Indian Journal of Chemical Technology
Vol. 23, July 2016, pp. 279-288

Optimisation of chitosan-honey composite film for wound dressing application

R Thenmozhi[1], R Rathinamoorthy[1,]* & G Thilagavathi[2]
[1]Department of Fashion Technology, [2]Department of Textile Technology
PSG College of Technology, Coimbatore, India.
E-mail: r.rathinamoorthy@gmail.com

Received 10 April 2014; accepted 17 February 2016

This research aims to develop honey loaded chitosan composite film based dressing for wound care application. The composite films have been prepared with different combination of chitosan, honey and cross-linking agent as per box and benhken design. The result analyses the effect of process parameters like chitosan percentage, crosslinking agent and honey percentage on the physical properties of the developed film. The findings indicate that increment in crosslinking agent percentage increases the brittleness of the film, the increment in honey and chitosan percentage helps to improve the important parameters like fluid absorption capacity, folding endurance and degradation of the film. The numerical optimization result shows that 1.5% of chitosan, 1.2% of acetic acid and 7.9% of honey as best combination for film development with respect to the selected responses. The developed wound dressing are characterised for their physical properties and also the biological evaluation. The antibacterial test and liquid culture test results confirm that the developed film dressing possess higher resistant against gram positive and negative bacterial strains.

Keywords: Chitosan-Honey composite, Wound dressing, Box and Benkhen design, Antibacterial test, Liquid culture test

Wound healing and microbe resistance are most important factors for the wound dressing. The wound occurs in many ways in the human skin such as skin disease, skin burn and surgical wound. A wound can be described as a defect or a break in the skin, resulting from physical or thermal damage or as a result of the presence of an underlying medical or physiological condition[1]. According to the wound healing society, wounds are physical injuries that result in an opening or break of the skin that a cause disturbance in the normal skin anatomy and function. They result in the loss of continuity of epithelium with or without the loss of underlying connective tissue[2, 3].

Chitosan film has the ability to inhibit the bacteria and much effective in wound healing activity[4]. The demand for film-based biodegradable wound dressing from the medical textile industry leads to development of new type of wound dressing. Chitosan, a naturally occurring polysaccharide, is a cationic polysaccharide composed of [α2-amino-2-deoxy-β-D-glucan] obtained by the alkaline deacetylation of chitin. This chitin is present in shells of insects like marine crustaceans such as shrimps and crabs[5]. The unique properties of chitosan including availability, biodegradability, biocompatibility, bioactivity, non-toxicity as well as good adhesion and sorption are the major reasons for its multiple applications[6,7]. Another main reason for this increasing interest of chitosan is its wide range of physical forms which can be obtained by using an appropriate technological process[8].

Honey has been used for its medicinal properties to treat a wide variety of ailment. In particular, it has been used in wound dressings and wounds owing to its promotion of rapid healing through a number of mechanisms[9]. Honey's high osmolarity, acidic pH, inhibin factor, and nutrient content contribute to the inhibition of bacterial growth and the promotion of wound healing[9]. Honey compound's oily content acts as a barrier, preventing excessive loss of body fluid and decreasing evaporative wound water loss. It thus prevents wound desiccation and provides an optimal moist ambience for wound healing[10].

The barrier effect also decreases local pain during and after wound manipulation. Honey is cheap, non-toxic and non-allergenic, it does not stick to the wound, and it provides a moist environment conducive to rapid wound healing and thus is suitable in wound dressing application[11]. Blending of chitosan along with honey as a cross-linking agent for biodegradable and microbe resistant film dressing improves the wound healing activity.

Manuka Honey is world famous for its unique properties. UMF (Unique Manuka Factors) in particular provides special benefits for the human body. Active Manuka is an antibacterial property which is naturally present in some strains of manuka honey, but it is not in all Manuka Honey[12]. UMF honey, the type of Manuka Honey which has the special UMF antibacterial property, is highly sought after for its health-giving qualities. UMF 10 is honey that has the same bacteria-killing power as 10% phenol. Generally, the UMF value of the honey will be in the range of 10 to 20[13].

In the present study, an attempt has been made to develop wound dressing with chitosan honey blended composite film based on the previous experience[14-15]. The effectiveness of developed film dressing was characterized by using various tests such % degradation, folding endurance, and fluid absorption capacities. The effect of above said process parameters on the film character was studied using Box and Behnken design. Numerical optimization technique was used to identify the best process parameter with higher folding endurance, fluid absorption capacity and lower degradation percentage. The optimised chitosan-honey composite films were characterized using SEM, FTIR, and liquid culture test for their suitability for wound dressing.

Experimental Section

Chitosan was purchased from Mahalakshmi chemicals, Coimbatore, India. Manuka honey with UMF 16+ was purchased from Australia.

Preparation of chitosan –honey films wound dressing

Homogenous chitosan-honey membranes were prepared by solution casting technique. 0.5, 1, 1.5% chitosan solution in 1, 1.5, 2% acetic acid were prepared and stirred in 10 mL distilled water for 1-2 hours. After thorough mixing, 5 mL of 10% aqueous sodium bicarbonate was added dropwise. The *p*H of the solution was ~6.Then 2 g of glycerol in 10 mL distilled water was added. The mixture was stirred and allowed to stand for 5-10 min. The foam on top was then skimmed off. 10 mL of this chitosan solution was stirred with 4, 6, 8% honey respectively, for 15-20 min. The solutions were then poured into the trays and dried at room temperature. When the films were in the stage of partial drying, the viscose nonwoven, bamboo nonwoven fabrics were placed on the films under slight pressure and allowed to dry.

Experimental design

Response surface methodology is an empirical modelization technique devoted to the evaluation of the relationship of a set of controlled experimental factors and observed results. It requires a prior knowledge of the process to achieve statistical model. Basically, this optimization process involves three major steps, performing the statistically designed experiments, estimating the coefficients in a mathematical model, and predicting the response and checking the adequacy of the model[16].

Box-Behnken is a spherical, revolving design, viewed as a cube and consists of a central point and the middle points of the edges. The resulting designs are usually very efficient in terms of the number of required runs, and they are either rotatable or nearly rotatable. It has been applied for optimization of several chemical and physical processes[17,18]. This design is generally used for fitting the second order polynomial model as given by Equation 1.

$$q = b_0 + \sum_{i=1}^{n} b_i x_i + \left(\sum_{i=1}^{n} b_{ii} x_i\right)2 + \left(\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} b_{ij} x_i x_j\right) \quad (1)$$

where q is the predicted response, b_0 = constant coefficients, b_i is the slope or linear coefficients of the input factor x_i, b_{ii} is the linear by linear interaction coefficients or quadratic coefficients between the input factor xi and x_j, b_{ij} is the interaction coefficients of input factor x_i[19].

The most important parameters which affect the properties of developed film like chitosan percentage, acetic acid (crosslinking agent) percentage and the honey percentage has been selected based on the literature and previous knowledge[14,15]. In order to study the combined effect of these factors, experiments were performed at different combinations of the physical parameters using statistically designed experiments.

The intervals of all three independent variables have been selected based on the preliminary work. Chitosan percentage (0.5, 1, 1.5%), Acetic acid percentage (1, 1.5, 2%) and honey percentage (4, 6, 8%) in the film were kept as variable input parameters (factors) within the ranges defined Table 1. The factors levels were coded as –1 (low), 0 (central point) and +1 (high) [20]. Table 2 shows the input parameters and experimental design levels used in the present work.

A multiple regression analysis is done to obtain the coefficients and the equation can be used to predict

Table 1 —Variables and their levels used in the process

	Coded level		
Variable	+1	0	-1
Chitosan % (X_1)	0.5	1	1.5
Acetic acid % (X_2)	1	1.5	2
Honey % (X_3)	4	6	8

Table 2—Process conditions according to Box-Behnken method

Exp No	Chitosan	Acetic acid	Honey
1	0	1	-1
2	-1	0	-1
3	0	-1	1
4	1	0	1
5	-1	0	1
6	0	0	0
7	0	0	0
8	1	0	-1
9	1	1	0
10	1	-1	0
11	0	1	1
12	0	-1	-1
13	-1	1	0
14	-1	-1	0
15	0	0	0

the response. The design is preferred because relatively few experimental combinations of the variables are adequate to estimate potentially complex response functions. A total of 15 experiments were necessary to estimate the 10 coefficients of the model using multiple linear regression analysis. The experimental data was analyzed using the statistical software, Systat 13.1 trial version, for regression analysis to fit the equations developed and also for the evaluation of the statistical significance of the equations.

The analysed data results were optimized using the statistical software, Design Expert 8.1 trial version. The software searches the grid space and identifies the numerical values which yield the desirable response values and corresponding process conditions.

Characterization of film

Scanning electron microscope (SEM) Study

The morphology and surface topography of the film is examined by SEM. Spherical samples (5 mm to 2 mm) were mounted on the SEM sample stab using a double-sided sticking tape. The samples are coated with platinum (200 A°) under reduced pressure (0.001 torr) for 2 minutes using an ion sputtering device. The platinum-coated samples are observed under the SEM and photomicrographs of suitable magnifications are obtained. The specimens are scanned with an electron beam of 1.2 kV acceleration potential, and images are collected in secondary electron mode.

Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectra of chitosan is carried out using the KBr disc technique

Physical properties of the composite film

Tensile strength and % of elongation, thickness and weight, folding endurance, degradation percentage and fluid absorption capacity of the composite film were evaluated as mentioned by Sakchai Wittaya-areekul *et al.*[21] and Hima Bindu *et al.*[22].

Biological evaluation of wound dressing

Liquid culture test (Optical Density Measurements) BS EN ISO -14119, 2003

For the liquid culture test, each film was cut into squares (1cm × 1cm). Three sample squares were immersed in 20 mL nutrient broth (Merck, Germany) in a 25 mL universal bottle. The medium was inoculated with 200 µL of *Eschericha Coli / S.aureus* in its late exponential phase, and then transferred to an orbital shaker and rotated at 37°C at 200 r.p.m. The culture is sampled periodically (0, 2, 4, 8, 12, 24 hours) during the incubation to obtain microbial growth profiles. The same procedure was repeated for the control starch-based film. The optical density (O.D. 600) was measured at λ = 600nm using a spectrophotometer (Model UV-160, Shimadzu, Japan) [23].

Antimicrobial property- Agar diffusion test (JIS L1902-2002)

Plate Count Agar (PCA) plate was prepared and 100 µL of the selected dilutions of respective bacterial cultures were spread plated in duplicate. The wound care product with the diameter of 2cm ± 0.1cm is taken for the analysis. Both the sides of samples are pre-sterilized under ultra-violet radiation for 15 min. Sterile bacteriostasis agar is dispensed in sterile petri dishes. Broth cultures (24 h) of the test organisms are used as inoculums. Using sterile cotton swab, the test organisms (*Escherichia coli & Staphylococcus aureus*,) are swabbed over the surface of the agar plate. Pre-sterilized sample are placed over the swabbed agar surface by using sterile spatula and forceps. After placing the samples, all the plates are incubated at 37ºC for 18 to 24 h. After incubation, the plates are examined for the zone of bacterial inhibition around the fabric sample. The size of the clear zone is used to evaluate the inhibitory effect of the fabric[24, 25].

Results and Discussion

Statistical analysis of process parameter on the characteristic of wound dressing

Response surface methodology was applied to the experimental data using statistical software, SYSTAT 13.1 (trial version). Linear and second-order polynomials were fitted to the experimental data to obtain the regression equations. The design matrix of the variables in the actual units along with their yarn properties are given in Table 3.

Model building and statistical analysis

The empirical relationships for Fluid absorption capacity (Y_1), Degradation percentage (Y_2) and Folding endurance (Y_3) of the tested variables were obtained by application of Response Surface Methodology. The final mathematical model in terms of uncoded factors as determined by SYSTAT software is shown in Table 4. The regression co-efficient (R^2) value indicates a correlation of selected experimental region with the physical properties of the film. In general, the p values are used as a tool to check the significance of each co-efficient, which also indicates the interaction strength between each independent variable. Smaller the p values, bigger the significant difference[26] and R^2 – represents the proportion that the model can explain the variation in the response. The model with the $R^2 \geq 0.6$ (60%) can be considered as a valid model[27].

The goodness of the model can be checked by the determination coefficient R^2 and the adjusted R^2 (multiple correlation coefficient R). The value of R^2 of 0.689, 0.677 and 0.726 suggests that the total variation of 68.7% for fluid absorption capacity, 67.7 % for degradation percentage and 72.6% Folding endurance are attributed to the independent variables and only 31.3%, 32.3% and 28.4% of the total variation cannot be explained by the model respectively. The closer the value of adjusted R^2 to 1, the better is the correlation between the experimental and predicted values[28].

Effect on fluid absorption (FA) capacity

The influence of process parameters like chitosan percentage, acetic acid percentage and honey percentage on the fluid absorption capacity were performed and shown in contour plots Figure 1a) shows that the increase in the chitosan percentage has

Table 3—Experimental results for the selected design

Standard order	Chitosan %	Acetic acid %	Honey %	Fluid absorption capacity g/g	Degradation %	Folding endurance in number of times
1	1	1.5	6	1.062	33.33	164
2	1.5	1.5	8	1.5	50	174
3	1.5	1	6	1.326	33.33	152
4	1.5	2	6	1.1	72.91	178
5	0.5	1	6	1.27	50	165
6	1	1	4	1.322	71.42	176
7	1	1	8	1.3	62.16	167
8	1	2	8	1.326	62.16	164
9	1.5	1.5	4	1.1	56.25	170
10	1	1.5	6	1.27	33.33	179
11	0.5	1.5	8	1.05	56.25	170
12	1	2	4	1.13	71.42	176
13	1	1.5	6	1.2	56.25	168
14	0.5	1.5	4	1.05	40	160
15	0.5	2	6	1.27	66.67	162

Table 4—Development of Empirical model

Response	Equation	R	R^2	F- Ratio	P - value
Fluid absorption capacity	$Y1=2.533-0.043X_1-1.151X_2-0.184X_3-0.226X_1X_2 +0.100X_1X_3+ 0.055X_2X_3-0.061X_1^2+0.317X_2^2+0.003X_3^2$	83%	68.9%	1.228	0.432
Degradation percentage	$Y2=253+4.907X_1-194.383X_2-25.984X_3+22.910X_1X_2-5.625X_1X_3+2.750X_2X_3-2.815X_1^2+61.845X_2^2+2.590X_3^2$	82%	67%	1.162	0.458
Folding endurance	$Y3= 176.042+3.083X_1+3.500X_2-4.812X_3+29X_1X_2-1.5X_1X_3-0.750X_2X_3-16.667X_1^2-7.667X_2^2+0.583X_3^2$	88%	72.6%	0.619	0.465

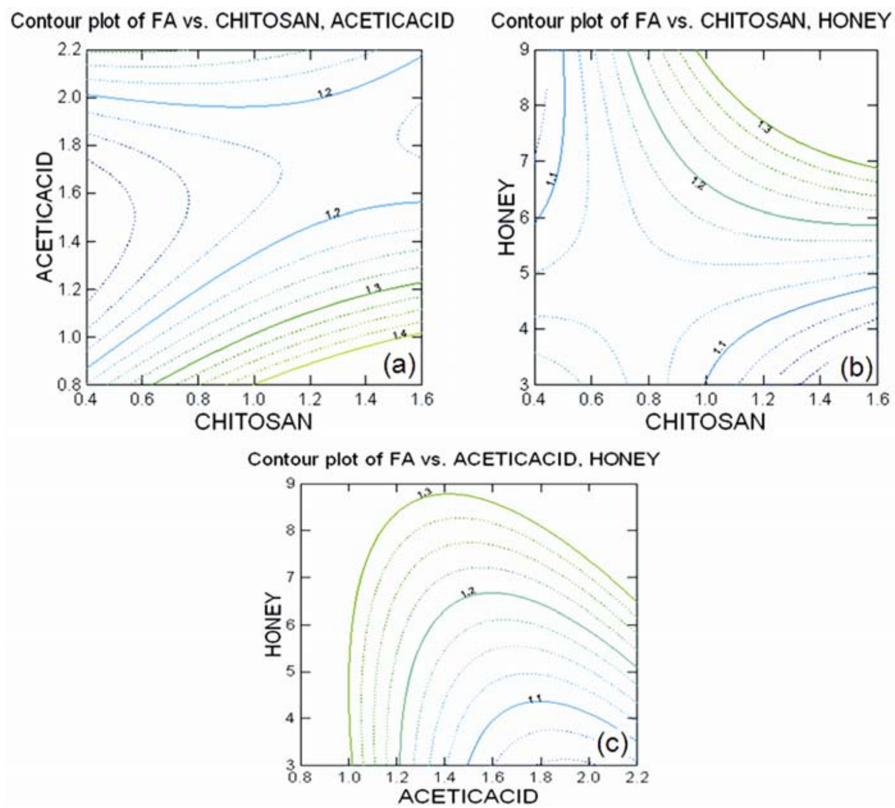

Fig. 1—Contour plots of (a) acetic acid with chitosan (b) honey with chitosan (c) honey with acetic acid on fluid absorption capacity of film

the direct influence on the fluid absorption capacity of the film. The contour plot shows that the fluid absorption capacity increases gradually from 1.1 g/g to 1.4 g/g during the chitosan incremental percentage due to the higher amorphous content in the chitosan material. The acetic acid was used as a cross-linking agent to improve the strength of the films. The increase in the acetic acid percentage decreases the fluid absorption percentage. This may be due to the cross-linking reaction of the acetic acid with chitosan. When the concentration of cross-linking agent increases the binding of the molecule by cross-linking process, it reduces the fluid absorption capacity of the developed film further it is also identified that higher percentage of cross-linking agent improves the brittleness of the film. These results were in line with the findings of Kienzle-Sterzer *et al.*, where they have reported the changes in mechanical properties of chitosan films with the type of acid solvent[29]. In their study, they estimated that acid type and concentration of chitosan may have affected both junction density and topological limitations in the films. This may have been due to the interactions between chitosan and acid solution. Bégin and Calsteren made antimicrobial films from chitosan with hydrochloric, acetic, lactic, and citric acids. They have reported that lactic and citric acid solutions formed softer films than did acetic acid solution with Young's moduli of 683 and 183 MPa, respectively, and stress at yield of 22.2 and 2.9 MPa, respectively[30].

But in the case of honey and chitosan interaction, the percentage of honey has no effect on the fluid absorption capacity of the film. It can be noted in the contour plot that after the addition of honey above 5%, there is a minimum amount of reduction the fluid absorption capacity. The amount of honey doesn't influence the fluid absorption. However, the percentage of honey is significant with respect to wound healing properties of the wound dressing.

Effect on degradation (D) of the film

For the evaluation of film degradation, the samples are immersed in solvents for different time periods using the area/volume ratio = 0.1 cm^{-1} , following each immersion the sample are carefully removed from the medium and weighed after drying at 40°C and constant final weight (Wf) being verified. The degradation index (Di) is calculated based on the mass loss. In Figs 1 and 2a, the increase in chitosan percentage reduces the degradation time after 1.2% of

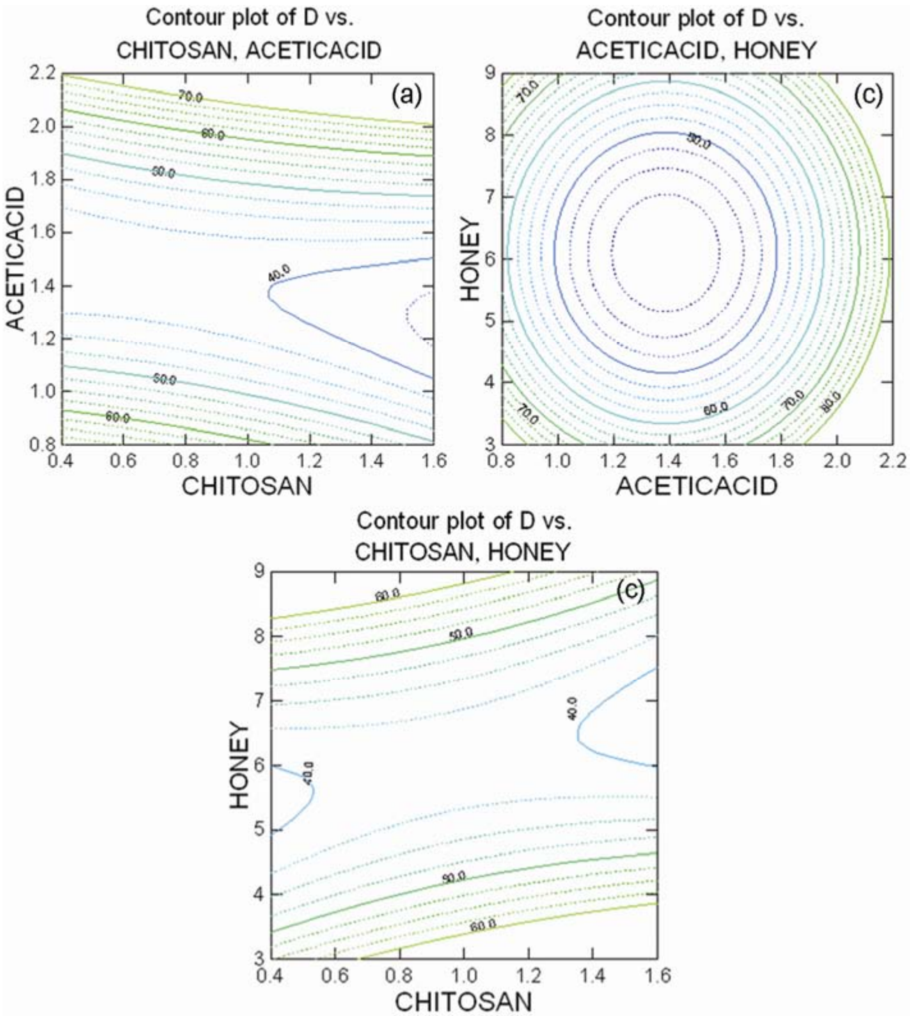

Fig. 2—Contour plots of (a) acetic acid with chitosan (b) honey with chitosan (c) honey with acetic acid on degradation capacity of film

concentration. After this, there is a reduction in degradation. In the case of acetic acid after 1.6% of the concentration, the film getting started degrades slowly. This phenomenon was noted as it increases along with the increment of the cross-linking agent. The addition of honey in film also increases the degradation percentage after 5%. This is may be because that, the addition of honey increases the fluid absorption after 5%. Hence, the fluid enters the film well; it degrades after a certain amount of honey percentage.

Effect on folding endurance (FE) of the film

The flexibility of film needed to handle the film easily and for comfortable, secured application of film on the wound. It is evaluated by repeatedly folding one film at same place till it breaks or folded upto 300 times manually. The number of times that the film withstands without breaking is the value of folding endurance.The contour plot Fig. 3a shows that the increase in the chitosan percentage has very less amount of influence on the strength or folding endurance of the film. But higher the acetic acid percentage gives greater the number of folding time. This is because the increment in acetic acid percentage binds the inter molecules of the chitosan film. But it is also observed that, the amount of acetic acid of the film increases and folding endurance gets reduced drastically as mentioned in Fig 3c. The increase in honey percentage has very less amount of increase in folding endurance but after 8% it slowly spoils the films endurance.

Numerical optimisation

The optimization of process parameter against the responses is performed by numerical optimization method, with the help of design expert software. The numerical optimization will search the design space,

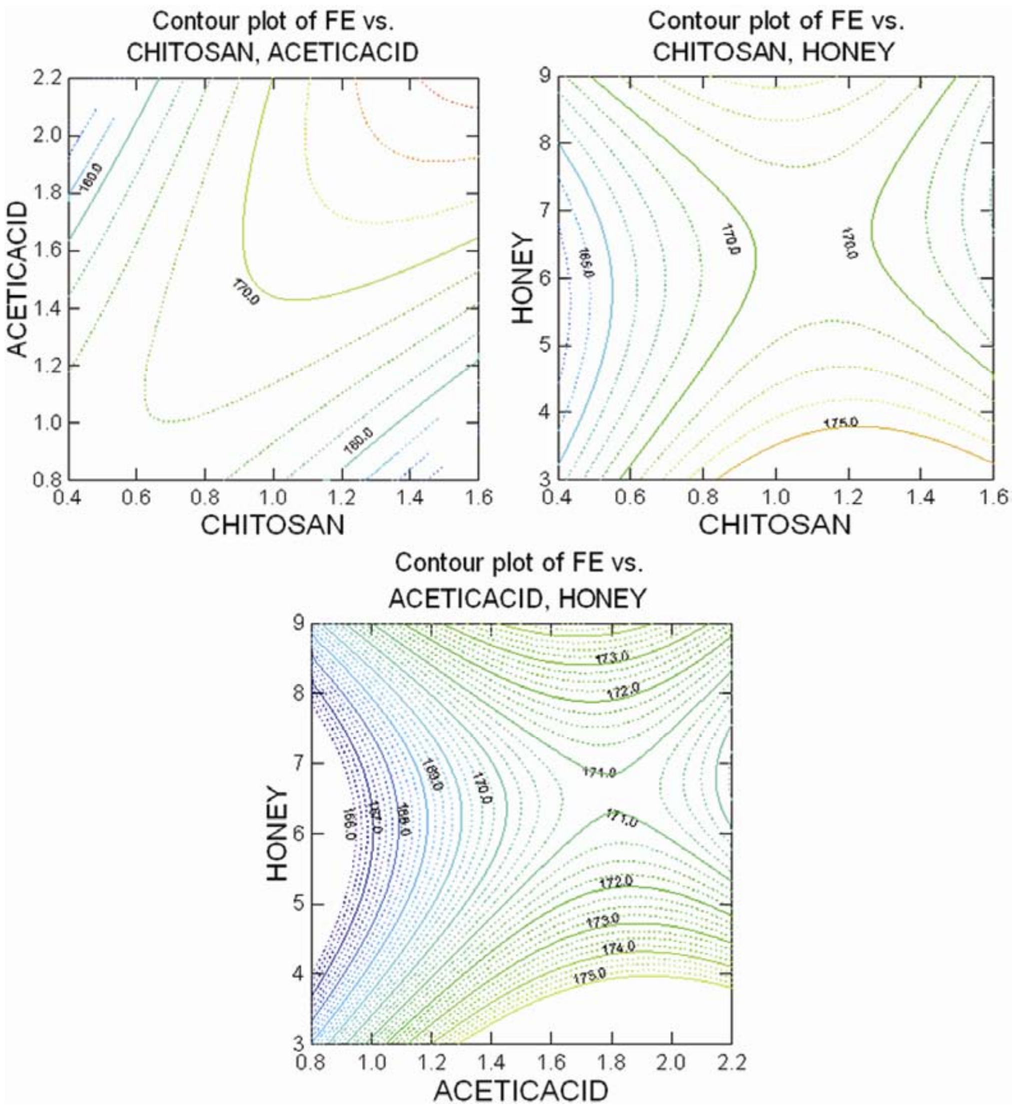

Fig. 3—Contour plots of (a) acetic acid with chitosan (b) honey with chitosan (c) honey with acetic acid on folding endurance of film

using the models created in the analysis to find factor settings that meet the goal. In this research achieving the improved fluid absorption capacity, higher folding endurance and less degradation percentage of the developed chitosan film for better handling purpose is the major objective. Based on the above-stated condition the numerical optimization analysis provides a set of top 10 combinations of the process parameters which yields improved physical properties (Table 5).

In all the selected response, the increment in the chitosan had positive results. The higher amount of chitosan helps to increase the fluid absorption capacity, reduced degradation percentage and improved folding endurance. Hence all the selected top ten experiment sets contains maximum amount of

Table 5—Numerically optimised top ten process parameter

CH	AA	H	FA	D	FE	Desirability
1.50	1.21	7.94	1.46377	41.2698	168.333	0.760746
1.50	1.21	7.93	1.46287	41.1845	168.333	0.760739
1.50	1.21	7.97	1.4656	41.4467	168.333	0.760728
1.50	1.22	7.93	1.46162	41.0722	168.333	0.760683
1.50	1.20	7.96	1.46607	41.4993	168.333	0.760666
1.50	1.22	8.00	1.46714	41.6056	168.333	0.760629
1.50	1.23	7.92	1.46062	40.9872	168.333	0.760603
1.50	1.20	7.99	1.46834	41.7215	168.333	0.760602
1.50	1.21	7.84	1.45698	40.648	168.333	0.760481
1.50	1.18	8.00	1.47058	41.9975	168.333	0.760122

chitosan percentage of 1.5%. In the case of acetic acid, the increment in acetic acid increases the cross-linking between chitosan and acid after a certain level increases the brittleness of the film and so it reduces the fluid absorption. Hence, an optimum of 1.21% was selected. Beyond that limit, the film loses its required property. Since honey percentage has very less effect on the selected response, the maximum of honey percentage selected to give better folding endurance. Higher the honey percentage is also good since it improves the antibacterial effect.

Characterisation of the optimized composite film

Physical properties

The optimized process parameters obtained from the numerical optimization were used to develop the better and improved chitosan-honey composite film. The physical parameters of the developed film were evaluated and compared with a plain chitosan film. The results were produced in Table 6.

The tensile strength and percentage of elongation at break of the film was tested in dry states and it was found that the tensile strength at break of the dry chitosan film is higher than the honey loaded composite film. However, the elongation percentage seems to be high in the case of honey loaded composite film. Higher the tensile strength of chitosan film may be due to the chitosan concentration which brings robustness to the films. In the case of honey added composite film, the addition of honey increased the elastic nature of the film a bit. In terms of weight and thickness, the composite film was higher than the chitosan film. The optimized parameters yielded improved folding endurance of 170 times which is higher than the predicted result. In the case of degradation percentage, the addition of honey improved the fluid handling capacity, which in turns helps the composite to degrade quicker than the chitosan film. All these factors are related with the reduction in the crystalline nature of the composite film than the chitosan film.

Surface morphology (SEM) analysis:

The surface morphology of the chitosan and chitosan honey composite film was studied using SEM. The scanning electron micrograph of the chitosan film revealed that the film is nonporous and the bit smooth texture. In the case of chitosan honey composite film, the surface was noted with irregularities and pores. This may be because of the addition of honey in the film. This indicates that the chitosan film was well crosslinked and brittle compared to the composite film. These result further supports the finding of optimisation process. The SEM photographs were provided in Fig. 4.

Figure 5 shows the FTIR spectroscopy of blended chitosan honey film. The characteristic peak of 1017.19 cm^{-1}, indicating the hydrogen bonded amide. The characteristic peak of chitosan was seen at 1742.48 and 1585.90 cm^{-1} corresponding to amide I and an amino group. C-H stretch region of FTIR spectrum, chitosan –honey shows the higher intensity

Table 6—The physical and mechanical properties of chitosan-honey composite film developed under optimized process condition

Film	Tensile strength (gf)	Elongation (%)	Thickness (mm)	Weight (gms)	Folding endurance (Nos)	Degradation %	Fluid absorption (g/g)
Chitosan	303.54	30.33	0.199	0.130	110	30	0.98
Chitosan honey composite	283.97	37.91	0.237	0.187	170	38	1.52

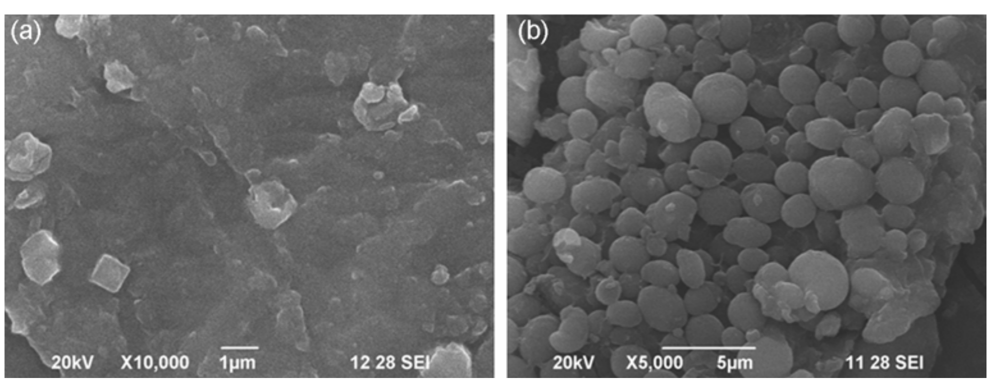

Fig. 4—SEM photographs of the a) chitosan film and b) chitosan honey composite film

peak at 2849.60 cm^{-1} is assigned to the asymmetric and the lower intensity peak at 2361.27 cm^{-1} is assigned to the symmetric modes of CH_2.Increase hydrogen bonding peaks 2920.67 cm^{-1} clearly suggest that honey compounds are efficiently bound by chitosan film through their –OH and $–NH_2$ functional groups.

Bio evaluation of chitosan honey film

Liquid culture test

Figure 6 shows the antimicrobial activity of chitosan honey film against *S.aureas* and *E.coli* in terms of OD value at 600 nm. The antimicrobial activities of chitosan-honey film were effective against Gram-positive bacteria than the Gram-negative bacteria. In fact, one of the reasons for the antimicrobial character of chitosan is because of the positively charge amino group interacts with negatively charged microbial cell membranes, leading to the leakage of proteinaceous and other intracellular constituents of the microorganisms[31]. The addition of Manuka honey also one of the reasons for the improved the overall antibacterial effect of the composite film.

Antimicrobial activity of composite dressing

The antibacterial activity of chitosan honey composite film and chitosan film were evaluated against *S.aureus* and *E.coli*. During the study the commercial soft cloth adhesive dressing was used as control sample. The results were provided in the Table 7. It is noted that the commercial cloth dressing has the maximum of inhibition against both gram positive and negative bacterial strains. With respect to the developed chitosan-honey loaded composite dressing, the zone of inhibition of 32 and 30mm was observed against *S.aureus* and *E.coli* respectively. But the chitosan film has lower zone of inhibition compared to the composite design. This can be

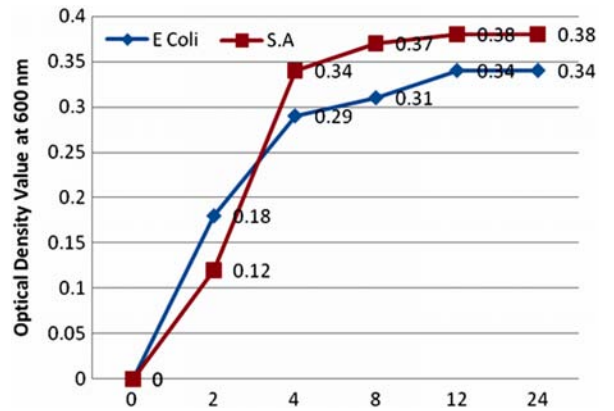

Fig. 6—Inhibition of control chitosan honey composite film against *E.coli* and *S.aureas*

Table 7—Antimicrobial activity of bilayer dressing

Sample	Particulars of bi-layer dressing	Zone of inhibition in (mm)	
		S.aureus	*E.coli*
Sample C	Pure chitosan film	28 mm	27 mm
Sample 1	Chitosan honey composite film (fixed on Non Woven)	32 mm	30mm
Control Dressing	3M soft cloth adhsive dressing-commercial product	38 mm	34 mm

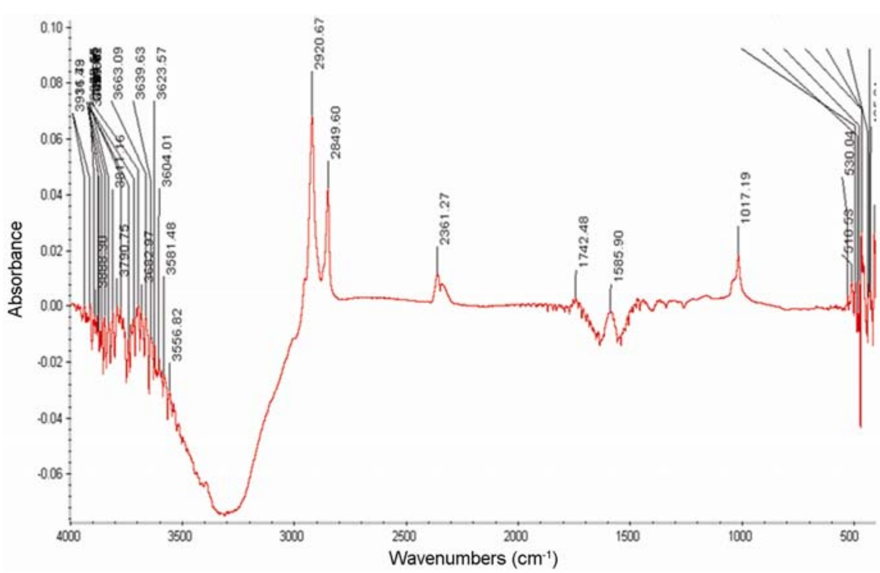

Fig. 5—FTIR – Spectrum of chitosan honey composite film

explained as the addition of honey might have improved the antibacterial activity compared to the chitosan film. This result suggests that even though the addition of honey increases the thickness and degradation percentage, the improved antibacterial activity will be one of the essential requirements.

Conclusion

The effect of process parameters like chitosan percentage, acetic acid concentration and the honey concentration percentage have been analysed on the yields like fluid absorption capacity, degradation percentage and folding endurance of the developed composite film. The results indicated that the increment in the acetic acid percentage brittles the film and improve the crystalline nature of the film. It ultimately leads to the reduction in the flexibility. The addition of honey in the chitosan film reduces the brittleness and improves the flexibility and so the degradation percentage. The chitoasn concentration of 1.5 %, acetic acid of 1.21% and Honey with 7.9% produced the film with optimum physical properties. A composite film was developed in the statistically arrived parameters and that film was characterized for wound dressing application. The results indicated that the developed composite film possess higher amount of folding endurance and fluid absorption capacity than the normal chitosan film. The liquid culture test and agar diffusion test results confirmed the higher potential of the chitosan honey composite film in the medical sector. However, the addition of honey increased the thickness of the film and reduced the mechanical strength considerably.

Reference

1 Sussman G, *Australian Doctor*, S1 (2001).
2 Ramzi S C, Vinay K and Stanley R, *Pathologic Basis of Diseases*, 5th Edn, (WB Saunders Company, Philadelphia), (1994) 86.
3 Strodtbeck F, *Nurs Rev*, 1 (2001) 43.
4 Niekraszewicz A, *Fibres Text East Eur*, 13(6) (2005) 16.
5 Wassila Arbia, Leila Arbia, Lydia Adour & Abdeltif Amrane, *Food Technol Biotechnol*, 51(1) (2013) 12.
6 Pillai C K S & Sharma C P, *Trends Biomater Artif Organs*, 22 (2009) 179.
7 Ravi Kumar M N V, *React & Funct Polym*, 1 (2000) 46.
8 Priwiya Peter S, Gopalakrishnan & Kannadasan T, *American J Engg Res*, 2 (5) (2013) 114.
9 Zumla A & Lulat A, *J R Soc Med*, 82 (1989) 384.
10 Osman O F, Mansour I S & El-Hakim S, *Annals of Burns and Fire Disasters*, XVI (3) (2003).
11 Subrahmanyam M, *Ann Burns Fire Disasters*, 20(3) (2007) 137.
12 http://www.manukahoney.com/
13 http://www.thehoneyfarm.com.au/acatalog/What_is_Manuka_Honey_.html
14 Rathinamoorthy R, Sasikala L & Parthiban M, *Colourage*, 59 (2012) 78.
15 Sasikala L, Bhaarathi Durai & Rathinamoorthy R, *Int J Pharm Tech Res*, 5 (4) (2013) 1774..
16 Box G E P & Draper N R, *J Am Stat Assoc*, 54 (1959) 622.
17 Tan I A W, Ahmad A L & Hameed B H, J *Hazard Mater*, 154 (2008) 337.
18 Hameed B H, Tan I A W & Ahmad A L, *J Hazard Mater*, 153 (2008) 324.
19 Box G E P & Hunter J, *Ann Math Statist*, 28 (1957) 195.
20 Rathinamoorthy R and Thilagavathi G, *Indian J Fib and Text Res*, 38 (2013) 293.
21 Sakchai W & Chureerat Prahsarn S, *AAPS Pharm Sci Tech*, E1 (2006).
22 Hima Bindu T V L, Vidyavathi M, Kavitha K & Sastry T P, *Int J App Biol Pharmaceut Tech*, 2 (1) (2011) 453.
23 BS EN 14119 - Testing of textiles. Evaluation of the action of microfungi, BSI, 24 (2003).
24 Yuan Gao & Robin Cranston, *Text Res J*, 78 (2008) 60.
25 Bartone F F & Adickes E D, *J Urol*, 140 (1988) 1134.
26 Cui F J, Li F, Xu Z H, Xu H Y, Sun K & Tao W Y, *Bioresour Technol*, 97 (2006) 1209.
27 Gong W J, Zhang Y P, Xu G R, Wei X J & Lee K P, *J Central South University Technology*, 14 (2) (2007) 196.
28 Fan G J, Han Y B, Gu Z X & Chen D M, *L W T*, 41 (2007) 155.
29 Kienzle-Sterzer C A, Rodriguez-Sanchez D & Rha C, *Makromol Chem*, 183 (1982) 1353.
30 Bégin A, Van Calsteren M R, *Int J Biol Macromolec*, 26 (1999) 63.
31 Shahidi F, Arachchi J K V &Jeon Y J, *Trends in Food Sci Tech*, 10 (2) (1999) 37.