TABLE 2: EVALUATION OF PHYSICAL PROPERTIES OF BUCCOADHESIVE CUPS.

Formulation	Weight variation* (%)	Thickness* (mm)	Depth of the core* (mm)	Hardness* kg/cm²	Friability* %	Biodhesive strength* (g)
A	0.79±0.18	1.93±0.05	1.80±0.04	6.93±0.25	0.40±0.05	22.22±2.81
B	0.77±0.10	2.50±0.05	2.00±0.08	9.20±0.16	0.31±0.06	44.76±3.66
C	0.80±0.25	2.06±0.48	1.92±0.24	10.00±0.17	0.25±0.05	39.76±1.98
D	0.79±0.13	2.00±0.00	1.90±0.36	7.86±0.09	0.38±0.03	23.29±4.73

* indicates mean of three estimations with standard deviation. Table summarizes the evaluation of the cups prepared with different ratios of carbopol and HPMC K4M viz., 1:0, 1:1, 1:2 and 0:1, respectively which are indexed as formulations A, B, C and D.

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In vitro Permeation of Verapamil Hydrochloride From Polymeric Membrane Systems Across Rat and Human Cadaver Skin

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In this article various polymeric membrane systems of poly vinyl pyrrolidone, ethyl cellulose, Eudragit RS100 and ethylene vinyl acetate, containing verapamil hydrochloride, along with glycerol and dibutyl phthalate as plasticizers have been fabricated for transdermal use. Both monolithic and membrane controlled systems were prepared by the method of casting on mercury surface and evaluated for thickness uniformity, drug content uniformity, tensile strength, Percentage of elongation and skin irritation. *In vitro* drug permeation through rat abdominal skin and human cadaver skin was performed using Keshary-Chien diffusion cells. Results indicated that, the order of permeation of the drug from different polymeric membranes was poly vinyl

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pyrrolidone>ethyl cellulose>Eudragit RS100>ethylene vinyl acetate. The drug release mechanism from all monolithic systems was diffusion controlled, where as membrane controlled systems followed nearly zero order kinetics, as the thickness of rate controlling membrane was increased from 100 to 200 μ, the drug release was decreased. Compared to rat skin, a low permeation rate of the drug was observed through human cadaver skin, this indicates meeting the target flux in rat skin does not guarantee it's good permeability in human skin.

In last few decades, novel drug delivery systems have been developed to deliver antihypertensive drugs into the body in order to maintain constant drug level in the blood, to reduce the frequency of administration and to avoid excess drug loading in the body[1-3]. Other than oral controlled release formulations, antihypertensive drugs can be delivered into the body using skin as a port of drug administration to provide continuous and constant drug infusion into systemic circulation[4]. With renewed interest in the transdermal therapy, more and more researchers are trying to develop systems suitable for dermal delivery of drugs[5-8]. It is exemplified by the development and marketing of scopolamine containing transdermal system for 72 h prophylaxis of motion induced nausea[9], nitroglycerine system for once a day medication of angina pectoris. Verapamil hydrochloride, a calcium channel blocker, is wildly used in the treatment of angina and hypertension[10]. It is 90 % absorbed from GIT, but its bioavailability is only 10-20 % indicating extensive first pass metabolism in liver[11]. In view of substantial first pass effect and it's shorter plasma half-life, verapamil hydrochloride is selected for incorporating in a transdermal drug delivery system.

The present research is concerned with fabrication of transdermal monolithic and membrane controlled systems, their characterization, and *in vitro* drug release studies through rat abdominal skin and human cadaver skin (selected formulae).

Gift sample of verapamil hydrochloride IP (VPH) was obtained from Torrent Pharmaceuticals, Ahmedabad. The polymers such as Eudragit RS100 (ED) was obtained from Rohm Pharma GmbH, Germany, poly vinyl pyrrolidone, K-30, mol wt 40,000 (PVP) and ethyl cellulose of 20 cps and ethoxy content 49.5 % (EC) were purchased from Loba Chemie Pvt. Ltd. Mumbai and ethylene vinyl acetate, 2806 (EVA) was obtained from Polyolefin Industries Ltd., Mumbai.

TABLE 1: DATA OBTAINED FROM EVALUATION OF POLYMERIC MEMBRANES.

Formulations	Drug content* (mg/cm²)	Tensile Tensile strength* (N/mm²)	Percentage of elongation*	Water vapour transmission rate* (g.cm/cm².24 h)	Skin irritation
PVP (M)	0.756 (0.31)	0.6898	9.89	--	--
EC (M)	0.753 (0.33)	0.0543	7.87	3.415×10^{-5}	-
ED (M)	0.754 (0.19)	0.3833	0.19	1.370×10^{-5}	+
EVA (M)	0.756 (0.41)	4.7157	650	2.286×10^{-6}	+
EC 100R	0.755 (0.28)	0.2626	13.99	7.110×10^{-5}	--
EC 200R	0.754 (0.25)	0.7441	17.77	4.618×10^{-5}	--
Ed 100R	0.755 (0.21)	0.9708	8.25	6.040×10^{-5}	--
EVA 100R	0.754 (0.23)	1.7533	417.8	7.181×10^{-6}	+

Data obtained from evaluation of various polymeric membrane systems, PVP (M), EC (M), ED (M) and EVA (M) are monolithic systems of poly vinyl pyrrolidone, ethyl cellulose, Eudragit RS 100, and ethylene vinyl acetate respectively. EC 100R, ED 100R, EVA 100R are membrane controlled systems (with RCM thickness of 100 μ) of ethyl cellulose, Eudragit RS 100 and ethylene vinyl acetate respectively, where as EC 200R is membrane controlled system of ethyl cellulose with RCM thickness of 200 μ. * Indicates values are average of three determinations, + indicates very slight erythema and -- indicates no erythema.

Method of casting on mercury surface was employed for the preparation of monolithic systems[12]. Eight percent w/v of PVP, EC, ED, and EVA were dissolved in alcohol, chloroform: dichloromethane (1:1), chloroform (at room temperature) and toluene (at 85°), respectively. Twenty milligrams of VPH was loaded into each formulation. Glycerol at a concentration of 12.5% w/w of dry polymer for PVP, dibutyl phthalate 50% w/w for EC and 15% w/w for ED were used as plasticizers, where as no plasticizer was added to EVA system. Polymeric solutions were mixed thoroughly with the help of magnetic stirrer, and then 5 ml was poured within a glass bangle (5.4 cm diameter) placed on the surface of mercury in a petridish. The rate of evaporation was controlled by inverting the cut funnel over petridish. After drying at room temperature for 24 h, membranes were taken out and stored in desiccator.

For the preparation of membrane controlled systems, drug free films of EC, ED and EVA were used as rate controlling membranes, PVP was used as the drug reservoir and aluminum foil as backing membrane. EC, ED, and EVA (in a concentration of 4% w/v) were dissolved in respective solvents along with plasticizers and they were casted on mercury surface to get the films of 100 μ thickness. Then 8 % w/v of PVP was dissolved in distilled water along with glycerol, and 5 ml of this solution was poured over previously prepared rate controlling membranes and dried in an oven at 45° for 5 h. After drying one surface of the drug reservoir matrix was slightly moistened with distilled water and a slightly oversized aluminum foil was placed on it and allowed to dry at room temperature for 24 h. Then dried systems were taken out and stored in desiccator. The fabricated membrane systems were evaluated for estimation of VPH in membrane systems[13], water vapor transmission (WVT) studies[14], tensile strength, percentage of elongation, skin irritation[13] and *in vitro* drug release.

Tensile strength and percentage of elongation were determined using a Universal Testing Machine (Shimadzu, Singapore). Films of 15 mm width and 40 mm length were cut and fixed to the machine jaws. Then load on the films was increased gradually to a maximum, at a speed of 50 mm/s and tensile strength and percentage of elongation was noted.

Vertically assembled Keshary-Chien diffusion cells (20 ml) were used for the study. The magnetic stirrer was set at 100 rpm, distilled water was used as receptor solution and whole assembly was maintained at 37±2°. The transdermal system of 3.631 cm^2 area was mounted on the donor com-

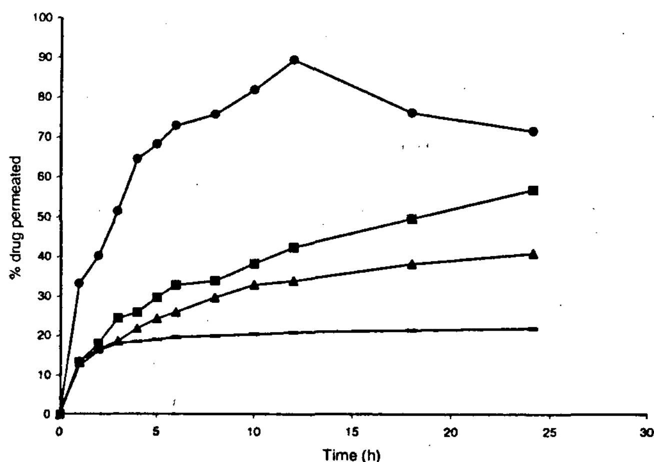

Fig. 1: Permeation profiles of verapamil hydrochloride from monolithic systems across rat skin.

Permeation profiles of verapamil hydrochloride across rat skin from monolithic systems of PVP (-●-), EC (-■-), ED (-▲-) and EVA (-). Study was carried out in Keshary-Chien diffusion cell at 37±2°, samples were withdrawn at regular intervals of time and analyzed spectrophotometrically at 229 nm.

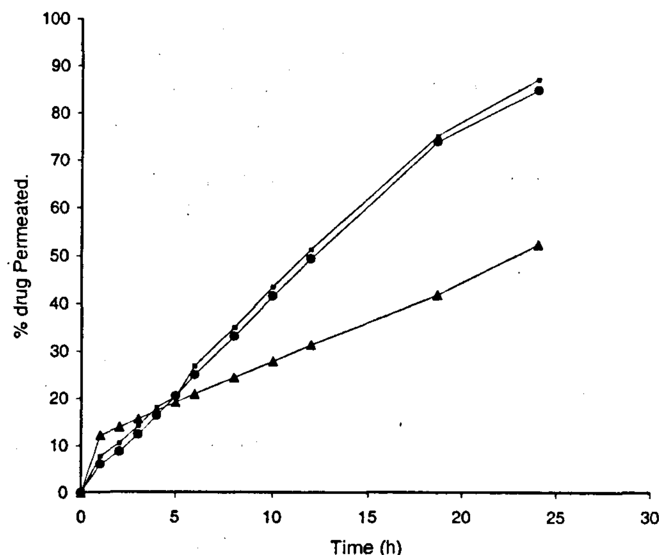

Fig. 2: Permeation profiles of verapamil hydrochloride from membrane controlled systems.

Permeation profiles of verapamil hydrochloride across rat skin from membrane controlled systems of EC 100R (-■-), ED 100R (-●-), EVA 100R (-▲-), Study was carried out in Keshary-Chien diffusion cell at 37±2°, samples were withdrawn at regular intervals of time and analyzed spectrophotometrically at 229 nm.

partment and then rat abdominal skin/human cadaver skin was fixed to the donor compartment with the help of an adhesive. The amount of drug release was determined by withdrawing 5 ml of samples at specific time intervals for 24 h, volume withdrawn was replaced with equal volume of fresh prewarmed (37°) distilled water, samples were analyzed in a UV spectrophotometer (Hitachi U-2000) at 229 nm[15].

Hair from the abdominal region of Wistar rats weighing between 150-200 g was carefully removed without damaging the skin. The dermal side of the excised skin was thoroughly cleaned of adhering tissues or blood vessels. The transdermal system was placed in intimate contact with stratum corneal side and was fixed to donor compartment of diffusion cell[13].

Cadaver skin of 23-25 y old male was obtained from Government Hospital, Raichur, samples of skin were removed from the abdomen of the cadavers within 48 h after death. Epidermal layers were separated from the remaining skin by immersing each skin section in water at 60° for 30 s. The epidermis was teased from dermis using forceps, the separated epidermal layer was used as such for the skin permeation studies[16].

The prepared membranes were smooth, thin and flexible. Thickness and drug contents were found to be uniform. All the films were permeable to water vapors and water vapor transmission (WVT) followed nearly zero order kinetics and it was decreased in the order of PVP>EC>ED>EVA. EVA membrane showed maximum tensile strength (TS) and percentage of elongation, EC and Ed showed low TS and percentage of elongation, it was in the order of EVA >PVP>ED>EC. Test films produced very slight erythema compared to control, there was no evidence of edema, and it indicated the skin acceptability of these test films for topical application. The release of drug from monolithic systems was decreased in the order of PVP>EC>ED>EVA. It was obvious from the permeation profiles that, PVP system offered very low resistance to the movement of the drug, PVP film showed maximum release of 89 % at the end of 12 h, a high permeation rate was observed in the initial hours and concentration gradient couldn't be maintained at the later hours. Fall of permeation rate is likely the result of drug depletion in the donor compartment. The permeation of drug from EC, ED and EVA films was 56.7, 40.8 and 21.9 %, respectively at the end of 24 h. Permeation profiles of these three films indicates that, the control of drug release was influenced by the characteristics of the polymers rather than stratum corneum, the slow release rate from these films may be attributed to relatively hydrophobic nature of polymers. The mechanism of drug release from all monolithic systems was found to be diffusion controlled.

Membrane controlled systems were fabricated with an aim to achieve constant release of drug. Rate controlling membranes and their thickness play a major role in controlling the drug release. We prepared PVP based systems containing EC, ED and EVA as rate controlling membranes

TABLE 2: DATA OBTAINED FROM *IN VITRO* PERMEATION OF VERAPAMIL HYDROCHLORIDE ACROSS RAT SKIN.

Formulations	Type	Thickness (μ)*	Diffusion Rate Constant** (mg/cm²/h)	Permeability coefficient** (mg/h. cm)
PVP (M)		150 (0.15)	4.8394×10^{-2}	1.5993×10^{-6}
EC (M)	Monolithic	200 (0.25)	1.5939×10^{-2}	8.7793×10^{-7}
ED (M)	Systems	200 (0.35)	1.1124×10^{-2}	6.1272×10^{-7}
EVA (M)		200 (0.26)	4.3294×10^{-3}	2.3846×10^{-7}
EC100R	Membrane	250 (0.18)	2.8337×10^{-2}	1.7189×10^{-6}
EC200R	controlled	350 (0.18)	1.9010×10^{-2}	1.6832×10^{-6}
ED 100R	Systems	250 (0.23)	2.8137×10^{-2}	1.7048×10^{-6}
EVA100R		250 (0.21)	1.3790×10^{-2}	8.3550×10^{-7}

Data obtained from evaluation of thickness uniformity and *in vitro* permeation across rat skin.
*Indicates values are average of five observations, **indicates values are average of three observations and figures inside the parenthesis are standard deviation (sd) values.

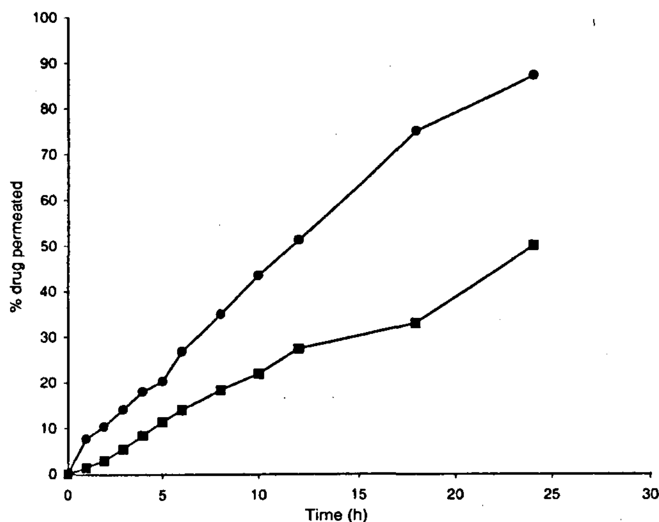

Fig. 3: Permeation of verapamil hydrochloride from EC 100R system through rat and human cadaver skin.

Permeation of verapamil hydrochloride from EC 100R system through rat skin (-●-) and human cadaver skin (-■-).

(RCM). The percentage of drug release from EC 100R, ED 100R, and EVA 100R was 87.0, 86.3 and 52.3 %, respectively at the end of 24 h. The permeation profiles of membrane controlled systems followed nearly zero order kinetics. Though an initial burst release was observed with EC 100R and EVA 100R, then the linearity was observed in the permeation profiles. There was a deviation from the zero order release with EC 100R and ED 100R systems at the end of 24 h may be due to exhaustion of the drug in the system. As long as excess drug concentration is maintained in the reservoir, a constant release is assured, once the system nears exhaustion of the drug, concentration gradient falls and deviation from zero order occurs. An increase in RCM thickness of EC system from 100 to 200 μ reduced the drug permeation rate appreciably. Further we carried out *in vitro* drug release from EC 100R system across human cadaver skin, the drug permeation across human cadaver skin was 50.08 %, which is lesser than the permeation across rat skin. Previously drug permeation was thought to takes place through two parallel pathways only, that is lipid and pore pathway[17], however a recent study about the mechanism of drug permeation through human skin revealed that lateral bilayer diffusion is the primary transport mechanism[18], *in this, drugs of low molecular weight travel through narrow* lipid slits that literally separates the keratinocytes. Hence it shows strong size dependence (< 300 Da), the poor permeability of the drug VPH in human cadaver skin could be related with its high molecular weight (491 Da). This study confirms the hypothesis of Zesch[19] that, data from animals are not transferable to man.

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