(Chitosan-Iodine) Bio-Films and Its Applications

[1] Duaa Abdul Rida, [2] Jaleel Kareem Ahmed ,[3]Auda Jabbar Braihi

Abstract

Films manufactured from chitosan polymerand iodine (I_2) for treating wounds, infected and burned areas. Chitosan-iodine composites are prepared by adding 14wt. % iodine element to the chitosan matrix. As a solution and film (coating and composite) states are used to investigate their healing actions. Evaluating healing action is done by applied these solution and films to the injured rabbits and comparing with the flamazine creamtreatmentmedical ointment. The antibacterial activity was checkedin Hilla General Teaching Hospital by expose the prepared samples to the action ofStaphylococcus aureus (gram positive) Escherichia coli (gram negative) andPseudomonas aeruginosa (gram negative) microorganisms. Agar well diffusion method was used to evaluate the antibacterial action by measuring the inhibition zone area. Results showed that,the prepared composite film is the best which have diameter zones 45, 35 and 15 mmantibacterial ability againstStaphylococcus aureus, Escherichia coli andPseudomonasmicroorganisms respectively.Density and wettability of chitosan film increased with addition of I_2 and the wettability of composite film as a function of time is more than the pure and coating film. Also the weight loss results proved that thelosses of iodine in coatedfilm (5.4%) is higher than in composite film (2.6%). Glass transition temperature (Tg) results proved presence of physical interactions between chitosan and I_2. From the calculations of Tg values shows that iodine gives energy to the chitosan film; to the composite film (11.69kJ mol^{-1}) and to the coated film (0.95kJ mol^{-1}).Complete healingof wound rabbits obtains within seven dayscomparing with negative control(without treatment).Both types of the films (coating and composite) show good healing power comparing with present medical ointment but composite film was better. Test is carried on to show the limited ability for area healing power of chitosan,iodine, chitosan-I_2 coated and composite films. Also tests are carried on to join the films with effected area i.e. to create a new skin.

Keywords:*Chitosan, Wound healings,flamazine cream,Iodine, Inhibitiondiameter zone.*

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1. Introduction

Chitosan films are biodegradable, biocompatible, flexible,durable, strong, tough and hard to break, have moderate valuesof water and oxygen permeability, decrease the respiratory rateof food and also inhibit the microbial growth[1].
Most of the mechanical properties of chitosan films are comparable to those of commercial polymers of medium strength such as cellulose [2].
The mechanical and permeable properties of chitosan films can becontrolled by selecting molecular weight, a suitable solvent system [3], addition of plasticizer agents, dispersants and compatibilizers, among others.However, the presence of such compounds can affect theantimicrobial activity of chitosan films [4-6].
Iodine (I_2) form Group I 7 of the Periodic Table; the halogens, and thus shares many of the common characteristics of the elements included arrange, like high electronegativity (2.66 to the degree that the Pauling mount) [7].

[1] *Polymer and Petrochemical Industries Department, College of Materials Engineering, University of Babylon, Iraq.*
duaa_rida@yahoo.com
[2] *Polymer and Petrochemical Industries Department, College of Materials Engineering, University of Babylon, Iraq.*
Jaleel_karim@yahoo.com
[3] *Polymer and Petrochemical Industries Department, College of Materials Engineering, University of Babylon, Iraq.*
auda_1964@yahoo.com

Drug resistance to pathogenic bacteria has led to an alarming situation worldwide due to the development of antibiotic resistance genes against current antibiotics. Misuse and overuse of antibiotics have led to new resistance patterns in several bacteria and the World Health Organization (WHO) focuses on control and prevention of such antibiotics. Multi-drug resistant (MRD) such as metallo-beta-lactamase (MBL) and methicillin-resistant Staphylococcus aureus (MRSA) bacteria is rapidly evolving causing an emerging threat to the community [8]. Wound can be defined as any process which leads to the disruption of the normal architecture of a tissue. They may be closed or open, e.g. abrasions, lacerations, avulsions, ballistic and excised, or surgical wounds. Open wounds are by far the most common and are characterized by a break in the skin[9]. Wound curing is the appeal of interactions by the whole of cytokines production factors, consanguinity and the extracellular grid [10].

The aim of this research is to assess the thermal, spectroscopic and mechanical properties, as well as the antibacterial activity of chitosan films, plasticized and non-plasticized with iodine as coating and composite film technologies.

2. Materials and Methods

2.1 Materials

Chitosan powder was provided by Shanghai Soyoung Biotech. Inc. China,with the following properties as shown in table (1)

Table (1): Properties of chitosan

Property	Value
Appearance	Off white powder
Viscosity (mpa.s)	(50 – 800)
Ash content	≤ 1.5%
Heavy metals (ppm)	≤10
Particle size(µm)	177

Iodine (I_2)
Iodine was purchased from Flucku, a Swiss company with the followingproperties (table 2).

Table (2): Properties of the used Iodine

Property	Data
Color	dark-gray/purple-black
Physical state	solid element
Density(g cm^{-3})	4.93
Meltingpoint (°C)	114
Boiling point(°C)	184
Covalent radius (nm)	0.133

Flamazine cream
Flamazine ointment was purchased fromSmith and Nephew Public Limited Companywith the following properties (table 3).
Table (3): Properties of the usedmedical Flamazine ointment

Property	Data
Composition	Silver sulphadiazine 1% (w/w) antiseptic, soothing and cleansing
Use	For external use in cases of wounds, abrasions and burns
Temperature store	Store between (15-25)°C

2.2 Experiment

Chitosan/I_2 films were prepared by casting method onto the glass surface.0.25 g of chitosan(which represented a critical weight) dissolvedin 2% aqueous solution of acetic acid with 49% DW and 49% diethyl etherby using magnetic stirrer for 10 min at40°C. The film is then left to dry at room temperature for 24 hours to obtain film with diameter of 8 cm, thickness 121.19micron, with area of two faces(100.4cm^2) for each cm^2 there is 0.025mg of chitosan.The methods of loading iodine on the chitosan film were done by two ways; coating method(in which the 14% iodine granules are sublimating on the dry chitosan film) and composite method (in which 14% iodine granules are grinded to powder then dissolved in 10 ml of ethanoland then add to the dry chitosan film and leave to dry at room temperature(RT).

2.3 Tests

Muller Hinton agar plates (Agar Well Diffusion Method)were used to determine the antibacterial activity. It was prepared and inoculated with tested organisms (Escherichiacoli, Staphylococcus aureus and Pseudomonas)microorganismsby spreading the inoculums on thesurface of themedia with the helpof sterile swaps [11].

Contact angle testwas carried out to evaluate effects of I2 addition on thewettability. The used device is SL 200C - Optical Dynamic I Static
Interfacial Tensiometer & Contact Angle Meter which manufactured in
KINO Industry Co., Ltd., USA with contact angle range from $0^o$to 180^o.
This device makes calculation and comparison of left andright contact angle as well as calculate their average value giving a Real time data graph monitoring changes of contact angle with video recording.
Thermogravimetric analysis of dry film samples was carried out using TGA-Polymer Laboratories, England.TGA measurements were carried out under a heating rate of 20°C/min at a temperature range 25 to 600 °C. About 10-11 mg of the sample was used for the analysis. This device consists of a furnace with a recording balance which holds the sample holder, a temperature programmer, an enclosed space for building up the required atmosphere, and hardware (computer) that records and displays the data.
Differential scanning calorimetry(DSC) device type Netzsch (200F3Maia), Germany according to ASTM D3418-03.The type of (sample & reference) pan is aluminum and the gas which used is N_2 (50 ml/min).In DSC test, two pans are used one as a reference and the other for the sample.The samples were heated at a heating rate 10°C/min. The onset and peak temperatures of T_g, and melting were determined.

3. Results

3.1 Antibacterial actions:

Chitosan/iodine system has the ability to inhibit the growth of the three kinds of microorganisms; Staphylococcus aureus, Escherichia coli and Pseudomonas microorganisms as shown in figure (1). It is clear that pure iodine is very active in inhibition the growth of these three microorganisms. The composite film shows higher bioactivity against the three kinds of microorganisms than the coated film because both of chitosan and iodine effected while in coating only iodine.
Figure (2) shows the effect of chitosan/I_2 film on the healing of injured rabbits. Rabbits were divided in four groups (each group with tow rabbits) to monitor this effect.
The healing action for injured rabbits for both types of the films (coating and composite) show good healing power comparing with present medical ointment but composite film was better and the complete healing achieved within seven days.

3.2 Weight Loss results

Figure (3) and table (4) show the weight loss for bothcoated and thecomposite films as a function of time and temperature due to the iodine sublimation.. Coated film shows higher loss (5.4%) than composite film (2.6%)which mean that thelosses of iodine in coated film higher than in composite film.

3-3 Density test

Figure (4) shows the densities values of the pure chitosan, chitosan-14 wt. % I2 coating and composite films. Density of the films coating and composite increased with addition of I_2,but in the case of composite film show small different comparing with the coating film.

3-4 Wettability results

Figure (5) shows that the contact angles of pure chitosan, coating andcomposite films. In short time (60 s), the behavior of the films are hydrophobic and slowly their absorptivity increased as a function of time with addition of iodine.The wettability of composite film as a function of time is more than the pure and coating film.

Differential scanning calorimetry (DSC)3.5

From figure (6) whichshows the glass transition temperatures (Tg's) of the pure chitosan film with Tg=90.05°C, which is higher than for the chitosan-14% I_2 films (coating and composite) (88.73, 73.16°C) respectively.

The following procedures were used to determine the energy arises from bond rupture:

$Ea = \Delta H^{\ddagger} + RT$ ……………………………… (1)[7, 8, 9]

The activation energy (Ea) is the summation of the activation enthalpy ($\Delta H^{\ddagger}$) and the work or the thermal energy (RT), as shown in equation (1).

According to polymer molecular models (the activation energy for chain rupture is about 60 kCal/mol (251 kJ mol^{-1}) at room temperature, so that the value of the decisive factor (D_f) for chain rupture as a thermal fluctuation process is about 101for one mole [7].

$\therefore Ea = \Delta H^{\ddagger} + RT = RTD_f$ ………………………………. (2)

Where:

R:the gas constant (0.0083 kJ $K^{-1}mol^{-1}$)

T: the absolute temperature (K)

$Ea_{at\ R.T} = 251$ kJ mol^{-1}

$Ea = \Delta H^{\ddagger} + RT = 251$

Since RT value small at room temperature which is equal to 2.47 kJ mol^{-1} comparing with $\Delta H^{\ddagger}$:

$\therefore \Delta H^{\ddagger} \gg RT \rightarrow Ea \cong \Delta H^{\ddagger}$…………................................. (3)

$Ea = \Delta H^{\ddagger} = RTD_f$……………………………….. (4)

$Ea = 0.0083$ kJ K^{-1} $mol^{-1} * 298.15$ $K * D_f = 251$ kJ mol^{-1}

$D_f = 101.43$

The energy of each unit of $D_f = 251/101.43 = 2.475$ kJ mol^{-1} …………(5)

For pure chitosan film:

T_g of pure chitosan film= 90.05 °C(from figure 6, a)

Since $Ea = R*T*D_f$ that means:

$251 = 0.0083 * 363.2 * D_f$

$D_f = 83.278$

$\Delta D_f = D_{f at\ R.T} - D_{f at\ 90.05°C}$

$\Delta D_f = 101.43 - 83.278 = 18.152$

Addition $Ea_{from\ (25\ to 107.41°C)} = \Delta D_f *$Each unit of D_f

$Ea_{from\ (25\ to 90.05°C)} = 18.152*2.475 = 44.92$ kJ mol^{-1}...............................(6)

$Ea_{at\ (90.05°C)} = 251 - 44.92 = 206.08$ kJ mol^{-1}

$\Delta T = 90.05 - 25 = 65.05°C$

$1°C = Ea_{at(25\ to 90.05°C)} / \Delta T$

$1°C = 44.92 / 65.05 = 0.69$ kJ mol^{-1}……………….....…. (7)

By addition 14wt% iodine to chitosan film as coating:

The temperature of chain rupture falls to 88.73°C due to addition 14wt%iodine$\therefore T_g = 88.73°C$ (from figure 6, b)

Since $Ea = R*T*D_f$ that means:

$251 = 0.0083*361.88*D_f$

$D_f = 83.58$

$\Delta T = 88.73 - 25 = 63.73°C$

Addition $Ea_{from\ (25\ to\ 88.73\ °C + 14\%iodine)} = 63.73°C*0.69$ $kJmol^{-1}$

$Ea_{from\ (25\ to\ 88.73\ °C + 14\%iodine)} = 43.97$ kJ mol^{-1}

Given energy from addition 14%iodine= 44.92-43.97=0.95 kJ mol^{-1} …..(8)

$Ea_{at\ (88.73°C)} = 206.08 - 0.95 = 205.13$ kJ mol^{-1}

The samecalculations were repeated for chitosan-14% I_2 composite film and tabulated in table 6.

4. Discussion

4.1 Antibacterial actions:

All samples were tested against the activity of Staphylococcus aureus (Staphella), Escherichia coli (E-coli) and Pseudomonas) microorganisms as shown in figure (1). It is clear that pure iodine is very active in inhibition the growth of these three microorganisms, where the inhibition zone diameters are 40 mm, 24 mm and 26 mm respectively, for iodine solution (table 4).In contrast, for pure chitosan film, the zone is zero; have no activity in inhibition the growth of three types of microorganisms. For chitosan/I_2coating film, the inhibition zone increased from 0 to 20 mm against Staphella and E-coli, while from 0 to 10 mm with Pseudomonas microorganisms. Similar increased happened for chitosan/I_2 composite film where the inhibition zone increased from 0 to 45 with Staphella, 0 to 35 with E-coli and 0 to 15 mm with Pseudomonas microorganisms. With addition of iodine in both solutionsand films, the inhabitation zones increased, which proved that iodine have good ability to inhibit the growth of these microorganisms.

Figure (2) shows the effect of chitosan/I_2 film on the healing of injured rabbits. Rabbits were divided in four groups (each group with tow rabbits) to monitor this effect. The first group (a) considered as negative control (without treatment) and the second group (b) as a positive control with medical ointment (Flamazine), (c) chitosan/I_2 coating film which put on the injured rabbits. (d) chitosan/I_2 composite film. The healing tendency for all samples was monitored daily by taking photos.

On the third day, there was an improvement in the healing treatedtreatment of wounds especially those treated with chitosan/I_2 films asshown images (c,d) in figure(2), while the animals without treatment (negative control) showed no healing improvement, the wound still wet, redness and there is some coagulation as shown in figure (2,a). On the fifth day after the treatment, the images showed that the wound treated with chitosan/I_2 composite film looked smaller and the wound healing was the best and skin returned to its normal color, light pink as shown in figure (2, d), while the wound appeared in the animal group of (b) is still in the coagulation stage despite of the using of a disinfectant wet as well as with the animals in the untreated group figure (2, a).

In the seventh day, complete healing of the wound is appeared as shown in figure (2, d) and the skin return to its nature, while other groups still in coagulation phase as shown in photos (a) and (b).

Both types of the films show good healing power comparing with present medical ointment (Flamazine) but composite film is better than the coated film because in composite both chitosan and I_2 contact directly on the wound while in case of coated only I_2 contact to the wounds.

4.2 Weight Loss results

Figure (3) and table (4) show the weight loss due to subjecting the prepared films (coating and composite) tosun light for 120 hr. at 30 °C. It is clear that, all samples lose their initial weights.

Bothcoated and thecomposite films show weight loss as a function of time and temperature due to the iodine sublimation. Coated film shows higher loss (5.4%) than composite film (2.6%) due to the lower interaction between chitosan and iodine in case of the former. This behavior proved the I_2 sublimation assumption, which means that I_2 with the time leaves the polymeric film towards the injured tissue and enhance the healing action.

4-3 Density test

Figure (4) shows densities valuesof the pure chitosan, chitosan-14 wt. % I2 coating and compositefilms. It is clear that density increased with addition of I2. Iodine is a dense element with 5 g/cm^3 comparing with the chitosan; thus the addition of 14 wt.% I_2 show increasing the density of the films in the case of composite and coating but composite film show small different comparing with the coating. Iodinediffusion between chitosan chains to fill the vacancy present in the chitosan network result increasing the composite density. While in the coated film, I_2 will be only on the surface of the chitosan film and vacancy remained exist.

4-4 Wettability results

Figure (5) shows that the contact angles of pure chitosan, coating andcomposite films. It is clear the composite wettability behavior with time. In short time (60 s), the behavior of the films are hydrophobic and slowly their absorptivity increased as a function of time as the angle of contact show that. Iodine enhances the water diffusion process, which is clear from figure (5).

4.5 Differential scanning calorimetry (DSC)

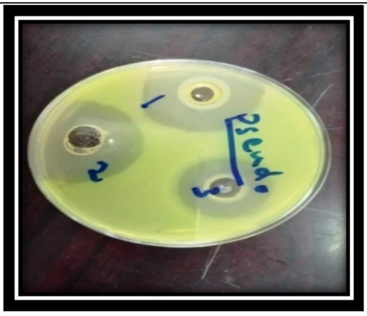
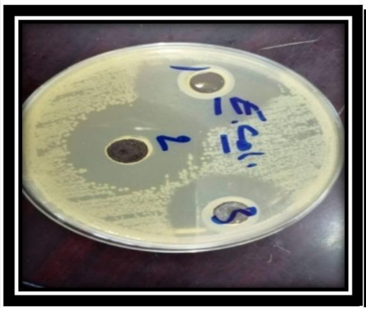
From figure (6) whichshows the glass transition temperatures (Tg's) of the pure chitosan film with Tg=90.05°C, which is higher than for the chitosan-14% I_2 films (coating and composite) (88.73, 73.16°C) respectively. This is due to the higher physical connection in the chitosan-I_2films than that in the pure chitosan film which effect on Tg. Addition of 14% I_2 an element which sublimes in room temperature under the normal atmosphere. The Tg resultsshow a decrease for both samples but for composite much decreasing than coated sample, this is due to that in composite ,iodine attacks the secondary bonds(engineering bonds) which formed by amino and hydroxyl group in chitosan result in highly decreasing of Tg (73.16 °C) while in case of coating system shows slightly decreases (88.73°C) because iodine present on the surface of the solid chitosan film and since iodine sublimes ,thus its leave to the atmosphere not to the inside chitosan film thus no effect on secondary bonds. It concludes that iodine gives energy to the composite film (11.69kJ mol^{-1}) and to the coated film (0.95 kJ mol^{-1}).

5. Conclusions:

1- Chitosan/iodine system has the ability to inhibit the growth of the three kinds of microorganisms;Staphylococcus aureus,Escherichia coli and Pseudomonas microorganisms.
2- Iodine acts as a vaporNanomolecule with covalent radius 0.133nm.
3- The composite film shows higher bioactivityagainst the three kinds ofmicroorganisms than the coated film because both of chitosan and iodine effected while in coating only iodine.
4- Losses of iodine in coated film higher than in composite film.
5- The healing action for injured rabbits for both types of the films (coating and composite)show good healing power comparing with present medical ointment but composite film was better (see point 3)and the complete healing achieved within seven days.
6- Tg results proved presence of physical interactions between chitosan and I_2.
7- Iodine gives energy to the composite film (11.69kJ mol^{-1}) and to the coated film (0.95 kJ mol^{-1}).
8- Density and wettability of composite increased with addition of I_2because I_2 is a dense element (5g/cm^3). The wettability of composite film as a function of time is more than the pure and coating film because in composite both of chitosan and I_2 effected.

Financial disclosure

There is no financial disclosure.

	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa
A			

Conflict of interest

None to declare.

Ethical Clearance

All experimental protocols were approved under the Polymer and Petrochemical Industries Department and all experiments were carried out in accordance with approved guidelines.

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B

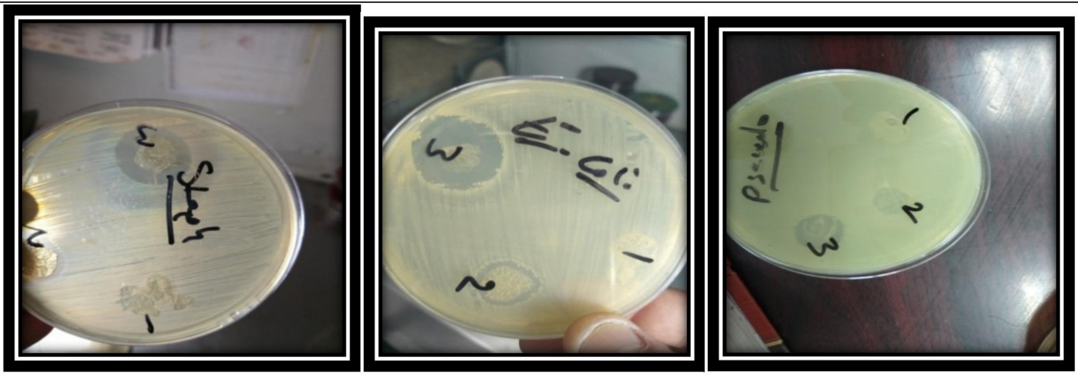


Figure (1): Antibacterial activity for (A) solutions (1)pure chitosan, (2) pure $I_2$and(3) chitosan/I_2solution (B) dry films:
(1) pure chitosan,
(2) chitosan-14% I_2 coating filmand (3) chitosan-14% I_2 composite film

Table (4): Inhibition zone diameters for pure iodine, pure chitosan and theircomposites

Sample	**Microorganism type**			**Inhibition zone diameter (mm)**		
Iodine	**STOPH**	**E- COLI**	**PSEUDO**	**40**	**24**	**26**
Pure chitosan solution	**STOPH**	**E- COLI**	**PSEUDO**	**33**	**23**	**23**
Chitosan-14%I_2 solution	**STOPH**	**E- COLI**	**PSEUDO**	**30**	**20**	**18**
Pure chitosan film	**STOPH**	**E- COLI**	**PSEUDO**	**0**	**0**	**0**
Chitosan-14%I_2 coating film	**STOPH**	**E- COLI**	**PSEUDO**	**20**	**20**	**10**
Chitosan-14%I_2 composite film	**STOPH**	**E- COLI**	**PSEUDO**	**45**	**35**	**15**

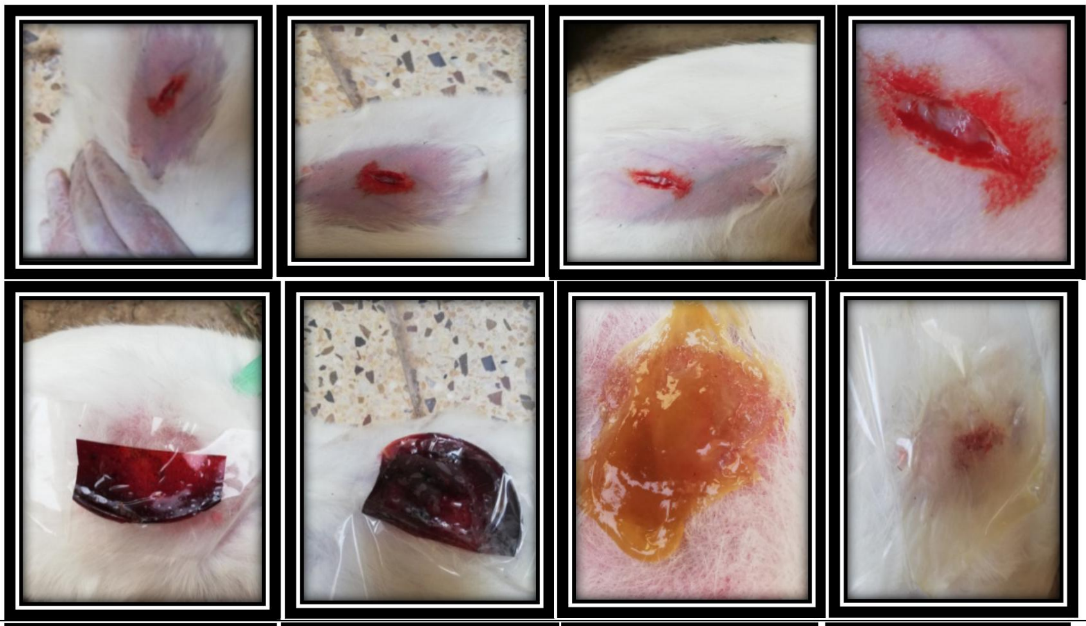
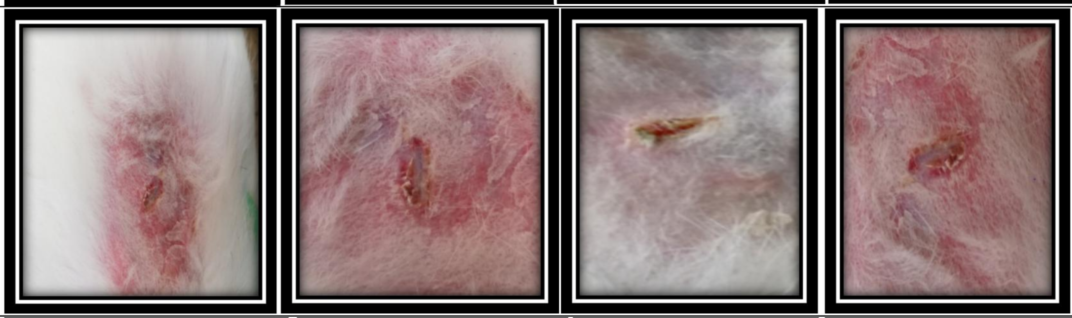
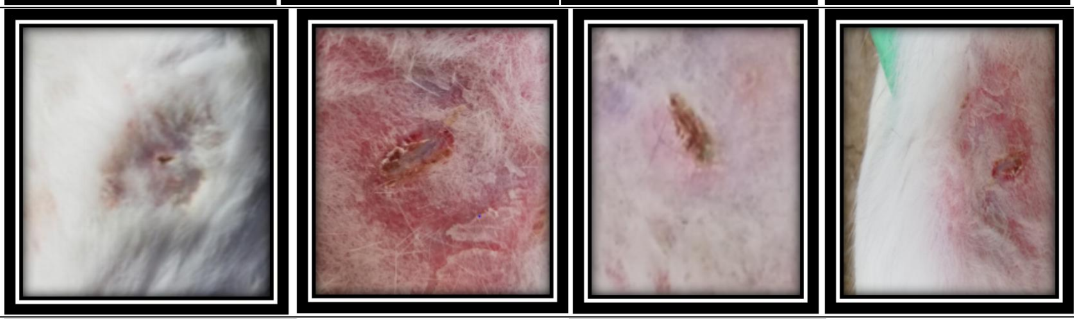
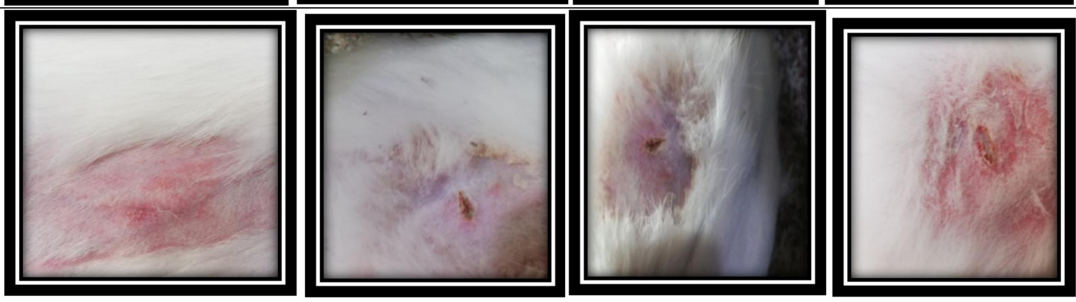
Day	(a)	(b)	(c)	(d)
1st				
3rd				
5th				
7th				

Figure (2): The effectof the prepared chitosan/I_2 films on the health of injured rabbits for (a) control negative (b) control positive (c) treated rabbits with coating film and (d) treated rabbits with composite film

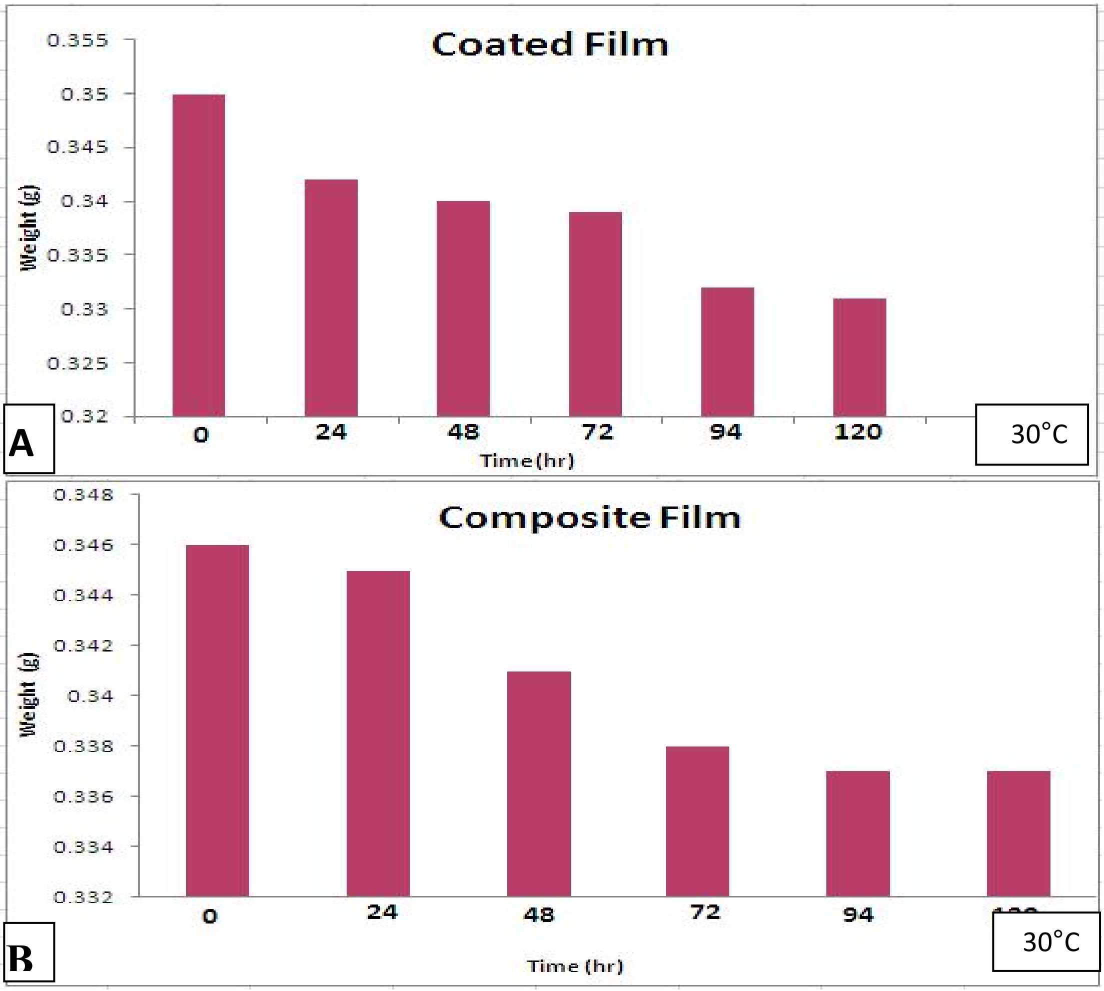


Figure (3): Weight loss for 14 wt. % I2 (a) coating (b) composite films

Table (5): Weight loss of iodine

Chitosan/I_2Sample	**I_2 g at 0 hr. 30 °C**	**$I_{2\ g}$ after 120 hrs. 30 °C**	**I_2 % lost after 120 hrs.30°C**
Coating film	0.049	0.04634	5.4
Composite film	0.04844	0.04718	2.6

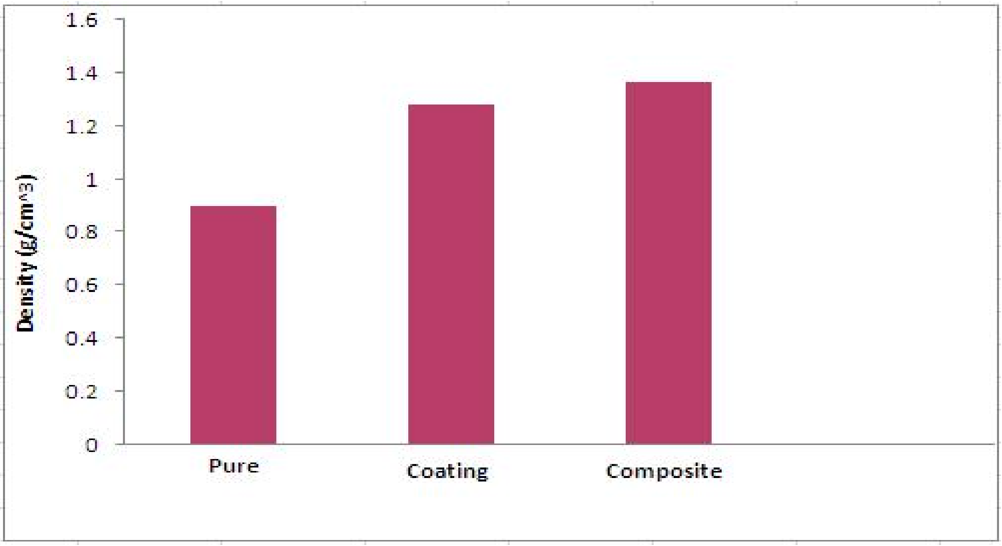
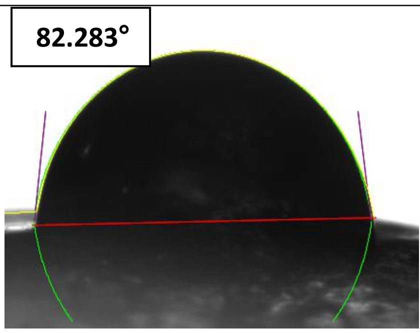
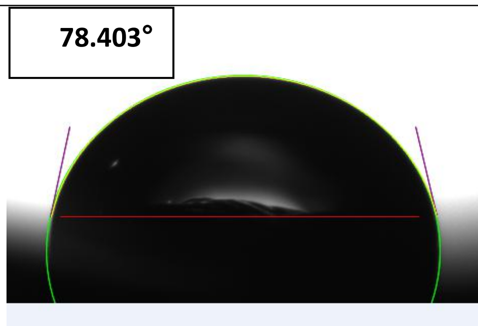
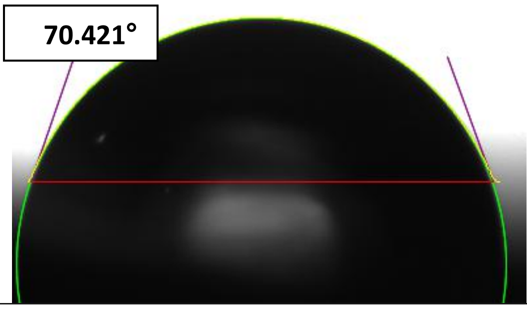
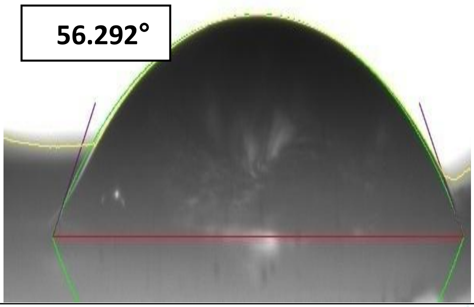
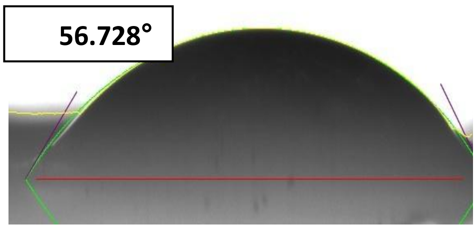
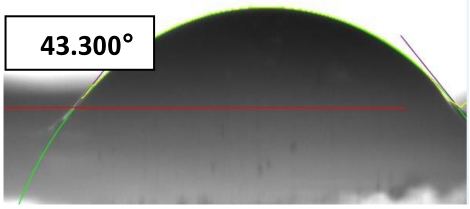


Figure (4): Density of the pure chitosan, chitosan -14wt% I2 coating and composite film

Sample	60 s	180s
Pure film		
Coating film		
Composite film		

Figure(5): Contact anglesat 60s and at 180s for (a) pure chitosan
(b) Chitosan/I_2 coating film with 14 wt.% I_2 (c) Chitosan/I_2 composite film with 14 wt.% I_2

Table (6): The Effect of 14wt% iodine on chitosan at R.T (25°C) & Ea (251 kJ mol^{-1}) with D_f (101.43)

Substance	**Tg (°C)**	**Df**	**Ea at Tg (kJ mol^{-1})**	**Addition Eafrom heating (kJ mol^{-1})**	**Given energy by addition iodine (kJ mol^{-1})**	**Secondary bond energy[12](kJ mol^{-1})**	**Expected type of bond**

Pure chitosan film	**90.05**	**83.278**	**206.08**	**44.92**	**-**	**-**	**-**
Coating film	**88.73**	**83.58**	**205.13**	**43.97**	**0.95**	**0.08-40**	(Van der Waals bonds) Dispersion(London) forces
Composite film	**73.16**	**87.45**	**194.39**	**33.23**	**11.69**		

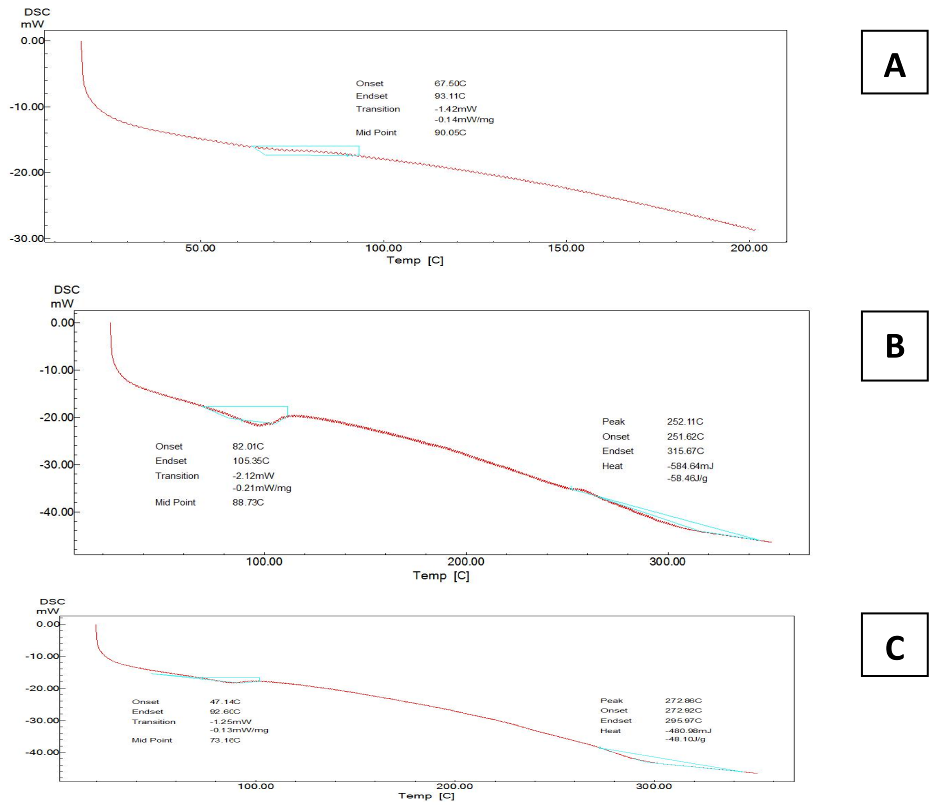


Figure (6):DSC curves of (a) pure chitosan film, (b) chitosan-14wt% I_2 coating film and (c)chitosan-14wt I_2% composite film