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In-Vitro and *In-Vivo* studies of Combination patch of Diclofenac Sodium and Tizanidine Hydrochloride

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ABSTRACT

Transdermal Patches of Combination Drugs Diclofenac sodium-Tizanidine Hydrochloride were prepared using solvent casting method. The Patches were evaluated for various Physicochemical Parameters including In-Vitro and In-Vivo Studies. In-vitro Permeation Studies were conducted using franz diffusion cell on the Rat abdominal skin. The Permeability Coefficient values and Flux were calculated. Kinetics for drug release were calculated by Zero, First, Higuchi Model and Koresmeyer-Peppas model indicate that release mechanism follows Fickian Diffusion as the value of release exponent is less than 0.5 ranging in between 0.0847-0.1690 for Koresmeyer-Peppas model. The percent of drug permeated in 12 h was found to be maximum 99.8± 0.01 and 98.98± 0.01 % from formulations FA2 and FA8 respectively. The two best formulations were subjected to In-Vivo studies on male albino wister rats by carragenan induced Paw edema method. The formulations were compared with marketed formulation. It was observed the on apply ANOVA no significant difference is found between the Marketed Nu-Patch and best formulations. It was found that formulation FA8 with DSMO as Plastisizer and Span 80 as Penetration Enhancher is the best formulation in all the Evaluation Parameters.

1. Introduction

NSAIDS play an important role in symptomatic management of osteoarthritis, rheumatoid arthritis and ankylosing spondylitis and other acute pain conditions. They produce anti-inflammatory and analgesic effects by inhibiting cyclooxygenase and this preventing the production of prostaglandins from arachidonic acid. They inhibit leukotriene production via lipooxygenase inhibition.[1] The diclofenac patch was approved to treat acute pain due to minor strains, sprains and contusions. It is primarily recommended for and used by patients who cannot tolerate oral diclofenac. One diclofenac patch is applied twice daily (every twelve hours).[2] A spasm is an involuntary contraction of the muscle for a sustained period of time. Usually a spasm occurs when the muscle has been suddenly stressed somehow which can include injury, sprains, tears or bruising.[3] Tizanidine is used for the management of Spasticity. A patch having Diclofenac and Tizanidine in combination may provide added advantages for patients suffering from pain and muscle spasm. Diclofenac Sodium and Tizanidine Hydrochloride may be better administered via transdermal route due to these properties short biological half life, low oral bioavailability value, dose, molecular weight and adequate solubility of the drugs.

2. Research Methodology

2.1 Analytical method for identification of drugs-

The ultraviolet spectrophotometric method was selected in the present study for estimation of Diclofeanc Sodium(reported by Mouraoa *et al*.)and Tizanidine Hydrochloride(reported by Shayeda *et al*) the drug solution was scanned in between the wavelength between of 200-400nm. [4-5]

Table 1. Absorbance data of standard solutions of Diclofenac Sodium in phosphate buffer saline pH 7.4

S.No.	Concentration (µg/ml)	Absorbance
1	5	0.150 ± 0.007
2	10	0.285 ± 0.007
3	15	0.444 ± 0.013
4	20	0.578 ± 0.010
5	25	0.723 ± 0.005

Each data represents Mean ± S.D of three determinations

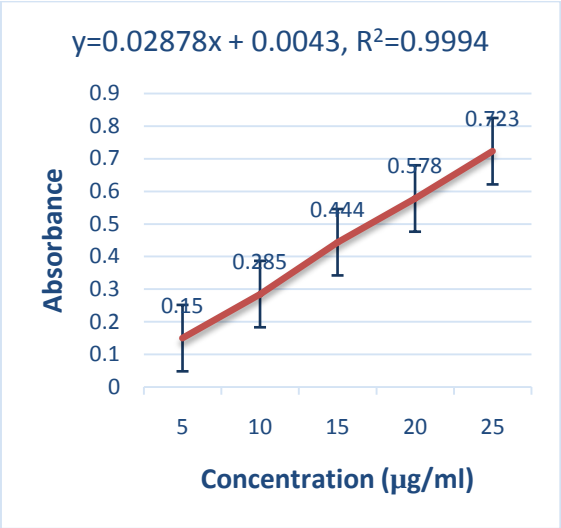

Fig.1-Standard Plot of Diclofenac Sodium in PBS 7.4

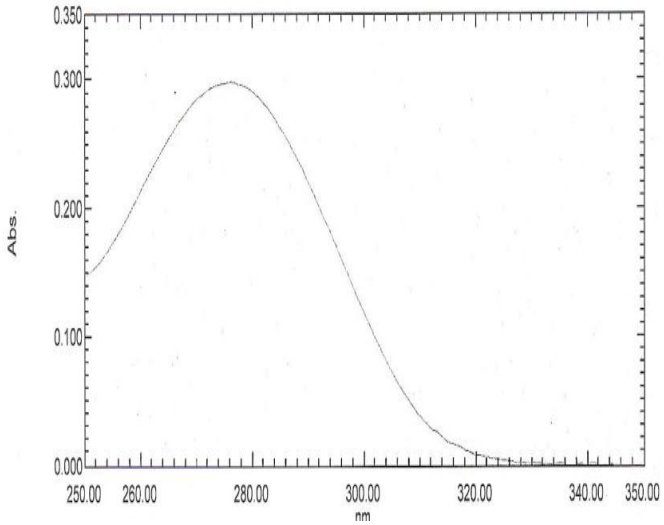

Fig.2- Spectrum of Diclofenac Sodium in PBS 7.4

The slope and the regression coefficient of the standard curve were found to be 0.029 and 0.9994 respectively.

Table 2-Absorbance data of standard solutions of Tizanidine Hydrochloride in 7.4 pH phosphate buffer saline.

S.No.	Concentration (µg/ml)	Absorbance
1	5	0.169± 0.002
2	10	0.34± 0.007
3	15	0.467± 0.005
4	20	0.634± 0.004
5	25	0.762± 0.006

Each data represents Mean ± S.D of three determinations

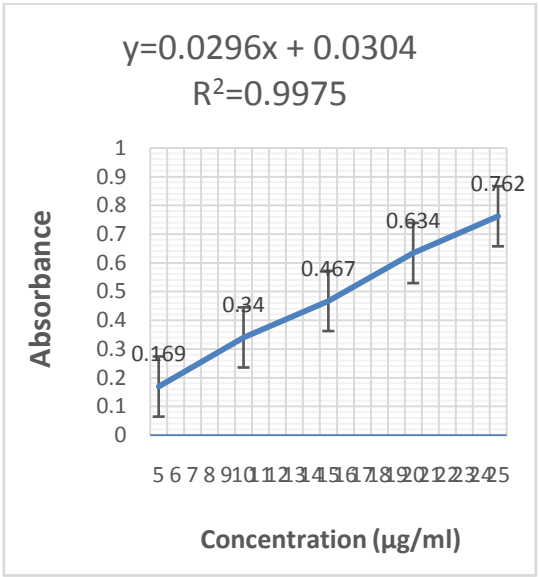

Fig.3 Standard Plot Of Tizanidine Hydrochloride in PBS 7.4pH

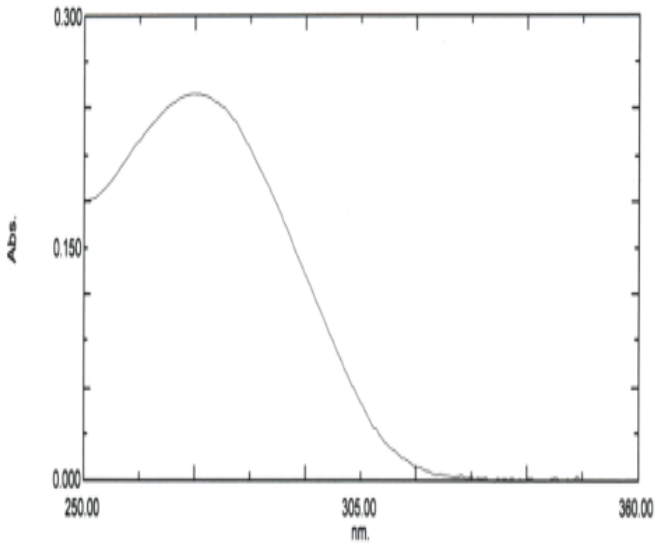

Fig.4- Spectrum of Tizanidine Hydrochloride in PBS 7.4pH

The slope and the regression coefficient of the standard curve were found to be 0.0304 and 0.9975 respectively.

2.2 Simultaneous estimation of Diclofenac Sodium and Tizanidine hydrochloride

In this method absorbances were measured at λ_{max} (λ_1 and λ_2) of both the drugs, i.e. Diclofenac Sodium (DICS) and Tizanidine hydrochloride (TIZH). Two equations were constructed based upon the fact that at λ_1=277 nm for Diclofenac Sodium DICS and λ_2 = 320.4 nm for Tizanidine hydrochloride (TIZH), the absorbance of the mixture is the sum of the individual absorbances of DICS and TIZH.(reported by Sanjay et al)[6]

$C_{DICS} = A_2ay_1\text{-}A_1ay_2 / ax_2ay_1\text{-}ax_1ay_2$

$C_{TIZH} = A_1ax_2\text{-}A_2ax_1 / ax_2ay_1\text{-}ax_1ay_2$

Where, A_1 and A_2 are absorbance of mixture at λ_1 and λ_2, ax_1 and ax_2 are absorptivities of DICS at λ_1 and λ_2 respectively; ay_1 and ay_2 are absorptivities of TIZH at λ_1 and λ_2 respectively and C_{DICS} and are C_{TIZH} concentrations of DICS and TIZH respectively.

2.3 Determination of partition coefficient[7-8]

20mg of Diclofenac Sodium was dissolved in 10ml of Phosphate Buffer Saline pH 7.4. A 10 ml of aqueous phase containing drug was equilibrated with 10 ml of n-octanol (organic phase)in a separating funnel with intermittent shaking for a time period of 24 hour at room temperature. The constituent phases were separated by centrifugation at 2000 rpm for 10 minutes and concentration of drug in aqueous phase was determined spectrophotometrically by measuring absorbance at 276nm. The partition coefficient was calculated from the equation (1). The experiments were performed in triplicates and analyzed.

2.3.1 Log P estimation

Log P of the drug was calculated by taking logarithm of experimentally obtained partition coefficient value.

Partition coefficient ($K_{O/W}$) was calculated by using the formula

$$\text{Partition Coefficient} = \frac{\text{amount of drug in organic layer (n-octanol)}}{\text{amount of drug in aqueous layer(PBS pH 7.4)}} \quad \ldots\ldots\ldots\ldots\ldots \quad (1)$$

Table.3: Data representing partition coefficient of Diclofenac Sodium and Tizanidine Hydrochloride in PBS 7.4 pH.

S.No	Drug	Partition coefficient (mean ± SD)	Log p
01	Diclofenac Sodium	13.10 ± 0.229	1.11
02	Tizanidine Hydrochloride	2.58 ± 0.219	1.06

Each data represents Mean ± S.D of three determinations.

2.4 Solubility Profile of Drugs

Solubility study was conducted to select a suitable media for Diclofenac Sodium and Tizanidine Hydrochloride according to the procedure described by Higuchi and Connors.[9] An excess amount of drug was added to 10ml of Phosphate Buffer Saline (7.4 pH) and methanol in a series of stoppered conical flasks. The samples were shaken for 24 hours at room temperature on a rotary flask shaker . After equilibration, samples were filtered using Whatmann filter paper no. 42 (0.45µm pore size) to separate the undissolved drug, diluted suitably and assayed for Diclofenac Sodium content by measuring the absorbance at 276 nm against the buffer as blank and against λ_{max} at 235 nm for drug Tizanidine Hydrochloride. The solubility experiments were carried out in triplicate to check the repeatability.

Table 4. Solubility of Diclofenac Sodium in different media

Tween 80	20.07 ± 0.85	Slightly soluble
PEG 200	90.07 ± 0.85	Soluble
Ethanol	87.21 ± 0.85	Soluble
PEG 400	43.35 ± 0.85	Sparingly soluble
Glycerin	9.01 ± 0.85	Very slightly soluble
Oleic acid	20.13 ± 0.85	Sparingly soluble
Methanol	95.56± 0.85	Freely soluble
Ethanol	85.34± 0.85	soluble
Water	42.78± 0.85	Sparingly soluble
Ether	1.01 ± 0.85	insoluble
Chloroform	1.01 ± 0.85	insoluble
Propylene glycol	17.71 ± 0.85	Slightly soluble

Mean ± SD, n = 3

Table 5.Solubility of Tizanidine Hydrochloride in different media

Phosphate Buffer	14.66 ± 0.85	Slightly soluble
Propylene Glycol	90.78 ± 2.25	soluble
Tween-80	100.00 ± 3.21	Freely soluble
DMSO	41.45 ± 1.56	Sparingly soluble
Ethanol 80%	50.81 ± 2.19	Sparingly soluble
Oleic acid	97.63 ± 3.26	Freely Soluble
Methanol	43.55± 1.56	Sparingly soluble
Ethanol	14.66 ± 0.85	Slightly soluble
Water	87.77± 0.95	Soluble
Glycerin	45.67± 0.88	Sparingly soluble
Polyethylene glycol 400	40.67± 0.34	Sparingly soluble

Mean ± SD, n = 3

3. Formulation Studies

The casting solutions For PVA and PVP Polymeric formulations FA1 to FA10 (Prabhu *et al*) were prepared by dissolving weighed quantities of various ratios of polymers in water. The drugs were dissolved in methanol and added to the above polymeric solution along with 10% Propylene Glycol 400 and 10% Dimethylsulfoxide as plasticizer separately, Tween 80(5% v/v) and Span 80(5% v/v) were add respectively and thoroughly mixed to form a homogeneous mixture by heating on heating mantle at 60°C. The volume 20 ml of this casting solution is poured into Petri plates and kept in the hot air open for drying.(Table 1)[10]

3.1 Preparation of transdermal patches

Twenty milliliter of the casting solution was poured into petri plates and dried in hot air for 24 hours for solvent evaporation. The patches were removed by peeling and cut into square dimension of 2 cm × 2 cm (4 cm^2). These patches were kept in desiccator for 2 days for further drying and wrapped in aluminum foil, packed in self-sealing covers.

Table 6-Preparation of transdermal patches using Propylene Glycol 400 & Tween 80

Code	Formulation (Polymeric Ratio)	PVA (mg)	PVP (mg)	Drug (100 mg+2mg)	Plasticizer (Propylene Glycol 400)	Penetration Enhancers (Tween 80)	Methanol	Water
FA1	10:0	1000	-	102	10% w/v	5% w/v	10	20
FA2	9:1	900	100	102	10% w/v	5% w/v	10	20
FA3	8:2	800	200	102	10% w/v	5% w/v	10	20
FA4	7:3	700	300	102	10% w/v	5% w/v	10	20
FA5	6:4	600	400	102	10% w/v	5% w/v	10	20

Table 7-Preparation of transdermal patches using Dimetylsulfoxide & Span 80

Code	Formulation (Polymeric Ratio)	PVA (mg)	PVP (mg)	Drug (100 mg+2mg)	Plasticizer (DMSO)	Penetration Enhancers (Span 80)	Methanol	Water
FA6	10:0	1000	-	102	10% w/v	5% w/v	10	20
FA7	9:1	900	100	102	10% w/v	5% w/v	10	20
FA8	8:2	800	200	102	10% w/v	5% w/v	10	20
FA9	7:3	700	300	102	10% w/v	5% w/v	10	20
FA10	6:4	600	400	102	10% w/v	5% w/v	10	20

4. Characterization Of Transdermal Patches

4.1 Physical appearance[11]

All the transdermal patches were visually inspected for color, clarity, flexibility, and smoothness.

4.2 Thickness of the films[12]

The thickness of the drug-loaded polymeric films were measured at three different places using a Vernier caliper and mean values were calculated.

4.3 Weight variation[13]

Weight variation was determined by weighing three patches individually, from each batch and the average weight was calculated.

4.4 Flatness [14]

The longitudinal strips were cut from the centre and both sides of each patch. The length of each strip was measured and the variation in length because of non-uniformity in flatness was measured as % constriction, and a 0% constriction was considered to be equivalent to 100% flatness.

4.5 Tensile strength[15]

Mechanical properties of the polymeric patches were determined by measuring their tensile strength. These mechanical properties were evaluated using Instron universal testing instrument (model F. 4026) with a 5 kg load cell. Film strips in special dimension and free from air bubbles or physical imperfections were held between two clamps positioned at a distance of 3cm. During measurement, the strips were pulled by the top clamps at a rate of 100 mm/min; the force and elongation were measured when the film broke. Results from film samples, which broke at and not between the clamps, were not included in the calculations. Measurements were run in triplicate for each film. Two mechanical properties, namely tensile strength and percentage elongation, were computed for the evaluation of the film.

Tensile strength =Force required to break the film/Intial cross sectional area (mm^2)' similarly,

Percentage Elongation = Increase in length/Original length × 100

Table. 8: Characterization of transdermal Patches for Physiochemical Parameters-I

Form .code	Thickness (mm)	Weight Variation(g)	Folding Endurance	Tensile Strength	Moisture Vapour Transmissionn	Percentage Elongation
FA1	0.29±0.03	31.32.±1.154	125-134	3.66±1.18	5.87×10^{-3}	83.724±15
FA2	0.24±0.02	30.33±1.156	125-130	4.69±0.23	4.27×10^{-3}	238± 0.002
FA3	0.25±0.03	31.60±0.144	126-134	5.13±0.13	4.17×10^{-3}	271± 0.100
FA4	0.23±0.01	32.23±1.154	121-134	4.76±1.18	13.77×10^{-3}	191± 0.03
FA5	0.19±0.02	31.33±1.155	126-137	4.89±0.23	6.87×10^{-3}	238± 0.02
FA6	0.27±0.01	32.66±1.165	121-134	4.13±0.13	7.77×10^{-3}	193±0.02
FA7	0.26±0.03	32.37±1.154	125-134	3.76±1.18	12.87×10^{-3}	83.91±15
FA8	0.25±0.03	31.78±0.111	121-134	4.59±0.23	13.77×10^{-3}	84.72±15
FA9	0.22±0.02	32.43±1.152	124-136	5.23 ±0.13	12.87×10^{-3}	85.72±15
FA10	0.23±0.01	31.36±1.154	121-129	4.66±1.18	10.77×10^{-3}	190.7±15

Mean ± SD, n = 3

4.6 Drug content [16-18]

Transdermal system of specified area (2 cm^2) was cut into small pieces and taken into a 100 ml volumetric flask and 100 ml of phosphate buffer pH 7.4 was added , and kept for 24 hours with occasional shaking. Then, the suitable dilution was made with phosphate buffer of pH 7.4. Similarly, a blank was carried out using a drug-free patch. The solutions were filtered and the absorbance was measured at 276 nm for Diclofenac sodium and 320 nm for Tizanidine Hydrochloride.

4.7 Water vapor transmission rate[19]

Conical flasks of equal diameter were used as transmission cells. These transmission cells were washed thoroughly and dried in an oven. About 1 gm anhydrous calcium chloride was placed ineach flask and the prepared transdermal patches of each formulation were fixed over the brimwith the help of adhesive. The cells were accurately weighed and kept in closed desiccators containing saturated solution of potassium chloride to maintain a relative humidity of 84%. The cells were taken out and weighed after 6, 12, 24, 36, 48 and 72 hrs of storage. Water vapor transmission rate is expressed as the number of grams of moisture gained /h/cm^2.

Table. 9: Characterization of transdermal Patches for Physiochemical Parameters-II

Form code	*Drug Content*		*Permeability coefficient*	*Flux*
	DLC	*TZN*		
FA1	99.99±0.8	99.99±0.8	0.88	0.0788
FA2	99.95±0.9	99.95±0.9	1.669	0.1539
FA3	95.99±0.10	96.99±0.10	2.418	0.2168
FA4	94.99±0.11	98.99±0.11	2.3933	0.227
FA5	99.07±0.12	98.07±0.12	2.848	0.2063
FA6	99.85±0.13	99.85±0.13	2.56	0.2294
FA7	93.55±0.14	94.55±0.14	2.28	0.2091
FA8	95.59±0.15	94.59±0.15	2.48	0.1686
FA9	97.99±0.16	98.99±0.16	2.824	0.2449
FA10	95.99±0.17	98.99±0.17	2.813	0.298

Mean ± SD, n = 3

5. *In Vitro* Skin Permeation Studies[20]

5.1 Preparation of the skin for permeation studies

A healthy Wistar Albino rat was selected.The hair from the abdominal region was shaved carefully with a safety razor and further cleaned with wet cotton to remove extra hairs. The rat was sacrificed by the proper method and the hairless clean skin was excised carefully with the help of a surgical blade. The procured skin was then cleaned thoroughly with distilled water and stored in Ringer solution with proper aeration.

5.2 Procedure

In vitro permeation studies were performed on Franz diffusion cells with an effective sectional area of 3.14 cm^2 and 15 ml of receiver chamber capacity. The rat abdominal skin was tightly secured between the donor and receptor compartments. The upper surface of the membrane was exposed to solution of the formulated films and covered with paraffin film. The receptor compartment was filled with isotonic phosphate buffer pH 7.4. The whole assembly was kept on a magnetic stirrer and solution in the receptor compartment was constantly and continuously stirred using a magnetic bead. The solution in the receptor compartment was constantly and continuously stirred using magnetic beads at 50 rpm; the temperature was maintained at 37±0.5°C. The 2 ml aliquots were withdrawal at different time intervals (0,2,4,8,10,12 hours) and analyzed the drug content by UV-Visible spectrophotometer (117 Systronics) at 305 nm. The receptor phase was replenished with an equal volume of phosphate buffer (37°C) at each sample withdrawal, the cumulative amount of drug permeated per square centimeter (µg/cm^2) of patches were plotted against time.

Permeability Coefficient (P)

Permeability coefficient is the velocity of drug passage through the membrane/skin in mcg/cm^2/hour. The permeability coefficient was calculated from the slope of the graph of percentage of drug transported vs. time as: P = Slope x Vd/S Where, Vd = volume of donor solution, S = surface area of tissue.

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 drug in donor solution, P = permeability coefficient.

TABLE.10 In-vitro Permeation Profiles Of Transdermal Patches Containing Both the Drugs with Penetration enhancers Propylene Glycol 400& Tween 80

Time (hrs)	FA1(%)	FA2 (%)	FA3 (%)	FA4 (%)	FA5 (%)
0	0	0	0	0	0
2	5.34 ± 0.01	6.81± 0.01	6.25± 0.01	5.35± 0.01	3.82± 0.01
4	17.4 ± 0.01	17.7± 0.01	18.2± 0.01	18.2± 0.01	16.2± 0.01
6	35.7 ± 0.02	35.2± 0.01	36.6± 0.01	30.8± 0.01	34.5± 0.01
8	67.6 ± 0.01	64.4± 0.01	66.3± 0.01	56.2± 0.01	50.4± 0.01
10	92.7 ± 0.02	86.2± 0.01	90.8± 0.01	78.5± 0.01	73.7± 0.01
12	99.6 ± 0.03	99.8± 0.01	99.3± 0.01	94.4± 0.01	97.3± 0.01

Mean ± SD, n = 3

TABLE.11: Invitro Permeation Profiles Of Transdermal Patches Containing Both the Drugs with Penetration enhancers Dimetylsulfoxide & Span 80

Time (hrs)	FA6 (%)	FA7 (%)	FA8 (%)	FA9(%)	FA10 (%)
0	0	0	0	0	0
2	6.02± 0.01	6.22± 0.01	7.84± 0.01	6.65± 0.01	6.28± 0.01
4	17.52± 0.01	16.92± 0.01	20.56± 0.01	17.78± 0.01	20.3± 0.01
6	32.64± 0.01	36.24± 0.01	34.32± 0.01	28.16± 0.01	45.8± 0.01
8	64.34± 0.01	63.56± 0.01	67.71± 0.01	55.32± 0.01	64.4± 0.01
10	83.28± 0.01	81.86± 0.01	83.04± 0.01	76.12± 0.01	84.7± 0.01
12	98.21± 0.01	98.52± 0.01	98.98± 0.01	97.62± 0.01	98.11± 0.01

Mean ± SD, n = 3

6. Drug Release Kinetics

Drug release mechanisms and kinetics are two characteristics of the dosage forms which play an important role in describing drug dissolution profile from a dosage form and hence to understand their *in vivo* performance. The dissolution data obtained is fitted to mathematical models and the best fit obtained describes the release mechanism of the drug. A number of mathematical models have been developed to describe the drug dissolution kinetics from drug delivery system *e.g*., Zero order (cumulative % drug release versus time), Higuchi (cumulative % drug release versus square root of time); First order (log cumulative % drug remaining versus time) and Korsemeyer - peppas model (log cumulative % drug release versus log time).

6.1 Analysis of drug release mechanism

The release kinetics was evaluated by making use of the following equations

1) Zero-order equation (Cumulative % drug release vs. Time)

$Q=k_0t$,Where Q is the amount of drug release in time t and k is the diffusion rate constant.

2) First order equation (Log Cumulative % drug unreleased vs. Time)

$C=Co.e^{-kt}$,Where C is the final concentration, Co is the initial concentration and k is the first order constant and t is the time.

3) Higuchi's diffusion equation (Cumulative % drug release vs. Square root of time)

$Q= k_H.t^{1/2}$

Where Q is the amount of drug release at a time t and k_H is Higuchi's square root of time release rate constant.

4) Korsmeyer's equation (log Cumulative % drug release vs. log time)

$Mt/M= k.t^n$

Where Mt/ M are the fraction of drug release at a time t, k is the characteristic constant of and n indicate the release order (slope).The 'n' (release exponent of Korsmeyer - peppas model) value could be used to characterize different release mechanisms. The interpretation of n values was done as per values given in table 12.

TABLE.12 : INTERPRETATION OF DIFFUSIONAL RELEASE MECHANISMS

Release exponent (n)	Drug transport mechanism
< 0.5	Fickian Diffusion
= 0.5	Diffusion mechanism
0.5 < n < 1.0	Anomalous (non-Fickian) Diffusion
1	Case II transport (Zero order release)
> 1	Super Case II transport

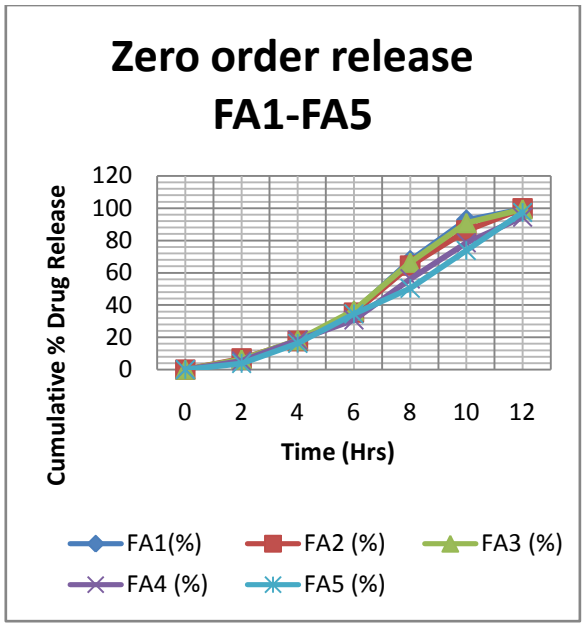

Fig.5 Zero Order drug release for FA1-FA5

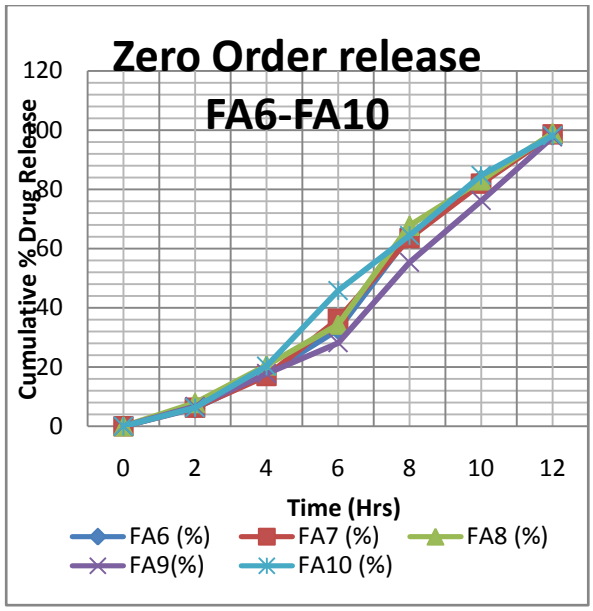

Fig.6 Zero Order release for FA6-FA10

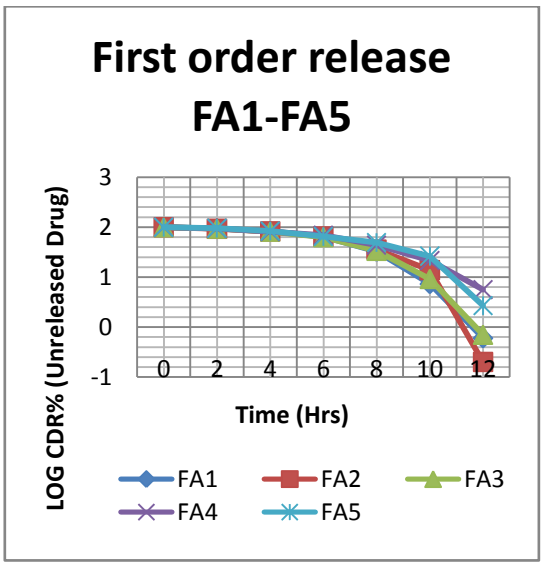

Fig .7 First Order release for FA1-FA5

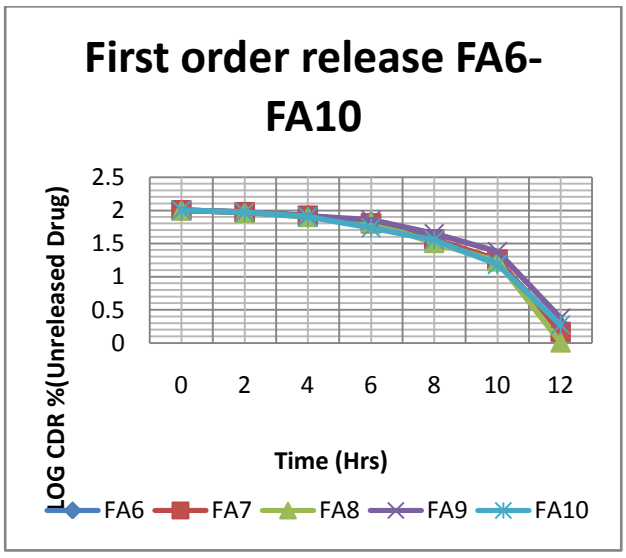

Fig .8 First Order release for FA6-FA10

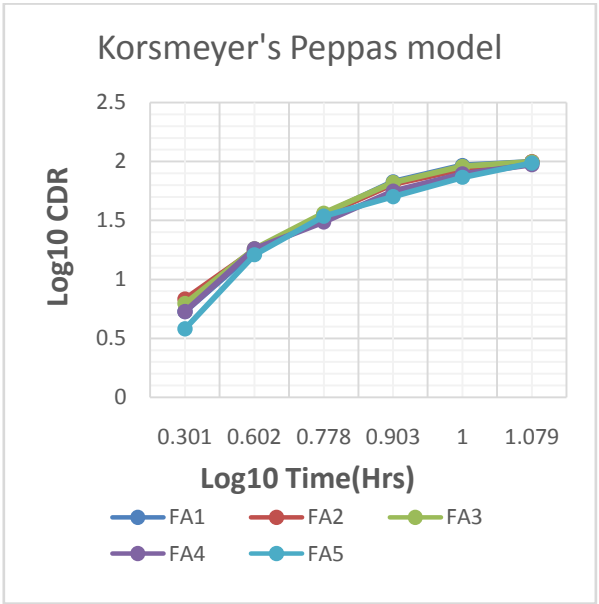

Fig 9-Korsmeyer's Plot for FA1-FA5

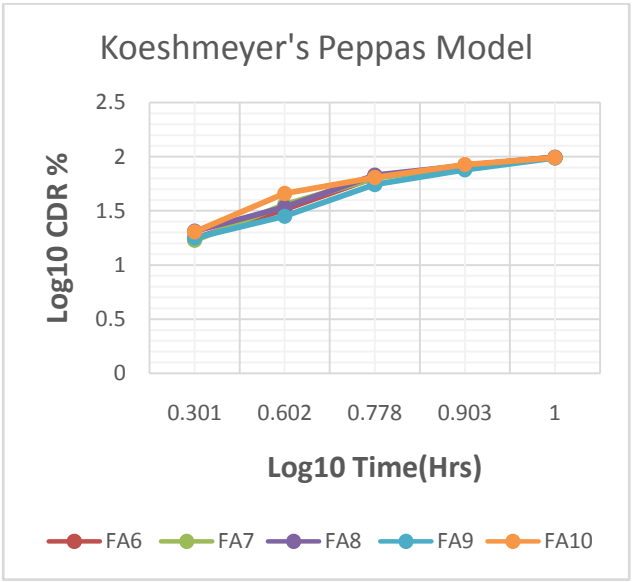

Fig.10.Korsmeyer's Plot for FA6-FA10

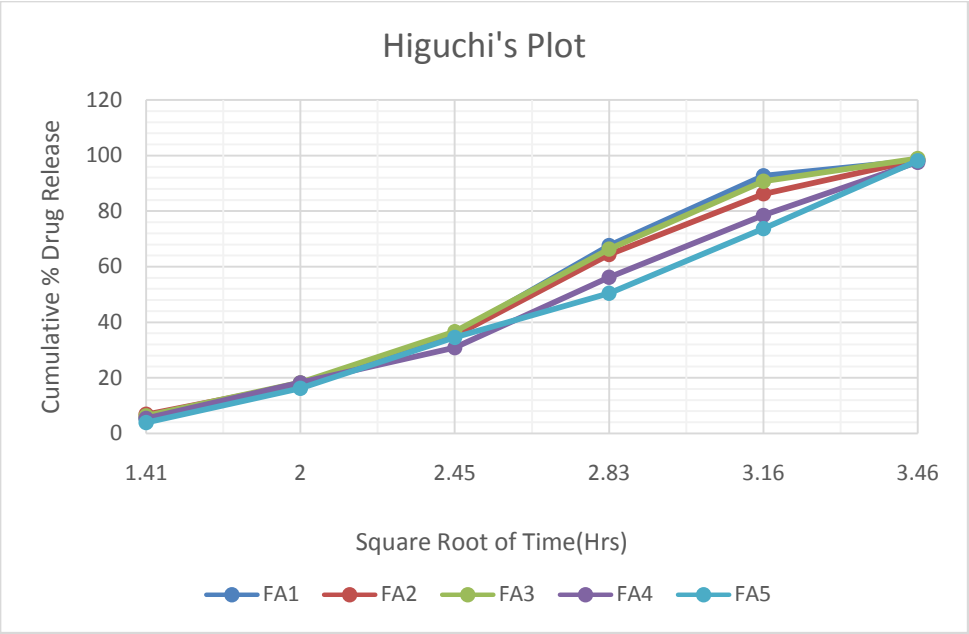

Fig.11- Higuchi's Plot for FA1-FA5

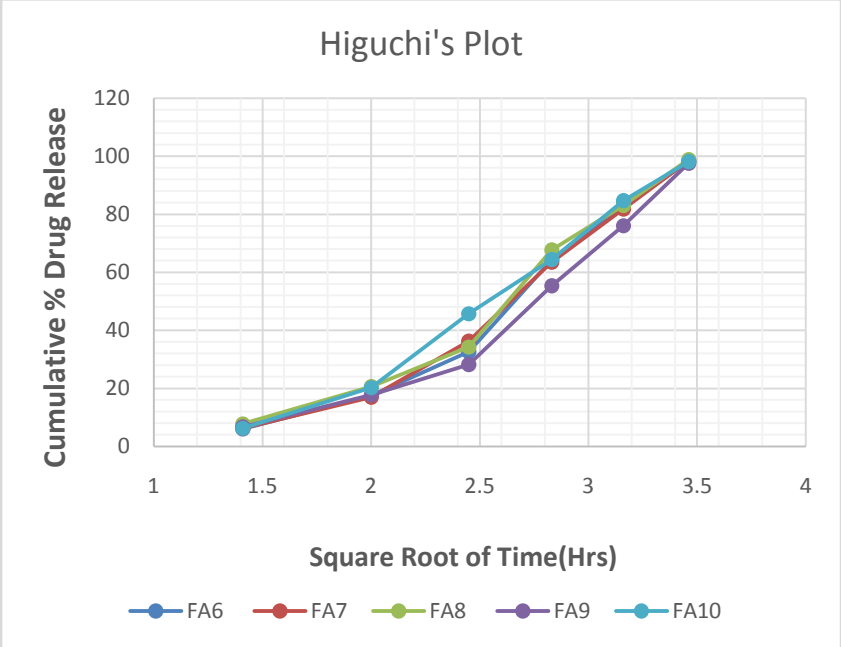

Fig.12 Higuchi's Plot for FA6-FA10

Table 13. Regression Values for In-Vitro drug release

S.no	Zero order plot	First Order plot	Higuchi's plot	Korsmeyer plot	
	R2	R2	R2	N	R2
FA1	0.9591	0.7611	0.7960	0.0847	0.9915
FA2	0.9687	0.6515	0.8036	0.1313	0.9936
FA3	0.9655	0.7529	0.8050	0.1174	0.9931
FA4	0.9680	0.8089	0.7961	0.0966	0.9963
FA5	0.9651	0.7034	0.7845	0.0333	0.9938
FA6	0.9655	0.7572	0.7980	0.1096	0.9937
FA7	0.97261	0.7362	0.8081	0.1156	0.9943
FA8	0.97108	0.7252	0.8163	0.1690	0.9915
FA9	0.9588	0.7095	0.7795	0.1299	0.9923
FA10	0.9838	0.7866	0.8421	0.1373	0.9881

7. *In- ViVo* Study-Carrageenan Induced Edema Model[21]

The anti-inflammatory activity was performed for the two best formulation by Carrageenan induced hind paw edema method in rats. In the present study nine male albino rats (approved by Institutional Animal Ethical Committee) were selected weighing approximately 200-250g. The hair on the abdominal skin of rats was removed 12 h prior to the application of patch. The groups of animals used for the anti-inflammatory studies are outlined in the table.14 .The first group (control) received orally 0.5 ml of normal saline solution, the second group (standard) received diclofenac patches (NuPatch), and the third group received best formulation FA2 and fourth group received the best formulation FA8 respectively.

Table.14 : Description of animals used for pharmacodynamic studies

Group	Route of administration
Group 1	Positive control
Group 2	Marketed formulation (NuPatch-100mg)
Group 3	Best Formulation FA2 (100mg Diclofenac and 2mg Tizanidine)
Group 4	Best Formulation FA8 (100mg Diclofenac and 2mg Tizanidine)

7.1 Preparation of 1% w/v carrageenan solution

About 1g of the carrageenan powder was transferred into a volumetric flask (100ml) dissolved and diluted suitably using double distilled water. The dispersion was sonicated in an ultrasonic bath until carrageenan was completely dissolved.

7.2 Procedure

Patches were applied on the shaved abdominal region of group 2, group 3 and group 4 (except the control group) half an hour before subplantar injection of carrageenan in the left paws.The volume of injected paw was measured immediately (0 h) and at 0, 1, 3, 5, 8 and 12 hours after injection using a plethysmometer. The amount of paw swelling was determined time to time and expressed as percent edema relative to the initial (0 min) hind paw volume

$$\%\ Inhibition = \frac{(C_t - C_o)_{Control} - (C_t - C_o)_{Treated}}{(C_t - C_o)_{Control}} \times 100$$

Where,

Ct=thickness of paw after carrageenan injection.

Co = thickness of paw before carrageenan injection.

Fig.13- Injection of 1% carrageenan in double distilled water in subplantar region

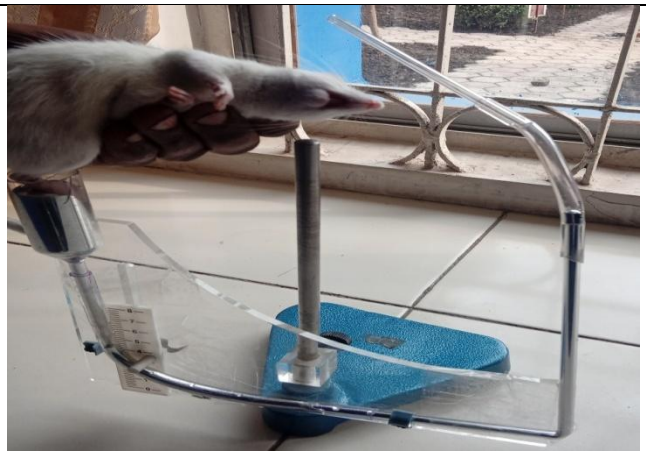

Fig.14 Deflection in Plethysmometer for measuring Paw volume (edema)

Table. 15: Mean Paw edema volume of the albino rats

Time in Hours (h)	Marketed Formulation	Best Formulation FA8	Best Formulation FA2	Control Group
0	4.34± 0.01	4.36± 0.01	4.35± 0.01	4.39± 0.01
1	5.19± 0.01	5.73± 0.01	5.88± 0.01	6.16± 0.01
3	6.43± 0.01	6.37± 0.01	6.32± 0.01	7.38± 0.01
5	5.35± 0.01	5.36± 0.01	5.97± 0.01	7.22± 0.01
8	4.74± 0.01	4.66± 0.01	5.73± 0.01	7.29± 0.01
12	4.34± 0.01	4.42± 0.01	4.48± 0.01	7.25± 0.01

The data represents mean ± S.E.M of six determinations (*n*=3).

Table. 16: % Inhibition of the edema in albino rats

Time in Hours (h)	Marketed Formulation	Best Formulation FA8	Best Formulation FA2
0	1.1389± 0.01	0.6833± 0.01	0.9111± 0.01
1	15.7467± 0.01	6.9805± 0.01	4.5454± 0.01
3	12.8726± 0.01	13.6856± 0.01	14.3631± 0.01
5	25.9002± 0.01	25.76177± 0.01	17.3130± 0.01
8	34.9794± 0.01	36.07681± 0.01	21.3991± 0.01
12	40.1379± 0.01	39.0344± 0.01	38.2068± 0.01

The data represents mean ± S.E.M of six determinations (*n*=3).

Table.17: ANOVA and T- test for % Inhibition of the edema in albino rats

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Best Formulation	6	130.775966	21.79599	214.0016		
Marketed Formulation	6	122.2226008	20.37043	247.1321		
Best Formulation	6	96.73885281	16.12314	177.0979		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	104.5059193	2	52.25296	0.245614	0.785309	3.68232
Within Groups	3191.157605	15	212.7438			
Total	3295.663524	17				

H_0 is accepted. No significant difference at p=0.05, T-test result=0.3806

8. Result and Discussion

Preformulation studies were performed by for the drugs Diclofenac Sodium and Tizanidine Hydrochloride as per specifications in Indian Pharmacopoeia. Parameters such as description and appearance, solubility, Partition Coefficient, Melting Point, Identification test of Diclofenac Sodium and Tizanidine Hydrochloride were perfomed .

Concentration of the drug was estimated using UV spectroscopic identification method at pH 7.4 using Phosphate saline Buffer which indicates pH of blood serum shown in (Table.1 and Table.2). Simultaneous equations for Diclofenac Sodium and Tizanidine Hydrochloride were also studied. Drug partition coefficient study was performed using n-octanol/Saline Phosphate buffer 7.4. Their log P values were also calculated as shown in Table.3.The Solubility of Diclofenac Sodium and Tizanidine Hydrochloride was found to be good with Methanol and Saline Phosphate buffer 7.4 (Table 4 and Table 5)

Formulation of TDDS using polymers, plasticizers and enhancers were done by solvent casting method. It was observed that PVA:PVP in combination with plasticizer Polyethylene glycol-400 dissolved in solvent system Methanol:Water in 30:20 ratio with enhancer Tween 80 at constant temperature of 60^{o}C shown in (Table 6).Another ratio of Plastisizer DMSO and enhancer Span 80 was combined with PVA and PVP in solvent methanol and water (Table 7). The enhancers were selected on the basis of HLB values. The plasticizers in a ratio of 10% w/w PEG400 and 10% w/w DMSO respectively for PVA and PVP were for good flexibility, clarity & elasticity. Characterization of Patches were done for Physiochemical Parameters as shown in (Table 8-9). *In-vitro* permeation release is shown in (Table 10 and table 11).Films of hydrophilic polymer PVA: PVP with different concentrations (10:0,9:1,8:2,7:3 6:4) were studied.To examine the drug permeation kinetics and mechanism, the data were fitted to models representing zero-order; first-order, Higuchi and Koresmeyer-Peppas. Permeation of the drug from a transdermal drug delivery system mainly involves the factor of diffusion. In our experiments the in vitro permeation profiles of all formulations did not fit into first-order (R^2 = to 0.6515 to 0.8089) they could be best expressed by the Korsmeyer plot for formulation FA1 to FA5 (0.9915 to 0.9963) and between (0.9937-0.9881) for formulations FA6 to FA10. For Formulations FA1 to FA10 the values of n<0.5, it means drug permeation followed Fickian diffusion. Similarly, for Zero order release (R^2 =0.9591 to 0.9838) and Higuchi equation (R^2 = 0.7960 to 0.8421). The percent of drug permeated in 12 h was found to be maximum 99.8± 0.01 and 98.98± 0.01 % for the formulations FA2 and FA8 respectively. The two best Formulations FA2 and FA8 were applied on Albino wister rat and Paw edema was induced using Carragenan 1% Solution subplanter region.The Percentage swelling was determined using Deflection in mercury in Plethysmometer Shown in Table.15-16.d results are verified with ANOVA and T-test shows that H_0 is Accepted that means no significant difference is found which could break the threshold of p=0.05.The formulated patches are similar Diclofenac in response to inflammation with added advantages.

9. Conclusion

This was concluded from the study that prepared transdermal patches of Tizanidine Hydrochoride-Diclofenac

Sodium transdermal patch using Polyvinylalcohol, Polyvinylpyyrolidone, plastisizer Polyethylene glycol-400 and Dimethylsulfoxide with penetration enhancers Span 80 and Tween 80 in solvent system methanol: water undergone various evaluation parameters such Physicochemical Studies and In Vitro and In Vivo studies. It is proved that all the preparations FA1 to FA10 shows good *in-vitro* properties. *In vivo* studies using pharmacokinetic and pharmacodynamic parameters confirm that the Formulation FA2 and FA8 are best among all Formulations. Hence the transdermal patch would prove to be a landmark in TDDS for Pain-Spasms. It is convincingly established that formulation FA2 and FA8 can be tried on commercial basis safely and will provide all the benefits of TDDS as discussed prior.

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