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ORIGINAL ARTICLE

Evaluating the efficacy of different curcumin polymorphs in transdermal drug delivery

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Abstract

Purpose Curcumin exists in three polymorphic forms: one monoclinic form and two orthorhombic forms. This work aims to investigate the efficacy of curcumin polymorphs in transdermal drug delivery by formulating curcumin polymorphs and their incorporation in polymeric films.

Methods Monoclinic form, Form 1, was precipitated by liquid antisolvent technique from acetone solutions, whereas orthorhombic form, Form 2, was obtained by vacuum evaporation of solutions of curcumin in a mixed solvent of chloroform and hexane (60:40%v/v). The other orthorhombic form, Form 3, was precipitated from dimethylsulfoxide solutions. All three curcumin polymorphs were incorporated into polymeric films made of low molecular weight hydroxypropyl methyl cellulose (HPMC E5LV) along with different plasticizers, and permeation enhancers. Radical scavenging activity and cytotoxicity of curcumin polymorphs on human melanoma cell lines were evaluated. Water uptake, in-vitro release, and in-vitro permeation studies on HPMC films loaded with curcumin polymorph were carried out.

Results Cytotoxicity studies on human melanoma cells (SK-MEL-28) showed that Form 2 results in the highest cell inhibition. Among all three curcumin polymorphs, the free radical scavenging activity of Form 3 was found to be the highest. HPMC films loaded with Form 3 exhibited higher water uptake and higher curcumin release profiles at pH of 5.5 (95.3% in 20 h) and pH 7.4 (79.8% in 20 h) as well as the highest in-vitro permeation compared to the other two curcumin forms.

Conclusion Overall, orthorhombic curcumin polymorphs (i.e., Form 2 and Form 3) showed a higher propensity for transdermal drug delivery as compared to the monoclinic curcumin (Form 1).

Keywords Curcumin polymorphs · HPMC films · Transdermal drug delivery · Human melanoma · Drug release · Drug permeation

Introduction

Many active pharmaceutical ingredients (API) exhibit low water solubility and poor dissolution rates despite possessing good medicinal properties, which limits their bioavailability. It has been shown that by increasing specific surface area by reduction in the particle size, dissolution rates of such APIs can be significantly enhanced (Kakran et al. 2012). A decrease in the particle size increases the surface-to-volume ratio, thereby increasing their dissolution rates (Merisko-Liversidge et al. 2003). A commonly used approach for particle size reduction is using either a top-down or a bottom-up technique. Compared to top-down approaches (such as milling, grinding, etc.), bottom-up techniques are not only simpler, more cost-effective, and easier to scale up but also offer better control over the process. Liquid antisolvent (LAS) precipitation is one of the bottom-up techniques used to produce nanoparticles of poorly water-soluble APIs. LAS precipitation can alter the physical properties of drug substances, including modification in the polymorphic form and particle size distributions (Thorat and Dalvi 2012). The other advantage is that water can be used as an antisolvent for many poorly water-soluble drugs since it is also highly

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miscible with many organic solvents. The use of ultrasound and additives during the crystallization process can easily give a greater control over the rate of nucleation, crystal growth, and polymorphism of APIs.

Several drug delivery routes have been investigated to deliver drug particles with efficacy (Bennet and Kim 2014). Transdermal drug delivery still remains limited to a narrow range of drugs. The drug has to be supplied at the site for an effective treatment. Also, stratum corneum's (SC) low permeability for macromolecular drugs poses significant challenges to transdermal drug administration via passive diffusion through the skin (Prausnitz and Langer 2008). Still, transdermal drug delivery via films has been well explored for the delivery of both hydrophobic as well as hydrophilic drugs (Karki et al. 2016). Since films are thin and flexible, their acceptance among patients is high (Maniruzzaman et al. 2012). Films are capable of accelerating the onset of drug action, reduce the required dose frequency, and enhance drug efficacy (Barbu et al. 2006). Ideal transdermal drug delivery films need to exhibit desirable features such as sufficient drug loading capacity, fast dissolution rate or long residence time at the site of administration, and acceptable formulation stability. They should also be non-toxic, biocompatible, and biodegradable (Achouri et al. 2013).

The availability of a wide array of suitable polymers has made it possible to develop films with a wide range of properties and applications (Nair et al. 2013). An ideal wound healing dressing or agent protects the wounded tissue from bacterial infection, reduces inflammation, and induces cell proliferation to aid in the reconstruction of the damaged tissue (Boateng et al. 2008). It should also contain antioxidant molecules since free radicals are considered as one of the major causes of inflammation during the wound healing process (Koh and DiPietro 2011).

Curcumin is a poorly water-soluble drug found in the herbal spice turmeric (*Curcumin longa*). It has potential antioxidant, anti-inflammatory, antitumor, anti-HIV, and antimicrobial properties (Aggarwal and Harikumar 2009). Curcumin has also been found to enhance cutaneous wound healing through tissue remodeling (Mohanty et al. 2012), tissue formation, and collagen deposition (Joe et al. 2004). Several reports have shown that curcumin assists in epithelial regeneration (Sidhu et al. 1998) and increases fibroblast proliferation (Thangapazham et al. 2013). The wound healing properties of curcumin has also been reported by some authors (e.g., Akbik et al. 2014; Joe et al. 2004).

Curcumin exists in three polymorphic forms, of which Form 1 is monoclinic and the other two (Form 2 and Form 3) are orthorhombic (Sanphui et al. 2011). Different curcumin polymorphs possess distinct functionality that could be utilized for several applications. Silva et al. (2019) have reported development of HPMC films loaded with curcumin mainly for food packaging applications. However, there are no reports on the evaluation of individual curcumin polymorphs for drug delivery applications, especially for transdermal drug delivery.

In this work, for the first time, the efficiency of different curcumin polymorphs for transdermal drug delivery has been investigated. The radical scavenging activity of curcumin polymorphs was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Cytotoxicity studies of curcumin polymorphs was performed on the human melanoma cell line (SK-MEL-28). The curcumin polymorphs were then incorporated in HPMC films and evaluated for their efficacy for transdermal drug delivery using in-vitro release and permeation studies.

Materials and methods

Materials

Curcumin (≥95%) was purchased from Mrida Greens Pvt. Ltd., India. All analytical grade solvents such as acetone (>99.9%) and DMSO (99.8%) were purchased from SRL, India. Hydroxypropyl methyl cellulose (HPMC) (80–120 cPs, F.C.C.), sodium dodecyl sulphate (SDS), bovine serum albumin (BSA), and polyvinylpyrrolidone (PVP) were purchased from Sigma-Aldrich Inc., India. HPMC (E5LV, E15LV, and K4000), used for film formulations, was purchased from Colorcon, India. Polyethylene glycol (PEG 400), triethyl citrate (TEC), triacetin (TRA) and propylene glycol (PG) were purchased from SRL, India. All chemicals were used without any further purification and deionized millipore water was used as an antisolvent.

Cell culture

The human melanoma cell line, SK-MEL-28, was obtained from the National Centre for Cell Science, Pune. These cells were cultivated in T75 tissue culture flasks in DMEM supplemented with 10% fetal calf serum, 100 µg/mL penicillin, 100 µg/mL streptomycin, 2 mM L-glutamine, and 20 mM hydroxyethyl piperazine ethane sulfonic acid and incubated in a humidified incubator containing 5% CO_2 at 37 °C.

Obtaining curcumin polymorphs

Form 1 and Form 3 were obtained by LAS precipitation. An organic solution of curcumin in 10 mL of acetone/DMSO was introduced in 100 mL water maintained at a constant temperature of 1 °C. An ultrasound horn (Sonics, Vibracell) was immersed in antisolvent at an immersion depth of 1.5 inch. The tip (1″ in internal diameter) of the ultrasound horn was directed over the surface of a solvent–antisolvent mixture solution such that the solution could be dispersed

instantaneously by ultrasound (105 W) for 10 min. The aqueous suspensions thus obtained were filtered using a vacuum filtration unit and particles were washed thrice with deionized water to remove any traces of the organic solvent. These particles were then freeze-dried using a freeze dryer (Alpha 2-4LD Plus, Martin Christ). The curcumin powders thus obtained were then used for further analysis.

Form 2 was obtained by vacuum evaporation of curcumin solutions dissolved in chloroform and hexane (60:40%v/v) and recovered at 150 mbar.

Evaluation of efficacy of solid curcumin polymorphs for drug delivery

Radical scavenging activity of curcumin polymorphs

The antioxidant activity of the curcumin polymorphs was estimated using the standard DPPH free-radical scavenging method. A specified amount of curcumin (6 µg/mL) was added to 5 mL of 0.1 mM DPPH solution in methanol. The difference in absorption was determined at 515 nm using a UV spectrophotometer after 30 min. All measurements were taken in triplicate, and the results were averaged to obtain a mean value. The radical scavenging activity was expressed as the inhibition percentage of free radicals by the sample and calculated as follows:

$$\text{Radical scavenging activity } (\%) = \frac{(A0 - A1)}{A0} \times 100 \quad (1)$$

where A0 is the absorbance value of the control, A1 is the absorbance value of the sample.

In-vitro cytotoxicity of curcumin polymorphs on human melanoma cell line

Cell viability of SK-MEL-28 cells was assessed using MTT assay. SK-MEL-28 cells were seeded in 200 µL of DMEM medium in two 96-well plates separately, and cultured overnight. Next day, the medium was replaced with fresh DMEM or DMEM containing different concentrations of curcumin. After further incubation for 48 h, 50 µL of MTT (2 mg/mL) was added to each well, followed by another 4 h of incubation. The medium was then discarded and 150 µL of dimethyl sulfoxide was added to each well and the culture was incubated for 20 min. The OD was measured at 570 nm, using which cell viability in percentage was calculated. In this study, cells treated with ethanol were used as vehicle control (VC) and cells treated with paclitaxel dissolved in ethanol (6 µg/mL) were used as a positive control (PC). Untreated cells were used as normal control (NC).

Preparation and characterization of films containing curcumin polymorphs

The polymeric films containing curcumin particles were prepared using the solvent casting method. Different polymers such as HPMC, chitosan, sodium alginate, polyvinyl alcohol (PVA), gelatin, and polyvinyl pyrrolidone (PVP) were optimized for their ability for film formation. During optimization, these polymers were dissolved at concentrations ranging from 1 to 10% (W/V) in double-distilled water at room temperature. Further, different plasticizers such as polyethylene glycol (PEG) 200, PEG 400, and glycerol were added at different concentrations ranging from 0.5% to 3% (W/V) to obtain an easily peelable film of adequate strength. It was found that HPMC (K4000) films could form acceptable quality films at all concentrations. Different permeation enhancers were optimized for an enhanced permeation of curcumin. The raw curcumin and precipitated curcumin powders (5 wt%) were dispersed in double-distilled water and mixed for 2 h with an aqueous solution of HPMC (10 wt%) that was stirred overnight. The HPMC solution also contained plasticizers (3 wt%) and permeation enhancers (3 wt%). This solution was then casted into the petridish and subjected to drying at 40 °C for 2.5 h in Mathis LabCoater, Switzerland.

X-ray diffraction was performed to determine the physical state of the precipitated curcumin particles and the films containing the particles were collected using D8 Discover, Bruker AXS GmbH, Germany. Sample collection was performed in the 2θ range from 5° to 50° with CuKα radiation at the scanning step of 0.2° and increment of 0.02°. The morphology of curcumin particles incorporated inside the films was characterized using field emission scanning electron microscope (FE-SEM; JSM 7600F, JEOL Japan). A small portion of the films containing curcumin polymorphs was cut and glued to the cross-sectional sample holder on the carbon tape. The films were then sputter-coated with platinum before analysis. The images were then recorded under 5 kV and 8 mm working distance at different magnifications.

Evaluation of curcumin polymorph loaded films for transdermal drug delivery

Water uptake ability of the films

At the start of the study, each film was weighed and subsequently immersed in a petri plate containing phosphate buffer saline (PBS), pH 7.4. The studies on film swelling were performed by measuring the increase in the weight of films when placed in contact with the buffer solution. At predetermined time intervals, films were removed, wiped to remove the excess water using filter paper, and weighed. This procedure was repeated until a constant weight was

observed. The percentage of water uptake was calculated using the equation below

$$\text{Water uptake ability } \% = \frac{\text{Wt} - \text{W0}}{\text{W0}} \times 100 \quad (2)$$

where, W0 is the initial weight of the film and W*t* is the weight of film at time *t*.

In-vitro release of curcumin from films

Dissolution experiments were performed using the USP dissolution apparatus V paddle over disc (Hanson Research, USA). The drug release study from the curcumin-loaded films (2×2 cm^2 equivalent to 14 mg of the drug) was performed in 500 mL phosphate buffer of pH 7.4 and acetate buffer of pH 5.5 separately, at the paddle speed of 50 rpm. The media temperature was maintained at 37 ± 3 °C. The release study was carried out for 22 h and samples of 2 mL were taken at regular intervals (every 15 min for the first hour and then every hour) and replaced with the same amount of media maintained at 37 ± 3 °C. UV spectrophotometer was then used to measure the concentration of curcumin. The experiments were performed in triplicate and the average drug release and standard deviation were plotted as a function of time.

In- vitro permeation of curcumin from HPMC films

In-vitro permeation of curcumin from the polymeric films were estimated using Franz diffusion cells and commercial cellulose acetate membranes (MWcutoff: 12,000 Da; Sigma–Aldrich) as a barrier mimicking the skin. The prehydrated membranes were mounted between the donor and receptor compartments of the diffusion cells. The receptor compartment was filled with 7 mL of PBS, pH 7.4, containing 20% (v/v) of ethanol and allowed to equilibrate at 32 ± 2 °C. The receptor medium was continuously stirred at 300 RPM. Films were placed on the donor compartment such that 1 cm^2 area was available for diffusion. Release studies were conducted for 24 h. At predetermined time intervals, samples of 0.5 mL were withdrawn from the receptor compartment and immediately replaced with the same volume of fresh medium maintained at 32 ± 2 °C. The permeated amount of curcumin was estimated using UV spectrophotometer. The cumulative % of the curcumin released into the receptor for 24 h was plotted as a function of time. All experiments were carried out in triplicate.

Results and discussion

Physical form of the curcumin particles

To confirm the polymorphs obtained in this work, the XRD patterns of the polymorphs were matched with the calculated XRD patterns of curcumin polymorphs, as shown in Fig. S1a. As already mentioned, Form 1 and Form 3 were obtained by LAS precipitation of curcumin from acetone and DMSO, respectively, whereas Form 2 was obtained by rotary evaporation of curcumin solutions in chloroform and hexane when subjected to the pressure of 150 mbar. Rietveld refinement was also performed on the recorded XRD patterns, which indicated the presence of no other polymorphs in the obtained powders (data not shown). DSC thermograms were recorded for all three curcumin polymorphs, which are presented in Fig. S1b. DSC thermogram for Form 1 showed a single endotherm at 180 °C, which is in agreement with the other literature reports (Sanphui et al. 2011; Thorat and Dalvi 2015; Pandey and Dalvi 2019). DSC thermograms for curcumin Form 2 and Form 3 showed two endothermic peaks. The first endothermic peaks in these thermograms correspond to the transformation of orthorhombic forms (Form 2 and Form 3) to monoclinic form (Form 1). This peak is followed by the second peak, which corresponds to the melting of Form 1 (Thorat and Dalvi, 2015; Pandey and Dalvi 2019). Thus, XRD and DSC studies confirmed the existence of specific polymorphs in the powders, which were further used to make polymeric films.

Particle size of Curcumin Particles

Table 1 presents the particle sizes of the curcumin polymorphs obtained in this work. The average particle size of curcumin Form 1 was 7.4 ± 1.5 µm at 0 h, which increased to 9.4 ± 3.1 µm at the end of 24 h. The particle size of Form 2 recovered by vacuum evaporation was found to be 5.9 ± 2.3 µm. Curcumin particles precipitated in DMSO resulted in Form 3 with an average particle size of 0.3 ± 0.2 µm at 0 h. However, the size of Form 3 particles increased to 2.6 ± 0.9 µm at the end of 24 h. The use of

Table 1 Particle sizes of curcumin polymorphs produced in this work

Curcumin polymorphs	Particle sizes (µm)			
	0 min	D10/D50/D90 at 0 min	24 h	D10/D50/D90 at 24 h
Form 1	7.4 ± 1.5	3.0/7.3/12.2	9.4 ± 3.1	2.3/8.2/18.6
Form 2	5.9 ± 2.3	0.2/0.7/21.1	–	–
Form 3	0.3 ± 0.2	0.2/0.3/0.6	2.6 ± 0.9	0.6/1.2/ 4.1

ultrasound during the precipitation resulted in the stabilization of particles both at 0 and 24 h. Ultrasound reduced the particle size due to enhanced micromixing, uniform supersaturation, and reduced particle growth (Thorat and Dalvi 2012). Ultrasound suppresses Ostwald ripening and prevents agglomeration of particles in the solution (Thorat and Dalvi 2012) and thereby stabilizes the particles in aqueous suspensions.

Solubility of curcumin polymorphs

Figure S2 shows the solubility trend of curcumin polymorphs in a typical organic solvent such as ethanol. The solubility of curcumin Form 3 was found to be higher, which was followed by Form 2 and then Form 1. Similar observation for curcumin polymorphs have been reported in the literature (Liu et al. 2015; Pandey and Dalvi 2019; Sanphui et al. 2011). It can be seen that Form 3 is the least stable form at all temperatures because it possesses a higher solubility than Form 2 and Form 1 at all temperatures. Form 1 is the most stable form of the three due to its lowest solubility at all considered temperatures. Higher solubility of curcumin would result in a higher amount of curcumin availability at the wound site for necessary action. It has been reported that increased solubility of drug and its availability at the wound site leads to higher cytotoxicity, which could be beneficial for the treatment of tumorous tissues (Din et al. 2017). This further suggests that Form 3 polymorph of curcumin can accelerate the process of wound healing and also provide cytotoxicity against cancer cells as compared to other curcumin polymorphs.

Radical scavenging activity of curcumin polymorphs

Generation of reactive oxygen species (ROS) is a part of the innate immune system, which helps to clean the wound of the invading bacteria. However, besides their beneficial role in microbial killing, ROS can have various harmful and negative effects as well. At high levels, ROS can lead to severe tissue damage, neoplastic transformation, and healing impairment (Ak and Gülçin 2008). The proliferating and migrating cells in the wound tissue are exposed to large amounts of ROS during the respiratory burst and thus have to develop strategies to protect themselves against such harmful exposures. Therefore, free radical scavenging activity of curcumin polymorphs was measured using the DPPH free radical scavenging test, as mentioned in the experimental section. The antioxidant properties of curcumin polymorphs were estimated using the DPPH method. It can be observed from Fig. 1, that the curcumin Form 1 and Form 2 showed 61% and 67% scavenging activity, respectively. However, curcumin Form 3 showed the highest scavenging activity of 88%. The minimum activity was obtained for the commercially available curcumin, i.e., 29%. The radical scavenging ability of curcumin is mainly due to its phenolic groups, which could scavenge DPPH· radical by donating their H atoms (Ak and Gülçin 2008). It has been reported that higher antioxidant activity could be due to the improved water-solubility and enhanced electron-donating capacity (Deng et al. 2019). This is in agreement with the activity of curcumin Form 3, which has enhanced aqueous solubility compared to other curcumin polymorphs. Thus, it can be surmised that the solubilization kinetics of curcumin plays an important role in governing the radical scavenging behavior of curcumin polymorphs. Also, all forms of

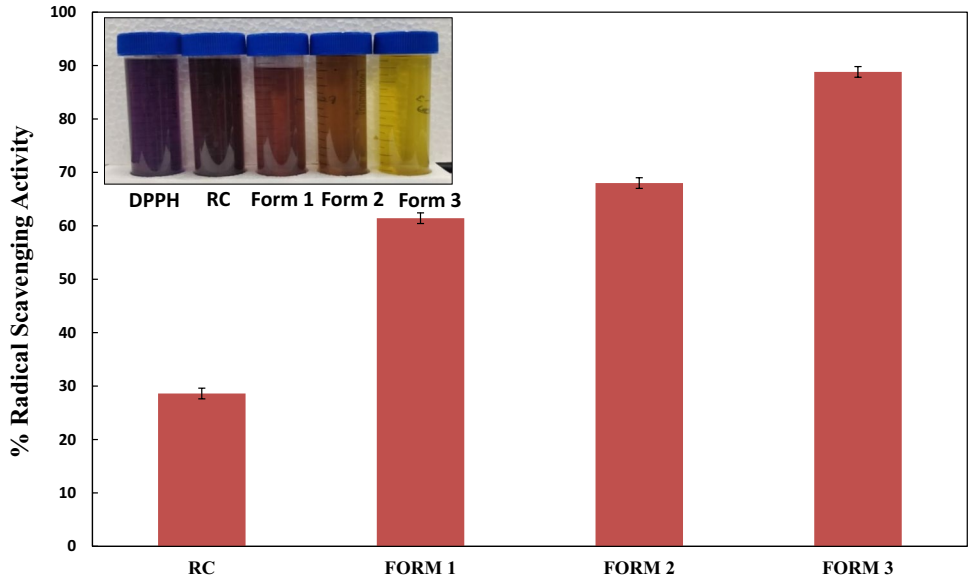

Fig. 1 DPPH scavenging activity of different curcumin polymorphs

curcumin seem to successfully reduce ROS concentration during the DPPH assay.

Cytotoxicity of curcumin polymorphs on human melanoma cell line

Curcumin has been shown to inhibit the growth of many cancer cell lines (Basnet and Skalko-Basnet 2011). However, there are no reports on the effect of individual curcumin polymorphs on cancer cells. In this study, for the first time, we have investigated the effect of curcumin polymorphs on human melanoma cell line, SK-MEL-28. Figure 2 shows the cell viability of the human melanoma cell line, SK-MEL-28 in percentage, when treated with different curcumin polymorphs. It can be observed that as compared to a positive control (PC), cell viability for normal control (NC) was significantly high. At concentrations lower than 12 µg/ml, curcumin polymorphs are less cytotoxic than PC. However, at the concentration of 12 µg/ml, all curcumin polymorphs as well as raw curcumin showed a higher cell cytotoxicity as compared to PC. Form 2 was found to be more cytotoxic (with only about 25% cell viability) as compared to all other curcumin forms (with about 55% cell viability for Form 1 and about 40% cell viability for Form 3). The order of cytotoxicity was Form 2 > Form 3 > Form 1 ($p < 0.05$ between the curcumin forms for the cytotoxicity against the melanoma cell lines). Thus it was clear that orthorhombic curcumin forms are more cytotoxic than the monoclinic curcumin form.

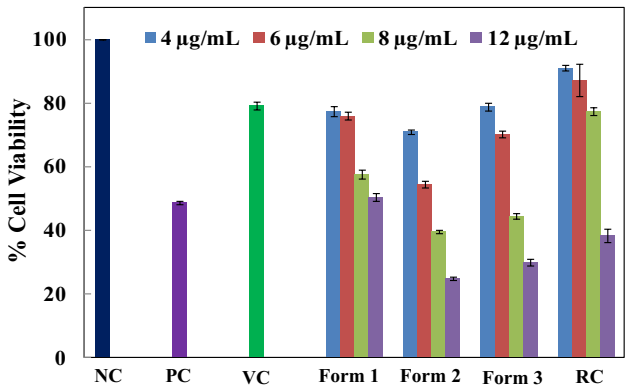

Fig. 2 Cytotoxicity of curcumin polymorphs on SK-MEL 28 cell line, where NC corresponds to untreated cells, PC corresponds to cells treated with paclitaxel, VC corresponds to cells treated with only ethanol, Form 1: cells treated with Form 1 curcumin, Form 2: cells treated with Form 2 curcumin, Form 3: cells treated with Form 3 curcumin, and RC corresponds to cells treated with commercially available curcumin, raw curcumin, (n = 3)

Optimization of HPMC formulation for film formation and loading of curcumin polymorphs on the films

The films were formed with different grades of HPMC, i.e., E5LV, E15LV and K4000 to evaluate the effect of different HPMC grades on curcumin release since the release of drugs from the HPMC matrix can be regulated by varying the degree of its substitution (Rahman et al. 2011). Commercially available curcumin was used during the film optimization process. Curcumin was loaded at 5 wt% on the films and its release was monitored in phosphate buffer saline of pH 7.4. Figure 3a shows the in-vitro release of curcumin from different HPMC matrixes at pH 7.4 and temperature of 37 °C. It can be observed that the highest curcumin release was obtained from the

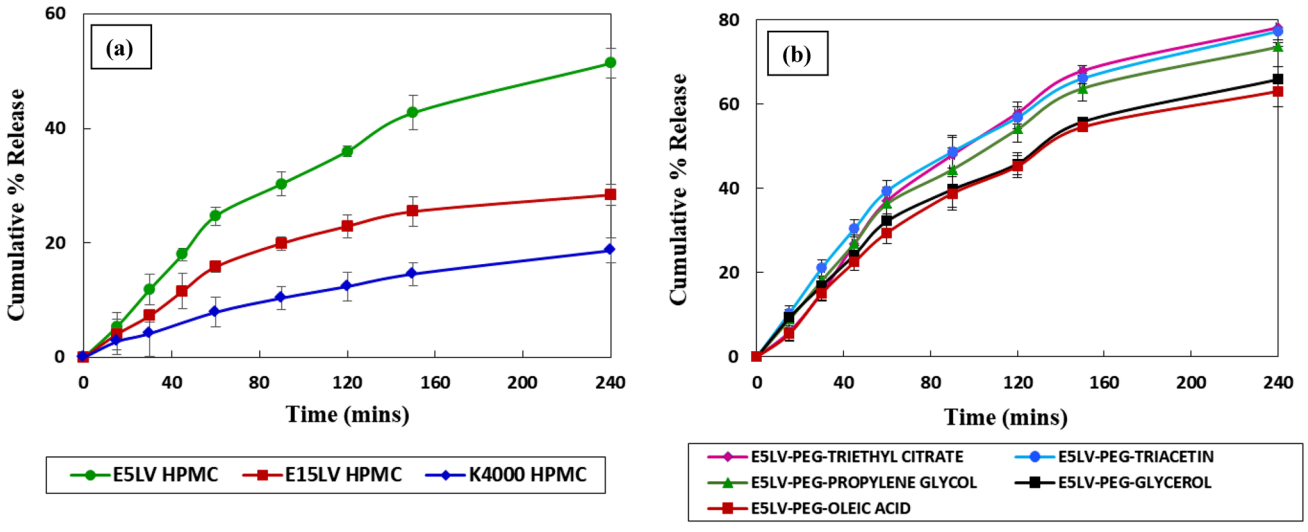

Fig. 3 In-vitro release of curcumin from films prepared from **a** different HPMC grades and **b** films made with HPMC E5LV containing different permeation enhancers at pH 7.4 and 37 °C

E5LV matrix, followed by the E15LV matrix. The least curcumin release was obtained from the K4000 matrix. Further, different plasticizers were also used since plasticizers significantly improve film properties, help in improving the flexibility of films, and reduce the brittleness during formulations. Apart from this, plasticizers also impart endurance, resistibility, and stability to the films. Glycerol and polyethylene glycol 400 (PEG 400) were used as plasticizers at different concentrations. Between, PEG400 and glycerol, PEG400 at the concentration of 3 wt% was found to be effective in obtaining good quality films (based on appearance and peelability). Therefore the E5 HPMC matrix with 3% PEG plasticizer and 5 wt% curcumin loading was chosen as the recipe for all film formulations.

The skin acts as the barrier for most of the hydrophilic as well as hydrophobic drugs and hence permeation enhancers are of utmost importance in transdermal drug delivery (Marwah et al. 2016). Therefore, different permeation enhancers were optimized to enhance the release of curcumin. Different permeation enhancers used in this study were triethyl citrate (TEC), triacetin (TRA), oleic acid (OA), propylene glycol (PG) and glycerol (G). The order of curcumin release from the films made with different permeation enhancers is TEC > TRA > PG > G > OA at pH 7.4 and temperature of 37 °C (Fig. 3b). The highest curcumin release and permeation were obtained for the films made with a TEC permeation enhancer. Hence the optimized recipe for film formulation was chosen to be 10 wt% E5 HPMC with 3 wt% PEG plasticizer and 3 wt%. TEC permeation enhancer loaded with 5 wt% of different curcumin polymorphs.

Characterization of films

Figure 4A presents the XRD diffractograms of curcumin polymorphs loaded films. The XRD patterns of all the films showed the characteristic peaks of the loaded polymorphs (i.e., Form 1, Form 2, and Form 3) confirming no change in the curcumin polymorphs at the end of film formulations. Figure 4B shows pictures of bare HPMC film and HPMC films loaded with curcumin polymorphs. It is interesting to note that the color of each film can be used to identify which polymorph is loaded on the film. Films loaded with Form 1 appear yellow whereas films loaded with Form 2 appear orange and Form 3 films appear red. The corresponding crosssectional SEM images showed that the curcumin particles were present within the layers of the HPMC films (Fig. 4e–h). Also, the particles seem to retain their morphology inside the films, which again indicates that there was no change in polymorphic form during formulation.

Water uptake by HPMC films

The HPMC films loaded with different curcumin polymorphs containing TEC were subjected to water uptake studies in PBS of 7.4 (Fig. 5). Overall, films loaded with Form 3 curcumin particles showed the highest water uptake capacity than the films with Form 1 and Form 2 particles. It has been shown that the drug solubility, chemical nature, and size play an important role in the film swelling behavior (Coughlan et al. 2004). The enhanced solubility of drug molecules was found to increase the swelling rate by facilitating a continuous water penetration through diffusion and dissolution (Coughlan et al. 2004). This suggests that films

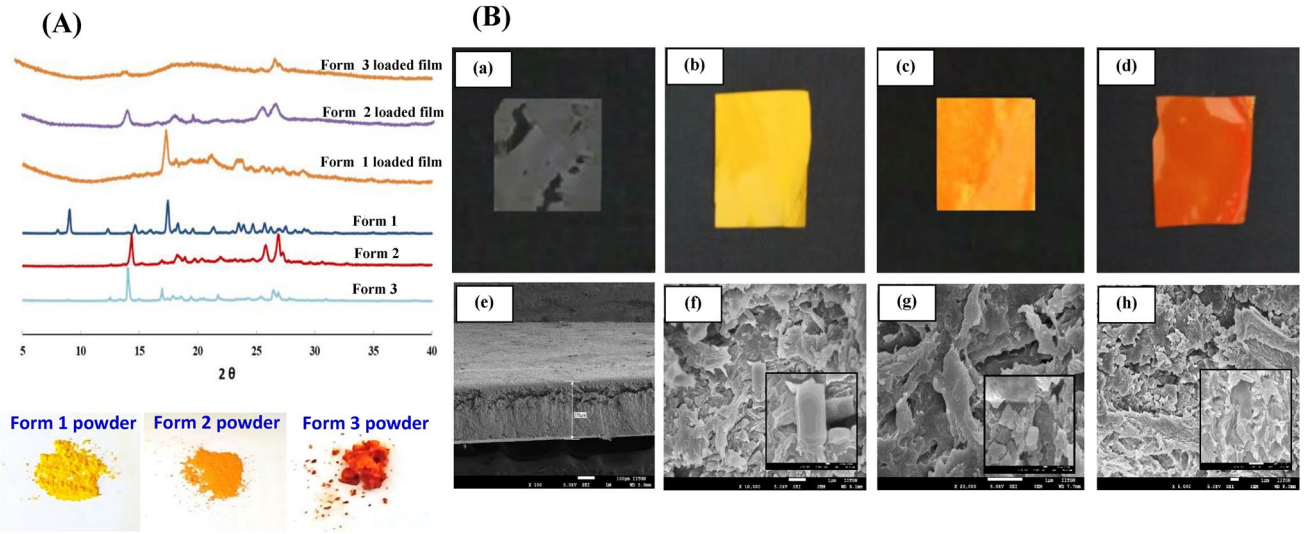

Fig. 4 **A** XRD patterns of the HPMC films containing different curcumin polymorphs and **B** Images of HPMC films loaded with curcumin **a** Blank HPMC, **b** Films loaded with Form 1, **c** Films loaded with Form 2, **d** Films loaded with Form 3 and crosssectional SEM images, **e** Blank HPMC films, **f** Films loaded with Form 1, **g** Films loaded with Form 2, **h** Films loaded with Form 1

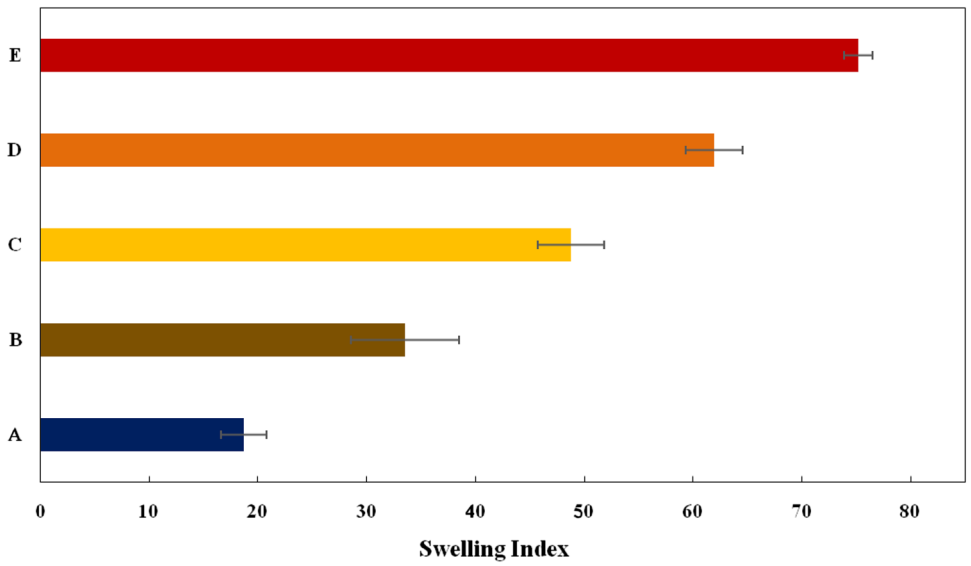

Fig. 5 Water uptake studies of films prepared with different curcumin polymorphs **A** Only HPMC film, and films containing, **B** Raw curcumin, **C** Form 1, **D** Form 2, **E** Form 3

loaded with Form 3 might be more effective in enhancing wound healing than films loaded with Form 1 and Form 2. Out of the four phases of wound healing process (hemostasis, inflammation, proliferation. and maturation), enhanced secretion of water and other fluids occur at the wound site during the inflammation phase. The drug delivery vehicle used for healing, if they are able to absorb the fluids secreted at the site, are found to heal the wound faster (Korting et al. 2011). Therefore, it is clear that films loaded with Form 3 would be able to absorb the extra fluids at the wound site and help accelerate the wound healing process.

In-vitro release of curcumin from HPMC films

The in-vitro curcumin release from HPMC films was estimated at different pH conditions. As the wound healing progresses, the pH of the wound environment changes from basic to neutral and then to acidic (Gethin 2007). The pH of a chronic wound mostly falls in the range of 7.15–8.9 (Gethin 2007). However, the ideal pH for the treatment of both acute and chronic wounds is 7.4 (Priyadarsini 2014).

In our study, we have considered both alkaline and acidic pH for estimating the release of curcumin, which could be useful in wound healing. For the alkaline pH, we have estimated curcumin release in the PBS of pH 7.4 and for the acidic pH, the release was estimated in acetate buffer of pH 5.5. Figure 6a shows the release profile of curcumin polymorphs from the HPMC films containing TEC, where films loaded with Form 3 show higher release followed by Form 2 and Form 1. Another observation from Fig. 6a is that films loaded with Form 3 exhibit higher release behavior, probably due to the lower size of Form 3 particles (Table 1).

Moreover, curcumin release from the films was found to be higher in the pH of 5.5 (continuous lines) than at the pH of 7.4 (dotted lines). This could be due to the enolic OH groups of curcumin, which is favorable for acidic pH conditions (Priyadarsini 2014). Thus, due to higher and sustained release, films loaded with Form 3 might accelerate the healing process at the wound site, followed by films loaded with Form 2 and Form 1.

In-vitro permeation of curcumin

The outermost layer of the skin, which is 10 mm thick, acts as a permeation barrier due to the presence of 79–90% of protein and 5–15% of lipids (Schneider et al. 2007). Therefore, for a transdermal delivery permeation enhancers are used, which help in permeation across the skin by disruption of the highly ordered structure of stratum corneum lipid and by interaction with an intercellular protein (Das and Ahmed 2008). Curcumin, belonging to BCS class IV, lacks the permeation ability. Hence, during the preparation of films different permeation enhancers such as OA, PG, TEC, TRA, and G were screened. The in-vitro curcumin permeation from HPMC films through cellulose membranes was performed with 20% v/v ethanol using Franz diffusion cell. Of all the permeation enhancers, TEC was found to be most effective for the permeation of curcumin through the cellulose membrane (Fig. 3). The enhanced permeation effect due to TEC could be attributed to the hydrogen bond interaction between TEC hydroxyl groups and keratin chain C=O groups, which enables solvation of keratin (Puri et al. 2019). Figure 6b shows the permeation profiles of curcumin through HPMC loaded with different curcumin polymorphs and made with a TEC permeation enhancer. Films loaded with curcumin Form 3 were found to have higher permeation as compared to films made with other curcumin polymorphs. Further, the drug permeation from the films loaded with

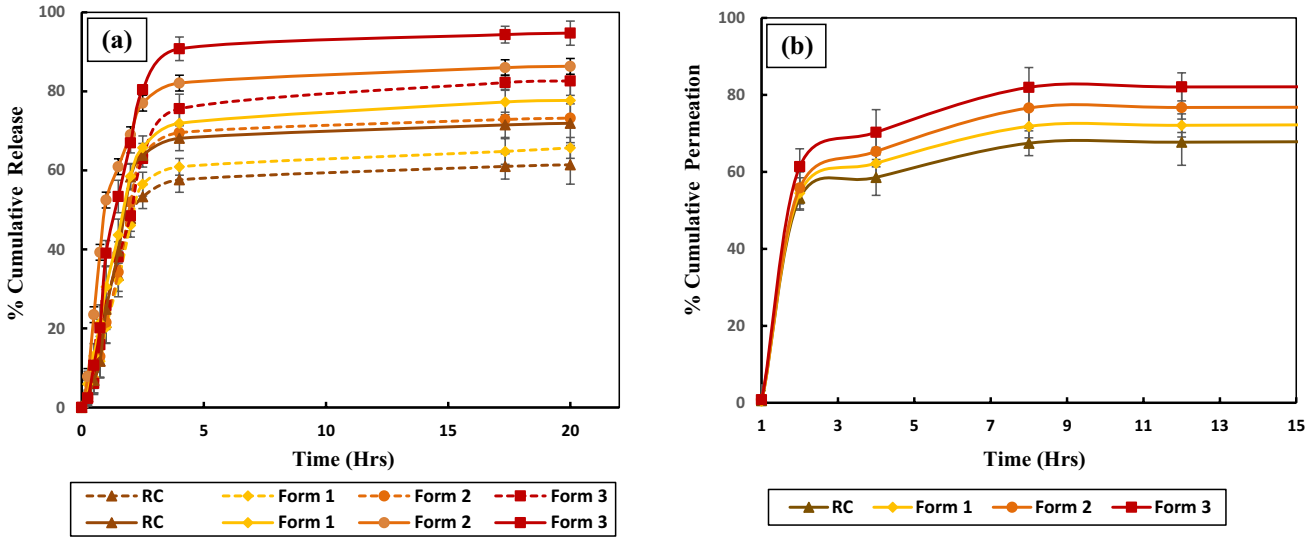

Fig. 6 **a** In-vitro release of curcumin through HPMC films at the temperature of 37 °C and in different buffer solutions of pH; 5.5 (continuous lines), 7.4 (dotted lines) and **b** In-vitro permeation of curcumin through HPMC films in 20% ethanol and at the temperature of 32 °C

Form 2 particles were found to be higher as compared to the films loaded with Form 1 particles. The synergistic effect of the enhanced solubility and the permeation enhancer seem to play a remarkable role in the overall permeation of curcumin through the films.

Conclusion

The main goal of this study was to investigate the efficacy of different curcumin polymorphs in transdermal drug delivery. The cytotoxicity studies showed that orthorhombic curcumin forms (Form 2 and Form 3 curcumin) caused lower cell viability for human melanoma cells (SK-MEL-28) with Form 2 being the most effective among all curcumin polymorphs. The free radical scavenging studies showed that the antioxidant activity of curcumin Form 3 was higher than that of Form 2 and Form 1. During the incorporation of curcumin polymorphs in different HPMC matrices, HPMC E5LV films were found to provide the highest release of curcumin as compared to other HPMC grades (E15LV and K4000). Among the different permeation enhancers used in film formulations, TEC was found to provide the highest release and permeation of curcumin through the films. The XRD and crosssectional SEM of films showed retention of specific polymorphic forms inside the formulations without any polymorphic transformation during the formulation. Water uptake capacity of films loaded with Form 3 was found to be higher than that of films loaded with Form 1 and Form 2. Also, films loaded with Form 3 were found to exhibit higher release and permeation efficiency than films loaded with Form 2 and Form 1. The in-vitro release profiles of curcumin at pH 5.5 were found to be higher than that of pH 7.4 for all film formulations with the highest release of curcumin from films loaded with Form 3. Overall, it could be concluded that orthorhombic curcumin polymorphs (i.e., Form 2 and Form 3) show a higher propensity for transdermal drug delivery as compared to the monoclinic curcumin polymorph (Form 1) and the commercially available curcumin.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human and animal rights This article does not contain any studies with human and animal subjects performed by any of the authors.

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