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Original Article

Cefixime trihydrate loaded chitosan-alginate transdermal patches

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ABSTRACT

Cefixime trihydrate is a third generation cephalosporin with a broad spectrum bactericidal activity. Due to its short biological half-life, it's required for frequent dosing, poor bioavailability and first pass metabolism. Transdermal matrix patches of Cefixime trihydrate were prepared by Solvent casting method using cross linked polymers with Chitosan and Sodium alginate with or without permeation enhancer and the patches were evaluated and found to be having good physicochemical properties. The cumulative percentage of drug permeation without and with permeation enhancer after 24 h through rat skin was found to be FS-1 to FS-4 was found 49.40% to 50.84% and FPE-1 to FPE-4 was found 75.48 % to 88.38 %. The kinetic profile show that the drug release followed zero-order kinetics and well fits with Higuchi's model and followed by non-fickian diffusion mechanism of drug release. The drug permeation rate (flux) and enhancement ratio was found to be maximum in the formulation FPE-2. Further, selected for invivo study of FPE-2 formulation and compared with oral suspension by using the rats, the results shows after 24 h 82.91% and 28.04%. The invitro-invivo correlation of FPE-2 was found to be 0.969, which indicates that linear and super-imposable.

Keywords: Skin permeability, Higuchi matrix, Controlled release, Cross linked polymers, Skin irritation study, Invitro-invivo correlation.

INTRODUCTION

Transdermal drug delivery system is topically administered medicaments in the form of patches that delivers the drug for systemic effects at a predetermined and controlled rate. The transdermal delivery offers several advantages, the skin represents a relatively large and readily accessible surface area for absorption, the application is a non-invasive procedure that allows a continuous intervention, and it is possible to cease the absorption preventing over dose or undesirable effects. Compared with the traditional oral administration route, transdermal delivery shows additional advantages it minimizes the first-pass metabolism, avoids drug degradation under the extreme acidity of the stomach, it prevents erratic delivery due to food interactions and it provides more controlled delivery. The delivery rate is controlled by the skin or membrane in the delivery system [1, 2]. Today trans dermal drug delivery system is a well-accepted means of delivering many drugs to the systemic circulation and currently trans dermal patch devices are used to treat motion sickness, hypertension, angina, female menopause, severe pain, nicotine dependence, and male hypogonism. US FDA approved the first transdermal patch in 1979, this patch delivered Scopolamine, a drug which suppresses nausea and vomiting in motion sickness. Over the last two decades, more than 35 transdermal products have been approved generating sales $ 3.2 billion in 2002, which is predicted to rise to $ 4.5 billion in 2008. This rapid increase in market value has lead to transdermal drug delivery becoming one of the fastest growing sectors within Pharmaceutical Industry [3].

The goal of the Trans dermal administration of drugs is not to achieve a bolus –type drug input rather, it is usually designed to offer a slow, sustained release of drug over longer period of time. Current trans dermal delivery system are capable to deliver drugs in cases that oral

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administration is limited by poor bioavailability, side effects associated with high peak plasma concentrations or poor compliance due to the need of frequent administration[4].

The transdermal absorption processes requires drug characteristics or an appropriate carrier which should be able to deliver the drug to the desired skin deepness to reach topical or systemic effects. A good transdermal delivery system must not only provide an adequate drug release from the formulation, but also allow considerable amounts of drug to overcome the skin barrier, ensure a non-irritancy of the skin, and also ensure that the drug will not be inactivated on the skin's surface or during the permeation processes [5]. An antibiotic (from the Ancient Greek: ἀντί – *anti*, "against", and βίος – *bios*, "life") is a substance or compound that kills, or inhibits the growth of bacteria. Antibiotics belong to the broader group of antimicrobial compounds, used to treat infections caused by microorganisms, including fungi and protozoa. Cephalosporin is the most frequently prescribed class of antibiotics. Cefixime trihydrate is an orally active third generation Cephalosporin with bactericidal activity against a wide variety of Gram-positive and Gram-negative organisms. This is commonly used for the treatment of Urinary tract infections, respiratory tract infections, typhoid fever etc [6, 7].

Cefixime trihydrate an orally active third generation cephalosporin with bactericidal activity against a wide variety of Gram-positive and Gram-negative organisms has been selected for the study to be formulated into a transdermal drug delivery system. It is the second largely used cephalosporin derivative in the treatment of respiratory, urinary and biliary tract infections. It is available as tablets and oral suspensions. It is rapidly and extensively absorbed from the gastro intestinal tract. Absolute bioavailability is in the range of 22% to 54% due to a significant degree of first-pass metabolism. Following oral administration, the apparent mean terminal elimination half-life of Cefixime generally ranges from 2.5 to 3.8 hours. Due to its short biological half-life, there is a need for frequent dosing which increase the burden on the metabolic system as well as increase in gastric irritation [8-10]. So an alternative route of administration like transdermal delivery system was chosen to deliver the drug directly to the systemic circulation thereby to reduce the dose as well as the frequency of dosing. Chitosan is a hydrolyzed (deacetylated) derivative of chitin, a biopolymer widely distributed in nature and biologically safe. This polymer exhibits several favorable properties, such as biodegradability and biocompatibility. Being a bio-adhesive polymer and having a good antibacterial activity, Chitosan is a good candidate for the site specific delivery, Chitosan has received considerable attention as a possible pharmaceutical excipients in recent decades due to its good biocompatibility and low toxicity properties in both conventional excipients applications as well as in novel application. And also its have unique properties like Wetting agent, and improvement of dissolution of poorly soluble drug substances [11, 12].

The film forming properties of Chitosan is used in the development of medicated transdermal patches along with a co-polymer like Sodium alginate. Combination of positively charged Chitosan with negatively charged bio-molecules like Sodium Alginate has been tested to yield novel matrices with unique characteristics for controlled release of the drug. Due to its cationic nature, Chitosan is capable of opening tight junctions in a cell membrane. This property helps in the usage of Chitosan as a permeation enhancer for drugs with poor oral bioavailability. The absorption enhancement is caused by interactions between the cell membrane and positive charges on the polymer. Thus, Chitosan can be served for a number of purposes like coating agent, gel former, controlled release matrix to improve the bioavailability of the drug [13-15].

EXPERIMENTAL

Dose Designing: Based on the Pharmacokinetic parameters of Cefixime trihydrate, the amount of drug required to achieve the effective plasma concentration was calculated using the equation [16],

$Kp = (Cplasma)ss \times Kel \times Vd$

Where, (Cplasma)ss - The drug level at steady state,

Kel - elimination rate constant and

Vd - the volume of distribution of drug.

Fabrication of Transdermal Patches: Solution of sodium alginate in distilled water containing Cefixime trihydrate in dispersed form was added drop wise to Chitosan solution in 2 % lactic acid under constant stirring. Glycerol solution was added as plasticizer. The coacervates formed were separated, poured in Petri dish and dried at room temperature to form films [17]. The concentration of the drug was kept constant and the concentration of the polymers Chitosan and Sodium alginate were altered.

Physicochemical Evaluations

Thickness of the patch: Thickness of the patch was measured by using a digital micrometer screw

gauge at three different places and the mean value was calculated [17].

Folding endurance: The folding endurance is expressed as the number of folds the patch is folded at the same place, either to break the specimen or to develop visible cracks. This test is important to check the ability of the sample patches to withstand folding. This also gives an indication of the brittleness. The folding endurance was measured manually for the prepared film .A strip of film was cut evenly and folded at the same place till it broke .The number of times the film could be folded at the same place without breaking gave the exact value of folding endurance[18].

Tensile strength: The patch was pulled by means of a pulley system; weights were gradually added to the pan to increase the pulling force till the patch was broken. The elongation i.e. the distance traveled by the pointer before break of the patch was calculated as kg/cm^2 [19].

Percentage of moisture absorption: To check the physical stability of the film in high humidity condition, accurately weighed film were placed in a desiccators containing saturated solution of aluminum chloride (79.5% relative humidity) for 3 days The film were reweighed and percentage moisture absorption were calculated.

Percentage of moisture loss: To check the extent of moisture loss from freshly prepared film. Accurately weighed film were placed in a desiccator containing fused anhydrous calcium chloride for 72 hrs .After 72 hrs the film were reweighed and percentage moisture loss was calculated[21].

Water Vapor transmission rate: The patches were fixed over the edge of the glass vial containing 3g of fused calcium chloride as a desiccant by using an adhesive. Then the vial was placed in a desiccator containing saturated solution of potassium chloride. The vial was taken out periodically and weighed for a period of 72 hours [22].

Drug content uniformity: The prepared patch was cut into small piece and put into 100ml dissolution or diffusion medium used respectively and Stirred continuously in an electrical shaker and sample was withdrawn at the end of three hours and the drug content was determined by uv-visible spectrophotomer at 288nm[23-25].

Stability Study: The prepared patches were subjected to stability study by storing the patches at storage conditions specified in the ICH guidelines. The patches were stored for three months at temperature $40^0C \pm 2^0C$ and at a relative humidity of 75% ± 5%. The stability study was conducted with regard to tensile strength, moisture content and drug content. The patches, which retained their physical properties, were further subjected to in-vitro permeation studies [26].

Skin Irritation Test: Ethical clearance for the handling of experimental animals was obtained from the Institutional animal ethical committee (IAEC) formed for this purpose. The protocol of the animal study was approved by the Institutional Animal Ethical committee, Protocol Number: SVCP/ IAEC/02/2009 dated, 7th November 2009. The experiment was conducted according to the guidelines of CPCSEA (Committee for the purpose of control and supervision of experiment on animals [27].

***Invitro* skin permeation studies of transdermal patch:** The experiments were performed in accordance with the guidelines for animal use specified by the Institutional Animal Ethical Committee (IAEC).

Preparation of rat skin: The full thickness rat abdomen skin was excised; all the subcutaneous fat and hairs are removed and cut into required size for experimental use. The skin was washed with purified water thoroughly, soaked in normal saline and stored in freezer at 30^0C until used. During the experiment, the skin was thawed at room temperature and checked for any damage and then used for the study [28].

***In vitro* skin permeation studies:** These studies were carried out using rat abdomen skin of required thickness. The permeation cell used for this study was a specially fabricated "Franz diffusion cell". A 3.14 cm^2 patch was placed in intimate contact with the stratum corneum side of the skin; the outer portion was covered with aluminum foil as a backing membrane. Teflon bead was placed in the receptor compartment filled with 60 ml of Phosphate buffer pH 7.2 solutions. The whole assembly was kept on a magnetic stirrer, at a speed of 100 rpm and the temperature conditions controlled at $32^0C \pm 0.5^0$ C. The cell contents were stirred with a magnetic stirrer. Sample of 1 ml was withdrawn at pre-determined time intervals and simultaneously replaced with equal volume of fresh medium. The samples were withdrawn and filtered through Whattman filter paper. The absorbance of the solution was measured by UV at 288nm [29].

Modified Franz diffusion cell: Modified Franz diffusion cell is a skin permeation system

developed by Franz and commercialized by crown glass has been frequently used for the studying the kinetics of percutaneous absorption. In the modified Franz diffusion cell has receptor compartment with an effective volume approximately 60ml and an effective surface area of permeation of 3.14cm^2. A rod shaped magnetic stirrer driven by a 3w synchronized motor stirs the solution in the receptor compartment. The stirring magnet rotates at constant rpm in a low viscosity receptor compartment.

Operation of diffusion cell: The skin was mounted and clamped between the receptor and donor compartment .The surface area exposed to the drug patch was 3.14 Sq. cm. The cell was maintained at $32^0 \pm 0.5^0$c using thermostatically controlled water bath. Water from the water bath was circulated through the cell, which was maintained at constant flow. The diffusion cell of 60ml capacity was filled with Phosphate buffer pH 7.2 and fixed to a thermostatically controlled magnetic stirrer, which was also maintained at the temperature $32^0 \pm 0.5^0$C [30].

Kinetic characteristics of the drug release: To know the mechanism of the drug release from the patches, the results obtained from the *In-vitro* skin permeation studies were fitted into different kinetic equations as follows [31, 32]

1. Zero - order drug release: Cumulative % drug release Vs Time
2. First Order drug release: Log cumulative % drug retained Vs Time
3. Higuchi's classical diffusion equation: Cumulative % drug release Vs Square root of time
4. Korsemeyer Exponential equation/ Peppa's model: Cumulative % drug release Vs Log time

Permeation Enhancement Ratio: The Permeation enhancement ratio was calculated from the kinetic study of the formulations with and without permeation enhancer (FS1-4 & FPE1-4). It was calculated from the following parameters [33].

Permeability coefficient (P): Permeability coefficient is the velocity of drug passage through the membrane in µg/cm ^{2} /h. The permeability coefficient was calculated from the slope of the graph of percentage of drug transported versus time as,

P = Slope x Vd / S

Where, Vd is the volume of donor solution and S is the surface area

Flux (J): Flux is defined as the amount of material flowing through a unit cross-sectional barrier in unit time. It is calculated by,

Flux (J) = P x CD

Where CD = concentration of donor solution and

P= permeability.

Enhancement ratio: Enhancement ratio was used to evaluate the effect of permeation enhancer on diffusion and permeation of selected drug molecules.

STATISTICAL ANALYSIS BY ANOVA: The in-vitro permeation data were statistically compared and analyzed using Graph Pad In stat – 3 software version. The Tukey – Kramer multiple comparisons test was utilized to find the 'q' and 'p' values and the significance of the formulations were studied [34],

If the 'p' is <0.05, the formulations are said to be significant

***INVIVO* STUDIES OF TRANSDERMAL PATCHES:** Six wistar rats of 180-220gm weight were to be utilized for the study. The rats were to be allowed to acclimatize for 1 week before the day of administration. The rats were fasted, but allowed access to water on the day before the study [35]. Six groups of rats were taken for the animal studies. Each group containing one rat Rats were to be anesthetized before the experiment. The hair on the abdominal site is clipped before the experiment. The skin was gently wiped with water and alcohol swab and patted dry. The patch of 3.14sq.cm size patch containing 5mg Cefixime trihydrate was fixed over the prepared skin. Blood samples of approximately 0.15ml were collected from the jugular vein in the dried heparinized tube at 0.5, 2, 4, 8, 16, 24 hrs after transdermal administration, and they are frozen at -20°C as soon as possible and stored until the analysis. The HPLC analytical reports of the blood samples collected at different hours were compared with that of standard

HPLC ANALYSIS:

Cefixime standard solution: Standard solution of Cefixime are prepared by dissolving 11.4mg of the compound (equivalent to 10 mg of Cefixime corrected for purity) in 1 ml of HPLC grade methanol and diluting the mixture to 10ml with HPLC water in volumetric glassware .This stock solution ,equivalent to 1 mg/ml was stored at 3^0C for up to one week. The 1mg/ml stock was further diluted 1:100 with HPLC water to prepare an additional standard that was 10µg/ml [36].

Mobile Phase: Mobile phase was prepared fresh on the day of analysis and was filtered and degassed by vacuum. Mobile Phase was prepared by combining 170ml of acetonitrile,1.36gm of monobasic sodium phosphate,2ml of 85% phosphoric acid and 828ml of HPLC water (Acetonitrile and buffer in the ratio of 17:83). The resulting pH of mobile phase solution was approximately pH7.2.

Serum sample preparation: A 250 µl aliquot of the serum of samples transferred in to a polypropylene 1.5 ml snap-cap conical bottom centrifuge tube to which were added 250µl of the working 6% TCA reagent for deproteinization .The tubes were vortexed at high speed for 15 s and centrifuged at 30,000g for 2 min. A 200µl portion of the clear supernatant was transferred to a auto sampler micro vial, 75µl of which were injected for each analysis. These samples were found to be stable for atleast 48hrs at room temperature [36].

***In vitro / In vivo correlation*:** *In-vitro / In-vivo* correlation is the demonstration of the direct relationship of *invitro* dissolution rate / diffusion rate of drugs and there *in vivo* bioavailability. Generally, the *in vitro* property is the rate or extent of drug dissolution or release while the in vivo response is the plasma drug concentration or amount of drug absorbed[86]. Correlation is used to ensure batch-to-batch consistency in the physiologic performance of a drug product by use of such *in vitro* values and to serve as a tool in the development of a new dosage form with desired in vivo performance [37, 38].

There are two basic approaches by which a correlation between dissolution/diffusion testing and bioavailability can be developed.

1. By establishing a relationship, between the in vitro dissolution/diffusion and the in vivo bioavailability parameters. If this relationship becomes linear with a slope of 1, then curves are super imposable, and there is a 1:1 relationship which is defined as point-to-point or level A correlation.
2. By using the data from previous bioavailability studies to modify the dissolution/diffusion methodology in order to arrive at meaningful *in-vitro / in-vivo* correlation.
3. The first approach was followed for studying *in-vitro / in-vivo* correlation.

RESULTS AND DISCUSSION

In the present study, transdermal matrix patches of Cefixime trihydrate were prepared by Solvent casting method using Chitosan and Sodium alginate as polymers. The patches were found to be having good film formation and smooth in their appearance. It was desired to develop a transdermal system that allows one to provide an optimum drug release via the most appropriate choice of rate controlling membrane and finally to produce overall constant /controlled release. The flexibility of formulations was also very good and fulfilled the physicochemical evaluations.

FTIR Spectroscopy: The compatibility studies were done by FT-IR Spectroscopy. The FT-IR spectral analysis showed that there were no physical and chemical interactions between the drug and polymers and they were found to be compatible with each other.

Physico- chemical evaluation: The moisture content in the preparations was found to be low. This helps the formulations to be stable and prevents them from drying and brittle. Moisture uptake ranged from 6.073 to 7.162%. Low moisture uptake protects the materials from microbial contamination and avoids bulkiness of the patches. The percentage moisture loss ranged between 0.280 to 0.795%, which prevents the films from drying.. A good tensile strength was found in all the films, ranging from 0.22 ±0.002 to 0.042±0.001g/cm $^{2.}$. The drug content Percentage yield was in the range 84.4 ± 2.00% to97.5 ± 0.10% [39, 40].

Skin irritation studies: The skin irritation test was conducted using rabbit on which drug loaded film was fixed, and observed that there is no oedema or erythema were produced. So the transdermal patches are free from skin irritation

***In vitro* skin permeation studies:** In vitro skin permeation studies were carried out using rat skin in a diffusion cell. Skin permeation profile demonstrated that, duration of sustained release of the patches appears to be depend on the characteristics and concentration of Chitosan polymer, the cumulative percentage drug permeation was found to be 49.404%, 55.413%, 57.766% and 50.840% (table-23) for formulations FS-1, FS-2, FS-3 and FS-4 respectively. The studies indicated that the transdermal patches of cefixime trihydrate showed lesser permeable. The in vitro skin permeation studies were carried out with Span 80 (1%) as permeation enhancer. The cumulative % drug permeation was found to be 77.967%, 88.385%, 79.419%, and 75.488% for the formulations FPE-1, FPE-2, FPE-3, and FPE-4 respectively. An ideal transdermal delivery system should show sustained release characteristics. Taken this in to account, the formulation FPE-2 met the above objective and hence, it was selected for in vivo studies. There is an increase in drug release with the increase in the concentration of

Sodium alginate two fold of the concentration of Chitosan. Formulations with altered concentrations of Chitosan and Sodium alginate show better results than the formulations with equal proportions of the polymers. This might be due to the cross linking and internal gelling capacity of Sodium alginate. The release patterns of the patches were controlled and spread over extended period of time. The values of the coefficient of correlation (r) were calculated and were found to be linear for the Zero order release. The kinetic profile show that the drug release followed zero-order kinetics and well fits with Higuchi's matrix model. The *In-vitro* drug release data were fitted to korse meyer Peppa's release model. The 'n' values were in the range of 0.5-1, so the drug release mechanism was diffusion and non fickian transport. Formulations also showed higher 'r' values for zero-order kinetics rather than first – order kinetic indicating that the release is by diffusion mechanism. The drug permeation profile suggests that it followed fick's law of diffusion. Linear relationship between cumulative percent drug permeated verses time indicate zero order permeation of drug through rat abdomen skin. The permeation co-efficient of the formulations FS-2 (without permeation enhancer) and FPE 2 (with permeation enhancer) were 17.4490 mg/cm^2 / day and 28.7315 mg/cm^2 / day respectively which indicate that the use of permeation enhancer like Span-80 (1%) improves the permeation of the drug considerably. The enhancement ratio was also found to be in the appreciable range 1.39 to 1.74.

Statistical analysis by ANOVA: The statistical analysis of the cumulative percentage of drug permeation data showed that the formulations are having 'p' value < 0.0001. This result suggests that the prepared transdermal patches are extremely significant for the approach.

Stability studies: Stability studies of the patches were performed in terms of stability against storage conditions and aging effect. The physical characterization like tensile strength, moisture absorption and drug content were main parameters for stability studies, which showed no appreciable changes occurred in patches. The formulations which were stable in their physical nature were selected and they were subjected to *In-vitro* skin permeation studies.

***In vivo* analysis:** The *invivo* analysis were performed for the best formulation selected from the *in vitro* permeation studies, (i.e FPE-2 formulation), The HPLC spectrophotometer was used for the analysis. The *invivo* drug absorption studies reveals that there is a controlled release of drug in to the systemic circulation and 82.91% of drug absorption after 24hrs time interval. The oral absorption the results shows, was prepared and rat dose was calculated

***In-vitro/ In-vivo* correlation**: In the current study of Cefixime trihydrate, the slope between the *invitro* skin permeation studies and *invivo* absorption studies were found to be 0.962 and the regression value will be 0.969. So, they appear to be well correlated, and the plots were not super imposable and are linear.

CONCLUSIONS

Cefixime trihydrate being a hydrophobic drug can be effectively transported through the skin by using suitable permeation enhancer. The characteristics and concentration of chitosan polymer can alter the permeation of the drug. The comparative statistical analysis done by ANOVA method proved that all the formulations are extremely significant for the transdermal route. The in vivo studies proved a controlled release effect of the drug in to the systemic circulation. The in vitro/ in vivo correlation were found to be not super imposable and said to be well correlated.

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Table 1: *In-vitro* skin permeation of various formulations (Without permeation enhancers)

Time (hrs)	Cumulative % of Drug Permeation			
	FS-1	FS-2	FS-3	FS-4
0	0	0	0	0
0.25	0.875	0.853	0.795	0.457
0.5	3.456	1.880	1.778	2.567
0.75	7.775	5.999	5.786	6.955
1	13.932	13.885	14.183	18.097
2	23.563	21.850	22.559	24.737

3	26.916	27.934	26.768	26.786
4	28.767	31.941	31.927	28.769
6	30.857	36.744	35.747	31.578
8	31.861	38.848	38.436	33.981
10	33.882	42.722	40.777	36.496
12	36.228	45.444	43.483	38.903
14	39.320	47.997.	44.924	41.880
16	40.564	49.970	48.843	44.074
20	43.872	52.813	54.823	47.854
24	49.404	55.413	57.766	50.840

Fig 1: *Invitro* skin permeation profile of various formulation (Without permeation enhancers)

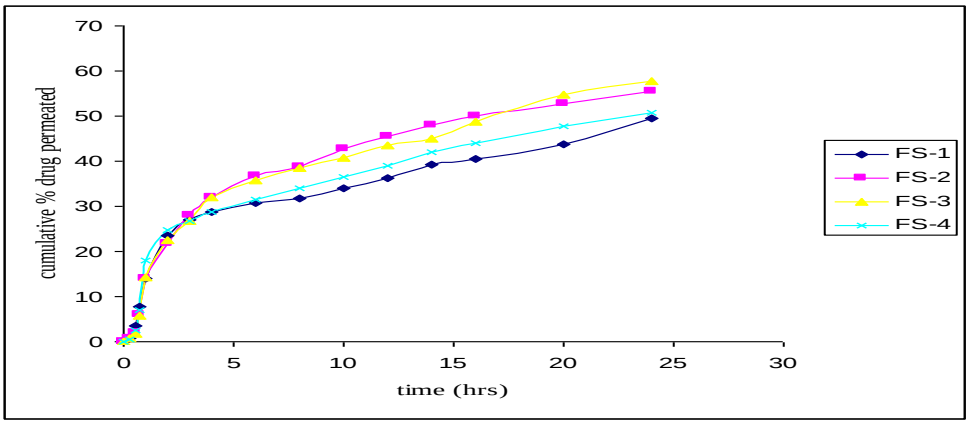


Table 2: *Invitro* skin permeation of various formulations (With permeation enhancers)

Time (hrs)	Cumulative % of Drug Permeation			
	FPE-1	FPE-2	FPE-3	FPE-4
0	0	0	0	0
0.25	1.105	4.845	2.272	2.67
0.5	6.892	10.998	9.199	8.649
0.75	12.449	15.479	13.598	12.780
1	18.024	22.522	20.570	22.477
2	29.933	32.921	32.737	31.264
3	38.578	38.847	38.525	35.646
4	43.880	45.896	45.844	42.754
6	52.412	56.328	50.665	48.331
8	58.547	62.693	55.867	54.404
10	61.008	70.075	58.189	59.245
12	62.859	76.659	60.331	63.708
14	66.589	77.518	65.340	66.440
16	68.425	80.311	70.242	70.235
20	73.345	85.451	75.481	71.515
24	77.967	88.385	79.491	75.488

Fig 2: *Invitro* skin permeation profile of various formulation (With permeation enhancers)

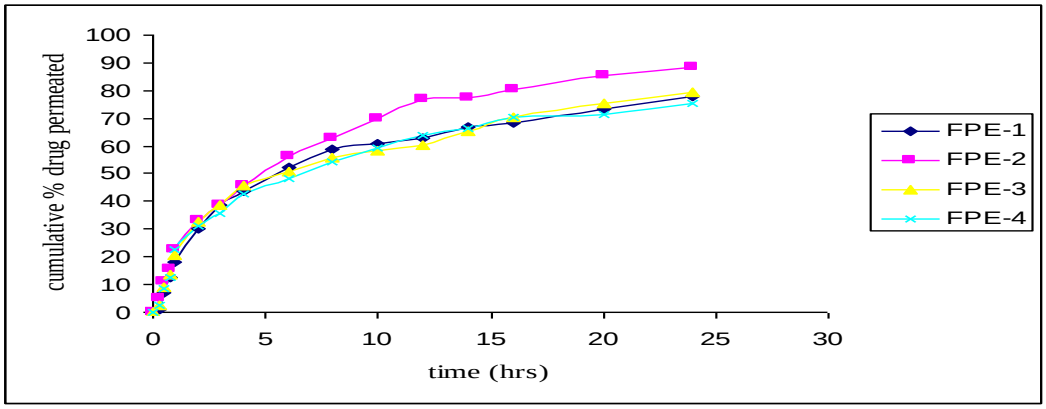


Table3: Kinetic values obtained from various formulations

Formulation Code	Zero order	First order	Higuchi	Korsemeyer		Possible mechanism of drug release
	R^2	R^2	R^2	n	R^2	
FS-1	0.7883	0.7323	0.9113	0.8357	0.8781	Higuchi diffusion
FS-2	0.7963	0.7423	0.9356	0.9953	0.8838	Higuchi diffusion
FS-3	0.8267	0.7223	0.9465	0.9803	0.8982	Higuchi diffusion
FS-4	0.7905	0.7111	0.9157	0.8549	0.8389	Higuchi diffusion
FPE-1	0.8018	0.7978	0.9412	0.9743	0.9419	Non-fickian release
FPE-2	0.8400	0.7979	0.9670	0.9989	0.9733	Non-fickian release
FPE-3	0.8225	0.7978	0.9533	0.9081	0.9475	Higuchi diffusion
FPE-4	0.8157	0.7998	0.9518	0.9073	0.9495	Higuchi diffusion

Table4: *In vitro* skin permeation kinetic data for transdermal patches (Without permeation enhancers)

SL.no	Formulation code	Slope of zero order plot	Permeation coefficient(p)	Flux(J)
1	FS-1	1.7135	2.7285	13.6425
2	FS-2	2.1916	3.4898	17.4490
3	FS-3	2.1792	3.4701	17.3501
4	FS-4	1.8520	2.9490	14.7452

Table5: In vitro skin permeation kinetic data for transdermal patches (With permeation enhancers)

SL.no	Formulation code	Slope of zero order plot	Permeation coefficient(P)	Flux(J)	Enhancement ratio(Er)
1	FPE-1	2.9940	4.7675	23.8375	1.7473
2	FPE-2	3.4087	5.7463	28.7315	1.6465
3	FPE-3	3.1478	4.8546	24.2730	1.3990
4	FPE-4	3.0456	4.8864	24.4320	1.6569

Table 6: *In-vivo* drug absorption of oral suspension

S. No	Time(hrs)	Mean Area under curve (AUC)	Concentration (µg/ml)	% drug absorption
1	control	59403.5	10	--
2	0.5	15184.83	2.564	5.13
3	2	25057.34	4.231	8.46
4	4	51233.99	8.651	17.30
5	8	61734.26	10.424	20.85
6	16	71677.84	12.103	24.21
7	24	83030.93	14.020	28.04

Fig 3: Percentage in-*vivo* oral absorption

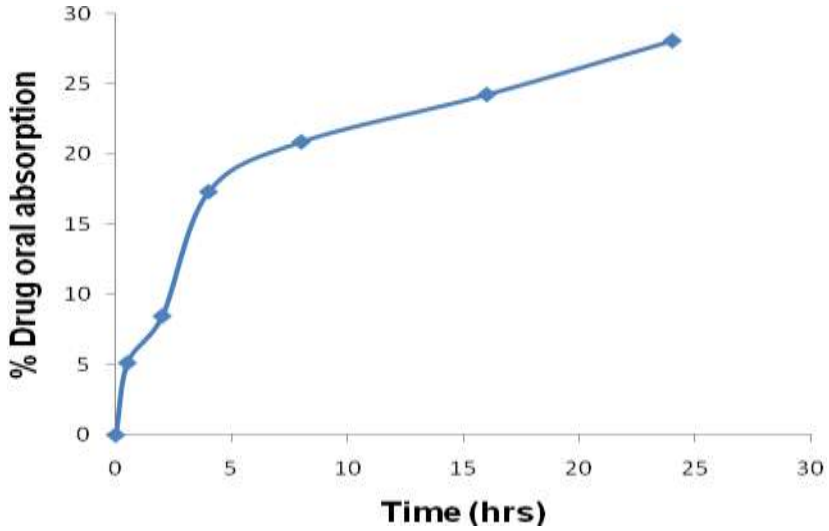


Table 7: *In-vivo* transdermal drug absorption (FPE-2)

S. No	Time(hrs)	Mean Area under curve (AUC)	Concentration (µg/ml)	% drug absorption
1	control	59403.5	10	--
2	0.5	52750.22	8.879	17.76
3	2	103529.4	17.76	34.86
4	4	124512.7	20.96	41.80
5	8	182526.7	30.72	61.45
6	16	221125.2	37.22	74.44
7	24	246373.1	41.47	82.91

Fig 4: Percentage *invivo* absorption

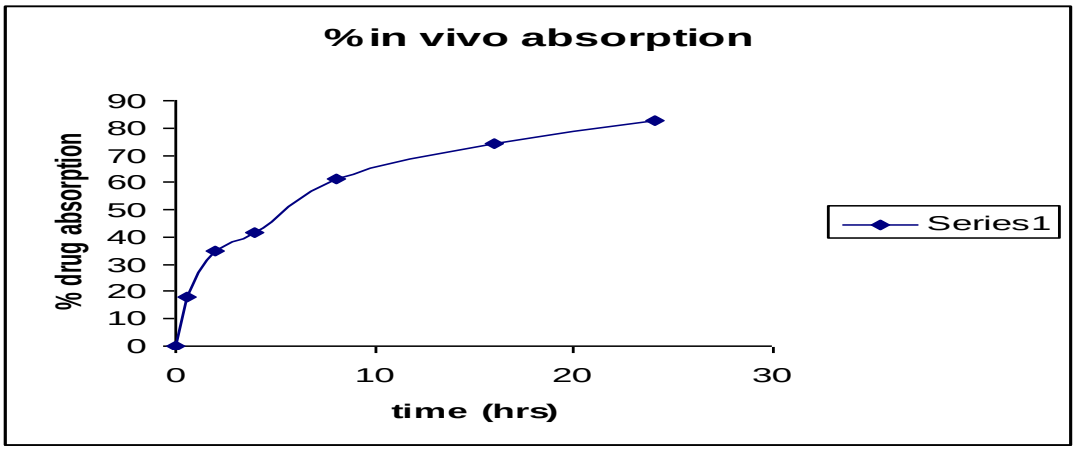


Table 8: *Invitro In vivo* correlation

Sl .No	Time (in hrs)	*Invitro* skin permeation (%) (FPE-2)	*In vivo* absorption (%) (FPE-2)
1.	0.5	10.998	17.76
2.	2	32.921	34.86
3.	4	38.847	41.80
4.	8	62.693	61.45
5.	16	80.311	74.44
6.	24	88.385	82.91

Fig 5: Comparative *In vitro-In vivo* drug absorption

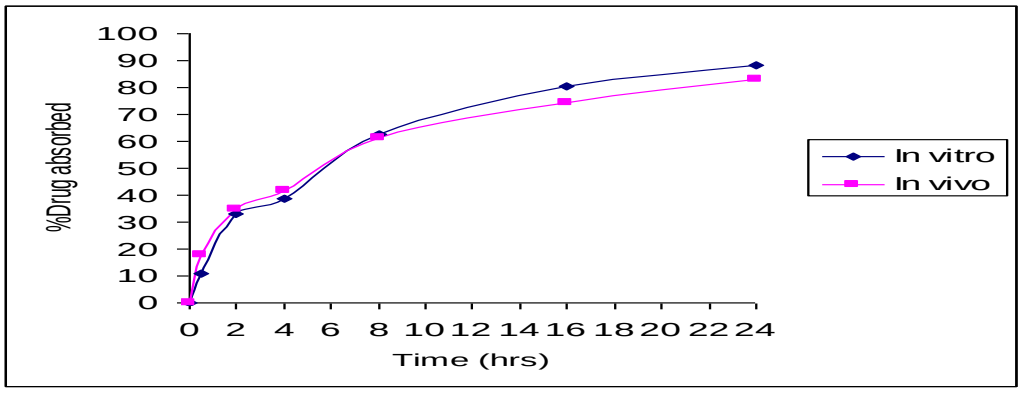
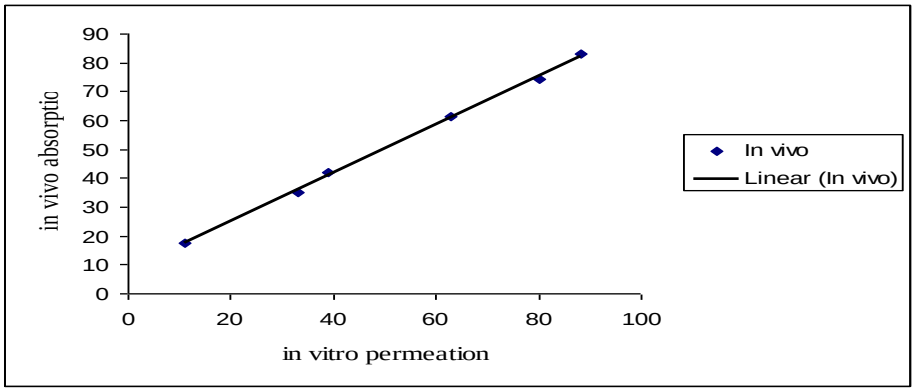


Table 9: *Invitro In vivo* correlation

In-vitro skin permeation (%) (FPE-2)	*In-vivo* absorption (%) (FPE-2)
10.998	17.76
32.921	34.86
38.847	41.80
62.693	61.45
80.311	74.44
88.385	82.91

Fig 6: *Invitro In vivo* correlation



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