

## INVESTIGATION OF ZETA POTENTIAL, PEEL ADHESION, SWELLING, PERMEABILITY AND TENSILE PROPERTIES OF CHITOSAN FILMS FOR WOUND HEALING

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### ABSTRACT

Chitosan is a biopolymer, made up of glucosamine and N-acetyl-D-glucosamine units which are constituents of mammalian tissues. Chitosan (1.4% w/v) in 1% acetic acid (Chit-S1-AA) and in 2% lactic acid (Chit-S1-LA) solutions were prepared and their zeta potential (ZP) determined before being cast to prepare films for wound dressing. The peel adhesion test and swelling behaviour of these films were studied. The ZP values of Chit-S1-LA and Chit-S1-AA solutions were differed significantly ( $p < 0.05$ ). The peel force ( $p=0.016$ ) and work of peel ( $p=0.004$ ) results were significantly different between Chit-S1-AA and Chit-S1-LA. The degree of swelling for Chit-S1-LA film in distilled water was higher than in phosphate buffer solution. Conversely, Chit-S1-AA films swelled to a comparatively greater extent in the phosphate buffer solution than in distilled water. There was a statistically significant difference in the rate of moisture permeability between Chit-S1-AA and Chit-S1-LA ( $p < 0.001$ ). The tensile strength of both Chit-S1-AA and Chit-S1-LA films decreased significantly with an increase in the relative humidity ( $p < 0.001$ ) or the exposure duration ( $p < 0.001$ ). In contrast, the elongation of both the films increased significantly with an increase in the relative humidity ( $p < 0.05$ ) or the exposure duration ( $p < 0.001$ ).

**Keywords:** Chitosan, Zeta potential, Peel adhesion, tensile strength.

### INTRODUCTION

Chitosan is a naturally occurring polysaccharide derived by partial deacetylation of chitin, which is a copolymer of glucosamine and N-acetyl-D-Glucosamine (Ling-Gibson *et al.*, 2003; Gan and Wang, 2007). Degree of deacetylation (DD) is considered an important characteristic which could significantly affect the various properties of Chitosan (Muzzarelli, 1977). The deacetylated chitosan backbone of glucosamine units has a high density of amine groups, allowing strong electrostatic interactions with proteins and genes (Gan and Wang, 2007). The change in zeta potential (ZP) value is mainly attributed to the electrical charge of the respective polymer (Takeuchi *et al.*, 1994). Since chitosan is positively charged and adheres readily to most of the tissues, enzymes and certain microbes (Sandford, 1992) the surface properties could be detected by measuring the ZP. Chitosan films have the ability to swell in water and its swelling characteristics could improve its biocompatibility (Nakatsuka and Andraday, 1992). Films with a greater swelling property allowed a greater extent of water penetration. The determination of the swelling behaviour of a chitosan film is essential to investigate its application as wound dressings.

Numerous wound dressing materials are available and are

also being investigated (Kane *et al.*, 1996; Azad *et al.*, 2004). Chitosan films combine the features of moist wound healing with good fluid absorbance and are transparent to allow the monitoring of healing. Humidity is an important factor which affects the tensile strength and elongation of the starch films (Lloyd and Krist, 1963). Different levels of moisture can cause either an increase or decrease in mechanical strength (Jerwanska *et al.*, 1997). The study of relative humidity could provide valuable information pertinent to the mechanical behaviour of the chitosan films when used as dressing for wet wound.

In our earlier papers, studies were carried out to determine the mechanical, *in vitro* bioadhesive strength and biological properties of chitosan films (Khan *et al.*, 2000). The influence of analytical methods on chitosan DD value (Khan *et al.*, 2002) as well as the relationship between the physical properties of chitosan solutions and their molecular weights were reported (Khan and Peh, 2003<sup>a</sup>). The chitosan films were further investigated on wound healing efficacy using punch biopsy wounds in rats. These films were evaluated in terms of transparency, flexibility, adherence property, ease of removal from wounds. Additionally, wounds were examined for dryness, exudation, contraction, period of epithelialization and scar formation (Khan and Peh, 2003).

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Some of the important parameters of the chitosan films were determined which had not yet been reported. The objective of this paper was to report the findings of the determination of the ZP of chitosan solutions, peel adhesion test and swelling behaviour of chitosan films. In addition, the effect of relative humidity and exposure duration on the tensile properties of chitosan films was also studied.

## MATERIALS AND METHODS

### Materials

Chitosan (practical grade from crab shells) was purchased from Sigma Chemical, St. Louis, USA. Sodium chloride solution (0.9% w/v) was a gift from B. Braun Pharmaceuticals, Penang, Malaysia. Glycerol and sodium nitrite were supplied by Fluka Biochemie, Buchs, Switzerland. Lactic acid 85% A.R., sodium chloride and sodium dichromate were purchased from Ajax Chemicals, Auburn, Australia. Potassium chloride was supplied by BDH, U.K. Acetic acid glacial, calcium chloride, potassium sulphate, and disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ) were obtained from R & M Marketing, Essex, U.K. Potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) was purchased from Merck, Darmstadt, Germany. All other chemicals used were of reagent grade. The materials were used as received.

### Zeta Potential Measurement

The electrophoretic mobility (EPM) of chitosan in sodium chloride (NaCl) was determined using a Rank Mark II microelectrophoresis apparatus (Rank Bros., Bottisham, Cambridge, UK). The technique involved an application of a known electric field across a suspension of polymer contained in a flat sample cell. Particles travelling in the sample under the applied electric field were monitored under the attached microscope. Chitosan (1.4% w/v) in 2% acetic acid (Chit-S1-AA) or 1% lactic acid (Chit-S1-LA) solutions was diluted with 0.001 M NaCl to produce the final concentrations of 0.014, 0.028 and 0.056 %w/v. About 5.5 ml of the respective chitosan sample (solution) was introduced into the electrophoresis flat cell. Both electrodes were fixed into the cell at a predetermined voltage of 40V. The time required for the charge particle to move across a fixed distance of 50  $\mu\text{m}$ , either from the right to left or left to right, depending on the charge of the electrodes was recorded.

The particle velocity could be determined by means of a micrometer scale in the eye-piece of the microscope, using a controlled timing device. The particle velocity was calculated using the equation as shown below:

$$v = d/t$$

$v$  = velocity of particles (m/s)

$d$  = length of the graticule on which particle across at time  $t$  (50  $\mu\text{m}$  = 50 x 10<sup>-6</sup> m)

$t$  = time travelled by particle (s)

The electrophoretic mobility ( $\mu$ ) was given by

$$\mu = v.L/E$$

$E$  = applied voltage (volt)/cell (electrode) distance (m)

$L$  = effective length of the electrode cell (6.614 cm = 0.066144 m)

From  $\mu$ , the zeta potential ( $\xi$ ) could be calculated with the aid of simple Helmholtz-Smoluchowski equation:

$$\xi = 4 \pi \times 9 \times 10^3 \times \mu \times \eta / \epsilon$$

$\xi$  = zeta potential (mvolt),

$4 \pi$  = Smoluchowski factor, the factor  $9 \times 10^3$  converts electrostatic units to practical electrical units and microns to centimeters,

$\mu$  = electrophoretic mobility ( $\text{m}^2 \text{s}^{-1} \text{volt}^{-1}$ )

$\eta$  = viscosity of suspending liquid (water) in poises at 25<sup>o</sup>C (8.903 x 10<sup>-4</sup>)

$\epsilon$  = dielectric constant of water (6.937 x 10<sup>-10</sup>)

Hence, for suspension in water at 25<sup>o</sup>C,

$$\xi = 12.869 \times \mu$$

### Preparation of Chitosan Films

Chitosan (1.4 %w/v) in 2 %w/v acetic acid (Chit-S1-AA) and 1 %w/v lactic acid (Chit-S1-LA) solutions were casted to fabricate films for wound dressing. The method for preparing the films was described in our earlier paper (Khan *et al.*, 2000). Briefly, chitosan (1.4%w/v) in 1%w/v lactic acid solution was prepared by stirring overnight using magnetic stirrer. The resulting solution was filtered through a sintered glass filter under vacuum to remove the extraneous matter, followed by casting on glass plate and dried in an oven (Memmert, Germany) at 60°C for 24 hr. After drying, the transparent film was carefully peeled off from the glass plate. The films were stored in an air tight glass container maintained at room temperature of 25°C and relative humidity of 60-65% until further investigation. The thickness of the film sample was measured using a micrometer at five locations and the mean thickness calculated. Samples with air bubbles, nicks or tears and having mean thickness variations of greater than 5% were excluded from analysis.

### Peel Adhesion 180<sup>o</sup> Test

The peel adhesion test was conducted using a texture analyzer equipment (TA.XT2, Stable Micro System, Haslemere, Surrey, UK) equipped with a 5 kg load cell. Glass petri dish was used as an adherent. A brief summary about the parameters used for evaluation of the given test are as under:

### Parameters

- Speed of the probe = 0.5mm/sec.
- Size of film (width = 2.54 cm width, and length = 2.54)
- Weight used for pressing the film = 100 gram
- Time to press the film by the proposed weight = 5 minute.

e. Solvent used to moisten the film surface = 10  $\mu$ l Normal saline.

A film sample was tied at one end (center point) with a 10 cm surgical thread, which was connected to the upper probe of the texture analyzer. During measurement, 10  $\mu$ l of distilled water was evenly spread on the surface of glass petri dish, which was held by the clamp. The film sample with a contact surface area of 6.45 cm<sup>2</sup> (2.54 cm width by 2.54 cm length) was applied onto the glass petri dish. A 100 g weight was then placed on top of the film sample for 5 min. The experimental setup is illustrated in figure 1. The film sample was pulled from the substrate at an 180° angle at a rate of 0.5 mm/s until the sample strip was detached completely from the glass petri dish. An acquisition rate of 200 points/s was chosen for the analysis. Data collection and calculation for peel force and work of peel were obtained using the XTRA Dimension software package of the machine. The experiments were run in four replicates.

#### Swelling Behaviour of Chitosan Films in Different medium

The film swelling measurement was conducted using the method proposed by Peh and Wong (1999) with slight modification. The swelling properties of the film were studied using two different media, namely, distilled water and phosphate buffer (3.39 g KH<sub>2</sub>PO<sub>4</sub> and 3.53 g Na<sub>2</sub>HPO<sub>4</sub> per litre of distilled water adjusted with phosphoric acid to pH 6.82). Each film sample (surface area: 2.49 cm<sup>2</sup>) was weighed and placed in a preweighed stainless steel wire mesh with a sieve opening of approximately 800  $\mu$ m. The mesh containing the film sample was then submerged into 30 ml medium contained in a glass container (diameter 9.0 cm, height 1.8 cm). Increase in weight of the film was determined at preset time intervals until a constant weight was observed. Each measurement was repeated four times. The swelling index was calculated using parameters,  $(W_t - W_0) / W_0$ , where 'W<sub>t</sub>' is the weight of film at time 't', and 'W<sub>0</sub>' is the weight of film at time zero.

#### Effect of Relative Humidity and Exposure Duration on Mechanical Properties

The mechanical properties of chitosan films that were exposed to different relative humidity and time intervals, were evaluated employing the method described in our earlier paper (Khan *et al.*, 2000) using a texture analyzer (TA.XT2, Stable Micro System, Haslemere, Surrey, UK) equipped with a 5 kg load cell.

Chit-S1-AA and Chit-S1-LA films (10 mm width x 50 mm length) were placed in desiccators at RH% 55±3, 65±2, 75±2, 85±2, and 95±5. The corresponding salts used to produce the various relative humidities were sodium dichromate, sodium nitrite, sodium chloride, potassium chloride and potassium sulphate. The films

were removed at different time intervals of 1, 6, 12 and 24 hours for evaluation of tensile properties.

#### STATISTICAL ANALYSIS

The results were presented as mean  $\pm$  standard deviation (SD). The results obtained from measurement of zeta potential, peel adhesion 180° test and swelling behaviour were statistically analyzed using one-way analysis of variance. The effects of relative humidity and exposure time on tensile properties of the chitosan films were analyzed using a general factorial test. When a statistically significant difference ( $p < 0.05$ ) was obtained, Tukey-HSD test was then performed.

#### RESULTS AND DISCUSSION

##### Zeta Potential

Table 1 shows the values of zeta potential (ZP) of different concentrations of chitosan in acetic acid (Chit-S1-AA) and lactic acid (Chit-S1-LA). It was found that the values of ZP increased as the concentration of chitosan in acetic acid or lactic acid solution was increased. The results were in good agreement with the report of Takeuchi *et al.* (1994). The ZP values of Chit-S1-LA and Chit-S1-AA at three different concentrations differed significantly ( $p < 0.05$ ). Post-hoc multiple comparisons using Tukey-HSD test showed a significant difference ( $p < 0.05$ ) between the means of the various groups (e.g. A&B, A&C, A&D) except between B&D ( $p = 0.894$ ) and C&E ( $p = 0.996$ ). When the zeta potential values of similar concentration of Chit-S1-AA and Chit-S1-LA solutions were compared statistically, a significantly higher value was obtained in Chit-S1-LA, suggesting that zeta potential of chitosan molecule was affected by the type of acid employed. The primary amino groups on the chitosan molecule are reactive and provide a powerful means to promote new biological activities and to modify its mechanical properties (Suh and Matthew, 2000).

Table 1. Zeta Potential values of Chitosan in Acetic Acid and Lactic acid. Mean  $\pm$  SD., N = 3.

Preparation of Chitosan Samples	Zeta Potential (+ mV)
A) Chit-S1-AA*-1	35.94 $\pm$ 1.60
B) Chit-S1-AA-2	50.37 $\pm$ 2.45
C) Chit-S1-AA-3	57.89 $\pm$ 1.78
D) Chit-S1-LA <sup>^</sup> -1	51.93 $\pm$ 2.56
E) Chit-S1-LA-2	57.19 $\pm$ 1.26
F) Chit-S1-LA-3	70.42 $\pm$ 0.63
Statistical Analysis	$p < 0.05$
Post-Hoc Test	B & D ( $p = 0.894$ )
(Tukey-HSD)	C & E ( $p = 0.996$ )

\*AA = Acetic acid, <sup>^</sup>LA = Lactic acid  
1 = 0.014%, 2 = 0.028%, and 3 = 0.056% w/v.

Table 2. Peel Adhesion 180° Test Values of Chitosan Films. Mean ± S.D., N= 4.

Films	Peel force (cN/cm)	Work of peel (m J)
A) Chit-S1-AA*	0.208 ± 0.024	0.126 ± 0.005
B) Chit-S1-LA^	0.284 ± 0.032	0.145 ± 0.003
Statistical Analysis	p < 0.05	p < 0.05
Post-Hoc Test (Tukey-HSD)	A & B (p = 0.016)	A & B (p = 0.004)

\*AA = Acetic acid, ^LA = Lactic acid

Table 3. Effect of Relative Humidity and Exposure Time on the Tensile Strength and Elongation of Chitosan Films Prepared in Acetic Acid (Chit-S1-AA). Mean ± S.D., N = 3.

Time (hr)	Tensile Strength (N/mm <sup>2</sup> )					Elongation (%)				
	RH% 55	RH% 65	RH% 75	RH% 85	RH% 95	RH% 55	RH% 65	RH% 75	RH% 85	RH% 95
1	65.13 (1.91)*	61.33 (2.10)	57.54 (1.00)	48.79 (1.44)	31.42 (1.05)	39.62 (0.86)	38.86 (0.75)	43.35 (0.46)	46.28 (0.61)	48.29 (0.67)
6	57.84 (1.40)	58.46 (1.60)	43.45 (1.31)	34.66 (1.37)	16.52 (1.14)	41.14 (0.59)	53.59 (0.38)	45.63 (0.72)	62.83 (0.90)	68.37 (0.81)
12	55.56 (2.15)	51.13 (1.02)	26.81 (1.56)	28.52 (1.34)	9.42 (0.94)	44.45 (1.07)	55.84 (1.04)	47.44 (1.16)	64.66 (0.71)	78.24 (0.89)
24	39.86 (2.11)	30.33 (1.88)	19.60 (1.56)	5.35 (1.03)	1.97 (0.26)	48.40 (0.81)	57.10 (0.96)	58.57 (0.82)	65.21 (0.79)	98.67 (1.32)

\*Figure in bracket = standard deviation (S.D).

Table 4. Effect of Relative Humidity and Exposure Time on the Tensile Strength and Elongation of Chitosan Films Prepared in Lactic Acid (Chit-S1-LA). Mean ± S.D., N = 3.

Time (hr)	Tensile Strength (N/mm <sup>2</sup> )					Elongation (%)				
	55%RH	65%RH	75%RH	85%RH	95%RH	55%RH	65%RH	75%RH	85%RH	95%RH
1	64.64 (3.15)*	57.35 (3.18)	44.49 (2.04)	41.09 (1.40)	3.10 (0.16)	40.31 (1.22)	49.24 (1.76)	57.83 (1.23)	56.55 (0.71)	86.34 (0.68)
6	58.59 (1.85)	44.02 (1.72)	34.23 (1.91)	15.57 (1.21)	1.31 (0.25)	40.38 (2.12)	67.09 (1.44)	75.58 (1.06)	75.52 (1.98)	92.47 (2.53)
12	54.30 (2.59)	41.46 (1.85)	18.67 (0.49)	13.54 (1.11)	1.02 (0.12)	66.42 (1.40)	75.23 (1.02)	83.08 (1.78)	81.71 (1.37)	96.66 (1.42)
24	44.20 (1.78)	36.98 (1.19)	11.00 (0.89)	4.78 (0.25)	0.71 (0.10)	71.63 (0.75)	84.15 (1.89)	89.21 (1.24)	99.40 (0.11)	99.27 (0.42)

\*Figure in bracket = standard deviation (S.D).

The zeta potential refers to the electrostatic charge on the particles, which causes them to move in an electric field towards a pole of opposite charge. ZP can be defined as the work necessary to bring unit charge from infinity to the surface of shear of the particle. Moreover, the ZP of polymers could provide the initial driving force for bioadhesion, followed by mutual interpenetration and subsequent establishment of secondary bonds through non-specific physical entanglement when pressure was applied. The higher surface charge could enhance interaction between chitosan and the glycoprotein network of the tissue, resulting in Chit-S1-LA having higher bioadhesion than Chit-S1-AA film (Khan *et al.*, 2000). Similarly, Chit-S1-LA was noted to have marginally higher peel adhesion than Chit-S1-AA film (Khan, 2001). A reasonable correlation was reported

between ZP of different polymers in saline and gastric media to their bioadhesive performance determined by the detachment experiment (Tur and Ch'ng, 1998). Much of the potential of chitosan as a biomaterial stems from its cationic nature and high charge density in solution (Suh and Matthew, 2000). The change in zeta potential value was mainly attributed to the electric charge of the polymers fixed on the surface (Takeuchi *et al.*, 1994).

#### Peel Adhesion 180° Study

Adhesion is an essential property of the dermal and transdermal therapeutic systems. Peel adhesion is the force required to peel away a strip of tape from a rigid surface (Minghetti *et al.*, 1999). The results of the peel adhesion 180° test on the glass petri dish for Chit-S1-AA, and Chit-S1-LA are depicted in table 2. Both the two

films were readily stripped from the glass petri dish with no visually noticeable residue. There was a statistically significant difference ( $p < 0.05$ ) in both the peel force and work of peel among the two different films. It was noted that Chit-S1-LA exhibited significantly greater peel adhesion force ( $p = 0.016$ ) as well as work of peel ( $p = 0.004$ ) than Chit-S1-AA. Similarly, significantly higher zeta potential values were obtained in Chit-S1-LA as compared to Chit-S1-AA solutions ( $p < 0.05$ ). The findings were in good agreement with those of *in vitro* bioadhesive strength of films as reported in our earlier paper (Khan *et al.*, 2000), where it was observed that Chit-S1-LA is consistently exhibited higher adhesion than Chit-S1-AA. The results suggested that glass petri dish and animal tissue could be used interchangeably as an adherent to evaluate the bioadhesive strength of film. The data obtained permitted a satisfactory differentiation between the adhesion of the different type of films.

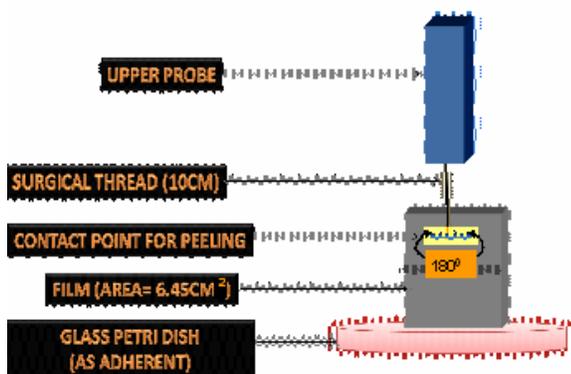


Fig. 1. Peel Adhesion 180° Test.

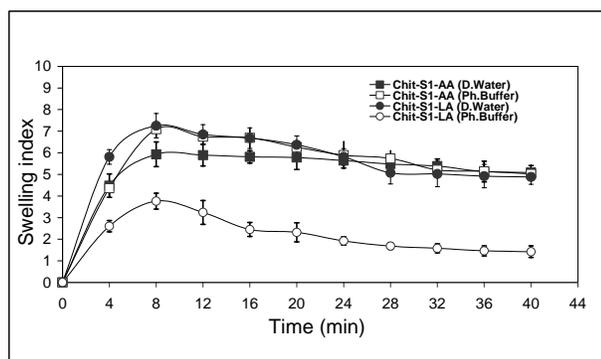


Fig. 2. Swelling Behaviour of Chitosan Films Prepared using Acetic Acid (Chit-S1-AA) and Lactic Acid (Chit-S1-LA) both in Distilled Water and Phosphate Buffer Solutions. Mean  $\pm$  S.D., N = 4.

### Swelling Behaviour of Chitosan Films in Different Media

When films were in contact with water, water molecule would penetrate into the free spaces between the polymer chains and cause the film to swell (Chan *et al.*, 1999). Figure 2 depicts the degree of swelling of Chit-S1-AA

and Chit-S1-LA films in both distilled water and phosphate buffer solution. The swelling profiles achieved maximum at approximately 8 min. It can be noted from the plots that the degree of swelling and swelling index for Chit-S1-LA film in distilled water were higher than in phosphate buffer solution. Conversely, Chit-S1-AA film swelled to a comparatively greater extent in the phosphate buffer solution than in distilled water. The results indicated that Chit-S1-LA and Chit-S1-AA films differed in their swelling behaviour in distilled water and phosphate buffer solution. It has been reported that the ionic strength and pH of the solution affected the swelling behavior of polymers (Park and Robinson, 1985; Peppas and Peppas, 1989; Yao *et al.*, 1994). In addition, the degree of swelling of chitosan depends on both characteristics of the chemical structure and properties of the medium (Singh *et al.*, 2006).

The results obtained in the present study demonstrated that ionic strength and pH might play an important role in affecting the swelling of Chit-S1-AA and Chit-S1-LA films. Macleod *et al.* (1999) conducted swelling studies on pectin/chitosan complex membranes, and concluded that the steady fall in the swelling of such membranes was due to the formation of interpolyelectrolyte bonds between the negatively charged pectin and chitosan which was cationic in nature. The swelling state of the polymer was reported to be crucial for its bioadhesive behaviour (Chen and Cyr, 1970; Huntsberger, 1971). Adhesion occurs shortly after the beginning of swelling but the bond formed is not very strong (Chen and Cyr, 1970). The adhesion will increase with the degree of hydration until a point where over hydration leads to an abrupt drop in adhesive strength due to disentanglement at the polymer/tissue interface.

### Measurement of Mechanical Properties

The tensile testing provides an indication of the strength and elasticity of the film, which can be reflected by tensile strength, and elongation at break. It is suggested that films suitable for wound dressing should preferably be strong but flexible. The mechanical properties of Chit-S1-AA and Chit-S1-LA were reported earlier in our paper (Khan *et al.*, 2000).

The tensile properties results of Chit-S1-AA and Chit-S1-LA films under different relative humidity and exposure duration are shown in tables 3 and 4, respectively. The tensile strength of both Chit-S1-AA and Chit-S1-LA films decreased significantly with an increase in relative humidity ( $p < 0.001$ ) or exposure duration ( $p < 0.001$ ). The decrease in tensile strength was significantly more dominant when the film was exposed to a high relative humidity over a long duration ( $p < 0.001$ ). In contrast, the elongation of both the films increased significantly with an increase in the relative humidity ( $p < 0.05$ ) or the exposure duration ( $p < 0.001$ ). The increase in elongation

was significantly predominant when the film was exposed to a high relative humidity over a long duration ( $p < 0.001$ ). Post-hoc tests showed that for Chit-S1-AA film, the tensile strength and elongation at break obtained under the various relative humidity and exposure duration, were in different subset. Similar findings were also obtained for Chit-S1-LA film.

Humidity and drying conditions have significant effect on the mechanical properties of free films. Repka *et al.* (1999) reported that humidity could be a relevant factor that affected the physico-mechanical properties of hydroxypropylcellulose films. The results demonstrated that the presence of moisture considerably affected the mechanical properties of the chitosan films. Lloyd and Kirst (1963) showed that moisture content was the most important factor that affected the tensile strength and elongation of the starch films.

## CONCLUSION

The Chitosan-lactic acid (Chit-S1-LA) film was more soft, flexible, pliable and bioadhesive than the chitosan-acetic acid (Chit-S1-AA) film. Zeta potential of chitosan molecule, peeling force and work of peel of chitosan films were affected by the type of acid employed. Increase in relative humidity as well as exposure period reduced the tensile strength but increased the elasticity of the two chitosan films. However, Chit-S1-LA film is recommended for wound dressing owing to their non-irritant nature to the skin.

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