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Preparation and Properties of Genipin-Fixed Polysaccharide of *Chamaedoris Auriculata* (CD_{SPS})

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INTRODUCTION

Chamaedoris auriculata is a green alga containing sulphated polysaccharides (CD_{sps}) consisting of arabinose, galactose and rhamnose in major amounts with minor presence of ribose. In an ongoing program of our laboratory on modification and value addition of seaweed polysaccharides for preparing hydrogels with improved properties (Prasad *et al*., 2005a; Prasad *et al*., 2005b; Prasad *et al*., 2006a; Prasad *et al*., 2006b; Meena *et al*., 2006a; Meena *et al*., 2007a; Prasad *et al*., 2007) we report herein preparation of crosslinked *Chamaedoris auriculata* (CD_{sps}) hydrogel, using the naturally occurring crosslinker genipin, crosslinked product revealed improved properties in comparison to the non-modified parent polysaccharide. The reaction mechanism of genipin with amino groups and schematic illustration (Figure 1) of the formation of genipin-fixed CD_{sps} has been proposed (Touyama *et al*., 1994).

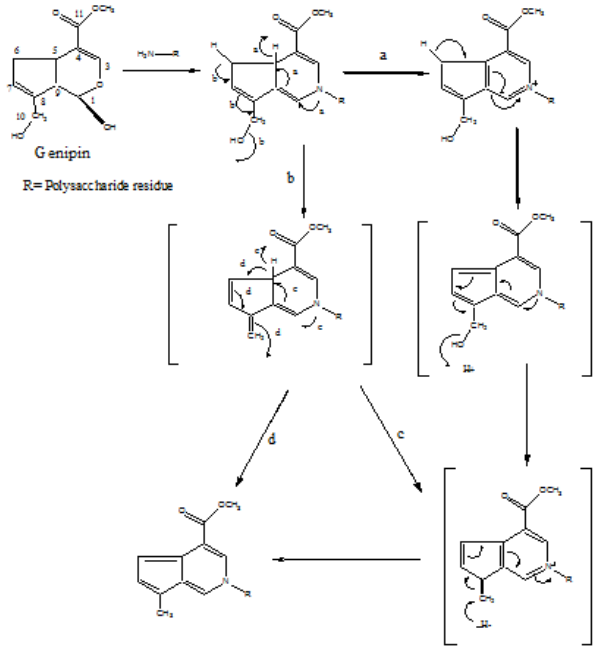


Figure 1. Schematic illustration of the formation of genipin-fixed CD_{sps}

MATERIALS AND METHODS

Materials

The polysaccharide was extracted from the green seaweed *Chamaedoris auriculata* collected from the Diu (020^o 042.364' N, 070^o 058.276' E), Veraval (20^o 54.932' N and 070^o 20.842' E) in the west coast of India and Kanyakumari (08^o 04 .822' N, 077^o 33.098' E), of southeast coast of India from inter-tidal zone during February-June 2005. Genipin was purchased from M/s Challenge Byproducts Co., Taiwan (*http*:// w3.to/cbc). Isopropanol (Laboratory Reagent grade) was purchased from Ranbaxy Chemicals, Mohali (Punjab), India. All other chemicals were analytical grade and were purchased from Ranbaxy Chemicals, Mohali (Punjab), India.

Preparation of CD_{sps} hydrogel

The purified sample of CD_{sps} its hydrogels was prepared employing the method as described by Siddhanta *et al*., 2001.

Preparation of genipin-fixed CD_{sps} hydrogel

In a typical batch, the CDsps solution was prepared by dissolving 1 g of CDsps in demineralized water, in separate experiments, at 100^{o} C for 120 s in a microwave oven (LG Domestic microwave oven); at pH 7. A stock solution (10%) of genipin was prepared in demineralized water. The aqueous solutions of the CDsps was then mixed with different aliquots of the genipin stock solution at 40oC to obtain the crosslinked product with weight percentages of genipin lying in the range of 0.001 to 1.5, having maintained the total weight of the experimental solution sample at 50g. The viscous homogeneous mixture was kept at room temperature (30oC) and allowed to react for different durations of time, up to 48 h in nine separate experiments. The reaction mixture started assuming light blue color after 90 minutes and the color intensified with the passage of time becoming deep blue in color after 30 h, the reaction product was obtained as follows. Isopropanol (IPA) was added to the reaction mixture (1:2 w/w) and the resultant mixture was allowed to stand for 24 h. The dehydrated products were then collected and washed twice with acetone (1:1, w/w) to remove the un-reacted genipin, if present. The product was air dried followed by drying in the oven at 50oC for 2 h.

Characterization of genipin fixed CD_{sps}

Bulk density, true density, pore volume and porosity

The bulk density, true density, pore volume and porosity of parent polysaccharide (CD_{sps}) and those of the genipin crosslinked product was calculated, followed the method described by Bai and Li (2006).

Swelling ratio measurement

The non-modified polysaccharides and genipin-fixed polysaccharides with different percentage of genipin with respect to the polysaccharide that is 0.50, 0.70 and 1.0 % (w/w), were used. In the swelling ratio (%) measurements, the dry hydrogel samples were weighed (W_1) and immersed in solutions of different pH e.g. 1.2 (dilute aqueous HCl), 7.0 (demineralized water) and 12.0 (aqueous NaOH) separately. After the designated soaking time had elapsed, the wet samples were wiped dry with filter paper to remove excess liquid and weighed (W_2). The swelling ratio (%) was calculated using the following Eq. (6). The results are the mean and standard deviation of four replicates.

$$\text{Swelling ratio } (\%) = [(W_2 - W_1) \div W_1] \times 100 \quad (6)$$

Gelation degree

The gelation degree *G* of parent CDsps and genipin-fixed CDsps was calculated following the method as mentioned by Lendlein *et al*., 2001. The results are the mean and standard deviation of four replicates.

Degradation rate measurement

The degradation studies of parent CD_{sps} and genipin-fixed CD_{sps} were performed using Ringer's solution followed the method as described by Yao *et al.*, 2004. The results are the mean and standard deviation of four replicates.

Optimization of the crosslinking reaction time and genipin quantity

Optimization of the reaction time and the genipin concentration were done based on swelling capacity of the genipin-fixed polysaccharide (CD_{sps}) samples in the different pH solutions.

Measurements of genipin-fixed CD_{sps} hydrogel

UV-Vis spectra were recorded on a Varian CARY 500 Scan UV-Vis-NIR spectrophotometer. Optical rotations were measured on a Rudolph Digi pol-781 Polarimeter (Rudolph Instruments Inc, NJ, USA). Optical microscopy was carried out on an Olympus model SZH 10, Japan with 70X magnification and thermal gravimetric analysis (TGA) were done on TGA Toledo Mettler TGA System Switzerland. Apparent viscosities were measured on Brookfield Viscometer (Synchrolectric Viscometer, Stoughton, MASS 02072) using Spindle No. 3 at rpm 60.

Nitrogen, protein, sulfur and metal ion contents

Total nitrogen, crude protein content and metal ions (K, Mg, Na, Zn, Mn, Co, As, Cd, Ba, Bi, Pb, Sr, Cr) and sulfur contents were measured as described by Wolnik, 1988.

Circular dichroism measurement

Circular dichroism (CD) spectra were recorded on a JASCO model J-815 CD Spectrometer, in the range 190-250 nm using sample concentration of ca. 0.8 mg/ml (800 ppm). Molar ellipticity values, [θ] are reported in units of deg cm^2 $dmol^{-1}$. All measurements were performed at room temperature using 1.0 cm quartz cells.

Scanning electron microscopy

Scanning electron microscopy (SEM) of vacuum oven dried powder of the non-modified CD_{sps} and genipin-modified CD_{sps} were mounted on a sample holder and coated with gold. The samples were examined with a scanning electron microscope (Model Carl-Zeiss Leo VP 1430) at an accelerating voltage of 20 kV and 202X magnifications.

MS/MS analysis

Tandem mass spectral analyses (MS/MS) were done on a Waters Q-Tof micro YA-260 mass selective detector using Waters Mass Lynx Version 4.0 software. Positive electron spray mode (ES+) was used for the analyses. For Mass spectrum analysis the following parameters were used: Capillary voltage 2700V, sample cone voltage: 30.0V, extraction cone voltage: 0.5V, desolation temperature150oC, source temperature: 80oC, syringe rate 0.5 µl/min, ion energy 2.0V, collision energy 7.0V. For the MS analysis the genipin-fixed CDsps was hydrolyzed in methanolic HCl for 1 h at 60oC. The resulting carbohydrate was precipitated in methanol (3v). The supernatant obtained by centrifugation evaporated to dryness. The residue was dissolved in 5ml HPLC grade methanol and subjected to MS analyses. In an additional experiment to establish the fact that genipin was present in the CDsps matrix through crosslinking but not as a physical mixture, genipin-fixed CDsps was heated in HPLC grade methanol at 60oC for 1 h under stirring condition without adding acid. The supernatant methanol leaving behind the blue colored product was evaporated to dryness and the residue was re-dissolved in HPLC grade methanol and was subjected to MS analyses.

Statistical analyses

Data were analyzed using analysis of variance (ANOVA). Results were considered statistically significant when $p<0.001$. Calculations were performed using Origin Software, Version 6 (Microcal Software Inc., MA. USA).

RESULTS

Physical properties

The results of bulk, true density and pore volume and porosity measurements for genipin-fixed polysaccharides and non-modified polysaccharides are given in (Table 1). The crosslinked CD_{sps} with genipin showed higher bulk density and lower true density, pore volume and porosity than that of the non-modified polysaccharide (Table 1). Apparent viscosities of the crosslinked polysaccharide product and the parent polysaccharide (CD_{sps}) were measured. The non-modified polysaccharide (CD_{sps}) showed apparent viscosities 200±3.51 cP at 60^{o}C.

The value of apparent viscosities for genipin-fixed polysaccharide was 300±3.70 cP, (at reaction time 30h, with 0.7 wt% genipin) (Table 1).

Table 1 Physical properties of the non-modified CD_{sps} and its crosslinked product

Products	Viscosity (cP)	Bulk density (d_o) (g/ml)	True density (d) (g/ml)	Pore volume (V_p) (g/ml)	Porosity (Φ)
Non-modified CD_{sps}	200	0.210	1.020	0.478	1.393
Crosslinked CD_{sps}	300	0.310	0.674	0.281	0.483

[a]Viscosities of polysaccharide and product were measured in 2% concentration in water, at 60°C.

Gelation degree

The gelation degree (G) values of the non-modified CD_{sps} decreased after crosslinking with genipin. The G values for CD_{sps} (0.3340±0.0015, 0.3340±0.002 & 0.1511±0.0015) were measured at 1.2, 7.0 and 12.0 pH media respectively. The gelation degree of the genipin-fixed polysaccharide decreased significantly with optimum 0.7 wt% genipin to 0.2105±0.0001, 0.0116±0.0079 & 0.0583±0.0001 at 1.2, 7.0 and 12.0 pH media, respectively in CD_{sps} (Figure 2).

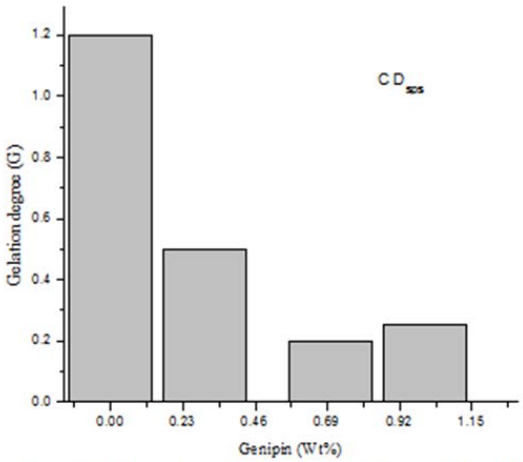
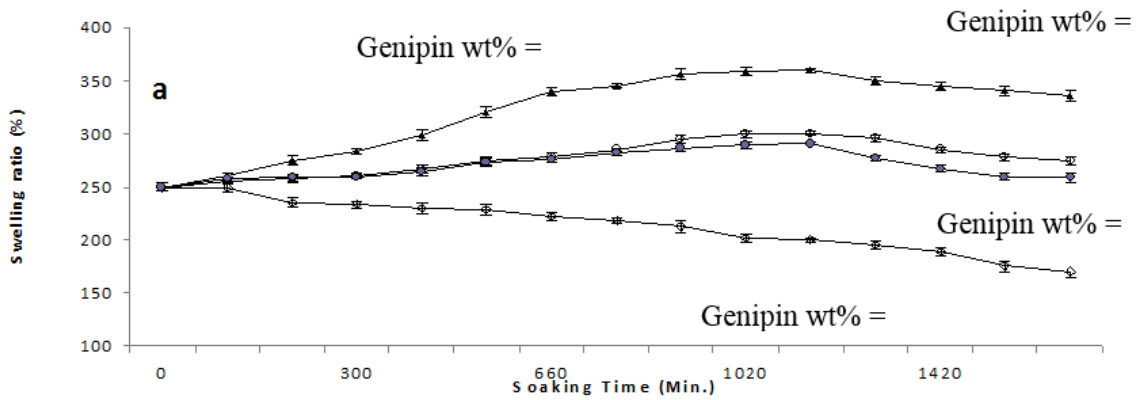


Figure V.2.2 Dependence of gelation degree of the cross linked CD_{sps} on the concentration of genipin (with 0.7 wt% genipin) at pH 7.

Swelling behavior and degradation

These genipin-fixed polysaccharide hydrogel containing 0.7 wt% genipin, showed swelling ratios (%) in CD_{sps}, 360±5.56 8400±10.02, 1600±12.16, (Figure 3) in pH media 1.2, 7.0 and 12.0, respectively. On the other hand, the parent polysaccharide CD_{sps} exhibited swelling ratios (%) at 200±5.21, 6000±25.11, 550±5.24 in pH 1.2, 7.0 and 12.0, respectively.



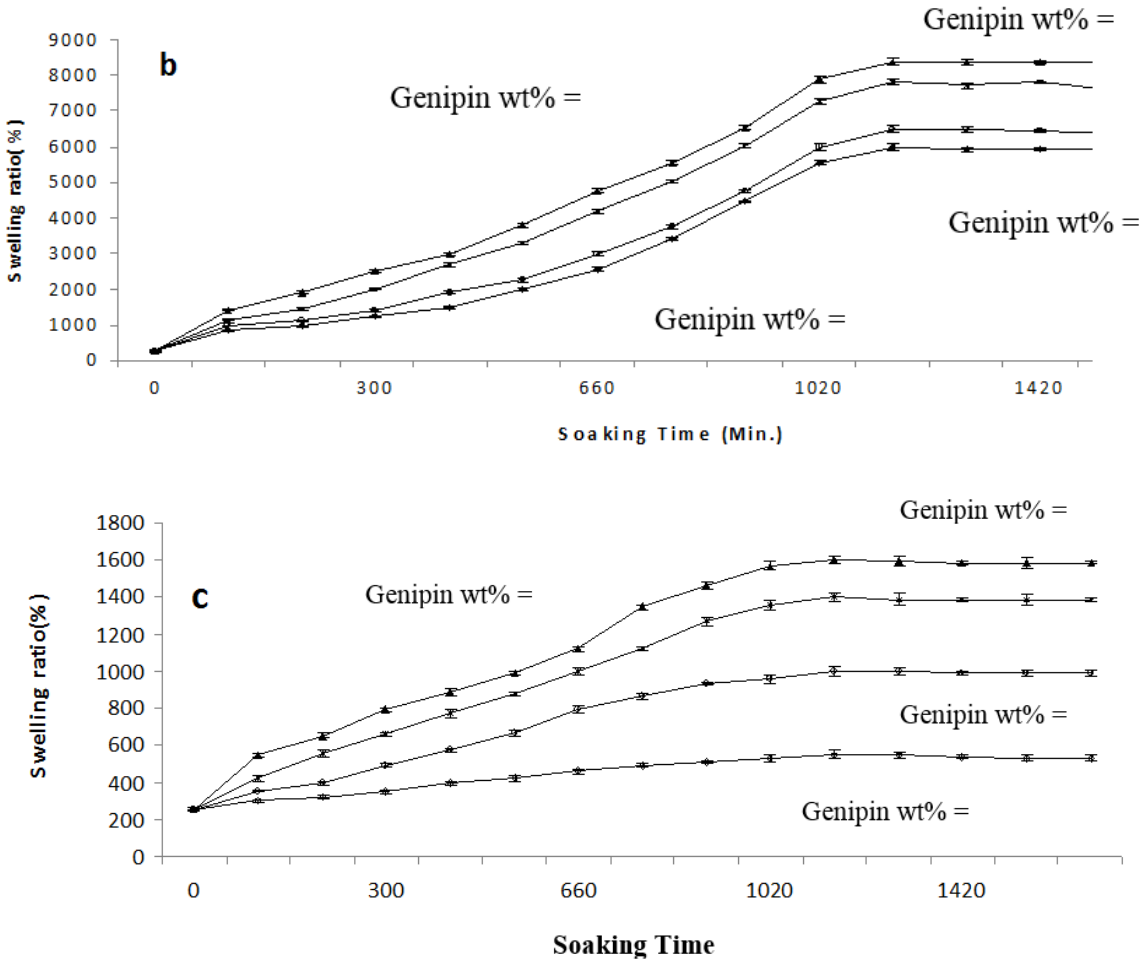


Figure 3. Swelling ability of CD_{sps} and genipin-fixed-CD_{sps} with different weight percentages of genipin in (a) pH 1.2; (b) pH 7 and (c) pH 12.0. Data represents the mean± standard deviation, n=4.

The effect of crosslinking reaction time on the swelling ratios of genipin-fixed polysaccharide is depicted in Figure .4. The degradation of the crosslinked products in Ringer's solution was also studied (Figure 5). The mass loss ratio of non-modified polysaccharide (CD_{sps}) in Ringer's solution was determined to be 70%, whereas the mass loss ratio of genipin-fixed polysaccharides (with 0.7 wt. % genipin) was 34%.

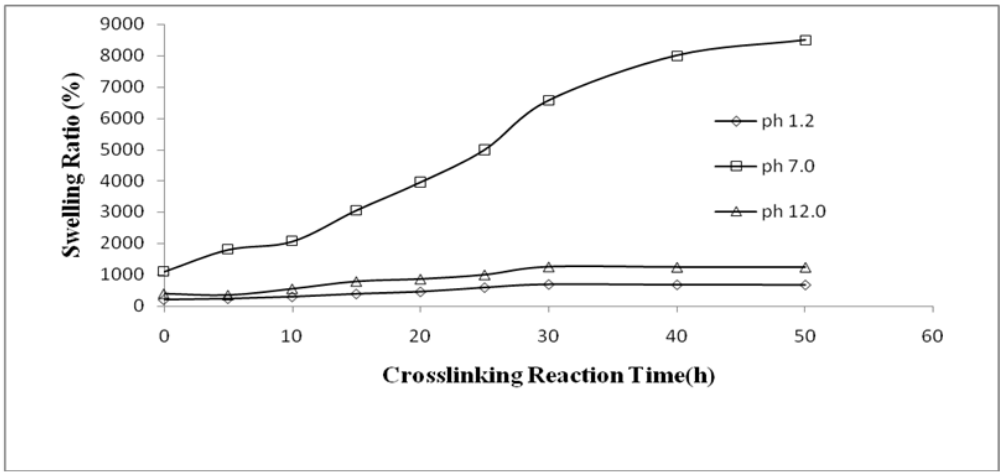


Figure V.2.4 Effect of cross-linking reaction time on the swelling ratio of genipin-fixed CD_{sps} (with 0.7 wt% genipin) at (a) pH 1.2 (b) pH 7.0 and (c) pH 12.0.

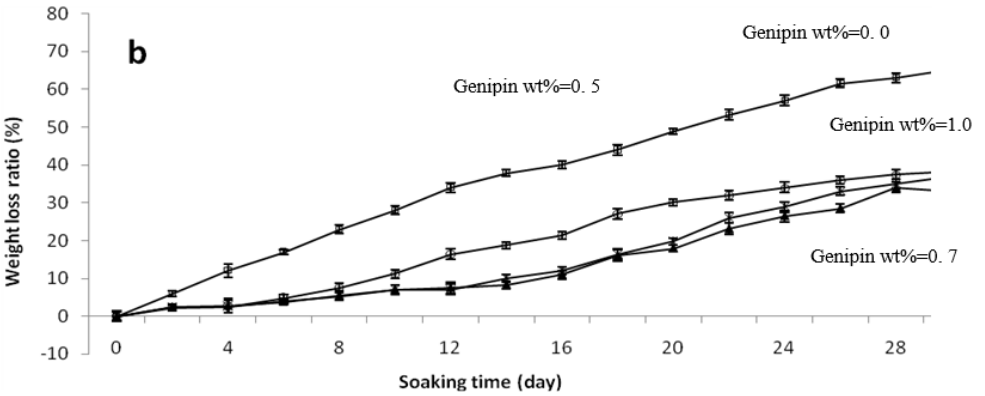


Figure V.2.5 Effect of weight percentage of genipin on the weight loss ratio of CD_{sps} and genipin-fixed CD_{sps} in Ringer`s solution, obtained after 30h cross-linking reaction.

Nitrogen, protein and metal ion contents

The non-modified polysaccharides (CD_{sps}) showed nitrogen and protein contents of 1.10±002% & 6.82±0.0029%. The nitrogen contents of genipin-fixed CD_{sps} were 1.08±0.0031%. Whenever required, the protein contents were calculated from these values by multiplication with the factor 6.20 (Wathelet *et al.*, 1999). The metal ion analyses using inductively coupled plasma spectrophotometry (ICP) of the parent polysaccharide samples, and were showed in Table V.2.

Table 2 Metal ion and sulfur contents in the non-modified polysaccharides[a]

Elements	CD_{sps}
K	20.42
Mg	18.85
Na	15.62
Zn	0.038
Sr	0.077
Mn	0.004
Co	Nil
As	Nil
Cd	Nil
Ba	Nil
Bi	Nil
Pb	Nil
Cr	Nil
S	1.1

[a]Values in ppm; [Wolnik,1988]

Thermogravimetric analysis

The TGA curves for the parent polysaccharide; genipin and genipin-fixed CD_{sps} are depicted in Figure V.2.6. The TGA curve of CD_{sps} showed a three-stage of mass loss. The first stage mass loss (16%) was observed between 41-200^{o}C, the second one (36%) between at 201-245^{o}C, and finally ca.79% mass loss was observed in the third stage between at 247-600^{o}C. The crosslinked CD_{sps} exhibited mass losses 10% between 41-192^{o}C, 30% between 195-218^{o}C and eventually 60% up to 650^{o}C.

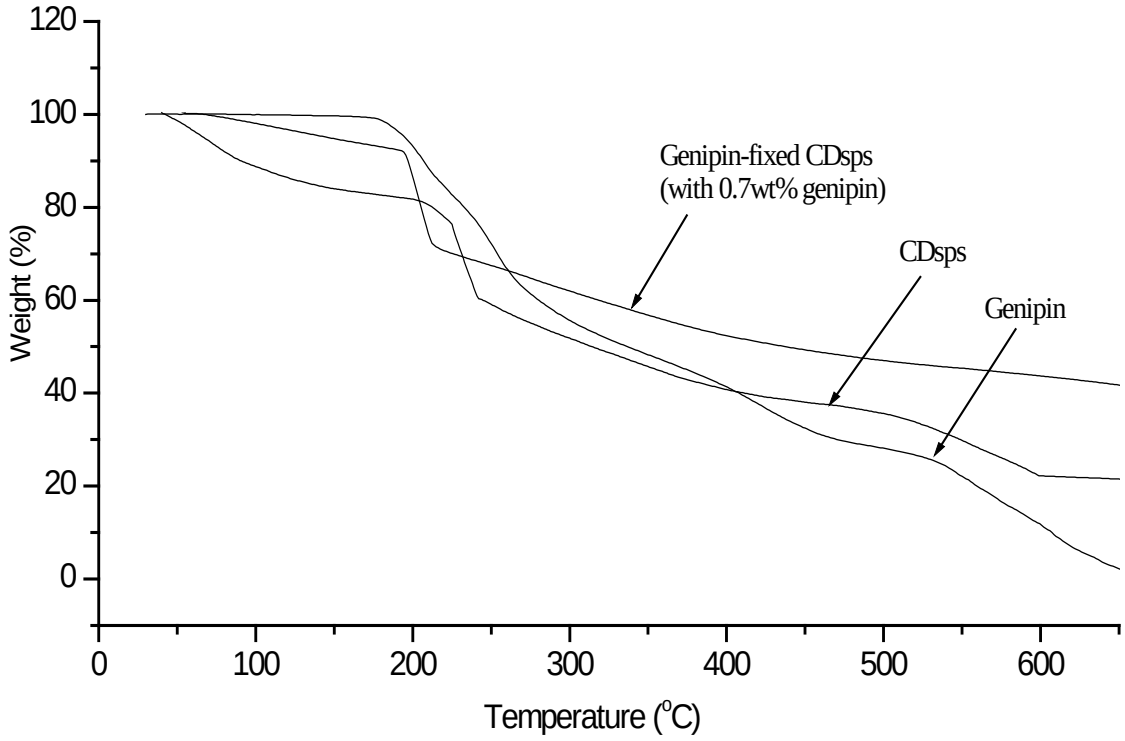


Figure 6 Thermogram (TGA) of CD_{sps}, genipin and genipin-fixed CD_{sps}

UV spectroscopy, optical rotation, and circular dichroism

The parent polysaccharides/blend did not exhibit any absorption maxima in the UV-Vis region. The genipin-fixed polysaccharides in aqueous solution (pH 7.16 at 30^{o}C) showed λ_{max} 589 nm, whereas genipin exhibited λ_{max} at 240 nm. The specific rotation $[\propto]_D$ values of parent CD_{sps} and genipin were + 114.4^{o} and +111.1^{o} (c. 0.20%, H_2O) respectively at 30^{o}C and 589 nm, while those of genipin-fixed CD_{sps} was +38.6^{o} (c. 0.20%, H_2O) (Table V 3).

The CD spectra of non-modified CD_{sps} was in the entirely negative region (peak 196 nm, [θ] -245.46 and trough 205.5 nm, [θ] -498.27) (Figure 7b). The genipin-fixed CD_{sps} exhibited a strong positive band (peak 203.0 nm, [θ] +437.05 and a trough 193.5 nm, [θ] +437.05) (Figure V.7c). The CD spectrum of genipin showed positive and negative bands, peak at 235 nm, [θ] +5749.63, and a trough at (c.a.203 nm, [θ] -3935.4 (Figure 7a).

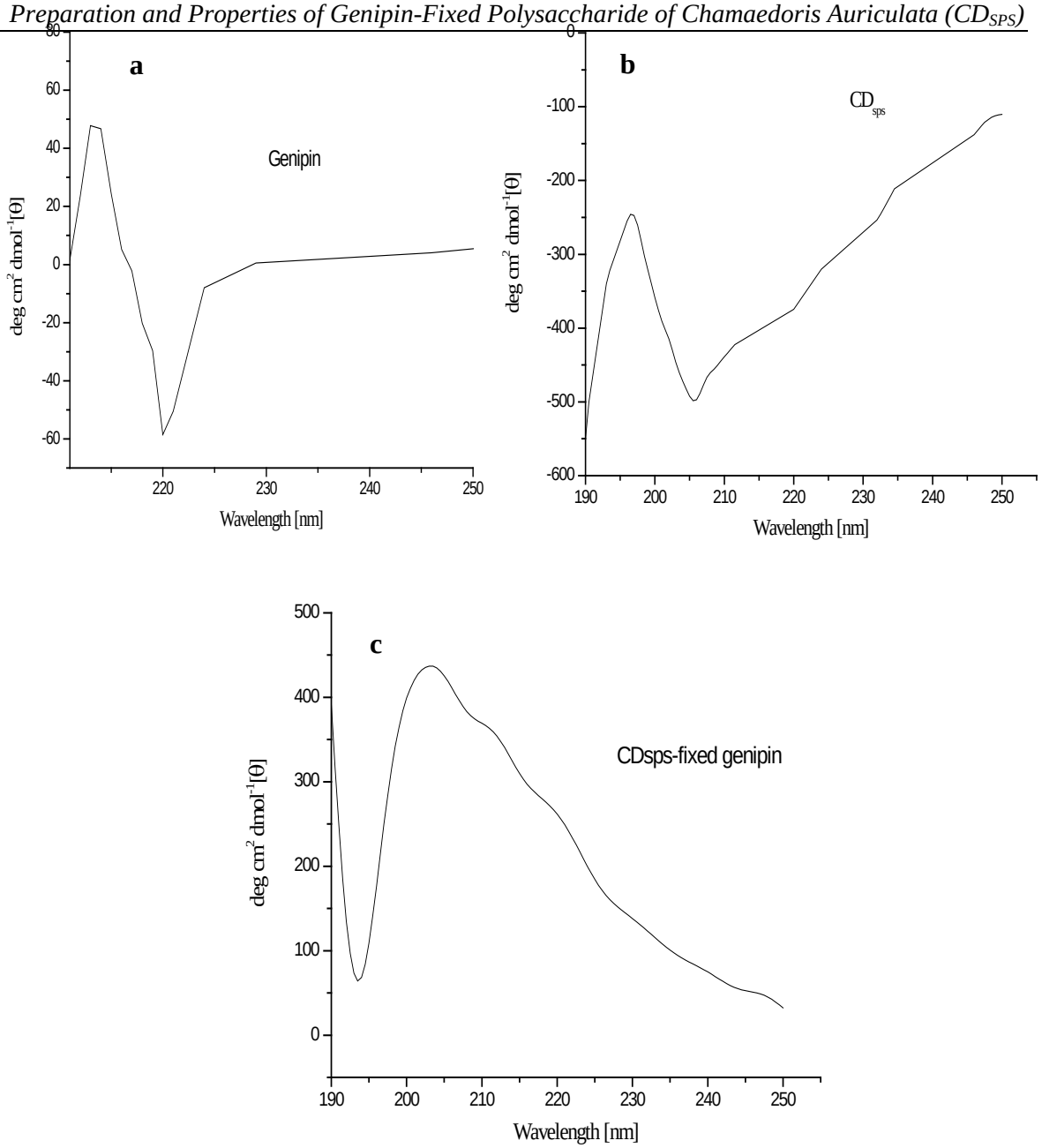


Figure 7**.** Circular dichroism spectra of genipin (a);CD_{sps} (b) and CD_{sps}-fixed genipin (c)

Properties	Agarose & cross linked agarose	Carrageenan and crosslinked carrageenan	CD_{sps} & cross linked CD_{sps}	Genipin
%N of A & B	0.07 & 0.07 [a]	0.32 & 0.30 [b]	1.10 & 1.08	N/A
Sulphate (%) in A	0.25[c]	17.0[d]	16.0	N/A
Carbohydrate composition in the repeating units	Galactose & 3,6-anhydro- galactose	Galactose & 3,6-anhydrogalactose	arabinose, galactose and rhamnose	N/A
Specific rotation values of A & B	-21.6^o & -12.2^o	+67.64 & +78.61^o	+114.4^o & +38.6^o	+111.1^o
Circular Dichroism Behavior of A & B (peak/trough)	<1 (0.52) & >1 (1.52)	>1 (1.03) & <1 (0.65)	<1 (0.64) & >1 (1.03)	>1 (1.45)

[a] Mwale *et al*.,2005; [b]Crini *et al*., 2005; [c]Prasad *et al*., 2007; [d]Unpublished data of author's laboratory; N/A=Not Applicable

Optical microscopy and scanning electron microscopy (SEM) analysis

The optical micrographs of parent CD_{sps} was off-white in color (Figure 8a) and genipin-crosslinked CD_{sps} (using 0.7 wt% genipin, after 20 h of crosslinking reaction) hydrogels were blue in color in appearance (Figure 8b.)

caused by the crosslinking reaction (Yueh-Sheng *et al*., 2005**)** between genipin (colorless) and polysaccharides. The scanning electron micrographs (SEM) of the modified and non-modified polysaccharides are depicted in Figure V 9a-b.

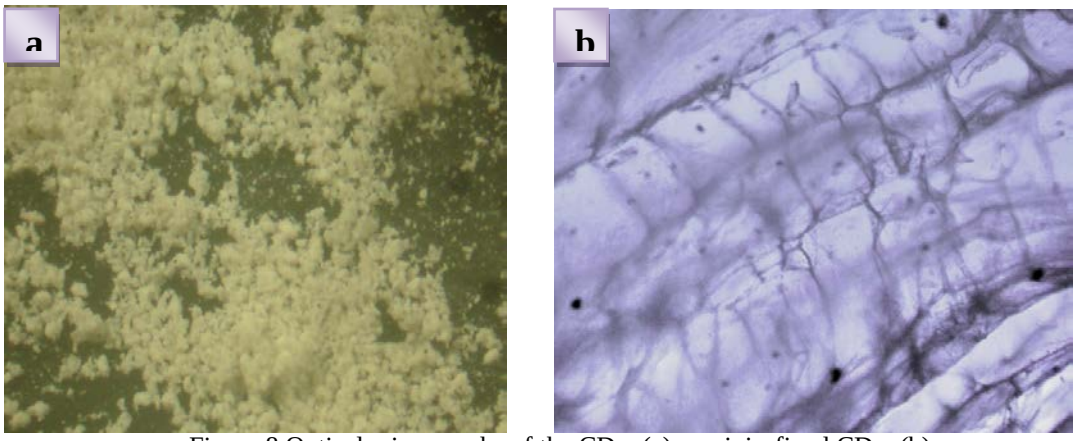


Figure 8 Optical micrographs of the CD_{sps} (a); genipin-fixed CD_{sps} (b)

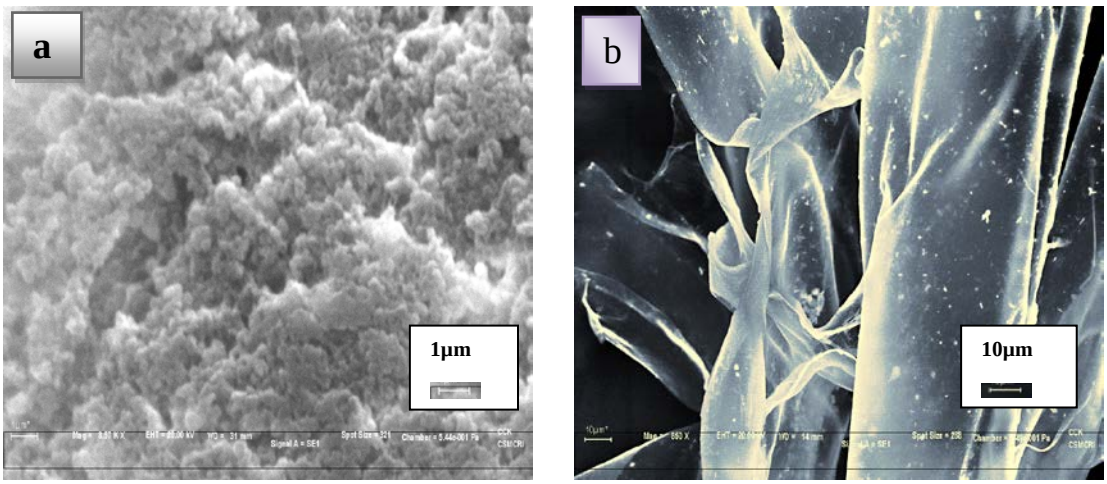


Figure 9. SEM images of CD_{sps} (a); genipin-fixed CD_{sps} (b)

Carbohydrate profile (GC-MS) and linkage pattern

The GC-MS analysis revealed that non-modified CD_{sps} contained arabinose, ribose, galactose and rhamnose. For linkage analysis the partially methylated alditol acetates (PMAAs) were characterized by GC-MS on the basis of their retention times and fragmentation patterns. The mass fragmentation pattern of the PMAAs were compared and validated with those of CCRC data bank (www.ccrc.uga.edu) as well as the ones reported by several authors in the literature. The CD_{sps} consists of 4-linked, 2,3-linked and terminal arabinose; 2,3,4-linked and terminal rhamnose; 2,6-inked, 6-linked, 4-linked and terminal galactose; 2,3,4-linked and terminal ribose residues.

MS/MS analysis

The mass spectral fragmentation pattern of standard genipin is presented in Figure 10. The *m*/*z* (%): 249.61 [M^+ +23(Na^+) 100%], 227.65 [M^+ +1 (12%)], 281.63 (85%), 177.61 (8%), 209.6 (20%) for standard genipin. The MS of the recovered genipin that was extracted with methanol from the acid hydrolysate of genipin-fixed CD_{sps} produced the following major ion fragments besides other contamination peaks (Figure 11) The *m*/*z* (%):249.63 [M^+ +23(Na^+) 100%], 227.65 [M^+ +1 (8%)], 281.63 (8%), 177.61 (22%), 209.6 (20%). The corresponding MS pattern of the control experiment did not show the peaks at *m*/*z* 249.63, 227.65, 177.61, 281.63 and 209.6, that were observed in the MS of standard genipin.

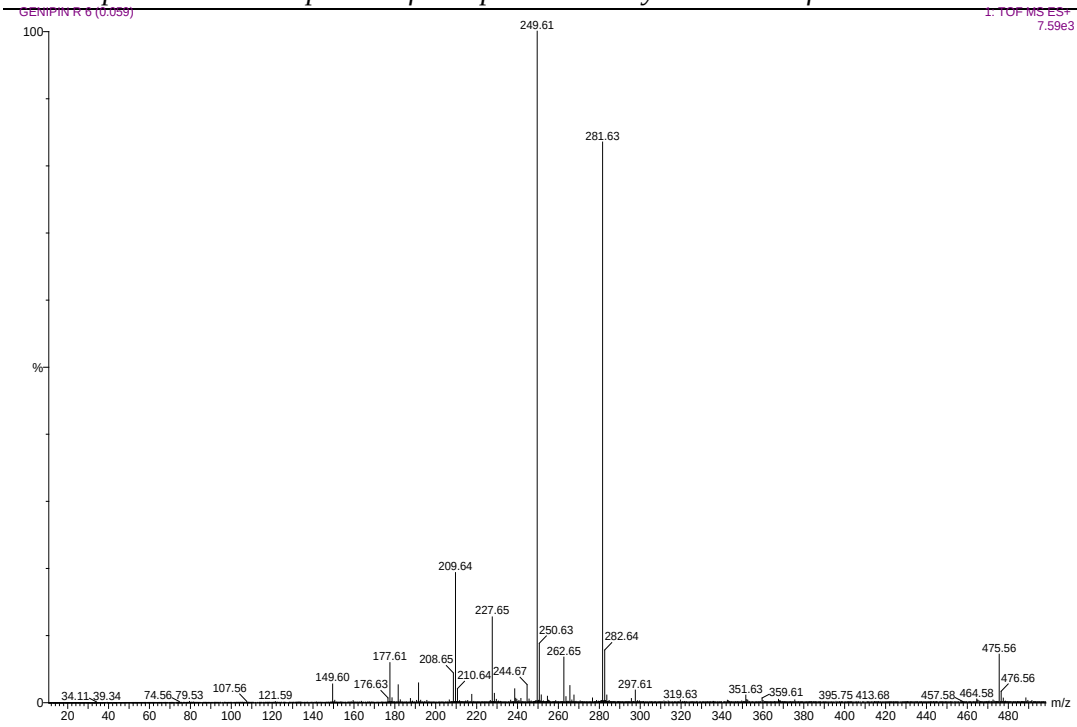


Figure 10 Mass spectrum of standard genipin

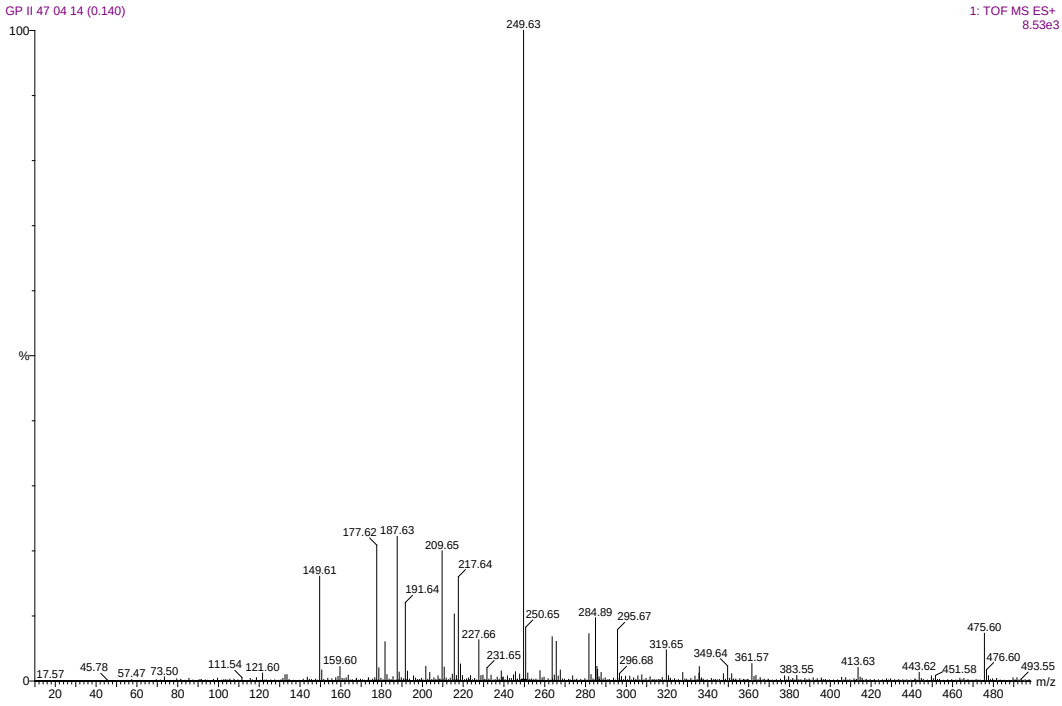


Figure 11 Mass spectrum of recovered genipin extracted from acid hydrolyzed genipin-fixed CD_{sps}

In this study the naturally occurring crossliker genipin (Figure 1) was employed on CD_{sps} in aqueous medium at ambient conditions to afford a stable hydrogel network. These genipin crosslinked polysaccharides exhibited significant absorbent capacity and stability. Gelation degree (G) of the crosslinked polysaccharides was lower than that of the respective parent polysaccharides (CD_{sps}), having an inverse relationship with the swelling ratio (cf. Meena *et al*., 2007b) (Figure 2) indicating lack of networking capability in the crosslinked products.

Swelling of the genipin-fixed polysaccharide was studied in a wide range of pH media viz. 1.2, 7.0 and 12.0 (Figure 3a-c). The parent polysaccharide swelled up to ca. 180 minute and then started dispersing, in all pH media. These genipin-fixed polysaccharides showed greatest water absorbency in neutral medium at pH 7.0 up to ca.1200 minutes and beyond this the absorbency remained virtually steady till ca.1400 minutes and then started dispersing. The swelling data indicated the consolidation of the network system in the crosslinked polysaccharide hydrogel. The genipin-fixed polysaccharide product revealed a statistically significant higher value of swelling ability in neutral pH, as compared to those in acidic and alkaline pH ($p < 0.001$). The crosslinked polysaccharides products with 0.7 wt% genipin, showed significantly higher value of swelling ability in all pH media, as compared to that with 1.0 wt% genipin ($p < 0.001$).

The swelling ratio increased gradually with crosslinking reaction time up to 30 h, beyond which no significant difference in swelling ability was observed (Figure 4). There was a decrease in true density, pore volume and porosity values of the genipin-fixed polysaccharide, while the apparent viscosity and bulk density values increased, in comparison to the respective parent polysaccharide. (Table 1). Similar observation was reported by Meena *et al.* (2007b).

The relatively greater stability of the crosslinked polysaccharide was also demonstrated in the degradation studies in Ringer`s Solution (Figure V 5). The mass loss ratios of non-modified polysaccharides ($CD_{sps}$) were observed 70%. Whereas the mass loss ratio of genipin-fixed polysaccharide (with 0.7 wt% genipin) were found to be 34% in Ringer`s solution.

The greater thermal stability of genipin-fixed polysaccharide compared to non-modified polysaccharide indicated generation of crosslinking in the polysaccharide network (Figure V 6). The TGA curve of genipin-fixed polysaccharide showed enhanced thermal stability than that of the parent polysaccharide. The initial mass loss (ca. 10%) was presumably due to the loss of bound water (moisture) to the polysaccharide. The parent polysaccharide showed ca. 79% mass loss while crosslinked products showed ca. 60% weight loss up to 650^{o}C, which may be the result of an acquired stability arising out of the rearrangement in the post crosslinking molecular architecture of the polysaccharide systems. It may be mentioned herein that the thermal stability of CD_{sps}, containing 4 different carbohydrates, was greater than that of CD_{sps}, containing only two carbohydrates (*vide* Figure 6 and Figure 7). The linkage study on the carbohydrate units in these polysaccharides indicated extensive branching in CD_{sps}, which presumably had certain bearing on its greater thermal stability. The crosslinked products of these two polysaccharides, however, had comparable thermal stabilities (*vide* Figure 6 and Figure 7).

The presence of molecules or substance in parent compound, able to induce conformational changes in the polymeric chain under appropriate physical conditions, leads to a more or less pronounced change in the Mol. ellipticity vs wavelength (Dentini *et al*., 2006) The peak-to-trough analyses were observed for CD_{sps} (0.64) i.e., peak/trough < 1 (Figure 7b), while for genipin (1.45) (Figure 7a), genipin-fixed CD_{sps} (1.03) (Figure 7c) i.e., peak/trough > 1. The CD spectrum and the peak-to-trough analysis (Table 3) indicated a reversal of chiroptical profiles that took place after insertion of genipin into the parent polysaccharide leading to alteration of symmetry elements. The overall chiral configurations of the parent polysaccharide have been deduced to be L for CD_{sps} by virtue of peak/trough ratios in the CD spectra.

The SEM images exhibited significant difference in the surface morphology of CD_{sps} after crosslinking with genipin which can be ascribed to crosslinked architecture. The non-modified polysaccharide appeared to have compact morphology having cloud-like clusters (Figure 9a) and can be distinguished easily from the modified polysaccharide. Each modified polysaccharide seemed to have very fibrous or thread-like morphology (Figure 9b), may be due to rearrangement of sugar unit in polysaccharide after crosslinking with genipin, indicating integrated molecular construct of the crosslinked system. This result appeared to have distinct positive correlation with the increased bulk density, apparent viscosity, and swelling ability of the crosslinked products relative to the respective parent systems.

The parent polysaccharide contained K, Na and Mg ions in significant amounts in varied proportions e.g. CD_{sps} contained K< Mg <Na. Amounts of K in these was 20.42 ppm, whereas the Mg and Na contents was 18.85 and15.62 ppm respectively in CD_{sps} (Table 2). The absence of some prominently toxic metal ions e.g. Cd, Pb, Ba and As in the parent polysaccharide suggest that these and the derivatives may be suitable for ingestible applications (Table 2). Sulphate contents of the polysaccharide and carrageenan were determined from the S contents (Table 3) (Meena *et al.,* 2007c). These sulphated polysaccharides harbor metal ions at the sulphate residues.

The decreased values of optical rotation in the crosslinked products (Table 3) suggested that crosslinking with genipin led to the modification of the original chiral disposition of the parent polysaccharides. This has been validated by the circular dichroism studies. The lowering of optical rotation values in crosslinked products suggested the enhancement of symmetry elements in these crosslinked polysaccharides. This

observation is in consonance with that happened with genipin crosslinked galactan polysaccharide reported by Meena *et al* (2007a).

The total nitrogen and protein values of non-modified polysaccharides and genipin-fixed polysaccharides were similar (Table 3) before crosslinking and after crosslinking with genipin, presumably because of the very low quantity of genipin that was involved in the crosslinking process with polysaccharides. The protein content in the parent polysaccharide (CD_{sps}) was estimated to be 7.44%. The absorption at 589 nm by the blue colored crosslinked polysaccharides indicated binding of genipin with the free amino acids or protein present that are present in the polymeric matrix of the parent polysaccharides' self-assembly (Lee *et al.,* 2003). The ESI-MS+ fragmentation patterns for standard genipin [m/z 249.61 [M^+ +23(Na^+)] and recovered genipin [m/z 249.63 [M^+ +23(Na^+)], which was extracted in methanol from the hydrolyzed crosslinked polysaccharides were similar confirming the presence of genipin in the grafted product. All these data confirmed that genipin was present in the cross linked polysaccharide matrix of CD_{sps} in chemically bound state imparting modified functional properties of the parent polysaccharide (cf. Figure. 1).

CONCLUSION

In this study the effect of genipin, a naturally occurring crosslinker, on the properties of CD_{sps} has been demonstrated in aqueous medium to impart functional stability. Genipin imparted thermal stability and enhanced swelling ability and lower gelation degree. The genipin-fixed hydrogel exhibited extraordinary stability in pH 7.0 medium as well as in Ringer`s solution. The genipin-fixed CD_{sps} products, exhibited higher viscosities, thermal stabilities and swelling abilities compared to the respective parent polysaccharide. Major toxic metal ions are absent in the parent polysaccharides. Thus, this naturally occurring crosslinking agent, which is relatively less cytotoxic than the others (Sung *et al*., 1999) can be exploited to prepare crosslinked polysaccharide, based materials. The resulting biodegradable polymer derived from seaweed, a renewable bioresource, may be of potential utility in the domain of biomedicine and catalysis as well as in the areas demanding the merits of such a unique asymmetric pool.

REFERENCES

[1]. Abad, L.; San Diedo, C., Relleve, L., Aranilla, C., Dela Rosa, A. M., Janik, I., Rosiak, J. In Proceedings of the 15th Philippine Chemistry Congress; Cebu City, Philippines, **1999**; p 466.
[2]. Akao, T., Kobashi, K., Aburada, M. Biol. Pharm. Bull. **1994,** 17,1573.
[3]. Aranilla, C. T., Yoshii, F., Dela Rosa, A. M., Makuuchi, K. Radiat. Phys. Chem.,**1999**, 55, 127.
[4]. Athawale, V. D., Padwaldesai, M. P. E. Polym. J. **1999,** 35, 1237.
[5]. Bai, Y.-X., Li, Y.-F. Carbohydrate Polym. **2006**, 64, 402.
[6]. Berger, J., Reist, M., Mayer, J. M., Felt, O., Peppas, N. A., Gurny, R. Eur. J. Pharma. Biopharm. **2004**, 57, 35.
[7]. Butler, M. F., Ng, Y.-F., Pudney, P. D. A. J. Polym. Sci: Part A: Polym. Chem. **2003**, 41, 3941.
[8]. Ciucanu, I., Kerek, F. J. Chromatogr.**1984**, 286, 179.
[9]. Crini, G., Morcellet, M. J. Sep. Sci. **2002,** 25, 789.
[10]. Dentini, M., Rinaldi, G., Barbetta, A., Risica, D., Skjak-Bræk. G. Carbohydr. Polym. **2006**, 63, 519.
[11]. Güven, O., Sen, M., Karadag, E., Saraydin, D. Radiat. Phys .Chem.**1999**, 56, 381.
[12]. Kume, T., Maekawa, Y., Eds. In Proceedings of the Takasaki Symposium on Radiation Processing of Natural Polymers, Takasaki, Gunma, Japan. **2001**, 300.
[13]. Lee, S.-W., Lim, J.-M., Bhoo, S.-H., Paik,Y.-S., Hahn, T.-R. Analytica Chemica Acta, **2003,**480, 267.
[14]. Lendlein, A., Schmidt, A. M., Langer, R. PNAS, **2001,** 98, 842.
[15]. Meena, R., Prasad, K., Siddhanta, A. K. Food Hydrocolloids, **2006a**, 20, 1206.
[16]. Meena, R., Prasad, K., Siddhanta, A. K. J. Appl. Polym. Sci. **2007a**, 104, 290.
[17]. Meena, R., Prasad, K., Siddhanta, A. K. Int. J. Biol. Macromol. **2007b**, 41, 94.
[18]. Meena, R., Siddhanta, A.K., Prasad, K., Ramavat, B. K., Eswaran, K., Thiruppathi, S., Ganesan, M., Mantri, V.A., Subba Rao, P.V. Carbohydrate Polym. **2007c**, 69, 179.
[19]. Mocanu, G., Vizitiu, D., Carpov, A. J. Bio. Comp. Polym. **2001**, 16, 315
[20]. Mwale, F., Iordanova, M., Demers, C. N., Steffen, T., Roughley, P., Antoniou, J. Tissue Eng. **2005,** 11 , 130.
[21]. Nickerson, M.T., Paulson, A.T., Wagar, E., Farnworth R., Hodge, S.M., Rousseau, D. Food Hydrocolloids, **2006**, 29, 1072.
[22]. Paik, Y.-S., Lee, C.-M., Cho, M.-H., Hahn, T.-R. J. Agric. Food Chem. **2001**, 49, 430.
[23]. Patrickios, C. S., Georgiou, T. K. Curr. Opin. Colloid. Int. Sci. **2003**, 8, 76.
[24]. Pourjavadi, A., Ghasemzadeh, H., Hosseinzadeh, H. e-Polymers, No. 027, **2004**. (www.e-Polymers.org).
[25]. Prasad, K., Meena, R., Siddhnata, A. K. J. Appl. Polym. Sci. **2006a,** 101, 161.
[26]. Prasad, K., Mehta, G., Meena, R., Siddhanta, A. K. J. Appl. Polym. Sci **2006b,** 102, 3654.
[27]. Prasad, K., Siddhnata, A. K., Ganeshan, M., Ramavat, B.K., Jha, B., Ghosh, P.K. Bioresource Technology, **2007**, 98, 1907.
[28]. Prasad, K., Siddhnata, A. K., Rakshit, A. K., Bhattacharya, A., Ghosh, P. K. Int. J. Biol.
[29]. Macromol. **2005a**, 35, 135.
[30]. Prasad, K., Trivedi, K., Meena, R., Siddhanta, A.K. Polymer Journal, **2005b**, 37, 826.
[31]. Relleve, L., Yoshii, F., dela Rosa, A. M., Kume, T. Radiat. Phys. Chem. **1999**, 273, 63.
[32]. Shiftan, D., Ravenelle, F., Mateescu, M. A., Marchessault, R. H. Starch/Stärke, **2000**, 52, 186.
[33]. Siddhanta, A. K., Sai Krishna Murthy, A. J. Indian Chem. Soc. **2001,** 78, 431.
[34]. Sung, H. W., Huang, D. M., Chang, W. H., Huang, L. L., Tsai, C. C., Liang, I. L. J. Biomater.Sci. Polym. **1999**, 10, 751.
[35]. Touyama, R., Takeda, Y., Inoue, K., Kawamura, I., Yatsuzuka, M., Ikumoto, T., Shingu, T., Yokoi, T., Inouye, H. Chem. Pharm. Bull. **1994**, 42, 668.
[36]. Wathelet, B. Biotechnol. Agron. Soc. Environ. **1999,** 3, 197.

[37]. Wolnik, K. A. Enzymology, **1988**, 158, 190.
[38]. Yao, C.-H., Liu, B.-S., Chang, C.-J., Hsu, S.-H., Chen, Y.-S. Mater. Chem. Phys. **2004**, 83, 204.
[39]. Yueh-Sheng, C., Ju-Ying, C., Chun-Yuan, C., Fuu-Jen, T., Chun-Hsu, Y., Bai Shuan, L.
[40]. Biomaterials, **2005**, 26, 3911.