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PREPARATION, *IN-VITRO* AND *EX-VIVO* CHARACTERIZATION OF MATRIX TYPE TRANSDERMAL PATCHES OF PALONOSETRON HYDROCHLORIDE

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ABSTRACT

Palonosetron Hydrochloride is a 5-HT3 antagonist used in the prevention and treatment of chemotherapy induced nausea and vomiting. It is the only drug of its class approved for this use by the many countries. The oral bioavailability of Palonosetron Hydrochloride is only 50% due to the first pass metabolism in liver. Drug delivery through transdermal patches bypasses the first pass metabolism and hence increases its bioavailability. It's characteristics like small molecular size, good solubility in both water and oil makes it as suitable drug candidate for transdermal drug delivery. The main goal of this investigation is to develop and evaluate matrix type transdermal patch of Palonosetron Hydrochloride that releases the drug constantly for a period of 70 hours. The matrix type transdermal patches of Palonosetron Hydrochloride were prepared by solvent evaporation technique. The tensile strength, elongation break, *in-vitro* drug release, *in-vitro* drug permeation and *Ex-vivo* permeation through rat abdominal skin were studied. All the formulations showed satisfactory physicochemical and mechanical characteristics. The optimized formulation F5 with permeation enhancer (5% v/w eucalyptus oil) showed maximum cumulative percentage of drug release, permeation and flux. Values of tensile strength and elongation at break revealed that formulation F5 was strong but not brittle.

INTRODUCTION:

Transdermal drug delivery systems are gorgeous because of their benefits over other routes of drug delivery. This system provides convenient, comfort, pain less, self-administrative and also eliminates frequent dosing of the drug and hence it is always an ideal choice for patients [1,2]. For potent drugs having extensive first pass metabolism this is preferable delivery system. It also has additional advantage that dosage forms can be removed in emergency (adverse/ side effect) or when action is to be stopped. Intensive research has reported that transdermal drug delivery is a probable mode of delivery for lipophilic drugs in systemic circulation [3-5]. Palonosetron Hydrochloride is most effective in controlling "delayed chemotherapy induced nausea and

vomiting" that appear more than 24 hours after the first dose of a course of chemotherapy. Palonosetron Hydrochloride is available in 1 mg tablets, 0.5 mg capsule etc,. It is very small molecule (332.87) and have good solubility (water and oil), good half life (40 hours), good protein binding (62%), but its poor bioavailability (50%) make us to look for transdermal patches [6]. Though the drug is lipid soluble, we added one permeation enhancer to the formulation as *stratum corneum* layer of skin is toughly organized [7-13]. The objective of present research was development of matrix type transdermal patches of Palonosetron Hydrochloride which releases the drug constantly up to 70 hours. This patch will be ideal for the patients who are under the chronic treatment of antibiotics. Objective also includes evaluating its physicochemical, mechanical properties, *in-vitro* drug release, *in-vitro* permeation and *Ex-vivo* permeation through rat abdominal skin.

MATERIAL AND METHODS

Materials

Palonosetron Hydrochloride was gifted by Yarrochem Chemicals, Mumbai. Polymers Hydroxy Propyl Methyl Cellulose (HPMC E15), Poly Ethylene Glycol (PEG-400), Menthol and Eucalyptus oil were purchased from S.D. fine chemicals.

Construction of calibration curve of Palonosetron Hydrochloride

Accurately weighed (Shimadzu; Model-AUW220D) 1 mg of Palonosetron Hydrochloride was carefully transferred into 10 ml volumetric flask. It is diluted with small amount of Phosphate Buffer Solution (PBS) pH 7.4 and then finally volume made up to the mark with same solvent. The Lamberts-Beers range of Palonosetron Hydrochloride found from literature as 4-20 µg/ml [23]. Hence, the resulted stock solution was diluted with same solvent to get serial concentrations of 4 - 16 µg/ml with 2 increments. Absorbance of all the solutions was measured at 304 nm (λ max) using double beam UV visible spectrophotometer (Elico; Model-SL244). The calibration data was showed in Table 1 and calibration curve was showed in Graph 1.

Development of matrix type transdermal system

Matrix type transdermal patches were prepared by solvent casting method with HPMC E15 as a polymer and PEG-400 as a plasticizer. Accurately weighed quantity of polymer dissolved in 8 ml of solvent mixture (1:1 ratio of Methanol: Dichloromethane) allowed for swelling for 6 hours, PEG-400 and drug were dissolved in solvent mixture and added to the polymeric solution. Measured quantity of Menthol and Eucalyptus oil (5% v/w) was added as a permeation enhancer. The composition of patches is shown in Table 2. This was set aside for 2 hours to exclude entrapped air, then transferred to a specially moulded ceramic pit plate, and dried at room temperature [14]. Each pit in ceramic plate is round shape and having diameter of 1 cm. The developed matrix type patches were carefully removed and stored in desiccators. Obtained each patch is round in shape having diameter 1 cm. The prepared patches were subjected to evaluation.

Evaluation physicochemical properties:

Six patches of each formulation were weighed and average weight was calculated. The thickness of the patch was measured at six different points of patch using laboratory screw gauze. Each patch taken into 50 ml volumetric flask allowed to dissolve in 1 ml DMF (Di Methyl Formamide) and made up to the mark with 0.1N hydrochloric acid. The obtained solution filtered using 0.50 µ membrane filters and the drug content was analyzed using UV-Visible spectrophotometer at 304 nm. Folding endurance of the patch was determined manually by repeatedly

folding the patch at the same point until broken [15].

Moisture absorption study: Patches were placed in desiccators containing 100 ml of saturated Aluminium Chloride solution. After 48 hours the patches were taken out and weighed. The percentage of moisture absorption was calculated as the difference between the final and initial weight with respect to the initial weight [16].

$$\% \text{ Moisture Absorbed} = \frac{(\text{Final Weight} - \text{Initial Weight}) \times 100}{\text{Initial Weight}}$$

Moisture content determination

Patches were placed in desiccators containing Calcium Chloride solution at 40ºC for 24 hours. The final weight patches were noted until there was no further increase in weight of patches. The percentage of moisture content was calculated using following formula [16].

$$\% \text{ Moisture Content} = \frac{(\text{Initial Weight} - \text{Final Weight}) \times 100}{\text{Initial Weight}}$$

Water Vapour Transmission Rate studies (WVTR)

These transmission cells were washed thoroughly and dried in oven. About 1 gm anhydrous Calcium Chloride was placed in the cells and the respective transdermal film was fixed over the brim. The cells were accurately weighed and kept in a closed desiccators containing saturated solution of Potassium Chloride to maintain a relative humidity of 84%. The cells were taken out and weighed after storage. The amount of water vapour transmitted was found using following formula. WVTR is expressed as the number of grams of moisture gained/hour/cm^2.

$$\text{Water Vapour Transmission Rate} = \frac{(\text{Final Weight} - \text{Initial Weight})}{\text{Time X Area}}$$

Measurement of mechanical properties

Mechanical properties of the films were evaluated using a microprocessor based advanced force gauze (Ultra Test, UK) which is equipped with a 25 kg load cell. Each patch was pulled the strips to a distance held between two clamps located at a distance of 3 cm. During measurement, the top clamp at a rate of 2 mm/s till the film broke. The force and elongation were measured, when the film broke. The mechanical properties were calculated according to the following formulae. Measurements were run in four replicates for each formulation [18].

$$\text{Tensile Strength (Kg/mm squere)} = \frac{\text{Force at Break (Kg)}}{\text{Initial Cross Section area of the sample (mm squere)}}$$

$$\text{at Break (\%mm squere)} = \text{Elongation } \frac{\text{Increase in length (mm)}}{\text{Original lenght (mm)X cross sectional area (mm square)}}$$

The tensile testing gives an indication of the strength and elasticity of the film, reflected by the parameters, tensile strength (TS) and elongation at break (E/B). A soft and weak polymer is characterized by a low TS, and low E/B; a soft and tough polymer is characterized by a moderate TS, and high E/B; where as a hard and tough polymer is characterized by a high TS, and E/B. Another parameter strain value indicates that the film is strong and elastic. Hence, it is suggested that a suitable transdermal film should have a relatively high TS, E/B.

***In- vitro* drug release studies:** *In-vitro* release studies were carried out using Franz diffusion cell. The transdermal patch was kept in the donor compartment and it was separated from the receptor compartment by dialysis membrane (Hi media M.W. cut off 1000). The donor and receptor compartment were held together using clamp. The receiver compartment contained 20 ml of PBS of pH 7.4 containing 20% v/v of PEG-400, stirred at 50 rpm and temperature was maintained at 37 ± 0.5^0C. One ml of samples were withdrawn at pre-determined time intervals

and replaced with an equal volume of fresh medium. The drug content in the samples was determined by double beam UV Visible spectrophotometer (Elico; Model-SL244) at 304 nm. Cumulative amount of the drug released were calculated and plotted against time.

Ex- vivo permeation studies

Preparation of rat abdominal skin: Albino rats weighing between 150-250 gm were sacrificed using anaesthetic Ether. The hair of test animals was carefully trimmed short, less than 2 mm with a pair of scissors and the full thickness skin was removed from the abdominal region. This abdominal skin is soaked in water at 60°C for 45 seconds followed by careful removal of the epidermis. The epidermis was washed with water and used for *Ex-vivo* skin permeability studies. For *Ex-vivo* permeation studies the skin was mounted between the two compartments of the Franz diffusion cell with *stratum corneum* facing the donor compartment. The *stratum corneum* side of the skin was kept in intimate contact with the release surface of the patch under test. A dialysis membrane (Hi Media, M.W. cut off 1000) was placed over the patch, in order to secure it tightly in the way that it will not get dislodged from the skin. The receiver phase contained 20 ml PBS of pH 7.4 containing 20% v/v PEG 400 which was stirred at 50 rpm on a magnetic stirrer and the whole assembly was kept at 37 ± 0.5^0C. Samples of 1 ml were withdrawn at pre-determined intervals up to 70 hours; the volume was replenished with an equal volume of fresh medium and analyzed by UV Visible spectrophotometer. Cumulative amounts of drug permeated in $\mu g/cm^2$ were plotted against time and drug flux ($\mu g/cm^2/h$) at steady state was calculated by dividing the slope of the linear portion of the curve by the area of the exposed skin surface (3.14 cm^2) and the permeability coefficient was deduced by dividing the flux by initial drug load [19-20].

Drug-polymer interaction studies: Fourier Transform Infrared Spectroscopy (FTIR) studies were carried out to determine possible interaction between drug and polymer using K Br pellet methodology (Shimadzu; Model-FTIR8400S). The prepared KBr pellets were scanned in FTIR, wave numbers ranging from 500 to 4000 cm^{-1}.

RESULTS AND DISCUSSION

Weight, thickness variation, drug content and folding endurance: The physicochemical characteristics like weight, thickness variation, drug content and folding endurance of the transdermal patches are shown Table 3. The weight and thickness increases with increasing HPMC E15 concentration in patches. The results showed that the transdermal films were uniform. Good uniformity in drug content was observed in all transdermal patches as evidenced by low RSD values. The folding endurance number gives the mechanical property of the patches; high folding endurance number indicates that the patches have high mechanical property. The folding endurance number was increased with increasing HPMC E15 content. These results indicated that the patches would not break and would maintain their integrity with general skin folding when applied.

Moisture absorption and moisture content studies: The results revealed that the moisture absorption and moisture content was found to increase with increasing the concentration of hydrophilic polymer (HPMC E15).

Mechanical properties: The results of mechanical properties (tensile strength, elongation at break) were shown in Table 4. The mechanical properties shows the film's strength and elasticity, as it was revealed by the parameters of tensile strength (TS), elastic modulus (EM) and elongation at break (E/B).

Table 1: Calibration Curve of Palonosetron Hydrochloride in PBS pH 7.4		
S No.	Concentration (µg/ml)	Absorbance (304 nm)
1	0	0
2	4	0.275
3	6	0.368
4	8	0.492
5	10	0.587
6	12	0.610
7	14	0.820
8	16	0.920

Table 2: Formulation Composition of Each Round Shape Transdermal Patch					
Ingredients Used	Formulation Codes				
	F1	F2	F3	F4	F5
Palonosetron Hydrochloride (mg)	4	4	4	4	4
HPMC E15 Grade (mg)	100	150	200	250	250
PEG 400 Grade (µl)	10	15	20	25	25
Methanol (ml)	4	4	4	4	4
Di Chloro Methane (ml)	4	4	4	4	4
Menthol Oil (µl)	-	-	-	15	-
Eucalyptus Oil (µl)	-	-	-	-	15

Table 3: Physico Chemical Evaluation of Transdermal Patches (Average; n=6)					
Formulation Code	Weight Variation (mg)	Thickness (mm)	Folding Endurance	Drug Content (%)	WVTR (g/cm^2)X10^{-3}
F1	122±0.5	1.5±0.2	98±2	99±0.2	4.00±0.5
F2	177±0.7	1.8±0.6	105±3	100±0.3	4.10±0.6
F3	232±0.2	2.2±0.3	108±4	98.5±0.4	4.18±0.4
F4	302±1.2	2.5±1.2	110±4	100±0.9	4.50±0.7
F5	302±1.5	2.5±1.6	110±5	99±0.5	4.60±0.7

Table 4: Mechanical Properties of Transdermal Patches (Average; n=3)				
Formulation Code	Tensile Strength (kg/mm^2)	Elongation Break (%mm^2)	Elastic Module (kg/mm^2)	Strain
F1	2.36±0.5	12.0±0.22	9.36±0.05	0.52±0.46
F2	2.40±0.7	13.0±0.25	9.56±0.05	0.32±0.01
F3	2.53±0.2	13.2±0.32	8.77±0.05	0.73±0.32
F4	2.66±0.9	13.8±0.44	9.98±0.05	0.86±0.96
F5	2.69±0.5	12.8±0.32	9.78±0.05	0.94±0.31

Table 5: *In-vitro* Drug Release Studies of Transdermal Patches (Average; n=3)					
Time (hr)	Cumulative % of Drug Released				
	F1	F2	F3	F4	F5
10	41.3	44.3	50.3	52.3	55.3
20	50	54.0	58.0	60.0	62.0
30	54.1	59.1	62.1	63.1	68.1
40	58.3	62.3	66.3	68.3	72.3
50	60.7	69.7	71.7	74.7	78.7
60	66.8	72.8	74.8	76.8	82.8
70	72.3	75.3	77.3	85.3	96.3

Table 6: Drug Release Kinetics of Transdermal Patches n value indicates anomalous transport mechanism					
Parameter	F1	F2	F3	F4	F5
Zero Order	0.92	0.97	0.88	0.97	0.98
First Order	0.98	0.92	0.89	0.92	0.89
Higuchi	0.80	0.93	0.82	0.94	0.94
Peppas	0.89	0.97	0.92	0.96	0.97
n Value	0.74	0.64	0.58	0.59	0.68

Table 7: *Ex-vivo* Drug Permeation Studies of Transdermal Patches					
Time (hr)	% of Drug Permeated				
	F1	F2	F3	F4	F5
10	31.3	34.3	40.3	43.3	46.3
20	40.9	44.0	48.0	51.0	53.0
30	44.2	49.1	53.1	54.1	59.1
40	48.4	52.7	56.3	59.3	64.7
50	50.8	56.7	62.7	65.7	68.7
60	56.7	62.2	65.8	66.8	73.8
70	62.2	65.7	68.3	78.3	90.6
Flux ($\mu g/cm^2/h$)	16.3	13.4	17.4	19.2	21.4

A low value of TS, EM, and E/B indicates polymer is soft and weak; a hard and tough polymer is characterized by high values of TS, EM and E/B. Another important parameter is strain, which has been used as an indicator of the polymer film's overall mechanical quality [21]. These observations indicate that as the polymer concentration increased the TS and EM also increased but E/B values decreased. These results revealed that optimized formulations were found to be strong and flexible but not brittle [14].

***In- vitro* drug release studies:** The drug release profile of prepared transdermal films are represented in Figure 2 and Table 5. Among the formulations without permeation enhancers, F3 formulation has higher drug release than F1 and F2. Formulations containing permeation enhancers F4 and F5 showed higher drug release respectively compared with formulation without permeation enhancers. From the results and graphs it is clear that the drug release was depends on polymer and permeation enhancer content. An increase in the content of polymer was associated with decrease in drug release rate. The *in-vitro* release data of all formulations of patches well fitted into zero order equation.

***Ex-vivo* permeation studies:** *Ex-vivo* permeation studies were carried out for all formulation and drug solution. The results showed in Figure 3 and Table 7. Use of permeation enhancer showed a good result in increase of drug permeation. Plotting the cumulative amount drug permeated per sq cm of the patches through the rat abdominal skin against time in hours showed that, the profile of drug permeation might follow zero order kinetics.

Drug- polymer interaction studies

No additional peaks were observed hence which indicates the absence of interaction of drug and polymers.

CONCLUSION

The present study showed that Palonosetron Hydrochloride patch containing HPMC E15 in the ratio of 1:12.5 with 15% v/w of PEG-400, achieved the desired objectives of TDDS, such as overcoming of first pass effect, extended release and may serve as better system for transdermal delivery. The polymeric films containing Palonosetron Hydrochloride were prepared and evaluated for physicochemical, *in-vitro* drug release and permeation characteristics. The formulations containing HPMC E15 and permeation enhancers (eucalyptus oil 5% v/w) were found to higher flux. Good *in-vitro* and *ex-vivo* correlation for optimized transdermal patch demonstrates the validity of the release test conducted. The transdermal patches of Palonosetron Hydrochloride with required flux could be prepared with suitable mechanical properties; further studies are recommended to find their therapeutic utility in humans by pharmacokinetic and pharmacodynamic studies.

ABRREVIATIONS USED

HPMC E15- Hydroxy Propyl Methyl Cellulose E15, DCM- Di Chloro Methane, PEG- Poly Ethylene Glycol, TDDS- Transdermal Drug Delivery System.

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REFERENCES:

1. Chien Y.W. Novel Drug Delivery Systems. Drugs and Pharmaceutical Sciences. Marcel Dekker; 2 (50), 201-220.
2. Chien Y. W. Transdermal Therapeutic systems. Robinson Joseph R, Lee Vincent HL. Editors. Controlled Drug Delivery: Fundamentals & Applications, New York: Marcel Dekker; 1987, 523.
3. Barry B.W. Dermatological formulation, New York: Mercel Dekker; 1983, 225-228.
4. Barry B. Transdermal Drug Delivery. Aulton M E editor. Pharmaceutics: The Science of Dosage Form Design, Churchill Livingston: 2002. 499-533.
5. Robinson J.R, Lee V.H. Controlled drug delivery fundamentals and applications, New York: Marcel Dekker: 1987. 52-55.
6. Sweetman S.C. Martindale. The complete drug reference, London: Pharmaceutical Press: 2007. 1556-1557.
7. Finnin BC, Morgan TM. Transdermal penetration enhancers: applications, limitations, and potential. Journal of pharmaceutical sciences. 1999; 88(10):955-958.
8. El-Kattan, The effect of terpene enhancer lipophilicity on the percutaneous permeation of hydrocortisone formulated in

HPMC gel systems. International journal of pharmaceutics. 2000; 198(2): 179-189.

9. Williams, Barry. Terpenes and the lipid–protein–partitioning theory of skin penetration enhancement. Pharmaceutical research. 1991; 8(1):17-24. https://doi.org/10.1023/A:1015813803205.
10. Pillai, Panchagnula R. Transdermal iontophoresis of insulin: V. Effect of terpenes. Journal of controlled release. 2003; 88(2):287-296.
11. Moghimi, Williams, Barry B.W. A lamellar matrix model for stratum corneum intercellular lipids. Effect of terpenes penetration enhancers on the structure and thermal behavior of the matrix. *Int J Pharm* 1997; 146: 41-54.
12. Lee , Ahmad, Langer R, Mitragotri, Shastri VP. Evaluation of chemical enhancers in the transdermal delivery of lidocaine. International journal of pharmaceutics. 2006;308(1):33-39.
13. Gao, Singh J. *In vitro* percutaneous absorption enhancement of a lipophilic drug tamoxifen by terpenes. Journal of controlled release. 1998;51(2):193-199.
14. Gannu R, Vishnu, Kishan V, Rao YM. Development of carvedilol transdermal patches: evaluation of physicochemical, *Ex vivo* and mechanical properties. PDA Journal of Pharmaceutical Science and Technology. 2008; 62(6):391-401.
15. Mandal, Bhattacharyya, Ghosal SK. *In-vitro* Release and Permeation Kinetics of Pentazociwe from Matrix-Dispersion Type Trawsdwul Drug Delivery Systw. Drug development and industrial pharmacy. 1994; 20(11):1933-41.
16. Dwarakanadha reddy Peram et al, Formulation and evaluation of ramipril transdermal patch, Int. J. Chem. Sci.: 8(1), 2010, 681-686
17. Kusum Devi , Saisivam S, Maria , Deepti . Design and evaluation of matrix diffusion controlled transdermal patches of verapamil hydrochloride. Drug development and industrial pharmacy. 2003;29 (5):495-503.
18. Gannu, Vamshi Vishnu Y, Kishan, Madhusudan Rao Y. Development of nitrendipine transdermal patches: *in vitro* and *Ex vivo* characterization. Current Drug Delivery. 2007; 4(1):69-76.
19. Friend. *In vitro* skin permeation techniques. Journal of Controlled Release. 1992;18 (3):235-48.
20. Franz. Percutaneous absorption. On the relevance of *in vitro* data. Journal of Investigative Dermatology. 1975; 64(3):190-5.
21. Aulton, The mechanical properties of hydroxypropyl methylcellulose films derived from aqueous systems Part 1: the influence of plasticisers. Drug Development and Industrial Pharmacy. 1981;7 (6):649-68.
22. Suddala Shirisha et.,Preparation and Evaluation of Matrix Type Transdermal Patches of Domperidone Maleate: in vitro and ex vivo Characterization; Indian Journal of Pharmaceutical Education and Research; Oct-Dec'2017; 4(51).
23. Atul Tripathi et.al.,; In-vitro evaluation of Transdermal Patch of Palonosetron for Antiemetic Therapy; International Journal of Drug Delivery; 9 (2017); 119-124