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# Calorimetric analysis of gelatine–glycosaminoglycans blend system

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ABSTRACT

Gelatine is one of the most valuable natural polymers used for drug delivery applications. Gelatine–GAGs based composite system has been shown to act as good scaffolds for tissue engineering. The objective of the present study is to investigate the calorimetric properties of microporous gelatine–GAGs based blend, which were modified by co-crosslinking with a naturally occurring crosslinking agent genipin. The melting temperature ($T_m$), enthalpy change ($\Delta H_m$) and heat capacity change ($\Delta C_p$) were systematically calculated over the experimentally observed systems using differential scanning calorimeter (DSC). The thermoporometry results suggest that the concentration of the glycosaminoglycans plays an important role in the pore size distribution of the blend matrices. The circular dichroism (CD) spectroscopy study, scanning electron microscopy (SEM) studies provide the valuable information about the structural features of the biodegradable blend that can be utilized for various biomedical applications. The results provide new insights into the thermal stability of blend and suggest potential strategies for its manipulation.



## 1. Introduction

Glycosaminoglycans (GAGs), including hyaluronan (HA) and chondroitin sulfate (CS), are the ubiquitous component of the intricate extracellular matrices (ECM) of all connective tissues [1]. HA is the only non-sulfated GAG [2] and is comprised of alternating units of β-1,4-linked D-glucuronic acid and (β-1,3) N-acetyl-D-glucosamine [3]. Another member of GAGs family is chondroitin sulfate, a high viscous mucopolysaccharide of high molecular weight (10,000–100,000 Da) consisting of alternating units of β-1,3-linked glucuronic acid and (β-1,4) N-acetyl-galactosamine (GalNAc) and is sulfated on either the 4- or 6-position of the GalNAc residues. CS is covalently linked to proteoglycans, which in turn have HA-binding modules [2] that form multivalent high-affinity interactions with HA. Protein–GAGs interactions regulate cell adhesion and motility and mediate cell proliferation and differentiation [4,5].

Gelatine, the hydrolyzed product of collagen, is being used for biomedical, pharmaceutical applications due to its biocompatibility and biodegradability properties. It is unique among hydrocolloids as it can form thermo-reversible with a melting point close to body temperature [6]. The disadvantages of gelatine namely the poor mechanical property and water sensitivity (poor water vapor barrier property) can be overcome by crosslinking it with different natural and synthetic crosslinkers [7]. It was already reported that gelatine derived bioadhesives display higher biocompatibility and less cytotoxicity when crosslinked with genipin, a naturally crosslinker than with other agents, such as formaldehyde, glutaraldehyde and epoxy compounds [8]. Interactions between gelatine and GAGs are already well investigated [9]. Gelatine–GAGs based scaffolds mimics the ECM of cartilage. GAGs enhance the cellular adhesion and chondrogenic differentiation. It promotes the secretion of proteoglycan and type II collagen. It facilitates the cell viability and differentiation [10]. Various parameters including thermal stability [11], pore size distribution [12], structural properties needs to be controlled for use of gelatine for drug delivery applications.

In this study, the thermal stability and pore size distribution of the gelatine–GAGs blend systems were studied by analyzing the experimental thermodynamic data and comparison between the families of homologous blend to design the optimal blend for drug delivery application. Gelatine–GAGs scaffolds are most suitable for thermo responsive drug release applications. The thermodynamic parameters, melting temperature ($\Delta T_m$), enthalpy change at the melting temperature ($\Delta H_m$) and heat capacity change ($\Delta C_p$) are sensitive to the composition of the material [13,14]. Thermally induced transformation of blends reflects the overall condition of the structure and crosslink in the blend network. Thermoporometry has been used to determine the pore size distribution of the gelatine–GAGs blend. Thermoporometry technique has been found to be an useful tool for studying the porosity parameters including pore size distribution and pore volume of biological materials in native state [15,16]. This technique can be employed to characterize closed meso-porosity of wet-spun fibers inaccessible by

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**Table 1**
Composition of prepared gelatine–GAGs blend.

| Ratio of gelatine:GAGs | Sample name | Genipin (%)[a] |
|---|---|---|
| 1:0.5 | WC-GG1[b] | – |
| | C-GG1[c] | 0.01 |
| 1:1 | WC-GG2 | – |
| | C-GG2 | 0.01 |
| 1:2 | WC-GG3 | – |
| | C-GG3 | 0.01 |

[a] Wt.% of gelatine.
[b] WC – without crosslinking agent.
[c] C – with crosslinking agent.

other standard porosimetry technique [17]. The average radius of the pores calculated by thermoporometry ranges 2 nm < *R*p < 65 nm [18]. Pores of this size could not be detected by SEM [18]. The analysis of the Gibbs–Helmholtz equation to study the interdependence of the thermodynamic parameters and their derivatives along with the results of investigations into the ATR-FTIR studies, circular dichroic (CD) spectroscopy studies, scanning electron microscopic (SEM) studies of the blend materials are presented in this study. The objective of the study is to throw light on the parameters, which can influence the blend properties for drug delivery applications.

## 2. Experimental

### 2.1. Materials

Gelatine type B extra pure from bovine skin (gel strength 225 bloom) of I.P. 5.05 was purchased from Himedia Laboratory, India. Chondroitin-6-sulfate of 90% purity (60 kDa) was obtained from Sigma–Aldrich, USA. Genipin (M.W. 226, 98% by HPLC) was purchased from Sigma Chemicals Co., USA. Water used for these studies was of Millipore grade.

### 2.2. Blend preparation

Gelatine stock solution was prepared on warming the water to about 40 °C. Blends were prepared by mixing suitable volumes of GAGs aqueous solutions into the gelatine solution at room temperature. The gelatine concentration (0.5%, w/v) into the blend was fixed and the different weight ratios of gelatine/GAGs used were 1:0.5, 1:1 and 1:2. These blends were stirred for 2 h. Genipin (0.01% on weight of gelatin) was added for the preparation of crosslinked blend system. After addition of genipin, the samples were stirred for 3 h at room temperature. Samples for SEM analysis were lyophilized under the pressure of 48 mTorr at −40 °C. The composition of the prepared blends is tabulated in Table 1. All other measurements were carried out in liquid solution of gelatine–GAGs.

### 2.3. Characterization of the blend systems

#### 2.3.1. Viscosity measurement

The viscosity of the blends was examined using Ostwald type viscometer of 2 ml capacity. The viscometer was thermostated at 25 °C. The flow times of water, protein solution, and protein solution with GAGs as well as with/without genipin were taken separately after a thermal equilibrium time of 15 min for the estimation of the effect of GAGs concentration on the absolute viscosity of protein. The flow time was measured with a digital stopwatch at least three times and the mean was taken. The viscosity contribution ($\eta$) due to gelatine was measured as a function of additive concentration. The viscosity was calculated from the relation,

$$\eta_{sp} = \frac{t - t_0}{t_0} \quad (1)$$

where $t_0$ is the flow time of water and $t$ is the flow time for each sample. Relative viscosity of protein ($\eta_{relative}$) was calculated by the following equation,

$$\eta_{relative} = \frac{\eta}{\eta_0} \quad (2)$$

where $\eta$ and $\eta_0$ are the viscosity of gelatine in the presence and absence of GAGs.

$\eta_{relative}$ versus gelatine/GAGs was plotted.

#### 2.3.2. Circular dichroic study

Circular dichroic spectra were measured using a Jasco 715 circular dichroism spectropolarimeter using a quartz cell with a light path 1 mm with 0.2 nm intervals, at 25 °C with computer-averaged trace of three scan averaged for each samples. CD spectra were recorded in the far UV region (190–280 nm) under $N_2$ atmosphere to estimate the conformational change of gelatine brought about by GAGs solution. An aqueous solution of gelatine was incubated with the GAGs solution of different concentration maintaining the different gelatine/GAGs weight ratio of the prepared blend with and without crosslinking agent. Signal due to solvent was subtracted. The data were normally plotted as mean-residue-weight ellipticity (deg. $cm^2$ $dmol^{-1}$) versus wavelength in nm.

#### 2.3.3. Thermoporometric studies for pore size distribution

Thermoporometric analysis was carried out using Q200 TA instruments between a temperature range from −40 °C to 10 °C with a heating rate of 1 °C/min. All the test samples were in liquid state. The stability of the baseline was checked before each measurement. The hermetically encapsulated aluminum pan containing the sample was placed in a heating block housed in a DSC cell mounted in calorimeter. The peak temperature ($T_m$) and total enthalpy ($\Delta H_m$) for the transition were computed using the system generated software. The pore size distribution was calculated as described earlier [15,16]. All the experiments were done in triplicate and the mean was taken.

#### 2.3.4. Differential scanning calorimetric (DSC) measurements

DSC measurements were performed using Q200 TA instruments in the temperature range of 10–200 °C using a heating rate of 5 °C/min. All the experiments were done in triplicate. The total enthalpy ($\Delta H_m$), onset temperature and peak temperature ($T_m$) of transition were noted down from the system generated software. The specific heat $C_p$ in J/g K of every sample was calculated from the DSC thermogram consisted of heat flow (W/g) by using Eq. (3) [19].

$$Cp = \frac{Q' \times 60}{r} \quad (3)$$

where $r$ is the heating rate of the process in K/min, $Q'$ is heat flow in W/g. The resulting data were used to construct specific heat versus temperature curves.

The endothermal processes seen in thermograms could be described by the Arrhenius equation. Hence, the energy of activation ($E_a$) for the process was calculated using the dependence of the enthalpy evolved with temperature [20].

$$ln\left(\ln\left(\frac{H_m}{H_m - H}\right)\right) = \frac{E_a}{R}\left(\frac{1}{T_m} - \frac{1}{T}\right) \quad (4)$$

where $H_m$ is the total enthalpy of the process, $H$ the enthalpy evolved at a given temperature $T$, $T_m$ the temperature at the peak of the endotherm and $R$ is universal gas constant. $H_m$ and $H$ were obtained from the area under the endotherm. A plot of $\ln(\ln(H_m/(H_m - H)))$ versus $1/T$ should give a straight line with the slope being $-E_a/R$.

The increment of specific heat $\Delta C_p$ was measured by calculating the difference between the extrapolations of the specific