Review Article

Factors Effecting the Gelling and Emulsifying Properties of a Natural Polymer

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ABSTRACT

Porphyran, sulfated polysaccharide, is derived from the cell wall and intercellular regions of *Porphyra* and known to be closely related to agarose in its basic structure, whereas it is very different in terms of having L-galactose-6-sulfate. Besides of various physiological effects, it is having wide pharmaceutical applications. Its structure-related gelling and emulsification properties have given birth to a new polymer in polymeric science. Porphyran uses are based on their unique properties to form strong gels after desulfation in an aqueous solution. This gel results from peculiar regular chemical structures, specific ordered molecular conformations, and aggregations. Now a days, new methodologies and instruments have provided a more accurate view of the relationships between the chemical structure and the gelling characteristics of these complex hybrid and heterogeneous polysaccharides. NMR is the single most powerful technique for solving the structures of intact polysaccharides. Developments in the NMR render the determination of structural distribution of this galactan more accessible. Such techniques also yield new information on the aggregate formation of these sulfated polysaccharides. These and other data question the existence of the generally assumed intertwined double helical conformations of these galactans during gel formation. Currently, porphyran availability is not known because of several problems such as its high molecular weight and viscosity that are suppressing its growth in world market. Hence, world market needs development of this novel compound to improve its pharmaceutical applications though this is an area of algal utilization that demands more research.

Introduction

Porphyra, commonly known as nori or laver, is important food source in many part of the world. It contains 11-13% water, 29-36% proteinaceous components, 39-40% carbohydrates including 5-7% crude fiber, 0.6-0.7% lipids, 8-11% ash, and some vitamins (total ascorbic acid 240 mg%).[1,2] The genus *Porphyra* has a worldwide distribution and more than 133 species have been described.[3] Earlier studies on the polysaccharides of *Porphyra* species showed that the extract called 'Porphyran' comprises alternating 3-linked-D-galactopyranosyl residues partially methylated at O-6 and 4-linked-L-galactopyranosyl residues occurring either as the 3,6-anhydride or sulfated at O-6 and that the composition with respect to these components varied widely.[4,5] Sugar analysis of dry material assist in differentiating polysaccharide content of different species of *Porphyra*.[6,7] Porphyran originates from the cell wall and intercellular regions of the raw laver, *Porphyra*, and is known to be closely related to agarose in its basic structure.[8,9] In the context of carbohydrates, it is recognized as valuable polymers for industrial applications and biological properties. Extensive uses of this algal polymer are based on their unique properties to form strong gels and emulsion that result from peculiar regular chemical structures, specific ordered molecular conformations and aggregations. Porphyran shows a relatively high viscosity and cannot form a gel due to the high amount of sulfate present as compared to agarose, which shows high viscosity and excellent gelling properties; porphyran has not received more general application. Matsuo *et al*., (1993) found that saccharide-6-sulfate could be converted to 3,6-anhydrosaccharide by 6-*O*-desulfation with N, *O*-bis(trimethylsilyl)acetamide, resulting in gelation of porphyran.[10] This gelation induced by desulfation means that the electrostatic repulsion among porphyran chains decreases with desulfation and chains can then associate by binding to each other through non-covalent bonds such as by the hydrophobic interaction or the electrostatic interaction via hydroxyl groups. If porphyran chains can potentially interact through the hydrophobic interaction, this may affect porphyran's amphiphilic properties, suggesting the possibility of its use as a new polysaccharide surfactant. Low-quality nori has no practical value, but have high carbohydrate content, suggesting high porphyran content. This review sparks factors influencing the emulsification and gelling properties of the natural polymer obtained from *Porphyra* sp.[11-21]

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Structural conformation

In general, polysaccharides are rich in OH groups that make them hydrophilic which allows them to establish an intra H-bond network implying the local stiffness of the molecules; from this rigidity, they get a high thickener character. Porphyran, a sulfated polygalactan, consist of the galactose and 3, 6 anhydrogalactose. It contains sulfated ester ranging from 6% to 11% and 3, 6-anhydrogalactose from 5% to 19%.

Chemically, porphyran is related to agarose, but inspite of structure similarity to agarose it has unique structural characteristics. It consists of linear backbone of alternating 3-linked β-D-galactose and 4-linked 3, 6-anhydro-α-L-galactose units. L and D-galactose, which are linked by beta 1 > 3 or alpha 1 > 4 glycosidic bonds form the basic pattern of agarose and porphyran, in the latter alternate L- and D-galactosyl residues. The L-residues are mainly composed of α-L-galactosyl 6-sulfate units and[3,6]-anhydrogalactosyl units are minor [Figures 1 and 2].

With that it carries high proportions of "precursor" 6-sulfate group which is stable when it is in salt form. Structural features of porphyran varies from genus to genus and from species to speices such as *Porphyra umbilicalis* is mainly produced in Japan, constituted of 4-linked 6-*O*-sulfo-α-L-galactopyranose residues and 3-linked 6-*O*-methyl-β-D-galactopyranose residue. The polymer was described as being composed for 49% of sulfated disaccharide units. The extract from *Porphyra capensis* has a linear backbone of alternating 3-linked β-D-galactose and 4-linked α-L-galactose 6-sulfate or 3,6-anhydro-α-L-galactose units. The polymer extracted from *Porphyra haitanensis* shows a typical porphyran structure with a linear backbone made of alternating 3-linked β-D-galactopyranosyl units and 4-linked α-L-galactosyl 6-sulfate and a minor amount of 3, 6-anhydrogalactosyl units. From *Porphyra yezoensis*, a polysaccharide with the following constituent units was found: Galactose, 3, 6-anhydrogalactose, 6-OSO_3-galactose, and xylose in ratio 25:15:10:1. These all features may make the backbone of the molecules quite stiff with a side chain giving an overall shape and a surface that are important for gelling properties after suitable chemical treatment.[11,22-28]

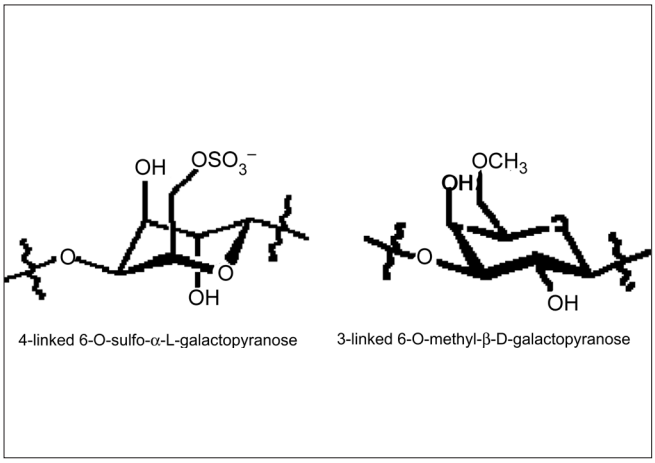

Figure 1: Structural conformation of porphyran

Determination of 3:6-anhydro galactose in porphyran

3:6-Anhydrogalactose is a constituent of the mucilaginous intercellular polysaccharides of the *Porphyra* sometimes occurring as the D- and sometimes as the L enantiomorph. Thus, these galactans are classified either as carrageenans if the 4-linked residue is in the D configuration or as agars if the 4-linked residue is in the L configuration. It was first discovered in commercial agar and has been found in an increasing number of these polysaccharides. For any polymer gelling and emulsification properties are based on their configuration and substituent's on 3:6-Anhydrogalactose [Figure 3].

As agar is referred as a queen of gelling agents, porphyran when treated by alkali exhibits similar properties to agar that proves that this polymer has high gelling potential. The gelling property is due to the three equatorial hydrogen atoms on the 3:6-Anhydro-L-galactose residues of agarose to form hydrogen bonds. Porphyran is related to agar, in that it contains residues of 3:6-anhydro-L-galactose. It also contains residues of L-galactose 6-sulphate. Compositional analysis of red seaweed galactans is complicated by the degradation of 3,6-anhydrogalactose occurring under the strong acid conditions needed for the total hydrolysis of the polymers. This problem has been circumvented by the use of a double-hydrolysis procedure (includes five main steps: Mild hydrolysis, reductive amination, strong hydrolysis, reductive amination and acetylation) in which a mild hydrolysis is used to cleave only the 3, 6-anhydrogalactosyl linkages followed by a reduction with sodium borohydride in order to produce stable terminal alditol residues, which are afterward hydrolyzed under the usual conditions. In another approach ('reductive hydrolysis'), an acid-stable reductant (4-methylmorpholine borane) is used, while the 3, 6- anhydrogalactosyl linkages are being cleaved. Further steps are then used to hydrolyze and derivatize the remaining components. The derivatives produced can then be separated and quantitated by GLC or HPLC.

Earlier partial reductive hydrolysis or methanolysis was used for the cleavage of the 3, 6-anhydrogalactosidic linkages and to obtain

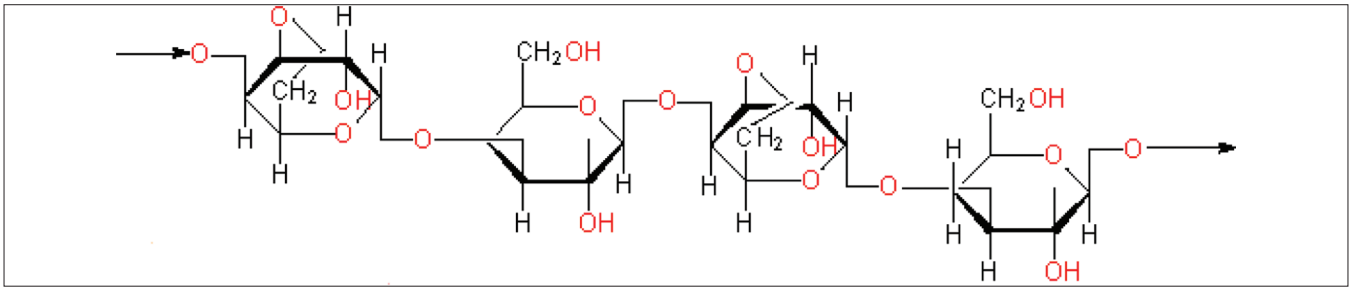

Figure 2: Structural features of agarose

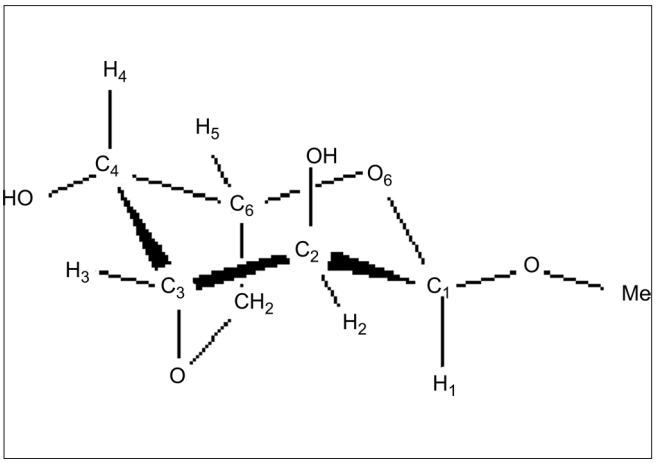

Figure 3: 2D view of 3, 6-anhydro-a-L-galactopyranose

the derivatives of representative disaccharides (carrabiose and/or agarobiose) amenable to GLC analysis. If consecutive carrabiose or agarobiose units are present in the polysaccharide, disaccharide alditols with a characteristic configuration are obtained. However, the method fails when no such consecutive units are present. Thus, no assessment of the configuration of the total 3, 6-anhydrogalactose is obtained, especially for polysaccharides with low 3,6-anhydrogalactose content. Another procedure developed for such purpose involves oxidative hydrolysis and derivatization of the 3,6-anhy drogalactonic acid terminals to esters with chiral alcohols. The technique is very laborious, as it requires many steps. Therefore, its sensitivity is very low.

Double hydrolysis procedure is simple and has a wider scope than previous techniques. Furthermore, as 3, 6-anhydrogalactose can be generated quantitatively from galactose 6-sulfate by an alkaline treatment, a one-pot procedure involving alkaline treatment, mild hydrolysis, reductive amination, total hydrolysis, and final derivatization was developed in order to quantitate both enantiomers of any polysaccharide.[29-40]

Gelation properties

The most important characteristic of the red algal galactans is their gel-forming ability, i.e. the ability to form well-ordered spatial structures during cooling of their hot polymeric solution. The gelling ability gives the basis for the vital functions of red algae as well as for their use in food industry, microbiology, chromatography, electrophoresis, etc.[41-43] Gel formation in an aqueous solution is a complex process that depends not only on polysaccharide structure, polymer concentration, and temperature, but also on the presence of co- and counter-ions.[44-48] Porphyran after alkaline treatment adopts a double-helix conformation in the solid state such as agarose; different types of helical structure were predicted by molecular modeling. It was shown that the double helix is the basis of gel formation, the main characteristic of porphyran. The gelation occurs from aggregation of double helices. The gelation temperature is related to the methoxyl and sulfate contents that can prevent gelation. The basis of gelation is the formation of double helices that phase-separate to form a gel; the gels are turbid due to the high degree of helix aggregation. Stronger gel corresponds with purer porphyran; these gels show syneresis (separation of water on aging) corresponding to the slow organization of the double helices.[49]

Improvement of gelation properties in porphyran

By alkaline treatment

Porphyran can be treated by alkali to increase the gelling ability. After alkaline treatment, the polysaccharides have the properties of agarose. The role of alkaline treatment on the reinforcement of the gel strength of porphyran decreases the yield in sulfate, increases the 3,6-anhydrogalactose, but does not change significantly the *O*-methyl and pyruvic acid contents. The analytical alkaline treatment was carried out as described by Ciancia *et al.* (1993).[50] Porphyran is treated with sodium hydroxide and reduced with sodium borohydride and after that it is neutralized with hydrochloric acid.[51] Formation of 3,6-anhydro-α-D-galactopyranose units from α-D-galactopyranose 6-sulfate residues by alkaline treatment is an important and well-known reaction undergone by porphyran which proves that sulfate groups are having great influence on cyclization rates.[50-58]

Effect of monovalent and divalent salt

Salts, as additives, are known to modify the properties of aqueous systems containing colloidal particles or macromolecules even in cases when the latter particles or molecules carry no net charge. As like the gelation properties of agarose, porphyran involves a conformational transition, induced by a lowering of the temperature, of randomly coiled polymer molecules to ordered double helices that are insoluble in water and hence the larger aggregated structures constituting the gel network. It was found that the effects of high concentration of salts affect these transitions significantly, shifting the gel setting and gel melting temperature of polymer, as well as the structure of the gel as evidenced by the sample turbidity. Porphyran contains few ionic (sulphate) groups. The various factors that influence the gel forming power are sulphate group content, the regularity of the alternation of D-galactose and 3,6-anhydro-L-galactose, and the molecular weight. The addition of the alkali metal ions screens the electrostatic repulsion between sulphate groups. The effects of salt addition on gelation of porphyran can be determined by melting transition temperature. The addition of monovalent salts (LiCl, NaCl, KCl) to native porphyran decreases the melting temperature. The gel strength in the presence of divalent salt follows the order: $Mg^{2+} > Ca^{2+} > Sr^{2+} > Ba^{2+}$, i.e. inversely proportional to the cationic radius. Addition of divalent salts to this polysaccharide promotes a melting temperature decrease. A model for the gelation was proposed that includes ionic interactions and hydrogen bonding. After alkaline treatment porphyran show good emulsifying and gelling properties.[59-66]

Effect of cationic and anionic polysaccharide

Crude porphyran has poor gelling property and show no linear viscosity region when it is examined for its rheological properties. So current research need a stable compound that is having good compatability and improves the rheological properties of porphyran. However, these modules are not applied yet but give the current knowledge of interaction between the two polymers.

In this regard, we are taking chitosan, cationic polyelectrolyte among all the natural polysaccharides. The presence of positively

charged amino groups in its molecule causes this polysaccharide to bind to metal ions via chelation and to be adsorbed at various interfaces, in most cases negatively charged. Porphyran is an anionic polysaccharide, unable to form gel. Its molecules contain many sulfo groups and anhydrogalactose residues. Mixing of the solutions of oppositely charged polymers, which causes the formation of polyelectrolyte or interpolyelectrolyte complexes (PECs), is a simple and convenient method to vary their properties and prepare new compositions. Because chitosan is a cationic polyelectrolyte, it is widely used to form PECs with various anionic polysaccharides and synthetic polymers. Its mixtures with porphyran can be studied in a number of works. The insoluble complexes of chitosan with porphyran can be use for the preparation of microcapsule shells and can be applied for chitosan separation from wastewater of food industry enterprises.[67]

Effect of different temperature

Crude porphyran when concentrated at high temperature gives a high viscous solution, on cooling give a semisolid compound that has poor gelling property but have high viscosity. Melting temperature of porphyran can be examined with the help of differential scanning calorimeter.[68]

Effect of adding skimmed milk and egg albumin

Protein ingredients derived from milk and eggs are the most commonly used food emulsifying agents, but these are not hydrocolloids. Porphyran is not yet applied for these models due to the lack of knowledge of interaction with proteins. This review sparks certain aspects of current protein interactions that can improve gelling and emulsifying properties of porphyran. This gelation is explained by the formation of cross-linked structure where the nodes are the casein micelles connected by polysaccharide chains. In many respects, milk is a natural unique colloidal system composed of fat emulsion and protein (casein) micelles. Casein micelles can withhold prolonged boiling and drying after which the system is restored by adding water. Porphyran does not form gel causing only an increase in the viscosity of aqueous solutions. Its molecules differ in the highest content of sulfo groups. The goal of this paragraph is to consider how the gelation ability and charge density in a porphyran affect the formation and mechanical properties of gels in skimmed milk.[68]

On the other part whole hen's egg or its constituents are key ingredients associated with unique sensory characteristics and excellent functionality for industrial applications. The egg yolk is mainly used for emulsion formation whose stability depends on the ability of the constituents to adsorb at the oil-water interface. The sensitivity of protein structure and interactions with processing conditions can be exploited by using changes in these conditions to alter protein functional properties in the formulation of foams, gels, and emulsions. The interaction with a porphyran may either give rise to stable high-molecular-weight complexes which increase the solubility of protein and inhibit protein-protein aggregation or perturb the protein structure and decrease the thermal stability of the protein.[69]

Effect of desulfation and microwave assisted desulfation

Desulfation process usually decreases the biological activity and increases the gelling properties of sulfated polysaccharide. The structural determination of red galactans has been a matter of study for many years. The problem arises when a polysaccharide also carries an alkali-stable group like most of the sulfate hemiester groups. In such circumstances, methylation analysis leads to ambiguities, as both the position of linkage and the position of sulfation will remain free after methylation. Methanolic hydrogen chloride was the first desulfation agent ever used. Red seaweed galactans are widely used as texturizing, viscosity-building and gel-forming ingredients in food and pharmaceutical industry. They represent a multi-million dollar market that grows every year. Depending on the configuration of the α-galactose units, red seaweed galactans were classified into carrageenans and agarans, whereas the whole sulfation pattern and the presence or absence of 3, 6-anhydrogalactose permit further classifications. The last factor is also related with their industrial applicability; thus, non-gelling polysaccharides may be transformed into gelling ones by an alkaline modification that increases the proportion of 3,6-anhydrogalactose. This reaction occurs when 6-sulfated α-galactose units are present: By heating the polysaccharide in strong alkaline media, the 3-OH group is ionized, and produces an intramolecular nucleophilic displacement of the sulfate group in position 6. The reaction is highly specific, as no other sulfate group is affected. This reaction is widely used in the lab and at industrial level in order to improve the gelling properties of carrageenan and agarans. In recent years, an increasing number of papers reporting the use of microwave irradiation to speed up chemical reactions have appeared some of them applied to polysaccharides achieving a complete cyclization of 6-sulfated units very rapidly and with no signs of degradation.[70]

Effect of enzyme treatment

Porphyran has enormous pharmaceutical uses but high-molecular weight featuring high viscosity limits its pharmaceutical application. Furthermore, many studies have demonstrated that molecular weight distributions of porphyran have great influence on their biological activities. Therefore, a simple procedure is needed to prepare different molecular weight porphyran to reduce the viscosity and find out more effective scopes of molecular weight. There are many researches on polysaccharides degradation. However, reports on porphyran degradation are scarce and mainly about agarase hydrolysis, such as β-agarase from *Arthrobacter* sp. S-22 and crude agarases from *Pseudomonas vesicularis*. Recent studies revealed that the relative distribution of the various sugar residues in porphyran can be studied by treating it with cytophaga enzyme that causes depolymerization of algal galactans. This enzyme system was most active on agarose, a polysaccharide component of agar, but it also had considerable action on porphyran, the galactan sulphate of the red alga *Porphyra*. Porphyran has a similar alternating structure, but the alternation is between either D-galactose or 6-O-methyl-D-galactose on the one hand and either 3, 6-anhydro-L-galactose or L-galactose 6-sulphate on the other hand. Thus, parts of the porphyran molecule could be identical in structure to agarose, but other parts will be different from it. This enzyme system is helpful in determining the degree of polymerization that can be determined by using the Peat, Whelan and Roberts (1956) method. In this method, the anthrone-H_2SO_4 reagent is used to determine total sugar, but 3, 6- anhydrogalactose produces a more intense color with the reagent than does galactose. The 3, 6-anhydrogalactose content of the oligosaccharide was therefore determined before and

after reduction in aqueous solution with $NaBH_4$ to calculate what proportion of this unit constituted terminal reducing groups. The appropriate allowances were then made to the extinctions produced with the anthrone reagent to calculate the degree of polymerization. Degree of polymerization can gives idea about the gelation properties of porphyran. Among all enzymes, β-agarase is the best-known enzyme used for the degradation of porphyran. Enzymatic processing of these marine polysaccharides is an essential step for the rational development of new drugs.[71-75]

Emulsification properties

Porphyran has a high emulsifying activity index and high emulsion stability over a wide pH and temperature range. Porphyran concentration decreases in aqueous phase after adding in oil-water emulsions and proves that porphyran show adsorption to the surface of oil droplets. Moreover decrease in ξ-potential of the O/W emulsions suggested that the sulfate groups of the adsorbed porphyran were oriented toward the external aqueous phase. It exhibits a biomolecular interaction by rapid binding to C_{16}-alkane, probably through 3,6-anhydrogalactose. Porphyran-coated liposomes were tolerant to digestion with phospholipase D. The increased molecular weight of the porphyran preparations had an increased effect on these characteristics. Emulsifying ability of porphyran can be derived from the adequate adsorption to the surface of oil droplets and that porphyran could be effectively applied to stabilize liposomes.[76]

Emulsifying ability of porphyran

Porphyran emulsification properties are molecular mass, 3,6-anhy drogalactose, protein, and sulfate content dependent. The sulfate content showed a tendency to increase with decreasing molecular mass. However, the[3,6]- anhydrogalactose content of each porphyran preparation was independent of molecular mass.3,6-anhydrogalactose content is based on the total saccharides as reported by Araki *et al*. (1977).[77] Because each porphyran preparation showed much higher absorbance, it was evident that porphyran had good emulsifying ability. The emulsifying ability of the porphyran preparation can be evaluated on the basis of the absorbance of their O/W emulsions at pH 7.0.[76,78]

The emulsifying ability was evaluated according to the method of Pearce and Kinsella (1978).[78] Porphyran was dissolved in buffer at a final concentration of 0.1% as saccharide. Corn oil was emulsified with this porphyran solution. Measured quantity of this emulsion was diluted with the sodium dodecyl sulfate solution prior to measurement of the absorbance at 500 nm. The emulsifying activity was determined as the emulsifying activity index (EAI), which was calculated by means of the following formula:

$$EAI = 2T/\emptyset c,$$

where $T = 2.3A/l$ [A being the absorbance at 500 nm immediately after emulsification and l (light pass) = 10-2 m] and c is the concentration of porphyran (1000 g/m^3), with Ø (oil phase volume) = 2. The emulsion stability (ES) was determined as the absorbance at 30 min after emulsification.

Moreover, a decrease in proteinaceous components showed high emulsifying stability. The porphyran itself is thus considered to exhibit the high emulsifying stability. Each emulsion of porphyran is a polydispersed system that is why the median, mode, and mean diameters of the oil droplets should be considered. Thus, emulsion with a small particle size could be prepared with a porphyran preparation having a suitable chain length or chemical composition. This will put the correlation between the median diameter of the oil droplets in the emulsions and the chemical structure of the porphyran preparations. The adequate correlation between the particle size of oil droplets and the 3, 6-anhydrogalactose content suggests that 3,6-anhydrogalactose may contribute substantially to the emulsification with porphyran. Because 3, 6-anhydrogalactose is considered to have high hydrophobicity as compared with galactose, galactose-6-sulfate and 6-O-methyl galactose, 3, 6-anhydrogalactose may interact with the surface of the oil droplets, resulting in the orientation of the porphyran chain.[78]

Effect of environmental factors on the emulsifying ability of porphyran

The effects of several environmental factors on the emulsifying ability of porphyran are reported by Takahashi *et al*. Emulsifying ability of porphyran depends on the factors that cause physical instability (flocculation, coalescence, breaking and creaming). This coarse disperse system having particle size greater than 1 μm is stable at different pH, temperature, and alkaline conditions.[76,79]

Effect of pH

The effect of pH on the emulsifying ability of porphyran can be evaluated by measuring the absorbance of the non-proteinaceous emulsions prepared at different pH.[76] It was observed that porphyran has good emulsification property over a wide pH range except in the acidic region. In the acidic region, porphyran emulsification ability decreases to some extent. Moreover, at pH 8 porphyran shows maximum emulsification property.[80]

Effect of temperature

Each of the porphyran preparations showed no essential change in the emulsifying ability and emulsion stability values in the temperature range of 4-60°C. Thus, the emulsifying ability of porphyran is considered to be independent of temperature.[76,81]

Effect of NaCl

The porphyran preparations exhibited a high emulsifying ability and stability over a wide range of pH and temperature in the presence of sodium chloride. Porphyran salt tolerance is considered due to its ion exchange capacity. The effect of NaCl at high pH was studied by examining the effect of a proteinaceous emulsification modifier such as β-lactoglobulin, loses almost all emulsifying ability in the presence of 1% NaCl, whereas β-lactoglobulin conjugated with porphyran retains emulsifying ability, possibly due to the ion-exchange capacity of side groups on the saccharide chain.[82-84]

Conclusion

Research into the properties of *Porphyra* sp. has led to the

discovery of Porphyran considered worthy of pharmaceutical application such as emulsification and gelation. In the past, pharmaceutical utility was limited due to its high molecular weight and high viscosity. Throughout this deep we realized that this polymer bears certain excellent pharmaceutical properties that need further attention (flow properties, instability and stabilization concepts, surface and interfacial phenomena and particle properties) of current researchers to commercialize it on a large scale. Recently, several inventions have been made on the degradation of this compound to improve its utility in this field. Although there are only a few porphyran products currently in the market, several robust new compounds derived from *Porphyra* products are now in the clinical pipeline, with more clinical development. Thus, the major breakthrough in drug discovery from the sea has so far not happened but is still eagerly awaited by those active in the field.

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