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Induction of PVP Functionality to the Polysaccharide from *Chaetomorpha Antennina*

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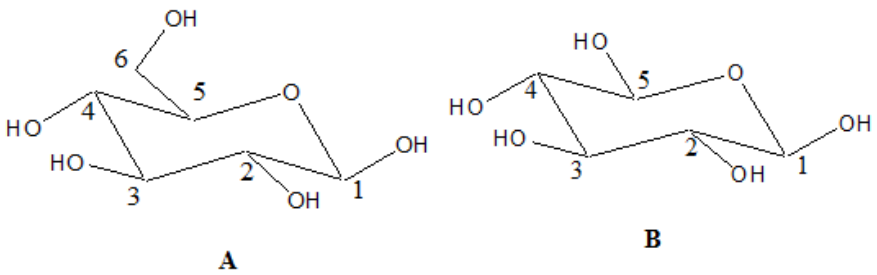
INTRODUCTION

In recent years, chemical modification of natural macromolecules has received considerable attention. Polysaccharides of marine origin are unique raw materials as they are abundant in nature, widely available in many countries, inexpensive, renewable resources, stable, hydrophilic and modifiable biopolymers. They offer tremendous potential for development of alternate materials (Chauhan *et al*., 2002; Okieimen, 2003; Sanghi *et al*., 2002; Singh *et al.*, 2004a; Singh *et al.,* 2004b). Grafting techniques have been widely used to prepare materials especially involving systems in which polysaccharides are used as substrate polymers (Gao *et al.,* 1994; Singh *et al*., 2004a, 2004b). In recent times microwave (MW) irradiation techniques have occupied an important position in grafting type modification reaction due to its simplicity and rapid nature. Hydrolyses of starch and plant seed gums have been achieved readily under MW irradiation with very mild reaction conditions (Xia *et al.,* 2000; Singh *et al.,* 2003; Singh *et al.,* 2004c). An important advantage of graft polymerization is that the grafted polymer chains are held together by chemical bonding, allowing the two polymers to be intimately associated. The polymer that is grafted is expected to be distributed on the backbone of the substrate polymer and also to impart beneficial effects on its properties. Synthesis of alkyl glycosides and graft copolymerization of acrylic acid with starch has been efficiently carried out with microwave irradiation (Nuchter *et al.,* 2001; Yanbin, *et al.,* 1999).

Seaweeds being abundant in nature are good source of polysaccharides and their use in the preparation of grafted materials having biomedical applications would add value to the seaweeds (Prasad *et al.,* 2006a). Seaweed polysaccharides are relatively less explored about such modifications. *Chaetomorpha antennina* is a green alga containing sulphated polysaccharide consisting of 4-linked arabinopyranose residues, as the backbone of the polysaccharide structure, 4-,6- and 2,6- linked galactose and 2,3-linked arabinosepyranose as well as terminal arabinopyranose residues (GC-MS, ^{13}C NMR). The sugar residues and linkage pattern were matched with the earlier report by Rao & Ramana., (1991). Structure of the basic units of the polysaccharide is depicted in Figure 1. Many of the copolymers of seaweed polysaccharides reported so far were synthesized by multi-step process involving prolonged reaction time (Paepe *et al*., 2002).



Figures IV.2.1 Structures of galactopyranose (A), and (B) arabinopyranose

As part of an ongoing program our laboratory on modification and value addition of seaweed polysaccharides for newer applications (Prasad *et al*.,2005a; Prasad *et al*.,2005b; Prasad *et al*., 2006a; Prasad *et al*, 2007; Meena *et al*.,2006a; Meena *et al*.,2006b; Meena *et al*., 2007a; Bajpai, *et al*., 1988) we are reported herein the synthesis of CM_{sps}-*graft*-PVP blends by a novel method, that is, microwave irradiation in the presence of water soluble redox initiator, potassium per sulphate, which enabled the formation of the desired products in a

shorter duration (120 s) and resulted in grafted product that were more crystalline than the controlled polysaccharides. The microwave irradiation conditions were also optimized in order to produce grafted product capable of forming hydrogels in water. The physicochemical properties of the hydrogels were studied and were compared with control CM_{sps}. Formation of the grafted products was confirmed by IR, optical rotation, circular dichroism (CD), X-ray diffraction (XRD), CP-MAS of ^{13}C-NMR and thermal studies (TGA). Because such hydrogels are not as a strong and have more spreadability (i.e., more gel thinning) and more water-holding capability, they are potentially useful in moisturizer formulation and active carriers of drugs. The use of blended PVP with agar in hydrogel dressings has also been reported (Lugao *et al*., 1998). The grafted product may have many different applications and may hold industrial promise for the future.

EXPERIMENTAL

Materials

Chaetomorpha antennina (Bory) Kuetz was collected from Diu (20o45′ N, 70o58′E) on the west coast of India during April 30, 2005. A herbarium specimen (AL-II-70-01) has been deposited with CSMCRI, Herbarium for future reference. The hot water-soluble sulphated polysaccharide (CMsps) was extracted following the method described by Siddhanta et al., (2001). The products were vacuum dried prior to reaction. Potassium persulphate, Polyvinylpyrrolidone (PVP), isopropanol, toluene used were of analytical grade and were purchased from Sigma-Aldrich, Mumbai and Ranbaxy Chemicals, Mumbai respectively. A domestic microwave oven (LG make; 2700 watt) operating over a temperature range 40-100^{o}C (Magnetrons are set at a frequency of 2450 MHz). A MILESTONE -START S microwave lab station for synthesis was also used (fixed frequency of 2,450 MHz) over set temperature parameters to prepare the hybrid material CMsps–PVP. This instrument had the programming facility to set the constant temperature and required reaction time. Apparent viscosity was measured on a Brookfield Viscometer (Synchrolectric Viscometer, Stoughton, MASS 02072). Spindle No.3 at 60 rpm was used for measuring the apparent viscosities of CMsps (2%) and CMsps-graft-PVP (2%) in (DMSO-water mixture (1:5v/v) at 70oC. For GC-MS analyses of the alditol acetates of CMsps and its charged and neutral fractions were used, which were prepared following the methods described in the literature (Siddhanta et al., 2001; Sen Sr et al., 2002). Hydrolysis of the grafted product for carrying out ESI-MS/MS analysis of PVP was done following the method reported by Harrison & Stimson (1968): The grafted product (25 mg) was hydrolyzed in 1 M HCl (10 ml) by heating at 70-80oC for 10 min. The hydrolysis reaction mixture (pH 3.1) was extracted with toluene to isolate the product. The toluene extract was evaporated to dryness and the white residue was dissolved in methanol, which was used in the mass spectrometric measurements.

GC-MS analysis

GC-MS analysis of the alditol acetates were carried out on a Shimadzu GCMS-QP2010 machine, using a SGE BP-225 capillary column (25 m, 0.25µm, 0.22mm), employing temperature programming (160^{o}C to 230^{o}C @10^{o}C per min).

Synthesis and purification of the graft copolymer

A MILESTONE-START *S* microwave labstation was used (fixed frequency of 2,450 MHz) over set temperature parameters to prepare the hybrid material CM_{sps}-PVP. This instrument has the programming facility to set the constant temperature and required reaction time. The reaction was carried out in a 100 ml narrow-mouth conical flask. CM_{sps} (1 g w/v, 3.22 mmol as per repeating arabinopyranose-galactopyranose repeating unit, the molecular weight was ca. 1.12 x 10^{6} D) was first dissolved in 100 ml of distilled water followed by the addition of methylmethacrylate (3.10 to 7.76 per mole equivalents of CM_{sps}) and 0.01g (0.00037 mol/L) of potassium persulphate.

The mixture was then irradiated under microwave at a set temperature 100^{o}C. The set temperature 100^{o}C was achieved in 1 min and sustained for remaining 1.5 min to carry out the reaction. The microwave instrument has inbuilt magnetic stirrer and the reaction mixture was continuously stirred throughout the course of the reaction. The reaction mixture turned milky white, presumably due to the polymerization of vinyl pyrrolidone (VP) to PVP, which was first cooled and the product was precipitated in isopropyl alcohol (1:2.25, v/v), followed by centrifugation at 8000 rpm for 3 min. The off-white precipitate was collected and air dried. The unreacted homopolymer (PVP) was extracted from the product by Soxhlet extraction with toluene for 1 h. The product was further washed with isopropyl alcohol and vacuum dried. The toluene solution containing PVP, was evaporated to dryness and the weight of unreacted PVP was measured. The hydrogel of the hybrid material (Figure 2) was prepared in DMSO water mixture (1:5 v/v) by heating at ca. 80^{o}C. The product obtained by using CM_{sps} : PVP as 1: 6.20 was solid in nature, very stable unlike those obtained using the proportions 1: 3.09 and 1:

4.68, which were not solid and was heterogeneous when viewed under a microscope. For the one obtained with 1: 6.46 (CM_{sps}: VP), the yield was low. Hence the product obtained by the optimized molar ratio (1: 6.20) of CM_{sps} and VP was used for further characterization.

Characterization of CM_{sps}-*graft*-PVP blends

Grafted product was characterized by FT-IR using a Perkin-Elmer Spectrum GX FT-IR system, by taking 10.0 mg of sample in 600mg KBr. All spectra were average of two counts with 10 scans each and a resolution of 5 cm-1. 13C NMR spectrum (noise-decoupled) was recorded on a Bruker Ultrashield Spectrometer, Switzerland at 125 MHz. Sample (10 mg/ml) was dissolved in D2O and DMSO (5:1) and spectrum was recorded at 70oC. The TGA of the parent polysaccharide (10 mg) and its graft copolymer (11.4 mg) were carried out on a Mettler Toledo, TGA/SDTA 851e system, Switzerland, using a temperature program 30o to 600oC at a heating rate 10oC min-1 in an air atmosphere. Powder X-ray diffractions were measured on a Philips X' pert MPD X-ray Powder Diffractomer using 2θ = 5 to 60o. The scanning electron micrograph (SEM) was recorded by applying 20 kV accelerating voltage and 9700X (for CMsps) and 2600 X (CMsps-graft-PVP) magnification using a Carl-Zeiss Leo VP 1430 instrument. Optical micrograph was recorded on an optical microscope of Olympus model SHZ 10, Japan with 70X magnification. The gel strength (gcm-2) was measured using a Nikkansui-type gel tester (Kiya Seisakusho Ltd. Tokyo, Japan).

Intrinsic viscosities [η] were determined at room temperature using an Ostwald viscometer with a flow time 1.39 min for 1:5v/v, DMSO-water mixture. For this, sols of samples were prepared in 1:5v/v DMSO-water mixture by heating until complete dissolution at a concentration 0.02% to 0.1% (w/v).

Bulk density

The bulk density of grafted polysaccharides before and after crosslinking was calculated , they were dried until a constant weight, and then transferred to a 10 ml volumetric flask (W_1), filling the same up to the mark and were weighed (W_2), for each samples. The bulk densities (d_o) of the polymers were calculated, using Eq. (1) (Bai & Li, 2006, Meena *et al.*, 2007b)

$$d_o = (W_2 - W_1) \div 10 \quad (1)$$

Where, W_2 is the total weight of the polymer and flask and W_1 is the weight of the flask.

True density

To estimate the true density of dried cross-linked (CM_{sps}-*graft*-PVP) and parent CM_{sps}, 0.35 g of grafted and non-grafted, was first dried until a constant weight (W_o) was reached and then placed into a 10 ml volumetric flask of known weight at 20°C. Into the flask was added 8 ml of cyclohexane and the mixture was kept at 20°C for 24 h. The flask was then filled with cyclohexane up to the mark and was weighed (W). The true density of grafted and non-grafted was calculated, using Equation (1) (Bai, & Li, 2006; Meena *et al*, 2007b).

$$d = W_o / [10-(W-W_o)/d_c] \quad (2)$$

Where W is the total weight of the polymer and the solvent, and W_o the weight of the dry polymer sample, d_c the density of the solvent ($d_{cyclohexane}$ = 0.778 g/ml).

Pore volume and porosity

The pore volumes of the parent CMsps and grafted polysaccharides (CMsps-graft-PVP) were studied by monitoring the weight gain of the products (Bai, & Li, 2006; Meena et al., 2007b). The dried parent CMsps and CMsps-graft-PVP were placed into tubes with a porous glass bottom. The tubes were kept inside a flask filled with cyclohexane for 48 h at 20oC. The excess cyclohexane was removed by centrifugation at 1500 rpm for 1

min. The volume of cyclohexane absorbed by the polymers was used to estimate the porosity of the polymer beads. The porosity of the polymers was determined by

$$Ø = Vp/Vo \quad (3)$$

Where Vp is the pore volume in the polymers beads and Vo is the true volume of polymers beads.

$$Vo = Wo /d \quad (4)$$

Where Wo is the weight of dry polymer beads, and d (the true density of dried polymers beads) was determined using Eq. (2).

CP-MAS ^{13}C NMR spectroscopy

The CP-MAS ^{13}C NMR measurements were performed at 20°C on a Bruker Advance 500 MHz, Spectrometer (Switzerland) at 52.3 MAS, Net spinning was kept 5000 rpm/min. The conventional CP/MAS method was used for high resolution solid-state ^{13}C measurements.

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) of Vacuum oven dried samples of the powder of the parent polysaccharides and CM_{sps}-*graft*-PVP were done as described in characterization section.

Circular dichroism measurement

Circular dichroism (CD) spectra were of CM_{sps} and grafted products were recorded as described in characterization section.

Specific surface area analysis (BET)

The specific surface area, pore volume, and pore size distribution of the calcined samples were determined from N_2 adsorption-desorption isotherms at 77.4 K using volumetric equation adsorption equipment (ASAP 2010, MicroMetrics, NH), using BET and BJH models (Gregg *et al*., 1982).

RESULTS AND DISCUSSION

Physicochemical properties

The physical properties of the polysaccharide-*graft*-PVP blend sol gel and a comparison with those of the control polysaccharide's sol and gel are given in (Table 1). It was observed that the apparent viscosity of the 2% (w/v) CM_{sps}-*graft*-PVP sol had decreased to 100 cP from the 250 cP exhibited by the 2% CM_{sps} sol. There was ca.50% decrease in the viscosity on grafting. It may be mentioned in this connection that the parent polysaccharide formed soft gel having gel strength 330gcm^{-2} (in 2% w/v concentration), at 20^{o} C, while grafted product formed soft gel having gel strength < 100 g/cm^{-2} under the same condition. Similar reductions in the gelling and melting temperature of the hydrogels were also observed. All the reports indicated that the grafted product formed weaker gel relative to the corresponding control polysaccharide gels (Table 1).

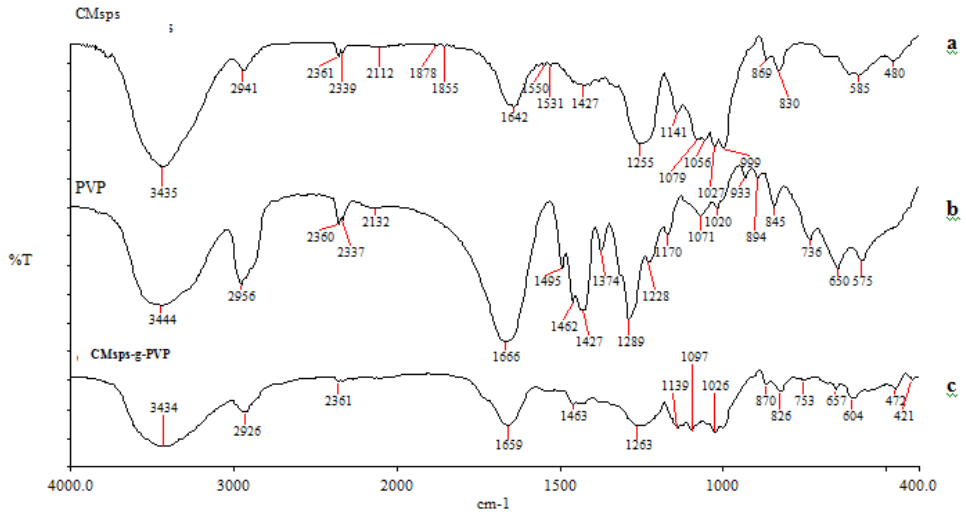
Table 1 Physicochemical properties of (CMsps) and CM_{sps}-*graft*-PVP

Properties	CM_{sps}	CM_{sps}-*graft*-PVP [c]
Apparent viscosity (cP) at 70°C[a]	250±10	100±05
pH (70°C)	6.9	7.3
Bulk density (do)(g/ml)	0.304	0.511
True density (d)(g/ml)	1.277	1.020
Pore volume (V_p) (g/ml)	0.544	0.352
Porosity(Φ)	1.985	1.026
Gel strength (g/cm^2) at 20°C	330±20	<100±10
%N	0.84	6.04
Gelling Temperature (°C) [b]	78±5	35±4
Melting Temperature T(°C) [b]	80±5	65±5
Specific rotation values	+177.8°	+56.51°
Circular Dichroism Behavior (peak/trough)	<1 (0.81)	>1 (1.34)
Chiral configuration of CM_{sps}	L	L

[a] Viscosity was measured in a 2% (w/v) concentration at 70°C
[b] Gel strength, gelling, melting temperature were measured in a 2% (w/v) gel of CM_{sps}
[c] Gel strength, gelling, melting temperature were measured in a 5% (w/v) gel of CM_{sps}-*graft*-PVP

Evidence of grafting

The formation of CM_{sps}-*graft*-PVP was indicated by its low solubility in water, while the non grafted homopolymer, PVP, was water soluble. Characteristic IR bands at 836,753, 993 cm^{-1} for β-skeletal bending of basic carbohydrate moieties in the copolymer indicated that during grafting reaction the polysaccharide did not get decomposed (Figures 2). Appearance of new IR bend of carbonyl group (PVP) at 1659 cm^{-1} and an additional band at 1463, 1426, 1370, 1263, cm^{-1} in the grafted product indicated the insertion of PVP into the polysaccharide structure. The broad band at 3200-3400 cm^{-1} is due to stretching of –OH group of CM_{sps}. PVP was grafted on to the sulphated polysaccharide in a homogeneous solution. Since the polymerization variables affected the grafting parameters and homopolymer quantity, certain critical parameters were investigated to have optimum condition for obtaining graft copolymer with minimum degradation of the parent polysaccharide. Presence of nitrogen in grafted product (6.04%) and parent polysaccharide (0.84%) was also determined (by Kjeldahl method), the higher percentage of nitrogen in grafted product indicated the presence of PVP.

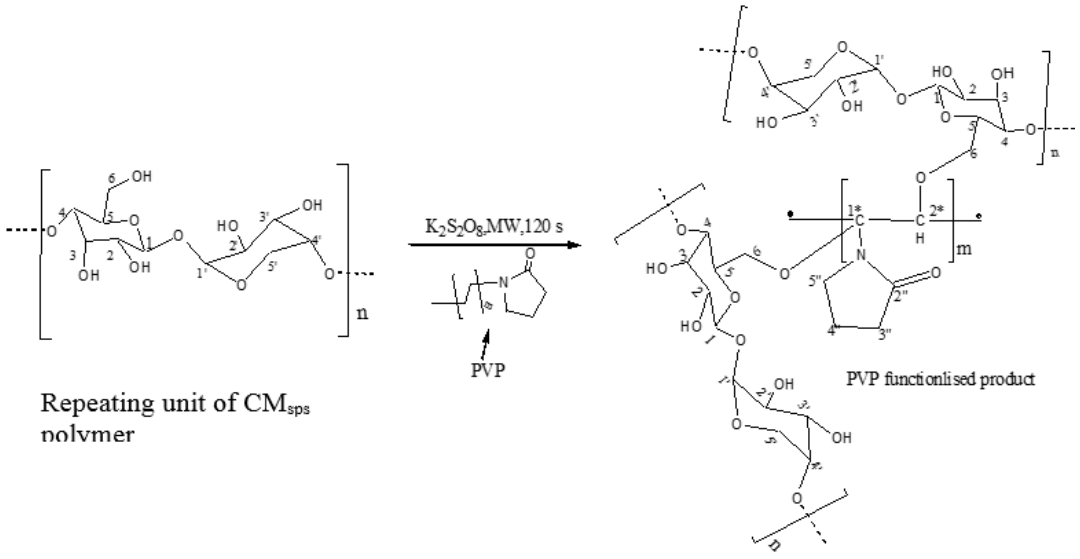


Figures 2 . FT-IR spectra of CM_{sps} (a), PVP (b), and CM_{sps}-*graft*-PVP (c)

GC-MS and CP-MAS ^{13}C NMR

The GC-MS analysis of the alditol acetates of the crude CM_{sps}, and its charged (≥95%) and neutral (≤5%) fractions (vide Figures in 6, Figures in 7, Figures in 8) revealed the presence of different carbohydrate moieties in varied distribution, which is presented in Table 2. Examination of Table 2 showed that the charged

fraction of CM_{sps} contained only arabinose and galactose units. The crude CM_{sps} contained ribose, arabinose, xylose, mannose and galactose units. Rao & Ramana (1991) described the crude hot water extract of *Chaetomorpha antennina* containing arabinose and galactose in major amounts with minor presence of rhamnose (3.8%). In the present investigation, however, no rhamnose was detected. It is presumed that the grafting reaction took place on the C-6 of the charged species (galactose moieties) bearing the sulphate group, as reported earlier by Prasad *et al*., (2006). Therefore, the structure of CM_{sps}-*graft*-PVP is proposed as given in Figures IV.2.3. The CP-MAS ^{13}C NMR data of CM_{sps} and CM_{sps}-*graft*-PVP are given in Table 3 and Table 4. The assignments of carbon shifts observed were done on the basis of the comparison with CP-MAS ^{13}C NMR spectrum (Figures 4.) and the δ values that were obtained from the ChemDraw Ultra 10.0 software on the proposed structure. The carbonyl carbon of PVP moiety appeared at δ176.029. In the grafted product anomeric carbons of arabinose and galactose moieties were merged and appeared at 97.3 ppm (Table 4 and Figures 3). Each of the chemical shift values of 74.3,71.3,66.0 and 65.0 ppm were assigned to C-2, C-3, C-4 and C-5 (arabinose) and C-2, C-5, and C-3 (galactose) in CM_{sps} as well as for grafted product, which appear to have similar anisotropic environment (Table 3 and Figure 4). These conclusions fitted well on the pyranose structures of galactose and arabinose in the copolymer.



Figures IV.2.3 Proposed formation of CM_{sps}-*graft*-PVP

Table 2 GC-MS profile of the carbohydrate moieties present in CM_{sps} and its charged and neutral fractions.

Carbohydrate moieties (%)	Crude CM_{sps}	Charged fraction of CM_{sps}	Neutral fraction of CM_{sps}
Ribose	7.06	ND[A]	15.51
Arabinose	71.48	50.32	75.80
Xylose	2.91	ND[a]	3.55
Mannose	4.14	ND[a]	ND[a]
Galactose	14. 41	49.68	5.40

[a] ND=Not detected

Table 3 CP-MAS ^{13}C NMR Data of CM_{sps} [a]

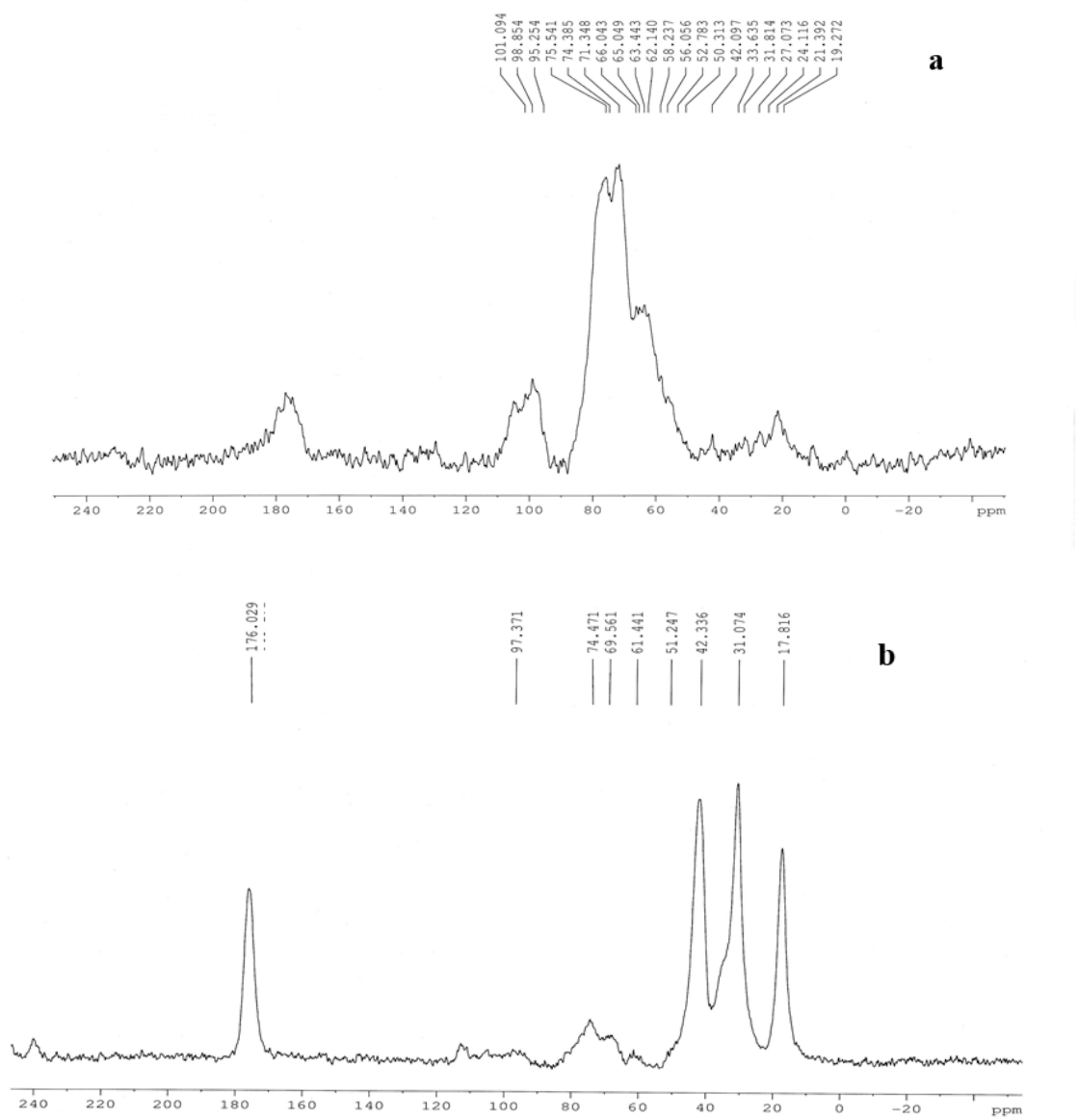
Compound	δ (ppm)	Assignment
CM_{sps} (Figure IV.2.4a)	98.8 (96.7)	C-1 of arabinose ring (anomeric carbon)
	74.3, 71.3 (70.8, 70.02)	C-2 & C-3 of arabinose moiety
	66.03, 65.0 (67.7, 66.3)	C-4 & C-5 of arabinose moiety
	95.2 (90.5)	C-1' of galactose moiety (anomeric carbon)
	74.38, 66.0, 71.3, (74.4, 67.5.69.9)	C-2', C-3' & C-5' of galactose moiety
	74.3, 62.1 (74.8, 59.8)	C-4' & C-6' of galactose moiety
	129, 107,104, 101	Not assign

[a]Values in the parentheses are obtained from ChemDraw Ultra Version 10.0 software, Cambridge Soft Corporation, USA, on the structure for CM_{sps}

Table .4 CP-MAS ^{13}C NMR Data of CM_{sps}-*graft*-PVP product [a]

Compound	δ (ppm)	Assignment
CM_{sps}-*graft*-PVP (Figure IV.2.4b)	176.02 (172.0)	C-2 of PVP moiety
	31.1 (32.2)	C-5 of PVP moiety
	17.81 (16.9)	C-4 of PVP moiety
	42.3 (37.1)	C-3 of PVP moiety
	84.03 (85.9)	N-linked aliphatic C-1* of PVP moiety
	84.03 (82.4)	N-linked aliphatic C-2* of PVP moiety
	97.3 (96.7)	C-1 of arabinose ring (anomeric carbon)
	69.5 (71.2, 67.7)	C-2 , C-3 of arabinose moiety
	74.4 (73.4)	C-4 of arabinose moiety
	61.4 (63.6)	C-5 of arabinose moiety
	97.3 (93.8)	C-1' of galactose moiety (anomeric carbon)
	74.4, 69.5 , & 69.5 (74.4, 67.5, 67.7)	C-2', C-3', C-5' of galactose moiety
	74.4, 61.4 (75.1, 60.0)	C- 4' & C-6' of galactose moiety

[a]Values in the parentheses are obtained from ChemDraw Ultra Version 10.0 software, Cambridge Soft Corporation, USA, on the structure for CM_{sps}-*graft*-PVP



Figures 4 CP-MAS ^{13}C NMR of CM_{sps} (a), and CM_{sps}-*graft*-PVP (b) follows; Ct = 1.25 (± 0.010); Gr = 2.30 (± 0.022); Ge = 0.92 (± 0.043); Ad = 0.56 (± 0.026); Hp = 0.15 (± 0.0011).

Yield and grafting parameters

Yield of CM_{sps}-*graft*-PVP was 2.50 g. Grafting parameters i.e., total conversion (Ct), Grafting ratio (Gr), grafting efficiency (Ge), add-on (Ad) and homopolymer content (Hp) were determined according to the known weight-basis expressions. (Pourjavadi *et al.*, 2002).

$Gr = W_3/W_o$ ---------- (2)

$Ge = W_3/W_2$ ---------- (3)

$Ad = (W_3-W_0)/W_3$ ---------- (4)

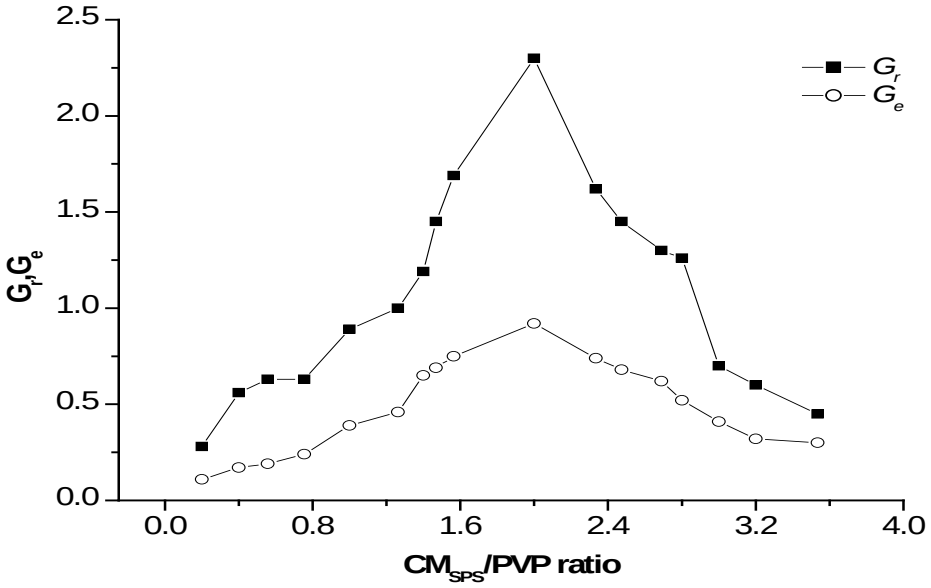
$Hp= (W_2-W_3)/W_2$ or $Hp = 1-Ge$ ---------- (5)

Where W_o, W_1, W_2 and W_3 are the weights of initial substrate, monomer used, the product mixture (i.e., copolymer and homopolymer) and pure graft copolymer respectively. In the present investigation the respective values for optimized reaction were W_o = 1g; W_1= 2 g; W_2 = 2.50 g and W_3 = (2.50-0.20) g =2.30 g (unreacted PVP homopolymer was 0.20 g). The grafting parameters calculated for the product were as follows; Ct = 1.25 (± 0.010); Gr = 2.30 (± 0.022); Ge = 0.92 (± 0.043); Ad = 0.56 (± 0.026); Hp = 0.15 (± 0.0011).

PVP was grafted on to the sulphated polysaccharide in a homogeneous solution. Since the polymerization variables affected the grafting parameters and homopolymer quantity, certain critical parameters were investigated to identify the optimum condition for obtaining graft copolymer with minimum degradation of the parent polysaccharide.

Effect of the ratio of PVP to polysaccharide

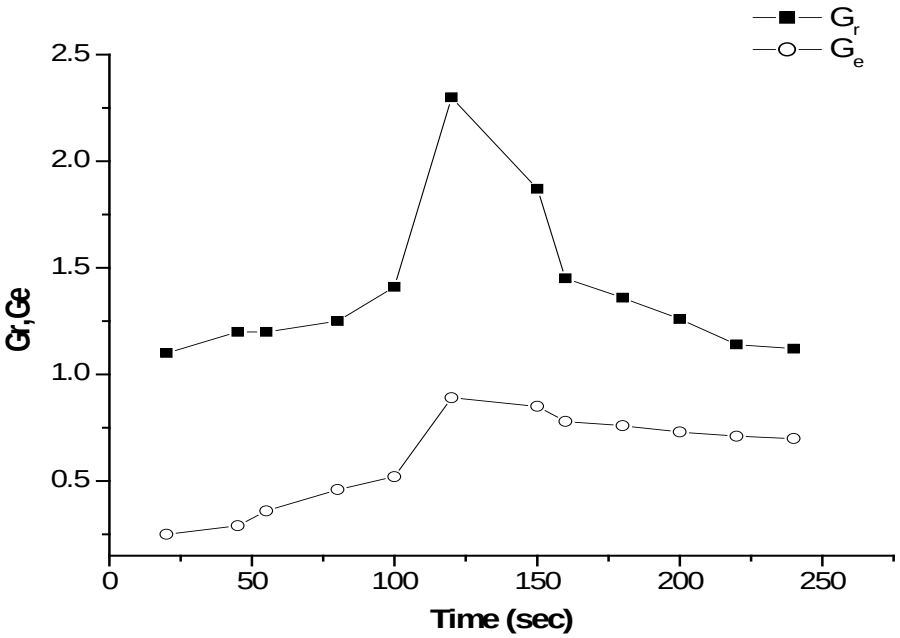
The influence of the ratio of PVP to CM_{sps} on the grafting parameters was studied. It was found that 'Gr' and 'Ge' reached a maximum value when the ratio of PVP to polysaccharide was 2:1 and then decreased gradually (Figures 5). In the absence of the water soluble initiator the reaction did not take place at all. In presence of the initiator in the aqueous solution of polysaccharide when the PVP to polysaccharide ratio increased then CM_{sps}-*graft*-PVP could play the role of self-emulsifier so as to absorb more monomers (PVP) on the polysaccharide surface, which subsequently enhanced the rate of the graft reaction thereby sharply increasing 'Gr' and 'Ge' till the PVP to polysaccharide reaches 2:1. When the ratio of PVP was higher which led to the acceleration of chain transfer reaction as a result 'Ge' decreased gradually (Prasad *et al*., 2006b). The possible mechanism of formation of the graft copolymer is presumably similar to that described by Prasad *et al*., (2006b) via radical ion polymerization.



Figures 5 Effect of ratio of PVP to Polysaccharide

Effect of reaction time

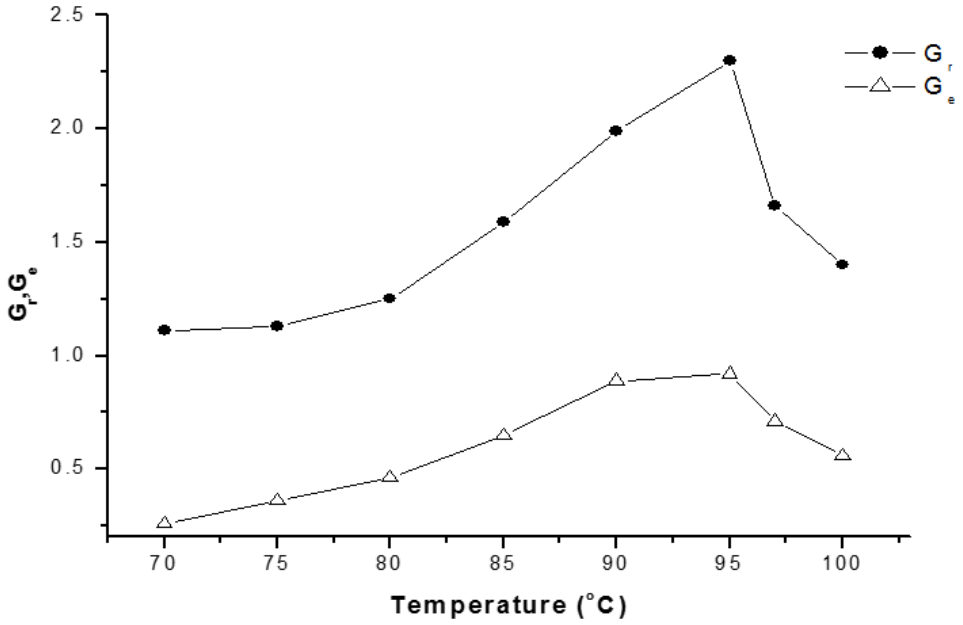
The influence of the reaction time or time of microwave irradiation on grafting parameters was optimized. As expected, up to 120 seconds of microwave irradiation the 'Ge' and 'Gr' increased. After 120 second and beyond, these values declined gradually (Figures 6). This phenomenon may be explained as follows. Microwave irradiation in presence of initiator produced and hydroxyl free radicals by vibrating water molecules. Simultaneously, it produced alkoxy radical on the polysaccharide backbone. The sulphate free radical was generated from potassium persulphate absorbed the hydrogen radical produced by vibrating water and polysaccharide molecules leading to the formation of sulphuric acid. The hydroxyl radical produced from water produced KOH by reacting with the potassium radical. At a smaller duration of microwave exposure there would be a balance in the formation of sulphuric acid and KOH leading to neutral pH of the reaction medium, which was really observed. As the duration of microwave exposure increased, the balance of sulphuric acid and KOH got tripped towards acidic pH, causing degradation of the product.



Figures 6 Effect of reaction time duration on grafting parameters

Effect of reaction temperature

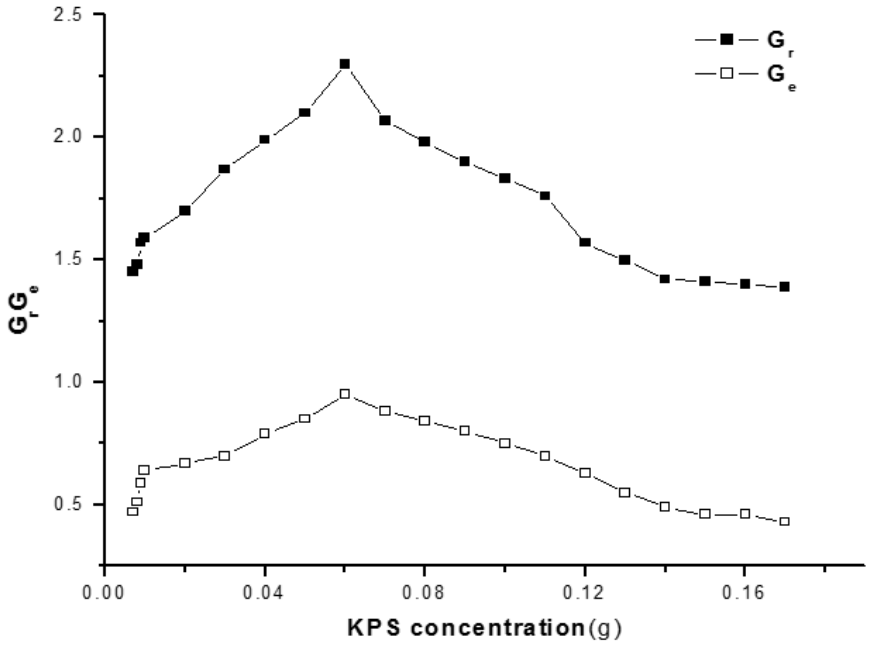
When the other reaction conditions were constant (microwave irradiation time duration 120 s, polysaccharide to PVP = 1:2), the grafting parameters at various temperatures were studied. In accordance with the general rule of radical polymerization, 'Ge' increased and then it leveled off (Figures 7). In this type of microwave induced reactions at 2450 MHz microwave frequency, the molecular rotation is affected without disturbing the molecular structure, increasing the reaction temperature and reaction rate. With further increase in temperature, excessive molecular vibration leads to the destruction of the polymer chain and as a result products with lower 'Ge' and 'Gr' values were obtained. In this study, no product was obtained in the reactions that were carried out at temperatures less than 70^{o}C. The reaction temperature was optimized to be 95^{o}C.



Figures IV.2.7 Effect of reaction temperature on grafting parameters

Effect of initiator concentration on grafting parameters

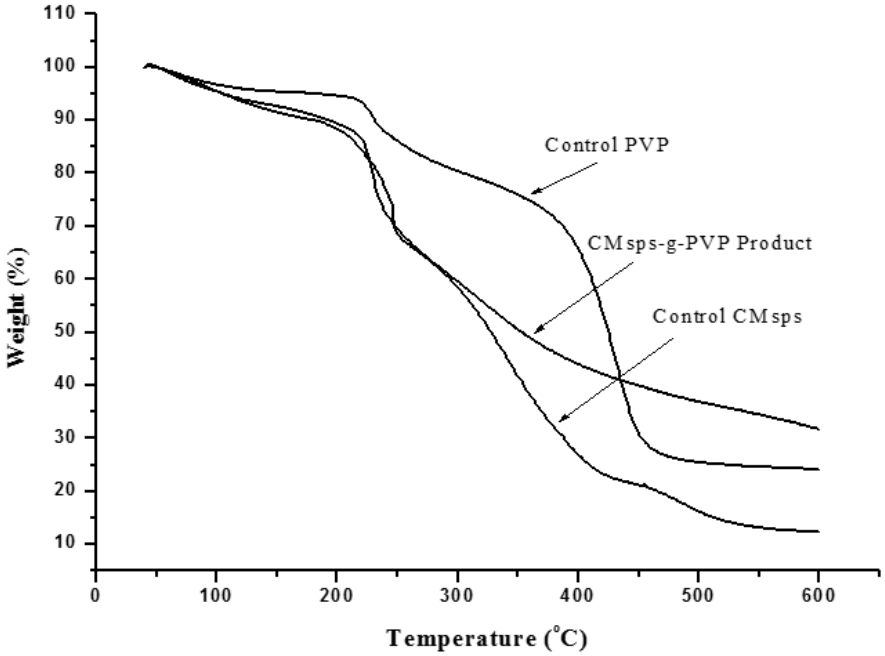
Potassium persulphate (KPS) was added in the range 0.007 to 0.15 g [2.5 $x10^{-4}$ to 5.5 $x10^{-3}$ mol/L] in the reaction mixture (100 ml). With low initiator concentrations, the 'Ct', 'Ge' and 'Gr' values were also low (Figures 8). This may be because of small amount of initiator is not sufficient to fully cleave the bond between O and H in the polysaccharide under microwave irradiation conditions. The values of grafting parameters gradually increased up to 0.06 g of initiator concentration [2.2 X 10^{-3} mol/L], beyond this grafting parameters declined. The amount of potassium persulphate was optimized to be 0.01g [3.7 x 10^{-4} mol/L] for 1 g of polysaccharide for the formation of the copolymer hydrogel. It was observed that at higher KPS concentration (i.e., 0.06 g), the grafted product obtained did not form hydrogel in water. The reaction did not take place in absence of (KPS).



Figures IV.2.8 Effect of KPS (Initiator) on grafting parameter

Thermal analysis

Thermogravimetric analysis (TGA) of polysaccharide and the grafted copolymer is shown in Figures 9. In the case of grafted product mass loss took place in three stages, ca.13% between 47-217^{o}C, 33% between 220-260^{o}C and finally ca. 70% mass loss observed up to 600^{o}C. The initial mass loss (ca.10%) was presumably due to the loss of bound water (moisture) to the polysaccharide. Non-modified CM_{sps} also showed mass loss in three stages, the first one (13%) occurring in the range 44-207^{o}C probably due to the loss of moisture. The subsequent mass losses were 33% and 88%, and occurred in the temperature ranges of 210-251^{o}C and 251-550^{o}C, respectively. In PVP, the mass loss took place in two stages: up to 105 and 600^{o}C corresponding to 12% and 97% mass losses, respectively. The TGA profile suggested that grafting of PVP on polysaccharide influenced the thermal behavior of the polysaccharide; it may be the result of the rearrangement in the post crosslinking molecular architecture of the polysaccharide systems.

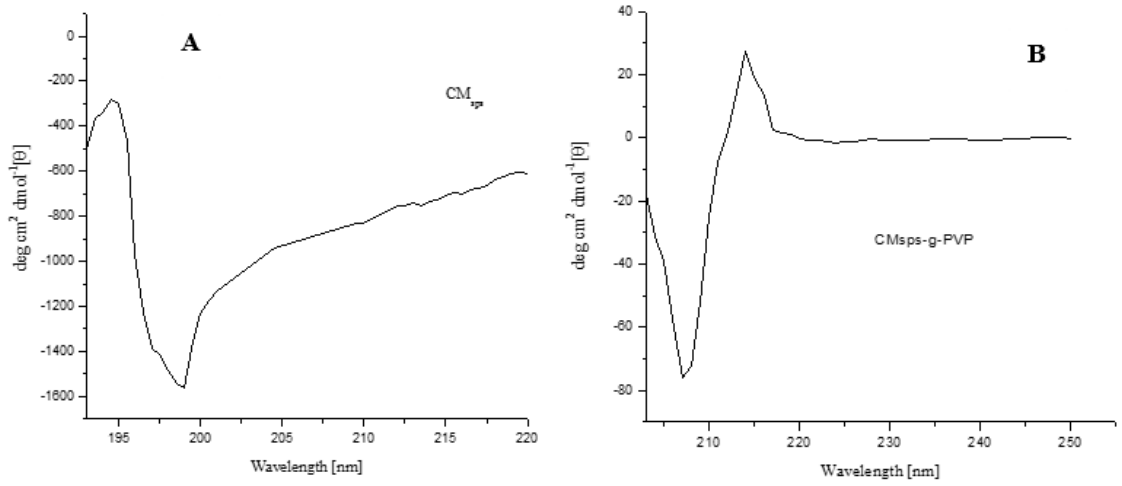


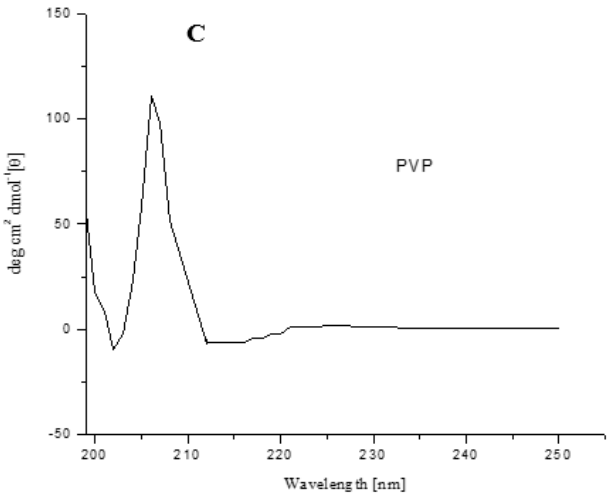
Figures IV.2.9 TGA profile for PVP, Control CM_{sps} and CM_{sps}-*graft*-PVP Product

Optical rotation and circular dichroism

The specific optical rotation values $[\propto]^{25}_{589}$ (c 0.20, H_2O) of the parent polysaccharide (CM_{sps}) and PVP were [+177.8°] and [+14.55°] respectively, while those of the grafted product (CM_{sps}-*graft*-PVP) was [+56.51°] under the same condition. The lowering of optical rotation values in grafted products suggested the enhancement of symmetry elements in these grafted polysaccharides which indicate the changes in the molecular symmetry profiles as a result of PVP functionalization (Table 1).

The CD spectra of non-modified CM_{sps}, were entirely in the negative region (peak 194.5 nm, [θ] -282.46 and trough 199 nm, [θ] -1562.66) (Figure 10A). However, CM_{sps}-*graft*-PVP showed a mixed pattern having positive and negative bands (peak 213.9 nm, [θ] +27.07 and trough 206.9 nm, [θ] -75.5 (Figure 10B). The CD spectrum of PVP showed positive and negative bands, peak at 205.9 nm, [θ] +110.2, and a trough at (c.a.202 nm, [θ] -9.25 (Figure 10C).





Figures IV.2.10 CD spectrum of (A) CM_{sps}, (B) CM_{sps}-g-PVP and (C) PVP

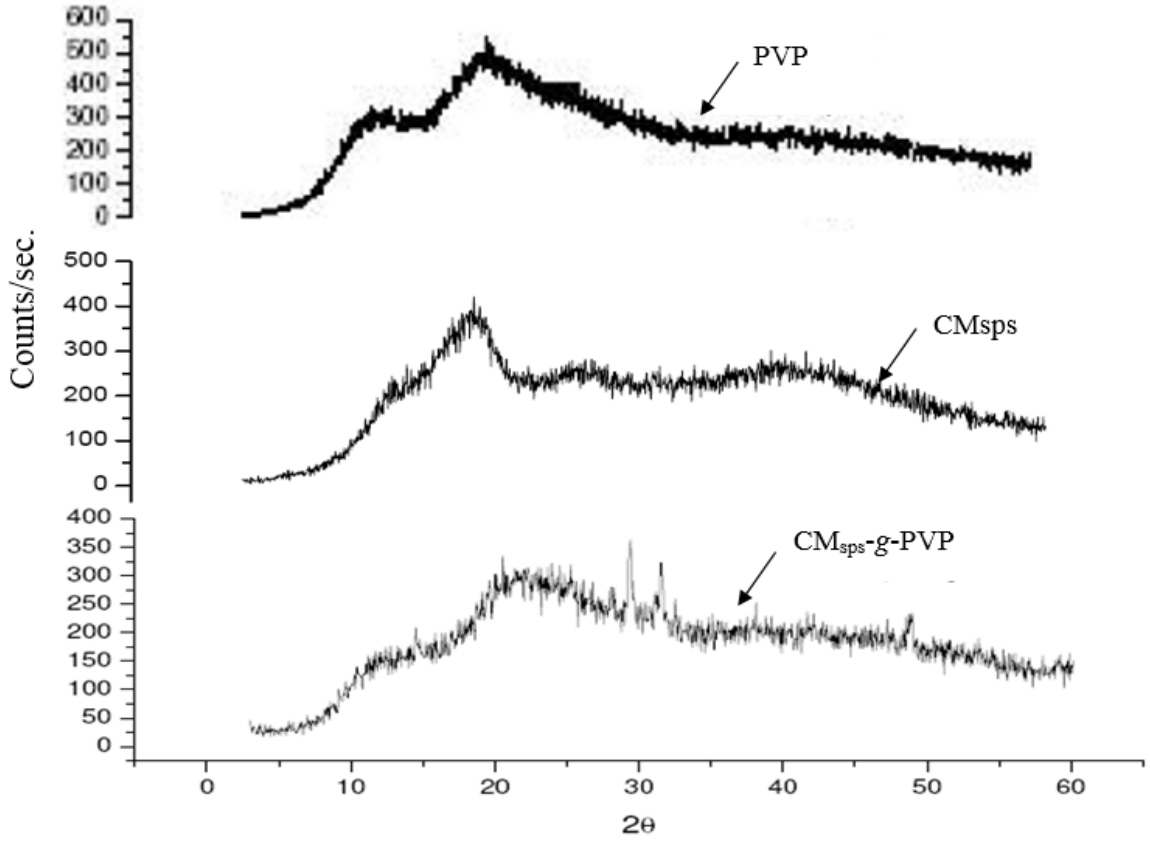
If the ratio of peak height to trough depth in CD spectrum were < 1, overall composition of any compound will show negative CD spectrum (i.e., peak/trough < 1). When the spectrum crosses the baseline, then the overall composition shows entirely positive spectrum (i.e., peak/trough >1) (Morris *et al.,* 1975; Morris *et al.,* 1980; Dentini *et al*., 2006). The use of the peak-to-trough analysis has been gainfully used to describe conformational changes that occur within polymers or modified polymers with varied chain lengths (McReynolds *et al.,* 2000). 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 CM_{sps} (0.81) i.e. Peak/trough < 1, while for PVP (8.0) and CMsps-*graft*-PVP (1.34), i.e. peak/trough > 1. The CD spectrum and the peak-to-trough analysis (Table IV.2.1) indicated a reversal of chiroptical profiles that took place after insertion of PVP into the parent polysaccharides leading to alteration of symmetry elements. The overall chiral configurations of the parent polysaccharides have been deduced to be L for CM_{sps} by virtue of peak/trough ratios in the CD spectra (Table IV.2.1).

Bulk density, true density pose volume and porosity

The results of bulk, true density and pore volume and porosity measurements for CMsps-*graft*-PVP and CM_{sps} are given in Table 1. The CMsps-*graft*-PVP showed higher bulk density and lower true density, pore volume and porosity than those of the non-modified polysaccharides (Table 1). Decrease in the porosity indicated small porous structure of the copolymer leading to the nano-porous material and hence this may be useful in applications wherein merits of nano-porous structure are in demand e.g. in catalysis and affinity.

X-ray diffraction analysis

The X-ray diffraction patterns of the parent polysaccharide (CM_{sps}), PVP and its copolymer were measured. The X-ray diffraction pattern of the parent polysaccharide and PVP showed that there was no sharp or narrow peak indicating its amorphous nature, but after grafting with PVP the copolymer exhibited two distinct sharp peaks (at $2\theta = 29.31^{o}$, 32.45^{o}) (Figures 11). Enhanced crystallinity suggested ordered molecular arrangement, in comparison to the parent polysaccharide, associated with a substantial change in the quantum of optical rotation values, e.g. from $+177^{o}.809$ (in CM_{sps}) to $-56^{o}.5$ (CM_{sps}-*graft*-PVP), indicating chiral modification, as one would expect. Similar observations i.e. enhanced crystallinity coupled with changes in the optical rotation values in the grafted product, were reported by Prasad *et al*., (2006b) for agar-and carrageenan-*graft*-PVP blends. The crystallinity index (C.I.) of the grafted product was determined using the equation described by Herman & Weidinger, (1948).



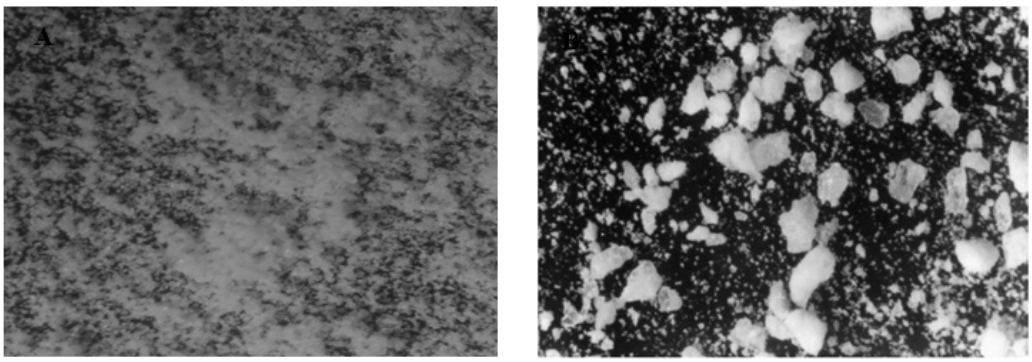
Figures 11. X-Ray diffraction pattern of Control C_{sps}, PVP and C_{sps}-*graft*-PVP product

C.I : Area of crystalline Peak / [Area of crystalline peak+ Area of amorphous peak].

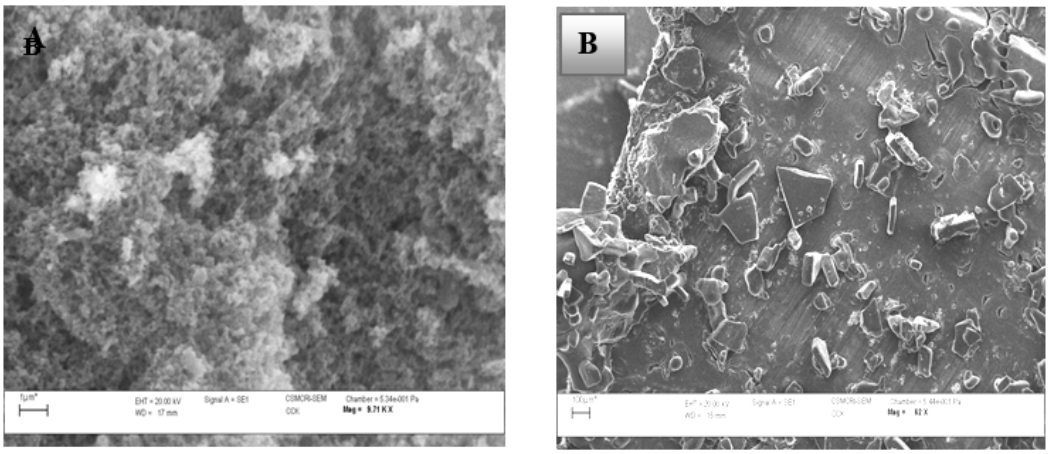
The C.I. value calculated for the grafted product was 0.25 while the parent polysaccharide was amorphous.

Optical microscopy

The optical microscopy of CM_{sps}-*graft*-PVP was taken with 70 X magnifications and compared with parent CM_{sps} (Figure 12). These images reveled that the morphology of the CM_{sps} got modified significantly in the copolymers. The optical micrograph of the parent polysaccharide (CM_{sps}) appeared fibrous in nature, while those of grafted copolymer (CM_{sps}-*graft*-PVP) appeared to have definite shapes to akin to rectangular and/or spheroid geometries.



Figures IV.2.12 Optical micrographs (A) CM_{sps} and (B) CM_{sps}-*graft*-PVP



Figures IV.2.13 Scanning electron micrographs of (A) CM_{sps} and (B) CM_{sps}-*graft*-PVP

Scanning electron microscopy (SEM) analysis

The scanning electron micrographs (SEM) images of the parent polysaccharide (CM_{sps}) and CM_{sps}-*graft*-PVP were distinctly different and indicated significant changes on the surface morphology of CM_{sps} after grafting with PVP and indicated that grafting had indeed taken place. The parent polysaccharides appeared to have compact morphology having cloud-like clusters while those of grafted copolymers appeared to have definite shapes similar to rectangular and/or triangular geometries (Figures 13) as revealed in the XRD profiles and can be distinguished easily from the parent polysaccharides. This fact points to a substantial build up of PVP on the grafted product (Figures 13B), substantiating difference in the structure of the parent polysaccharide (Figures 13A). The SEM images were supportive evidence for grafting which indicated integrated molecular construct of crosslinked systems.

Average pore diameter and surface area (BET)

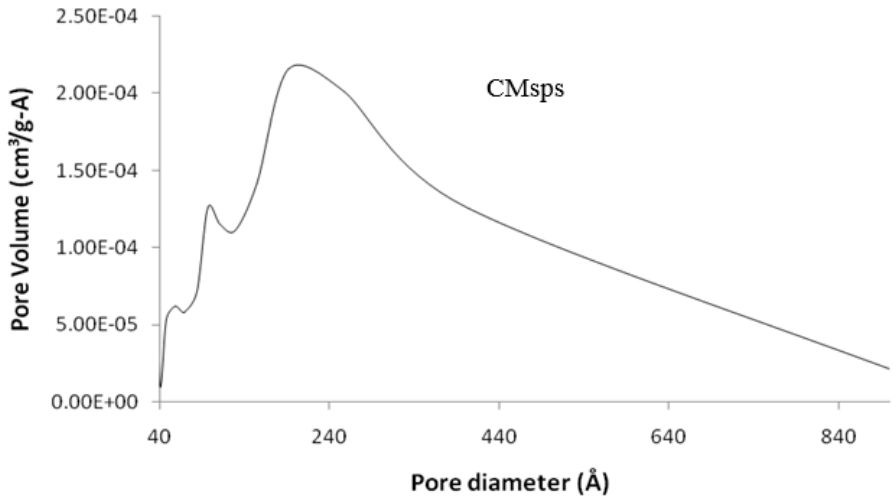
The pore size distribution of CM_{sps} and CM_{sps}-*graft*-PVP were determined from the desorption branch of the isotherm as shown in Figures 14 and Figures 15. The values of average BET surface area and pore diameter (BET) of CM_{sps} and those of the grafted products were depicted in Table 5. Two types of mesopores were observed in grafted product and the hysteresis shown by grafted product was slightly different from that of the parent polysaccharide indicated that grafting had indeed taken place.

Table 5 Surface area and pore diameter measured by N_2 adsorption-desorption isotherms using BET and BJH models.

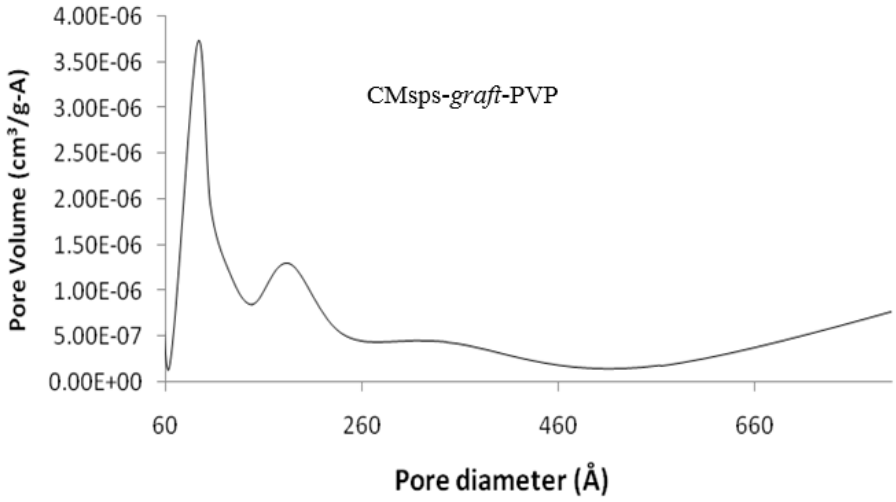
Sample	Surface area (m^2/g)			Pore diameter (nm)		
	BET	BJH adsorption	BJH desorption	BET	BJH adsorption	BJH desorption
CM_{sps}	8.47	3.41	6.41	9.23	64.15	35.01
CM_{sps}-PVP hybrid material	1.97	1.51	0.79	2.39	10.49	18.06

Proposed mechanism of the formation of graft copolymer

The above results indicate the PVP was grafted on polysaccharides. Grafting of PVP onto the backbone of CM_{sps} was carried out in an aqueous medium using KPS as a free-radical initiator and PVP as a crosslinking agent. The proposed mechanism by which this crosslinking process occurred is illustrated in Scheme 1 for the formation of CM_{sps}-*graft*-PVP blend. The sulphate anion radical that would be produced as a result of thermal decomposition of KPS in the presence of microwave irradiation would abstract hydrogen from the hydroxyl group of CM_{sps} to form the corresponding alkoxy radical on the C-6 of galactopyranose moiety of the polysaccharide (CM_{sps}); free radical of PVP was linked with alkoxy radical of the CM_{sps} under MW condition. The consecutive steps of these linking result in the formation of polymer. The sulphate free radical was generated from potassium persulphate absorbed the hydrogen radical produced by vibrating water and polysaccharide molecules leading to the formation of sulphuric acid. The hydroxyl radical that was generated from water produced KOH by reacting with the potassium radical. At a smaller duration of microwave exposure there would be a balance in the formation of sulphuric acid and KOH leading to neutral pH of the reaction medium, which was actually observed.

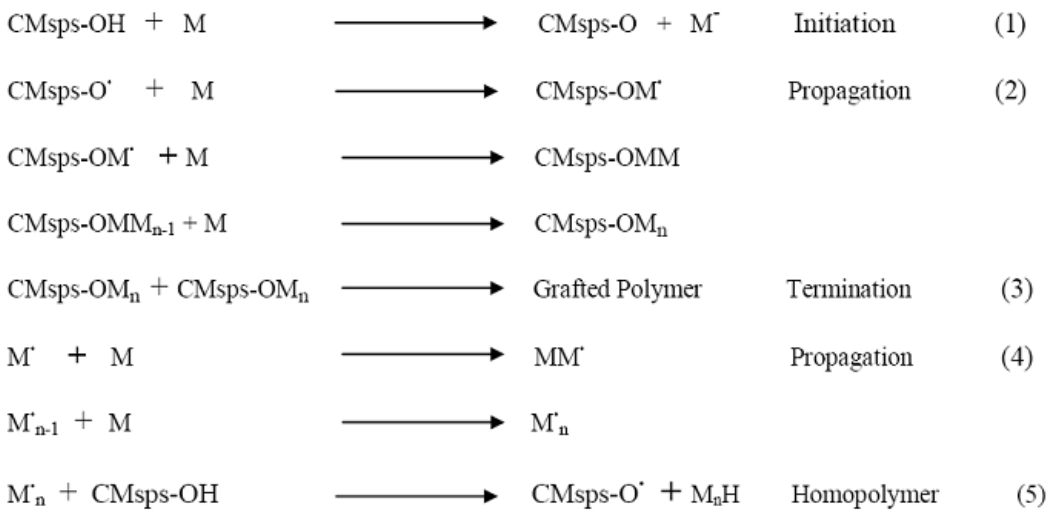


Figures IV.2.14 Pore size distribution plot of the CM_{sps}



Figures IV.2.15 Pore size distribution plot of the CM_{sps}-graft-PVP

Scheme 1. Represents a proposed mechanism of formation of the graft copolymer under microwave irradiation



Where CM_{sps}-OH stands for polysaccharide from *Chaetomorpha antennina*, M stands for Poly-vinylpyrrolidone, MW for microwave irradiation and KPS is potassium persulphate.

CONCLUSION

A rapid water-based synthesis of a copolymer hydrogel of the sulphated polysaccharide of the green seaweed *Chaetomorpha antennina* and poly-vinylpyrrolidone has been achieved using microwave conditions in presence of a free radical initiator, KPS. The optimum grafting percent was obtained with polysaccharide-PVP ratio 1:2 and initiator (KPS), 2.2 X 10^{-3} mol/L, in the reaction mixture. FT-IR, CP-MAS ^{13}C-NMR, scanning electron microscopy, circular dichroism, TGA and viscosity measurements substantiated that grafting indeed had taken place. The proposed structure of the repeating units of the copolymer CM_{sps}-*graft*-PVP is given in Figures IV.2.3 (^{13}C NMR and GC-MS). The carbohydrate units (galactopyranose and arabinopyranose) present in the parent polysaccharide (CM_{sps}) were first identified by GC-MS analysis of their alditol acetates (Siddhanta *et al*., 2001). This PVP grafted copolymer (CM_{sps}-*graft*-PVP) was biodegradable; it exhibited enhanced crystallinity, porosity and was partially hydrophobic, compared to the parent polysaccharide (CM_{sps}), forming weak gel in water. The copolymer may be potentially useful in catalytic and affinity chromatographic applications.

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