

Mucoadhesive Buccal Films Embedded with Antiviral Drug Loaded Nanospheres

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Acyclovir loaded nanospheres embedded mucoadhesive film have been developed as potential drug delivery system and evaluated both *ex vivo* and *in vivo*. Poly (lactic-co-glycolic acid) nanospheres were formulated and impregnated in films prepared with varying concentrations of hydroxyethyl cellulose and eudragit RS PO. The prepared formulations (F1-F4) were evaluated by *ex vivo* permeation studies using rabbit mucosa in order to select the optimum formulation for further studies. *In vivo* studies were carried out in rabbits to assess the pharmacokinetic profile of acyclovir when delivered from mucoadhesive buccal films. *Ex vivo* studies (6 h) indicate significant difference in permeation rate of acyclovir from mucoadhesive films (F3 > F4 > F2 > F1), suggesting the release and/or permeation of acyclovir is influenced by the film composition used. SEM images showed the nanospheres were embedded uniformly in the films. *In vivo* data demonstrated significant absorption ($C_{max} \sim 360.93$ ng/mL; $P < 0.0001$), prolonged duration (T_{max} 6 h) and greater $AUC_{0-\infty}$ (~5 folds, $P < 0.0001$) with buccal films in comparison to oral solution (control). The results showed the prospective of employing nanospheres loaded mucoadhesive film (F3) for buccal delivery of acyclovir.

Key words: Acyclovir, Buccal delivery, Eudragit, *Ex vivo*, *In vivo*.

Antiviral Etken Madde Yüklü Nanokürelerin Yerleştirildiği Mukoadhezif Bukkal Fimler

Asiklovir yüklü nanopertillerin yerleştirildiği mukoadhezif film potansiyel ilaç salım sistemi olarak geliştirildi ve *ex vivo* ve *in vivo* olarak değerlendirildi. Poli(laktik-ko-glikolik asit) nanoküreler formüle edildi ve farklı konsantrasyonlarda hidroksipropil selüloz ve eudragit RS PO ile hazırlanan filmlere eklendi. Hazırlanan formülasyonlar (F1-F4) daha sonraki çalışmalar için optimum formülasyonun seçilmesi için tavşan mukozası kullanarak *ex vivo* olarak değerlendirildi. Asiklovirin mukoadhezif bukkal filmlerden salındığında farmakokinetik profilini belirlemek için tavşanlarda *in vivo* çalışmalar yapıldı. *Ex vivo* çalışmalar (6 saat) mukoadhezif filmlerde asiklovirin permeasyonunda anlamlı farklılık gösterdi (F3 > F4 > F2 > F1), bu da asiklovir salım ve/veya permeasyonunda kullanılan film kompozisyonunun etkili olduğunu gösterir. SEM görüntüleri nanokürelerin filmlere homojen yerleştiğini gösterdi. *In vivo* veriler oral çözeltiye (kontrol) göre bukkal filmler ile anlamlı absorpsiyon ($C_{max} \sim 360.93$ ng/mL; $P < 0.0001$), uzatılmış etki süresi (T_{max} 6 h) ve daha büyük $AUC_{0-\infty}$ (~5 kat, $P < 0.0001$)'yi kanıtlamıştır. Sonuçlar, asiklovirin bukkal salımı için nanoküre yüklü mukoadhezif film (F3) kullanımının muhtemel olduğunu göstermiştir.

Anahtar kelimeler: Asiklovir, Bukkal salım, Eudragit, *Ex vivo*, *In vivo*.

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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, 2). Moreover, the buccal mucosa is relatively permeable with a rich blood supply and is readily accessible for self-medication. The potential of this route to augment the transmucosal transport of drugs into the systemic circulation 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, the 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 (5-7). 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 (8). Recently, our research group has demonstrated the prospective of drug loaded nanospheres embedded films in enhancing the bioavailability of selegiline when delivered through buccal route (9).

Acyclovir is a potent, selective inhibitor of herpes viruses and is most widely used to treat recurrent cold sores on the lips and face, genital herpes, shingles and chicken pox (10). Topical application of acyclovir is found to be unsuccessful probably because of its low permeation potential across the stratum corneum (11, 12). Alternatively, oral route has been widely investigated as it provides numerous advantages and is favored over other routes. However, the efficiency of oral therapy of acyclovir is limited by the physicochemical properties and slow, variable and incomplete

absorption with low oral bioavailability (15-30%) (13). Reports in the literature suggest that there have been few attempts to formulate an effective delivery system for the successful therapy of acyclovir (14). Effort has been made to formulate buccal tablets of acyclovir and deliver it through the buccal mucosa, but the application issue like very short residence time has limited its achievement (15). Alternatively, inclusion complex of acyclovir with cyclodextrin was also assessed to enhance the oral absorption (14, 16). Indeed, the reduced efficiency of acyclovir following oral delivery is primarily because of this drug's inherent low solubility and permeability (17). Considering the biological classification system, the intrinsic permeability of acyclovir through the buccal epithelium is likely to be low. It has been postulated that the incorporation of acyclovir loaded nanospheres in buccal film could enhance the efficiency of this drug molecule. In the present investigation, PLGA copolymer was selected for preparing nanospheres owing to its distinguished properties such as biodegradability, biocompatibility and controlled drug release (18).

The present study investigated the feasibility of developing mucoadhesive buccal films embedded with biodegradable polymeric nanoparticles of acyclovir with the objective of improving bioavailability. Totally different from the earlier study, the mucoadhesive buccal films for the controlled delivery of acyclovir were fabricated using varying concentrations of hydroxyethyl cellulose (HEC) as mucoadhesive agent, Eudragit RL PO as a film former, propylene glycol as plasticizer, polyvinyl alcohol as backing membrane, in addition to higher amount of acyclovir (2 mg/cm²). The prepared films were evaluated by *ex vivo* permeation studies. *In vivo* studies were carried out in rabbits to evaluate the potential of optimized buccal films embedded with drug loaded nanospheres to improve bioavailability of acyclovir.

MATERIALS AND METHOD

Materials

Polyvinyl alcohol, poly (D,L-lactide-co-glycolide) (PLGA, 50:50, Mw: 7000-17000), hydroxyethyl cellulose (HEC), propylene glycol (PG), dichloromethane, acetonitrile, ethanol and acetic acid were obtained from Sigma Aldrich St. Louis, MO, USA. Acyclovir was received from Ind-Swift Laboratories (Chandigarh, India). Eudragit RL PO (Evonik, Darmstadt, Germany) and polyvinylpyrrolidone (PVP K30; Loba Chemie, Mumbai, India) were purchased commercially.

Preparation of nanospheres

Nanospheres were prepared by double emulsion-solvent evaporation method reported before with slight modifications (19). Briefly, PLGA (200 mg) was added to dichloromethane (10 mL) and stirred to obtain a clear solution. Acyclovir (1 mL; 10 mg/mL) in water was added slowly to the polymeric solution under high speed stirring and sonicated to provide primary emulsion. After emulsification (~2 min), the primary emulsion was immediately added to PVA solution (4% w/v, 20 mL; optimized from concentrations ranging from 1-5% w/v) and was homogenized at high speed for 2 min. The residual solvent in the multiple emulsion was removed by stirring for 12 h at room temperature. The resulting dispersion was centrifuged (20,000 rpm, 20 min) and the supernatant was decanted. The nanospheres prepared were washed (3 times) with deionized water to remove the surface residue and freeze dried for 24 h. The supernatant separated was analyzed for drug content to determine the entrapment efficiency (EE) by using following equation (20).

$$\% \text{ EE} = (\text{amount of drug entrapped} / \text{amount of drug incorporated}) \times 100$$

Particle size characterization

The prepared nanospheres were characterized for particle size, distribution as well as Zeta potential using Zetasizer (Nano-ZS, Malvern,

Westborough, MA). The freeze dried nanospheres were suspended in deionized water prior to measurement.

Preparation of mucoadhesive films

Required quantity of eudragit was added to ethyl alcohol with constant stirring to obtain a clear solution. HEC was dispersed separately in water containing PG (1% w/v) by stirring for 12 h using a mechanical stirrer to obtain uniform dispersion. Then the eudragit solution was added to the HEC dispersion under stirring to form a homogeneous mixture. Drug loaded nanospheres (corresponding to 2 mg acyclovir/cm²) were added to the above mixture and stirred to ensure uniform distribution of nanoparticles. Then, the dispersion was sonicated in an ultrasonicator to remove the entrapped air bubbles (~10-20 s) and the properties of nanoparticles were not affected by this short period of sonication. Accurately measured volume of the dispersion was then casted on custom made glass mold (area 6 cm²) and covered with inverted glass funnel and allowed controlled evaporation of the solvent at room temperature for 24 h. The compositions of various mucoadhesive buccal films are summarized in Table 1.

Backing membrane was prepared by mixing polyvinyl alcohol dispersion (4% w/v) and casted on a custom made glass mold and allowed to dry at room temperature for 24 h. The dried membrane was attached to the mucoadhesive film using polyvinylpyrrolidone (PVP K30) solution (5% w/v). The prepared films adhered to backing membrane were then cut into 1 cm² sizes, wrapped and stored in desiccator until used.

Analytical method

The concentration of acyclovir in samples was measured by high performance liquid chromatography (HPLC) system (Cyberlab, LC P100). The HPLC system consisted of a Symmetry C18 analytical column (4.6 X 150 mm, 5.0 µm) with a LC UV-100 detector. Chromatographic separation of acyclovir was achieved by a mobile phase comprising of 0.1 % acetic acid and acetonitrile (98:2). The

column temperature was maintained at 25°C while the flow of the mobile phase was regulated at 1.2 mL/min. Samples of 25 µL were injected and the area under the curve was measured at a wavelength of 207 nm (21). The calibration curve was linear in the range of 10-500 ng/mL ($r^2=0.991$). The retention time of acyclovir was about 2.6 min. The coefficient of variation and accuracy ranged 1.29-7.10% and -1.77 to -8.45, respectively.

compartment. Drug loaded films were punched using biopsy punch (0.6 cm²) and placed on the donor compartment. Receptor compartment with a capacity of 5 mL was filled with simulated saliva (22). Thereafter, the donor and receptor compartments of the Franz diffusion cell were clamped together. The diffusion cell was configured at a temperature of 37 ± 0.2°C. The release medium in the receptor compartment was stirred at 50 rpm.

Table 1. Composition of the prepared mucoadhesive buccal films.

Formulation code	Acyclovir (mg)*	HEC (% w/v)	Eudragit RL PO (% w/v)	PG (% w/v)	PVA (% w/v)
F1	2	5.0	15.0	1.0	4
F2	2	7.5	12.5	1.0	4
F3	2	10.0	10.0	1.0	4
F4	2	12.5	7.5	1.0	4

* Amount of nanoparticles equivalent to 2 mg of acyclovir in each square centimeter area of film.

Drug content in film formulations

The content uniformity test was performed to ensure the uniform drug distribution in the film. Six films were randomly selected for each formulation. Films were punched with a biopsy punch (1 cm²) and soaked in ethanol-1 mM sodium hydroxide solution (50:50; 50 mL) and stirred using vortex mixer. The extraction period was selected (24 h) as the next sampling point (30 h) did not improve the extraction of acyclovir. The solution was centrifuged at 10,000 rpm (5 min) and the supernatant was collected and filtered (0.2 µm). The amount of acyclovir in the supernatant was determined by HPLC methods described in analytical method before.

Permeability studies

Freshly excised rabbit buccal mucosa was mounted between the donor and receptor compartments of a Franz diffusion cell ensuring that smooth surface of the mucosa faces donor

The samples withdrawn were analyzed using HPLC as mentioned in the analytical method before. The calibration curve was linear in the range of 1-1000 ng/mL ($r^2=0.996$). The retention time of acyclovir was about 2.6 min.

Scanning electron microscopy (SEM)

SEM was used to observe the selected mucoadhesive buccal film. Fabricated mucoadhesive films were fixed in place by means of a double sided silver electrical tape and gold coated in SCD005 Baltek Sputter Coater in a neutral environment of argon maintained at a low pressure. SEM images were obtained under different magnification using a Jeol 457V (Tokyo, Japan) at an intensity of 15 kV and photographed.

In vivo study

White male rabbits (3-5 kg) which were maintained on a 12/12 h light/dark cycle in an animal facility with unlimited access to food

and water (IAEC/SSP/14/PR-054) were used. Animals were divided into two groups, group I animals were anaesthetized for 3-4 h by intramuscular injection of ketamine (40 mg/kg) and xylazine (5 mg/kg) (23). Mucoadhesive buccal film (1 cm²) was wetted (~30 µL of water) and applied to the buccal mucosa of the rabbits while the group II (control) animals received oral solution of acyclovir equivalent to 2 mg dose (1 mL). The blood samples were collected (~500 µL) from the marginal ear vein at predetermined time intervals (1-24 h) using 25 gauge 1 inch needle, and injected into HPLC.

Data analysis

The cumulative amount of drugs permeated per unit buccal surface area was plotted against time and the slope of the linear portion of the plot was estimated as the flux (24, 25). Statistical analysis was performed by unpaired t-test using GraphPad Prism (version 5, Graphpad software, San Diego, California, USA) to test the level of significance. P value less than 0.05 was considered as the level of significance.

RESULTS

Four different films (F1-F4) with varying concentrations of polymers (HEC and Eudragit RL PO) were formulated to evaluate the permeation through buccal membrane. The composition of different mucoadhesive films fabricated is summarized in Table 1. The amount of hydrophilic (HEC) and hydrophobic polymer (Eudragit RL PO) was varied from 5-12.5% and 7.5-15%, respectively. Based on preliminary studies, PG (plasticizer) content (1% w/v) was fixed. Different amount of acyclovir loaded nanospheres (equivalent to ~1-5 mg/cm² of drug) were incorporated into the films and the physical characteristics were examined. Indeed, the film embedded with 2 mg/cm² (amount of drug) exhibited excellent physical characteristics, among the various drug level studied. The prepared backing membrane was then attached to the films using PVP

solution (5% w/v). The measured thickness of various mucoadhesive buccal films suggests significant difference between different formulations (F1-F4) (Table 2).

Figure 1 depicts the amount of acyclovir permeated through the buccal membrane from various films (F1-F4). It is apparent from Figure 1 that drug permeation was significantly low with films F1 and F2, when compared to F3 and F4 films. However, the permeation rate was comparable in formulation F3 and F4. Among the tested mucoadhesive films, the cumulative amount of drug permeated at the end of the study period (6 h) decreases in the following pattern, F3 > F4 > F2 > F1. The observed flux values are summarized in Table 2.

Topographical and morphological features of film F3 was examined using SEM images. It is apparent from Figure 2 that nanospheres were embedded uniformly in the films and were smooth and nonporous. Thus, the image shows that the prepared film possess all the desired characteristics of an ideal film for buccal application.

Given promising *ex vivo* results, final phase of the study assessed the potential of selected film (F3) in delivering acyclovir *in vivo*. Mucoadhesive buccal film contain 2 mg of acyclovir was applied to buccal mucosa of test animals while same dose (2 mg) was administered orally in control groups. Various pharmacokinetic parameters i.e. area under the concentration time curve from time 0 to ∞ (AUC_{0-∞}) (using trapezoidal method), time to reach maximum drug concentration (T_{max}) and peak plasma drug concentration (C_{max}) (from plasma drug concentration curves) were calculated using non-compartmental pharmacokinetic model. Figure 3 compares the plasma level profiles of acyclovir following administration of nanosphere loaded mucoadhesive buccal film and acyclovir solution. The measured pharmacokinetic parameters of acyclovir following administration of mucoadhesive buccal film and oral solution are summarized in Table 3. Plasma half-life and elimination rate constant of acyclovir following oral therapy (control) was found to be ~ 3.5 h and ~0.19 /h, respectively.

Table 2. Thickness, drug content and flux values of different mucoadhesive buccal films.

Formulation code	Thickness (nm)	Percentage drug content/cm ²	Flux (µg/cm ² /h)
F1	651 ± 72	93.27 ± 3.14	72.80 ± 14.59
F2	546 ± 49	97.22 ± 3.61	116.44 ± 27.15
F3	430 ± 39	95.73 ± 3.94	148.69 ± 20.83
F4	320 ± 25	96.29 ± 2.05	138.31 ± 24.05

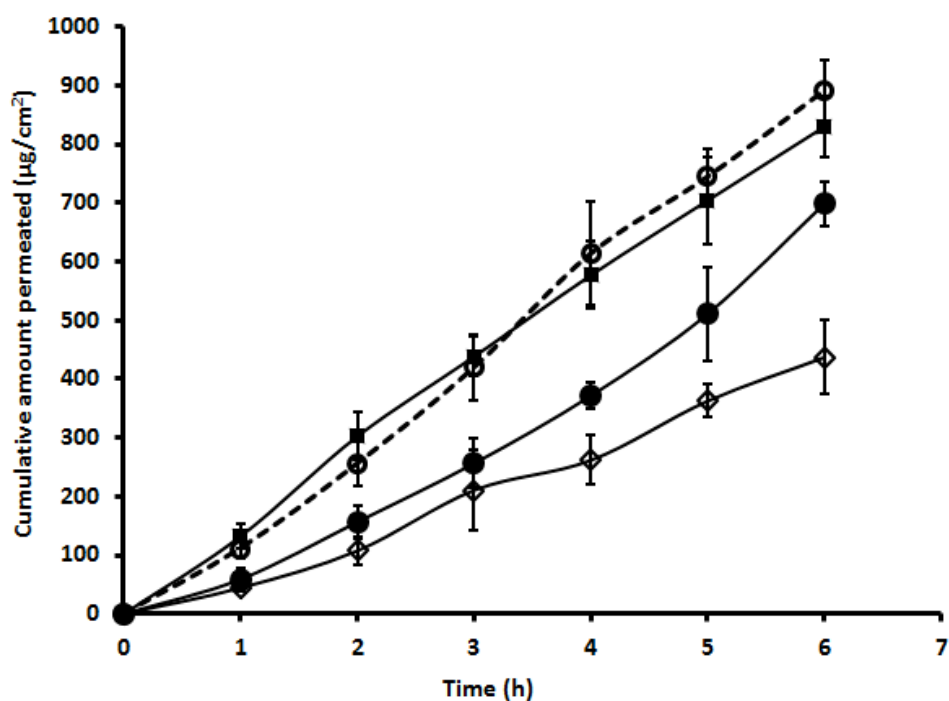


Figure 1. Amount of acyclovir permeated across the excised rabbit buccal mucosa at different time intervals. *Ex vivo* permeation study was carried out by applying mucoadhesive buccal films F1 (◇), F2 (●), F3 (○) and F4 (■) for a period of 6 h in Franz diffusion cell. Data represents average of six trials ± SD.

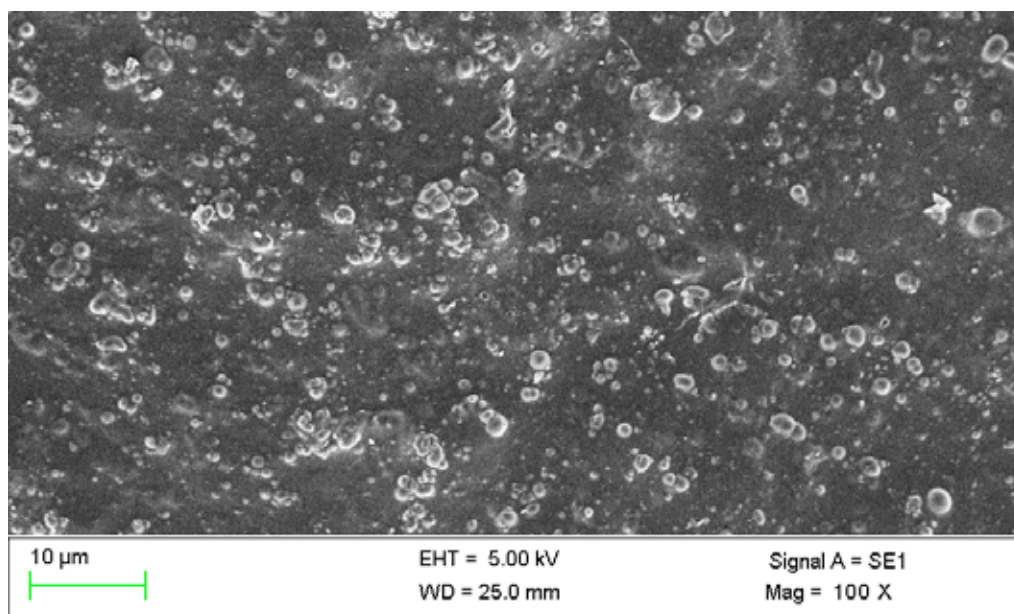


Figure 2. Representative SEM image of buccal film F3 embedded with acyclovir loaded nanospheres.

DISCUSSION

Preliminary investigation was carried out to optimize the drug concentration to obtain greater entrapment efficiency in nanospheres. Different concentrations (1-15% w/v) of acyclovir were added to the polymeric solutions and amount of drug entrapped were determined. The data observed in this set of experiment indicate that entrapment efficiency increases when acyclovir content was increased from 1% w/v to 10% w/v (~63%). However, further attempt to increase the drug level yielded in significant reduction in entrapment efficiency, although the quantity of acyclovir loaded was comparable. This data suggest that the drug entrapment in PLGA has attained saturation in the current experimental condition. This observation could be related to the fact that drug entrapment potential of a polymer mainly depends on partition coefficient of pharmaceutical active as well as its solubility in specific polymer (20). In addition, solubility of drug molecule in a polymer depend on molecular weight, chemical nature as well as

possible drug-polymer interaction. The size of prepared particles was in nano range (240-800 nm) with an average size of 450 nm. Further, the nanospheres also exhibited appropriate zeta potential (~24.11 mV) and polydispersity index (~0.19).

The films were fabricated using two renowned polymers such as HEC and Eudragit RL PO, based on literature for their renowned contribution in developing various mucoadhesive films (5, 26). In this study, we used HEC as mucoadhesive polymer (nonionic, molecular weight ~250,000 and viscosity of ~120 cP), while eudragit RL PO (water insoluble and most water permeable polymer among the eudragit polymers) as a film former.

The thickness of prepared mucoadhesive buccal films (F1-F4) decreases with increase in eudragit content (Table 2). This is most likely due to difference in the content of Eudragit RS PO, whose density (0.3 g/mL) is much low than HEC (0.6 g/mL). As seen from Table 2, the thickness of films (~300- 700 nm) is suitable for buccal application in human. Moreover, the percentage drug content was good (~93-97%) in

Table 3. Mean pharmacokinetic parameters of acyclovir in plasma following buccal application of film F3 (2 mg) and oral solution (control) in rabbits.

Parameter	Buccal Film (F3)	Control
T _{max} (h)	6	2
C _{max} (ng/mL)	360.93 ± 91.52	164.08 ± 59.61
AUC _{0-∞} (ng.h/mL)	3820.87 ± 351.64	699.32 ± 71.29

all the formulated mucoadhesive films (Table 2) and was not influenced by the polymers used. Furthermore, Fourier transform infrared spectroscopy spectrum of films also denoted the absence of any drug polymer interaction (data not shown).

In next phase of study, the release and transport of acyclovir from the prepared films across rabbit buccal mucosa was determined. Indeed, assessment of permeation of acyclovir following application of a buccal patch is likely to provide better insight into the absorption kinetics and bioavailability. The permeation of drug moieties across the biological barriers is directly influenced by the rate and extent of drug release from the matrix (27). It should be emphasized that the release rate of a drug in film is mainly governed by its solubility in the polymer as well as its diffusion coefficient in polymer. Rabbit buccal mucosa was used in current investigation as this membrane is the primary choice among animal models and is considered to be an alternative to human buccal mucosa due to the presence of para-keratinized buccal membrane (21). The data observed in *ex vivo* experiments concluded that the release and/or permeation of acyclovir is influenced by the film composition used. The observed difference in acyclovir transport across the buccal membrane from formulated films could be due to the difference in content of water insoluble polymer (eudragit RS PO), which was low in film F1 (7.5 % w/w) and high in film F4 (15 % w/w). Thus it is speculated that increase in concentration of hydrophobic polymers will slow down the release and/or permeation of acyclovir. Based on these results, film F3 was selected for *in vivo* studies.

Figure 3 revealed that plasma profiles of acyclovir following buccal application of the nanospheres loaded mucoadhesive buccal film (F3) and the control solution by oral route in rabbits were different. In both cases, acyclovir was detected in plasma in the initial hour itself, although the drug concentration was significantly different ($P= 0.0010$). However, the profile indicates that the application of mucoadhesive buccal film prolonged the acyclovir delivery and increases the duration of absorption which in turn enhanced the drug level in body (measured from plasma concentration) in comparison to oral solution (control) (Figure 3). The values of C_{max} (360.93 ± 91.52 ng/mL) and T_{max} (6 h) were significantly high following buccal delivery in comparison to oral solution. Followed by peak plasma concentration, the drug level decreased rapidly in both cases suggesting short biological half-life of acyclovir. The overall mean value of AUC_{0-∞} by buccal route (3820.87 ± 351.64 ng.h/mL) was found to be ~5 folds higher as compared to oral suspension, demonstrating improved bioavailability of acyclovir by the optimized mucoadhesive buccal film embedded with drug loaded PLGA nanoparticles (Table 3). The high AUC values observed with mucoadhesive buccal film signifies increased rate and extent of acyclovir absorption (relative bioavailability ~500%) compared to the control oral suspension. In addition, the pharmacokinetic data observed in the current study was comparable to our earlier study (28).

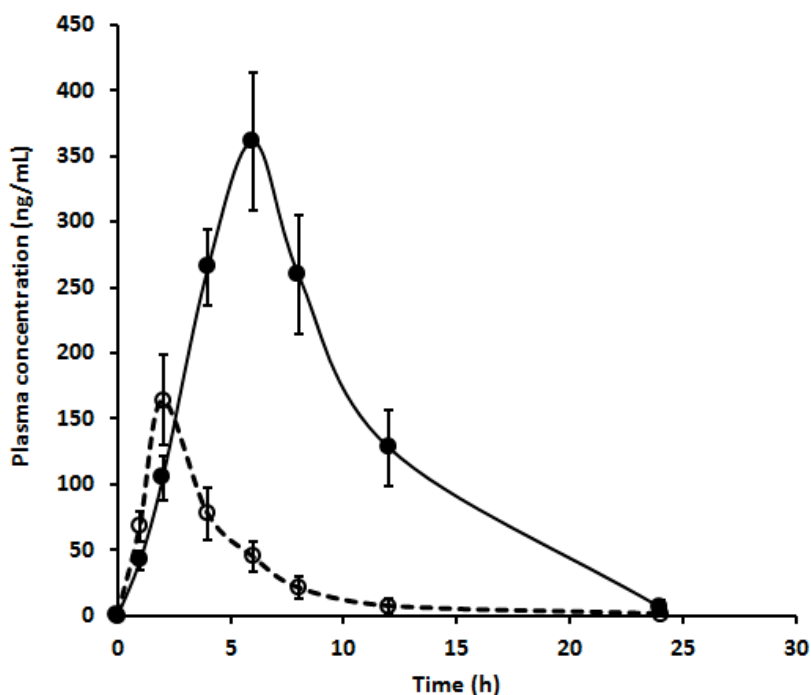


Figure 3. Comparison of the plasma profiles of acyclovir following buccal application of the nanospheres loaded mucoadhesive buccal film F3 (●) and the control solution (○) by oral route in rabbits (The value represents average of six trials \pm SD).

CONCLUSION

Mucoadhesive buccal films embedded with acyclovir PLGA nanospheres were fabricated and evaluated *ex vivo* and *in vivo* in rabbits. Films were prepared making use of HEC and eudragit RS PO. *Ex vivo* permeation studies substantiate the potential of the prepared films to transport acyclovir in a sustained manner. *In vivo* data indicate the ability of prepared mucoadhesive film (F3) to prolong the absorption of acyclovir and enhance its bioavailability. This improvement in bioavailability via buccal application of nanospheres could be due to the direct transmucosal transport of acyclovir into the systemic circulation as against the oral route which is known to show poor oral

bioavailability (15-30%). Also the T_{max} value of 6 h (mucoadhesive film) as against 2 h after oral administration suggest that the buccal route is able to prolong the drug delivery and may reduce the frequency of drug administration. In nutshell, the developed sustained release mucoadhesive buccal film (F3) can be a good alternative for administering acyclovir.

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