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FORMULATION AND EVALUATION OF TRANSDERMAL THERAPEUTIC SYSTEM OF ONDANSETRON HYDROCHLORIDE

[1]Malay K Das and Ravi N Choubey
Department of Pharmaceutical Sciences,
Dibrugarh University, Dibrugarh, Assam 786004 INDIA

ABSTRACT

The problems in peroral bioavailability of Ondansetron hydrochloride can be attenuated using the skin as a port for its systemic delivery. The present work is aimed for development and evaluation of monolithic polymeric patch for transdermal delivery of Ondansetron hydrochloride at therapeutic level. The monolithic matrix patch containing Ondansetron hydrochloride was prepared by solvent evaporation technique using different rate controlling polymers. The prepared patch was subjected to various physicochemical evaluations with respect to drug content, moisture content, moisture uptake, water vapor transmission rate, thickness, folding endurance and weight uniformity. The *ex vivo* permeation of Ondansetron was investigated across pig ear epidermis using Keshary-Chien glass diffusion cell. The films were uniform with respect to drug content, thickness and weight and with low moisture content and moisture uptake capacity. The FTIR and DSC analyses confirmed the stability of Ondansetron in the polymeric film. The SEM and XRD analyses confirmed that the drug was uniformly distributed in amorphous state. The permeation of Ondansetron seemed to follow zero order kinetic and the drug was released from the formulation independent of concentration and by diffusion mechanism. The patch formulation having polymeric composition of EC:PVP at 1:1 with 15% w/w of lemon oil as permeation enhancer produced desired transdermal target flux of Ondansetron hydrochloride at 179.93 µg/cm²/h.

Key words: Transdermal, Ondansetron, Cancer chemotherapy, Lemon oil, Zero order kinetic.

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[1] **Corresponding author's e-mail:** du_mkd@yahoo.co.in, **Tel: +919954229317.**

INTRODUCTION

Ondansetron hydrochloride is a serotonin (5-hydroxytryptamine) subtype 3 (5-HT3) receptor antagonists is used to prevent and control nausea and vomiting after cancer chemotherapy, radiotherapy, and surgery. Unlike other antiemetic, ondansetron is known not to block dopamine subtype-2 receptors, and therefore not to induce the undesirable side effects like extrapyramidal reactions. Even though ondansetron is thought to be a good candidate for patients receiving highly emetogenic agents, its use has been limited in patients who have difficulty in swallowing after chemotherapy. The oral bioavailability of ondansetron hydrochloride is about 60-70 % [Tripathi 2008]. It undergoes extensive hepatic metabolism by the cytochrome P 450 enzyme system and its elimination half-life is 3 – 5 h. Due to its short half-life and low bioavailability, it is administered orally 3 to 4 times a day, wherein the patient compliance is low. Oral administration has disadvantages associated with the need for frequent administration during the day and is difficult to use as a means of drug delivery to a patient who is already suffering from severe nausea and vomiting. Likewise, oral administration is not desirable for patients having difficulty in swallowing, such as very young and elderly patients, as well as patients suffering from neck, mouth or head cancer. Rectal administration, as in suppository form, avoids, to some extent, the disadvantages of oral administration, but is not convenient or a widely accepted approach to drug delivery. Rectal and nasal administration of ondansetron is also considered to have low patient compliance. Even with oral controlled-release dosage forms, fluctuation in plasma concentration of drug is high, which in turn results in low patient compliance. Intravenous administration must be performed under medical supervision, however, and causes significant discomfort to the patient. Therefore, an effective transdermal delivery system for ondansetron hydrochloride would be very convenient to use. The usual oral dose of Ondansetron hydrochloride is 16 mg a day in two divided doses which can be about 10 mg a day for an effective transdermal delivery system, considering its bioavailability and metabolism. The physicochemical properties of ondansetron hydrochloride (MW 365.67 D, MP 179 – 189 and logP 1.94) indicate its feasibility to deliver through skin [Chandrasekhar and Shobha 2008].

The use of skin as a port for ondasetron hydrochloride delivery have been attempted by several research workers and reported its potential transdermal delivery in the

presence of permeation enhancers (Takahashi and Rytting 2001, Gwak, Oh and Chun 2004, Dallas, Dimas and Rekkas 2004, Krishnaiah *et al* 2008a, Krishnaiah *et al* 2008b, Krishnaiah *et al* 2009, Obata *et al* 2010). However, the extensive research is required to develop transdermal therapeutic system in the area of passive patch technology. The present work is aimed for development and evaluation of monolithic polymeric patch for transdermal delivery of ondansetron hydrochloride at therapeutic level.

MATERIALS AND METHODS

Materials

Ondansetron hydrochloride (Gift sample from Ozone Pharmaceutical Ltd., Guwahati), Polyethylene glycol 400 (RFCM Ltd., New Delhi), Sodium bromide (Central drug house Pvt. Ltd New Delhi), n-Octanol (Merck specialties Pvt. Ltd., Mumbai), Propylene glycol (Merck India Ltd., Mumbai), Chloroform (Spectrochem Pvt. Ltd., Mumbai), Lemon oil (Wilson Brothers Lab., Mumbai), Potassium Dihydrogen Phosphate (RFCL Ltd. , New Delhi), Try Ethyl Citrate (E- Merck Ltd., Mumbai), Ethyl Cellulose (Central drug house Pvt. Ltd New Delhi), Poly Vinyl Alcohol, Cold Water Soluble (HiMedia Laboratories Pvt. Ltd. Mumbai), Poly Vinyl Pyrrolidone K-30 (Burgoyne Burbidges and Co. India Pvt. Ltd. Mumbai).

All the reagents and chemicals used were of analytical grades (AR) and complied with pharmacopoeial standards.

Methods

Preformultion studies

Solubility study

Excess amount of Ondansetron hydrochloride was added to different vehicles (Water, Ethanol (95% v/v), Polyethylene glycol (PEG) 400, Aqueous PEG 400 (40%v/v), Phosphate buffer saline (PBS) (pH 5.8, 6.0 and 7.0) and the contents were shaken continuously in a mechanical shaker for 24 hours at 37± 0.5°C until equilibrium was achieved. After 24 hours the solutions were filtered through filter paper. The filtrate was diluted with respective vehicles and the amount was quantified spectrophotometrically at 249.8 nm using Chemito Spectrascan UV 2600 Spectrophotometer.

Partition coefficient

The partition coefficient was determined between equal volumes (10 ml) of distilled water as an aqueous phase and 1-octanol as an organic phase (10 ml) at ambient temperature of 37± 0. 5°C in different separating funnels. The funnels were shaken gently for 4 h and then kept for 24 h for achieving mutual separation. After the achievement of equilibrium between water and 1-octanol, they were separated and 25 mg of drug was added to the aqueous phases. Aqueous drug solutions and organic phase were mixed again and shaken gently for 4h and then kept for 48 h to attain the equilibrium. 1ml of the aqueous drug solution was then recovered and amount of drug was quantified spectrophotometrically at 249.8 nm after proper dilution with the same aqueous phase. Partition coefficient was then determined by the formula: $P = (C_a - C_b)/ Cb$, Where, P = Partition coefficient, C_a = Initial concentration of drug in aqueous phase (µg/ml) and C_b = Final concentration of drug in aqueous phase after equilibrium (µg/ml).

Partition of drug through skin

The pig ear was obtained from local slaughter house. The ear was washed in tap water to remove the debris and foreign particles. The hairs were removed first by using scissors and then by gently shaving so that the skin surface remains intact. Subcutaneous fat and other extraneous tissue adhering to dermis were completely removed and trimmed, by using forceps and scissors. Then it was soaked in 2 M sodium bromide solution for 36 h. The pig epidermis was obtained by peeling off the dermis with a piece of moistened cotton. The epidermis so obtained was washed with distilled water repeatedly and was kept in 0.9% sodium chloride solution containing 1% formalin in freezing condition at -20°C.

A piece of whole, excised pig ear epidermis was weighed accurately (200 mg) and put into separate glass stopped conical flasks containing 0.5 mg, 1mg and 2 mg of drug in 10 ml solution of PEG 400 (40% v/v). The pieces of skin were equilibrated with the PEG 400 (40% v/v) for 24 hours at 37 ±1°C in a water bath shaker. The solutions were filtered through membrane filter of (pore size 0.22 µm) and drug amount was quantified spectrophotometrically at 249.8 nm. The partition coefficient through the pig ear epidermis was calculated by using the formula [Hansen *et al* 2008]: P_{ds} =

$[\{C_{in} - C_{eq}\} / C_{eq}] \times (1000/ W)$, Where, P_{ds} =Partition coefficient of drug through skin, C_{in}=Initial concentration of the drug, C_{eq} = Equilibrium concentration of the drug (µg/ml), W = Weight of skin (mg).

Target flux

The target flux is calculated based on the mean pharmacokinetic parameters of ondansetron hydrochloride (Clearance, CL = 21,240 ml/h) to obtain a mean steady-state plasma drug concentration (Css) of 26.2 ng/ml in humans using the equation: J = [(Css × CL) / (area of the membrane)] [Krishnaiah *et al* 2008b]. Area of the membrane=3.14 cm^2, Css= 26.2 ng/ml, Therefore, J=177.22 $\mu g/cm^2/h$ (Desired flux).

Preparation of transdermal matrix patch

A 4% Poly vinyl alcohol (PVA) backing membrane is prepared by adding PVA in cold water, stirred at 800 rpm for 3 hour .Then poured on flat bottomed aluminum foil mould. And then kept for evaporation at 60°C for 6 h. The dried backing membrane was stored in desiccator until use in same mould.

Transdermal matrix patch composed of different ratio (1:4 (RNF1), 2:3 (RNF2), 4:1 (RNF 4), 1:1 (RNF6), 3:2 (RNF7)) of Ethyl cellulose (EC) and Poly vinyl pyrrolidone (PVP) as matrix forming polymers containing ondansetron hydrochloride (2.10 mg/cm^2 patch) were prepared by solvent evaporation technique using casting solvents. Initially, the polymer was added in stoppered conical flask with chloroform as casting solvent [Pattnaik *et al* 2011], stirred at 500 rpm for 2 h, after that Triethyl citrate (TEC) was incorporated as a plasticizer at a concentration of 30% (w/w) of dry weight of polymers and stirred for 1.5 h. For optimized formulation (RNF6) the lemon oil 15% w/w was added after incorporation and mixing of TEC comprising the formulation RNF8. Then the drug (55 mg) was added and again stirred up to 1.5 h for proper mixing. The matrix was prepared by pouring the homogeneous dispersed solution on 4% PVA backing membrane in a flat bottomed aluminum foil mould and dried at room temperature for 24 h. The dried patches were removed and stored in desiccators until use [Mukherjee *et al* 2005].

Physicochemical evaluation of patch

Drug Content: In 100 ml Phosphate buffer saline solution (pH 5.8), patch was placed and shaken for 5 h on a mechanical shaker. The complete solution was filtered and

then analyzed by Chemito Spectrascan UV 2600 UV-VIS Spectrophotometer with proper dilution. [Lewis, Pandey and Udupa 2006]

Moisture content (%): The films were weighed and kept in a desiccator containing Fused silica for 36 h until it showed a constant weight. The moisture content was the difference between the constant weight taken and the initial weight [Mukherjee *et al* 2005].

Moisture Content = (Initial weight - Final Weight) × 100 / Initial weight.

Moisture Uptake (%): The films were weighed accurately and placed in the Humidity chamber, which maintains 80-90% RH. After 3 days, the films were weighed until it showed constant weight. The study was performed at room temperature. The percentage moisture uptake was calculated using the formula [Devi *et al* 2003]:

Moisture Uptake = (final weight - Initial weight) × 100/ Initial weight

Water vapor transmission rate (WVTR): Equal diameter and weight, glass vials as transmission cells were washed thoroughly and dried in an oven. About 1g anhydrous calcium chloride was placed in the cells and the respective polymer film was fixed over the brim with the help of adhesive tape. The cells were accurately weighed and kept in humidity chamber of 80-90 % RH condition. The cells were taken out and weighed after 24 and 36 hours. of storage. The amount of water vapor transmitted was determined using the formula:

Water vapor transmission rate = (Final weight - Initial weight) / time × Area

Water vapor transmission rate is usually expressed as the number of grams of moisture gained/h/cm^2 [Saettone *et al* 1995].

Thickness Uniformity (TU): The thickness of the film was measured at three different points using a screw gauge and average thickness recorded.

Folding endurance (FE): This was determined by repeatedly folding the film at the same place until it broke. The number of times the film could be folded at the same place without breaking/cracking gave the value of folding endurance [Devi *et al* 2003].

Weight Uniformity (WU): It is carried out for the uniformity of the films after pouring on the moulds. By selecting the three random patches of same ratio were weighed. And the values were noted with Standard Deviation for all the formulations.

Ex vivo permeation study

The prepared pig ear epidermis and film were mounted carefully between the donor and receptor compartment in the Keshary-Chien diffusion cell. The transdermal film of an appropriate size was applied to the epidermal side of the skin and mounted on the donor side (area 3.14 cm^2) of the cell. The dermal side was in contact with the receptor compartment fluid. Receptor compartment cell were filled with 40% PEG 400 solution [Gwak, Oh and Chun 2004] and the bathing solution were stirred at 500 rpm by Teflon coated magnetic bar to keep them well mixed. The permeation media were maintained at 37±0.5°C thermostatically with the help of water bath and pump (Remi Instruments Ltd. Mumbai).

Aliquots of 1ml from the receptor solution were withdrawn periodically for 12 h and replaced with fresh PEG 400 (40% v/v) solution. The aliquots withdrawn were diluted with respective PEG 400 (40% v/v) solution and estimated for drug content spectrophotometrically at 249.8 nm.

Skin retention

The skin samples after permeation study was washed with distill water repeatedly to remove the drug particles attached to the surface. Then the epidermis was cut into small pieces and put into stopper conical flask containing 10 ml of water and stirred for 24 h. The aliquot of 1ml was then taken and diluted with water and filtered through membrane filter of pore size 0.22µm. The drug amount was quantified spectrophotometrically.

Scanning electron microscopy (SEM)

The film of about 2-10 mm size was cut and dried in oven at 40°c. Then the specimen is mounted on brass or aluminium stubs and sputter coating is carried out using silver or gold. The coating should be of about 35nm thick.

Surface morphologies of the blank films, drug loaded films, and the films used in *ex-vivo* studies were investigated using Jeol JSM 6360 Scanning Electron Microscope at 10 KV at magnifications of 500 X (Zang *et al* 2007).

Fourier transform infrared spectroscopy (FTIR)

For IR spectra pure drug, a mixture of drug with the polymers (PVP, EC), blank film and drug containing film were mixed separately with IR grade KBr in the ratio of

100:1 and corresponding pellets were prepared by applying 5.5 metric ton of pressure in a hydraulic press. The pellets were scanned over a wave number range of 4000–400 cm^{-1} in Perkin-Elmer model 883 IR Spectrophotomer and the spectrums were recorded.

Differential scanning calorimetry (DSC)

The thermal analysis of the pure drug, pure polymers (EC, PVP), a mixture of drug with the polymers, blank film and drug loaded film was performed using a Jade DSC (Perkin Elmer, Switzerland). All samples were weighed about 10 mg and heated at scanning rate of 20°C/min between 35°C and 250°C.Temperature calibrations were performed periodically using indium as standard. DSC evaluates the internal structure modifications after drug incorporation and measure quantitative enthalpy change that occurs in a sample as a function of temperature [Llacer *et al* 1999].

X-ray diffraction (XRD)

Samples of pure drug, blank film and drug loaded transdermal films were assessed for crystallinity using X-ray Diffractometer. The voltage and current were 30 KV and 15 mA, respectively. Measurements were carried out in the angular scan range from 5° to 40° (2è) at a scan speed of 1°/min.

Data analysis

All experiments were replicated at least three times. The amount of drug permeating through the skin during a sampling interval was calculated based on the measured receptor-phase concentration and volume. Drug concentration in the skin permeates was corrected for sampling effects according to the following equation [Hayton and Chen 1982]: $C^1n = Cn(V_T/V_T$ " Vs)(C^1n"1/Cn"1), where 'C^1n is the corrected concentration of the nth sample, 'Cn' is the measured concentration of ondansetron hydrochloride in the nth sample, 'Cn"1' is the measured concentration of the ondansetron hydrochloride in the (n"1)th sample and C^1n-*1*is the corrected concentration in (n-*1*)th sample, 'V_T' is the total volume of the receiver fluid, and 'V_S' is the volume of the sample drawn.

The corrected cumulative amount of drug permeating per unit area versus time was plotted. All data were calculated and presented as mean ± S.D. The slope of the linear portion of the plot was calculated as the flux (J, μg/cm²/h). The lag-time was determined by extrapolation of the linear portion of the cumulative amount of drug permeated versus time plot to the abscissa. The permeability coefficient Kp was calculated by dividing the flux by the solubility of Ondansetron hydrochloride in the individual donor vehicles. The enhancement ratio (ER) was calculated by dividing the flux of drug loaded film (with enhancer) by that without enhancer.

RESULTS AND DISCUSSION

Solubility study

The drug Ondansetron hydrochloride shows considerable solubility in both polar and non-polar solvent. The maximum solubility was observed at 64.30 ± 3.20 mg/ml in Phosphate buffer saline (pH 5.8). The solubility in polar solvent like water is due its salt form. Ondansetron is weak base (pKa = 7.4) and under the acidic conditions is soluble in aqueous solvent [Salem, Lopez and Galan 2001]. The natural pH of Ondansetron hydrochloride is found to be 4.6±0.03. The solubility pattern of the drug depicts it's hydrophilic and lipophilic nature and indicates its suitability for transdermal delivery. The drug having optimum hydrophilicity and lipophilicity can only be permeated through the lipid bilayer of the skin [Gwak, Oh and Chun 2004].

Partition coefficient

The partition coefficient of Ondansetron hydrochloride was determined in n-octanol/water, n-octanol/aqueous PEG 400 (40%v/v), n-octanol/PBS pH7.0, n-octanol/PBS pH 6.0 and n-octanol/PBS pH 5.8 system at 37 ± 10°C. Ondansetron HCl is weakly lipophilic drug having partition coefficient at 1.64±0.08 in octanol/water. The optimum partition coefficient of the drug between -1 and 4 in octanol/water system are considered suitable for transdermal delivery [Hansen *et al* 2008].

Partition of drug through skin

The Partition coefficient of the drug did not change on changing the amount of drug from 0.5 – 2 mg in aqueous PEG 400 (40% v/v). This shows that the drug partition

through pig ear epidermis is a constant property of the drug molecule irrespective of the amount of drug in the medium. The mean value of the partition coefficient was found to be at 2.52 ± 0.33.

Physicochemical evaluation of patch

The physicochemical data of the prepared patches are shown in the Table 1.

Drug Content: The drug content of all the formulations was found at ~97±0.54%. It indicates that the drug was uniformly distributed throughout the films.

Moisture content (%): The moisture content of the patches was found to be ranged from 2.7±0.50 to 7.35± 0.20 %. Moisture content increases with increase in PVP concentration in the film [Mutalik and Udupa 2004]. The formulation with low moisture content indicates its stability against the microbial contamination.

Moisture Uptake (%): The percentage moisture uptake was found to be in the range of 17.83±0.35 % to 25.92±2.4 %. It was increased with increase in PVP level in the patch.

Water vapor transmission rate (WVTR): The water vapor transmission was found to be greater in case of the formulation having greater level of PVP.

Thickness Uniformity (TU): By measuring the thickness of the patch with screw gauge shows no or very small changes. Thickness ranges from 169.66±4.1 to 172.0±1.0 ìm.

Folding endurance (FE): After folding the patches number of times shows good tolerability to that movements from 11.00±1.1 to 22±1.0.

***Ex vivo* permeation study**

The drug permeation curve of different formulation was plotted by taking cumulative amounts of drug permeated against time (Figure 1). The curve showed that the permeation profiles seem to follow apparent zero order kinetics. It indicates that the drug was released from the formulation independent to the concentration and by diffusion mechanism. The desired flux of ondansetron HCl was determined at 177.22 µg/cm²/h. The permeation profile of various formulations of different polymeric ratio showed good result to optimize the ODH therapeutic film for further

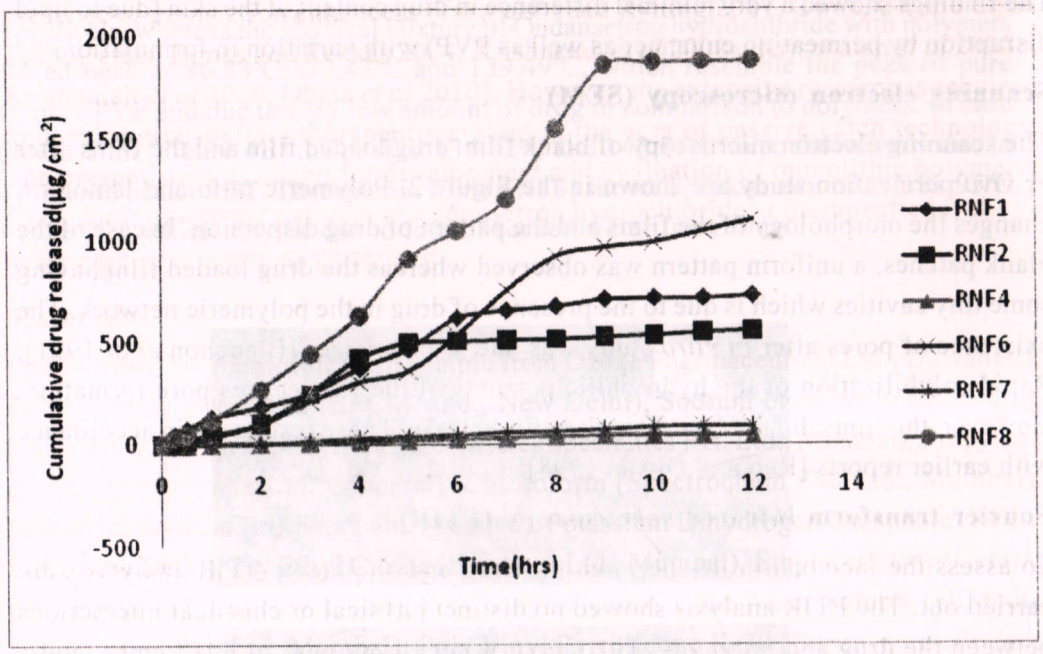

Figure 1: Permeation profile of Ondansetron hydrochloride across pig ear epidermis from different formulations

study. The flux of an optimized formulation (RNF6) having maximum flux was found to be 104.97 µg/cm²/h. The flux of RNF8 was increased from 104.97 µg/cm²/h to 179.93 µg/cm²/h due to presence of 15 % w/w of lemon oil as permeation enhancer (Table 2). Because lemon oil changes the properties of the skin by disrupting intercellular lipid bilayers to increase flux [Barry 1991]. The flux rate was also increased with increase in the concentration of PVP in the formulation. It may be due to that the PVP decreases the crystallinity of the drug in the patch and also fluidized the lipid of stratum corneum which accounts for the increased release of the drug [Yeneto *et al* 1995].

Skin retention

The drug content of the pig ear skin in some of the selected permeation study was determined. The drug content in skin is a constant property and does not change with variation of the polymeric ratios which is confirmed by the results shown in Table 2.

The findings showed a very minimal difference in drug content of the skin (due to lipid disruption by permeation enhancer as well as PVP) with variation in formulation.

Scanning electron microscopy (SEM)

The scanning electron microscopy of blank film, drug loaded film and the films after *ex vivo* permeation study are shown in the Figure 2. Polymeric ratio and lemon oil changes the morphology of the films and the pattern of drug dispersion. In case of the blank patches, a uniform pattern was observed whereas the drug loaded film having some tiny cavities which is due to the presence of drug in the polymeric network. The existence of pores after *in vitro* study was due to plasticizer [Blanchon *et al* 1991]. Rapid solubilization of the hydrophilic portions of the film causes pore formation. However, the films did not lose integrity after release. These results are in accordance with earlier reports [Rao and Diwan 1998].

Fourier transform infrared spectroscopy (FTIR)

To assess the incompatibility between the drug and excipients FTIR analysis were carried out. The FTIR analysis showed no distinct physical or chemical interactions between the drug and polymers. The lattice water shows peak at 3412 cm^{-1}, and a prominent peak was observed at 1639.49 cm^{-1} for C=O and C=N in case of pure drug (Figure 3A) and physical mixture. But in the case of drug loaded film this peak was found at 1641.42cm^{-1}due to weak or medium intensity hydrogen bonding between polymers and drug (Figure 3C). The peak at 1531.48cm^{-1} for aromatic C=C bond was present in the pure drug, drug loaded film and physical mixture which was not seen in the case of blank film (Figure 3B). The C-H stretching was only seen for aromatic ring of the drug in the range of 3050-3000 cm^{-1}. The CH_3, aromatic amines and O-substituted benzene groups of ondansetron showed peak at 1456.26 cm^{-1}, 1280.73 cm^{-1}and 756 cm^{-1}, respectively, only in the case of physical mixture and drug loaded films. Therefore, the FTIR studies confirm no major incompatibility between drug and polymer.

Differential scanning calorimetry (DSC)

The pure Ondansetron showed a sharp endothermic peak at 192.66°C (Figure 4A), corresponding to its melting point (179-210°C) [Salem, Lopez and Galan 2001]. Pure Ethyl cellulose and pure PVP K-30 showed peak at 84.12°C and (136.03°C, 140.22°C),

respectively, whereas the physical mixture of Ondansetron hydrochloride with polymers showed peak at 86.83°C,132.42°C and 139.49°C, which resemble the peak of pure EC, pure PVP and due to very low amount of drug in comparison to polymers. In case of blank film (Figure 4B) the peak at 134.12°C and 142.83°C were shown only due to low intense peak of EC. Therapeutic film (RNF8) showed peak at around <"181.95°C (Figure 4C), which may be due to loss of sorbed water from films

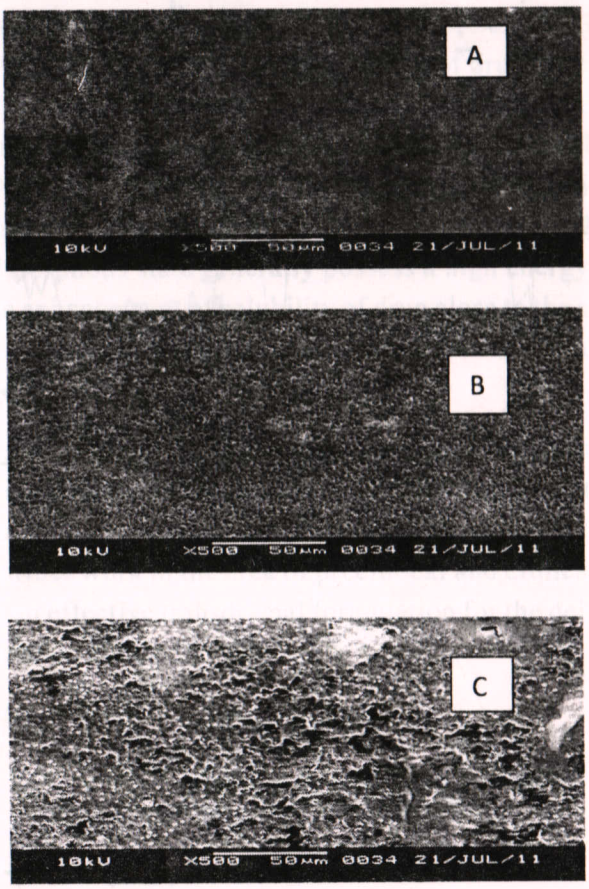

Figure 2: SEM of Blank film (A), Drug loaded film (B) and film after *ex vivo* permeation study (C)

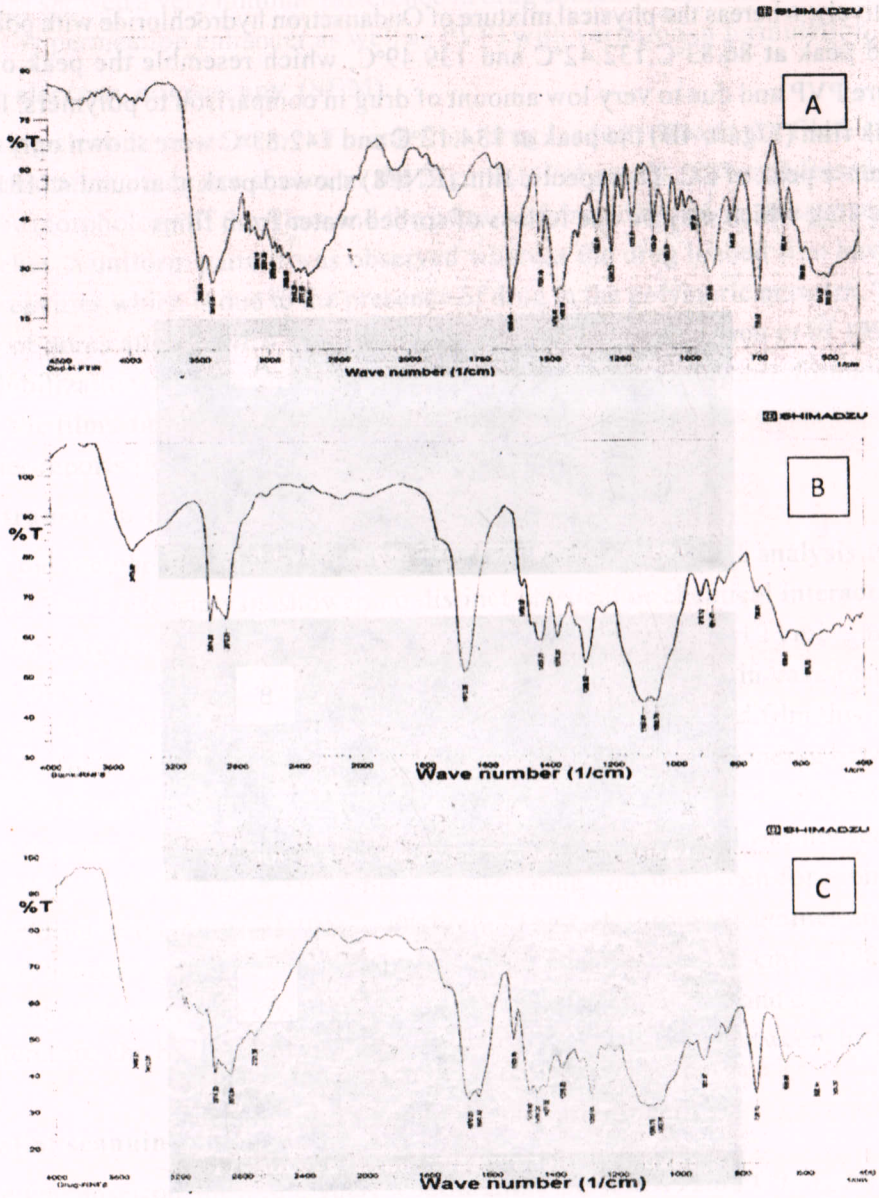

Figure 3: FTIR Spectrum of Ondansetron hydrochloride (A), Blank film (B) and Drug loaded film (C)

(expected due to hygroscopic property of PVP in the films). The significantly decreased intensity and sharpness of the endothermic peaks corresponding to the melting point of Ondansetron in the formulations also indicated partial dissolution of drug crystals in the polymeric film, i.e. the presence of drug in amorphous state [Pattnaik *et al* 2011].

X-ray diffraction (XRD)

X-ray diffraction is generally used for investigating the internal structure and it is non-destructive method. The pure Ondansetron hydrochloride (Figure 5A) exhibited the diffraction peaks at 2θ values of 11.66°, 12.10°, 16.66°, 20.53°, 23.76°, 23.76°, 25.42°, 27.63° and 31.89°, which revealed the crystallinity of drug. Interestingly, there were found no crystalline peaks of Ondansetron hydrochloride in the therapeutic film (Figure 5C). The pattern of diffractogram of drug loaded film resembles to the blank film (Figure 5B). Therefore, the drug may be dispersed at the molecular level or in amorphous form and the crystallinity of the drug was not shown [Pattnaik *et al* 2011]. Compounds in amorphous state generally possess a high energy state with improved solubility and the enhancement of solubility of drug close to the skin surface increases thermodynamic activity that facilitates the permeation rate through the skin.

CONCLUSION

The results of *ex vivo* permeation study across pig ear epidermis with transdermal formulation having polymeric composition of EC:PVP at 1:1 with 15% w/w of lemon oil produced desired transdermal target flux of Ondansetron hydrochloride at 179.93 $\mu g/cm^2/h$. The further work in the area of preclinical and clinical studies is needed in order to develop an effective transdermal formulation for the delivery of Ondansetron HCl at clinical level in human.

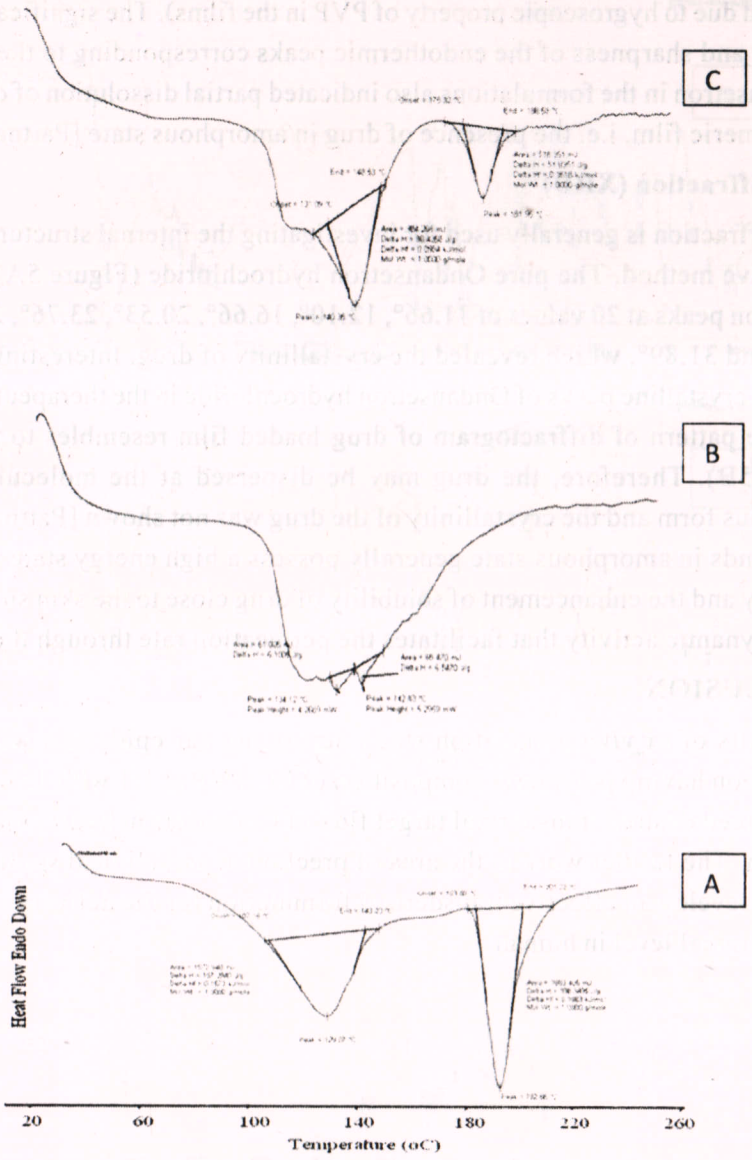

Figure 4: DSC Thermograms of Pure drug (A), Blank film (B) and Drug loaded film (C)

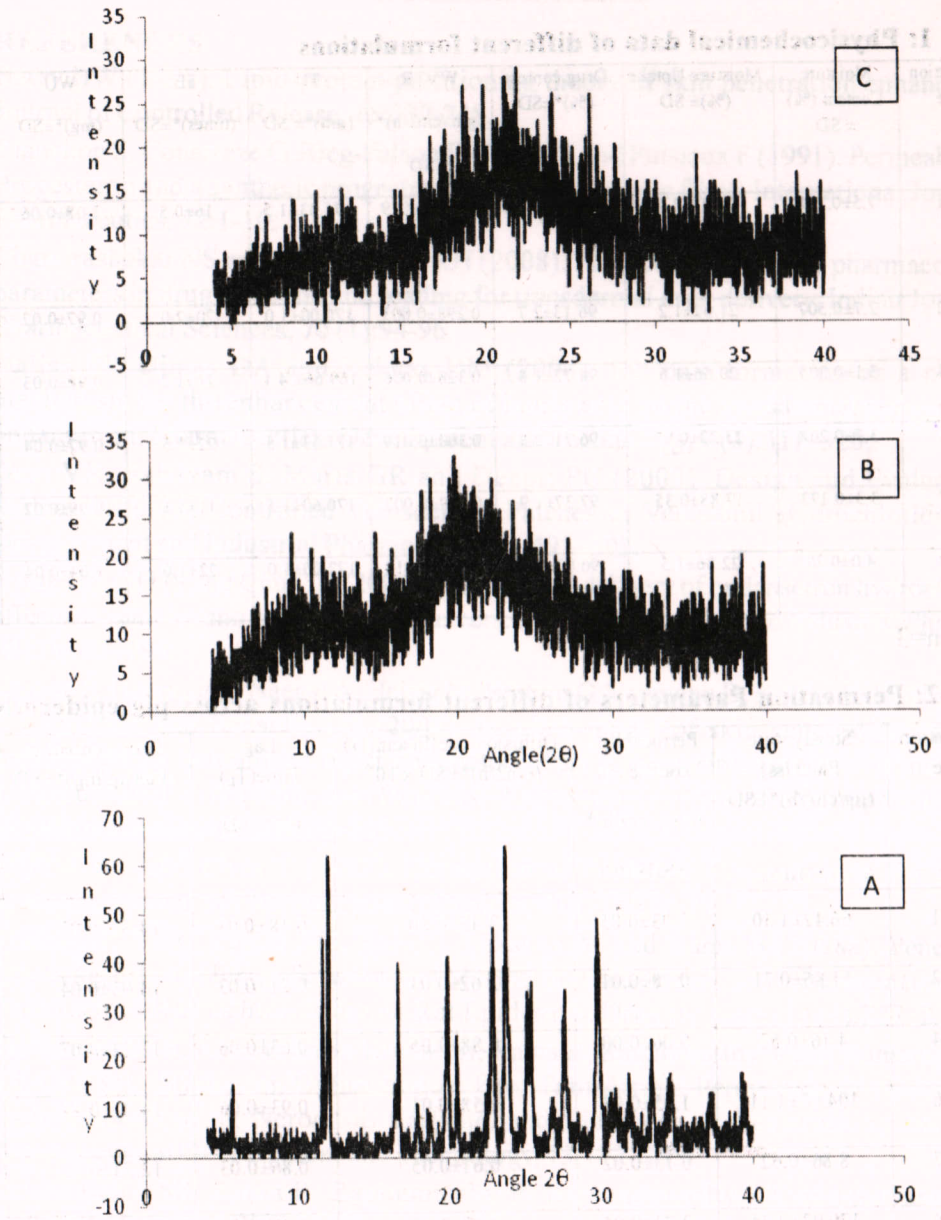

Figure 5: X-Ray Diffractograms of pure drug (A), blank film (B) and drug loaded film (C)

Table 1: Physicochemical data of different formulations

Formulation Code	Moisture Content (%) ± SD	Moisture Uptake (%)± SD	Drug content (%)*±SD	WVTR (gm./cm2/h)* ± SD	TU (μm)*± SD	FE (times)*±SD	WU (mg)*±SD
RNF1	7.5±0.208	25.92±2.4	96.10±0.58	0.449±0.019	171.33±1.5	16±0.5	1.08±0.06
RNF2	2.7±0.507	21.62±1.2	96.13±2.7	0.298±0.003	170.00±1.0	20±2.0	0.97±0.02
RNF4	3.1±0.305	20.66±1.6	96.72±1.8	0.328±0.006	169.66± 4.1	21±1.2	0.98±0.03
RNF6	3.7±0.208	23.23±0.98	96.71±2.8	0.360±0.019	171.33±1.5	22±1.5	0.97±0.04
RNF7	3.3±0.173	17.83±0.35	97.37±1.9	0.209±0.002	170.60±1.5	11±1.1	0.99±0.02
RNF8	4.0±0.20	22.86±1.5	96.93±2.9	0.285±0.012	172.00±1.0	22±1.0	1.04±0.04

*±SD, n=3

Table 2: Permeation Parameters of different formulations across pig epidermis

Formulation code	Steady state Flux(Jss) (μg/cm^2/h)*±SD	Permeability coefficient (Kp) (cm/h)* ±SDx10^{-2}	Diffusion coefficient(D) (cm2/h)*±SD x 10^{-5}	Lag Time(T_L) (h)*±SD	Drug content in skin(μg/mg)*±SD
RNF1	66.42±1.30	1.03±0.05	3.43±0.20	0.18±0.03	14.32±0.07
RNF2	51.86±0.71	0.78±0.01	2.62±0.01	0.21±0.03	14.03±0.04
RNF4	4.16±0.52	0.06±0.00	0.88±0.05	0.63±0.06	13.22±0.07
RNF6	104.97±1.11	1.55±0.03	0.58±0.01	0.93±0.06	14.21±0.21
RNF7	8.86±0.62	0.13±0.02	0.61±0.05	0.86±0.03	12.73±0.02
RNF8	179.93±2.34	2.72±0.04	1.32±0.03	0.43±0.06	15.97±0.12

*±SD, n=3

REFERENCES

Barry BW (1991). Lipid-protein-partitioning theory of skin penetration enhancement. Journal of Controlled Release, 15:237-248.

Blanchon S, Couarraze G, Rieg-Falson F, Cohen G and Puisieux F (1991). Permeability of Progesteron and a synthetic progestin through methacrylic films. International Journal of Pharmaceutics, 72:1-10.

Chandrashekar NS and Shobha Rani RH (2008). Physicochemical and pharmacokinetic parameters in drug selection and loading for transdermal drug delivery. Indian Journal of Pharmaceutical Sciences, 70 (1):94-96.

Dallas PP, Dimas DA and Rekkas DM (2004). Ion pair formation as a possible mechanism for the enhancement effect of lauric acid on the transdermal permeation of ondansetron. Pharmaceutical Development and Technology, 9 (3):311–320.

Devi VK, Saisivam S, Maria GR and Deepti PU (2003). Design and Evaluation of Matrix Diffusion Controlled Transdermal Patches of Verapamil Hydrochloride. Drug Development and Industrial Pharmacy, 29 (5):495–503.

Gwak HS, OH IS and Chun IK (2004). Transdermal delivery of ondansetron hydrochloride: effects of vehicles and penetration enhancers. Drug Development and Industrial Pharmacy, 30 (2):187–194.

Hansen S, Henning A, Naegel A, Heisig M, Wittum G, Neumann D, Kostka K-H, Zbytovska J, Lehr C-M and Schaefer UF (2008). *In-silico* model of skin penetration based on experimentally determined input parameters. Part I: Experimental determination of partition and diffusion coefficients. European Journal of Pharmaceutics and Biopharmaceutics, 68 (2):352–367.

Hayton WL and Chen T (1982). Correction of Perfusate Concentration for Sample Removal. Journal of Pharmaceutical Sciences, 71 (7):820-821.

Krishnaiah YSR, Kumar MS, Raju V, Lakshmi M and Rama B (2008a). Penetration-Enhancing Effect of Ethanolic Solution of Menthol on Transdermal Permeation of Ondansetron Hydrochloride across Rat Epidermis. Drug Delivery, 15 (4):227–234.

Krishnaiah YSR, Raju V, Shiva Kumar M, Rama B, Raghumurthy V and Ramanamurthy KV (2008b). Studies on Optimizing *In Vitro* Transdermal Permeation of Ondansetron Hydrochloride using Nerodilol, Carvone, and Limonene as Penetration Enhancers. Pharmaceutical Development and Technology, 13:177–185.

Krishnaiah YSR, Rama B, Raghumurthy B, Ramanamurthy KV and Satyanarayana V (2009). Effect of PEG 6000 on the In Vitro and In Vivo Transdermal Permeation of Ondansetron Hydrochloride from EVA1802 Membranes. Pharmaceutical Development and Technology, 14, (1):50-61.

Lewis S, Pandey S and Udupa N (2006). Design and evaluation of matrix type and membrane controlled transdermal delivery system of Nicotine suitable for use in Smoking Cessation. Indian Journal of. Pharmaceutical Sciences, 68 (2):179-184.

Llacer JM, Gallardo V, Parera A and Ruiz MA (1999). Formation of ondansetron polymorphs. International Journal of Pharmaceutics, 177 (2):221-229.

Mukherjee B, Mahapatra S, Gupta R, Patra B, Tiwari A and Arora P (2005). A comparison between povidone-ethylcellulose and povidone-eudragit transdermal dexamethasone matrix patches based on *in vitro* skin permeation. European Journal of Pharmaceutics and Biopharmaceutics. 59 (3):475–483.

Mutalik S and Udupa N (2004). Glibenclamide Transdermal Patches: Physicochemical, Pharmacodynamic, and Pharmacokinetic Evaluations. Journal of Pharmaceutical sciences, 93(6):1577-1594.

Obata Y, Ashitaka Y, Kikuchi S, Isowa K and Takayama K (2010). Statistical approach to the development of a transdermal delivery system for ondansetron. International Journal of Pharmaceutics, 399 (1-2):87-93.

Pattnaik S, Swain K, Mallick S and Lin Z (2011). Effect of casting solvent on crystallinity of ondansetron in transdermal films. International Journal of Pharmaceutics, 406 (1-2):106–110.

Rao PR and Diwan PV (1998). Formulation and *in vitro* evaluation of polymeric films of Diltiazem Hydrochloride and Indomethacin for transdermal administration. Drug Development and Industrial Pharmacy, 24(4):327-336.

Saettone MF, Perini G, Rijli P, Rodriguez L and Cini M (1995). Effect of different polymer plasticizer combinations on in vitro release of theophylline from coated pellets. International Journal of Pharmaceutics, 126:83-88.

Salem II, Lopez JR and Galan AC (2001). Ondansetron hydrochloride. In Florey K, ed. Analytical Profiles of Drug Substances and Excipients, Vol. 27. London: Academic Press Inc., pp. 301-337.

Takahashi K and Rytting JH (2001). Novel approach to improve permeation of ondansetron across shed snake skin as a model membrane. Journal of Pharmacy and Pharmacology, 53(6):789-794.

Tripathi KD (2008). Essential of Medical Pharmacology, 6th Edition. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd, pp. 639-664.

Yeneto K, Ghanem AH, Higuchi WI, Peck KD and Li SK (1995). Mechanistic study of the 1-alkyl-2 pyrrolidone as skin permeation enhancers. Journal of Pharmaceutical sciences, 84 (3):312-317.

Zang C-F, Yang Z-L, Luo J-B, Zhu Q-H and Zhao H-N (2007). Effects of cinnamene enhancers on transdermal delivery of ligustrazine hydrochloride. European Journal of Pharmaceutics and Biopharmaceutics, 67:413–419.