



# Budesonide-Cyclodextrin in Hydrogel System: Impact of Quaternary Surfactant on *in vitro-in vivo* Assessment of Mucosal Drug Delivery

SUNIL PATTANAIK, SOUVIK NANDI, RUDRA NARAYANA SAHOO, ASHIRBAD NANDA, RAKESH SWAIN, SHUBHASHREE DAS, SUBRATA MALLICK\*

School of Pharmaceutical Sciences, Siksha 'O' Anusandhan (Deemed to be University), Khandagiri, Bhubaneswar, Odisha, India, 751003

**Abstract:** Budesonide, a glucocorticosteroid is generally used to treat chronic inflammation and asthma. Hepatic first-pass metabolism and poor solubility are the major causes of its limited oral bioavailability. Present work was undertaken for the preparation of hydrogel film formulation with cyclodextrin complexation of budesonide containing quaternary surfactant for possible enhancement of mucosal permeation. FTIR study confirmed drug-polymer hydrogen bonding. Almost complete amorphization of the drug was pronounced by SEM, DSC and XRD studies. The film containing benzalkonium and hydroxypropyl beta-cyclodextrin exhibited *in vitro* dissolution and mucosal permeation to the highest extent of 87.2 and 95.8 % respectively in contrast to the others. Film formed hydrogel in aqueous mucin and enhanced the mucosal tissue residence time due to the mucoadhesive nature of the polymer. Acute inflammation in the rabbit eye was controlled within 3 h by applying the film in the cul-de-sac. The presence of cyclodextrin and quaternary surfactant brought about significantly improved drug release and mucosal permeation compared to their absence in the HPMC film. Hydrogel formed in aqueous mucin enhanced the mucosal residence time and controlled acute inflammation in the rabbit eye within 3 h after topical application.

**Keywords:** Budesonide, cyclodextrin inclusion, mucosal delivery, ocular anti-inflammation.

## 1. Introduction

Budesonide, a highly potent glucocorticosteroid is used to control asthma by decreasing swelling and irritation in air pathways for easier breathing. It is also used to treat inflammatory bowel disease (IBD), ulcerative colitis (UC), and Chron's disease (CD) [1]. It is available in the market as dry powder inhaler (DPI), tablet, and capsule formulations. According to the biopharmaceutical classification system (BCS) the drug is having high permeability and low solubility, with a log P of 3.2. The oral bioavailability of budesonide is highly affected due to its poor dissolution and high hepatic first-pass metabolism (11 %). Acid hydrolysis and enzymatic degradation in the GI tract affect the absorption process after oral administration. Budesonide also shows low inhalation bioavailability of 6 % with a very short elimination half-life of 2-3 h [2]. Fluidization and dispersion characteristics may be affected due to problems like upper airway deposition associated with budesonide DPI [3].

Transmucosal routes like ocular, buccal, nasal, rectal, vaginal give more discrete advantages for the administration of a drug over other non-parenteral routes. These mucosal routes also offer many times greater permeability rather than of skin [4]. Drug delivery through buccal route leads to absorption to the systemic circulation effectively bypassing the first-pass metabolism with minimal fluctuations in plasma concentration [5]. Buccal mucosal tissue is highly vascularised with blood vessels. Mucin concentration in the buccal mucosa is high for facilitating more effective mucoadhesion compared to inhalational therapy and many other mucosal deliveries [6]. Moreover, buccal tissue recovery is quicker than other delivery routes and could be appropriately explored for better drug delivery [7].

\*email: [profsmallick@gmail.com](mailto:profsmallick@gmail.com)



Nowadays hydrophilic vehicles play a great role in incorporating various kinds of drugs in mucosal delivery. Hydrogels as the semisolid vehicle can also be adapted easily in sustained and controlled release and thus minimizing the side effect and toxicity [8-12]. Gaikwad et al have developed an enteric-coated self nano emulsifying capsule formulation for colonic delivery of budesonide [13,14]. The main limitations associated with these types of delivery systems are drug loading efficiency and bulkiness of the final volume of the formulation.

Inclusion agents are used to achieve better bioavailability and dissolution due to improved physicochemical stability and increased solubility [15-17]. Cyclodextrins, a non-toxic cyclic oligosaccharide has particularly drawn attention for improving the solubility and stability of drugs [18]. Cyclodextrin complexation increased the solubility and stability of lansoprazole [19]. The use of cetrimide and hydroxypropyl beta cyclodextrin independently improved the solubility of valdecoxib [20]. Oral pediatric formulation of budesonide was prepared by inclusion complexation using cyclodextrin for increased bioavailability [6]. Transbuccal diffusion of omeprazole was increased in the presence of cyclodextrins [21]. Presence of surfactants also in the delivery system is very much promising in improving mucosal permeation. Transmucosal permeation of fluorescein isothiocyanate dextran has been improved through cornea by incorporating benzalkonium chloride in the solution [22]. Another cationic surfactant, cetrimide was also found to increase the buccomucosal permeation of peptides and proteins [23].

The solubility of a surfactant can be increased by forming an inclusion complex with cyclodextrin [24]. Critical micelle concentration of surfactants increases markedly in the presence of cyclodextrin [25]. The impact of quaternary surfactants and cyclodextrin in combination on mucosal permeation of budesonide has probably not been reported yet and could be the potential for significant improvement of bioavailability of the drug. In our study, hydrogel film has been prepared with cyclodextrin complexation of budesonide containing quaternary surfactant for possible enhancement of mucosal permeation. Film formulation was chosen because of its better patient compliance, ease of application, and other advantages like transport and storage [26]. Solvent casting method was employed for the preparation of budesonide-in-HPMC films using  $\beta$ -cyclodextrin or HP- $\beta$ -cyclodextrin as an inclusion agent. Benzalkonium chloride (BZK) or cetrimide (CET) was also used as quaternary surfactants in combination with inclusion agent for possible enhancement of transmucosal permeation of budesonide. Budesonide films were tested for *ex vivo* buccal mucosal tissue permeation. *In vivo* anti-inflammatory activity has also been examined after topical application of film on carrageenan induced rabbit eye model [27,28]. The utility of *ex vivo* permeation (using mucosal biomembrane) was demonstrated to predict the ability of budesonide *in vivo* (in life system) performance.

## 2. Materials and methods

### 2.1 Materials

Budesonide was obtained from Cipla Laboratories (Goa, India) as a gift sample. HPMC K 15M and Cetyl Trimethyl Ammonium (cetrimide) were procured from Burgoyne Burbidges & co. Laboratories (Mumbai, India). Triethanolamine and benzalkonium chloride (50 % solution) were purchased from SRL laboratories and MERCK laboratories India respectively. Beta cyclodextrin and hydroxypropyl beta-cyclodextrin were obtained from Dr. Reddy's Laboratories (Hyderabad, India) as gift samples. Carrageenan was purchased from TCI Laboratories Pvt. Ltd. Japan.

### 2.2 Preparation of budesonide film

HPMC K 15M was taken in a 100 mL beaker and 40 mL of distilled water was added and left for swelling at 2-8 °C for 24 h.  $\beta$ -cyclodextrin and HP- $\beta$ -cyclodextrin, triethanolamine, and surfactants were incorporated into the viscous polymer solution with continuous stirring. Budesonide in 10mL of methanol solution was added to the mixture and stirring continued for 2-3 h until a fully transparent solution was obtained. The drug-polymer solution was then poured on Tarson Petri dish and left to dry at 40-50 °C for 36-48 h. Complete drying was assured when a constant weight was achieved of the

prepared film. Films were separated from the Petri dish and packed in the zip lock Tarson pouch and preserved in an airtight container until further studies [29] (Table 1). A standard calibration curve of Budesonide was prepared by recording the absorbance of various dilutions of the drug (1.44, 2.88, 4.32, 5.76, 7.20, 8.64, and 10.08 µg/mL) at 247 nm (JASCO V-630 spectrophotometer). Assay of the film formulations was estimated by placing a preweighed piece of film in a volumetric flask. Sufficient methanol was added and the flask was shaken for 24 h at laboratory ambient condition in an Orbital shaker (Remi Elektrotechnik Ltd, Vasai, India). Volume was made up to the 100 mL mark for the complete solubilization of film with methanol. The resulting solution was filtered (Whatman Uniflo 0.45µm, PVDF) and a serial dilution was made with aqueous phosphate buffer (pH 6.8) and checked spectrophotometrically at 247 nm [30,31]. Film thickness was measured 5-10 random portions by Mitutoyo Digimatic Micrometer (Japan) and the average values were recorded. Folding endurance of the film was observed by repeatedly folding a small strip of 2cm x 2cm till it breaks. The number of times the film folded was measured as the folding endurance value.

**Table 1.** HPMC hydrogel film formulation of budesonide-cyclodextrin containing quaternary surfactant<sup>a</sup>

Formulation code	HPMC k15M (mg)	Surfactant (0.1 %)	Inclusion agent (molar ratio 1:1)	Budesonide Assay mean ± sd (%)
BHT	900	-	-	3.78 ± 0.194
BHT <sub>b</sub> C <sub>1</sub>	900	BZK	β – cyclodextrin	2.48 ± 0.065
BHT <sub>b</sub> C <sub>2</sub>	900	BZK	HP-β–cyclodextrin	2.55 ± 0.03
BHT <sub>c</sub> C <sub>1</sub>	900	CET	β – cyclodextrin	2.66 ± 0.03
BHT <sub>c</sub> C <sub>2</sub>	900	CET	HP-β–cyclodextrin	2.49 ± 0.05

<sup>a</sup> Budesonide (40 mg) in HPMC matrix using triethanolamine (20 % of polymer) as a plasticizer

### 2.3 Moisture content and Moisture uptake

Prepared films were cut into small pieces and put into the desiccator containing activated silica gel at least for 24 h. The percentage of weight difference value of the films with respect to the initial was estimated as moisture content (%). For evaluation of moisture uptake film pieces were placed for equilibration in a closed desiccator containing the supersaturated solution of sodium chloride for maintaining 75 % RH up to a constant weight. The weight difference value between final weight and initial weight was used % of moisture uptake of the films [32].

$$\text{Moisture Content} = \frac{(\text{Initial weight} - \text{dryweight after 24 h})}{\text{Initial weight}} \times 100$$

$$\text{Moisture Uptake} = \frac{(\text{Final weight} - \text{Initial weight before putting in dessicator})}{\text{Initial weight}} \times 100$$

### 2.4 Swelling and erosion study

Dynamic hydration of swelling and erosion of the film was determined from the percentage hydration and matrix erosion using the following equations. Randomly selected film sample (≈1 cm × 1 cm) was placed in Petri dishes containing 40 ml of simulated buccal fluid (phosphate buffer of pH 6.8) [33]. The weight gained by the films was noted at regular intervals after removing excess liquid by tissue paper swabbing [34].

$$\text{Dynamic hydration} = \frac{(\text{Hydrated weight} - \text{Initial dry weight of the film})}{\text{Weight after hydration}} \times 100$$

$$\text{Matrix erosion} = \frac{(\text{Initial dryweight} - \text{Dried final weight after swelling})}{\text{Initial dryweight}} \times 100$$

## 2.5 FTIR

FTIR study was done for the prepared films and pure budesonide to observe drug-excipient interactions. Samples were placed on the diamond ATR crystal (JASCO ATR PRO ONE) in the FTIR spectrometer (JASCO FT/IR 4600). All the scans were done between 4000-400  $\text{cm}^{-1}$  and an average of 80 scan accumulations at a resolution of 4 $\text{cm}^{-1}$  were recorded.

## 2.6 DSC (thermal analysis)

Calorimetric study of budesonide and the formulations were performed by differential scanning calorimetry (Model: DSC-1, Mettler Toledo; Switzerland). The study was conducted at a temperature range of 30-300  $^{\circ}\text{C}$  under constant nitrogen flow (20.0  $\text{mL}\cdot\text{min}^{-1}$ ) and a heating rate of +10  $^{\circ}\text{C}\cdot\text{min}^{-1}$ .

## 2.7 X-ray diffractometry

X-ray diffraction pattern of pure budesonide and formulations were acquired by using X-ray diffractometry (Model: Rigaku Ultima IV). The voltage and current were 40 kV and 15 mA respectively. The X-ray source anode material used was CU. 1 $^{\circ}$ /min scan speed was maintained and X-axis values ranging between 5-70 $^{\circ}$  specifications test was carried out.

## 2.8 SEM analysis

Surface morphology of budesonide and the films were examined by scanning electron microscopy (Model: JEOL JSM-6510). Sputter coating with platinum was applied as a prior treatment before subjecting the samples in the SEM. Samples were scanned under an accelerated voltage of 30kV at room temperature. The film surfaces and crystal size of pure drug visualizing at 3,000-10,000X magnification.

## 2.9 In-vitro drug release study

Prepared budesonide films were attached on the surface of a glass slide with cyanoacrylate adhesive and fully merged it into the dissolution vessel containing 200 mL phosphate buffer (6.8 pH) in a USP Type-II dissolution apparatus (Dissolution Tester USP, Electrolab TDT06L, India). The paddle was adjusted to 50 rpm along with the temperature at 32 $\pm$ 2  $^{\circ}\text{C}$  and the study was carried out for 6 h [35]. The release of the drug was estimated spectrophotometrically.

## 2.10 Mucosal permeation study

Fresh buccal skins were collected from local chicken meat shop within an hour of its sacrifice. Buccal mucosal membranes were made free from fat and then washed with distilled water and dipped into phosphate buffer pH 6.8 at room temperature. The dissected tissue was then attached to the diffusion tube in modified Franz diffusion cell. Buccal permeation was carried out for 6h using phosphate buffer 6.8, 200 mL as the diffusion media maintaining 34  $\pm$  0.2 $^{\circ}\text{C}$ . The samples were withdrawn at particular time intervals and assayed by UV Spectrophotometer to calculate the percentage of drug permeated [36].

## 2.11 Anti-inflammatory activity study

For in vivo anti-inflammatory study the ocular environment was much convenient because of its easy access and for the fact that mucin is present in both buccal and ocular environment [33]. New Zealand white rabbits of 1.5-2 Kg were used for the study. Animals were held for 24h in the laboratory before the experiment for environmental adaptation. This anti-inflammatory study was properly assessed and approved by the animal ethical committee of Siksha O Anusandhan (Deemed to be University) (IAEC no. IAEC/SPS/SOA/07/2019). Local ocular anesthesia was given with 0.5 % Proparacaine HCl (Ophthalmic Solution) USP. Carrageenan (200  $\mu\text{L}$ , 3 % w/v) was injected in the upper palpebral region of the eye of the rabbit by BD U-40 syringe to induce acute inflammation. The film (BHT<sub>b</sub>C<sub>2</sub>) was sterilized under UV radiation by keeping it for 10 mins at a distance of 25 cm from

the UV source. The sterilized film then placed at the cul-de-sac and ocular anti-inflammatory activity was visualized and photographed.

### 3. Results and discussions

#### 3.1 Physical characterization

The average thickness of the films was found in the range of 160-195  $\mu\text{m}$ , well acceptable for mucosal application [37]. Films were showing good folding-endurance between 192-210 indicating sturdy and plasticized enough and not fragile due to the presence of triethanolamine [38]. The content of moisture in the films was found to be in the range of 1.25-2.41 % in laboratory conditions.

The main role of moisture content in a film is to deliver enough plasticity and keeping it from being brittle. But excess moisture content affects the properties of the films. Moisture uptake study of the films was carried out at 75% RH condition showed in the range of 5.59 - 5.69 % [39]. The physical properties of the films are illustrated in Table 2.

**Table 2.** Physical properties of polymeric film

Formulation Code	Moisture uptake Mean $\pm$ sd, (n=4) (%)	Moisture content Mean $\pm$ sd, (n=4) (%)	Thickness ( $\mu\text{m}$ ) Mean $\pm$ sd, (n=4)	Folding endurance
BHT	5.67 $\pm$ 0.26	2.41 $\pm$ 0.66	166.0 $\pm$ 3.3	192
BHT <sub>b</sub> C <sub>1</sub>	5.59 $\pm$ 0.40	1.25 $\pm$ 0.63	178.1 $\pm$ 7.5	203
BHT <sub>b</sub> C <sub>2</sub>	5.69 $\pm$ 0.30	1.70 $\pm$ 0.44	196.6 $\pm$ 7.7	209
BHT <sub>c</sub> C <sub>1</sub>	5.64 $\pm$ 0.36	1.67 $\pm$ 0.48	174.6 $\pm$ 4.9	196
BHT <sub>c</sub> C <sub>2</sub>	5.67 $\pm$ 0.377	2.12 $\pm$ 0.37	196.0 $\pm$ 8.0	201

#### 3.2 Swelling and erosion study

The swelling profile of the films was depicted in Figure 1. Hydration of water in the polymeric matrix was seen followed by matrix erosion. Rate of swelling increased significantly in the presence of cyclodextrin compared to its absence in the films. BHT showed swelling rate up to 2.402  $\text{min}^{-1}$  whereas the film containing inclusion agent (BHT<sub>b</sub>C<sub>1</sub>, BHT<sub>b</sub>C<sub>2</sub>, BHT<sub>c</sub>C<sub>1</sub>, and BHT<sub>c</sub>C<sub>2</sub>) showed 3.543, 3.656, 3.624, 3.134  $\text{min}^{-1}$  (Table 3). Cyclodextrin in the polymeric film played a vital role in hydration to loosen the polymer network and increased water retention capacity [40,41].

**Table 3.** Swelling behaviour and permeability parameter of budesonide film

Formulation n Code	Rate of swelling (K <sub>s</sub> ) (min <sup>-1</sup> ) (mean $\pm$ sd; n=4)	Erosion (%), (mean $\pm$ sd; n=4)	Flux (J <sub>s</sub> ) ( $\mu\text{g}/\text{min}$ ) (mean $\pm$ sd; n=4)	Permeability co-efficient (P <sub>ss</sub> ) (cm/min) *10 <sup>5</sup> (mean $\pm$ sd; n=4)
BHT	2.4 $\pm$ 0.4	72.16 $\pm$ 2.8	0.82 $\pm$ 0.15	1.6 $\pm$ 0.57
BHT <sub>b</sub> C <sub>1</sub>	3.5 $\pm$ 0.3	72.65 $\pm$ 12	1.41 $\pm$ 0.47	4.7 $\pm$ 1.4
BHT <sub>b</sub> C <sub>2</sub>	3.6 $\pm$ 0.1	72.39 $\pm$ 2.3	1.67 $\pm$ 0.74	8.3 $\pm$ 3.7
BHT <sub>c</sub> C <sub>1</sub>	3.6 $\pm$ 0.3	79.01 $\pm$ 3.5	2.12 $\pm$ 0.49	6.5 $\pm$ 2.0
BHT <sub>c</sub> C <sub>2</sub>	3.1 $\pm$ 0.1	77.80 $\pm$ 1.3	1.44 $\pm$ 0.21	4.8 $\pm$ 1.6



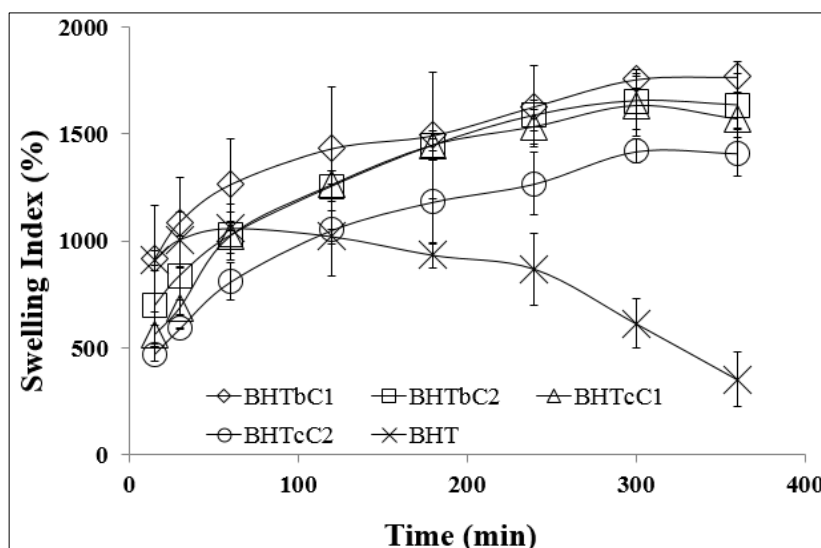


Figure 1. Swelling index profile of budesonide film

### 3.3 FTIR study

Polymer and drug intermolecular interactions were analyzed by the FTIR spectra (Figure 2A). Characteristic peaks of budesonide were showed at 3490, 2956, 1723, 1666, and 888  $\text{cm}^{-1}$  due to the stretching of O-H, C-H, C=O, and C=C [42,43]. In the formulated films the peaks are masked, the characteristic C=O stretching peak at 1666 shifted to 1656 with a decreased intensity. Broadening of the peaks at 1200-1000 regions was found. Masking, shifting and broadening of the parent peak intensity are the clear evidence of interaction of drug with polymers and cyclodextrins due to H-bond formation [30].

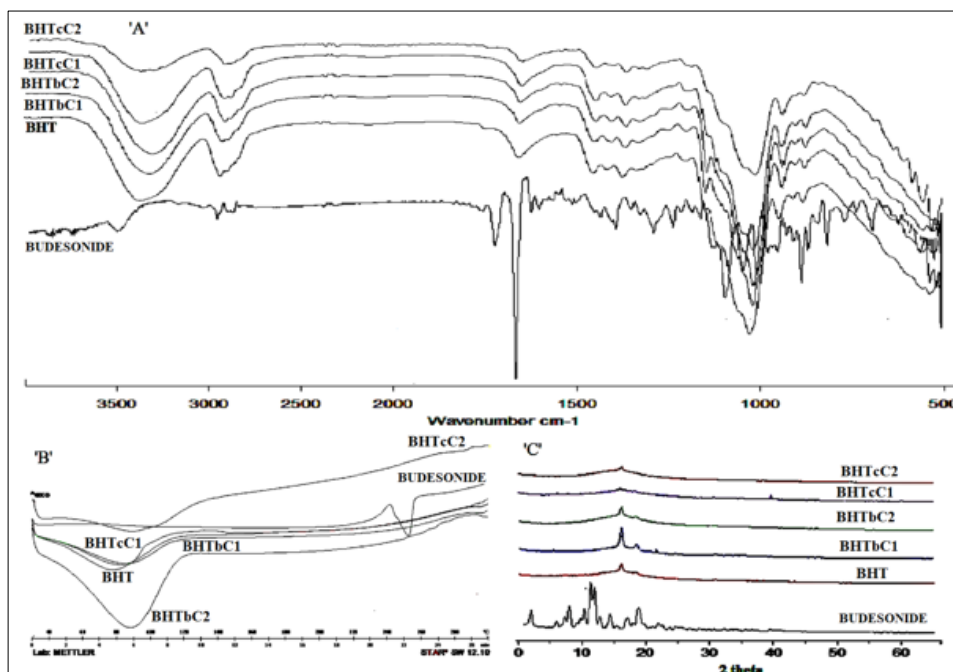


Figure 2. (A) FTIR study (B) DSC study (C) XRD study of pure drug and films

### 3.4 DSC study

DSC study was carried out to find the endothermic peak, crystalline behavior, and degradation of pure drug. Budesonide showed (Figure 2B) the sharp endothermic peak at 252.57°C due to the melting point of the drug. Polymeric films showed only a wide endothermic shouldering within 50-100°C

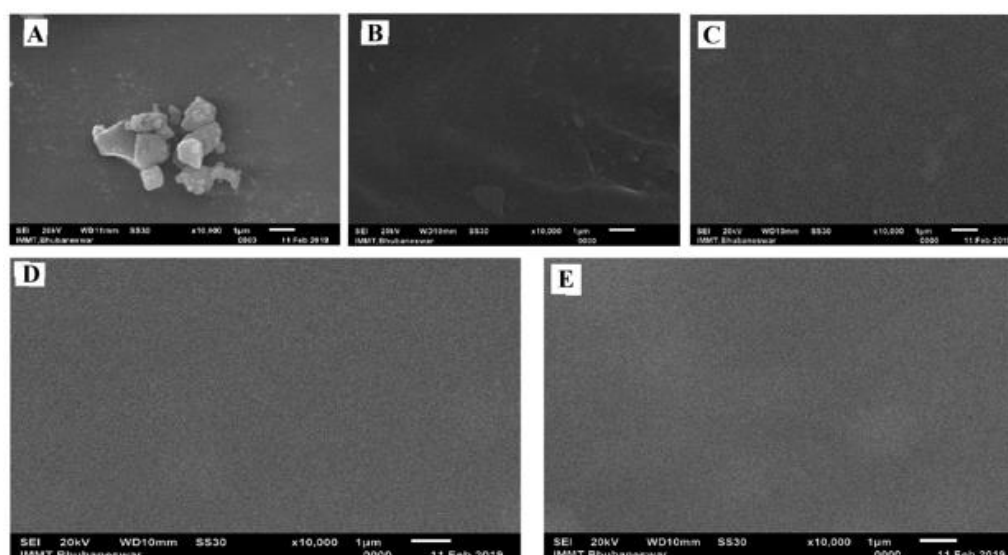
specifying water evaporation of water from the hydrogel matrix. Complete disappearance of the melting peak demonstrated complete dispersion of budesonide in the polymer matrix and almost complete amorphization [44].

### 3.5 XRD analysis

X-ray diffractogram of pure budesonide (Figure 2C) showed high-intensity peaks at 6.21, 10.18, 11.49, 12.17, 15.53, 16.10, 22.16  $2\theta$  which can only be observed if the drug is in crystalline form [42]. The disappearance of the diffraction peaks in the polymeric matrix indicated intermolecular complexation between drug and polymer and almost complete amorphization of the drug crystal.

### 3.6 SEM analysis

Figure 3 depicts the SEM images of pure budesonide as regular geometric crystal form [45] and loss of geometry were clearly observed in the formulated polymeric films. All the film formulations showed smooth and homogenous surfaces may be due to interpenetration and considerable solubility of budesonide into the matrix system. The crystal grains are not visible even after  $\times 10000$  magnification of the micrographs which confirms the lowering of crystalline intensity to a great extent. XRD and DSC report also confirmed the diminished crystallization of drug markedly in the polymeric film. Presence of HPMC arrested the crystal growth significantly in the film [38].



**Figure 3.** SEM images (A) Budesonide, (B) BHT<sub>b</sub>C<sub>1</sub>, (C) BHT<sub>b</sub>C<sub>2</sub>, (D) BHT<sub>c</sub>C<sub>1</sub>, (E) BHT<sub>c</sub>C<sub>2</sub>

### 3.7 In-vitro dissolution study

Improved in-vitro cumulative drug release profiles have been observed from all the film formulations for an extended period of 6 h in Figure 4A. Drug release has been more effective due to the presence of inclusion agents and quaternary surfactants [46]. BHT formulation showed a release up to 63 % whereas, the combination of cyclodextrin ( $\beta$ -cyclodextrin or, HP- $\beta$ -cyclodextrin) as an inclusion agent and surfactant (cetrimide or, benzalkonium chloride) improved the dissolution of the drug (77.8 to 87.2 %). BHT<sub>b</sub>C<sub>2</sub> formulation exhibited the in vitro release to the highest extent (87.2 %) due to the presence of benzalkonium chloride and HP- $\beta$ -cyclodextrin compared to other formulations (BHT<sub>b</sub>C<sub>1</sub>: 84.12; BHT<sub>c</sub>C<sub>1</sub>: 77.8; and BHT<sub>c</sub>C<sub>2</sub>: 80.2 %). In a literature report, it was noticed that the rate and extent of budesonide dissolution have become significantly effective by the use of lung surfactant [47]. Akkari et al 2016 have claimed that the addition of HP- $\beta$ -cyclodextrin in the hydrogel matrix has increased the solubility of budesonide [48]. Lansoprazole dissolution was increased by the addition of cyclodextrin [19]. In a study presence of cetrimide or cyclodextrin has improved the solubility of valdecoxib [20]. Sodium lauryl sulphate (0.5%) was used for maintaining sink condition in dissolution

testing of a hydrogel containing budesonide tablet at pH 6.8 [48]. Solubility of the budesonide-cyclodextrin complex has further been improved in the presence of quaternary surfactants during the *in vitro* release study of the present film formulation. The difference and similarity factor ( $f_1$  and  $f_2$ ) of the formulations (against BHT) has been tabulated (Table 4).

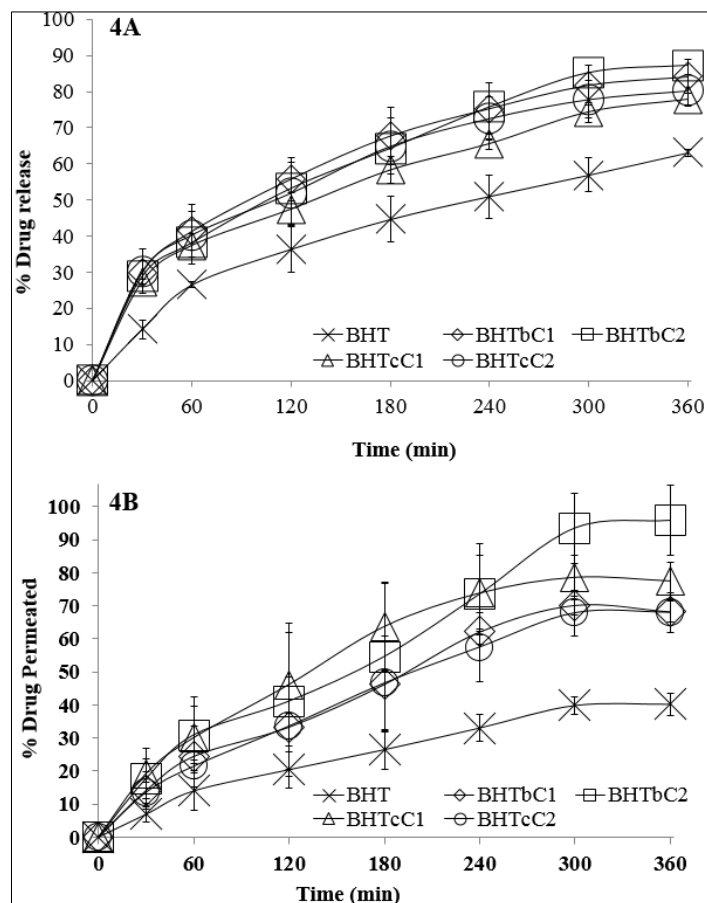


Figure 4. (A) In-vitro dissolution study (B) Buccal permeation study

Table 4. Comparison of *in-vitro* dissolution and mucosal permeation profiles

Comparison with BHT		<i>in-vitro</i> dissolution		mucosal permeation		Similarity or equivalence value
		Difference factor ( $f_1$ )	Similarity factor ( $f_2$ )	Difference factor ( $f_1$ )	Similarity factor ( $f_2$ )	
BHT vs. BHTbC1		49.054	34.024	76.176	33.234	$f_1 \leq 15$
BHT vs. BHTbC2		47.911	34.079	125.124	21.981	
BHT vs. BHTcC1		32.651	42.932	114.997	24.985	$f_2 \geq 50$
BHT vs. BHTcC2		42.847	37.046	70.520	34.805	

### 3.8 Mucosal permeation study

Mucosal permeation was carried out also for 6 h by using chicken buccal tissue as a mucosal membrane (Figure 4B). Presence of inclusion agent ( $\beta$ -cyclodextrin or, HP- $\beta$ -cyclodextrin) and surfactant (cetrimide or, benzalkonium chloride) significantly improved also the permeation (BHTbC1: 70.1; BHTbC2: 95.8; BHTcC1: 78.5 and BHTcC2: 67.9 %) compared to its absence in the film (BHT: 40.2 %). Among all the formulations, the benzalkonium chloride-containing film along with HP- $\beta$ -cyclodextrin showed the highest result (BHTbC2: 95.8 %). Benzalkonium chloride or cetrimide in the



film (BHT<sub>b</sub>C<sub>2</sub> and BHT<sub>c</sub>C<sub>1</sub> respectively) increased the release of drug on the mucosal surface because of its surfactant property and facilitated the better permeation via paracellular route compared to BHT [23]. Additionally, the cationic surfactants overcame the physiological and mucosal barrier for the increase of permeation and BZK exhibited better ability rather than CET [49]. Cyclodextrin exhibited increased transmucosal diffusion of omeprazole [21]. BZK was found to be facilitating the mucosal permeation of fluorescein isothiocyanate dextran [22].  $f_1$  and  $f_2$  of the formulations with respect to BHT are tabulated in Table 4.

### 3.9 Kinetics of drug release and permeation

Korsmeyer-Peppas, Higuchi, and first-order model equations were used to describe the drug release and permeation kinetics of the drug [50,51]. The parameters were reported in Table 5.

Korsmeyer-Peppas:

$$\frac{W_t}{W_\infty} = K_k t^n$$

Higuchi Kinetics:

$$H = K_h \sqrt{t}$$

First-order:

$$\log H = \log H_0 + \frac{kt}{2.303}$$

Here,  $W_t$  is the fraction of drug released/permeated at time “t”, and  $W_\infty$  maximum amount of drug available at the release/permeation site,  $K_k$  is the constant related to system structure and geometry and  $n$  is the drug release exponent.  $H$  and  $K_h$  signify the amount of drug released per unit area of the film and Higuchi rate constant for release/permeation respectively. The drug release of all the formulation could be described as a diffusion-controlled process ( $n = 0.40-0.56$ ) [52].

$$P_{ss} = \frac{J_{ss}}{C}$$

$C$  = drug concentration remaining in the formulation ( $X/V$ ) [where  $X$  = cumulative drug amount in receiver compartment]

$J_{ss}$  = Flux of permeation at steady state calculated from the slope of  $X$  vs time plot.

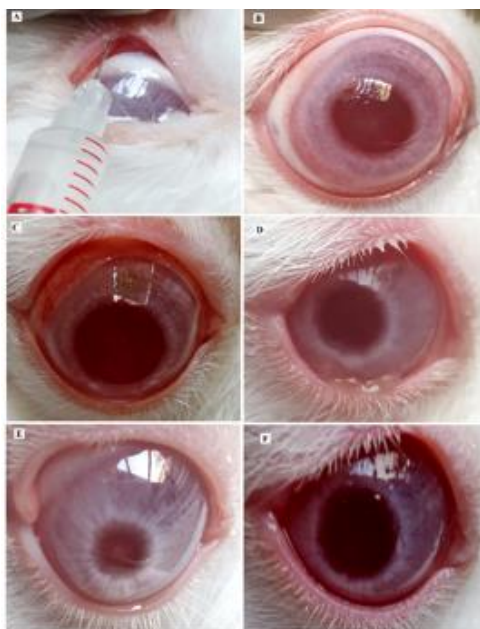
The amount of drug permeated or permeability co-efficient ‘ $P_{ss}$ ’ of BHT<sub>b</sub>C<sub>2</sub> film ( $8.311 \times 10^{-5}$ ) was better than the other formulations demonstrated in Table 3 [53,54]. A low amount of drug was permeated from the BHT formulation ( $1.675 \times 10^{-5}$ ). According to the Korsmeyer-Peppas equation, the ‘ $n$ ’ value lies in between 0.59 to 0.7 indicating majorly diffusion-controlled and partially erosion controlled release (Table 5) [50]. The erosion of the film (BHT) was higher relative to other formulations (BHT<sub>b</sub>C<sub>1</sub>, BHT<sub>b</sub>C<sub>2</sub>, BHT<sub>c</sub>C<sub>1</sub>, and BHT<sub>c</sub>C<sub>2</sub>). On the other hand, BHT<sub>c</sub>C<sub>1</sub> has shown mostly diffusion-controlled compared to other films. The erosion of the films were in the order of: BHT > BHT<sub>b</sub>C<sub>2</sub>, BHT<sub>c</sub>C<sub>2</sub> > BHT<sub>b</sub>C<sub>1</sub> > BHT<sub>c</sub>C<sub>1</sub>.

**Table 5.** Kinetic parameter of in-vitro drug release and ex-vivo buccal permeation

Formulation code	Release					Permeation				
	First order	Higuchi		Peppas		First order	Higuchi		Peppas	
	$r^2$	k	$r^2$	n	$r^2$	$r^2$	k	$r^2$	n	$r^2$
BHT	0.978	3.372	0.995	0.569	0.981	0.976	2.295	0.980	0.71	0.986
BHT <sub>b</sub> C <sub>1</sub>	0.980	4.503	0.988	0.425	0.995	0.964	3.996	0.970	0.68	0.985
BHT <sub>b</sub> C <sub>2</sub>	0.988	4.699	0.996	0.463	0.996	0.966	5.307	0.960	0.69	0.984
BHT <sub>c</sub> C <sub>1</sub>	0.977	4.06	0.994	0.422	0.997	0.957	4.55	0.978	0.55	0.982
BHT <sub>c</sub> C <sub>2</sub>	0.970	4.262	0.986	0.406	0.995	0.984	3.919	0.978	0.69	0.995

### 3.10 Anti-inflammatory activity

Budesonide (1 mg equivalent) containing film formulation (26.4-40.3 mg) has been used for anti-inflammatory study after topical application. The probable methanol content in the film used for the anti-inflammatory study was well below the permissible daily exposure according to the European Medicines Agency – ICH guideline Q3C (R6) on impurities (30 mg/day). Carrageenan was found to be more convenient than the Freund's adjuvant for its chronic inflammation which can elongate up to 14 days but carrageenan causes an acute inflammation which gives effect up to 24 h [55]. After 30 minutes from the carrageenan injection (Figure 5A), frequent lacrimation, meibomian secretion, reddening, and swelling of the conjunctiva appeared in comparison to the normal eye (Figure 5B, 5C). But there were no signs and symptoms in the normal/control eye. (Figure 5E). The film, BHT<sub>6</sub>C<sub>2</sub> has exhibited the highest extent of *in-vitro* dissolution and also *ex vivo* mucosal permeation and selected for *in vivo* anti-inflammatory activity study. After complete inflammation, the film (BHT<sub>6</sub>C<sub>2</sub>) was placed in the cul-de-sac region of the rabbit eye. The symptoms of inflammation like redness were decreased within 3 hours after the film was applied. But inflammation was not decreased in the positive controlled eye and redness not decreased within the 3 h (Figure 5F) [32,56].



**Figure 5.** (A) Injecting carrageenan in the upper palpebral region; (B) Normal rabbit eye (right) before carrageenan injection; (C) Acute inflammation in the rabbit eye (right) 30 min after carrageenan injection; (D) Inflammation of the eye (right) significantly reduced after application of film formulation (BHT<sub>6</sub>C<sub>2</sub>) (E) Rabbit normal eye (left) without carrageenan injection and without application of film; (F) Acute inflammation in the rabbit eye (right) after 3 h of carrageenan injection without film application.

## 4. Conclusions

Hydrogel forming film formulation has been prepared with cyclodextrin complexation of budesonide containing quaternary surfactant and *in vitro-in vivo* assessment of mucosal drug delivery was carried out. Inclusion agents in the film increased the drug release and swelling rate. The erosion rate of the hydrogel film has been decreased in presence of inclusion agent. Enhanced mucosal permeation was also observed due to the increased release of drug on the mucosal surface and the surfactant property of the quaternary compounds which facilitated better permeation probably via paracellular route compared to others. A majorly diffusion-controlled mechanism was observed in the process of drug release and mucosal transport with minimal erosion. Budesonide hydrogel has finally shown ocular anti-inflammatory activity after topical application *in vivo*. Inflammatory conditions have believably been encountered after reaching of budesonide to the target site bypassing the first-pass metabolism and could be the cause of improved bioavailability.

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