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Original Article

MUCOADHESIVE POLYMERIC FILMS OF ACYCLOVIR PRONIOSOMES FOR BUCCAL ADMINISTRATION

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ABSTRACT

Objective: The aim of the present work was to formulate and evaluate proniosomes of the poorly soluble drug, acyclovir incorporated in mucoadhesive polymeric films for improved buccal mucosal permeability of the drug while achieving prolonged release.

Methods: Acyclovir was formulated as proniosomes using Span 60 and cholesterol. The prepared proniosomes were loaded into mucoadhesive polymeric films prepared with varying quantities of carbopol 934P and HPMC K15M. The proniosome incorporated films were evaluated for physicomechanical characters, mucoadhesion, swelling index, drug content, *in vitro* drug release and *ex vivo* permeation through porcine buccal mucosa.

Results: Hydration of the proniosomes produced spherical vesicles or niosomes, which was confirmed by Scanning Electron Microscopy. The optimized formulation selected on the basis of vesicle size, entrapment efficiency PDI, Zetz potential and *in vitro* drug release was selected for incorporation into mucoadhesive polymeric films. All the films showed excellent physicomechanical characters. Formulations with higher proportions of carbopol produced slower *in vitro* drug release. The kinetics of release of drug from all the formulations appeared to be zero-order based on their regression coefficient values. Comparative evaluation of *ex vivo* permeation from niosomal and non-niosomal films indicated that the former demonstrated improved mucosal permeation and drug release was also sustained for the 8 h period.

Conclusion: Mucoadhesive films impregnated with acyclovir loaded proniosomes could be a potential approach for buccal delivery of acyclovir for improving its absorption and bioavailability.

Keywords: Cholesterol, Carbopol, Mucoadhesive, Proniosomes, Buccal, Permeation

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INTRODUCTION

The oral therapy of acyclovir, an antiviral, has several limitations, such as poor oral bioavailability of only 15-30% due to its poor aqueous solubility. It has a short half-life of 2.5-3.3 h and therefore requires frequent dosing [1, 2]. Hence the chances of patient noncompliance with respect to adhering to the dosing schedule by the conventional oral route would be high. Acyclovir immediate-release tablets and capsules are associated with cholinergic side effects like nausea, vomiting, dizziness, dyspepsia and acute renal failure [3]. Therefore, there is a need to improve the efficacy of acyclovir by improving absorption while reducing its toxicity. Several approaches have been investigated to enhance the oral bioavailability of acyclovir, such as prodrugs, self-emulsifying drug delivery systems and nanoemulsions to name a few [4, 5].

An alternative approach to oral administration is the buccal mucosal delivery of acyclovir. Of all the transmucosal routes for the systemic delivery of drugs, administration through the buccal mucosa has some well-defined advantages. Besides its easy accessibility, excellent patient acceptance rate, the buccal mucosa is highly permeable and rich in blood supply with rapid recovery after damage or stress [6]. This route of drug administration is a means of avoiding first-pass metabolism and presystemic elimination in the gastrointestinal tract. Moreover, by the use of mucoadhesive controlled release buccal dosage forms, patient compliance can be improved by eliminating the need for frequent administration [7].

Several studies have been reported wherein acyclovir has been formulated as buccal tablets or films [8, 9]. However, the mucoadhesive buccal tablets of acyclovir currently in the market have been approved for the topical or local delivery of the drug in the oral cavity for the treatment of herpes labialis and is expected to be minimally absorbed through the oral mucosa. Thus the buccal mucosal delivery for systemic absorption of this drug is limited by its poor aqueous solubility and permeability. Generally, drugs which shows significant problems in absorption after oral administration due to limited solubility and poor permeability are likely to show similar problems even after buccal administration unless the drug is incorporated in the solubilized form first. One way of overcoming this problem is to use vesicular systems such as liposomes and niosomes, which can entrap both hydrophilic and hydrophobic drugs while their nanosize ensures permeation through the mucosa [10]. However, they are associated with physical and chemical instabilities due to aggregation, fusion or leakage upon storage, degradation of phospholipids by hydrolysis, and oxidation [11]. To avoid these problems, proniosomes, which are provesicular systems that are converted into niosomes upon hydration, can be an alternative. Proniosomes are actually surfactant coated water-soluble carriers in the form of a dry, free-flowing powder that on agitation with water, are converted to nonionic surfactant vesicles or niosomes. The use of proniosomes can solubilize the drug within the nonionic surfactant vesicles and improve its permeability through the buccal mucosa [12]. We hypothesize that the buccal absorption of acyclovir can be enhanced due to the combined effect of buccal and vesicular drug delivery system.

Hence, the aim of our investigation was to formulate proniosomes of acyclovir which were incorporated into mucoadhesive buccal films fabricated from mucoadhesive polymers and investigate them for their efficacy in buccal permeation.

MATERIALS AND METHODS

Materials

Acyclovir was a gift sample from Sanofi Aventis Goa, while span 60 and cholesterol were procured from Loba Chemie Pvt Ltd Mumbai. Hydroxypropyl methylcellulose K15M (HPMC K15M) was obtained from Himedia Lab Pvt, Ltd, Mumbai and carbopol 934P was procured from Yarrow Chem Products, Mumbai. All other chemicals were of analytical grade.

Preparation of proniosomes

Span 60 and cholesterol are required for the preparation of the proniosomes. Span 60 is chosen among other spans because it is

reported to possess greater drug encapsulation efficiency owing to its long alkyl chain [13].

Coacervation phase separation method was used in the preparation of proniosomes. Accurately weighed amounts of span 60 and cholesterol (the composition is given in table 1) were taken in a glass vial and acyclovir (50 mg) was added to the mixture. About 0.16 ml of ethanol was admixed, and the vials were covered and warmed in a water bath at 50 ° C–65 °C until a clear solution was obtained. To this clear solution about 2 ml of the aqueous phase (distilled water) at 65 °C was added and warmed for 5 min. The resultant solution was allowed to cool until a white creamy gel of proniosomes was obtained. Obtained proniosomal system was lyophilized for stabilization. Prior to lyophilization, the dispersion was frozen at -30 °C for 1 d. The frozen sample was subjected to freeze drying for 6 h and immediately used for formulation [14].

Table 1: Composition of the acyclovir loaded proniosomes

Formulation code	Drug (mg)	Span 60 (mg)	Cholesterol (mg)
N1	50	43	38.6
N2	50	86	38.6
N3	50	43	77.2
N4	50	86	77.2
N5	50	43	193
N6	50	86	193

Characterization of niosomes derived from acyclovir loaded proniosomes

About 30 mg of prepared proniosomes from each formulation was taken in a glass tube, to which 10 ml of distilled water at 80 C was added and vortexed for about 15 min to get a niosomal dispersion. The formed niosomes were observed under an optical microscope at 45X magnification [15].

The niosomal dispersion so obtained after hydration was characterized for vesicle size distribution, zeta potential, surface morphology by SEM, entrapment efficiency (EE) and *in vitro* release of acyclovir.

For the entrapment efficiency, 3 ml of the niosomal dispersion was centrifuged at 18000 rpm for 15 min at 4 °C to separate the un-entrapped drug. The supernatant layer was separated, of which 1 ml was withdrawn and diluted with Phosphate Buffer Saline (PBS) of pH 7.4 to 10 ml and the concentration of drug was determined using an UV spectrophotometer at 253 nm [16].

The entrapment efficiency was calculated using the formula:

$$\% EE = (Ct - Cf)/Ct \times 100 \quad \ldots\ldots\ldots (1)$$

Where C_t = total drug incorporated and C_f = un-entrapped drug in the supernatant

The vesicle size distribution and zeta potential measurement was carried out using the Zetasizer nano ZS (Malvern Instruments Ltd., UK).

The surface morphology and vesicle shape of proniosomes before and after hydration was studied using SEM [17].

In vitro drug release from niosomes was determined using a dialysis bag of pore size, 0.22-0.45 micron and molecular weight cut-off (MWCO) of 14000-18000 (Himedia) [18]. Niosomal dispersion equivalent to 10 mg of drug was placed in the bag which was sealed and immersed in 200 ml of phosphate buffer of pH 7.4, maintained at temperature of 37±0.5 °C and stirred at 50 rpm on a magnetic stirrer. At suitable time intervals (15, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330 and 360 min), samples were withdrawn from the dissolution medium and absorbance was measured using UV spectrophotometry at 253 nm.

Preparation of buccal films

Based on the results obtained from the characterization of the proniosomes, the optimal formulation was selected for incorporation into the buccal films.

The mucoadhesive polymers HPMC K 15M and carbopol 934P were used in the ratio of 1:1, 1:3 and 3:1 and the buccal films were prepared by solvent casting. The required amounts of HPMC K 15M and carbapol 934P were dissolved in sufficient distilled water and stirred on a magnetic stirrer for 6 h. The pH of the polymeric solution was adjusted to neutral with triethanolamine (TEA). As a plasticizer, propylene glycol was mixed with the polymeric solution and the volume was made up to 25 ml using distilled water and the solution was kept overnight to remove air bubbles. Accurately weighed proniosome powder equivalent to 325 mg of drug was dispersed uniformly in the above solution and stirred slowly on a magnetic stirrer until a clear solution was obtained. The resultant solution was poured into circular glass moulds having a surface area of 70.84 cm^2 and was allowed to dry at 40 °C in a temperature-controlled hot air oven. The top of the mould was covered with a funnel to permit controlled evaporation of water during the entire period of drying. The dried films were cut into circular dimension of 1.5 cm diameter and area of 1.766 cm^2, so that each film contained about 8 mg of Acyclovir. For the sake of comparison, films of the same composition were also prepared with acyclovir in the free form. In such films, the drug was incorporated by dissolving in 2 ml of dimethyl sulphoxide (DMSO) and then added to the polymeric solution before solvent casting. The obtained films were wrapped in aluminum foil and stored in a desiccators [19]. The composition of prepared buccal films is summarized in table 2.

Table 2: Composition of casting solution for buccal films containing acyclovir without proniosomes

Formulation	F1	F2	F3	F4	F5	F6
Acyclovir (mg)	325	325	325			
Weight of proniosomes equivalent to 325 mg of acyclovir (mg)				1100	1100	1100
DMSO (ml)	q. s	q. s	q. s	q. s	q. s	q. s
HPMC K 15M (mg)	100	50	150	100	50	150
Carbapol (mg)	100	150	50	100	150	50
Propylene glycol (ml)	1	1	1	1	1	1
Water (ml)	25	25	25	25	25	25

Evaluation of drug-loaded films

Weight, thickness, surface pH and drug content

The thickness of films was determined using a micrometer screw gauge and about 10 films were weighed individually.

Drug content was determined by dissolving one film of 1.766 cm^2 area containing 8 mg of acyclovir in 100 ml of ethanol for 1 h with continuous stirring. From this solution, 10 ml was diluted to 50 ml using a simulated salivary fluid of pH 6.8 and the absorbance was measured using an UV spectrophotometer.

A desirable buccal film should be non-irritant to the oral mucosa and therefore, it is important to determine the surface pH when brought in contact with moisture. The film to be tested was placed in a petri dish and moistened with 0.5 ml of distilled water and kept for 30 s. The electrode of the pH meter was brought in contact with the surface of the formulation and allowed to equilibrate for 1 min and the pH was measured [20, 21].

Mechanical properties of the films–folding endurance, tensile strength and percentage elongation

Folding endurance was determined by repetitively folding a film at the same place until the film was broken when folded up to 300 times. The number of times the films could be folded without breaking gives the value of the folding endurance [22].

Tensile strength is the maximum stress applied to a point at which the film breaks. The Linus Bursting/tensile strength apparatus was used to determine the tensile strength. The pressure gauge was selected by turning a gauge selector switch, depending on the sample to be tested. Film strips free from air bubbles and other imperfections were cut into dimensions of 3 cm x 1 cm. One strip was placed on the diaphragm plate and the wheel on the top of the diaphragm plate was rotated till it fitted securely on the sample and did not rotate any further. The push-button was pressed till the sample broke. The tensile strength was directly read from the pressure gauge in Kg/cm^2. Measurements were run in triplicate for each film [23].

The percentage elongation can be obtained by the following equation.

$$\% \text{ Elongation} = (L1 - L2)/L2 \times 100 \quad \ldots\ldots (2)$$

Where L_1 is the final length of each strip, and L_2 is the initial length of each strip.

Swelling index

The swelling index of the formulated films in the simulated salivary fluid was determined by a method documented by several researchers. A pre-weighed buccal film of area 1.766 cm^{2}was placed in a stainless steel sieve (800 µm mesh) and immersed in a china dish containing 15 ml of simulated salivary medium. At regular intervals, the film was taken out, blotted with a tissue to remove excess moisture and then the film was reweighed. The increase in the weight of the film was noted in 15 min intervals until a constant weight was observed [24]. The swelling index was calculated using the equation below:

$$\% SI = (W_t - W_0)/W_0 \times 100 \ldots. (3)$$

Where SI is the swelling index, W_t is the weight of the film at time t and W_0 is the initial weight of the film.

***In vitro* residence time**

Using USP disintegration apparatus, the *in vitro* residence time was determined. The disintegration apparatus was filled with 900 ml of phosphate buffer of pH 6.8 maintained at 37±2 °C. Porcine buccal mucosal membrane of 3 cm length was glued to the surface of a glass slab, which was then fixed vertically in one of the tubes of the basket rack assembly. A film of 1.5 cm diameter was moistened on one side with a few drops of the phosphate buffer and was pressed briefly onto the mucosal membrane to bring about adhesion. The apparatus was switched on, to allow the glass slide with the attached mucosa to move up and down in the medium. The time required for complete erosion or detachment of the film from the mucosal surface was recorded [25].

Measurement of *ex vivo* mucoadhesive strength

In order to prolong the residence time at the site of application and to provide prolonged drug release, satisfactory bioadhesion of the bioadhesive drug delivery system is essential. The tensile strength required to detach the bioadhesive films from the mucosal surface was taken as a measure of the bioadhesive performance. For the measurement of bioadhesive strength, several methods have been reported in the literature. In the present work, a specially designed or fabricated assembly based on published literature was used. For bioadhesion testing, a porcine check pouch was used as the model membrane.

The basis of the bioadhesion test assembly is the working of a modified physical balance. A plastic cap (4 cm diameter) was fixed vertically to the left arm of the balance using a wire. To the lower surface of the cap, a buccal film of 1.5 cm diameter was attached using cyanoacrylate glue. A piece of freshly excised porcine buccal mucosa was attached to the upper end of a cylindrical Teflon block of 4 cm diameter with the mucosal side up. The block was fixed vertically onto the bottom centre of a 250 ml capacity beaker, which was positioned in such a way that the plastic cap was directly above the membrane-covered Teflon block with a gap of 0.5 cm between. The beaker was then filled with a sufficient buffer of pH 6.8 until the liquid level just skims the mucosal surface. The horizontal beam of the balance was balanced using a weight of 5 g on the right pan.

For the measurement, the 5 g weight on the right pan was removed, allowing the left arm of the balance to lower, so that the cap with the film attached came in contact with the mucosa. A 20 g weight was placed on the cap to apply pressure on the mucosa for 3 min and then removed. Slowly, weights were added to the right pan, starting from the least, at 30 s intervals until the film was detached from the surface of the mucosa [26, 27]. The total weight at which the detachment occurred was noted and the bioadhesion force was calculated using the following equation:

$$F = (W_W \times g)/A \ldots. (4)$$

F= bioadhesion force (kg/m/s^2)

W_w = mass applied (g)

G = acceleration due to gravity (cm/s^2)

A = surface area of the film (cm^2)

***In vitro* drug release studies from films**

The buccal films were subjected to dissolution studies using the paddle-over-disc method with the USP Type II apparatus. A film of diameter 1.5 cm was cut and fixed to a glass slide using cyano-acrylate glue. The glass slide was placed in the bottom of the dissolution vessel, which was later filled with 900 ml of simulated saliva (phosphate buffer, pH 6.8) maintained at 37±1 °C; the paddle was rotated at 50 rpm. Samples of dissolution medium were withdrawn at predetermined time intervals, filtered, and analyzed by UV Spectrophotometry after suitable dilution [28]. To maintain sink conditions the samples were replaced with equal volumes of fresh buffer.

The drug release data were further subjected to kinetic analysis by fitting to different mathematical models such as zero order, first order and Korsmeyer-Peppas to predict the kinetics and release mechanism of the drug.

***Ex vivo* permeation studies**

Porcine buccal mucosa for this study was procured fresh from the local slaughterhouse and the underlying connective tissue was separated and removed from the buccal mucosa membrane with surgical scissors, washed with phosphate buffer of pH 6.8 and used immediately [24].

The *ex vivo* permeation studies of buccal films of Acyclovir were performed using the Franz Diffusion Cell. The porcine mucosa was clamped between the donor and receptor compartments. The receptor compartment contained 25 ml of phosphate buffer of pH 7.4, which was stirred continuously at 100 rpm and maintained at 37±0.5 °C to mimic physiological conditions. At appropriate time intervals, aliquots (1 ml) were withdrawn and replaced with an equal volume of fresh buffer to maintain sink conditions. The samples were filtered and analyzed by HPLC.

Reconstitution of films for confirming the presence of niosomes

Reconstitution of the prepared films was done to understand whether properties of proniosomes are affected by the film formation method.

The Acyclovir loaded films were reconstituted by taking three buccal films of 1 cm×1 cm diameter in a conical flask containing 50 ml distilled water. This was shaken and stirred for 1 h until a colloidal system was obtained. The reconstituted proniosomal dispersion was then examined under the optical microscope using 45 X magnification for the presence of vesicles. These results were compared with the corresponding data of the initial formulation (hydrated proniosomes) in terms of vesicle size, PDI and zeta potential.

RESULTS AND DISCUSSION

Proniosomes of Acyclovir were successfully prepared and were observed to be white, free-flowing powders.

Characterization of niosomes derived from acyclovir loaded proniosomes

Observation of the dispersion obtained after hydration of the proniosomes, under an optical microscope at 45x magnification, revealed spherical niosomes which were homogeneously dispersed.

The drug entrapment efficiency (EE) was observed to be in the range of 13.63-53.76 % as shown in table 3. Significant increase in entrapment efficiency was observed when the concentration of span 60 was increased, which is due to an increase in the formation of niosomes as a consequence. At low concentrations of cholesterol, the encapsulation efficiency was also found to be low and initially increases with increasing concentrations of cholesterol. This is because cholesterol is the key element for improving the stability of the bilayer, increasing its hydrophobicity and reducing its permeability to the entrapped drug. But when the cholesterol content exceeded beyond a particular point, the entrapment efficiency decreased. This observation is attributed to the fact that cholesterol beyond a certain level disturbs the regular bilayer structure of proniosomes resulting in the expulsion of the drug from the bilayer due to overcrowding, during the vesicle formation [29]. Among all the formulations, N4 was found to have maximum entrapment efficiency (53.76 %), indicating that its composition was optimal.

The results for vesicle size, PDI and Zeta potential are displayed in table 3. Vesicle size is important for stability and can influence drug encapsulation, membrane permeation as well as cellular uptake and bioavailability. The vesicle size for all formulations was less than a micron with the exception of N1 and N2. The highly negative values for Zeta potential indicate high physical stability of the dispersions due to electrostatic repulsion between particles. The PDI values indicate homogeneity of the dispersions of all formulations.

SEM studies showed that the acyclovir proniosomes were irregularly shaped particles with a rough surface. Morphology of proniosomes of acyclovir before hydration was shown in fig. 1(A). After hydration, the resulting niosomes were spherical and uniform and the surfaces were found to be smooth as shown in fig. 1(B).

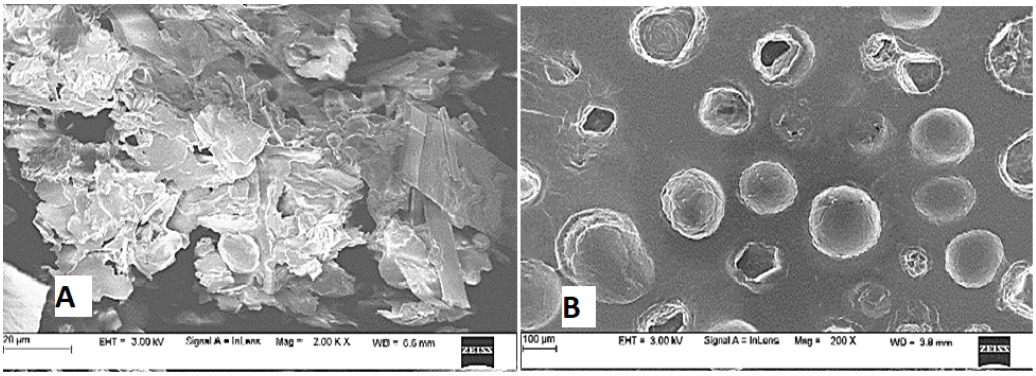

Fig. 1: A) SEM of proniosomes before hydration B) SEM of proniosomes after hydration

In vitro drug release studies using dialysis bag, indicate that the formulation N4 showed maximum drug release (74.09 %) at the end of 6 h when compared to other formulations. Drug release was markedly decreased in N5 and N6 since the increased cholesterol content could reduce the gel to the liquid phase transition of niosomal bilayers leading to decrease in the fluidity of the niosomal membrane and enhancing its rigidity and thereby decreasing its permeability and release rate [30]. The results for drug release from the niosomes is shown in table 3.

On the basis of results obtained from the characterization studies of niosomes, N4 was considered as the optimal formulation and was selected for incorporation into buccal films.

Table 3: Data for particle size, PDI, zeta potential, EE and maximum % CDR for all niosome formulations

Product code	Vesicle size (nm)	PDI	Zeta potential (mV)	EE (%)*	Maximum CDR (%)* at 6 h
N1	1045	0.251	-46.82	28.56±1.12	64.74±0.12
N2	1024	0.316	-31.48	34.76±0.74	59.50±0.14
N3	972	0.332	-35.12	46.32±1.31	61.73±0.11
N4	962	0.234	-42.40	53.76±1.32	74.09±0.14
N5	836	0.253	-37.19	13.63±1.54	44.93±0.10
N6	845	0.205	-33.12	19.54±1.32	42.00±0.15

EE–Entrapment efficiency; CDR–Cumulative drug released; *Average of 3 measurements with standard deviation

Preparation of buccal films

Buccal films were prepared using HPMC K15M and carbopol 934P and a comparison was made between buccal films containing free drug and buccal films incorporated with proniosomes.

The films prepared were then evaluated for various physico-mechanical properties. The films from all the batches were smooth and flexible without any signs of roughness, air bubbles and other deformities. Films with proniosomes (F4, F5, and F6) were comparatively more smooth and translucent than films with free drug (F1, F2, and F3). This may be because in case of films with proniosomes, the drug was entrapped within the niosome vesicles; therefore the drug remained solubilized and did not precipitate. But films in which the drug was directly added are comparatively

whitish because of poor solubility of the drug, which may have caused some degree of precipitation on the surface of the film.

Evaluation of drug-loaded films

Weight, thickness, drug content and surface pH

The data for weight and thickness determined for all formulations is given in table 4. The weights of films with higher polymer content were found to be greater. It was observed that the thickness of all film samples was uniform in each formulation. The average thickness was found to be in the range of 0.221 to 0.311 mm. The films with larger amounts of HPMC K 15M were slightly thicker. The percentage drug content of the niosomal formulation was found to be in the range of 93-95 % as shown in table 4, whereas for the non-niosomal films, it was approximately 90-93%. The lower drug content in formulations, F1-F3 could be due to loss of the precipitated form of the drug during the drying of the films.

In order to confirm the ideal pH for the application of buccal films orally, the pH of the buccal films was measured to avoid any irritation to the oral mucosa. It was observed that the surface pH of the films was close to the buccal pH of 6.8 and this means that they may have less potential to irritate the buccal mucosa and hence more comfortable. The results of surface pH are displayed in table 4.

Table 4: Data of weight, film thickness, surface pH, and drug content for film formulations

Formulation code	Weight (mg)#	Thickness (mm)#	Surface pH*	Drug content uniformity (%)*
F1	22.62±1.14	0.234±0.1	6.74±0.1	93.12±1.23
F2	27.46±1.16	0.229±0.27	6.66±0.3	90.97±0.94
F3	29.92±1.12	0.301±0.23	6.73±0.2	92.08±1.25
F4	23.75±0.93	0.221±0.25	6.70±0.4	93.72±0.826
F5	25.97±1.19	0.231±0.29	6.78±0.5	94.75±0.903
F6	30.12±1.15	0.311±0.003	6.52±0.8	95.78±1.321

*Mean of 3 determinations±SD #Mean of 5 determinations±SD

Mechanical properties of the films–folding endurance, tensile strength and percentage elongation

The ability of the films to sustain mechanical handling as well as have adequate flexibility when used in the oral cavity, was evaluated by determining folding endurance. The films containing free drug showed folding endurance (table 5) less than 300 (F1, F2, F3), probably due to the undissolved drug, which produced a non-homogenous polymer mass. On the other hand, the films with the niosomal form of the drug produced a folding endurance of 300 and more (F4, F5, F6). The drug being in the solubilized form in the niosomes, produced a homogenous polymer mass which yielded more flexible films. Such films are better able to conform to the contours of the buccal mucosa during application without cracking or breaking and therefore with lesser chances of mechanical irritation or drug loss.

The tensile strength gives an indication of the strength and elasticity of the film. The results for the measured tensile strength and percentage elongation of all the formulations are shown in table 5. It is observed that the film formulations F3 and F6 demonstrated the highest tensile strength and the least percentage elongation. These formulations possessed the lowest content of carbopol 934P, which is known to be a soft polymer that increases the softness, flexibility and elasticity of the film but decreases tensile strength [31]. On the other hand film formulations with lower content of HPMC resulted in lower tensile strength but higher percentage elongation. A suitable buccal film should have high tensile strength and percentage elongation, which means that the proportions of HPMC and carbopol need to be balanced to yield films which are flexible and comfortable to wear but have sufficient strength to withstand mechanical handling.

Swelling index

The ability of the water-soluble polymer to take up water upon hydration can be determined using the swelling index. Swelling property of polymer is an important parameter which is responsible for establishing intimate contact of the film with the buccal mucosal surface through the formation of weak bonds and ultimately its bioadhesion [32]. Percentage swelling index of all the formulations was found to be in the range of 65.87-75.87% (table 5). Formulation F2 and F5 showed higher values of swelling index due to the presence of higher concentrations of carbopol 934P. This may be attributed due to the high water uptake capacity of carbopol 934P [33]. The water permeability increased as the quantity of carbopol 934P increased in the film. The presence of the drug in the free or niosomal form did not have a significant effect on the percentage swelling index among the formulations.

Table 5: Data for tensile strength measurement, percentage elongation, folding endurance and percentage swelling index for buccal films of acyclovir

Formulation code	Tensile strength (kg/cm²)*	Percentage elongation*	Folding endurance*	Percentage swelling index*
F1	2.3±0.2	37.8±0.1	236	65.87±0.12
F2	2.0±0.4	43.1±0.4	254	75.87±0.02
F3	2.8±0.1	35.9±0.2	245	69.76±1.23
F4	2.9±0.4	40.1±0.4	>300	66.98±0.43
F5	2.5±0.1	45.7±0.2	>300	73.87±1.43
F6	3.2±0.2	37.8±0.4	>300	68.91±0.02

*Mean of 3 determinations±SD

***In vitro* residence time**

Residence time is the time taken for the films to detach or erode completely from the mucosa and will therefore determine how long the film will remain in the oral cavity after administration. Since the mucoadhesive polymer in the composition is responsible for the adhesive interaction of the film with the mucosa, the concentrations of the former play an important role in the retention time of the latter on the mucosal surface. Both HPMC K15M and Carbopol 934P are classified as mucoadhesive polymers of which the latter is reported to have greater mucoadhesive properties. The carboxylic groups in carbopols are capable of forming strong hydrogen bonds with the oligosaccharide chains present in the mucin [34].

It was observed that as the amount of carbopol in the film composition increased, there was an increase in the mucoadhesion time; therefore, F2 and F5 exhibited the longest residence time among the non-niosomal and niosomal films, respectively. As shown in fig. 2, the residence time was slightly lesser for the non-niosomal or free drug-containing films as compared to that of niosomal films.

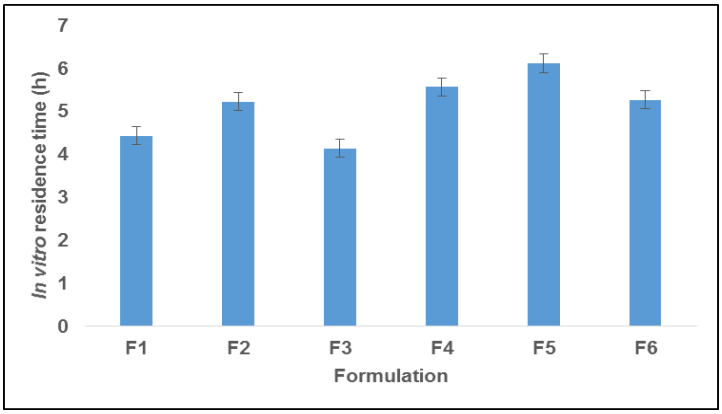

Fig. 2: Comparison of *in vitro* residence time of all formulations

Measurement of *ex vivo* mucoadhesive strength

In order to increase the residence time at the site of application and to provide prolonged drug release, satisfactory bioadhesion of bioadhesive drug delivery is essential. As described earlier, although both carbopol 934P and HPMC K15M are mucoadhesive, the contribution of the former to the total mucoadhesive strength of the film onto the porcine mucosa appears to be greater. As fig. 3 clearly indicates, the mucoadhesive strength of films increases with increasing concentrations of carbopol. Like the *in vitro* residence time, the measured mucoadhesive strength appeared to be greater for the niosomal films as compared to the non-niosomal films.

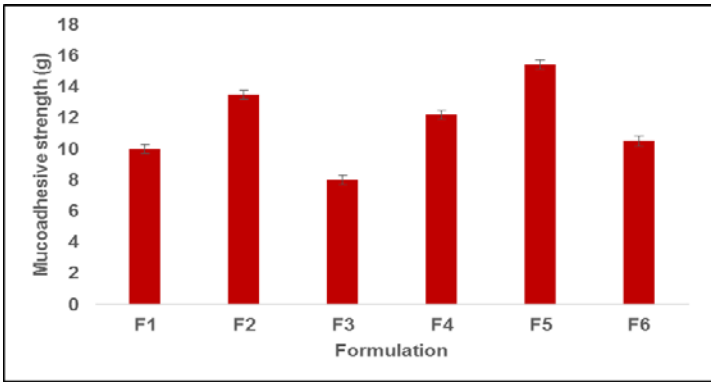

Fig. 3: Comparison of *ex vivo* mucoadhesive strength of all formulations

***In vitro* drug release studies from films**

Fig. 4, 5 and 6 compare the *in vitro* drug release profiles from the noisome incorporated films with their corresponding non-niosomal films. Formulations with free acyclovir showed lesser release as compared to the proniosome incorporated films. As described earlier, the drug in the former formulations was present in the precipitated form due to decreased solubility in the polymer mass during the drying of the films. This form of the drug was also not very soluble in the release medium. On the other hand, the niosomal films, F4-F6 showed better release since acyclovir was solubilized in the polymer mass by virtue of the niosomes formed by the hydration of the proniosomes during the preparation of films. In the case of all the formulations (F1-F6), irrespective of the presence or absence of proniosomes, the drug release profiles indicate that as the ratio of carbopol 9334P to HPMC in the film composition increases, there is a decline in the rate of drug release as exhibited by F2 and F5. This may be due to the fact that higher proportions of carbopol tend to increase the swelling effect of the films upon hydration and consequently produce a thicker diffusional barrier to the release of the drug. This effect of carbopol concentrations on drug release was observed from the formulations for both niosomal and non-niosomal films. The formulation with a higher proportion of HPMC has demonstrated greater drug release at the end of the 8 h. The maximum percentage drug release (93.2%) was recorded from F6, which had the lowest concentration of carbopol and the highest concentration of HPMC among the niosomal films. All formulations exhibited sustained release of acyclovir during the 8 h period.

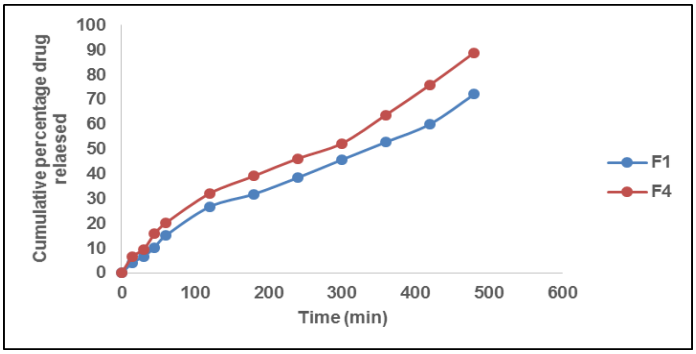

Fig. 4: *In vitro* drug release profiles from F1 and F4

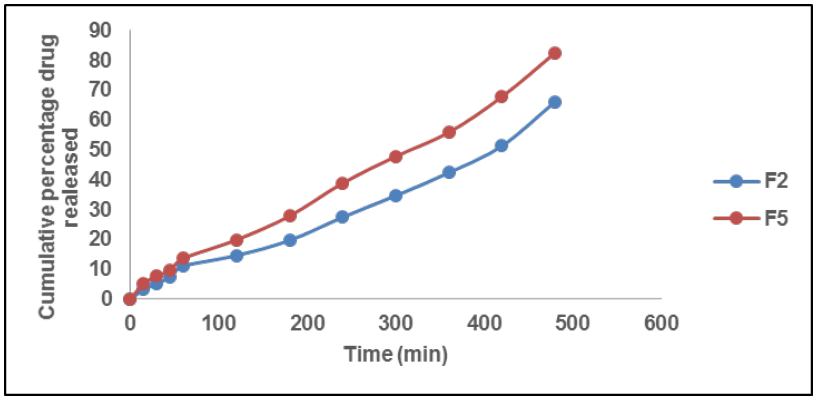

Fig. 5: *In vitro* drug release profiles from F2 and F5

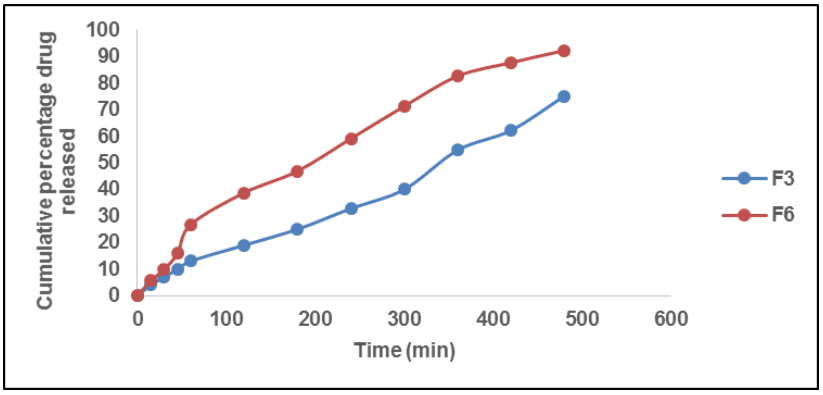

Fig. 6: *In vitro* drug permeation profiles from F3 and F6

The data from kinetic analysis using various models is given in table 6. Based on the regression coefficient value (R^2) values determined for different models, it is evident that drug release from the formulations followed zero-order kinetics. The value of release exponent "n" obtained by applying Korsmeyer-peppas equation for all the formulations was greater than 0.5 and less than 1, hence the mechanism of drug release from these formulations followed anomalous or non–Fickian release, that is a combination of diffusion and polymer surface erosion [35, 36].

Table 6: Data for kinetic analysis of drug permeation from films

Formulation code	Zero-order R^2	First-order R^2	Korsmeyer-peppas	
			R^2	n
F1	0.9243	0.9037	0.9673	0.6741
F2	0.9261	0.9430	0.9845	0.6914
F3	0.9550	0.9507	0.9897	0.7244
F4	0.9858	0.9107	0.9723	0.7256
F5	0.9913	0.8768	0.9725	0.7805
F6	0.9775	0.7445	0.9822	0.7595

R^2–Regression coefficient; n–Release exponent

Ex vivo permeation studies

On the basis of the mechanical film properties, drug content, mucoadhesive strength, *in vitro* residence time and *in vitro* drug release data, formulation F5 was considered optimum and was selected for *ex vivo* permeation studies using porcine buccal mucosa.

Porcine buccal mucosa was selected as the membrane model for the permeation studies since it is similar to human buccal mucosa in structural characteristics and permeability. On comparing the drug permaetion profiles of niosomal film F5 and its corresponding free acyclovir containing film, F2, the drug permeation was found to be higher from the former. The maximum percentage of drug permeated from F5 was 80.24 %, whereas, from F2, it is only 59.72 %. The comparison of their drug permeation profiles is shown in fig. 7. This was further confirmed by the determination of permeation flux of acyclovir through the porcine mucosal membrane from F2 and F5. For this purpose, the amount of drug permeated through the membrane in 8 h was determined and plotted against time. Using regression analysis the slope of the linear portion of the plot was calculated and divided by the mucosal surface area to give the permeation flux. The steady-state permeability coefficient was calculated by dividing the permeation flux by the donor phase drug concentration [37,38]. The permeation flux of acyclovir from F2 and F5 was 11.27 µg/cm^2/h and 13.97 µg/cm^2/h, respectively. The permeability coefficient of the drug from F2 and F5 was calculated to be 0.00141 and 0.00175 respectively. This comparison reveals that the incorporation of acyclovir into proniosomes has improved its permeation through the membrane as compared to free acyclovir films.

From the above results it can be concluded that our objective of improving the permeation of acyclovir by incoparating in proniosomes and further embedding into a buccal films was achieved.

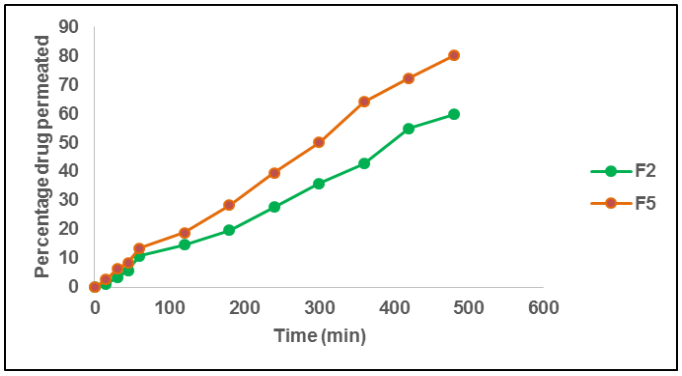

Fig. 7: *Ex vitro* drug permeation profiles from F2 and F5

Reconstitution of films for confirming the presence of niosomes

Formulations F4, F5 and F6 were reconstituted as per the method described earlier. On dissolution of the film, a clear solution was formed, which when observed under the optical microscope, showed intact vesicles. These results concluded that the vesicle size and shape have been retained during the film formation. The measured vesicle size, PDI and zeta potential of the reconstituted film were comparable to that of the initial dispersion obtained by hydrating the proniosomes. Therefore, it can be concluded that the niosomes retained their properties even after film formation and the process of incorporation into the polymer mass did not affect the integrity of the proniosomes.

CONCLUSION

Proniosomes of acyclovir were successfully formulated by using Span 60 and cholesterol, which were then loaded into mucoadhesive buccal films formulated from various ratios of carbopol 934P and HPMC K 15M. The present study has established the fact that it was possible to formulate stable and uniform mucoadhesive buccal films from poorly soluble drugs such as acyclovir. The use of proniosomes helped to avoid some of the inherent problems associated with the preparation and use of niosomes or liposomes. Further, this investigation revealed that it was possible to improve permeation of acyclovir through the buccal mucosa when proniosomes of the drug was incorporated into the mucoadhesive films, which could potentially improve the bioavailability of this drug. Thus the dose of the drug could be reduced, and therefore the toxicity associated with it, while improving patient compliance. Hence these films could be promising formulations in the buccal delivery of acyclovir for the treatment of viral infections.

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AUTHORS CONTRIBUTIONS

Marina Koland: Conceptualization, Methodology, Writing–review and editing, Deeksha U Suvarna: Investigation, Writing–original draft preparation, Ananth Prabhu: Formal analysis, Sindhoor S. M: Validation

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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