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Synthesis, thermal and antimicrobial studies of chitosan/starch/poly(vinyl alcohol) ternary blend films

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ABSTRACT

The ternary blend films of chitosan/starch/PVA(poly vinyl alcohol) are prepared by solution casting method using glutaraldehyde as cross-linking agent and PEG (polyethylene glycol) as plasticizer. Films are prepared in the varying ratio of chitosan, starch and PVA. Fourier transform infrared (FTIR) spectroscopy is used to characterize and study the interaction present in blended materials. To study their thermal behavior, thermogravimetry (TG) and derivative thermogravimetry (DTG) under air and argon atmospheres have been applied from ambient temperature to 500°C at heating rate of 10°C min^{-1} and this study indicates that blended films are stable upto 190°C, excellent for food packaging. The antimicrobial activities of these films are tested against three pathogenic microorganisms Bacillus subtilis, Pseudomonas aeruginosa and Candida albicans against the measurement of clear zone diameter. These films show fair activity against Bacillus subtilis. Therefore such films can be used to prolong shelf life of food.

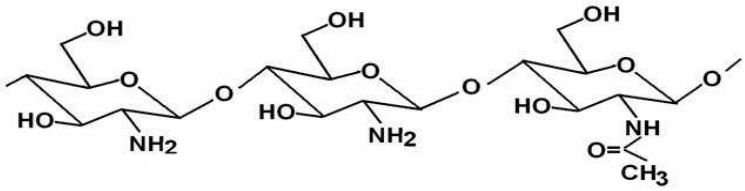
Key words: Chitosan, Blending films, TGA, Antimicrobial activity.

INTRODUCTION

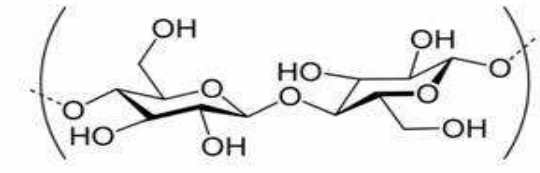
Chitin (β-(1-4)-2-acetamido-2-deoxy-D-glucopyrnaose) is second most abundant natural biopolymer generally obtained from exoskeletons of crustaceans such as crabs, shrimp, lobsters and krill. The most important derivative of chitin is chitosan ((1, 4)-2-amino-2-deoxy- β -D-glucose) obtained by excessive deacetylation of chitin with alkali [1]. In present time food packaging is serious problem for scientists to protect and preserve all type of foods from dehydration and mainly from microbial spoilage. Synthetic and non-biodegradable materials are generally used for food packaging that can create serious problem to the environment. This problem can be controlled by using environmental friendly packaging material based on natural

biopolymers [2, 3]. So, we have chosen chitosan, starch and PVA, ecofriendly materials for film formation, which can be used for active packaging.

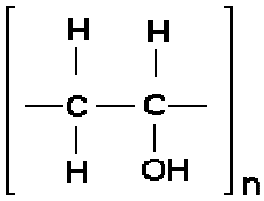
There is considerable research on synthesis of edible films for food packaging using natural biopolymers and expanding their uses as biomaterials. Chitosan is non-toxic, biodegradable and biocompatible having good-film forming ability and antimicrobial activity [4]. Due to these properties chitosan have many pharmaceutical applications and generally used as natural preservative for the safety of food [5, 6]. PVA is non-toxic biodegradable and water-soluble synthetic polymer and has been used on large scale in biomaterial application. Since, it has large number of hydroxyl groups that helps in formation of H-bonding with amino and hydroxyl group of chitosan [7]. Starch is naturally occurring biopolymer mainly composed of two homopolymers amylase and amylopectin, which are attractive raw materials having low cost and renerability used to produce biodegradable films for packaging materials [8]. Chemical structure of chitosan, starch and PVA (poly vinyl alcohol) are shown in Fig.1. PEG (polyethylene glycol) was used as plasticizer to increase the flexibility of chitosan films and facilitate the polymer processing. Several studies have shown that chitosan films plasticized by PEG increase the elastic properties and stability of films [9]. Glutaraldehyde has been used as cross-linking agent in chitosan films [10]. From the structure of chitosan it is clear that it has many functional groups and can be used as blending material with other polymer compounds to be useful for many applications. It is also reported that chitosan form clear and homogenous blends with PVA [11, 12]. Binary blend films of chitosan with starch, PVA and polyurethanes are already studied [8, 10, 13, 14] but study of ternary blends of chitosan/starch/poly (vinyl alcohol) is still rare. Thermo gravimetric analysis (TGA) and derivative thermogravimetry (DTG) are the techniques to investigate the thermal behaviour of substances as a function of mass loss with heat. These techniques are widely used to investigate the thermal stability and thermal decomposition behavior of polymers in different conditions [15-17]. TGA studies of these blended films were performed in atmospheres of air and argon. The *in vitro* antimicrobial activities of blended films were evaluated on pathogenic microorganisms such as *Bacillus subtilis, Pseudomonas aeruginosa* and *Candida albicans.*



Chitosan



Starch



PVA

Fig. 1 Chemical structures of chitosan, starch and PVA

Main objective of this study was to prepare, characterize and undergo antimicrobial study of chitosan/ starch/poly (vinyl alcohol) ternary blends films and also to check out their thermal stability so that they might be used in food packaging at higher temperature.

MATERIALS AND METHODS

Chitosan (molecular mass 100000-300000 and 89% deacetylation calculated by ^{1}H NMR spectroscopy [18]) was purchased from Acros Organic USA. Starch, PVA and PEG (molecular mass 4000) were obtained from Himedia Lab. India. Glutaraldehyde (25% solution in water) and glacial acetic acid was purchased from Rankem India. Three microbial strains, one Gram-positive bacteria (*Bacillus subtilis* MTCC 121); one Gram-negative bacteria (*Pseudomonas aeruginosa* MTCC 741) and one fungi, *Candida albicans* (MTCC 227) were screened for evaluation of antibacterial and antifungal activity of the films. All the microbial cultures were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh. The bacteria were subcultured on Nutrient agar whereas fungi on Malt yeast agar

Preparation of chitosan/starch/PVA ternary blend films

Chitosan solution 2wt%(w/v) was obtained by adding 10g chitosan into a 500ml of 0.1M acetic acid followed by stirring and heating at 60°C up to 4hr. The solution was filtered to remove dust and other impurities. 2wt% (w/v) solution of PVA and starch were prepared at 80-90°C using distilled water. Similarly 2wt% (w/v) solution of PEG and glutaraldehyde were prepared in distilled water.

Table 1. The different compositions of the chitosan, starch and PVA ternary blends

Films	Chitosan (wt%)	Starch (wt%)	PVA (wt%)
CSP-1	100	0	0
CSP-2	90	5	5
CSP-3	80	10	10
CSP-4	70	15	15
CSP-5	60	20	20
CSP-6	50	25	25

Chitosan, starch and PVA solutions were mixed in different composition (Table 1) and continuously stirred for 15min at 95°C. Then glutaraldehyde solution was added in the above mixture in 1:1 molar ratio with NH_2 group of chitosan. PEG solution used was 25% of total mixture and equal volume of PEG solution has been taken for all films. After adding the PEG solution the mixture was further stirred for 20min at same temperature. The mixtures were

filtered, degassed under vacuum and casted onto polypropylene Petri dish. The casted films were dried at 80°C overnight and then dried under vacuum for 24hr at 70°C to remove last traces of water and acetic acid. Films were peeled off and stored in desiccator.

Instrumentation

FTIR spectra of blended films were recorded on ABB spectrophotometer. TGA study was performed using Perkin Elmer diamond TG/DTA analyzer. Dried alumina powder was used as reference material for taking the thermograms. Samples were heated from ambient temperature to 500°C at heating rate of 10°Cmin^{-1} in air and argon atmosphere at flow rate of 20ml min^{-1}.

RESULTS AND DISCUSSION

FTIR spectroscopy

FTIR spectroscopy was used to study the interaction between the chitosan and other chemical used in the film formation. In the FTIR spectra of pure chitosan bands appeared at 895 and 1026 cm^{-1} was mainly due to saccharide group [19] and band at 2924 cm^{-1} corresponded to C-H stretching. The band at 1589 cm^{-1} (-NH_2 vibration), weak band at 1655 cm^{-1} (amide band) and broad band in the region 3250-3400 cm^{-1} (N-H and OH stretching) are the characteristics of chitosan. In the FTIR spectrum of films, band at 1650 cm^{-1} (amide group of chitosan, OH groups of polysaccharides and water molecules [20]) and bands around 3400-3650cm^{-1} were due to the N-H and OH stretching. In the chitosan films peak at 1730 cm^{-1} was characteristic of carboxylic acid dimmer formed due to acetic acid used for dissolving the chitosan [21] and peak at 1695 cm^{-1} was probably due to presence of carbonyl group of glutardehyde that interact with hydroxyl groups of starch and PVA through hydrogen bonding. A sharp peak in all composite films at 1520 cm^{-1} was mainly due to (C=N) group formed by combination of NH_2 group of chitosan and carbonyl group of glutaraldehyde by nucleophilic addition reaction and simultaneously decrease in content of amine (-NH_2) vibration band. The changes in the characteristic peaks replicate the chemical interaction between blended materials [22, 23]. In the spectra of films, amide band of chitosan shifted from 1655 cm^{-1} to 1650 cm^{-1}, change in band due to N-H and OH stretching and new peaks around 1730, 1695 and 1520 cm^{-1} in all films. These results indicated that the cross-linking were present in blended compounds through hydrogen bonding and covalent bond formation, preferably with amine group of chitosan and glutaraldehyde.

Thermogravimetric analysis

TG curves of chitosan and its blended films in different atmospheres are shown in Fig. 2(a-h). Mass loses in different stages with corresponding temperature range and DTG maximum are summarized in table 2. The moisture vaporization causes a mass loss of 2-5% in chitosan and 8-9% in films. Chitosan have a large mass loss of about 39-42% between 250-340°C in argon and at 220-330°C in air atmosphere. This mass loss is mainly due to thermal degradation of polymeric chain with vaporization of voltaic compounds and cross linking reaction occurring with destruction of amino group [24]. Chitosan films show a large mass loss of about 35-38% in argon and mass loss of about 43-48% in air atmospheres in between 190-380°C is due to thermal degradation of chitosan, starch, PVA and PEG. Second mass loss occurs in between 380-440°C is due to degradation of residual cross-linked chitosan [25] and by products generated by PVA during the thermal degradation process [26]. Chitosan have higher mass loss in air atmosphere as

compared with argon atmosphere in between 440-500°C is mainly due to oxidation of residual mass.

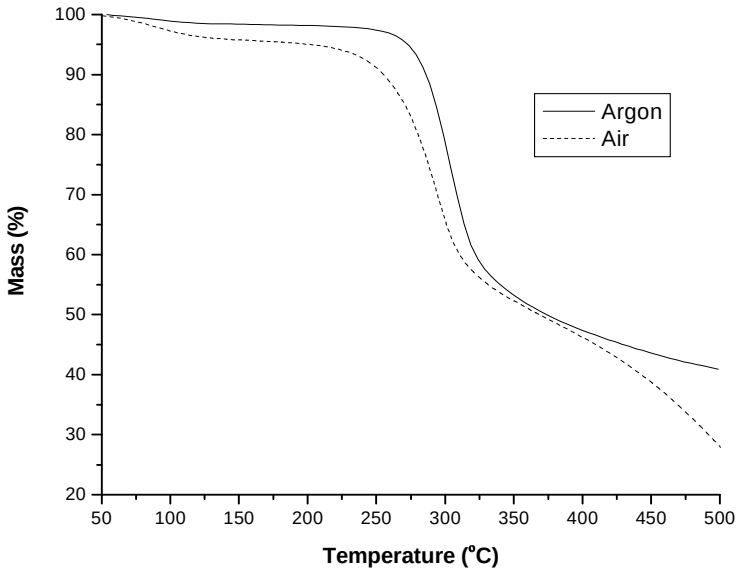


Fig. 2a TG curves of chitosan at heating rate of 10°C min^{-1}

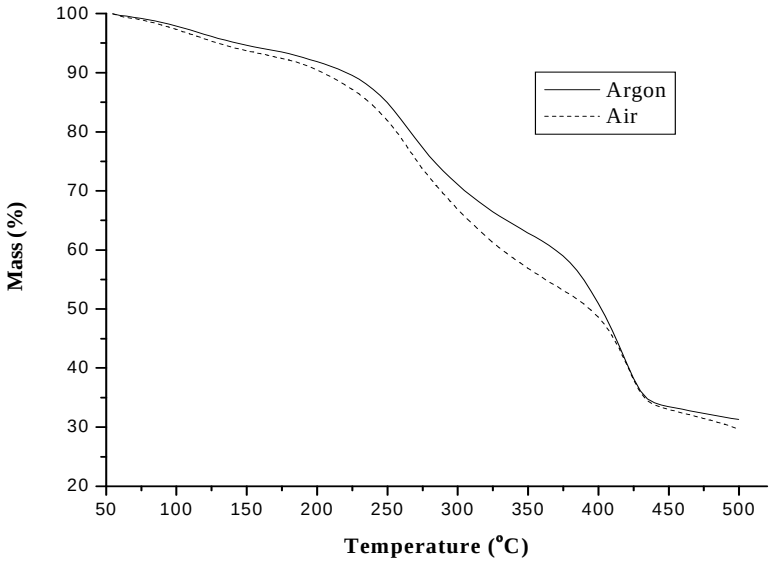


Fig. 2b TG curves of film CSP-1 at heating rate of 10°C min^{-1}

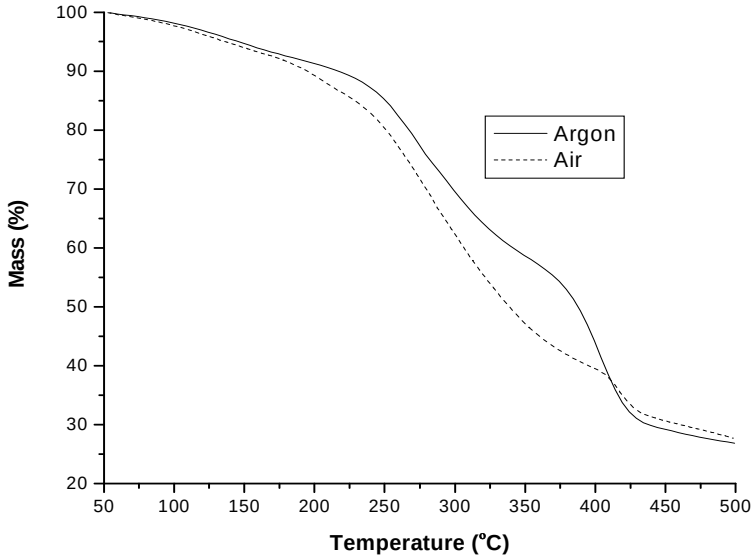


Fig. 2c TG curves of film CSP-2 at heating rate of 10°C min^{-1}

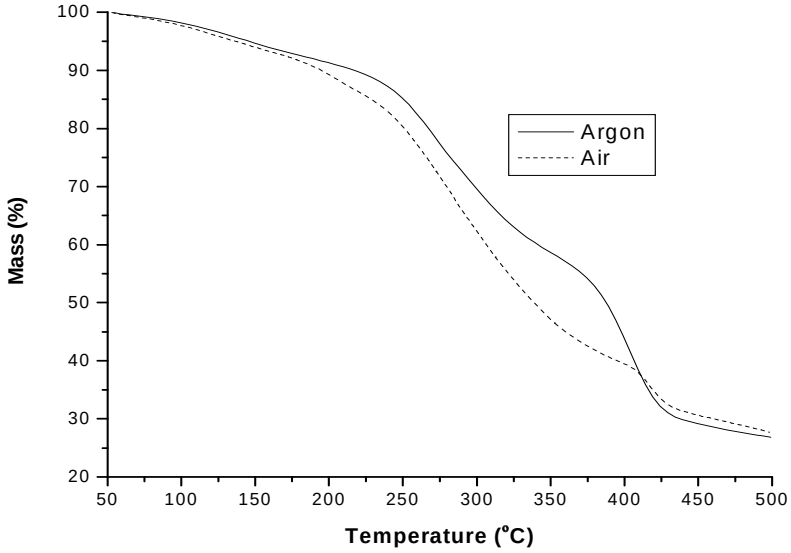


Fig. 2d TG curves of film CSP-3 at heating rate of 10°C min^{-1}

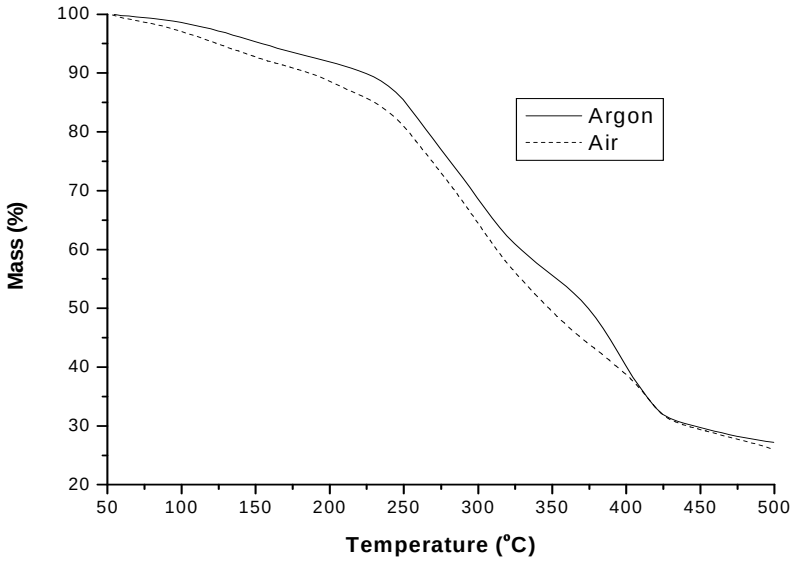


Fig. 2e TG curves of film CSP-4 at heating rate of 10°C min^{-1}

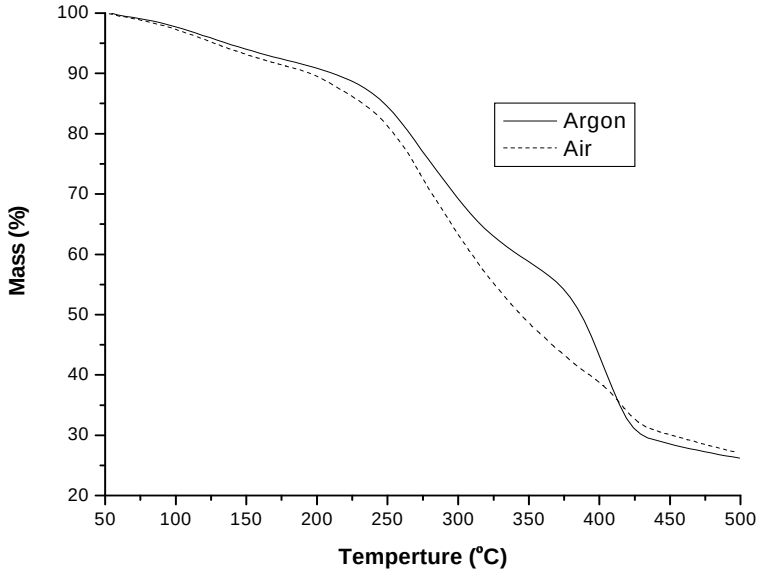


Fig. 2f TG curves of film CSP-5 at heating rate of 10°C min^{-1}

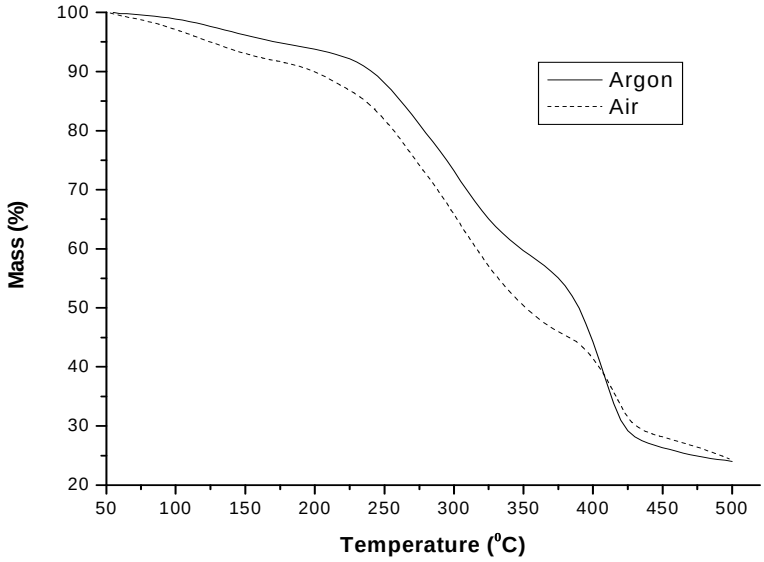


Fig. 2g TG curves of film CSP-6 at heating rate of 10°C min^{-1}

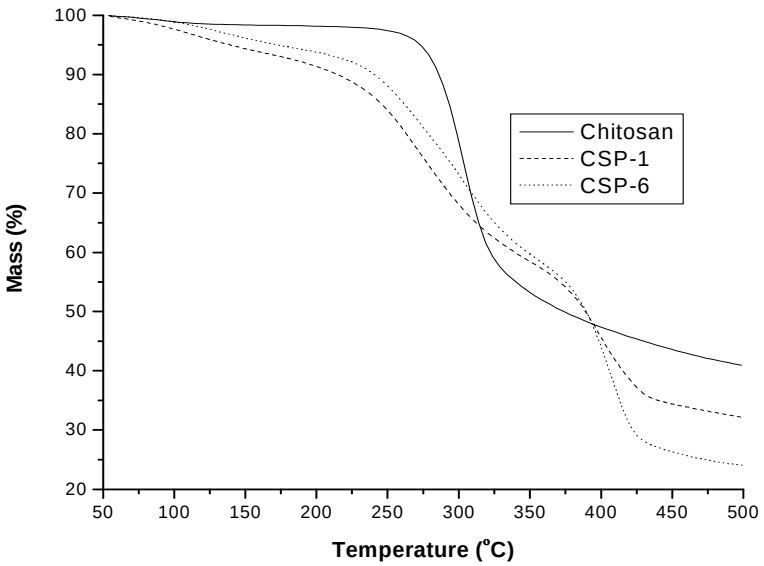


Fig. 2h TG curves of films CSP-1, CSP-6 and chitosan in argon atmosphere at heating rate of 10°Cmin^{-1}

Table 2 Data taken from TG/DTG curves of chitosan and its blended films under argon and air atmosphere

Compounds	Argon			Air		
	Temperature Range (°C)	Mass loss or (residue) %	DTG maxima(°C)	Temperature Range (°C)	Mass loss or (residue) %	DTG maxima(°C)
Chitosan	55-250 250-340 340-500 >500	2.5 42.3 14.3 (41.2)	302.0	55-220 220-330 330-500 >500	5.3 39.1 27.2 (28.5)	294.8
CSP-1	55-190 190-360 360-440 440-500 >500	7.7 35.1 22.5 2.9 (32.1)	271.2 401.2	55-185 185-380 380-440 440-500 >500	8.9 45.6 11.2 4.3 (29.5)	269.0 419.9
CSP-2	55-185 185-360 360-440 440-500 >500	7.0 31.3 28.0 2.8 (31.3)	261.7 418.8	55-180 180-380 380-440 440-500 >500	7.6 43.2 15.8 4.1 (29.6)	262.9 421.5
CSP-3	55-190 190-370 370-440 440-500 >500	7.9 36.6 26.0 2.9 (26.8)	271.8 403.9	55-185 185-380 380-440 440-500 >500	8.6 47.6 12.9 3.7 (27.5)	287.1 415.2
CSP-4	55-190 190-370 370-440 440-500 >500	7.3 38.3 24.5 3.2 (27.1)	302.8 397.9	55-180 180-380 380-440 440-500 >500	8.7 47.6 12.7 4.1 (26.4)	302.6 415.0
CSP-5	55-190 190-375 375-440 440-500 >500	8.3 37.4 24.9 3.0 (26.9)	269.5 403.5	55-185 185-380 380-440 440-500 >500	8.9 48.7 12.2 3.8 (26.2)	268.3 416.1
CSP-6	55-190 190-365 365-440 440-500 >500	5.7 37.1 30.0 3.0 (24.0)	304.3 408.4	55-185 185-380 380-440 440-650 >500	8.5 45.8 16.4 4.6 (24.1)	306.7 419.2

Antibacterial activity

All the blended films were screened for their antibacterial and antifungal activities. Out of six, first four (CSP 1-4) films showed fair antibacterial activity against Gram-positive (*B. subtilis*) bacteria while none of the tested film exhibits any antibacterial activity against Gram negative (*P. aeruginosa)* bacteria. All the tested films were unable to exhibit any antifungal activity against *C. albicans*. In blended films chitosan was in solid state so, only those organisms were inhibited which were in direct contact of active sites of chitosan because it was not diffused through agar media [27, 28]. Inhibitory effects of these films against *B. subtilis* are shown in Fig. 3(a-f). The inhibitory effect was measured based on clear zone surrounding circular filmstrips. Measurement of clear zone diameter included diameter of filmstrips, therefore, the values were always higher than the diameter of filmstrips whenever clearing zone was present. If there is no clear zone surrounding, we assumed that there is no inhibitory zone, and furthermore, the

diameter was valued as zero. The method commonly used to examine antimicrobial activity is agar diffusion test regarding the diffusion of the compound tested through agar plate. The diffusion changes with the size, shape and polarity of the diffusion material [27].

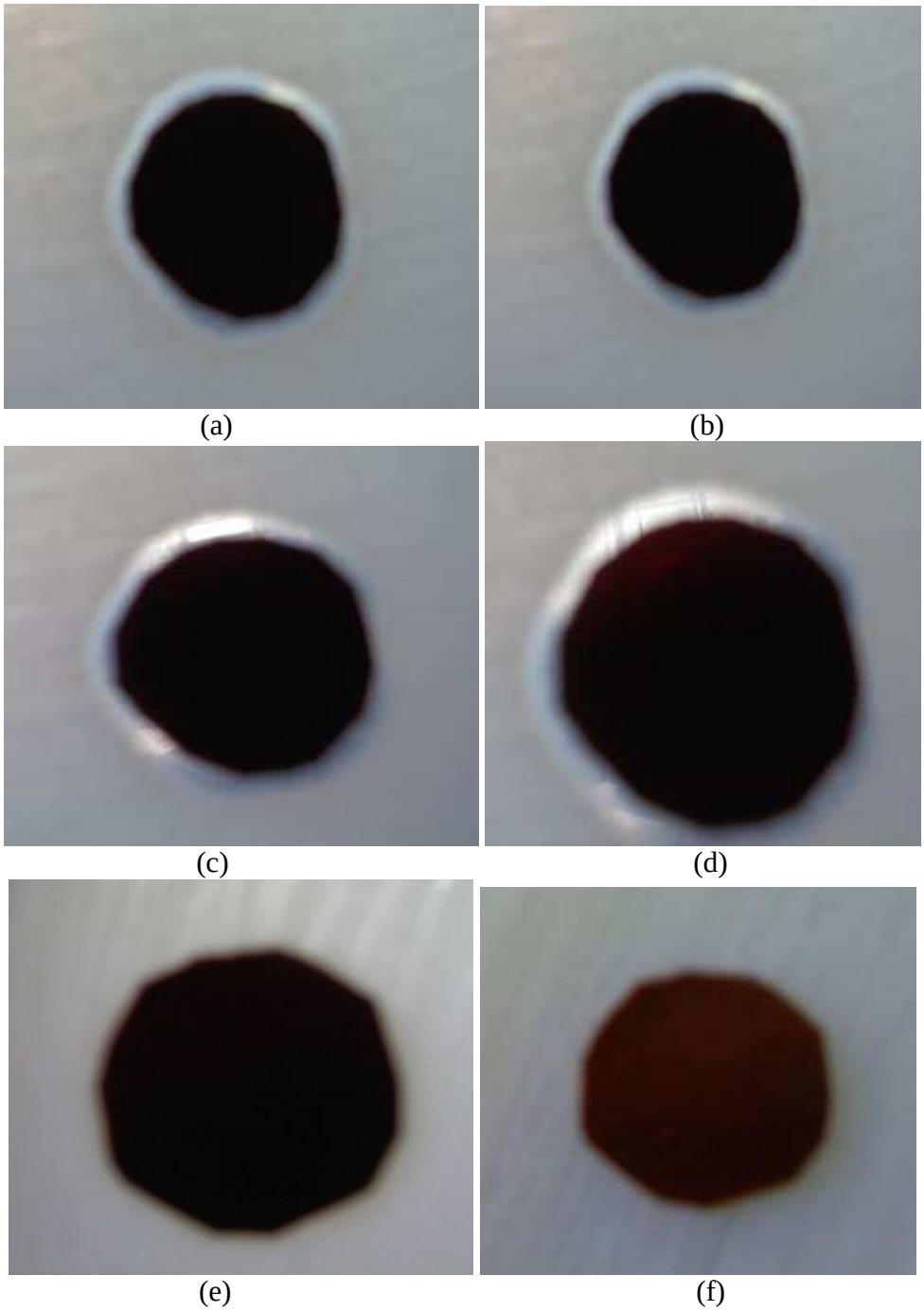


Fig. 3. Inhibitory effect of chitosan/starch/poly(vinyl alcohol) ternary blend films
(a)CSP-1, (b) CSP-2, (c) CSP-3, (d) CSP-4, (e) CSP-5, (f) CSP-6 against B. subtilis.

CONCLUSION

Glutaraldehyde was superior binding agents for the preparation of ternary blended films of chitosan/starch/PVA. The FTIR results showed that there was physical and chemical interaction present between the used materials. All the blended films are thermally stable up to 190°C that are reasonably good in food packaging application. Four films (CSP1-4) displayed fair activity against Gram-positive (*B. subtilis*) bacteria. However, none of the tested films showed any activity against the tested Gram-negative bacteria and fungi. Thermal and antimicrobial study reveals that these ternary blend films might be used as potential materials in food packaging.

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