Curr. Pharm. Res. **2019**, *9(3),* 2894-2903; doi: 11.258369/jcpr-342

Current Pharma Research
ISSN-2230-7842
CODEN-CPRUE6
www.jcpronline.in/

Research Article

Chitosan - Polyvinyl alcohol Blend Transdermal Patches Consist of *Curcuma longa L., Tridax procumbens L., Aloe vera (L.)* Ethanol-water Extract for Healing of Wounds.

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Received 14 January 2019; received in revised form 29 January 2019; accepted 05 February 2019
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ABSTRACT
The research has been carried out for the development of transdermal drug delivery systems with the traditional Indian medicine. Specific transdermal drug delivery system which is composed with chemically modified chitosan based hydrogels patches. The traditional Indian medicine - Ayurveda, describes various herbs, fats, oils and minerals with anti-aging as well as wound healing properties. Wound healing can be defined as a complex dynamic process results in the restoration of anatomic continuity and function. Healing of wounds, whether from accidental injury or surgical invention, involves the activity of an intricate network of blood cells, tissue types, cytokines, and growth factor. Wound healing herbal extracts promote blood clotting, inhibit infections, and accelerate the healing of wounds. . In our study we improve the properties of the chitosan by blending it with polyvinyl alcohol and incorporate wound healing herbal medicines into it. Further it was cast in to transdermal patches. Antiseptic and antifungal activities of loaded hydrogel patches were studied.

KEYWORDS
Hydrogel, chitosan modification, *Curcuma longa L.,* Tridax procumbens L. *Aloe vera (L.),* Transdermal patches.

1. INTRODUCTION

A hydrogel is a cross-linked network formed from a macro- molecular hydrophilic polymer[1]. It is stable upon swelling in water and capable of absorbing a large amount of water, varying from 10% to thousands of times of its own volume. The physical properties, including swelling, permeation, mechanical strength, and surface characteristics, can be modulated through structural modification. Hydrogels based on natural polymers are currently receiving a great deal of interest, and are notable for controlled delivery drug[2-5].

Chitosan is a hetero-polymer of glucosamine and N-acetyl glucosamine residues (Fig. 1), and is obtained by de-acetylation of chitin[5]. It is a weak base, soluble in acidic solution (pH o 6.5) and insoluble in water and organic solvents. It forms a hydrogel in the presence of multi-valent anions, such as tri-polyphosphate (TPP) anions by ionic interaction between the positively charged amino groups of chitosan and the negatively charged counter-ion of TPP. Due to their hydrophilic nature and greater solubility in acidic medium, chitosan hydrogels exhibit relatively low mechanical strength and limited ability to control the release of encapsulated com- pounds 5, thus necessitating chemical modification facilitated by its hydroxyl and amino groups.

Modified chitosan hydrogels have been proven to be a potential carrier for delivery of different drug molecules with respect to size and type [6–8]. As few reports were found on modified chitosan hydrogel, this review summarizes recent developments in its properties and applications. Transdermal patches and ointment with petroleum wax made from the cross-liked modified chitosan and the leaf extract of plant *Curcuma longa L.,Tridax procumbens L*., *Aloe vera (L.)* Extract in ethanol-water were loaded through diffusion method. This forms modified gel casted in to transdermal patches is used to carry ethanol-water extract of *Curcuma longa L.,Tridax procumbens L*., *Aloe vera (L.)* as antiseptic, antifungal for wound healing.

In addition, antibacterial activity and wound healing properties of chitosan make it a suitable candidate as a dressing to be used in burn and wound care. However, the low mechanical strength of chitosan necessitates the need for water-soluble, non-toxic polymers such as cellulose derivatives poly ethylene oxide (PEO) and poly vinyl alcohol (PVA) to be blended with. In this study, PVA was chosen because of its good mechanical properties, excellent chemical resistance, biodegradability, easy preparation and film forming ability.

1.1. Properties of Chitosan

Chitosan is commercially obtained by hydrolysis of the amino acetyl group of chitin, a straight chain homo polymer composed of b-(1,4)-2-acetamido-2-deoxy-D-glucose units[9]. Chitosan has one primary amino and two free hydroxyl groups for each glucose unit. The cationic amino groups react with a number of multi-valent anions to form hydrogels. In physiological environments various enzymes, such as chitosanase and lysozyme, degrade chitosan and form harmless products. Increased de-acetylation enhances the bio compatibility of chitosan[10]. Entrapment of viable cartilage cells into the chitosan hydrogel does not produce a significant untoward effect[11]. Thereby improving the biocompatibility. Practical use of unmodified chitosan has been limited due to its poor solubility in acid solutions. Sugimotoetalv [12] reported that the water solubility of chitosan was improved by its modification with polyethylene glycol. Similarly, the properties of chitosan, such as complexation, bacteriostatic effect, absorb ability and antioxidant properties; have been shown to be enhanced by its modification[13].

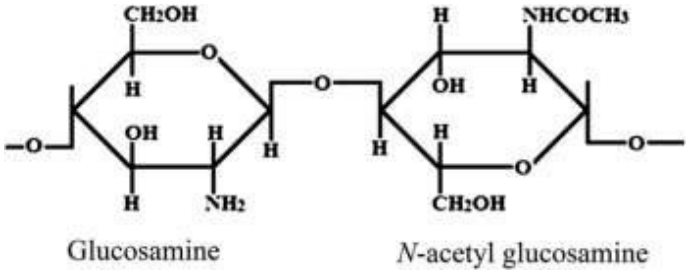

Figure 1. Chemical structure of chitosan.

2. MATERIALS AND METHODS

2.1 Materials

Chitosan (Cs) with deacetylation degree of 97% and viscosity grade of < 25 cp was purchased from Loba-Chemie Industrial Co., Mumbai, India. , methanol, salicylic acid and glutaraldehyde (GA) were obtained from Poly vinyl alcohol (PVA) (MW 72000) was obtained from Merck Limited, Mumbai, India. All other materials used in this experiment were of analytical reagent (AR) grade.

2.2 Methods

2.2.1. Preparation of the films

The films were prepared using casting and solvent evaporation. Cs was dissolved in acetic acid 1.8% v/v under gentle agitation to produce Cs solution 3% w/v, followed by addition of propylene glycol 1.43% as a plasticizer. In order to prepare drug-loaded Cs films, aqueous solutions of PVA (0%, 2%, 3% and 4%) and ethanol-water extract of *Curcuma longa L., Tridax procumbens L*., *Aloe vera (L.)* were added to equal volumes of chitosan hydrogels followed by stirring for 15 min at room temperature. The resulting mixtures were allowed to stand until air bubbles disappeared, and then 35 ml portions of solution were cast into glass Petri dishes and dried at 40°C, overnight. After cooling, all films were carefully detached from the glass Petri dishes and stored in airtight desiccators containing saturated magnesium nitrate solution (relative humidity of 50%) until used.

2.2.2. Swelling degree (Sw)

The Swelling degree of the films was measured by gravimetric method. The completely dried films (2 × 2 cm2) were weighed. Then, they were submerged in phosphate buffer solution (PBS) and incubated at 37°C for 24 hr. The resultant swollen films were removed; the excess water was omitted carefully with filter paper and weighed immediately. The swelling degree of the film is the increase in weight, expressed as percentage [11].

2.2.3. Differential scanning calorimetric (DSC)

The thermal properties of PVA and the chitosan films were characterized by a differential scanning calorimeter (DSC, Mettler Toledo CH-8603, Switzerland). Dried samples were exposed to nitrogen gas while being heated between 25 to 300°C at the rate of 30°C/min.

2.2.4. In vitro drug release

Release of salicylic acid as a model drug from chitosan films (1.5×1.5 cm2) was evaluated by the modified diffusion cell apparatus Fig. 4. (Franz diffusion cell) 2 in 30 ml PBS at 32 ±0.5°C. The

rotary paddles were adjusted to 50 rpm. At appropriate time intervals the amount of salicylic acid released from the drug-loaded films was evaluated by UV spectrophotometer at 377 nm.

2.2.5. Antibacterial activity

The zone inhibition test was carried out with a modified agar diffusion assay. The films were cut into 7 mm diameter discs. The discs were placed on Meuller Hinton agar in Petri dishes which had been seeded with bacterial cell suspensions (*Pseudomonas aeroginosa* or *Staphylococcus aureus*) adjusted to Mcfarland's standard. The Petri dishes were examined for zone of inhibition after 48 hr incubation at 37°C. To obtain ethanol-water extract of *Curcuma longa L.,Tridax procumbens L.*, *Aloe vera (L.)* paper disc containing equal concentration of drug to the blend films, 10 ml ethanol-water extract of *Curcuma longa L.,Tridax procumbens L.*, *Aloe vera (L.)* (3 mg/ml) was added to each paper disc (7 mm-diameter) allowing them to dry at room temperature.

3. RESULTS & DISCUSSION

3.1. Differential scanning calorimeter (DSC)

Thermo-gram of pure chitosan showed two exothermic peaks at 255 and 259 ºC. PVA powder and Cs- films showed endothermic peaks at 216 and 176 ºC, respectively. In the thermo-gram of blend films (Figure.2.), only one broad endothermic peak was observed between the above two temperatures that moved to higher levels with increasing PVA proportion in films

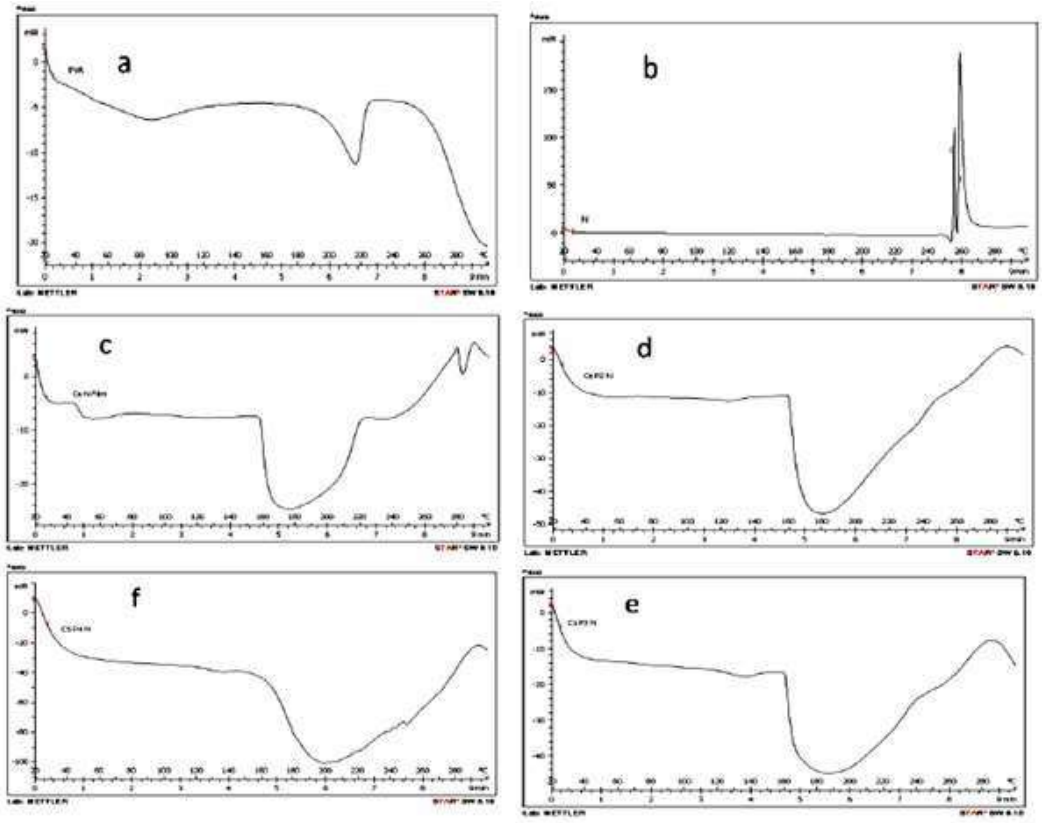

Figure 2. DSC thermo-grams: **a:** PVA, **b:** salicylic acid **c:** CsN ilm, **d:** CsP2N film, **e:** CsP3N film, **f:** CsP4N film.

DSC thermo-gram of pure salicylic acid showed two exothermic peaks at 255 and 259°C which may be attributed to the degradation of the drug. In this study, CsN films showed a very broad endothermic peak at 176°C which might be related to the glass transition temperature (Tg) of Cs film. Our findings are in line with the study of Yinyong Li et al. who reported a small transition area at the range of 160 -170°C on DSC thermo-gram of a glycerol-plasticized Cs film [28]. In other studies, higher value of Tg (205°C) reported for un- plasticized Cs films. This difference could be attributed to the effect of plasticization by glycerol or propylene glycol that was used in our study [29, 30]. We found an endothermic peak at 216°C for PVA powder which agrees well with the finding of Kenawy *et al* who reported Tm of 217°C for virgin PVA [31].
In each chitosan-PVA composite film containing salicylic acid, only one wide endothermic peak was observed between 176 and 216°C that shifted to higher levels with increasing PVA proportion in films. The changes of Tm with respect to that of pure PVA suggest the miscibility and interphase interaction between the components of a polymer blend [32]. This miscibility can be attributed to hydrogen bonding between hydroxyl groups of PVA and the partially protonated amine groups of chitosan.

3.2. In-vitro drug release

As w e can be seen in Figure 3. a burst release of drug occurred during the first 30 min but then the rate of drug release slowed down and continued overnight. Cs -PVA films showed lower burst release.

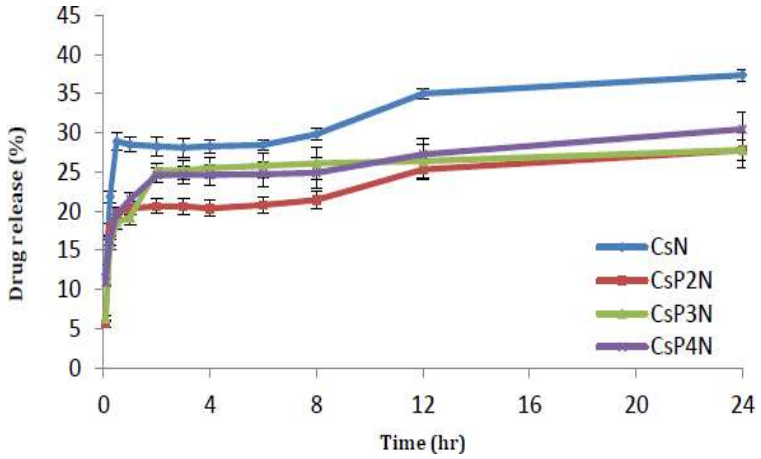

Figure 3. Profile of salicylic acid release from different Cs-PVA blend films in PBS (Mean ±SD, n=3)

Drug release test showed a burst release of drug during the first 30 min but then the rate of drug release slowed down and continued overnight. However, Cs -PVA films showed lower burst release. The strong intermolecular interactions between chitosan and PVA molecules resulted in more cross-linked regions in films that showed as impenetrable barriers to the movement of drug molecules. It seems that the drug release continues by polymer gradual erosion. When the ratio between Cs and PVA was 3:2 (in CsP2N formulation), the blend films showed the lowest drug

release rate. This result may be attributed to the increase in PVA which causes a decrease in compatibility and enhancing electrostatic repulsion between Cs and PVA. Similar results were reported by Rao et al. who found that the highest cross linking takes place between Cs and guar agar at the lowest volume ratio used in their study [25-28].

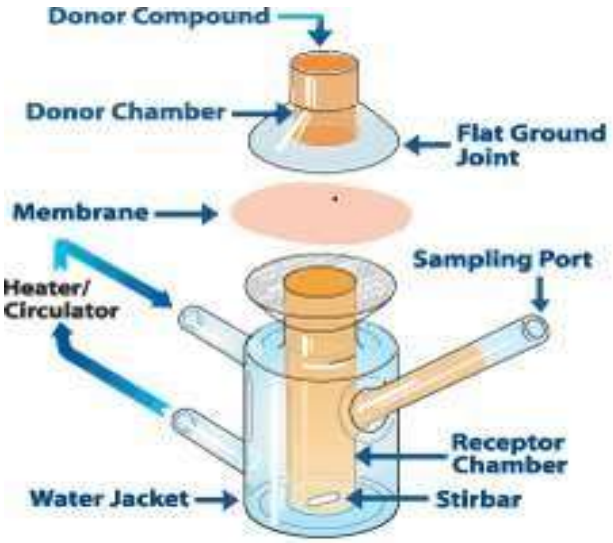

Fig. 4. Franz diffusion cell.

3.3. Antibacterial activity

As displayed in Table 3, ethanol-water extract of *Curcuma longa L., Tridax procumbens L.*, *Aloe vera (L.)* effectively inhibited the growth of *S. aureus*, but did not inhibit the growth of *P. aeroginosa*. Conversely, chitosan markedly affected *P. aeroginosa* but was ineffective ethanol-water extract of *Curcuma longa L., Tridax procumbens L.*, *Aloe vera (L.)* with a broad spectrum of antibacterial activity with weak activity against *Pseudomonas* spp. [29]. As expected, in this study, nitrofurazone effectively inhibited the growth of *S. aureus*, but did not inhibit the growth of *P. aeroginosa*. Conversely, chitosan was markedly effective only against *P. aeroginosa*. However, CsN film showed antibacterial effect against both microorganisms.

Table 3. Antibacterial activity of the prepared films and ethanol-water extract of *Curcuma longa L., Tridax procumbens L.*, *Aloe vera (L.)* disc (mean ± SD, n=5)

Formulations	**Inhibition zone (mm)**	
	Pesudomonas Aeruginosa	*Staphylococus aureus*
N	NE[a]	10.20 ± 1.30
Cs	22.60 ± 6.23	NE
Cs N	25.80 ± 4.02	8.20 ± 0.84
CsP_2N	Whole Petri dishes	NE
CsP_3N	21.20 ± 1.79	NE
CsP_4N	8.00 ± 0.5	8.00 ± 1.00

3.4. Swelling degree (Sw)

Table. 2. shows the water uptake of the films after 24 hr. Cs films showed the highest increase in swelling degree, while salicylic acid caused a significant decrease in this value (P<0.05).

Table 2. Swelling degree of films at 24 hr (Mean±SD, n=3)

Formulations	Sw_{24} (%)
Cs	102.58±1.5
Cs N	73.24±1.29
CsP_2N	86.92±8.13
CsP_3N	75.84±3.97
CsP_4N	76.18±8.03

4. CONCLUSIONS

Transdermal films of chitosan/PVA blend containing ethanol-water extract of *Curcuma longa L., Tridax procumbens L*., *Aloe vera (L.)*were developed for wound dressing applications. the chitosan films containing ethanol-water extract of *Curcuma longa L.,Tridax procumbens L*., *Aloe vera (L.)* showed a significant inhibitory effect against the growth of this microorganism which was even higher than that of the drug-free chitosan films. This is the benefit of application of Cs as a carrier for ethanol-water extract of *Curcuma longa L., Tridax procumbens L*., *Aloe vera (L.)* in treatment of burn wounds. As *P. aeruginosa* remains a cause of serious wound infection and mortality in burned patients, clinical trial is proposed to evaluate the usability of the films. *In vitro* evaluation revealed that PVA can be incorporated into chitosan film to improve its mechanical properties.

While substantially maintaining good vapor penetration, water swelling, and oxygen penetration properties. These properties are desirable for burn wound dressing materials.

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