Preparing chitosan–Polypyrrole nanocomposite film and evaluation of its mechanical, electrical, and antimicrobial properties

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ABSTRACT

In this study, chitosan–Polypyrrole film was prepared with a combination of different concentrations of Polypyrrole and various synthesis times to produce antimicrobial and biodegradable packaging film. The physical, electrical, and mechanical properties of the films were investigated. The interaction between chitosan and Polypyrrole was confirmed by Fouriertransform infrared spectroscopy (FTIR) and X-ray diffraction patterns. The size and morphology of the synthesized particles were examined by scanning electron microscopy. The results indicated that the synthesized Polypyrrole particles had a spherical shape (45–100 nm). The antimicrobial and antifungal activity of the films against Aspergillus niger fungi (antifungal area: 61.47 mm2) and Escherichia coli bacteria (antimicrobial area: 187.27 mm2) increased with increasing the concentration of Polypyrrole. The results obtained from the study of the effect of Polypyrrole on the electrical conductivity of the chitosan film showed that increasing the Polypyrrole concentration and synthesis time resulted in decreased electrical resistance of the film, for which the film with the highest Polypyrrole concentration and the highest synthesize time had the lowest resistance. According to the mechanical property results, tensile strength (TS) and elastic modulus were increased due to the addition of the Polypyrrole to the polymer matrix. The chitosan blank film had a lower TS than nanocomposites. As the final results, the chitosan–Polypyrrole film has good electrical conductivity, indicating thatthe produced film could be used in intelligent food packaging.

Keywords: Chitosan, Polypyrrole, conducting polymer, nanocomposite, biodegradability

1. INTRODUCTION

Recently, polymer composites especially bio-composites and intrinsic conducting polymers (CPs) with conjugated double bonds have been attracted much attention. Due to the increased production and consumption of petroleum polymers and plastics in the daily life of humans, the diseases caused by food poisoning have become a major threat to human health and the environment and have contributed to the emergence of the packaging industry. Since the bulk of the packaging industry is made up of plastics, thepackaging industry can be linked to petroleum products. Therefore, the increasing growth of population, pollution caused by packaging materials derived from oil derivatives, and problems caused by various methods of disposal of these contaminations, including burning, burial, and recycling, have attracted more attention toward biopolymers and biopackaging. Biodegradable films and coatings are good alternatives to synthetic films in the packaging industry due to their eco-friendliness and low dependence on nonrenewable resources and, therefore, have attracted the attention of many researchers. Synthetic plastics that are used to pack different types of foods are causing serious environmental problems. The environmental impacts of plastic consumption in the food industry have encouraged the packaging industry to produce packaging out of renewable materials. Biodegradable packaging protects food products against mechanical, physical, and chemical damage and prevents their quality loss. It can also prevent microbial activity as an antimicrobial carrier in the form of antimicrobial packaging and increase the shelf life of food products. In recent years, many studies have been carried out to replace synthetic polymers with biodegradable biopolymers, especially for food packaging. Most disadvantages and problems with synthetic polymers are not raised for biopolymers due to their biodegradable nature, and this has made many researchers in the food packaging industry study the use of a variety of biopolymers to make biodegradable packaging.[1] Nanotechnology is one of the most important and the fastest growing parts of advanced technology. Products containing nanoparticles can be used in various industrial, medical, personal, and military applications. Nanocomposite is a compound substance with at least one of its phases having nanoscale dimensions (between 1 nm and 100 nm). [2] Nanocomposites are new alternatives to traditional methods of improving the properties of polymers. Nanocomposites are currently being used for the packaging of nonalcoholic beverages and foodstuffs because of their thermal properties, improved resistance, and conductivity[3] Chitosan is a linear polysaccharide composed of D-glucosamine and N-acetyl D-glucosamine units, prepared from acetylation of chitin. In fact, it is a natural polysaccharide having unique properties, such as biodegradability, biocompatibility, antimicrobial, and antioxidant properties. The main source of chitin is marine resources, such as crustaceans, shrimps, and crabs. The hard structure and the presence of hydrous bonds in chitin lead to its poor solubility in organic solvents, while chitosan is dissolved in dilute acid solvents because of the presence of amine groups and the formation of cationic polyelectrolyte. Various factors such as temperature, degree of deacetylation, particle size, and molecular weight affect the chitosan solubility. Low cost, biocompatibility, biodegradability, adhesion, nontoxic, antimicrobial, antifungal, and film-forming ability can be referred to as some of the properties of this polymer[4] Various mechanisms have been proposed for the antimicrobial property of chitosan. It is argued that the reaction between chitosan molecule with a positive charge and the cell membrane of microbe with negative charge results in the leakage of proteinaceous compounds and other intracellular compounds into the extracellular space[5] Chitosan also behaves as a chelator restricting low-grade metals and consequently preventing toxin production and bacterial growth. As a water-binding agent, chitosan activates various processes in the host tissues and prevents the activity of different enzymes[6] Hydrophilic and poor mechanical properties of chitosan in the presence of water and wet environments, as well as the inadequacy of thermal resistance, hardness, and its prevention against gases and the cost of its production, have made its single application in the packaging industry very difficult. Therefore, the preventive properties of chitosan can be improved in combination with other polymers such as polypyrrol. Conducting polymers are polymers with conjugated electrons that have electronic properties. Some of the properties of conducting polymers include electrical, magnetic, optical, mechanical properties, absorption of electromagnetic waves, and wettability. So far, many studies have been carried out on conducting polymers, amongwhich Polypyrrole has attracted much attention, due to its high conductivity, chemical stability, and its electrochemical properties. Recently, Polypyrrole has been considered for its use in electrical and industrial appliances[7] Conducting polymers are polymers withconjugated electrons that have electronic properties. Some of the properties of conducting polymers include electrical, magnetic, optical, mechanical properties, absorption of electromagnetic waves, and wettability. So far, many studies have been carried out on conducting polymers, amongwhich Polypyrrole has attracted much attention, due to its high conductivity, chemical stability, and its electrochemical properties. Conducting polymers, such as polyaniline (PANI) and polypyrrole (PPy), have been studied extensively.Polypyrrole (PPy) is one of the most widely used conductive polymers (CPs) in commercial applications due to the capability to conduct electricity and its special characteristics, such as; good environmental stability, facile synthesis, and high conductivity with mammalian cells [8] PPy can be used in production of biosensors, gas sensors, microactuators, wires, solid electrolytic capacitors, electrochromic windows and displays, anti-electrostatic coatings, polymeric batteries, functional membranes etc9 PPy has triggered enormous research activities because of its fascinating features such as intrinsic high electrical conductivity and stability compared to the other conductive organic polymers. In addition, PPy can be used in the fabrication of light emitting diode , polyFETs , EMI shielding6 , sensors , and anhydrous electro-rheological fluids .In this study, new conducting antibacterial chitosan–Polypyrrole film was provided by chemical polymerization and studied its mechanical, antibacterial, and electrical characteristics. In fact, the provided film has antibacterial properties and electrical conductivity that can be used in food packaging as a smart film[10] .

1.1 Materials and methods

Chemicals and equipments
The following chemicals were used: chitosan (medium molecular weight, DD 99%, Mark, India), iron (III) chloride (FeCl3),glycerol of 98% purity (Merck, Germany), acetic acid 1% (Merck), distilled water, Polypyrrole (Sigma-Aldrich, USA), ethanol (Merck), concentrated hydrochloric acid (Merck), agar nutrient culture medium (Sigma).

1.2 Equipments

RADVAG digital scale with an accuracy of 0.001, model WTB 200, PECO sterile heater, Iran; UVVIS Spectrophotometer, model UNICO 2100 (UNICO-US), Glassware (B´echer, Erlenmeyer flask, pipette, thermometer, etc.) Sina Glass, Iran; multipurpose X-ray diffraction (XRD; MPD) Model B.V (made in The Netherlands); HIOKI multimeter machine, model DT4252, XRD device (MPD) model (Philips, Xpert), made in The Netherlands; electronic scanning microscope, Tescan Vega-3 incubator (Shimax, Iran); and spectrometer (Fouriertransform infrared spectroscopy (FTIR)) model 2002, Germany.

Table 1. list of experiments in the central composite design.

Film	Polypyrrol concentration(mol/lit)	Polymerization Time (min)
1	47.5	0.055
2	60	0.01
3	22.5	0.055
4	35	0.0325
5	35	0.055
6	60	0.1
7	35	0.055
8	35	0.0775
9	35	0.055
10	35	0.055
11	35	0.055
12	10	0.01
13	10	0.1
14	1	0.1
15	5	0.1

1.3 Synthesis of chitosan film

First, 5 g of chitosan powder (DD, 99, Mark) was dissolved in 300 mL solution of acetic acid (vol/vol 1%). Then, to improve the flexibility of the film, 2 mg of glycerol (Merck with 98% of purity) was added to the solution as a plasticizer, and then, the solution was stirred at 90_C for 8 h, and after complete uniformity, 150 mL of the solution was added to plastic molds (20 _ 30 cm2) and placed at ambient temperature for 72 h to dry.

1.4 Preparation of chitosan– Polypyrrole film

Polypyrrole/Chitosan Nanocomposite preparation The reaction was carried out in aqueous media at room temperature for 5 hours. In a typical experiment, 0.5g chitosan was added to 100 mL of acetic acid (1M) and then uniform solution was resulted by using magnetic mixer. Then, 5 g of FeCl3 was added to solution and 1 mL fresh distilled pyrrole monomer was added to stirred solution. The reaction was carried out for 5 h at room temperature. Consequently, the product was filtered and to separate the impurities, product was washed several times with deionized water and dried at temperature about 60 oC in oven for 24 h.

1.5 X-Ray diffraction analysis

X-Ray diffraction analysis The XRD test was carried out on the films by MPD (Philips model, made in The Netherlands) located in the X-ray lab of Tarbiat Modarres University. At first, the samples were compressed onto disks. The nickel-filtered copper Ka ray with a wavelength of 0.145 nm was produced. To do the test, the X-ray producing generator was set at 40 kV and 40 mA, and the samples were exposed to X-rays. Refractive radiations from the sample were measured at ambient temperature and at an angle 2θ ranging from 2◌̊ to 7◌̊. Crystallite size was measured via the Scherrer formula.

$$crystalitte\ size = \frac{\lambda \times 0.9}{(\beta \cos\theta)} \qquad (1)$$

Where λ is the wavelength of X-ray (0.154 nm),β is the peak width of diffraction in half of the height of the sample and q is the angle of reflection.

1.6 FTIR spectroscopy

To carry out the FTIR test, Perkin Elmer instrument (USA) was used at Urmia University. Thin tablets with the thickness of less than 1 mm were obtained for 10 min in a tablet machine through mixing water and milling samples of the film or nanoparticles coated with dry bromide potassium to the ratio of 1:20 and by applying a pressure of about 60 kPa. Transmission spectrum of the samples was analyzed in the wavelength range of 400–4000 cm_1 and with a resolution of 0.5 cm_1

1.7 Scanning electron microscopy

The microstructure of the produced films was investigated using a Tescan Vegan3 scanning electron microscopy (SEM) in the Razi Metallurgical Institute (Tehran). To investigate the effect of the addition of Polypyrrole on the microstructure of the produced films, electron microscopic images were prepared from the film surface. First, the films were adhered to the aluminum base by silver glue. The bases are dried up in a coating/disperser device to the critical point and were covered with gold for 5 min. Imaging of the specimens was carried out using a SEM with an applied force of 20 kW and a magnification of 6μм[11]

1.8 Measuring the electric resistance of films

Conductivity or electrical resistivity of films was measured by HIOKI multimeter model DT4252. First, the device was set on the resistive meter and two electrodes of the device were placed on the film 1 cm apart from each other. Then, theelectrical resistance value was recorded. Electrical resistance was measured at five points on the back and front of thefilms and the average was calculated.

1.9 Measuring antimicrobial properties of the film

The antimicrobial properties of the film were measured using a diffusion method at the agar level. The antimicrobial properties of the film were measured in a solid culture medium in Escherichia coli bacteria at the Nutrient Agar (NA) levels. At first, the culture medium was prepared for bacterium, the solution containing the bacteria was added to the medium, and pieces of the film were cut as large as 6 mL and were put in the plates. Then, the plates were placed in an incubator for 72 h at 37◌̊C, so that the bacteria could grow completely. After the end of growth, the mean diameter of the created halo was measured with a caliper and the area of the generated halo was calculated. [12]

1.10 Measurement of the mechanical properties of the films

Tensile tests were performed using TA.XT Plus, stable microsystems, UK, and standard brush (ASTM D882) at the Faculty of Agriculture, Urmia University. Three mechanical films were selected for optimum performance and a blank film. For this purpose, films in the 5 × 1 cm2dimensions were cut with a special film cutter. The distance between the jaws was 5 cm, and the jaw speed was 0.83 mm s_1. The maximum failure factors (in terms of newton) of the gradient were expressed per second and the distance to the breaking point (millimeter) by the device, and the strain coefficients of the breakdown (STB) and the tensile strength (TS) were determined by the following relations. In this regard, TS in MPa, force in terms of newton, and thickness (D) and film width (W) in meters[13]:

$$\text{Strain to Break(STB\%)} = \frac{\text{distance to break}}{\text{film length}} \qquad (2)$$

$$\text{tensile strength(ts)} = \frac{\text{F}}{\text{Wd}} \qquad (3)$$

2. Statistical analysis

In this work, statistical analysis was done in two stages: in stage 1, central composite design (at 95% probability level) was used for studying Polypyrrole concentration (in five levels) and polymerization time (in five levels) effects on the chitosan– In stage 2, to study aniline concentration (0.01 and 0.1 mol L_1) and polymerization time (10 and 60 min), a factorial design with three replications was used. In stage 1, statistical analysis was carried out based on a central composite design at the 95% probability level and using statistical software of Design expert-7, and in stage 2, Minitab (Version 17) software was used for data analysis; Tukey's test was used to compare the means. Also, significant levels of data were considered at the 5% probability level ($p < 0.05$). Meanwhile, the data in tables and forms were considered as a mean ±standard deviation (SD).

3. Results and discussion

3.1 XRD study

Figure 1 displays the results of XRD analysis for pure chitosan film (a), Polypyrrole (b), and chitosan–Polypyrrole nanocomposite (c). The XRD pattern of chitosan film in Figure 1(a) exhibits the weak peaks at diffraction angle $2\theta=12.7^{\circ}$ and sharp peaks at $2\theta=19.37^{\circ}$ that indicate the high degree of crystallinity morphology of chitosan.14 The XRD pattern of Polypyrrole in Figure 1(b) broad peak was observed at about $2\theta=26^{\circ}$. The broadpeak is characteristic of amorphous PPy 15 and are due to the scattering from PPy chains at the interplanar spacing 16 . This decrease in intensity of the peaks in chitosan–Polypyrrole can beassociated with a disorder in the planes of Polypyrrole, suggesting the formation of new chemical bonds between chitosan and Polypyrrole. In other words, the penetration of Polypyrrole particles into the chitosan film matrix and its uniform distribution throughout the matrix will reduce the chitosan crystalline mode. According to Bragg's law, the lower the diffraction angle is, the better the nanoparticle diffusion has occurred in the polymer matrix. The results are consistent with the findings of other researchers. [17], [18]

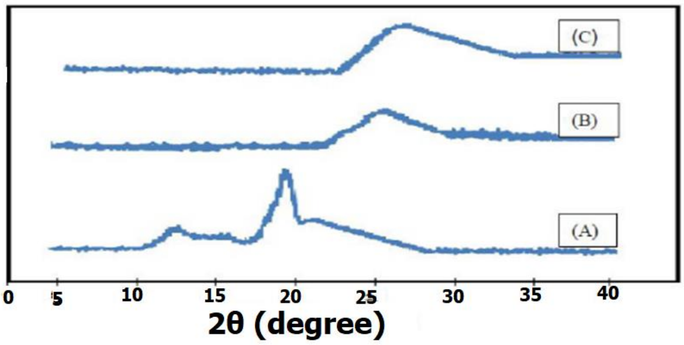


Fig. 1. XRD spectrum patterns of(a)chitosan, (b)ppy, and (c)chitosan –ppy.XRD: X-ray diffraction

3.2 FTIR study

Figure 2(a)shows the FTIR spectrum for pure chitosan. Pure chitosan has distinct absorption at a frequency of 3442cm-1due to symmetric and asymmetric-NH2 tensile vibrations and -NH2 bending bonds at a frequency of 1557 cm-1.the peak appearing at a frequency of 2850 cm-1is related to the C-H tensile bond(central carbon –hydrogen bonding)in the chitosan film, which could be considered a reason for the high relative strength of these films. [16] ,[24] the peak at a frequency of 1200 cm-1 is due to the asymmetric stretch of C-O-C and the peak at a frequency of 1600cm-1is due to symmetric and asymmetric C-O tensile vibrations indicating sucrose structure for chitosan. [19], 20the chemical structures of the obtained products were determined by FTIR spectroscopy, which has provided valuable information regarding the formation of polypyrrole nanocomposite. As can be seen in Fig.2, the broad peak at 3445 cm-1can be attributed to the O-H stretching vibrations of chitosan and N-H stretching vibrations of polypyrrole . the characteristic peak at 1598 cm -1is assigned to the stretching vibrations of the –CONH- groups of chitosan . the characteristic peak at 1383 and 1329 cm-1are attributed to the C-N stretching vibration of the secondary amine and C-H in –plane bending vibration of pyrrole rings, respectively[21]

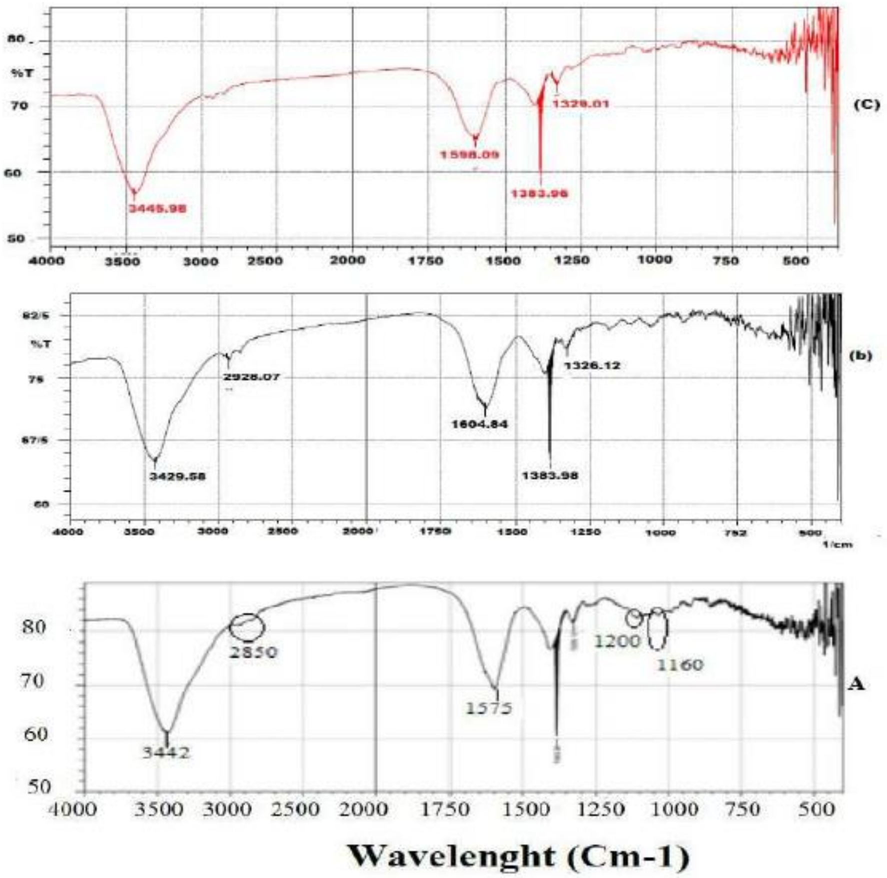


Fig. 1. *FTIR spectrum patterns of the (a)chitosan, (b), Polypyrrole and (c) chitosan– Polypyrrole. FTIR: Fouriertransform infrared spectroscopy*

3.3 SEM study

Homogeneity of the composite, the presence of the cavity, the level of dispersion of nanoparticles in the matrix, and nanoparticles orientation can be detected via SEM. Figure 3(a) shows the SEMimage of pure chitosan film, and Figure 3(b) shows the SEM image of chitosan filmmodifiedwith Polypyrrole particles (0.1Mat 60min). The SEMimage of pure chitosan film showed that the film was smooth without pores, which indicated the homogeneity of the prepared film[11]. the chitosan–polypyrroleSEM image showed that nanocomposite has irregular sphere-like structure the plypyrrol particles were synthesized no uniformly and the particle shapes were almost spherical and in nanodimensions (90–100nm).Although some aggregationswere observed, it should be noted that the particles were dispersed in the chitosan matrix in a nonuniform manner. The results are consistent with the findings of other researchers. [22]

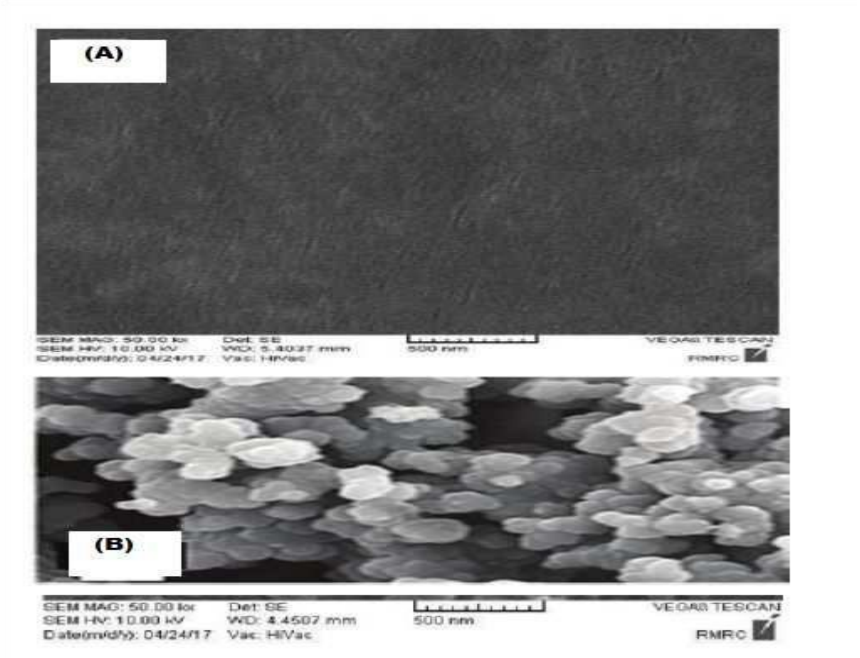


Fig. 3. *SEM image of (a) pure chitosan film and (b) chitosan film modified with polypyrrol (0.1 M, 60 min). SEM: scanning electron microscopy.*

3.4 Measuring electrical resistance of the films

Figure 4 shows the effect of synthesis time and ppy concentration of Polypyrrole on conductivity or electrical resistance. As the synthesis time and the concentration of monomer of Polypyrrole increase, electrical resistance decreases and electrical conductivity increases. The reason for this is that Polypyrrole is conductive and by increasing the time, more nanopolymers are synthesized on the film leading to the decrease of electrical resistance and increase of electrical conductivity. Furthermore, as the Polypyrrole concentration increases, due to conductivity, electrical resistance decreases. The results show that the produced films have a high capacity for use in smart food packaging. It should be mentioned that the SD for each film electrical resistance was below 8%. In addition, the electrical resistance of films is a very important factor that can be used in biosensors and gas sensors, and so on. The results are consistent with the findings of other researchers[23]

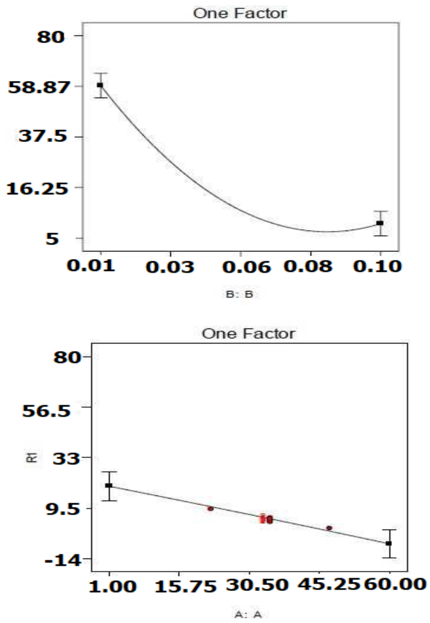


Fig. 4. *the chart of film electrical resistance against the synthesis time (A: A) and aniline concentration (B: B).*

3.5 antibacterial property

The results of the antimicrobial properties of the control film and optimal films on E. coli bacteria are reported in Table 2. illustrates antimicrobial property of the control film and optimal films against E. coli. The control film (pure chitosan) and F6,2 showed nongrowth halo against E. coli bacteria. The results indicate that the polypyrrol concentration, in comparison to the synthesis time, has the greater effect on antimicrobial properties of films, so that the highest antibacterial effect is on the film with high polypyrrol concentration, and the lowest antibacterial effect is on the film with low polypyrrol concentration. According to the results, the higher polypyrrol concentration results in the production of the greater amount of synthesized polymer and therefore increases electrical conductivity. According to the results, increasing the amount of synthesized polymer increases the antibacterial property of films, so it can be concluded that the film that has less electrical resistance (more electrical conductivity) has a better antibacterial effect. There are different reported research studies that discussed the reasons for antimicrobial properties of chitosan–nanoparticle films[24], [25].

Table 2. Comparison of average (+SD) of antimicrobial activity of chitosan–polypyrrol film against Escherichia coli bacteria.a

Film	Synthesis time (min)	Polypyrrol time (mol L_1)	Film diameter (mm)	Antimicrobial area (mm2)	Film
blank	-	-	6	68.78±1.1a	blank
2	60	0.01	6	103.78±1.4b	2
6	60	0.1	6	183.42±1.9c	6
Film	Synthesis time (min)	Polypyrrol time (mol L_1)	Film diameter (mm)	Antimicrobial area (mm2)	Film

[a] Data are means of three replicates. Means with different letters within a column indicate significant differences ($p < 0.05$).

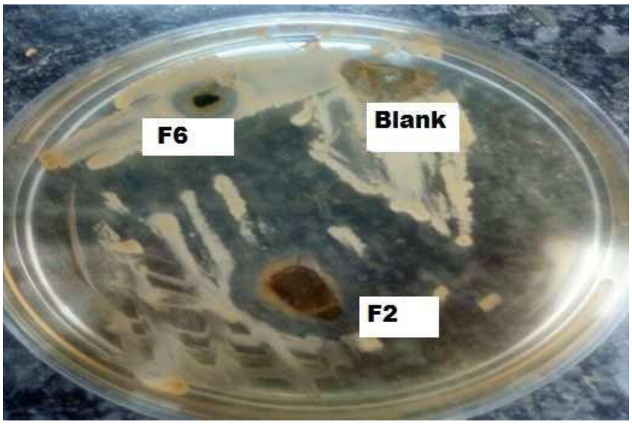

F6
Blank
F2

Fig.5 . *Effect of chitosan–polypyrrol film on the growth of Escherichia coli.*

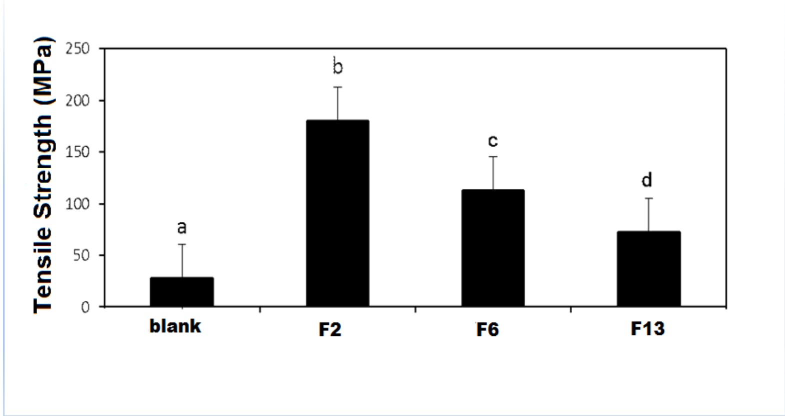
3.6 Mechanical properties of the films

The results of the evaluation of mechanical properties are depicted in Table 3. As can be seen, TS and elastic modulus are increased due to the addition of the polypyrrol to the polymer matrix. Figure 7(a) shows the TS of the film samples chitosan. The blank sample has a lower TS than nanocomposites. The highest TS and elastic modulus are for F2, F6, and F13, respectively. This could be due to the uniform dispersion of polypyrrol nanoparticles in the chitosan polymer matrix, a strong interaction between chitosan and polypyrrol by an ion bond that creates new and strong bonds between chitosan and polypyrrol. Figure 7(b) shows the STB of the film samples. According to the results, blank sample has the highest strain at the fracture point relative to the optimal specimens and is flexible and has less TS. The other results indicate improved mechanical properties and increased TS of biopolymer films due to the addition of nanoparticles[34]

Table 3. Comparison of average (±SD) of mechanical features of the films.[a]

Film	Gradient (g s^{-1})	Maximum force (N)	Elongation (mm)	STB (%)	TS (MPa)
blank	506.405	6.9	1.714	3.42 ±0.12a	27.700 ± 0.50d
F2	4080.105	42.26	1.214	2.428 ± 0.18b	178.292±2.3a
F6	3278.925	22.31	0.985	1.96 ±0.9c	111.240±2.1b
F13	3127.807	16.85	0.509	1.18±0.08d	73.654±1.4c

[a]Data are the means of three replicates. Means with different letters within a column indicate significant differences ($p < 0.05$).



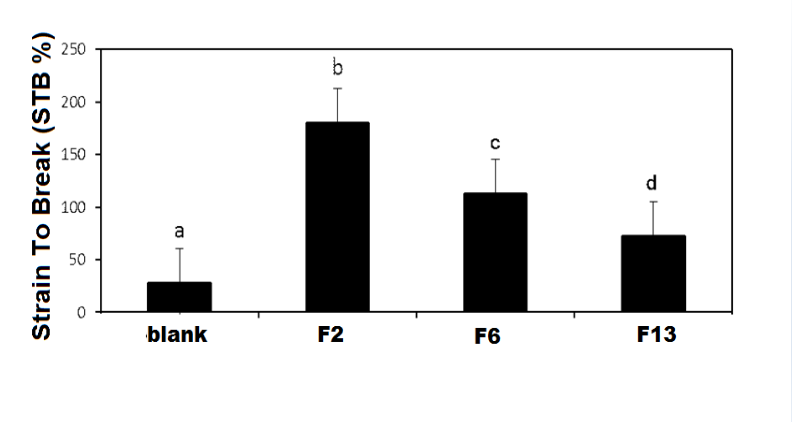


Fig.6. (a) Strain-to-break and (b) the tensile strength of films

4. Conclusion

The biodegradable/antifungal–antibacterial/conducting film based on chitosan–polypyrrol was prepared by chemical polymerization method. The crystallinity, morphology, chemical structure, conductivity, and mechanical properties of prepared films were studied by different techniques. The nanostructure of polypyrrol and the interaction between chitosan and polypyrrol was confirmed by XRD, SEM, and FTIR pattern. According to the results, the addition of different polypyrrol concentration to the chitosan film improved the antimicrobial, electrical, and mechanical properties of chitosan film. The antimicrobial properties of nanocomposites were satisfactory and showed that the film had a high potential for antibacterial packaging. The study of mechanical property results showed that the addition of polypyrrol improves the mechanical properties of chitosan film. Electrical resistance study showed that pure chitosan film had the highest electrical resistance; however, increasing polypyrrol concentration enhanced electrical conductivity, which could be used in food packaging as a smart film.

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