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Development of local, mucoadhesive, sustained release patches of tetracycline hydrochloride for treatment of mouth infections: a preliminary *in vitro* study

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The aim of this study was to develop tetracycline hydrochloride sustained drug delivery system for local treatment of mouth infections, and to demonstrate the feasibility of xanthan gum as a film forming material that can be used in buccal drug delivery systems. The bilayered patches were prepared using ethyl cellulose as a backing layer, and xanthan gum as a matrix mucoadhesive layer. The patches were prepared by solvent-casting method with different loading amounts. *In vitro* drug release was performed using Franz-diffusion cells. Antibacterial activity was assessed for all prepared patches using disc-diffusion method against *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus* and *Bacillus bronchisepti*. *Ex vivo* mucoadhesive force, swelling studies, physical characteristics, and Fourier transform infra-red spectroscopy were performed for all the patches. The sustained action was achieved for 8h, with effective microbial activity against the tested microbes. The polymer reserved acceptable swelling with average value 400% for water uptake. The mucoadhesion force was $50*10^3$ dyne/cm^2 and it was adhesive for more than 8h. The addition of selected loading amounts of tetracycline hydrochloride did not result in a change in swelling or mucoadhesive properties. The patches were smooth, elegant in appearance, uniform in thickness, weight, drug content, and possessed good folding endurance (>200). In this study, preparation of sustained release buccal mucoadhesive patches was obtained. This study illustrated the feasibility of xanthan gum as a mucoadhesive film forming polymer. The patches were elastic and easy to prepare and can be used as a model for buccal drug delivery system of any water soluble drug.

Key words: buccal drug delivery, water uptake, antibacterial, tetracycline hydrochloride, FTIR, mucoadhesion

INTRODUCTION

Bacterial mouth infections may cause recurrent aphthous stomatitis, and periodontitis. Treatments of these bacterial infections require antibacterial therapy[1-3]. Treatment for aphthous ulcers is usually empiric; however, for children older than 8 years, a tetracycline mouth rinse (125mg/5ml) used four times daily produces good results and prevents secondary infection[2]. On the other hand, the major method of eliminating bacteria in periodontitis has been the mechanical removal of bacterial deposits and the use of antibacterial agents[3-4].

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Antibacterial agents can be used as systemic or local dosage forms. Traditional local treatments include mouth rinses, and irrigation solutions[5-7]. Local therapy provides much higher drug concentrations at the diseased site with lower total doses than systemic treatment. However, the drug level cannot be maintained for the required time in these formulations[8-9]. This necessitates the development of local oral sustained antibacterial drug delivery systems. Several drug delivery systems for various antibacterial agents have been studied and evaluated for the treatment of mouth infections such as strips, gels, and films[9-17].

In the present study, tetracycline was selected because of its wide application, both locally and systemically, in the treatment of aphthous ulcer and periodontal disease. Tetracycline hydrochloride has been shown to be effective against many of the common periodontopathic bacteria[18-20], in particular against

Prevotella intermedia and *Porphyromonas gingivalis*[21-24]. Although tetracycline is usually contraindicated for children younger than 8 years, it is the drug of choice if *Haemophilus* infection is confirmed[2].

The use of excellent buccoadhesive polymers such as xanthan gum gives a great opportunity to meet the challenges of drug delivery[25-28]. Xanthan gum is a polysaccharide with a β-D-glucose backbone like cellulose. It is produced by the bacterium *Xanthomonas campestris*, which is found on cruciferous vegetables such as cabbage and cauliflower[27].

The objectives of this study were development of tetracycline hydrochloride sustained release buccal patches for local treatment of mouth infections, *In vitro* release evaluation, mucoadhesiveness, studying bacterial activity, and evaluation of different physical characteristics. Also this study aims to determine the feasibility of xanthan gum as a buccal mucoadhesive film forming polymer.

MATERIALS AND METHODS

Materials:

Tetracycline hydrochloride- batch no. 189341 was kindly donated by Dar Al-Dawaa Company, Amman, Jordan. Ethyl cellulose CAS number 9004-57-3 by Sigma-Aldrich. Xanthan gum–batch no. 250451- supplied by Jangbazler was kindly donated by JPM, Amman-Jordan. Propylene glycol-batch no. 001746333150. All chemicals were used as supplied.

Micro-organisms were obtained from Dar-Aldawa Products, Amman, Jordan. Two strains of gram-negative bacteria *Escherichia coli* (ATCC 8739) and three strains of gram-positive bacteria. These include *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (ATCC 14579), and *Bacillus bronchisepti* (ATCC 4617) were used. The cultures of bacteria were maintained in their appropriate agar slants at 4°C throughout the study. Mueller-Hinton broth from Oxoid, England. Tetracycline antibacterial discs (Oxoid, England).

Methods:

Preparation for the bilayered mucoadhesive patches:

Bilayered films were produced by a casting/solvent evaporation technique using different combinations of polymers and drugs. The backing membrane was prepared by dissolving ethyl cellulose (5%) in chloroform. 20ml of ethyl cellulose solution was poured into a 10cm^2 glass mould on a level surface and solvent was allowed to evaporate.

The mucoadhesive layer was prepared using xanthan gum as the polymer forming matrix. 80ml water was added to 2g of xanthan gum and stirred at 40°C, and 2ml propylene glycol was added to the mixture. Mixing using a mechanical stirrer was performed for 3h. Various loading amounts of tetracycline (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.7mg) were added to 20ml to obtain various required formulae. Then 20ml of the plasticized polymer was poured on the ethyl cellulose layer and oven dried at 30°C for 96h. The dried films were kept at 25°C in desiccators of 50% relative humidity until further use. The thickness of the prepared films was measured using a screw gauge at six different areas.

In vitro *drug release:*

In vitro release of tetracycline hydrochloride from the matrices through dialysis membrane (MWCO 25 kDa, previously soaked in the buffer solution for 12h) was investigated using Franz-type diffusion cells with an effective diffusion area of 2.3cm^2. 15ml of pH 7.4 phosphate buffered solution at 37°C and stirred at 100rpm was used as the receptor medium. The matrices (donor phase) were put in contact with the dialysis membrane. At predetermined time intervals, samples of the receiving phase were withdrawn for analysis and replaced with an equal volume of fresh buffer to maintain the sink conditions. The amount released of tetracycline hydrochloride was determined by UV analysis at 275nm using a UV spectrophotometer (CAVY 1E VARIAN No. 94101153). Each release test was replicated at least three times.

Unidirectional permeation across the backing layer was studied by taking one sample (1ml) from the donor compartment after 24h of starting the experiment. The percent of drug permeated was calculated by dividing the total amount of drug permeated in the donor cell to the initial amount of drug loaded in the mucoadhesive film.

A study for the release mechanism for the selected patches according to Peppas equation (6):

$$M_t/M_\infty = K\, t^n \qquad (1)$$

where M_t/M_∞ is the fractional release of the drug, t denotes the release time, K is a constant incorporating structural and geometric characteristics of the controlled release device and n is the release exponent, indicative of the drug release mechanism.

The linear form of this equation is:

$$Log\left(\frac{M_t}{M_\infty}\right) = Log\,(K) + nLog\,(t) \qquad (2)$$

Kinetic parameters are obtained from a plot of Log (M_t/M_∞) versus Log (t), where n represents the slope, and Log (K) represents the intercept.

Ex vivo *mucoadhesion:*

The mucoadhesive strength was determined by measurement of the force of detachment or force of adhesion and *ex vivo* adhesion time. These parameters are the most frequently studied for determination of adhesive properties.

A) Mucoadhesive force:

A two-arm balance method reported by Parodi[29], with minor modifications, was also used to assess the bioadhesiveness of the films.

Fresh rabbit buccal mucosa 2mm thick, 2×1cm were washed with cold Tyrode's solution, and blotted with

tissue paper. The mucosa was fixed on the bottom of a smaller beaker attached to the bigger beaker. Tyrode's solution was added to the beaker up to the upper surface of the mucosa. The film was attached to the upper clamp and the platform was slowly raised until the film surface came in contact with mucosa. After a preload time of 5 minutes, specific pre-weighed loads were added until the film was detached from the buccal mucosa. The total weight was calculated and expressed as force required for the detachment. The experiments were performed in triplicate, and average values were reported.

B) Mucoadhesive time:

The mucoadhesive performance of the patches was evaluated using rabbit buccal mucosa tissue (2mm thick, 2×1cm). The time for film to detach from the mucosal section in a well-stirred beaker was used to assess the mucoadhesive performance. The fresh cut tissue was fixed on the side of the beaker with cynoacrylate glue. Before addition of the buffer, the films were attached to buccal mucosal tissue by applying light force with a fingertip for 20s. The beaker was then filled with 800ml phosphate buffer and kept at 37°C. A stirring rate of 150rpm was used to simulate buccal and saliva movement. The time for the film to detach from the mucosal tissue was recorded as the mucoadhesion time.

Water uptake:

Patches were weighed individually (designated as W_1) and placed separately above a test tube filled with simulated saliva (2.38g disodium hydrogen phosphate [Na_2HPO_4], 0.19g potassium dihydrogen phosphate [KH_2PO_4], and 8g sodium chloride [NaCl] per litre of distilled water adjusted with phosphoric acid to pH 6.7) incubated at 37°C ±1°C, and examined for any physical changes. After 2h, patches were removed from the test tube and excess surface water was removed carefully using filter paper. The swollen patches were then reweighed (W_2), and the water uptake (%) was calculated using the following equation:

$$\text{Water uptake (\%)} = \frac{(W_2 - W_1)}{W_1} * 100 \qquad (3)$$

The experiments were performed in triplicate, and average values were reported.

Antibacterial activity:

The aim of the antibacterial study was to check whether or not the prepared formulae affected tetracycline activity. Antibacterial activity was studied using a disc diffusion method[30-31]. Results were compared to 5mm standard 30μg/disc tetracycline discs as a positive control. Bacterial cultures were prepared using an 18h culture at 37°C in 10ml of Mueller-Hinton broth with sterile saline solution. The plates were then incubated at 37°C for 24h.

Each prepared formula film was cut into 5mm discs. The prepared discs and the standard tetracycline discs were attached to the agar plates. The agar plates were incubated for 24h after which the mean inhibition zone diameter (MID) was determined. The experiments were performed in triplicate, and average values were reported.

Physical characteristics of the selected patches:

A) Fourier Transform Infrared Spectroscopy (FTIR):

The FTIR spectra for various formulae were measured using FTIR spectrometer (FTIR PerkinElmer FT-I Insf. USA).

B) Folding endurance:

The folding endurance of patches was determined by repeatedly folding one patch at the same place until it broke or was folded up to 200 times without breaking. The experiments were performed in triplicate, and average values were reported.

C) Surface pH of films:

For determination of surface pH three films of each patch were allowed to swell by keeping them in contact with 1ml of distilled water for 2h at room temperature. The pH was noted by bringing the electrode into contact with the surface of the patch and allowing it to equilibrate for 1m. The experiments were performed in triplicate, and average values were reported.

D) Content uniformity, thickness, and weight uniformity:

Drug content uniformity was determined by dissolving each patch in 30ml of ethyl alcohol and filtering with Whatman filter paper (0.45μm). The filtrate was evaporated and drug residue dissolved in 100ml of phosphate buffer (pH 6.8). The 5ml solution was diluted with phosphate buffer (pH 6.8) up to 20ml, filtered through a 0.45μm Whatman filter paper, and analysed at 275nm using a UV-visible spectrophotometer equipped with a photodiode array detector (Multispec-1501, Shimadzu Corporation, Tokyo, Japan). Experiments were performed in triplicate, and average values were reported.

The thickness of the selected patches was measured using a screw gauge at selected pieces. Pieces for thickness, and weight variation were obtained from six different areas including edges and the middle parts of the selected patch. The dimension of each piece was 1cm*2cm, rectangular in shape with an area of 2cm^2. Readings were performed in triplicate, and average values were reported.

Stability testing was done by testing drug content, physical properties of the films and release characteristics of the stored films (at 25°C in desiccators of 50% relative humidity) after 6 months and during swelling experiments.

One-way analysis of variance was used to compare the weights and thicknesses. A comparison of tetracycline release was analysed using a t-test.

RESULTS

In vitro *drug release:*

Figure 1 illustrates the percent tetracycline release profiles from the various patches versus time. It can be clearly seen that in all patches the amount released over time decreased. The slowest release was observed for F2, since sustained action was obtained for 8h. On the other hand, a greater cumulative amount of drug released was observed from patches with a higher loading of tetracycline hydrochloride reaching 100% released within 8h.

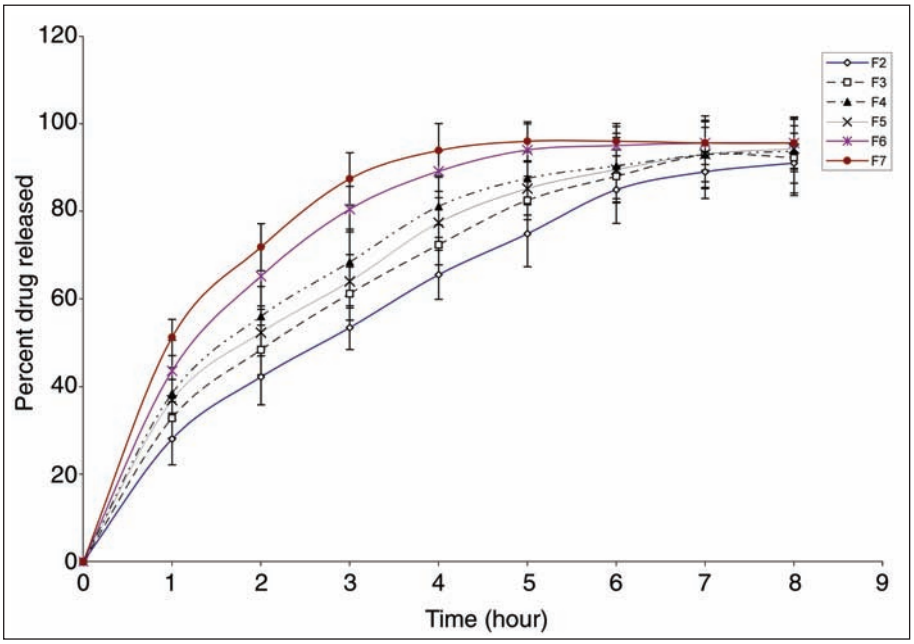

Figure 1. Cumulative percent *in vitro* release of tetracycline hydrochloride at 37°°C in Franz Diffusion cell (n=3, ±standard deviation).

Table 1. Summary of the release data analysis of the selected patches (n=3).

Form.	Tetracycline loading amount (g) per Petri-dish	R^2	n	K
F2	0.1	0.99	0.59	0.52
F3	0.2	0.99	0.55	0.44
F4	0.3	0.99	0.50	0.37
F5	0.4	0.99	0.53	0.39
F6	0.5	0.99	0.53	0.33
F7	0.6	0.99	0.45	0.28
		Average	0.52 (±0.05)	0.39 (±0.08)

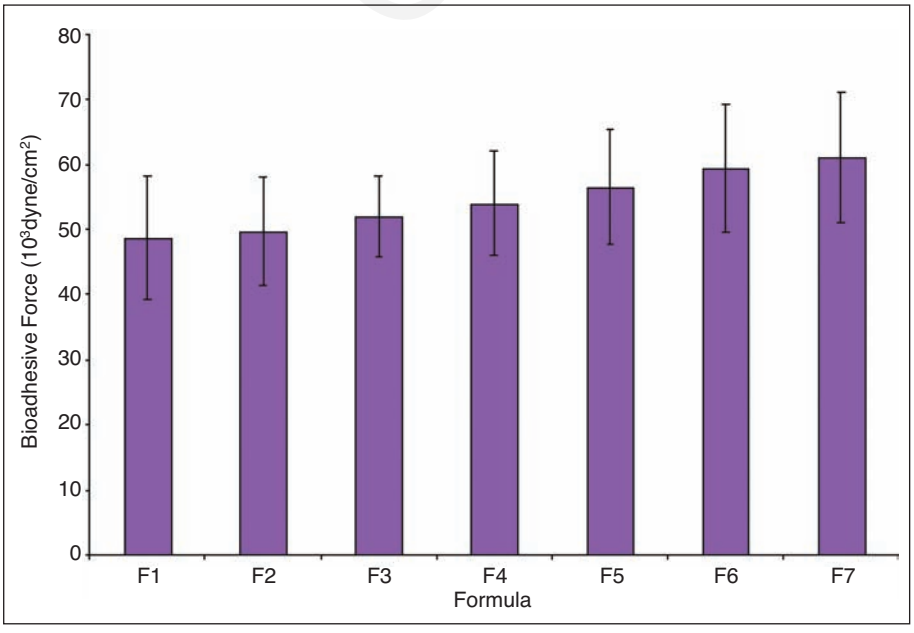

Figure 2. *Ex vivo* mucoadhesion force of patches, average values (n=3, ±standard deviation).

The unidirectional drug permeability of tetracycline through the backing layer of ethyl cellulose was assured, where the amount of tetracycline in the donor compartment after 24h of starting the permeation was insignificant.

Kinetic parameters obtained from a plot of Log (M_t/M_∞) versus Log (t) results are presented in **Table 1**. The diffusional exponent (n) average value was 0.52 ±0.05, with average (k) value 0.39 ±0.08. This indicates a mechanism of release in between Fickian (n=0.5), and an anomalous (n≥0.55) which indicates that the release occurs through coupling of diffusion and erosion mechanisms. In swellable systems, factors affecting release kinetics are the liquid diffusion rate and the polymeric chain relaxation rate. When the liquid diffusion rate is slower than the relaxation rate of the polymeric chains, the diffusion is Fickian; whereas when the relaxation process is very slow compared with the diffusion, the case II transport occurs. When the liquid diffusion rate and the polymer relaxation rate are of the same order of magnitude, anomalous or non-Fickian diffusion is observed.

Ex vivo *mucoadhesive force:*

The mucoadhesive strength was determined by measurement of the force of detachment or force of adhesion. **Figure 2** shows the *ex vivo* mucoadhesion force for various patches prepared. Xanthan gum produced good adhesion to the mucosal surface with force of mucoadhesion equals $50*10^3$ dyne/cm^2. Statistical analysis revealed no significant difference between the data of force of mucoadhesion with addition of tetracycline hydrochloride (P>0.5). This indicates that tetracycline hydrochloride does not affect the bioadhesiveness of xanthan gum.

During *in vitro* mucoadhesive time studies, no films were dropped from the mucosal tissue within 8h of experiment time.

Water uptake:

Xanthan polymers have very good water sorption properties. The value of water uptake reached 400% (**Figure 3**). Addition of tetracycline hydrochloride did not affect the sorption properties of the polymer.

Patches did not show any appreciable changes in their shape and form during 3h soaking in simulated salivary solution.

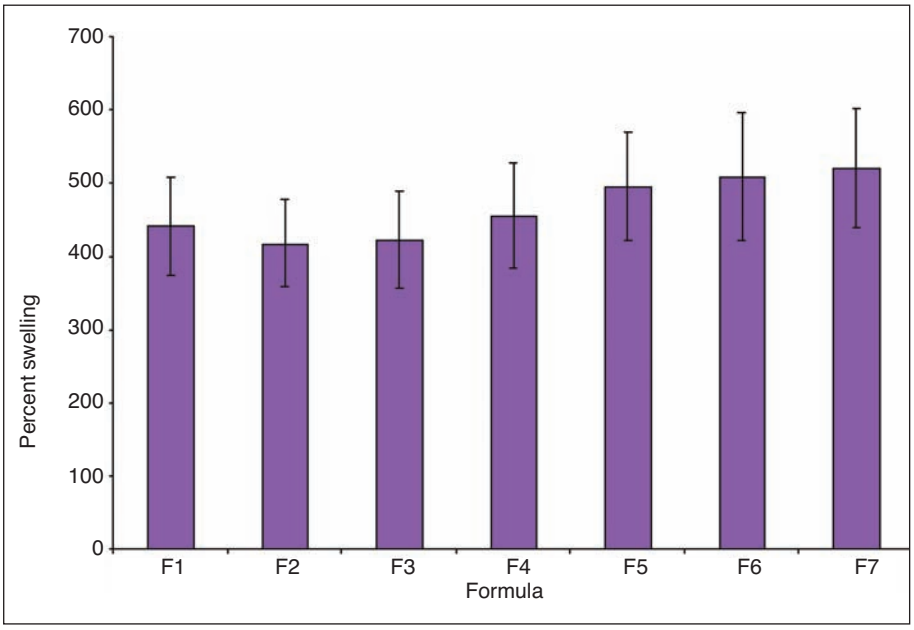

Figure 3. Water uptake of patches, average values (n=3, ±standard deviation).

Table 2. Mean inhibition zone diameter for various prepared patches (n=3).

Formula	Mean inhibition zone diameter (MID) (mm)			
	Staphylococcus aureus	*Bacillus cereus*	*Bacillus bronchisepti*	*Escherichia coli*
Control Disc	30	20	15	15
F1	0	0	0	0
F2	>30	20	15	15
F3	>30	20	15	15
F4	>30	20	15	15
F5	>30	20	20	15
F6	>30	20	20	15
F7	>30	20	20	15

Antibacterial activity:

Antibacterial activity for the patches was tested against *Escherichia coli, Bacillus cereus, Staphylococcus aureus* and *Bacillus bronchisepti*. The antibacterial activity was assessed by determining MID using the disc diffusion method[30-31]. **Table 2** summarises the results. It is clear that there is no significant difference in the effective MID between the patches and the control disc. All the prepared tetracycline hydrochloride patches had MID of more than 30mm against *Staph. aureus*, not less than 20mm against *B. cereus*, more than 15mm against *B. bronchisepti*, and 15mm for *E. Coli*.

Physical characteristics of the selected patches:

A) FTIR Studies

In this work, FTIR spectrums of xanthan and tetracycline samples are shown in **Figures 4** and **5** respectively. The most characteristic region of the tetracycline spectrum occurred between 1,200 and 1,800cm^{-1}. The FTIR spectra of the patch and the physical mixture are shown in **Figures 6** and **7**.

B) Folding Endurance

The folding endurance was measured manually, by folding the film repeatedly at a point until it broke. The breaking time was considered as the end point. The folding endurance test did not develop any visible cracks or breaks (>200).

C) Surface pH

The average pH value was 6.51 ±0.73 for various patches.

D) Content uniformity, thickness, weight uniformity, and stability of the selected patches:

The thicknesses of the patches were (0.15 ±0.06mm). Weight variation was uniform in the patches with an average value (0.05 ±0.02g). Drug content in formulations was uniform, and the standard deviation did not exceed 0.75% of the prepared concentration.

Stability of the patches was tested after 6 months. Results showed that the decrease in drug content did not exceed 2%. None of the patches exhibited colour or shape changes.

DISCUSSION

The sustained release action obtained in the prepared patches can be explained by the extensive swelling of the xanthan polymer, which created a thick gel barrier, making drug diffusion more difficult. Due to the presence of glucuronic acid and pyruvate in its side chains, xanthan is considered to be an anionic polyelectrolyte. The negatively charged carboxyl groups on the side chains forced the

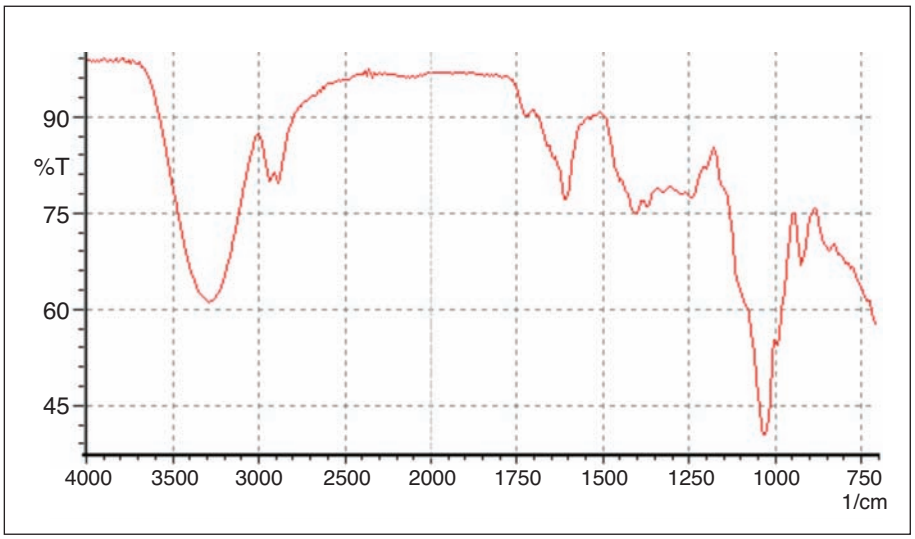

Figure 4. FTIR spectrum of xanthan gum.

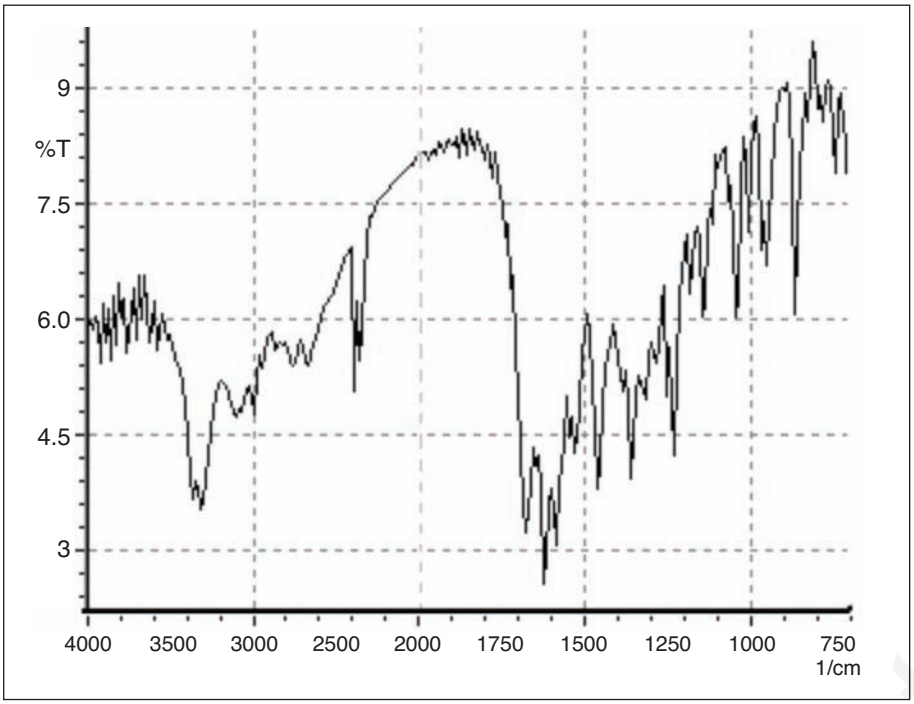

Figure 5. FTIR spectrum of tetracycline hydrochloride.

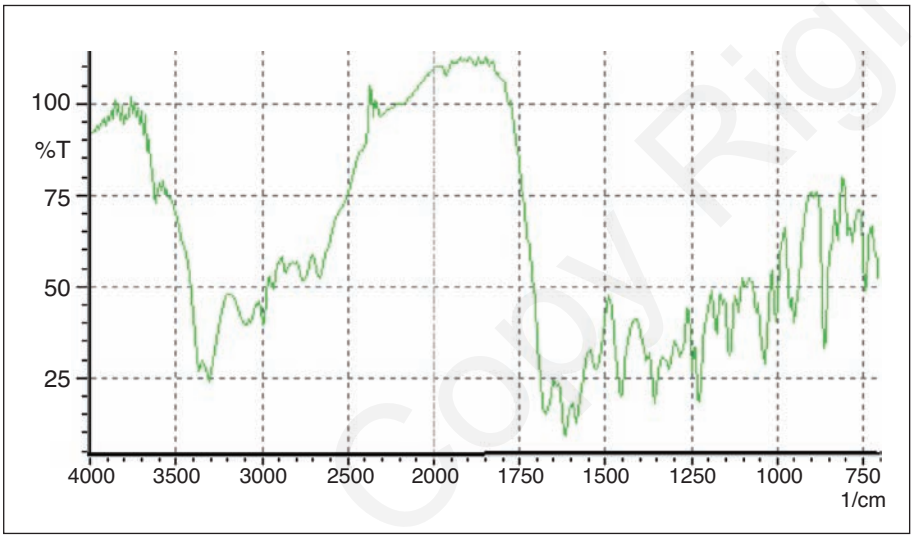

Figure 6. FTIR spectrum of physical mixtures of xanthan gum and tetracycline hydrochloride.

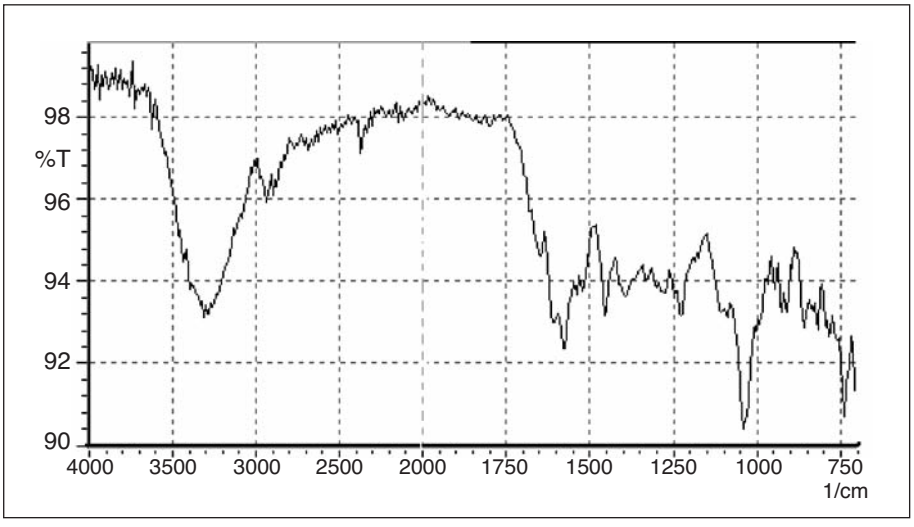

Figure 7. FTIR spectrum of a film of xanthan gum containing 0.3g tetracycline hydrochloride.

molecules to form very viscous fluids when mixed with water[32]. On the other hand, the increase in the release correlated to the concentration of tetracycline hydrochloride can be explained by the water solubility of tetracycline hydrochloride which increased the swelling of the polymer and facilitated drug diffusion.

The ethyl cellulose backing layer produced insignificant release of the drug, providing unidirectional release through xanthan gum. This result agreed with previous studies[33] which assure the backing layer capability of ethyl cellulose.

Xanthan gum was shown to have mucoadhesive characteristics. Richardson and Ross-Murphy[32] demonstrated the anionic nature of the polymer, which allows the possibility of a hydrogen bond between carboxylic groups of the polymer and the sugar residues in oligosaccharide chains in the mucus membrane, resulting in the formation of a strengthened network between the polymer and the mucus membrane. Tetracycline addition did not affect the force of mucoadhesion. This assessed the ability of xanthan gum to retain its mucoadhesiveness in the presence of the drug. At the same time, all the patches were retained on the mucosal surface during evaluation of *in vitro* muco-adhesion time. This indicated that the bioadhesive strength exhibited by films was satisfactory for maintaining them in the oral cavity.

Hydration is required for a mucoadhesive polymer to expand and create a proper macromolecular mesh of sufficient size, and also to induce mobility in the polymer chains in order to enhance the inter-penetration process between polymer and mucin. Polymer swelling permits a mechanical entanglement by exposing the bioadhesive sites for hydrogen bonding and/or electrostatic interaction between the polymer and the mucous network. However, a critical degree of hydration of the mucoadhesive polymer exists where optimum swelling and bioadhesion occurs. On the other hand, swelling of xanthan polymer and formation of a thick gel barrier plays a very important role in sustained release

action for the incorporated drug. As shown in the results, addition of various loading amounts of tetracycline hydrochloride did not affect the polymer swelling.

Also the polymer's ability to retain its stability and physical appearance during various experiments indicates that this polymer is a good candidate for formulation in buccal drug delivery systems.

The antibacterial results indicate that tetracycline hydrochloride dispersed in the patches retained its antibacterial activity.

According to the results, Formula (F2) produced maximum sustained action of more than 8h, acceptable mucoadhesive force, as well as mucoadhesiveness of more than 8h, with maintained drug antibacterial efficiency.

To study any interaction between the drug and the polymers used in the preparation of patches FTIR spectroscopy was carried out for the test preparations. **Figure 5** shows the FTIR spectrum of tetracycline hydrochloride. It is compared to **Figures 6** and **7**, which represent spectra of (drug-polymer) physical mixture and (drug-polymer) prepared patches, respectively. The figures showed the same drug characteristic bands. This illustrates an absence of chemical interaction between tetracycline hydrochloride and xanthan gum polymer. It suggests that the drug molecule is present in an unchanged state in the patch.

All the prepared patches showed good folding endurance (>200), and thus excellent elasticity. All prepared films showed no visible cracks. This indicates that xanthan gum is an excellent candidate for buccal drug delivery system since the obtained elasticity with mucoadhesiveness will not affect buccal cavity movement or cause discomfort to the patient.

Considering the fact that acidic or alkaline pH may cause irritation to the buccal mucosa and influence the degree of hydration of polymers, the surface pH of the buccal films was determined to optimise both *in vitro* drug permeation and mucoadhesion. The surface pH of the patches was determined in order to investigate the possibility of any side effects in the oral cavity. Statistical analysis revealed that there is no significant difference between the pH of the patches and the mouth pH (6.68 ±0.55)[34].

The drug thickness was uniform for all the patches. Results proved that the prepared patches had acceptable thickness and content uniformity. All prepared patches were stable in terms of patch physical stability in shape and appearance.

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CONCLUSION

This study describes the development of tetracycline sustained local drug delivery patches for the treatment of mouth infections. The mucoadhesive bilayered film consists of ethylcellulose as the backing layer and xanthan gum as a matrix forming layer. This work illustrates the feasibility of using xanthan gum as a film forming material. The prepared patches were smooth, elegant in appearance, uniform in thickness, weight, and drug content. They showed no visible cracks, and possessed good folding endurance which proved the elasticity of the patches for buccal drug delivery system. The results revealed that tetracycline activity was maintained in the patches with the ability to be sustained for 8h. However, future clinical investigations are necessary to validate its efficiency.

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