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Preparation and Characterization of Polyvinyl Alcohol–Gelatin Hydrogel Membranes for Biomedical Applications

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ABSTRACT

The purpose of this research was to design and develop hydrogels by esterification of polyvinyl alcohol (PVA) with gelatin. The membranes were characterized by Fourier Transform Infrared (FTIR) spectroscopy, x-ray diffraction (XRD), and differential scanning calorimetry. The viscosity of the esterified product (as solution) was compared with the mixture of PVA and gelatin of the same composition. The mechanical properties of the hydrogels were characterized by tensile tests. Swelling behavior and hemocompatibility of the membrane were also evaluated. The diffusion coefficient of salicylic acid (SA), when the receptor compartment contained Ringer's solution, through the membrane was determined. SA was used as a model drug. FTIR spectra of the membranes indicated complete esterification of the free carboxylic groups of gelatin. XRD studies indicated that the crystallinity of the membranes was mainly due to gelatin. The comparison of viscosity indicated an increase in segment density within the molecular coil. The membrane had sufficient strength and water-holding capacity. Hemocompatibility suggested that the hydrogel could be tried as wound dressing and as an implantable drug delivery system. The diffusion coefficient of SA through the membrane was found to be 1.32×10^{-5} cm^2/s. The experimental results indicated that the hydrogel could be tried for various biomedical applications.

KEYWORDS: Hydrogels, diffusion coefficient, hemocompatibility.

INTRODUCTION

Hydrogels are one of the most promising types of 3-dimensional cross-linked hydrophilic polymeric networks being used for various biomedical applications. These polymeric materials do not dissolve in water at physiological temperature and pH but swell considerably in an aqueous medium. Applications of hydrogels in the biomedical field include contact lenses, artificial corneas, wound dressing, coating for sutures, catheters, and electrode sensors. The application of hydrogels in such a wide range of fields leads to the manipulation of their physical properties. Since the hydrogels have absorbed water, they are usually biocompatible in nature and are nonirritating to the soft tissues when in contact with them.[1-3] The increasing importance of hydrogels in areas such as pharmaceuticals, food chemistry, medicine, and biotechnology has stimulated theoretical and experimental work on the properties of hydrogels in aqueous solutions. Chemically cross-linked gels can be obtained by radical polymerization or cross-linking agents (eg, glutaraldehyde, epichlorohydrin) or can be induced by radiation (eg, UV radiation, gamma radiation). The hydrogel characteristics, including the swelling properties and strength, can be modulated by the amount of cross-linking agent. Environmentally sensitive gels can be obtained by the addition of special monomers.[4,5] Water-soluble polymers owe their solubility properties to the presence of functional groups (mainly OH, COOH, NH_2) that can be used for the formation of hydrogels. Covalent linkages between polymer chains can be established by the reaction of functional groups with complementary reactivity, such as an amine-carboxylic acid or an isocynate-OH/NH_2 reaction.[6]

Polyvinyl alcohol (PVA) blends have long been used with other natural polymers because of PVA's ability to form films. The performance properties of PVA are influenced by the molecular weight and the degree of hydrolysis. The molecular mass of PVA is ~160 kDa.[7] PVA has a planar zigzag structure like polyethylene.[8] All PVA grades are readily soluble in water and are dependent on factors like molecular weight, particle size distribution, and particle crystallinity. As a hydrophilic polymer, PVA exhibits excellent water retention properties.[9] Optimum solubility occurs at 87% to 89% hydrolysis. For total dissolution, however, PVA requires water temperatures of ~100°C with a hold time of 30 minutes.

Gelatin is obtained by the thermal denaturation of collagen from animal skin, bones, and, rarely, fish scales. It contains mainly the residues of 3 amino acids—glycine (arranged every third residue), proline, and 4-hydroxyproline—in its

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structure. Gelatin contains extended left-handed proline helix conformations incorporated with 300 to 4000 amino acids. The presence of higher levels of pyrrolidines in gelatin results in the formation of stronger gels.[10] The strength of the gelatin film is due to the presence of triple helixes. The greater the triple-helix content, the higher the strength of the film and the lower the swelling property in water.[11] The gelling properties of the gelatin can be altered by the introduction of chemical cross-links by using transglutaminase to link lysine to glutamine residues[12] or glutaraldehyde to link lysine to lysine.

Like PVA, gelatin possesses a film-forming property. Accordingly, it is used for making hard and soft gelatin capsules. Considering the film-forming property, a PVA-gelatin film was thought to be a good candidate for artificial skin. Hence, attempts were made to develop a hydrogel membrane by esterifying the hydroxyl group of PVA with the carboxyl group of gelatin. Attempts were also made to characterize the membrane by FTIR, x-ray diffraction (XRD), differential scanning calorimetry (DSC), tensile strength, hemocompatibility, swelling studies, and diffusion studies.

Materials and Methods

Materials

PVA (molecular weight 125 000) was obtained from SD Fine Chem Ltd (Mumbai, India). Gelatin (for bacteriological purposes) and salicylic acid (SA) were obtained from Loba-Chemie Indoaustranal Co (Mumbai). Hydrochloric acid 35% pure was obtained from Merck Ltd (Mumbai). Double-distilled water was used throughout the study.

Preparation of Hydrogel

The preparation of the hydrogel has been described elsewhere.[13] In short, 2.5 g of gelatin was dissolved in 100 mL of a 10% aqueous solution of PVA. Concentrated hydrochloric acid (HCl, 0.05 mL) was added, and the resulting dispersion was stirred (using a overhead stirrer at 100 ± 5 rpm) at 70°C for a half-hour to carry out the esterification reaction between PVA and gelatin. The thick dispersion so obtained was converted into a membrane by the conventional solution casting method. The resultant membrane was washed thoroughly with distilled water to remove the HCl and was stored in a dessicator at 37°C.

Characterization

Gelatin, PVA, and the hydrogel membrane were subjected to FTIR spectroscopy in the range of 4000 to 400 cm^{-1}. An FTIR spectrophotometer (NEXUS-870, Thermo Nicolet Corporation, Waltham, MD) was used for the study.

The raw materials and the membrane were subjected to XRD (XRD-PW 1700, Philips, Rockville, MD) using CuKα radiation generated at 40kV and 40 mA; the range of diffraction angle was 10.00 to 70.00° 2θ.

The viscosity of the esterified product in solution and the mixture of PVA-gelatin (taken in the same ratio as was taken in the preparation of the esterified product) was measured using a TA rheology instrument (Model AR 1000, New Castle, DE).

A PerkinElmer DSC-2 (Waltham, MD) was used to study the melting and crystallization behavior of the polymeric membrane. The temperature and energy scales were calibrated with the standard procedures. The melting studies were performed in the temperature range of 50 to 400°C at a heating rate of 10°C/min in an N_2 atmosphere.

The tensile strength of the membrane was tested in a Hounsfield H10KS tensile testing machine (Horsham, PA). The cross-head speed was kept at 5 mm/min.

The hemocompatibility test was performed as described elsewhere.[14] In place of citrated goat blood, chelated human blood (with EDTA) was used. The rest of the procedure was the same. The hemolysis percentage is defined as

$$\% \text{ Hemolysis} = \frac{OD_{test} - OD_{negative}}{OD_{positive} - OD_{negative}} \times 100 \qquad (1)$$

where OD is optical density.

Swelling Behavior

The membrane was immersed directly in buffers of pH 1.4, 5.4, or 7.4 (prepared as per Indian Pharmacopoeia 1996, Ministry of Health and Social Welfare, New Delhi, India) at room temperature for 72 hours; after that, the swollen product was dried at 37°C under vacuum to a constant weight. The equilibrium percentage of swelling (% swelling) of the product was calculated as follows:

$$\% \text{ Swelling} = \frac{W_e - W_d}{W_d} \times 100 \qquad (2)$$

where W_e is the weight of the product after hydration for 72 hours, and W_d is the weight of the dried product.

Measurement of Diffusion Coefficient

A diaphragm cell[15] was used to measure the diffusion coefficient. The cell consisted of 2 chambers separated by a film (0.2-mm thick) of the hydrogel. The hydrogel membrane was equilibrated in water for 2 hours before the experiment began. The first chamber (donor) contained 10 mL

of SA solution (0.8 mg/mL). The other chamber (receptor) contained 200 mL of Ringer's solution. Then the donor chamber was lowered so that the hydrogel just touched the receptor fluid kept under stirring. The system was placed in a constant-temperature water bath (30°C). A pipette was used to draw 0.1 mL of solution from the donor chamber and 1.0 mL of sample from the receptor chamber periodically. The withdrawn samples were replaced with an equal volume of distilled water. The samples were analyzed spectrophotometrically at 294 nm to determine the concentration of SA in each chamber as a function of time. The diffusion coefficient, *D*, was calculated from these results.

At any time, *t*, the concentration values in the 2 chambers can be used to calculate the diffusion coefficient, *D*, of the drug in the hydrogel from the following equation[16]:

$$D = \frac{1}{\beta t} \times \ln \frac{C_D(t) - C_R(t)}{C_D(0) - C_R(0)} \quad (3)$$

with

$$\beta = \frac{A_H}{W_H} \times \left[\frac{1}{V_1} + \frac{1}{V_2}\right] \quad (4)$$

where $C_D(0)$ is the initial concentration of drug in the donor chamber;, $C_R(0)$ is the initial concentration of drug in the receptor chamber; $C_D(t)$ is the concentration of drug in the donor chamber after time *t*; $C_R(t)$ is the concentration of drug in the receptor chamber after time *t*; A_H is the effective cross-sectional area of diffusion in the hydrogel sample; W_H is the width of the hydrogel sample; V_1 is the volume of drug solution in the donor chamber; and V_2 is the volume of the receptor chamber media.

Results and Discussion

FTIR Characterization

The FTIR spectra of gelatin (Figure 1) showed peaks at 3450 cm^{-1} and 3423 cm^{-1} due to -NH stretching of secondary amide, C=O stretching at 1680 cm^{-1} and 1640 cm^{-1}, -NH bending between 1550 cm^{-1} and 1500 cm^{-1}, -NH out-of-plane wagging at 670 cm^{-1}, and C-H stretching at 2922 cm^{-1} and 2850 cm^{-1}. The FTIR spectra of PVA (Figure 1) showed a broad peak around 3425 cm^{-1} indicating stretching of hydroxyl groups and peaks at 2923 cm^{-1} and 2850 cm^{-1} due to C-H stretching. The spectra of the membrane (Figure 1) showed a peak at 3398 cm^{-1}, indicating the presence of a hydroxyl group with polymeric association and a secondary amide. It can be observed from the spectra of the membrane that the peak of the gelatin at 1680 cm^{-1} shifted to 1758 cm^{-1} (Figure 1), indicating the formation of an esterified product (after esterification bond length is shortened,

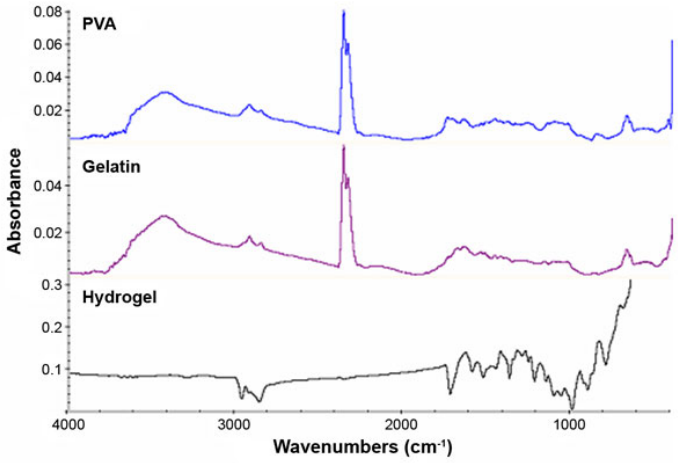

Figure 1. FTIR spectra of PVA, gelatin, and hydrogel. PVA indicates polyvinyl alcohol.

resulting in the shift of the peak to a higher wave number). Since there are no peaks at 1680 cm^{-1} it can be concluded that all the free carboxylic groups of gelatin have been esterified. The peaks at 1088 cm^{-1} and 1277 cm^{-1} indicated the C-O stretch of secondary alcoholic groups and the ester. The spectra of the membrane also showed a peak at 1635 cm^{-1} (Figure 1), indicating the presence of a secondary amide group. The peak at 2947 cm^{-1} (Figure 1) indicates the presence of a hydrocarbon chromophore in the esterified product. In short, the esterified product has an ester linkage, a secondary alcoholic group, and secondary amide groups in addition to the hydrocarbon chromophore.

XRD Characterization

The XRD patterns of PVA film and gelatin (Figure 2) revealed that the PVA peak was at around 22.5° 2θ, while that of gelatin was at around 20° 2θ, having intensities of ~3300 and 1200, respectively. The XRD pattern of the membrane (Figure 2) revealed a prominent peak at around 20° 2θ, having an intensity of 1600. From this we can infer that the crystallinity of the membrane is mainly due to gelatin rather than PVA. From the XRD patterns the percent crystallinity of PVA, gelatin, and the hydrogel membrane was found to be 5.27, 9.84, and 2.46, respectively. Thus, there appears to be a reduction in crystallinity of the membrane.

Viscosity Measurement

The viscosity of the esterified product was measured at varying shear rates (0-50 1/s) and was compared with the mixture of PVA and gelatin having the same composition as that of the esterified product.

From Figure 3, it can be observed that at every shear rate the viscosity of the esterified product is lower than that

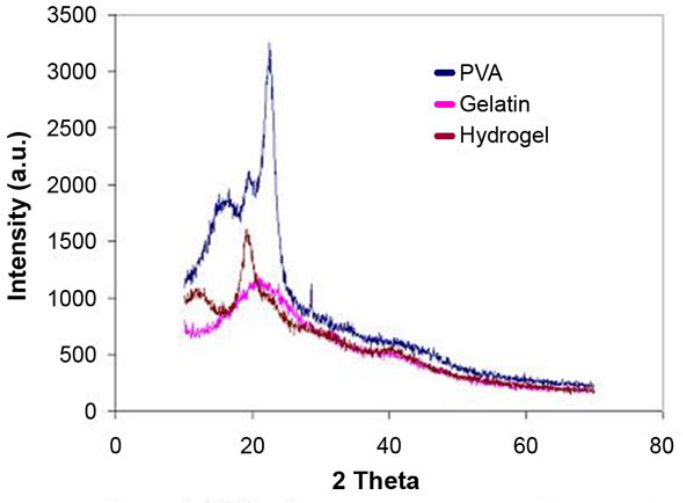

Figure 2. X-ray diffraction pattern of PVA, gelatin, and hydrogel. PVA indicates polyvinyl alcohol.

of the PVA-gelatin mixture having the same composition. This indicates that some interaction has taken place among the 2 polymers. Esterification results in an increase in segment density within the molecular coil, which in turn results in a smaller hydrodynamic volume and a lower intrinsic viscosity.[17]

DSC Characterization

The DSC thermogram of PVA indicated a glass transition temperature of 92°C and a melting isotherm at 292°C. The thermogram of gelatin indicated a glass transition temperature of 60°C. The glass transition temperature of the membrane was found to be 145°C, indicating the formation of a new product. No melting endotherm was found for gelatin. As we have already discussed, gelatin is available in a coiled structure, while PVA has a planar zigzag structure. Because of this mismatch in crystalline structure, the polymer matrix might behave as an amorphous polymer complex, and hence there was no sharp melting point in the thermograms of the membrane.

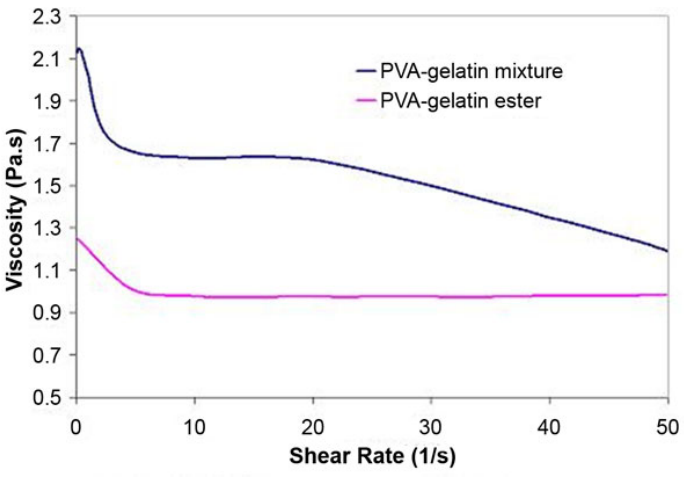

Figure 3. Comparison of viscosity of PVA-gelatin mixture and esterified product. PVA indicates polyvinyl alcohol.

Table 1. Hemocompatibility Test (Human Blood)*

	OD at 545 nm	% Hemolysis	Remarks
Positive	0.648	—	—
Negative	0.005	—	—
PVA-gelatin	0.005	0.00	Highly hemocompatible

*OD indicates optical density; PVA, polyvinyl alcohol.

Tensile Strength of Membrane

The tensile strength of the PVA membrane was found to be 19 ± 1.92 MPa, while the strength of the gelatin film was found to be 1.29 ± 0.50 MPa. The strength of the membrane was found to be ~14 ± 2.81 MPa. So, this membrane could be used for wound covering, as it can withstand some frictional stresses during day-to-day activities. The membrane is stitched around the wound surface so as to cover the wound. If there are any frictional stresses, the membrane absorbs the energy without breaking and thus protects the wound.

Hemocompatibility Test

Because no hemolysis or nearly 0% hemolysis (Table 1) occurred in the presence of the sample, the sample could be considered highly hemocompatible and could be tried as a

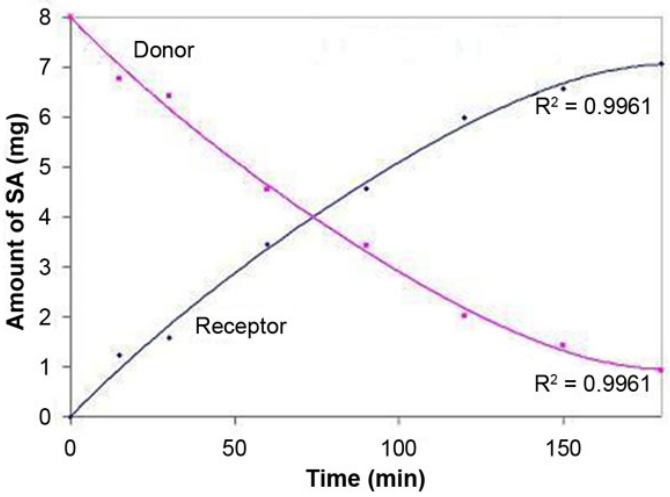

Figure 4. Change in amount of SA in receptor and donor chambers during diffusion experimentation. SA indicates salicylic acid.

moist wound-dressing material or for implantable drug delivery systems.

Swelling Behavior

The water retention capacity of the hydrogel membrane prepared was ~260% of the dried weight, so it can be categorized as superabsorbent. From the FTIR result for the membrane, it is clear that the whole carboxylic group of the gelatin has been esterified but the free primary amine groups are present. From this fact it can be hypothesized that these free amino groups play an important role in water uptake because of their hydrophilic nature.

Measurement of Diffusion Coefficient

The change in the amount of SA in the donor and receptor chambers is shown in Figure 4. A plot of $-\ln \frac{C_D(t)-C_R(t)}{C_D(0)-C_R(0)}$ (denoted by –lnX) vs time yielded a straight line. The slope of this line was used to calculate the diffusion coefficient, D, as indicated in Equation 3. The diffusion coefficient of SA through the membrane was found to be 1.32×10^{-5} cm^2/s.

CONCLUSION

PVA-gelatin membranes were made by esterification of the hydroxyl group of PVA with the carboxyl group of gelatin. The hydrogel developed was found to be superabsorbent, to be hemocompatible with human blood, and to allow diffusion of SA. Hence, it could be tried for various biomedical applications, such as drug delivery systems and moist wound dressings.

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