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Research Article

ENHANCEMENT OF TRANSDERMAL PERMEATION OF CARVEDILOL BY SONOPHORESIS TECHNIQUE: IN VITRO EVALUATION

Grace Rathnam*[1], B. Rama[1], A. Shanta[1], U. Ubaidulla[1]

1. Department of Pharmaceutics, C.L.Baid Metha College of Pharmacy, Jyothi Nagar, Thorapakkam, Chennai.

***Corresponding Author: Email**: d_rathnam@vsnl.net

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ABSTRACT

In this study, various matrix type transdermal patches containing carvedilol of variable combination of polyvinylpyrrolidone (PVP) and ethylcellulose (EC) were prepared by solvent evaporation technique. The patches were prepared by adding surfactant (sodium lauryl sulphate) to promote drug penetration which was found to increase transdermal transport. Therapeutic frequency ultrasound (1 MHz) and surfactant have been individually shown to enhance transdermal drug transport. In this study, we investigated the synergistic effect of ultrasound and surfactants on transdermal drug delivery. This study provides an investigation of the dependence of therapeutic-frequency sonophoresis on various ultrasound parameters, including ultrasound pretreatment time and the distance of the horn from the skin. Based on the *in vitro* release and *in vitro* permeation profile the formulation Fs1 (PVP/EC 1:2) shows the best release. Ultrasound was applied to pretreat the skin using a sonicator operating at a frequency of 1 MHz at an intensity 2 Watts/cm^2. The optimum ultrasonication time of 50 min at a distance of 1 cm shows the best release. The skin conductivity enhancement was found to be inversely proportional to the distance of horn from the skin and it is directly proportional to ultrasonication pretreatment time. These findings will be useful in optimizing therapeutic-frequency sonophoresis.

Keywords: Sonophoresis, Carvedilol, Transdermal.

INTRODUCTION

Transdermal drug delivery offers a beneficial alternative to injections[1] although its applications are limited by low skin permeability. Specifically, stratum corneum is responsible for the primary barrier of the skin. Stratum corneum is relatively thin (10-15 μm) impermeable membrane that consists of flat, dead cells, which are filled with keratin fibers (keratinocytes) that are surrounded by lipid bilayer. Various approaches including chemical enhancers[2], sonophoresis[3], iontophoresis[4] and electoporation[5] have been investigated to enhance transdermal drug transport. Investigations of sonophoresis can be classified into three categories based on the ultrasound frequency used: low frequency[6] (below 1 MHz), therapeutic frequency[7] (1-3 MHz), high frequency ultrasound[8] (above 3 MHz). Ultrasound enhances the transdermal transport of drugs by oscillation and cavitation which disorder the lipid bilayer of the stratum corneum. Although each method has an effect of its own on skin permeability, some methods have been shown to work synergistically[9]. Chemical enhancers such as surfactants can work synergistically with therapeutic-frequency ultrasound. The main aim of this work was to formulate matrix type transdermal patches of carvedilol using ethylcellulose and polyvinylpyrollidone. The release of the drug is optimized for improved release to the skin by using surfactant and ultrasonication. Carvedilol is a lipophilic β-blocker drug with low therapeutic dose, low bioavailability due to extensive first pass effect, poor solubility and low molecular weight, which makes it suitable to be formulated into a transdermal patch.

MATERIALS AND METHODS

Ethylcellulose and chloroform were purchased from S.D. Fine Chemicals, Mumbai. Polyvinylpyrrolidone (K value: 30) was purchased from Rainbow Laboratories, Chennai. Di-n-butyl phthalate was purchased from Lab Chemicals, Chennai. Octanol was purchased from Spectrochem (P) Ltd., Mumbai. Carvedilol was a gift sample from Orchid Pharmaceuticals Ltd., Chennai. All the chemicals purchased or received were of high purity.

Preparation of the transdermal patches

Matrix type transdermal patches (F1, F2, F3, Fs1, Fs2 and Fs3) were prepared by solvent evaporation technique containing carvedilol using different ratios of polyvinylpyrrolidone and ethylcellulose (Table 1). The two polymers were weighed in requisite ratios and they were then dissolved in chloroform. Di-n-butyl phthalate 30%w/w of polymer composition was used as a plasticizer. The drug was added as 20% w/w of the total weight of the polymers, in the homogeneous dispersion, by slow stirring with a mechanical stirrer. Sodium lauryl sulphate 0.5% of polymer composition was used as a surfactant in Fs1, Fs2 and Fs3. This uniform dispersion (10 ml) was cast on a petridish of area 39.6 sq. inch and then dried in a hot air oven at 50° for 6 h[10].

Determination of partition coefficient of drug

The partition coefficient study was performed using n-octanol as oil phase and phosphate buffer saline pH 7.4 as aqueous phase. The two phases were mixed in an equal quantity and were saturated with each other on a mechanical water bath shaker (Indian scientific, India) at 32° for 24 h. The saturated phases were separated by centrifugation at 2000 rpm on a RM-12c centrifuge. Standard plots of drug were prepared from both the phosphate buffer saline and octanol. Equal volumes (10 ml) of the two phases were taken in triplicate in conical flasks and to each 100 mg of weighed amount of drug were added. The flasks were shaken at 32^0 for 6 h to achieve a complete partitioning at 100 rpm. The two phases were separated by centrifugation at 1000 rpm for 5 min and they were then analyzed for respective drug contents[12] and the partition coefficient of drug Ko/w was calculated using the formula

$$Ko/w = \frac{\text{Concentration in octanol}}{\text{Concentration in phosphate buffer saline pH 7.4}}$$

Drug-Excipient Interaction study

The compatibility between carvedilol, ethylcellulose and polyvinylpyrollidone was evaluated using infrared spectroscopy (IR). Physical mixtures were prepared to study the effect of sample manipulation. In addition the samples of physical mixture were heated at 55^0 for three weeks to obtain more reliable conclusions[11].

Moisture content

The prepared films were marked, then weighed individually and kept in a desiccator containing activated silica at room temperature for 24 h. The films were weighed again and again individually until it showed a constant weight. The percentage of moisture content was calculated as difference between initial and final weight with respect to final weight[12].

Moisture uptake

The weighed film kept in a desiccator at normal room temperature for 24 h was taken out and exposed to 84% RH in a humidity chamber until a constant weight for the film was obtained. The percentage of moisture uptake was calculated as the difference between final and initial weight with respect to initial weight[12].

In vitro dissolution studies

The release rate determination is one of the most important studies to be conducted for all controlled release delivery systems. The dissolution studies of patches are very crucial because one needs to maintain the drug concentration on the surface of stratum corneum consistently and substantially greater than the drug concentration in the body to achieve a constant rate of drug permeation.

Dissolution of patches was performed using USP Basket Type Dissolution Apparatus. The patches were placed in respective baskets with their drug matrix exposed to phosphate buffer saline pH 7.4. All dissolution studies were performed at 50 rpm with each dissolution jar carrying 900 ml of phosphate buffer saline pH 7.4. Samples were withdrawn at different

time intervals and analyzed using a Shumedzu Spectrophotometer at 240 nm taking phosphate buffer saline solution pH 7.4 as blank. Cumulative amounts of drug released were plotted against time for different formulations[12].

In vitro permeation studies

The permeation studies were performed using full thickness albino rat skin. The skin was then immediately mounted onto a Franz Diffusion cell, which consists of two compartments, a donar and a receiver compartment. The receiver compartment volume was 15.0 ml and the skin area was 1.77 cm^2. The skin was mounted with the stratum corneum side facing the donor compartment.

The holder containing the skin and formulation was then placed on the receiver compartment containing phosphate buffer saline pH 7.4. The whole assembly was kept on a magnetic stirrer and solution in the receiver compartment was constantly and continuously stirred during the whole experiment using magnetic bead.

The samples were withdrawn (1 ml each time) at different time intervals and an equal amount of phosphate buffer saline pH 7.4 was replaced each time. Absorbances of the samples were read spectrophotometrically at 240 nm taking phosphate buffer saline solution pH 7.4 as blank. The amount of drug permeated at each time interval was calculated and plotted against time.

Ultrasound application

Ultrasound was applied for skin pretreatment using a sonicator operating at a frequency of 1 MHz at an intensity of 2 watts/cm^2. Before each experiment, the sonicator was tuned according to the instruction manual. The ultrasound was applied to the skin at various time intervals such as 10, 30, and 50 min and also at varying distances such as 1, 2 and 3 cm between the sonicator horn and the skin. After ultrasound application the skin was placed on the receiver compartment and the patch was placed on the dermal side of the skin in the donor compartment facing the matrix side of the patch to the skin. The whole assembly was kept on a magnetic stirrer and solution in the receiver compartment was constantly and continuously stirred using magnetic bead.

The concentration of Carvedilol in the receiver compartment was measured for 24 h. The skin permeability following ultrasonic pretreatment, P_{us}, as well as that without ultrasonic pretreatment (passive permeability), P_p, was calculated using equation[13]

$$P = \frac{V.\ \Delta C_r}{A.\ \Delta t.\ C_d}$$

where V is the volume of the receiver compartment, A is the skin area, ΔC_r is the change in carvedilol concentration of the solution in the receiver compartment in a time Δt (24 h), and C_d is the carvedilol concentration of the solution in the donor compartment. The carvedilol permeability enhancement (CPE) is defined as the ratio of P_{us} to P_p.

Measurement of skin electrical resistance:

In order to measure the electrical resistance of skin, two Ag disk electrodes were introduced in the diffusion cell, one in the receiver compartment and the other in the donor compartment. A 100 mV AC electric field (10 Hz) was applied across the skin for a short period of time typically 5s using a signal generator and the electrical resistance was measured. In order to obtain the actual skin resistance, the Phosphate buffer saline pH 7.4 resistance was measured separately using the same assembly but without mounting the skin and was subtracted from the measured skin resistance. The skin conductivity following ultrasound C_{us} and as well as that without ultrasound (control) $C_{control}$ was calculated based on the equation[13]

$$C = \frac{1}{(R_m - R_{PBS})\ A}$$

where R_m is the measured resistance of skin, R_{PBS} is the resistance of Phosphate buffer saline pH 7.4 resistance, A is the skin area. The skin conductivity enhancement (SCE) is defined as the ratio of C_{us} and $C_{control}$

$$SCE = \frac{C_{us}}{C_{control}}$$

RESULTS AND DISCUSSION

Carvedilol is a lipophilic β- blocker drug, which undergoes substantial hepatic first pass metabolism and it has aabsolute bioavailability of 25-35%. Therefore, there is a need to search for an alternative route of administration, which may

Table 1: Composition of Carvedilol Transdermal Patches

S. No	Formulation Code	Ratio of PVP/EC	Total Weight of PVP/EC (mg)	Chloroform (ml)	Di-n-butyl Phthalate	Drug (carvedilol)	Surfactant
1.	F1	1:2	600	10	30% w/w polymers	20% w/w of polymers	-
2.	F2	1:5	600	10	30% w/w polymers	20% w/w of polymers	-
3.	F3	1:3	600	10	30% w/w polymers	20% w/w of polymers	-
4.	Fs1	1:2	600	10	30% w/w polymers	20% w/w of polymer	0.5%w/w of polymers
5.	Fs2	1:5	600	10	30% w/w polymers	20% w/w of polymers	0.5%w/w of polymers
6.	Fs3	1:3	600	10	30% w/w polymers	20% w/w of polymers	0.5%w/w of polymers

Table 2: percentage of moisture content and moisture uptake of transdermal patches

Formulation	Percentage of moisture content (%)	Percentage of moisture uptake (%)
F1	1.57	5.5
F2	1.204	4.06
F3	1.245	4.11
Fs1	1.236	4.53
Fs2	1.031	3.53
Fs3	1.233	4.11

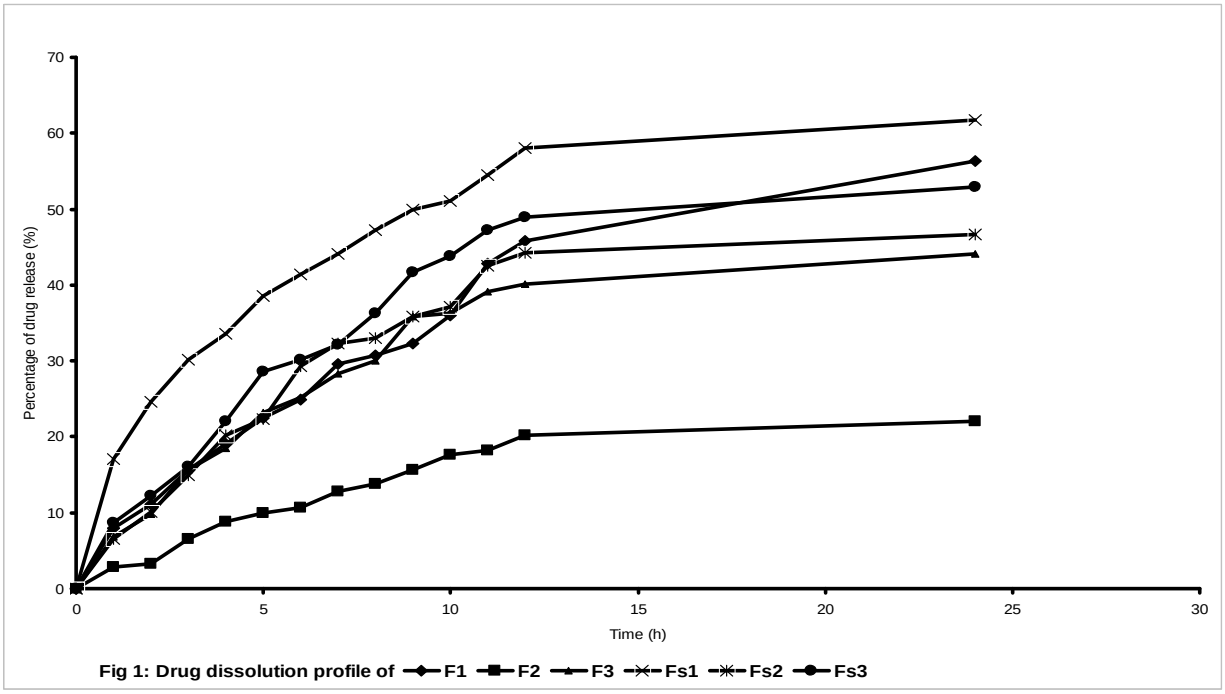

Fig.1: In vitro dissolution profile of transdermal patches

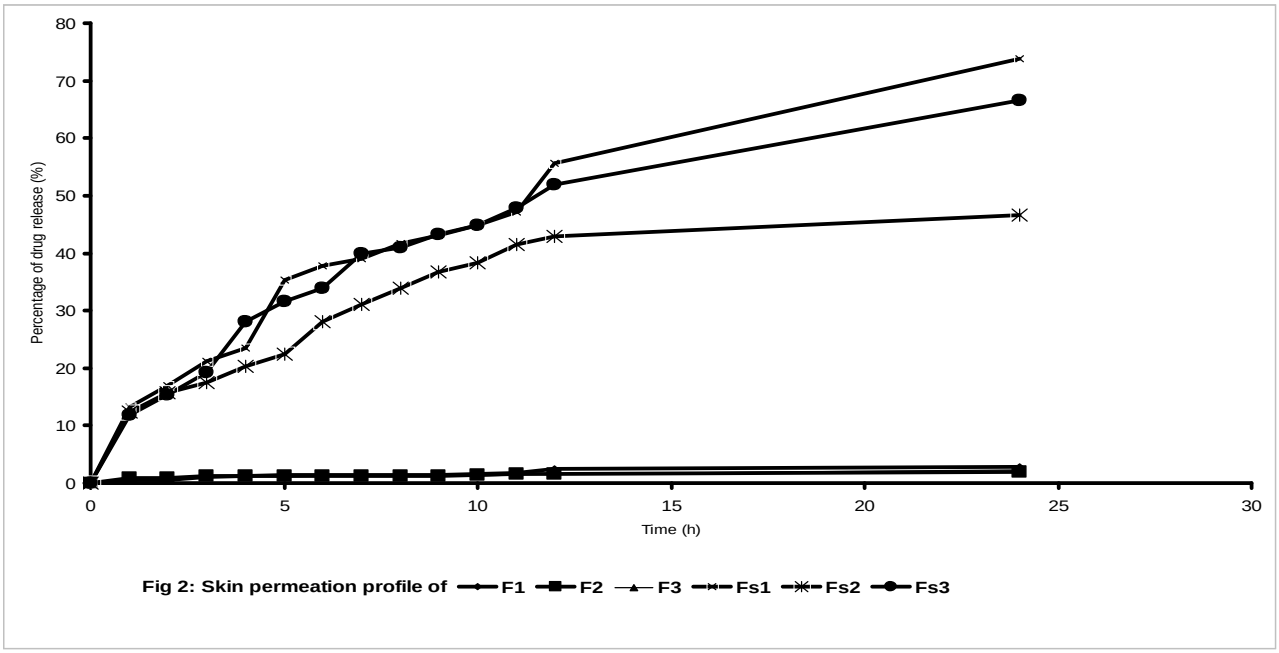

Fig.2: In vitro skin permeation profile of transdermal patches

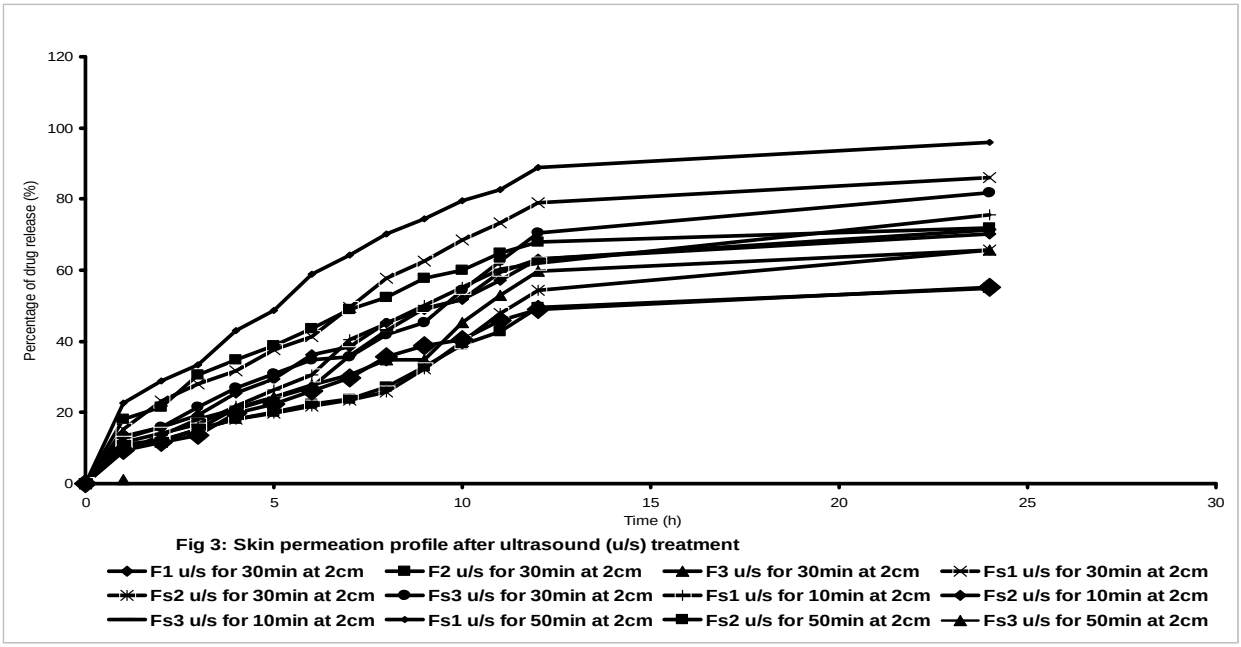

Fig.3: Carvedilol skin permeation profiles after ultrasound application with different time period

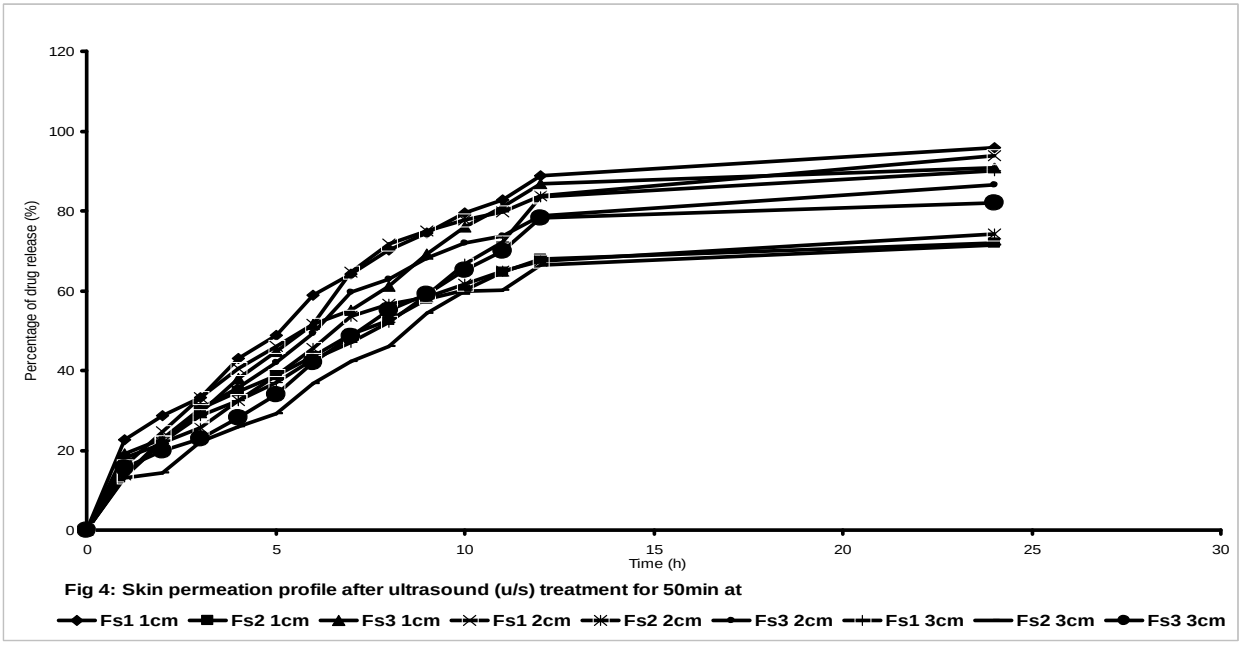

Fig.4: Carvedilol skin permeation profiles after ultrasound application with different height

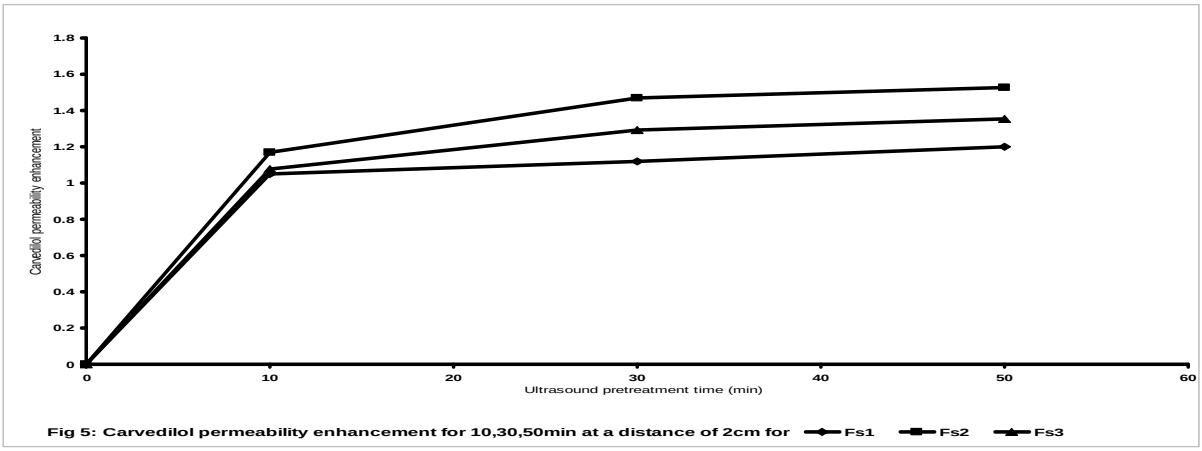

Fig.5: Enhancement of skin permeation profiles of carvedilol by sonophoresis

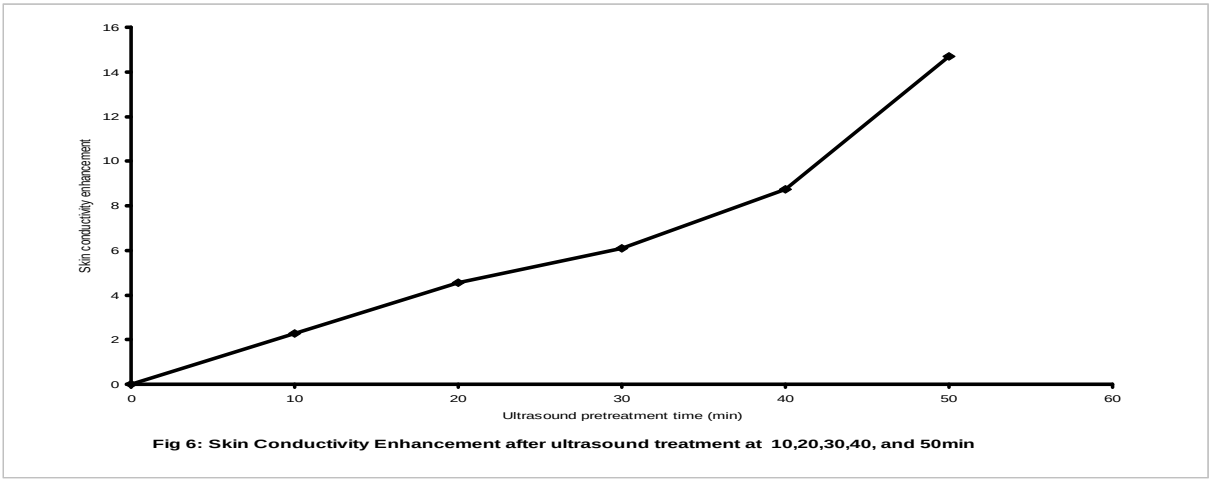

Fig.6. Effect of Skin conductivity enhancement by ultrasound time

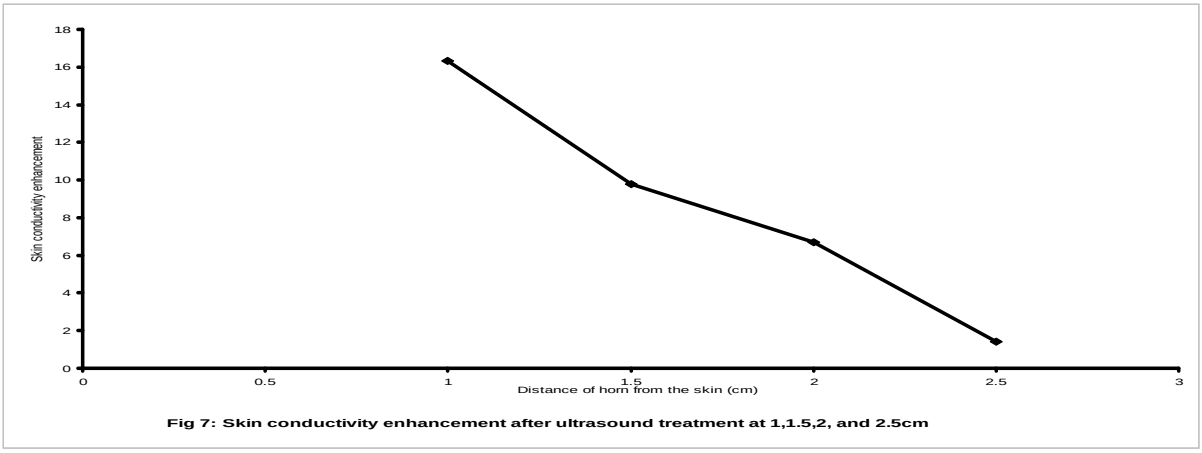

Fig.7. Effect of Skin conductivity enhancement after ultrasound applications at various heights

bypass the hepatic first pass metabolism. The transdermal patch delivery system is an attractive choice of an alternative route of administration.

Drug content determined for the prepared patches was found to be between 92.62% to 95.77%.

The logarithmic value of the partition coefficient of the drug in octanol-saline phosphate buffer pH 7.4 system, in this study was found to be 0.912 which shows that the value is well within the range of 0.8-3.0 required for a transdermal patch delivery system. Drug-excipient interactions were studied using Infrared spectroscopy. The results show that there was no interaction between the drug and excipients.

The moisture content and moisture uptake of the various formulations showed that with the increase in concentration of hydrophilic polymer, PVP, both the percentage of moisture content and the moisture uptake increased. The small moisture content in the formulations helps them to remain stable and from being a completely dried and brittle film. Again a low moisture uptake protects the material from microbial contamination and bulkiness of the patches. The results are shown in Table. 2.

In vitro dissolution results are shown in Fig. 1. Maximum Percentage of drug released was found for the formulations Fs1 (PVP/EC 1:2) and minimum percentage of drug released was observed for the formulation F2 (PVP/EC 1:5). It shows that increase in the concentration of EC in the formulations decrease the rate of dissolution. The results of *in vitro* permeation are shown in fig. 2. The results show that maximum percentage of drug released (73.86%) was found for the formulation Fs1 and minimum percentage of drug released (1.91%) was observed for the formulation F2. It shows that surfactant increases the permeation and the effect of the surfactant on the dissolution as well as permeation of the drug is evident. Ultrasound was applied at frequency of 1 MHZ and at an intensity of 2 W/cm^2. The permeation studies were carried out after subjecting the skin to ultrasonication for various time intervals of 10 min, 30 min and 50 min and the distance of horn from the skin was maintained at 2 cm.

The skin was also subjected to ultrasonication for 50 min at a distance of 1, 2 and 3 cm from the horn. The results are shown in Fig.3 and 4 respectively. Carvedilol permeability enhancement was calculated and the results are shown in Fig. 5. In case of formulation with PVP: EC in ratio 1: 2 *in vitro* permeation without surfactant or ultrasound application was found to be 2.86%, with the addition of surfactant it was found to be 73.86%, and with surfactant and ultrasound application for 50min at a distance of 1cm was found to be 95.96% which is optimum. Fig. 3 corresponds to ultrasound application when the transducer is 2 cm from the skin. SCE is proportional to pretreatment time. As the time of application increases the SCE also increases. The results are shown in Fig. 6. Fig. 5 shows the Carvedilol permeability enhancement measured over a period of 24 h after ultrasound pretreatment under the same ultrasound condition of Fig.6. Fig.7 shows the dependence of skin conductivity enhancement on the distance of the horn from the skin. Skin conductivity enhancement increases with a decrease in the distance of the horn from the skin. The results suggest that the distance of the horn from the skin is an important parameter in determining the efficiency of sonophoresis. Bringing the horn close to the skin may increase the pressure amplitude at the skin and hence increase the effectiveness of low frequency. The data presented here offer information for optimizing ultrasound parameters used in sonophoresis and to study the synergistic effect of surfactant and ultrasound on drug permeation. We show the dependence of sonophoresis on time of application and distance of horn from the skin. The synergistic effect of ultrasound and use of surfactant has been established.

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