ORIGINAL ARTICLE

Structural characterization and pharmaceutical properties of porphyran

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Marine polysaccharides remain an untapped reservoir for development of novel biomaterials. Algae derived sulfated polysaccharides (SPs) have their interesting pharmaceutical and biological properties. Degree and pattern of sulfation of such biopolymers favors their binding property with tissues when compared with non-SPs. Due to the gel formation potential, hydrocolloids such as agar, carrageenan, fucoidan, and alginate are extensively studied food and nonfood applications. Degree of sulfation and favorable physical properties are essentially required for tissue engineering applications. Therefore, our investigation explores the structural and gelling properties of novel polysaccharide porphyran (POR) isolated from *Porphyra vietnamensis* by alkali hydrolysis. Percentage yield of POR was found to be 19.7%. The sulfate content of the polysaccharide was 11.1% and the main sugars present were D-galactose (16.1%), 3, 6-anhydro galactose (3, 6-AG) (10.1%) and 6-O-methyl D-galactose (7.81%). After hydrolysis D-galactose was again confirmed by paper chromatography (Rf: 0.8) and phenol-sulfuric acid method. Gelling properties, including gelling strength (241 g/cm^2), gelling temperature (35.8°C), melting temperature (70.7 $\pm$ 0.4) and apparent viscosity (56.2 η) were also explored. Differential scanning calorimeter analysis showed purified fraction has gel melt between 70°C and 80°C and show glass transition between 35°C and 38°C. Viscometric analysis was examined to analyze the different behavior of SPs fraction under the influence of cationic and anionic salts and polysaccharides. Molecular mass of POR was determined (16,280). SPs were characterized by Fourier transform infrared and nuclear magnetic resonance spectroscopy, which showed the presence of linear backbone structure called as POR. The rheological behavior of POR exhibits a gel-like behavior close to the one observed in commercial agar.

Key words: *Gelling, Porphyra, porphyran, red alga, sulfated polysaccharides*

INTRODUCTION

Biomedical field is constantly looking for new bio-macromolecules, with novel applications. Due to their excellent biocompatibility and biodegradability natural polymers appear as materials of election for this goal. In this concern, polysaccharides obtained from the marine environment are of great interest since the chemical and biological diversity found in this environment is almost uncountable and continuously growing with the research in deeper waters. Moreover, there is also a slower risk of these materials to pose illnesses to humans. Biopolymers have a great demand in remodeling of the injured tissue related health problems such as trauma or tissue related diseases. To overcome the drawbacks of the current clinical practices such as prosthesis, autografts, etc., biopolymers are the best regenerative medicine that can use in tissue engineering. Several natural and synthetic biopolymers or conjugate of both have been proposed as porous structures called as scaffolds.[1,2]

Sulfated polysaccharides (SPs) are the anionic polymers that are present widely in nature with a wide range of significant biological and pharmaceutical properties. Degree and pattern of sulfation of such biopolymers favors their binding property with tissues when compared with non-SPs. Nonanimal SPs are chiefly obtained from marine algae. SPs from macroalgae, mainly carrageenans (red algae), ulvan (green algae) and fucoidan and alginate (brown algae) are known to be having the various potential biomedical applications. Pharmaceutical and biological properties

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of these algal polysaccharides are much dependent on their structure which is usually varies according to the species of algae.[3,4] Algal SPs posses certain important pharmacological properties such as anti-inflammatory, antioxidant, antipeptic, immunomodulatory, anticoagulant, antiproliferative, antiviral, antitumoral, anticomplementary, and antiadhesive.[5-7] Exploration of structure dependent biological properties is rare. However, some structural moieties or groups are essential for biological activities, e.g. sulfate clusters in red galactans ensures interactions with cationic proteins.[8] Furthermore, several reports related molecular weight (MW) dependent gelling and biological properties are recently reported.[9,10]

Porphyran (POR), anionic polysaccharide obtained from the hot water (alkali, acid, enzyme, radical hydrolyzed) soluble portion of cell wall of *Porphyra*.[11] Gelling properties of all the red galactans is dependent on the concentration of 3, 6AG which is a fine honeycomb-like network participates as a "hidden" precursor structure in galactan solutions at significantly high temperature. Polymeric formulations that exhibit sol-to-gel transition at physiological conditions are good candidates for tissue engineering applications. One of the main limitations with using SPs to engineer tissues *in vitro* is their overlooked physical properties. Therefore, the evaluation of physical properties like viscosity and gelation essentially required for the development of suitable scaffold or injectable hydrocolloid system. Various biological properties of *Porphyra* sp. were explored in our previous studies however structure, and gelling properties of POR are not explored yet.[11-15] Hence here in this work, we have successfully explored the structural as well as gelling properties of POR isolated from *Porphyra vietnamensis*.

EXPERIMENTAL

Isolation of sulfated polysaccharides from *Porphyra* sp.

Porphyra vietnamensis was collected from different locations of Ratnagiri, Maharashtra and authentified. POR was prepared according to protocol adopted in our previous work.[13]

Chemical analysis

Total sugar, galactose, 3, 6-AG, content of each fraction was determined according to the method of Dubois *et al*.[16] Lipid content was determined by Bligh and Dyer, 1959.[17] Protein content was measured by Bradford's method.[18] Sulfate content in polysaccharides was determined by the barium chloride gelatin method.[19] After hydrolysis D-galactose content in hydrolyzed polysaccharide fraction (0.5 M sulfuric acid) was confirmed by paper chromatography, which was performed by the descending technique on Whatman No. 1 paper; using 10:3:3 (v/v) l-butanol: Pyridine: Water, 9:2:2 (v/v) ethyl acetate-acetic acid-water, 18:3:1:4 (v/v) ethyl acetate-acetic acid-formic acid-water; spots of methylated sugars were made visible by spraying with 1-anisidine hydrochloride in aqueous 1-butanol, and heating for 5 min. Sugars were detected with p-anisidine hydrochloride or silver nitrate sodium hydroxide sprays.[20,21] Concentration of D-galactose was confirmed by phenol-sulfuric acid method. Method was adopted for the estimation of dissolved carbohydrates especially D-galactose in the *Porphyra* sp. This method depends on color development and the amount of phenol added. The intensity of the developed color varies with the amount of phenol added for D-galactose. In phenol-sulfuric acid method absorption maxima for D-galactose occur at a lower concentration of phenol decreases at higher phenol concentration.

Determination of gelling strength, gelling temperature, melting temperature (MT) MW determination.

Five percentage of POR solution (100 ml) was prepared in an autoclave at 100°C. Gel formation took place in the dark place at 25°C after which the gel was kept at 10°C overnight in the refrigerator.[22] Strength of the gel was measured at 20°C using a model TA-XT2 Texture analyzer (Stable Micro System, Surrey, UK). The gelling and MTs were measured according the method described by Craigie and Leigh.[23] MW of POR was determined by Rochas and Lahaye method.[24]

Viscometric studies of sulfated polysaccharides fraction at different temperature

For the determination of viscometric behavior of POR under the influence of cationic and anionic compounds (salts and polysaccharides), 10% of purified POR sample was prepared in water. Different salts and polysaccharides were added in the different ratios to the prepared 10% POR solution and coded from P_1 to P_6 [Table 1]. After sample preparation each sample were analyzed at different temperature (10°C–60°C). Apparent viscosity of POR was measured by the brook field

Table 1: Description of samples and their methods of preparation

Code number	Method of preparation
P_1	Effect of hydrolysis with sodium borohydride 3M sodium hydroxide (1 ml) was added to the sample (10%), previously dissolved in water (2 ml) and reduced with sodium borohydride (10% w/w), reaching the final concentration of M sodium hydroxide. This solution was then heated at 80°C and neutralized with 1M hydrochloric acid
P_2	Effect of cationic salt addition To 10% of sample add 0.5 g of $CaCl_2$ and make up the volume up to 10 ml with distilled water
P_3	Effect of addition of NaCl To 10% of sample add 0.5 g of NaCl and make the volume upto 10 ml with distilled water
P_4	Effect of addition of cationic polysaccharide 1% of chitosan is added to 10% of sample
P_5	Effect of addition of anionic polysaccharide 1% of gum acacia is added to 10% of sample
P_6	Pure sample purified in methanol and acetone

viscometer (Synchrolectric Viscometer, Stoughton, MASS 02072, USA). Spindle No. 1 at 60 RPM was used for measuring apparent viscosities of agar samples (5% in deionized water) at 60°C. Some parameters are adjusted such as RPM is at 50, shear rate at 666 and time interval is adjusted at 10.2 s.

Nuclear magnetic resonance and Fourier transform infrared spectroscopic analysis

The organic functional groups of the polysaccharides preparations were identified by using a Fourier transform infrared (FTIR) spectrophotometer (FTIR-8400S, Shimadzu Co., Japan) via the KBr 141 pressed-disc method. ^{1}H-nuclear magnetic resonance (NMR) and ^{13}C-NMR studies were performed in the Department of Chemistry, University of Pune, Pune. For this study, 500 mg of lyophilized sample was dissolved in $CDCl_3$ for ^{13}C-NMR and for ^{1}H-NMR D_2O was used as a solvent. In$^{[13]}$ C-NMR, the sample was also studied for hydrolysis after treatment with NaBr. Spectroscopic analysis was performed according to our previous study.

Differential scanning calorimeter analysis

In this experiment, nitrogen gas was used as a reference material. A heat flux differential scanning calorimeter (DSC) was used by setting the machine to heat both the sample and reference material at a specific rate (10°C/min). For DSC polysaccharide fraction obtained from the *Porphyra* was lyophilized and the lyophilized sample (100 mg) was treated with NaOH (3 M) and $NaBH_4$ (0.6 M) at 80°C for 24 h and then again lyophilized.

Preparation of gel

Twenty % w/v of purified NaBr hydrolyzed fraction of POR treated with one drop of triethanolamine. After that make up the volume with 10 ml of water and homogenized on the magnetic stirrer at 60°C for 12 h. Each dispersion was refrigerated until a clear solution was formed (5 h).

Rheological characterization

Rheological parameters were determined after its uniform mixing. From different concentrations of 20% w/v of POR only was selected for further rheological studies Rheological characterizations of all samples were conducted using a controlled stress rheometer: Viscotech rheometer (rheologica instruments AB, Lund, Sweden) using cone-plate geometry with angle of the cone being 0.8 mm and operating in the oscillation mode. The gap was maintained at 0.05 mm. all the Rheological measurements were performed at 37°C ± 0.5°C. The Rheological characterizations of the gel were obtained by performing the following set of rheological tests [Table 2].

RESULTS AND DISCUSSION

Extraction and chemical analyses

Through compositional analysis it was confirmed that *P. veitnamensis* contains significant amount of proteins (18.82 ± 3.53), lipids (1.25 ± 0.05), Vitamin C (7.87 mg/100 g) whereas moisture (15.43 ± 0.17) and ash values (6.1 ± 0.2) are almost similar as reported earlier. *Porphyra* sp. contains plenty amount of polysaccharides of which structural characterization and pharmaceutical properties evaluation should be essential. Whole aqueous fraction (excluding crude fibers) contains dietary fiber 73.56% and POR 19.7%±1.6%. It means that Indian algae constitute plenty amount of polysaccharide in comparison to the polysaccharide isolated from *Porphyra* Columbia and many other species. POR contains significant amount sulfur, sugar, 3, 6-AG as mentioned in Table 3. These features favor the gelling property of POR. Different gelling properties and its molecular mass are mentioned in Table 3.

To improve the pharmaceutical uses and for the better estimation of its polysaccharide fraction it's very essential to hydrolyze with $NaBH_4$. Moreover, while determining pharmacological uses of this polysaccharide fraction, it is suggested to not to hydrolyze with any chemical. In fact, it was taken as whole crude sample. 3, 6-AG and methyl galactose are essential for the gelling property of POR. Therefore, after hydrolysis galactose estimation and detection was again confirmed by phenol-sulfuric acid method and paper chromatography. After treatment of POR with 0.5M sulfuric acid paper chromatogram produces two band of RF value 0.8 and 0.7 which matches with the theoretical RF values of D-galactose and methyl galactose. Estimation of D-galactose was confirmed by phenol-sulfuric acid method. Color responses given by standard galactose (0.51/40 micrograms) and sample are similar.

H^{1}-nuclear magnetic resonance and Fourier transform infrared spectroscopic analysis

Structural characterization of polysaccharide fraction obtained from *Porphyra* sp. was already performed in previous study through H^{1}, C^{13} NMR and FTIR spectroscopic analysis. Since this *Porphyra* sample was collected from new location then sample used in previous study, therefore it is important to confirm the level of degradation of POR (which may not affect the important functional elements and groups) collected from new location.

Two strong signal at 3.30 and 4.8 ppm were observed which may be due attributed to the methyl group of 6-O-methyl-D-galactose and 3,6-anhydro--l-galactose units [Figures 1 and 2]. These spectral findings are slightly different from the previous report. This difference may be due to the polysaccharide degradation due to change in environmental conditions of algae.

Fourier transform infrared spectral analysis demonstrated the absorption spectrum of POR with functional peaks 3383, 1635, 1200, 1063, 930 and 767 cm^{-1} [Figure 3]. Alkali hydrolyzed sample showed very little shift in the peaks, which proved this type of hydrolysis does not interfere with the arrangement of functional groups in POR sample [Table 4].

Table 2: Rheological conditions

Test	Purpose	Test condition
Oscillation stress sweep	It is performed to determine the LVR and the viscoelastic properties of the POR gel. The LVR gives information about the critical stress beyond which the sample may show significant structural changes. In the oscillation stress sweep test, the gel samples were exposed to a growing applied stress at constant frequency. Usually while performing the test in this range, the stress is applied until the structure of the sample under study breaks down. The three main parameters determined in this test were the storage modulus G', loss modulus G" and loss tangent tan δ. The end point of the LVR was determined as a stress, when the G' value was dropped 10% from the linear level that indicated a significant change in the structure of gel samples being studied	5-500 Pa; at constant frequency of 1 Hz
Oscillation frequency sweep	Dynamic oscillation frequency sweep test was used to determine the capability of the gel samples to resist structural changes under the increased frequency	0.1-100 Hz; at a selected averaged stress of the stress sweep mode that falls well within the LVR of each sample
Creep recovery	The creep recovery test was used to determine the viscoelastic properties of the gel samples. The creep compliance Jc (defined as the ratio of measured strain to the applied stress) is monitored against time. Percentage recovery of the gel was calculated in this test	The samples were exposed to the selected averaged stress of the stress sweep mode within LVR. for 100 s. and allowed to relax for 200 s

LVR: Linear viscoelastic region, POR: Porphyran

Table 3: Compositional analysis of *Panax vietnamensis*

Types of compound	Total percentage and concentration	GS[g] (g/cm^2)	GT(°C)	MT (°C)	Apparent viscosity at 80°C	MMS	pH
Protein	18.82±3.53	-	-	-	-	-	-
Lipid	1.25±0.05	-	-	-	-	-	-
Carbohydrate	Dietary fiber 73.56%	-	-	-	-	-	-
	% yield of POR 19.7±1.6						
	Sulfura 11.1±0.14	241±4.6	33.1±0.1	68.7±0.4	56.2±0.7	16.280±4.2	6.1±0.08
	D-galactose 16.1±2.7						
	Methyl galactose 7.81±1.1						
	3, 6 AG 10.4±1.1						
	Total sugar 60.36±0.9						
Vitamin C	7.87 mg/100 g	-	-	-	-	-	-
Ash	6.1±0.2	-	-	-	-	-	-
Moisture	15.43±0.17	-	-	-	-	-	-

[a]Data are mean value of triplicate determinations±SD. [y]Yields were determined on the basis of bone dry as received seaweed. GS: Gelling strength, GT: Gelling temperature, MT: Melting temperature, POR: Porphyran, AG: Anhydogalactose, MMS: Molecular mass

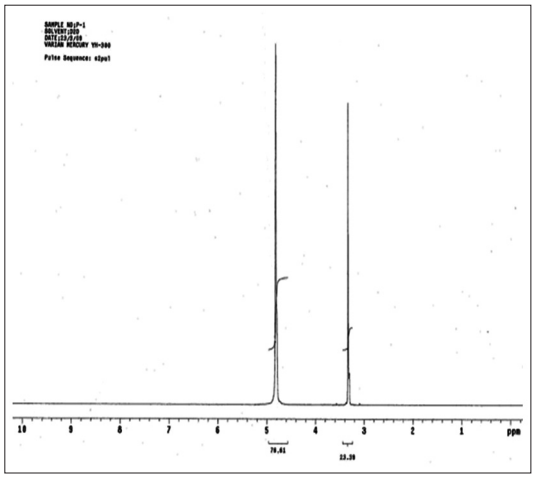


Figure 1: ^{1}H-nuclear magnetic resonance spectrum of polysaccharide fraction from *Porphyra*

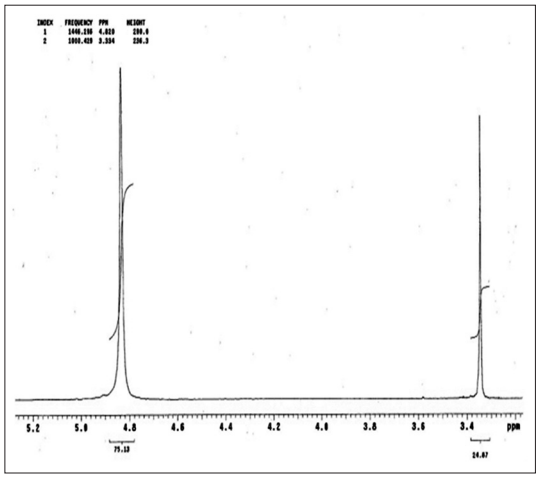


Figure 2: ^{1}H-nuclear magnetic resonance spectrum of polysaccharide fraction from *Porphyra*

Fourier transform infrared and ^{1}H-NMR analysis confirmed the presence of POR with little degradation in the present sample *P. vietnamensis*.

Differential scanning calorimeter analysis

Polysaccharide fraction was treated by alkali to increase the gelling ability. After alkaline treatment, the polysaccharides have the properties like agarose. It has somewhat same glass transition and melting as like agarose. The gelation occurs from aggregation of double helices. The gelation temperature is related to the methoxyl and sulfate contents, which can prevent gelation. This purified fraction has gel melt between 80°C and 90°C and show glass transition between 35°C and 38°C and its neutral fraction gives the stronger gels [Figure 4]. It was reported that after alkaline treatment gel strength of polysaccharide can be increased by decreasing the sulfate content and by increasing the 3, 6-AG, but does not change significantly the O-methyl and pyruvic acid contents.

Viscometric studies

Viscosity synergism has been proposed as an *in vitro* parameter to measure the mucoadhesive properties of various polymers. The aim of this study was to investigate the interaction of various polymers and different salts with the polysaccharide fraction obtained from the *Porphyra* sp. The main components of the *Porphyra* is an anionic polysaccharide, that is, POR which is SPs. To study the effectiveness of this SPs fraction it was mixed with both types of cationic and anionic salts and cationic and anionic polysaccharides. The purpose behind this addition was to study the effect on the viscosity of this polysaccharide due to either formation of poly-electrolyte complexes or either due to increase in mucoadhesive joint. As it is generally accepted that chain interlocking, conformational changes and chemical interactions, which occur between a polymer and this SPs are likely to produce changes in the rheological behavior of the two macromolecular species. As in SPs sulfated group and several methyl group substitution gives a new range of viscosity. In this context, the viscosity of a molecular dispersion of a different polymers and this fraction may be considered as a reflection of the strength of the mucoadhesive joint, which could be either increased due to removal of sulfated group or any other structural changes. In this study, some interactions likewise treatment with sodium borohydride and calcium chloride would give a synergistic increase in viscosity, whereas addition of sodium chloride leads to decrease in viscosity in comparison with purified fraction of SPs [Figure 5]. And in case of polysaccharide treatment, addition of similar charged polysaccharide (gum acacia) would lead to decrease in viscosity, whereas increase in viscosity was observed when a putative mucoadhesive (chitosan) polymer and this fraction were mixed together [Tables 5 and 6].

Table 4: Summarize data of spectra of purified and alkali hydrolyzed POR

Sample	Wave number (cm^{-1} sample)					
	OH stretching	C=O (amide 1)	Sulfate group	C-C	C-O	3-6 AG
POR	3383	1635	1200	-	1031	930
POR NaBr	3383, 3131	-	1185	1320	-	930

POR: Porphyran, AG: Anhydogalactose

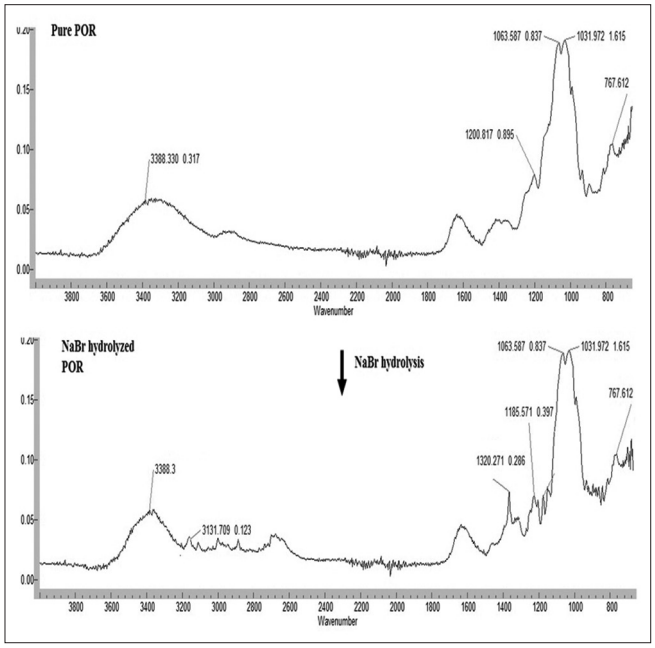


Figure 3: Fourier transform infrared spectral analysis of porphyran

Rheological characterization

As marine sources give wide range of polymers out of which some polymers are having excellent gelling property. In this context attempt was made to develop new gelling agent from *Porphyra* sp. after determining its essential rheological properties. Knowledge of the rheological properties is very important because the micro-structural environment or mobility and other gel properties can be indirectly probed using these measurements. The results of rheological tests

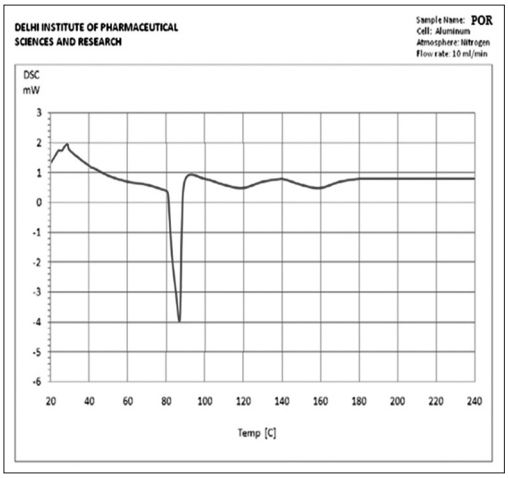


Figure 4: Differential scanning calorimeter of porphyran

can give information about their behavior during production, storage, and application (rheological manual). The rheological studies assist to explore the viscoelastic properties of gel system under study. Rheological studies can be performed by static (rotational) and dynamic (oscillatory) measurement mode. The dynamic rheology provides a more direct correlation with microstructure than steady rheology since the materials can be examined in their at-rest state without causing any disruption of their underlying structures. Effect of addition of the other excipients on the gel properties and its performance can be predicted from systematic rheological studies.

Oscillation stress sweep

Oscillation stress sweep is conducted to find out the linear viscoelastic region (LVR) for all tested samples under study at a constant frequency. In-phase diagram stress is related to amplitude and frequency is related to the phase length. In oscillation stress sweep a series of amplitudes are given and phase length remains same. An oscillation stress sweep test is a dynamic test where the complex modulus G* is measured as a function of stress at a constant frequency. The complex modulus (G*) is a measure of the total resistance of the system against the applied stress. Mathematically, G* is given as $G^* = G'' + I\,G''$. In this equation G′ is the in-phase (elastic modulus) component and G″, is the out-of-phase (viscous modulus) component (Rheology manual). The range of stress over which G″ is independent of the applied stress amplitude is called the LVR.

Stress sweep study for POR sample was carried at stress range of 5–500 Pa and at a constant frequency of 1 Hz [Figure 6]. A LVR range was obtained from which a stress of 75 Pa was selected and was continued for further tests.

Oscillation frequency sweep

To obtain information about viscous and the elastic behavior of an investigated system and the network structure formed by interactions, oscillation frequency sweep test has to be conducted. An oscillation frequency sweep test is a dynamic test measuring the response of a system as a function of frequency at constant stress amplitude (within LVR). Elastic modulus (G′) and viscous modulus (G″) were determined as a function of frequency. G′ is a measure of energy stored and recovered per cycle of deformation and reflects the solid like component of the viscoelastic material. The G′ will be large if a material is predominantly elastic or highly structured. The G″ is a measure of the energy lost per cycle and reflects the liquid-like component. The G″ will be large when the sample is predominantly viscous. If performed within the LVR a frequency sweep provides a fingerprint of a viscoelastic system under nondestructive conditions. Thus, the systems are examined in their rheological ground state without disrupting the structure. In oscillation frequency sweep we also monitor the change in-phase degree with applied

Table 5: Relative viscosity measured by brook field DV-2 + viscometer of POR at different temperature

Temperature	Spindle number	RPM	Percentage torque
10	4	50	17.4
20	4	50	19.0
30	4	50	11.0
40	4	50	12.2
50	4	50	10.2
60	4	50	7.8

POR: Porphyran, RPM: Revolution per minute

Table 6: Absolute viscosity by Capcalc V2.2 brook field of POR

Temperature	P_1	P_2	P_3	P_4	P_5	P_6
10	0.50589	0.5123	0.5969	1.1834	0.2783	0.0416
20	0.58731	0.6921	0.5941	1.0981	0.4747	0.2589
30	0.76210	1.2833	0.2922	0.2331	0.6681	0.3281
40	1.10433	1.3699	0.2788	0.3790	0.6874	0.4099
50	1.17645	1.5873	0.2161	0.5128	1.1231	0.5165
60	3.34135	1.6746	0.1543	0.9830	0.0831	0.6131

Data are mean value of triplicate determinations±SD. SD: Standard deviation, POR: Porphyran, P_1:Sodium borohydride, P_2: Cationic salt addition, P_3: Addition of NaCl, P_4: Cationic polysaccharide, P_5: Anionic polysaccharide, P_6: Pure POR

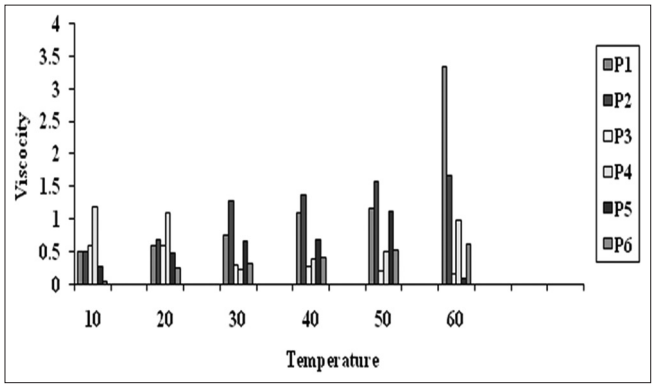


Figure 5: Viscosity analysis of different samples of porphyran by brook field viscometer at different temperature

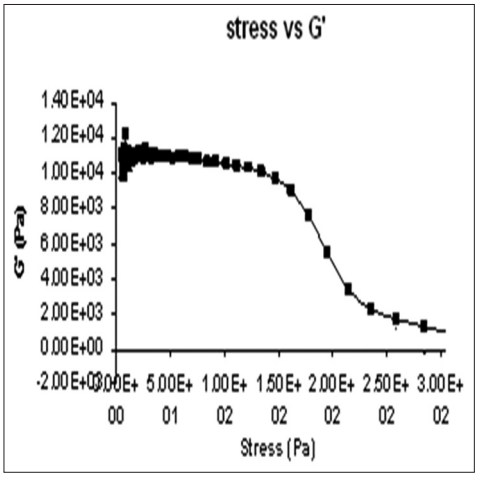


Figure 6: Effect of stress on G' (elasticity modulus) on porphyran gel

frequency. When phase degree is between 0°C and 45°C, then system is mainly elastic and phase degree between 45°C and 90°C indicates mainly viscous system. At 0°C and 90°C system is perfectly elastic and viscous respectively, while at 45°C both viscous and elastic components are in equal proportion. In oscillation frequency sweep stress is constant and frequency varies, it means that the amplitude is constant and phase length changes. In this test one stress value is selected within the LVR and frequency range is applied in other words, one amplitude is selected and a varying phase length is applied. As expected the gel followed an elastic behavior rather than viscous. The tan δ values for POR gel were all below 0.4 and G′ was more dominant than G″ thereby giving idea about elasticity of the gel nature. Similarly for extract incorporated gels except for 18% G′ was dominant than G″ representing elastic behavior and thus indicating stability. For 20% the G″ value was slightly less than that of G′ value indicating some instability. The tan δ value for all the POR gels were well below 0.8 indicating elastic nature [Figures 7 and 8].

Creep-recovery

In the creep test a constant stress within LVR is applied for a fixed time (100 s) and then removed (200 s). In this the strain of a sample is determined as a function of time. During the creep test the strain is measured as a function of applied stress and presented in terms of compliance, *J*. The compliance is calculated from the measured strain Ý, and the applied stress, σ, see Eq. (1).

$$J(t) = \acute{Y}(t)/\sigma. \quad (1)$$

Typical graph of creep-recovery test is given in Figure 9 the creep curve can usually be split up into three separate regions. The instantaneous elastic region representing rapid elastic response (A-B), the curved viscoelastic region representing retarded response (B-C), and the viscous flow (C-D). Stress is removed at point D and recovery is observed. The instantaneous elastic region recovers totally and the viscoelastic region partly while the viscous region does not recover. In the recovery curve, in Figure 9 the region D-E corresponds to the instantaneous elastic recovery and it is equivalent to the region A-B. The region E-F represents the elastic recovery and it is equivalent to B-C. The unrecoverable part of the curve corresponds to the viscous flow. The unrecoverable viscous flow represents the irreversibly broken internal bonds in the material. Second parameter is creep-recovery percent (δJ) which is calculated as follows.

The creep-recovery percent can be calculated with the Eq. (2).

$$\delta J = \left[\frac{J(100\,s) - J(300\,s)}{J(100\,s)} \right] \times 100 \quad (2)$$

The creep-recovery values for 20% POR gel were found to be 30 and Figure 10 gives idea about the recovery of gel after a cycle of stress and relaxation for 100 and 200 s respectively.

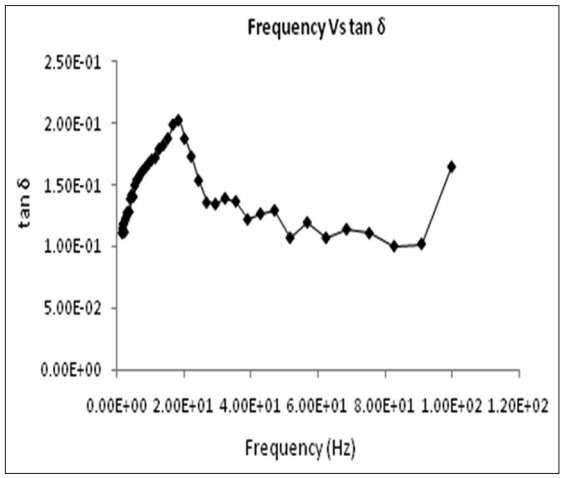


Figure 7: Tan δ value for all the porphyran gels

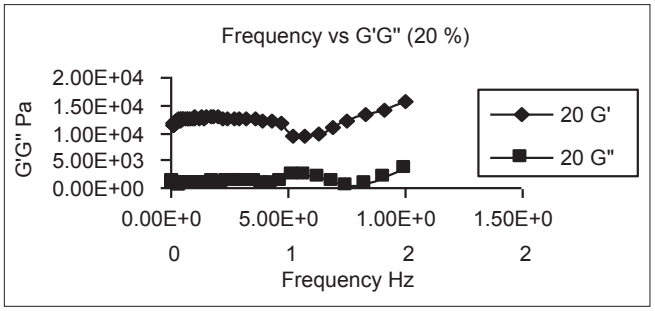


Figure 8: Effect of changing frequency on nature of porphyran gel

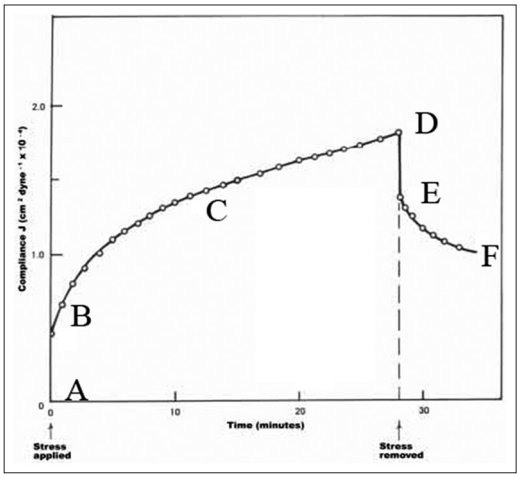


Figure 9: Standard plot for creep-recovery

As above study gives a brief idea that polysaccharide fraction which was purified and isolated from *Porphyra* sp. exhibits certain gelling properties which can be improved by studying more related aspects of its gelation.[25-28]

CONCLUSIONS

The SPs from marine nutritious red algae *P. vietnamensis* is composed of significant amount of galactose, and methyl-galactose. This polysaccharide also presents a high

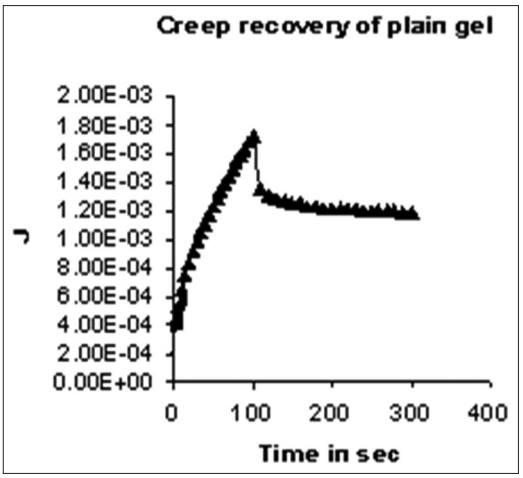
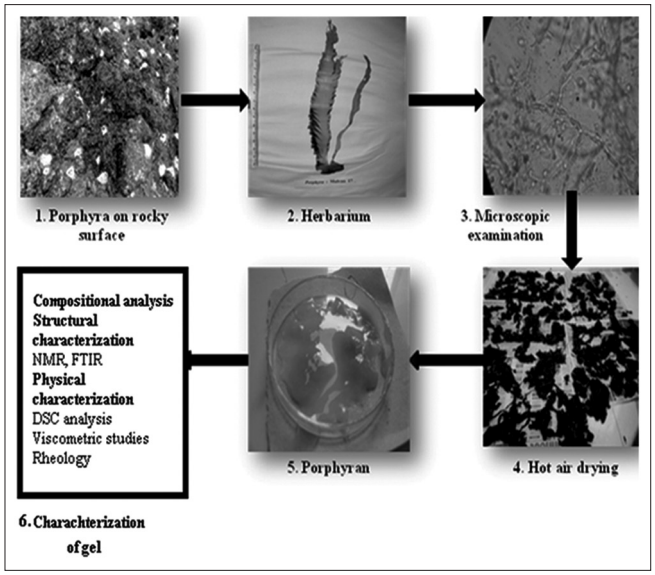


Figure 10: Creep-recovery of porphyran gel



Graphical abstract

content of 3, 6-AG and has a sulfate content. The SPs was characterized by ^{1}H-NMR and FTIR, findings of which proved that this SP is POR. Viscosity analysis suggested that NaBr hydrolysis would give a synergistic increase in viscosity, whereas addition of sodium chloride leads to decrease in viscosity. In addition conjugation of POR and similar charged polysaccharide (gum acacia) would lead to decrease in viscosity whereas increase in viscosity was observed with putative mucoadhesive polymer like chitosan. Rheological characterization explored that the further optimization of various parameters or poly-electrolyte complex development are essentially required for their utilization in drug delivery and biomedical field.

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