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DEVELOPMENT OF MATRIX TYPE TDD SYSTEM OF CARVEDILOL & HYDROCHLOROTHIAZIDE AND ITS PHARMACOKINETIC AND PHARMACODYNAMIC EVALUATIONS

[*]Sumithira G, Agarwal S S

Delhi Institute of Pharmaceutical Sciences & Research, Sector 3, Pushp vihar,
New Delhi, India – 110 017.

Abstract

The aim of the present study was to develop transdermal delivery system to deliver two cardiovascular drugs in combination of carvedilol and hydrochlorothiazide, as they have synergistic effect. Matrix type transdermal delivery system with both the drugs was prepared by film casting method using chitosan polymer. Fourier transform infra-red (FTIR) spectrometry, differential scanning calorimetry (DSC) were applied to investigate the physicochemical characteristics of the drugs in formulation. Characterization studies indicate that there were no changes in the physical state of the drug between either the drugs or polymer in the formulation. The permeations of drugs were analyzed *in vitro* and *in vivo*. The results revealed that the transdermal film released the drugs at a controlled rate for prolonged period of time 72 h and *in vivo* release study also showed prolonged therapeutic concentration of carvedilol and hydrochlorothiazide available in the plasma as compared to the 2 days of oral dosage form. The formulations were subjected to stability studies at 40°C/ 75% RH and 25°C/ 60%RH as per the ICH guidelines and it was found to be stable at all temperature conditions. Pharmacodynamic studies were performed in rats against fructose-induced hypertension and 0.9% normal saline-induced diuretics. In vivo pharmacodynamic data shows that similarity in therapeutic efficacy compared to multiple dose oral suspension.

Keywords: Chitosan matrix, Carvedilol, Hydrochlorothiazide, Pharmacodynamic, Transdermal delivery.

Introduction

Hypertension is one of the major risks associated with heart disease. Carvedilol is non-selective β and α_1 adrenergic antagonist due to its high first pass metabolism[1] only 22-24% of the orally delivered dose is bioavailable. Its plasma half life is 6 h causing frequent administration. Also frequent doses are uncomfortable to patients, transdermal delivery offer potential as an alternative therapy to injection and to avoid first-pass metabolism. Fluid retention may occur during up-titration of carvedilol, in such cases, diuretic should be administrated along with therapy[2]. Carvedilol in combination with a hydrochlorothiazide (HCTZ) is an effective and safe treatment for patients with severe hypertension[3]. The addition of HCTZ led to further increase in anti hypertensive efficacy[4].

Author for Correspondence:
Sumithira G,
Delhi Institute of Pharmaceutical Sciences & Research,
Sector 3, Pushp vihar,
New Delhi, India – 110 017.
Email: gsumithira@ymail.com

Recently much attention has been focused on developing controlled drug delivery system using natural polymers. TDDS are designed to provide controlled continuous delivery of drugs directly through the skin into systemic circulation maintaining consistent efficacy and minimizing side effects[5,6] . Chitosan [(1-)2amino,2-deoxy-β-D-glucan] has inspired much interest in the past decade with a view to high value products for medical and pharmaceutical purposes due to its high biocompatibility, non-toxicity and non-antigenicity[7] that offer advantages for possible clinical use. We have developed polymer matrix controlled transdermal carvedilol and hydrochlorothiazide delivery films using chitosan polymer with different concentrations. The physicochemical properties of the membranes have been well characterized. This paper deals with development and *in vitro*, *in vivo* evaluations and Pharmacodynamic studies of chitosan-based TDDS films. *In vitro* evaluations of the films while supported on rat and cadaver skin were carried out in modified Franz diffusion cells.

Materials and Methods

Gift sample of carvedilol was obtained from Zydus Cadilla, Mumbai; Hydrochlorothiazide was from Microlabs, Hosur; while chitosan was from central fisher Ltd, Cochin and all other chemicals used were of analytical grade.

Preparation of Transdermal Films

Method used for the preparation of film by solvent casting technique[8]. The various concentration of polymer (Table 1) was taken in 20 ml beaker and 10 ml of 4 % v/v lactic acid was added and allowed to stir continuously until clear solution was obtained. The drugs were then added and stirring was continued. Then resulting solution was poured into Teflon mould cover with backing membrane (aluminium foil) the rate of evaporation of the solvent was controlled by placing an inverted funnel over Teflon mould and dried. The films were packed in aluminium foil and kept in desiccators until use.

Physical evaluation of prepared films

The following parameters of the films were studied; physical appearances (Transparent, smooth, flexible, sticky, homogeneous and opaque).

Thickness

The thickness of the films was measured by screw gauge. The mean of the five observations were calculated.

Folding endurance

This was determined by repeatedly folding the film at the same point until it broke. The maximum number of times the film could be folded at the same point without breaking/cracking gave the value of folding endurance[9].

Drugs content uniformity

The uniformity of weight was calculated and percentage deviations were determined. The content uniformity was evaluated by dissolving 1 cm^2 of film in 10ml of Phosphate buffer saline (PBS) pH 7.4 containing 40%v/v PEG and the contents were kept for 72h with occasional shaking (added PEG to achieve a sufficiently high concentration of drugs in the donor phase). Then this solution was quantitatively transferred to volumetric flasks and appropriate dilution was made with receptor fluid. The resulting solution was filtered and analyzed for drug content by spectrophotometer at 332, 275 nm for carvedilol and hydrochlorothiazide respectively. Data obtained from the above evaluations were shown in Table 2.

Percentage moisture absorption

The films were weighed accurately and placed in the desiccators containing 100mL of saturated solution of potassium chloride, which maintains 79.50% RH. Three days later, the films were taken out and weighed again. The study was done at room temperature. The percentage moisture absorption was calculated using formula:

$$\% \text{ moisture absorption} = \frac{\text{final weight-Initial weight}}{\text{Initial weight}} \text{X } 100$$

Percentage moisture loss[9]

The films were weighted accurately and kept in a desiccators containing anhydrous $CaCl_2$. After 3 days, the films were weighed. The moisture loss was calculated using the formula

$$\% \text{ moisture loss} = \frac{\text{final weight-Initial weight}}{\text{Initial weight}} \text{X } 100$$

Moisture content

The prepared films were marked, then weighed individually and kept in the desiccators containing activated silica at room temperature for 24 h. the films were weighed individually until showed a constant weight. The percentage of moisture content was calculated as a difference between initial and final weight with respect to final weight and results are summarized in Table 3.

$$\% \text{ moisture content} = \frac{\text{final weight-Initial weight}}{\text{Initial weight}} X\ 100$$

Investigation of physiochemical compatibility of drugs and polymer

Fourier Transform- Infrared Spectroscopic Analysis (FT-IR)

Fourier Transform Infrared measurement was performed using a Shimadzu, Japan instrument in order to find out any kind of chemical interaction between drugs and polymer. The samples of pure carvedilol, HCTZ, polymer, and formulated film were analyzed in the spectral region (4000-400cm^{-1}) with 25 scans recorded at a 4cm^{-1} resolution.

Differential Scanning Calorimetry Studies

DSC thermograms were obtained by using (Q10, TA Instruments, Japan) differential scanning calorimeter. Sample sizes were in the range 3-5 mg and were sealed in an aluminium pan. Thermograms were recorded from 50-300º C at a rate of 10º C/min.

Stability study of optimized TDDS

The optimized TDD film of carvedilol and HCTZ was packed in aluminium foil and then kept at different temperature and relative humidity (25º C, 65 % RH and 40º C, 75 % RH) for 3 months as per ICH guideline and the percentage of cumulative drugs release, FTIR, DSC were studied in sample withdrawn at monthly intervals[10].

Skin irritation test

The film containing the drugs were placed on the dorsal area on the both sides of the rabbit, secured it firmly in place with adhesive plaster. The rabbits were observed for 7 days for any sign of oedema and erythema[11].

***In vitro* drug permeation studies**

The *in vitro* skin permeation of drugs from the optimised film through the rat abdominal skin was tested by using a modified Franz diffusion cell, fastened with an O-ring having diffusion area of 1cm^2. The full thickness of abdominal skin of male Wister rat was used. Hairs on the abdominal area were removed by a razor and washed with distilled water one day before to the experiment. Rats were sacrificed by using high dose of anaesthetic ether, abdominal skin was excised, and the fatty material attached to the dermis was peeled off. Then the skin piece was mounted between the two compartments of the diffusion cell with the epidermis facing upward to the donor compartment. The skin was stabilized for 4 h (by stirring pH 7.4 PBS containing 40%PEG in receptor compartment) before in vitro permeability study[12]. The film to be tested was placed on the skin. Phosphate buffer pH 7.4 containing 40%v/v PEG[13] was used as receptor medium (60 ml) and agitated with a magnetic stirrer at temperature of 32 ± 2º C, the top of donor compartment was covered with aluminium foil to avoid the drugs photosensitivity. Samples (3ml) were withdrawn at regular interval through the sampling port and fresh receptor fluid was replaced to maintain the constant volume of the receptor phase. The content was analyzed by UV spectrophotometer. The flux values were calculated from the linear portion of the plots. The same procedure was adopted for *in vitro* permeation through cadaver skin also performed.

Feasibility of optimized TDDS for transdermal delivery

Optimized film of carvedilol and HCTZ was studied for its feasibility in transdermal drug delivery. In vitro permeation through rat skin was carried out to determine flux, and film size required to deliver carvedilol and HCTZ through skin was determined to satisfy pharmacokinetic criteria.

Pharmacokinetic Evaluation

In vivo studies

In vivo evaluation of optimized film was done on the albino rats using HPTLC method. 12 healthy animals were used for this study. Six rats received formulated transdermal film and six animals received an oral suspension at the interval of 24 h upto 72 h. Film was applied to abdomen skin of rats and blood specimen were taken at specific time intervals in an eppendorf tubes containing Acid Citrate Dextrose solution and centrifuged at 3000rpm for 1 h to obtain plasma and frozen until

analyzed. Mobile phase was mixture of chloroform: methanol: glacial acetic acid (8:1:1) and ethyl acetate: cyclohexane (9:1) for carvedilol and HCTZ respectively. The peak height and area were detected at 254 and 270 nm for carvedilol and HCTZ respectively. The C_{max}, T_{max} values were determined from the individual serum concentration-time profiles, the AUC_{0-96} was also calculated.

Pharmacodynamic evaluation

Evaluation of antihypertensive activity

The male Wister rats were divided into five groups (n=6), group I, II, III, IV, V. Hypertension was produced by feeding 10% w/v (F 10) fructose in distilled water[14]. Blood pressure (BP) was recorded by using a Tail cuff method (NIBP, ADI'S Instrument, Australia). Group I (control) animals were given distilled water to drink throughout the experimental period and all other group received 10%w/v fructose. Systolic blood pressure (SBP) of the rats were measured before the start of fructose treatment (day 0), and at days 1, 2, 3, 4, 5, 6, 7 during the period of fructose treatment and after the drug treatment. At the end of 7th day, group III and IV were applied medicated and blank films respectively on the dorsal portion of rats, and group V received both the drugs made into oral suspension at the interval of 24 h and systolic blood pressure were measured at interval of 12 h upto 84 h.

Evaluation of diuretic activity

For diuretic activity[15], male albino rats weighing about 200-225 grams were selected and divided into four groups each group consisting of six rats. All the rats were fasted and deprived of water for 18 h before the experiment. Control group received 0.9%w/v saline in dose of 5% body weight and 2% starch solution in a dose of 5ml/kg throughout the experiment. Optimized medicated and blank films were applied in dorsal portion of animals to group II and III respectively, and for group IV besides saline, same quantity of drugs equivalent to medicated film in 2% starch in dose of 5ml/kg were given at 24 h interval. The rats were placed in metabolic cages and urine was collected at the end of 5 h, 24 h intervals. The volume, pH, Na^+, K^+ were measured by flame photometry (ELICO, model CL-360).

Statistical data analysis

All data were analyzed using one-way analysis of variance (ANOVA). P values < 0.05 were considered significant. Results are expressed as the means of at least three experiments ± SD.

Results & Discussion

Film preparation and characterization

All the films prepared were flexible, wrinkle free transparent, smooth, sticky, homogenous and opaque. From the results of table 3 revealed that the percentage moisture absorption, percentage moisture loss and percentage moisture content were maximum for the transdermal film formulated with chitosan 200 mg**(CCH1)**, which may be attributed to its hydrophilic nature of polymer. Increment in the concentration of polymer showed the maximum percentage of moisture absorption, moisture content and also thickness of the films varied ranging from 0.090-0.135 mm. From the result of folding endurance study showed that formulation CCH4 have highest folding endurance while CCH1 have the least among the different formulations. FTIR spectra of the drugs, polymer and drugs-loaded films are presented in Fig 3. The characteristic FTIR absorption peak of Carvedilol[16] (3a) showed the stretching of the N-H group was at 3344 cm^{-1} and SO_2 of aromatic ring gave peaks at 1168 and 1180 cm^{-1} for HCTZ[17] (3b). FTIR spectra of the carvedilol and HCTZ loaded chitosan film (3c) show a prominent peak for carvedilol and HCTZ at 3344 cm^{-1} and 1180 cm^{-1} respectively. This indicates that carvedilol and HCTZ are not involved in any chemical interactions with either the polymer or within drugs.

DSC scans of the drugs, polymer and drugs-loaded film are presented in Fig 4. The endothermic peaks of pure carvedilol and HCTZ appeared at its melting points 116.4º C (4a) and 268.1º C(4b) respectively, which were also appeared in DSC plots of drugs- loaded film (4c). This further confirms no chemical interactions with either the polymer or within drugs.

The formulations exhibited good stability at all storage condition after 3 months (Table 4). The skin irritation test results indicated that no oedema and erythema was observed for film developed during the observed period of 7 days.

The effect of different experiment variables on the amount of drug penetrating through the unit diffusion surface release of carvedilol and hydrochlorothiazide from the drugs loaded transdermal film was calculated and plotted as a function of time, the percentage cumulative amount (upto 72h) is represented in Fig 1.

All the formulations showed release of carvedilol upto 72 h, except the formulation CCH4 showed the highest release of 96.9% carvedilol within 30h, formulation CCH1 showed lowest only 24.3% of drug release at the end of 72h; however release of hydrochlorothiazide almost in all the formulations showed same rate. The flux (J) was calculated by the slope of the linear portion of percentage cumulative release-time plots for zero order and expressed as the mass of drug passing across $1cm^2$ of skin overtime.

The Fig 2 indicates that, rat skin and cadaver skin showed remarkable similarities in permeation for carvedilol and HCTZ respectively. In vitro skin permeation through rat skin and cadaver skin is found to be in accordance with zero-order kinetic procedure.

Pharmacokinetic studies

For the purpose of studying the pharmacokinetic aspects of transdermal absorption of drugs carvedilol and HCTZ, one of the prerequisites is that the pharmacokinetic parameter after the oral administration should correlate with that after transdermal absorption of carvedilol and HCTZ. The pharmacokinetic parameters after administration of the oral and film are shown in Table 5.

The calculated AUC_{0-96} values were significantly different between ($P < 0.05$) the oral and film for both the drugs. Both the drugs in film showed a significant higher AUC_{0-96} value in comparison with the oral administration. The C_{max} value of the film for both the drugs was 39.41ng/ml, 13.43 ng/ml of carvedilol and HCTZ respectively were higher compared to the oral administration. The T_{max} value for film also observed higher in this study.

Pharmacodynamic studies

An attempt has been made to measure the systolic blood pressure (SBP) in terms of its pharmacodynamic activities. (Fig 5) shows progressive increase in body weight in all groups of rats during the one week experimental period. Neither fructose treatment at 10%w/v concentration nor fructose withdrawal significantly affected the body weight gain. From the observation (Fig 6) indicates that treatment of rats with film and oral suspension significantly prevents the development of fructose induced hypertension and also no statistically significant (P.> 0.05) difference between film and oral treated groups in preventing the fructose induced hypertension.

Table 6 illustrates 84 h urinary Na^+ and K^+ excretion rates obtained after end of every 5 and 24hs intervals. It indicates statistically no significant difference in Na^+ and K^+ execration were found between film and oral administration. When compared to control, the film treated animals showed significant higher execration of Na^+ and K^+ level in urine.

Conclusion

The investigated in this study to develop transdermal films composed of both hydrophilic and lipophillic drugs into hydrophilic polymer such as chitosan. Using this process, we have successfully developed membrane controlled transdermal composed of carvedilol and hydrochlorothiazide in a chitosan film. In vitro, in vivo transport of drugs across skin without any permeation enhancer was greater. It was shown in earlier studies that chitosan increases the paracellular absorption[18], it is important for the transport of hydrophilic compounds. The release profile obtained with rat skin and human cadaver skin were found to be comparable[19].Primary skin irritation study has shown no signs of erythema or oedema, indicating that the transdermal films were safe for using highly biocompatible and non-antigenic biopolymer like chitosan[20].The stability study data shows that no significant P>0.05 variation in percentage cumulative amount, FTIR and DSC were observed at mentioned conditions. From the results observed that the film was found to be chemically stable at 25 ± 2 ºC / 65 ±5% RH, 40 ±2ºC / 75 ±5% RH for 90 days, however the hydrophilic polymers had become slightly soft due to moisture absorption when stored at higher RH[21]. The pharmacokinetic results showed there were no significant differences between formulated films and oral suspension. The mean serum carvedilol

and HCTZ concentration-time profiles for formulated films shows that little higher plasma concentration and steady state for longer period compared to oral administration. From this result it also shows formulated films for carvedilol has better bioavailability than oral administration. A film size of 15 cm^2 was predicted that would maintain carvedilol and HCTZ concentration within a therapeutic window. The films containing carvedilol β-adrenergic receptor antagonist significantly reduced SBP equal to values observed in control rats. The data observed from diuretic evaluation, the formulated films and oral suspension shows no significant differences in urinary volume, pH, Na^+ and K^+ excretion. The use chitosan clearly has great potential as an aid to transdermal drug delivery and has been shown to mediate a good degree of transdermal drugs availability both *in vitro* and *in vivo* [22]. From these results it can be concluded that formulated films containing carvedilol and HCTZ shows significantly similar therapeutic effect to multiple dose oral suspension, such monolithic transdermal formulation are advantageous in providing effect treatment for hypertension with enhanced patient compliance, it is however important to conduct experiment to prove the safety and efficacy by conducting phase-I clinical trial using human volunteers.

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Table No. 01: Compositions of drugs and polymer for transdermal formulations

Formulation code	Polymer (mg)	Carvedilol (mg)	Hydrochlorothiazide (mg)
CCH1	200	25	12.5
CCH2	200	6.6	12.5
CCH3	150	7	13
CCH4	100	3.3	2.5

Table No. 02 illustrates the drug content, thickness and weight of the various formulations (n=6)

Formulation Code	Drug content (%)/cm^2 (± SD)		Thickness (mm)	Weight/cm (mg)
	Carvedilol	hydrochlorothiazide	(± SD)	(± SD)
CCH1	94.27⊥ 4 .05	93.21⊥ 3.75	0.135⊥ 0 .22	19.14 ⊥ 1.08
CCH2	97.56± 6 .82	92.53 ± 5.82	0.116± 0.01	15.04± 0.65
CCH3	98.54± 3.25	93.63± 5.07	0.103 ± 0.01	14.23± 1.70
CCH4	98.75± 3.25	92.18± 5.48	0.090± 0.02	11.96± 0.93

Table No. 03: Physiochemical properties of transdermal films

Formulation code	%MA	%ML	%MC	Fold Endurance
CCH1	14.34±0.33	6.20±2.36	13.94±1.33	18.71±0.50
CCH2	9.73±0.87	4.22±0.68	10.51±1.66	19.00±0.81
CCH3	6.89±0.38	3.84±0.63	05.89±1.23	19.92±0.55
CCH4	5.38±0.55	2.35±0.32	08.32±0.25	20.10±0.56

Table No. 04: Shows in vitro % cumulative release of carvedilol and HCTZ

Formulation code	Cumulative percentage of drugs released			
	Carvedilol		HCTZ	
	30h	72 h	30h	72 h
CCH1	12.8±0.7	24.3±2.0	42.43±6.6	71.38±0.6
CCH2	52.2±4.5	84.6±1.1	34.70±3.3	71.26±1.1
CCH3	56.8±1.7	96.9±2.4	39.35±4.5	75.62±4.4
CCH4	99.1±2.1	--	44.30±3.0	89.40±0.8

Values are mean ± S.D. of n=6 rats in each group.
*P< 0.05 vs group IV (Oral suspension)

Table No. 05: Stability studies of optimized TDD films

Days	Storage conditions			
	25 ± 2 °C / 65 ± 5%RH		40 ± 2 ° C / 75± 5% RH	
	Carvedilol	HCTZ	Carvedilol	HCTZ
	(% cumulative amount at 72 h)		(% cumulative amount at 72 h)	
30	74.20 ± 1.1	58.99±0.88	70.13±0.37	53.26±0.18
60	70.20±0.11	55.21±0.14	68.21±0.91	53.36±0.03
90	68.52±0.72	53.39±0.11	64.70±0.79	50.94±0.06

Table No. 06: Pharmacokinetics of carvedilol and hydrochlorothiazide from the film and oral administration in rats

Formulation	C_{max}(ng/ml)	T_{max} (h)	$AUC_{(0-96)}$ (ng h/ml)
Carvedilol			
CCH3 Film	39.4 ± 5.4	36	2238.8 ± 272.3
Oral suspension	17.5 ± 7.9	06	1329.6 ± 258.6
HCTZ			
CCH3 Film	13.4 ± 12.3	36	964.1 ± 88.6
Oral suspension	10.3 ± 8.6	06	648.1 ± 139.5

Each value represents the mean S.D. of six determinations. AUC, area under plasma concentration-time curve from the time zero to ninety six; C_{max}, maximum plasma; T_{max}, time of C_{max}.

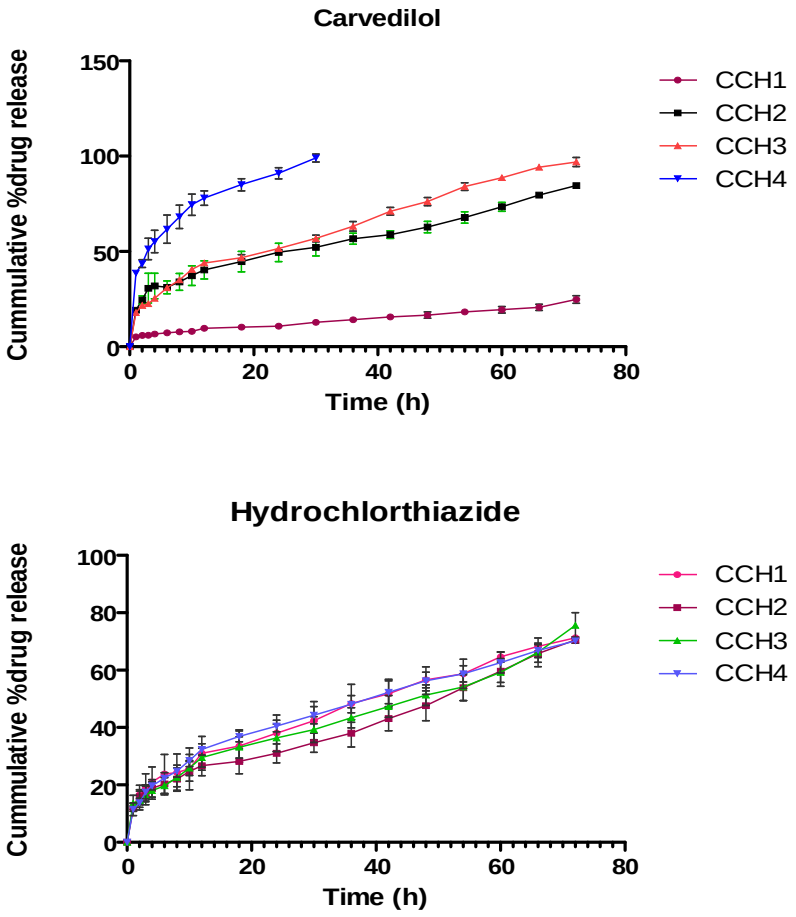

Fig. No. 01: In vitro release profile of carvedilol and hydrochlorothiazide from formulations CCH 1-4 across rat dorsal skin

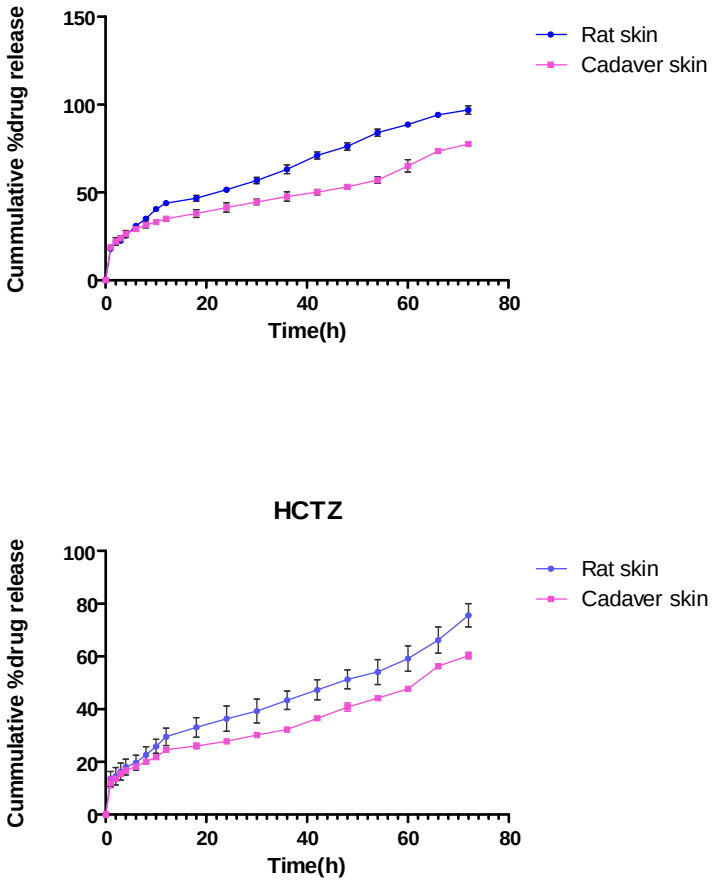

Fig. No. 02: illustrates the permeation of carvedilol and hydrochlorothiazide from formulations CCH3 across rat dorsal and cadaver skin.

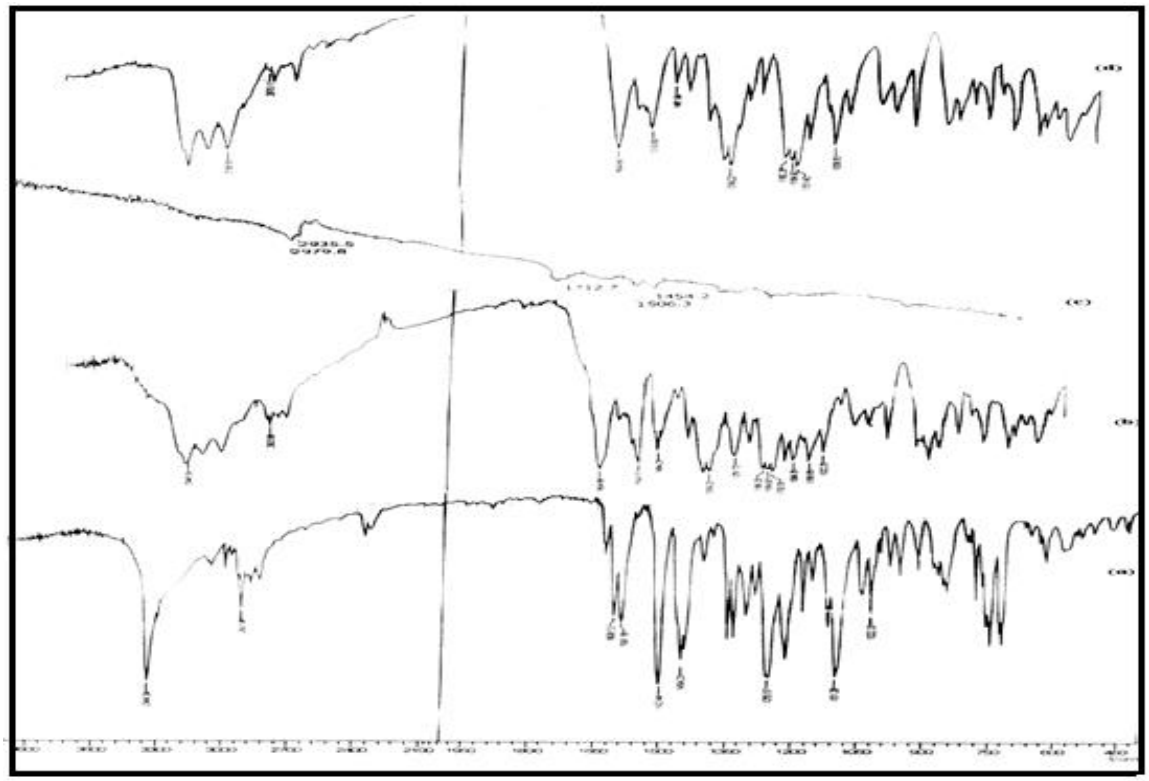

Fig. No. 03: FTIR spectra of (a) carvedilol; (b) HCTZ; (c) chitosan; (d) drugs containing film

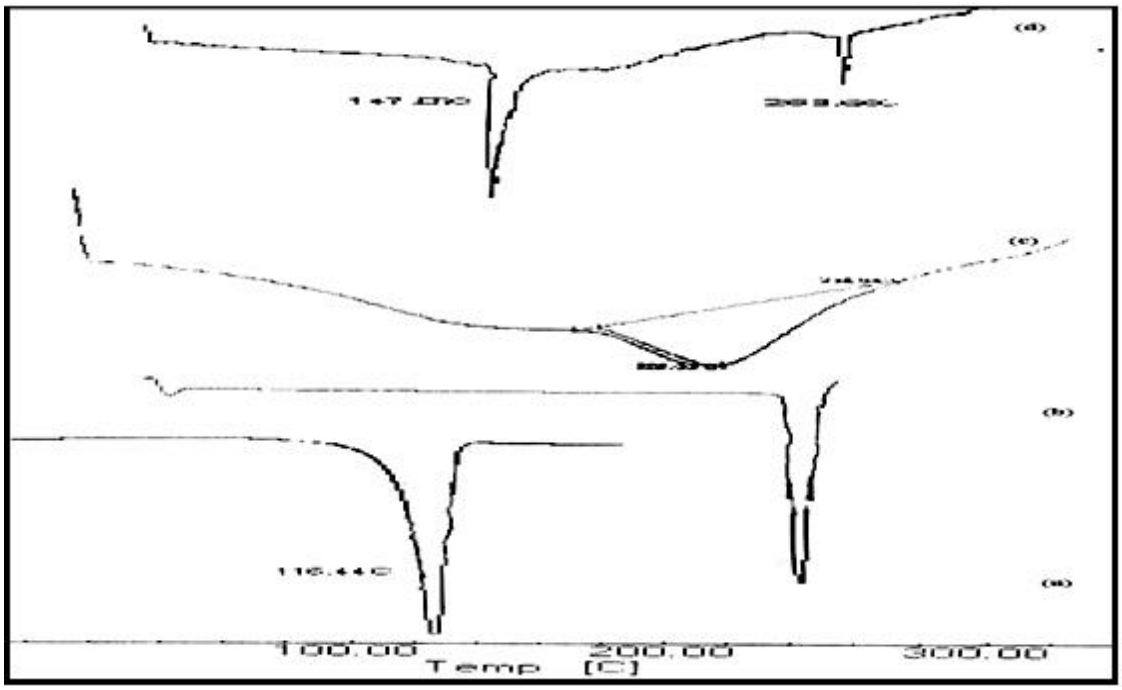

Fig. No. 04: DSC scans of (a) Carvedilol; (b) HCTZ; (c) Chitosan; (d) Drugs containing film

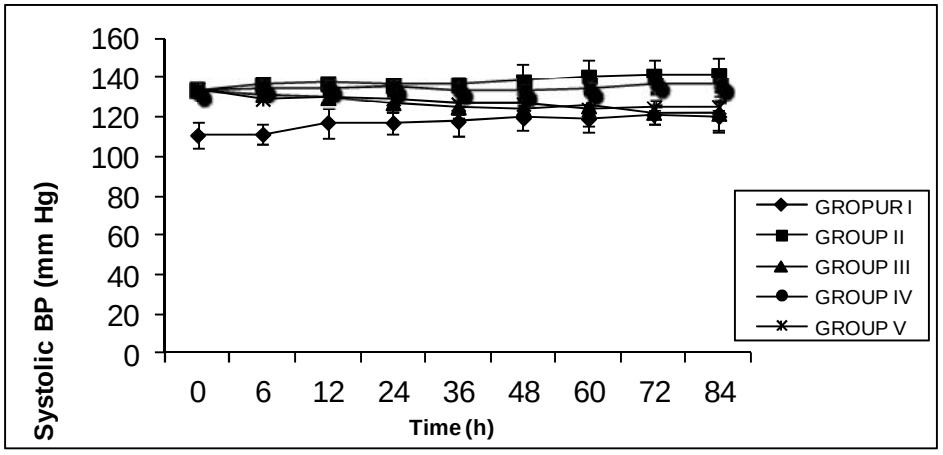

Fig. No. 05: Reduction of Systolic blood pressure (systolic BP) of rats measured at 0-84 h. during the experimental period. Data, expressed as mean ± S.D.

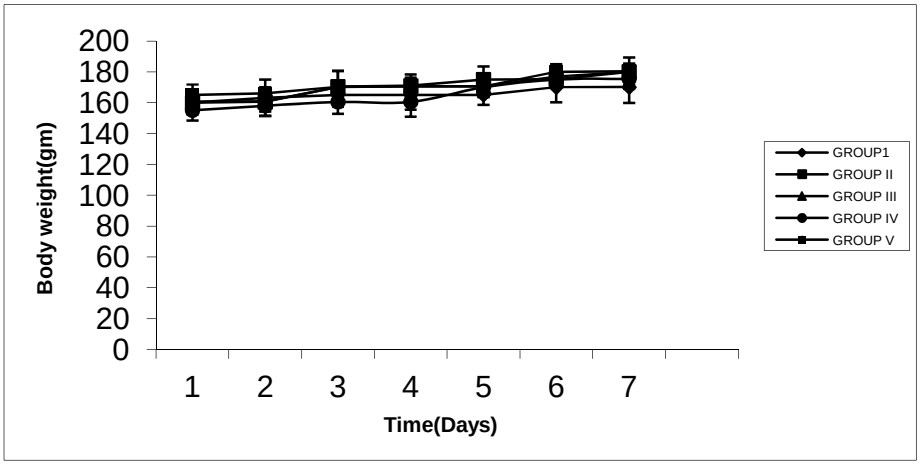

Fig. No. 06: Depicts body weight of rats fed with ordinary water (CON), 10 %w/v (F10) fructose solution during one week period of fructose treatment.

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