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Buccoadhesive erodible disk for treatment of oro-dental infections: design and characterisation

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Abstract

Buccoadhesive erodible disks of cetylpyridinium chloride were prepared using different bioadhesive polymers along with excipients like mannitol. The purpose of designing the erodible disk was to obviate the need for removal of exhausted device. The optimized disk containing 5.0 mg of cetylpyridinium chloride, 2.0 mg of magnesium stearate and 6.0 mg of mannitol along with sodium carboxy methyl cellulose DVP and hydroxypropylmethylcellulose K4M in the ratio of 1:3 was found to release the drug for a period of over 6.0 h without getting dislodged. Maximum in vitro drug release was found to be 94.78% in 6.0-h study. In situ release characteristics were evaluated using a 'flow-through assembly', which simulated the conditions of the human buccal cavity. The drug concentrations in the in situ samples were found to be above minimum inhibitory concentration (MIC) of the drug. The bioadhesive performance and the surface pH of the disks were satisfactory. Cetylpyridinium chloride disks were tested against microorganisms commonly found in oro-dental infections namely *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus mutans*. The disk as well as the in situ samples showed inhibition of growth of microorganisms. Approval was taken from Jamia Hamdard Review Board (Ethical Board) to perform in vivo studies in healthy human volunteers. In vivo evaluation of buccoadhesive disks revealed adequate comfort, taste, and non-irritation and none of the volunteers reported severe dry mouth/severe salivation or heaviness at the place of attachment. Salivary concentrations were maintained above MIC for 8.0 h. Correlation was found between the drug concentration in situ and concentration of drug in saliva collected in healthy human volunteers. The correlation was found to be positive with a correlation coefficient of 0.9596. It was found to be statistically significant at 5% confidence level ($P < 0.05$).

Keywords: Erodible buccoadhesive disk; Cetylpyridinium chloride; In vitro human studies; In vivo human studies

1. Introduction

Most localized or progressive oro-dental infections occur when bacteria from dental plaque invade surrounding tissues. Plaque covers the fissures and pits of the teeth adjacent to gingivae causing gingivitis (Macfarlane, 1989). Supragingi-

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val and subgingival plaque play an essential role in the causation of dental caries and periodontal disease, respectively (Ross, 1992). Control of bacterial plaque helps in slowing or arresting oro-dental infections. Conventional therapy has long sought the use of mechanical plaque control procedures, which are time consuming, require highly trained personnel to carry them out and result in varying amounts of discomfort to the patients (Heasman and Seymour, 1994). Cetylpyridinium chloride, a quaternary antiseptic has been found effective in controlling the accumulation of bacterial plaque on the teeth, with a subsequent decrease in gingivitis. The drug has bactericidal activity against Gram-positive and at higher concentration against Gram-negative organisms. It has a good activity against *Candida albicans* (Pitcher et al., 1980). The bacterial composition of early plaque consists of mainly aerobic or facultatively anaerobic organisms, which later becomes colonized by strict anaerobes. Most oral infections attribute exclusively to facultative pathogens such as Staphylococci, Coliform bacteria and oral streptococci (viridans Streptococci). The use of cetylpyridinium is most desirable since the drug is effective against these facultative pathogens chiefly present at the sites where the infection initiates. At the later stage of infections, i.e. periodontitis, the organisms are combinations of anaerobic Gram-negative bacilli, facultative and strict anaerobic species chiefly *Bacteroides*, *Fusobacterium* and *Actinomyces* species and *P. gingivalis* (Ross, 1992; Pitcher et al., 1980). Lozenges and mouthwashes are available but none of these dosage forms are able to release the antiseptic drug to the oral cavity for a prolonged period because of short residence time. Multilayered bioadhesive lozenges containing cetylpyridinium chloride are available but these were not erodible and require removal after 3.0 h. Moreover formulation requires multiple steps for preparation (Collins and Deasy, 1990). In this study, we attempted to formulate a low dose matrix buccoadhesive erodible disk which would release the drug in a sustained manner using sodium carboxy methyl cellulose DVP (SCMC DVP) and hydroxypropylmethylcellulose K4M (HPMC K4M), with no need to remove any residual fragment thus claiming clinical usefulness. It would release the drug at the targeted site at concentration above minimum inhibitory concentration (MIC) for a prolonged period of time and provide an overall improvement in the treatment of oro-dental infections.

2. Materials and methods

2.1. Materials

Cetylpyridinium chloride was procured from BDH chemicals (New Delhi, India). HPMC K4M was obtained as gift sample from M/s Panacea Biotec Ltd (New Delhi, India). SCMC DVP was obtained as gift sample from M/s Ranbaxy Labs Ltd. Mannitol was procured from BDH chemicals. Isotonic phosphate buffer of pH 6.6 was prepared according to USP XX (1980). Water (HPLC grade), methanol (HPLC grade), tetramethyl ammonium hydroxide pentahydrate and Lichorsphere 100 RP-18 μm was obtained from E. Merck (India) Ltd (Mumbai, India). All other materials used during the study were of analytical reagent grade.

Shimadzu UV spectrophotometer UV 1601 (Japan) was used for spectrophotometric analysis. The HPLC system was of LC-10 Shimadzu model (Japan). Mechanical shaker was supplied by Veego (New Delhi, India). BOD incubators were also supplied by Veego.

2.2. Preparation of buccoadhesive disks

The disks were prepared using different combinations of polymers as shown in Table 1. The various components in each formula were mixed by trituration in a glass pestle and mortar. The mixture was then compressed using a 13 mm diameter die on an infra-red hydraulic press (Spectra Lab-SL-89, Mumbai) using a compression force of 5 tons and a compression time of 15 s. The disks were prepared with or without the addition of mannitol. The prepared disks were 13 mm in diameter and 1.15–1.21 mm in thickness. The mean weight was 205.80 mg and maximum variation from the mean was found to be 4.47%.

The thickness was found to be in the range of 1.15–1.21 mm with an average of 1.17 mm.

2.3. Evaluation of erodible buccoadhesive disks

2.3.1. Content uniformity

Representative disks were crushed and aliquots of the crushed disks were weighed and the required amount of distilled water was added to extract the drug. This suspension was shaken in mechanical shaker for 6 h. Samples were withdrawn as required, filtered through Whatman filter paper no. 42 and analyzed spectrophotometrically at 259 nm at λ_{max} of the drug in distilled water on UV 1601 (Shimadzu, Japan). Reference solution consisted of placebo tablets. The mean drug content (mg) was 5.09 ($\pm$0.13) with maximum variation of 1.8%.

2.3.2. Measurement of bioadhesive strength

Bioadhesive strength of the disks was measured on a modified physical balance (Gupta et al., 1992). The apparatus consisted of a modified double beam physical balance in which the right pan had been replaced by a lighter pan and the left pan had been replaced by a teflon cylinder (1.5 cm diameter and 3 cm height) suspended by teflon rings and copper wire. The left-hand side of the balance was exactly 5 g heavier than the right side. The height of the total set up was adjusted to accommodate a glass container of 4.2 cm height below the left side, leaving a head space of about 0.5 cm in between. Another teflon block of 3.8 cm diameter and 2 cm height was fabricated with an upward protrusion of 2 cm height and 1.5 cm diameter on one side. This was kept inside the glass container which was then placed below the left-hand set up of the balance.

Bovine cheek pouch was used as the model membrane and isotonic phosphate buffer pH 6.6 was used as the moistening fluid (Ahuja et al., 1995). The bovine cheek pouch obtained from slaughter house was kept in isotonic phosphate buffer at 37 °C for about 2.0 h. The underlying mucous membrane was separated and was washed thoroughly with IPB pH 6.6. It was then tied over the protrusion in the teflon block using a thread. The block was lowered into the glass container filled with IPB pH 6.6 at 37 $\pm$ 1 °C such that the buffer just touched the sides of the mucosal membrane. Two sides of the balance were made equal, before the study, by keeping a 5.0 g weight on the right pan. A glass container was kept below the left-hand set up of the balance. The disk was stuck on to the lower side of the hanging teflon cylinder. A modification in the earlier method was done which used distilled water as moistening fluid. In the modification the surface of the mucosal membrane was blotted with a Whatman filter paper and 25 l of IPB pH 6.6 was added to the mucosal surface. This was done to obtain reproducible results.

Table 1
Composition of various bioadhesive erodible disks

Formula code	Ingredients (mg)				
	CPC	SCMC DVP	HPMC K4M	MgS	M
A-1	5	19	171	2	–
A-2	5	38	152	2	–
A-3	5	57	133	2	–
A-4	5	76	114	2	–
A-5	5	95	95	2	2
A-6	5	38	152	2	4
A-7	5	38	152	2	6
A-8	5	38	152	2	8
A-9	5	38	152	2	10

CPC, cetylpyridinium chloride; HPMC K4M, hydroxypropylmethylcellulose; SCMC DVP, sodium carboxy methyl cellulose DVP; MgS, magnesium stearate; M, mannitol.

Five grams weight from the right pan was then removed. This lowered the teflon cylinder along with the disk over the membrane with a weight of 5.0 g. This was kept undisturbed for 3 min. Then the weights on the right-hand side were slowly added in increments of 0.5 g till the disk just separated from the membrane surface. The excess weight on the right pan, i.e. total weight minus 5 g was taken as a measure of the bioadhesive strength. From the bioadhesive strength, the following parameters were calculated:

$$\text{Force of adhesion (N)} = \frac{\text{Bioadhesive strength}}{1000} \times 9.81$$

$$\text{Bond strength (N/m}^2\text{)} = \frac{\text{Force of adhesion (N)}}{\text{Surface area of disk (m}^2\text{)}}$$

2.3.3. Surface pH of the bioadhesive disks

The disks were first allowed to swell in contact with 1 ml of distilled water (pH 6.5 ± 0.05) for 2 h in specially fabricated glass tubes. The surface pH was noted by bringing a combined glass electrode near the surface of tablets and allowing it to equilibrate for 1 min. The surface pH of the disks was determined in order to investigate the possibility of any side effects, in the oral cavity. As acidic or alkaline pH is bound to cause irritation to the buccal mucosa, hence attempt was made to keep the surface pH close to the neutral pH (Bottenberg et al., 1991).

2.3.4. In situ release studies

In situ studies were carried out on a self designed flow-through apparatus consisting of a cavity at the lower base (1.6 mm diameter and 1.5 cm depth) for placement of mucosal membrane (bovine cheek pouch) and the disk. IPB pH 6.6 simulating the salivary pH was continuously pumped at a flow rate of 0.65 ml/min using a small pump and flow regulators (Ali et al., 1998b). The flow rate chosen corresponded to the mean resting salivary flow rate (Schneyer and Levin, 1955). The whole assembly was maintained in a hot air oven at 37 °C. After stabilization the tablet was stuck on the mucosal membrane using 25 μl of IPB and a weight of 10 g for 30 s. Five milliliters of the sample was collected at different time intervals, till the formulation completely eroded or dislodged which ever was earlier (Ali et al., 1998a). Each sample was filtered through Whatman filter paper no. 42 and analyzed spectrophotometrically at 260 nm at λ_{max} of the drug in IPB pH 6.6 on UV 1601 (Shimadzu, Japan). The duration of bioadhesion or erosion was determined by measuring the time required for the formulation to erode completely or the time for which the formulation was maintained at its position without dislodging in flow-through assembly.

2.3.5. In vitro drug release studies and determination of duration of bioadhesion/erosion

The apparatus consisted of an internal compartment which consisted of 150-ml beaker (diameter: 50 mm) with a teflon block having a depression of 13 mm diameter and 1.5 mm depth for holding the disk. The dissolution fluid used was 100 ml isotonic phosphate buffer (pH 6.6), containing 2.25% glycoproteins with a stirrer speed of 50 rpm. The sample (5 ml) was withdrawn every half an hour for 6 h (Ahuja and Khar, 1999). Glycoproteins were added in order to simulate our buffer with the salivary fluid. The percentage used was as given in the composition of human saliva and mucus (Shellis, 1978; Smart, 1991). From the release studies carried out we saw that the presence of glycoproteins does not hinder release of drug from the matrix. This was confirmed using control samples.

The interference studies were done in order to ascertain that the glycoproteins do not interfere with the analysis of the drug. UV scans of 0.1% drug, 0.1% glycoproteins and combination of glycoproteins and drug (0.1% each) revealed that the drug exhibited λ_{max} at 260 nm in IPB pH 6.6 and glycoproteins showed no absorbance at the same wavelength. The concentration of the sample was determined and graph was plotted between absorbance at 260 nm on UV 1601 (Shimadzu, Japan) and time (h). The assembly was kept at 37 ± 1 °C.

2.3.6. Microbiological evaluation

Cetylpyridinium chloride disks were tested against *C. albicans*, *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus mutans* (Balanyk and Sandham, 1985; Magrex et al., 1993). Molten agar media was transferred to sterilized petridishes and when the temperature was around 40 °C, 0.2 ml of an overnight culture of strains mentioned were added to the petridishes. The evaluation was carried out in different ways.

(a) Nutrient agar plates inoculated with *S. aureus*
(b) Blood agar plates inoculated with *S. mutans*
(c) Nutrient agar plates inoculated with *E. coli*
(d) Sabouraud agar plates inoculated with *C. albicans*.

The culture was evenly distributed and the medium was allowed to solidify. Wells equidistant from each other were made in the solidified medium using a well borer, which was sterilized in the flame. The bottom of the wells were sealed with a drop of sterilized molten agar to prevent irregular creeping of the fluid from the wells.

All microbiological studies were performed in an aseptic area in the laminar flow hood.

2.3.6.1. Study with the buccoadhesive disks. Optimized bioadhesive disk containing 5.0 mg cetylpyridinium chloride were kept on medium petriplates and the drug was allowed to diffuse for a period of 1.0 and 2.0 h after which they were removed with the help of a sterilised spatula. The removed disks were shaken in distilled water for 1 h on a mechanical shaker. Filtered samples were analysed spectrophotometrically at 259 nm at λ_{max} of the drug in distilled water. Difference of loaded drug and drug remaining in the disk showed that only 48.85% of drug diffused in the media after 2 h of study. The plates were inoculated with the strains mentioned above. The nutrient agar plates were kept in an incubator for a period of 18 h at 35–37 °C. The sabouraud agar plates were kept in an incubator for a period of 18 h at 25–28 °C. Control test was also done without the tablet.

2.3.6.2. Evaluation of in situ release study samples. The samples collected from the in situ study at different time intervals (0.25, 0.5, 1.0, 2.0, 4.0 and 6.0 h) were tested on the above mentioned strains. The solutions collected were filtered through sterilized millipore membrane filters (0.2 μm). One hundred microliters of the samples collected from in situ study at different time intervals were carefully filled into the wells. The samples were allowed to diffuse for 2 h at room temperature. The plates were incubated in the similar way. The diameter (mm) of zone of growth inhibition surrounding each agar well was measured with the zone finder.

2.3.7. In vivo evaluation of erodible bioadhesive disks

The disks were tested on healthy volunteers to

(i) determine the time of erosion/adhesion of disks
(ii) investigate the acceptability of mucoadhesive polymers
(iii) measure salivary levels.

The study was conducted on six (four males, two females; age group 21–28 years) healthy human volunteers. Written consent was obtained from the volunteers before the study. Disk A-7 was used in the study. The volunteers were not allowed to take water and food starting from half an hour before the study till the end of the study. Drinking was allowed ad libitum from 30 min after administration of the disk. However no drinking was allowed 10 min before the collection of the salivary samples. The volunteers were given disks along with the written instruction sheets. They were instructed to press the disks against the cheek for about 30 s without moistening the disks before application. They were asked to record the time of disk insertion and the time and circumstances at the end of adhesion (erosion or dislodgement of the disks) (Parodi et al., 1996). The volunteers were also asked to complete a questionnaire after trial period for scoring irritation, discomfort if any, taste, dry mouth, salivation and heaviness in the buccal cavity.

The adhesion time was defined as the interval between the application till the time the disk was no longer visible. The tablet was checked after every 30 min. Samples of saliva were taken prior to the application of the tablet (blank) and 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0 and 8.0 h after the application of the tablet.

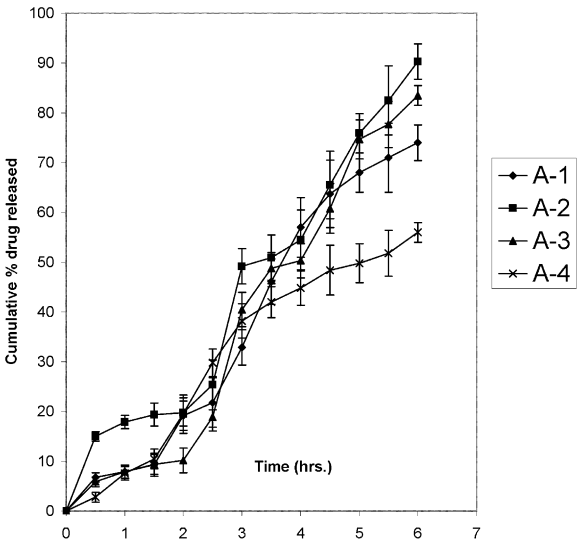

Fig. 1. In vitro release from formula code A-1 to A-4.

The disk was developed with the intention that the drug released locally would maintain the concentration of drug above MIC. Hence the amount of drug ingested with swallowed saliva was not calculated. Saliva (2 ml) was collected by spontaneous flow into a borosilicate tube over a 2.0 min period (1.0 min before and 1.0 min after the given time). Care was taken that the tongue did not contact the disk 10.0 min before sampling, in order to avoid abnormally high drug levels (Bouckaert et al., 1996). The salivary samples were diluted with water (HPLC grade) and filtered through millipore membrane filter (0.45 μm). The mobile phase was a mixture of methanol and 0.02 M tetramethyl ammonium hydroxide pentahydrate (60:40) adjusted to a pH of 3.5 with acetic acid. Flow rate was adjusted to 1.0 ml/min. Lichorosphere 100 RP-18, 5 μm protected by precolumn cartridge was used as the stationary phase. Blank saliva and salivary samples were injected and the column was run for 20.0 min. Area obtained for different samples was noted at 260 nm. Concentration of drug was calculated from, the calibration curve of the drug prepared in similar manner. The maximal salivary concentration (C_{max}) and the time to reach the maximal salivary concentration (T_{max}) for the drug was determined from the concentration time curve. When the salivary concentration at the last sampling time was still above 5 μg/ml, the same was taken as the end point of $T^{>\,MIC}$ (Khanna et al., 1996).

3. Results and discussion

The present study was an attempt to develop erodible buccoadhesive disks of cetylpyridinium chloride for treatment of oro-dental infections. SCMC DVP and HPMC K4M used in our disk have not been used earlier. This combination formed an erodible matrix which had a better release profile and patient compliance than earlier formulations containing other polymer combinations. The advantage of this disk was its erodible character as compared to the tablets prepared earlier (Collins and Deasy, 1990) which either dislodged or disintegrated during studies. Our new matrix system had better patient compliance because of the decrease in the frequency of administration. The disk released the drug in a sustained manner leaving no undisintegrated residual fragments. This was a significant achievement as patients did not have to remove the disk after predetermined time intervals.

3.1. In vitro release rate studies

Different polymers were chosen on the basis of their compatibility, bioadhesive performance, non-toxicity and non-irritant behaviour. The disks were prepared using different polymer combinations of SCMC DVP with HPMC K4M as given in Table 1. Optimization of the disks were carried out on the basis of in vitro release studies, in situ studies, Surface pH and bioadhesive strength. Drug release from the formulation A-2 which contained 38.0 mg SCMC DVP and 152.0 mg HPMC K4M was found to be 91.56% in 6.0 h (Fig. 1). An attempt was made to improve the mouthfeel, release of drug and to impart erodible character to the disk by addition of mannitol to the polymer matrix (A-2). Addition of 6.0 mg mannitol to the formulation further increased the release to 94.78% in 6.0 h time and imparted erodible character to the disk A-7 (Fig. 2). Opti-

mized disk along with suitable magnesium stearate (lubricant), aspartame (sweetener) and dried peppermint flavour (flavouring agent) gave maximum in vitro release of 94.78% over a period of 6.0 h. Fig. 3 represents schematic representation of liberation of drug from disk. The log % drug remaining against time gave a linear curve revealing first order rate kinetics. The first order release rate constant (K_r) was found to be 0.3447/h according to first order rate equation.

3.2. Bioadhesion study

None of the disks dislodged before complete erosion. The bioadhesive strength exhibited by disks is satisfactory for maintaining them in oral cavity. This aspect was further confirmed by simultaneously carrying out in vivo evaluation in healthy human volunteers. Greater bioadhesion was exhibited by disks containing mannitol, which could be related to its spatial conformation, and linear configuration, which facilitated interactions between the adhesive sites (–OH groups) and the mucus layer. Bioadhesive strength of optimized disk was found to be 45.13 g which was measured according to method given in Section 2.3.4. Force of adhesion and Bond strength were found to be 0.43 N and 3353.8 N/m^2. The important characteristics of different bioadhesive disks are given in Table 2.

3.3. In situ release rate study

In situ release study revealed that the concentration of the drug released was maintained well above its MIC (5 μg/ml) over a period of 6.0 h (Fig. 4). Concentration of drug released was in a range of 6.58–18.38 μg/ml. The important parameters obtained in situ are shown in Table 3. The maximum drug released at 4.5 h (18.38 μg/ml). MIC was maintained from 0.25 to 6.0 h.

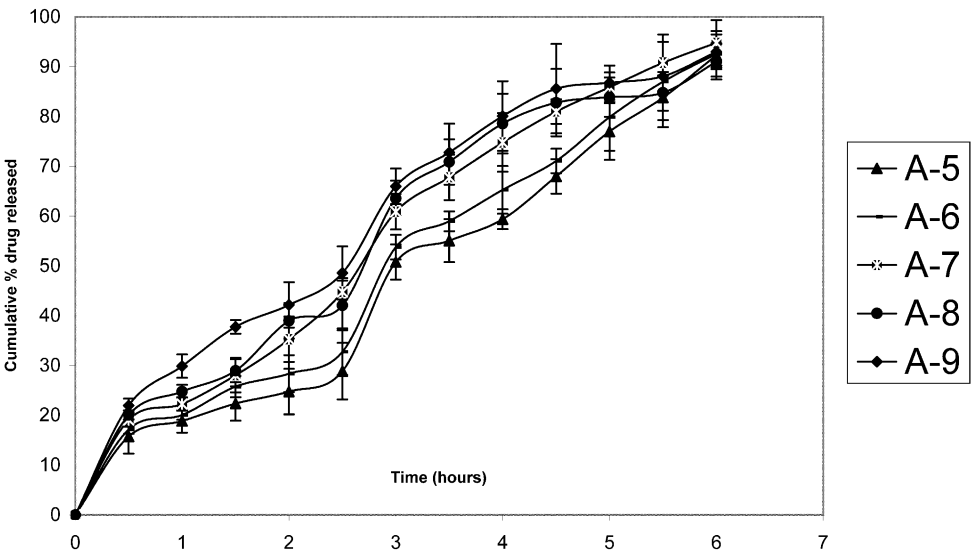

Fig. 2. In vitro release from formula code A-5 to A-9.

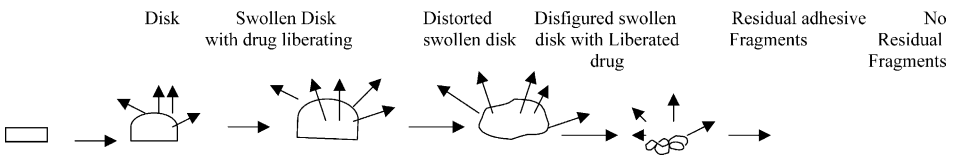

Fig. 3. Schematic representation of drug liberation from buccoadhesive disk.

Table 2
Important bioadhesive parameters of cetylpyridinium chloride disks

Formula code	Bioadhesive strength (g) ($\pm$S.D.) ($n=3$)	Force of adhesion (N)	Bond strength (N/m^2)	Surface pH ($\pm$S.D.) ($n=3$)
A-1	35.86 ($\pm$1.17)	0.3517	2664.39	6.66 ($\pm$0.01)
A-2	40.10 ($\pm$1.17)	0.3933	2979.54	7.03 ($\pm$0.02)
A-3	41.66 ($\pm$0.92)	0.3997	3021.21	6.76 ($\pm$0.04)
A-4	43.16 ($\pm$1.12)	0.4233	3206.81	6.73 ($\pm$0.03)
A-5	42.01 ($\pm$0.69)	0.4086	3095.45	6.63($\pm$0.06)
A-6	42.36 ($\pm$0.84)	0.4155	3147.72	6.60 ($\pm$0.03)
A-7	45.13 ($\pm$0.92)	0.4327	3353.88	6.91($\pm$0.04)
A-8	44.63 ($\pm$1.15)	0.4248	3316.66	7.03 ($\pm$0.05)
A-9	45.56 ($\pm$0.46)	0.4469	3385.60	7.16 ($\pm$0.03)

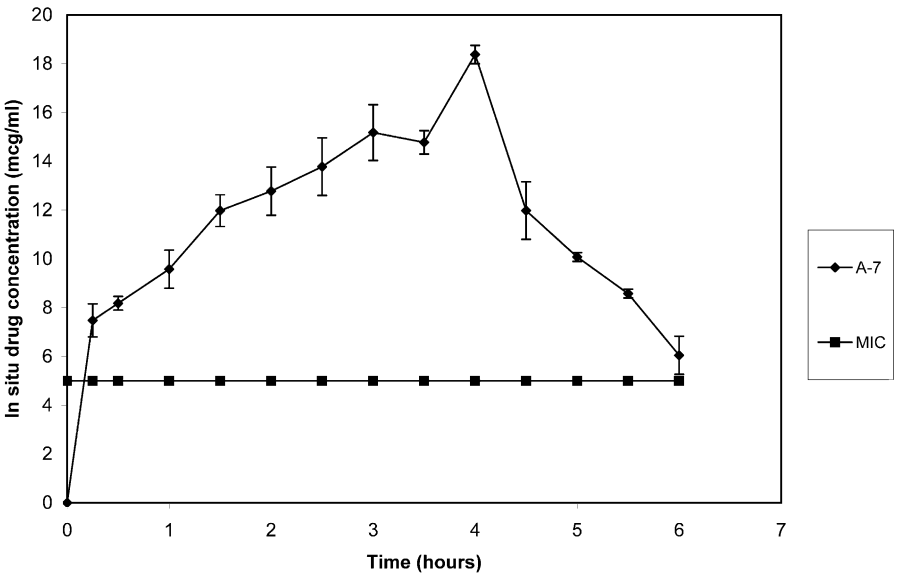

Fig. 4. In situ release of cetylpyridinium chloride from optimized disk.

Table 3
In situ parameters of optimized disk

C_{max} (μg/ml)	T_{max} (h)	AUC_{0-8} (μg/ml h)	$T^{>MIC}$ (h)	Adhesion time (h)
18.38 ($\pm$1.18)	4.5 ($\pm$0)	83.247($\pm$3.93)	0.25–6.0	6.0 ($\pm$0)

3.4. Surface pH

Surface pH of disk of cetylpyridinium chloride was taken into consideration so that the disk does not cause irritation to the buccal mucosa. This concept has not been considered by earlier workers and no mention of surface pH of cetylpyridinium chloride disks has been made. This formulation exhibited surface pH of 6.91. The surface pH of disks is shown in Table 2.

3.5. Microbiological studies

Optimized disks were tested against strains commonly implicated in oro-dental infections and were found to be very effective in inhibiting the growth of all the strains. The disk inhibited the growth of the strains as shown in Table 4. Samples released from in situ studies also showed inhibition of growth of the strains as shown in Tables 4 and 5, thus proving the effectiveness of the disk.

3.6. In vivo studies in healthy human volunteers

In vivo evaluation of the optimized disks on healthy human volunteers revealed that the disks eroded completely and none had to be removed due to irritation. The disks did not cause any discomfort to the volunteers and the taste was acceptable. No side effects like taste alteration, heaviness or severe salivation was observed with the disks. A lot of inter-individual variation was observed for the C_{max}, T_{max}, AUC_{0-8} and time of erosion (adhesion time) as shown in Table 6. This was probably due to the variation in the individuals with respect to the salivary flow rate, the oral anatomy and the individuals movement pattern of the tongue (Bouckaert et al., 1992; Khanna et al., 1997). Salivary drug levels revealed that the optimized disk was able to maintain concentration of the drug well above MIC (≈ 5 μg/ml) over a period of 8.0 h in the oral cavity as shown in Fig. 5. The drug release can also be attributed after complete erosion of the formulation, i.e. after 8.0 h possibly due to reversible binding of the drug to the oral mucosa (Ali et al., 2000). The scatter diagram (Fig. 6) shows a positive correlation between the percentage drug release attained in situ

Table 4
Effect of mucoadhesive tablets on the strains

Time of diffusion	Effect on the strains			
	S. aureus	*E. coli*	*S. mutans*	*C. albicans*
1.0	NG	NG	NG	NG
2.0	NG	NG	NG	NG

NG, no growth. Growth was seen in all the plates without cetylpyridinium disks.

Table 5
Inhibition of growth of in situ samples against different tested strains

Time (h)	Zone mean diameter *S. aureus* ($\pm$S.D.)	Zone mean diameter *E. coli* ($\pm$S.D.)	Zone mean diameter *S. mutans* ($\pm$S.D.)	Zone mean diameter *C. albicans* ($\pm$S.D.)
0.25	2.63 ($\pm$0.12)	1.40 ($\pm$0.16)	5.03 ($\pm$0.12)	2.33 ($\pm$0.12)
0.5	2.70 ($\pm$0.14)	1.43 ($\pm$0.12)	5.10 ($\pm$0.16)	2.40 ($\pm$0.16)
1.0	2.76 ($\pm$0.12)	1.47 ($\pm$0.20)	5.23 ($\pm$0.12)	2.43 ($\pm$0.12)
2.0	3.00 ($\pm$0.08)	1.53 ($\pm$0.20)	5.53 ($\pm$0.20)	2.47 ($\pm$0.20)
4.0	3.03 ($\pm$0.12)	1.63 ($\pm$0.16)	5.67 ($\pm$0.24)	2.57 ($\pm$0.20)
6.0	2.66 ($\pm$0.20)	1.40 ($\pm$0.21)	4.97 ($\pm$0.24)	2.33 ($\pm$0.24)

Table 6
Important parameters of optimized disk in healthy human volunteers ($n = 6$)

C_{max} (μg/ml)	T_{max} (h)	AUC_{0-8} (μg/ml h)	$T^{>MIC}$ (h)	Adhesion time (h)
16.84 ($\pm$2.63)	3.08 ($\pm$1.33)	87.13 ($\pm$4.17)	>8 ($\pm$0)	5.58 ($\pm$0.33)

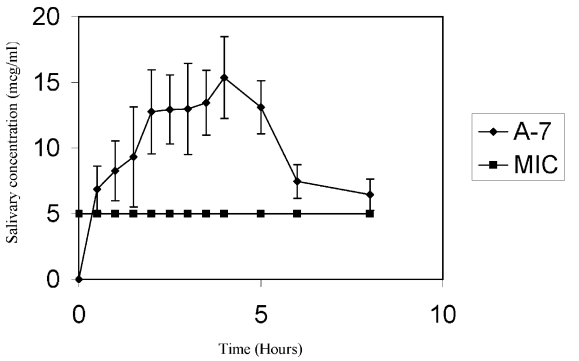

Fig. 5. Salivary concentration of the drug at various time intervals in healthy human volunteers.

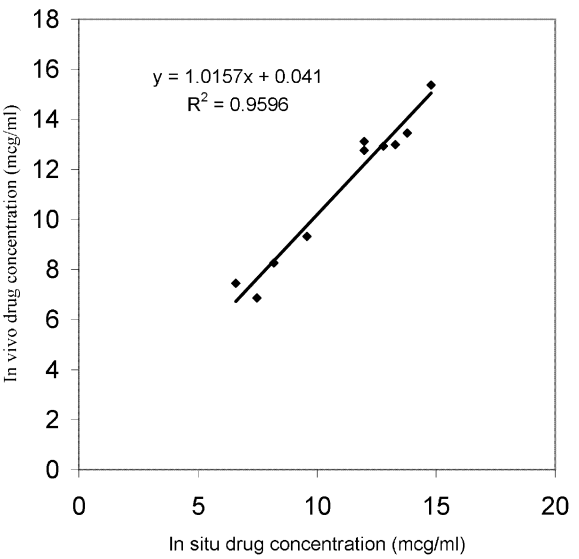

Fig. 6. Scatter diagram showing correlation between in situ and in vivo drug concentration.

and in vivo (salivary drug levels). The correlation coefficient of 0.959 was obtained, which was statistically significant at 5% confidence level ($P < 0.05$).

4. Conclusions

Bioadhesive erodible disk of cetylpyridinium chloride was successfully developed. It exhibited good controlled and delayed release pattern, which is effective for overnight therapy. With this system targeting could be achieved at the diseased site over a longer period of time thus increasing efficacy, compliance and better clinical usefulness.

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