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Design of PVA/Chitosan Loaded with Chromolaena Odorata Extract as Wound Dressings

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ABSTRACT

This current study was conducted to investigate the optimal fabrication variable that would give desirable properties in designing hydrogel wound dressings. This new PVA/chitosan hydrogel loaded with *Chromolaena odorata* were fabricated using solvent-casting method. Variables including PVA concentration (3, 5, 7 and 10% w/v), chitosan concentration (1, 2, 3, 4 and 5% w/v) and PVA/Chitosan ratio (10:90, 30:70, 50:50, 70:30 and 90:10) were investigated and elucidated. Hydrogel properties including tensile strength, swelling capacity, erosion rate and porosity were measured and effects of the fabrication variables towards these properties were elucidated. In conclusion, desirable PVA/Chitosan hydrogel loaded with *Chromolaena odorata* extract as wound dressing was favorly designed. The optimal range for PVA concentration, chitosan concentration and PVA/Chitosan ratio were 5% to 7%, 2% to 3% and 50:50 to 70:30 respectively.

1. Introduction

Wound related problem has remarkable impact towards socioeconomic and quality of life. Based on latest current market report [1,2], wound dressing market was estimated to be increased to $USD5 billion worldwide. It is worth noted that proper management of wound would be beneficial in order to overcome this issue. Wound could be categorized into two major categories which are acute wound and chronic wound. Major problems usually came from chronic wound as it is a depiction of non-healing wound.

Wound dressing, including bandages, mats and creams are preparation to provide protection to the wound site. It is well accepted that due to the nature of pathophysiology of the skin, wound

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needed a good moisture balance and oxygen transfer at the wound site to heal properly [3]. Therefore, it is agreed that an ideal wound dressing, should not only provide protection to the wound site, but also need to possess certain enhanced properties such as breathable, promotes good moisture and oxygen balance, antibacterial action and accelerate wound healing [4-6].

Hydrogels are among the most often used dressing materials, and research has demonstrated that they are useful in the treatment of wounds. Hydrogels' three-dimensional polymer networks have the ability to absorb huge amounts of water, ensuring not only a moist environment that is conducive to wound healing, but also a good biocompatibility. Because of the presence of hydrophilic groups in the polymer chains, hydrogel dressings are capable of retaining more water, resulting in a porous, soft, and elastic structure that is more compatible with biological tissues [7] .

Chitosan, a biopolymer, is widely employed as the structural backbone of hydrogels and other gel-like substances. Additionally, due to chitosan's intrinsic antibacterial property, it is frequently used in antimicrobial hydrogels [7]. Due to the fact that using chitosan alone reduces the hydrogel's mechanical strength during swelling, it was recommended that polymer mixing may be utilised to improve the hydrogel's physicochemical qualities (mechanical strength). Many previous works had shown chitosan-based hydrogels cross-linked with polyvinyl alcohol (PVA) had higher mechanical strength than a chitosan-only system [8-10].

The design and development of effective wound dressing materials has become a primary focus in aiding a faster wound healing process. Researchers are increasingly interested in incorporating medicinal plant benefits [11] into the polymeric structure of wound dressings. A great number of researchers from many regions of the world have investigated the impact of plant extracts on wound healing [12,13].

Chromolaena odorata is one of herbs belongs to Asteraceae, sunflower family. Several parts of this plant widely used to treat wound, burns, skin infections as well as possess anticancer, antidiabetic, anti-hepatotoxic, anti-inflammatory, antimicrobial, and antioxidant properties [12,14-18].

The current study was conducted with the aforesaid rationale in order to create an effective PVA/Chitosan hydrogel wound dressing loaded with *Chromolaena odorata* extract. The formulation for the hydrogel fabrication was optimized by investigating the effect of varying the PVA and chitosan concentrations and the PVA/Chitosan mixing ratio on the mechanical tensile strength, swelling capacity, percentage of erosion and porosity for the fabricated hydrogel dressings.

2. Methodology

2.1 Materials

Polyvinyl alcohol (PVA) MW900000 (R&M), chitosan (low molecular weight) high acetylation (Sigma-Aldrich), glycerol (R&M), $NaCl_2$ (Sigma-Aldrich), $CaCl_2$ (Merck) and absolute ethanol (R&M) of research grade were used throughout this study.

2.2 Preparation of Plant Extract

Dried leaves of *Chromolaena odorata* was extracted using ethanol at 10% w/v composition, macerated at room temperature for 24 hours. Then, the extract was filtered through Whatman #1 filter paper and further concentrated under vacuum at 60°C. The resulting extract in brownish paste was stored at 4°C until usage.

2.3 Optimization of the Hydrogel Formulation.

In order to determine the best formulation for the hydrogel fabrication, parameters such as PVA concentration, chitosan concentration and PVA/Chitosan mixing ratio was optimized using "One-factor-at-a-time" or OFAT approach. A series of 14 different formulations were developed accordingly (Table 1).

Table 1
Hydrogel Formula Composition

Hydrogel Formula	PVA Concentration (%)	Chitosan Concentration (9%)	PVA/Chitosan Ratio
H1	3	2	50:50
H2	5	2	50:50
H3	7	2	50:50
H4	10	2	50:50
H5	5	1	50:50
H6	5	2	50:50
H7	5	3	50:50
H8	5	4	50:50
H9	5	5	50:50
H10	5	2	10:90
H11	5	2	30:70
H12	5	2	50:50
H13	5	2	70:30
H14	5	2	90:10

2.4 Fabrication of Hydrogel

The hydrogels were prepared by solvent-casting technique. Briefly, PVA and chitosan solution were prepared in separate in which PVA were dissolved in distilled water and chitosan were dissolved in 2% v/v acetic acid at different concentration according to the formulation in Table. The pre-prepared solutions were allowed to aging for 24 hours before mixing. Then, both PVA and Chitosan were mixed at different ratio as depicted in Table 1 (10:90, 30:70, 50:50, 70:30, 90:10) for 2 hours at 60°C.

2.5 Tensile Strength Analysis

All fabricated hydrogels with different formulation were subjected to tensile strength analysis using Autograph AG-X Plus Universal Test Machine (Shimadzu, Japan) according to ASTM D 1708-66. Samples were cut into 15 mm x 60 mm and the thickness were recorded individually. Samples were stretched at constants speed of 10mm/sec until breaking point.

2.6 Erosion Test

Erosion percentage represents the crosslinking strength of hydrogel [19]. Samples with the size of 10 mm x 10 mm were weighed and immersed in PBS, pH 7.4. The samples were incubated at 60°C and weighed at certain time intervals until constant weight were achieved. The percentage of erosion were was calculated according to the following equation.

$$\% \, Erosion = \frac{Wi - Wf}{Wi} \times 100\% \quad (1)$$

where *Wi* is the initial weight of the hydrogel and *Wf* is the final constant weight of the hydrogel.

2.7 Swelling Capacity Test

The samples of size 10 mm x10mm were cut and weighed and its initial weight were recorded according to the method described by Eakwaropas [19]. Samples were immersed in simulated wound fluid (SWF) pH 7.4 and incubated at 37°C. The weight of each sample was recorded at several time intervals up to 24 hours. The swelling capacity of the samples were calculated according to the following equation:

$$\% \, Swelling = \frac{Wi - Wt}{Wi} \times 100\% \quad (2)$$

where *Wi* is the initial weight of the hydrogel and *Wt* is the weight of the hydrogel at time t.

2.8 Porosity Test

The samples of size 10 mm x10mm were cut and weighed and its initial weight were recorded. Samples were immersed in simulated wound fluid (SWF) pH 7.4 and incubated at 37°C. The weight of each sample was recorded at several time intervals up to 24 hours. The swelling capacity of the samples were calculated according to the following equation.

$$Porosity, \phi = \frac{Ws - Wd}{\rho V} \times 100\% \quad (3)$$

where *Ws* is the saturated weight, *Wd* is the dry weight of the hydrogel, ρ is the density of ethanol at 37°C and *V* is the volume of hydrogel.

3. Results

The prepared hydrogel films were evaluated using various physicochemical test and these tests are the indicators of quality and reproducibility of the method employed in this formulation [20].

3.1 Effects of PVA Concentration

The effect of PVA concentration on tensile strength, swelling capacity, erosion and porosity are represented in Figure 1(a)-(d). As depicted, the tensile strength of the fabricated hydrogel wound dressing shows increasing trend with the increase of PVA concentration. However, a decreasing in tensile strength could be seen when PVA concentration exceed 7% w/v. Similarly, for the swelling capacity of different PVA concentration had shown increased swelling with the increase of PVA content but the swelling behaviour start to dropped when the concentration of PVA exceed 7%. This finding is similar to a work done by Park and Kim [21] in which they reported that the tensile and swelling of the hydrogels are lower at higher PVA content. Although it is clear that increased in PVA concentration would lead to stronger tensile strength, however, the rearrangement of the hydrogel

matrix with higher loading of PVA might changes the coordination of molecular forces and bonding between molecule in the hydrogel. For erosion, it could be seen that the similar trend of increasing erosion rate as the PVA concentration increases. As being reported by Eakwaropas [19], erosion rate of the fabricated hydrogel were corresponding with the increased of PVA concentration as well as higher extract loading in the formulations.

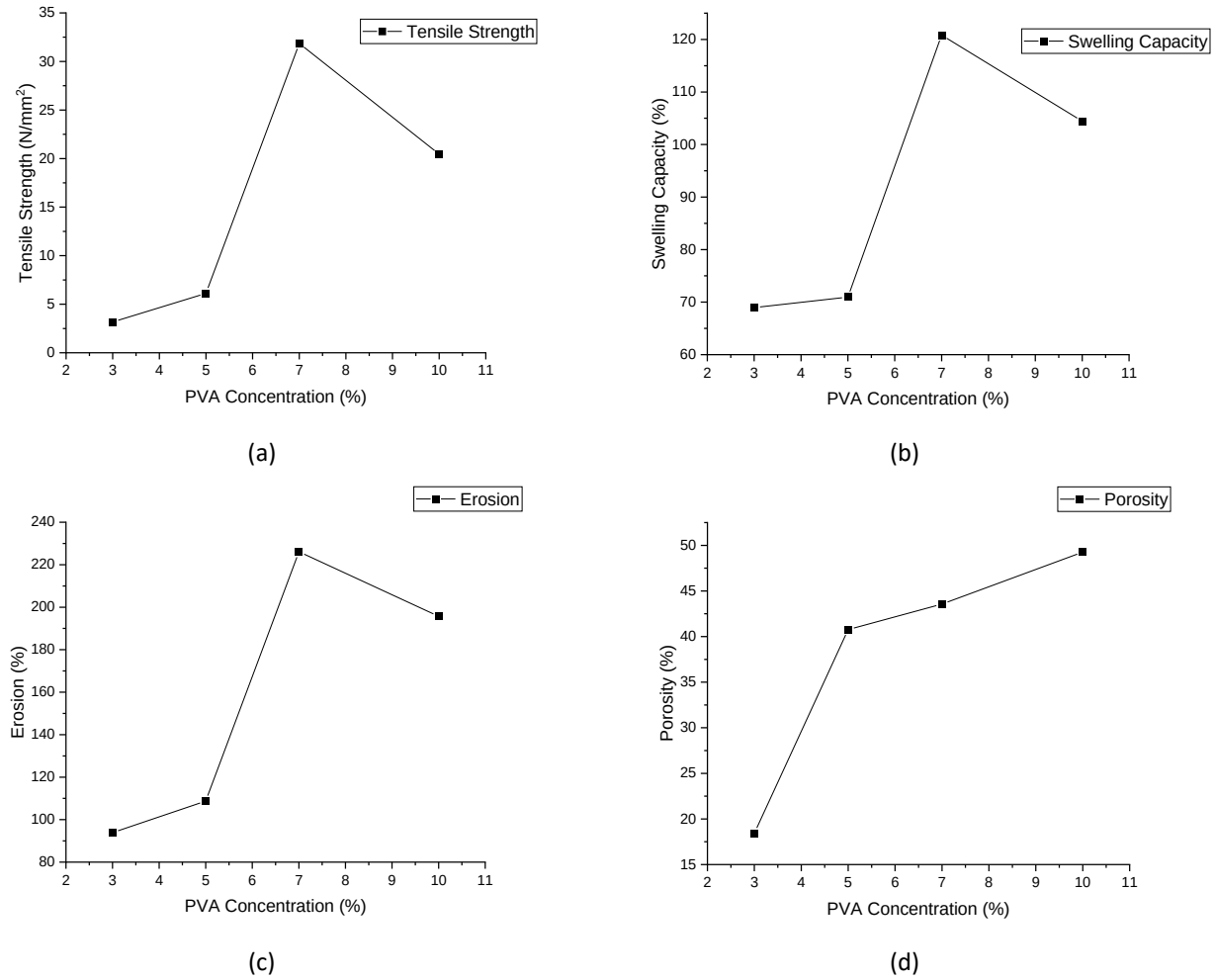

Fig. 1. Effect of various PVA concentration towards physical properties of the formulated hydrogel (a) tensile strength (b) swelling (c) erosion (d) porosit

3.2 Effects of Chitosan Concentration

Figure 2 shows the effect of chitosan concentration towards tensile strength, swelling capacity, erosion and porosity of the formulated hydrogel (H5-H9). As depicted in the figure, in general, tensile, swelling and porosity of the hydrogel decreases as the chitosan concentration increases. On the other hand, erosion rate of the formulated hydrogel exhibit increasing trend with the increase of chitosan formulation. A similar finding had been reported by Chopra [20] where higher chitosan concentration of the formulated hydrogel exhibit higher erosion rate and release of the active compounds.

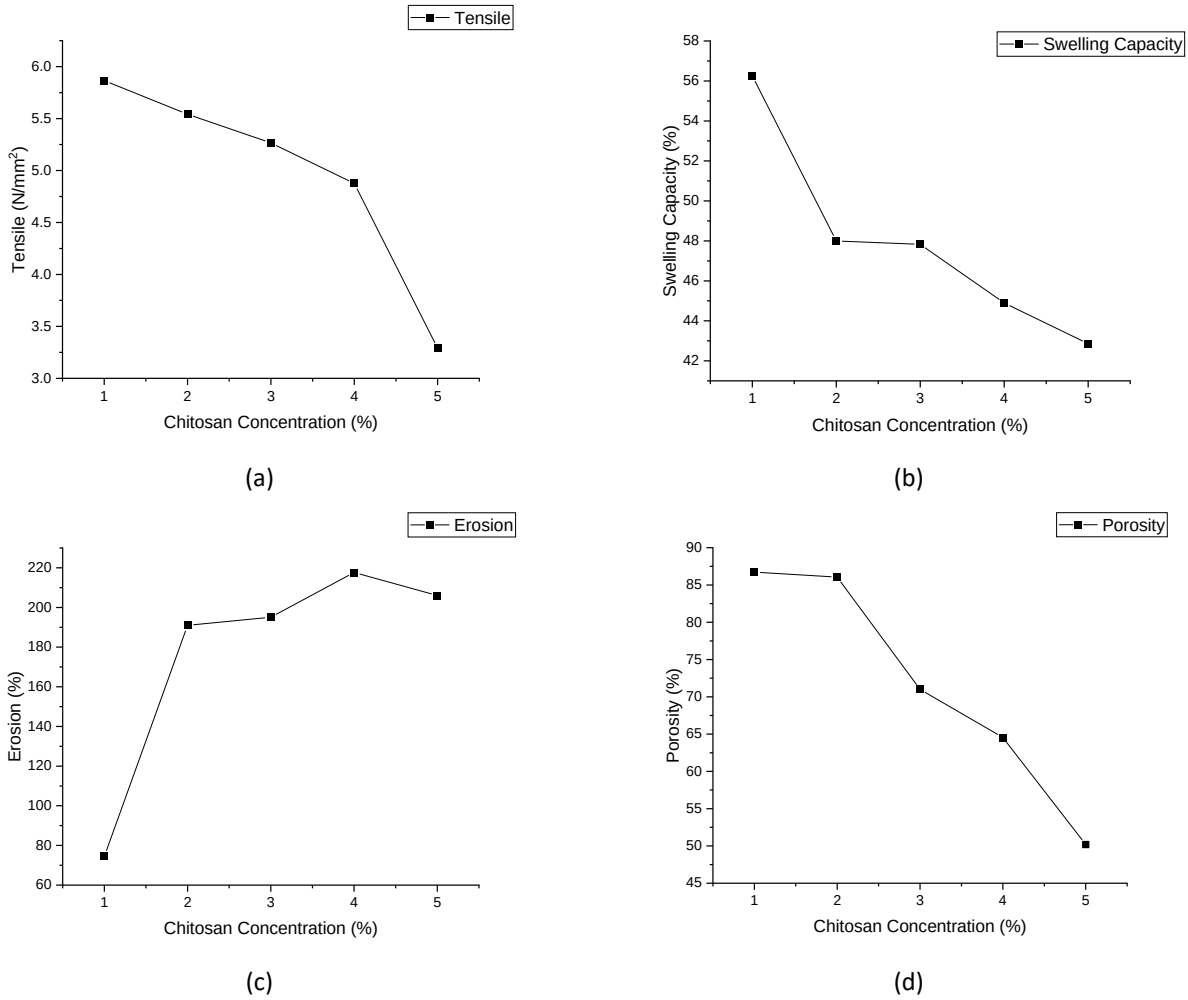

Fig. 2. Effect of various chitosan concentration towards physical properties of the formulated hydrogel (a) tensile strength (b) swelling (c) erosion (d) porosity

3.3 Effects of PVA/Chitosan Mixing Ratio

Figure 3 represents the effect of PVA/Chitosan mixing ratio on tensile, swelling, erosion and porosity of different hydrogel formulations (H10- H14). In general, it could be seen that higher PVA/Chitosan ratio gives better tensile strength and better porosity. On the contrary, swelling of the hydrogel increased with the higher ratio of chitosan. This finding is in consistency with the result as depicted in the previous section in which tensile and porosity of the formulated hydrogel proportionately increased with the increment of PVA concentration and the swelling of the hydrogel are better at higher chitosan content. Erosion of the formulated hydrogel are minimum at equal ratio of the polymeric mixture (50:50). Higher PVA and chitosan content in the formulation leads to higher erosion rate of the hydrogel. It is suggested that this phenomenon could be as a result of various cross-linking density of the polymeric chain within the hydrogel matrix.

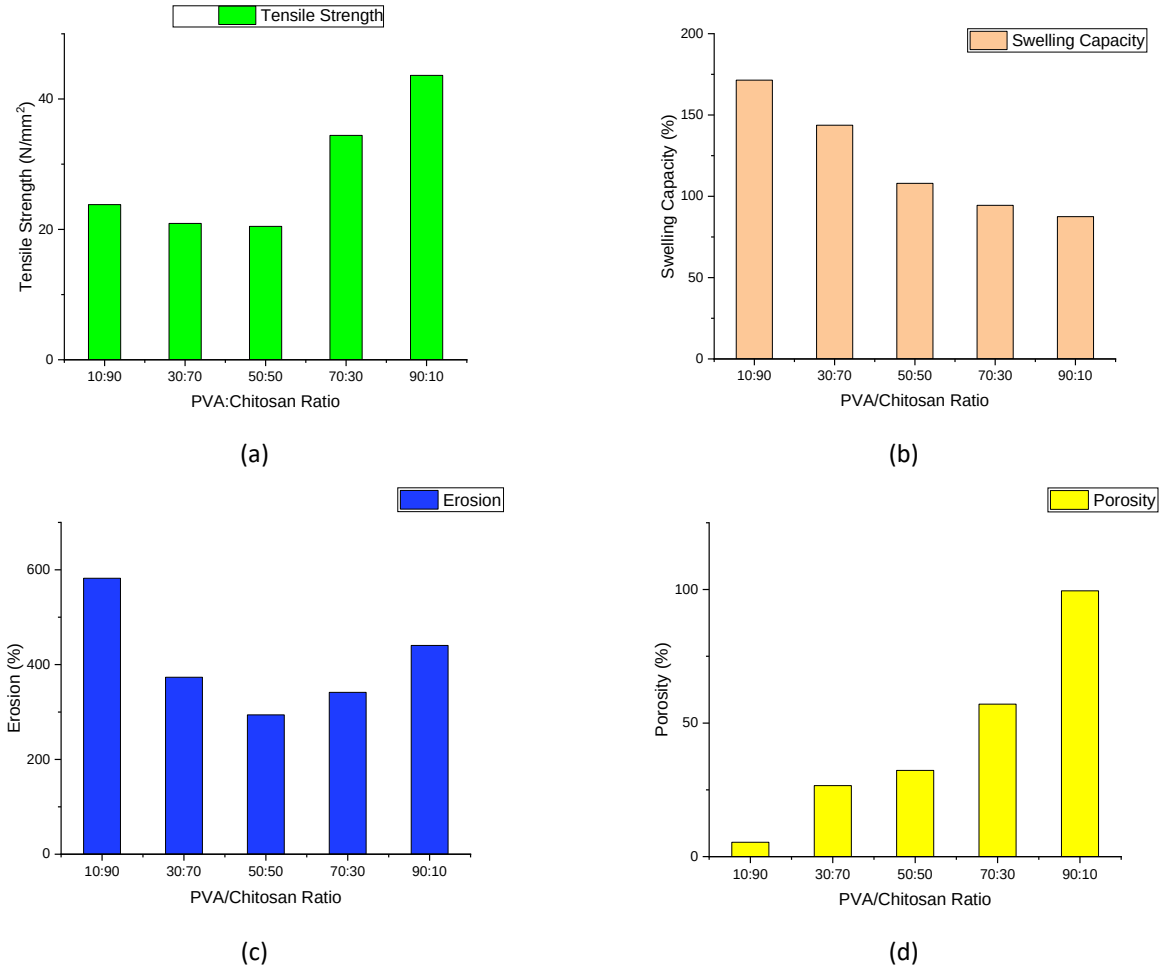

Fig. 3. Effect of various chitosan concentration towards physical properties of the formulated hydrogel (a) tensile strength (b) swelling (c) erosion (d) porosity

Good mechanical properties of the hydrogel including its tensile strength, swelling capacity, erosion and porosity are favourable for commercialization. Adequate tensile strength is important for ease of manufacturing, packaging, transportation and end-user applications. For wound treatment, high tensile strength values are required to maintain film integrity and high elongation at break values show flexibility in order to make the application easy on skin and ability to endure certain amount of stress to avoid deformation [22].

Another important feature for a good hydrogel based wound dressing are its swelling capacity. Hydrogel dressings interact with aqueous solutions by swelling to an equilibrium value. As they swell, they also trap wound debris and bacteria in the gel matrix, thus potentially reducing wound bioburden [23]. They have water vapour permeability comparable to a semipermeable membrane , thus providing some dynamic moisture balance in the wound [24]. Hydrogel dressings are easily and painlessly removed from the wound bed because the moist interface between the dressing and the wound prevents dressing adherence [23].

The loss of polymer mass through hydrogel degradation, also known as erosion, can proceed simultaneously in the bulk or preferentially on the surface of the hydrogel. Many hydrogels undergo bulk erosion, as the network is permeable to water or enzymes that mediate degradation [25]. Hydrogels consisting of oxidized polysaccharides such as alginate and chitosan typically undergo bulk erosion, and the degradation rate can be mediated by the degree of oxidation [26]. For a variety of hydrogels, one can tune the degradation reaction and erosion mechanism to obtain desirable release

kinetics ranging from weeks to months, thereby allowing for long-term release. However, one must consider that degradation products should be nontoxic and small enough for natural clearance.

Also, the porosity of the hydrogel is one of the important features to be considered in designing advance wound dressings. Porosity of the biopolymer matrix is important in the healing process because it enables cell filtration and high permeability, as well as oxygen and nutrients diffusion [27]. The use of natural polymer such as chitosan had been reported in various study to have good porosity [4,28,29] and making it as an excellent candidate for drug delivery system.

4. Conclusions

Although natural polymer had excellent biocompatibility to the skin, the mechanical properties of the biopolymer is unsatisfactory for domestic use. Ongoing research continuously conducted in developing ideal wound dressing that address both wound healing activity and practicability. In this current work, a novel PVA/Chitosan based hydrogel loaded with *Chromolaena odorata* extract were designed and developed. The effect of PVA and chitosan concentration as well as the blending ratio was investigated to rule out the best combination of PVA/Chitosan in the formulation that gives satisfactory mechanistic features. Tensile strength, swelling capacity, erosion and porosity are among important features of hydrogel and investigation on its value are deemed necessary when designing hydrogel based wound dressings. Based on the current work, the best PVA concentration, chitosan concentration and PVA/Chitosan ratio was 5% to 7%, 2% to 3% and 50:50 to 70:30 respectively. Future optimization study should be conducted to pinpoint the optimal value for the formulation using response surface methodology approach.

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