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FABRICATION, APPRAISAL AND PHARMACODYNAMICS OF CURCUMIN LOADED POLYMETHYL METHACRYLATE NANOPARTICLES

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ABSTRACT

The objective of present study was to develop nanocurcumin for potential application in inflammatory diseases. The particles were prepared by nanoprecipitation with high speed homogenization and ultrasonication. Two polymers namely Eudragit RS 100 and RL 100 were used in combination, with varying ratios. The nanodispersion was further converted to nanocomposite film by solvent casting technique, to make feasible for topical application on skin. The formulations were evaluated in terms of particle size, entrapment efficiency, content uniformity, stability and drug excipient interaction. The permeation properties of nanocomposite films were investigated across pig epidermis in Keshary-Chien glass diffusion cell. The pharmacodynamics properties of the films were evaluated in carrageenan induced rat paw edema model. The SEM and TEM data confirmed the deposition of spherical nanocurcumin in the size range of 55-89 nm. The FT-IR and DSC data exhibited no distinct physical or chemical interactions between the drug and polymers. The XRD data indicated the amorphous nature of nanocurcumin in the film. The skin permeability of nanocurcumin was found to be more when compared to plain curcumin. The pharmacodynamics studies in rat model showed that nanocurcumin reduced inflammation more effectively than the oral formulation of same dose.

KEY WORDS: Nanocurcumin, Nancomposite film, Pharmacodynamics.

INTRODUCTION

Curcumin, a natural polyphenolic phytoconstituent, is isoalated from *Curcuma longa* Linn. (Zingiberaceae) and has diverse pharmacological activities. [1] Curcumin is a well-established

natural anti-inflammatory agent, due to the inhibition of cyclooxygenase-2 (COX-2), lipoxygenase, and inducible nitric oxide synthase (iNOS) enzymes. [2-6] However, the therapeutic benefit is hampered due to its poor water solubility. It exhibits solubility limited bioavailability, which makes it a class II drug in the Biopharmaceutics Classification System (BCS). [7] Furthermore, due to its rapid intestinal and hepatic metabolism after oral administration, approximately 60–70% of an oral dose of curcumin gets eliminated in the feces. [8] Curcumin is now in phase II clinical trial and a lot of work has been done on curcumin, but it is difficult to correlate all the data for exact prediction of the pharmacokinetic profile. Still from the available reports it is found that curcumin has poor oral bioavailability, short biological half-life (1.7 ± 0.5 h). [9]

Enhancing the absorption of poorly water-soluble drugs is a real challenge for pharmaceutical research. The advent of nanotechnology has been exploited for the development of various nanoparticulate drug delivery systems that can enable formulation and delivery of hydrophobic drugs, which earlier was a conundrum for the formulation scientists. These delivery systems have gained immense popularity in the last decade due to their potential to improve the therapeutic index of the encapsulated drugs either by protecting them from enzymatic or pH mediated degradation, by altering their pharmacokinetics, by blunting their toxicity or by providing controlled release over extended periods of time, drug targeting and easy large scale production. According to the National Nanotechnology Initiative (NNI), nanoparticulate (NP)-delivery systems contain encapsulated, dispersed, adsorbed, or conjugated drugs within a particle size range of 1–100 nm. [10-17]

Curcumin (MW 368.38 D, MP 183^{o}C) is lipophilic in nature with octanol/water partition coefficient (logP) value at 2.5. [18] The nanotechnology based drug delivery system will overcome the problems associated with curcumin delivery. [19] The purpose of the present work was to develop curcumin nanocomposite polymeric films and evaluation of skin permeation and pharmacodynamics studies in animal model for potential application in inflammatory diseases. The major problems associated with per oral curcumin can be avoided via topical application of the nanocomposite polymeric film and sustained anti-inflammatory action is possible due to accumulation of nanoparticles within the skin compartment. The magnetic-based core-shell particles with an average diameter of 296 nm have been found to have great potential to target, treat and monitor skin melanoma for targeted drug delivery to reduce side effects of chemotherapeutic agents. [20] The sodium carboxymethyl cellulose

silver nanocomposite films impregnated with curcumin have been reported for synergistic antimicrobial activity against *Escherichia coli.* [21]

Considering the fact that most inflammatory diseases occur locally and near the surface of the body, topical application of curcumin on the inflamed site can offer the advantage of delivering a drug directly to the disease site and producing its local effect. [22,23] However, the barrier properties of intact skin limit the permeability of wide variety of substances, including pharmaceutical active agents. [24] The topical anti-inflammatory properties of curcumin have been investigated from transdermal matrix film and gel using chemical permeation enhancers. [25,26] Some other approaches developed include terpene microemulsion, [27] niosomes, [28] liposomes [29] and nanoemulsions. [30] The present study was undertaken to investigate the potential application of topical nanocurcumin to target and treat inflammatory diseases in a better way.

MATERIALS AND METHODS

Materials

Curcumin (purity 99 %), Pluronic F-68, Polyvinyl alcohol, Polyethylene glycol 400, Isopropyl alcohol, Sodium bromide (Hi Media Laboratories Pvt. Ltd. Mumbai India); Eudragit RL100, Eudragit RS100, Eudragit NE 30D (Yarrow Chem Product, Mumbai, India); Methanol, Acetone, Dichloromethane, n-Octanol, Dibutylphthalate (Merck India Ltd., Mumbai, India) were used.

Methods

Preparation of nanocurcumin

The curcumin nanodispersions were prepared by nanoprecipitation method [31] with slight modification using varying proportions of Eudragit RL 100 and Eudragit RS 100 [1:1 (FF1), 1:2 (FF2), 1:3 (FF3), 1:4 (FF4), 2:1 (FF5), 3:1 (FF6) and 4:1 (FF7)]. The curcumin and polymers at 1:10 proportions were dissolved in a binary mixture of acetone and isopropanol (1:1). The organic phase solutions were slowly injected into external aqueous phase solution containing 1 % w/v of Pluronic F-68 under mild magnetic stirring (300 rpm). The organic phase solutions are always composed of solvents, making the drug and polymer soluble completely and external aqueous phase comprises antisolvent with surfactant in it. The solvent and antisolvent ratio was kept constant at 1:5. The mixture was homogenized (11000 rpm, 5 min) using a high speed homogenizer (T25 Digital Ultra Turrax homogenizer, IKA India Pvt. Ltd., Bangalore, India) followed by ultrasonication (40 KHz, 4 min) using a bath

sonicator (UCB 30D, Spectralab Instruments Pvt Ltd., Mumbai, India)). It was then magnetically stirred (500 rpm) at room temperature for 4 h to form the curcumin nanodispersions. Blank formulations were prepared following the above method without curcumin. All the formulations were prepared in triplicate.

The mean particle size and polydispersity index of the nanocurcumin was determined using 90-Plus dynamic light scattering particle size analyzer (Brookhaven Instruments Corporation, New York, USA). Each sample was diluted 100 times with distilled water for analysis. The particle size analyzer could measure sizes ranging from < 1 nm to 6000 nm. Samples should be diluted to suppress interparticulate interactions. The measurement was made three times with data acquisition for nine minutes at room temperature.

Preparation of nanocomposite films

The bilayer backing membrane composed of 5 % w/v polyvinyl alcohol and 5% w/v Eudragit NE 30D [32,33] was prepared in aluminum petridish (25 cm^2). The nanocomposite films were developed from curcumin nanodispersions by solvent casting technique. The plasticizer triethyl citrate (20 % w/w of dry weight of polymer) was mixed with the prepared nanodispersion under magnetic stirring. The homogeneous dispersion was cast onto the backing membrane into aluminum petridish and dried at room temperature for 60 h to leave stable nanocomposite film and kept in desiccators until further use.

The thickness of the prepared films was measured at nine different places with the help of a screw gauge micrometer (Western Electronic and Scientific Works Ltd., India) and the average value was considered.

For determination of drug content in the films, 1 cm^2 of the films were taken into small pieces. The pieces were transferred in stopper conical flasks containing 10 ml of aqueous PEG solution (50 % v/v). The flasks were subjected to vigorous stirring with the help of magnetic stirrer for 12 h. The whole solutions were ultrasonicated for 15 min. The extracted solutions were then filtered and replaced with 10 ml of fresh aqueous PEG solution (50 % v/v) in each of the flasks. The drug contents were quantified spectrophotometrically at 437 nm after appropriate dilution with freshly prepared aqueous PEG solution. The drug content was determined using the calibration curve in aqueous PEG solution (Absorbance = 1.01 + 0.168 × Concentration, R^2 = 0.998). The process was repeated until the absorbance was found to be zero. The total amount was calculated by adding the amount of curcumin released after

each interval. The total drug content in the formulation was calculated by multiplying the total area of the film with the total amount of drug extracted from 1 cm^2 patch. The films were stored in desiccators until further use.

Scanning electron microscopy (SEM)

The surface morphology of the drug loaded formulation was investigated using scanning electron microscope (Jeol, JSM 6360, Japan) at 15 kV. The sample was gold coated under vacuum using an ion sputter (JFC 1100) before being observed under microscope.

Transmission electron microscopy (TEM)

The external morphology of nanocurcumin was determined using a transmission electron microscope (Jeol JEM 2100 TEM, Japan). A drop of fresh nanoformulation was placed onto a carbon coated copper grid forming a thin liquid film and air dried. The dried films were observed under the transmission electron microscope without being stained and photographed. The analysis was done at 200 kV accelerated voltage.

The *in vitro* permeation study across porcine ear epidermis

The full thickness porcine ear skin was obtained from local slaughter house. The epidermis was separated from the full thickness skin according to the process described elsewhere. [34]

For *in vitro* permeation studies, skins were mounted on the modified Franz diffusion cell with the stratum corneum (SC) facing the donor compartment. The receptor compartment was filled with 43 ml aqueous solution of PEG 400 (50% v/v) and receptor phase was maintained at 37 ± 0.5^oC to ensure the skin surface temperature at 32 ± 0.5^oC to mimic the *in vivo* conditions. The curcumin nanocomposite film of appropriate size (3.47 cm^2) or plain curcumin (pure curcumin suspension in water at equivalent amount in the film) was applied on the SC side in the donor compartment. The receptor phase was stirred magnetically at 400 rpm. The receptor samples were analyzed for drug content spectrophotometrically at 437 nm by removing 1 ml aliquot through a hypodermic syringe fitted with a 0.22 µm membrane filter (Sartirius Stedim Biotech Gmbh, Germany), at designated time intervals for 24 h. The volume was replenished with the same volume of prewarmed receiver solution to maintain sink condition. A similar set was run simultaneously using the blank film (without drug) at the donor compartment as a skin film control system to avoid the influence of inherent extracts from the skin or leaching of any material from the film without drug and absorbance was taken at 437 nm, at which the sample aliquots were analyzed spectrophotometrically.

After the permeation experiment, the skin area of 3.47 cm^2 was cut and washed with distilled water and blotted dry. The treated skin area was weighed, cut into small pieces and placed in 5 ml of aqueous PEG solution (50 % v/v) with occasional stirring for 24 h. The desorbing solution was filtered through membrane filter (pore size 0.22 µm) and the amount of drug in the filtrate was determined spectrophotometrically.

All experiments were replicated at least three times. The concentrations of curcumin in the samples were calculated using the calibration curve described earlier and corrected to compensate the loss due to sample withdrawal using the equation proposed by Hayton and Chen 1982. [35] The amount of drug permeated through the skin during a sampling interval was calculated based on the measured receptor phase concentration and volume.

The permeation profiles were constructed by plotting the cumulative amount of drug permeated (Q, µg/cm^2) against time (t, h). The flux (Jss, µg/cm^2/h) of curcumin was calculated from the slope of the plot at steady-state using linear regression analysis. The lag time (t_L, h)) of penetration is defined as the corresponding time when extrapolated from the slope of the steady-state region intersects the time axis. Diffusion coefficient (D) was calculated by the formula [36]: $D = h^2/(6 \times t_L)$ where, h = Thickness of the skin (µm), t_L = Lag time (h). The enhancement ratio (ER) was calculated by the equation: ER = Permeation flux with nanocomposite film/Permeation flux with plain curcumin.

Pharmacodynamics evaluation

The pharmacodynamics evaluation was based on the anti-inflammatory properties of the optimized formulation in carrageenan induced rat hind paw edema model. [37] The study was approved by the Institutional Animal Ethical Committee (IAEC) Dibrugarh University (approval no. IAEC/DU/19 dated 17/02/2012, registration no. 1576/GO/A/11/CPCSEA). The Wister albino rats of both sexes (100-130 g) were obtained from M/S. Chakrobarty Enterprises, Kolkata India and the animals were kept in institutional animal house. The animal house was well ventilated and the animals were maintained on a 12:12 h light/dark cycle in large spacious cages throughout the experimental period. The animals were provided with food and water ad libitum. All efforts were made to minimize animals suffering and to reduce the number of animals for the study. The animals were divided into four groups and each group contained 3 rats.

The group one was served as inflammation control and received double distilled water. The group two (placebo control) was applied with the blank film (without drug) of 3.47 cm^2 on the plantar surface of the hind paw with the help of adhesive tape. The group three was orally administered 2 mg/ml of curcumin suspension in double distilled water. The group four was applied with the curcumin nanocomposite film of 3.47 cm^2 (equivalent to 2 mg of curcumin) on the plantar surface of the hind paw with the help of adhesive tape. The hair from the plantar surface of the rats in group two and group three were removed with the help of a marketed depilatory cream preparation twelve hours before the beginning of the experiment. At one hour after drug administration, the animals in all the groups were injected subcutaneously with 100 µl of 1 % w/v homogeneous suspension of carrageenan in double distilled water into the sub-plantar region of the right hind paw beyond the tibia tarsal region and the paw was marked properly. The paw volume was measured at 1, 2, 4, 8, 12 and 24 h after carrageenan administration by volume displacement method [38] using a plethysmometer (PLM 01, Orchid Scientific, Nashik, India). The percentage edema rate (E %) and anti-inflammatory activity (I %) were calculated for each animal group from the mean volume with respect to inflammation control group using following equations [32]: E % = (Vt/Vo – 1) × 100 and I % = (1 – Et/Ec) × 100; where Vt is the mean paw volume after carrageenan injection, Vo is the mean paw volume before carrageenan injection, Et is the edema rate of treated group and Ec is the edema rate of control group.

Stability study

The optimized nanocomposite films were subjected to stability study at ambient room temperature and accelerated conditions (40°C/75 % RH) for 3 months. The films were packed in aluminum foil and kept at ambient and accelerated conditions. The films were analyzed for drug content and physical changes, if any, at 0, 1, 2 and 3 months. FT-IR study was carried out to find out any chemical changes in the IR spectra after 3 months of storage.

Fourier transforms infrared spectroscopy (FT-IR)

The FT-IR spectrums for curcumin, polymers (Eudragit RL100 and Eudragit RS100), physical mixture of curcumin with polymers and curcumin loaded film were recorded by KBr pellet technique using a FT-IR spectrophotometer (Alpha, Bruker Optik GmbH, Germany) over a range of 4000 cm^{-1} to 500 cm^{-1}.

Differential scanning calorimetry (DSC)

The DSC thermograms were obtained for the pure curcumin, polymers (Eudragit RL100, Eudragit RS100) individually, a physical mixture of curcumin with each polymer and drug loaded films using a Jade DSC (Perkin Elmer, Switzerland). All samples were weighed about 10 mg and heated at scanning rate of 20°C/min between 35 and 250°C.

X-Ray Diffraction Analysis

The samples of pure curcumin, blank film and curcumin loaded films were assessed for crystallinity using X-ray Diffractometer (X'Pert Pro, PANalytical). The voltage and current were 30 kV and 15 mA, respectively. Measurements were carried out in the angular scan range from 5° to 40° (2θ) at a scan speed of 1°/min.

Statistical analysis

The results are expressed as mean ± standard deviation (SD) (n = 3). Statistical analysis was carried out by Student's *t-test* comparison at a significant level of $P < 0.05$ using MS Excel software.

RESULTS AND DISCUSSION

The curcumin nanodispersion prepared by nanoprecipitation with homogenization and ultrasonic dispersion showed uniform appearance. The mean particle size of all the formulations were varied between 78.8 ± 0.5 nm and 97.3 ± 0.9 nm, when diluted 100 times with distilled water. Our result is in accordance with the nanoparticle definition (dimension 1-100 nm) designated by the National Nanotechnology Initiative adopted by the American Standards Institute. [39] This is a highly desirable property in terms of their topical application to improve their penetration into the deeper skin layers. In all cases the polydispersity index (PI) felt within the range of 0.142 ± 0.002 to 0.251 ± 0.003. As known, the PI is a parameter used to define the particle size distribution of nanoparticles, it is a dimensionless number and ranges from a value of 0.01 for monodispersed particles up to values of around 0.5 – 0.7. Values greater than 0.7 are characteristic of samples with a very broad size distribution. [40] Therefore, it can be stated that the Eudragit based nanocurcumin prepared by nanoprecipitation technique were characterized by a homogeneous size distribution. The curcumin-loaded nanoparticles were small, uniform and spherical and there was no adhesion between particles seen on transmission electron microscope (Figure 1). The particle size and polydispersity index of different formulations are presented in the Table1. The size of the nanocurcumin, as observed on transmission electron microscope, was smaller

than the size measured by light scattering technique using particle size analyzer. It can be explained with the fact that the light scattering technique measured the hydrodynamic diameter of nanocurcumin, whereas the sample was completely dried before being observed on transmission electron microscope to get the actual diameter of the nanocurcumin. The selection of nanoprecipitation technique for curcumin nanodispersion was based on its advantages of being relatively simple and rapid to perform. It has been demonstrated in the literature that this nanoparticle delivery system can substantially transform the original physicochemical properties of drugs and greatly improve their bioavailability. [41,42]

In the nanoprecipitation technique, the internal organic phase solution was composed of the binary mixture of acetone and isopropanol in the proportion of 1:1, making the drug and polymer soluble completely. The organic solvent used was water miscible. The polymer precipitated in the form of nanospheres in which the drug was finely dispersed in the polymer matrix network. Nanoprecipitation occurred as a result of diffusion of internal organic solvent phase into the external aqueous phase in presence of surfactant Pluronic F-68 (1% w/v). The surfactant can penetrate the curcumin nanoparticles during the nanoprecipitation process to form a stable nanodispersion. The addition of isopropanol to acetone reduced the fast diffusion rate. When diffusion rate of solvent is too fast, the solvent may diffuse into the aqueous phase before stable nanodispersion is developed or formed causing the aggregation of nanoparticles.

The effect of various ratios of polymers on nanocurcumin formation was investigated. A significant different ($P < 0.05$, Student's *t*-test) in mean diameter of nanocurcumin was found in the FF1 formulation as compared to all other formulations. The lowest particle diameter was observed with the formulation FF6 composed of higher proportion of Eudragit RL 100 (Table 1). The Eudragit RL 100 contains higher proportion of quaternary ammonium groups than the Eudragit RS 100, making it more hydrophilic[43]. The hydrophilic behavior of Eudragit RL 100 probably ensured the polymer chain dissolution during nanocurcumin formation by nanoprecipitation method, resulting in smaller particle diameter. From the result it can be concluded that the mean diameter of nanocurcumin was markedly affected by the quarternary ammonium groups of the Eudragit polymers.

The nanodispersions were converted to nanocomposite films to make feasible for topical application on skin. The nanocomposite films were prepared from curcumin nanodispersion by solvent casting technique. The films were brittle without addition of plasticizer. Triethyl

citrate was chosen as a water soluble plasticizer, which initiated fusion of the two polymers and reduced the minimum film formation temperature. The plasticizer diffuses into and softens the nanoparticles. This softening promotes particle coalescence and film formation. The SEM photomicrograph (Figure 2) confirmed the deposition of uniform spherical nanocurcumin in the film. The Eudragit RL 100 is much more permeable than the Eudragit RS 100 and combination of these two polymers in a suitable proportion can be used to achieve prolonged release of the drug in a controlled manner. The Eudragit RL 100 results in rapid hydration due to higher content of quaternary ammonium groups, whereas lower proportion of quaternary ammonium groups in the Eudragit RS 100 is expected to control the release of drug from the prepared formulations.

The method of casting on aluminum dish was found to give good films. Low standard deviation values were found in the thickness of the films, which ensured uniformity of thickness of each film. Good uniformity of drug content among the batches was observed with all formulations. The results (Table 2) indicate that the process employed to prepare nanodispersions and films in this study was capable of producing films of uniform drug content and minimal film variability.

The *in vitro* skin permeation study is predictive of *in vivo* performance of a drug. The study was carried out in a modified Franz diffusion cell across porcine ear epidermis. The results obtained for the nanocomposite films were compared with that of plain curcumin with respect to various permeation parameters tabulated in the Table 2. The permeation profiles are shown in Figure 3. The cumulative percentage of curcumin permeated from different nanocomposite films were varied from 29.08±0.04 % to 44.35±0.02 % for 24 h. The results showed that the flux was markedly increased ($P < 0.05$, Student's t-test) in case of the curcumin nanocomposite films when compared to plain curcumin. The enhancement ratio was varied between 3.08 to 6.00. The lag time was found to be more for the plain curcumin as compared to nanocomposite film. The maximum flux was obtained with the formulation FF7 at 14.43±0.86 µg/cm^2/h and the minimum flux obtained with the plain curcumin at 2.40±0.95 µg/cm^2/h. The lag time for the formulation FF7 was found to be 0.16±0.04 h, which was significantly different ($P < 0.05$, Student's t-test) from the lag time observed with the plain curcumin (0.53±0.09 h). A significant different was observed in diffusion coefficient with nanocurcumin, when compared to the plain curcumin (Table 2). These results suggest that the skin permeability and diffusivity of nanocurcumin was more than the plain curcumin. The

reason may be the large surface area provided by the nanocomposite film and very small particle size allowing easy penetration of nanocurcumin into the deeper skin layers.

The total amount of curcumin retained in the skin showed a higher order with the nanocomposite films than the plain curcumin. The maximum retention (17.54±0.03 µg/mg) was observed with the formulation FF3 with nanocurcumin size of 86.5±0.3 nm and showed 1.36 fold higher retention than the plain curcumin. The formulation FF7 (nanocurcumin size 91.5±0.5 nm) showed 1.25 fold higher retention properties than the plain curcumin. The results indicated the nanocurcumin exerted a positive effect on the skin penetration and retention of curcumin.

It was observed that drug permeation from nanocomposite film containing higher amount of Eudragit RS 100 were slow as compared to the formulation containing higher amount of Eudragit RL 100. Overall the faster drug permeation rate from the formulation with higher concentration of Eudragit RL 100 was probably due to the high water permeability and swellability of Eudragit RL 100. The presence of a high content of quaternary ammonium groups makes the polymer more permeable. The contemporary presence of two polymers created a more compact matrix, hindering drug desorption and the following diffusion into the dissolution medium.

Early report suggested that the potential sites for nanoparticles penetration include the surface of the skin, furrows and hair follicles. [39] When the nanocomposite film was applied on the skin, it retarded the loss of moisture as a result of evaporation with consequent occlusive action and skin hydration. This resulted in reduction of corneocyte packing and opening of inter-corneocyte gaps and thus facilitated the drug penetration into the skin [44] resulting in higher permeation rate. Also the nanoparticles could penetrate into the superficial layers of the stratum corneum and from there the encapsulated drug released at controlled manner into the deeper skin layers resulting in higher permeation flux. In addition, the nanoparticles could accumulate in the hair follicles and created high local concentration of drug which improved the controlled diffusivity of the drug and thus higher permeation flux. Follicular penetration of solid particles, including liposomes, minoxidil loaded, and fluorescent polystyrene nanoparticles has already been demonstrated. [45-47]

The results of pharmacodynamic evaluation are reported in the Figure 4 & 5. Statistical analysis showed that the E % and I % of the formulation FF7 were significantly different

from oral standard group and control groups ($P < 0.05$, Student's *t*-test). The less inhibition of edema in the rats of oral standard group was due to the fast pass metabolism of curcumin into inactive metabolites in the gastrointestinal tract of rats. The placebo control group showed no anti-inflammatory activity. The curcumin nanocomposite film could significantly improve the inhibition effect of carrageenan induced hind paw edema of rats for nanocurcumin with particle size of 73.26 nm. The occlusive behavior of nanocomposite film promoted easy skin penetration of nanocurcumin and accumulation in the hair follicles, creating high local drug concentration. The controlled diffusion of the drug into the inflammation site showed the sustained anti-inflammatory effect as observed from the anti-inflammatory activity profile in the Figure 5.

Stability study

One of the major criteria for any rational design of a dosage form is its stability. Drug instability in pharmaceutical formulations may be detected by the changes in the physical appearance, color, odor, taste or texture of the formulation. The chemical changes may occur, if any, may only be ascertained through chemical analysis. The results of stability studies of the nanocomposite film (formulation FF7) are shown in the Table 3. There were no significant changes in their physical appearance. It was observed that the initial drug content and the drug contents of the samples analyzed after 1, 2 and 3 months of storage at various conditions were similar indicating there was no significant changes in the physical as well as chemical characteristics of the formulations. FT-IR study after 3 months showed similar spectrum with that of the spectrum observed initially. The IR spectrum of curcumin loaded film mainly exhibited the characteristic peaks for polymers indicating that the curcumin was molecularly embedded in the polymer matrix. There was no change in permeability of the film across porcine skin after 3 months of storage. Based on the observations, it was concluded that the developed curcumin nanocomposite film was physically and chemically stable and retained their pharmaceutical properties at various temperature and humidity conditions over a period of 3 months.

Drug–Excipient Interaction Studies

To study the compatibility of drug with the polymers used for the preparation of formulations, it is important to study the drug-polymer interaction. For this purpose various analytical techniques were used like FT-IR, DSC and XRD.

Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR analysis exhibited no distinct physical or chemical interactions between the drug and polymers. The IR spectrums for pure curcumin, physical mixture of curcumin and polymers, curcumin loaded formulation and blank formulation were recorded (Figure 6). The peaks of curcumin were observed in the physical mixture indicating no interaction. The IR spectrum of formulation mainly exhibited the characteristic peaks for polymers, with few overlapping peaks of curcumin. The IR peaks of blank formulation showed the peaks for polymers, which were present in the spectrum of individual polymers.

Differential Scanning Calorimeter Analysis (DSC)

From the DSC thermogram (Figure 7) of pure curcumin, it is observed that curcumin is crystalline in nature. It exhibited a sharp melting endotherm at an onset temperature of 181.71°C, a peak temperature of 186.77°C and end temperature of 189.06°C and heat of fusion was 138.0514 J/g. The thermogram of physical mixture exhibited three different peaks. The peaks at 88.07°C and 209.14°C resemble the peak of pure Eudragit RL100 and Eudragit RS100 whereas the peak at 177^{o}C resembles the peak of pure drug, which confirms that no interaction took place. The thermogram of blank formulation exhibited two peaks at 87.81°C and 209.94°C, resembles with peak of the two pure polymers. The thermogram of formulation exhibited single peak at 165.26°C, which was less than that of pure drug due to lowering of crystallinity of drug, as drug was encapsulated in polymer particle and became amorphous in nature.

X-Ray Diffraction (XRD)

In order to investigate the physical nature of the encapsulated drug in the transdernmal film, the powder XRD technique was used. The XRD data (Figure 8) of pure curcumin exhibited sharp peak intensities at 2θ diffraction angle of 30.265^{o}, 33.205^{o}, 64.795^{o}, 64.855^{o}, 64.915^{o}, 77.995^{o}, 78.055^{o}, which indicates the crystalline nature of curcumin. The intense peaks at the same 2θ diffraction angle were also present in the diffractogram of physical mixture of curcumin, Eudragit RL100 and Eudragit RS100, which reveals absence of any interaction. There was no sharp intense peak observed at 2θ diffraction angle in the diffractogram of drug loaded formulation, which indicates the amorphous curcumin dispersed in the polymeric nanocomposite film.

Table 1: Particle size and polydispersity index by dynamic light scattering technique

FN Code (mean±SD)	Polymer ratio (ERL100:ERS100)	Particle size (nm) (mean±SD)	Polydispersity index
FF1	1:1	97.3±0.9	0.251±0.003
FF2	1:2	82.2±0.2	0.163±0.005
FF3	1:3	86.5±0.3	0.185±0.011
FF4	1:4	87.8±0.5	0.210±0.004
FF5	2:1	85.4±0.3	0.184±0.019
FF6	3:1	78.8±0.5	0.142±0.002
FF7	4:1	91.5±0.6	0.213±0.005

Table 2: Permeation parameters of different formulations across porcine ear skin

FN Code	Jss ($\mu g/cm^2/h$) (mean±SD)*	t_L (h) (mean±SD)*	D ($cm^2/h \times 10^{-4}$) (mean±SD)*	Q at 24 h (%) (mean±SD)*	ER (mean±SD)*	Skin retention (µg/mg) (mean±SD)*	Film thickness (µm) (mean±SD)*	Drug content ($\mu g/cm^2$) (mean±SD)*
FF1	9.47±0.98	0.18±0.01	1.17±0.05	36.23±0.07	3.94±0.09	16.12±0.08	258±3	432±23
FF2	8.16±0.78	0.23±0.06	0.91±0.07	32.25±0.06	3.39±0.07	15.83±0.04	287±6	423±17
FF3	7.78±0.91	0.22±0.05	0.95±0.40	33.25±0.03	3.24±0.03	17.54±0.03	275±5	429±34
FF4	7.05±1.09	0.24±0.21	0.88±0.32	29.08±0.04	3.08±0.08	16.21±0.09	267±6	408±16
FF5	10.84±0.77	0.21±0.02	1.00±0.01	35.94±0.05	4.51±0.12	14.54±0.10	277±7	418±22
FF6	11.36±0.59	0.20±0.07	1.05±0.30	40.60±0.03	4.73±0.28	13.17±0.07	272±4	430±29
FF7	14.43±0.86	0.16±0.04	1.31±0.03	44.35±0.02	6.00±0.19	16.13±0.06	269±8	435±32
CRCMN Plain	2.40±0.95	0.53±0.09	0.39±0.12	10.59±0.04	1	12.82±0.02	252±5	432±25

Table 3: Stability study of nanocomposite film formulation FF7

Conditions	Parameters	Observation (days)			
		0	30	60	90
Ambient room temperature	Physical appearance	Yellow-brown	No change	No change	No change
	Drug content (µg/cm^2)	435±32	434.98±3.9	434.87±3.6	434.79±4.0
	FT-IR	Performed	-	-	NSC
	Jss (µg/cm^2/h)	14.43±0.86	-	-	14.33±0.75
Accelerated condition	Physical appearance	Yellow-brown	NSC	NSC	NSC
(40^{o}C/75 % RH)	Drug content (µg/cm^2)	435±32	433.57±2.7	432.98±3.7	432.54±3.8
	FT-IR	Performed	-	-	NSC
	Jss (µg/cm2/h)	14.43±0.86	-	-	14.23±0.80

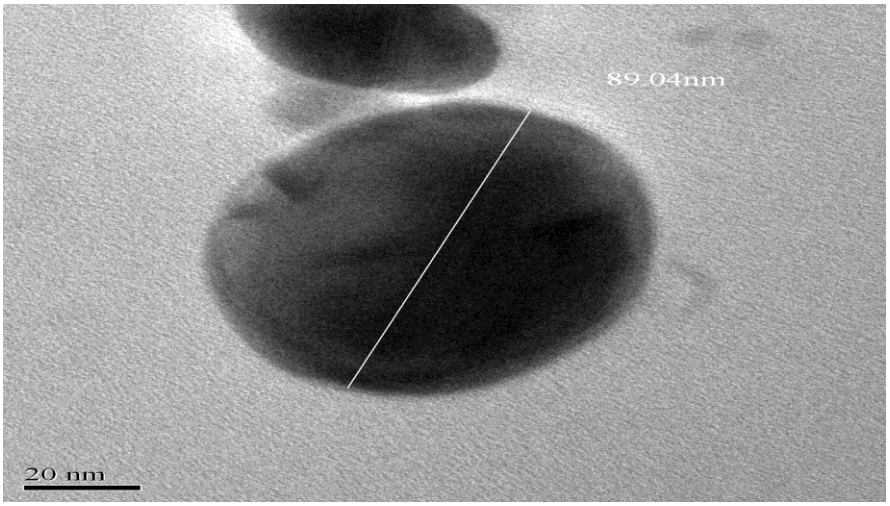


Fig. 1: Transmission electron microscopy of nanocurcumins. Scale length 20 nm.

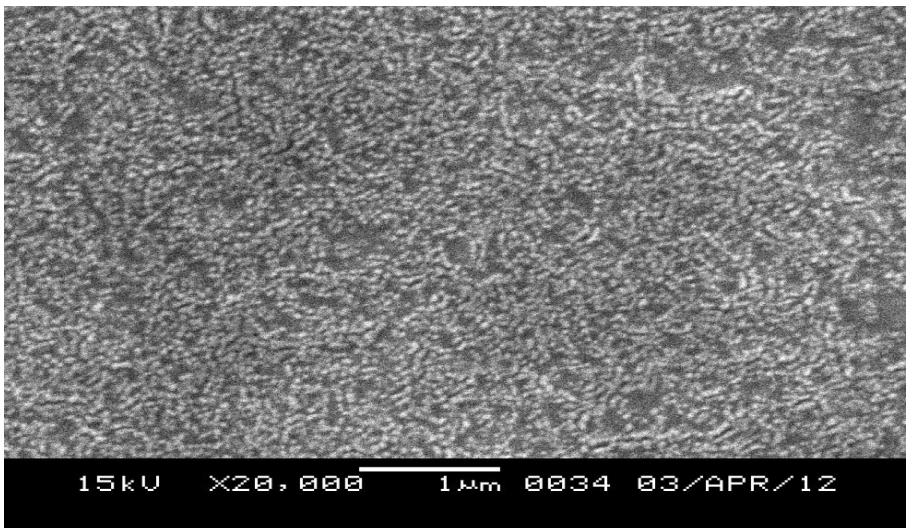


Fig. 2: The SEM photomicrograph of curcumin nanocomposite film.

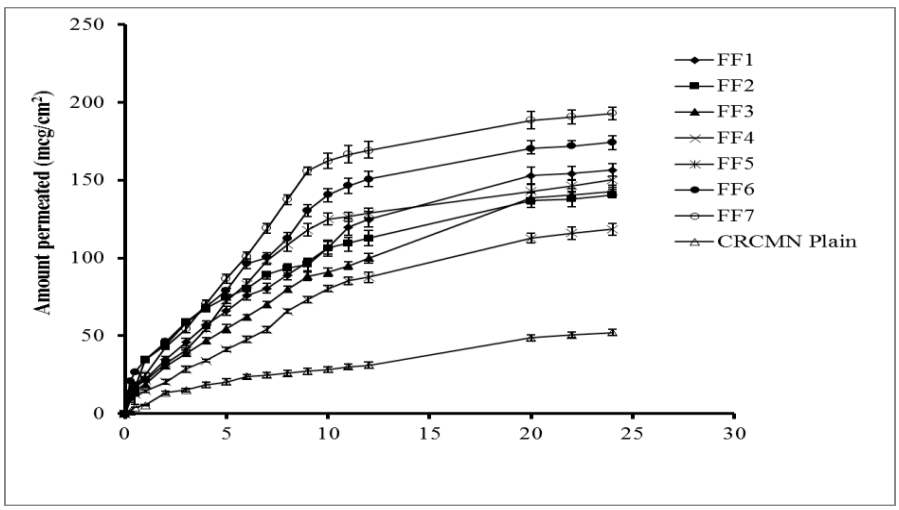


Fig. 3: *In vitro* permeation profile of nanocurcumin across porcine ear skin. Each data point represent the mean±SD (n = 3).

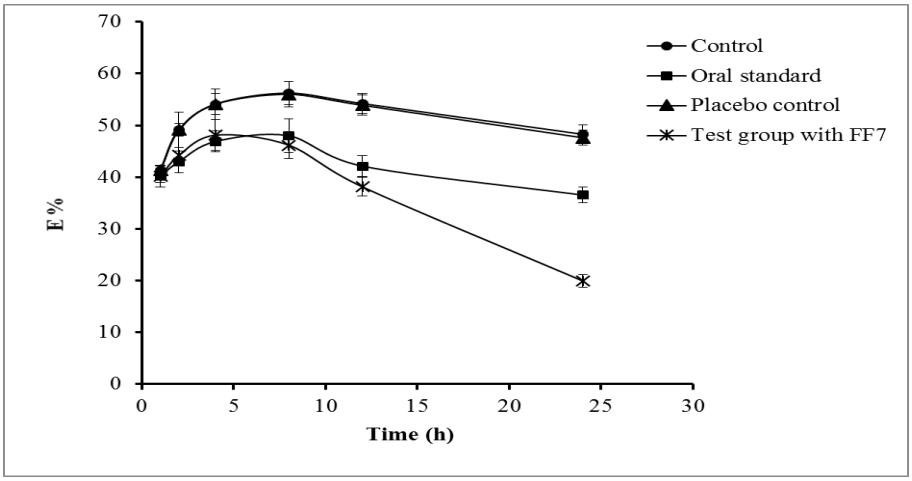


Fig. 4: Edema rate profile in different groups. Each data point represents the mean±SD (n = 3).

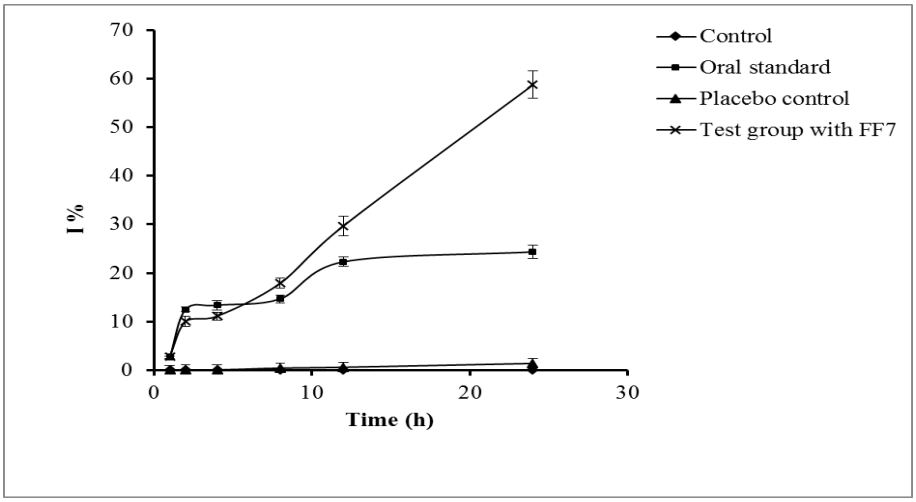
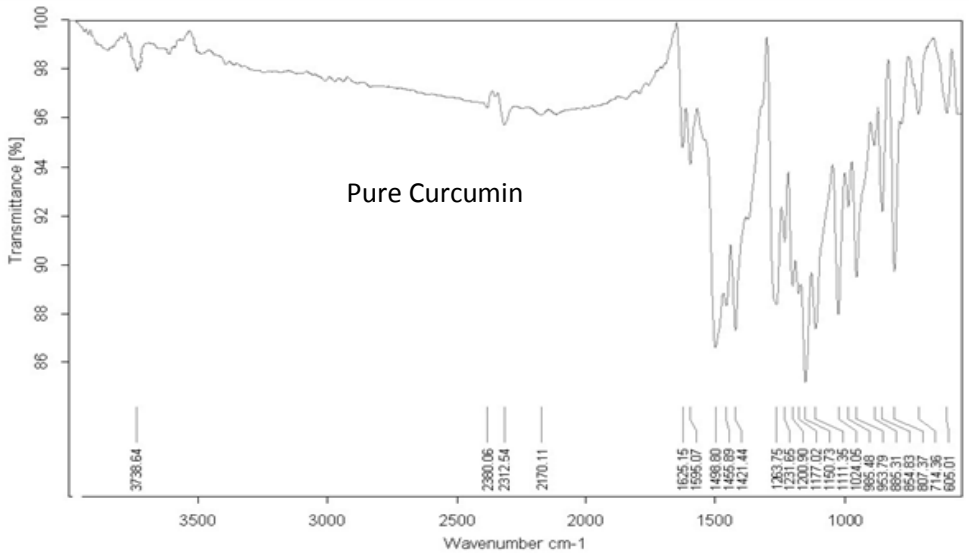


Fig. 5: Anti-inflammatory activity profile in different groups. Each data point represents the mean±SD (n = 3).



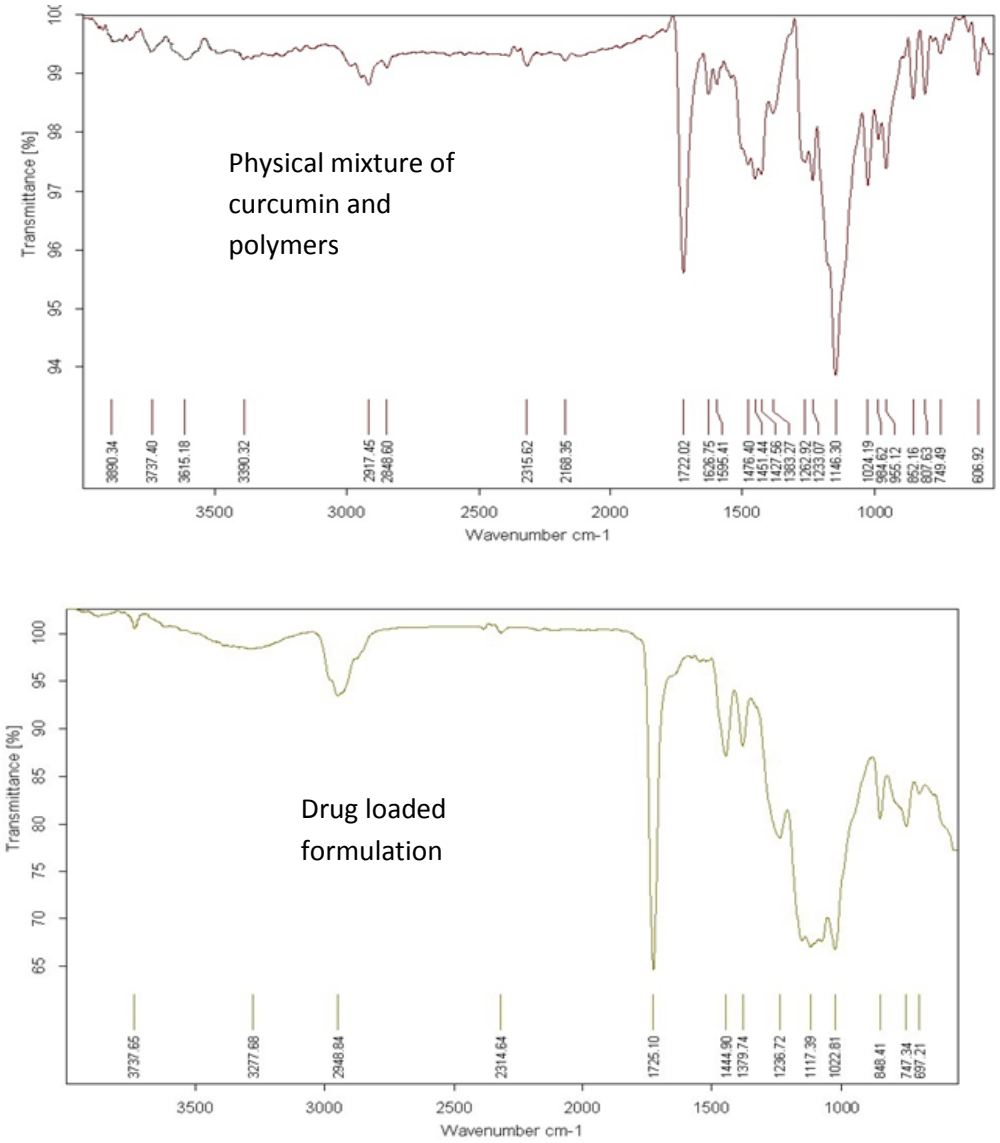
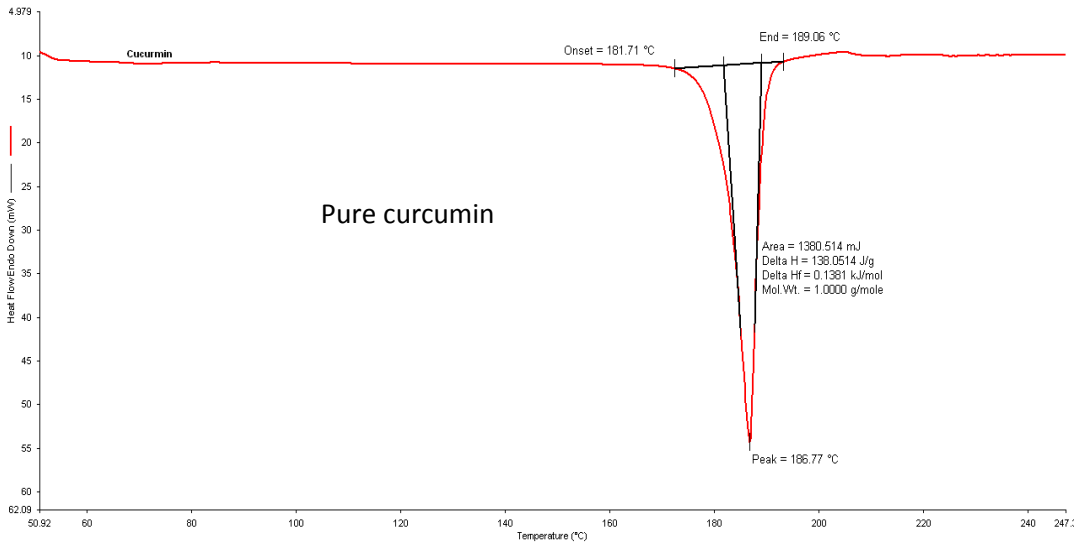


Fig. 6: FT-IR spectrums for pure curcumin, physical mixture of curcumin, polymers and drug loaded formulation.



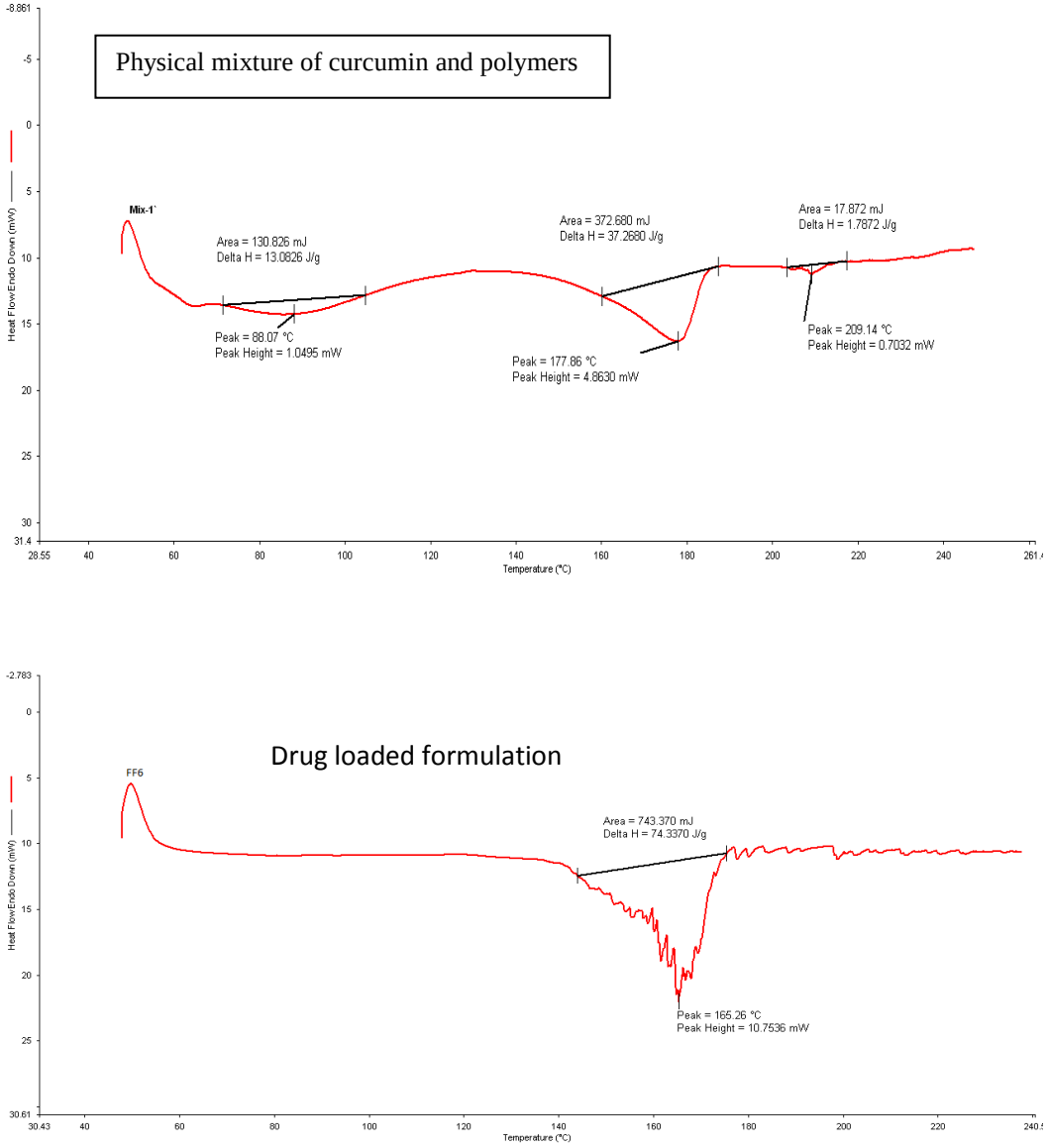
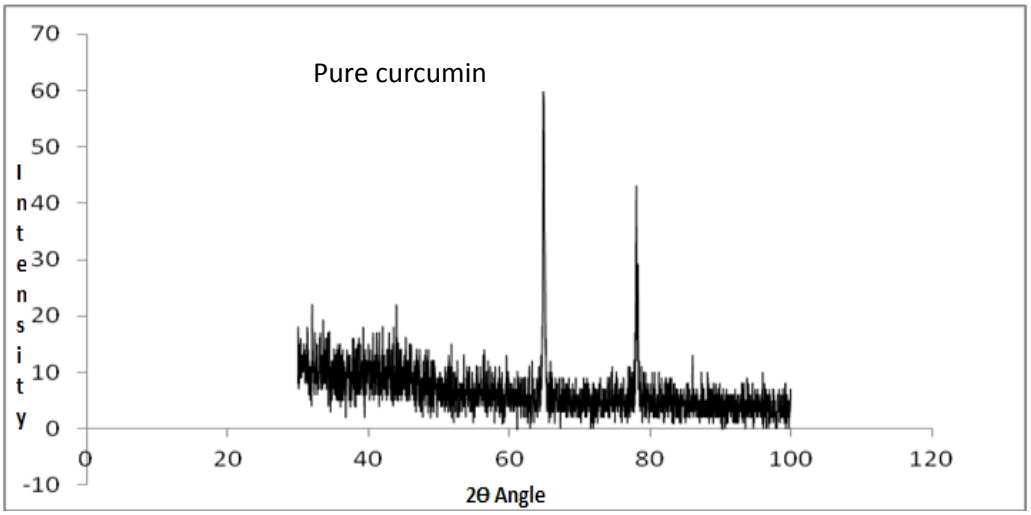


Fig. 7: DSC thermograms of pure curcumin, physical mixture of crucumin, polymers and drug loaded formulation.



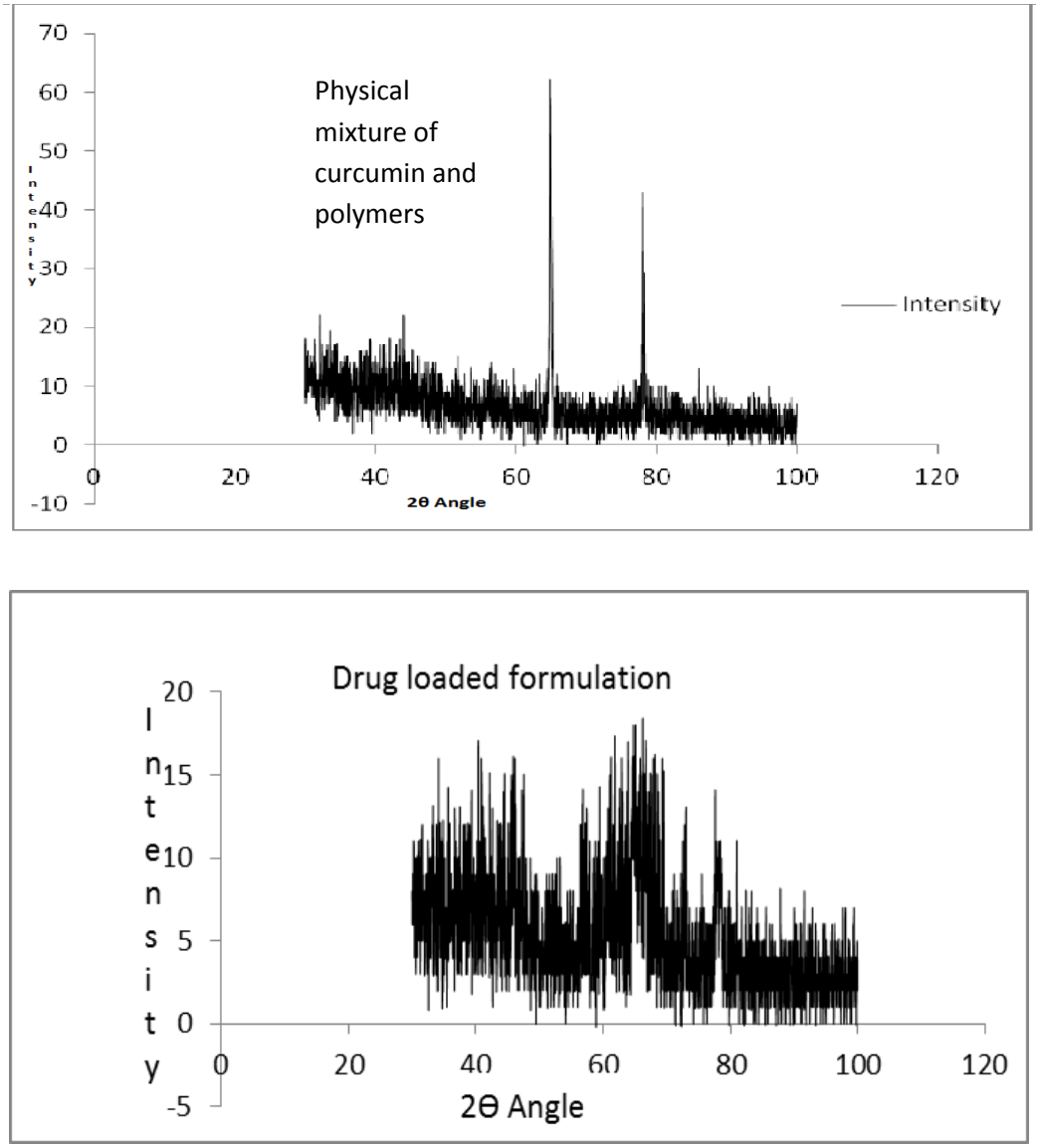


Fig. 8: XRD spectrums of pure curcumin, physical mixture of curcumin and polymers, drug loaded formulation.

CONCLUSION

The curcumin nanocomposite films were developed for higher topical bioavailability to treat the inflammatory diseases in a better way. The nanocomposite formulation FF7 showed the best *in vitro* skin permeation and anti-inflammatory activity in carrageenan induced rat paw edema model as compared to other film formulations. The skin toxicities by the use of the chemical permeation enhancers in film based curcumin formulation can be avoided using the developed nanocomposite formulation. Based on the present results, trials may be performed on humans.

REFERENCES

1. Anand P, Thomas SG, Kunnumakkara AB, Sundaram C, Harikumar KB, Sung B, et al. Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. Biochem Pharmacol, 2008; 76: 1590-1611.
2. Basnet P, Skalko-Basnet N. Curcumin: An anti-inflammatory molecule from a curry spice on the path to cancer treatment. Molecules, 2011; 16: 4567-4598.
3. Jurenka JS. Anti-inflammatory properties of curcumin, a major constituent of Curcuma longa: a review of preclinical and clinical research. Altern Med Rev, 2009; 14: 141-153.
4. Kohli K, Ali J, Ansari MJ, Raheman Z. Curcumin: A natural antiinflammatory agent. Indian J Pharmacol, 2005; 37: 141-147.
5. Sharma RA, Gescher AJ, Steward WP. Curcumin: The story so far. Eur J Cancer, 2005; 41: 1955–1968.
6. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: problems and promises. Mol Pharm, 2007; 4: 807-818.
7. Kasim NA, Whitehouse M, Ramachandran C, Bermejo M, Lennernas H, Hussain AS, et al. Molecular properties of WHO essential drugs and provisional biopharmaceutical classification. Mol Pharm, 2004; 1: 85–96.
8. Pan MH, Huang TM, Lin JK. Biotransformation of curcumin through reduction and glucuronidation in mice. Drug Metab Dispos, 1999; 27: 486–494.
9. Xie L, Li XK, Takahara S. Curcumin has bright aspect for the treatment of multiple sclerosis. Int Immunopharmacol, 2011; 11: 323-330.
10. Bawarski WE, Chidlowsky E, Bharali DJ, Mousa SA. Emerging nanopharmaceuticals. Nanomedicine, 2008; 4: 273–282.
11. Khan JA, Kainthan RK, Ganguli M, Kizhakkedathu JN, Singh Y, Maiti S. Water soluble nanoparticles from PEG-based cationic hyperbranched polymer and RNA that protect RNA from enzymatic degradation. Biomacromolecules, 2006; 7: 1386–1388.
12. Schluep T, Hwang J, Hildebrandt LJ, Czernin J, Choi CH, Alabi CA, et al. Pharmacokinetics and tumor dynamics of the nanoparticle IT-101 from PET imaging and tumor histological measurements. Proc Natl Acad Sci U S A, 2009; 106: 11394–11399.
13. Italia JL, Bhatt DK, Bhardwaj V, Tikoo K, Kumar MN. PLGA nanoparticles for oral delivery of cyclosporine: nephrotoxicity and pharmacokinetic studies in comparison to Sandimmune Neoral. J Control Release, 2007; 119: 197–206.

14. Grabovac V, Bernkop-Schnurch A. Development and *in vitro* evaluation of surface modified poly (lactide-co-glycolide) nanoparticles with chitosan-4-thiobutylamidine. Drug Dev Ind Pharm, 2007; 33: 767–774.
15. Koo OM, Rubinstein I, Onyuksel H. Role of nanotechnology in targeted drug delivery and imaging: a concise review. Nanomedicine, 2005; 1: 193–212.
16. Jiang W, Kim BY, Rutka JT, Chan WC. Advances and challenges of nanotechnology-based drug delivery systems. Exprt Opin Drug Deliv, 2007; 4: 621-633.
17. Suri SS, Fenniri H, Singh B. Nanotechnology-based drug delivery systems. J Occup Med Toxicol, 2007; 2: 16. Doi: 10.1186/1745-6673-2-16.
18. Curcumin, The Merck Index, Fourteenth Ed., New Jersey: Merck & Co. Inc, 2006.
19. Sun M, Su X, Ding B, He X, Liu X, Yu A, Lou H, Zhai G. Advances in nanotechnology-based delivery systems for curcumin. Nanomedicine (Lond.), 2012; 7: 1085-1100.
20. Wadajkar AS, Bhavsar Z, Ko CY, Koppolu B, Cui W, Tang L, Nguyen KT. Multifunctional particles for melanoma-targeted drug delivery. Acta Biomater, 2012; 8: 2996-3004.
21. Varaprasad K, Vimala K, Ravindra S, Reddy NN, Reddy GVS, Raju KM. Fabrication of silver nanocomposite films impregnated with curcumin for superior antibacterial applications. J Mater Sci Mater Med, 2011; 22: 1863-72.
22. Arellano A, Santoyo S, Martin C, Ygartua P. Surfactant effect on the *in-vitro* percutaneous absorption of declofinac sodium. Eur J Drug Metab Pharmacokinet, 1998; 23: 307–312.
23. Arellano A, Santoyo S, Martin C, Ygartua P. Influence of propylene glycol and isopropyl myristate on the *in-vitro* percutaneous penetration of diclofenac sodium from carbopol gels. Eur J Pharm Sci, 1999; 7: 129–135.
24. Shah VP, Behl CR, Flynn GL, Higuhi WI, Schaefer H. Principles and criteria in the development and optimization of topical therapeutic products. J Pharm Sci, 1992; 81: 1051–1054.
25. Patel NA, Patel NJ, Patel RP. Design and evaluation of transdermal drug delivery system for curcumin as an anti-inflammatory drug. Drug Dev Ind Pharm, 2009a; 35: 234-42.
26. Patel NA, Patel NJ, Patel RP. Formulation and evaluation of curcumin gel for topical application. Pharm Dev Technol, 2009b; 14: 80-89.
27. Liu CH, Chang FY. Development and characterization of eucalyptol microemulsion for topical delivery of curcumin. Chem Pharm Bull, 2011; 59: 172-78.

28. Rungphanichkul N, Nimmannit U, Muangsiri W, Rojsitthisak P. Preparation of curcuminoid niosomes for enhancement of skin permeation. Pharmazie, 2011; 66: 570-75.
29. Basnet P, Hussain H, Tho I, Skalko-Basnet N. Liposomal delivery system enhances anti-inflammatory properties of curcumin. J Pharm Sci, 2012; 101: 598-609.
30. Wang X, Jiang Y, Wang YW, Huang MT, Ho CT, Huang Q. Enhancing anti-inflammatory activity of curcumin through O/W nanoemulsions. Food Chem, 2008; 108: 419-424.
31. Jingling T, Xu N, Hongyu J, Hongmei L, Wang Z, Linhua W. Eudragit nanoparticles containing genistein: formulation, development, and bioavailability assessment. Int J Nanomedicine, 2011; 6: 2429–2435.
32. Arora P, Mukherjee B. Design, development, physicochemical, and *in vitro* and *in vivo* evaluation of transdermal patches containing diclofenac diethylammonium salt. J Pharm Sci, 2002; 91: 2076-2089.
33. Das MK, Bhattacharya A, Ghosal SK. Transdermal delivery of Trazodone Hydrochloride from acrylic films prepared from aqueous latex. Indian J Pharm Sci, 2006; 68: 41-46.
34. Das MK, Ahmed AB. Formulation and *ex vivo* evaluation of rofecoxib gel for topical application. Acta Pol Pharm, 2007; 63: 461-467.
35. Hayton WL, Chen T. Correction of perfusate concentration for sample removal. J Pharm Sci, 1982; 71: 820-821.
36. Hansen S, Henning A, Naegel A. In-silico model of skin penetration based on experimentally determined input parameters. Part I: Experimental determination of partition and diffusion coefficients. Eur J Pharm Biopharm, 2008; 68: 352–367.
37. Winter CA, Risley EA, Nuss GW. Carrageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med, 1962; 111: 544–547.
38. Harris JM, Spencer PSJ. A modified plythesmographic apparatus for recording volume changes in rat paw. J Pharm Pharmacol, 1962; 14: 464–466.
39. Prow TW, Grice JE, Lin LL, Faye R, Butler M, Becker W, Wurm EMT, Yoong C, Robertson TA, Soyer HP, Roberts MS. Nanoparticles and microparticles for skin drug delivery. Adv Drug Deliv Rev, 2011; doi:10.1016/j.addr.2011.01.012.
40. Lopedota A, Trapani A, Cutricgnelli A, Chirantini L, Pantucci E, Curci R, Manuali E, Trapani G. The use of Eudragit RS 100/cyclodextrinnanoparticles for the transmucosal administration of glutathione. Eur J Pharm Biopharm, 2009; 72: 509-520.

41. Ratnam DV, Ankola DD, Bhardwaj V, Sahana DK, Kumar MN. Role of antioxidants in prophylaxis and therapy: A pharmaceutical perspective. J Control Release, 2006; 113: 189–207.
42. Dai J, Nagai T, Wang X, Zhang T, Meng M, Zhang Q. pH-sensitive nanoparticles for improving the oral bioavailability of cyclosporine A. Int J Pharm, 2004; 280: 229–240.
43. Rowe RC, Sheskey PJ, Owen SC. Handbook of Pharmaceutical Excipients, fifth ed., London: Pharmaceutical Press, 2006.
44. Wissing SA, Müller RH. Cosmetic applications for solid lipid nanoparticles (SLN). Int J Pharm, 2003; 354: 65–68.
45. Lieb LM, Ramachandran C, Egbaria K, Weiner N. Topical delivery enhancement with multilamellar liposomes into pilosebaceous units: I. *In vitro* evaluation using fluorescent techniques with the hamster ear model. J Invest Dermatol, 1992; 99: 108–113.
46. Shim J, Seok Kang H, Park WS, Han SH, Kim J, Chang IS. Transdermal delivery of mixnoxidil with block copolymer nanoparticles. J Control Release, 2004; 97: 477–484.
47. Alvarez-Roman R, Naik A, Kalia YN, Guy RH, Fessi H. Skin penetration and distribution of polymeric nanoparticles. J Control Release, 2004; 99: 53–62.