

Formulation and evaluation of curcumin loaded nanofilms for the treatment of wounds

Apurvaa Deb, Aboli Arun Mandurnekar, Varsha Rani, Tejpratap Chauhan, Brahmeshwar Mishra, Ruchi Chawla

Department of Pharmaceutics, Indian Institute of Technology, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Correspondence: Dr. Ruchi Chawla, Department of Pharmaceutics, Indian Institute of Technology, Banaras Hindu University, Varanasi - 221 005, Uttar Pradesh, India. Phone: +91-9336077707. E-mail: rchawla.phe@iitbhu.ac.in

ABSTRACT

The activity of curcumin loaded nanofilm was evaluated for wound healing potential in the present studies. Hydrophobicity and extensive first-pass metabolism make curcumin a suitable candidate for topical application. Nanofilms of curcumin were prepared by solvent evaporation method using chitosan, glutaraldehyde, and hydroxypropyl methylcellulose. The morphology of film was characterized by scanning electron microscopy. Compatibility studies were performed using Fourier transform infrared (FTIR). Swelling capacity and *in vitro* drug release studies were performed to evaluate and understand the drug release characteristics. *In vivo* wound healing studies on rats were also performed. Wound healing effect with prepared nanofilms was observed and found to be similar to that produced by marketed Neosporin formulation. The better healing efficiency of prepared films can be attributed to the presence of curcumin and chitosan.

Keywords: Chitosan, curcumin, nanofilms, swelling capacity, wound healing

Introduction

Medicinal plants are a precious source of bioactive compounds, despite established popularity of pharmaceuticals in the health care management. The therapeutic efficacy of the bioactive components is enhanced when incorporated in a suitable drug delivery system. Especially for the purpose of wound healing, drug delivery scaffolds/carriers which can create an intimate contact between the bruised tissues and the drug will facilitate the healing process. Nanofilms which are polymer based nanosheets/films with a surface area up to tens of cm² and thickness ranging up to few hundred nanometers^[1] can be used as inert and non-reactive scaffolds to act as an interface between the tissues and the eluting drug.^[2] The release of drugs from the biodegradable and biocompatible nanofilms can be modified based on composition, morphology, permeability, degradation, functionalization, and charge characteristics of film, keeping in view the physiological characteristics such as pH, temperature, and binding properties. The diverse pharmaceutical and biomedical applications of the nanofilms include regenerative therapy, wound healing and transcutaneous delivery of drugs, and adjuvants and the

like.^[3-5] Nanostructuring adds special properties to the delivery system like enhanced drug permeability, cell adhesion, proliferation protein synthesis,^[6] and self-cleaning effect.^[7] Furthermore, the nanofilms show very good adhesion to moist wounds^[8] facilitated by the large specific surface area of up to 100 m²g⁻¹ which complements the adsorption of liquids, causing local release of drugs on the skin, making these materials suitable for application in hemostatic wound closure.^[9]

An injury to the skin with torn, cut or punctured surface is generally referred to as open wound. However, a closed wound is a contusion caused due to blunt force trauma. It is often observed that recovery of wounds is slow, associated with excruciating pain and develop secondary infections. Incorporation of herbal bioactive components into nanofilms for the dressing of wounds immensely enhances their healing efficiency as the porous nanostructure assures the exchange of liquids and gases with the environment and the dimensions of the films prevent bacteria from entering the wounds. Turmeric (*Curcuma longa*), a perennial herb belonging to the Zingiberaceae family, is cultivated extensively in the south and southeast tropical Asia. Natural yellow

Access this article online

Website: sjpr.sperpublications.com

E-ISSN: ***

How to cite this article: Deb A, Mandurnekar AA, Rani V, Chauhan T, Mishra B, Chawla R. Formulation and evaluation of curcumin loaded nanofilms for the treatment of wounds. SPER J Pharm Biol Res 2018;1(1):5-9.

Source of Support: Nil. **Conflict of Interest:** None declared.

This is an open access article distributed under the terms of the Creative Commons Attribution NonCommercial ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non commercially, as long as the author is credited and the new creations are licensed under the identical terms

curcumin, a component of turmeric, has a wide range of beneficial properties including anti-inflammatory and antioxidant properties.^[10] It also plays an important role in wound repair by ensuring the induction of transforming growth factor beta 1 within the wound thus the healing process gets significantly enhanced.^[11]

Chitosan (CS), a natural polymer and a derivative of chitin, besides being nontoxic, provides excellent biodegradable and biocompatible properties for induction of the healing process.^[12] Patient compliance is always an essential requirement for the success of a therapy and repetitive daily applications of conventional topical dosage forms such as ointment, creams or gels, and leads to poor compliance. Therefore, polymeric films can be a potential alternative for drug delivery via topical route to the skin. Their application convenience and cosmetic attributes, superior to conventional semisolids, may offer improved patient compliance in addition to the reduction of dosing frequency.^[13]

Materials and Methods

Materials

Curcumin powder (pure) and chitosan were obtained from Sigma-Aldrich Chemicals Pvt. Ltd. Glycerol, glutaraldehyde, citric acid, hydroxypropyl methylcellulose (HPMC), and acetic acid were procured from S.D. Fine Chemical, Mumbai, India. All other chemicals/solvents used were of analytical reagent grade.

Preparation of nanofilm

The nanofilms were prepared by the method described elsewhere with modifications.^[14] The aqueous solution of glutaraldehyde (0.25% w/v) was added to a solution of Chitosan (3.5% w/v) in citric acid (5% w/v) with constant stirring. Aqueous HPMC solution (1.0% w/v) containing curcumin (1% w/v) was blended with the cross-linked chitosan solution, followed by addition of 1 mL of glycerol. After probe sonication for 10 min twice, the solution was left for overnight stirring to form a homogenous blend. Films were formed by solvent evaporation, at room temperature under a fume hood by pouring 10 mL of the blend into a rectangular mold (710 × 260 mm). After drying, nanofilms were removed and stored at 30°C in a glass container till used further.

Physicochemical characterization of nanofilm

Drug-excipient compatibility study

Drug-excipient incompatibility can alter the physicochemical, pharmacokinetic, and pharmacodynamics characteristics of a drug which in turn affects its safety and efficacy. Therefore, the study of drug incompatibility is an important process in the development of stable dosage forms. Physical mixtures of curcumin, chitosan, HPMC, glutaraldehyde, and curcumin loaded nanofilm were analyzed by FTIR spectroscopy (FTIR, Shimadzu, Model 8400S, Tokyo, Japan) by conventional KBr disc/pellet method. The spectra were measured over the range of 4000-400 cm⁻¹.

Morphological evaluation

Surface topographical properties of the dry nanofilms were observed using scanning electron microscopy (FEI, Quantum 200E). Samples

were mounted onto metal stubs and coated with gold using SPI-Module Sputter Coater (SPI Supplies, Division of Structure Probe Inc., West Chester, PA, USA).

Percentage swelling capacity of nanofilms

The swelling capacity gives an idea about the rate of absorption of water by polymeric films when in contact with the skin surface. Film samples (n=6) were cut into size (2 × 2 mm) and initial weights were recorded. The films were immersed in 15 mL of phosphate buffer solution; pH 6.8 and allowed to equilibrate at room temperature for 24 h. Samples were removed periodically and weighed to determine the rate of water absorption using the formula:

$$\% \text{ swelling capacity} = ([\text{final weight} - \text{initial weight}] / \text{initial weight}) \times 100$$

Determination of water vapor transmission rate (WVTR) from the nanofilms

During the wound healing process, the rate of water vapor transmission plays a key role. A low transmission rate leads to an excessive build-up of exudates. However, a very high value can lead to wound dehydration that decelerates the healing process. Film samples were tied firmly on the mouth of the glass tubes containing 10 mL of phosphate buffer (pH 6.8). The setup was pre-weighed and placed in an oven at 35°C for 24 h. The water loss due to transmission was calculated by reweighing the samples at regular intervals and weight loss due to vapor transmission was deduced using the formula:

$$\text{WVTR} = ((W_i - W_t) / A) \times 106 \text{ g/m}^2 \text{ day}^{-1}$$

Where A = area of the beaker opening (mm²), W_i and W_t = the weight of the beaker before and after being placed in the oven, respectively.

In vitro drug release studies

The pattern of release of curcumin from the nanofilms was studied using modified Franz diffusion cell. Circular sections of nanofilm were placed on the receptor compartment filled with 12 mL of phosphate buffer pH 6.8 at 37°C with constant stirring on a magnetic stirrer. At specified time periods of 0.5, 1, 3, 6, 9, 12, 15, 18, 21, and 24 h, samples (0.1 mL) were withdrawn from the donor compartment, with the replacement of equivalent volume of fresh media. The samples in triplicate were analyzed spectrophotometrically at 427 nm for drug content, after filtration through 0.2 µm syringe filters. The experimental data were fit into kinetic models: Zero-order, first-order, Higuchi, and Korsmeyer-Peppas and based on correlation coefficient (R²) values, drug release pattern was predicted.

Animal experiments

Experimental animals

The study protocols for animal experimentation were approved by Animal Ethical Committee, Institute of Medical Science, Banaras Hindu University (Varanasi, India). The study required albino rats (female) of average weight of 200 ± 20 g, which were obtained from Central Animal House, BHU, Varanasi, and were housed in the animal

house facility conditioned at $25 \pm 2^\circ\text{C}$ and 50-60% RH with alternate 12-h dark and light cycle and free access to food pellets and distilled water *ad libitum*.

Induction and evaluation of wound in rats

The dorsal surface animals were depilated and cutaneous wound of 1.0 cm^2 was inflicted on the pre-shaved area of each animal under ether anesthesia.^[15] The animals were divided into three groups of six animals each. Group I served as a control (untreated); Group II was the treated group, applied with nanofilm and; Group III was the standard group, treated with topical antibiotic formulation Neosporin USP 2% (ointment). The wounds of animals were treated once daily with the drugs after cleaning with surgical cotton wool. The healing of wounds was observed for 18 days, by measuring the length and breadth of the wounds using a vernier caliper. The healing was measured in terms of percentage wound contraction calculated as:

(Initial area of wound - area of wound on the nth day/initial area of wound) $\times 100$

Results and Discussion

Drug-excipient compatibility study

FTIR spectra of pure curcumin, a physical mixture of curcumin, chitosan, HPMC, glutaraldehyde, and curcumin loaded nanofilm have been shown in Figure 1a-c. The characteristics peaks of curcumin at 3508 cm^{-1} corresponding to $-\text{OH}$ stretch, 2920.32 cm^{-1} (aromatic C-H stretching), 2848.96 cm^{-1} (aliphatic C-H stretching), and $1429.30\text{--}1377.22\text{ cm}^{-1}$ corresponding to C-O stretching were present in the physical mixture and the nanofilm, indicating no incompatibility between the drug and the excipients.

Morphological evaluation

The scanning electron micrographs of the nanofilms have been shown in Figure 2a and b. The surface morphology of dummy nanofilm (without curcumin) was relatively smooth, however, due to the addition of bioactive (curcumin), rough surface texture with the existence of pores and cracks was observed.

Swelling capacity of nanofilm

The percentage swelling capacity of the prepared nanofilms was found to be 172% as shown in Table 1 within the first hour after immersion. The swelling capacity is generally influenced by crosslinker concentration; lower is the concentration lesser is the covalent bond formation between the polymeric chains. Lower crosslinking provides porosity to the matrix and enhanced hydrodynamic free volume to accommodate water molecules, also, facilitating faster initial rates of liquid uptake. Thus, it may be proposed that rate of swelling regulates the release of the rate of curcumin from the nanofilm.

WVTR from nanofilm

The values for water vapor transmission from the nanofilm have been shown in Table 2. In the case of wounds, the WVTR gives a fair idea about the moisture balance that needs to be maintained in the wounds throughout the repair process. A very high transmission rate can lead to the dehydration of wounds, and a low rate would lead to a build-up of exudates. In both the cases, the healing process is decelerated. Hence, an optimal concentration of the polymer and crosslinker is required to regulate the WVTR.

In vitro drug release study

In vitro release studies were conducted using modified Franz Diffusion cell in freshly prepared phosphate buffer (pH 6.8) at $37 \pm 1^\circ\text{C}$. Figure

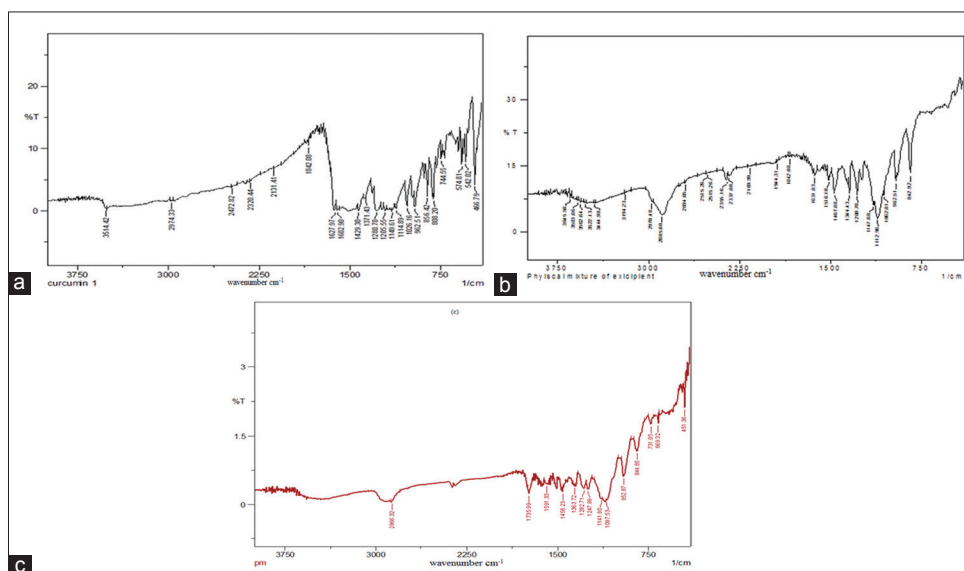


Figure 1: Fourier transform infrared spectrum of (a) curcumin, (b) physical mixture of curcumin, chitosan, HPMC, glutaraldehyde, and (c) curcumin loaded nanofilm

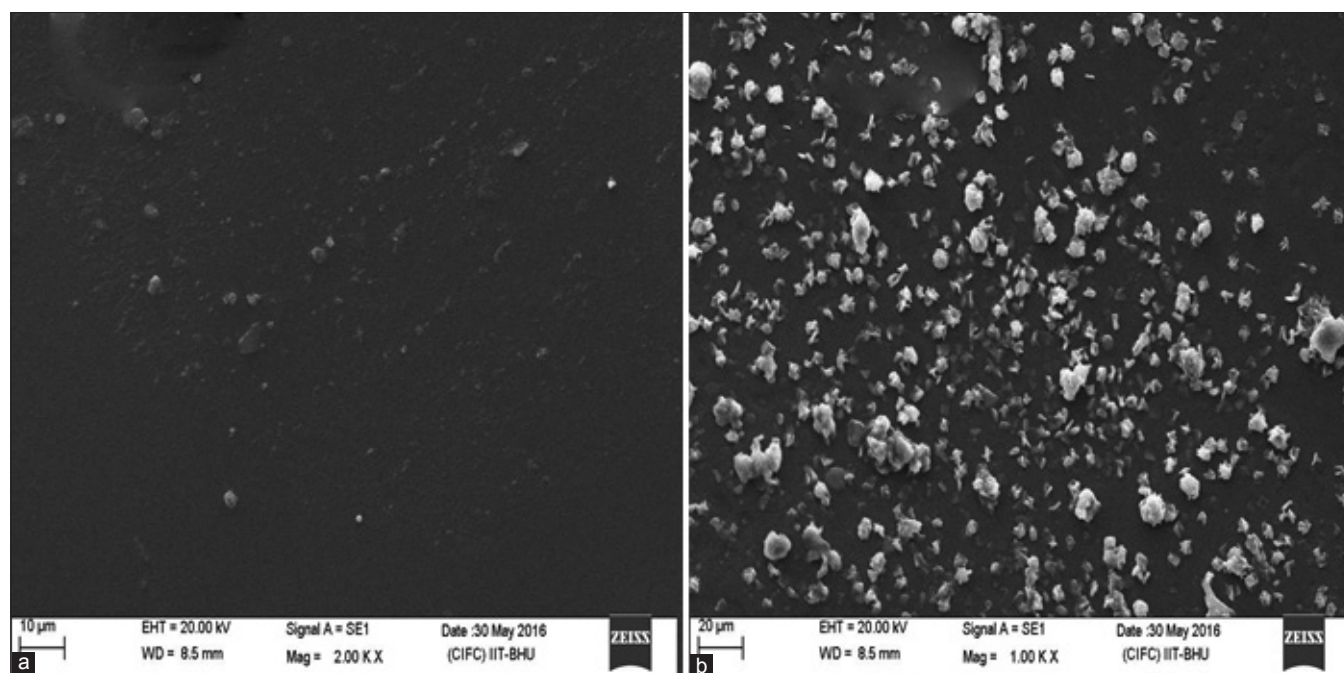


Figure 2: Scanning electron micrographs of (a) placebo film, and (b) curcumin loaded nanofilm

Table 1: Percentage swelling capacity of nanofilm in PBS pH 6.8 at room temperature ($n=6$)

Time points (in h)	1	3	5	10	15	20	24
% Swelling capacity (mean \pm SD)	172.0 \pm 5.61	191.1 \pm 6.69	206.7 \pm 4.90	163.0 \pm 4.25	151.8 \pm 5.52	134.8 \pm 8.54	112.0 \pm 4.01

PBS: Phosphate buffer solution

Table 2: Percentage WVTR from the nanofilm at 35°C for 24 h ($n=6$)

Time points (in h)	0	5	10	15	20	25
WVTR g/m ² day ⁻¹ (mean \pm SD)	0 \pm 0.0	63.33 \pm 3.51	61.20 \pm 2.98	60.56 \pm 4.01	60.67 \pm 2.25	54.16 \pm 3.08

WVTR: Water vapor transmission rate

Table 3: Kinetic modeling of drug release data from the nanofilm

Drug	Zero-order kinetics (R^2)	First-order kinetics (R^2)	Higuchi kinetics (R^2)	Korsmeyer–Peppas model		Release mechanism
				(R^2)	n	
Curcumin	0.893	0.9517	0.996	0.994	0.503	Non-Fickian

Table 4: Percentage wound contraction during the 18 days treatment period

Groups/day	% Wound healing effect (mean \pm SEM) wound					
	3	6	9	12	15	18
I (control)	0.449 \pm 0.203	5.091 \pm 0.504	11.689 \pm 0.975	23.111 \pm 0.778	33.564 \pm 0.621	52.769 \pm 0.57
II (treated)	13.956 \pm 0.241	35.478 \pm 0.503	44.653 \pm 0.032	57.229 \pm 0.306	67.453 \pm 0.587	88.829 \pm 0.332
III (standard)	7.742 \pm 0.298	27.886 \pm 0.250	43.173 \pm 0.522	57.056 \pm 0.410	70.176 \pm 0.194	83.549 \pm 0.672

Group I: Untreated control, Group II: Treated with curcumin nanofilm, Group III: Treated with marketed neosporin ointment

3 summarizes the release profile of curcumin from the nanofilm. All the data shown are a mean of triplicate results. A maximum of 60.39% of curcumin was released from the nanofilm over a 24 h period.

Kinetic modeling

Kinetic modeling was performed to assess the pattern of release of curcumin. The drug release data were fit into kinetic models: Zero-order,

first-order, Higuchi, and Korsmeyer–Peppas. The regression coefficient (R^2) was used as an indicator of the best fitting model. The value of regression coefficient for zero-order, first-order, Higuchi kinetics, and Korsmeyer–Peppas model was found to be 0.893, 0.961, 0.996, and 0.994, respectively (Table 3). Based on the value of regression coefficient, Higuchi model was the best fit model indicating drug release from the nanofilm was proportional to the square root of time, indicating diffusion

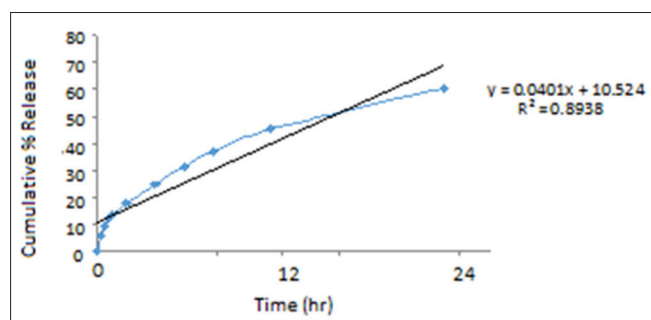


Figure 3: *In vitro* release studies of curcumin from nanofilm

controlled release mechanism. However, a similar regression coefficient for Korsmeyer–Peppas model was also observed. The value of “n” as determined by applying Korsmeyer–Peppas model was found to be 0.504, indicating non-Fickian diffusion controlled the release of the drug ($0.45 < n < 0.89$ corresponds to non-Fickian transport).

Evaluation of wound healing

The wound was induced on the dorsal surface of the rats and monitored for contraction during the treatment period of 18 days. Significantly lesser wound contraction and healing were observed in Group I (untreated control). However, in Group II (nanofilm treated) healing was fastest among the experimental groups, but comparable to that of Group III in which Neosporin ointment was applied. The results of the study have been shown in Table 4. The results of the study suggest that curcumin incorporated in the nanofilms can be a suitable alternative for regenerative therapy of wounds.

Conclusion

Nanofilms of chitosan cross-linked with glutaraldehyde, incorporated with curcumin prepared for use as a potential wound healing delivery system, exhibited considerable swelling index and released curcumin in a sustained manner over a period of 24 h. The therapeutic potential as evaluated by wound healing studies indicated the healing effect of the nanofilm better than the marketed ointment. Thus, formulation of therapeutic films containing a bioactive like curcumin will

allow comfortable healing of wound along with the biocompatible, bioadherent, nontoxic and biodegradable properties for effective recovery.

References

1. Fujie T, Okamura Y, Takeoka S. Ubiquitous transference of a free standing polysaccharide nanosheet with the development of a nanoadhesive plaster. *Adv Mater* 2007;19:3549-53.
2. Zelikin AN. Drug releasing polymer thin films: New era of surface-mediated drug delivery. *ACS Nano* 2010;4:2494-509.
3. Hammond PT. Form and function in multilayer assembly: New applications at the nanoscale. *Adv Mater* 2010;16:1271-93.
4. Baron R, Willner I, Willner I. Biomolecule-nanoparticle hybrids as functional units for nano-biotechnology. *Chem Commun* 2007;3:323-32.
5. Sahoo S, Parveen S, Panda JJ. The present and future of nanotechnology in human health care. *Nanomedicine* 2007;3:20-31.
6. Thangaraj M, Mills D. Design of smart nano-films for regenerative medicine. *FASEB J* 2010;24 1 Suppl:181-4.
7. Barthlott W, Neinhuis C. Purity of the sacred lotus, or escape from contamination in biological surfaces. *Planta* 1997;202:1-8.
8. Venugopal J, Ramakrishna S. Applications of polymer nanofibers in biomedicine and biotechnology. *Appl Biochem Biotechnol* 2005;125:147-58.
9. Huang ZM, Zhang YZ, Kotaki M. A review on polymer nanofibers by electrospinning and their applications in nanocomposites. *Compos Sci Technol* 2003;63:2223-53.
10. Chattopadhyay I, Biswas K, Bandyopadhyay U. Turmeric and curcumin: Biological actions and medicinal applications. *Curr Sci* 2004;87:44-53.
11. Mani H, Sidhu GS, Kumari R, Gaddipati JP, Seth P, Maheshwari RK. Curcumin differentially regulates TGF- β 1, its receptors and nitric oxide synthase during impaired wound healing. *Biofactors* 2002;16:29-43.
12. Dash M, Chiellini F, Ottenbrite RM, Chiellini E. Chitosan-a versatile semi-synthetic polymer in biomedical applications. *Prog Polym Sci* 2011;36:981-1014.
13. Frederiksen K, Guy RH, Petersson K. Formulation considerations in the design of topical, polymeric film-forming systems for sustained drug delivery to the skin. *Eur J Pharm Biopharm* 2015;91:9-15.
14. Mayet N, Kumar P, Choonara YE, Tomar LK, Tyagi C, du Toit LC, et al. Synthesis of a semi-interpenetrating polymer network as a bioactive curcumin film. *AAPS PharmSciTech* 2014;15:1476-89.
15. Sainuddin T, Haneefa KP. Formulation and pharmacological evaluation of herbal gel of *Pothos scandens* Linn. *Web Med* 2010;1:WMC001344.