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Graphical Abstract

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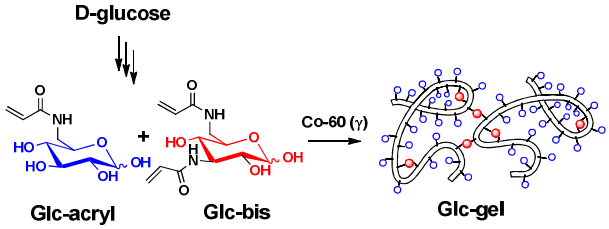

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Abstract

The ability of sugar pendants in glycopolymers to mimic that on the cell surface can be used as a reliable method for the site specific delivery of drugs. In the search for new possibilities, we herein report a D-glucose based synthesis of hitherto unknown bisacrylamide crosslinker (Glc-bis), a related acrylamide monomer (Glc-acryl), with hemiacetal functionality. A one pot process to transform the synthesized Glc-acrylamides to sterilized homogeneous glycopolymeric gel (Glc-gel) was investigated using radiation induced polymerization technique. The Glc-gel was subjected to swelling studies, viscoelastic, and thermal analysis. The *in vitro* cytotoxicity test revealed that the constituents (Glc-acryl and Glc-bis) as well as the Glc-gel were non toxic to the cells and the cells grew normally in their presence. The synthesized Glc-gel was found to show good affinity to lectin Con A demonstrating that replacing hydroxyl group in D-glucose moiety with amide bond does not largely affect its interaction with Lectins.

Keywords: Glycopolymer, Radiation, Acrylamide, Bisacrylamide, Hydrogel, Biocompatible

Introduction

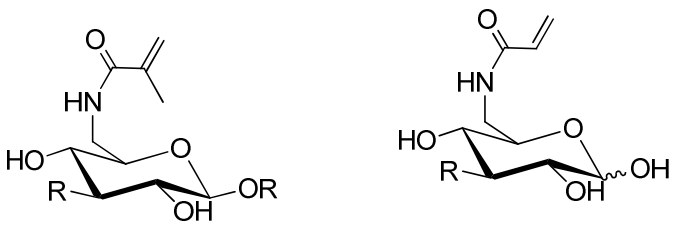
A new class of biocompatible and biodegradable materials containing sugar moieties called as glycopolymers has received great attention in the scientific community. This is largely due to their wide range of applications, which include, the synthesis of macromolecular drugs, matrices for cell culture, model biological systems, surface modifiers, chromatographic purposes and so on.[1] The advanced polymerization techniques have facilitated the synthesis of glycopolymers of different types required for specific applications. In general the application of polymers widens, with the scope of achieving it in different forms like gels.

The last three decades saw a vast and more creative development in the field of hydrogels directed towards a more precise/selective application.[2] The most attractive and important aspect of hydrogel is its bio-compatibility and bio-degradability which ensures its application in biomedical field.[3] This is largely promoted by its high water content and a similar physiochemical nature of hydrogels to the native extracellular matrix.[4] Even though extensive work have been carried out in the area of glycopolymers,[5] very little is known about the synthesis and study of purely sugar based hydrogels.[6] The significance of carbohydrate based polymers/hydrogels in the biomedical field is owing to the glycotargeting ability of carbohydrate pendants present in the polymer network.[7] The carbohydrate pendants in the glycopolymeric framework can be recognized by the cell surface carbohydrate binding proteins-lectins [8] and this makes them a unique class of materials for targeted drug delivery applications.[9]

Generally, sugar based hydrogels are synthesized from low molecular weight gelators (LMWG).[10] However, it has been reported that hydrogels derived from LMWG possess several disadvantages that include aggregation, crystallization or precipitation with time.[10d] One way to

overcome this is to synthesize hydrogel from low molecular weight carbohydrate derivative by radiation polymerization. This technique has the potential to overcome most of the limitations that arises from LMWG, as the radiation crosslinked hydrogels possess more lifetime stability due to covalent crosslinking. An added advantage of radiation induced synthesis is that, a sterilized hydrogel can be achieved in a single step process by applying appropriate radiation dose.

Recently, studies towards the synthesis of biodegradable materials revealed that incorporation of biodegradable crosslinkers into a non-biodegradable but biocompatible polymer could transform the latter to a biodegradable material.[11] This observation triggered efforts to make biodegradable crosslinkers based on peptides/saccharides. In this context, it is of interest to have a crosslinker with functional groups similar to that on the monomer, so that the functional homogeneity is maintained throughout the polymeric network. Currently, for the synthesis of glycopolymeric hydrogels, commercially available crosslinking agents with non-sugar residue are being used.[11c] It was Dordick and coworkers who demonstrated for the first time a chemoenzymatic method for the synthesis of sugar containing polyacrylate hydrogels.[11d] To the best of our knowledge there exists only one report on the chemical synthesis of a sugar based cross linker *i.e.*, bis(methacrylamido) derivative of D-glucose **1 (**Figure 1). The utility of **1** to form hydrogel has been tested successfully by synthesizing PHEMA based biocompatible hydrogel.[11c]



1, R = $(CH_2)_2NHC(O)C(Me)=CH_2$ **2a**, R = $NHC(O)CH=CH_2$
2b, R = OH

Figure 1: Acrylamides derived from D-glucose

Citing the significance of glycopolymeric hydrogels and the relevance of having a sugar based crosslinker, we here by describe the synthesis of a D-glucose based bisacrylamide cross linker substituted at C-3 and C-6 carbon of sugar (Glc-bis) **2a**, (Figure1) with hemiacetal functionality. To check the feasibility of Glc-bis to form homogeneous glycopolymeric gel (Glc-gel), a related monoacrylamide substituted at C-6 position (Glc-acryl) **2b** was also achieved and their gelation was studied using radiation polymerization. The targets selected are also interesting by the fact that D-glucose derived polymers substituted at C-6 position showed specific binding to the Asialoglycoprotein receptor of mouse primary hepatocytes.[12]

The synthesized Glc-bis and Glc-acryl were characterized by ^{1}H and ^{13}C-NMR. The molecular structure, water content, viscoelasticity, thermal stability, cytotoxicity and lectin recognition of the synthesized hydrogels (Glc-gel) were studied using the techniques like Fourier Transform Infra-Red (FT-IR) spectroscopy, oscillatory rheology, Thermogravimetric-Differential Scanning Calorimetric (TG-DSC) analysis, MTT assay and UV-vis spectroscopy.

Experimental

Materials

Methanol, dichloromethane, pyridine, triethylamine, were purified and dried before use. The n-hexane used was the fraction distilling between 40–60 ºC. All the chemicals including acryloyl

chloride were procured from either Aldrich or Fluka. DMEM cell cuture medium, Penicillin and Streptomycin were purchased from HiMedia, Mumbai, India. Fetal Bovine Serum (FBS) was procured from Invitrogen BioServices India Pvt. Ltd. MTT and Staurosporine were procured from Sigma Aldrich. Water, with conductivity 0.6 μS cm^{-1}, from Millipore Milli-Q system, was used for the preparation of aqueous solutions. Con A (lyophilized powder) from *Canavalia ensiformis* and BSA (Bovine Serum Albumin) were purchased from Sigma and was used directly. 0.01M phosphate-buffer saline (PBS) at pH 7.4 was prepared by diluting 10X concentrated PBS purchased from sigma into distilled water with 150 mM NaCl, 1 mM NaN_3, 1mM $CaCl_2$ and 1 mM $MnCl_2$.

Methods

The IR spectra were recorded using diamond single reflectance ATR in IR Affinity-1 spectrometer. The samples were analyzed over the range of 400-4000 cm^{-1}, operating at 4 cm^{-1} resolution. The ^{1}H (200 MHz, 600 MHz, 700 MHz) and ^{13}C (50 MHz, 126 MHz, 176 MHz) NMR spectra were recorded with a Brüker Oxford instrument in $CDCl_3$, CD_3OD or D_2O as solvents. A Co-60 gamma radiation source was used with a dose rate of 1.23 KGy/h. Irradiation was carried out in 1 cm x 1 cm closed glass vials in nitrogen atmosphere. Thermogravimetric analysis (TGA) and Differential Scanning Calorimetry (DSC) were performed, using Mettler Toledo TG/DSC star^{e} system. All the dynamic rheological measurements were carried out using the parallel plate geometry (Anton Paar physica MCR101; PP25-4, diameter 24.972 mm). The temperature was controlled by a circulating water bath and maintained at 37 ^{o}C with a thermoelectric peltier device. Ultraviolet-visible (UV-vis) absorption spectra of the interaction of synthesized hydrogel (Glc-gel) with Con A and BSA were recorded on Jasco V-650 spectrophotometer.

(2R,3S,4S,5S)-4-acrylamido-6-(acrylamidomethyl)-tetrahydro-2H-Pyran-2,3,5-triol (Glc-bis, 2a).

A pre-cooled solution of TFA-H_2O (3:2, 10 mL) was added dropwise to a RB charged with bisacrylamide **6** (1.30 g, 3.98 mmol) (synthesized as shown in Scheme 1) at 0 ^oC. The reaction mixture was stirred at same temperature for 30 min, slowly brought to 25 ^oC and stirred for additional 10 h. After completion of reaction (*cf*. TLC) TFA was coevaporated with toluene and dried under vaccum. The residue was precipitated using dry EtOAc (20 mL) and washed well with EtOAc (5 x 10 mL). The residue was vaccum dried, redissolved in double distilled water, filtered through Millex (25 mm, 5 μm) and lyophilized to afford bisacrylamide **2a** as a white amorphous powder (0.78 g, 68%). In the ^1H NMR spectrum of **2a** the anomeric protons H_{1e} and H_{1a} appeared as two distinct doublets at δ 5.27 and 4.76 with $J_{1e,2a}$ = 3.6 Hz, and J_{1a2a} = 7.8 Hz, respectively. The three sets of multiplets at δ 6.41 – 6.31, 6.29 – 6.21, and 5.88 – 5.78 were due to protons attached to the olefinic carbons. The ^{13}C NMR spectrum confirmed the presence of two amide bonds with the appearance of peaks at δ 169.5, 168.8, while peaks at δ 129.9, 129.7, 127.7, 127.5, accounted for four olefinic carbons of the bisacrylamide moiety.

***N*-(((3S,4S,5S,6R)-tetrahydro-3,4,5,6-tetrahydroxy-2H-pyran-2-yl)methyl) acrylamide (Glc-acryl, 2b)**.

A pre-cooled solution of TFA-H_2O (3:2, 10 mL) was added dropwise to RB charged with acrylamide **12** (1.30 g, 4.75 mmol) (synthesized as shown in Scheme 2) at 0 ^oC. The reaction mixture was stirred at same temperature for 30 min, slowly brought to 25 ^oC and was stirred for additional 10 h. After completion of reaction (*cf*. TLC) the reaction was worked up as mentioned for the synthesis of bisacrylamide **2a** to get **2b** as a white amorphous powder (0.73 g, 66%). In the ^1H NMR anomeric protons of **2b**, H_{1e} and H_{1a}, appeared as two doublets at δ 5.20 and 4.62

with J_{1e2a} = 3.6 Hz and J_{1a2a} = 7.0 Hz, respectively. The multiplets at δ 6.61 – 6.42, and 5.85 – 5.73 accounted for three olefinic protons of acrylamide functionality. In the ^{13}C NMR spectrum, the peak appeared at δ 168.9 was due to the amide functionality and the peaks at δ 129.6, 127.6 were attributed to olefinic carbons of the monoacrylamide **2b**.

Preparation of Glc-gel

The aqueous solutions of **2a** and **2b** prepared in different compositions were irradiated upto 29.5 KGy in Co-60 γ-source (dose rate 1.23 KGy/h), under ambient conditions (Figure 3). The synthesized gels were washed thoroughly with deionized water and vacuum dried at 40 ^{o}C to constant weight. These dried gels were used for different studies.

Characterization of hydrogels

Swelling kinetics and equilibrium degree of swelling

The swelling studies were carried out gravimetrically by immersing the dried hydrogel discs of known weight in 50 mL of double distilled water at 25 ^{o}C. The hydrogel discs were removed from water at regular intervals and weighed after wiping off the free water on the surface with tissue paper. After weighing, the samples were replaced into the same aqueous medium. The samples were swollen and reweighed until they attained a constant weight. The percentage of swelling at time‘t’ (%S) of the swollen hydrogels were calculated using the relation (1):

$$S(\%) = (W_t - W_d) / W_d \times 100 \qquad \text{----------} \rightarrow \qquad (1)$$

Where, W_t is the weight of swollen gel at time‘t’ while W_d is the weight of dried gel. The values reported are average of three repeated experiments.

The percentage equilibrium degree of swelling (%EDS) of the gel was calculated using the relation (2):

$$\%EDS = (W_e - W_d) / W_d \times 100 \quad \dashrightarrow \quad (2)$$

Where, W_e is the weight of the gel at equilibrium swollen state.

Dynamic rheological analysis

All the dynamic measurements were performed in the linear viscoelastic region. Viscoelastic properties were measured in 0.1–100 Hz frequency range at a constant deformation strain (5%).

Thermal Analysis of Glc-gel

Thermogravimetric analysis (TGA)

About 5–10 mg of the dried hydrogel samples were heated in an alumina crucible and the thermogravimetric profiles were recorded from 25 to 900 °C, at a scan rate of 10 °C/min, under nitrogen atmosphere, with a flow rate of 50 mL/min. The weight loss profiles of the hydrogel samples were studied from the thermogram.

Differential Scanning Calorimetry (DSC)

The biomedical and pharmaceutical activity of the hydrogel is decided by the manner in which the water molecules are associated with the polymer in the matrix. It is well established that water exists in three different physical states in polymeric networks: free water, freezing bound water and non-freezing bound water. The composition of different states of water in the hydrogel matrix can be determined by DSC analysis. For this the vaccum dried hydrogel samples were brought to equilibrium swollen state and then sealed in aluminium crucibles. These samples were

initially subjected to a cooling run from 30 ^{o}C to –50 ^{o}C and then a heating run from –50 ^{o}C to 40 ^{o}C at a rate of 5 ^{o}C/min in nitrogen atmosphere.

The fractions of the freezing water (free water and freezing bound water (W_f)) within the hydrogels were calculated from the area under the endothermic melting peak (ΔH_m) during the heating run and heat of fusion of pure water (ΔH_w = 333.3 J/g) according to the relation (3):[13]

$$W_f(\%) = \Delta H_m / \Delta H_w \times 100 \quad \dashrightarrow \quad (3)$$

Non-freezing bound water content (W_{nf}) was determined by subtracting W_f from the equilibrium water content of the hydrogel (W_∞) (relation (4)), which can be calculated from the fraction of equilibrium degree of swelling (EDS) of the corresponding hydrogel using the equation (5).

$$W_{nf} = W_\infty - W_f \quad \dashrightarrow \quad (4)$$

$$W_\infty(\%) = EDS / (EDS+1) \times 100 \quad \dashrightarrow \quad (5)$$

***In vitro* cell cytotoxicity test**

Cell cytotoxicity test was carried out with two different cell lines INT407 and L929 both qualitatively and quantitatively by MTT assay. Staurosporine (1 μM) induced cell death in both cell lines was used as control. The detailed procedure is given in supporting information S11.

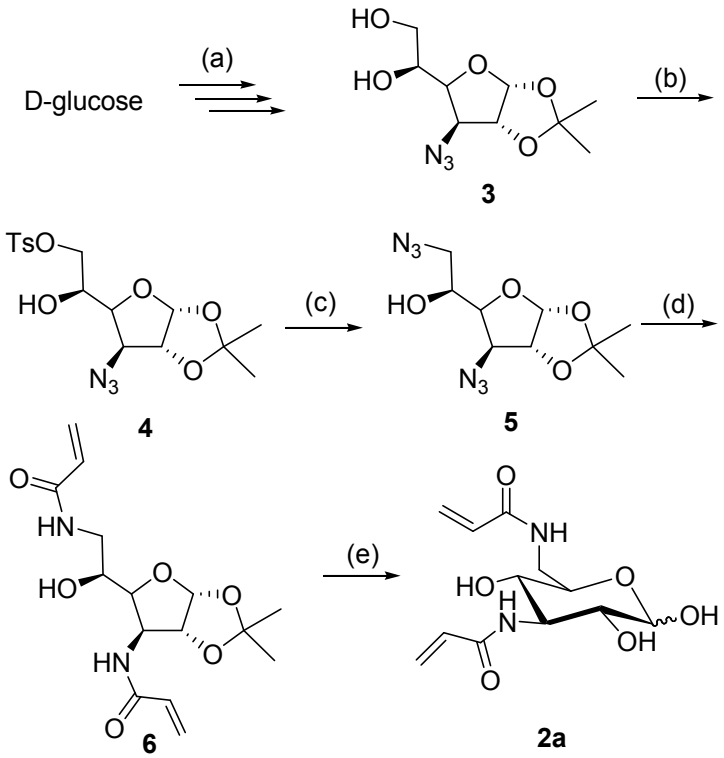
Lectin recognition studies

The interaction of Glc-gel with lectin Con A and BSA was studied by measuring the absorbance at λ = 420 nm and 278 nm, respectively, of the corresponding buffer solution before and after treatment with the hydrogel.

Results and Discussion

Synthesis of Glc-bis (2a)

In the synthesis, as shown in Scheme 1, easily available and cost effective monosaccharide D-glucose was transformed to azidodiol **3** as reported before.[14] Selective tosylation of primary hydroxyl group in **3,** using TsCl and pyridine, afforded monotosylated product **4** in 87% yield. Heating tosylate **4** with sodium azide in DMF furnished the desired diazide **5** in 86% yield. In the next step, both azide groups in **5** were reduced to diamine under hydrogenation condition which, without purification, was subjected to acrylation using acryloyl chloride and DIEA in CH_2Cl_2 at –40 ^oC to obtain bisacrylamide **6** as a thick liquid. Unmasking of 1,2-hydroxyl group in **6** using TFA-H_2O (3:2) afforded the required fully unprotected Glc-bis **2a** in 68% yield (8% overall yield from azido diol **3**).



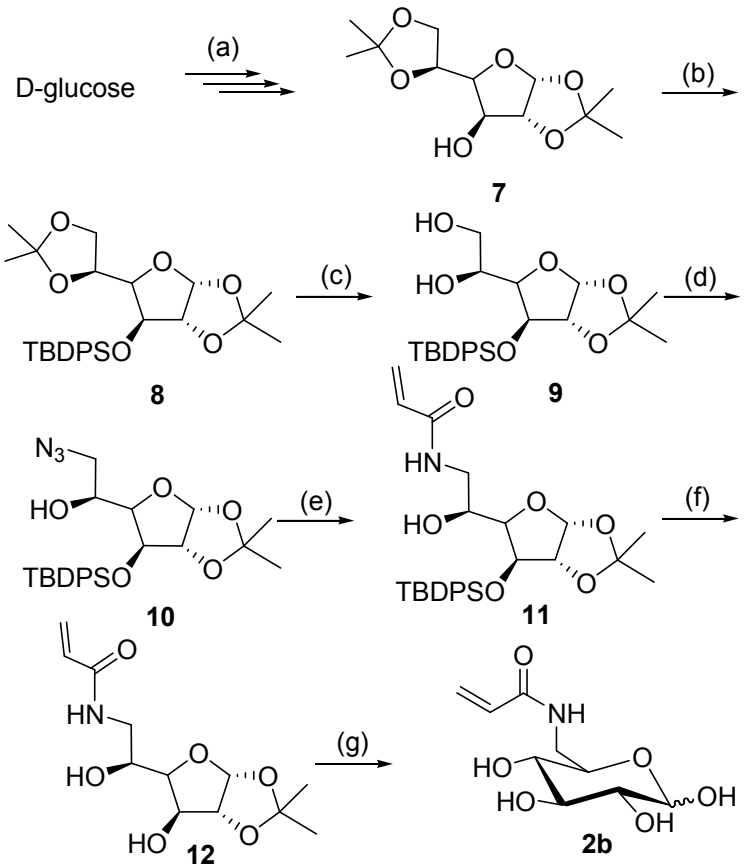
Scheme 1: *Synthesis of bisacrylamide*: (a) ref. 14; (b) TsCl, Py, CH_2Cl_2, 0 ^oC-25 ^oC, 3 h; (c) NaN_3, DMF, 80 ^oC, 3 h; (d) i) 10% Pd/C, H_2 (80 psi), 12 h, ii) Acryloyl chloride, DIEA, CH_2Cl_2, -40 ^oC, 20 min; (e) TFA-H_2O (3:2), 0 ^oC-25 ^oC, 10 h.

The Glc-bis **2a** is the only sugar based crosslinker wherein, two hydroxyl groups in the sugar ring is substituted with bis-reactive site in the form of bisacrylamide with the hemiacetal functionality intact, which could find usefulness in further functionalization to make materials of different properties.

Synthesis of Glc-acryl (2b)

In order to study and understand the ability of **2a** to function as crosslinker for the synthesis of Glc-gel it is required to have a suitable glycomonomer. Most of the known sugar based monomers have the active group (olefinic) located at the secondary carbon.[15] Since the distance of the sugar pendent from the carbon chain frame work is also an important factor for the

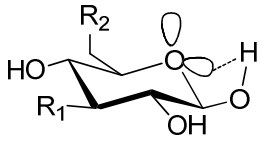
glycopolymer to show affinity to lectins, we thought of synthesizing a new C-6 acrylamide derivative of D-glucose, which would place the sugar residue at an optimum distance from the main skeleton without using a spacer.[16]



Scheme 2: *Synthesis of monoacrylamide*: (a) ref.14a; (b) TBDPSCl, DMF, Imidazole, 0 ^{o}C-25 ^{o}C, 24 h; (c) 30% $HClO_4$, THF, 0 ^{o}C-10 ^{o}C, 2.5 h; (d) i) MsCl, TEA, CH_2Cl_2, 0 ^{o}C-25 ^{o}C, 2 h; ii) NaN_3, DMF, 80 ^{o}C, 3 h; (e) i) 10% Pd/C, H_2 (20 psi), MeOH, 5 h; ii) Acryloyl chloride, DIEA, CH_2Cl_2, –40 ^{o}C, 20 min; (f) TBAF, THF, 0 ^{o}C to 23 ^{o}C, 1.5 h; (g) TFA-H_2O (3:2), 0 ^{o}C-25 ^{o}C, 10 h.

Thus, as shown in Scheme 2, C-3 hydroxyl functionality in **7** [14a] was protected using TBDPS to yield fully protected furanose **8**. The 5, 6-acetonide in **8** was selectively deprotected using 30% $HClO_4$ in THF to furnish the diol **9** in 77% yield. Mono mesylation of 1^{o} hydroxyl group in diol

9 followed by heating of the resultant mesylate with sodium azide in DMF afforded the azido compound **10** in 53% yield (over two steps). The azide **10** was reduced and acrylated *vide infra* to afford the monoacrylamide **11** in 83% yield, (over two steps). Further, deprotection of TBDPS group in **11** using TBAF in THF yielded the azido diol **12** in 83% yield. Finally, deketalization of monoacrylamide **12** using TFA-water generated the Glc-acryl **2b** in 66% yield. ^{1}H NMR studies of **2a/b** revealed the predominance of α-anomer over β-anomer, due to anomeric effect, however the ratio didn't differ (55:45) too much, largely due to the possible H-bonding between anomeric hydroxy group and ring oxygen, as shown in Figure 2.[17]

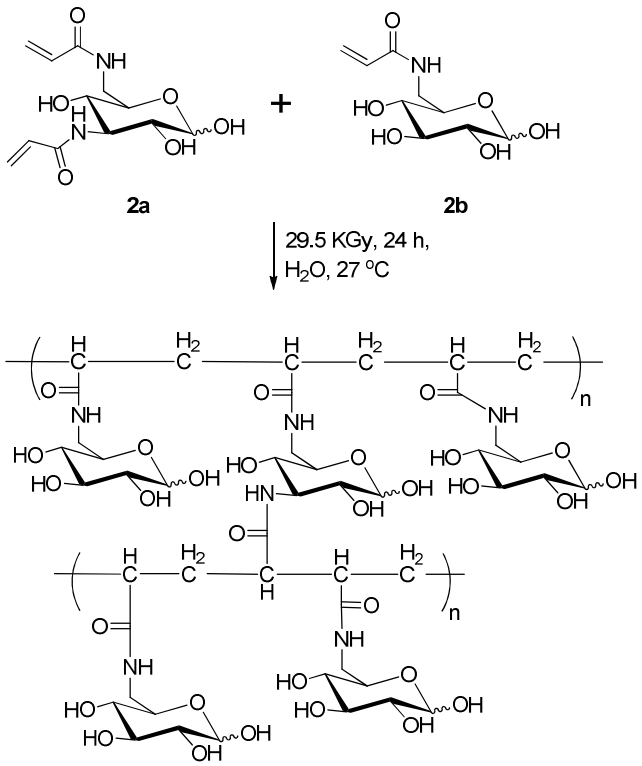


2a, $R_1 = R_2 = NHC(O)CH=CH_2$

2b, $R_1 = OH$; $R_2 = NHC(O)CH=CH_2$

Figure 2: Possible H-bonding between anomeric OH and lone pair on ring oxygen

Glc-acryl and Glc-bis thus obtained were dissolved in water (in suitable proportions) and irradiated with γ-source at a radiation dose of 29.5 KGy to yield transparent Glc-gel (Figure 3).



Scheme 3: *Synthesis of D-glucose derived glycopolymeric hydrogel*

Figure 3: Synthesis of Glc-gel (above). Photograph of (A) freeze dried Glc-gel (B) swollen Glc-gel formed by radiation induced polymerization (below).

FT-IR analysis

Figure 4 shows the FT-IR spectra of Glc-bis (B) and Glc-acryl (C) in the powder form and also of the dry Glc-gel (A). The characteristic peaks of Glc-acryl are slightly broadened due to the hygroscopic nature of the material. The typical peaks of amide I (~1651 cm^{-1}) and amide II (~1552 cm^{-1}) in the monomer (Glc-acryl) and the crosslinker (Glc-bis) remains unaffected in the

polymerized dry gel. The peak attributed to $CH=CH_2$ group (~1640 cm^{-1}) in the FT-IR spectra of Glc-acryl and Glc-bis, disappeared in the polymerized gel. This observation suggest that the polymerization has taken place *via* the C=C groups in the Glc-acryl and the Glc-bis.[18]

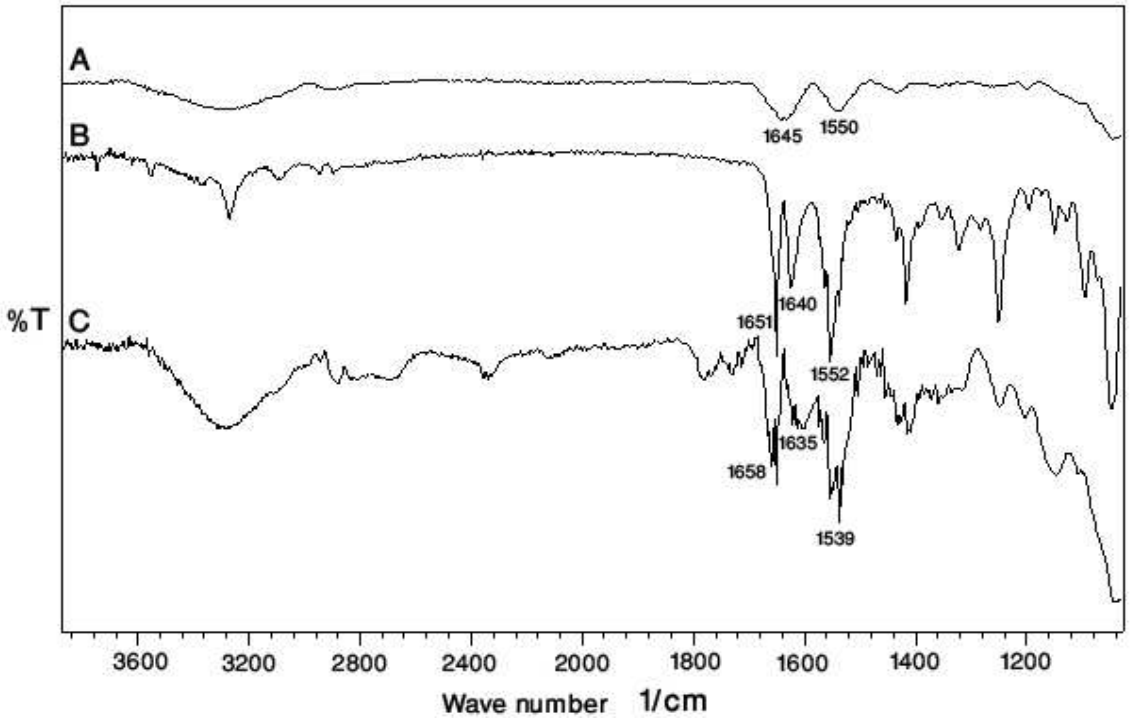


Figure 4: FT-IR spectrum of (A) dried Glc-gel, (B) Glc-bis and (C) Glc-acryl powder.

Swelling studies

The rate of swelling (Figure 5) was found to decrease with the increase in Glc-bis concentration in the hydrogel. This is because as the Glc-bis concentration increases the rigidity of the hydrogel increases and hence the degree of freedom between the chains decreases. Therefore, the polymeric network swells up slowly as compared to the gel with less concentration of Glc-bis. The %EDS (Table in Figure 5) calculated using relation **2** shows that the equilibrium swelling is dependent on the Glc-bis content.

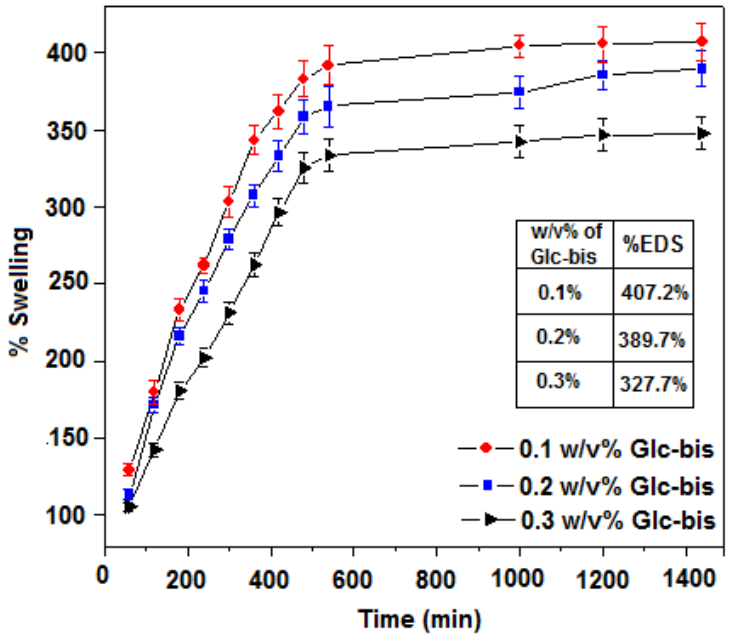


Figure 5: The effect of Glc-bis concentration on the rate of swelling of the Glc-gel (8% w/v Glc-acryl at radiation dose of 29.5 KGy) (left). Variation in %EDS at different Glc-bis concentration in the hydrogel formed with, 8% w/v Glc-acryl at radiation dose of 29.5 KGy (right).

Effect of Glc-bis concentration on viscoelastic properties

In order to evaluate the effect of crosslinker concentration on the viscoelastic response, the hydrogels derived from different Glc-bis compositions at 8% w/v Glc-acryl and a radiation dose of 29.5 KGy, were used to investigate the viscoelastic parameters in the linear viscoelastic range.

The strain amplitude sweeps as shown in figure 6(A) was carried out at a fixed angular frequency of 10 rad/s and 5% strain was chosen as the deformation strain for frequency sweep experiments since it lies in the LVE region. Figure 6 (A) also shows the linearity in the stress strain response at the fixed applied angular frequency. The frequency sweep experimental data is presented in figure 6(B).

The complex viscosity (η*) was found to increase with increase in Glc-bis concentration at a fixed Glc-acryl concentration (8% w/v). This indicates the rise in gel strength with crosslinker

content. At low frequencies the rate of molecular rearrangement exceeds the rate of oscillation, hence the entanglement of polymer chains can occur easily during long period of oscillation. However, it was observed that during the frequency sweep the value of complex viscosity decreases with increasing frequency, which could be due to the faster oscillation rate than the rate for entanglement of polymer chains (Figure 6).[19]

The complex viscosity η* is given by the relation (6)[19a]:

$$\eta^* = \eta' - i\eta'' = G''/\omega - iG'/\omega \qquad \text{----------} \rightarrow \qquad (6)$$

Wherein, G' = storage modulus (elastic component) (Pa), G'' = loss modulus (viscous component) (Pa)

ω = angular frequency (rad/sec), η' = Dynamic viscosity (Pa-sec) and η'' = in phase component of dynamic viscosity (Pa-sec).

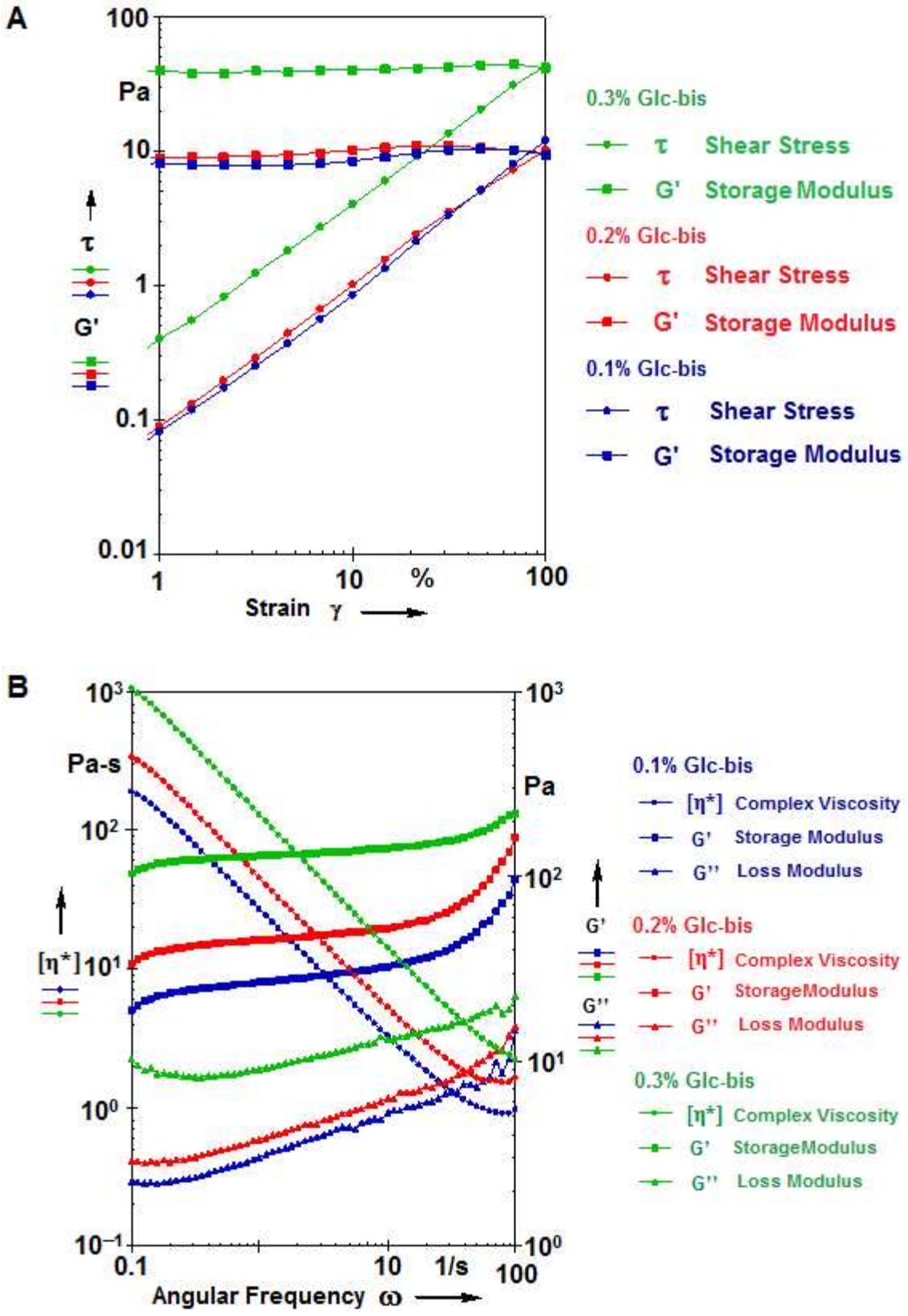


Figure 6: (A) Strain sweeps for Glc-gel samples with varying Glc-bis concentration at a fixed angular frequency of 10 rad/s. (B) The evolution of G', G" and η* for Glc-gel samples with varying Glc-bis concentration at 5% deformation strain.

Figure 6 also represents the variation of G' with oscillatory frequency. All the Glc-gels (measured) exhibits a plateau in the range 0.1–10 Hz, which indicates a stable, strong crosslinked gel network. At higher frequencies, all gels showed an increase in G', with the rate of increase highest for the gel with lowest crosslinker concentration (0.1% Glc-bis) and lowest for the gel with highest crosslinker concentration (0.3% Glc-bis). The loss modulus (G") also exhibited a similar behavior. This is because the magnitude of the viscoelastic response of a polymeric network depends on length of the flexible polymer chains and the nature of the imposed mechanical motion. The relaxation times are longer for longer polymeric chains, which depend on the crosslinker content. In the case of less crosslinked networks the polymeric chain segments between the crosslinks are longer, which gives lower molecular motion frequencies than those arising from highly crosslinked networks.

This implies that, at higher frequencies, long chains fail to rearrange themselves at the imposed time scale and they assume more stiff and 'solid- like ' behavior which is characterized by a sharp increase in G' in this region.[20] In other words, for highly crosslinked networks even higher applied frequencies are required for a similar response which is the reason for gradual rate of rise in G' in case of Glc-gel with 0.3% Glc-bis concentration.

Thermogravimetric analysis

The thermal degradation profiles of the dried hydrogels at various Glc-bis concentrations (0.1, 0.2, 0.3% w/v) and Glc-acryl concentrations (4, 6, 8, and 10% w/v) at a radiation dose of 29.5 KGy, were studied and were found to be similar. A typical degradation profile is shown in supporting information-S39. Degradation takes place in two different steps, first step from 180-320 ^{o}C and second step from 370-520 ^{o}C, which is characteristic of glycopolymers.[18] The effect of Glc-acryl concentration on the thermal stability of Glc-gels, is shown in Figure 7.

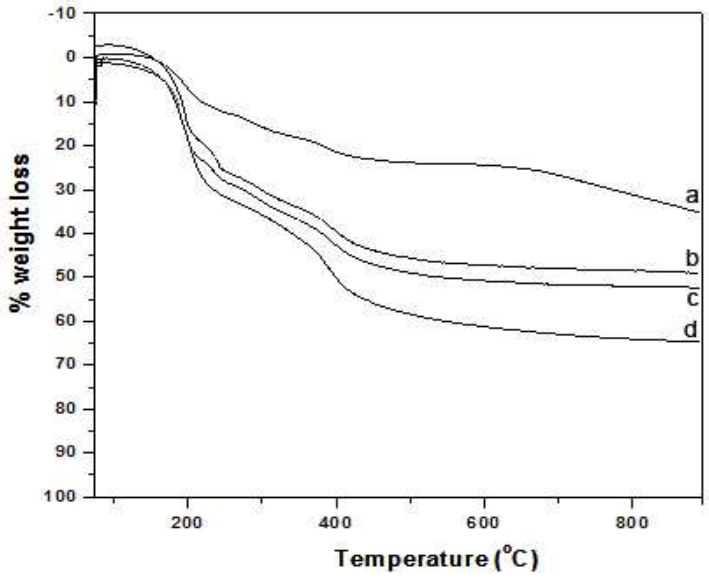


Figure 7: Thermal degradation curves for Glc-gel with varying Glc-acryl concentration (**a**) 4 (**b**) 6 (**c**) 8 and (**d**) 10% w/v, at 0.1% w/v Glc-bis and at an applied radiation dose of 29.5 KGy.

From the percentage weight loss at 900 ^{o}C we can deduce that the gels with higher Glc-bis to Glc-acryl ratio are thermally more stable. Thus, the thermal stability of Glc-gel decreases with increase in Glc-acryl concentration keeping all other factors constant, due to the decrease in crosslinking density of the hydrogel.

Influence of Glc-bis concentration upon the states of water

The DSC curves of the swollen hydrogels with varying Glc-bis concentration at equilibrium swelling are shown in Figure 8.

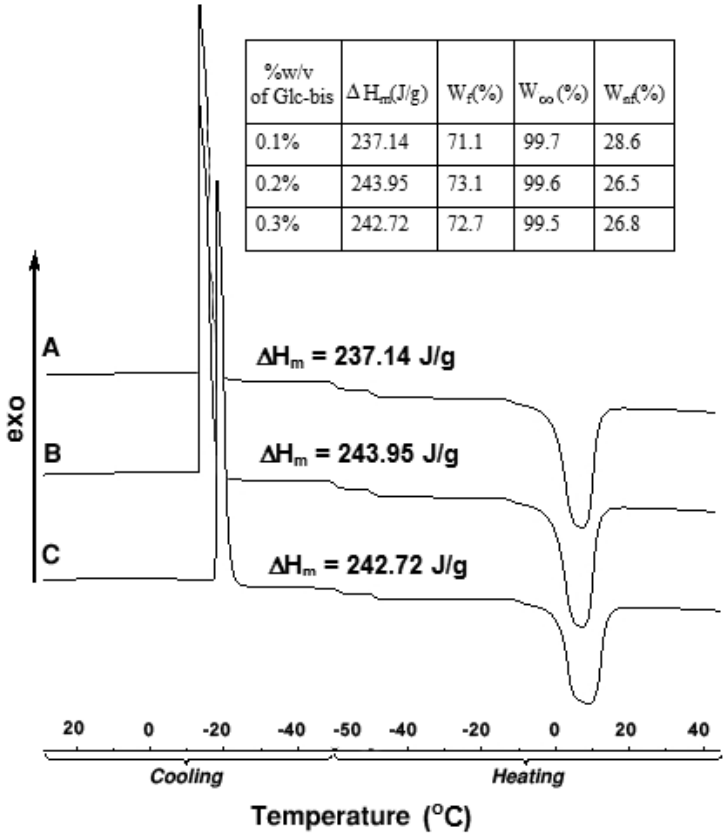


Figure 8: Study of states of water in the Glc-gel with varying Glc-bis concentration.

The ΔH_m values were calculated from the area under the corresponding endothermic melting peaks, while the different states of water in the various hydrogel compositions were calculated using the equations (3), (4), and (5). The Table in Figure 8 shows the amount of various states of water in the hydrogels with varying Glc-bis concentration (0.1, 0.2 and 0.3% w/v), at 8% w/v Glc-acryl and 29.5 KGy radiation dose. It was observed that the amount of freezing water (W_f) and non-freezing water (W_{nf}) was almost same at all the studied Glc-bis concentrations.

Influence of Glc-acryl concentration upon states of water

States of water in the hydrogels were also determined with varying Glc-acryl concentration. It was observed that with an increase in Glc-acryl concentration the amount of freezing water increased, while the proportion of non-freezing water decreased (Figure 9).

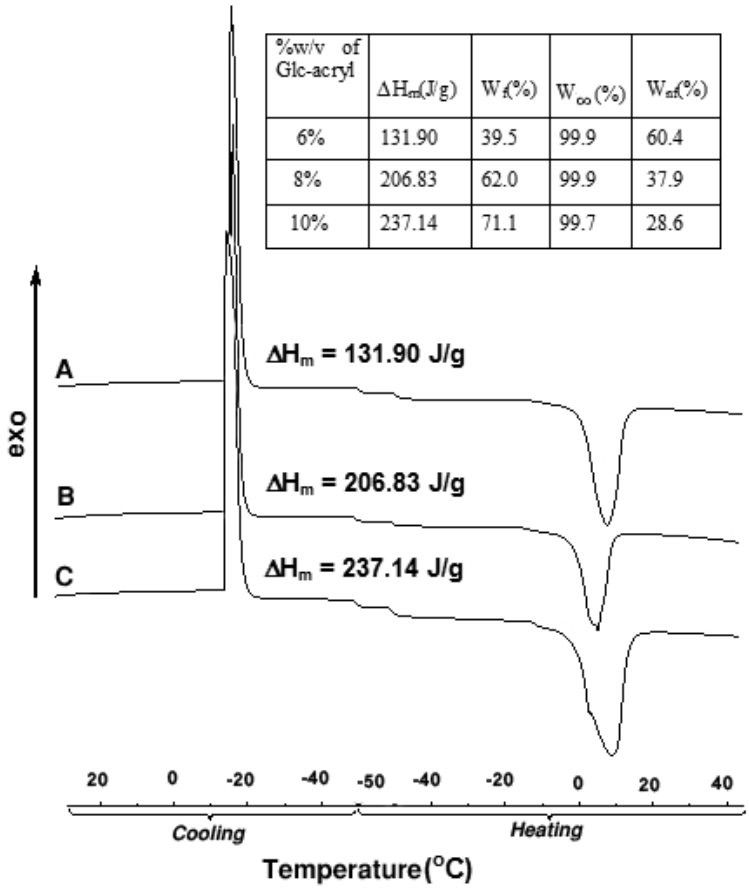


Figure 9: Study of states of water in the Glc-gel with varying Glc-acryl concentration.

This suggests that the water absorbed by the Glc-gel, with high Glc-acryl concentration, is slightly structured with its freezing occurring in a temperature range close to that of pure water. This could be due to the lower crosslinking density with the increase in Glc-acryl content. Hence unlike Glc-bis the variation in Glc-acryl concentration could decide the amount of various states of water in Glc-gel.

***In vitro* cytotoxicity of Glc-acryl, Glc-bis and Glc-gel**

The *in vitro* cytotoxicity of Glc-acryl, Glc-bis and Glc-gel were evaluated qualitatively and quantitatively.[21] At a concentration of 1 mg/mL of Glc-acryl and Glc-bis as well as Glc-gel (20 mg) did not exhibit any toxicity to both the cell lines tested while 1 μM Staurosporine killed both the cell lines completely.

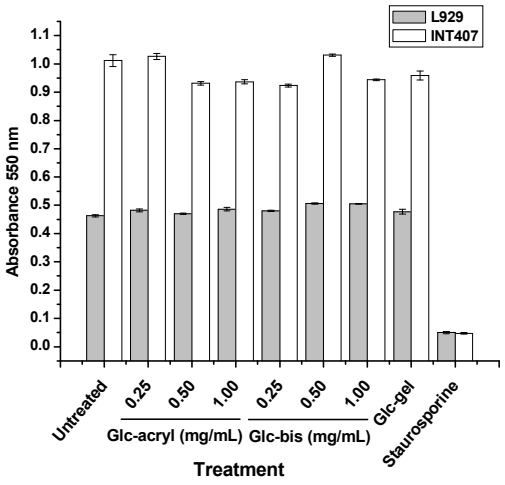


Figure 10: Quantification of viable cells by MTT assay after treatment for 48 h with different test samples. The test samples were not different from untreated sample ($p < 0.05$) as evaluated by unpaired student's t –test in case of both the cell lines.

As evident from the Figures shown in supporting information-S39 and S40, both the cell lines grew normally in the presence of the test materials without any reduced growth or cell death when observed under a microscope. At the end of 5 days all the samples attained confluence and the surface of the well was completely covered with the growing cells. In the quantitative MTT assay different concentrations (0.25 to 1 mg/mL) of Glc-acryl, Glc-bis and 50 mg of Glc-gel were tested.[22] In this assay too both the cell lines grew in a comparable manner to the untreated sample at all the concentrations tested (Figure 10). To demonstrate death in the cells 1 μM staurosporine was used which killed the cells completely. This clearly indicated that the test

samples were nontoxic to the cells as they neither retarded the growth nor induced cell death in both the cell lines.

Recognition study of Glc-gel towards Con A

To determine the amount of protein that can be precipitated by Glc-gel, UV/vis spectroscopy was performed.[23] Therefore Con A (0.25 mg) in PBS-buffer (1 mL) was mixed with swollen gel pieces (20 mg) and recorded the spectra at regular intervals after separation of the gel pieces. Comparison of UV/vis spectra recorded before and after treatment with gel showed a decrease of peak height at λ = 420 nm of 43.8% after an interaction period of 10 minutes which can be attributed to the protein adsorbed on Glc-gel (Figure 11). The amount of protein left in solution is therefore calculated to be 0.14 mg (Δm = 0.11 mg) which means, that 20 mg of swollen Glc-gel is able to precipitate 0.11 mg of Con A. Similar studies in the presence of BSA solution showed a negligible decrement in the absorbance at λ = 278 nm.

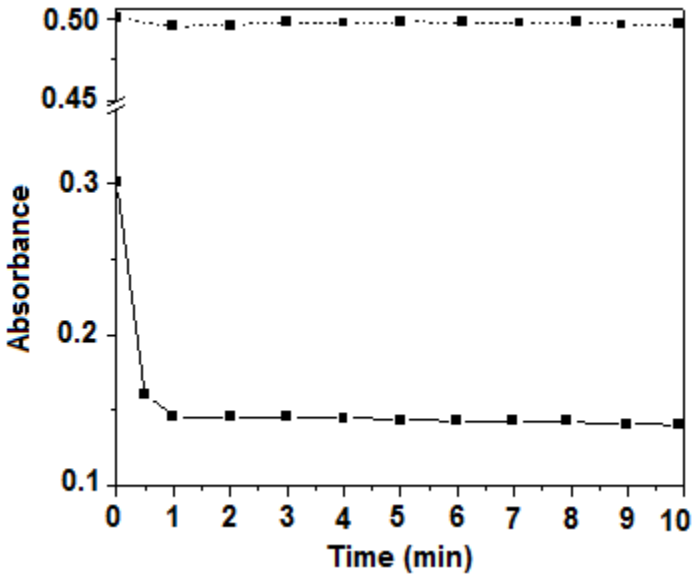


Figure 11: Interactions of Glc-gel with Con A (solid line) and BSA (dotted line).

Conclusion

In summary we have devised for the first time a D-glucose based bisacrylamide (Glc-bis) and monoacrylamide (Glc-acryl) with hemiacetal functionality. The high yielding reaction sequence proves the strategy is good enough to make them in gram quantities. The acrylamides were converted to a sterilized, noncytotoxic and homogeneous Glc-gel using radiation induced polymerization, which also showed strong interaction to lectin Con A. The thermal stability, states of water and viscoelastic properties of the gels were studied using thermogravimetry, variable temperature DSC and oscillatory rheology technique, respectively. Further studies to synthesize functionalized gels and their application in drug delivery are in progress.

Acknowledgment

AKS and MS are thankful to Prof. S Chattopadhyay for his valuable suggestions and support. JKA and MMK are thankful to Dr. D. K. Palit, Head, RPCD, and Mr. Nilanjal Misra (RTDD, BARC) for DSC analysis. The authors are thankful for the constant support and encouragement from Dr. B. N. Jagtap, Director chemistry group.

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