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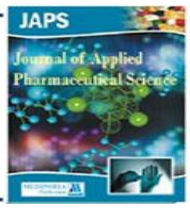

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Development and in vitro characterization of metronidazole loaded chitosan microspheres for delivery to periodontal pocket

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ABSTRACT

The aim of the present work was to develop drug delivery system for localized controlled release of metronidazole following insertion into and/or around the periodontal pocket. This would ensure increased local drug concentration at the periodontal site to maintain an effective concentration over an extended period of time and a decrease in superfluous distribution of the drug to other body organs with a subsequent decrease in side effects. In the present work, metronidazole loaded chitosan microspheres were prepared by external gelation technique using tripolyphosphate as the cross-linker. The drug to polymer ratio was chosen at three levels: 1:4, 1:5 and 1:6 (by weight) and tripolyphosphate concentration also at three levels: 6, 12 and 18 (%). The microspheres were characterized for surface morphology, particle size, drug entrapment efficiency, swelling, erosion, bioadhesion and drug release profile. Nearly spherical, rough and porous particles (size ~ 800 μm) were obtained. Drug entrapment efficiency was found to be in the range of 60-75%. Percentage swelling, erosion and bioadhesion ranged from 10-25%, 5-15% and 43-59% respectively. The results indicated that formulation of metronidazole in chitosan microspheres could be utilized as a potential drug delivery system to periodontal pocket.

Key words: Biodegradable polymer, external gelation, local delivery, microparticles, mucoadhesive, periodontitis.

INTRODUCTION

Periodontal pocket provides ideal conditions for the proliferation of microorganisms: primarily Gram negative, facultative anaerobic species. Systemically applied antimicrobials have been advocated for the treatment of severe forms of periodontal infections (Loesche, 1999). Control of bacterial plaque helps in slowing or arresting periodontal 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). However, systemic antibiotic administration can be essential in eliminating pathogenic bacteria that invade gingival tissue and in helping control periodontal pathogens residing in various domains of the mouth from where they may translocate to periodontal sites. Multiple systemic doses of antibiotics have shown several drawbacks including inadequate antibiotic concentration at the site of the periodontal pocket (Pitcher *et al*., 1980). Adequate concentration at the site of action is not achieved as the drug gets diluted thousand fold times, thus leading to low benefit to high-risk ratio (Pandit, 1997); a rapid decline of the plasma antibiotic concentration to subtherapeutic levels (Gates, *et al.*, 1994); development of microbial resistance; and high peak-plasma antibiotic concentrations, which may be associated with side effects like hypersensitivity, gastrointestinal intolerance, depression, and

tachycardia (Slots and Rams, 1990). Due to disadvantages of the systemic administration, of late considerable attention has been given to local delivery systems of antimicrobial agents for treatment of periodontal diseases. Conventional antimicrobial formulations for the mouth, such as toothpaste and mouthwash, have short duration of action and very low penetration into periodontal pocket. Each system tested had release half life ranging from minutes to hours necessitating their replacement at regular intervals. A targeted sustained release device which could be inserted in the periodontal pocket and prolong the therapeutic level at the site of action at a much lower dose is the need of the hour. The advantages offered by a targeted sustained release device include freedom from undesirable side effects to different parts of the body as the device is targeted to the periodontal pocket. Moreover, patient acceptability is higher; the device can be placed within the periodontal pocket in a short time with minimal pain and discomfort and is therapeutically active at a much lower concentration (Goodson *et al*., 1985; Kornman, 1993; Schwach Abdellaoui *et al*., 2000).

Chitosan, a deacetylated product of chitin is widely used in drug delivery devices. Since it exhibits favorable biological properties such as non-toxicity, biocompatibility, biodegradability and wound healing traits (Muzzarelli *et al.*, 1988), it has attracted great attention in the pharmaceutical and biomedical fields. Chitosan based drug delivery systems apart from these properties also stay longer in the oral cavity, exhibit adequate drug penetration, shows excellent antimicrobial activity, and high efficiency and acceptability (Tharanathan and Prashanth, 2007). Chitosan based drug delivery system represent an attractive and strategy for achieving the therapeutic concentration of drug in periodontal pocket.

Chitosan microspheres have been successfully employed as a potential periodontal drug delivery carrier. They could preferably be formulated as a chip or could be part of a dental paste formulation, or otherwise be directly injected into the periodontal cavity (Jain *et al*. 2008). Chitosan microsphere of tetracyclines (Govender *et al*., 2005), doxycycline (Babu *et al*., 2008), natamycin (Sargon et al., 2003), ofloxacin (Zhang *et al*., 2006) have been reported.

Metronidazole, a broad spectrum antibiotic, was selected in the present study owing to its efficacy on dental bacteria specifically on Gram negative bacteria, its sparing solubility in water leading to poor dissolution and diffusion from microspheres and hence more extended drug release can be expected. It doesn't cause teeth staining like tetracycline, chlorhexidine.

MATERIAL AND METHODS

Materials

Metronidazole was kindly provided as a gift sample from Aarti Drugs Ltd., Mumbai. Chitosan (degree of deacetylation, 85.2%) particles with a viscosity 50 cps was obtained as a gift sample from CFTI, Kochi, India. All other chemicals used were purchased from Himedia (India) and were of analytical grade and were used as received.

METHODS

Preparation of chitosan microspheres

In the present study, the microspheres were prepared by external gelation technique (Fig. 1). The characteristic feature of this technique include ease of preparation, higher drug entrapment, less frequency of administration required, higher amount of polymer ensures extended drug release, large particle size than those prepared by other methods, but these particle can be inserted by number according to the depth of pocket. It minimizes an additional process like in films, of cutting appropriate size film to fit periodontal pocket. The process variables selected for the present study were drug and polymer ratio and concentration of crosslinking agent. The composition of the various formulations is given in Table 1.

Table 1: Composition of various microspheres.

Formulation code	Drug [mg]	Chitosan [mg]	Drug : polymer	TPP in 50 ml water [g]
a1	50	200	1:4	3
a2	50	250	1:5	3
a3	50	300	1:6	3
b1	50	200	1:4	6
b2	50	250	1:5	6
b3	50	300	1:6	6
c1	50	200	1:4	9
c2	50	250	1:5	9
c3	50	300	1:6	9
d1 (blank)	0	200	0	3

Table 2: Characterization of metronidazole loaded microspheres.

Formulation code	% Drug entrapment	Particle size (µm)	Percentage swelling	Percentage erosion	Percentage bioadhesion
a1	60.78 ± 0.41	825.38 ± 0.85	18.34 ±1.47	9.96 ± 1.32	58.66 ± 1.57
a2	69.88 ± 0.31	875.84 ± 0.82	22.68 ±0.82	8.07 ± 0.26	55.25 ± 1.15
a3	71.57 ±.0.32	894.88 ± 1.54	24.78 ±1.09	18.07 ± 0.99	48.34 ± 1.52
b1	59.92 ± 0.49	815.86 ± 0.59	12.25 ±0.48	8.97 ± 0.64	57.85 ± 1.00
b2	72.74 ± 0.33	855.84 ± 0.82	15.25 ±1.04	4.93 ± 0.78	53.33 ± 1.08
b3	75.45 ± 0.45	882.50 ± 1.13	19.38 ±0.98	13.70 ± 0.36	47.12 ±1.35
c1	61.11 ± 0.40	801.58 ± 0.76	10.30 ±0.37	8.773 ± 0.87	57.34 ± 1.42
c2	69.6 ± 0.05	841.56 ± 0.94	16.97 ±0.93	7.273 ± 0.44	51.66 ± 1.21
c3	72.48 ± 0.55	871.08 ± 1.07	21.08 ±0.98	14.85 ± 0.52	43.33 ± 0.87
d1	0	788.25 ± 0.39	13.18 ±0.66	1.75 ± 0.24	68 .00 ± 1.73

Values represent mean ± Std. deviation (n=3)

Characterization

Surface morphology of microspheres

Scanning Electron Microscopy (SEM) (JEOL JSM-6390, Japan) was used to evaluate the surface texture, shape and size of the microspheres. The photomicrographs are presented in Fig. 2 and 3.

Particle size

The shape and size of particle and their size distribution are of profound importance with regards to the physicochemical

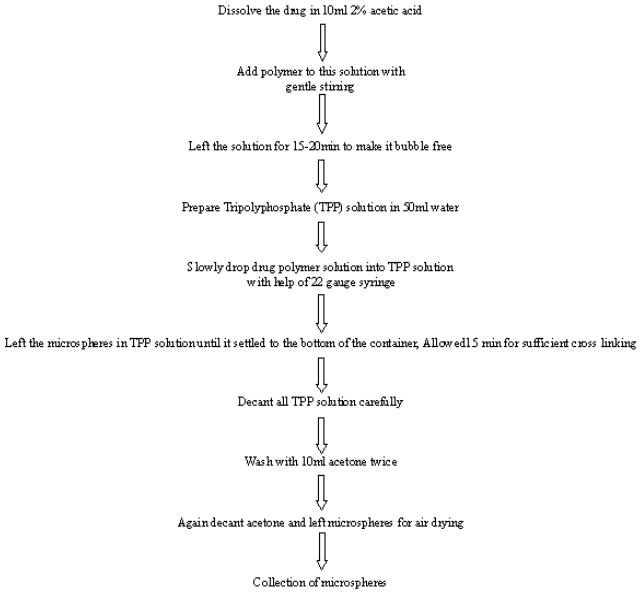

Figure 1: Flow chart for the method of preparation of chitosan microspheres

properties of the prepared granule. Particle size of the prepared microspheres was examined by optical microscopy under 10X. This measures the particle size as projected diameter (Table 2).

Drug entrapment efficiency

50 mg of chitosan microspheres loaded with metronidazole were transferred into glass mortar and crushed and further digested in 0.1N HCl for 10min to dissolve the drug. The solution was filtered and an aliquot was assayed sphectrophotometrically to quantify for drug content (Table 2).

$$\text{Drug entrapment} = \frac{\text{Amount of drug in known amount of spheres}}{\text{Intial drug load}} \times 100$$

Swelling and Erosion

Accurately weighed quantity of microspheres (50mg) was immersed in phosphate buffer (pH 6.8) and at regular intervals of time the beads were reweighed after carefully wiping off excess of liquid with a tissue paper. It was observed that 1hr was sufficient for maximum swelling of microspheres. For determing erosion, microspheres were dried at 70 ^{o}C for 15 min and reweighed after maximum swelling. Water uptake and mass loss were determined according to the following equations:

$$\text{Degree of swelling (water uptake)} = \frac{\text{Wet weight - original dry weight}}{\text{Original dry weight}} \times 100$$

$$\text{Erosion (\% mass loss)} = \frac{\text{Original weight - remaining dry weight}}{\text{Original weight}} \times 100$$

The results are tabulated in Table 2.

Bioadhesion study

The bioadhesive profile was studied in terms of the adhesion number. 50 microspheres were dropped on excised goat buccal mucosa immediately hydrated with phosphate buffer pH 6.8) from a fixed distance. Adhesion number for microspheres was determined as the ratio of the number of particles attached to the substrate to the total number of applied particles, expressed as a percentage (Table 2). The adhesion strength increases with an increase in the adhesion number.

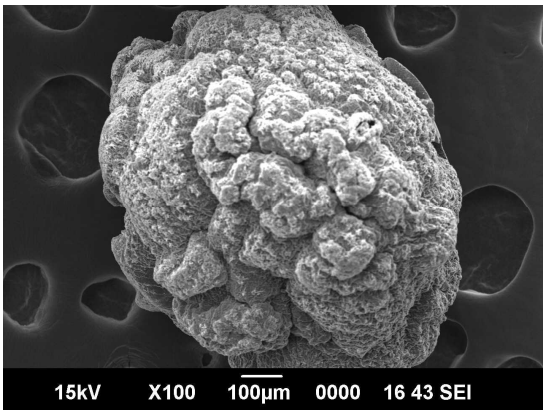

Fig 2: Cumulative % drug release from various formulations.

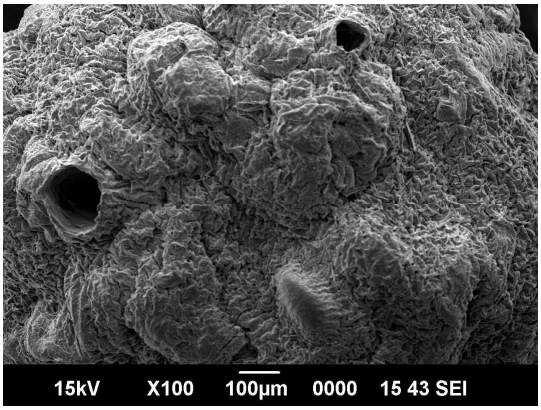

Fig 3: Scanning electron microscopy of chitosan microspheres prepared by external gelation method.

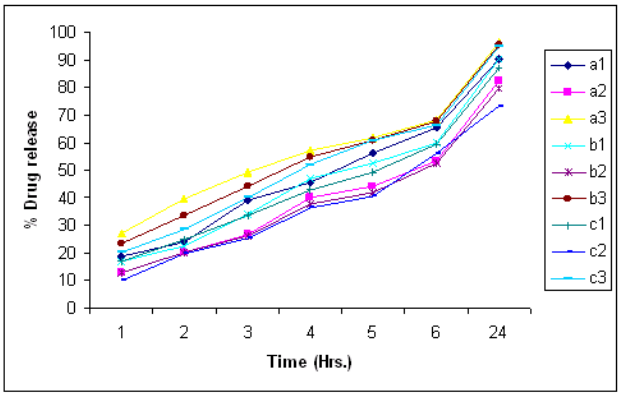

Fig 4: Scanning electron microscopy of chitosan microspheres prepared by external gelation method.

Drug release study

50mg accurately weighed microspheres were used to study drug release profile using USP Type II Paddle dissolution apparatus. Dissolution flask was filled with 90ml of 6.8 phosphate buffer and rpm of the paddle stirrer was adjusted to 20. At frequent

time intervals, 1ml aliquot was withdrawn and after sufficient dilution was analyzed spectrophotometrically. The sample withdrawn was replaced by an equal quantity of fresh phosphate buffer. The drug release profile is presented graphically in Fig. 4.

RESULT AND DISCUSSION

In the present study, the microspheres were prepared by external gelation technique. The effect of two process variables viz., drug and polymer ratio, and cross-linking time was studied. It was observed that 45-60 minutes in TPP solution were sufficient for proper crosslinking of microspheres. In this period all the microspheres settled to the bottom of the container. Short period produced fragile microspheres, whereas on keeping them overnight, leaching of metronidazole from microparticle was observed. Overnight cross-linking led to ruptured microspheres and crystals of metronidazole were clearly seen in the TPP solution.

SEM of chitosan microspheres illustrated their nearly spherical shape with very rough, heterogeneous and porous surface. The surface morphology may have important implications for bioadhesion. It has been reported (Vasir *et al.*, 2003) that microspheres with a coarser and more porous surface may offer enhanced bioadhesivity as compared to those with a more smoother texture. Therefore, the rough, coarse structure observed in this study may have led to the observed bioadhesion. SEM of the optimized formulation b2 indicated that surface had crenations. On considering formulation b3, pores may be because of coiling of chitosan polymer on increasing the concentration and also due to immediate crosslinking at very high concentration of TPP.

Drug entrapment was increased with the higher polymer concentration. The data revealed that in chitosan microspheres entrapment increases with the higher drug:polymer ratio. On evaluating the effect of crosslinking agent on microspheres preparation it was evident that there was no direct relationship between crosslinker concentration and entrapment efficiency. But the magnitude of enhancement of entrapment is not as such for b3 and c3 as their polymer concentration increased. This may be because at higher polymer concentration coiling of polymer occurs which leads to pore formation, permitting leaching of the drug through these pores and in this way the final drug entrapment decreases. A minor increment in the drug entrapment of the b2 and c2 indicate that 12% concentration of TPP was sufficient for cross-linking and efficient drug entrapment.

On increasing the concentration of chitosan, microspheres showed higher swelling characteristics as well as erosion kinetics. Formulations a2, b2 and c2 showed lower swelling than a3, b3 and c3 respectively that indicate swelling increased with higher concentration of the polymer. As the concentration of crosslinking agent increased swelling and erosion decreased in a1, b1, c1.

Particle size of the microspheres obtained were increased with the chitosan concentration. Plane microspheres d1 had particle size approx. 700µm. It seems that particle size were large so it could be administered as by number according to the depth of the periodontal pocket. Sizes of particle increased with the higher polymer concentration.

At a higher chitosan concentration, coiling of the polymer molecules may have occurred reducing the flexibility of the polymeric chain thereby reducing the bioadhesive strength. At lower chitosan concentrations, the polymer structure of the microspheres may have been looser and the polymer chains therefore had more space to extend within the mucin. It can be seen that TPP concentration had a positive effect on bioadhesion. It has been documented that flexibility of the polymer chains is required for interpenetration and entanglement with mucin. Highly crosslinked polymers decrease the mobility of the individual polymer chains and can therefore lead to decreased bioadhesive strength (Vasir *et al.*, 2003). Therefore, these findings were contrary to what was expected; namely a decrease in the bioadhesive force with an increase in TPP due to an increased crosslinking and hence decrease in the effective length of the polymeric chain for penetration into the mucus layer and hence bioadhesion. Rather, the increased bioadhesion with an increase in crosslinking could be attributed to the fact that an increased interaction of chitosan with the negatively charged TPP instead of mucin may have led to more sites on the negatively charged sialic acid residues of mucin being additionally available for interaction with the positively charged drug (Govinder *et al.*, 2005).

Highest % bioadhesion was obtained in formulation a1, b1, and c1 but the studies suggested that on increasing drug polymer ratio no significant change was observed but little bit decrease with increasing crosslinking agent was evident. This can be attributed to the neutralization of the positive charge of the chitosan polymer by the phosphate molecule, and thus attraction of the chitosan microspheres toward negative charged membrane is reduced.

Dissolution studies revealed that formulation b2 and c2 gave slowest drug release profile. The microsphere with a drug: polymer ratio of 1:6 released the drug more rapidly then others having drug: polymer ratio 1:4 and 1:5 in chitosan microsphere. This can be attributed to the coiling of polymer at higher concentration and due to pore formation. Drug release retarded as the cross-linking concentration of TPP increases.

CONCLUSION

The limitations of systemic therapy with antibiotics have evoked an interest in the development of localized drug delivery systems that can provide an effective concentration of antibiotic at the periodontal site for the duration of the treatment with minimal side effects. The delivery system prepared in the present study illustrated a good capacity to entrap high percentage of drug as well as ease of transformation into film and strips. It can also be administered in the form of solution and or applied as such.

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