ORIGINAL ARTICLE

Skin Permeation of Candesartan Cilexetil from Transdermal Patch Containing *Aloe Vera* Gel as Penetration Enhancer

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Abstract

Aim: To prepare and evaluate the matrix type transdermal films of candesartan cilexetil (CC) containing *Aloe vera* gel as penetration enhancer by solvent evaporation technique to enhance its bioavailability. **Material and Methods:** Eudragit-RL 100 and hydroxypropyl methylcellulose (HPMC) were used as film former and dibutyl phthalate (DBP) as a plasticizer. *A. vera* gel was employed as a penetration enhancer to improve the skin penetration of CC by a probable pull effect of complexes formed between the drug and the enhancing agent (lignin). The film preparations were characterized in physicochemical properties such as thickness, folding endurance, uniformity of drug content, stability, and tensile strength. *In vitro* drug release and skin permeation kinetics studies of CC from film preparations were examined using a Franz-type diffusion cell. Infra-red spectroscopy and differential scanning colorimetry were performed to follow drug-carrier interactions. **Results and Discussion:** The uniformity of drug content was evidenced by the low standard deviation values for each film preparation. Enhancers examined in this study increased the moisture uptake capacity and release rate of CC, as by increasing the concentration of *a. vera* gel from 5% to 10% w/w in the film enhanced the release rate of CC substantially. Skin irritation studies revealed that there was no erythema and edema at the site of application. **Conclusion:** The study indicates that the candesartan could be administered transdermally for the effective control of hypertension through the matrix type TDDS having suitable mechanical properties and high bioavailability. Based on the observation, we can reveal that a11 formulation is better suited for development with compositions ERL 100: HPMC (5:5), DBP 5 ml with *a. vera* gel 10% (w/w) provided with a high skin permeation rates.

Key words: *Aloe vera* gel, candesartan cilexetil, penetration enhancer, permeation transdermal patch

INTRODUCTION

Hypertension is a chronic disease, which requires long-term treatment with drug therapy at steady state drug blood concentration. Candesartan cilexetil (CC) is a prodrug that is rapidly converted to candesartan (its active metabolite) during absorption from the gastrointestinal tract and confers blood pressure lowering effects by antagonizing the hypertensive effects of angiotensin II. It has a molecular weight of 610.66 with poor oral bioavailability due to which its usual recommended starting dose is 16 mg once daily when it is used as a monotherapy in patients who are not volume-depleted. This can be administered once or twice daily with total daily doses ranging from 8 to 32 mg. The patients requiring further reduction in blood pressure should be titrated to 32 mg.[1] The major disadvantages of such a drug therapy are more frequency of administration, extensive first pass metabolism and variable bioavailability which make it an ideal candidate for transdermal drug delivery systems. This explains the need of anti-hypertensive transdermal patches in the perspective of enhancing the bioavailability as well as in improving patient compliance.[2]

Transdermal drug delivery system allows delivery of contained drug into the systemic circulation via permeation through skin layers at a controlled rate. These systems are easy to apply and remove as and when desired. However, the protective upper layer of the skin, stratum corneum (SC) behaves like a

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Received: 17-02-2016
Revised: 25-03-2016
Accepted: 05-04-2016

challenging barrier for the penetration of majority of drugs. It offers a formidable physical barrier to molecular transport. This layer is very specific with regards to the type of molecule that can be transported across the skin, and therefore, only molecules with certain physicochemical properties can readily cross the skin.[3] This limits the range of potential drugs that can be administered transdermally, which emphasizes the need for formulations to incorporate penetration enhancers to assist in the effective delivery of a larger variety of drugs across the skin.

The use of herbal penetration enhancers which penetrate into human skin and shows reduction in the barrier resistance is widely accepted in transdermal drug delivery. The mechanism behind enhanced penetration rate of drugs across the skin is through two possible mechanisms of action. First, the penetration enhancer can work by altering the solubility properties of the skin, thereby increasing the solubility of the drug within the SC; second, the enhancer disrupts the ordered nature of the skin lipids, which consequently influences diffusion across the SC. Nowadays, many herbal penetration enhancers are included in generally recognized as safe substances list, and they possess low side effects and irritancy in comparison with synthetic chemicals such as solvents and azones or surfactants.[4]

One such a natural product, *Aloe vera* (*Aloe barbadensis* Miller) gel, has shown potential to enhance the permeation of certain drug molecules through skin membranes. *A. vera* gel is the viscous, transparent, and colorless mucilaginous gel obtained from the parenchymatous cells in the fresh leaves. It was suggested that the mucilaginous gel of the aloe, consisting mainly of polysaccharides, holds the secret to some of the medicinal properties and biological effects of this family of plants, which was confirmed for drug absorption enhancement across intestinal epithelial cells. *A. vera* has an element called "Lignin" which helps it to penetrate right down to the cellular level. It also has another element called "Saponin" which works as a natural cleansing agent. Both these elements working in conjunction reach the cellular level of the skin. In addition to this, it also nourishes the skin and replenishes it with the much-needed nutrition that it requires. Along with this, it increases the *in vitro* skin penetration of some compounds depending on their molecular weights with an apparent inverse correlation between enhancement ratio and molecular weight of the compound. This penetration enhancement effect of the aloe gel was explained by a probable pull effect of complexes formed between the compound and the enhancing agent within the aloe gel but it was stated that the proposed mechanism of action has to be further investigated and confirmed.[5]

MATERIALS AND METHODS

Materials

CC was obtained as a gift sample by Sun Pharma (Gurgaon, India). Eudragit RL 100 and Eudragit RS 100 was obtained from Evonik Degussa Ltd., (Mumbai, India). Hydroxypropyl methylcellulose (HPMC) was purchased from Rankem India. *A. vera* gel was procured from Plunkett. All other chemicals used in the study were of analytical grade.

Characterization of CC

The drug was checked for parameters such as appearance, odor, melting point, partition coefficient, solubility, and identification tests. Ultraviolet (UV) spectroscopy, infrared (IR) spectroscopy, and differential scanning calorimetry (DSC) were conducted and compared with that of standards as reported in the literature.

Partition coefficient

The partition coefficient of the drug was determined by shaking equal volumes of organic phase (n-octanol) and the aqueous phase in a separating funnel for 10 min and allows standing for 2 h with intermittent shaking. Then, the concentration of CC in both phases was determined using UV spectrophotometer at 256 nm to get the partition coefficient value.[6]

Preparation of standard plot of CC

Standard plot of CC was prepared in 20% isopropyl alcohol in isotonic phosphate buffer pH 7.42.

For making, the standard curve of CC serial dilutions was made. Initially, a solution of the concentration of 100 mcg/ml was made by weighing 10 mg of drug using digital balance and dissolving in 100 ml of 20% isopropyl alcohol in isotonic phosphate buffer pH 7.4 in a volumetric flask. From this stock solution, different concentrations ranging from 10 to 50 mcg/ml were made, and the absorbance of the resulted solution was taken on a double beam UV spectrophotometer using 20% isopropyl alcohol in isotonic phosphate buffer pH 7.4 as a blank at 256 nm.[7] A graph was plotted between the concentration (X-axis) and absorbance (Y-axis).

Drug - excipient interaction studies

The possible interaction between CC and the polymers used in the formulation of the transdermal film was found out by UV spectral analysis, Fourier transform IR (FT-IR) spectroscopy, thin layer chromatographic studies, and DSC studies on the pure substance and their physical mixtures.[8]

Preparation of transdermal films

Matrix-type transdermal films of candesartan were prepared by solvent evaporation technique. A mercury floor film casting assembly was used for fabrication of the films. Over this floor a glass ring of 6.0 cm diameter was placed. The film casting

solution, comprising ERL 100, HPMC, dibutyl phthalate (DBP), and *A. vera* gel as penetration enhancer was poured. The series of A formulations were prepared using different ratios of polymers along with 16.77% (w/w) of candesartan, 10% (w/w) of plasticizer DBP, and 5% and 10% (w/w) *A. vera* gel (based on total polymer weight) in a mixture of dichloromethane and methanol (50:50 v/v) as shown in Table 1. An inverted funnel with cotton plug is placed over this assembly and is kept in a humidity control oven to dry at 32 ± 2°C and 45 ± 5% RH, reason for placing inverted funnel over the Petri dish is to prevent rapid evaporation of the solvent. This also helped in preventing cracking or wrinkling of film.[9]

The solvent was allowed to evaporate undisturbed. The dried films were then cut with a circular metallic die of 2.93 cm internal diameter to form a circular film with an area of 6.74 cm^2. An adhesive tape was then laminated on the backing film (leaving a flange along the circumference for easy sticking).[10] Finally, a wax paper was placed on the other side as the release liner (which could be easily removed before application of the patch on the skin).[11] The transdermal patch was then finally sealed packed into laminated aluminum foil and was kept in desiccators until used.

Characterization of the transdermal films

Physical appearance

The films were observed visually for their color, clarity, completeness, uniformity, surface texture, and flexibility.

Thickness of the film

The thickness of the film was measured at three different places using a micrometer and mean values were calculated.

Flatness studies

Longitudinal strips were cut out from each film, one from the center and two from either side. The length of each strip was measured, and the variation in the length because of non-uniformity in flatness was measured by determining percent constriction, considering 0% constriction is equivalent to 100% flatness.[12]

Weight variation

Three films of size 1 cm^2 from each batch were weighed individually and average weight determined.

Moisture content

The prepared films were marked, then weighed individually and kept in a desiccator containing activated silica at room temperature for 24 h. The films were weighed again and again individually until it showed a constant weight. The percentage moisture content was calculated as a difference between initial and final weight with respect to final weight.[13]

Percentage moisture uptake

A weighed film kept in desiccators at normal room temperature for 24 h was taken out and exposed to 84% relative humidity (saturated solution of potassium chloride) in desiccators until a constant weight for the film was obtained. The percentage of moisture uptake was calculated as the difference between final and initial weight with respect to initial weight.

Folding endurance

A small strip of the film (1 cm^2) was folded repeatedly at the same place till it broke. The number of times the film could be folded at the same place without breaking is the folding

Table 1: Formulation composition

Formulation code	ERL 100 (mg)	HPMC 15 cps (mg)	Dibutyl pthalate (ml)	*Aloe vera* gel (%w/w of polymer)	Dichloro methane (ml)	Methanol (ml)
A1	0	600	5	5	3	3
A2	100	500	5	5	3	3
A3	200	400	5	5	3	3
A4	300	300	5	5	3	3
A5	400	200	5	5	3	3
A6	500	100	5	5	3	3
A7	600	0	5	5	3	3
A8	0	600	5	10	3	3
A9	100	500	5	10	3	3
A10	200	400	5	10	3	3
A11	300	300	5	10	3	3
A12	400	200	5	10	3	3
A13	500	100	5	10	3	3
A14	600	0	5	10	3	3

HPMC: Hydroxypropyl methylcellulose

endurance. This was repeated six times and the mean values plus standard deviation was calculated.

Tensile strength and % elongation

A strip of the film of 1 cm × 0.5 cm was selected and attached to a clip at one end of a flat wooden surface and was pulled using a pulley system. Weights were added to the pan to increase the pulling force till the film was broken. The elongation of the film at the point of break up was measured.[14]

The tensile strength was calculated as per the formula given below:

Tensile strength = [break force/a×b] [(1+ΔL)/L]

Where a = Thickness of the film, b = Width of the film, DL = Length at breaking point, L = Length of the film.

% Elongation was calculated using the formula

% Elongation = {(Lb− L0)/L0}×100

L0 = Original length of the film, Lb = Length of the film when stress is applied.

Hardness

Hardness was determined using an apparatus designed in our laboratory as per literature report. The film was placed between the metal and sharp end of the rod of the apparatus. Weights were added gradually at an interval of 10 s for the stabilization of the force till the bulb glows. The final weight was considered as the measure of hardness.[15]

Drug content

A film of size 6.74 cm^2 cut into small pieces (1 cm^2) and put in a 100 ml (60:40 isopropyl alcohols:phosphate buffer pH 7.4). This was then shaken in a mechanical shaker for 2 h to get a homogeneous solution and filtered. The drug was determined spectroscopically at 256 nm after suitable dilution. Similarly, a blank was prepared from transdermal films without the drug.[16]

Stability studies

The transdermal films were sealed in polyethylene coated aluminum foils and kept at 10°C, room temperature and 45°C for a period of 3-month. During this period, the films were tested for any change in color, texture and analyzed periodically for its drug content.[17]

In vitro drug release studies

A horizontal glass diffusion cell (Franz-type) fabricated by a local glass fabricator was used for the release study. The cell consisted of two half cells, donor and receiver cell, each of capacity 100 ml. The donor and receiver compartments were held with springs and the area of diffusion between the two half cells was 1.76625 cm^2.

The transdermal film was placed on cellophane membrane which was mounted on the donor compartment of the diffusion cell.[18] The donor compartment was kept in contact with the receptor compartment which was filled with 100 ml phosphate buffer solution pH 7.4 at a temperature of 37 ± 1°C, in such a way that the membrane just touches the solution. The elution medium was stirred magnetically at 50 rpm. The aliquots (1 ml) were withdrawn at predetermined time intervals for 12 h and replaced with the same volume of the buffer.[19] The samples were analyzed for drug content using UV spectrophotometer at 256 nm.

In vitro skin permeation studies

In vitro skin permeation study with rat abdominal skin was performed using Franz-type cells with effective permeation area of 1.76625 cm^2. Before the experiment, the skin was taken out and thawed until it reached room temperature and was kept soaked in phosphate buffer solution for 1 h. It was gently blotted dry with filter paper. The integrity of the skin was tested microscopically before use in the diffusion cell to detect any histological change.[20]

The prepared rat abdominal skin was fastened carefully between the donor and receptor compartment in the diffusion cell so that the SC faced the donor side. The transdermal films to be studied were placed in between the donor and the receptor compartment in such a way that the drug releasing surface faced toward the receptor compartment. The elution medium was magnetically stirred for uniform drug distribution at a speed of 50 rpm. The temperature of the whole assembly was maintained at 37 ± 1°C by thermostatic arrangements.[21]

An aliquot of the receptor fluid was withdrawn periodically and replaced with the same volume of 80:20 phosphate buffers to isopropyl alcohol (pH 7.4). The concentration of candesartan in the receptor was determined by UV-visible double beam spectrophotometer at λmax 256 nm. The cumulative amount of candesartan transferred into the receptor side and permeation kinetics parameters were calculated. To examine the drug permeation kinetics and mechanism, the data were tabulated and fitted to models representing zero-order, first-order and Higuchi diffusion model.[22]

RESULTS AND DISCUSSION

The drug CC was characterized as per the monograms specified for melting point 171°C as shown in Figure 1, FT-IR spectral analysis as shown in Figure 2, partition coefficient

(Log P of 4.06), solubility and permeability at various values of pH with phosphate buffer.

The DSC thermo grams of CC and physical mixture of the drug with the polymers are displayed with the characteristic peak at 171.74°C corresponding to its melting point. The drug peak appeared in the thermo gram for the physical mixture of the drug and polymers, confirming the chemical integrity of the drug.

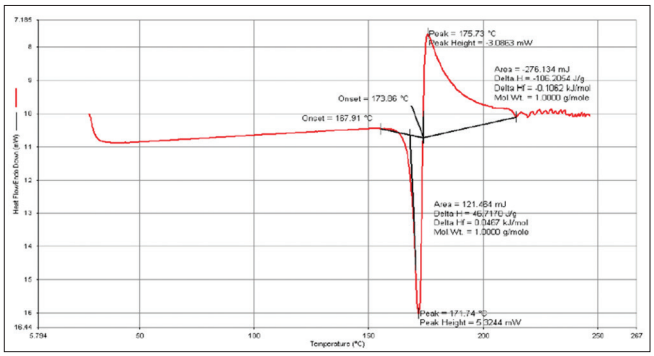

Figure 1: Differential scanning calorimetry of candesartan cilexetil drug

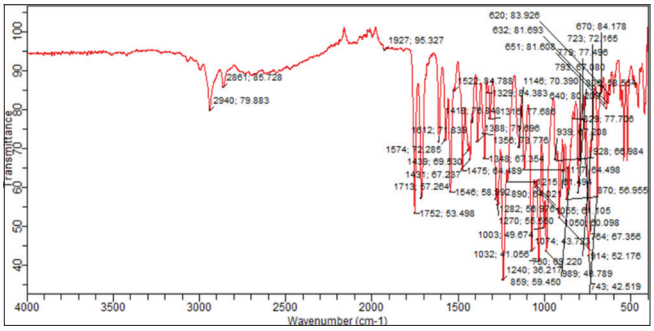

Figure 2: Fourier transform infrared of candesartan cilexetil drug

Matrix type transdermal films of CC with different compositions of Eudragit RL 100: HPMC films were prepared using *A. vera* gel as penetration enhancer using solvent casting technique. Adhesive tape and release liner were then suitably positioned to complete the transdermal patch. The polymers used for the fabrication of the transdermal system showed good film forming properties. The method adopted for casting the film was found to be satisfactory, the prepared films were further subjected to physical characteristics such as folding endurance, percentage elongation at break, percentage moisture uptake, percentage moisture content, weight, thickness, and percentage drug content results are as shown in Table 2.

The physical parameters, such as folding endurance and percentage elongation at break, were found to decrease with the increasing amount of HPMC polymers, but no sign of cracking in films was observed, which might be attributed to addition of plasticizer DBP (10% w/w of polymer weight). The percentage moisture uptake and percentage moisture content were found to increase with the increasing amount of *A. vera* gel in the films.

The low values for SD in the case of thickness of the films indicate physical uniformity of the films. The drug content analysis of the prepared formulations showed that the process used to prepare the films is capable of giving uniform drug content and minimum batch variability. The weights are ranged from 18.2 ± 1.37 to 24.1 ± 2.16 mg. Thickness ranged from 120 ±1 to 141 ± 2 µ. The weights are found to be high with films prepared with higher proportions of HPMC as one of two polymers. As the proportion of HPMC was decreased, the thickness was also decreased.

Table 2: Physicochemical evaluation of the transdermal films of candesartan cilexetil

Formulation code	Mean thickness (µ)	Weight (mg)	Folding endurance	Moisture content	Moisture absorption	Drug content (%)
A1	126±1	19.9±1	200±1	2.5±1	3.2±1	90.5±1
A2	132±2	22.4±2	204±2	2.7±1	3.9±2	89.9±2
A3	137±2	23.6±2	206±1	2.6±1	4.1±2	91.5±1
A4	141±2	24.1±2	207±2	3.4±1	7.8±1	90.2±2
A5	138±2	23.4±2	208±1	3.6±1	5.8±2	88.3±2
A6	129±2	20.1±1	207±2	2.5±1	4.3±1	87.3±1
A7	125±1	19.9±1	204±1	2.6±1	3.5±2	84.8±2
A8	120±1	18.2±1	200±2	1.5±1	1.3±1	88.7±2
A9	122±1	18.9±1	203±1	1.5±1	2.5±2	91.6±1
A10	128±2	20.3±2	206±2	1.9±1	4.9±1	94.6±1
A11	131±2	21.2±1	210±2	3.8±1	8.8±2	98.8±2
A12	139±2	23.9±2	205±2	3.5±1	7.3±1	96.7±1
A13	125±1	19.8±1	202±1	2.7±1	5.6±2	95.3±2
A14	121±1	18.6±1	201±1	1.8±1	2.1±2	93.3±1

Table 3: Physical parameters of stability samples of formulation A11

Time (days)	Weight (mg)	Thickness (μ)	Folding endurance	% Moisture uptake	% Moisture content	Drug content (mg)
0	21.2	131	210	8.81	3.84	16
30	22.7	135	216	8.86	3.86	15.8
60	24.6	138	221	8.88	3.9	15.5
90	24.8	138	228	8.89	3.96	15.3

Table 4: Release kinetic data of candesartan cilexetil containing matrix type of A1-A14

Formulation code	Mathematical models (r^2)		
	Zero order	First order	Higuchi
A1	0.8337	0.8421	0.9533
A2	0.8224	0.8143	0.9765
A3	0.8242	0.8235	0.8956
A4	0.8954	0.9865	0.9765
A5	0.8951	0.9834	0.9376
A6	0.8272	0.8342	0.9785
A7	0.8321	0.8432	0.9234
A8	0.8418	0.8587	0.8956
A9	0.8645	0.8976	0.9122
A10	0.8756	0.9456	0.9563
A11	0.8967	0.9875	0.9564
A12	0.8656	0.9154	0.9754
A13	0.8854	0.9487	0.8953
A14	0.8675	0.8967	0.9765

The results of flatness study showed that none of the formulations had the difference in the strip lengths before and after their cuts, thus indicating 100% flatness. It shows that no amount of constriction in the patches and thus they could maintain a smooth surface when applied onto the skin. The folding endurance was found to be ranged from 200 ± 1 to 210 ± 2 and it was found to be satisfactory. An inverse relation was observed between tensile strength and elongation at break. These observations indicate that formulation A11 patches were found to be strong, not brittle and flexible.

All formulations were selected for stability studies and observed for changes in color, appearance, flexibility, and drug content. Temperature and humidity values were selected as per the ICH guidelines and the tests were carried out in a stability chamber. Films were analyzed at an interval of 30 days for a period of 3-month as shown in Table 3.

In vitro drug release profiles showed that as the concentrations of *A. vera* gel increased in the formulations, the drug release rate increased substantially however with a very nominal decrease in some formulations. The addition of hydrophilic component to an insoluble film former tends to enhance the release rates. The process of drug release in most controlled

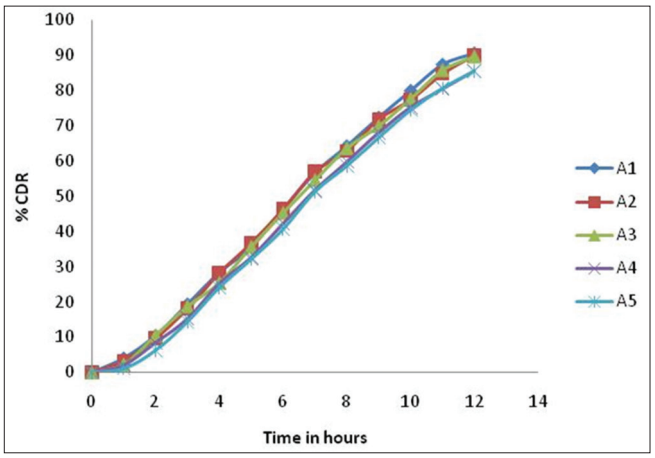

Figure 3: *In vitro* drug release profile of films (A1-A5)

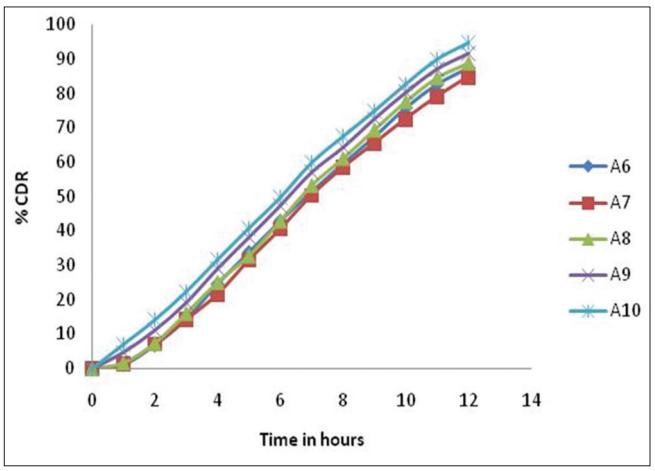

Figure 4: *In vitro* drug release profile of films (A6-A10)

release devices is governed by diffusion, and the polymer matrix has a strong influence on the diffusivity as the motion of a small molecule is restricted by the three-dimensional network of polymer chains. Release rates were increased when the concentration of *A. vera* gel increased in the formulations. This is because as the proportion of this gel in the matrix increased; there was an increase in the amount of water uptake and hydration of the polymeric matrix and thus more drugs were released.

From the *in vitro* drug release profiles, it can be shown in Figures 3-5 that the films which contain less amount of penetration enhancers showed the least drug release. This explains the fact that penetration enhancement was

Table 5: *In vitro* permeability of candesartan cilexetil from formulation (A11)

Time (h)	Concentration (µg/ml)	Cumulative amount of drug permeated (µg)	Cumulative percentage of drug permeated	Cumulative amount of drug permeated (µg/cm²)	Flux (µg/cm²/h)
1.0	18.1	1808.0	11.3	268.2	195.6
2.0	30.7	3072.0	19.2	455.8	
3.0	43.7	4368.0	27.3	648.1	
4.0	57.1	5712.0	35.7	847.5	
5.0	73.0	7296.0	45.6	1082.5	
6.0	87.4	8736.0	54.6	1296.1	
7.0	103.0	10304.0	64.4	1528.8	
8.0	116.0	11600.0	72.5	1721.1	
10.0	138.6	13856.0	86.6	2055.8	
12.0	158.1	15808.0	98.8	2345.4	

Table 6: *In vitro* permeability of candesartan cilexetil from formulation without *Aloe vera*

Time (h)	Concentration (µg/ml)	Cumulative amount of drug permeated (µg)	Cumulative percentage of drug permeated	Cumulative amount of drug permeated (µg/cm²)	Flux (µg/cm²/h)
1.0	0.9	88.0	0.6	13.1	30.56
2.0	1.4	135.2	0.8	20.1	
3.0	2.0	198.4	1.2	29.4	
4.0	2.5	247.2	1.5	36.7	
5.0	2.9	292.8	1.8	43.4	
6.0	5.4	540.0	3.4	80.1	
7.0	6.5	650.0	4.1	96.4	
8.0	8.8	879.0	5.5	130.4	
10.0	18.7	1870.0	11.7	277.4	
12.0	23.4	2336.0	14.6	346.6	

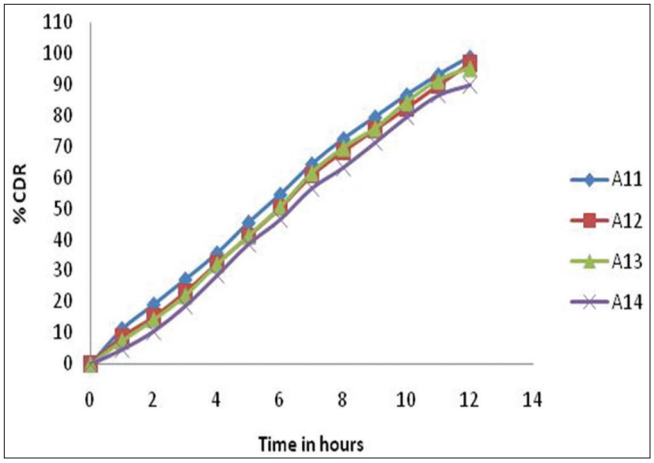

Figure 5: *In vitro* drug release profile of films (A11-A14)

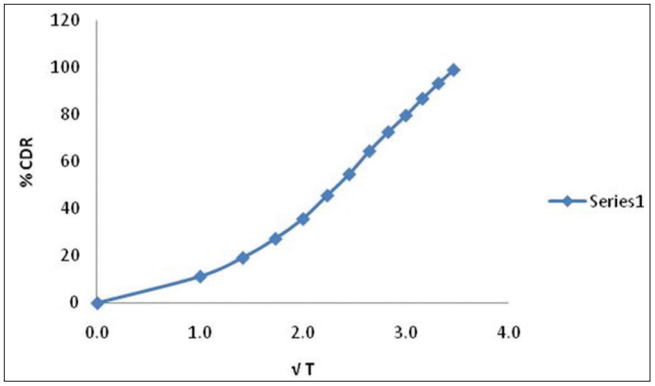

Figure 6: Higuchi plot for formulation A11

required for drug release. The cumulative amounts of drug permeated per square centimeter of release (zero order release model, $R^2 = 0.896$) following diffusion mechanism from the matrix system (Higuchi square root time plot, $R^2 = 0.976$, Figure 6 and Table 4). The diffusion process follow anomalous non Fick's diffusion (Fick's plot, slope $n = 0.826$), i.e., the rate of solvent penetration and drug release are in the same range. This deviation is due to increased drug diffusivity from the matrix by the solvent induced relaxation of the polymer. Various release parameters for drug release of candesartan with and without penetration enhancer are shown in Tables 5 and 6.

CONCLUSION

From this study, it can be concluded that the films of CC could be prepared by solvent casting technique having suitable mechanical properties. The *in vitro* drug release and *ex vivo* skin permeation performance shows that the candesartan could be administered transdermally through the matrix type TDDS for effective control of hypertension and thus enhance the bioavailability. *Ex vivo* permeation of candesartan (formulation A11) shows that patches of ERL 100: HPMC with *A. vera* gel are more effective in delivering candesartan to that of formulation without *A. vera* gel, therefore, the formulation A11 was selected as the best formulation for delivering candesartan through transdermal patch containing *A. vera* as penetration enhancer.

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Source of Support: Nil. **Conflict of Interest:** None declared.