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RELEASE ENHANCEMENT OF MELOXICAM FROM TRANSDERMAL GEL THROUGH CYCLODEXTRIN COMPLEXATION

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Keywords:

Meloxicam, *In- situ* complexation, Transdermal gel, β-cyclodextrin, Inclusion complex

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ABSTRACT

The purpose of the study was to determine the in vitro as well in vivo efficiencies of 1% carbopol hydrogel different preparations containing the meloxicam, a cyclooxygenase-2 inhibitor anti-inflammatory drug with β-cyclodextrin. The release of plain drug was then compared with release of from trans dermal gel containing physical mixture of drug and β-cyclodextrin, inclusion complex of drug and β-cyclodextrin obtained by kneading method and in situ complex of drug and β-cyclodextrin obtained by reacting the drug and β-cyclodextrin within the gel. All solid inclusion complex abstained were then characterized by x-ray diffractrometry, infrared spectroscopy, differential scanning colorimetry and scanning electron microscopy, where as the in situ complex formation was evidenced only by release and permeation studies. Although many other derivative of cyclodextrins such as hydroxy propyl-β-cyclodextrin and methylated-β-cyclodextrin are better skin permeating enhancers but in this study β-cyclodextrin was employed because of ease of availability and low cost. Solid inclusion complex was prepared by kneading method. Gel formulations were prepared by incorporating pure drug, physical mixture, inclusion complex and separately drug and β-cyclodextrin dispersed in propylene glycol (to get *in situ* complex) to the gel base. Prepared gels were subjected to physical evaluation for its viscosity, pH and drug content. *In vitro* drug release and in vitro drug permeation experiments were carried out on Franz diffusion cell using cellophane membrane and human cadaver skin respectively. Selected formulations were evaluated for anti-inflammatory activity using the carrageenan-induced paw oedema in rats. Differential scanning colorimetry and infrared spectroscopy studies indicate the complexation where as x-ray diffractrometry studies indicate conversion of crystalline drug to porous, spherical and fluppy structures. The release rates when compared were found to be highest with gel containing inclusion complex than the gels containing pure drug, physical mixture and the in situ complex. A lag period was observed with all formulations. Physical stability was performed by freeze thaw cycling. The complex containing *in situ* complex was more stable. The overall data suggest that the prepared hydrogel of meloxicam is highly efficient transdermal vehicle for the delivery of the drug at the site of action.

INTRODUCTION: Cyclooxygenase-2 (COX-2) inhibitors constitute a new group of NSAIDs, which at recommended doses block prostaglandin production by inhibiting cyclooxygenase-2, but not by Cyclooxygenase-1 (COX-1). Meloxicam [1] is 4-Hydroxy-2-methyl-N- (5-methyl-2-thaizolyl)-2-H, 1, 2-benzothiazine-3-carboxamide, 1, 1-dioxide, is a new non-steroidal anti-inflammatory drug together with low gastrointestinal and renal toxicity [2]. It is official in B. P., 1999. Meloxicam is used in arthritis and other rheumatoid disorder [3-5]. Meloxicam is yellow crystalline powder, soluble in DMF. However, meloxicam along with the other COX-2 inhibitors shows poor aqueous solubility, which hinders its easy formulation and dissolution. Therefore, a possible percutaneous administration of the meloxicam was considered worth investigating. The stratum corneum (SC) is the rate-limiting barrier to percutaneous delivery for most of the drugs, and therefore investigations were made to achieve an optimized formulation for better release.

Cyclodextrins (CDs), a group of structurally related cyclic oligosaccharides, have attracted interest for topical use as penetration enhancers [6-14] on account of their ability to affect absorption by means of two mechanisms, indirectly, by influencing physicochemical properties of the drugs, and/or directly, by influencing the biomembrane permeability [15].

Nevertheless, CDs have been poorly studied on transdermal absorption, and their mechanisms of action still have not been clarified. It is obvious that the solubilizing properties of the CDs can improve drug release, absorption and bioavailability, but their enhancing effects on the percutaneous absorption of drugs by altering the skin permeability has been a controversial issue. On the one hand, CDs may affect the permeation of drugs via the interaction with some SC components [12], inclusion of phospholipids and cholesterol [16], by extraction proteins [17], by removal and disorganization of the lipids matrix resulting from complexation [9, 18, 19], and by interaction with keratin in an increased order in the lipid lamella [14]. While on the other hand, it would appear that such large, relatively hydrophilic molecules as the CDs would not readily permeate into the skin, and in fact it has been reported that CDs permeate lipophilic biological membrane with considerable difficulty and at a very slow rate [20]. In this regard William et al. also suggested that CDs do penetrate into SC lipid to some extent [14]. Gels have gained more and more importance because the gel-based (hydrogel) formulations are found to be better percutaneously absorbed than cream and ointment bases.

MATERIALS AND METHODS: Lupin Laboratories (India) had kindly provided meloxicam. β-Cyclodextrin was obtained from Cavitron (USA) carbopol 940 (carboxypolymethylene) and propylene glycol were purchased from Roig Pharma, Barcelona (Spain) and sodium hydroxide was procured from Merck (India). All other reagents and chemicals used were of analytical grade. Carbopol Gel (0.5-1.5%) was prepared by dispersing of carbopol in 700 ml water with stirring. The dispersion was then neutralized to pH 7.4±0.5 and made viscous by adding 10% sodium hydroxide solution. The final volume was then made up to 1000 ml with distilled water and the resulting gel was then stored at room temperature for 24 h before use.

Preparation and Characterization of complexes:
The inclusion complex of meloxicam was prepared by kneading method. Weighed quantity of β-cyclodextrin was mixed with one-third quantity of water to make a homogenous paste. In this homogenous paste the drug was added and continuously mixed for 30 min. The preparation was dried at 45, pulverized and finally sieved through mesh #100. Complexes were prepared at various molar concentrations viz. 1:0.25, 1:0.5, 1:1, 1:2, 1:3, 1:4, and 1:5 of meloxicam: β-cyclodextrin. Physical mixture was also prepared by mixing the corresponding substances in a mortar. Inclusion complex formation in solid state was assessed by x-ray diffractrometry (XRD), infrared spectroscopy (IR) and differential

scanning colorimetry (DSC). X-ray powder diffractrograms were obtained using a Phillips PW 1729 powder X Ray Diffractometer using Ni-filtered CuK_α radiation, a voltage of 30 kV and current of 30 mA. The instrument was operated in the continuous scan mode web of 2θ ranges 2-50^o with a chart speed of 5mm/2cm/2θ.

Studies of the IR spectra of rofecoxib and its complexes were conducted using potassium bromide pellets on Jasco FTIR spectrophotometer in range of 400-4000 cm^{-1}. Thermal analyses were carried out using a Toledo (Japan) differential scanning calorimeter. Each sample (pure component, physical mixture and inclusion complex) was sealed in 40 μl aluminum pans, the lids were pierced and scanned at a speed of 50-350 rpm for 10 min, using nitrogen as purging gas. Formation of in situ complexes were evidenced only by drug release and drug permeation study. The molecular volume of β-cyclodextrin and meloxicam were determined by SERINA Software's, PC model 5.01.

Transdermal Gel Formulations: Four different carbopol gels formulations of each drug were prepared by levigating the carbopol gel base with drug, physical mixture or its complex.

i) The gel bearing pure drug was prepared by dispersing the drug (1g) in 10 ml propylene glycol and then the dispersion was levigated into the gel base.

ii) The gel bearing physical mixture was prepared by incorporating the physical mixture of drug (1 g) thoroughly mixed with 3.6 g β-Cyclodextrin, then passed through #100 and then dispersed in 10 ml propylene glycol before levigating.

iii) The third formulation was prepared with solid drug: β-cyclodextrin inclusion complex (1:2) equivalent to 1 g prepared by kneading method was dispersed in 10 ml propylene glycol before levigation, i.v.). In the fourth formulation the drug (1g) and β-cyclodextrin (7.2 g) were dispersed in propylene glycol and added to gel base. The complex (1:2) was allowed to occur *in situ*. Here the drug and β-cyclodextrin dispersed separately in 10 ml propylene glycol and added to the gel base. After through mixing the gel and drug complexes, the final weight of the gel was adjusted to 100g with plain carbopol gel (drug concentration 1% in the vehicle) and stored in a proper container and set aside at room temperature for 24 h before use.

Evaluation of the formulations: These gels were evaluated for pH, viscosity, drug content; in vitro drug release, *in vitro* drug permeation and some selected gels were tested for anti-inflammatory activity using the carrageenan- induced paw edema in rats. pH measurements were done by using a digital pH meter(Electrolabs, India) by dipping the glass electrode and reference electrode completely, into the gel system so as to cover the electrodes. The viscosities of the prepared gels were measured with a Brooke field programmable Rheometer (model no DVIII) using spindle no. 7. All the properties of characterizations are recorded in **Table 1**.

TABLE 1: FORMULATIONS OF THE MELOXICAM TRANSDERMAL GELS AND THEIR PHYSICAL CHARACTERISTICS

Ingredients	MGPD	MGPM	MGIC	MGIS
Carbopol	1g	1g	1g	1g
Meloxicam	1g	1g	1g	1g
Pro.Glycol	20g	-	20g	20 gm
Purified. Water (Q. s.)	100g	100g	100g	100g
pH	7.59	7.22	7.47	7.28
Viscosity (cps)	7120	7220	7330	7210
Drug content (%)	99.0	99.5	99.7	99.4

MGPD-gel containing plain meloxicam, MGPM-gel containing physical mixture (meloxicam: β-CD, 1:1), MGIC- gel containing meloxicam: βCD (solid inclusion complex, 1:2), MGIS - gel containing meloxicam: βCD (*in situ* formed complex, 1:2)

***In vitro* release studies:** Meloxicam release rate from different gels was measured through cellophane membrane (no. 1, Spectrum Laboratories, Houston, TX) using a Franz diffusion cell (Crown Glass Co. NJ., USA) with a diffusional area of 1.76 cm^2. The gel (0.5 g) was placed on the treated cellophane membrane surface in the

donor compartment while the receptor side was filled with 10 ml of saline phosphate buffer (pH 7.4) as the diffusion media maintained at 37±1°C. The contents of the receptor compartment were stirred at 50 rpm with the help of a magnetic stirrer. At designated time intervals, 300 µl, aliquot samples were withdrawn from the receptor phase and replaced with equal volume of fresh buffer solution. The samples were analyzed spectrophotometrically for the drug content using saline phosphate buffer as blank, and the release rates (k) were calculated using first order kinetics (**Table 2**). The *in vitro* release profiles for different transdermal system are also graphically presented.

TABLE 2: *IN VITRO* RELEASE PROFILE OF MELOXICAM FROM DIFFERENT CONCENTRATION OF CARBAPOL

Conc. Time (Hr)	Drug release with different carbopol concentration (%)		
	0.5%	1.0%	1.5%
0.25	1.98	1.51	2.13
0.50	4.11	3.42	3.01
0.75	5.79	5.60	4.79
1.00	8.27	7.86	6.81
1.50	11.25	10.14	8.92
2.00	13.68	12.66	10.22
3.00	16.55	15.49	13.46
4.00	19.23	18.36	16.57
5.00	22.78	21.36	20.29
6.00	25.56	24.82	13.18
24.00	35.27	33.31	32.59

N=3

***In vitro* Skin Permeation Studies:** The *in vitro* skin permeation study of the system was performed on Franz diffusion cell [21] using human cadaver skins. The adhering tissues to the skin and other visceral debris were scrapped off by a blunt knife. The skin was then delipidized by placing it in the mixture of Chloroform: methanol: 0.1 M potassium chloride in the ratio of 1.0:2.0:0.8 for 20 min. The delipidized skin was washed and equilibrated in saline phosphate buffer solution for 1 h before mounting on the diffusion cell. This membrane mounted on the Franz-type diffusion cell with the dermis facing the receptor compartment while the donor side was charged with the gel (0.5 g). Sample of receptor fluid (300 µl) were withdrawn at the scheduled time intervals for 6 h, and replenished with fresh buffer solution. The samples were assayed spectrophotometrically for the drug content using saline phosphate buffer as blank. The release kinetics was established by determining the diffusional release exponent from the plot of log of cumulative drug release versus log time. The slope of the straight line was recorded as value of diffusional release exponent. The cumulative amount of drug permeate through skin was plotted as a function of time.

The correlation coefficient of in vitro drug release and in vitro drug permeation were also established. Various plots between in vitro drug release and in vitro drug permeation were constructed and determined the correlation coefficient. The correlations between the in vitro release profiles and in vitro skin permeation profiles for different transdermal system are graphically presented.

Analytical method: The amount of drug in the receptor phase was assayed spectrophotometrically using Shimadzu UV-2101PC UV-VIS scanning spectrophotometer at 350 nm. The absorbances were measured against the blank solution, prepared by same method except using drug solution. The linearity intervals established was 2 to 20 µg/ml (r>0.9999)

Pharmacological Screening: Screening for anti-inflammatory activity was carried out by carrageenan-induced paw edema method on rats as reported by Writer *et al*., [22], edema was produced by injecting 0.1 ml of carrageenan solution (1% w/v in normal saline) under the sub planner region of the left hind foot of rats. After half an hour of carrageenan injection the gel was applied at the hair cleaned abdominal area of the rat. Paw volume was measured using plethysmometer for 3 h.

RESULTS AND DISCUSSION: Based on the method used, it was verified that meloxicam could form complexes in solid phase with β-cyclodextrin. The DSC thermograms are depicted in, **Fig. 1**.

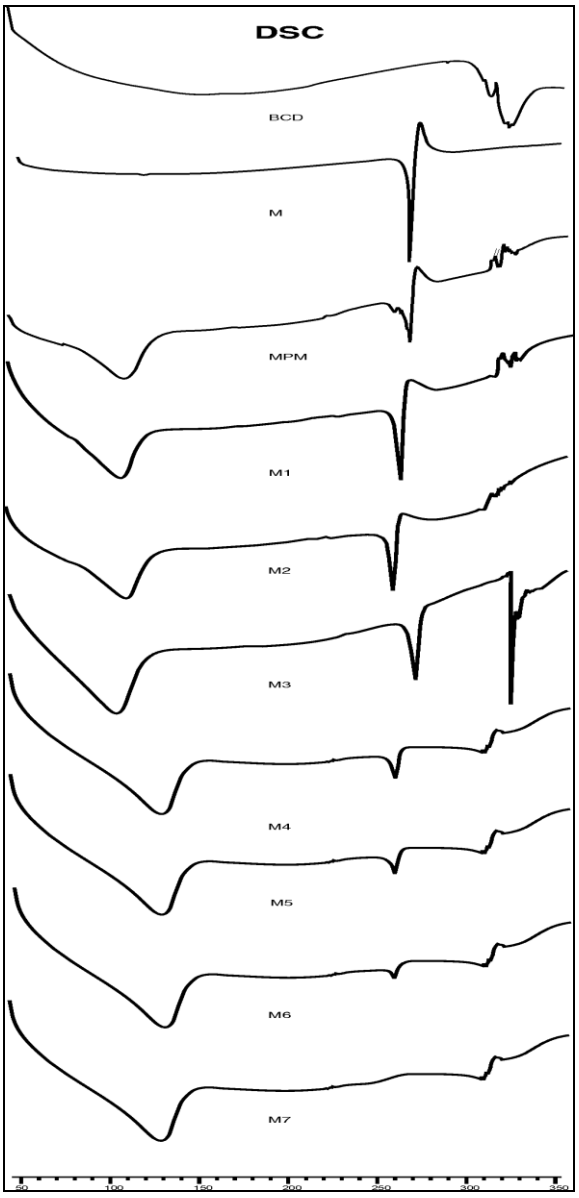

FIG. 1: DSC THERMOGRAMS OF INCLUSION COMPLEXES OF MELOXICAM AND β-CD IN DIFFERENT MOLAR CONCENTRATIONS

i) BCD:DSC thermogram of β-cyclodextrin,
ii) M:DSC thermogram of plain meloxicam,
iii) MPM:DSC thermogram of meloxicam: βcyclodextrin physical mixture (1:1),
iv) M1:DSC thermogram of meloxicam β-cyclodextrin (1:0.25) inclusion complex,
v) M2:DSC thermogram of meloxicam: β-cyclodextrin (1:0.5) inclusion complex,
vi) M3:DSC thermogram of meloxicam: β-cyclodextrin (1:1) inclusion complex,
vii) M4:DSC thermogram of meloxicam: β-cyclodextrin (1:2) inclusion complex,
viii) M5:DSC thermogram of meloxicam: β-cyclodextrin (1:3) inclusion complex,
ix) M6:DSC thermogram of meloxicam: β-cyclodextrin (1:4) inclusion complex,
x) M7:DSC thermogram of meloxicam: β-cyclodextrin (1:5) inclusion complex

The DSC thermograms of meloxicam exhibited an endothermic peak at 260 corresponding to its melting point. β-cyclodextrin alone showed a broad endothermic representing a loss of water molecule, a dehydration process. The thermograms of physical mixture and complexes are different from the pure drug; thereby giving clear evidence that there is formations of the complex.

As the concentration of the β-cyclodextrin increased in the in the complex it was observed that the height of endothermic peak at 260 diminished gradually and it disappeared completely at the concentration of 1:5, indicating complex formation at all these concentrations. However, maximum deflection in the peak height was found to occur while changing the concentration ratio from 1:1 to 1:2, indicating maximum inclusion at this concentration. Hence, other characterizations were performed in complexes bearing drug: β-cyclodextrin, 1:3, 1:4 and 1:5 in molar concentration.

The molecular volume of β-cyclodextrin is 346 $Å^3$ where as molar volumes of, meloxicam is 412 $Å^3$, which is greater than the molecular volume of β-cyclodextrin. Therefore two molecules of β-cyclodextrin may be required for making true inclusion complex. Practically, it has been found that for making inclusions, two molecules of β-cyclodextrin were to enclose one molecule of drug.

On the basis of molecular volume itself it is suggestive that drug could not be covered by the cavity of one molecule of β-cyclodextrin. So it is presumed that for H-bonding with β-cyclodextrin, the drug entered into the cavity of β-cyclodextrin, **Fig. 2**. The lateral combinations with the primary OH groups were also involved in this transfer. Therefore for further studies 1:2, drug: β-cyclodextrin ratio were used for further studies studied.

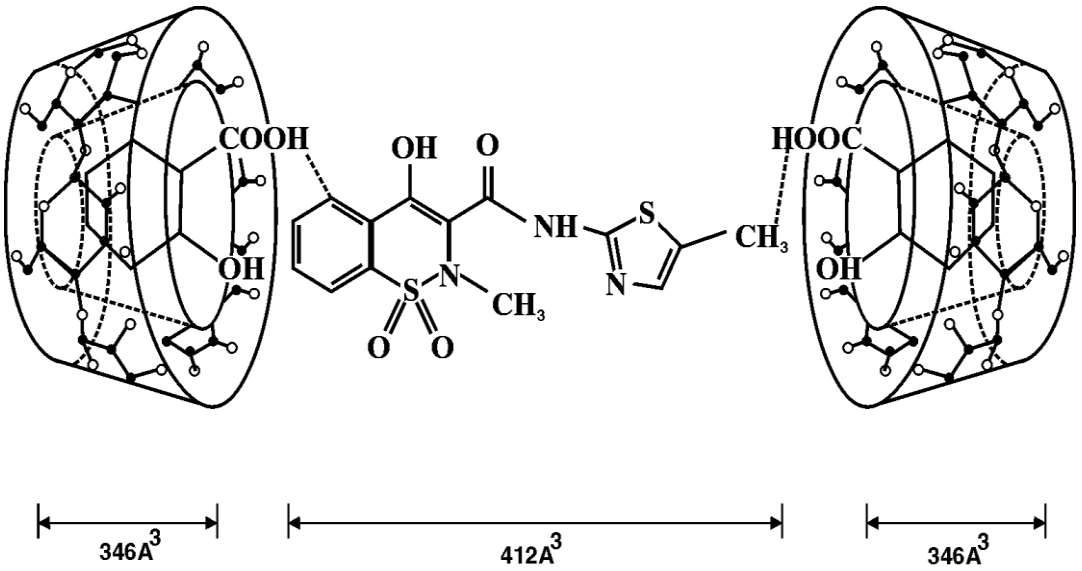

FIG 2: PROPOSED STRUCTURE FOR MELOXICAM: βCYCLODEXTRIN (1:2) INCLUSION COMPLEX

A remarkable difference between the inclusion complex, physical mixtures and pure drug were found by x-ray diffractrometry, **Fig. 3**. The diffraction pattern of the physical mixture was simply the sum of those of the components, whereas the reductions in crystallinity via the inclusion complex indicate complexation with β-cyclodextrin, which results in an amorphous powder. In the same manner, the IR spectra of meloxicam-β-cyclodextrin complexes show considerable differences when compared with those of the physical mixture and pure component.

All these modifications can be observed with the inclusion complexes clearly indicate the presence of host-guest interactions, **Fig. 4**. The formation of stable hydrogen bonds between meloxicam and the β-cyclodextrin is a possible reason for these observed spectral changes. Though a good drug release was observed with 0.5% carbopol (Table 1), but the gel was too soft and less viscous in nature, hence 1% carbopol was selected for the incorporation of the drug and the inclusion complexes. All the properties studied are recorded in Table 2. The release kinetics was established by determining the diffusional release exponent from the plot of log of cumulative drug release versus log time. The slopes of the straight line were recorded as values of diffusional release exponent (η)[23]. The slope of diffusional release exponent for all transdermal gels was calculated to be 0.48 to 0.51 that is very near to 0.5, which is indicating that the release of drug from these systems followed Fikian diffusion pattern (square root time dependence of solute release). Hence linear relationship exists between cumulative drug releases and square root of time, **Fig. 5**.

The drug release rate was lowest with pure drug formulations, k=0.92 mg/min$^{0.5}$/cm^2 mg/min$^{0.5}$/cm^2, Physical mixture exhibited drug release rate 0.89 mg/min$^{0.5}$/cm^2, gel bearing inclusion solid complexes exhibited drug release rate, 1.03 mg/min$^{0.5}$/cm^2. The release rate of transdermal gel containing the in situ inclusion complex, were 1.16 mg/min$^{0.5}$/cm^2.

The in vitro skin permeation study of the system was performed on Franz diffusion cell using human cadaver skin. The linear relationship was obtained after a lag time of 30-60 min in every case. The lag time could be accounted for the time taken by the drug to diffuse across the skin. The linear relationship indicated zero order permeation of the drug through skin. The slope of the graph was used to calculate skin permeability rate, **Fig. 6**.

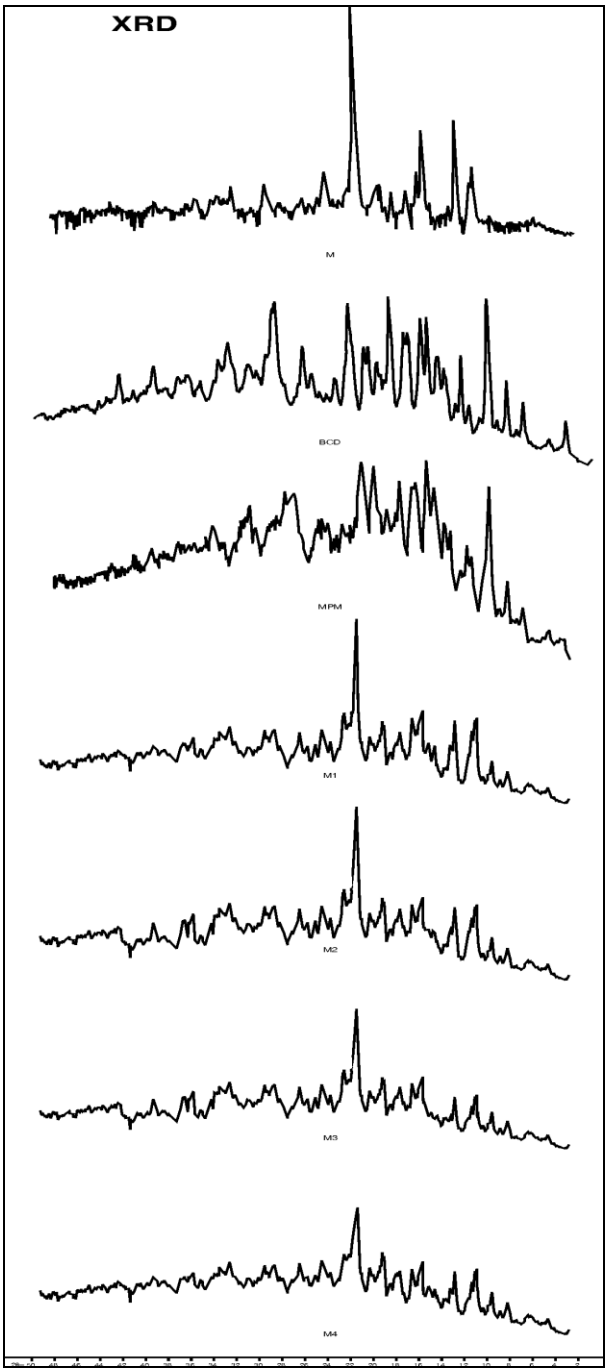

FIG. 3: **POWDER X-RAY DIFFRACTION PATTERN OF MELOXICAM AND β-CD INCLUSION COMPLEX IN DIFFERENT MOLAR CONCENTRATIONS**

i) BCD: powder x-ray diffraction pattern of β-cyclodextrin,
ii) M: powder x-ray diffraction pattern of plain meloxicam,
iii) MPM: powder x-ray diffraction pattern of meloxicam: β-cyclodextrin physical mixture (1:1),
iv) M1: powder x-ray diffraction pattern of meloxicam: β-cyclodextrin (1:0.25) inclusion complex,
v) M2: powder x-ray diffraction pattern of meloxicam: βcyclodextrin (1:0.5) inclusion complex,
vi) M3: powder x-ray diffraction pattern of meloxicam: βcyclodextrin (1:1) inclusion complex,
vii) M4: powder x-ray diffraction pattern of meloxicam: βcyclodextrin (1:2) inclusion complex,

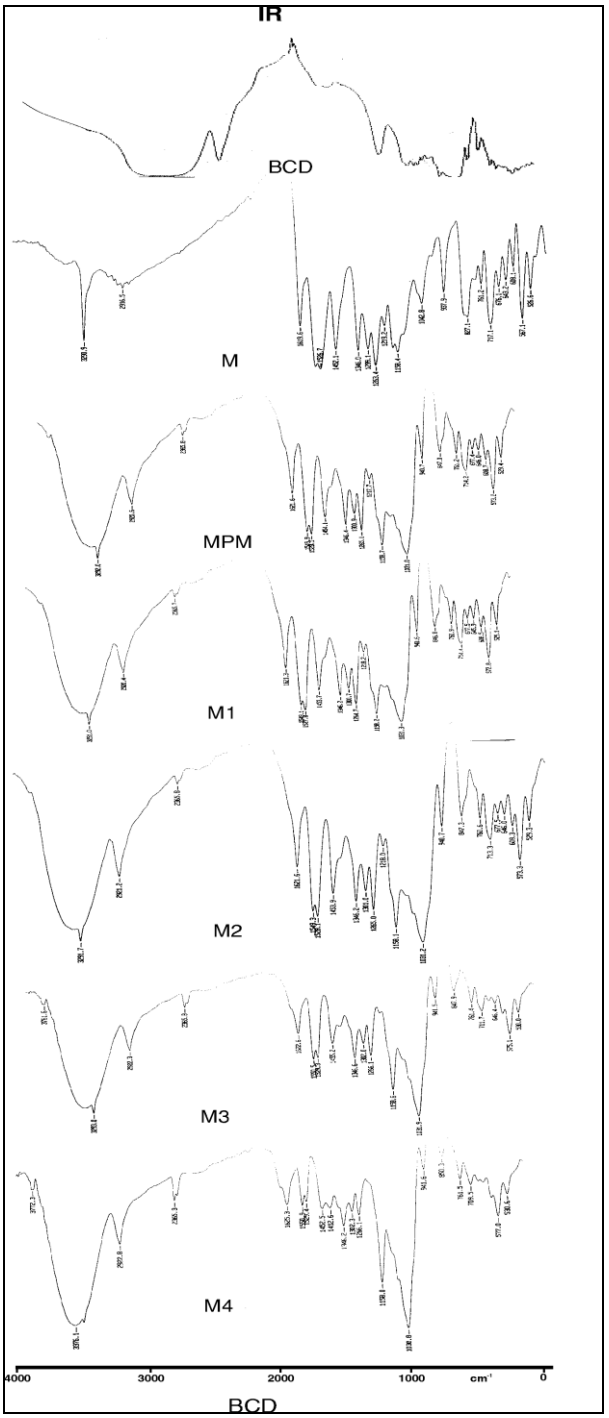

FIG. 4: **IR SPECTRA OF MELOXICAM AND β-CD INCLUSION COMPLEXES IN DIFFERENT MOLAR CONCENTRATIONS**

i) BCD: : IR spectrum of β-cyclodextrin,
ii) M: : IR spectrum of plain meloxicam,
iii) MPM: : IR spectrum of meloxicam: β-cyclodextrin physical mixture (1:1)
iv) M1: : IR spectrum of meloxicam: βcyclodextrin (1:0.25) inclusion complex,
v) M2: : IR spectrum of meloxicam: βcyclodextrin (1:0.5) inclusion complex,
vi) M3: : IR spectrum of meloxicam : βcyclodextrin (1:1) inclusion complex,
vii) M4: : IR spectrum of meloxicam: βcyclodextrin (1:2) inclusion complex

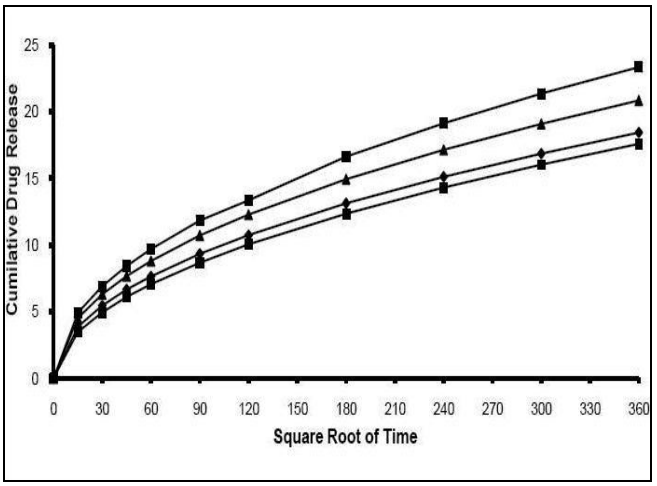

FIG. 5: ***IN VITRO*** **DRUG RELEASE PROFILE OF MELOXICAM TRANSDERMAL GEL THROUGH CELLOPHANE MEMBRANE**

MGPD-gel containing plain meloxicam (-□-), MGPM-gel containing physical mixture (meloxicam: β-CD, 1:1, -♦-) MGIC- gel containing meloxicam: βCD (solid inclusion complex, 1:2, -□-), MGIS - gel containing meloxicam: βCD (*in situ* formed complex, 1:2, -□-)

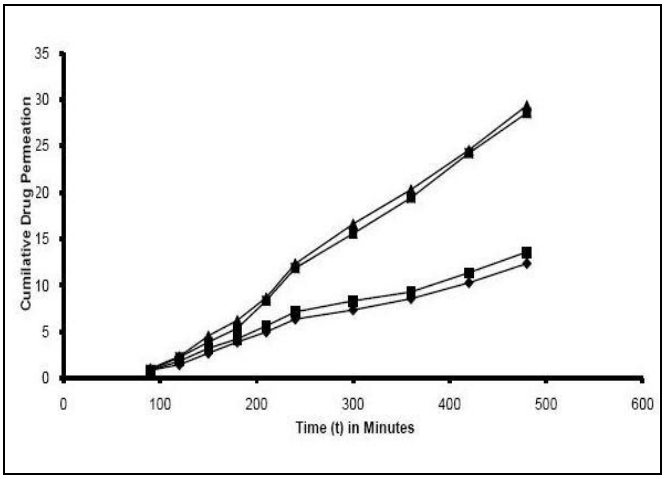

FIG. 6: ***IN VITRO*** **SKIN PERMAEATION PROFILE OF MELOXICAM TRANSDERMAL GELS**

MGPD-gel containing plain meloxicam (-□-), MGPM-gel containing physical mixture (meloxicam: β-CD, 1:1, -♦-) MGIC- gel containing meloxicam: βCD (solid inclusion complex, 1:2, -□-), MGIS - gel containing meloxicam: βCD (*in situ* formed complex, 1:2, -□-)

The skin permeation was poor with the pure drug transdermal gel formulations. The skin permeation rate for formulations containing pure drug was found to be 1.36 μg/min/cm^2. The transdermal gels containing inclusion complex of the drug showed best drug skin permeation rate (3.55 mg/min/cm^2). The skin drug permeation rates were found to be 1.59 μg/min/cm^2 for formulations containing physical mixture and transdermal gel containing the in situ complexes exhibited intermediate skin permeation rate of 3.42 μg/min/cm^2.

The differences in the release from different drug formulations might be attributed to three different factors: the stability constant, drug solubility and the gel viscosity. The release rates were higher in formulations, which contain inclusion complexes, than the pure drug, physical mixtures or in situ complex. This could be explained by believing that β-cyclodextrin might function as effective permeation enhancer by influencing the distribution and partitioning the drug in the skin or by changing the structure of the impermeable barrier.

Here, a statistical correlation was also attempted between the mean percent drug released at time 't' and quantities of drug permeate at time 't', **Fig. 7**. The correlation coefficient 'r^{2}' values lie within 0.94 to 1.00. This indicates a strong positive correlation between the in vitro drug release through cellophane and in vitro drug permeation of the drug through human cadaver skin.

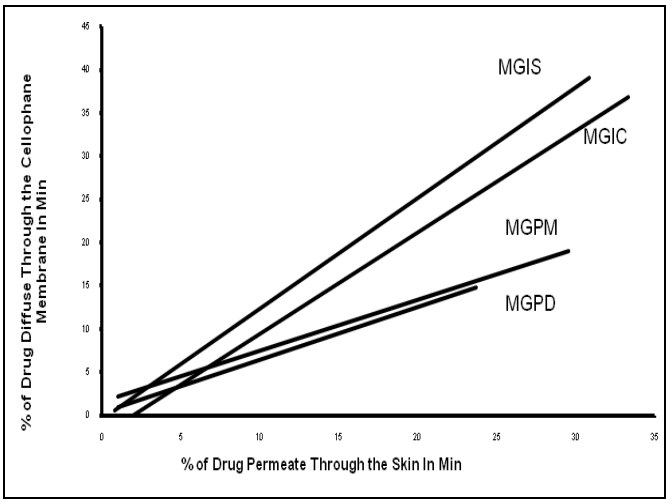

FIG. 7: CORRELATION OF ***IN VITRO*** **PERMEATION AND** ***IN VITRO*** **DIFFUSION OF MELOXICAM TRANSDERMAL GELS**

MGPD-gel containing plain meloxicam, MGPM-gel containing physical mixture (meloxicam: β-CD, 1:1), MGIC-gel containing meloxicam: βCD (solid inclusion complex, 1:2), MGIS - gel containing meloxicam: βCD (*in situ* formed complex, 1:2)

In vivo anti-inflammatory activity by % inhibition in oedema carrageenan-induced paw edema in rats determined after the topical application of transdermal gel bearing plain drug, physical mixture or solid inclusion complexes (drug: β-cyclodextrin) The observations are graphically represented in **Fig. 8**.

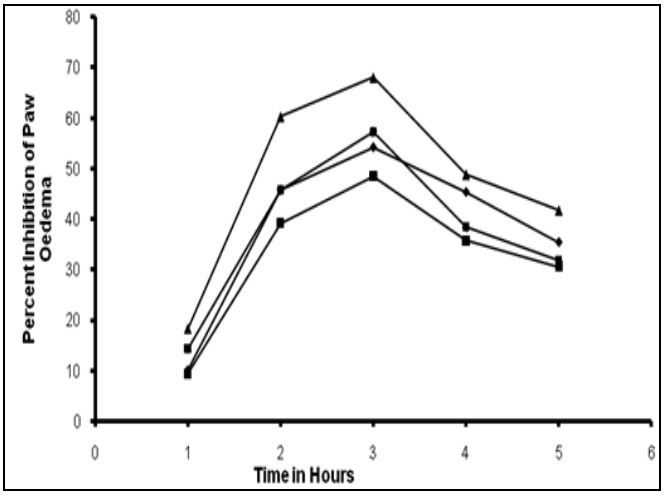

FIG 8. EFFECT OF TOPICAL APPLICATION OF MELOXICAM TRANSDERMAL GELS ON PAW OEDEMA INHIBITION

MGPD-gel containing plain meloxicam (-□-), MGPM-gel containing physical mixture (meloxicam: β-CD, 1:1, -♦-) MGIC- gel containing meloxicam: βCD (solid inclusion complex, 1:2, -□-), MGIS - gel containing meloxicam: βCD (*in situ* formed complex, 1:2, -□-)

The results exhibited that the maximum % inhibition in oedema was observed at 3rd hour. The transdermal gels bearing solid inclusion and in situ complex of drug showed 74.60% (±0.025) and 68.44 (±0.077) % inhibition in oedema, respectively, while its plain drug inhibited oedema only 59.70% (0.032) and its physical mixture exhibited % inhibition in oedema only 65.41% (±0.025).

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