Chitosan inserts for periodontitis: Influence of drug loading, plasticizer and crosslinking on *in vitro* metronidazole release

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Chitosan based metronidazole (MZ) inserts were fabricated by the casting method and characterized with respect to mass and thickness uniformity, metronidazole loading and *in vitro* metronidazole release kinetics. The fabricated inserts exhibited satisfactory physical characteristics. The mass of inserts was in the range of 5.63 ± 0.42 to 6.04 ± 0.89 mg. The thickness ranged from 0.46 ± 0.06 to 0.49 ± 0.08 mm. Metronidazole loading was in the range of 0.98 ± 0.09 to 1.07 ± 0.07 mg except for batch CM3 with MZ loading of 2.01 ± 0.08 mg. The inserts exhibited an initial burst release at the end of 24 h, irrespective of the drug to polymer ratio, plasticizer content or cross-linking. However, further drug release was sustained over the next 6 days. Cross-linking with 10% (*m/m*) of glutaraldehyde inhibited the burst release by ~30% and increased the mean dissolution time (MDT) from 0.67 to 8.59 days. The decrease in drug release was a result of reduced permeability of chitosan due to cross-linking.

Keywords: chitosan inserts, cross-linking, metronidazole, periodontitis, sustained release

Periodontitis, a disease involving supportive structures of the teeth prevails in all groups, ethnicities, races and both genders. The relationship between bacterial plaque and the development of periodontal disease and caries is well established (1). Antibacterial agents have been used effectively in the management of periodontal infection. The effectiveness of mechanical debridement of plaque and repeated topical and systemic administration of antibacterial agents are limited due to the lack of accessibility to periodontopathic organisms in the periodontal pocket (2). Systemic administration of drugs leads to therapeutic concentrations at the site of infection, but for short periods of time, forcing repeated dosing for longer periods (3). Local delivery of antimicrobials has been investigated for the possibility of overcoming the limitations of conventional therapy. The use of sustained release formulations to deliver antibacterials to the site of infection

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(periodontal pocket) has recently gained interest. These products provide a long-term, effective treatment at the site of infection at much smaller doses (4). Biodegradable polymers are extensively employed in periodontal drug delivery devices because of their abundant source, lack of toxicity, and high tissue compatibility (5). A major advantage of natural polymers is that they do not affect periodontal tissue regeneration. Amongst various natural polymers, chitosan, a deacetylated product of chitin is widely used in drug delivery devices. Since it exhibits favourable biological properties such as non-toxicity, biocompatibility (6), biodegradability and wound healing traits (7), it has attracted great attention in the pharmaceutical and biomedical fields.

Metronidazole (MZ), a nitroimidazole derivative, is effective against periodontal pathogens (8–10). It is the drug of choice in the case of pathogenic Gram negative anaerobes. Systemic metronidazole has been employed in the control of chronic periodontal diseases (11, 12). Topical metronidazole therapy has been used as an alternative (13) and as an adjunct to scaling and root planning (14). Dental gels containing metronidazole (25%, *m/V*) in glyceryl monooleate have been studied (15–17). The present study is aimed at developing chitosan biodegradable inserts of metronidazole for local long-term treatment of periodontal disease. The fabricated inserts were evaluated for their physical properties and i*n vitro* drug release characteristics.

EXPERIMENTAL

Materials

Metronidazole was obtained as a gift sample from M/s Ranbaxy Laboratories (India). Chitosan (viscosity 0.05 Pa s), was generously donated by Cochin Fisheries Ltd (India). All other reagents used were of analytical grade.

Methods

Preparation of inserts. – Drug loaded and placebo inserts were prepared by the method described by Oungbho and Muller (18), with modification. A homogeneous gel--like solution of chitosan was prepared by dispersing weighed amount of chitosan in 2% (*V/V*) acetic acid under constant stirring. Metronidazole was passed through a 53-μm sieve and dispersed in 1% (*V/V*) acetic acid solution. Drug dispersion was mixed with chitosan solution for 6 h. A measured amount of cross-linking agent (glutaraldehyde/formaldehyde) was added to the solution and stirred for 6 h. Plasticizer (propylene glycol, PG) was then added to the drug-polymer mixture (Table I). After deaerating under vacuum for 6 h, the drug-polymer mixture was poured on leveled glass moulds at 50 °C and dried for 10–12 h. The films were subdivided into inserts (2 x 6 mm) by punching out. Dried films were wrapped in butter paper and stored in amber colored glass vials in a desiccator until further use.

Physical properties of inserts. – The fabricated inserts were evaluated for thickness, mass and drug content uniformity (19). Drug content uniformity, between batches and within batches, was determined by dissolving individually weighed inserts (*n* = 6) in 5 mL

Table I. Composition of the fabricated metronidazole inserts by casting method in 2% (V/V) glacial acetic acid

Batch code	Chitosan (mg cm^{-2})	Metronidazole (mg per insert)	Plasticizer PG (%, *m/m*)	Cross-linking agent (%, *m/m*)
CM1	40	1	10	–
CM2	40	1	40	–
CM3	40	2	10	–
CM4	40	1	40	5 (HCHO)
CM5	40	1	40	10 (HCHO)
CM6	40	1	40	5 (glutaraldehyde)
CM7	40	1	40	10 (glutaraldehyde)

PG – propylene glycol

of glacial acetic acid in a volumetric flask. After gentle swirling for an hour, the volume was made up to 100 mL with McIlvaine's buffer pH 6.6 (potassium dihydrogen orthophosphate and citric acid in water). The final pH of the buffer solution was adjusted using either 1 mol L^{-1} potassium dihydrogen orthophosphate solution or 0.5 mol L^{-1} citric acid solution, as required. The resultant solution was filtered through a G-2 glass filter (40 μm). An aliquot of the filtrate was suitably diluted and analyzed for metronidazole content at 316 nm on a UV spectrophotometer (UV-1601, Shimadzu, Japan). The thickness of the inserts was measured at 5 different randomly selected spots with a screw gauge. For mass uniformity, 10 inserts from each batch were weighed individually and their average was determined.

In vitro *release studies.* – Weighed inserts were placed in a stainless steel wire mesh (250 μm) holder of dimensions 4 x 8 mm and suspended in amber colored vials containing 10 mL of McIlvaine's buffer pH 6.6 (to simulate pH of gingival crevicular fluid *in vitro*) as the dissolution medium. The vials were stopped and placed on a vial holder fitted in a water bath thermostated at 37 ± 1 °C. At pre-determined time intervals, the dissolution medium was completely withdrawn and replaced with a fresh 10 mL portion of pre--warmed buffer, which ensured sink conditions. Since the formulations provide a high initial burst release of metronidazole, during the first 24 h drug release study, samples were collected every hour to maintain sink conditions. Metronidazole released was determined at 316 nm after suitable dilution. A dissolution study of film devoid of metronidazole was performed concurrently to nullify any interference by film materials.

Drug release kinetics. – The drug release kinetics from the fabricated inserts was evaluated using Higuchi's equation: $Q = kt^{1/2}$ (20), where Q is the amount of drug released, k is the release constant and t is time. Further, the data was fitted to Korsmeyer-Peppas' power law equation: $M_t/M_\infty = Kt^n$ (21), where, M_t/M_∞ is the fraction of drug released in time t, K is the structural and geometric constant, and n is the release exponent.

Statistical evaluation. – The *in vitro* data were analyzed for statistical significance using Student's *t*-test.

RESULTS AND DISCUSSION

The inserts were prepared by the casting method with controlled solvent evaporation. However, incorporation of plasticizer (propylene glycol) was necessary to obtain films with sufficient flexibility and allow subsequent sub-division into inserts. The plasticizer concentration was optimized to 40% (*V/V*). Although a lower level of propylene glycol could have been used, on drying harder inserts with little or no flexibility were obtained. The formulated inserts were evaluated for various physico-chemical properties and the results are given in Table II. The mass and thickness of the prepared inserts was in the range of 5.63 ± 0.42 to 6.04 ± 0.89 mg and 0.46 ± 0.06 to 0.49 ± 0.08 mm, respectively. The drug content was found to be in the range of 0.98 ± 0.09 to 1.07 ± 0.07 mg except for batch CM3 with 2.01 ± 0.08 mg MZ.

Table II. Physico-chemical characterization and release rate constants of fabricated insert

Batch code	Mass (mg)[a]	Thickness (mm)[a]	Drug content (mg)[a]	k (μg h$^{-1/2}$ cm^{-2})	R
CM1	5.63 ± 0.42	0.48 ± 0.07	0.98 ± 0.09	0.78	0.9783
CM2	5.94 ± 0.53	0.49 ± 0.08	1.06 ± 0.06	0.91	0.9281
CM3	5.97 ± 0.89	0.47 ± 0.07	2.01 ± 0.08	0.95	0.8131
CM4	5.86 ± 0.87	0.46 ± 0.06	0.99 ± 0.03	0.85	0.9917
CM5	6.01 ± 0.87	0.47 ± 0.07	1.02 ± 0.08	0.80	0.9882
CM6	6.04 ± 0.89	0.46 ± 0.09	1.07 ± 0.07	0.92	0.9627
CM7	5.98 ± 0.91	0.48 ± 0.07	1.05 ± 0.07	0.98	0.9738

[a] Mean ± SD, n = 3.

Drug release studies were performed by a static method. The inserts released the drug in a biphasic manner with an initial burst release followed by slow release for the next 7 days. The lowest burst release of 68% was observed with glutaraldehyde (10%, *m/V*) crosslinked inserts batch CM7 (1 mg MZ, PG 40%, *m/m*). However, further drug release was sustained for the next 7 days of the release study. The burst effect in the initial stages could be due to the release of drug entrapped on the surface of inserts. Considering the initial pathological load in the periodontal pocket, an initial burst release of anti-microbial is always desirable.

In vitro release studies show that the metronidazole to chitosan ratio plays an important role in the modulation of metronidazole release from the inserts (Fig. 1). Drug release decreased as the chitosan ratio in the inserts increased. The release of metronidazole from batch CM3 (2 mg MZ per insert, PG 10%, *m/m*) was at a rate of 39 μg h^{-1} on day 1 in comparison with 33 μg h^{-1} from batch CM1 (1 mg MZ per insert, PG 10%, *m/m*). Thus, nearly 94 and 80% of drug was depleted, respectively, from inserts CM3 and CM1 with no cross-linking agent, in 24 h. The increased release rate from CM3 might partly due to the drug entrapped in the superficial layer of the insert. When the insert comes in contact with dissolution medium, the drug from the surface leaches into the surrounding medium, leaving a more porous polymer structure, which enables faster drug diffusion
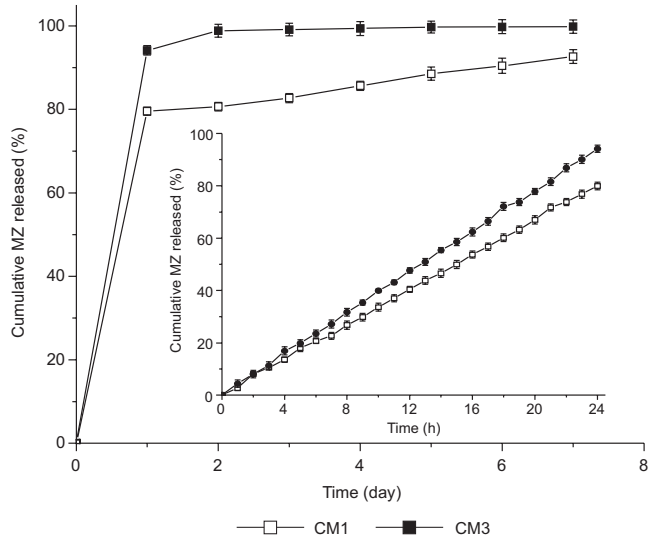
Fig. 1. Effect of metronidazole loading on *in vitro* release rate from inserts at 24-h time intervals; inset graph represents drug release in the first 24 h (Mean ± SD, *n* = 3).

from the matrix. The release rate was found to decrease with increasing the polymer concentration. As shown in Fig. 2, the concentration of propylene glycol in the inserts seems to affect the release rate. It was observed that as the concentration of propylene glycol increased from 10 to 40%, *m/m*, the rate of metronidazole release increased from 33 µg h^{-1} in CM1 (10%, *m/m*, PG) to 38 µg h^{-1} in CM2 (40%, *m/m*, PG). The change in the observed release rate was possibly due to alteration in membrane permeability caused by the modification of film hydrophilicity by the plasticizer (22).
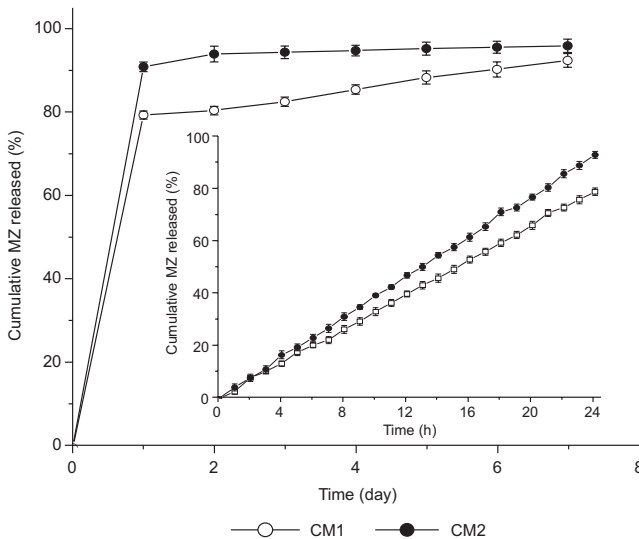

Fig. 2. Effect of plasticizer content on *in vitro* metronidazole release at 24-h time intervals; inset graph represents drug release in first 24 h (Mean ± SD, *n* = 3).
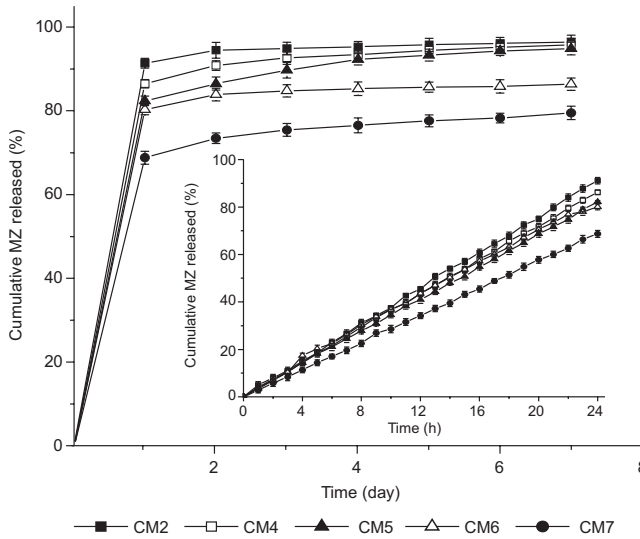
Fig. 3. Effect of cross-linking with formaldehyde and glutaraldehyde on *in vitro* release rate of metronidazole in comparison to uncross-linked inert; inset graph represents drug release in first 24 h (Mean ± SD, *n* = 3).

The release rate was affected by the cross-linking of chitosan inserts (Fig. 3). Cross-linking of the inserts with either formaldehyde or glutaraldehyde decreased the drug release rate in comparison with uncross-linked inserts (*e.g.*, CM2, 1 mg MZ, PG 40%, *m/m*). However, even after cross-linking, there was no significant decrease in the burst release. Of the two aldehydes tested, glutaraldehyde as cross-linking agent was more effective in decreasing both the burst release and subsequent release rate. Chitosan-metronidazole insert, cross-linked with 10% (*m/m*) glutaraldehyde (CM7, 1 mg MZ per insert, PG 40%, *m/m*) had an initial burst release rate of 29 μg h^{-1}, which gradually decreased to around 1 μg h^{-1} at the end of 48 h. Further release rate was maintained at 1 μg h^{-1} to the end of day 7. The mean dissolution time (MDT) increased to 6.4 and 8.6 days, respectively, for CM6 and CM7 both containing 1 mg MZ per insert but crosslinked with 5 and 10% (*m/m*) glutaraldehyde, respectively. This was a significant change since the not cross-linked insert (CM2) exhibited MDT of 0.7 days. This difference in release rate is due to the decrease in the permeability coefficient of the cross-linked membrane resulting from the cross-link points (23). A similar decrease in meloxicam release from cross-linked gelatin inserts has been reported recently (24).

Table II shows the treatment of release data according to the Higuchi equation. The results indicate that the amount of drug released per unit area (*Q*) increased with the square root of time. Furthermore, the release coefficient *n* < 0.45 for all prepared batches, suggests diffusion as the dominant release mechanism, and not a combination of swelling and erosion. The release rate constant (*k*) was > 0.7 for all batches. The high release rate constant was a result of the initial burst release exhibited by the inserts.

CONCLUSIONS

The inserts exhibited burst release, which is desirable considering the initial pathogenic load in periodontal pockets, but provided sustained release for subsequent six days. The *in vitro* evaluation results are encouraging and need further evaluation of the inserts in patients suffering from periodontitis.

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S A Ž E T A K

Kitozanski umetci za periodontitis: Utjecaj količine lijeka, plastifikatora i umrežavanja na oslobađanje metronidazola *in vitro*

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Umetci metronidazola na bazi kitozana načinjeni su kasting metodom. Proučavana je ujednačenost mase i debljine, količina ljekovite tvari i kinetika oslobađanja metronidazola *in vitro*. Fizičke karakteristike umetaka bile su zadovoljavajuće: masa je bila u rasponu od 5,63 ± 0,42 – 6,04 ± 0,89 mg, debljina od 0.46 ± 0.06 – 0.49 ± 0.08 mm, količina metronidazola od 0,98 ± 0,09 – 1,07 ± 0,07 mg osim u pripravku CM3 s MZ 1,07 ± 0,07 mg. Nakon 24 h, neovisno o omjeru ljekovite tvari i polimera, količini plastifikatora ili umrežavanju, dio metronidazola se naglo oslobodio iz svih umetaka. Međutim, daljnje je oslobađanje bilo polagano, tijekom 6 dana. Umrežavanje s 10% (*m/m*) otopinom glutaraldehida spriječilo je naglo oslobađanje za ~30% i povećalo srednje vrijeme oslobađanja (MDT) s 0,67 na 8,59 dana. Smanjenje u oslobađanju ljekovite tvari posljedica je smanjenja permeabilnosti umreženog kitozana.

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