

## DEVELOPMENT AND EVALUATION OF NEBIVOLOL HYDROCHLORIDE NANOCRYSTALS IMPREGNATED BUCCAL FILM

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### Abstract

Clinical efficacy of nebivolol is restricted primarily because of its low aqueous solubility and extensive hepatic metabolism. This study investigated the prospective of enhancing the systemic delivery of nebivolol HCl by formulating nanocrystals impregnated films for the buccal application. Nanocrystals of nebivolol HCl were prepared by acid-base neutralization method. Placebo films (N1-N3) were optimized by varying concentrations of chitosan. Further pure nebivolol HCl and its nanocrystals were incorporated to the films at two levels (2.5 mg/cm<sup>2</sup> and 5 mg/cm<sup>2</sup>). The prepared films (N4-N7) were assessed for physicochemical properties, swelling, drug release and permeation by standard protocols. Selected films were evaluated *in vivo* on rabbits. Physicochemical properties displayed by the prepared films (N4-N7) were found to be adequate for the buccal application. Rapid swelling of films was noticed which would be beneficial and may contribute to immediate and effective bioadhesion with the buccal mucosa. The rate of drug dissolution was rapid and complete in 4 h from nanocrystals embedded films (N6 and N7) when compared to films loaded with pure drug. The permeation data indicates greater transport when drug concentration was higher (Film N7; 36.31 ± 6.54 µg/cm<sup>2</sup>/h; p < 0.0001). *In vivo* data signified rapid absorption (C<sub>max</sub> ~ 24 ng/mL) and higher AUC<sub>0-4</sub> (~400 ng \* h/mL) with ~ 2.5 folds improvement in the bioavailability. In conclusion, registered data suggests that the buccal application of nanocrystals embedded film improved the efficacy of nebivolol HCl, and necessitates further clinical studies.

### Rezumat

Eficacitatea clinică a nebivololului este limitată în primul rând din cauza hidrosolubilității sale scăzute și a metabolismului hepatic intens. Acest studiu a investigat perspectiva creșterii biodisponibilității sistemice a clorhidratului de nebivolol prin formularea de filme impregnate cu nanocristale pentru aplicarea bucală. Nanocristalele de nebivolol HCl au fost preparate prin metoda de neutralizare acido-bazică. Filmele placebo (N1-N3) au fost optimizate prin utilizarea diferitelor concentrații de chitosan. Mai mult, nebivololul HCl pur și nanocristalele acestuia au fost încorporate în filme la două concentrații (2,5 mg/cm<sup>2</sup> și 5 mg/cm<sup>2</sup>). Filmele preparate (N4-N7) au fost evaluate privind proprietățile lor fizico-mecanice, umflarea, eliberarea substanței active și permeabilitatea, folosind protocoalele standard. Filmul selectat a fost evaluat *in vivo* pe iepuri. Proprietățile fizico-mecanice afișate de filmele preparate (N4-N7) s-au dovedit a fi adecvate pentru aplicarea bucală. S-a observat umflarea rapidă a filmelor, care ar fi benefică și ar putea contribui la bioaderența imediată și eficiență pe mucoasa bucală. Procesul de dizolvare a fost rapid și complet, în 4 ore, pentru filmele încorporate în nanocristale (N6 și N7), în comparație cu filmele încărcate cu substanța pură. Datele de permeabilitate indică un transport mai eficient atunci când concentrația nebivolol HCl este mare (film N7; 36,31 ± 6,54 µg/cm<sup>2</sup>/h; p < 0,0001). Datele *in vivo* au indicat o absorbție rapidă (C<sub>max</sub> ~ 24 ng/mL), o valoare mai mare a AUC-α (~ 400 ng \* h/mL) cu o îmbunătățire de 2,5 ori a biodisponibilității. În concluzie, datele obținute sugerează că aplicarea bucală a filmului preparat prin încorporarea de nanocristale a îmbunătățit eficacitatea nebivolol HCl și necesită studii clinice suplimentare.

**Keywords:** nanocrystals, buccal; nebivolol; chitosan, rabbit; film; *in vivo*

### Introduction

The recent reports by World Health Organization (WHO) suggests that the hypertension is one of the global epidemics with serious medical, psychological and economic impact [31]. Hypertension is a silent, invisible killer which ranks among the third leading causes for morbidity and mortality, and accounts to ~ 10 million deaths a year worldwide [16].

Epidemiological studies showed that more than 1.5 billion individuals are living with hypertension and the prevalence is projected to rapidly increase in the next decade [5]. In addition, raised blood pressure is also a public health challenge and the global economic burden due to this disorder was estimated to be ~ 10% of overall healthcare expenditures [9]. If uncontrolled, hypertension may lead to catastrophic events such as coronary artery disorder, stroke, renal failure or

blindness [17]. Several categories of antihypertensive agents are currently available for the management of hypertension including thiazide diuretics, calcium channel blockers, beta blockers, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers. However, most of the drugs possess several potential adverse effects including hypotension, fatigue, exercise intolerance, erectile dysfunction and glucose intolerance [27].

Beta blockers represent a primary choice in the management of hypertension. However, the major side effects of beta-blockers are their non-cardioselective activity along with other adverse effect [30]. Nebivolol is a unique, extremely cardioselective, 3<sup>rd</sup> generation beta adrenergic receptor blocker that was approved by the US FDA for the management of hypertension [7]. This drug is reported to possess the highest  $\beta_1$  receptor affinity among  $\beta$  blockers which signifies its high tolerability in patients with lung disease [18]. Further, nebivolol applies nitric oxide mediated vasodilation as well as beta blocking effects [29]. However, the efficacy of nebivolol HCl in oral therapy is limited by poor and variable bioavailability (~ 12%) due to low aqueous solubility and high hepatic metabolism [15]. The physicochemical properties of nebivolol HCl such as molecular weight (441 g/mol), water solubility (~ 50  $\mu\text{g/mL}$ ), partition coefficient ( $\log P$  3.2),  $pK_a$  (13.5) as well as biopharmaceutics classification (Class II) suggest that solubility is one of the issues which limit its therapeutic efficacy [4]. On the other hand, oral delivery of lipophilic drugs like nebivolol is a great challenge for drug delivery scientists again due to their poor aqueous solubility [28]. Few studies have been carried out to overcome the current challenges associated with oral administration of nebivolol, and that can provide a controlled and effective delivery of nebivolol. Fast dissolving tablets, nanoparticles, self-nanoemulsifying delivery systems, transdermal systems etc were attempted to improve bioavailability of this drug but resulted with limited success [8, 11]. Thus the therapeutic efficacy of nebivolol for the successful therapy remains elusive. The buccal drug delivery systems offer several advantages and have captivated many research scientists around the world and are being considered as a research topic of great interest [2]. The buccal films are capable to provide higher residence time in oral cavity which in turn improve therapeutic efficacy as well patient adherence [24]. Furthermore, the buccal films can provide better bioavailability, minimize the dose dependent side effects, in addition to its potential to release the drug at a controlled rate. Being a hydrophobic molecule, intrinsic solubility of nebivolol HCl is low. In this context, efficiency of nanocrystals in enhancing bioavailability of poorly soluble drugs has already been demonstrated in various preclinical and clinical studies [10]. Hence it is presumed that preparing nanocrystals of nebivolol and incorporating

the same in the buccal film could be a relevant approach to enhance its therapeutic efficacy. Thus, the objective of this study was to formulate nebivolol nanocrystals impregnated buccal film and evaluate *in vitro* and *in vivo* using an animal model. Systemic availability of nebivolol HCl following buccal application of film embedded with nebivolol HCl nanocrystals and oral therapy was also compared.

## Materials and Methods

### Materials

Nebivolol HCl, chitosan, medium and very low molecular weight (degree of de-acetylation of 75 - 85%), polyvinyl alcohol, glycerol, sodium hydroxide, acetic acid, sodium dihydrogen phosphate, potassium dihydrogen phosphate and acetonitrile were purchased from Sigma Aldrich St. Louis, MO, USA. All other used chemicals and reagents were of analytical grade.

### Quantification of nebivolol

Chromatographic separation and quantification of nebivolol HCl was carried out using a high performance liquid chromatography (HPLC) system (Shimadzu, Tokyo, Japan) comprising a 20 AT pump, along with UV detector and column oven. Samples were measured using a system consisting of a Symmetry C18 analytical column (4.6  $\times$  150 mm, 5.0  $\mu\text{m}$ ). Chromatographic separation of nebivolol HCl was achieved using methanol:acetonitrile:0.02 M potassium dihydrogen phosphate (60:30:10, pH 4.0). The flow of mobile phase was adjusted to 1.2 mL/min at room temperature and drug concentration was measured at 280 nm wavelength [25]. Specific volume of samples (25  $\mu\text{L}$ ) were injected (1 - 1000 ng/mL,  $r^2 = 0.996$ ) and the retention time was found to be 2.6 min.

### Preparation of nebivolol nanocrystals

Nanocrystals of nebivolol HCl were prepared by acid-base neutralization process. Nebivolol (50 mg) was solubilized in a blend of hydrochloric acid (10%, w/w) and methanol (20 mL). Chitosan (1% w/w, very low molecular weight) was added to drug solution as a stabilizer. Then, this solution was slowly introduced into 100 mL 0.1% (w/v) sodium hydroxide solution under stirring at 400 rpm. The formed suspension was homogenized in an Ultra-High pressure homogenizer. The homogenized nano-suspension was centrifuged at 8,000  $\times$  g for 15 min. The supernatant was removed and the nanocrystals were re-dispersed in an aqueous solution of chitosan. The re-dispersed nanosuspension was lyophilized to obtain the nanocrystals [32].

### Preparation of films

Preliminary studies were carried to prepare the buccal films using chitosan. For this, the amount of chitosan varied (0.5 g - 1.5 g) and three films (N1-N3) were prepared (Table I). Briefly, placebo films (N1-N3) were prepared by dissolving the required amount of chitosan in aqueous solution of acetic acid 1% (v/v) and glycerol 2.5% (v/v) was used as a plasticizer.

The solution was casted on to a borosilicate Petri dish and dried at 37°C for 24 h. The dried films were carefully removed and neutralized using 1% (w/v) sodium hydroxide solution and dried in an oven for 30 min. Backing membrane was prepared using polyvinyl alcohol aqueous solution (4% w/v), casted on a Petri plate and then allowed to dry for 12 h at 40°C in an oven. The film was casted over this backing membrane [12].

**Table I**  
Compositions of placebo films

Film code	Chitosan (g)	Film characteristics
N1	0.50	No peelable film formed
N2	1.00	Thin film formed
N3	1.50	Peelable film with good mechanical characteristics

#### Preparation of drug/nanocrystals loaded films

For preparing nebivolol HCl loaded films, first the chitosan was dissolved in an aqueous solution of acetic acid 1% (v/v). Then glycerol was added to the polymer solution. Nebivolol was separately dissolved in a blend of different proportion of methanol and acidic buffer (pH 1.2). The polymer and drug solution were mixed, homogenized and sonicated for 15 min. The mix was casted on to a borosilicate Petri dish and dried at 37°C for 24 h. The dried films were carefully removed and neutralized using 1% (w/v) sodium hydroxide solution and dried in an oven for 30 min. Drug loading was done at two different concentrations (Table II) to evaluate the effect of the drug loading on physico-mechanical properties of the prepared films. Similarly, nebivolol nanocrystals were also loaded into the films at these two concentrations as shown in Table II. The nebivolol nanocrystals were suspended in the polymer solution, mixed and casted on a Petri dish.

**Table II**  
Composition of nebivolol hydrochloride impregnated films

Film code	Chitosan (g)	Nebivolol (mg/cm <sup>2</sup> )	Size range of crystal impregnated in the film
N4	1.50	2.5	3 - 5 µm
N5	1.50	5.0	3 - 5 µm
N6	1.50	2.5	380 - 450 nm
N7	1.50	5.0	380 - 450 nm

#### Evaluation of mucoadhesive films

The prepared films were evaluated for various physico-mechanical characteristics. For the pH determination, a film of size of 1 x 1 cm was taken and dipped in water (5 mL) for 30 min. Then the film was removed and the surface liquid was cleaned and pH was measured using a flat surface electrode [2]. Thickness of the film was noted at five different locations using screw gauge [13]. For drug content estimation, six films were randomly selected from each formulation and film size of 1 x 1 cm were punched from different regions and soaked in 10 mL of acidic buffer solution

(pH 1.2) with stirring. The solution was then centrifuged at 10,000 rpm for 2 min and the supernatant was filtered and analysed using the HPLC. To determine the folding endurance, six films were randomly selected from each formulation and films sized 4 x 4 cm were cut and folded at the same location repeatedly making an angle of 180° till they break.

Swelling measurements were conducted using an accurately weighed portion of the film (1 x 1 cm) and was immersed in 10 mL of simulated saliva (pH 6.2) at 37 °C. At predetermined intervals of time, the film was carefully removed from the medium, excess moisture was cleaned and weighed. Percentage swelling (% Qs) was calculated using the following formula:

$$\% Q_s = [(W_t - W_o)/W_o] \times 100,$$

where,  $W_o$  and  $W_t$  are the weights of dry film and wet film, respectively.

#### Surface morphology

Scanning electron microscopy (SEM) photographs of film (N7) were captured by mounting it on an aluminium stud by applying double adhesive carbon tape. The film was sputter coated with gold-palladium in presence of argon gas (under vacuum) using the "POLORON" E5100 SEM coating system. The scanning electron micrographs were recorded at HT-15 KV accelerating voltage using LEO 435VP (LEO Electron Microscopy Ltd, UK).

#### Mucoadhesive properties

Buccal membrane was fixed on the static platform of a texture analyser (Stable Micro Systems Ltd., Surrey, UK) and the assembly was bathed with buffer solution to keep the tissue moist. A film of size 1 x 1 cm was fixed to the probe of the texture analyser. Probe of texture analyser was brought down to touch the mucus membrane. The probe speed (0.5 mm/s), contact time (60 s) and the applied force (1N) were maintained in all the trials [14]. The amount of force needed to pull the film from the rabbit mucosal epithelium was considered as the mucoadhesive strength.

#### Drug release

The *in vitro* studies of the prepared drug loaded films (loaded with drug crystals/nanocrystals) were performed by paddle over disc method using USPXXIV Type II apparatus (Electro Lab TDC 50, Mumbai, India). Six films were selected from each formulation. Films measuring 2 x 1 cm were cut and attached on a glass slide and immersed in buffer (pH 6.2; 900 mL) and the paddle was rotated at 50 rpm [2]. At predetermined intervals of time, samples were withdrawn and replaced with fresh media. Then, the samples were filtered and analysed by using HPLC.

#### Ex vivo permeation studies

The permeation studies provide information regarding the mechanisms of drug diffusion into and through the biological membranes. Rabbit buccal membrane was used as the barrier for assessing nebivolol delivery. Freshly prepared rabbit buccal mucosa, with smooth

surface facing the donor compartment was mounted between the donor and receptor compartment of the Franz diffusion cell. The buccal film (size; 0.6 cm<sup>2</sup>) was placed over the rabbit mucosa in donor compartment and both the donor and the receptor compartment of the cell were clamped together. The receptor compartment was further filled with simulated saliva (5 mL) [2]. The diffusion cell was maintained at a temperature of 37 ± 0.2°C and the diffusion medium was stirred at 50 rpm. Samples were withdrawn at pre-determined time points and replaced using fresh simulated saliva maintained at 37 ± 0.2°C. The samples withdrawn were filtered and analysed using HPLC.

#### *In vivo study*

Male white rabbits (2.5 - 3.0 kg) housed in an animal house facility were used for carrying out the *in vivo* studies. Animals used for the study were fasted overnight with free access to water and the guidelines of Institutional Animal ethical committee (IAEC/SSP/16/PR-009) were strictly adhered to. Animals used in the study were anaesthetized using intramuscular injection of ketamine and/or xylazine [20]. The buccal film measuring 0.5 cm<sup>2</sup> was slightly wetted (water 30 µL) and applied to the buccal mucosa of the rabbits. Control animals were given an oral suspension of nebivolol HCl equivalent to 2.5 mg, and the dose was determined based on its standard human dose [19]. At pre-determined points of times, blood samples (500 µL) were withdrawn from the marginal ear vein of the rabbit. These samples were mixed with an equal amount of acetonitrile and centrifuged at 10,000 rpm for 10 min and the supernatant was filtered and analysed by HPLC.

#### *Data analysis*

A plot of total amount of nebivolol HCl transported through the membrane *versus* time was made and the slope was projected as the flux [23]. Unpaired t-test was carried out for conducting statistical analysis using GraphPad Prism (version 5, Graphpad software, San Diego, California, USA). A P-value of less than 0.05 was considered as the level of significance. Mean

value and standard errors were calculated using values of six trials.

## Results and Discussion

Table I summarize the composition of placebo films prepared with different amounts of chitosan. The characteristics of films observed in this study suggest that a low concentration of chitosan (in case of film N1) could not provide adequate peeling. In case of film N2, films were formed but these were very thin and could not withstand peeling. However, the mechanical strength of films increased as the amount of chitosan increased. Films N3 had a good thickness and mechanical strength. Hence, film N3 was selected for drug loading and films N4, N5, N6, and N7 were formulated. Films N4 and N5 were loaded with drug solution which upon drying formed drug crystals. Film N4 was prepared to have 2.5 mg/cm<sup>2</sup> of drug while N5 was designed to have 5.0 mg/cm<sup>2</sup> of the drug. Films N6 and N7 were loaded with nanocrystals containing 2.5 mg/cm<sup>2</sup> and 5.0 mg/cm<sup>2</sup> of drug, respectively (Table II). It is apparent from Table II that the size ranges of crystals impregnated in films were significantly different in pure drug and nanocrystals.

It is well known that the accuracy of dose as well as bioadhesion is influenced by the film thickness [21]. Table III indicates that the thickness of drug loaded films varied from 354 ± 52 nm (placebo film) to 531 ± 64 nm [nebivolol HCl (5 mg/cm<sup>2</sup>) loaded film], signifies the prepared films were thin and appropriate for buccal application. Furthermore, standard deviation in the film thickness was found to be relatively low suggesting their uniform nature and drug concentration. However, the thickness of films increased when nebivolol HCl was incorporated, in relative to the placebo films (Table III). On the other hand, there was no significant difference between pure drug loaded or nanocrystals incorporated films as observed with films N4-N7. In addition, an increase in the drug concentration in the films have little influence on its thickness as observed with films (N4 and N5 or N6 and N7).

**Table III**  
Physicomechanical properties of prepared films

Film code	Thickness (nm)	pH	Folding endurance	% Drug Content	Muoadhesive strength (N)
N3	354 ± 52	6.65 ± 0.54	210 ± 18	-	5.9 ± 0.88
N4	467 ± 55	6.35 ± 0.40	225 ± 12	94.88 ± 5.26	6.5 ± 1.22
N5	531 ± 64	6.58 ± 0.51	237 ± 19	97.57 ± 4.22	6.2 ± 1.31
N6	485 ± 75	6.75 ± 0.64	256 ± 10	95.34 ± 4.20	6.4 ± 1.75
N7	515 ± 88	6.87 ± 0.72	247 ± 17	96.58 ± 3.08	6.5 ± 1.62

All values are expressed as mean ± S.D; n = 6

The film pH measurement is equally important to ensure ideal pH for buccal application and to evade any irritation or destruction of mucosa. In this study, the pH of nebivolol HCl loaded films was found to be 6.3 - 6.9. Thus, it is likely that prepared films may

not cause any local irritation when it is applied on the buccal mucosa.

Flexibility and mechanical strength of the prepared films are generally assessed by the folding endurance test [21]. It is important to measure actual strength of

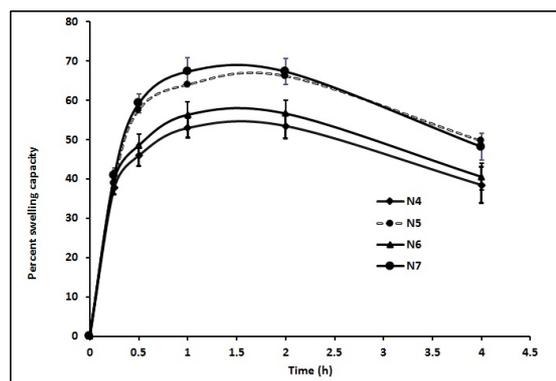
films to avoid accidental damage during storage and handling. The values in Table III signify that the prepared films have good folding endurance and are mechanically strong which in turn could prevent any damage during handling and packing. The data suggest that the formed films were flexible and had good mechanical strength which is a desirable attribute as the films would resist breaking or tearing during application [3]. It should also be emphasized that the amount of drug loaded into the films as well as size of drug crystals loaded in film have not affected the folding endurance value of prepared films.

Content uniformity is another significant criterion to be evaluated in any pharmaceutical preparation as it confirms the availability of active substances. Table III summarizes the drug concentration in the films (N4 to N7) which was found to be between  $94.88 \pm 5.26\%$  and  $97.57 \pm 4.22\%$ . The data suggest a sufficient drug amount in the prepared films and in the range of standard limits.

Preclinical studies of the buccal film generally investigate mucoadhesive strength which is crucial for application of formulations [21]. Data from this study will provide sufficient information regarding the binding potential as well as retention of films at the application site (mucosal layer). The mucoadhesive strength was determined for the placebo film N3 and the nebivolol HCl loaded films (N4-N7) with a texture analyser using the rabbit cheek mucosa as substrate. It was observed that mucoadhesive strength of drug loaded films was slightly higher than that of the placebo film N3. Maximum mucoadhesive strength was found for films N4 and N7 (~6.5 N) followed by film N6 and N5 (Table III). The data signifies that the films possess good mucoadhesive strength and could remain adhered to the buccal mucosa for a prolonged period of time. These films would probably be able to withstand the flow of saliva and the biological movement of the cavity without being detached. Mucoadhesive strength of chitosan has been reported in the literature and is due to the interaction between positively charged amino groups of chitosan with negatively charged mucin glycoprotein forming an ionic bond [1].

Swelling studies are conducted to assess the bio-adhesive property of the film and to gain an insight into the drug release kinetics and release rate which depend on the film composition, film matrix characteristics and the type of polymer used [12]. Swelling is required for mucoadhesive polymer to expand and thereby creates a macromolecular mesh of an appropriate size and also to mobilize the polymer chains so as to enhance the interpenetration between polymer and mucin. Swelling is also important for prolonged and controlled release of drug as well as for an effective mucoadhesion. Polymer swelling enables mechanical entanglement by exposing the mucoadhesive sites for hydrogen bonding or for

electrostatic interaction between the mucosa and the polymer [6]. Probably upon application of film, molecules of water diffuse into the film, hydrate and bind to the mucosal membrane and this bonding advances as hydration progresses. As polymer disentangles, the mucoadhesive strength of the film decreases [12, 21]. Simultaneously, water entering the matrix enables the diffusion of drug molecules into liquid matrix which subsequently enter the mucosa. Swelling studies of the drug loaded films (N4-N7) were carried out for a period of 4 h (Figure 1). The graph indicates that the rate of swelling capacity was the highest during the first 0.25 h of the study as depicted by the steep curve during this phase (Figure 1). This rapid swelling in the initial phase may contribute to the good bioadhesion with the buccal mucosa. After 0.25 h, greater percent swelling of films N5 and N7 were noticed when compared to films N4 and N6. However, after 2 h, the swelling capacity decreased, in all the cases. A similar swelling behaviour has been observed by Giovino *et al.* where an increase in the swelling capacity followed by a decrease was witnessed in chitosan films [6].

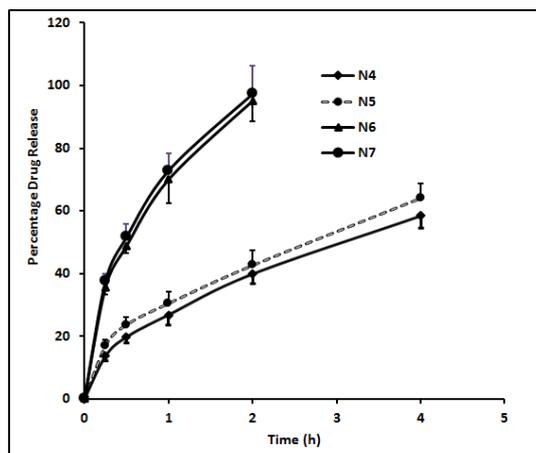


**Figure 1.**

The percentage hydration pattern of the prepared buccal films for a period of 4 h determined using 1 cm × 1 cm of the film. The value represents the average of six trials ± SD

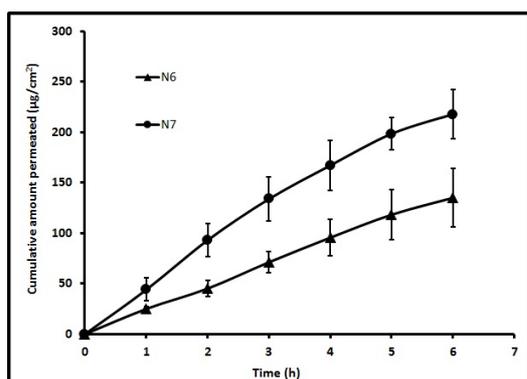
Drug release from film is essential for the permeation of the drug across the biological membranes. This study was conducted in films N4-N7. Figure 2 depicts the drug release from the prepared films at different time intervals. It is evident from Figure 2 that the cumulative percent drug release profile of the films N4 and N5 is different from films N6 and N7, in which drug is present as nanocrystals. The drug release observed in films N4 and N5 is relatively slower when compared to films N6 and N7 which exhibit a faster drug release. In film N6 and N7, the drug release was the highest during the first hour of the study while the drug release was low and incomplete in film N4 and N5. These profiles clearly indicate that nanocrystals impregnated films exhibited a considerably higher

drug release and was nearly complete in the 4 h of the study.



**Figure 2.**

Comparison of the cumulative percentage of nebigivol hydrochloride released from different buccal films at various time intervals. The value represents the average of six trials  $\pm$  SD



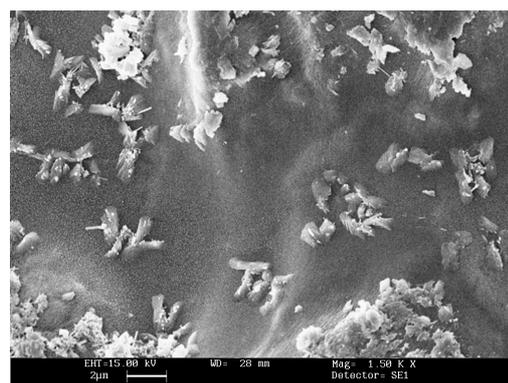
**Figure 3.**

Comparison of *ex vivo* permeation of nebigivol hydrochloride from selected buccal films N6 and N7 using rabbit buccal mucosa for a period of 6 h in Franz diffusion cell. The value represents the average of six trials  $\pm$  SD

The permeability of nebigivol released from the chitosan films was investigated in an *ex vivo* environment using the multi-layered rabbit buccal tissue. The rabbit is the only laboratory rodent that has non-keratinized mucosal lining similar to human tissue and it has been used extensively in experimental studies [21]. *Ex vivo* permeation studies of drug loaded films across biological membranes are helpful to predict the absorption of drug molecules and to study the kinetics of drug absorption [26]. The permeation of drug molecules across the biological membranes is a function of physicochemical properties of drug molecules and the physiological properties of the biological membrane [22]. Permeation studies were carried out for the drug loaded films, N6 and N7 to study the transport of nebigivol across the rabbit cheek mucosa using the

Franz diffusion cell. The amount permeated across the rabbit cheek mucosa was determined by sampling the media in the receptor compartment and is depicted in Figure 3. The cumulative amount of drug permeated was found to be significantly higher in film N7 relative to N6. Drug permeated in 1 h amounted to  $24.96 \pm 2.29 \mu\text{g}/\text{cm}^2$  in film N6 and  $44.01 \pm 11.38 \mu\text{g}/\text{cm}^2$  in film N7. The flux was found to be  $22.50 \pm 4.21 \mu\text{g}/\text{cm}^2/\text{h}$  and  $36.31 \pm 6.54 \mu\text{g}/\text{cm}^2/\text{h}$  ( $p < 0.0001$ ) from films N6 and N7, respectively. The study clearly indicated that the permeation across biological membranes is greater when the drug concentration is high.

The external morphology of the buccal films impregnated with nanocrystals was carried out using scanning electronic microscopy (SEM). The buccal film was found to be smooth, non-porous and uniform (Figure 4). Nanocrystals were uniformly distributed in the films and were found to be impregnated in the chitosan films. The images also confirm that nanocrystals loaded film have the required morphological features of an ideal film and hence can be used for buccal delivery of nebigivol.



**Figure 4.**

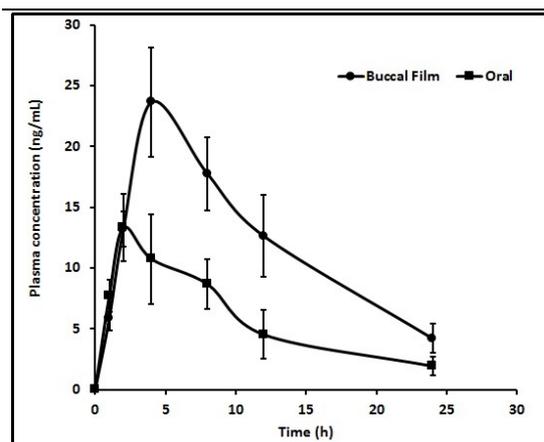
Scanning electron microscopy image of buccal film N7 impregnated with nebigivol hydrochloride nanocrystals

Various pharmacokinetic properties like area under plasma drug concentration time profile (AUC), maximum concentration of drug ( $C_{\text{max}}$ ) and time to reach maximum concentration ( $T_{\text{max}}$ ) were analyzed by using a non-compartmental method. Figure 5 depicts the mean plasma concentration *versus* time profiles of nebigivol HCl of nanocrystals loaded N7 buccal film and control (suspension). The estimated pharmacokinetic parameters are summarized in Table IV.

**Table IV**

Mean pharmacokinetic parameters of nebigivol hydrochloride in plasma following buccal application of film N7 and oral suspension (control) in rabbits

Parameter	Buccal Film (N7)	Control
$T_{\text{max}}$ (h)	4	2
$C_{\text{max}}$ (ng/mL)	$23.67 \pm 4.47$	$13.33 \pm 3.70$
$\text{AUC}_{0-\infty}$ (ng.h/mL)	$401.03 \pm 62.35$	$167.83 \pm 39.24$



**Figure 5.**

Comparison of the plasma profiles of nebivolol hydrochloride following buccal application of the nanocrystals loaded buccal film (N7) and oral solution in rabbits. The value represents the average of six trials  $\pm$  SD

It is evident from Figure 5 that the kinetic profiles are distinct for buccal application and oral therapy (control). Indeed, nebivolol absorption was rapid and the drug was available in plasma in the first hour and the two administration routes were comparable (buccal;  $5.86 \pm 0.97$  ng/mL, oral;  $7.73 \pm 1.29$  ng/mL). Considering the local administration, drug absorption was rapid, extended and attained  $C_{max}$  value of  $\sim 24$  ng/mL in 4 h ( $T_{max}$ ), showing greater efficiency (Table IV). In control, the drug level achieved the maximum level ( $C_{max} \sim 13$  ng/mL) in 2 h ( $T_{max}$ ) which is considerably lower than the buccal route, suggesting a low absorption. The  $C_{max}$  values for buccal delivery ( $\sim 24$  ng/mL) were also found to be  $\sim 2$  folds higher than the control ( $p < 0.005$ ). Furthermore,  $AUC_{0-\infty}$  values were  $\sim 400$  ng \* h/mL and  $\sim 165$  ng \* h/mL ( $p < 0.0001$ ) for buccal and oral delivery, respectively. The bio-availability of nebivolol was increased  $\sim 2.5$  fold following buccal application in comparison to oral therapy (nebivolol suspension). Overall, these findings suggest that a higher plasma drug concentration is attained when the drug is administered *via* the buccal route when compared to oral route.

## Conclusions

Placebo films using different concentrations of chitosan were formulated and evaluated to optimize the film forming potential. Further, drug loaded (nebivolol) films were prepared and their evaluation suggests that all films possess adequate physical characteristics. Drug loaded films (N4-N7) exhibited uniformity in drug concentration and desirable physicochemical properties such as pH, film thickness, folding endurance, swelling capacity and mucoadhesive strength. Drug release profiles suggest higher release rate from nanocrystals incorporated films. Indeed, a greater bio-

availability of nebivolol through the buccal mucosa was observed for the nanocrystals loaded film. Our findings therefore suggest that the nanocrystals impregnated buccal film could be a promising approach for the delivery of nebivolol, although need to be proved in human.

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## Declaration of interest

The authors report no conflict of interest.

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