Carbohydrate Polymers 83 (2011) 876–882

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Cryogenic fabrication of savlon loaded macroporous blends of alginate and polyvinyl alcohol (PVA). Swelling, deswelling and antibacterial behaviors

Amita Chhatri [a], J. Bajpai [a], A.K. Bajpai [a,*], S.S. Sandhu [b], Nitika Jain [b], Jayeeta Biswas [b]

[a] Bose Memorial Research Laboratory, Department of Chemistry, Government Autonomous Science College, Civil Lines, Jabalpur (M.P.) – 482001, India
[b] Fungal Biotechnology and Invertebrate Pathology Lab, Rani Durgawati University, Jabalpur (M.P.) – 482001, India

ARTICLE INFO

Article history:
Received 6 November 2009
Received in revised form 22 July 2010
Accepted 31 August 2010
Available online 9 September 2010

Keywords:
Wound dressing
Antibacterial activity
Freeze–thaw
Deswelling

ABSTRACT

The present investigation deals with designing of savlon loaded blend hydrogels (termed cryogels) of polyvinyl alcohol (PVA) and alginate by repeated freeze–thaw method and their characterization by SEM and FTIR techniques. The FTIR spectra clearly reveal that savlon loaded alginate and PVA blends are bonded together through hydrogen bonding. The SEM analysis suggests that cryogels show a well defined porous morphology. The prepared cryogels were also investigated for swelling and deswelling behaviors. The results reveal that both the swelling and deswelling processes greatly depend on the chemical composition of the cryogels, number of freeze–thaw cycles and pH and temperature of the swelling bath. The savlon loaded blends were also investigated for their in vitro blood compatibility and antibacterial activity.

1. Introduction

Wound healing is a dynamic process and performance requirement of a dressing can change as healing progresses. However, it is widely accepted that a warm and moist environment encourage rapid healing and most modern wound care products are designed to consider these conditions (Barnett & Irving, 1991; Fwu-Long et al., 2001; Vanessa, Grey & Harding, 2006; Winter, 1962). It is known that the body's natural processes also regenerate dermal and epidermal tissues and the healing cascade is activated when platelets come into contact with exposed collagen leading to platelet aggregation and the clearer of clotting factors resulting in the deposition of a fibrin clot at the site of injury (Nayak, Raju & Ramsubhag 2008). Factors such as nutrition, infection associated illness, diabetes, mellitus, malignancy and vascular insufficiency, cytotoxicity treatments also affect wound healing (Kavanagh & Alette de Jong, 2004).

An ideal wound dressing is designed to provide the following capabilities: control of water-loss through evaporation, inhibition of drainage and prevention of exudates, build-up protection from external contamination and sufficient bactericidal effect to inhibit infection (Lee et al., 2001). Various biological, biosynthetic and synthetic wound dressings are used in burn care and selection and use of which depend on the condition of the wound (Carrougher, 1998).

A hydrogel dressing prevents the wound from microbial contamination, inhibits the loss of body fluids and provides free flow of oxygen to the healing process (Pal, Banthia, & Majumdar, 2006; Sen & Avc, 2005). The hydrogel dressing removal is almost painless because it does not stick to the wound but remains intact in the wound site due to the presence of hydrophilic groups, which form secondary bonds with those at the wound surface (Pal, Banthia, & Majumdar, 2006).

Alginate is a natural biopolymer derived from brown seaweed (Mohan & Nair, 2005). It acts via an ion exchange mechanism absorbing exudates and forming a non-adherent gel (Somers et al., 1992). It contains calcium and sodium. It is well established that calcium plays an important role in wound healing (Valerie, Herlick & Kidd, 2005) and sodium has the ability to kill bacteria. (Gilchrist & Martin, 1983; Ichioka, Harii, Nakahara, & Sato, 1998; Motta, 1989).

PVA is a well known biologically friendly synthetic polymer and has been developed for biomedical applications such as artificial pancreas (Giusti, Lazzeri, & Barbani, 1993; Young, Chuang, Yao, & Chen, 1998), synthetic vitreous body (Inoue et al., 1992), wound dressings, artificial skin and cardiovascular devices (Burczak, Gamian, & Kochman, 1996; Hassan & Peppas, 2000; Hoffman, 2002; Lee & Mooney, 2001; Razzak, Zainuddin, Dewi, Lely, & Taty, 1999; Rosiak & Ulanski, 1999; Wan, Campell, Zhang, Hui, & Boughner, 2002).

Thus, the present study aims to design a savlon loaded wound dressing patches of PVA and alginate by repeated freeze–thaw method and study their water sorption and antibacterial behaviors.

* Corresponding author. Fax: +91 761 2625514.
E-mail addresses: akbmrl@yahoo.co.in, akbajpailab@yahoo.co.in (A.K. Bajpai).

0144-8617/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2010.08.077

2. Experimental

2.1. Materials

Polyvinyl alcohol (PVA) 98.6% hydrolyzed, Mol. Wt. 1×10^5 Da was purchased from Merck, India and used without any pretreatment. Alginate was purchased from Central Drug House (CDH, New Delhi, India) while Savlon from Johnson & Johnson (Mumbai, India). All chemicals were of analytical grade and doubly distilled water was used throughout the experiments.

Sources of bacteria: 5 bacterial cultures namely *Staphylococcus aureus*, *Vibrio cholerae*, Gram positive *Bacilli*, *Bacillus subtilis* and *Pseudomonas* sp. were provided by Fungal Biotechnology and Invertebrate Pathology Lab, Department of Biosciences, R.D. University, Jabalpur (M.P.), India.

2.2. Preparation of savlon loaded cryogels

The repeated freeze–thaw method was adopted for preparing blend hydrogels of PVA and alginate containing savlon as an antiseptic liquid. In a typical experiment, 2 g of PVA was dissolved in 25 mL of water under hot condition (80 °C) and 1 g alginate was dissolved in cold water. Solutions of PVA and alginate, and 10 mL savlon were homogenized and kept in a petridish at −20 °C for 24 h. The frozen gels were then thawed for 2 h at room temperature (25 °C) and again kept at −20 °C for freezing. Such freezing thawing cycles were repeated at least thrice so that the whole mass was converted into a soft spongy yellowish orange coloured cryogel. It is worth mentioning here that the time of 24 h was found to be sufficient for freezing of the gel while that of 2 h was enough for complete melting. In this way, a complete cycle required about 24 h and by repetition of these cycles the cryogels were prepared. The gels so prepared were purified by equilibrating them in distilled water for 24 h, so that all unreacted chemicals were leached out. The swollen gel was cut into small discs and dried at room temperature for a week. The dried cryogel pieces were stored in air tight polyethylene bags.

2.3. Reswelling study in PBS

The dry gel was allowed to swell in phosphate buffer saline (PBS, pH 7.4) and taken out after 72 h. It was noticed that after swelling, the gel again becomes slightly spongy. The swollen gel was gently pressed in between the filter papers to remove excess water and then weighed. The swelling ratio was calculated by the following equation:

$$\text{swelling ratio} = \frac{W_s}{W_d} \quad (1)$$

where W_s and W_d are the swollen and dry weights of the gels, respectively.

2.4. Deswelling study

The kinetics of deswelling behavior of the hydrogel was monitored at room temperature. Prior to study the deswelling kinetics, the hydrogels were allowed to swell till equilibrium in the PBS. After wiping off excess water with filter papers present on the surface the weight of the deswelling gel was recorded at desired time intervals and percent deswelling was calculated by the following Equation:

$$\%\,\text{Deswelling} = \frac{(W_i - W_t) \times 100}{W_i} \quad (2)$$

where W_i and W_t are the weights of swollen gels at time zero time (start) and time t, respectively.

3. Characterization

3.1. FTIR spectral analysis

The spectral analysis of the prepared cryogels were performed on an IR spectrophotometer (Perkin Elmer, 1000 Paragon) by recording the IR spectra of a dry thin film of the blend.

3.2. SEM analysis

For scanning electron microscopy, samples were coated with 100 Å thick layer of gold by using a Denton II (Denton Vacuum, Moorestown, NJ) vacuum sputter coater to minimize charging of the sample and then mounted onto aluminum stubs using conductive carbon tape and conductive paint to ensure efficient charge dissipation. Scanning electron microscopy images were obtained using a STEREO SCAN, 430, Leina, SEM (USA) set at 20 keV accelerating voltage at 1×10^{-9} Torr vacuum.

3.3. In vitro blood compatibility

The in vitro blood compatibility of the prepared cryogels was determined by methods described as below

3.3.1. Clot formation test

The antithrombogenic potential of the cryogel surface was judged by the blood clot formation test, as described elsewhere (Maitz, Tsyganoy, & Pham, 2003). In brief, the specimens were equilibrated with saline water (0.9% (w/v) NaCl) at 37 °C for 24 h and to these swollen samples were added 0.5 mL of ACD blood and 0.03 mL of $CaCl_2$ solution (4 mol L^{-1}) to start the thrombus formation. 4.0 mL of deionized water was added to stop the reaction and the thrombus formed was separated by soaking in water for 10 min at room temperature and then fixed in 36% formaldehyde solution (2.0 mL) for another 10 min. The fixed clot was placed in water for 10 min and after drying, its weight was recorded. The same procedure was repeated for glass surface, blood bags and for the gels of varying compositions and respective weights of thrombus formed was recorded.

3.3.2. 2% haemolysis tests

Haemolysis experiments were performed on the surfaces of the prepared PVA/alginate cryogels following a method described elsewhere (Singh & Ray, 1994). In a typical experiment, a dry gel piece (4 cm^2) was equilibrated in normal saline water (0.9% (w/v) NaCl) at 37 °C for 24 h and human ACD blood (0.25 mL) was added into the gels. After 20 min 2.0 mL of saline water was added into the specimens to stop haemolysis and the sample was incubated for 60 min at 37 °C. Positive and negative controls were obtained by adding 0.25 mL of human ACD blood and 9% NaCl solution, respectively to 2.0 mL of bidistilled water. Incubated samples were centrifuged for 45 min, the supernatant was taken and its absorbance was recorded at 545 nm. The percentage haemolysis was calculated using the following relationship:

$$\text{Haemolysis}\,(\%) = \frac{A_{\text{test-sample}} - A_{(-)\text{Control}}}{A_{(+)\text{Control}} - A_{(-)\text{Control}}} \quad (3)$$

where A is absorbance. The absorbance of positive and negative controls was found to be 1.73 and 0.048, respectively.

3.4. Antibacterial assay

The study was carried out using zone of inhibition method (Awodele, Agbamuche, & Akintonwa, 2007) as described by WHO (2003). Nutrient agar plates (5 g peptone, 5 g NaCl, 3 g Beef extract, 1000 mL, Agar 2%) were prepared and seeded with 200 μL of test

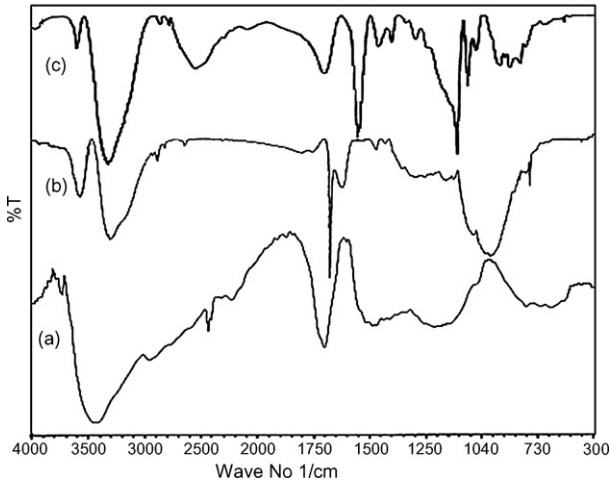

Fig. 1. FTIR spectra of savlon loaded cryogel.

bacteria. Discs of cryogel of different concentrations were prepared with the help of sterile cork borer (4 mm) and transferred on to the inoculated plates. They were incubated at 37 °C for 24 h. The zone of inhibition was determined by measuring the diameter in millimeters of zone to which the disinfectant inhibited the growth of the organism. Three replicates of each test were conducted (Azoro, 2000).

3.5. Statistical analysis

All experiments were done at least thrice and figures and data have been expressed along with the respective error bars and standard deviations, respectively.

4. Results and discussion

4.1. Characterization of savlon-containing PVA-alginate cryogel

The FTIR spectra of native PVA, savlon and savlon loaded cryogels are presented in Fig. 1(a)–(c), respectively. It is clear from the spectra (a) and (b) that hydroxyl groups of polyvinyl alcohol and savlon appear at 3449 and 3404 cm^{-1} respectively. Whereas in the savlon loaded cryogels, –OH groups (hydroxyl) appear at 3452 cm^{-1} (Bajpai & Saini, 2006) which clearly indicates a shifting of hydroxyl frequency because of the interaction of hydroxyls of native PVA and savlon. Likewise, the –OH bending of native PVA appears at 1107 cm^{-1} (spectra a) whereas in the savlon loaded cryogels the –OH bending is seen at 1147 cm^{-1} thus suggesting an interaction between the components of savlon loaded cryogels. The IR spectra also shows sharp peaks at 1741 cm^{-1} (C=O stretching) due to the presence of alginate (Roy, Bajpai, & Bajpai, 2009) and at 1589 cm^{-1} (N–H bending) due to chlorohexidiene (CHX) of savlon group (Young et al., 2008), which is also confirmed by the spectra (b) of pure savlon. Thus, on comparing all the three spectra, the spectra (c) of prepared cryogels suggest the presence of PVA, alginate and savlon in the prepared cryogels.

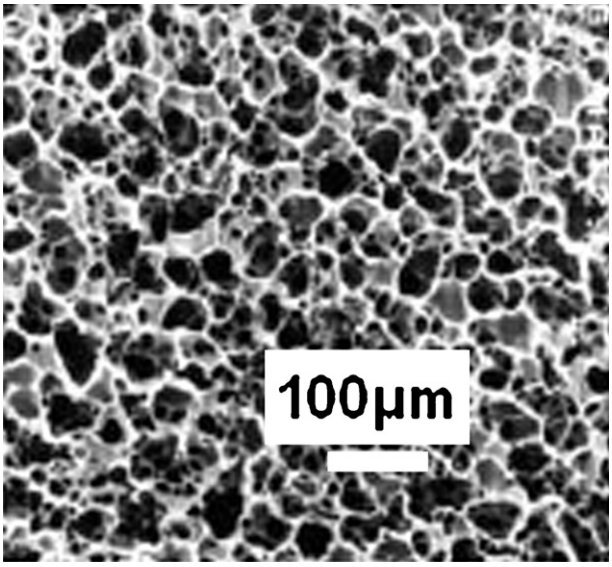

Fig. 2. SEM images of savlon loaded cryogel.

Table 1
Data showing the influence of composition of the cryogels on its swelling behavior.

PVA/alginate % (W/W)	Savlon(mL)	Freeze–thaw cycles	Eq. swelling ratio
40/60	10	3	2.7 ± 0 .1
50/50	10	3	3.2 ± 0.1
66/34	10	3	4.1 ± 0.2
75/25	10	3	3.5 ± 0.2
80/20	10	3	3.4 ± 0.2
66/34	7	3	4.5 ± 0.2
66/34	10	3	4.3 ± 0.2
66/34	13	3	3.2 ± 0.1
66/34	10	2	3.0 ± 0.09
66/34	10	3	2.9 ± 0.09
66/34	10	5	2.7 ± 0.08

The SEM image of the cryogel is shown in Fig. 2 which reveals that the cryogels is macroporous in nature and the size of the pores varies in the range 35–100 μm.

4.2. Water sorption measurements

4.2.1. Effect of PVA/alginate

In the present work the two hydrophilic components of cryogels, i.e. PVA and alginate, are nonionic and ionic respectively and the influence of wt. fraction of PVA and alginate varying from 40/60 to 80/20 has been investigated on the water sorption capacity of the cryogel. The results are summarized in Table 1 which reveals that the swelling ratio increases when the PVA/alginate wt. fraction increases from 40/60 to 66/34, while beyond this wt. fraction the equilibrium swelling constantly decreases up to 75/25 and thereafter becomes almost constant. The results may be explained as below

When the PVA/alginate wt. fraction increases from 40/60 to 66/34, the overall hydrophilicity of the cryogel increases due to increasing amount of PVA and, therefore, the equilibrium swelling also increases. Here it is worth mentioning that in the lower wt. fraction range the nonionic PVA is dominating over the ionic polymer (alginate) while in the higher wt. fraction range the ionic polymer (alginate) dominates over the PVA. The reason behind this supposition is that at the experimental pH (7.4) the nonionic polymer PVA is present as fully expanded conformation and, therefore, results in a porous cryogel with large pores. On the other hand, at high wt. fractions of alginate (ionic polymer), its chains will be present as fully extended conformation due to the existing repulsion forces between the anionic –COO– groups present along the alginate chains.

4.2.2. Effect of savlon

The effect of increasing amount of savlon in the cryogel has been investigated on the water sorption characteristics of the cryogel by adding its increasing volume in the range from 7 to 13 mL to the feed

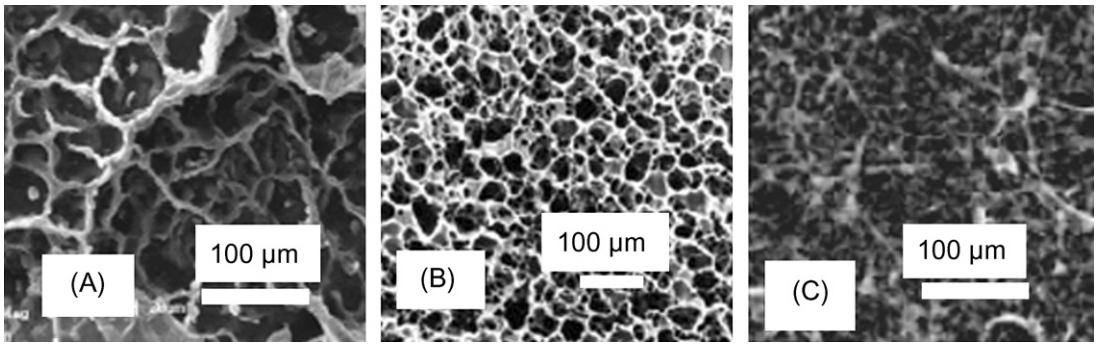

Fig. 3. Influence of FTC on pore size of the cryogel (a) 2 FTC, (b) 3 FTC and (c) 5 FTC.

mixture. The results are presented in Table 1 which reveals that the equilibrium swelling ratio decreases with increasing savlon content in the cryogel. The results are quite obvious and may be explained by the fact that increasing amount of savlon may block the pores of the cryogel and results in less water sorption.

4.2.3. Effect of number of freeze–thaw cycles (FTC)

The effect of number of freeze–thaw cycles on the swelling ratio of cryogel has been studied and shown in Table 1. The results clearly reveal that the swelling ratio decreases with increasing number of FTC. The observed results may be attributed to the fact that at 3rd cycle, the cryogel does not possess large crystalline regions and, therefore, the chains of disinfectant loaded PVA and alginate cryogels keep on relaxing with increasing swelling time. However, with increasing number of FTC, the gel acquires increasing crystallinity which restricts the mobility of disinfectant loaded PVA and alginate chains, thus resulting in a suppressed equilibrium swelling ratio. Peppas and co-workers (Hassan & Peppas, 2000b) also noticed that the swelling ratio of cryogel decreases with increasing number of FTC due to an increase in crystallinity and crosslinking density within the cryogel. An alternate explanation may be that with increasing number of FTC, the pore sizes of the cryogel decreases which also results in lower water sorption. In order to provide evidence for this logic, SEM images of cryogels were recorded with increasing number of FTC. The results are shown in Fig. 3 which clearly indicates that the pore sizes of the cryogel networks decrease with increasing number of FTC. This obviously explains the reduced swelling with increasing number of freeze–thaw cycles.

4.2.4. Effect of pH

In order to examine the role of pH in influencing water sorption capacity of the cryogels, the experiments were performed in three pH solutions viz. 4.0, 7.4 and 9.2, respectively. The results are shown in Table 2 which reveals that the equilibrium swelling decreases with increasing pH of the swelling bath. The results may be explained as below

In acidic medium, i.e. at pH 4.0, the cryogel shows larger swelling which may be explained by the fact that chlorohexadiene, an essential component of savlon, is positively charged and produces repulsion with H^+ ions which have diffused into the porous cryogel from the acidic swelling bath. Now due to these repulsion forces the polymer chains of the cryogel get expanded and allow more water molecules to enter the gel. This obviously results in an optimum swelling of the cryogel (Bajpai & Kankane, 2007).

With increase in pH of the swelling bath, the relaxation of polymer chains decreases which consequently results in a reduction in pore sizes of the cryogels. Thus bringing about a fall in the equilibrium swelling. It is important to mention here that alginate is anionic in nature and, therefore, may be expected to show repulsions among the cryogels chains at alkaline pH and should clearly result in larger swelling. However, the results obtained are just opposite and this is due to the reason that positively charged chlorohexadiene molecules might have bound to the anionic groups of alginate thus neutralizing their charge. This is the reason why the alginate chains of the network do not show relaxation at high pH.

4.2.5. Effect of temperature

The influence of temperature on the swelling of the cryogel is of much significance because it directly controls diffusion of water molecules into the gel, segmental mobility of the network chains and water-polymer interactions.

In the present work the effect of temperature on the degree of water sorption has been investigated by carrying out water sorption experiments in the range 5–25 °C. The results are presented in Table 2 which clearly indicate that the swelling ratio constantly increases with increasing temperature of the swelling medium. The results may be explained by the fact that when the temperature is increased the segmental mobility of cryogel chains also increases which causes widening of the pores of the cryogel network. This eventually results in greater swelling of the cryogels (Bajpai & Mishra, 2005).

4.2.6. Effect of biological fluids

It is well established that the equilibrium swelling behavior of a polymer network in a solvent is a result of a balance between osmotic and restoring elastic pressure. The presence of salts in the surrounding aqueous medium is capable of tilting this balance, which may result in either an increase or decrease in the swelling ratio. The effect of biological fluids on swelling has been examined by performing swelling experiments in the presence of urea, D-glucose (5% w/v), potassium iodide (15% w/v) and in physiological fluids such as saline water (0.9% w/v) and artificial urine. The

Table 2
Effect of experimental parameters on the swelling behavior of the cryogels.

Composition	Equilibrium swelling ratio										
	pH			Temperature (°C)			Biological fluids				
	4.0	7.4	9.2	5 °C	10 °C	25 °C	Syn. urine	NaCl	Urea	D- Glucose	KI
66/34	5.4 ± 0.2	3.6 ± 0.2	3.2 ± 0.1	2.9 ± 0.1	3.4 ± 0.2	4.2 ± 0.2	3.3 ± 0.1	5.6± 0.2	4.2 ± 0.2	4.3 ± 0.2	5 ± 0.2

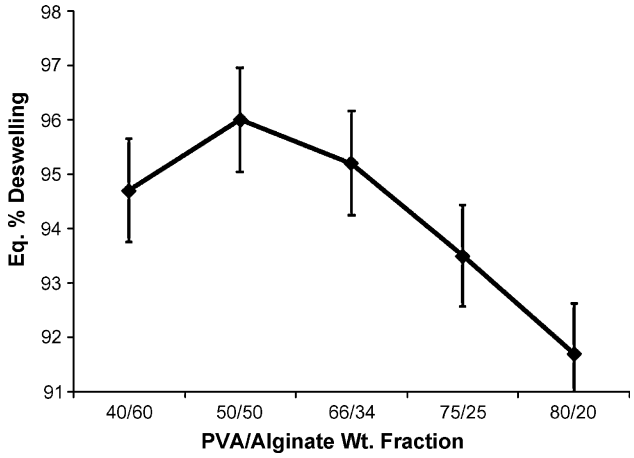

Fig. 4. Effect of wt. fraction of PVA/alginate on deswelling of the cryogel.

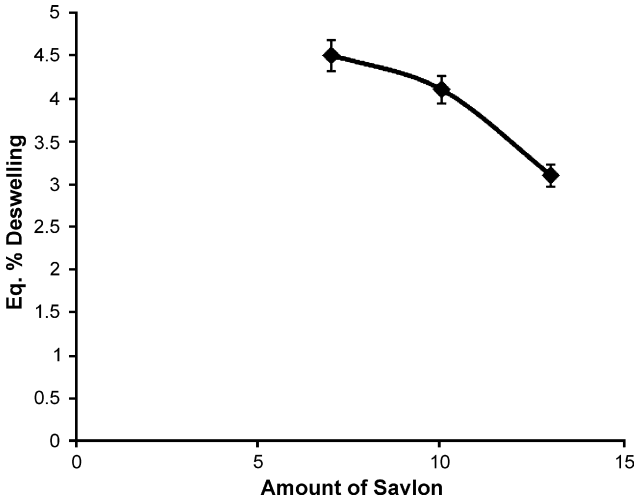

Fig. 5. Influence of amount of savlon (mL) on the deswelling of cryogel.

results are depicted in Table 2, which clearly show that the presence of solutes in swelling medium suppresses the swelling ratio due to a decrease in osmotic pressure of external solution (Bajpai & Saini, 2005).

4.3. Deswelling of cryogels

The capacity to imbibe water and retaining it for longer time periods are important parameters to determine performance of a hydrogel dressing. The percent deswelling equilibrium of a hydrogel is related to the chemical composition of the hydrogel as discussed below

4.3.1. Effect of PVA/alginate

When the composition of cryogel is varied by varying PVA/alginate wt. fraction in the range 40/60–80/20, the equilibrium deswelling initially increases up to 50/50 wt. fraction of the cryogel and then decreases as depicted in Fig. 4. The increase observed in deswelling of cryogels indicates that up to 50/50 wt. fraction of PVA/alginate, the water loosing capacity of cryogel increases while beyond this wt. fraction, the equilibrium deswelling decreases. Thus a cryogel containing greater PVA and lesser alginate shows greater water retention capacity and this may be explained by the fact that due to greater hydrophilic nature of PVA, water molecules are tightly bound to PVA chains and, therefore, equilibrium deswelling is less. The results also reveal that for 50/50 wt. fraction of PVA/alginate an optimum deswelling is noticed which could be attributed to the reason that water molecules bound to both the PVA and alginate chains get detached thus causing deswelling of the cryogel.

4.3.2. Effect of savlon

The influence of the amount of savlon present in the cryogel on equilibrium deswelling has been studied by monitoring extent of deswelling of the cryogel loaded with varying amounts of savlon, i.e. 7, 10, and 13 mL. The results are shown in Fig. 5, which indicates that an optimum deswelling is obtained when the cryogels was loaded with 10 mL savlon while minimum equilibrium deswelling is seen with cryogel loaded with 13 mL savlon. The results may be explained as below

When the savlon content increases in the cryogel from 7 to 10 mL, the extent of deswelling also increases which may be due to the reason that the decreasing hydrophilicity of the cryogel matrix tends to loose water rather rapidly. However, beyond 10 mL of savlon content the equilibrium deswelling drops suddenly and this may be explained by the fact that the added savlon fills up the pores of the cryogel and thus restrains the evaporation of water molecules through the pores. This clearly lowers the equilibrium deswelling of the cryogel. An alternate explanation may be that since chlorohexadiene molecules in savlon are positively charged, they can get attached to anionic centers of alginate chains and thus reduce the mesh size of the cryogel network which lowers the evaporation rate of water molecules.

4.3.3. Effect of freeze–thaw cycle (FTC)

When the number of FTC varies from 2 to 5 the equilibrium deswelling initially increases up to 3rd cycle and then decreases as shown in Fig. 6. The results may be explained on the basis of fact that with increasing FTC the cryogel develops increasing porosity, which facilitates water evaporation and causes an increase in equilibrium deswelling. However, after 3rd cycle the cryogels are greatly crosslinked and the network becomes so compact that water evaporation rate is suppressed due to much narrow size of the cryogel pores. This obviously lowers the equilibrium deswelling.

4.4. Evaluation of biocompatibility

A biomaterial is a substance used in medical devices to contact with the living body for the intended period. In order to be biocompatible, materials used in medical applications must meet certain criteria and regulatory requirements. The surface of biomaterials is believed to play an important role in determining biocompatibility. For materials that come into contact with flowing blood, the

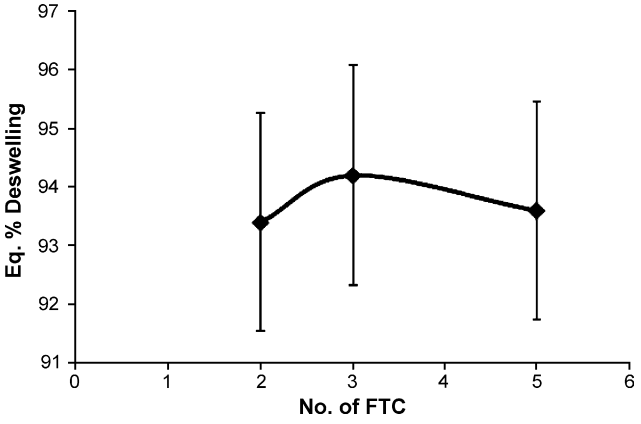

Fig. 6. Effect of number of FTC on deswelling of the cryogel.

Table 3
Data showing the biocompatibility parameters with varying composition of cryogel.

PVA/alginate% (W/W)	Savlon (mL)	Freeze–thaw cycles	% Haemolysis	Blood clot (g)
40/60	10	3	10.1	nil
50/50	10	3	5.2	nil
66/34	10	3	6.6	nil
75/25	10	3	18.9	nil
80/20	10	3	20.4	nil
66/34	7	3	17	nil
66/34	13	3	17.8	nil

formation of clot is the most undesirable but frequently occurring even that restricts clinical acceptance of the material as biomaterial. In the present study, the assessment of biocompatibility has been made on the basis of in vitro tests viz. clot formation and haemolysis assay. The results are summarized in Table 3 and may be discussed as below

4.4.1. Blood clot formation

It was noticed from the blood clot formation experiments that almost no blood clot was formed onto the surfaces of any of the gels and this conforms excellent antithrombogenic nature of the cryogels. The results can be explained by the fact that both PVA and alginate are hydrophilic and biocompatible polymers, so the cryogels will also be biocompatible. Moreover, as no chemical crosslinks are present in the blend mixture, the cryogels showed excellent antithrombogenic property.

4.4.2. Haemolysis study

Haemolysis studies were performed on the cryogel surfaces of varying compositions and the results are summarized in Table 3. It is clear from the % haemolysis data that the haemolysis values vary

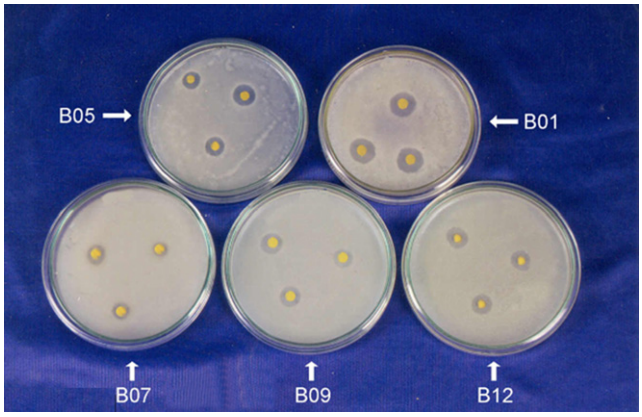

Fig. 7. Photographs showing anti bacterial activity of savlon loaded cryogel. *Staphylococcus aureus* (B01), *Vibrio cholerae* (Bo5). Gram positive *Bacilli* (B07), *Bacillus subtillus* (B09). *Pseudomonas aerugenosa* (B012).

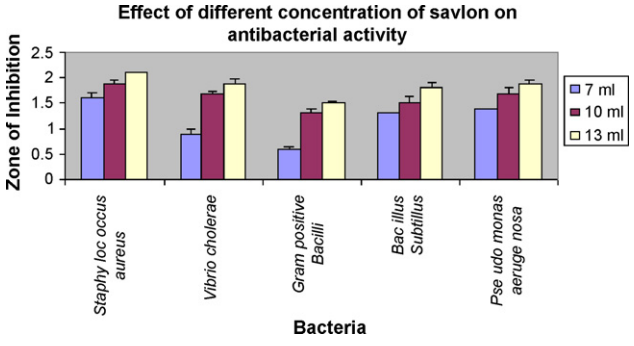

Fig. 8. Bar diagram showing the antibacterial effect of savlon on various bacteria's.

Table 4
Antimicrobial activities of savlon loaded gel against organisms at different.

	Bacteria	Zone of inhibition (mm)		
		7 mL	10 mL	13 mL
1.	*Staphylococcus aureus* (B01)	15 ± 0.09	18 ± 0.04	21
2.	*Vibrio cholerae* (Bo5)	9 ± 0.09	16 ± 0.04	18 ± 0.08
3.	Gram positive *Bacilli* (B07)	5 ± 0.04	12 ± 0.08	14 ± 0.04
4.	*Bacillus subtillus* (B09)	13	13 ± 0.12	16 ± .09
5.	*Pseudomonas aerugenosa* (B012)	14	16 ± 0.09	18 ± 0.04

in the range 5.2–20.4%, which suggest for a good blood compatibility of the cryogels. As no systematic correlation is seen between the chemical composition of the cryogel and percent haemolysis, the results have not been discussed in detail.

4.5. In vitro antibacterial activity

Antibiotics are molecules that stop microbes (bacteria and fungi) from growing or killing them outright. However, antibiotics are sometimes associated with adverse effects on host, which include hypersensitivly, depletion of beneficial gut and mucosal microorganism, immunosupressoin and allergic reaction (Ali & Jabri, 2005). Antimicrobial drugs, antiseptic and disinfectant have played an important role in the control of infectious diseases and they are essential parts of infection control practices (Awodele et al., 2007). However, the antimicrobial activity of these agents may be influenced by their formulation effects, level of organic load synergy, temperature, dilution rate and test method (Rutala, 1996; Larson, 1996).

In the present investigation, the bactericidal effect of disinfectant loaded cryogel was evaluated using Disc Diffusion method. The antibacterial activity of disinfectant loaded cryogel has been demonstrated in Figs. 7 and 8 and the repetitive data have been summarized in Table 4. The results obtained in this study indicate that disinfectants have broad activity against pathogenic organism like *S. aureus* (B01), *V. cholerae* (B05), Gram positive *Bacilli* (B07), *B. subtilis* (B09) and *Pseudomonas* sp. (B012). These results clearly show that the prepared gels possess fair antibacterial activity. The results also depict that antibacterial potential increases with increasing amount of savlon.

5. Conclusions

Cyclic freeze–thaw method, also coined as cryogenic, has emerged as a promising approach to fabricate well-designed macroporous architectures which may find novel applications in biomedical fields. In the present study, the cryogenic route has been adopted to prepare antibacterial wound dressing type of materials from savlon, PVA and alginate.

The FTIR spectra of the cryogel clearly mark the presence of constituent components like PVA, alginate and savlon. The SEM studies reveal that the cryogel has porous morphology with pore sizes varying in the range 35–100 μm

The savlon loaded cryogels to show clear inhibition zones around the loaded gels, which suggest for their fair antimicrobial activity. The cryogels also show adequate water intake capacity that varies with chemical composition of the gel and external experimental conditions. It is found that when PVA/alginate wt. fraction increases from 40/60 to 66/34, the swelling ratio increases, while from 66/34 to 75/25 wt. fraction, the water sorption capacity decreases. The swelling ratio of prepared cryogels also decreases with increasing savlon content and number of freeze–thaw cycles. The pore sizes of the cryogel also decrease with increasing number of freeze–thaw cycles.

The cryogels show decreasing water sorption capacity with increasing pH of the swelling bath whereas the swelling ratio of the cryogel increases with increasing temperature of the swelling medium. The cryogels also imbibe simulated biofluids and do not disintegrate upon swelling.

For wound healing applications the water retention capacity is an equally important parameter and this property has been evaluated in terms of deswelling of gel when kept standing at room temperature. The extent of deswelling depends upon the chemical composition of the gel.

The cryogels show lower deswelling when both the polymer components, i.e. PVA and alginate are either in low or in high content. The amount of savlon also affects the extent of deswelling. The degree of deswelling also increases with increasing number of freeze–thaw cycles.

The cryogel blends offer extremely fair blood compatibility as evident from in vitro blood clot formation and haemolysis tests. In the former test no blood clots are observed on the surfaces of the cryogel of different compositions.

References

Ali, A., & Jabri, A. L. (2005). Honey milk and antibiotics (review). *African Journal of Biotechnology*, *4*(13), 158–187.

Awodele, E., Agbamuche, P. M., & Akintonwa, A. (2007). The antimicrobial activities of some commonly used disinfectants on *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Candida albicans*. *African Journal of Biotechnology*, *6*(8), 987–990.

Azoro, C. (2000). Antibacterial activity of crude extract of *Azadirachita Indic* on *Salmonella typhi*. *World Journal of Biotechnology*, *3*, 347–351.

Bajpai, A. K., & Kankane, S. (2007). Preparation and characterization of macroporous poly(2-hydroxyethyl methacrylate) based biomaterials; water sorption property and invitro blood compatibility. *Journal of Applied Polymer Science*, *104*, 1559–1571.

Bajpai, A. K., & Mishra, A. (2005). Preparation and characterization of tetracycline-loaded interpenetrating polymer networks of carboxymethyl cellulose and acrylic acid): Water sorption and drug delivery study. *Polymer International*, *54*, 1347–1356.

Bajpai, A. K., & Saini, R. (2005). Preparation and characterization of biocompatible spongy cryogels of poly (vinyl alcohol)-gelatin and study of water sorption behaviour. *Polymer International*, *54*(9), 1233–1242.

Bajpai, A. K., & Saini, R. (2006). Preparation and characterization of novel biocampatible cryogels of poly (vinyl alcohol) and egg–albumin and their water sorption study. *Journal of Material Science: Materials in Medicine*, *17*, 49–61.

Barnett, S. E., & Irving, S. J. (1991). Studies of wound healing and the effect of dressing. *Lancaster:Technonic*, 583–620.

Burczak, K. E., Gamian, E., & Kochman, A. (1996). Long-term in vivo performance and biocompatibility of poly (vinyl alcohol) hydrogel macrocapsules for hybrid type artificial pancreas. *Biomaterials*, *17*, 2351–2356.

Carrougher, G. (1998). Burn wound assessment and topical treatment. In *Burn care and therapy*. Missouri: Mosby., 133–165.

Fwu-Long, M., Shin-Shing S., Yu-Bey, W., Sung-Tao, L., Jen-Yeu, S., & Rong-Nan, H. (2001). Fabrication and characterization of a sponge-like asymmetric chitosan membrane as a wound dressing. *Biomaterials*, *22*, 165–173.

Gilchrist, T., & Martin, A. M. (1983). Wound treatment with sorbsan an alginate fibre dressing. *Biomaterials*, *4*(4), 317–320.

Giusti, P., Lazzeri, L., & Barbani, N. J. (1993). Hydrogels of Poly(vinyl alcohol) and collagen as new bioartificial materials. *Journal of Materials Science: Materials in Medicine*, *4*, 538–542.

Hassan, C. M., & Peppas, N. A. (2000a). Structure and applications of poly (vinyl alcohol) hydrogels produced by conventional crosslinking or by freezing/thawing methods. *Advances in Polymer Science*, *153*, 37–65.

Hassan, C. M., & Peppas, N. A. (2000b). Cellular PVA hydrogels produced by freeze/thawing. *Journal of Applied Polymer Science*, *76*, 2075–2079.

Hoffman, A. S. (2002). Hydrogels for biomedical applications. *Advanced Drug Delivery Reviews*, *43*, 3–12.

Ichioka, S., Harii, K., Nakahara, M., & Sato, Y. (1998). An experimental comparison of hydrocolloid and alginate dressing, and the effect of calcium on the behaviour of alginate. *Journal of Plastic Reconstructive Hand Surgey*, *32*(3), 311–316.

Inoue, K., Fujisato, T., Gu, Y. J., Burezak, K., Sumi, S., Kogire, M., et al. (1992). Experimental hybrid islet transplantation: Application of poly (vinyl alcohol) membrane for entrapment of islets. *Pancreas*, *7*, 562–568.

Kavanagh, S., & Alette de Jong. (2004). Care of burn patient in the hospital. *Burns*, *30*, A2–A6.

Larson, E. L. (1996). Antiseptics. In R. N. olmmstad (Ed.), *APIC infection control and applied epidemiology: Principals and practices*. St. Louis, MO: Mosby-Year Book, Inc., 19–1, pp. 19–7 G1–G17.

Lee, J. S., et al. (2001). Preparation of wound dressing using hydrogel polyurethane foam. *Trends in Biomaterials & Artificial Organs*, *15*, 4–6.

Lee, K. Y., & Mooney, D. J. (2001). Hydrogels for tissue engineering. *Chemical Review*, *101*, 1869–1880.

Maitz, M. F., Tsyganoy, I., Pham, M. T., & Wieser, E. (2003). Blood compatibility of titanium oxide with various crystal structures and element doping. *Journal of Biomaterials Applications*, *17*(4), 303–319.

Mohan, N., & Nair, P. D. (2005). Novel porous polysaccharide scaffolds for tissue engineering application. *Trends in Biomaterials & Artificial Organs*, *18*(2), 219–224.

Motta, G. J. (1989). Calcium alginate topical wound dressing: A new dimension in the cost-effective treatment for exudation dermal wounds and pressure sores. *Ostomy Wound Management*, *25*, 52–56.

Nayak, B. S., Raju, S. S., & Ramsubhag, A. (2008). Investigation of wound healing activity of *Lantana camara* L. in Sprague dawley rats using a burn wound model. *International Journal of Applied Research in Natural Products*, *1*(1), 15–19.

Pal, K., Banthia, A. K., & Majumdar, D. K. (2006). Starch based hydrogel with potential biomedical application as artificial skin. *African Journal of Biomedical Research*, *9*, 23–29.

Pal, K., Banthia, A. K., & Majundar, D. K. (2006). Preparation of transparent starch based hydrogel membrane with potential application as wound dressing. *Trends in Biomaterials & Artificial Organs*, *20*(1), 59–67.

Razzak, M. T., Zainuddin, E., Dewi, S., Lely, H., & Taty, S. (1999). The characterization of dressing component materials and radiation formation of PVA–PVP hydrogel. *Radiation Physics and Chemistry*, *55*, 153–165.

Rosiak, J. M., & Ulanski, P. (1999). Synthesis of hydrogels by irradiation of polymers in aqueous solution. *Radiation Physics and Chemistry*, *55*, 139–151.

Roy, A., Bajpai, A. K., & Bajpai, J. (2009). Dynamics of controlled release of chlorpyrifos from swelling and eroding biopolymeric microspheres of calcium alginate and starch. *Carbohydrate Polymers*, *76*(2), 222–231.

Rutala, W. A. (1996). APIC guidelines committee. APIC guideline for selection and use of disinfectants. *American Journal of Infection Control*, *24*, 313–342.

Sen, M., & Avc, E. N. (2005). Radiation synthesis of poly (N-vinyl-2-pyrrolidone)-K-Carrageen a hydrogels and their use in wound dressing application. *Journal of Biomedical Materials Research*, *74A*, 187–196.

Singh, D. K., & Ray, A. K. (1994). Graft copolymerization of 2-hydroxy ethyl methacrylate onto chitosan films and their blood compatibility. *Journal of Applied Polymer Science*, *53*, 1115–1121.

Somers, S. S., Lyons, C., Brown, A. F., Klein, J., & Serriff, H. M. (1992). The flexipore 6000 membrane as a wound dressing for use in accident and emergency department. *Archive of Emergency Medicine Journal*, *9*, 246–248.

Valerie, A. J., Herlick, S., & Kidd, K. (2005). Use of alginates and for management of symptoms of and their impact on quality of life. *Wound Care Canada*, 3.

Vanessa, J., Grey, J. E., & Harding, K. G. (2006). Wound dressing. *British Medical Journal*, *332*, 777–780.

Wan, W. K., Campell, G., Zhang, Z. F., Hui, A. J., & Boughner, D. R. (2002). Optimizing the tensile properties of polyvinyl alcohol hydrogel for the construction of a bioprosthetic heart valve stent. *Journal of Biology Matters Research*, *63*, 854–861.

World Health Organization (WHO) (2003). Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of Public Health Importance in the developing world. WHO/CdS/RMD/2003.6.

Winter, G. D. (1962). Formation of the scab and the rate of epithelialization of superficial wound in the skin of the young domestic pig. *Nature*, *193*, 293–294.

Young, T. H., Chuang, W. Y., Yao, N. K., & Chen, L. W. (1998). Use of a diffusion model for assessing the performance of poly (vinyl alcohol) bioartificial pancreases. *Journal of Biomedical Materials Research*, *40*, 385–391.

Young, A. M., Ng, P-Y. J., Gbureck, U., Nazhat, S. N., Barralet, J. E., & Hofmann, M. P. (2008). Characterization of chlorohexidiene-releasing fast-setting brushite bone cements. *Actabiomaterialia*, *4*, 1081–1088.