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Fabrication and in vitro evaluation of subgingival strips of calcium alginate for controlled delivery of ofloxacin and metronidazole

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Review Paper

Artículo de Revisión

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El estudio se ha realizado con los medios habituales de que dispone el Departamento de Nutrición y Bromatología y a través de los recursos aportados por el Máster Universitario en Atención Farmacéutica (EuropharmNES) de la Universidad de Granada.

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RESUMEN

Objetivos: Elaborar y evaluar tiras subgingivales combinadas compuestas por Ofloxacin y metronidazol in vitro con alginato de calcio biodegradable

Métodos: las tiras se prepararon utilizando el método de evaporación del disolvente. Se usó una concentración del 10% de CaCl_2 para la gelificación de las tiras.

Resultados: el grosor de las tiras se encuentra dentro de las recomendaciones ($>320 \mu\text{m}$). In vitro, la liberación de la droga siguió una cinética bifásica que fue suficiente para alcanzar la CMI e inhibir el crecimiento de microorganismos durante 5 días. La "tasa de liberación de la droga" es inversamente proporcional a la concentración de polímero de la formulación. La liberación de la "droga" fue por difusión y en segunda fase por disolución

Discusión: Las preparaciones OM1 y OM2 que contienen un 90 y un 75% de polímero respectivamente, podrían ser empleadas en liberación controlada durante cinco días en infecciones sublinguales. Siendo el alginato cálcico biodegradable una buena elección como polímero retardante

PALABRAS CLAVE: Biodegradable, Liberador de droga bifásico, Alginato de calcio, Liberación controlada, Tiras subgingivales

ABSTRACT

Aim: Subgingival strips of combined ofloxacin (OFX) and metronidazole (MET) were fabricated and evaluated in vitro using biodegradable calcium alginate.

Methods: Strips of drug:polymer (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 strips.

Results: The thickness of strips were at par of recommended thickness ($<320 \mu\text{m}$). In vitro release of drugs followed a biphasic kinetics which was sufficient to maintain the minimum inhibitory concentrations (MIC) to inhibit the growth of the microorganisms for 5 days. The rate of drug release was inversely proportional to polymer concentration in the formulations. The drug release was by diffusion in second phase of dissolution.

Conclusions: The formulations OM1 and OM2 which contain 90 and 75%w/w of polymer could be employed for controlled delivery of combined OFX and MET for 5 days in subgingival infections. Calcium alginate, being a biodegradable is a good choice as drug retarding polymer.

KEY WORDS: Biodegradable, Polymer Biphasic drug release, Calcium Alginate, Controlled Release, Subgingival strips.

INTRODUCTION

Periodontitis is a tooth destructive disease that affects about 15% of the population. Apart from scaling and planing, systemic antibiotic therapy is employed in treating periodontitis¹. Systemic antimicrobial therapy requires high doses over a prolonged period to achieve required concentrations in the sulcular fluid which results in adverse effects². 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 fibers³, strips^{4,5} and gels⁶ were studied. Various biodegradable polymers such as poly(glycolide-co-dl-lactide)⁷, polyester poly(β -caprolactone)⁸, glycerol monooleate⁹ and cross linked atelocollagen¹⁰ 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 polymer and has controllable porosity, bioadhesivity and biocompatibility^{11,12}. Calcium alginate is prepared by displacing sodium ion from sodium alginate. It has been used as controlled release drug delivery device¹³⁻¹⁵ and employed to treat orthodontic constructions in periodontal diseases¹⁶ and bone marrow implants¹⁷. In our previous work for the first time we have reported the design and evaluation of calcium alginate subgingival films of cephalixin¹⁸.

Ofloxacin (OFX) is an oral synthetic intermediate spectrum fluoroquinolone antibacterial agent active against enteric bacteria and other eubacteria. It is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria^{19,20}. It is also effective against *Streptococcus mutans* and *Porphyromonas gingivalis* that are present in periodontal infections²¹ and was investigated for its local delivery to periodontal pockets²². Resorbable hydroxypropylcellulose based devices for the delivery of ofloxacin was reported earlier^{22,23}. Metronidazole is an oral synthetic nitroimidazole antimicrobial agent, active against anaerobic bacteria and against protozoa^{24,25}. Metronidazole (MET) is bactericidal against many gram-positive and most gram-negative anaerobic bacteria. It is also effective against

*Porphyromonas gingivalis*²⁶ that are present in periodontal infections with MIC of 0.125 $\mu\text{g}/\text{ml}$ and was investigated for its local delivery to periodontal pockets^{27,28}. Literature survey revealed that subgingival strips of calcium alginate for controlled delivery of ofloxacin and metronidazole was not reported except our earlier work for the first time¹⁸. The aim of the present investigation was to fabricate and evaluate the controlled release subgingival strips containing combination of ofloxacin and metronidazole using calcium alginate as biodegradable carrier.

MATERIALS AND METHODS

Materials

Ofloxacin and Metronidazole were gratis samples from Alkem Laboratories, Mumbai, India. Sodium alginate (200-600 cps and 120 mesh size), anhydrous CaCl_2 , propylene glycol, monobasic potassium phosphate were purchased from BDH Chemicals Ltd., London.

Spectrophotometric method for the estimation of OFX and MET

The spectrophotometric method was developed to estimate OFX and MET simultaneously in pH 7.5 phosphate buffer. A stock solution containing 100 $\mu\text{g}/\text{mL}$ of each drug was prepared and scanned using UV-Visible spectrophotometer (Jenway 6505, UK, No. 2177). The λ_{max} and absorbance were measured. Further serial dilutions were made to obtain linearity range of concentration of OFX and MET. Standard graph was constructed; the regression equation and correlation coefficients (r) were calculated using MS Excel software.

Preparation of subgingival strips

The subgingival strips of sodium alginate were prepared using solvent casting technique on leveled glass plate and glass tubes¹⁸ providing a surface area of 3.142 cm^2 . 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 1 cm Teflon® coated stirrer bar. Propylene glycol (10%w/w)

Table 1. Composition of cast subgingival strips

Formulation	Drug: Polymer	Drug (mg)	Polymer (mg)	Propylene glycol(mg)	Water (ml)	Drug ^a in strip (mg)
OM1	10:90	20	180	18	2	1.11
OM2	25:75	50	150	15	2	2.775
OM3	50:50	100	100	10	2	5.55
OM4	75:25	150	50	5	2	8.325

^aTheoretical amount of ofloxacin and metronidazole discretely present in each strip of size 0.5 \times 0.7 cm.

was added to the bottle as a plasticizer. Then OFX and MET were 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 (distilled water) was allowed to evaporate slowly at 24°C for 24-48 h. After complete evaporation of solvent, cast strips were collected. Then the strips were placed separately in food grade aluminum foil cups of 2 cm diameter containing 0.5ml solution of 10%w/v CaCl₂ so that they are immersed completely. The cups are left aside at room temperature for complete drying. Finally the dried calcium alginate strips were cut into pieces of 0.5×0.7 cm (area of 0.35 cm²) size and wrapped in an aluminium foil and stored in a desiccator at room temperature in a dark place until further studies.

Evaluation of subgingival strips

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 strips using micrometer (Mitutoyo, Japan) and the average value was noted. Individual weights of three strips 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 strip of 1×1 cm size at same place until it broke. Percentage moisture loss was determined by placing the strip in a desiccator containing anhydrous CaCl₂. After 72 h, the strips were weighed and the percentage moisture loss was calculated using the following formula.

Drug content estimation

Three strips were powdered using mortar and 10 ml pH 7.5 phosphate buffer was added to dissolve the drug. The solution was filtered through Whatman No. 44 filter paper and the drugs contents were determined at λ_{\max} of 288 and 321 nm for OFX and MET respectively after suitable dilution using UV/Vis spectrophotometer. The extract of strips without drugs was used as blank.

Differential scanning calorimetry (DSC)

The main principle involved in determination of the enthalpy of caloric process by measuring the heat flow between samples and reference with linear or isothermal heating or cooling and calibration of the heat capacity with fusion heat standards. DSC study of pure OFX, MET, calcium alginate free strip, and prepared formulation (OM2) was performed using a JEOL JSM 5200 DSC to determine the drug excipient compatibility. The analysis was conducted at a rate 50°C min⁻¹ from 0° to 350°C temperature range under nitrogen flow of 25 ml min⁻¹.

2.7. In vitro drug release studies

The strips (0.5×0.7 cm size) were placed separately in 5 ml glass vials with polyethylene cap containing 1 ml of pH 7.5 phosphate buffer. As the pH of crevicular fluids vary from 7.5-8.5 depending on pathological condition²⁹, the pH 7.5 was selected in the present study. The vials were capped and 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 absorbance was measured after suitable dilutions at λ_{\max} 288 for OFX and 321 nm for MET using standard graphs. The dissolution kinetics was calculated using Microsoft Excel Software.

Stability studies

Stability studies were conducted by placing triplicates of the strips of size 0.5×0.7mm wrapped 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 strips were observed for physical changes like colour, texture, and drug content at periodical intervals of 10 days each. The change in absorbance of OFX and MET was also studied during the study.

RESULTS

A UV method for simultaneous determination of OFX and MET in pH 7.5 phosphate buffer was developed. The λ_{\max} were obtained as 288 for OFX and 321 nm for MET. From the standard graph the regression equations and correlation coefficients (r) were calculated and found to be $y=0.047x-0.228$ ($r=0.998$) and $y=0.087x-0.022$ ($r=0.999$) for OFX and MET respectively. The linearity was obtained as 5–50 µg for OFX and as 0.5–20 µg for MET. The equipment necessary for the present investigation was successfully fabricated and employed for casting of sodium alginate subgingival strips with the help of a mould made of glass cylinder. All the prepared strips were found to be uniform in thickness with rough to smooth surface as the polymer concentration increased. The strips containing 25% w/w of polymer have shown granular surface. Upon treatment of the strips with a 10%w/v CaCl₂ solution resulted in slightly wrinkled strip structure upon drying to the formulations containing 90% of the polymer. All the formulations resulted in gelation after treatment with CaCl₂ solution.

The strip properties such as size, thickness, weight variation, folding fortitude, percentage moisture loss and

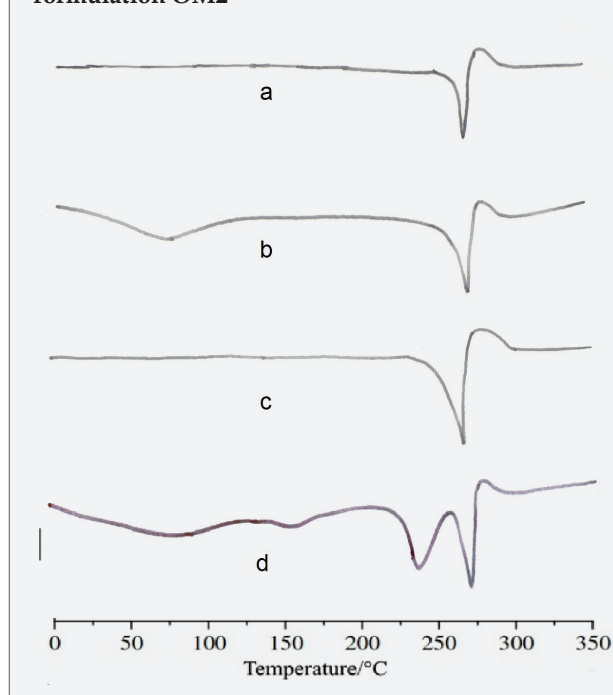
Table 2. Evaluation data of ofloxacin and metronidazole subgingival strips

Batch No. ^a	Thickness (µm) (n=6)	Weight (mg) (n=3)	Drug Content (mg) (n=3)	% Drug Content	Folding fortitude ^b	Moisture Loss (%)
OM1	127±0.6	15.19±0.35	0.419±0.31 ^c 0.810±0.81 ^d	37.75 ^c 72.97 ^d	31±5	19±5.2
OM2	171±0.9	17.42±0.63	0.592±0.97 ^c 1.500±1.04 ^d	21.33 ^c 54.05 ^d	23±6	17±4.6
OM3	224±1.1	21.06±0.77	1.994±0.69 ^c 6.560±0.28 ^d	35.93 ^c 76.35 ^d	18±5	12±3.4
OM4	289±1.8	19.47±0.94	2.544±0.74 ^c 7.301±0.29 ^d	30.56 ^c 87.70 ^d	11±3	9±1.5

All the values are mean with ± standard deviation; ^aEach strip has size of 0.5×0.7 cm; ^bnumber of times folded; ^cdrug content of ofloxacin; ^ddrug content of metronidazole

content uniformity were determined and summarized in Table 2. The thickness of strips varied from 127±0.6 to 289±1.8 µm. The thickness increased proportionally to drugs concentration in the strips. The average weight of the strips was found to be between 15.19±0.35 and 21.06±0.77 mg for the strip size of 0.5×0.7 cm and area of 0.35 cm². The folding fortitude of the strips was directly proportional to the polymer concentration. The percent moisture loss after drying of the strips was found to be between 9±1.5 and 19±5.2% and increased as drug concentration was increased. The percentage of OFX and MET content ranged from 21.33–37.75 and 54.05–87.70% respectively. DSC thermograms of calcium alginate free strip (a), ofloxacin (b), metronidazole (c) and controlled release subgingival formulation containing drug:polymer of 25:75 (OM2) (d) were shown in Figure 1. The DSC results demonstrated a sharp endothermic peak for OFX and MET at 278.2 and 252.1°C correspondingly. Similar endothermic peaks were observed in the controlled release subgingival strip (OM2).

In vitro drug release studies performed using pH 7.5 phosphate buffer which simulates gingival crevicular fluid²⁹. All the strips showed an initial burst release for the first 8 h, followed by controlled release of OFX and MET (>0.25 µg/ml and >0.125 µg/ml respectively) up to 120 h. The rate of drugs released was inversely proportional to polymer concentration in the formulations. The Figures 2 - 5 summarize the release patterns and kinetics of OFX and MET from alginate subgingival strips. A phase of sharp release of OFX and MET was observed within 8 h followed by a steady release plateau phase from all the formulations. The log% of drug remaining vs time plots resulted in regression curves as shown in Figures 4 and 5. The regression equations of OFX obtained were; $y = -0.015x + 1.172$, $y = -0.010x + 1.212$, $y = -0.010x + 1.509$ and $y = -0.012x + 1.477$ from the formulations OM1, OM2, OM3 and OM4 respectively. Similar regression equations were found for MET such as, $y = -0.011x + 1.271$, $y = -0.009x + 1.431$,

Figure 1. Thermograms of (a) calcium alginate free strip, (b) ofloxacin, (c) metronidazole and (d) formulation OM2

$y = -0.011x + 1.270$ and $y = -0.009x + 1.407$; where x is time in h and y is log% of drug released from the formulations.

The dissolution kinetic parameters are summarized in Table 3. Higher OFX and MET release of >97.80 and 97.27% respectively, were found from all the strips after 120 h. The formulations followed first order release kinetics with K_1 values for OFX and MET obtained in a range of 0.02303–0.034545 and 0.020727–0.025333 respectively. A similar observation of half-lives were found between 20.06–30.09 and 27.35–33.43 h for OFX and MET correspondingly. The low values of correlation coefficient (r) of <0.9165 and <0.9252 for OFX and MET respectively were obtained. From the Higuchi equation values of <0.789338 for OFX and <0.751076 for MET were found. The strips have shown to have integrity even after 5 days of dissolution studies.

Table 3. Evaluation data of ofloxacin and metronidazole subgingival strips

Formulation	First order rate constant (K_1) h^{-1}	Half life T_{50} (h)	Correlation coefficient (r)	Higuchi constant	Drug released after 120 h (%)
OM1 ^a	0.034545	20.06	0.8938	0.789338	97.80±5.7
OM2 ^a	0.02303	30.09	0.8111	0.698072	99.32±4.1
OM3 ^a	0.02303	30.09	0.9549	0.572701	98.75±4.8
OM4 ^a	0.027636	25.07	0.9165	0.570397	99.80±3.6
OM1 ^b	0.025333	27.35	0.8643	0.751076	97.41±2.9
OM2 ^b	0.020727	33.43	0.9252	0.674419	97.27±5.3
OM3 ^b	0.025333	27.35	0.8349	0.62623	98.75±4.6
OM4 ^b	0.020727	33.43	0.8385	0.611265	98.95±2.5

^adrug content of ofloxacin; ^bdrug content of metronidazole.

Figure 2. Cumulative % of ofloxacin remaining vs time from subgingival strips of formulations; OM1 (-♦-), OM2 (-■-), OM3 (-▲-) and OM4 (-x-).

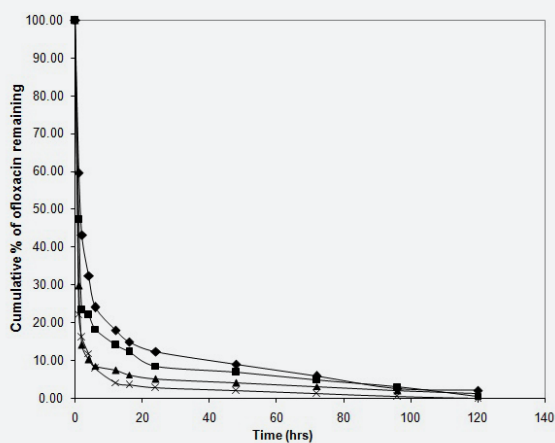


Figure 3. Cumulative % of metronidazole remaining vs time from subgingival strips of formulations; OM1 (-♦-), OM2 (-■-), OM3 (-▲-) and OM4 (-x-).

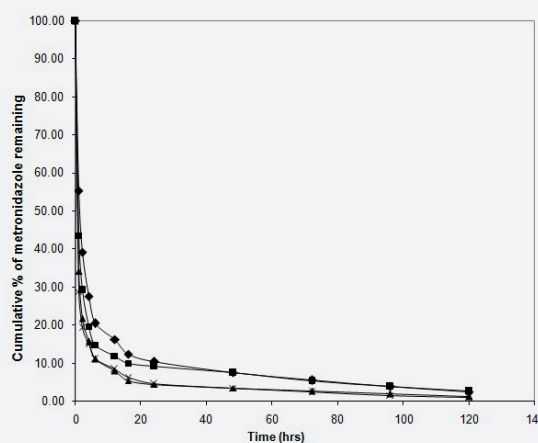


Figure 4. Log % of ofloxacin remaining vs time from subgingival strips of formulations; OM1 (-♦-), OM2 (-■-), OM3 (-▲-) and OM4 (-x-).

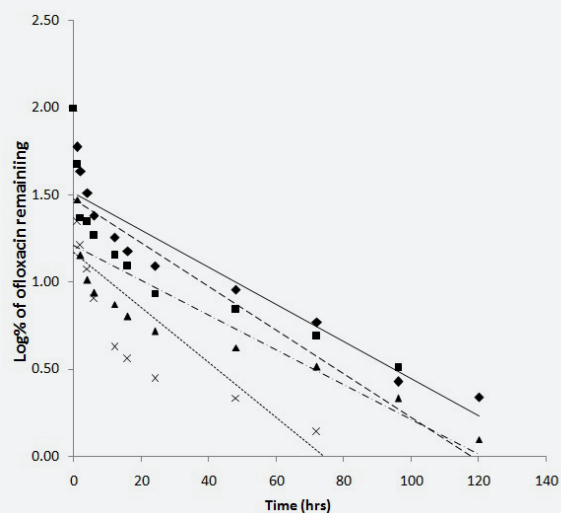
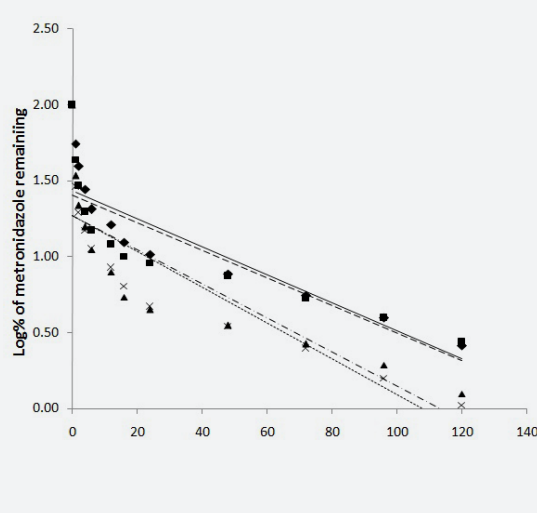


Figure 5. Log % of metronidazole remaining vs time from subgingival strips of formulations; OM1 (-♦-), OM2 (-■-), OM3 (-▲-) and OM4 (-x-).



During the stability studies the strips did not show any significant change in physical properties. The drug content did not alter by more than 3.7% for all the formulations. Stability studies indicated that there was no change in physical properties and drug content throughout the period of observation for all the formulations.

DISCUSSION

The developed UV method for simultaneous determination of OFX and MET in pH 7.5 phosphate buffer has indicated good correlation and linearity for both the drugs. Strips can be prepared using solvent casting technique. As drug concentration was increased the smoothness of the films decreased due to their presence in the form of dispersion as crystalline form. The decreased folding fortitude of the films with increased drug concentration might be due to decreased binding of polymer molecules with the presence of drugs in the strips. Propylene glycol used as plasticizer contributed for the strips to have improved flexibility. The formulation OM1 has resulted in slightly wrinkled strips after treatment with CaCl₂ solution. This may be due to the fact that the formulations containing high concentration of polymer form hydrogel when wetted and then upon drying might have resulted in formation of folds. The observed gelation of all the formulations after treatment with CaCl₂ solution indicated that the resultant strips were converted into calcium alginate.

The thickness of strips was found well below the recommended thickness of <320 μm of the subgingival strips which facilitates them to be inserted into the subgingival space for delivery of drug³⁰. The thickness increased proportionally to drugs concentration in the strips because of aggregation of drug particles. The folding fortitude of the strips was directly proportional to the polymer concentration which is essential for structural integrity and robustness of the prepared strips. The high moisture content (18±5.9%) found in the formulation OM1 may be due to high polymer concentration which is hydrophilic in nature and contributes for more absorption of moisture. All the formulations contained less drugs content than expected theoretical values which might have resulted from the loss of drugs during treatment of sodium alginate strips with CaCl₂ solution. It was also found that there was more loss of OFX during gelation process which is due to its high aqueous solubility compared to that of MET. The DSC results have shown no variation in the endothermic peaks of OFX and MET in pure form and in formulation indicate that the drugs present inside the strips as crystalline form with no significant physical or chemical interaction between drugs and polymer.

For invitro drug release studies pH 7.5 phosphate buffer was used to simulates gingival crevicular fluid²⁹. The biphasic drug release pattern showing initial burst release followed by controlled release of OFX and MET (>0.25 μg/ml and >0.125 μg/ml respectively) up to 120 h showed that it is sufficient to inhibit the growth of the microorganisms^{26,31}. From second day onwards the calculated OFX release was more than 40 times of its MIC and that of MET was more than 160 times of its MIC. The faster drug release from the formulations containing low polymer concentration might be due to the formation of more pores and channels in the strips by the drug itself. A phase of sharp release of OFX and MET was observed within 8 h followed by a steady release plateau phase from all the formulations. This may be due to the sudden release of drug from the surface of the strips into dissolution medium followed by a slow release from the inner layers of the strip by diffusion mechanism.

The OFX and MET release from the strips showed that calcium alginate has the ability to retard the drugs upto 120 h. The observed low K₁ values might have resulted from the biphasic drug release pattern of the drug from the strips. A similar observation of half-lives for OFX and MET correspondingly due to the same reason. The low values of correlation coefficient (r) of <0.9165 and <0.9252 for OFX and MET respectively were justified due to biphasic drug release with initial sharp followed by plateau phase. The formulations did not fit into Higuchi equation because of low values of <0.789338 and <0.751076 which correspond to OFX and MET. This indicates that the drugs release from strips might be due to only diffusion in the second phase of dissolution process. The integrity of the strips even after 5 days of dissolution studies indicates 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 drugs. The strips were stable during stability period which indicate that there was no change in physical properties and drug content throughout the period of observation for all the formulations.

Local subgingival controlled delivery of antibiotics using biodegradable polymers is of recent interest to formulation scientist. The present investigation shows that the formulation OM1 and OM2 which contain 90 and 75%w/w of polymer could be employed for controlled delivery of ofloxacin and metronidazole together for 5 days in subgingival infections. Calcium 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 strips.

RECOGNITIONS

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