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DESIGN AND *IN-VITRO* EVALUATION OF CONTROLLED RELEASE CEPHALEXIN SUBGINGIVAL FILMS USING NATURAL BIODEGRADABLE POLYMER

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Abstract

The aim of the present investigation was to prepare and evaluate cephalexin controlled release subgingival films using biodegradable sodium alginate. The equipment necessary for the present investigation was fabricated and employed for casting of sodium alginate subgingival films. Subgingival films of drug:polymer in various proportions (10:90, 25:75, 50:50 and 75:25) were prepared using solvent casting method. A 10%w/v $CaCl_2$ solution was used for gelation of the films. As polymer concentration is increased the smoothness of the films increased. The thickness of films varied from 146±5 to 312±15 µm which is well below the recommended thickness (<300 µm) of the subgingival films. The average weight of the films was found to be between 14.21±0.18 and 20.42 ±0.49. The percentage of drug content ranged from 53.14 to 56.81%. The low values may be due to loss of drug during treatment of the films with $CaCl_2$ solution. In vitro release studies of all the films showed an initial burst release for the first 24 hrs, followed by controlled release of cephalexin (>0.24 µg/ml) up to 120 hrs which is sufficient to inhibit the growth of the micro-organisms. The rate of drug release was inversely proportional to polymer concentration in the formulations. The low K_1 and 'r' values obtained may be due to biphasic drug release pattern. The formulations did not fit into Higuchi equation because of low values of <0.788025 which indicate that the drug release might be due to diffusion only in second phase of dissolution. All the films have shown to have integrity even after 5 days of dissolution studies. The formulations C1 and C2 which contain 90 and 75%w/w of polymer could be employed for controlled delivery of cephalexin for 5 days in subgingival infections. Sodium alginate, being a biodegradable polymer is a good choice as drug carrier in the present study.

Keywords: Controlled Release; subgingival films; cephalexin; biodegradable polymer; sodium Alginate.

Introduction

Periodontitis is a tooth destructive disease that affects about 15% of the population [1]. Apart from scaling and planing, systemic antibiotic therapy is employed in treating periodontitis [2]. Systemic antimicrobial therapy requires high doses over a prolonged period to achieve required concentrations in the sulcular fluid which results in adverse effects. [3]. Development of local delivery devices which can be placed directly into the periodontal pocket promise a relief to the patient. These devices can maintain extremely high local concentrations of drug for period of up to one to two weeks. Several implantable devices like fibers [4], films [5, 6] and gels [7] were studied. Various biodegradable polymers such as poly(glycolideco dl-lactide) [8], polyester poly(-caprolactone) [9], glycerol monooleate [10], cross linked atelo-collagen [11], hydoxypropylcellulose [12, 13] were employed as drug carriers.

The disadvantages of synthetic polymers insist the researcher to explore new avenues in drug delivery systems using natural biopolymers. Sodium alginate is natural hydrophilic, biodegradable [14, 15] polymer and has controllable porosity, bioadhesivity and biocompatibility. It has been used as controlled release drug delivery device [16-19] and employed to treat orthodontic constructions in periodontal diseases [20] and bone marrow implants [21]. Literature survey revealed that sodium alginate was not used as carrier in subgingival delivery.

Cephalexin monohydrate is an oral semisynthetic first generation cephalosporin, effective against both gram-positive and gram-negative organisms [22, 23]. It is effective against *Streptococcus mutans* and *Porphyromonas gingivalis* that are present in periodontal infections [24, 25]. It is used in prophylaxis in dental procedures for individuals allergic to penicillins [26, 27]. Therefore cephalexin is a drug of choice for local delivery into subgingival cavity. The aim of the present investigation was to prepare and evaluate the controlled release subgingival films containing cephalexin using sodium alginate as carrier.

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Materials and Methods

Materials

Cephalexin (as monohydrate) was gratis sample from Alkem Laboratories, Mumbai, India. Sodium alginate (200-600 cps and 120 mesh size), anhydrous calcium chloride, propylene glycol, monobasic potassium phosphate were purchased from BDH Chemicals Ltd., London.

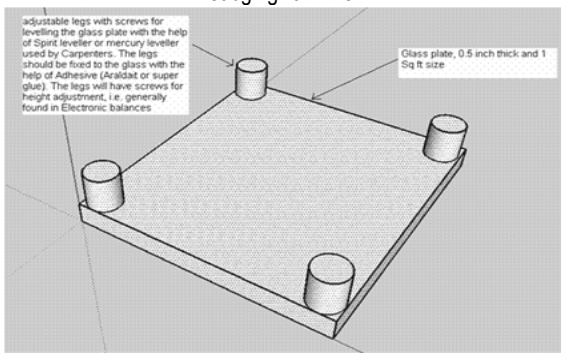
Spectrophotometric method for the estimation of cephalexin

The spectrophotometric method reported by Patel et al [28] was modified by estimating cephalexin in pH 7.2 phosphate buffer. A stock solution containing 100µg/mL cephalexin was prepared and scanned using UV-Visible spectrophotometer (Jenway 6505, UK, Sl.No. 2177) and the λ_{max} and absorbance were measured. Further serial dilutions were made to obtain 0.1 – 300 µg/mL of drug. Standard graph was constructed; the regression equation and correlation coefficients (r) were calculated using MS Excel software.

Fabrication of equipment for preparation of films

A glass plate with 1 ft side (1 sq ft size) and about ½ inch thickness was cut using glass cutter. The sides of the plate were ground so that it becomes smoother on all sides. Holes of 1/6 inch were bored on the four corners of the glass plate. Legs with adjustable screws were fixed to the glass plate (Fig. 4.1). The apparatus was leveled using a Spirit Leveller. A glass tube with 2 cm internal diameter (surface area of 3.142 cm^2) was cross-sectionally cut into pieces of 2 cm height and used for the casting of the subgingival films on the glass plate. Fevistick® paper glue was used to fix the die-tubes to the glass plate for temporary attachment during the process of casting of the films.

Figure1. Fabricated glass plate used for casting of subgingival films



Preparation of subgingival films

The subgingival films of sodium alginate were prepared using solvent casting technique. The formulations containing drug:polymer ratios of 10:90, 25:75, 50:50 and 75:25 were designed as summarized in Table 1. Sodium alginate was dissolved in deionized water in a glass bottle (5mL) by stirring for 1 h using a magnetic stirrer (Heidolph MR 3002, RPM 500, Germany) with 1cm Teflon® coated stirrer bar. Propylene glycol (10%w/w) was added to the bottle as a plasticizer. Then cephalexin was added to get required concentrations as above. After complete mixing, a 2 ml solution was poured into glass cylinder fixed on the fabricated glass plate placed on a horizontal plane. The glass plate was loosely covered so that the solvent (water) was allowed to evaporate slowly at 24° for 24-48 h. The casted films were collected after complete evaporation of the solvent. Then the films were placed separately in aluminum foil (domestic grade) cups of 2 cm diameter containing 0.5ml solution of 10% calcium chloride so that they are immersed completely. The cups were left aside at room temperature for complete drying. Finally the dried films were cut into pieces of 0.5×0.7 cm (0.35 cm^2) size and wrapped in an aluminium foil and stored in a desiccator at room temperature in a dark place until further studies.

Table 1. Composition of cast subgingival films containing Cephalexin

Formulation	Drug:Polymer ratio	Drug (mg)	Polymer (mg)	Propylene glycol mg	Water (ml)	Drug # in film (mg)
C1	10:90	20	180	18	2	2.22
C2	25:75	50	150	15	2	5.55
C3	50:50	100	100	10	2	11.1
C4	75:25	150	50	5	2	16.65

Theoretical amount of drug present in each film strip of size 0.5×0.7 cm.

Evaluation of subgingival films

Various properties such as size, thickness, weight variation, folding fortitude, percentage moisture loss and content uniformity were determined on the formulations. The thickness was measured at 6 locations on the films using micrometer (Mitutoyo, Japan) and the average value was noted. Individual weights of three films were measured on top loading electronic balance (Sartorius BP 310S, Germany) and the mean weight was measured. Folding fortitude was measured by repeatedly folding the film of 1×1 cm size at same place until it broke. Percentage moisture loss was determined by placing the film in a desiccator containing anhydrous calcium chloride. After 72 h, the films were weighed and the percentage moisture loss was calculated using the following formula.

$$\%\text{Moisture loss} = \frac{\text{Initial wt - Final wt}}{\text{Initial wt}} \times 100$$

Drug content estimation

Three films were powdered using mortar and 10 ml pH 7.2 phosphate buffer was added to dissolve the drug. The solution was filtered through Whatman No. 44 filter paper and cephalexin content was determined at λ_{max}263 nm after suitable dilution using UV/Vis spectrophotometer. The extract of films without drug was used as blank.

In vitro drug release studies

The films (0.5×0.7 cm size) were placed separately in 5 ml glass vials with polyethylene cap containing 1 ml of pH 7.2 phosphate buffer. As the pH of crevicular fluids vary depending on pathological conditions, pH 7.2 was selected in the present study. The vials were placed in an incubator to maintain body temperature of 37°C during dissolution study. A 1mL of the buffer containing dissolved drug from the vial was withdrawn using a micropipette (Gilson 462046C, France) at time intervals of 1, 2, 4, 8, 12, 16, 24, 36, 48, 72, 96 and 120 h. The sample was collected into a 10mL volumetric flask and an equal volume (1mL) of fresh buffer was replaced immediately into the vial after the withdrawal of the sample. The drug content was estimated by measuring the absorbance after suitable dilutions at λ_{max}263 nm using standard graph. The dissolution kinetics were calculated using Microsoft Excel Software.

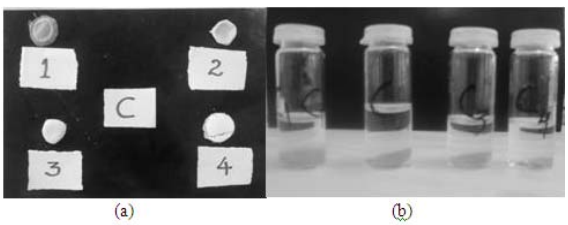
Stability Studies

Stability studies were conducted by wrapping the films in triplicate (size 0.5 x 0.7 cm) in aluminium foils and placing them at room temperature (25±5°C), in oven (40±2°C) and in refrigerator (4±2°C) over a period of three months. The films were observed for physical changes like colour, texture, and drug content at periodical intervals of 10 days each. The change in absorbance of cephalexin was also studied during the study.

Results and Discussion

A modified UV method for cephalexin in pH 7.2 phosphate buffer was developed. The λ_{max} was found to be 263nm. From the standard graph the regression equation and correlation coefficient (r) were calculated and found to be y=0.028x+0.024 and 0.998 respectively indicating good linear relationship between concentration and absorbance. The Linearity was found to be 5-100 µg for cephalexin. The equipment necessary for the present investigation was successfully fabricated and employed for casting of sodium alginate subgingival films with the help of a mould made of glass cylinder which shows that such formulations could be prepared with an easy technique. All the prepared films were found to be uniform in thickness with rough to smooth surface as the polymer concentration increased. The granular nature of the films containing above 25% w/w of polymer was attributed to high concentration of the drug present on the surface of the films. Using of propylene glycol (10%w/w of polymer) as plasticizer contributed to the flexibility of the prepared films. The treatment of the films with a 10%w/v calcium chloride solution resulted in slightly wrinkled film structure upon drying to the formulations containing 90% of the polymer (Fig. 2). This may be due to the fact that the formulations containing high concentration of polymer which forms hydrogel when wetted and then upon drying might have resulted in formation of folds. All the formulations resulted in gelation of the film after converted into calcium alginate.

Figure 2 Showing (a) the dried films of various formulations; (b) Dissolution vials



(a) (b)

Table 2. Evaluation data of cephalexin subgingival films

Formulation#	Mean Thickness (μm) (n=6)	Mean Average Weight (mg) (n=3)	Drug Content (mg) (n=3)	Drug Content %	Folding fortitude*	% Moisture Loss
C1	146±5	14.24±0.18	1.26±0.41	56.76	46	16±4.5
C2	166±14	15.21±1.04	3.153±0.12	56.81	35	13±2.8
C3	243±9	19.96±1.31	7.241±0.08	65.23	29	9±3.2
C4	312±15	20.42 ±0.49	8.847±0.28	53.14	19	7±1.4

All the values are mean with ± standard deviation.
Each film has size of 0.5×0.7 cm
* number of times folded.

The film properties such as size, thickness, weight variation, percentage moisture loss and content uniformity were determined and summarized in Table 2. The thickness of films varied from 146±5 to 312±15 μm which is well tolerated to the recommended thickness (<300 μm) of the subgingival films. The films can easily be inserted into the subgingival space for delivery of drug. The variation in thickness of the films may be due to change in drug content. The average weight of the films was found to be between 14.21±0.18 and 20.42 ±0.49 mg. All the formulations exhibited uniform thickness with low standard deviation values. Folding fortitude of the films showed that as the polymer concentration increased the folding strength increased. The percent moisture loss after drying of the films was found to be between 7±1.4 and 16±4.5%. The high moisture content in the formulation C1 may be due to high polymer concentration. The percentage of drug content ranged from 53.14 to 56.81%. The low values may be due to loss of drug during treatment of the films with $CaCl_2$ solution.

In vitro release studies performed using pH 7.2 phosphate buffer. All the films showed an initial burst release for the first 24 hrs, followed by controlled release of cephalexin (>0.24 g/ml) up to 120 hrs which is sufficient to inhibit the growth of the micro-organisms as reported by Higashi et al. (1990). The rate of drug release was inversely proportional to polymer concentration in the formulations. The rate of cephalexin release was more in formulations containing low polymer concentration that might be due to formation of more pores and channels.

The Figs. 3 and 4 summarize the release patterns and kinetics of drugs from alginate subgingival films. The log% of drug remaining vs time plots resulted in regression curves as shown in Fig. 4. The dissolution kinetic parameters are summarized in Table 3. Higher drug release (>96.64%) was found from all the films after 120 h. The formulations followed First order release kinetics with K_1 values from 0.013818 to 0.043757 which may be due to biphasic drug release pattern. Half-life of drug release was found to be between 15.83 and 50.15 h. The correlation coefficient (r) is obtained from 0.8062 to 0.9116. The low 'r' values in the formulations may be due to biphasic drug release with initial sharp followed by plateau phase.

Figure 3. Cumulative % of cephalexin remaining vs time from subgingival films showing formulations C1 (-♦-), C2 (-■-), C3 (-▲ -) and C4 (-×-).

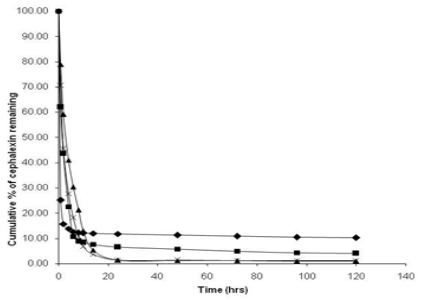


Figure 4. Log % of cephalexin remaining vs time from subgingival films showing formulations C1 (-♦-), C2 (-■-), C3 (-▲ -) and C4 (-×-).

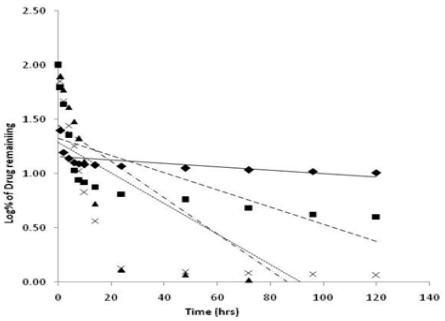


Table 3. Dissolution kinetics of cephalexin subgingival films

Formulation	First order rate constant (K_1) h^{-1}	Half life T_{50} (h)	Correlation coefficient (r)	Higuchi constant	% Drug released after 120 h
C1	0.013818	50.15	0.9116	0.585246	96.64
C2	0.032242	21.49	0.8062	0.652076	99.44
C3	0.04301	16.37	0.9022	0.788025	99.54
C4	0.043757	15.83	0.8854	0.730703	99.63

The formulations did not fit into Higuchi equation because of low values of <0.788025 which indicate that the drug release might be due to only diffusion in second phase of dissolution. All the films have shown to have integrity even after 5 days of dissolution studies which shows that the formed calcium alginate gel was strong enough to be placed in the subgingival cavity for sufficient period of time for controlled release of the cephalexin. During the stability studies the films did not show any significant change in physical properties. The drug content did not alter by more than 4%.

Conclusions

Local subgingival controlled delivery of antibiotics using biodegradable polymers is of recent interest to the formulation scientist. The present investigation showed that the formulations C1 and C2 which contain 90 and 75%w/w of polymer could be employed for controlled delivery of cephalexin for 5 days in subgingival infections. Sodium alginate, being a biodegradable polymer is a good choice in the present study. It has other advantages like biocompatibility, natural origin, hydrogel-property and bioadhesivity which culminate to make this polymer a judicious choice as drug carrier for controlled release subgingival delivery films.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the manuscript.

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