Research Paper

Synthesis and Characterisation of Chitosan conjugate; Design and Evaluation of Membrane Moderated Type Transdermal Drug Delivery System

B. K. SATHEESHABABU* AND R. SHRUTHINAG
Department of Pharmaceutics, Government College of Pharmacy, Bengaluru-560 027, India

The purpose of the present research investigation was to synthesis, characterisation of chitosan conjugates and its effect on drug permeation from transdermal rate controlling membrane. Chitosan conjugate was synthesised by conjugation with thioglycolic acid. The prepared chitosan conjugate was characterised by determining the charring point, Fourier transmission-infrared and differential scanning calorimetric analysis. The rate controlling membranes were prepared by various proportions of chitosan and chitosan conjugate, to moderate drug permeation through rate controlling membrane. The membrane moderated transdermal system consists of reservoir to hold the drug gel was prepared by 20% w/v ethylcellulose with a cavity in its center. An impermeable backing layer was prepared by 2% w/v ethylcellulose. Gel consists of carvedilol was prepared by sodium alginate and sodium carboxymethylcellulose in ethanol:water solvent system The rate controlling membranes prepared were evaluated by various parameters like thickness, folding endurance, swelling index, moisture content, moisture uptake, water vapor transmission rate, tensile strength test, measurement of gel strength, *in vitro* permeation study, *ex vivo* permeation study, compatibility study using differential scanning calorimetry and stability studies. All physical parameters evident that prepared membranes have good folding endurance and sufficient tensile strength. As the proportion of chitosan conjugate increases in membrane swelling index, moisture content, moisture uptake and permeability coefficient increases. The gel strength of chitosan conjugate was considerable less compared with chitosan.

Key words: Chitosan conjugate, membrane moderated transdermal system, rate controlling membrane

The moderated transdermal system essentially consists of rate controlling membrane, reservoir, impermeable backing membrane and drug loaded gel. The diffusion behavior of rate controlling membrane will be moderated by preparing rate controlling membrane with various proportions of chitosan and chitosan conjugate. These devices have following advantages over the oral route, in long term treatment it improves patient compliance, no hepatic first-pass metabolism, reduction in side effects, offers sustain release of the drugs, maintains constant and prolonged drug level in blood and minimizing inter and intra patient variability and interruption of treatment when necessary[1,2].

Carvedilol, a non-cardioselective beta blocker is a drug of choice for the management of hypertension, its absorption through the gastrointestinal tract well but under goes considerable first-pass metabolism in the liver and its absolute bioavailability is about 25% and needs repeated dosing this leads in increased side effects[3,4]. Due to above pharmacokinetic properties of the drug leads to develop a formulate membrane moderated transdermal system of carvedilol using chitosan and chitosan conjugate[5]. The main objectives of the present research work were synthesis, characterisation of chitosan conjugates and to prepare a rate controlling transdermal membranes by various proportions of chitosan and chitosan conjugate to study the effect of conjugation on permeation of drug from transdermal rate controlling membrane.

***Address for correspondence**
E-mail: bksatishbabu@gmail.com

Accepted 26 July 2015
Revised 21 January 2015
Received 03 July 2014
Indian J Pharm Sci 2015;77(4):405-412

MATERIALS AND METHODS

Carvedilol was received as a gift sample from Dr. Reddy's Laboratories Limited Hyderabad, India. Chitosan and sodium alginate were purchased from Sigma-Aldrich Chemicals, USA, Carbodiimide hydrochloride and thioglycolic acid were purchased from S.D. Fine Chem. Ltd., India, Sodium CMC was purchased from Loba Chemie Pvt. Ltd., India. All other chemicals used were of analytical grade.

Synthesis and characterisation of chitosan conjugates:

The synthesis of chitosan conjugates involved following procedure, initially 5 g of chitosan was hydrated in 40 ml of 1 M HCl to get a 1% solution of chitosan hydrochloride in demineralized water. To activate the carboxylic acid group of the chitosan hydrochloride, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC) was added in a final concentration of 50 mM. To this mixture subsequently 3.95 ml of thioglycolic acid (TGA) was added for conjugation with chitosan, the pH of the reaction mixture was maintained at 9 by adding 1 M NaOH solution. The resulting mixture was incubated under permanent stirring for 3 h at room temperature to facilitate conjugation between chitosan and TGA. After incubation period was over the reaction mixture was subjected for dialysis to remove any unbound TGA and to obtain a polymeric conjugates, in tubing's for 3 days at 10º in the dark against 5 mM HCl. To reduce the ionic interactions between the cationic polymer and the anionic sulfhydryl compound, the reaction mixture was subjected dialysis against same medium containing 1% NaCl for 2 times. Later polymeric conjugates were vacuum dried and stored in desiccator[6].

The synthesised chitosan-TGA conjugates were characterised by determining the charring point, Fourier transform-infrared (FT-IR) spectroscopic study and differential scanning calorimetric (DSC) analysis. To determine charring point for the chitosan and conjugate, the 5 mg sample was placed in one end sealed glass capillary tube then this tube was kept in melting point apparatus. The temperature was gradually increased and temperature at which the sample completely charred was noted as the charring point in degree. For FT-IR studies the both the samples were prepared in the form of KBr pellets and analysed in the scanning range from 4000 to 400cm^{-1} in FT-IR spectrophotometer (FT-IR-5300, JASCO, Japan). The DSC analysis was done by using differential scanning calorimeter (DSC-60, Shimadzu, Japan). Approximately 2 mg of both the sample were placed in an aluminum pan sealed by aluminum cap under nitrogen atmosphere. The samples were scanned from 0-400° at scanning rate of 10°/min.

Compatibility study:

To establish the compatibility between drug and polymer the physical mixture (1:1) were subjected to DCS analyses. The conditions for the study were same as mentioned in characterisation.

Preparation of membrane moderated type transdermal drug delivery system:

The membrane moderated transdermal drug delivery system essentially consists of the following components, rate controlling membranes, reservoir for placing the gel, backing membrane to seal the one side of the reservoir to facilitate unidirectional drug diffusion through the rate controlling membranes and gel containing drug.

Preparation of rate controlling membranes using chitosan conjugates:

The rate controlling membranes were prepared by using various proportions of chitosan and chitosan conjugate as mentioned in Table 1. The polymers were dissolved in 35 ml of 2% lactic acid solution by vigorous stirring to get a homogenized solution. To make polymeric solution free from entrapped air bubbles solution was subjected to sonication. Polyethylene glycol (PEG) 400 (10%) was added as plasticizer. The rate controlling membranes were obtained by casted on circular glass mould and allowed to dry over a level surface in hot oven at temperature of 40º for 48 h[7].

Preparation of transdermal reservoir:

The transdermal reservoir was prepared using ethylcellulose. The polymeric solution 20% was

TABLE 1: FORMULATION OF RATE CONTROLLING MEMBRANES

Code	Chitosan conjugate	Chitosan	Drug	PEG 400
J-1	105	595	70	70
J-2	210	490	70	70
J-3	350	350	70	70
J-4	490	210	70	70
J-5	595	105	70	70

Values are given in mg. J-1 to J-5 are formulation codes. PEG: Polyethylene glycol

prepared in ethanol: isopropyl alcohol (1:1) where the polymer was first dissolved, along with (10% w/w) plasticizer di-butyl phthalate. From this solution, 35 ml was then poured into a polyurethane coated glass petridish. The solvent was allowed to evaporate at room temperature for 48 h to get 2.5 mm thick monolithic layer. The reservoir was made in this layer by punching the adhesive layer using suitable punch having the diameter 1.5 cm to form a reservoir of depth 2.5 mm[7].

Preparation of adhesive membrane and impermeable backing membrane:

Impermeable backing membrane was casted by ethyl cellulose. The polymeric solution 2% was dissolved in ethanol:isopropyl alcohol (1:1) along with (10% w/w) plasticizer di-butyl phthalate. From this solution, 35 ml was then poured into a glass mould. The solvent was allowed to evaporate at room temperature for 48 h and this impermeable backing membrane was stick to the bottom of the reservoir to facilitate the unidirectional diffusion through the rate controlling membrane which was stick to the other side of the reservoir after placing the gel within the reservoir well[7].

Preparation of transdermal gel:

The transdermal gel was prepared using carvedilol (2% w/w), sodium alginate and sodium CMC (3% w/w) in ethanol:water solvent system (1:1). Polymers were soaked in water to form a solution. Carvedilol dissolved in ethanol was added to this solution, along with continuous stirring to form a gel[8]. The prepared gel 500 mg was placed by syringe and reservoir was closed by placing a rate controlling membrane, through which the drug was allowed to permeate.

Folding endurance:

To measure the folding endurance capacity of the prepared rate controlling membranes a modified United States Pharmacopoeia (USP) tablet disintegrating tester was employed. The modification was done for USP tablet disintegrating tester such way that it consisted of fixed and movable jaws that could be moved up and down at the rate of 30 strokes/min. The gap between the 2 jaws at their farthest and closest was 6 cm and 0.5 cm, respectively. The membrane (7 cm length) was clamped between the jaws in such a way that the jaws were at their closest, the membrane bends across its middle and when at their farthest, the membrane was in a stretched condition. Thus for every stroke of the movable jaw the membrane went through one cycle of bending and stretching. The folding endurance is expressed as the number of strokes required to either break or develop visible cracks on the membrane. The test was conducted for 30 min equating 600 strokes[9].

Swelling index:

The rate controlling membranes cut into 3 cm^2 were weighed accurately and placed on the plate containing 2% w/w of agar. The each rate controlling membranes were weighed at regular time intervals until they showed a constant weight[8]. The swelling index (SI) of each patch was calculated using the following equation: Percentage of $SI=(W_2-W_1)/W_1\times100$.

Percentage of moisture content and moisture uptake:

The specimen of rate controlling membrane used for the above studies was of size 3 cm^2. For moisture content samples were pre weighed and placed in desiccator filled with fused calcium chloride at room temperature for 24 h. Each samples were weighed regularly until they attain constant weight. Percent of moisture content was calculated as the difference between initial and final weight with respect to final weight. Percent *t* moisture content=(initial weight–final weight)/final weight×100. For moisture uptake study the samples were placed in desiccator with desiccant for 24 h at room temperature to remove any residual moisture could be their within the membranes. Then samples were exposed to 84% RH (a saturated solution of potassium chloride in desiccator) until they attain a constant weight. The percentage of moisture uptake was calculated as the difference between final and initial weight with respect to initial weight. Percentage of moisture uptake=final weight-initial weight/initial weight×100[8].

Water vapor transmission study:

To assess the water vapor permeability through the rate controlling membranes, the samples of the size 3.142 cm^2 were fixed over the brim of a glass vials, consists of 2 g of fused calcium chloride as desiccant. The vials were weighed and placed 84% RH. For period of 7 days the vials were taken out and note down the weight at every 24 h intervals. The flux, i.e., the amount of water vapor transmitted through 1 cm^2/24 h and permeability coefficient were calculated using the formula $P=slope/p\times24$, where P is permeability coefficient and '*p*' is the

vapor pressure of saturated solution of potassium chloride[10,11].

Tensile strength and percent elongation:
The tensile strength and percent elongation of rate controlling membranes was measured with house field universal machine (Tinius Olsen Ltd, England). This machine had two load cell jaws, the upper one is movable and lower one fixed. Sample of specific size specific size (4 cm×1 cm) was fixed between these grips and upper jaw was moved at a speed of 100 mm/min applying force gradually until the membrane break. The tensile strength of the membrane was taken directly from the dialed reading in kilogram and extension of membrane in millimeter.

Gel strength:
Gel strength of the chitosan and conjugated chitosan was determined using locally fabricated instrument, having free moving piston with pointed conical tip (tip length-10 mm; tip angle-60°) along with the provision to apply the load over the piston. The 10% w/v gels of both the polymers were prepared individually using 4% v/v hydrochloric acid as a solvent. The homogenized gel was filled in sample holder and stored below 10° in a refrigerator for 24 h. The gel strength of the polymer was determined by placing the piston tip over gel surface and the load was applied over the piston at a constant rate by adding the water using i.v. infusion set at a constant flow rate (100 ml/min). The load required to pierce the piston tip up to 4 mm in the gel was taken as the gel strength of that polymer. The temperature of the gel was maintained below 10° throughout the study[12].

***In vitro* skin permeation study:**
The *in vitro* skin permeation study of the rate controlling membranes were carried out in a modified Franz diffusion cell having donor compartment, receptor compartment having capacity of 16 ml and effective diffusion area of 1.5 cm^2 and receptor compartment had a water jacket for temperature controlling and sampling port. The donor and receptor compartment were separated by dialysis membrane-110 above this the transdermal reservoir was placed such way that the rate controlling membrane facing toward the dialysis membrane. The receptor compartment was filled with ethanol and phosphate buffer pH 7.4 (1:1) to its capacity. The whole assembly was fixed on a magnetic stirrer, and the solution in the receptor compartment was constantly and continuously stirred using magnetic beads at 50 rpm. The care was taken during complete diffusion study the dialysis membrane was always contact with the diffusion medium of the receptor compartment. The aliquots were withdrawn at different time intervals up to 24 h and same volume was replenished by fresh diffusion medium. The absorbance of withdrawn samples was measured at 241.5 nm using U.V spectrophotometer. During throughout the diffusion study the temperature of the receptor compartment was maintained at 37±0.5º by circulating hot water inside the water jacket[13].

***Ex vivo* permeation studies:**
For *ex vivo* permeation studies the Wistar albino rat's skin epidermis consists of stratum corneum was excised from dorsal surface after obtaining approval from Institutional Animal Ethics Committee (IAEC) of National College of Pharmacy, Shivamogga. (REG. NO.144/1999 date: 05/07/1999 under the rules 5(a) of the "Breeding of and experiments of animals (control and supervision) rules 1998"). The same procedure was followed as explained above and the dialysis membrane was replaced by rat's skin epidermis containing stratum corneum.

Stability study:
The stability study indicates the capacity of any formulation in a suitable package, to maintain within its physical, chemical, therapeutic and toxicological specifications throughout its shelf period. The best rate controlling membrane was considered for stability study for 2 months the conditions were maintained according to International Conference on Harmonistion (ICH) guidelines. The membrane was packed in aluminum foils, which were kept in wide mouth bottle closed tightly. They were then stored at 40º/75% RH for 2 months and evaluated for their permeation study.

RESULT AND DISCUSSION

The DSC thermogram of pure carvedilol showed a sharp endothermic peal at 116° which is the melting point of the pure drug. The DSC thermogram of carvedilol with sodium alginate and sodium CMC demonstrated negligible change in the melting point of carvedilol (105°), which evidenced that the polymer do not interact with the drug. The DSC thermograms of carvedilol and carvedilol with sodium

alginate, sodium carboxymethylcellulose are shown in fig. 1.

To charcterise the prepared chitosan conjugate was subjected to following studies, charring point of chitosan and chitosan conjugate were found to be 258° and 190°, respectively. This might be due to conjugation of TGA with chitosan. The FT-IR spectra of chitosan conjugate, showed the bands representing the -C=O stretching of amide bond (at 1521.97 cm^{-1}) and -SH stretching (at 2058.23 cm^{-1}) which were absent in the FT-IR spectra of the chitosan. The additional peaks in the FT-IR spectra of the chitosan conjugate have confirmed the conjugation of chitosan with TGA[14]. The FT-IR spectra of chitosan and chitosan conjugate are shown in fig. 2. The DSC thermogram of chitosan conjugate, have shown one extra endothermic peak at 202.12° represented the thiol moiety, which was absent in the DSC thermogram of chitosan. This has further confirmed the conjugation of chitosan with TGA. The DSC thermograms of chitosan and chitosan conjugate are shown in fig. 3.

The folding endurance was measured in triplicate; the results were shown in Table 2. Swelling index was determined by keeping patches in an agar gel plate containing 2% agar. The percentage swelling index was calculated as the difference between wet weight and initial weight with respect to wet weight. The results of the swelling index studies for different formulations are shown in Table 2 and fig. 4. The moisture content was determined by keeping membranes in a desiccator containing fused calcium chloride. The percentage moisture uptake was determined by exposing the membranes to 84% RH, both moisture content and percentage moisture uptake were calculated as the difference between initial and final weight with respect to final weight. The results of the moisture content studies and percentage moisture uptake for different membranes are shown in Table 2 and fig. 5. The permeation coefficient of different membranes was found by water vapour transmission test and the results for different membranes were shown in Table 2 and fig. 6. The tensile strength was found to be highest in membrane J3 and lowest in J5. The results of percent elongation and tensile strength are shown in Table 2. Gel strength of the chitosan was found to be 237.85±1.16 g whereas the conjugated chitosan showed gel strength of 4.672±0.02 g (n=3). The

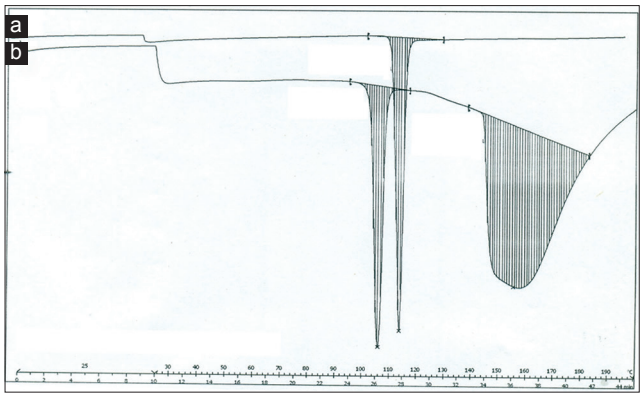

Fig. 1: DSC thermograms.
Differential scanning calorimetric (DSC) thermogram of (a) carvedilol and (b) carvedilol with sodium alginate and sodium carboxymethylcellulose.

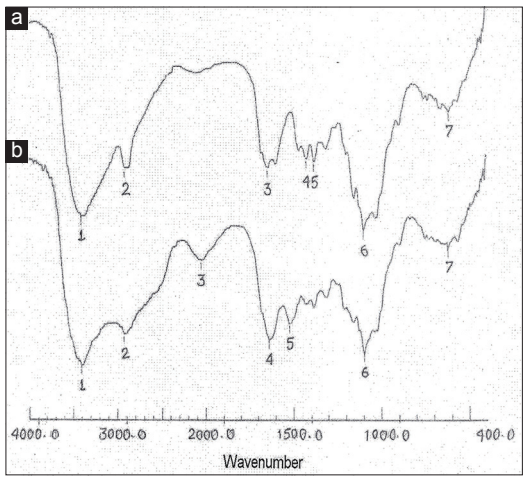

Fig. 2: FT-IR spectra.
Fourier transform-infrared (FT-IR) spectra of chitosan (a) and conjugated chitosan (b).

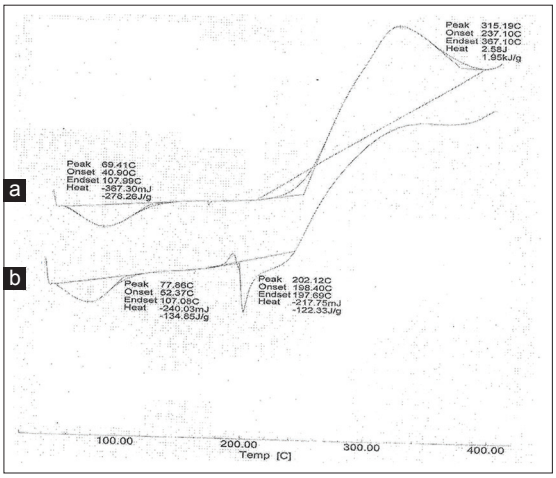

Fig. 3: DSC thermograms of chitosan and formulation.
Differential scanning calorimetric (DSC) thermograms of chitosan (a) and conjugated chitosan (b).

in vitro permeation studies are predictive of *in vivo* performance of a drug. These studies were performed

TABLE 2: CHARACTERISATION OF RATE CONTROLLING MEMBRANES

Code	Folding endurance	Swelling index	Percent moisture content	Percent moisture uptake	Permeation coefficient	Tensile strength (kg/cm²)	Percent elongation
J1	No cracks	34.84±2	3.01±2	9.08±1.7	0.95±0.04	0.382±0.03	33.08±1
J2	No cracks	55.73±0.4	3.42±1.8	10±1	1.18±1	0.243±0.03	5.92±0.01
J3	No cracks	50±0.64	5.3±1	11.5±0.42	1.28±0.14	0.747±0.03	11±0.93
J4	No cracks	57±0.09	7.6±0.4	12.7±1.47	1.56±0.09	0.33±0.05	3.33±0.64
J5	No cracks	66.34±0.4	7.87±1.85	13±0.42	1.8±0.58	0.125±0.01	1.85±0

The values are expressed as mean±SD (*n*=3). SD: Standard deviation

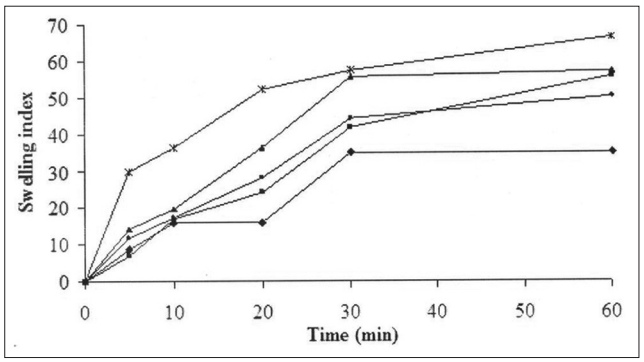

Fig. 4: Swelling index of different membranes.
Swelling index of different rate controlling membranes J1 (-♦-), J2 (-■-), J3 (-•-), J4 (-▲-), and J5 (-×-).

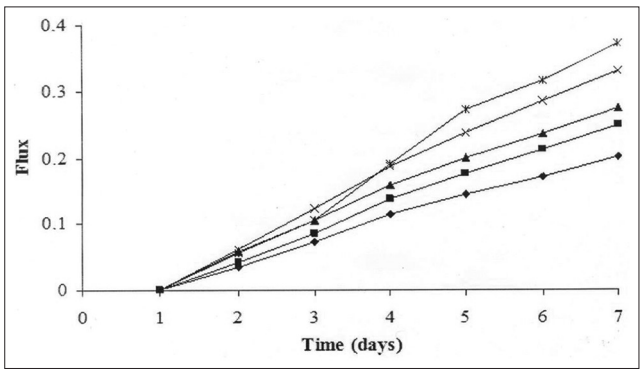

Fig. 6: Flux versus time plots for conjugated chitosan membranes.
J1 (-♦-), J2 (-■-), J3 (-▲-), J4 (-×-), and J5 (-*-).

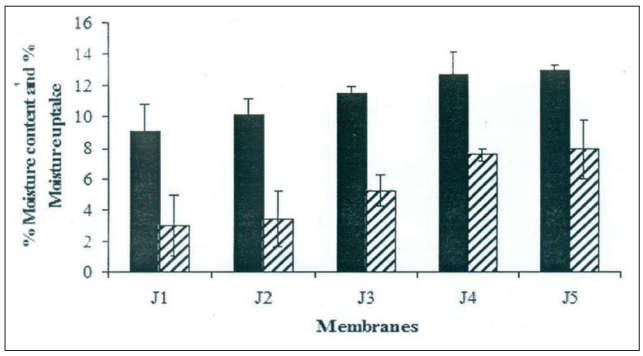

Fig. 5: % Moisture content and moisture uptake of membranes J1 to J5.
Moisture uptake (■) and moisture content (▨).

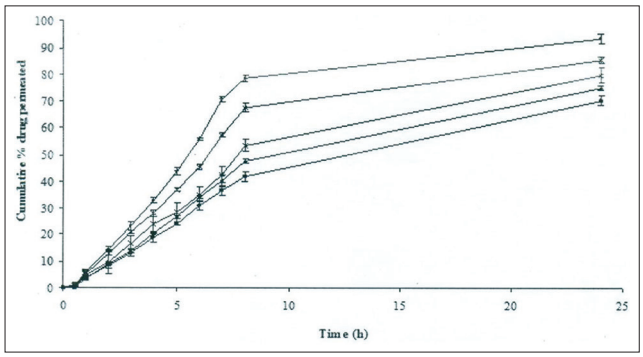

Fig. 7: *In vitro* permeation of drug across dialysis membrane.
J1 (-■-), J2 (-♦-), J3 (-×-), J4 (-*-), and J5 (-•-).

for different membranes across dialysis membrane no 110 using phosphate buffer, pH 7.4: ethanol (1:1) mixture as an *in vitro* study fluid in the receptor compartment of a modified Franz diffusion cell. The results of these studies are given in Table 3 and fig. 7. *Ex vivo* permeation studies were performed for different membranes across wistar albino rat's skin epidermis containing stratum corneum excised from the dorsal surface using phosphate buffer, pH 7.4: ethanol (1:1) mixture for the receptor compartment of a modified Franz diffusion cell. The results of these studies are given in Table 3 and fig. 8. The stability study was performed for J5, the conditions of the study maintained was 40º/75%RH for 60 days, *in vitro* permeation study was performed at 0, 30 and 60 days. Cumulative percent of drug permeated was found to be 93±1.77, 92±0.56 and 92.12±0.29, respectively. Results suggested that there was negligible change in permeation of profile after study. The other physical parameters were also not affected, the membranes remained smooth, flexible and no change in physical appearance.

The procured pure sample of carvedilol was tested for its compatibility with polymers by DSC analysis and it reveals that the polymers do not interact with the drug and compatible with each other. The significant difference in charring point of chitosan and chitosan conjugate has suggested that there might be the conjugation of TGA with chitosan. The FT-IR spectra of chitosan and

TABLE 3: DRUG PERMEATION KINETICS PARAMETERS OF PERMEATION STUDIES THROUGH DIALYSIS MEMBRANEA AND EXCISED RAT SKIN

Formulation code	Zero order plot (R^2)	First order plot (R^2)	Higuchi's plot (R^2)	Korsmeyer's-Peppas plot (n)
J1	0.8945	0.9548	0.9506	1.133
J2	0.8712	0.9716	0.9429	1.5503
J3	0.8657	0.9737	0.9433	1.0936
J4	0.7623	0.9219	0.9045	1.2478
J5	0.7063	0.9212	0.8735	1.1972
J5#	0.853	0.967	0.9514	1.0174

Values are expressed as mean of triplicate readings, #J5 is excised rat skin

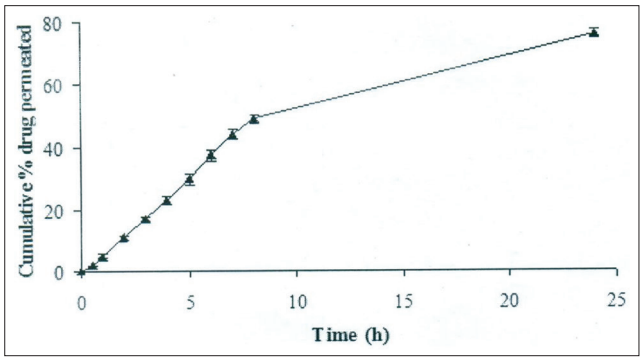

Fig. 8: *Ex vivo* **permeation profile of membrane J5.**

chitosan conjugate have shown bands represented S-H stretching (at 2058.23 cm^{-1}) and band represented -C=O stretching of amide bond (at 1521.97 cm^{-1}) which has confirmed that the conjugation has occurred through the amide linkage only. The DSC thermogram of chitosan conjugate, have shown one extra endothermic peak at 202.12° represented the thiol moiety, which was absent in the DSC thermogram of chitosan. This has further evident that the conjugation of TGA with chitosan. The folding endurance results showed that the membrane would not break and would maintain their integrity with general skin folding when applied. Moisture content and moisture uptake studies suggested that increase in ratio of chitosan conjugate resulted in increased moisture content and moisture uptake of the membrane. This is due to replacement of primary amino group by thiol group in chitosan conjugate, which opens the polymeric network and allows to absorb more amount of moisture, as a result of this the swelling of polymer was also found to be increased. The moisture content of the prepared membranes was low, which could help the formulations remain stable and reduce brittleness during long term storage. The moisture uptake of the formulations was also low, which could protect the membranes from microbial contamination and reduce bulkiness. Water vapor transmission studies showed that all the membranes prepared were permeable to water vapor. The value of permeation coefficient of the rate controlling membranes were increased with increase in proportions of chitosan conjugate in the formulation, as the proportions of chitosan conjugate increases polymer chain become more opened and offer less resistance to permeation of moisture due to presence of thiol moiety. The rate of water vapor transmission in different membranes was found to be decreased in following order J5>J4>J3>J2>J1. The results reveal that the membranes have reasonable tensile strength and moderate percentage elongation. Due to presence of thiol group in place of primary amino group it creates breakage in the polymer chains, as a result the percentage elongation decreased with increasing concentration of conjugated chitosan whereas tensile strength increased with increase in concentration of conjugated chitosan from membrane J1 to J3, but decreased in J4 and J5. A very significant change in the gel strength of chitosan was observed after the conjugation with TGA. This might be due to opening of the polymer chains after conjugation which resulted in the formation of a very soft gel as it retained more amount of moisture. Whereas in the chitosan formed a hard gel as the amount of moisture retained was less due to the rigidity of the matrix. The reduction in the gel strength of chitosan after the conjugation has further supported the results of swelling index, moisture content, moisture uptake and *in vitro* drug permeation of the membranes which contained different proportion of conjugated chitosan. The *in vitro* release and *ex vivo* profile is an important tool that predicts in advance how a drug will behave *in vivo*. The *ex vivo* permeation study for J5 was carried out in the same manner as the *in vitro* permeation study except that Wistar albino rat's skin epidermis containing stratum corneum excised from the dorsal surface was used instead of dialysis membrane. The cumulative amount of drug permeated from membranes increased with increase in amount of chitosan conjugate. This is due to the property of chitosan conjugate in which the primary amino group is replaced by thiol group of thioglycolic acid, which results in opening of polymer chain and increasing swelling index, moisture content, moisture uptake, permeation coefficient and results in increased drug permeation. When the cumulative amount of drug permeated per square centimeter of membrane through dialysis

membrane was plotted against time, the permeation profiles of the drug followed first order kinetics. Since many release processes can be represented by a coupling of a Fickian and non-Fickian mechanism, Ritger and Peppas introduced the power law equation $Mt/M\infty=K^{tn}$ to characterize the controlled-release behavior of a drug from polymer matrices. The value of n can be calculated from the slope of In Mt/ M∞ vs In t and can be indicative of the operating release mechanism. The n values more than 1 obtained by this equation indicated that the amount of drug permeation followed Non Fickian super case II diffusion in all the membranes. The membrane J5 was subjected to accelerated stability studies for 60 days at 40ºC/75% RH, showed negligible change in permeation profile. The membranes subjected for stability studies were found to be smooth and flexible and no change in the physical appearance of the membrane was observed.

Thus, this research study could be concluded that the inclusion of chitosan conjugate in the formulation of rate controlling membranes for transdermal devices has the considerable effect on drug permeation. Studies have shown promising results in this regard and there is a scope for further pharmacokinetic and pharmacodynamics investigation. There is a need to conduct toxicity study of selected formulations to establish safety and efficacy.

Financial support and sponsorship:
Nil.

Conflicts of interest:
There are no conflicts of interest.

REFERENCES

1. Das MK, Bhattacharya A, Ghosal SK. Transdermal delivery of trazodone hydrochloride from acrylic films prepared from aqueous latex. Indian J Pharm Sci 2006;68:41-6.
2. Sanap GS, Dama GY, Hande AS, Karpe SP, Nalawade SV, Kakade RS, *et al*. Preparation of transdermal monolithic systems of indapamide by solvent casting method and use of vegetable oils as permeation enhancer. Int J Green Pharm 2008;2:129-33.
3. Ubaidulla U, Reddy MV, Ruckmani K, Ahmad FJ, Khar RK. Transdermal therapeutic system of carvedilol: Effect of hydrophilic and hydrophobic matrix on *in vitro* and *in vivo* characteristics. AAPS PharmSciTech 2007;8:2.
4. Tanwar YS, Chauhan CS, Sharma A. Development and evaluation of carvedilol transdermal patches. Acta Pharm 2007;57:151-9.
5. Kast CE, Bernkop-Schnürch A. Thiolated polymers – Thiomers: Development and *in vitro* evaluation of chitosan-thioglycolic acid conjugates. Biomaterials 2001;22:2345-52.
6. Kast CE, Frick W, Losert U, Bernkop-Schnürch A. Chitosan-thioglycolic acid conjugate: A new scaffold material for tissue engineering? Int J Pharm 2003;256:183-9.
7. Thacharodi D, Rao KP. Development and *in vitro* evaluation of chitosan-based transdermal drug delivery systems for the controlled delivery of propranolol hydrochloride. Biomaterials 1995;16:145-8.
8. Bagyalakshmi J, Vamsikrishna RP, Manavalan R, Ravi TK, Manna PK. Formulation development and *in vitro* and *in vivo* evaluation of membrane-moderated transdermal systems of ampicillin sodium in ethanol: PH 4.7 buffer solvent system. AAPS PharmSciTech 2007;8:7.
9. Satishbabu BK, Srinivasan BP. Preparation and evaluation of buccoadhesive films of atenolol. Indian J Pharm Sci 2008;70:175-9.
10. Sciarra JJ, Gidwani RN. Evaluation of selected physical constants of polymeric films and proposed kinetics of drug release. J Pharm Sci 1972;61:754-61.
11. Gannu R, Vishnu YV, Kishan V, Rao YM. Development of nitrendipine transdermal patches: *In vitro* and *ex vivo* characterization. Curr Drug Deliv 2007;4:69-76.
12. Satheeshababu BK, Shivakumar KL. Synthesis of conjugated chitosan and its effect on drug permeation from transdermal patches. Indian J Pharm Sci 2013;75:162-70.
13. Padula C, Nicoli S, Colombo P, Santi P. Single-layer transdermal film containing lidocaine: Modulation of drug release. Eur J Pharm Biopharm 2007;66:422-8.
14. Bhalekar MR, Desale SS, Madgulkar AR. Synthesis of MCC-PEG conjugate and its evaluation as a superdisintegrant. AAPS PharmSciTech 2010;11:1171-8.