RESEARCH PAPER

Fabrication of curcumin-loaded soluble soy bean polysaccharide/ TiO_2 bio-nanocomposite for improved antimicrobial activity

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ABSTRACT
Objective(s): Bioactive compounds like curcumin can be incorporated into food packaging formulation either to enhance physico-mechanical properties or to improve the biological activity of the packaging systems. Furthermore, it enables the packaging to monitor the changes in food quality.
Materials and Methods: In the present study, the effect of curcumin concentration (0.2, 0.4 and 0.6%) on physico-mechanical and biological activity of soluble soy bean polysaccharide (SSPS)/TiO2nanocomposite films was investigated. Additionally, the release behavior of this bioactive compound from the developed film was tested. Finally, the anti-bacterial activity and pH Sensitivity of SSPS/TiO2 /curcumin nanocomposites was examined.
Results: When the curcumin concentration increased up to a certain point (0.4 %), the physical and mechanical properties of the film improved, but beyond this point, an opposite effect was observed. SSPS/TiO2 nanocomposite showed strong antibacterial activity against both gram positive and negative bacteria. Curcumin was released at low rate in ethanol as a food simulant.
Conclusion: The films incorporated by curcumin can be used as promising packaging systems for non-destructively detecting quality and freshness of foods.

Keywords: *Curcumin, Nanocomposite, SSPS, TiO2 nanoparticles*

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INTRODUCTION

The primary functions of packaging are to preserve food quality over the storage period and facilitate distribution and marketing [1]. Recent efforts have been focused on introducing new active packaging systems to play an active role in food preservation [2, 3, 4]. The incorporation of beneficial bioactive ingredients into packaging systems is one of the most effective techniques in the preservation of the food quality and further improvement of food safety [5, 6]. Active packaging systems can extend the shelf-life and preserve the quality of food products by the controlled release of antioxidants and antimicrobial agents. Still, they cannot show the spoilage, safety, and quality of foods. The ever-increasing progress in the food industry has been accompanied by the development of intelligent packaging systems, which provide useful information about the quality and safety of foods during storage [7].

Biopolymers such as carbohydrates, proteins, lipids, and their complexes have extensively been employed for the formation of eco-friendly biodegradable films [8]. Among them, polysaccharides are known as excellent matrices for film-forming due to their renewability, easy

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availability, biodegradability, biocompatibility, and having excellent film-forming properties with high mechanical performance [9]. Soybean polysaccharide (SSPS) with a pectin-like structure, is one of the polymeric polysaccharides introduced for making transparent, water-soluble, and edible films [10]. Soybean polysaccharide (SSPS) is an acidic polysaccharide exhibit limitation including high water and moisture absorption and mechanical weakness. However, in attempt to overcome these problems, they have been used in combination with other compounds such as nanoparticles (NP) and other material to modify and improve these physicochemical characteristics [11].

Among NP, titanium dioxide (TiO2) is a biocompatible, non-toxic, and inert substance that is potentially active against a wide range of pathogens [10]. It has been reported that the incorporation of NP, like titanium dioxide (TiO2) into polymer matrix such as a packaging material, it will modify the physical properties of the films, such as tensile strength and heat resistance, gas and vapor permeability. Salarbashi et al. (2018) reported that addition of TiO2 NP to SSPS led to enhance the heat-sealing strength and antimicrobial activity of developed film [10].

Besides nanoparticles, other active additives have been tested in the mentioned eco-friendly food packaging systems [1]. The addition of curcumin has shown to lead to the development of the packaging materials with improved food preservation. Curcumin, as a natural bioactive agent derived from Curcuma longa, has been known as a treatment in a wide range of diseases [12]. The structure of curcumin consists of bis-α, β-unsaturated β- diketone [13]. It can be observed that curcumin has a symmetric structure with two phenolic groups in the form of aromatic rings containing o-methoxy phenolic groups with a seven carbon linkers and α, β-unsaturated β-diketone moiety. In this context, the biological activity curcumin can be correlated with the presence of phenolic OH groups and double bonds, as well as diketone. When the pH increases above 7, the phenolic hydroxyl groups of curcumin can react with OH and consequently form phenoxide anion, which results in further changes in the color of curcumin. At the same time, this property is used for the fabrication of intelligent packaging systems to monitor the quality of seafood like fish and shrimp [14].

In a previous study conducted by researchers in this group, it was observed that TiO2 NP concentration of 15% incorporation in the SSPS film preparation could improve various physical, mechanical and biological properties suitable for food packaging purposes. The objectives of the present work were to fabricate SSPS/TiO2-15%-based nanocomposites incorporated by curcumin and subsequently examine its physicochemical and biological properties. The anti-bacterial activity of the films was also investigated. Finally, the pH sensitivity of the developed intelligent packaging system was tested.

MATERIALS AND METHODS

Materials

Soluble soybean polysaccharide (SSPS) was purchased from Fuji Oil Company (Osaka, Japan). TiO2 nanoparticles were purchased from Rockwood Additives Ltd. Microbial cultures were obtained from Himedia. For antimicrobial activity, culture media for Gram-negative bacteria (P. aeruginosa ATCC 27853) and Gram-positive bacteria (Staphylococcus aureus ATCC 25923) were purchased from the institute of standards and industrial research of Iran. All other reagents were analytical grade and obtained from Sigma-Aldrich.

Fabrication of smart nanocomposite based on SSPS/TiO2/curcumin

The SSPS/TiO2 nanocomposites were fabricated as reported by Salarbashi, Tafaghodi, and Bazzaz (2018) [10]. The SSPS/TiO2 composite films were fabricated based on casting method. First, SSPS (1/5% w/v) and TiO2 nanoparticles (15% w/w to SSPS) (based on the results of a previous study by researchers in this group) were dispersed in deionized water separately on a magnetic stirrer at 25 °C. Sorbitol was added into SSPS solution as a plasticizer at 30% w/w ratio and mixed for further 30 min. The prepared nanoparticles were suspended in deionized water using a high Intensity Ultrasonic Processor (Model VCX 750, Sonics & MaterialsInc., Newtown, CT, USA). Then, SSPS/TiO2 mixtures were subjected to homogenized at 13500 rpm for 15 min.

For those of SSPS/TiO2 nanocomposite containing the curcumin, ethanolic solution of curcumin (5 mg. mL-1) was added to the SSPS/TiO2 suspension at 0.2, 0.4 and 0.6% weight ratios under the above-mentioned homogenizing condition. The prepared suspensions were poured onto a plate and dried in an air dryer at 25 °C. The formed films were finally peeled carefully from the casting surface after 48 h and stored at zip-kips until further experiments.

Physical characteristics

Films thickness

The films thickness was recorded using a hand-held digital micrometer (Elcometer A300 FNP 23, Elcometer Instrument Ltd., Manchester, England). For each test, 10 random points were selected and the average values were recorded.

Water solubility (WS) and water vapor permeability (WVP)

To determine the WS in the developed composites, pieces of films (2.0 × 2.0 cm2) were dried at 105 ºC using an oven-drier until they reached a constant weight, followed by weighting (W0). Then, the samples were dipped in 50 mL distilled water for 6 h and the remaining pieces were taken out and dried (at 105 °C) until they reached to a constant weight (W1). WS was calculated using following equation:

$$\mathrm{WS}\ (\%) = \frac{W_0 - W_1}{W_0} \quad (1)$$

WVP was measured as reported by ASTM E96/E96M (ASTM) standard test [15]. First, the films were cut and mounted horizontally on cups containing calcium anhydride, followed by placing in a desiccator containing saturated sodium chloride solution (NaCl –75% RH). In the next step, the weight of samples were periodically determined for 4 days. WVP of the samples was calculated using Eq. 2:

$$\mathrm{WVP} = \frac{\Delta m}{A \Delta t} \frac{X}{\Delta p} \quad (2)$$

here, Δm/Δt, X and A are the weight of moisture gain per unit of time (g/s), the film thickness (mm) and the films surface area (m2), respectively.

Scanning electronic microcopy (SEM) analysis

The morphological properties of the films' specimens were investigated by scanning electron microscopy (SEM) (VEGA II, TESCAN, Czech Republic) using an accelerating voltage of 20.0 kV. Before the test, samples were cryo-fractured by immersion in liquid nitrogen and then the nanocomposite surface was coated by gold layer with a sputter coater (K-450X, EMITECH, England).

Mechanical properties

The mechanical performance of the developed films was examined by an M350-10CT Machine (Testometric Co., Ltd., England) at a crosshead speed of 10 mm.min−1 and an initial grip distance of 6 cm at 25 °C. To prepare the samples, the film specimens were cut into 1×8 cm2 rectangular strips after conditioning for 48 h at 25 °C and 50% RH in desiccators containing saturated solutions of Mg(NO3)2. The test was conducted three times and the average values were reported. Tensile strength (TS) and elongation at break (EB) were calculated as follow:

$$\mathrm{TS} = \frac{F_{max}}{A_{min}} \quad (3)$$

$$\mathrm{EB}\ (\%) = \frac{L_{max}}{L_0} \times 100 \quad (4)$$

where, Fmax, Amin, Lmax, and L0 present the maximum load, the cross-section area, the extension at rupture point and the original length of the specimen, respectively.

Antibacterial activity

The antibacterial activity of SSPS/TiO2/curcumin nanocomposites were examined using disk diffusion method. In this process, samples were cut into 5mm discs and placed on the microbial plates, where an appropriate volume of MHA medium containing the bacteria (P. aeruginosa, and Staphylococcus aureus) at approximately 108 CFU/ml was already poured and left to solidify. The plates were incubated at 30 °C for 24 h and subsequently, the inhibition zone was measured as the indicator of the antibacterial activity [16].

Evaluation of the curcumin migration

The migration of curcumin from the developed composites into ethanol was tested by dipping nanocomposite films in simulant solution. The curcumin content in the food simulant (ethanol) was determined according to using spectophotometery methods as reported by Rostami and Esfahani (2019). Korsmeyer's Peppas model was employed to describe the release of curcumin from the SSSPS/TiO2 matrix [17].

Korsmeyer's Peppas model:

$$M_t/M_\infty = k.\ t^n \quad (5)$$

here, Mt/M∞, n, and k are the fractions of the ingredient released in time t, release exponent and release constant, respectively.

The response of smart film to pH changes

pH sensitivity of the developed intelligent nanocomposites was investigated using a Digital camera (Kodak M853, USA) under the identical lighting environment and camera setting. First, each smart film was exposed to solutions with pH of 4, 7 and 10. Second, Hunter's color indexes (L* and a*) were examined by Image J software.

Statistical analysis

The experimental data were analyzed using complete randomized design using SPSS software (Version 16). Duncan test was used (α = 0.05) to compare the mean values. The measurements were carried out in triplicate.

RESULTS AND DISCUSSION

Film thickness

The thickness of a nanocomposite is directly related to the microstructure, the orientation of the molecules, mechanical properties and moisture permeability of film [18]. The thickness of SSPS/TiO2 nanocomposite contains different concentrations of curcumin is shown in Table 1. The addition of different concentrations of curcumin (0.2-0.6%) to SSPS/TiO2 nanocomposite increased the thickness of the film, which ranged from 0.140 to 0.152 mm (Table 1). However, this effect was significant (p <0.05) only at concentrations of 0.6% curcumin. Low concentration of curcumin incorporated in the formulation showed no impact on thickness of caseinate/zein nanocomposite film [19]. Also, these results were similar to those of Musso et al. (2016) who reported that the thickness of gelatin film increased significantly with addition of curcumin [20]. This increase is probably due to the combination of TiO2 nanoparticle and curcumin in the SSPS polymer network and the increase in the solid content of the produced film.

Table 1. Physical characteristics of SSPS/TiO2 nanocomposites incorporated with various concentrations of curcumin

Curcumin conc (%)	Thickness (mm)	Water solubility (%)	WVP ($\times10^{-6}$ g^{-1} s^{-1} pa^{-1})
0	0.140 ± 0.005^{a}	63.11±2.21^{a}	7.33±0.76^{a}
0.2	0.145 ± 0.003^{a}	19.18±0.64^{b}	6.18±0.61^{a}
0.4	0.146 ± 0.002^{a}	16.14±0.82^{c}	4.53±0.033^{b}
0.6	0.152 ± 0.002^{b}	16.27±0.79^{c}	5.19±0.011^{ab}

Data reported are mean values. Different letters in the same column indicate significant differences ($p < 0.05$).

Water solubility (WS) and water vapor permeability (WVP)

Water solubility is a crucial characteristic of the films that indicates the film's hydrophilicity. The determination of WS is useful for choosing a film for specific applications [11]. For instance, low WS is required to protect food from moisture absorption. However, in other cases, like the encapsulation of phytochemicals, the high value of WS may be preferred [21].

As shown in Table 1, with the increase in curcumin level up to 0.4 %, the WS decreased; however, with further increase in the curcumin concentration, a slight increase in WS was observed (Table 1). The permeability and WS of films depend on their structural and morphological properties. In similar study by Rostami and Esfahani (2019), in comparison with control sample (onmucilage of Melissa officinalis seed incorporated by 5% MMT), the WS decreased with addition of curcumin, which is expectable due to the hydrophobic nature of curcumin [11]. Based on SEM analysis, the structure of the SSPS/TiO2/curcumin films containing low amount of curcumin had a higher density than the film incorporated by higher content (Fig 1).

The WVP of films, a parameter to show the moisture transfer between the packaging materials and the food, is a critical capacity to should be as low as possible [22]. In this study, WVP in SSPS/TiO2 nanocomposite (7.33 $\times10^{-6}$ g^{-1} s^{-1} pa^{-1}) (Table 1) is comparable to the SSPS/TiO2 nanocomposite prepared in the previous study by Salarbashi et al (2018) (7.0 $\times10^{-6}$ g^{-1} s^{-1} pa^{-1}) [10]. Similarly, with the increase in curcumin content up to a certain point, WVP of the film slightly decreased, which has been attributed to the hydrophobic nature of curcumin. It was expected that the presence of hydrophobic compounds acts as an obstacle for hydrogen interactions, and it causes a decrease of WVP [19]. However, with further increase in the curcumin concentration, a slight increase in WVP was observed (Table 1). This observation is related to the higher compaction structure of the composites with lower concentration of curcumin (Fig 1). Comparatively, the WVP of the developed films is lower than the value reported for cellphone (0.84 × 10-10 g m-1 Pa-1 s-1) as a synthetic film [23]. On the other hand, with further increasing in the curcumin concentration, WVP increased. This increasing trend is due to the decrease of structural compactness of the film arisen from poor dispersity of agglomerated curcumin. It should be noticed that despite high curcumin concentration used in this study, a homogenous and continuous structure was obtained from SEM images (Fig 1-A-C), revealing an excellent compatibility between

SSPS/TiO2 and curcumin.

Morphology of active film

Fig. 1 shows the SEM micrographs of the surface microstructure of SSPS/TiO2 films and curcumin incorporated SSPS/TiO2 films. As shown in Fig. 1, SSPS/ TiO2 and SSPS/TiO2/curcumin composites have a smooth, compact and continuous structure without cracks and pores. Based on SEM analysis, the structure of the SSPS/ TiO2/curcumin composite containing low amount of curcumin had a higher density than the film incorporated by higher content of curcumin. Wang et al (2019), reported that the higher concentration of curcumin in the nano dispersions of zein film induced a greater extent of aggregation because of their hydrophobic nature, thus inducing the irregular appearance of film microstructure [19]. Rostami and Esfahani (2019) reported that the addition of curcumin into mucilage of Melissa officinalis seed/montmorillonite (MMT) did not result in porous structure [11]. Similarly, the cross sections of the mucilage of Melissa officinalis seed/ montmorillonite (MMT) film and that containing curcumin were homogenous and continuous, which indicates a good compatibility between curcumin and Melissa officinalis seed.

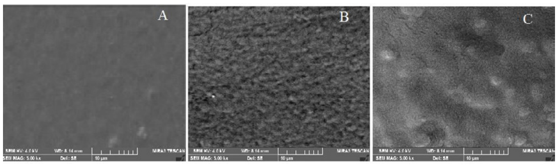

Fig 1. Surface images of SSPS/TiO2/curcumin films with various curcumin concentrations (A = 0 %, B = 0.4 % and C = 0.6 %)

Mechanical properties

Since the mechanical property of films is known as one of the most notable effective factors in their application as packaging systems, the mechanical's assessment of films is the point of interest in recent years. Different studies have been evaluated the mechanical characteristics of the nanocomposites incorporated with curcumin [11, 19, 24]. Here, we incorporated curcmin into the SSPS curcumin//TiO2 (15 %) nanocomposites, and then their mechanical charactristices were analyzed. In this study, TS and EB in SSPS/TiO2 nanocomposite (17.15 and 32.15, respectively) (Table 2) can be compared with TS and EB (17.17 and 29.90, respectively) of SSPS//TiO2 films prepared by casting method in perevious study by Salarbashi et al (2019) [21]. With increasing in curcumin concentration up to a certain point (0.4 %), TS and EB significantly improved, but beyond this, these parameters fell off. The improved mechanical properties are associated with, the H-bonding between polymer matrix and curcumin. Moreover, the observed decrease in TS and EB at high curcumin concentration is attributed to poor dispersion of agglomerated curcumin. Similar observations have been reported about the effect of curcumin on mechanical parameters of agar films [22].

The TS of SSPS/curcumin (0.4 %)/TiO2 composite incorporated by curcumin is close to most of synthetic packaging materials such as low-density polyethylene (11.8 MPa), ethylene-vinyl acetate (13.8 MPa), and high-density polyethylene (17.3-34.6 MPa). Accordingly, SSPS/ curcumin/TiO2 composite can be introduced as a promising packaging system to apply in food and pharmaceutical systems. As a suggestion for future research, mathematical modeling should be employed to predict the reaction between curcumin and SSPS matrix and the mechanical performance of SSPS films incorporated by curcumin.

Table 2. Mechanical properties of SSPS/TiO2 nanocomposites incorporated with various concentrations of curcumin

Curcumin conc (wt. %)	Tensile strength (MPa)	Elongation at break (%)
0	17.15±1.8[c]	32.15±3.14[c]
0.2	17.57±1.82[bc]	36.222±1.22[b]
0.4	25.17±1.33[a]	39.00±1.10[a]
0.6	19.11±1.41[b]	33.12±2.39[c]

a Data reported are mean values. Different letters in the same column indicate significant differences ($p < 0.05$).

The antimicrobial activity

The films with improved antimicrobial activity lead to extending the shelf-life of food products and delaying the microbial growth on the surface of foods [1]. As mentioned above, curcumin is a naturally occurring polyphenolic compound with antimicrobial activity against a broad spectrum of pathogenic microorganisms [25]. Antimicrobial activity of the developed film incorporated by curcumin against two strain of bacteria (P. aeruginosa, and Staphylococcus aureus) was showed in Fig 2. The pure SSPS films showed no antimicrobial activity against the bacteria, since no inhibition zones were found on agar plates. The results demonstrated that SSPS/TiO2 film loaded by curcumin effectively suppressed the growth of tested bacteria. The greatest inhibitory effect was observed on S. aureus with a zone area of 391.2

mm2, followed by S. aureus with a zone area of 290.3 mm2 (Fig 2). The antibacterial activity of curcumin is due to the interaction between this phenolic compound with membranal proteins of bacterial cells and as a result inhibition of bacterial growth [25]. Similarly, Varaprasad, Vimala, Ravindra, Reddy, Reddy, and Raju (2011) exhibited that curcumin loaded CMC films could effectively suppress the growth of E. coli. Gram-negative P. aeruginosa was more resistant to the curcumin [26]. This might be attributed to the presence of an additional external membrane surrounding the cell wall in Gram-negative bacteria, which can restrict the diffusion of hydrophobic material across lipopolysaccharide layer [27]. The inhibition zone for pure SSPS, SSPS/TiO2, SSPS/TiO2-0.2% curcumin and SSPS/TiO2-0.6% curcumin composites against S. aureus were found to be as 0, 25.3, 110.1, and 290 mm2, respectively (Fig 2), revealing a synergistic effect between curcumin and TiO2 nanoparticles. The reason for that is the interaction of Ti nanoparticles with microbial DNA, that inhibit bacterial replication [28]. The synergistic effect of combining Ti nanoparticles and curcumin in preventing the bacterial growth. Overall, SSPS/TiO2 (15%)/curcumin nanocomposite films can be introduced as packaging system to delay or prevent the bacterial growth.

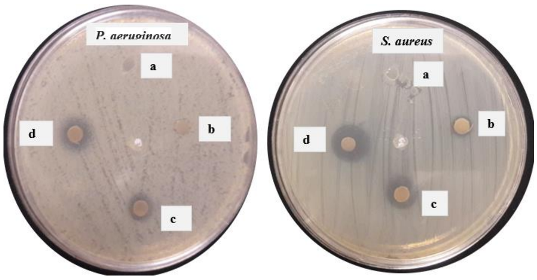

Fig 2. Antibacterial activity (inhibition zone) of SSPS/TiO2/curcumin. (a) pure SSPS, (b) SSPS/TiO2, (c) SSPS/TiO2-0.2% curcumin and (d) SSPS/TiO2-0.6% curcumin

Migration properties

The mathematical modeling revealed that Korsmeyer's Peppas model could effectively describe the release profile of curcumin from that SSPS/curcumin/TiO2 nanocomposite. According to Korsmeyer's Peppas model, a n value equal with 0.43 shows Fickian release mechanism, and n values between 0.43 to 0.85 reveal the anomalous or non-Fickian transport. n value > 0.85 presents case II transport [29]. The release exponent value for curcumin released from SSPS-curcumin/TiO2 film was 0.99, demonstrating an anomalous transport mechanism.

In conclusion, the developed composites could be used as promising packaging systems because the curcumin released at a low rate that leads to restricting the bacterial growth for a prolonged time.

pH sensitivity of the SSPS/TiO2 (15%)/curcumin nanocomposite

pH is commonly employed to recognize bacterial growth. Diverse biological activities and sensitivity against pH change, make curcumin ideal for application in intelligent packaging systems. The films containing curcumin can inform consumers that the product is suitable for consumption or not. Different investigations have been performed to fabricate intelligent packaging systems incorporated with curcumin. [30, 31, 32 33, 34]. The color of SSPS/ TiO2/curcumin composites in contact with acid, neutral and alkali liquids is presented in Fig. 3. The developed intelligent composites had the ability to sense pH changes, and thus can be utilized to monitor the quality of foods during storage. When the developed composites were dipped in basic medium, the color changed from yellow to orange-red, which has been due to the deprotonation of some of the functional groups present in curcumin structure at alkaline medium, which increase the polarity of molecule molecules [11].

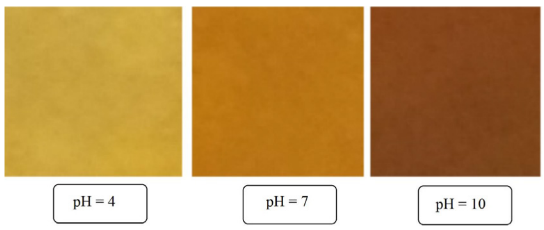

Fig 3. pH sensitivity of SSPS/ TiO2/curcumin composites in contact with acid, neutral and alkali liquids

The color properties of the SSPS/TiO2/curcumin composites in contact with the mediums with different pHs are shown in Fig. 4-A-B. L* value was 39.25 at acidic medium (pH = 4) and declined to 20.38 when the pH increased to 10, revealing the color of films becomes darker. With increasing pH from 4 to 10, a significant increase in a* value was observed, showing the intensification of the red color. These results are in agreement with those observed in previous [20, 35]. Overall, SSPS/TiO2/curcumin films can be used as a useful tool in the food industry to inform customers about the freshness without purchase and opening of the pre-packaged item.

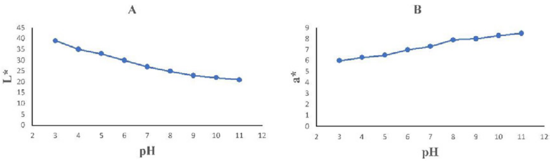

Fig 4. The color properties of the SSPS/TiO2/curcumin composites in contact with the mediums with different pHs

CONCLUSION

In the present study, the potential of curcumin as a pH sensing bioactive compound in the fabrication of SSPS/TiO2/curcumin composites was explored. The films incorporated by curcumin could be used as promising packaging systems for non-destructively detecting quality and freshness of foods. Considering the marked tendency for increasing the application of pH sensing compounds to both synthetic and biodegradable packaging systems, more investigations should be carried out on their safety and possible side effects before their application in the industrial scale. As another suggestion for future studies, further investigations should also be conducted to improve the pH-sensitivity of curcumin-loaded films.

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