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Aloe vera Loaded (Polyvinyl Alcohol) Cryogel: Potential Wound Healer

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The present work describes the preparation methodology of polyvinyl alcohol and *Aloe vera* hydrogels and their potential role in wound healing. *Aloe vera* is frequently used in treating many diseases due to its spectacular properties (anti-inflammatory, antiviral, antitumor and antibacterial) which assist in wound healing and help in treating many diseases a range of ailments. The designing of *Aloe vera* loaded polyvinyl alcohol (PVA) blend hydrogels (coined as cryogels) was done following repeated freeze-thaw cycles method. Characterization of the cryogels was done using some analytical techniques to study its properties and possible applications. The FTIR spectra shows that *Aloe vera* loaded PVA cryogels are interconnected by hydrogen bonding. Scanning electron microscope analysis established the porous nature of crogels. These hydrogels show water imbibing capacity, which depends on the experimental conditions and the chemical composition of the gel. The factors affecting the swelling ratio of crogels are amount of PVA, *Aloe vera*, number of Freez-Thaw cycles, pH and medium. The pore size of the cryogels also decreases with increasing number of freeze-thaw cycles. The *Aloe vera*-PVA cryogel is healing compatible with blood as there is less than 2% hemolysis.

Keywords: Biocompatible, Deswelling, Swelling freeze-thaw method, Hydrogel, Healing of wounds.

INTRODUCTION

Cream, solutions, dressing and skin tissue engineering substitutes are widely available as wound care products in the market. An efficient wound healing treatment system should be cost effective and quality oriented. Wound healing at site of damage is a complex, dynamic and continuous process. Now-a-days polymer based wound dressings have proved to be effective in wound healing. They are ideal to be used in wound healing due to their chemical efficiency and lower manufacturing cost. Wound dressings form the central part of the medical and pharmaceutical wound care market worldwide. For some infected wounds the use of polymeric dressing alone is not sufficient thus in order to overcome this limitation, dressings, based on natural polymers, have been developed which include encapsulation of synthetic/natural drugs/substitutes to reduce the growth of microorganism in wounds [1,2]. They help to protect the wound site from contamination and aces of infection healing process. Removal of a wound dressing should be an easy, painless process and should enable and accelerate wound healing. These dressings should irrigate and moisten the wound and delay and accelerate the healing process [3].

Hydrogels are emerging as one of the prime preferences in wound healing process hence extensively studied in healing. These cryogels absorb wound exudates and protect the wound from further infections, so it promotes the healing process, also provides a conducive wound healing environment and can be easily removed without causing distress [4]. Polymeric materials are being extensively used in wound dressing. Synthetic polymers like collagen, alginate, poly(vinyl alcohol), polyurethane, polyethylene, gelatin, polycaprolactone, poly-(lactic acid), polyacrylonitrile, poly(amino acid), silicone rubber and natural polymers such as chitin, chitosan, *etc*. have been studied extensively [5]. Specific hydrophilic functional groups *viz.*, -OH, $-CONH_2$, $-SO_3H$, -CONH, -COOR present in these polymers help in absorption of water and biological fluids. The hydrogels resemble the nature of human tissue in having a soft and rubbery surface and similarity in structure and physiochemical properties. These characteristic features of resemblance make the hydrogels probable candidate for various biomedical applications [6].

Poly(vinyl alcohol) (PVA) is non-toxic, biocompatible, biodegradable, low-cost polymer and used as a matrix for sorption of ions of metal or salts [7,8] PVA based hydrogels prepared by freeze-thaw technique has great potential in biomedical field [9]. In freeze-thaw method, physical crosslinking takes place between hydroxyl groups in PVA thus forming crysta-llites by strong inter-chain hydrogen bonding [10]. The PVA based hydrogels have been investigated for their water imbibing capacity, strength and gel fraction [11].

Aloe vera is a naturally occurring wound healer containing enzymes, amino acids and other active ingredients. It is an important medicinal plant, a source of 'aloin' [12] belonging to the family, Liliaceae [13]. The sap from *Aloe vera* reduces pain and inflammation and its antiseptic and antibiotic properties account for its application in treatment of cuts, buries and abrasions. The sap is used for its medicinal, health, beauty and skin care properties Aloe's watery composition increases the migration of epithelial cells so that wound healing is improved [14]. It has been widely used for the manufacture of topical products like ointments, gel, tablets and capsules in the pharmaceutical industry. *Aloe vera* plant being a rich source of many chemical compounds plays a pivotal role in the international markets [15-18].

Thus being fascinated by the great medicinal potential of *Aloe vera*, an attempt is made to formulate *Aloe vera* loaded cryogel of PVA as a wound healing dressing and study its characterization, water sorption and desorption properties and antibacterial and biocompatible nature.

EXPERIMENTAL

Polyvinyl alcohol (mass 1 × 10^5 Da, 98.6% hydrolyzed) Merck, India was used as purchased. Other chemicals used were of analytical grade and double distilled water was used throughout the experiments. Aqueous solution of *Aloe vera* gel was prepared by mixing *Aloe vera* and distilled water with the help of magnetic stirrer.

Preparation of cryogel loaded with *Aloe vera*: Physical cross-linking of polymer can be achieved by using freeze-thaw method. The complexing process of freezing and thawing involves the heat transfer and certain physical and chemical changes, which can alter the quality of product [19]. In a typical experiment, an aqueous extract was prepared by mixing *Aloe vera* gel and distilled water with the help of magnetic stirrer. For preparing blend hydrogels of PVA containing *Aloe vera* as an antiseptic repeated freeze-thaw method was used. PVA (1 g) was dissolved in distilled water (25 mL) under hot condition (80 ºC). *Aloe vera* gel (5 mL) and dissolved PVA were mixed in beaker and kept on a Petridish at –20 ºC left for 24 h. The frozen gel was thawed at room temperature (25 ºC) for 2 h followed by freezing at –20 ºC. After three continuous freezing thawing cycles, the mass was converted into a soft, spongy and transparent gel. Optimal time for sufficient freezing was found to be 24 h and for complete melting 2 h. Purification of the gels was achieved by equilibration in for 24 h in distilled water. The gel swelled which was cut into pieces, dried and stored in air tight polyethylene bags.

Antibacterial assay: The bactericidal effect of *Aloe vera* loaded cryogels against *Microbacterium luteus* and *Citrobacter* sp. was estimated using disc diffusion method [20]. *Aloe vera* extract was increased in the range of 7 to 9 mL and the zone of inhibition was measured with its increasing amount.

FTIR: FTIR spectrophotometer (Model: SHIMADZU 800 series) was used for the study of spectral analysis in the range of 4000-400 cm^{-1} by recording the IR spectra of *Aloe vera* loaded PVA cryogel.

Morphology studies (FESEM analysis): FESEM (model: SEM A1S2300C) technique was used to compare the classical SEM regarding the surface images of the material. Samples of the cryogels were dried in vacuum desiccators and subjected to analysis.

Water sorption study: In this method, dry gel of known weight was allowed to swell in phosphate buffer saline (PBS, pH 7.4) and taken out after 72 h. After swelling, the gel again becomes slightly spongy. Excess water was removed after pressing the gel between two filter papers and then following equation was used to calculate the swelling ratio [21]:

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

where W_s = weight of swollen gel; W_d = weight of dry gel.

Deswelling study: Deswelling kinetic studies were performed at room temperature. The hydrogels were allowed to swell till equilibrium in the PBS and then deswell. At fixed time intervals, the gels were weighed by removing excess of water from the gel surface. The following equation was used to calculate the deswelling [22] and to study the kinetics:

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

where W_i = initial weights of swollen gels; W_t = t time weight of gel.

Evaluation of biocompatibility

Formation of clot: For clot formation, acid citrate dextrose (ACD) blood was dropped on the swollen gel and 0.03 mL solution of calcium chloride and distilled water were poured on the gel after an interval of 10 min. Formaldehyde solution (36%) was poured on the gel and kept for 10 min. The clotted gel was then dried gently between two filter papers and finally weighed. The blood clots were formed on the cryogel surfaces of varying compositions.

Haemolysis: In present study, dry cryogel piece of 4 cm^2, 0.25 mL of human ACD blood and saline water (0.9% w/v NaCl) was added and left for 24 h at 37 ºC. Specimen were taken in which haemolysis was initiated for 20 min then 2.0 mL of saline water was added to stop haemolysis. The specimen were then incubated for 60 min at 37 ºC. Use of human ACD blood and saline water gave positive and negative control. The incubated samples were centrifuged for 45 min, the specimen liquid was separated and its absorbance was measured at 545 nm [23]. The following equation was used to calculate the percentage haemolysis:

$$\text{Hemolysis (\%)} = \frac{A_{\text{test-sample}} - A_{(-)\text{control}}}{A_{(+)\text{control}} - A_{(-)\text{control}}} \times 100 \quad (3)$$

RESULTS AND DISCUSSION

Formation of cryogel: During the cryogelation monomers or polymers precursors were subjected to sub zero temperature when they freeze leading to the formation of macroporous cryogel matrices. It results in the formation of large and interconnected pores in the range of 60-100 µm diameter. Ice crystals were formed at subzero temperature and get connected with each other [24]. It has also been revealed from unconfined compression analysis that even after 90% compression they regain their original length. The disinfectant loaded PVA mixture forms ice crystals on freezing which melts on thawing, thus creating large pores and eventually a porous gel. Porosity enhances and pore size widens by repetitive freeze-thaw cycle.

FTIR: Fig. 1a and 1b shows the FTIR spectra of PVA and *Aloe vera* loaded cryogel, respectively. The hydroxyl groups of poly (vinyl alcohol) and *Aloe vera* appear at 3600 cm^{-1} and 3424 cm^{-1}, in the spectra (a) and (b). A peak at 3300-2500 cm^{-1} is due to –OH (carboxylic) group of *Aloe vera* (Fig,. 1b). Moreover a sharp peak at around 2700 cm^{-1} (C-O groups) shows the presence of emodine group of *Aloe vera*. Sharp peak at 1740 cm^{-1} is due to –COO- stretching indicates the presence of >C=O group of *Aloe vera*. A absorption peak at 1400 cm^{-1} is due to symmetrical –COO- stretching of carboxylate group of *Aloe vera*. When the two spectra are compared spectra (b) of cryogels clearly confirmed that *Aloe vera* has been loaded in the cryogel.

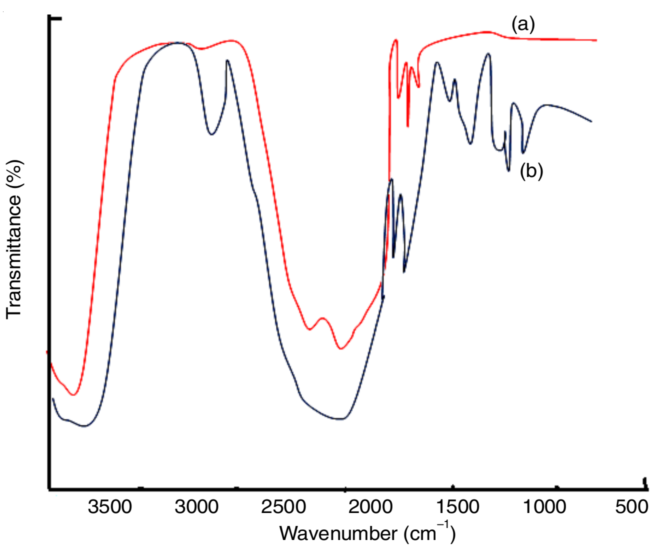

Fig. 1. FTIR of alone PVA and *Aloe vera* loaded PVA cryogels

FESEM studies: Fig. 2 shows the image of cryogel, which indicates that the macro porous nature of the cryogel. Its pore size is approximately 1 to 5 µm. The macroporous nature due to the melting of ice crystals crystals lying between the PVA chains.

Swelling kinetics

Effect of PVA: PVA amount varied from 1.0 to 4.0 g and its influence on the water sorption capacity of the cryogel was studied. Fig. 3 indicates that an increase in amount of PVA, the swelling ratio decreases. The degree of interaction

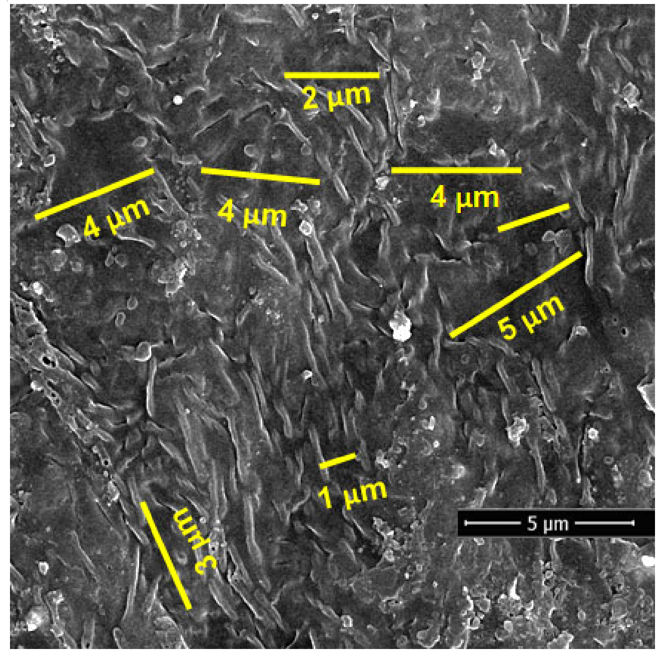

Fig. 2. FESEM image of PVA-*Aloe vera* cryogel

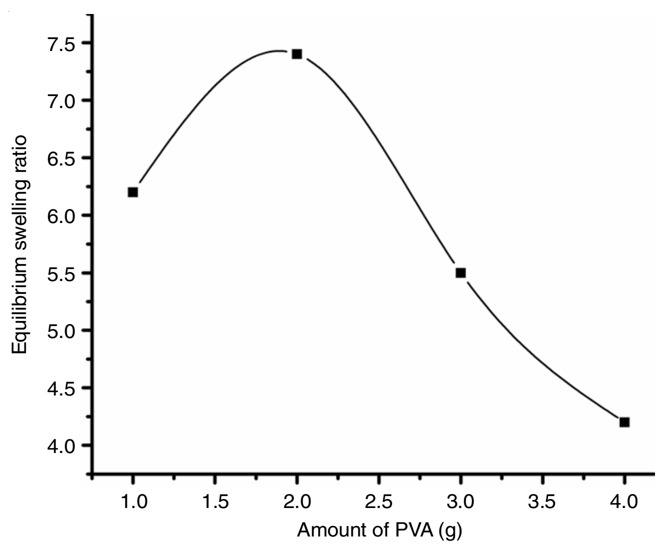

Fig. 3. Effect of PVA on the equilibrium swelling ratio of the cryogel

between the PVA-PVA macromolecules enhances with increasing amount of PVA (polymer fraction of polymer). A decrease in the swelling ratio is due to the increase in crosslinking, which decreases the porosity of gel [25].

Effect of *Aloe vera*: To study the effect of *Aloe vera* on the formation of cryogel, the volume was varied from 3.0 to 9.0 mL in the feed mixture. Fig. 4 clearly shows that the equilibrium swelling ratio decrease with increase in *Aloe vera* content. The observed decrease in swelling is due to an increase in the quantity of *Aloe vera*, resulting in the decrease of absorption of the liquid medium by PVA [26].

Effect of number of freeze-thaw cycle: Fig. 5 shows that if freeze-thaw cycle increased then the swelling ratio decreases. The results revealed that when the number of freeze-thaw cycle increases from 3 to 9, a constant fall in equilibrium swelling ratio is observed, which may be attributed to the fact

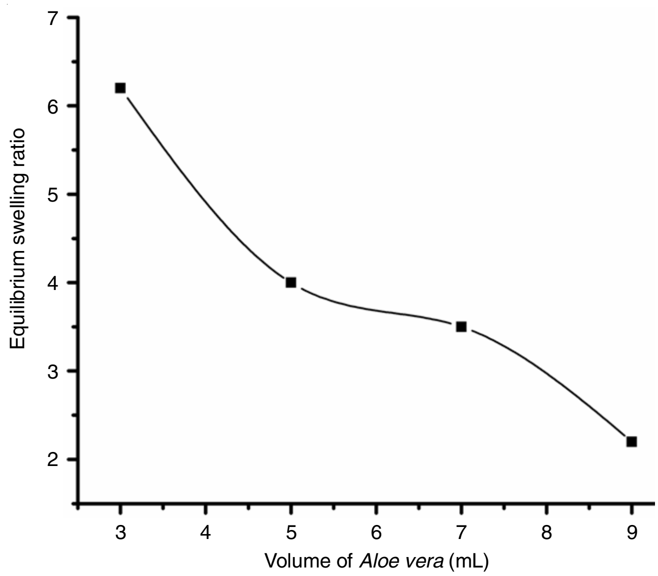

Fig. 4. Influence of varying amout of *Aloe vera* on swelling ratio of the cryogel

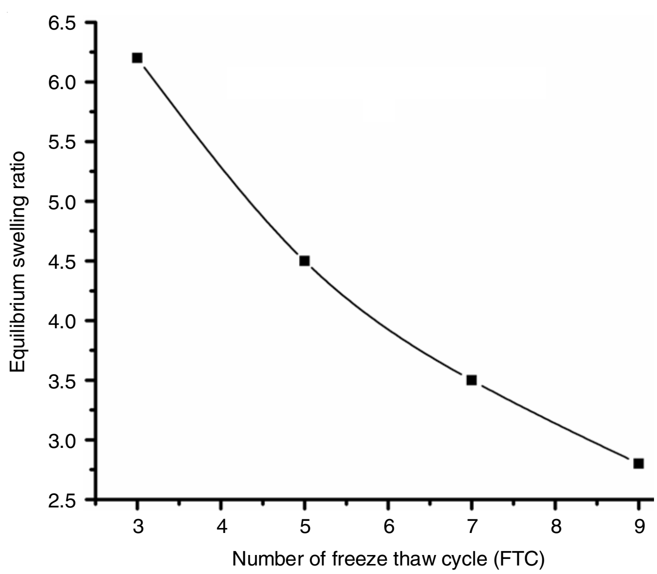

Fig. 5. Effect of freeze-thaw cycle on swelling ratio

that during the 3rd cycle, the macromolecular chains of cryogel keep relaxing with increasing swelling time due to the lack of large crystalline regions in cryogel. Increased number of freeze-thaw cycle enhances the cryogel crystallinty, restricting the mobility of disinfectant loaded PVA and chains [27], consequently suppressing the equilibrium swelling ratio. Another possibility is that with increasing number of freeze-thaw cycle, the pore size decreases and thus resulting in the lower water sorption by cryogel.

Effect of pH: Fig. 6 shows that the swelling ratio decreases in 4.0 to 9.0 pH. The swelling ratio was large at acidic pH 4.0 but as pH increases, the swelling ratio decreases. Moreover, as pH is increased, the swelling capacity of cryogels increases. Beyond 7.4, there is a decrease in the equilibrium swelling ratio. *Aloe vera* loaded cryogel immersed in the medium having low pH causes the diffusion of hydronium ion (H^+) in cryogels network due to which a repulsive force are initiated in the matrix.

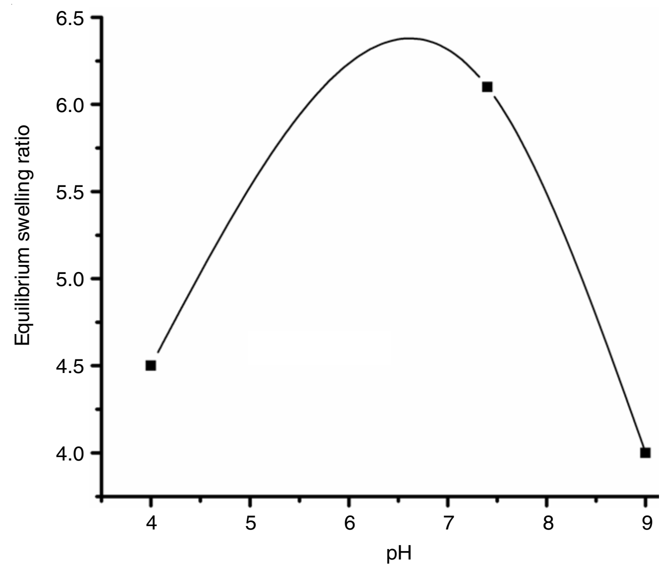

Fig. 6. Effect of pH on swelling ratio

These repulsive forces cause a relaxation of cryogel chains thus allowing a large number of water molecules inside the gel ultimately increasing their water intake capacity. Above pH 7.4, the OH^- ions in alkaline medium enter the gel and neutralize the H^+ ions, thus decreasing the equilibrium swelling of gel. This causes the minimization of repulsive forces inside the gel and thereby decreasing its water sorption capacity.

Effect of temperature: The effect of temperation variation was conducted in the range of 5-25 ºC. Fig. 7 shows that as temperature increases, the mobility of segments of cryogel chains and diffusion of water molecules inside also increases, which causes the greater swelling [28].

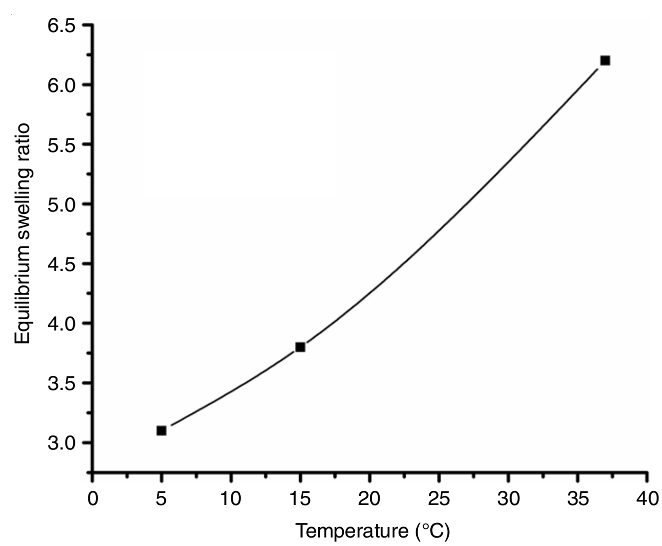

Fig. 7. Data showing the effect of temprature on swelling ratio

Deswelling kinetics: Water holding capacity of polymer matrix depends on the chemical composition of the cryogels and is prime parameter for a polymer material to be employed as hydrogel or wound dressing The progress of the deswelling process was monitored for cryogels of different compositions during the study.

Effect of PVA: On varying PVA from 1 to 4 g, a continuous decrease in the deswelling percentage was observed (Fig. 8). It was found that higher PVA content in cryogels shows greater the water retention capacity. Lowering of equilibrium deswelling was observed as the water molecules were tightly bound to PVA chains due to its hydrophilic nature [29].

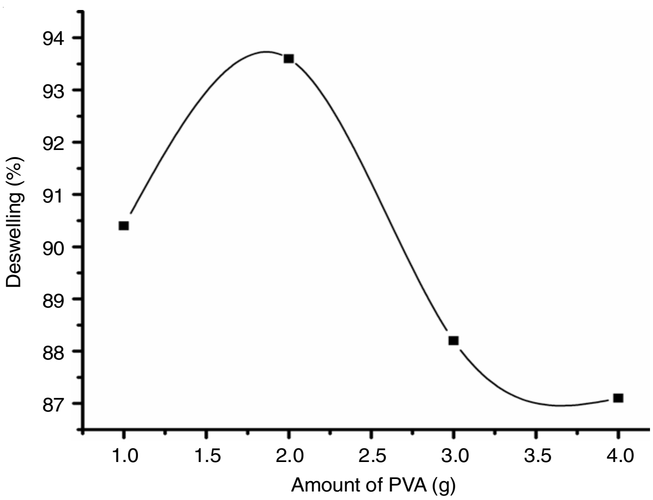

Fig. 8. Effect of varying amount of PVA on % deswelling of the cryogel

Effect of *Aloe vera*: The quantity of *Aloe vera* in the hydrogel matrix also affects its deswelling nature. This was studied by varying *Aloe vera* between 3 to 9 mL. Fig. 9 shows that the deswelling constantly increases with increasing amount of *Aloe vera*, the lowest equilibrium deswelling was observed at 3 mL, while an optimum deswelling when 5 mL *Aloe vera* was used.

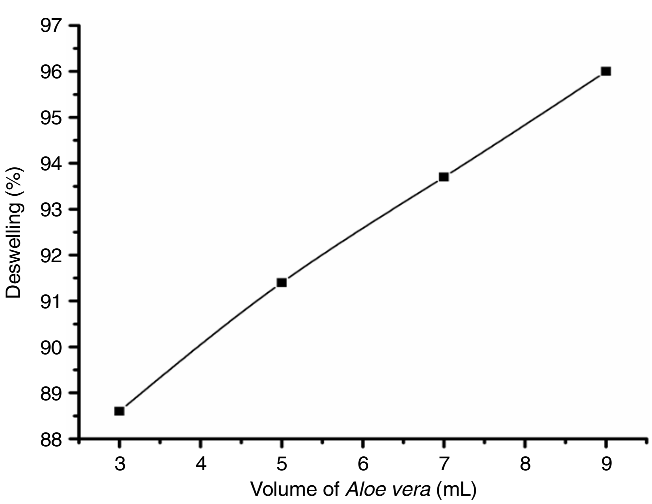

Fig. 9. Effect of *Aloe vera* on % deswelling of the cryogel

Effect of number of freeze-thaw cycle: It was observed that on varying freeze-thaw cycle from 3 to 9, there was a decrease in the equilibrium deswelling (Fig. 10). Increasing the number of freeze thaw cycle increases the crosslinkingin cryogels causing a compact network buildup. This sup-presses the evaporation rate of water as the pores of cryogel become small and hence decrease in the equilibrium deswelling.

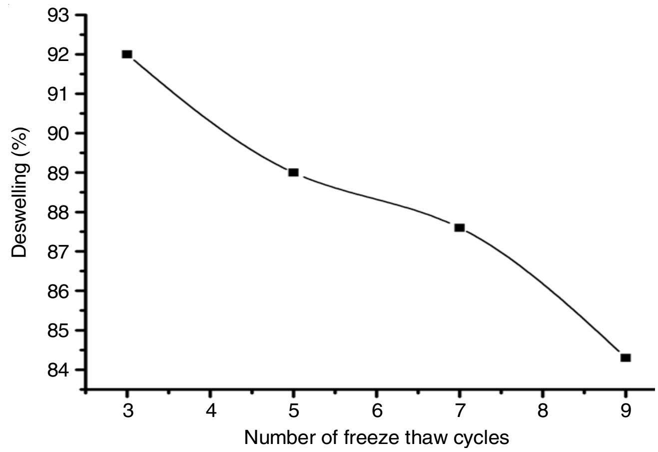

Fig. 10. Effect of FTC on % deswelling

Blood compatibility

Clot formation and hemolysis: The blood clot experiments of *Aloe vera* cryogels were performed and weights after clotting were recorded. The effect of PVA, *Aloe vera* contents and number of freeze-thaw cycle was investigated on the amounts of blood clot formed. It is clear from the results (Table-1) that in most cases almost no blood clots were formed, which indicated that the cryogels prepared are fully blood compatible in nature. The fairly high blood compatibility observed it may be due to the biocompatible nature of *Aloe vera* and PVA [30,31]. Similarly, the hemolysis tests were also conducted on the cryogels of varying compositions and percent hemolysis was determined. The results revealed that for all compositions the percent hemolysis was below 2%, which suggests the high blood compatible nature of cryogels.

TABLE-1
EFFECT OF VARYING AMOUNTS OF PVA, *Aloe vera* AND NUMBER OF FREEZE-THAW CYCLE ON THE AMOUNTS OF BLOOD CLOTS FORMED AND % HAEMOLYSIS

Amount of PVA (g)	Volume of *Aloe vera* (mL)	No. of freeze thaw cycles	Weight of the blood clot (mg)	Haemolysis (%)
1.0	5.0	3	0.0	1.92
2.0	5.0	3	0.0	1.51
3.0	5.0	3	2.1	1.59
4.0	5.0	3	0.0	1.71
2.0	3.0	3	0.0	1.50
2.0	5.0	3	2.2	1.62
2.0	7.0	3	0.0	1.64
2.0	5.0	3	0.0	1.92
2.0	5.0	5	2.0	1.52
2.0	5.0	7	0.0	1.48
2.0	5.0	9	0.0	1.51

Antibacterial study: The germicides generally exhibit selective toxicity based on the activity, which may be either viricides, bacteriocides, algicides or fungicides. In present study, the antibacterial activity of *Aloe vera* cryogels was performed and the results indicate that the cryogels have broad activity against *Microbacterium luteus* and *Citrobacter* sp. It was observed that as the amount of *Aloe vera* increases the zone of inhibition in the cryogel in the range of 3 to 7 mL (Fig. 11).

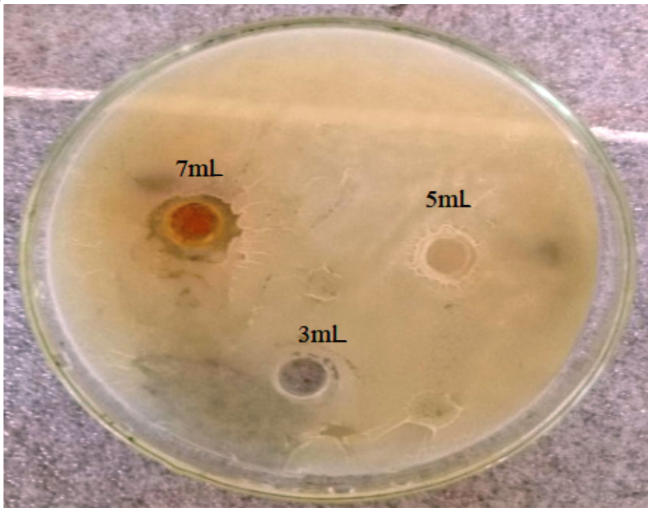

Fig. 11. Formation of inhibition zones on the PVA-*Aloe vera* cryogel surface

Conclusion

The preparation of cryogels of poly(vinyl alcohol) (PVA) containing *Aloe vera* as an antibacterial agent has proved to be useful for the healing of wounds. From the studies, it is concluded that dermal application of *Aloe vera*-PVA cryogel has no side effects and can be used effectively in treatments of wounds. The repeated freeze thaw method was used for preparation of hydrogels of *Aloe vera* and PVA. The FTIR spectra shows that *Aloe vera* loaded PVA cryogels were inter-linked through H-bonds. These hydrogels show the water absorption capacity, which may change due to the composition and external experimental conditions. The swelling ratio of cryogels was found to decreases with increasing amount of PVA, *Aloe vera* gel, number of freeze thaw cycles, pH and temperature of the swelling medium. The pore size of cryogels also decreases with increasing number of freeze-thaw cycles. The *Aloe vera*-PVA cryogels also show fairly high blood comp-atible nature as evidenced from almost nil blood clot formation and less than 2% hemolysis. Thus, *Aloe vera*-PVA cryogels show great inhibitory effects against the pathogenic bacteria causing different diseases in humans. The study also shows anti-bacterial activity of the prepared cryogels. Hence, the prepared material is subjected to *in vivo* studies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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