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Fabrication of Buccal Dissolving Tetrahydro Curcumin Loaded Polyvidone Fiber Mat: Synthesis, Characterization, and *In Vitro* Evaluations

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ABSTRACT

A simple high molecular weight polyvidone (PVP) with tetrahydro curcumin (THC) loaded nanofiber was prepared and in-vitro dissolution was evaluated. Various polymeric concentration solution of PVP was prepared, from which 10% w/v PVP solution was used to load 10% w/w THC. The morphology of the prepared nanofibers was evaluated using scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier-transform-infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and ultraviolet-visible (UV-Vis) spectroscopy. The prepared mat released around 91% of the loaded THC within the first 5 minutes of the dissolution evaluation, which might be suitable for a buccal delivery system with a higher dissolution rate.

INTRODUCTION

In recent decades, research on nanoformulations such as nano-particles, polymeric micelles, liposomes, and nanofibers has increased in order to provide effective drug delivery systems (Balamurugan *et al.*, 2011). Recently, the drug delivery system with oral dissolving property is emerging to treat various ailments (Deng *et al.*, 2009). The nanofibrous membranes are extremely beneficial with classical features such as the high surface area to volume ratio, porosity, ease of surface functionalization, and applicability as mouth dissolving mats (Balamurugan *et al.*, 2011; Deng *et al.*, 2009).

Though there are various techniques available for the fabrication of nanofibers, electrospinning is the more versatile technique used to fabricate polymeric nanofibers under high electric potentials with required diameter ranges and surface morphologies (Zander *et al.*, 2013). The influencing electric potentials make a polymeric solution or melt into the fibers and are collected on a collector surface (Huang *et al.*, 1996). The process variability involved in this technique can form polymeric mixtures containing non-spinnable components into nanofibers and allows a high degree of flexibility in fabricating various functional mats (Dasari *et al.*, 2012). In general, the electrospun nanofibers can be fabricated by using various synthetic or natural polymeric materials. The as-fabricated nanofibers will have unique properties like high encapsulation efficiency, high pay-loading capability, and simultaneous delivery of the payloads at the desired site of action. Electrospun nanofibers are attractive for its bio-application features like wound dressing fabrics, tissue engineering scaffolds, and drug delivery (Xiuli *et al.*, 2014; Huaimin *et al.*, 2015; Ravikumar *et al.*, 2018). Since the electrospun nanofibers are structured with pores, they can be used seamlessly in wound healing applications because it can prevent the bacterial penetration by aerosol particle filtration from the atmosphere while simultaneously allowing the transport of vapor from wounds (Khil *et al.*, 2003; Zong *et al.*, 2003; Kenawy *et al.*, 2003; Min *et al.*, 2004; Sukhwinder *et al.*,

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2017).

Various types of natural or synthetic biodegradable and rate controlling polymers are used as blends or individually for fabricating electrospun nanofibers, and as delivery systems in cancer diseases, wounds, and other skin ailments (Shrestha *et al.*, 2016; Kalaipriya *et al.*, 2013; Nasouri *et al.*, 2015). Among the various polymers, poly (vinyl pyrrolidone) (PVP) is a highly biocompatible polymer, soluble in most of the organic solvents (Komeil *et al.*, 2015). PVP has potential application in various products such as paints, adhesives, detergents, medical devices, and biological materials for drug delivery (Ignatova *et al.*, 2007). PVP as cross-povidone used as superdisintegrants to attain the hasty disintegration of the drugs and also to enhance the elasticity of the prepared film (Liew *et al.*, 2014), and as mucoadhesive agents in buccal drug delivery films (Semalty *et al.*, 2010). A lot of works has been devoted to making formulations of buccal films using PVP as a plain polymer or as a co-film forming agent in various buccal film formulations. In buccal film or transdermal film formulations, optimization of film thickness is one of the most important hurdles, which may lead to forming a less uniformed film which consequently disturbs the drug distributions and contents within the film (Salehi *et al.*, 2017). Nanofiber formulation can overcome the aforementioned limitation of thickness and drug distribution problem. Hence recently *PVP* has also been electrospun as nanofibers either individually or as composites with cellulose phthalate and can be used as transdermal patches (Castillo *et al.*, 2011; Bai *et al.*, 2008). Furthermore, PVP can be used as a superior capping agent or stabilizer, since it can protect various metal nanoparticles from agglomeration during electrospinning (Wang *et al.*, 2007). Accordingly, a PVP nanofiber with gold nanoparticles has been prepared by reducing $HAuCl_4$ by trisodium citrate in the ethanolic solution of PVP (Jia *et al.*, 2015). Similarly, PVP nanofiber embedded with silver nanoparticles has also been prepared with good antimicrobial property (Mahendrakumar *et al.*, 2017). The PVP with higher molecular weight can form silver nanoparticles with smaller size ranges (Kan *et al.*, 2004).

Tetrahydro curcumin (THC) is a metabolite of curcumin with similar biological activities as curcumin. THC is also a polyphenolic compound similar to curcumin, but, it is colorless compared to curcumin (Sugiyama *et al.*, 1996; Pidaran *et al.*, 2006). It has been shown that THC is more advantageous than curcumin in terms of anti-oxidant, anti-inflammatory, anti-diabetic, and anti-tumor effects (Sugiyama *et al.*, 1996; Pidaran *et al.*, 2006). By triggering the wound healing factors, THC also possesses effective wound healing property (Wu *et al.*, 2014; Aggarwal *et al.*, 2015; Trivedi *et al.*, 2017). Though THC is highly polar than curcumin, still THC has limited biological effects due to lack of aqueous solubility, which may hinder the dissolution which eventually reduces its bioavailability (Saipin *et al.*, 2011).

The aim of this study is to develop a PVP (high molecular weight) nanofiber loaded with THC for rapid dissolution and absorption. The use of the higher molecular weight PVP in this work was to further extend the present work for fabricating *in-situ* silver nanoparticles with ultra nano sizes (Kan *et al.*, 2004). The prepared nanofibrous formulation was evaluated for its morphological features using scanning electron microscopy (SEM). The concentrations of the polymers and drugs were also evaluated for their impact on the ideal morphology of the formulations. The interactions, if any, were evaluated by Fourier-transform infrared spectroscopy (FT-IR), a crystallinity check was carried out using X-ray diffraction (XRD), and the thermal degradation and integrity were analyzed using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA), respectively. The *in vitro* drug release was also performed to evaluate the rate of release of the drug from the polymers.

MATERIALS AND METHODS

Materials

PVP (Av. Mw ~ 1,300, 000) was purchased from Sigma-Aldrich (South Korea). Acetonitrile and ethanol were purchased from Daejung Chemicals & Metals Co. Ltd (South Korea). THC was purchased from Sabinsa Korea Corporation (South Korea). All other solvents used in the experiments were of an analytical grade.

Methods

Pre-formulation studies

The electrospinning solution was prepared according to the method described by (Ming *et al.*, 2007) with little modifications. A series of PVP (high molecular weight) concentrated solution was prepared with concentrations between 5 and 15% w/v in a solvent mixture of ethanol and acetonitrile (9:1). The PVP (high molecular weight) 10% w/v solution was appeared to have uniform size and shapes. A series of THC solutions were prepared by individually extracting 5, 10, and 15% w/w towards the dry weight of PVP in the above-fixed PVP solutions. These solutions were subjected to electrospinning to determ endothermic peak of PVP with ine the suitable THC concentration for preparing nanofibrous mats. Finally, 10 mL of 10% w/v PVP solution was extracted, to which the above-fixed amount of THC was added.

Electrospinning of nanofiber solution

All the solutions including plain PVP NF and THC loaded PVP NF were fabricated using the same electrospinning processes. The prepared solutions were loaded in a 10 mL plastic syringe attached to a blunt metallic needle (21”). A distance of 15 cm was maintained between the needle tip and the rotary collector. The fabrication was initiated by applying high potential DC of around 12 kV at the needle, and the rotary drum was grounded for safe deposition of the non-woven fibers (Nicole, 2013). A 2 mL/h feeding rate of the solution was maintained throughout the fiber fabrication along with 25 rpm of the rotary drum. The fabricated fiber was then carefully collected on the drum and dried at room temperature for the complete removal of the residual solvents. The dried fibers were stored at room temperature in a light protected container until used for further characterizations.

Characterizations

The morphology and diameters of the prepared nanofibers were measured using scanning electron microscopy (SEM) (JEOL JSM 5600, Japan). Before performing the scanning electron microscopy (SEM) analysis a small piece of sample fiber was sputtered with gold after mounting over the scanning electron microscopy (SEM) stub then the samples were scanned

at different positions. The structural interactions of the drug and the polymers were evaluated using Fourier transform-infrared spectroscopy (FT-IR, Nicolet 6700) by holding samples with KBr pellets and scanned in the range of 4,000 to 400 cm^{-1}. The scan cycles were repeated for 20 cycles with a 4 cm^{-1} resolution. The X-Ray diffraction (XRD) was used to identify the crystallinity of the samples after forming into nanofibers. The Rigaku Miniflex instrument was used with λ = 1.54 Å of Cu Kα radiation at the 2θ range of 10-80° with the step size and step time of 0.1° and 1-s, respectively. Differential scanning calorimetry (DSC, SCINCO DSC, N-650) was also used to evaluate the crystallinity changes in the samples after nanofibers have been fabricated. The change in thermal behavior was also measured by thermogravimetric analysis (TGA, SCINCO N-1000) in the nitrogen atmosphere with heating ranges between 25 and 500°C at a rate of 10°C/min.

The efficiency of drug entrapment (%)

The electrospun nanofiber mat with THC was dissolved in the solvent used for electrospinning and the entrapment was calculated using UV-Vis spectroscopy at 280 nm. The entrapment efficiency (%) was calculated using Eq. (1) as follows (Abdelrazek *et al.*, 2013):

$$\text{Drug entrapped efficiency (\%)} = \frac{\text{Am. of dru release}}{\text{Am. of drug added}} \text{X } 100 \quad (1)$$

In vitro dissolution evaluations

After fabrication, the as-prepared NF mat was carefully removed and stored. It was then subjected to *in vitro* release study evaluation for the release of THC. 100 mg of nanofiber was placed in 50 mL of phosphate buffer solution, a physiologically mimicking pH 6.8 (±0.1), and at the physiologically mimicking body temperature (37 ± 0.5)°C (Sahni *et al.*, 2008). The fiber in the buffer container was maintained at 75 rpm using a magnetic stirrer. The sample solutions of 5.0 mL each were withdrawn from the buffer solution at regular intervals of 0.0, 5.0, 10.0, 15.0, 20.0, and 30.0 minutes. Following the sampling, the same volume of sample was replenished with fresh buffer solution. The drug amount released in the buffer solution was evaluated using UV-Vis spectroscopy at 280 nm. The experiment was conducted in triplicate and the average values were reported. The calibration curve was used to calculate the cumulatively released drug.

Statistical evaluation

All the experimental data were collected before performing data analysis, and the analysis of variance mean ± standard deviation (SD) were specified. The commercial software Origin 10.0 (Origin Lab Inc., Northampton, MA, USA) was used to obtain the fitting values.

RESULTS AND DISCUSSIONS

Scanning electron microscopy (SEM)

Figure 1 depicts the morphologies of the polymeric solutions with different concentrations. Among the various concentrations, 10% w/v PVP nanofiber (Figure 1a) exhibited a finer and narrow distribution, above which the fiber appeared clumsy, possibly leading to decreased encapsulation efficiency (Saravanakumar *et al.*). A work (Yang *et al.*, 2004) portrayed the effects of solvents on PVP nanofibers' morphology, in which, ethanol, ethanol: DMF, ethanol: dichloromethane as solvent for electrospinning of PVP and observed that the fiber formed with ethanol alone gave a fine morphology with 4% PVP concentration, while using with other solvents like dichloromethane and dimethylformamide, the NF morphology was highly affected. Further, the 4% PVP will not be sufficient enough for high drug loading. In this study, the ethanol/ACN (9:1) was used for better spinning along with fine morphology. These would be depended on the dielectric constant (ε) and the vapor pressure (Pa) of the solvents used. Ethanol has Pa = 65.92 mm HG @ 27°C and ε = 24.5 whereas ACN has Pa = 100 mm Hg 27°C and ε = 37.5 The vapor pressure and dielectric constant play the vital roles, such as to improve the spinnability and spinner jet formation (Olaru *et al.*, 2010). The 10% w/v PVP nanofibers with 10% w/w of THC have shown a fine morphology, with an average diameter of around 600 nm (±50 nm) (figure 1d).

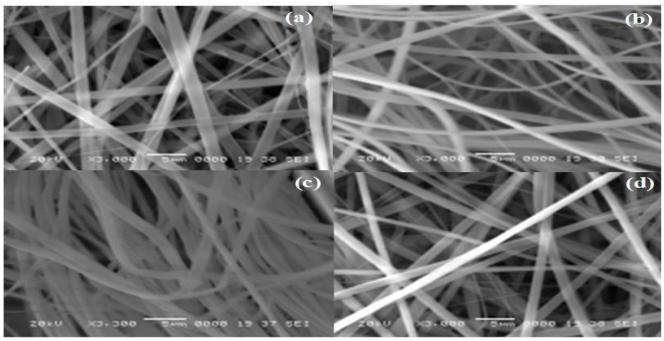

Fig. 1: (a). 5% PVP NF, (b). 10% PVP NF, (c). 15% PVP NF, and (d). THC loaded PVP NF.

Fourier Transform – Infra-Red Spectroscopy (FT-IR)

The interactions, if any, between the polymer and payloads, can be evaluated using the FT-IR technique. Figure 2 depicts the FT-IR spectrum of the polymer and THC. The –OH band of PVP appeared at 3449 cm^{-1}, whereas the C-H asymmetric stretching vibration of PVP appeared at 2919 cm^{-1}, the C-N band was appeared at around 1300 cm^{-1} and the peak at 1638 cm^{-1} was assigned to C=O stretching vibrations (Rayna Bryaskova *et al.*, 2011). Similarly, the characteristic peaks of THC were assigned to the –OH stretching at 3423 cm^{-1}, the C-H bending peak appeared at 869 cm^{-1}, the stretching of the C=C-C aromatic ring appeared at 1516 cm^{-1}, the C=C bending appeared at 1602 cm^{-1}, the saturated ketone appeared as a small spike at 1708 cm^{-1}, and the methoxy moiety appeared as stretching at 2934 cm^{-1} (Ravikumar *et al.*, 2018). In case of NF formulations, the characteristic peaks of PVP were dominantly visible with slight shifting and with a decreased intensity, which might be due to the interactions between carbonyl group of PVP and hydroxyl group of THC (Silvana *et al.*, 1997).

Differential Scanning Calorimetry (DSC) and Thermo Gravimetric Analysis (TGA)

Figure 3 shows the TGA thermograms of (i) PVP NF, (ii) THC, and (iii) PVP + THC NF, depicting the thermal stability of the as-prepared nanofiber with and without drugs.

The loss has shown within 100°C of the plain PVP NF and THC containing PVP NF indicate moisture loss from

the polymer. The degradation of THC started at around 290°C (Ravikumar *et al.*, 2018), and for degradation of PVP started at around 410°C (Anwar *et al.*, 2017). The degradation of PVP NF with THC initiated at 370°C; this demonstrates the dispersion of THC within the polymer. The **Differential Scanning Calorimetry (DSC)** thermograms of pure THC, PVP NF, and PVP with THC NF are shown in figure 4. The melting endotherm of THC has appeared at 98°C in case of pure THC. The characteristic degradation endothermic peaks of THC are at around 290°C (Ravikumar *et al.*, 2018) and PVP has at 400°C (Jing *et al.*, 2010), respectively. The endothermic peak of PVP with THC is also at around 400°C, as like in the **Thermo Gravimetric Analysis (TGA)** thermogram. The THC melting endothermic peak is not visible in the PVP + THC NF, which shows the clear amorphous dispersion of the payload within the polymer without decreasing the thermal decomposition of the payload. As the polymer was dominant in the formulation the degradation peak for THC was also not observed here. These results proved that the THC was finely distributed in the NF system as expected.

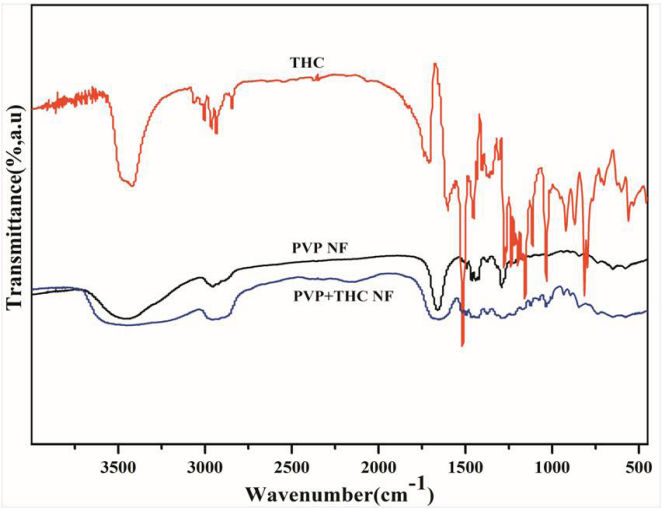

Fig. 2: FT-IR spectrum of THC, PVP NF, and the composite NF.

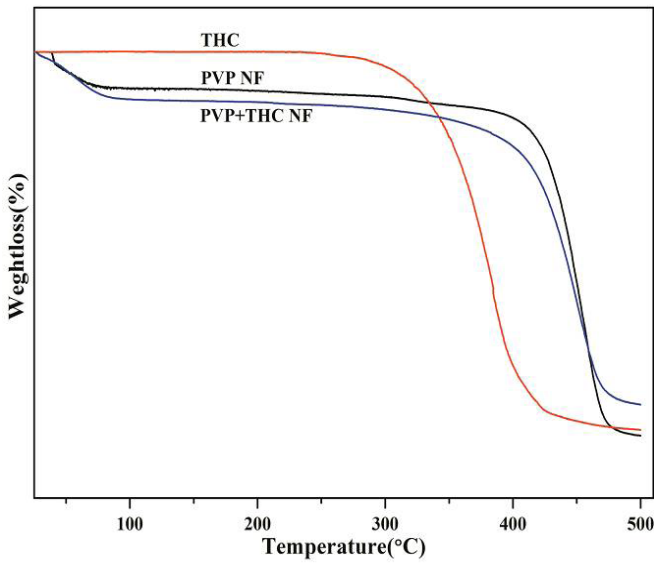

Fig. 3: TGA thermograms of THC, PVP NF, and THC containing PVP NF.

X-Ray Diffraction (XRD)

Figure 5 shows the XRD patterns of PVP NF, THC, and PVP with THC NF. The PVP only NF exhibited a broad diffraction XRD peak at 2θ = 22.1, confirming the amorphous nature of the polymer (Reda *et al.*, 2017). The characteristic 2θ diffraction peaks for pure THC where observed at 17.51, 23.08, and 23.96 due to the crystallinity of THC (Abdelrazeka *et al.*, 2013). The THC loaded PVP NF showed none of the THC peaks from the formulation, because of the complete dispersion of the payload within the polymer without changing the amorphous nature of the polymer.

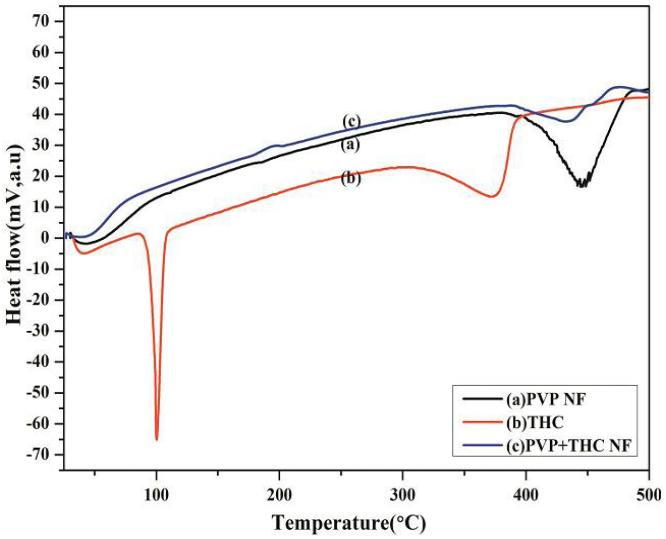

Fig. 4: DSC thermograms of THC, PVP NF, and THC containing PVP NF.

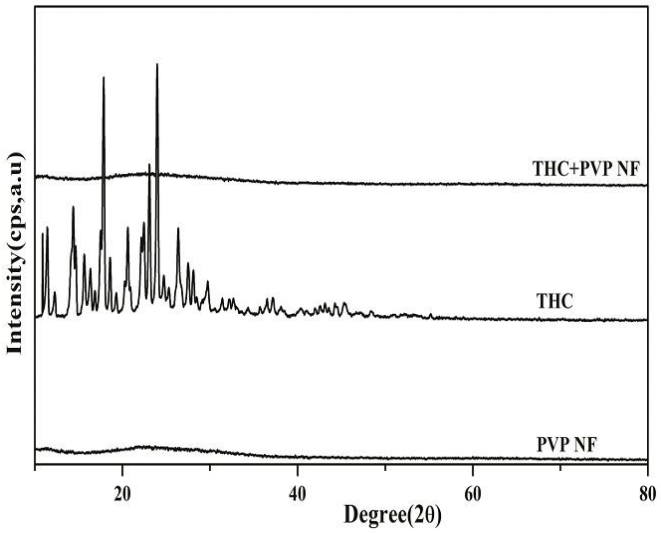

Fig. 5: XRD spectra of THC, PVP NF, and THC loaded PVP NF.

Drug entrapment efficiency

The as-prepared THC loaded PVP NF showed an entrapment efficiency of about 94%. The passive loading of the payload could contribute to the high accommodation of THC within the polymer. This higher entrapment value might be due to the larger surface areas

and the solidification payloads due to the electrospinning processes within the polymeric matrix (Bahijja *et al.*, 2015).

In vitro drug release studies

The *in vitro* dissolution study of THC from PVP is shown in figure 6. The release pattern showed that around 94% of the THC was released cumulatively within 30 min. The maximum rapid release of THC (91%) was observed within 5 minutes of the total *in vitro* release evaluation time which comparable with that of other drug loaded PVP based buccal films (Deng *et al.*, 2009). As notified earlier, the limitations due to thickness variation in a buccal film can easily be circumvented while using the same as nanofiber. This faster release of THC was attributed to the hydrophilic and hygroscopic nature of PVP, which tends to form an amorphous solid dispersion of THC because the transient concentration will be higher for the amorphous solid dispersed system than for the crystalline counterparts (Deng *et al.*, 2009). Therefore, the THC loaded PVP NF can be applied to the buccal delivery system with higher dissolution ability for treating ailments such as acute mouth ulcers.

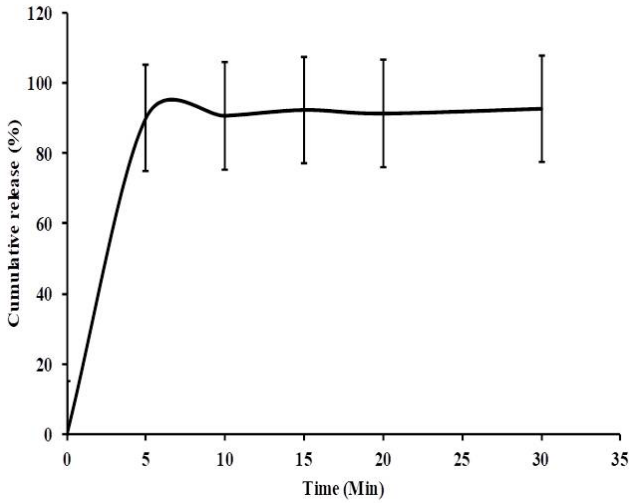

Fig. 6: Cumulative *in vitro* THC release.

CONCLUSION

High molecular weight PVP-THC composite nanofiber mats were prepared and evaluated for increased solubility and release of THC in shorter duration and could be applied in ailments such as oral ulcers. The higher molecular weight PVP was used to extend the work for *in situ* synthesis of the silver nanoparticles embedded drug delivery system for the enhanced healing property. The prepared PVP-THC composite NF mat had an average diameter of around 600 (±50) nm and the drug entrapment value of about 94%. The characterization value suggested that the as-prepared mat can be applied as a buccal delivery system with higher dissolution ability and can be applied for ailments such as mouth ulcers.

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