INH IMPREGNATED CHITOSAN-PVA COMPOSITE FILMS: EVALUATION OF RELEASE PROFILE

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Abstract: Composite films of chitosan and polyvinyl alcohol (PVA) cross-linked with glutaraldehyde, impregnated with antimicrobial drug isonicotinic acid anhydrides (INH) were successfully prepared using a solvent casting method. The effect of composition on the property of several composite films formulations using different ratios of chitosan obtain from crab shell and PVA containing INH were studied. The films were evaluated for tensile strength, swelling ability (water uptake capacity) and *In-vitro* drug release profile. The films were further characterized with FTIR. Fourier transform infrared spectroscopy (FTIR) studies revealed a broad band at 3286.34 cm^{-1} (OH) indicative of intermolecular hydrogen bonding between chitosan and PVA. The films were found to exhibit good tensile strength, flexibility and water uptake capacity relative to the concentration of PVA and cross-linker. Drug release studies via UV spectroscopic analysis shows that the observed rate involves diffusion process which is dependent on the film morphology with potential application as vehicles for drug delivery systems and wound dressing materials.

Keywords: Chitosan, composite, drug-release., films, INH, PVA

Introduction

The need to develop new biomaterials with film-forming ability for drug delivery has attracted the attention of researchers to polymer blend/composite. Polymer blend/composite consists of physical mixture of two or more polymers with or without chemical bonding between them. A composite have superior and controllable properties that are different from the component homopolymer. Blends of synthetic and natural polymers combine the good mechanical properties, easy of transformation to variety of different shapes and low production costs of synthetic polymers (Sahoo *et al*., 2010) with the good biocompatibility of natural polymers (Sahoo *et al*., 2011). Poly (N-vinyl-2-pyrrolidone)-kappacarrageenan (PVP/KC), poly(N-vinyl-2-pyrrolidone)-iota-carrageenan (PVP/IC) (Relleve *et al*., 1999), poly (ethylene oxide)-hydroxypropyl methylcellulose (PEO/HPMC) (Sawatari and Kondo, 1999), chitosan and poly(ethylene oxide) (PEO) (Abdul Kadir *et al*., 2009), poly(vinyl alcohol)-chitosan (PVA/C) etc (Abraham *et al*., 2016), are polymeric materials based on the blends of synthetic and natural polymers that have previously prepared.

Polysaccharide derivatives such as cellulose esters (Edgar, 2007), chitosan (Percot *et al*., 2003; Koide, 1998; Nagahama *et al*., 2008) e.t.c are useful natural polymers for drug delivery. They are generally nontoxic, are not absorbed from the gastrointestinal (GI) tract, and can be readily modified to enhance properties critical for drug delivery.

Chitosan (poly-β-(1 → 4) N-acetyl-D glucosamine), is the N-deacetylated derivative of chitin that has been employed in various fields such as the edible film industry (Ansorena *et al*., 2011), as additives to enhance nutritional quality of foods (Chatterjee *et al*., 2008; Kanauchi *et al*., 1994), for the recovery of solid materials from food processing wastes, as separators in medicine and biotechnology (Aiba *et al*., 1986) and in the purification of water (Bhatnagar and Sillanpää, 2009; Camci-Unal and Pohl, 2009) and more recently for preparation of drug-loaded films for wound dressing owning to its film forming properties, homeostasis, biodegradability, biocompatibility, antimicrobial and wound healing activity and its ability to absorb exudates Alsarra, 2009; Wang *et al*., 2007).

Poly vinyl alcohol (PVA), a semi crystalline synthetic polymer with carbon chain backbone and hydroxyl groups attached to the methane carbons (Rajendran and Mahendran, 2001), is highly hydrophilic, biocompatible, and low cost polymer with good mechanical strength, thermal stability, chemical stability and excellent film forming properties (Yang and Wu, 2009). PVA membranes exhibits minimal cell adhesion and protein absorption, hence has found application in the biomedical field (Koyano *et al*., 2000). Chitosan contains hydroxyl and amine groups which can interact with PVA. Some aspects, of their blend properties have been studied (Goel *et al*., 2008). Cross-linking treatment improves the performance of chitosan/PVA films.

The aim of this work is to describe a facile approach to obtain stable, good sorption and cost effective Chitosan/PVA impregnated with INH (antimicrobial agent) which can accelerate wound healing processes by preventing fluid loss and bacterial infection.

Materials and Methods

Materials

Crab shell was used source of chitin/chitosan. Other materials used include acetic acid, sodium hydroxide (NaOH), polyvinyl alcohol (PVA), distilled water, glutaraldehyde and glass slides.

Synthesis of chitosan

Extraction of chitin

Crab shells obtained from Lagos Lagoon were washed, dried, crushed and pulverized to fine powder. The pulverized samples were then demineralized using HCl. Briefly; 25 g of pulverized sample was soaked for 24 h in HCl (100 ml, 0.01M) to remove calcium carbonate. The resulting demineralized sample was boiled in NaOH (50mls, 0.02M) for 1 h to decompose the albumen into water soluble amino acid. The obtained chitin was washed with de-ionized water and air dried. The chitin was further converted to chitosan by the deacetylation process.

Deacetylation of chitin to chitosan

Chitin (20 g) was deacetylated to chitosan by refluxing in 40% sodium hydroxide (80 ml) at 100ºC for two hours using a hot plate. The suspension obtained was allowed to cool and stand for 30 min before decanting the supernatant. The residue wet chitosan was washed with distilled water by steeping and decantation process until neutral (pH 7), filtered, dried in the oven and stored in an air tight container in a dark place.

Preparation of composite films

Chitosan-PVA composite films were prepared by mixing aqueous PVA solution (10%, w/v) with chitosan solution (1%, w/v in acetic acid) at ratios (1:1, 1:2, 1:3, and 1:5). In a typical polymer blend, 1:1 mixture of the polymers were stirred thoroughly using the magnetic stirrer. The resulting solution was kept for an hour to remove bubbles, casted on a clean dry glass slide in a dust free atmosphere at room temperature and allowed to dry for 3 days. The dried films were carefully removed from the glass slides and stored in a well closed, dust-free container for further analysis. Glutaraldehyde cross-linked Chitosan-PVA Films were prepared using similar procedure except that 0.5 ml of glutaraldehyde was added to each mixture prior to stirring.

INH impregnated Chitosan- PVA composite films and Glutaraldehyde cross-linked INH impregnated Chitosan-PVA Films were also prepared. Typically, 74mg of antimicrobial drug isonicotinic acid anhydrides (INH) was added while stirring the polymer mixtures of chitosan, PVA or Chitosan, PVA and Glutaraldehyde respectively prepared using the procedure above. The resulting films known as isoniazid loaded films were used for the release studies.

Characterization of films

The prepared films were characterized with Fourier Transform Infrared spectroscopy (FTIR) recorded using BRUKER (vector 22) spectrophotometer over the range of approximately 4000-400 cm^{-1}. The tensile strength study was carried out using silver weights. The tensile strength and elongation at break were calculated as follows (10):

Tensile strength (N/mm^2) = Breaking force (N)/Cross-sectional area of sample (mm^2)

Elongation at break (%) = [Increasing in length at breaking point (mm)/original length (mm)] × 100

The swelling ability was measured by gravimetric method. The completely dried films (7 × 2 cm) were weight, then submerge in 20 ml distilled water at room temperature and the resultant swollen films removed at different time interval were weighed immediately after carefully removing excess surface water by blotting with a tissue paper. The specimens were returned to the distilled water immediately after weighing. The swelling ratio Z was calculated using the formula given below.

$$Z = \frac{X2}{X1}$$

Where: X1 and X2 are the dry and swollen sample weights respectively. The swelling degree of the film is the increase in weight.

InVitro Drug Release

In-vitro drug release study was carried out by immersing the films in distilled water in a beaker. The amount of INH release from the drug loaded films was evaluated using the calibration curves, constructed from the reference standards by analyzing films in $T80^+$ UV-Vis spectrophotometer at 262 nm.

Results and Discussion

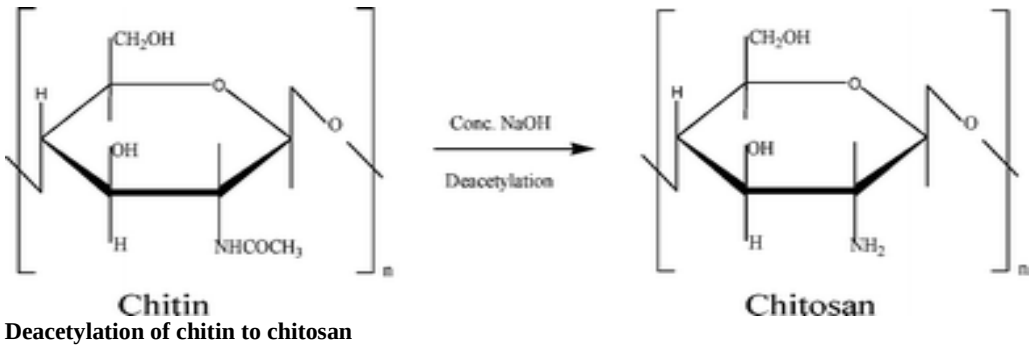
Extraction of Chitin and conversion to Chitosan

Chitin was obtained from crab shell shown in Fig 1a through washing, drying, pulverization, demineralization, deproteinization, washing and drying. Chitin (Fig 1c) was deacetylated (Scheme 1), washed and dried to obtain chitosan (Fig 1d).

Preparation of composite films

Different formulation ratios (1:1, 1:2, 1:3 and 1:5) of free and INH loaded films of chitosan-PVA and chitosan-PVA-glutaraldehyde were prepared. The precursor solutions were mixed, stirred and allowed to stand for 60 min. The solutions were readily miscible probably due to the hydrophilic groups present on the surface of the precursors which interact through hydrogen bonding (Scheme 2) to form a gel network (Zhang *et al*., 2007). The resulting solution which did not show any layer separation was casted and allowed to stay for 72 h in a dust free environment (Fig 2). The films were carefully removed, labeled and stored. The drug (INH) loaded films were prepared by adding the drug to the mixed precursor solution followed by stirring and casting on a glass slides. The prepared composites of chitosan-PVA films were clear and transparent while that of chitosan-PVA-glutaraldehyde were cloudy and opaque. The films were stable i.e. there were no microbial growths or fungal development on the films on storage. Physically, there was no difference between the loaded and unloaded films.

Fig. 1: (a) Crab Shell (b) Pulverized crab shell (c) Chitin (d) Chitosan



Scheme 1: Deacetylation of chitin to chitosan

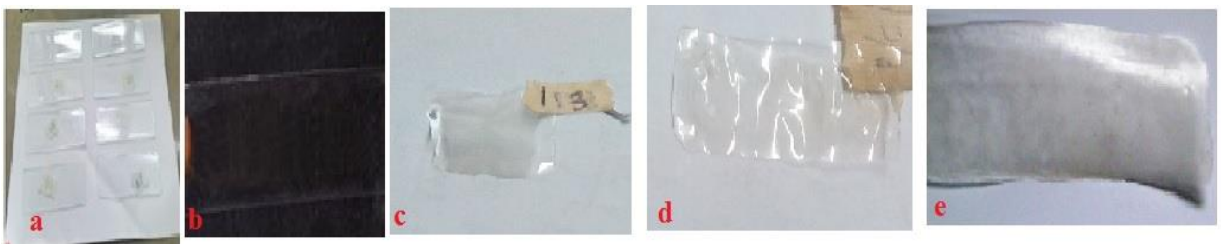
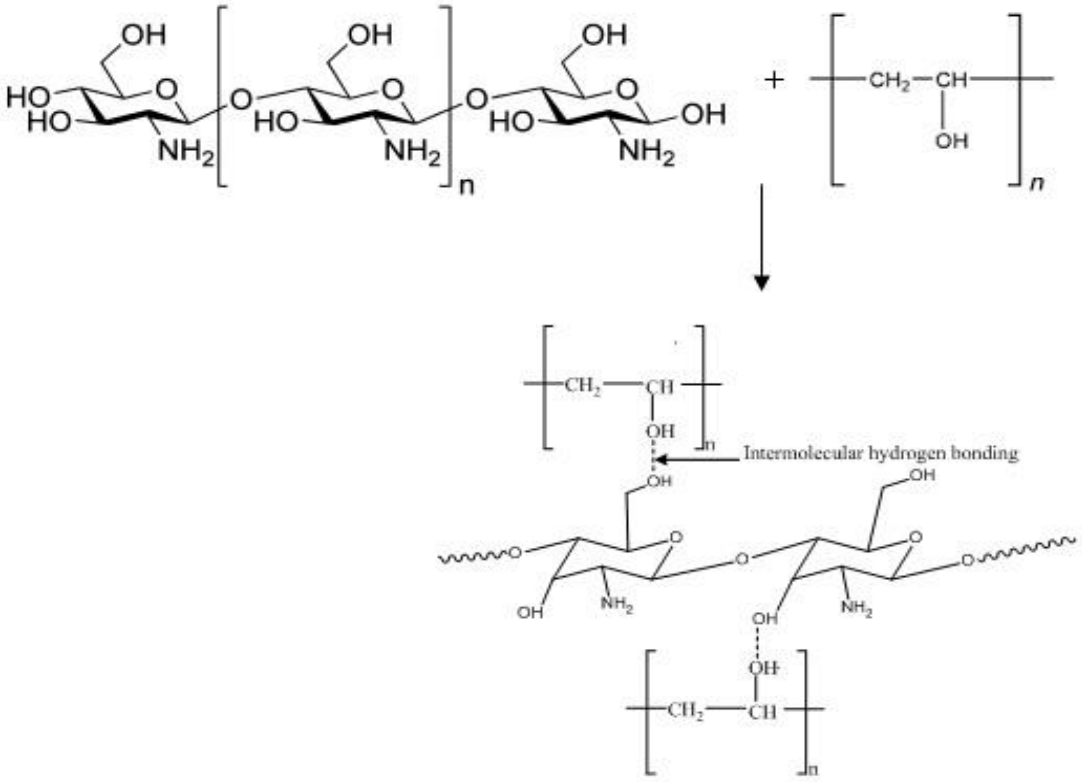


Fig. 2: (a) Casted chitosan-PVA films on glass slides PVA. Chitosan-PVA Films (b) 1:2 (c) 1:3 (d) 1:4 (e) 1:6



Scheme 2: Intermolecular hydrogen bonding between chitosan and PVA

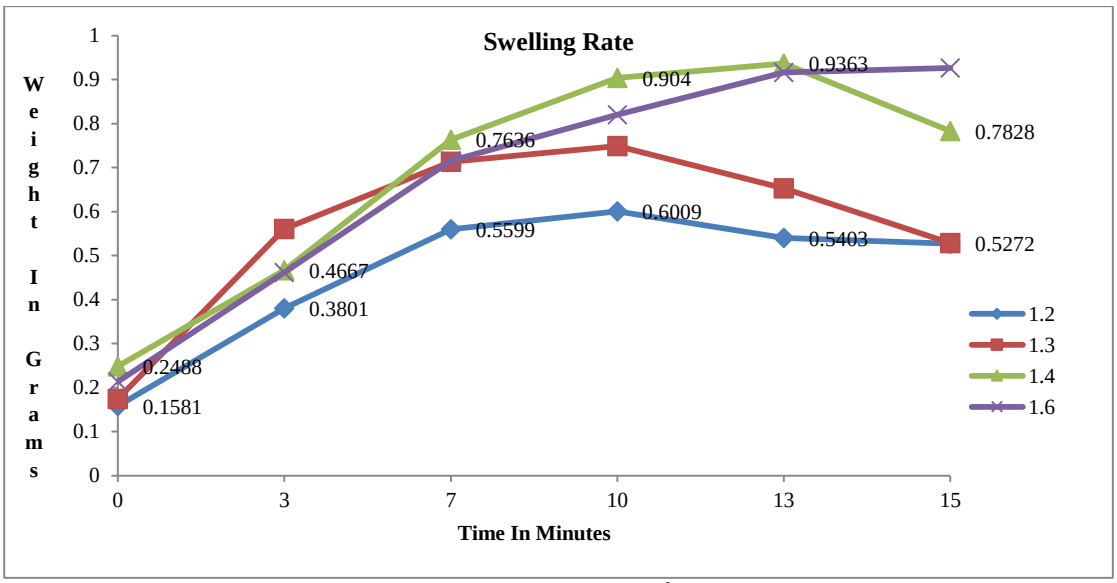


Fig. 3: Swelling kinetics of various film formulations up to 15 min at 37^{0}C

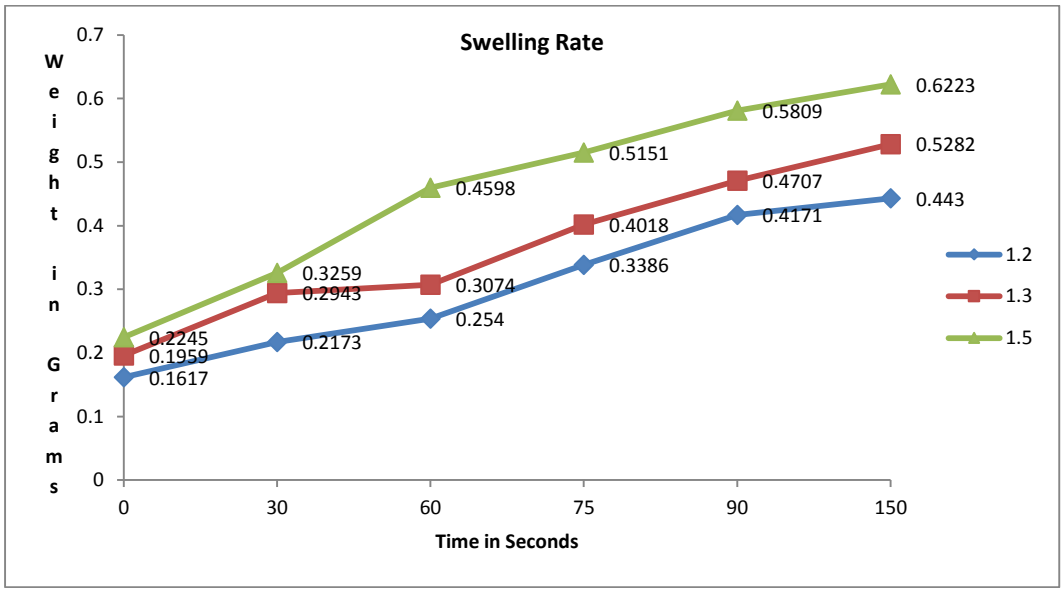


Fig 4: Swelling kinetics of composite film formulations of glutaraldehyde cross-linked chitosan-PVA and chitosan-PVA up to 150 second at 37^0C

Swelling study

The swelling property of the composite films formulations were obtained from the weight difference relative to the final weight at various time intervals (37^0C) up to 15 min and 150 seconds. Chitosan-PVA film formulations were observed to swell progressively until they were unable to absorb further, thus results in reduced weights while the cross-linked chitosan-PVA film formulations swell progressively with time. The chitosan-PVA film formulations shows increase swelling degree as the composition of PVA increases with time. This implies that the PVA content in the blend influences the swelling rate of the samples (Fig 3). This may be attributed to increase in the hydrophilic groups available for hydrogen bonding. It was also found that the cross-linked composite film formulations did not showed reduce weights at any point which implies that the addition of the glutaraldehyde in the blend further influences the swelling rates of the samples (Fig. 4).

Tensile Strength

Tensile properties give information on the strength and elasticity of the films, determined by their ability to withstand load and strain-at- break. The tensile strength and strain-at-break of different film formulations in dry state was measured by their ability to withstand silver weights (10 & 20 grams). The result obtained is summarized in Table 1 which indicates that increase in the composition of PVA increases the strength of the composite film. The film with 1:1 formulation could not be used for this test because it breaks while removing it from the glass slides. The films exhibit considerable degree of stretching before it finally breaks.

Table 1: Tensile strength of film formulations

S/N	Composition of Chitosan:PVA	Weight (g)	Tensile strength (kg/cm^2)
1	1:2	0.1331	7.14
2	1:3	0.17	14.29
3	1:5	0.2122	27.14

In-vitro release study

The INH drug release profile for different chitosan-PVA film formulations is presented in Tables 2 and 3 while Fig. 5 shows the cumulative percentage release with time. As can be seen from the Table, the rate of drug release continued with increase in time. Also, increase in concentration of PVA decreases the amount of drug release with sample (1:2) releasing more at every time interval than the other samples and sample 1:5 with least (Fig. 2). This may be attributed to the degree of crosslinking in the film arising from the strong interaction between chitosan and PVA with increase in the concentration of PVA, resulting in reduction of pore size. From the swelling studies, sample 1:5 showed relatively high degree of swelling compared to sample 1:2 which is the reverse of the release studies. This further revealed that the release rate of the drug from the film possibly occurs by a solution-diffusion mechanism which depends on the film pore size and compact structure.

Table 2: Showing absorbance of INH drug release of formulations chitosan-PVA Films and cross-linked chitosan-PVA films up to 15 min

S/N	Blend Ratio	5 min		10 min		15 min	
		Chitosan-PVA Films	Cross-linked PVA-Chitosan Films	Chitosan-PVA Films	Cross-linked PVA-Chitosan Films	Chitosan-PVA Films	Cross-linked PVA-Chitosan Films
1	1:2	0.862	0.473	0.564	0.510	0.748	0.746
2	1:3	0.672	0.549	0.790	0.596	0.762	0.437
3	1:5	0.192	0.549	0.103	0.506	0.145	0.606

Table 3: Showing concentration and absorbance of INH drug release of chitosan-PVA Films formulation

S/N	Blend Ratio	3 min		7 min		10 min		13 min	
		Chitosan-PVA Films		Chitosan-PVA Films		Chitosan-PVA Films		Chitosan-PVA Films	
		Abs	Conc. (mg/ml)	Abs	Conc. (mg/ml)	Abs	Conc. (mg/ml)	Abs	Conc. (mg/ml)
1	1:2	0.316	8.8643	0.409	11.4464	0.439	12.2794	0.497	13.8897
2	1:3	0.163	4.6165	0.176	4.9974	0.420	11.7517	0.519	14.5005
3	1:4	0.135	3.6328	0.146	4.1444	0.161	4.5609	0.168	4.7553
4	1:6	0.099	2.8396	0.173	4.8940	0.224	6.3100	0.303	8.5034

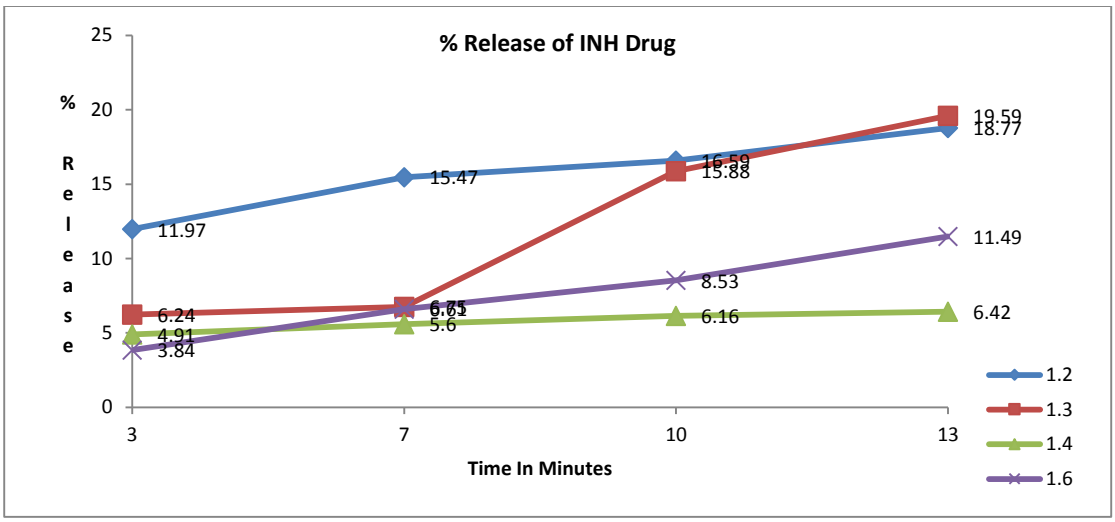


Fig. 5: Percentage (%) release of INH drug of Chitosan-PVA film formulations up to 13 min

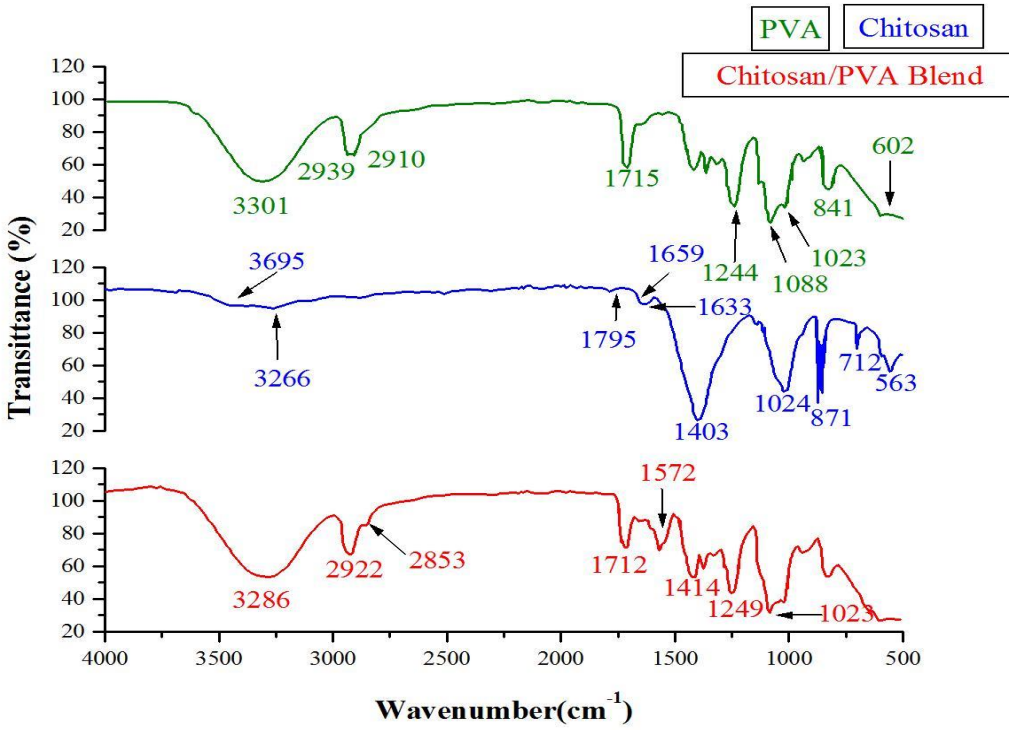


Fig. 6: FTIR Spectrum of Chitosan (blue), polyvinyl alcohol; PVA (Olive), and Chitosan/PVA blend (1:1) (Red)

Fourier infrared spectroscopy (FTIR)

FTIR was used to probe the functional groups that decorated the surface of the composite film in comparison with that of the extracted chitosan and PVA. The broad peak in PVA spectrum (Fig. 6) centered at 3300 cm^{-1} is attributed to O-H stretching vibrations (Jia *et al.*, 2007). Peaks at 2940 and 2909 cm^{-1} relate to C-H stretch of sp^3 while the sharp peaks at 1717, 1425, 1087 cm^{-1} is associated with C=O stretching, O-H bending and C-O stretching of secondary alcohol respectively. Several vibrational bands at 3694, 3265, 1659, and 1633 cm^{-1} in chitosan spectrum (Fig. 6) are due to free O-H stretching of alcohol, N-H stretch , amide 1 and amide II (N-H bending) bands respectively (Ibekwe *et al*., 2017). Peaks observed at 1403, 1153, 1024, and 871 cm^{-1} corresponds to characteristic O-H bending, C-O stretching, aliphatic C-N stretching and N –H wagging vibrations respectively of chitosan. In comparison with Chitosan and PVA, the spectrum of chitosan/PVA composite (1:1) (Fig. 6) shows

frequency shift to lower wavenumber in O-H stretching band (3286 cm^{-1}). In addition, N-H bending vibration deceased (1571 cm^{-1}). The broadness and reduction in O-H and N-H band frequency as well as the disappearance of the N-H stretching vibration band is indicative of intermolecular and intermolecular hydrogen bonding (Zhang *et al*., 2007). All other characteristics peaks of chitosan and PVA were observed. The sharp peak at 1713 cm^{-1} refers to C=O and C-O stretching of acetate groups. The presence of identified groups on the composite film in comparison to pure precursors indicates interaction.

Conclusion

This study demonstrates the development of INH impregnated chitosan-PVA composite film cross-linked with glutaraldehyde by the solvent casting method. The films produced were clear, cloudy (cross-linked), transparent, and flexible and are stable on storage. The results obtained reveals that the strength and water uptake ability of the film is dependent on the PVA or PVA/ glutaraldehyde composition in the film. While the drug release study indicates that the release mechanism from the film involves more of diffusion process which is a function of the film pore size and compact structure. The infrared spectroscopy showed that there exist some intermolecular interactions between chitosan and PVA. These results suggest that the films incorporated with INH drug have potential use for delivery systems, as dressing material for wounds.

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