] reported no changes in the F concentrations in the serum and urine of human subjects after fitting the polymeric devices. Kula et al.[6] demonstrated that intraoral fluoride releasing devices could significantly elevate salivary F concentrations in adolescents without having the same effect on the systemic concentrations. Alacam et al.[23] reported that fluoride releasing removable appliances could be useful for the prolonged release of low concentrations of fluoride without any systemic effect.

In the systems developed earlier, the fluoride release was not affected by environmental factors. In our study we used a 3-layered pH-sensitive controlled release system carrying fluoride which achieved fluoride release into the medium when the pH was 5.5 or less. In this manner, the systemic and local side effects typical of long term fluoride release were eliminated while the unnecessary release of fluoride to the environment was prevented. As a result of fluoride disrupting the mechanism of acid production by bacteria in the environment, the number of bacteria rapidly decreased. At the same time, lasting anti-caries effects were achieved due to the regulated levels of fluoride. One limitation of the study was that it was carried out *in vitro*. Future studies that examine the *in vivo* effects of the fluoride carrying system developed for this report are indicated.

## CONCLUSIONS

We prepared a fluoride-containing anti-caries pH-sensitive release system for intraoral use. When the pH in the medium decreased below 5.5 after 6 hr, the fluoride ion release increased rapidly. With the 30 and 45 mg NaF groups, the bacterial count reached zero after 8 hr. The system appears to have promise for future *in vivo* use in preventing dental caries.

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