*Original Article*

# Mucoadhesive Film Embedded with Acyclovir Loaded Biopolymeric Nanoparticles: *In vitro* Studies

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**ABSTRACT**

**Objective:** The efficiency of acyclovir, a potent antiviral agent, is mainly limited by its low permeability. The purpose of this investigation was to formulate and assess nanoparticle impregnated buccal films to improve acyclovir permeability and thereby enhance bioavailability. **Methods:** Acyclovir was incorporated into the polymeric materials and formulated as nanoparticles. The prepared nanoparticles were then loaded into various films (F5-F7) prepared with varying quantities of hydroxyethyl cellulose and Eudragit RL 100. The prepared films were evaluated for physico-mechanical characters, mucoadhesion, swelling, *in vitro* acyclovir release and *ex vivo* diffusion. **Results:** The films so prepared showed adequate mucoadhesive strength and excellent physico-mechanical properties. Rapid hydration of films (>35%) were observed in 15 min, followed by a relatively slower phase of hydration which continued till 60 min. *In vitro* release profile exhibited biphasic pattern, while the rate of acyclovir release was higher with film F7. Prepared films exhibited either first order (F5, control) or Higuchi model (F6, F7) kinetics. The enhancement in acyclovir permeation in F5, F6 and F7 loaded films were 2.2, 4.1 and 6.7 folds, respectively, when compared to control. **Conclusion:** This study concludes that the drug loaded nanoparticles impregnated buccal film could be an alternative approach to enhance the oral bioavailability of acyclovir, and need to be proved *in vivo*.

**Key words:** Acyclovir, buccal, film, Nanoparticles, permeation, release studies.

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## INTRODUCTION

Buccal route of drug delivery offers most promising alternative to oral route because of its potential to overcome first-pass hepatic metabolism as well as gastrointestinal-tract limitations.[1] In addition, the buccal mucosa is comparatively permeable and is readily accessible for self-medication.[2] The potential of this route to augment trans-mucosal transport of therapeutic agents and delivering it into blood has been demonstrated for several poorly oral bioavailable drugs.[3,4] Many types of mucoadhesive dosage forms have been designed and investigated for buccal delivery in the past decade. However, efficiency of these formulations primarily depend on their adhesion with mucosa to enable effective drug release and transport across the membrane. The concept of mucoadhesive polymeric buccal films has garnered considerable importance among drug delivery scientists owing to their advantages like ease of adherence to mucosa, high flexibility, higher patient compliance, controlled release of drug, prolonged residence time etc.[5,6] In this context, polymeric nanoparticles impregnated buccal films have been extensively studied and proved to be an effective approach to enhance bioavailability of drug molecules in addition to its potential to provide sustained release over an extended period of time.[7,8] Recently, the potential of polymeric nanospheres of selegiline impregnated buccal films in enhancing the oral bioavailability has been demonstrated.[9]

Acyclovir is one of the potent antiviral drugs used for management of herpes simplex viral infections, Varicella zoster as well as Herpes zoster.[10] Different dosage forms such as tablets (oral), creams (topical) and suspensions (parenteral) of acyclovir are available with limited efficiency. The systemic availability of acyclovir following oral and topical therapy

is very low than the therapeutic requirement. The treatment of herpes infections with acyclovir involves repeated oral administration of acyclovir due to very poor oral bioavailability (15%) and small elimination half-life (3 h).[10] The delivery of this active is primarily limited by its biopharmaceutical properties and physiological nature of the membrane. Few approaches have been attempted in the last decade to overcome issues with this drug, but resulted with limited success.[11-13] In this context, the buccal route could be explored as an alternate to oral therapy of acyclovir by enhancing the absorption, bioavailability and provide effective systemic delivery. It is hypothesized that embedding polymeric nanoparticles of acyclovir in buccal film could enhance the efficiency of this drug molecule. The present study assessed the potential of developing mucoadhesive buccal films embedded with biodegradable polymeric nanoparticles of acyclovir with the objective of improving bioavailability. The mucoadhesive buccal films of acyclovir was fabricated using varying concentrations of hydroxyethyl cellulose (HEC) as mucoadhesive agent, Eudragit RL 100 as a film former, propylene glycol as plasticizer and polyvinyl alcohol as backing membrane.

## MATERIAL AND METHODS

### Materials

Acyclovir, hydroxyethyl cellulose (HEC), poly (D,L-lactide-co-glycolide) (PLGA, 50:50, Mw 7000-17000 dalton), polyvinyl alcohol (PVA; Mowiol® 40-88, Mw ~205,000), polyvinylpyrrolidone (K 30, Mw 40,000), propylene glycol (PG), dichloromethane, acetonitrile, ethanol and acetic acid

were purchased from Sigma Aldrich St. Louis, MO, *USA*. Eudragit RL 100 was obtained from Evonik, Darmstadt, Germany.

### Analysis

Amount of acyclovir in samples were quantified by Ultraviolet (UV) spectroscopy (Shimadzu, Japan). Calibration curve of acyclovir was prepared by dissolving accurately weighed amount of drug and dissolving in phosphate buffered saline (PBS, pH 7.4) in volumetric flask. This stock solution (PBS) was further diluted to various concentrations ranging from 1- 500 µg/ml. For sample analysis, absorbance was measured at 250 nm ($\lambda_{max}$) by UV Spectrophotometer, using PBS as blank. The method is validated to avoid any interefence of extracts in permeation studies.

### Preparation of nanoparticles

Nanoparticles were prepared by solvent evaporation technique using chloroform-dichloromethane.[14] Briefly, chloroform: dichloromethane (20:30 V/V) were measured accurately and mixed well.[15] Acyclovir and PLGA (1:20 W/W) were weighed and dissolved in the solvent mixture (10 mL) and added to known volume of PVA solution (2% W/V, 20 mL) and homogenized for 2 min at 13,000 rpm. The system was further stirred for 2 h (600 rpm) and allowed to stabilize for 1 h. Then the supernatant liquid was decanted and centrifuged (2 min at $13416 \times g$ at 25 °C) to obtain nanoparticles. The product was washed few times and freeze dried for 24 h.

### Evaluation of nanoparticles

The percentage yield of nanoparticles was determined using the formula:[16]

$$\% \text{ Yield} = \frac{\text{Practical Yield}}{\text{Theoretical Yield}} \times 100$$

Entrapment efficiency was determined by weighing required amount of nanoparticles (50 mg), added to PBS and sonicated for 24 h. Then the solution was centrifuged ($13416 \times g$), supernatant was used for UV analysis. The amount of drug loaded was determined as; Amount of drug/ Amount of nanoparticles × 100. The percentage entrapment efficiency (%EE) was computed by the equation:[16]

$$\% \text{ EE} = \frac{C_t - C_r}{C_t} \times 100$$

where $C_t$ is total and $C_r$ is free acyclovir concentration.

Particle size and distribution of nanoparticles were measured using Zetasizer (Malvern, USA).

### Preparation of mucoadhesive films

The required amount of Eudragit RL 100 was dissolved in ethyl alcohol. Amount of Eudragit RL 100 added for film forming was gradually increased (as shown in Table 1) to optimize the Eudragit content required to form a film with desired physicomechanical properties. HEC was dispersed separately in water containing PG and added to Eudragit solution under magnetic stirring. Drug loaded nanoparticles was added to the above mixture and stirred to ensure uniform distribution of nanoparticles. The dispersion so formed was sonicated for 15 min and casted on to a petridish and incubated at 37 °C for drying (24 h).

### Preparation of backing membrane

PVA aqueous solution (4% W/V) was prepared and casted on a thin sheet and allowed to dry for 12 h at 40 °C in an oven. Drug loaded buccal films were casted over the backing membranes by solvent casting.[17]

### Evaluation of films

Quantification of drug in the prepared films were carried out by cutting a film of size 1×1 cm$^2$ and kept in glass vial contain PBS. The solution was mixed for 5 h by continuous shaking on a water bath, filtered and amount was measured using UV spectrometer. Thickness of the film was noted making use of a screw gauge. The thickness was measured at six different locations.[18] Folding endurance was measured by folding films (4x4 cm²) at same location repeatedly making an angle of 180° till it breaks. For pH determination, film of size of 1x1 cm² was taken and dipped in water (5 mL) for 30 minutes. Then the film was removed, surface liquid was cleaned and pH was measured using a flat surface electrode.[18]

Mucoadhesive studies were carried out using a standard texture analyzer.[18] Male white rabbits (2.5-3.0 kg) cheek pouch was used to measure the mucoadhesive strength (IAEC/SSP/16/PR-013). Rabbit buccal mucosa was surgically removed from the oral cavity using scalpel and scissors to separate the connective tissue. The AC electrical resistance of the buccal mucosa was measured using a wave form generator and a digital multimeter (Agilent Technologies, Santa Clara, CA, USA). The prepared films were cut into small pieces and fixed to probe of a texture analyzer using cyanoacrylate adhesive. The rabbit epithelium mucosa was mounted on the stationary platform. The assembly containing the rabbit cheek mucosa was filled with 2 mL of the buffer solution to keep the mucosa wet during the contact period. The movable probe of the texture analyzer was lowered until it made contact with the mucosa. The contact time between the cheek mucosa and film was 1 min. Measurements were obtained using the following parameters: pre-test speed: 0.5 mm/s; test / post-test speed: 0.5 mm/s; applied force 1N. The force needed to separate film from the membrane was measured. The mucoadhesive strength was taken as the maximum amount of force required to detach the film from the rabbit mucosa.

Percentage hydration of prepared films were measured by cutting films (1×1 cm²), weighed ($w_1$) and kept on a stainless steel mesh. This was then immersed in PBS for specific periods (15, 30, 45, 60 min). At these time intervals the wire mesh was removed from the buffer, extra water was wiped and determined the weight ($w_2$).[4] Percent hydration was calculated as;

$$\text{Percentage hydration} = \frac{W_2 - W_1}{W_2} \times 100$$

### In vitro release

The film size (2 × 1 cm²) was cut and pasted on a glass plate and placed inside a beaker (100 mL) contain PBS (50 mL). The medium was retained at 37 ± 1 °C and stirred at speed of 50 rpm. Samples were withdrawn at scheduled time periods, exchanged with new medium, filtered and analyzed.[19] The acyclovir release data was then evaluated using mathematical models like zero-order, first-order, Hixson–Crowell, Higuchi, and Korsmeyer–Peppas models.

### Ex-vivo permeation studies

The rate and extend of mucosal permeation of acyclovir and/or nanoparticles across the rabbit buccal mucosa (permeation barrier) was determined on a Franz diffusion cell.[20] The excised rabbit buccal mucosa membrane of specific integrity was fixed on the diffusion cell such that the smooth surface facing donor. The prepared films were cut into small sections (0.6 cm²) and positioned on the surface of buccal epithelium. Receptor compartment with a capacity of 5 mL was filled with PBS. The temperature was maintained at 37 ± 0.2°C, while the media was stirred at 50 rpm. Samples were drawn (1 mL) at specific time periods, sonicated for 24 h and centrifuged ($13416 \times g$) for 10 min to avoid the interference of some biological extracts from excised buccal tissues in UV absorbance during analysis. The samples were further diluted and analyzed using UV spectrophotometer.

**Table 1: Composition of the prepared mucoadhesive buccal films**

| Formulation code | Acyclovir (mg) | HEC (mg) | Eudragit RL 100 (mg) | PG (mg) |
|---|---|---|---|---|
| F1 | - | 750 | 500 | 100 |
| F2 | - | 500 | 1000 | 100 |
| F3 | - | 250 | 1500 | 100 |
| F4 | - | 100 | 2000 | 100 |
| F5 | 14* | 100 | 2000 | 100 |
| F6 | 28* | 100 | 2000 | 100 |
| F7 | 42* | 100 | 2000 | 100 |
| Control | 28** | 100 | 2000 | 100 |

*Incorporated nanoparticles equivalent to acyclovir. ** Incorporated free acyclovir.

**Table 2: Physico-mechanical properties of films (mean ± SD)**

| Formulation code | Thickness (nm) | pH | Folding Endurance | % drug content | Mucoadhesive strength (N) |
|---|---|---|---|---|---|
| F4 | 203 ± 22 | 6.2 ± 0.35 | 287 ± 16 | - | 6.8 ± 0.35 |
| F5 | 251 ± 39 | 6.4 ± 0.26 | 306 ± 19 | 92.37 ± 3.52 | 6.5 ± 0.42 |
| F6 | 283 ± 32 | 6.5 ± 0.30 | 311 ± 24 | 93.91 ± 1.65 | 5.9 ± 0.46 |
| F7 | 311 ± 40 | 6.3 ± 0.22 | 333 ± 35 | 95.15 ± 2.26 | 6.3 ± 0.51 |
| Control | 264 ± 26 | 6.8 ± 0.41 | 277 ± 15 | 94.62 ± 3.91 | 6.7 ± 0.58 |

**Table 3: Regression coefficients assessed by different kinetic models for acyclovir release from films**

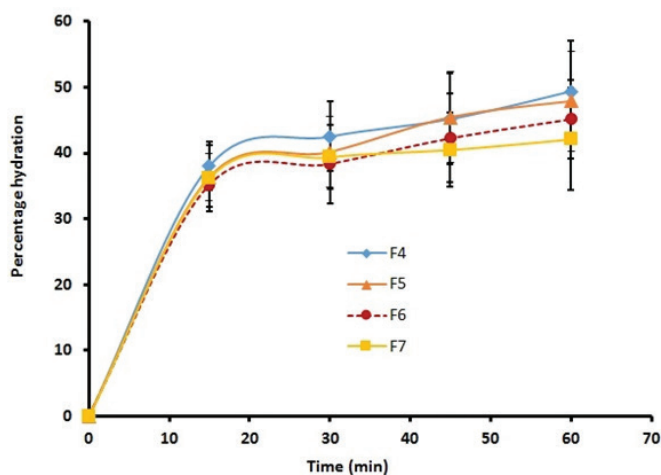| Formulation code | Zero order | First Order | Higuchi | Hixson-Crowell | Korsmeyer-Peppas |
|---|---|---|---|---|---|
| F5 | 0.9235 | 0.9922 | 0.9804 | 0.8043 | 0.9715 |
| F6 | 0.9379 | 0.9641 | 0.9869 | 0.8251 | 0.9787 |
| F7 | 0.8990 | 0.9763 | 0.9935 | 0.8103 | 0.9768 |
| Control | 0.8724 | 0.9909 | 0.9423 | 0.7984 | 0.9451 |



**Figure. 1:** The percentage hydration pattern of the prepared buccal films for a period of 1 h determined using 1 cm×1 cm of the film. The value represents average of six trials ± SD.
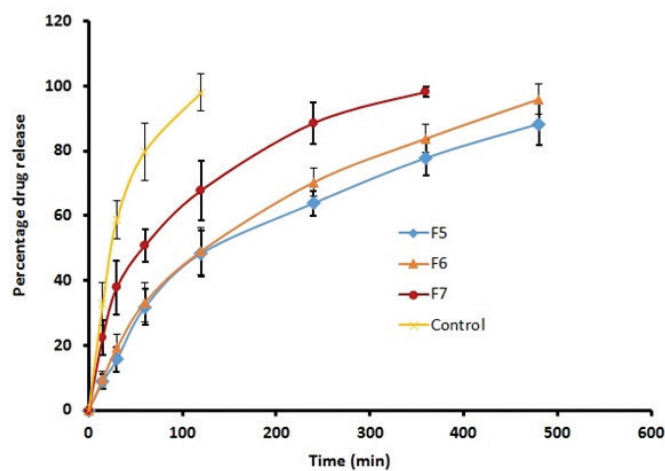


**Figure. 2:** Comparison of the cumulative percentage of acyclovir released from different buccal films at various time intervals. The value represents average of six trials ± SD.
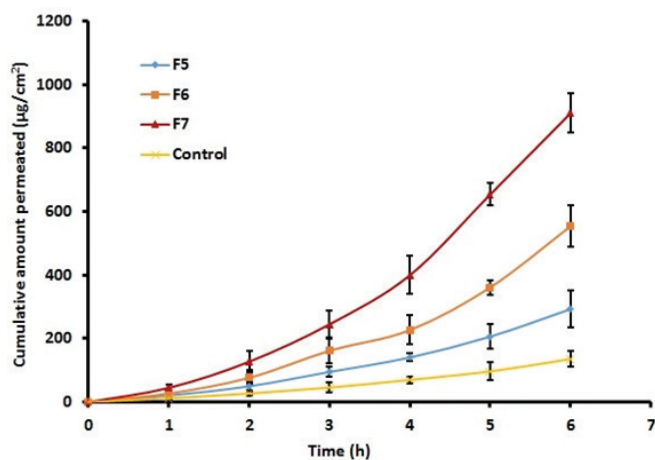
**Figure. 3:** Comparison of *ex vivo* permeation of acyclovir from different buccal films at various time intervals. The value represents average of six trials ± SD.

### *Data analysis*

A plot of total amount of acyclovir transported through the membrane versus time was made and slope was projected as flux. Unpaired t-test was carried out for conducting statistical analysis using GraphPad software. *P* value of less than 0.05 was considered as the level of significance.[21] Mean value and standard errors were calculated using values of six trials.

## RESULTS AND DISCUSSION

Acyclovir is an amphoteric low molecular weight (MW=225 Da) drug having both weak acidic and basic groups with reported partition coefficient (log P= -1.56), aqueous solubility (1.5 mg/mL) and pKa values (2.16 and 9.04).[22] In this context, it is presumed that permeation of acyclovir through the biological membrane could be relatively low. Hence, the drug was first enclosed in a biodegradable polymer (PLGA) by formulating into nanoparticles. It is known that the percentage entrapment efficiency gives the indication of entrapped drug in nanoparticles. The highest yield and entrapment was observed when a weight ratio of 1:20 (drug: polymer) was used. In this case, the percentage yield, entrapment efficiency and drug loading were 82.31 ± 5.91%, 64. 81 ± 4.72% and 4. 06 ± 1.12%, respectively, and this formulation was used for further studies. In addition, the particle size of the prepared nanoparticles were in the range of 150-400 nm with a typical size of 220 ± 40 nm.

In the next stage, buccal films were prepared using HEC as mucoadhesive agent and Eudragit as film former. The selection of these polymers in the current investigation is based on earlier studies.[16,17] Moreover, HEC is a common excipient used in various pharmaceutical formulations.[21] The composition of the prepared films is summarized in Table 1. The concentration of HEC (1-7.5% W/V) and Eudragit (5-20% W/V) were varied to obtain an appropriate buccal film for clinical use. In case of formulation F1, the film formed was defective as it could not be peeled, while F2 and F3 do not provide adequate strength and was sticky as well. However, composition of F4 was ideal and form a good film. In this formulation the concentration of HEC was 1% (W/V) while the Eudragit content was 20% (W/V). This composition was further incorporated with three different amounts of acyclovir loaded nanoparticles (0.5 mg/cm$^2$ area, 1 mg/cm$^2$ area and 1.5 mg/cm$^2$ area in F5, F6 and F7, respectively) as described in Table 1. Control films were prepared using same polymers in a similar experimental conditions, while the nanoparticles were exchanged with free drug. The concentration of propylene glycol was optimized by varying concentrations between 0.5 - 2% w/v. Thus

formulated films were further fixed to the backing membrane prepared using aqueous solution of PVA (4% W/V).

The films prepared were then evaluated for various physico-mechanical properties. It was found that the prepared films (F4-F7 and control) were transparent, soft, homogenous and peelable. Determination of film thickness is also important to assure good adhesion of film with mucous membrane as well as the uniformity of drug in the film. The prepared films thickness were in the range of 180 to 350 nm (Table 2). These results showed that formulation F4 (without nanoparticles) had a thinnest film and formulation F7 had thick film. Among nanoparticles loaded films (F5, F6 and F7), the thickness increases with increase in drug content of acyclovir loaded nanoparticles. Certainly, the measured thickness of prepared films (F4-F7 and control) is ideal for application on the buccal mucosa and for bioadhesion.[18] pH of the buccal film was measured to ensure optimal pH for oral application and avoid any damage to the buccal mucosa. Indeed, prepared films (F4-F7 and control) showed a pH near to 6.

Mechanical strength and flexibility of prepared films were evaluated by measuring the folding endurance. The data observed in Table 2 suggest that the prepared buccal films (F4-F7 and control) possess adequate folding endurance and can provide easy application on the site of administration. Further, formulations were evaluated for drug content i.e., the amount of drug loaded in the buccal films. Uniform dispersion of nanoparticles in film means the drug is homogeneously distributed in the film. It can be seen from Table 2 that the drug content do not vary among the formulations and is in the range of limits (100 ± 10% w/w). This comparable values in drug content suggest that the variation in acyclovir loaded nanoparticles have little or no effect on drug content, in the present experimental settings. The low standard deviation also signify that the drug was uniformly dispersed within the film.

The measurement of mucoadhesive strength is likely to provide real time effect of binding of films with buccal mucosa. This is important as the film has to adhere and retain on buccal mucosa for effective delivery of actives. The mucoadhesive strength measured with various formulations were summarized in Table 2. No significant difference (*P*>0.05) between mucoadhesive strength (5.9 -6.8 N) of films (F4, F5, F6, F7 and control) also suggest that the incorporation of free drug or nanoparticles have little or no effect. Based on the evidences from literature, it is likely that the values exhibited by the prepared films possess adequate buccoadhesive strength to withstand pressure due to movements in the buccal cavity.[7]

Hydration of films is a prerequisite for adhesion and release of drug molecules.[23] Following application, the films absorb water and hydrate itself to provide good adhesion in addition helps in separation and diffusion of particles (nanoparticles in this case). However, hydration is highly influenced by polymer, crosslinking as well as interaction between nanoparticles and polymers.[24,25] The swelling index of all the prepared films (F4, F5, F6 and F7) were comparable (Figure 1). As indicated from Figure 1, all the prepared films showed rapid hydration in the initial 15 min of the study, which is in agreement with several earlier studies.[9, 26] This was followed by a relatively slower phase of hydration which continued till 60 min. The prepared films comprised of a combination of hydrophilic (HEC) and hydrophobic (Eudragit) polymer and the hydrophilic polymer is responsible for hydration. However, the greater amount of Eudragit (in the composition) prevent higher hydration, which in turn maintain the integrity and mechanical stability of films.

The prepared films were impregnated with nanoparticles, and as a prerequisite, these nanoparticles must be released from the film and transport through the membrane. The release of nanoparticles from formulations is governed by the separation of nanoparticles from the polymer matrix and their diffusion from the matrix.[27] Percentage of drug released from different films [F5, F6, F7 and control (drug incorporated films)]

are depicted in Figure 2. It is evident from Figure 2 that all formulations exhibited a biphasic release pattern. The drug release was rapid in the initial 2 h and were 48.37 ± 7.12%, 49.05 ± 7.24%, 67.79 ± 9.16% and 97.98 ± 5.63% in F5, F6, F7 and control (drug incorporated films), respectively. The high release observed could be attributed to the presence of hydrophilic polymer (HEC) in the films, which would have allowed the diffusion of water more rapidly and helped in release of nanoparticles. The rapid release was followed by slower release in nanoparticles loaded films, while in control drug release was complete in 2 h. Among nanoparticles loaded films, the percentage release was relatively high with film F7 (with highest drug content). However, the percentage drug release was comparable (*P*>0.05) in film F5 and F6.

The *in vitro* release data were applied to various kinetic models such as zero-order, first-order, Hixson–Crowell, Higuchi, and Korsmeyer–Peppas models to predict the drug release mechanism and kinetics. It is evident from Table 3 that the calculated regression ($r^2$) values were different for prepared films. Briefly, when log percent of drug remaining to be release versus time was plotted in accordance with first order equation, a straight line was obtained ($r^2$ >0.99) for formulation F5 and control. These values indicated that the drug release followed first order kinetics, where the drug release was dependent upon acyclovir concentration in the polymer matrix. However, in case of formulations F6 and F7, the drug release mechanism was diffusion controlled as plots of the amount releases versus square root of time was found to be linear. Overall, the kinetics data suggest that the release was first order when the acyclovir concentration was low (0.5 mg/cm²) while it followed Higuchi model at high drug concentration (1 mg/cm²).

Permeation study is done usually to confer the transport of drug across permeation barriers. The permeation of drug molecules into and through a biological barrier is influenced by thickness of the membrane and its composition, and the specific passage accessible for drug permeation.[28] The amount of acyclovir incorporated in the films was determined based on its standard human dose.[29] The permeation profiles of drug loaded films (F5, F6, F7 and control) are shown in Figure 3. It is evident from Figure 3 that the drug permeation increased with duration of time. Similarly, the increase in drug content in the films from F5 (14 mg) to F6 (28 mg) and further F7 (42 mg), consistently increased the acyclovir permeation. The amount of drug permeated across the membrane at 1 h was 20.65 ± 4.26 μg/cm², 26.99 ± 5.18 μg/cm², 44.70 ± 8.63 μg/cm² and 11.62 ± 4.29 μg/cm², in F5, F6, F7 and control, respectively. This observation also support the drug release studies in which the amount of drug release was higher when drug content was increased. Significant difference (*P*<0.05) in acyclovir permeation between the formulations was observed from 4 h onwards. The flux values in F5, F6, F7 and control were 48.81 ± 7.93 μg/cm²/h, 92.33 ± 11.01 μg/cm²/h, 151.67 ± 16.43 μg/cm²/h and 22.60 ± 4.23 μg/cm²/h, respectively. Moreover, the enhancement in acyclovir permeation in F5, F6 and F7 loaded films were 2.16, 4.09 and 6.71 folds, when compared to control. Overall the data observed here substantiate our objective of enhancing the permeation of acyclovir by incorporating the drug in nanoparticles and further embedding into a buccal film.

## CONCLUSION

The present study aimed to develop and evaluate buccoadhesive films to enhance acyclovir permeation. Acyclovir loaded biopolymeric nanoparticles were prepared and loaded into mucoadhesive films. The films were evaluated for physico-mechanical properties, *in vitro* and *ex vivo* studies. Drug loaded films (F5, F6 and F7) exhibited uniformity in drug content and desirable physico-mechanical properties such as pH, film thickness, folding endurance, swelling capacity and mucoadhesive strength. Data observed with *in vitro* release studies substantiate the potential of de-

veloped films to provide an adequate amount of acyclovir for diffusion across the buccal membrane. Permeation data further confirmed the potential of acyclovir loaded nanoparticles to transport into and across the buccal epithelium and provide adequate delivery of acyclovir for systemic therapy.

## CONFLICT OF INTEREST

The authors report no conflict of interest.

## ACKNOWLEDGEMENT

## ABBREVIATIONS

HEC: hydroxyethyl cellulose, PBS: phosphate buffered saline, PG: propylene glycol, PLGA: poly (D,L-lactide-co-glycolide)

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