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RESEARCH PAPER

Design and Evaluation of Matrix Diffusion Controlled Transdermal Patches of Verapamil Hydrochloride

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ABSTRACT

Transdermal patches of verapamil hydrochloride were prepared using four different polymers (individual and combination): Eudragit RL100 (ERL100), Eudragit RS100 (ERS100), hydroxypropyl methylcellulose 15 cps (HPMC), and ethyl cellulose (EC), of varying degrees of hydrophilicity and hydrophobicity. The effect of the polymers on the technological properties, i.e., drug release, water vapor transmission rate (WVTR), and percentage moisture loss (ML), percentage moisture absorption (MA), folding endurance, and thickness, was investigated. Different formulations were prepared in accordance with the 2^3 factorial design, with ERL100 being the parent polymer. The patch containing ERL100 alone showed maximum WVTR, % MA, and % ML, which could be attributed to its hydrophilic nature. As expected, substitution with ERS100, HPMC, and EC decreased all the above values in accordance with their decreasing degree of hydrophilicity. In vitro release studies showed zero-order release of the drug from all the patches, and the mechanism of release was diffusion mediated. Moreover, the release of the drug was sustained and it extended over a period of 24 hr in all formulations. A12 emerged as the most satisfactory formulation insofar as its technological properties were concerned. Further, release and permeation of the drug from the most satisfactory formulation (A12) was evaluated through different biological barriers (shed snake skin, rabbit skin, and rat skin) to get an idea of the drug permeation through human skin. Shed snake's skin was found to be most permeable (82.56% drug release at 24 hr) and rat skin was least permeable (52.38%). Percutaneous absorption studies were carried out in rabbits. The pharmacokinetic parameters calculated from blood levels of the drug revealed a profile typical of a sustained release formulation,

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with the ability to maintain adequate plasma levels for 24 hr. [AUC: 3.09 mg/mL hr, C_{max}: 203.95 μg/mL, T_{max}: 8 hr]. It can therefore be concluded that the patch containing ERL100 and HPMC in the ratio 8:2 has achieved the objectives of transdermal drug delivery system, such as avoidance of first pass effect, extended release, and reduced frequency of administration.

INTRODUCTION

The concept of delivering drugs through the skin for systemic treatment of diseased states is gaining increasingly great importance due to its numerous advantages.[1] Several important advantages of transdermal drug delivery are limitation of hepatic first pass metabolism, enhancement of therapeutic efficiency, prolonged duration of action of potent drugs with short plasma half-life, and maintenance of steady plasma level of the drug. However, transdermal delivery is limited to drugs having low doses, low melting points and molecular weights, and a solubility of greater than 1 mg/mL in both water and mineral oil.

Verapamil hydrochloride is a calcium ion influx inhibitor. It is widely used in the treatment of angina, hypertension, and supraventricular tachyarrhythmias.[2] The plasma half-life of verapamil hydrochloride is 2–7 hr, which necessitates multiple dosing. It is approximately 90% absorbed from the gastrointestinal tract but is subject to considerable first pass metabolism and its bioavailability is around 20–30%.[3] In order to improve the bioavailability of verapamil hydrochloride and reduce its frequency of administration, it was proposed to make transdermal patches of the drug. It is suitable for transdermal delivery owing to its low molecular weight (491.07) and low melting point (144°C).[4] Both water soluble polymer like hydroxypropyl methylcellulose (HPMC) and water insoluble polymers, ERL 100, ERS 100, and ethylcellulose (EC), were selected as carriers into which the drug was incorporated. The present study is aimed at the design of matrix dispersion type transdermal systems followed by the evaluation of their technological properties [effect of polymer ratios on patch thickness, water vapor transmission rate (WVTR), percentage moisture loss (ML), percentage moisture absorption (MA), drug content, folding endurance, in vitro drug release] and in vivo studies (in rabbits) including evaluation of pharmacokinetic parameters.

MATERIALS AND METHODS

Verapamil hydrochloride B.P was obtained from JRSF Ltd., Bangalore. Eudragit RL 100 (15 cps) and Eudragit RS 100 (15 cps) were procured from Rohm Pharma, West Germany. HPMC (15 cps) and EC (20 cps) were purchased from SD fine chemicals, Boisar. Eudragit RL and RS are copolymers of acrylic and methacrylic acid esters containing 10 and 5% trimethylammonium methacrylate chloride, respectively. The RL polymer swells more than RS due to its higher concentration of hydrophilic quarternary groups. RL films are more permeable than RS films. HPMC is a film former and is soluble in cold water, whereas EC forms films which are insoluble in water. The polymers selected were nontoxic and nonabsorbable and they did not lose their film forming properties when formulated with the drug and the excipients. Sodium acetate G.R. anhydrous was procured from E-Merck, Mumbai. All other chemicals such as acetonitrile, solvent ether, dibutyl phthalate, anhydrous calcium chloride, aluminum chloride, potassium dihydrogen phosphate, sodium hydroxide, and hydrochloric acid were of laboratory grade.

Preparation of Transdermal Films

The films were cast on mercury surface contained in a petri dish using fabricated glass rings. The rings had a diameter of 5.8 cm and 5 mL capacity. The required amount of drug was dissolved in ethanol and respective polymers were added to it (Table 1). To this, the plasticizer dibutyl phthalate (30% w/w of the polymer) was added and stirred well to get a homogeneous solution. The volume was made up to 5 mL with ethanol and it was pipetted onto the mercury surface contained in a glass ring so that it formed a well.

The films were dried for a period of 48 hr, and the rate of evaporation was controlled by inverting a funnel over the petri dish. The dried films were stored

Table 1. Transdermal films: polymers and composition.

	Formulation code							
Polymers	A11	A12	A13	A14	A15	A16	A17	A18
	Parts							
Eudragit RL 100	10	8	8	8	8	8	8	8
Eudragit RS 100	—	—	2	—	1	—	1	0.66
HPMC	—	2	—	—	1	1	—	0.66
EC	—	—	—	2	—	1	1	0.66

Note: Plasticizer, dibutyl phthalate (30% w/w of the polymer). The drug content in 2 cm^2 of the film is 120 mg.

in a desiccator for a week until further evaluation. There was no change in the physical appearance or texture of the films after storage.

Physicochemical Properties of the Films

The films were evaluated for the following physicochemical properties:

- Percentage moisture absorption: The films were weighed accurately and placed in the desiccator containing 100 mL of saturated solution of aluminum chloride, which maintains 79.50% RH. After 3 days, the films were taken out and weighed. The percentage moisture absorption was calculated using the formula

$$\text{Percentage moisture absorption: } \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

- Percentage moisture loss: The films were weighed accurately and kept in a desiccator containing anhydrous calcium chloride. After 3 days, the films were taken out and weighed. The moisture loss was calculated using the formula

$$\text{Percentage moisture loss: } \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

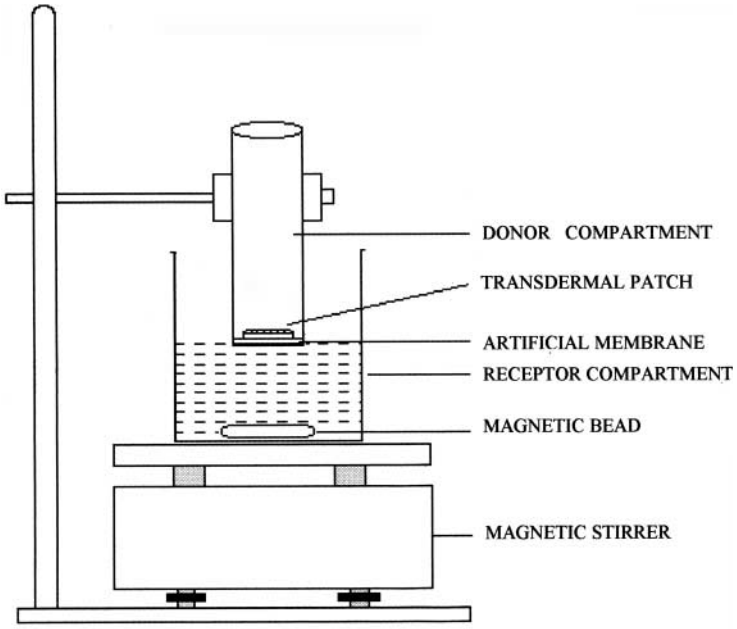
- Water vapor transmission rate: Glass vials of equal diameter were used as transmission cells. These transmission cells were washed thoroughly and dried in an oven. About 1 g anhydrous calcium chloride was placed in the cells and the respective polymer film was fixed over the brim. The cells were accurately weighed and kept in a closed desiccator containing saturated solution of potassium chloride to maintain a humidity of 84%. The cells were taken out and weighed after 6, 12, 24, 36, 48, and, 72 hr of storage. The amount of water vapor transmitted was found using the formula.

$$\text{Water vapor transmission rate: } \frac{\text{Final weight} - \text{Initial weight}}{\text{Time} \times \text{Area}}$$

  Water vapor transmission rate is usually expressed as the number of grams of moisture gained/hr/cm^2.
- Thickness: The thickness of the film was measured at three different points using a screw gauge and average thickness recorded.
- Folding endurance: 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.
- Drug content: A film of size 2 cm^2 was cut into small pieces and put in a 100-mL buffer (pH 7.4). This was then shaken in a mechanical shaker for 2 hr to get a homogeneous solution and filtered. The drug was determined spectroscopically at 278 nm after suitable dilution.

In Vitro Drug Release Studies

Commercial semipermeable membrane was employed in the study as the permeation barrier. The membrane used was transparent and regenerated cellulose which was permeable to low molecular weight substances. A film of size 2 cm^2 and semipermeable membrane was mounted carefully between the donor and receptor compartment and secured.



DIFFUSION CELL

Volume of receptor medium:	50 mL
pH of the receptor medium:	7.4
Area of the transdermal patch used for the study:	2 cm^2
Temperature of the study:	$32 \pm 2^{\circ}C$
Degree of turbulence in the receptor medium:	45 rpm

Figure 1.

The donor compartment was empty and open to the atmosphere, but the receptor compartment contained 50 mL buffer of pH 7.4. The contents of the receptor compartment were maintained at $32 \pm 2^{\circ}C$ and stirred at 45 rpm by placing it on a magnetic stirrer (Fig. 1). Samples of 1 mL were withdrawn from receptor compartment every hour and replaced with equal volumes of fresh receptor medium, and the concentration of the drug permeated was determined spectrophotometrically at 278 nm after suitable dilution.

Treatment of In Vitro Data to Obtain Mathematical Constants

The following parameters were determined from the in vitro data obtained for the release of verapamil hydrochloride through semipermeable membrane.

- Kinetics of permeation
- Diffusion coefficient
- Flux
- Permeability
- Mechanism of release

In Vitro Diffusion Studies Using Different Animal Skins

Release and diffusion of verapamil through various biological barriers was investigated to get an idea of drug penetration through human skin. The study was carried out with the most promising formulation (A12) through shed snake skin, rat skin, and rabbit skin using the same assembly as used for in vitro studies. Animals (rat and rabbit) were sacrificed using chloroform anesthesia. Freshly excised rat

and rabbit abdominal skin was used for the studies. After excision, the skin was thoroughly washed to remove the adhering tissue and fat, trimmed to the required size, and used as the permeation barrier. However, shed snake skin was procured and used without any further treatment as permeation barrier.

Pharmacokinetic Evaluation of the Films on Animals

Rabbits have been used as models for bioavailability studies among the various animal models. The study was conducted on six rabbits. Before the commencement of the experiment, the skin of every rabbit was thoroughly examined for any abnormality and only those having no structural abnormality of the skin were selected.

The skin of around 50 cm^2 was shaved covering both sides of the vertebral column of each rabbit and care was taken to avoid damage to the skin during shaving. Before application of the patches, rabbits were kept for 24 hr under observation for any untoward effects of shaving and they were fasted over this period. Only rabbits weighing between 1.2 and 1.5 kg were selected for the study. The dose of verapamil hydrochloride was calculated according to the body weight, i.e., 4 mg was selected for in vivo studies (minimal variation in the weights of individual rabbits). The patch was affixed on the shaved dorsal surface and blood samples were collected at hour 1, 2, 4, 8, 16, and 24 after patch application from the marginal ear vein. Before collection of the blood samples, the syringe and the test tubes were rinsed with a dilution of the anticoagulant heparin. The blood samples were centrifuged at 5000 rpm and the plasma separated and refrigerated until analyzed.[6]

High Pressure Liquid Chromatographic Analysis

Verapamil was assayed by an HPLC procedure employing a C-18, 100 × 4.6 mm column. The HPLC apparatus was fitted with a SPD-10A UV-Vis detector, LC-10AD pump, and a C-R7A Plus integrator from Shimadzu-Japan.[7] The mobile phase was a mixture of 60:40 v/v mixture of acetonitrile and 0.1 N sodium acetate buffer and a flow rate of 1.5 mL/min was used. Drug was analyzed at a wavelength of 278 nm. Sodium hydroxide (0.2 mL of 2 M solution) and solvent ether (5 mL) were added to the plasma and the solutions swirled for 2 min followed by centrifugation for 15 min at 1500 rpm. The organic layer was separated and the extraction procedure was repeated with the plasma layer as described above except for the addition of sodium hydroxide. The pooled organic phases were dried under a gentle stream of nitrogen at 40°C. The residue was dissolved in 0.5 mL of mobile phase and vortexed for 1 min just before the injection (20 μL) into the column. The concentrations of the samples were calculated from a standard graph prepared from peak area vs. concentrations of verapamil.

Skin Irritation Studies

Skin irritation studies were carried out on six healthy rabbits weighing 1.2 to 1.5 kg. The dorsal surface (50 cm^2) of the rabbits was cleared and the hair was removed by shaving. The skin was cleared with rectified spirit. The patches (A12) were placed over the skin with the help of adhesive tape. They were removed after 24 hr and the skin was examined for any untoward reaction. No signs of erythema, edema, or ulceration was observed.

Stability Studies

Accelerated stability testing was conducted for 30 days at different temperatures: 4, 45, and 60°C. At specific intervals of time (Day 5, 10, 15, 20, 25, and 30), patches were taken out to assay their drug content, appearance, and texture.

RESULTS AND DISCUSSION

The formulations were prepared according to the 2^3 factorial design. Hence, eight different formulations were obtained with Eudragit RL100 being the parent polymer. The physicochemical properties of the patches are recorded in Table 2. The thickness of the patches varied from 0.37 to 0.48 mm. The drug content analysis of the prepared formulations have shown that the process employed to prepare films in this study was capable of giving films with uniform drug content and minimum batch variability.

The patch formulated with Eudragit RL100 alone showed maximum %WVTR, %MA, and %ML, which can be attributed to its hydrophilic nature. As expected, substitution of Eudragit RL100, with HPMC, Eudragit RS100, and EC decreased the

Table 2. Physicochemical properties of transdermal films.

Formula code	WVTR ($g/cm^2/hr$) $\pm$ SD[a]	% MA $\pm$ SD[a]	% ML $\pm$ SD[a]	Thickness $\pm$ SD (mm)[a]	Folding endurance[a] (No. of folds)	Drug content (mg) $\pm$ SD[a]
A11	$4.731 \times 10^{-4} \pm 0.0002$	15.752 ± 2.1342	6.172 ± 1.5105	0.43 ± 0.0608	6	119.33 ± 0.8563
A12	$2.077 \times 10^{-4} \pm 0.0001$	10.730 ± 1.7363	4.112 ± 1.0256	0.46 ± 0.04	4	119.33 ± 1.1467
A13	$1.653 \times 10^{-4} \pm 0.0005$	8.143 ± 1.2492	4.018 ± 0.9982	0.45 ± 0.03	5	118.60 ± 0.3736
A14	$1.905 \times 10^{-4} \pm 0.0003$	6.113 ± 0.7128	2.314 ± 0.3630	0.37 ± 0.0265	2	118.66 ± 1.0967
A15	$1.592 \times 10^{-4} \pm 0.0003$	7.349 ± 1.3106	3.756 ± 0.5249	0.42 ± 0.0529	6	118.30 ± 1.0623
A16	$2.071 \times 10^{-4} \pm 0.0005$	9.176 ± 1.3100	3.595 ± 1.2746	0.46 ± 0.0173	4	119.33 ± 0.4230
A17	$1.076 \times 10^{-4} \pm 0.0002$	4.113 ± 1.9662	1.672 ± 0.6326	0.48 ± 0.0529	4	119.33 ± 0.7301
A18	$0.974 \times 10^{-4} \pm 0.0004$	3.710 ± 0.7518	0.793 ± 0.1603	0.42 ± 0.02	3	119.30 ± 0.5813

[a]Average $\pm$ SD of three determinations has been reported.

Table 3. In vitro release of verapamil hydrochloride from transdermal films.

Time (hr)	Cumulative % drug release $\pm$ SD							
	A11	A12	A13	A14	A15	A16	A17	A18
2	10.38 ± 0.20	4.78 ± 0.15	4.77 ± 0.18	5.51 ± 0.84	4.82 ± 0.08	6.33 ± 0.40	4.21 ± 0.42	4.71 ± 0.15
4	18.01 ± 0.78	12.91 ± 0.52	11.49 ± 1.04	12.43 ± 0.38	12.48 ± 0.50	12.28 ± 1.11	8.09 ± 0.32	9.88 ± 1.52
6	25.22 ± 0.89	19.53 ± 0.75	19.31 ± 0.39	18.85 ± 0.86	19.95 ± 0.44	19.11 ± 0.29	12.84 ± 0.36	15.4 ± 2.27
8	30.06 ± 0.61	27.05 ± 0.64	25.32 ± 0.84	25.60 ± 0.52	25.06 ± 0.59	27.11 ± 0.72	20.20 ± 0.72	22.55 ± 2.38
10	36.76 ± 0.20	35.34 ± 1.36	30.14 ± 0.58	32.22 ± 1.24	32.36 ± 1.47	33.72 ± 0.13	28.79 ± 0.23	29.46 ± 0.55
12	41.65 ± 0.14	41.69 ± 0.26	37.61 ± 0.53	37.66 ± 1.06	37.99 ± 0.53	40.49 ± 1.35	33.58 ± 0.07	35.94 ± 2.20
14	45.68 ± 0.47	49.24 ± 0.64	42.12 ± 0.05	42.08 ± 0.18	42.41 ± 0.14	47.40 ± 2.34	41.22 ± 0.16	41.01 ± 1.08
16	57.84 ± 1.46	55.59 ± 1.28	49.32 ± 1.90	53.58 ± 2.08	50.42 ± 1.84	56.99 ± 0.84	45.06 ± 0.48	45.08 ± 3.86
18	66.78 ± 0.50	65.94 ± 0.49	59.86 ± 1.68	62.38 ± 0.85	56.48 ± 1.06	65.78 ± 0.72	53.08 ± 0.96	51.43 ± 3.36
20	79.22 ± 1.14	72.64 ± 0.96	72.35 ± 0.72	70.11 ± 1.60	65.35 ± 0.49	74.59 ± 0.17	60.48 ± 1.47	61.04 ± 3.80
22	92.29 ± 0.16	81.62 ± 1.36	80.08 ± 0.49	76.16 ± 1.29	78.03 ± 0.49	82.56 ± 1.92	69.56 ± 1.45	69.00 ± 2.79
24	94.08 ± 0.56 (23rd hr)	94.02 ± 0.64	87.68 ± 0.83	89.36 ± 0.42	86.33 ± 0.49	89.26 ± 0.42	77.80 ± 1.47	77.11 ± 1.82

[a]Average $\pm$ SD of three determinations has been reported.

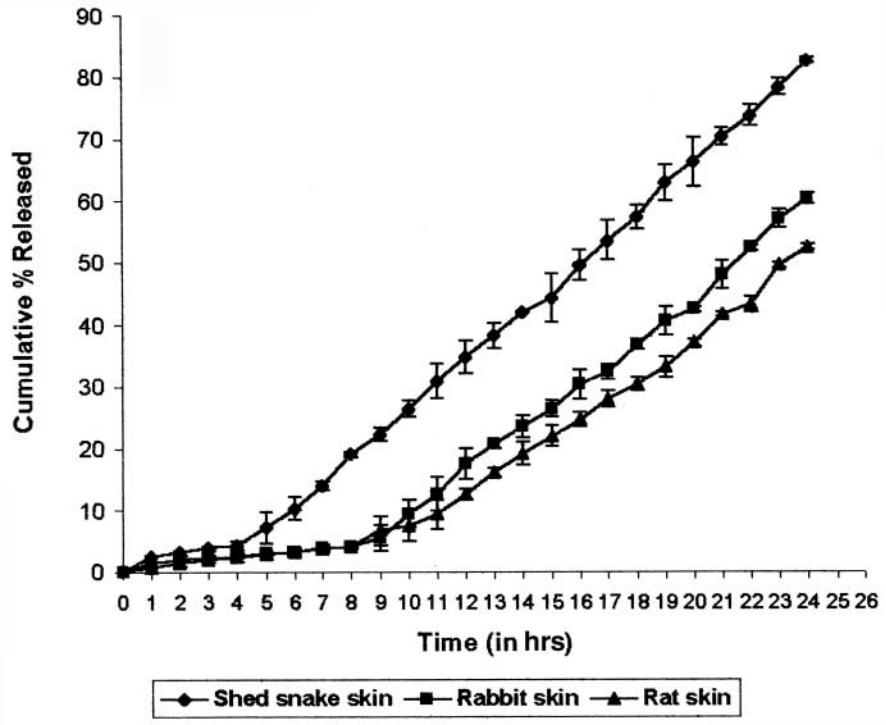
values of percentage water vapor transmission rate, percentage moisture absorption, and percentage moisture loss in accordance with their reducing hydrophilic nature. The patch having all the polymers showed the least water vapor transmission, %MA, and %ML.

Drug release from in vitro diffusion studies are indicated in Table 3. All the formulations showed zero-order release and mechanism of release was diffusion mediated (Higuchi plot). Regression analysis of the in vitro permeation curves was carried out. The slope of the straight line obtained after plotting the mean cumulative amount released per patch vs. $\sqrt{}$time was taken as the experimental flux for verapamil. The flux obtained for all the formulations was in the range of 9–12 mg/hr, but formulation A12 showed not only the maximum flux, i.e., 11.930 mg/hr, but it also exhibited sustained release up to 24 hr. Although the other formulations also exhibited release for 24 hr, the flux obtained was lesser than that of A12. Incidentally, A11 showed a flux similar to A12 but the release extended only up to 23 hr. It was therefore decided to carry out further studies with formulation A12. The results are presented in Table 4. The diffusion coefficient was calculated according to the modified Higuchi's equation.[8] Formulation A12 had the maximum diffusion coefficient and flux. The above results indicate the effect of polymers, their combinations, and concentrations on the technological properties of the films.

Table 4. Release kinetics of verapamil diffusion from transdermal patches.

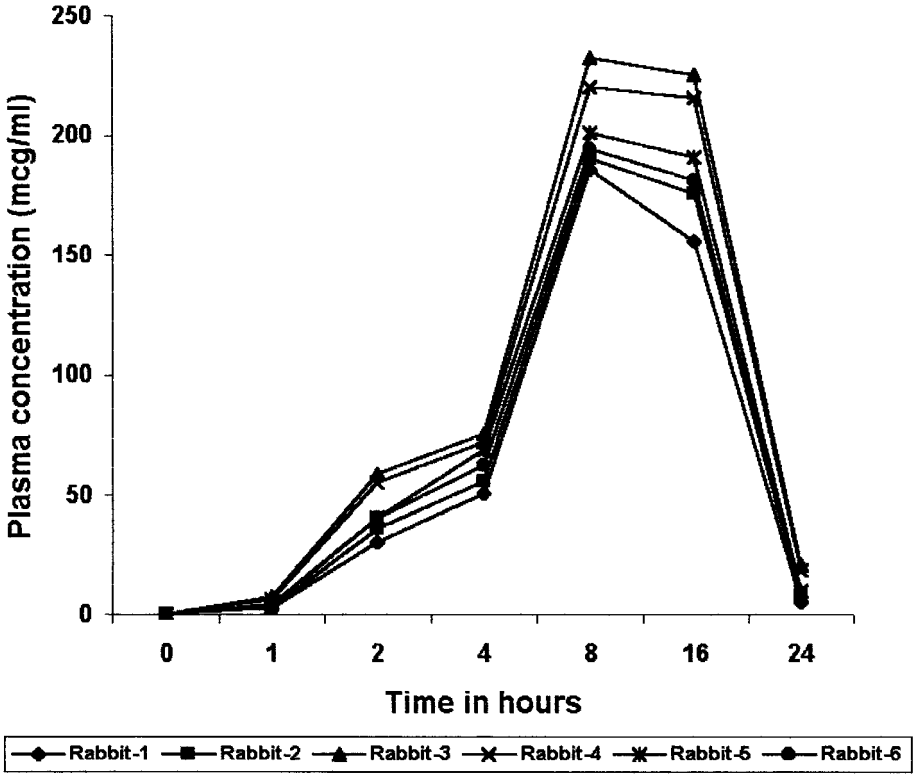
Formula code	Kinetics of drug release	Mechanism of drug release (diffusion)	Diffusion coefficient (cm^2/hr) ± SD	Flux ($mg/cm^2/hr$) ± SD	Permeability (cm/hr) ± SD
A11	Zero order	Swelling type	$7.55 \times 10^{-3} \pm 0.0002$	11.316 ± 0.255	3.801 ± 0.180
A12	Zero order	Non-Fickian type	$7.22 \times 10^{-3} \pm 0.0001$	11.93 ± 0.057	3.836 ± 0.181
A13	Zero order	Non-Fickian type	$6.28 \times 10^{-3} \pm 0.0005$	11.278 ± 0.117	3.684 ± 0.212
A14	Zero order	Swelling type	$6.53 \times 10^{-3} \pm 0.0002$	11.200 ± 0.178	3.638 ± 0.125
A15	Zero order	Non-Fickian type	$6.09 \times 10^{-3} \pm 0.0003$	10.784 ± 0.144	3.487 ± 0.168
A16	Zero order	Non-Fickian type	$6.51 \times 10^{-3} \pm 0.0005$	11.775 ± 0.100	3.817 ± 0.103
A17	Zero order	Non-Fickian type	$4.94 \times 10^{-3} \pm 0.0002$	10.045 ± 0.072	3.260 ± 0.065
A18	Zero order	Swelling type	$4.86 \times 10^{-3} \pm 0.0003$	9.842 ± 0.867	3.181 ± 0.162

Note: Diffusion coefficient, flux, and permeability were calculated taking all 24 hr into consideration. The flux was calculated from the slope of the straight line obtained after plotting the mean cumulative amount released per cm^2 vs. $\sqrt{}$time.



Graph 1. Permeation of verapamil through biological membranes.

Literature review indicates that there was a decrease in the drug release as the concentration of polymer increased when verapamil hydrochloride was formulated as a transdermal patch using sodium carboxymethyl guar as polymer matrix.[9] It is also indicated in the literature that the permeation of the drug across guinea pig skin can be enhanced using propylene glycol as permeation enhancer.[10] Before carrying out in vivo studies, permeation studies were carried out using biological barriers like rat skin, shed snake's skin, and rabbit skin with the formulation A12. Graph 1 shows permeation kinetics of verapamil through biological barriers. Maximum drug release was obtained through shed snake skin (82.56%) and least was obtained through rat skin (52.38%) after 24 hr.



Graph 2. Blood level profiles of verapamil from transdermal films in rabbits.

A good correlation was shown for drug release from formulation A12 through shed snake skin and artificial membrane ($r = 0.9962$).[11]

Percutaneous absorption studies were carried out using rabbits as animal models. Graph 2 shows blood levels of verapamil in rabbits. Pharmacokinetic parameters were calculated from the plasma concentration of the drug and recorded in Table 5. Measurable concentrations of the drug were obtained within an hour of application of the patch and the drug persisted in the blood of the rabbit up to the 24th hour. This indicated that the release was sustained for a period of 24 hr. The maximum drug concentration was obtained at the 8th hour. C_{max} obtained in rabbits was 203.9513 µg/mL. The minimum effective concentration of verapamil hydrochloride in humans is 120 ± 0.20 ng/mL. In view of the encouraging results obtained in rabbits, it can be predicted that the required minimum effective concentration could be achieved in humans in spite of the greater barrier properties of human skin when compared to rabbit skin. However, further studies should be carried out on human volunteers.

Table 5. Mean pharmacokinetic parameters of verapamil from transdermal patches.

S. no	Parameters[a]	Values obtained in rabbit plasma[b]
1	C_{max} (mg/mL)	0.2039 ± 0.0186
2	T_{max} (hr)	8 ± 0.0000
3	K_e (hr^{-1})	0.0656 ± 0.0146
4	AUC ($0 \rightarrow 24$) (mg/mL hr)	3.091 ± 0.388
5	AUC ($0 \rightarrow \infty$) (mg/mL hr)	3.647 ± 0.4115
6	MRT (hr)	11.5737 ± 1.7228

[a]The parameters were calculated from blood levels of the drug in rabbits.
[b]Average ± SD of six rabbits has been reported.

It can therefore be concluded that the formulation A12 has achieved the objectives of the transdermal drug delivery system, i.e., extended release and reduced frequency of administration, and has also avoided the first pass effect. Skin irritation studies carried out on six rabbits revealed that the formulation A12 shows no erythema, edema, and ulceration.

Table 6. Accelerated stability studies.

	4°C		45°C		60°C	
Time in days	R.D.C.[a] (mg)	P.A.	R.D.C.[a] (mg)	P.A.	R.D.C.[a] (mg)	P.A.
0	119.33 ± 0.307	+	119.33 ± 0.307	+	119.33 ± 0.307	+
5	119.25 ± 0.128	+	119.22 ± 0.211	+	117.80 ± 0.197	+
10	119.00 ± 0.076	+	118.98 ± 0.076	+	116.20 ± 0.096	+
15	118.80 ± 0.106	+	118.60 ± 0.069	+	115.80 ± 0.122	+
20	117.85 ± 0.189	+	117.61 ± 0.102	+	114.70 ± 0.115	−
25	116.90 ± 0.080	+	116.70 ± 0.067	+	112.80 ± 0.053	−
30	115.80 ± 0.067	+	113.80 ± 0.056	+	111.603 ± 0.06	#

Abbreviations: R.D.C.: remaining drug content; P.A.: physical appearance; +: good, transluscent; −: hard; #: rigid, brittle.
[a]Average ± SD of three determinations.

Accelerated stability studies (Table 6) indicated that the shelf life of the above formulation was 177 days at 25°C.[12] It is therefore preferable to store the patches in the refrigerator. In view of the encouraging results obtained, further studies can be carried out using the formulation A12 on healthy human volunteers.

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