



## Formulation and evaluation of medicated chewing gum as antiplaque and antibacterial agent

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### ABSTRACT

**Aim:** The aim of this study was to formulate chewing gum using chlorhexidine and chitosan and to prove its antibacterial and antiplaque properties effectively at low doses of chlorhexidine. **Materials and Methods:** Chewing gums were prepared by using chlorhexidine and various proportions of gum base and chitosan. Hot melt technique was used to prepare chewing gums. The prepared gums were evaluated for physical parameters, compatibility studies, drug content, moisture content, stability studies and *in vitro* drug-release testing. To carry out *in vivo* study on humans double-blind, crossover clinical trial was conducted on 18 adult volunteers from Department of Oral Medicine and Radiology, JSS Dental College, Mysore. The test group administered chewing gums containing chlorhexidine, with and without the polymer, while the control group administered dummy chewing gums. The volunteers were visually examined for presence or absence of gingival erythema, gingival edema and gingival bleeding and the antibacterial activity was assessed by the reduction in bacterial count in the plaque samples. **Result:** As the proportion of gum base was increased, an increase in hardness of the chewing gum was observed. The formulation, which contains highest concentration of chitosan, showed more *in vitro* release compared to other formulation. Analysis of variance revealed significant differences between subjects receiving F1 and F5 for antiplaque activity. The post plaque samples results showed significant reduction in microbial count ( $P < 0.05$ ). **Conclusion:** The *in vivo* studies on human volunteers were carried out for optimized formulation F5 containing chitosan and antibacterial agent which showed a better reduction in the bacterial count and gingival index. The dose of chlorhexidine can be reduced significantly when used along with chitosan. Chitosan containing chewing gum has a greater antibacterial effect compared with gums containing only chlorhexidine.

**Key words:** Chewing gum, chitosan, chlorhexidine, dental caries

### INTRODUCTION

In the recent years, chewing gum being a tasty and enjoyable confectionery has been proven its potential as an effective delivery vehicle for pharmaceutical and nutraceutical ingredients. As far as patient convenience is concerned chewing gum is discrete, and its administration without water promotes higher compliance. Since it can be taken

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anywhere, a chewing gum formulation is an excellent choice for acute medication. Studies have shown that even non-medicated chewing gums stimulates saliva flow and increases plaque pH, which prevents tooth decay.<sup>1</sup> Today, improved technologies have made it possible to develop and manufacture medical chewing gum with pre-defined properties, such as release of the active substance, taste, and texture.

A number of active substances in chewing gum formulations are available commercially. Medicated chewing gum is now a well-established dosage form described in the European pharmacopoeia. It offers a number of advantages, including fast onset of action, avoidance of hepatic first pass metabolism for substances absorbed via buccal route, and possibly lower dosage requirements and hence a fewer side-effects.<sup>2</sup>

Dental plaque, a bacterial biofilm, is one of the major etiologic agents involved in the initiation and progression of dental caries, gingivitis and periodontal disease. Due to the strong association between oral microorganisms and caries, as well as periodontal diseases, the dental profession has been increasingly interested in the topical use of antimicrobial agents. Chlorhexidine was the first antimicrobial agent shown to inhibit dental plaque and chronic gingivitis. Numerous studies have followed that chlorhexidine is one of the best investigated compounds in dentistry and to date remains the gold standard compared to other antimicrobials. For dental usage, chlorhexidine is marketed in various galenic forms such as mouth washes, sprays, and gums.<sup>3</sup>

In the case of mouth rinse only 30% of the dose is retained, which leads to a persistent antibacterial milieu in the mouth. Cessation of chlorhexidine concentration in mouth rinse results in re-growth of normal salivary bacterial count. Plaque inhibition by mouthwash appears to be dose related. Chlorhexidine at 0.2% concentration shows oral toxic effects such as oral mucosa desquamation, soreness and bitter taste in conventional dosage form as reported. Further, cosmetic problems associated with teeth coloration due to chlorhexidine are a factor decreasing patient compliance. Teeth color is a combination of intrinsic color of the teeth and the presence of extrinsic stains that might accumulate on the tooth surface. Extrinsic stains are linked with the adsorption of materials into the acquired pellicle on the enamel surface.<sup>4</sup> The amount of staining seems to be dependent on the mode of application, concentration, and presence of potential discoloring agents within the diet.<sup>3</sup> These effects can be minimized if the dose of chlorhexidine is reduced.

To overcome the above mentioned problems in the present study medicated chewing gums were prepared using chlorhexidine along with chitosan as it has antibacterial and antiplaque properties, to give better patient compliance compared with mouth rinse in lesser dose when compared with the mouth wash.<sup>5</sup>

## MATERIALS AND METHODS

### Materials

Chlorhexidine gluconate, chitosan was purchased from Strides Arcolab Ltd., Bangalore. Chitosan medium molecular weight (190,000-310,000 Da; 75-85% deacetylated) was purchased from Sigma Aldrich, Mumbai. Gum base, dibutyl phthalate, sucrose, sorbitol were purchased from the Lobachemie, Mumbai.

### Method

#### Preparation of chewing gum

Synthetic gum base was weighed accurately and melted. To this plasticizer was added and then mixed thoroughly. The melting process was carried out in a porcelain dish at about 50-60°C on the steam bath and was allowed to cool at 15-20°C.<sup>6,7</sup>

#### Formulation of medicated chewing gum

Chewing gums were prepared by using 10, 20, 30, 40, 50% gum base, and the formula was optimized at particular percentage of gum base composition. Required quantity of gum base was melted at 50-60°C. To this sorbitol was added and mixed well. Then, the physical homogenous mixture of chlorhexidine gluconate, talc and sucrose was added with continuous stirring to ensure even distribution of drug. Flavor and color were added in the end. The mixing mass was allowed to cool at room temperature on steel plate; the mass was rolled evenly and cut into the pieces of uniform size. The weighed pieces were removed and wrapped in aluminum foil.<sup>8,9</sup> Formulation chart is given in Table 1.

**Table 1:** Formulation chart of medicated chewing gum

Excipients	F1	F2	F3	F4	F5
Chlorhexidine gluconate	5 mg	5 mg	5 mg	5 mg	5 mg
Chitosan %	-	0.25	0.50	0.75	1
Gum base	300 mg	300 mg	300 mg	200 mg	200 mg
Dibutyl phthalate	20 mg	20 mg	20 mg	20 mg	20 mg
Sucrose	625 mg	600 mg	575 mg	650 mg	625 mg
Sorbitol	15 mg	15 mg	15 mg	15 mg	15mg
Talc	20 mg	20 mg	20 mg	20 mg	20 mg
Color and flavor	15 mg	15 mg	15 mg	15 mg	15 mg

### Evaluation of medicated chewing gum

All prepared formulations were physically evaluated for parameters such as appearance, color, stickiness, hardness and plasticity. Weight variation test for chewing gums was carried out as per the guidelines in I.P using 10 randomly selected chewing gums. Chewing gum was cut into small pieces, and moisture content was determined by using moisture balance.

### Fourier transformation infrared spectroscopy (FT-IR) and stability analysis

FT-IR spectrophotometer (Shimadzu-8400S, Japan) was used to study FT-IR spectra by KBr pellet method. FT-IR spectrum of chlorhexidine was compared with FT-IR spectra of formulations. Disappearance of peaks or shifting of peaks in any of the spectra was studied.

### Differential scanning calorimetry (DSC)

The DSC thermograms of drug, prepared formulation were studied, which were performed using shimadzu-thermal analyzer DSC-60, Japan. Sample was put in aluminum pans, thermograms were obtained at a scanning rate of 10°C/min conducted over a temperature 30-300°C in a liquid nitrogen environment (flow rate 10 mL/min).

### Stability study

Stability testing was performed according to ICH guidelines by storing at 25°C/60% RH and 30°C/65% RH for 30 days to find out the extent of deterioration and to ensure that the degradation has not exceeded an acceptable level assuring the safety of the patient and the activity of the product.

### Drug content

One gram of the formulation was taken in mortar, to this about 20 mL of pH 6.8 phosphate buffer was added and triturated. This was transferred to a conical flask. About 30 mL of pH 6.8 phosphate buffer was added to this and shaken well for about 3 h using orbital shaking incubator at 100 rpm. Then it was filtered and the filtrate was made up to mark with the same buffer. Suitable dilutions were made, and the drug concentration was determined by measuring the absorbance at 254 nm.

### In vitro drug release studies

*In vitro* drug release testing apparatus for chewing gum, was a self-fabricated apparatus which was a modification of disintegration test apparatus. Here rigid basket-rack assembly is supporting six cylindrical glass tubes was replaced with a

detachable stainless steel rod fitted with a Teflon bead which moves up and down vertically at 40 cycles/min inside the glass cylinder of 50 mL capacity. Length of the stainless steel rod can be adjusted by the adjusting screw which is positioned at the top of the stainless steel rod and is also used to fix the rod to the disintegration apparatus. The base of the glass cylinder was made up of Teflon. Temperature was maintained at  $37 \pm 0.5^\circ\text{C}$ .<sup>10</sup>

About 1 g of formulation was placed in a glass tube between the Teflon bead and Teflon base. To this about 20 mL of pH 6.8 phosphate buffer was added. 0.5 mL of sample was withdrawn and replaced with 0.5 mL of fresh pH 6.8 phosphate buffer at intervals of 5, 10, 15 and 20 min. Collected samples were estimated by ultraviolet spectrophotometer at 254 nm.<sup>10</sup> The release data were fitted into various mathematical models using *planar cell polarity* Disso v2.08 – Poona College of Pharmacy, Pune, Maharashtra, India. Sigma plot software 10.0 – Systat Software Inc. software to know, which mathematical model fits best to the obtained release profile.

### In vivo studies on human volunteers

#### Study population

A total of 18 subjects (14 male and 4 female) from Department of Oral Medicine and Radiology, JSS Dental College, Mysore were taken for the study. Screening and selection of volunteers were carried out by a single investigator who explained the study and obtained signed consent to participate. The subjects were selected from 25 individuals on the basis of having at least 20 natural teeth free from conservative restorations, who experienced bleeding of gums and erythematous gingiva. The exclusion criteria included systemic problem, not on any medication, not a medically compromised patient, wearing of any form of intra oral prostheses or appliances, recession, other signs of periodontitis, temporomandibular joint disorders, mal occlusions and chronic onychophagia. The female subjects enrolled were not pregnant or nursing. All the volunteers were in good general health. In particular, no subject had received mouth rinses, gels, or chewing gums containing antimicrobial agents in the preceding 3 months.

#### Study design

The effect on plaque growth of a chewing gum containing chitosan and chlorhexidine was assessed in a 4-day double-blind, crossover clinical trial with 18 adult volunteers. The control group comprised of 6 volunteers while the remaining 12 were categorized under test group. Chewing gums containing 5 mg of chlorhexidine gluconate and varying

concentration of chitosan (F1 and F5) were administered twice (total daily dose = 10 mg) to the test group subjects, while control group was aided with dummy chewing gums.

On day 1 the subjects received scaling to remove any extrinsic stain, calculus, and the plaque samples were collected. Then, the formulation group were provided with individual packets of chewing gum labeled with his/her name, group of study along with verbal and written instructions of their use (time of use etc.). The subjects were instructed to chew one piece of gum at a time, twice a day, after breakfast (9:00-9:30 a.m.) and after lunch (2:30-3:00 p.m.). The chewing time was set as 20 min each time. During this test period, the volunteers suspended the use of mouth rinses, toothpastes, candies, other gum products and avoided eating and drinking up to 1 h of post-commencement of the gum.

On day 5, the volunteers received an oral soft tissue examination, and any side effects were noted, plaque samples were collected from the subjects. The volunteers were also visually examined for presence or absence of gingival erythema, gingival edema, gingival bleeding and stain indices. The antibacterial activity of the medicated chewing gum was assessed by the reduction in the bacterial count in the plaque samples collected after the test period. Plaque index was also recorded at baseline after 4 days of study.

The study design was approved by the local Ethical Committee and conformed to the requirements of the "Declaration of Helsinki" as adopted by the 18<sup>th</sup> World Medical Assemblé in 1964 and subsequently revised.

## Statistics analysis

The data were analyzed for normality of distribution through the use of Kolmogorov-Smirnov test. Since the data was normally distributed, an analysis of variance (ANOVA) was performed to determine the difference among the test products. The level of significance was set at  $P < 0.05$ .

## RESULTS

As a proportion of gum base was increased, an increase in hardness of the chewing gum was observed. At 20% and 30% gum base composition homogeneous mass was obtained, and the prepared chewing gums exhibited good elasticity with good texture.

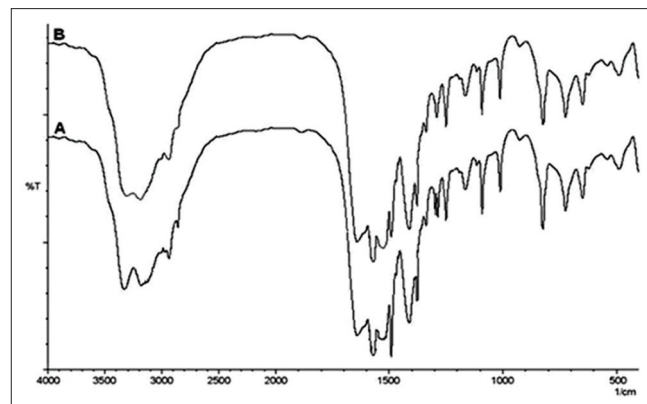
### FT-IR

When the FT-IR spectra of chlorhexidine and F5 were compared, they showed similar characteristic peaks

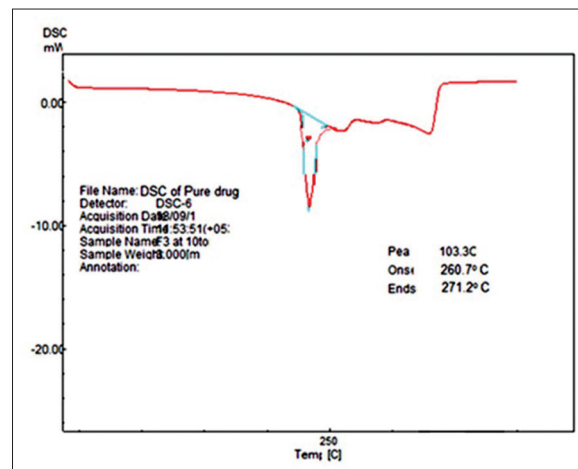
with minor differences (Figure 1). Important peaks of chlorhexidine at 3325.30, 1097.68, 2937.42, 1492.04, 660.12  $\text{cm}^{-1}$  corresponding to N-H stretching vibration in amines, C-N stretching in amines, C-H stretching in alkane, C=C stretching vibration in aromatic compound, C-Cl stretching respectively remained intact in the FT-IR spectra of the F5 also.<sup>11</sup>

### DSC

Thermogram of pure drug (chlorhexidine) is shown in Figure 2. Melting transition of chlorhexidine HCl was observed from 260.7°C (Onset) to 271.2°C (Endset). Sharp melting transition of chlorhexidine HCl was observed at 266.8°C. In the optimized formulation drug and excipients melting endotherm were observed at 264.3°C (Onset) to 274.8°C (Endset). Sharp melting transition of chlorhexidine HCl in optimized formulation was observed at 268.5°C, which is shown in Figure 3. There was a significant difference in the melting point of the drug in all the thermograms. Hence it was

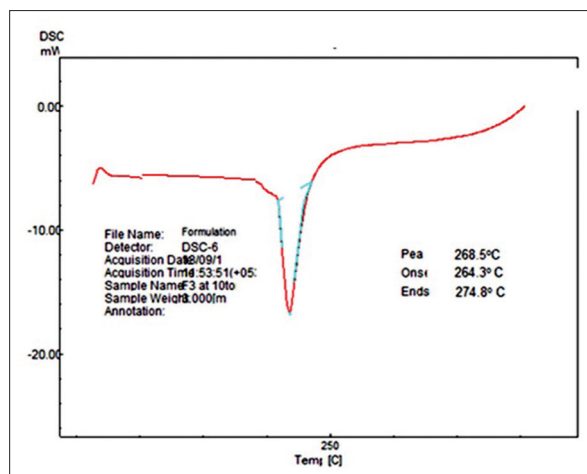


**Figure 1:** Fourier transformation infrared spectroscopy spectra of (a) chlorhexidine, (b) formulation F5



**Figure 2:** Differential scanning calorimetry of chlorhexidine





**Figure 3:** Differential scanning calorimetry of formulation

concluded that there is no interaction between drug and polymer.

### Drug content

All the formulations were found to contain almost uniform quantity of drug as per content uniformity studies. The percentage of drug content of F5 was found to be highest of all the formulations i.e., 98.58%.

### Moisture content

The mean moisture content of all the formulations were found to be 1.33 which was in the limit prescribed by the prevention of food adulteration act, 1954 (not more than 3.5% by weight).

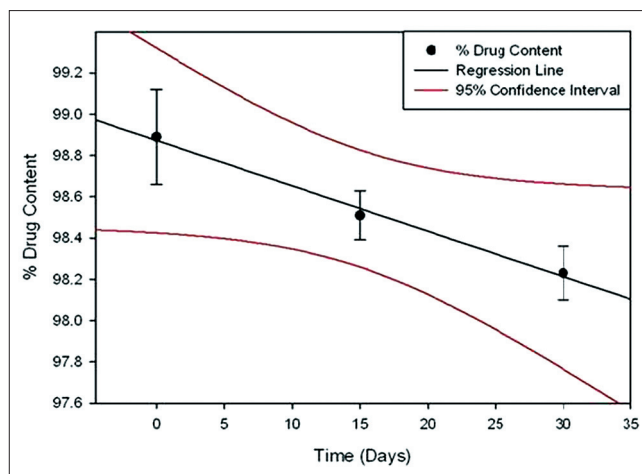
### Stability study

There was no marked change in the physical property and the drug content during the stability study period. The chlorhexidine content in the formulation along with 95% confidence limit is plotted using sigma plot software 10.0. The observations of conditions are shown in Figures 4 and 5.

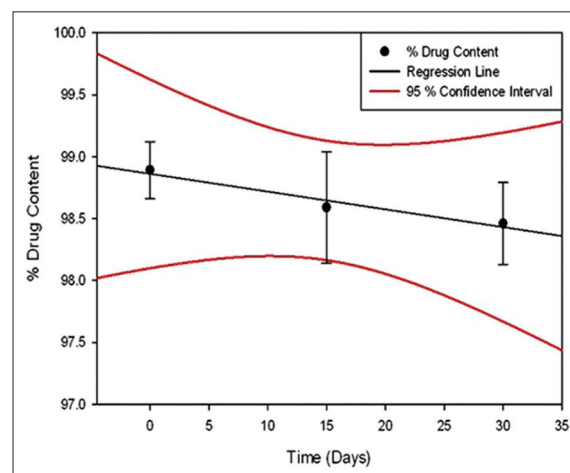
### *In vitro* drug release studies

As shown in Figure 6, among all formulations, F5 showed good sustained release effect (98.4%), based on release profile. The results indicated that, the best-fit model was the Peppas model. Koresmeyer-Peppas equation is given as  $\log \% R = \log K + n \log t$ , where  $R$  is the amount of drug released in given time  $t$ ,  $K$  is the release rate constant and  $n$  is the diffusion exponent of Peppas model.<sup>12</sup>

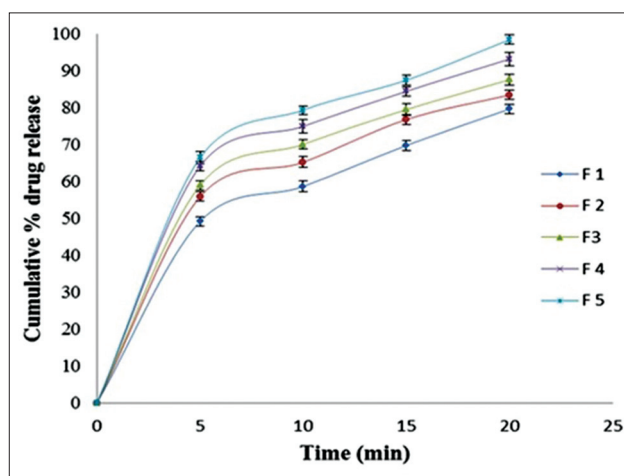
To determine the release mechanism, the parameter  $n$  and  $k$  were calculated. The value of “ $n$ ” for all the



**Figure 4:** Graph showing drug content in chewing gum when stored at 25°C/60% RH for 30 days



**Figure 5:** Graph showing drug content in chewing gum when stored at 30°C/60% RH for 30 days.



**Figure 6:** *In vitro* drug release from medicated chewing gum

formulations lied in range of 0.43-0.71. This indicates that drug release from chewing gum is governed by non-Fickian diffusion.

## In vivo studies on human volunteers

### Visual examination

The results obtained by visual examination for the presence or absence of gingival erythema, gingival edema, gingival bleeding were carried out.

Further, no intraoral staining or discolorations on the teeth were observed in the test group subjects who received F1 and F5 chewing gums.

### Plaque index

The mean and standard deviations for the individual plaque index of the control and study group are studied. ANOVA showed significant difference in the antiplaque activity among the control and test groups, for smooth and occlusal surfaces ( $P < 0.05$ ). During the study, no side effects were reported by the volunteers.

### Bacterial count

The microbial count in the plaque samples of both control and test group subjects were measured as given in the Tables 2 and 3 respectively. ANOVA showed that pre samples of control, F1 and F5 were statistically insignificant ( $P > 0.05$ ), whereas the post samples results showed statistically significant differences ( $P < 0.05$ ).

## DISCUSSION

The use of chlorhexidine in chemical therapy for maintaining oral hygiene is well established.<sup>13-16</sup> Traditional

methods of application of chlorhexidine such as rinses or gels have not proved its antibacterial activity to acceptable standards.<sup>17-19</sup> Thus, chewing gum containing chlorhexidine has been explored for the treatment of gingivitis, periodontitis and infections in the oral cavity or throat.<sup>3</sup> Chewing gum containing chlorhexidine resulted in significant reduced plaque values compared with the sorbitol-and xylitol-containing gums.<sup>20</sup> The authors concluded that the use of chlorhexidine containing gum twice daily provided significant benefits to oral hygiene and gingival health.<sup>21</sup> The daily recommended dose of chlorhexidine gum is 4-6 pieces, 2 pieces at a time, chewed for 20 min. One piece of chlorhexidine chewing gum contains 5 mg of chlorhexidine (total daily dose = 20-30 mg).<sup>10</sup> However, the main risk is the appearance of light brown discolorations of teeth, and some restorative materials on the tongue. The following explanations have been proposed:

- Degradation of the chlorhexidine molecule to release parachloraniline
- Catalysis of mallard reactions
- Protein denaturation with metal sulfide formation
- Precipitation of anionic dietary chromogens.<sup>22,23</sup>

To overcome all the above problems, the study was designed to formulate chewing gums containing chlorhexidine along with chitosan and to prove its antiplaque activity by lowering the daily dose of chlorhexidine.

When chewing gums were prepared by varying the concentration of gum base, 10% gum base composition did not produce homogeneous mass, and the prepared chewing gums were brittle in nature. At 40% and -50% gum base composition homogeneous mass was obtained but it was highly viscous, and it was difficult to mix other ingredient. Hence, prepared chewing gums were hard in nature. Hence, polyisobutylene gum base at 20% and -30% gum bases were used.

Chitosan being a polymer may interact with the drugs or other excipients in the formulation. But the FT-IR studies proved that there was no chemical interaction between drug and chitosan, and it can be inferred that the characteristics bands of pure drug were not affected by the method used to prepare chewing gum.

Chitosan facilitates the sustained release of drugs by a process of slow erosion from a hydrated compression matrix. The *in vitro* drug release studies showed that presence of 1% chitosan (F5) enhanced the sustained release of chlorhexidine (98.4%) when compared to F1 that had no chitosan. The *in vitro* drug release of F5 was

**Table 2:** Pre and post sample values for control group

Subject	Pre-treatment (cfu/mL)	Post treatment (cfu/mL)
1	1000	100
2	10000	1000
3	100000	1000
4	10000	1000
5	100000	100
6	1000	1000

**Table 3:** Pre and post sample values for formulation group

Subjects	Pre Rx (cfu/ml)	Post Rx (cfu/ml)
1 (F1)	10000	10 <sup>1</sup>
2 (F1)	1000	10 <sup>1</sup>
3 (F1)	1000000	10 <sup>1</sup>
4 (F1)	1000	10
5 (F1)	10000	100
6 (F5)	100000	1 <sup>1</sup>
7 (F5)	100000	1
8 (F5)	1000	10
9 (F5)	100000	10
10 (F5)	10000	100

66.43% after 5 min and 87.45% after 15 min. This shows a promising long oral presence of chlorhexidine in presence of chitosan. The drug-polymer ratio was found to affect the drug release characteristics of the prepared chewing gums. At higher drug-polymer ratio, the drug release from the chewing gums was faster as compared to lower drug-polymer ratio. This was because the high drug-polymer ratio promotes the increase saliva uptake and lead to the greater solubilization of the drug present in the polymer matrix causing faster diffusion of drug through the gum base. It was observed that decreasing the distance between the chewing surfaces from 1.8 to 1.6 mm leads to an increased rate of release, since the force acting on the gum was larger with a 1.6 mm setting. At a constant stroke frequency, the twisting angle setting influences the release rate.

The 4 day, double-blind, crossover clinical trial with 18 adult volunteers corroborate the antiplaque activity of the chlorhexidine and chitosan chewing gum at low daily doses. The antimicrobial activity of chlorhexidine was enhanced by the presence of chitosan. Hence, the post samples of F1 and F5 group showed statistically significant difference in the bacterial count. The biological effects of chitosan depend on a wide range of molecular weights and the degree of deacetylation (DDA). The DDA of the chitosan oligomer is presumably a more critical factor than the chain length of an oligomer for the growth inhibition. The chitosan oligomer significantly inhibits bacterial growth at various concentrations. A nearly complete inhibition can be obtained at 2% (w/v) of chitosan solution. The reduction in the amount of chlorhexidine resulted in the significant lowering of staining intensity and plaque index. The present findings strongly indicate that even by reducing the dose of chlorhexidine, the antiplaque activity and inhibitory effects can still be retained. Further, the cosmetic problems associated with the chlorhexidine mouthwash can be avoided. This study also purports the application of natural materials such as chitosan for both oral health and improving the quality-of-life.

## CONCLUSION

The *in vivo* studies on human volunteers were carried out for optimized formulation F5 containing chitosan and antibacterial agent, which showed a better reduction in the bacterial count and gingival index. The dose of chlorhexidine can be reduced significantly when used along with chitosan. Chitosan containing chewing gum has a greater antibacterial effect compared to gums containing only chlorhexidine. The present findings

indicate that the application of natural materials such as chitosan is useful for both oral health and improving the quality-of-life.

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