Formulation and *in-vitro* evaluation of Chitosan films containing tetracycline for the treatment of periodontitis

Mohammed Gulzar Ahmed, R Narayana Charyulu, N M Harish, Prabhakar Prabhu

Department of Pharmaceutics, NGSM Institute of Pharmaceutical Sciences, Paneer, Deralakatte, Mangalore - 574 160, Karnataka, India

Tetracycline is a broad spectrum antimicrobial agent, which is formulated into films and evaluated for the treatment of periodontitis. Chitosan films containing tetracycline in three different concentrations (10, 20, and 30% w/w to the weight of polymer) were prepared by the solution casting method, using 1% v/v acetic acid solution. The prepared films were evaluated for various properties such as weight variation, tensile strength, stability studies, *in-vitro* release, and mass balance studies. Further films were cross-linked in order to extend the drug release. Average weight and thickness among the different films was uniform. Tensile strength was maximum for plain films and minimum for films containing the highest percentage of the drug or cross-linked films. The stability studies did not show any significant changes. Static dissolution studies showed a burst release initially followed by a progressive fall in the release of the drug, and also showed extended release when cross-linking was attempted. The *in-vitro* release kinetics of tetracycline followed the zero order pattern. The fact that the mass balance studies done after *in-vitro* dissolution did not deviate by more than 3% from the experimental drug content, confirms that the drug is in free form rather than bound to the polymer.

Key words: *Films, Chitosan, cross-linking, periodontitis, tetracycline*

INTRODUCTION

Periodontitis, the severely debilitating disease of the periodontium, is characterized by the loss of bone, collagen support of the affected teeth and accumulation of bacterial pathogens, mainly within the periodontal pockets.[1,2] The treatment relies on mechanical and antimicrobial suppression of the etiologic bacteria. To eliminate bacterial infections, antibiotics are administered either locally or systemically, but the systemic therapy dilutes the drug several thousand-folds before it reaches the disease site. Therefore, a large dose and/or prolonged administration are often necessary to maintain an effective drug concentration in the periodontal pocket. Repeated long-term use of systemic antibiotics is associated with potential adverse effects at higher dose levels makes systemic administration unacceptable; therefore, a safe and effective low-dose drug delivery device is highly desirable.[3,4]

One particular problem in the treatment of oral cavity diseases is the short residence time, such as, oral gels, which are easily washed and removed by saliva from the site of application. This may be resolved by using mucoadhesive polymers, which will stay firmly on the applied site. Moreover, polymeric strips are also suitable for protecting the infected surfaces, thus reducing the pain and increasing the treatment effectiveness.[5] The preferred drug, therefore, is the one that does not cause development of resistant bacterial strains.[6] The main advantage of this local route of drug delivery is the reduction of the dose of a drug and the possibility of increasing the concentration of the drug in the periodontal pockets, at the same time keeping a comparatively low systemic concentration of the drug.[7] This appears to be holding some promise in periodontal therapy.

Chitosan is a (1,4)-2 amino-2-deoxy β-D glucan, with similar structural characteristics as that of glucosaminoglycans. It is tough, biodegradable, inert, and non-toxic in nature.[8,9] Drugs dispersed in Chitosan were found to be released at a constant rate, suggesting that Chitosan is a useful matrix for sustained release of drugs.[9] The tetracyclines comprise a group of broad spectrum antimicrobial agents, bacteriostatic in nature, and most of the subgingival

Address for correspondence:
*Dr. Mohammed Gulzar Ahmed, Department of Pharmaceutics,
NGSM Institute of Pharmaceutical Sciences, Paneer, Deralakatte,
Mangalore - 574 160, Karnataka, India.
E-mail: mohammedgulzar@rediff.com*

microorganisms are susceptible at a concentration of less than 1-2 μg/ml. The minimum inhibitory concentration required to inhibit growth of 90% of strains is less than 6 μg/ml.[10] Tetracycline hydrochloride is a semi-synthetic tetracycline, effective against all Gram positive and many Gram negative bacteria and exerts its antibacterial activity by inhibiting microbial protein synthesis.[10] It is the first antibiotic, whose efficacy was evaluated in periodontal studies.[11] Apart from its antibacterial activity, it also exhibits additional pharmacological properties such as collagenase inhibition, that is, it inhibits mammalian collagenolytic enzyme to condition the root surfaces. The drug is acidic and demineralizes the cementum and dentin, which may enhance attachment of fibroblasts to the root surface.[12] These properties are of significance in the management of periodontal diseases. The main objective of the present study is to develop a low-dose delivery system in the form of a film, for local delivery of tetracycline for treating periodontitis.

MATERIALS AND METHODS

A gift sample of Tetracycline was obtained from Karnataka Antibiotics Pvt Ltd., Bangalore, Chitosan (85% deacetylated with viscosity of 8000-11000 cps) from Central Institute of Fisheries Technology, Kochi and all other chemicals used were of analytical grade.

Preparation of plain and drug loaded Chitosan films
Chitosan (2% w/v) was soaked in acetic acid (1% v/v in water) for 24 hours to get a clear solution. This dispersion was filtered through a muslin cloth to remove the undissolved portion of the polymer, and the required amount of the drug (0, 10, 20, and 30% w/w of the drug to the weight of polymer) was added and vortexed for 15 minutes, to dissolve the drug in Chitosan solution. This dispersion was kept aside for 30 minutes for expulsion of air bubbles. The films were cast by pouring the dispersion into the center of leveled glass moulds, which were allowed to dry at room temperature for 24 hours.[13] After drying, the films were cut into strips of the required size (7 × 2 mm). These were wrapped in aluminium foil and stored in a desiccator until further use.

Preparation of cross-linked Chitosan films
For cross-linking, 2% v/v glutaraldehyde was selected as a cross-linking agent. The time duration of cross-linking was selected as two and four hours. On increasing the concentration of glutaraldehyde or time of cross-linking, the films were found to be brittle and hard. The general procedure for cross-linking was followed according to the literature, with little modification.[14] The films containing 30% tetracycline were prepared and subjected to cross-linking by exposing to glutaraldehyde vapors in a chromatography chamber, which was previously saturated with 2% v/v glutaraldehyde vapors for 24 hours. The films were exposed for two and four hours of cross-linking and then dried. After drying, the films were wrapped in aluminum foil and stored in desiccator for further study.[14]

Characterization of the films
Compatibility studies were conducted using Fourier transform infrared (FTIR) spectroscopy of the drug alone, polymer alone, and polymer along with the drug. Physicochemical properties such as size, thickness, content uniformity, weight variation, folding endurance, tensile strength, and percentage moisture loss of the prepared films were determined.

Thickness measurement
The thickness of the polymer films (1 × 1 cm) was determined by using a film thickness tester (Model Mitutoyo 4026F, Japan). The thickness of each strip at six different places was determined and a standard deviation was calculated.[15]

Weight determination
Twenty films of the same size (7 × 2 mm) were weighed on an electronic balance and the average weight was calculated. The results were recorded as one set. Six sets of such films were weighed and the standard deviation was calculated.[16]

Tensile strength measurement
This mechanical property was evaluated using the Instron universal tensile strength measurement instrument (Model 2046, Instron Ltd., Japan), with a 5-kilogram load cell. Film strips in special dimensions and free from air bubbles or physical imperfections were held between two clamps positioned at a distance of 3 cm. During measurement, the strips were pulled by the top clamp at a rate of 100 mm/min, and the force and elongation were measured in triplicate when the film broke.

Two mechanical properties, namely tensile strength and percent elongation were computed for the evaluation of the film. Tensile strength is the maximum stress applied to a point at which the film specimen breaks, and can be computed from the applied load at rupture and cross-sectional area of fractured film, as described from the following equation,[17]

$$\text{Tensile strength} = \frac{\text{Force at break (N)}}{\text{Initial cross sectional area of the sample (mm}^2)}$$

$$\% \text{ Elongation} = \frac{\text{Increase in length}}{\text{Original length}} \times 100$$

Estimation of drug content
The drug-loaded films of known weight (7 × 2 mm) were taken in 10 ml of acetic acid 1% (v/v) and crushed until dissolved. The drug solution was suitably diluted with acetic acid 1% (v/v) and the amount of drug present was estimated spectrophotometrically at 357 nm.[16]

Moisture loss studies

The percentage moisture loss study was carried out as per the literature.[18] The films of known weight and of predetermined size (2 × 2) were placed in a desiccator (containing anhydrous calcium chloride) for three days. The films were removed and re-weighed, and the percentage moisture loss was calculated as per the formula.[18]

$$Percentage\,moisture\,loss = \frac{(IW - FW)}{IW} \times 100$$

Where IW = Initial weight, FW = Final weight.

Folding endurance studies

This study was determined by repeatedly folding a small strip of film, 2 × 2 cm in size, at the same place, till it broke.[18]

In vitro release studies

Since the pH of the gingival fluid lies between 6.5-6.8, phosphate buffer pH 6.6 was used as the simulated gingival fluid for the dissolution studies, and a static dissolution model was used as the film remains immobile in the periodontal pocket.[19] Sets of six strips of known weight and dimension were placed separately into small test tubes containing 1.0 ml of the phosphate buffer, pH 6.6. The tubes were sealed and kept at 37°C for 24 hours. The buffer was then drained off and replaced with a fresh 1.0 ml phosphate buffer of pH 6.6. The concentration of the drug was determined by a UV-Visible spectrophotometer and this procedure was continued for seven consecutive days.[19]

Mass balance study

Following the *in-vitro* release studies, the test films were further analyzed for the drug content left in the strip. Each strip was dissolved in acetic acid 1% v/v and diluted suitably. The amount of drug released into the dissolution medium plus residual drug content in the films were accounted and compared for the actual drug content.[19]

Stability studies

The stability of the entire drug loaded polymer films were studied at different temperatures using the reported procedure. The films of size (7 × 2 mm) were weighed in three sets (12 strips in each set). The films were wrapped individually in aluminum foil and also in butter paper and placed in petri dishes. These containers were stored at room temperature (27 ± 2°C), oven temperature (40 ± 2°C) and in a refrigerator (5–8 ± 2°C) for a period of three months. All the polymeric films were observed for any physical changes, such as color, appearance, flexibility, or texture, and the drug content was estimated at an interval of one week.

Furthermore, the amount of drug in the films was estimated spectrophotometrically. The drug solutions were further scanned to observe any possible spectral changes. The drug content data obtained showed that the content did not differ from the initial drug content by more than 5%.

In vitro antibacterial activity

In vitro antibacterial activity was performed on all formulations by placing the film, cut into 0.5 × 0.5 sq cm, on agar plates seeded with oral bacteria, *Streptococcus mutans*. After 48 hours of incubation at 37°C, the films were transferred onto freshly seeded agar plates for an additional 48 hours for incubation. This procedure was repeated until no inhibition of bacterial growth was detected on the agar plate. The growth inhibition area on the agar plate was measured.[18]

RESULTS AND DISCUSSION

The optimum loading for good, flexible films was found to be 30% or less than that. For the present investigation, Chitosan films containing tetracycline with three different concentrations, that is, 10, 20, and 30% to the weight of the polymer, were prepared using the solvent casting method. The prepared films containing tetracycline 30% were cross linked with 2% gluteraldehyde for two-hour and four-hour duration, in order to extend the drug release. As the concentration of gluteraldehyde or time of cross-linking was increased, changes in the basic properties of the film were observed.

The FTIR studies from the spectra [Figure 1] confirmed the absence of any chemical interaction between the drug and the polymer. Macroscopical features revealed that the drug had dissolved in the polymer matrix rather than dispersing.

The physicochemical evaluation data presented in Table 1 showed that the average weight of the films ranged from 1.37 mg to 1.82 mg. The maximum weight was observed with 30% drug-loaded films, where as the average weight of the cross linked films ranged from 1.9 mg to 1.95 mg for two hours and four hours cross-linking, respectively. The thickness of the films ranged from 0.14 to 0.18 mm for non-cross-linked films and 0.185 mm and 0.190 mm for

Table 1: Physical characterization of Chitosan films containing different concentrations of tetracycline

Film type	Tensile strength (kg/mm²) ± SD	% elongation	Weight (mg)*	Thickness* (mm)
Plain film	1.44 ± 0.922	35.01 ± 2.09	0.99 ± 0.06	0.035 ± 0.008
TC-I	1.97 ± 0.08	17.12 ± 1.98	1.34 ± 0.93	0.14 ± 0.004
TC-II	2.36 ± 0.414	9.57 ± 0.85	1.58 ± 0.75	0.15 ± 0.006
TC-III	2.98 ± 0.375	5.76 ± 0.78	1.82 ± 0.85	0.18 ± 0.014
TC-III2	2.68 ± 0.452	5.16 ± 0.843	1.90 ± 0.93	0.18 ± 0.014
TC-III4	2.46 ± 0.658	4.56 ± 0.90	1.95 ± 0.43	0.18 ± 0.009

*Each value is a mean and standard deviation of six determinations TC-I: Tetracycline 10%; TC-II: Tetracycline 20%; TC-III: Tetracycline 30%; TC-III2: Tetracycline 30% two-hour cross-linked; TC-III4: Tetracycline 30% four-hour cross-linked
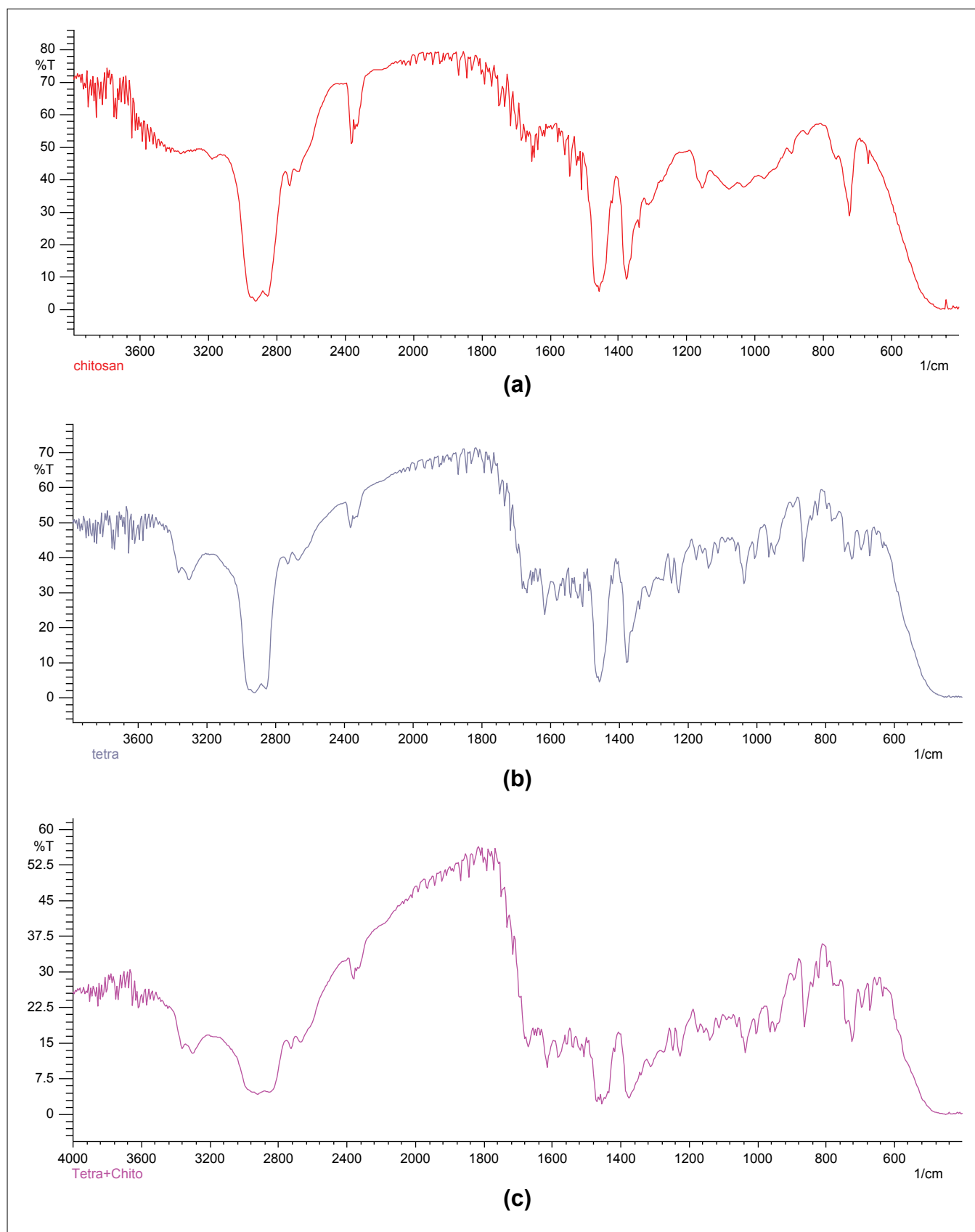
Figure 1: (a) FTIR spectra of plane Chitosan; (b) FTIR spectra of pure sample of tetracycline; (c) FTIR spectra of Chitosan film containing tetracycline

two-hour and four hour cross-linking, respectively. There was no significant difference in the thickness and weight among the different films.

The tensile strength of the strips ranged from 0.927 to 2.98 kg/sq mm, tensile strength was minimum for plain films and maximum for films containing 30% of the drug. The tensile strength of the cross-linked ciprofloxacin films ranged from 2.68 to 2.46 kg/sq mm for a two-hour and four-hour cross-linking, respectively. In contrast to tensile strength the percent elongation of the films decreased as drug loading increased, and increased the time of cross-linking, which ranged from 8 to 22%.

All the films were found to contain an almost uniform quantity of the drug, as per content uniformity studies [Table 2], indicating reproducibility of the technique. The percentage moisture loss varied between 7.98 ± 0.69 and 12.13 ± 0.51. The uncrosslinked films showed more moisture loss compare to crosslinked films and all the films exhibited more than 70 folding endurance, the data is shown in Table 3.

The concentration of the drug in the samples remained well above the minimum inhibitory concentration (3 mg/ml) of the drug for a period of seven days. The *in vitro* antibacterial activity demonstrated a significant antibacterial profile of all the films, for all the previously mentioned microorganisms, as shown in Figure 2. Films without the drug were also tested

and it was found that the films were not effective against the microorganisms.

The release time profile for different concentrations of tetracycline from Chitosan films are shown in Figure 3. The release profile showed that there was rapid initial release of the drug on day one, that is, 48.06, 56.93, and 62.36 for 10, 20, and 30% of the drug-loaded films, respectively. A perusal of Figure 1 indicated that the initial rapid release must have been because of the burst effect, due to elution of the drugs from the outer surface and cut edges of the matrix. Once the burst effect was completed, slow and sustained release was seen up to seven days. At the end of seven days the amount of drug release was found to be 95.89, 93.95, and 92.43% for 10, 20, and 30% of the drug-loaded films, respectively. In comparison, the cross-linked films also showed a burst effect initially followed by sustained release of the drug, but up to 21 days, with more uniformity of drug release per day. The two-hour and four-hour cross-linked films showed 85.15 and 77.23% drug release, respectively, at the end of 21 days.

Since the films were intact during the static dissolution studies, the dissolution rate-controlled release was ruled out. Hence data was fit according to Higuchi's diffusion model as the plots showed linearity (R^2: 0.913 to 0.9533). Thus, the cumulative percentage of drug release per square millimeter area versus square root of the time in days was calculated and there was a near linear relationship from the third day to the seventh day and the third day to the eighteenth day, in case of the non-cross-linked and cross-linked films, respectively. The *in vitro* release

Table 2: Data for drug content estimation of Chitosan films containing tetracycline

Chitosan film	Theoretical drug loading* (mg)	% drug loading
TC-I	140	91.03
TC-II	210	97.39
TC-III	280	96.31
TC-III2	280	90.67
TC-III4	280	88.62

*Each value is a mean and standard deviation of six determinations TC-I: Tetracycline 10%; TC-II: Tetracycline 20%; TC-III: Tetracycline 30%; TC-III2: Tetracycline 30% two-hour cross-linked; TC-III4: Tetracycline 30% four-hour cross-linked
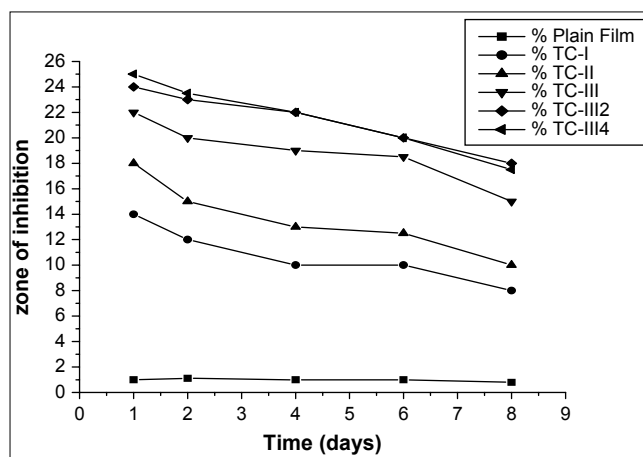
Table 3: Percentage moisture loss and folding endurance studies

Formulation	Percent moisture loss ± SD	Folding endurance
TC-I	12.13 ± 2.51	112 ± 9.51
TC-II	11.36 ± 1.29	109 ± 9.44
TC-III	11.00 ± 1.11	91 ± 8.19
TC-III2	8.21 ± 1.33	79 ± 9.44
TC-III4	7.98 ± 1.69	70 ± 8.16

TC-I: Tetracycline 10%; TC-II: Tetracycline 20%; TC-III: Tetracycline 30%; TC-III2: Tetracycline 30% two-hour cross-linked; TC- III4: Tetracycline 30% four-hour cross-linked
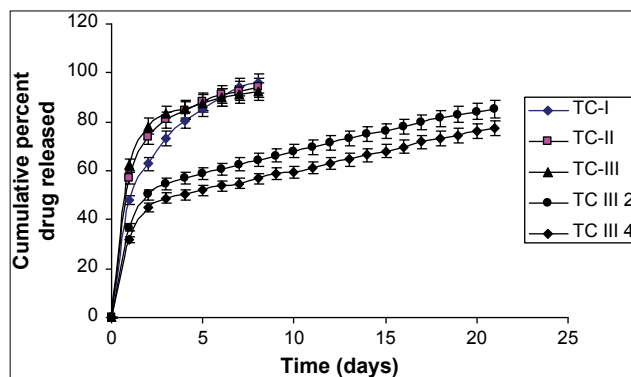

Figure 2: *In vitro* anti microbial activity of films containing tetracycline

Figure 3: Cumulative percent drug released from both uncrosslinked and crosslinked Chitosan films

profile of the drug from all the films could be further confirmed by Korsmeyer *et al*.'s equation. All the films showed good linearity (R^2: 0.9566 to 0.9958), with slope (n) values ranging from 0.176 to 0.304, indicating that fickian diffusion is the prime mechanism of drug release. All the kinetic parameters and correlation values of various mathematical models for all the films were taken and compared as shown in Table 4.

Mass balance studies were attempted and the total amount of drug loaded in the films was accounted for in the *in vitro* evaluation. It indicated that the drugs were physically bound to the polymer by weak forces. The stability studies carried out for a period of 10 weeks showed that there were no significant physical changes. The drug content did not deviate by more than 5% from the initial drug content.

CONCLUSION

A number of delivery systems have been investigated for use in periodontal disease, but still an ideal targeted delivery system is yet to be developed. The greatest advantages associated with the use of intra-pocket delivery systems over systemic delivery are that the administration is less time consuming than mechanical debridement and a lesser amount of drug is sufficient to achieve effective concentration at the site.

Compatibility studies showed no interaction between the drug and polymer, by FTIR studies. The drug was incorporated into Chitosan and fabricated as a film in three different concentrations 10, 20, and 30% w/w to the weight of the polymer. The films containing the highest drug content (30% w/w) were further cross-linked with glutaraldehyde 2%, which was aimed to extend and control the drug release for more number of days.

The drug loaded Chitosan films were flexible and possessed good tensile strength, and the physicochemical evaluation parameters were found to be satisfactory. As the films were cross-linked, the tensile strength of the films was reduced, but cross-linking had a definite influence on the release rate of the drug. The films were stable in all respects and did not show any signs of degradation, when stored in the refrigerator or at room temperature. The *in-vitro* antibacterial activity studies explained the positive effect on periodontal pathogens.

The *in-vitro* release studies showed an initial burst release of the drug by more than 40% and the release was sustained up to seven days and 21 days for the non-cross-linked and cross-linked films, respectively. Throughout the release study, the films remained intact, without any disintegration. The order of release of the drug was found to be zero order and followed Highuchi's diffusion model.

From the above studies it can be concluded that the drug(s) were found to release at a constant rate, so Chitosan may

Table 4: Kinetic values obtained from different plots of Chitosan films containing tetracycline

Film type	First order plots	Higuchi's plots	Korsmeyer *et al*.'s plots	
	Regression coefficient (R^2)	Regression coefficient (R^2)	Slope (n)	Regression coefficient (R^2)
TC-I	0.9889	0.9533	0.304	0.9958
TC-II	0.9388	0.8849	0.232	0.9566
TC-III	0.8591	0.8277	0.176	0.9266
TC-III2	0.9196	0.9063	0.247	0.9782
TC-III4	0.9406	0.9130	0.254	0.9669

*Each value is a mean and standard deviation of six determinations TC-I: Tetracycline 10%; TC-II: Tetracycline 20%; TC-III: Tetracycline 30%; TC-III2: Tetracycline 30% two-hour cross-linked; TC- III4: Tetracycline 30% four-hour cross-linked

be a useful matrix for sustained release of drugs, and cross-linking of the polymer is essential for the management of adult periodontitis. The advantage of Chitosan is that it has a wound healing property, which is a positive effect in anti-bacterial therapy. Since Chitosan is bioadhesive and biodegradable, it is immobile when left in-situ and degrades as the time advances. There is a need to extend the formulations reported in this study, for commercial exploitation. Furthermore, studies are in progress to evaluate the clinical efficacy, patient acceptability, and compatibility of the designed Chitosan films for the effective treatment of periodontitis.

ACKNOWLEDGEMENTS

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