Original Research Article

In vitro and *in vivo* evaluation of chitosan buccal films of ondansetron hydrochloride

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Abstract

Buccal films of ondanstron hydrochloride were fabricated from mucoadhesive polymer, chitosan, and polyvinyl pyrrolidone (PVP K30) for the purpose of prolonging drug release and improving its bioavailability. All fabricated film formulations prepared were smooth and translucent, with good flexibility. The weight and thickness of all the formulations were found to be uniform. Drug content in the films ranged from 98 – 99%, indicating favorable drug loading and uniformity. The inclusion of PVP K30, a hydrophilic polymer, significantly reduced the bioadhesive strength and *in vitro* mucoadhesion time of the films, although the degree of swelling increased. *In vitro* drug release studies in simulated saliva showed a prolonged release of over five to six hours for all formulations, except C4, with 99.98% release in 1.5 hours. Kinetic analysis of the release data indicated that the best fit model with the highest correlation coefficient for all formulations was the Peppas model. *In vivo* studies, on selected films in rabbits, were conducted, to determine the pharmacokinetic parameters such as C_{max}, T_{max}, and $AUC_{0-\infty}$, using model-independent methods with nonlinear least-squares regression analysis. The AUC and values of C_{max} of ondansetron hydrochloride were found to be significantly greater ($P < 0.005$) than the selected films C2 and C3, as compared to those from the oral solution, thereby confirming improved bioavailability via the buccal route. The T_{max} values were also significantly greater ($P < 0.005$), indicating the slower release of the drug from buccal films, thereby, providing prolonged effects. Good *in vitro–in vivo* correlation was observed with R^2 values exceeding 0.98, when the percentage of drug released was correlated with the percentage of drug absorbed.

Key words: Buccal, chitosan, mucoadhesive, ondansetron, polyvinyl pyrrolidone

INTRODUCTION

The rich vascularization of the oral mucosa and its permeability to many drugs makes the buccal route an attractive alternative to the oral and parenteral routes, for systemic drug delivery. Absorption of therapeutic agents from the oral mucosa overcome premature drug degradation due to enzyme activity, the pH of the gastrointestinal tract avoids active drug loss due to first-pass hepatic metabolism, and the therapeutic plasma concentration of the drug can be rapidly achieved.[1] The buccal mucosa permits a prolonged retention of a dosage form especially with the use of mucoadhesive polymers without much interference in the activities, such as speech or mastication, unlike the sublingual route.[2] Mucoadhesive buccal films or patches are preferred in terms of flexibility, comfort, patient compliance, and better adhesion of the system to the oral mucosa.[3] Ondansetron hydrochloride, a $5HT_3$ antagonist is a potent antiemetic drug used for control of nausea and vomiting associated with cancer chemotherapy. It exhibits only 60 – 70% of oral bioavailability due to first pass metabolism and has a relative short half-life of three to five hours.[4] Buccal permeation studies by Mashru R.C *et al.* have indicated the ability of Ondansetron hydrochloride to diffuse through the buccal mucosa to an appreciable extent.[5] With a view to optimize the therapeutic effect of Ondansetron, the objective of this investigation is to formulate mucoadhesive buccal films using chitosan for sustained release of the drug, and evaluate them for physical characteristics such as swelling behavior, bioadhesive strength, and mucoadhesion time. The formulations will also be evaluated for drug release both *in vitro* and *in vivo*, and thus an attempt is made in this study to investigate their feasibility as alternative dosage forms to oral therapy.

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MATERIALS AND METHODS

Materials

Ondansetron hydrochloride was obtained as a gift sample

from Sun Pharma Pvt. Ltd., Ahmedabad. Chitosan, 85% deacetylated was procured from CIFD, Cochin; ethyl cellulose and polyvinylpyrolidone K-30 from Ozone international, Mumbai. Glycerine was of laboratory grade, obtained from Loba Chemie, Mumbai.

Preparation of ondansetron hydrochloride film from chitosan

The buccal films were prepared by solvent casting, using chitosan as the mucoadhesive polymer, and to improve the release properties, different proportions of polyvinylpyrrolidone (PVP K-30) were incorporated. with glycerine as a plasticizer. Chitosan was dissolved in 40 ml of 1% v / v acetic acid as solvent, to produce a 2% w / v solution, which was filtered to remove the debris and undissolved matter. To 5 ml of 1% v / v acetic acid, glycerin was added as plasticizer, and then the drug and PVP were dissolved in it. The drug solution was then poured into the chitosan filtrate. The polymer solution was stirred well and kept overnight for deaeration and swelling of the chitosan. The solution was poured into a glass mould of diameter 9 cm. The films were dried in a hot air oven at 45°C and cut into circular films of 15 mm diameter. The composition of the various films is shown in Table 1. The films were packed in aluminum foil and stored in an air tight glass container, to maintain their integrity and elasticity.

Physical characterization of buccal films

Weight and thickness

The individual weight of 10 samples of each formulation was determined using a calibrated digital balance. The individual thickness of 10 films of each type of formulation was measured using a micrometer screw gauge and the average was calculated with a standard deviation.

Content uniformity

Drug content uniformity was determined by dissolving the film, by homogenization, in 15 ml of 1% v / v acetic acid for five hours, with occasional shaking, and diluted to 100 ml with distilled water. After filtration through a 0.45 μm Whatman filter paper to remove the insoluble residue, 1 ml of the filtrate was diluted to 10 ml with simulated saliva of pH 6.75. The composition of the salivary fluid, as reported by Peh KK and Wong CF, is given in [Table 2].[3] The absorbance was measured at 248 nm, using a UV spectrophotometer.[6] The experiments were carried out in triplicate for the films of all formulations and the average values were recorded, and are given in Table 3.

Table 1: Composition of films loaded with ondansetron hydrochloride

Ingredients	Formulation code			
	C1	C2	C3	C4
Ondansetron hydrochloride (gm)	0.35	0.35	0.35	0.35
PVP K30 (gm)	--	0.07	0.09	0.11
Chitosan (gm)	0.80	0.80	0.80	0.80
Glycerine (ml)	0.40	0.40	0.40	0.40

Folding endurance

This test helps to reveal the flexible properties of the films, and therefore, their ability to conform to the contours of the oral cavity after application. A brittle film may fragment soon after application or during use, which may lead to mechanical irritation and a source of discomfort to the user and also drug loss. The folding endurance is a measure of the mechanical strength and flexibility of the films that is necessary for handling. This property was determined by mechanically folding one patch at the same place repeatedly till it broke or at least up to 300 times, which is considered suitable for revealing satisfactory film properties. The number of times the film could be folded at the same place without breaking gives the value of the folding endurance.[7,8]

Measurement of swelling index

This measurement is used to determine the extent of water uptake or the degree of hydration by the hydrophilic polymers used in the fabrication of the films. Most of the mucoadhesive polymers undergo some degree of swelling after hydration, which is necessary to initiate intimate contact of the film with the mucosal surface.[9] The studies for determination of the Swelling Index of the films were conducted in the simulated salivary fluid of pH 6.75. The film sample (surface area : 1.75 cm^2) was weighed and placed in a preweighed stainless steel wire sieve of approximately 800 μm mesh. The mesh containing the film sample was then submerged in 15 mL of the simulated salivary medium contained in a porcelain dish. At definite time intervals, the stainless steel mesh was removed from the dish and the excess moisture was removed by carefully wiping it off with absorbent tissue, after which it was reweighed. Increase in weight of the film was determined at each time interval until a constant weight was observed. The degree of swelling was calculated using the formula:

$$S.I = (w_t - w_0) / w_0$$

where S.I is the Swelling Index, w_t is the weight of film at time 't' and w_0 is the weight of the film at time 0.[3]

Measurement of bioadhesive strength

The force required to detach the bioadhesive film from the mucosal surface was applied as a measure of the bioadhesive performance. Several techniques have been reported in literature for the measurement of bioadhesive strength. In the present study a specially fabricated assembly based on published literature was used. A porcine cheek pouch was used as the model surface for bioadhesion testing. After the cheek pouch was excised and trimmed evenly, it was then washed in simulated salivary

Table 2: Composition of the simulated salivary fluid

Ingredients	Quantity
Disodium hydrogen phosphate	2.382 g
Potassium dihydrogen phosphate	0.19 g
Sodium chloride	8.00 g
Distilled water	Up to 1 liter
Phosphoric acid	q.s to pH 6.75

Table 3: Physical characterization of film formulations

Formula code	Weight* (mg)	Thickness* (mm)	Drug content * (%)	Bioadhesive force* (Kg / m / s²)	Mucoadhesion time (minutes)*	Folding endurance*
C1	42.2 ± 1.30	0.52 ± 0.03	98,23 ± 0.17	15.67 ± 0.13	295 ± 2	> 300
C2	44.3 ± 2.51	0.55 ± 0.06	99.52 ± 0.12	11.06 ± 0.20	270 ± 6	230 ± 10
C3	45.6 ± 1.52	0.58 ± 0.07	98.89 ± 0.23	9.82 ± 0.56	160 ± 4	205 ± 13
C4	47.1 ± 2.33	0.62 ± 0.11	98.79 ± 0.14	6.45 ± 0.22	144 ± 5	195 ± 16

* The values are represented as mean ± S.D and n = 10 for weight and thickness and n = 3 for others

fluid and then used immediately. The working of a double beam physical balance formed the basis of the bioadhesion test assembly.[10]

The left pan was removed and hung with a stainless steel chain. A Teflon block, 1.5 inches in height and 1.5 inches in diameter was hung with the stainless steel chain, to balance the weight of the other pan. The height of the total set-up was adjusted to accommodate a glass container or beaker below it leaving a head space of about 0.5 cm in between. Another Teflon block, 2 inches in height and 1.5 inches in diameter was kept inside the glass vessel, which was then positioned below the top hung Teflon block. Suitable weights were added (15.0 gm) on the right pan to balance the beam of the balance. The porcine cheek membrane was attached with the mucosal side up on the lower Teflon block, which was then placed in the glass vessel. Sufficient simulated salivary fluid was filled into the beaker so that the surface of the fluid just touched the mucosal surface to keep it moist. The beaker was positioned below the upper Teflon block. The film under test was fixed to the surface of the upper block with glue. The 15.0 gm weight on the right pan was removed and this lowered the upper Teflon block with film, so it was in contact with the mucosal surface. A load of 20.0 gm was placed as the initial pressure on the upper block for three minutes. Slowly weights were added onto the right pan, starting from 500 mg, at 30-second time intervals. The total weight at which detachment of the film from the mucosal surface took place was noted and the bioadhesion force was calculated per unit area of the film, as follows:

$F = (W_w \times g) / A$

Where F is the bioadhesion force (kg / m / s^2), W_w is the mass applied (gm), g is the acceleration due to gravity (cm / s^2) and A is the surface area of the patch (cm^2). The results are tabulated in Table 3 for all films.

Ex vivo mucoadhesion time

The residence time for the formulation, that is, the time taken for the film to detach or erode completely from the mucosa was measured *ex vivo,* by application of the film on freshly excised porcine buccal mucosa. The porcine mucosa was cut to an appropriate size of a 3 cm × 3 cm square patch and fixed on the internal side of a beaker with cyanoacrylate glue. The film was first wetted with 50 µl of simulated saliva fluid and attached to the porcine buccal tissue by applying light pressure with a finger tip for 20 seconds. The beaker was filled with 200 ml simulated saliva fluid and kept at 37° C on a magnetic stirrer. After two minutes, a 50 rpm stirring rate was applied to simulate the buccal cavity environment, and during the test, the time taken for the film to completely erode or detach from the mucosa was observed as the *ex vivo* mucoadhesion time.[10]

In vitro drug release studies

In vitro release studies were carried out by a slight modification of the method suggested by Perioli L., *et al.* and Ilango *et al.* A buccal film was attached to the wall of the dissolution vessel of the USP Dissolution Test Apparatus, midway from the bottom, with instant adhesive or cyanoacrylate glue.[10,11] After two minutes, the vessel was filled with 500 ml of simulated saliva. The temperature of the dissolution medium was maintained at 37 ± 0.5ºC and stirred at 50 rpm. Samples of 5 ml were withdrawn at predetermined time intervals and replaced with a fresh medium. The samples were filtered and drug concentrations were determined using a *high-performance liquid chromatographer* (HPLC) with an ultraviolet (UV) detector. A C-18 column was used at 25°C and the mobile phase utilized was a mixture of methanol and PBS (pH = 7.5) in the ratio of 65 : 35, delivered at a flow rate of 1.0 ml / minute. The injection volume was 20 µl and the drug was detected at 310 nm. The calibration curve range was 4.62 – 104.3 µg / ml (r = 0.9996). The detection limit was 0.122 µg / ml and daily RSD ≤ ± 2.0%.

In vivo buccal permeation studies of ondansetron hydrochloride from mucoadhesive sustained release films in rabbits

The following study in rabbits was conducted after obtaining approval from the Institutions Animal Ethics Committee of the K.S. Hegde Medical Academy, Derelakatte, Mangalore, Karnataka State.

Based on *in vitro* mucoadhesion and drug release studies, optimized formulations that were selected were used in this study. The films used in the animal study were formulated to contain 4 mg of ondansetron hydrochloride each. To ensure one way flux after application to the mucosa, the films were backed with a membrane made from ethyl cellulose, thus producing patches. This backing membrane was prepared by dissolving 5% ethyl cellulose in a mixture of acetone, isopropyl alcohol (65 : 35), and dibutyl phthalate, equal to a 20% dry weight of the polymer, which was included as plasticizer.[12]

Procedure

New Zealand white rabbits of either sex and body weight of 2.5 – 3.0 kg were used for the test. To carry out the study each formulation was applied to the buccal mucosa of the anesthetized rabbits. Prior to the test, the rabbits were fasted overnight with

ad libitum water, having stored them in individual cages for an acclimatization period of one week before the experiment was carried out. The rabbits were divided into three groups of four each. The rabbits were weighed and anesthetized by an intramuscular injection of ketamine HCl (40 mg / kg) and xylazine (10 mg/ kg).[13,14] The rabbits remained anesthetized for four hours without respiratory depression, when an additional dose of the anesthetic combination was administered after one-and-a-half hours. After 10 minutes of initiation of the anesthesia, the buccal patches containing the drug were moistened with 30 μl of simulated saliva of pH 6.75 and applied to the buccal pouch of one group of rabbits. To the second group, 5 ml aqueous solution containing the same amount of drug as the films was administered orally through an infant feeding tube. The placebo films were used on the control group. At time intervals, initially of 0.5 hours for 1.5 hours followed by 1 hour intervals for the remaining period, 0.5 – 1.0 ml of blood was removed from each rabbit via the marginal ear vein, using 22 gauge needles, through a butterfly cannula. Blank blood samples were removed from each group before initiation of the treatment. Before use, the cannula and blood collection tubes were rinsed with 3.8% w / v sodium citrate solution as an anticoagulant. The procedure for rabbit bleeding was carried out as per Laboratory Animal Science Association (LASA) Good Practice Guidelines.[15] The last blood sample was collected at the end of 4.5 hours. The blood samples were subjected to centrifugation at 10,000 rpm for 10 minutes, to separate the plasma, thereafter they were immediately stored frozen at -20°C, until analysis. At the end of 4.5 hours all films were removed and analyzed for the remaining drug content. This procedure was repeated with the second formulation after a wash-out period of two weeks.

Quantification of ondansetron hydrochloride from rabbit plasma

Ondansetron hydrochloride was estimated from the plasma samples by a method reported by Hidy B.J *et al*.[16] Drug concentrations were determined using the LCMS / MS API-3000(SCIEX). The extraction method used involved precipitation with acetonitrile as the protein precipitating agent. The mobile phase utilized was a combination of acetonitrile and 0.1% Formic acid (40:60 v / v) controlled by gradient elution. The samples were injected into a C-18 column (Chromolith, RP-18e, 100-4.6) and a flow rate of 0.8 ml / minute was maintained. The drug was detected by a quadrupole mass spectrometer system, using positive ion electrospray. Bupropion was used as the internal standard (IS), as a solution of strength 5 μg / ml. Standard solutions for the calibration curve were prepared by spiking pooled rabbit blank plasma with 20 μl of Ondansetron hydrochloride stock solution. Good linearity was obtained in the concentration range of 4.0 – 1051 ng / ml with a correlation coefficient of 0.9995. The detection limit was 0.371 ng / ml. In the case of both standard and test, 20 μl of the plasma was mixed with 20 μl of the IS, and while vortexing, 250 μl of acetonitrile was added, to precipitate the proteins. The mixture was centrifuged at 4500 rpm for 10 minutes and 10 μl of the supernatant was injected into the column. The calibration curve was constructed by plotting the measured peak area ratios of ondansetron hydrochloride to IS (Internal Standard) versus concentration of the standard samples. Drug concentrations were determined and the data were subjected to statistical analysis by one way analysis of variance (ANOVA) using the software, Graph Pad Prism 5.0. Statistical differences were considered significant at $P < 0.005$.

RESULTS AND DISCUSSION

Physical characterization of buccal films

All the fabricated film formulations prepared were smooth and almost opaque. The individual weight of each of the 10 samples, of each type formulation, was found to be consistent within the formulation. Between formulations, the weight increased with increased content of the polymers used. The thickness of all film samples was uniform within each formulation. The films with increased polymer content showed a slight increase in thickness.

C1 exhibited good folding endurance exceeding 300, indicating good flexibility. However, the folding endurance of the films, C2 – C4, was found to be less than 300 and it decreased with the increasing content of PVP. Thus it appears that the inclusion of PVP decreased the flexibility of chitosan films, as the former is a brittle polymer. The results for weight, thickness, and folding endurance of films are given in Table 3.

Content uniformity

All film formulations were found to be of uniform drug content, as seen in the results given in Table 3.

Measurement of the swelling index

The swelling behavior of the polymer influences its bioadhesive character. The adhesion increases with the degree of hydration until a point where overhydration leads to an abrupt drop in adhesive strength, due to disentanglement at the polymer tissue interface.[17] The rate and the extent of film hydration and swelling also affect film adhesion and consequently the drug release from the film. The chitosan formulations (C2 – C4) show a slower rate of swelling. The presence of PVP, a hydrophilic polymer, increases the extent of swelling; therefore, maximum swelling among the chitosan films is obtained for the formulation C4, which contains higher amounts of PVP. The poor solubility of chitosan limits the swelling of the films; hence the swelling index measured is the least for C1, in which PVP is absent, with an SI value of 2.07. The swelling profile of the four formulations is shown in Figure 1.

Measurement of bioadhesive strength

Porcine buccal mucosa was used as the model membrane for this study, owing to its similarity to the human oral mucosa, both in structure and composition, as also the presence of non-keratinized epithelia. Moreover, a larger expanse of the mucosa was available as compared to that of the rabbit, for conducting multiple simultaneous experiments using the same animal, which minimizes individual biological variation.[18]

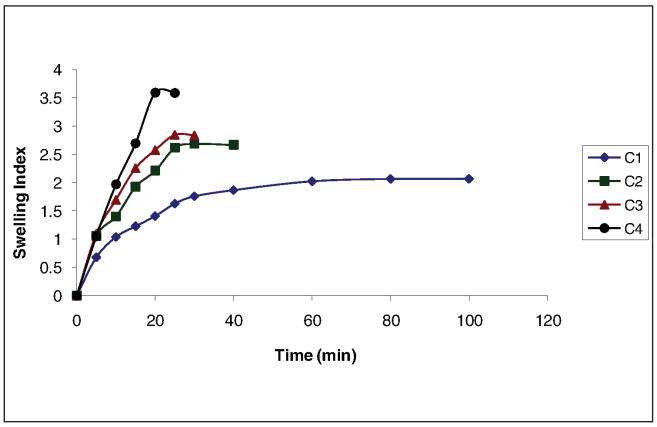

Figure 1: Swelling profile of chitosan film formulations in simulated saliva

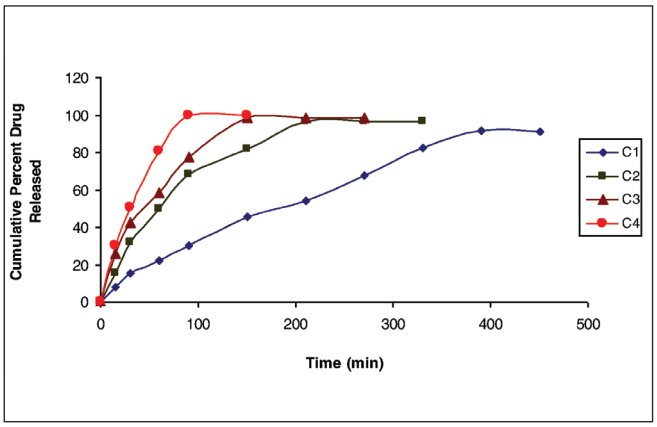

Figure 2: *In vitro* release profile of ondansetron hydrochloride from films in simulated saliva

Satisfactory bioadhesion is essential for the successful application of bioadhesive drug delivery systems in order to increase the residence time at the site of the application, and hence, to provide prolonged release of the drug. Chitosan is a mucoadhesive polymer. Incorporation of PVP K30, a hydrophilic polymer, significantly reduced the bioadhesive strength of the films, and hence, the bioadhesive strength among the chitosan films decreased with the increasing content of PVP. Therefore, a C4 film with the highest PVP content exhibited the least bioadhesive force of 6.44 kg / m / s^2. The results are tabulated in Table 3 for all films.

Ex vivo mucoadhesion time

It was observed that with an increasing content of PVP in the formulations, the mucoadhesion time decreased. Thus, C1 and C4 films showed the longest and the least adhesion time, respectively, as PVP tended to decrease the mucoadhesive strength of chitosan. This was attributed to the fact that the rapid uptake of water by PVP brought about the disentanglement of the chitosan polymer chains from the mucin chains in the mucus of the buccal mucosa, and therefore, gradual reduction in mucoadhesive bonding. The results of *ex vivo* mucoadhesion time of the formulations is demonstrated in Table 3.

In vitro drug release studies

In vitro drug release studies in simulated saliva showed that drug release increased with the increasing content of PVP, with respect to both rate and extent, hence a maximum release of 99.98% in one-and-a-half hours was observed for C4. This higher release was attributed to the higher rate and extent of water uptake, with an increase in the amount of the water soluble polymer PVP, resulting in increased wetting and penetration of water into the film matrices, and hence, increased diffusion of the drug. PVP was also responsible for the swelling, as it increased to a maximum rapidly and then declined, as overhydration led to dissolution and erosion of the polymer. Comparatively the drug release profile from C2 and C3 appeared to be more prolonged for four to five hours, with an extent of 96.5 and 98.5%, respectively. The drug release profiles for all formulations are shown in Figure 2.

Kinetic analysis of in vitro release data

The data from the *in vitro* release studies was subjected to kinetic analysis, that is, zero-order and first-order. To determine the mechanism that best described the release of the drug from the formulations, the data was also fitted to the Higuchi matrix model and Korsmeyer–Peppas equation. The release exponent (n) describing the mechanism of drug release from the matrices was calculated by regression analysis, using the Peppas equation.[19]

$$Mt / M\infty = kt^n$$

where $Mt / M\infty$ is the fraction of drug released (using values of $M / M\infty$ within the range 0.10 – 0.60) at time t, and k is a constant incorporating the structural and geometric characteristics of the release device. When $n = 0.5$, Case I or Fickian diffusion is indicated, $0.5 < n < 1$ for anomalous (non-Fickian) diffusion, $n = 1$ for Case II transport (Zero order release), and $n > 1$ indicates Super case II transport.[20] The values of k, n, and R^2 (coefficient of determination) have been obtained using the software PCP Dissolution v 2.08, as presented in Table 4.

The values of n obtained by the linear regression of log ($Mt / M\infty$) versus log t, were between 0.5 to 1 for all formulations, indicating non-fickian diffusion as the release mechanism, and close to 0.5 in the case of C3. Drug release from all films appeared to follow first order kinetics. The best fit model with the highest correlation coefficient or coefficient of determination, R^2 for all formulations, was the Peppas model. The values of R^2 for the Higuchi matrix model and First order model for all films were greater than those of the Zero order model, indicating matrix-diffusion controlled release from the hydrophilic polymer matrices by first order kinetics.

In vivo buccal permeation studies of ondansetron hydrochloride from mucoadhesive sustained release films in rabbits

Based on the results obtained from *in vitro* as well as from mucoadhesion studies, suitable formulations that were selected for the study were C2 and C3, which showed a slower, but greater extent of release in four to five hours and better mucoadhesive properties than other film formulations.

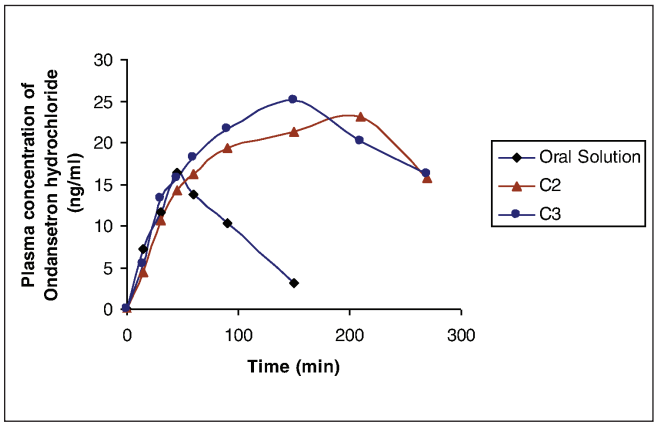

Figure 3: Mean plasma drug concentration–time profile of ondansetron hydrochloride from C2, C3, and oral solution

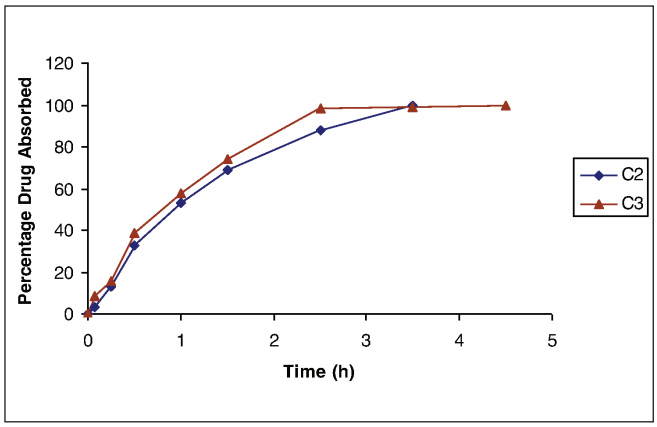

Figure 4: Plot of percentage ondansetron hydrochloride absorbed from patches with time after deconvolution of plasma level data, in rabbits

Table 4: Kinetic analysis of *in vitro* drug release data from ondansetron films

Release model		Formulation code			
		C1	C2	C3	C4
Zero Order	R^2	0.8837	0.8570	0.816	0.8613
	k	0.2514	0.3048	0.079	0.0747
First Order	R^2	0.9639	0.9378	0.9778	0.974
	k	0.0882	0.0762	0.0673	0.0873
Higuchi Matrix	R^2	0.9719	0.9813	0.9396	0.9900
	k	4.0955	5.1464	1.3016	12.135
Peppas	R^2	0.9981	0.9865	0.9885	0.9985
	k	1.1251	3.5130	2.2898	4.992
	n	0.7349	5721	0.4978	0.6724
Best fit Model		Peppas	Peppas	Peppas	Peppas

During the study it was observed that all patches remained intact and adhered well to the buccal mucosa of the rabbit. There were also no noticeable signs of any irritation or redness at the sites of application.

The HPLC method used for the measurement of the concentrations of ondansetron hydrochloride from plasma was sufficiently sensitive and suitable for the analysis. From the calibration curve, the plasma drug concentrations were determined for each rabbit and the mean plasma drug concentrations were calculated, with a standard deviation for each treatment group, and the drug concentration-time profiles were plotted. The mean plasma concentration of the ondansetron-time profiles following the application of buccal patches and oral administration of the solution in each group of rabbits, is shown in Figure 3.

Pharmacokinetic parameters such as C_{max}, T_{max}, and $AUC_{0-\infty}$ were determined using model-independent methods, with nonlinear least-squares regression analysis using the software, WinNonlin®, Pharsight, from the plasma drug concentration-time profiles of each individual rabbit. C_{max} was the peak plasma drug concentration, T_{max} was the time required to reach peak plasma drug concentration, and AUC was the area under the curve. The average values of these pharmacokinetic parameters were determined as given in Table 5.

Table 5: Pharmacokinetic parameters of ondansetron hydrochloride from C2, C3, and oral solution administered in rabbits

Formulation	Pharmacokinetic parameters*		
	Cmax (ng / ml)	Tmax (hour)	AUC 0-∞ (ng-hour / ml)
Oral solution	16.442 ± 2.342	0.75 ± 0.08	37.012 ± 1.038
C2	23.184 ± 0.127	3.5 ± 0.2	148.739 ± 2.453
C3	25.112 ± 1.041	2.5 ± 0.3	155.361 ± 2.231

*All values are represented as mean ± S.D and n = 4. The oral solution and the patches administered each contained 4 mg of the drug

Statistical analysis of data from rabbit plasma drug concentrations

The mean plasma drug concentration data from the different treatment groups were subjected to statistical analysis by one way ANOVA; it was found that differences between the groups that received the oral solution and the other groups that received the patches containing the same dose of the drug, were statistically significant with respect to C_{max}, T_{max}, and AUC.

From published literature it is known that ondansetron suffers from significant bioavailability problems after oral administration due to the first pass effect.[4] In this study, the AUC and values of C_{max} of ondansetron hydrochloride were found to be significantly greater ($P < 0.005$) from all patches, as compared to those from the oral solution containing the same dose of drug. This confirmed that the bioavailability of this drug could be improved by buccal administration.

The T_{max} values from the formulations, C2 and C3 were significantly greater ($P < 0.005$) as compared to those from the oral solution indicating the slower release of the drug from the patches, thereby providing prolonged effects. Therefore these formulations could be considered suitable for sustained release of the drug.

In vitro – in vivo correlation

According to the BCS classification, ondansetron hydrochloride can be considered as a Class I drug, and incorporating this drug in a sustained release formulation will place it in Class II (low solubility, high permeability). Hence a Level A correlation was

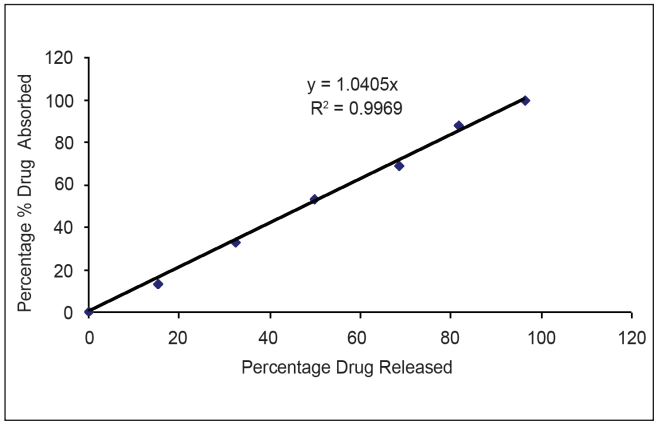

Figure 5: *In vitro–in vivo* correlation of percentage drug absorbed and percentage drug released from C2

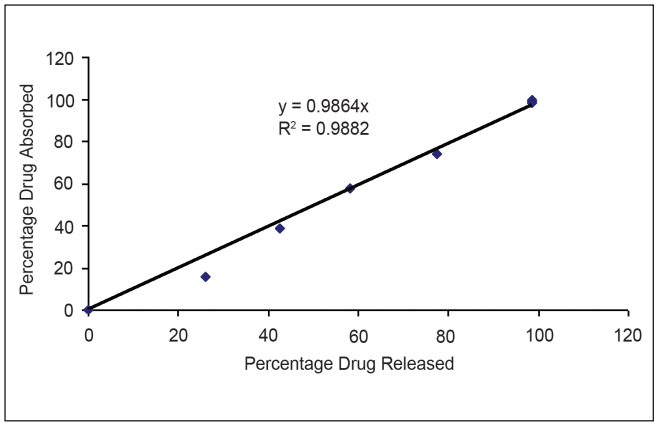

Figure 6: *In vitro–in vivo* correlation of percentage drug absorbed and percentage drug released from C3

undertaken. Level A correlation is a point-to-point relationship between the *in vitro* dissolution and *in vivo* absorption rates of a drug from the dosage form.[21] Here, the *in vivo* percentage of the drug absorbed was plotted against the *in vitro* percentage of the drug released, to determine the correlation coefficient.

The percentage of the drug absorbed was determined using the Wagner Nelson method by the deconvolution of the plasma level data, using the following equation.[22]

$$F_a = [(C_t + k_e AUC_{0-t}) / k_e AUC_{0-\infty}] \times 100$$

where F_a is the fraction of drug absorbed, C_t is the plasma drug concentration at time t, k_e is the overall elimination rate constant obtained by the least squares regression analysis of the terminal phase of the first order plot, AUC_{0-t} and $AUC_{0-\infty}$ are areas under the curve between time zero and time t and between time zero and infinity, respectively. The drug absorption-time profile obtained is shown in Figure 4.

The values thus obtained were correlated with the *in vitro* percentage of the drug released at the same time intervals as shown in the Figures 5 and 6. Good *in vitro–in vivo* correlation was obtained for all the formulations.

CONCLUSION

Thus it was possible to successfully formulate mucoadhesive buccal films using chitosan, for the purpose of achieving sustained release of ondansetron hydrochloride, with better bioavailability than oral formulations. The results of drug absorption studies in rabbits could be easily extrapolated to human beings, and therefore, formulations C2 and C3 could be considered promising for clinical application. To support the data from *in vivo* animal studies an extensive clinical investigation is required with respect to the optimized films

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