each addition. After the final addition of hydrogen peroxide the solution (pale yellow) was cooled and 5.0 ml per chloric acid was added and heated at 180° for 1 h. The watch glass was removed and heated until approximately 1 ml remained in the beaker. The solution was cooled for 1min and 2 ml hydrogen peroxide was taken, adding 1 ml at a time. After each 1 ml addition the solution was kept on the hot plate until effervescence has ceased. The solution was cooled and 15.0 ml deionised water was added. It was gently heated for up to 5 min to get a clear solution. The solution was allowed to cool and transferred to the volumetric flask and diluted with deionised water to the required level and then the sample was analyzed by ICP-MS. A 100 PPb multi element mixture (NPL-100) was used as the calibration standard and also as an unknown. NIST standard 1643 b that was a highly dependable standard was also run along with the sample. Ruthenium and bismuth were used as internal standards and so the values cannot be used.

The sample, which was analyzed, contained a number of elements at different concentration as reported in Table 2. Some metals like V, Cu, Pb, Sr, Ba, Zn, Cd, Fe, As, Ni, Se, Bi were found to be present in concentrations higher than the acceptable levels of the same metals in drinking water (assuming a person consumes 2 l of water a day). As the marketed ayurvedic preparation is intended for use in infants and young children, proper care should be taken to keep the concentration of the above mentioned elements at low levels. Since it is true that plants grown in polluted water, polluted atmosphere, hills and rocky areas take up a number of elements, it is necessary that the medicinal plants should be grown in a good environment.

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In vitro Permeation of Ionized and Unionized Diclofenac: Comparison of Chitosan Membranes with Rat Skin

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To predict permeability of drugs through the skin chitosan membranes have been formulated. The *in vitro* permeability of ionized and unionized diclofenac through chitosan membranes has been compared with that of dorsal skin of Wistar rats. The chitosan membranes were prepared by cast drying to achieve thickness of hairless-dorsal-skin of Wistar rats. The thickness of chitosan membrane varied with change in concentration of chitosan and sodium tripolyphosphate. The

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chitosan membrane has been observed to mimic the flux of ionized and unionized diclofenac through rat skin. Chitosan membranes are easy to prepare with controlled composition and reproducibility.

The noninvasive delivery of drugs through the skin increases patient acceptability by avoiding first-pass metabolism and gastrointestinal disturbances[1]. The transdermal drug delivery systems used for noninvasive delivery of drugs are evaluated *in vitro* and *in vivo* by estimating permeation through human and animal skin. The experiments with skin involve ethical considerations and because of limited availability of skin, the experiments are expensive and time consuming. To predict the permeability of drugs through the skin, researchers have developed polymeric membranes as models for the skin[2-5]. The polymeric membranes of controlled composition can be prepared easily and reproducibly[6].

Chitosan[7], a natural hydrophilic polymer, has well known film-forming property. The authors have formulated chitosan membranes to mimic the barrier properties of skin. The *in vitro* permeability of ionized and unionized diclofenac through chitosan membranes has been compared in the present study with that of dorsal skin of Wistar rats.

The chitosan membranes were prepared by cast drying[5]. The solution of chitosan (2-5% w/v in 10 mM acetic acid) was homogenized using Teflon pestle at 2000 rpm and filtered through cheesecloth to remove debris. The chitosan solution was degassed and the membranes were casted on polycarbonate petridish to achieve thickness of hairless-dorsal-skin of Wistar rats. The membranes were dried at 60° for 40 h and stored in polyethylene bags till use. The chitosan membranes were treated with sodium tripolyphosphate (10 ml of 3-12% w/v) solution for 3-40 min to crosslink chitosan membranes. The counter ion diffuses into the polymeric film and the amino group of chitosan reacts with negative group of sodium tripolyphosphate forming the intermolecular or intramolecular linkage[8]. The chitosan membranes were washed thrice with water to remove excess of tripolyphosphate. Freshly crosslinked membranes that retained flexibility and integrity for more than 24 h were used for *in vitro* permeation study.

To prepare animal skin for permeation study the dorsal skin of Wistar rats was shaved with mechanical hair clipper and subsequently, a non-irritating depilatory cream was applied to remove any remaining fur. The rat was sacrificed after 24 h and the dorsal skin was excised. After removing the adhering fat, visceral debris and washing with physiological saline, the whole dorsal skin was clamped on diffusion-cell with stratum corneum facing the donor compartment. The skin was stabilized for 4 h in the presence of phosphate buffer (pH 7.4) before *in vitro* permeation study. The animal experiment protocol described here has met with the approval of the Institutional Animal Ethics Committee.

Vertical Franz-diffusion-cell apparatus was used to study the permeability of diclofenac (ionized and unionized) employing infinite dose technique[9]. The apparatus assembly consisted of clamped stabilized rat skin or crosslinked chitosan membrane onto glass diffusion-cell (3.63 cm^2) between donor and receptor compartments. In the receptor compartment phosphate buffer pH 7.4 (25 ml) was stirred (600 rpm) and maintained at 37±1°[10]. The donor compartment contained saturated solution of diclofenac in phosphate buffer pH 7.4 (ionized diclofenac) or in PEG 4000 (unionized diclofenac). The aliquots (1 ml) withdrawn at various intervals were immediately analyzed for drug concentration spectrophotometrically at 276 nm, directly or after appropriate dilution with phosphate buffer. The volume of receptor compartment was replenished with phosphate buffer.

The permeation parameters such as flux and lag time (Table 1) were calculated from the linear portion of graph (cumulative amount of diclofenac permeated versus time) and intercept of the extrapolated linearity on the time axis, respectively. The thickness of chitosan membrane varied with change in concentration of chitosan and sodium tripolyphosphate. The chitosan membranes H1 and H9 exhibited minimum difference from rat skin with respect to flux and lag time respectively of ionized diclofenac. A point-to-point correlation was established for cumulative permeation of ionized diclofenac through membrane H9 and rat skin (fig. 1). The chitosan membranes H1 and H9 showed minimum difference from rat skin with respect to flux and lag time respectively of unionized diclofenac (Table 2). A point-to-point correlation was established for cumulative permeation of unionized diclofenac through membrane H9 and rat skin (fig. 2).

The chitosan membrane (H9) has been observed to mimic the flux of ionized and unionized diclofenac through

TABLE 1: FORMULATION OF CHITOSAN MEMBRANES AND PERMEATION PARAMETERS OF IONIZED DICLOFENAC THROUGH RAT SKIN AND CHITOSAN MEMBRANES (H1-H15)

Membrane code	X (% w/v)	Y (% w/v)	Z (min)	Flux* X 10^{-5}±S.D. ($mg/cm^2.s$)	Thickness* X 10^{-1}±S.D. (cm)	Lag time* X 60±S.D. (s)
H1	3.0	5.0	10	6.95±0.016	0.650±0.010	5.88±0.031
H2	3.0	5.0	30	7.29±0.023	0.693±0.006	4.66±0.014
H3	3.0	10.0	10	6.60±0.004	0.723±0.006	8.11±0.011
H4	3.0	10.0	30	6.21±0.015	0.743±0.006	6.66±0.013
H5	4.0	5.0	10	5.76±0.008	0.823±0.006	3.65±0.003
H6	4.0	5.0	30	5.55±0.008	0.837±0.006	10.21±0.025
H7	4.0	10.0	10	5.17±0.019	0.857±0.006	6.62±0.009
H8	4.0	10.0	30	4.55±0.004	0.857±0.006	6.76±0.004
H9	3.5	12.0	20	4.61±0.005	0.867±0.006	10.17±0.011
H10	3.5	3.5	20	5.74±0.016	0.683±0.006	5.44±0.009
H11	4.5	7.5	20	4.55±0.012	0.923±0.006	10.29±0.016
H12	2.5	7.5	20	5.79±0.015	0.703±0.006	5.71±0.007
H13	3.5	7.5	40	5.30±0.001	0.840±0.006	7.19±0.003
H14	3.5	7.5	05	5.43±0.001	0.797±0.006	9.89±0.004
H15	3.5	7.5	20	4.84±0.005	0.833±0.006	7.30±0.009
Rat skin	-	-	-	4.66±0.012	0.710±0.006	5.99±0.007

X represents concentration of chitosan, Y indicates concentration of sodium tripolyphosphate and Z shows cross-linking time. *values are expressed as mean ±S.D. (n=6).

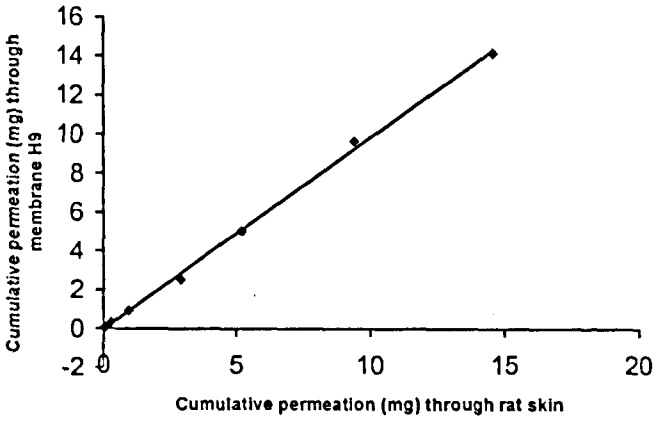

Fig. 1: *In vitro* permeation profile of ionized diclofenac through rat skin versus membrane H9.

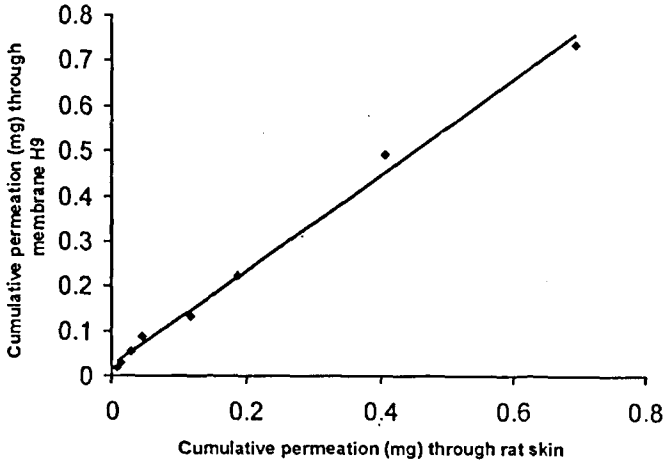

Fig. 2: *In vitro* permeation profile of unionized diclofenac through rat skin versus membrane H9.

TABLE 2: PERMEATION PARAMETERS OF UNIONIZED DICLOFENAC THROUGH RAT SKIN AND CHITOSAN MEMBRANES (H1 AND H9).

Membrane code	Flux* X 10^{-6}±S.D. ($mg/cm^2.s$)	Thickness* X 10^{-1}±S.D. (cm)	Lag time* X 60±S.D. (s)
H1	2.75±0.008	0.65±0.010	14.67±0.005
H9	2.22±0.013	0.87±0.006	28.76±0.009
Rat skin	2.14±0.007	0.71±0.006	15.00±0.003

*Values are expressed as mean± S.D. (n=6).

rat skin, however lag time in case of membrane H9 was more than that of rat skin. The increased lag time can be attributed to difference in thickness of chitosan membrane (H9) and rat skin. A composite membrane can be designed to match lag time, the top layer consisting of membrane H1 and the bottom layer of membrane H9. On the basis of above findings it is concluded that optimized chitosan membranes can be used to imitate skin barrier in the evaluation drug permeation from transdermal drug delivery systems.

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