



CARRAGEENAN: EXTRACTION AND ITS APPLICATION

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ABSTRACT

Carrageenan is a naturally originated carbohydrate (polysaccharide) derived from edible red seaweeds. The name Carrageenan derives from the seaweed species *Chondrus crispus* known as Carrageen Moss or Irish Moss. In addition to these functions, carrageenans are used in pharmaceutical formulations, experimental medicines, cosmetics, food and dairy industries.

KEYWORDS: Carageenan, biological activity, toxicity, applications.

INTRODUCTION

Natural polymers are inert polymer and are safe, non-toxic, biocompatible, biodegradable, low cost and chiefly available in nature. Carrageenans are economically important biopolymers used in the pharmaceutical, chemical, and food industries.^[26] Carrageenan is a natural anionic linear sulfated polysaccharides derived from some red algae.^[6,16,30] These are commonly used for gelling, thickening and stabilising properties in the food industry.^[21,30] Carrageenan, first identified as 'carrageenin' discovered in 1862 by Stanford, a British pharmacist who extracted it from Irish moss (*Chondrus crispus*).^[36] The name was changed later to carrageenan so as to comply with the '-an' suffix for the names of polysaccharides. The word carrageenan is derived from the colloquial Irish name for this seaweed, carrageen, means 'little rock'.^[12] Carrageenan is a galactoserich polymer isolated from various species of the class Rhodophyceae marine red seaweed among carbohydrate polymers. Carrageenan can be derived from several types of red seaweed, including Kappaphycus, Gigartina, Eucheuma, *Chondrus* and *Hypnea*, containing as much as 50% of the dry weight.^[19,36] There are three main varieties of carrageenan which differ in their degree of sulfation depending on the method and the algae from which carrageenan is extracted. There is one sulfate group per disaccharide in kappa-carrageenan, two in iota-carrageenan and three in lambda-carrageenan.^[28] The kappa form comes mainly from *Kappaphycus alvarezii* and it forms solid, rigid gels in the presence of potassium ions.^[17] Iota form is obtained from *Eucheuma denticulatum* and it forms soft gel in presence of calcium.^[17] Lambda form is used to thicken the milk product. All three carrageenan forms are known differently but their base structure remains the same; which mainly consist of D-galactopyranose units bound together with alternating α -1,3 and β -1,4 linkages.^[36]

Carrageenan Kappa and iota are the sources of fiber to improve elasticity of dry noodles.^[10] Carrageenans are large, highly flexible molecules and they have an ability to form a variety of different gels at room temperature.^[8] Chemically, carrageenans are a family of linear hydrophilic sulphated galactans which are found primarily in red marine algae cell walls.^[6,12,16] Carrageenan helps to improve the quality of meat products. They are used in the field of pharmaceuticals, cosmetics, printing and textile formulation industries.^[12] Carrageenan possesses some biological activities including antitumor, immunomodulation, anticoagulant and antioxidant properties.^[14,31,32] Because of its biocompatibility and consolidation behaviour, carrageenan is commonly used by pharmacists to improve the properties of formulations of drugs.^[3] All form of carrageenan can be easily dissolved in hot water but only carrageenan lambda form is soluble in cold water. Because of their different chemical structure and physical properties of carrageenan, this natural source can be used in the various applications, ranging from tissue engineering to drug vehicle preparation for controlled release of drugs.^[1,31] Some important carrageenic characteristics, such as adhesivity and positive surface load, provide an additional advantage in prolonging the release of drugs in mucosal / epithel tissues.^[3] Interaction between Carrageenan and other polymers is used to create an optimal profile for drug release. This review article is prepared to concentrate on carrageenan extraction, separation, and various applications in the pharmaceutical and food industries.

PHYSICAL AND CHEMICAL PROPERTIES

There are several different form of carrageenans with slightly difference in their chemical structures and properties. Carrageenan can be in 3 forms according to the literature survey based on the sulfate group size and

position, based on their families and properties.^[1] Based on the sulfate group quantity and location carrageenan are classified into (λ), (κ), (ι), (ν), (μ),

(θ) and (κ). Based on family carrageenan mainly classified into three types.

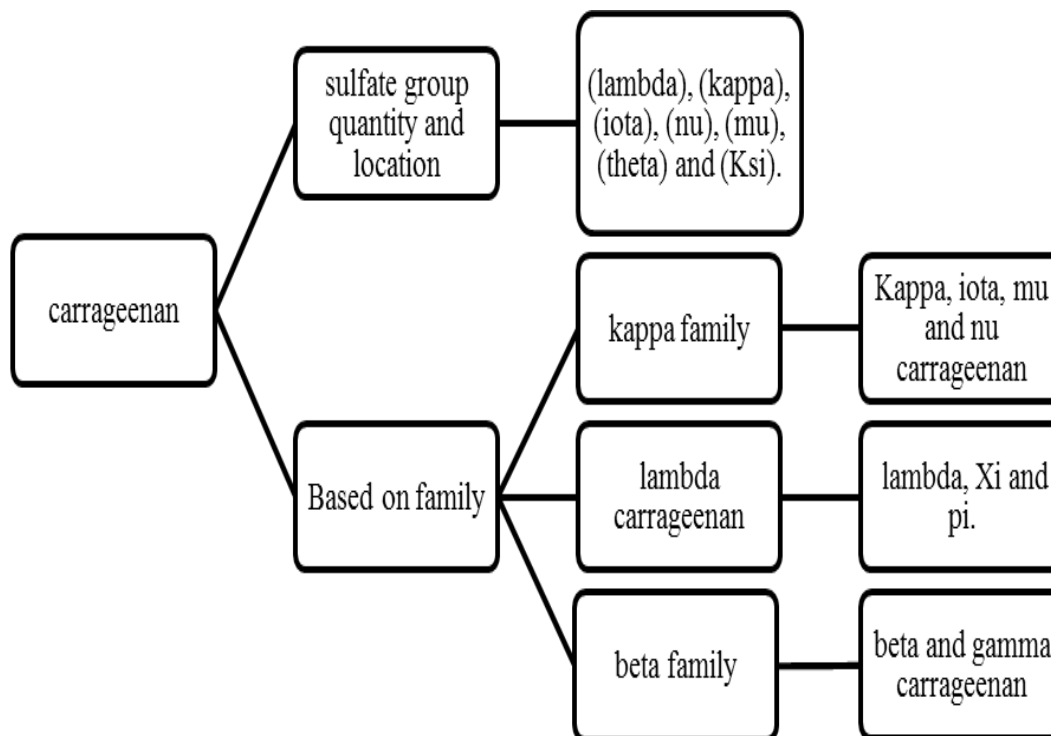


Fig 1: classification of carrageenan.

The first family involves kappa family which contain a subclass such as Kappa, iota, mu and nu carrageenan. The second family class comprises lambda carrageenan which contains subclasses such as lambda, Xi and pi. Third class includes beta family that comprises subclasses such as beta and gamma carrageenan.^[1]

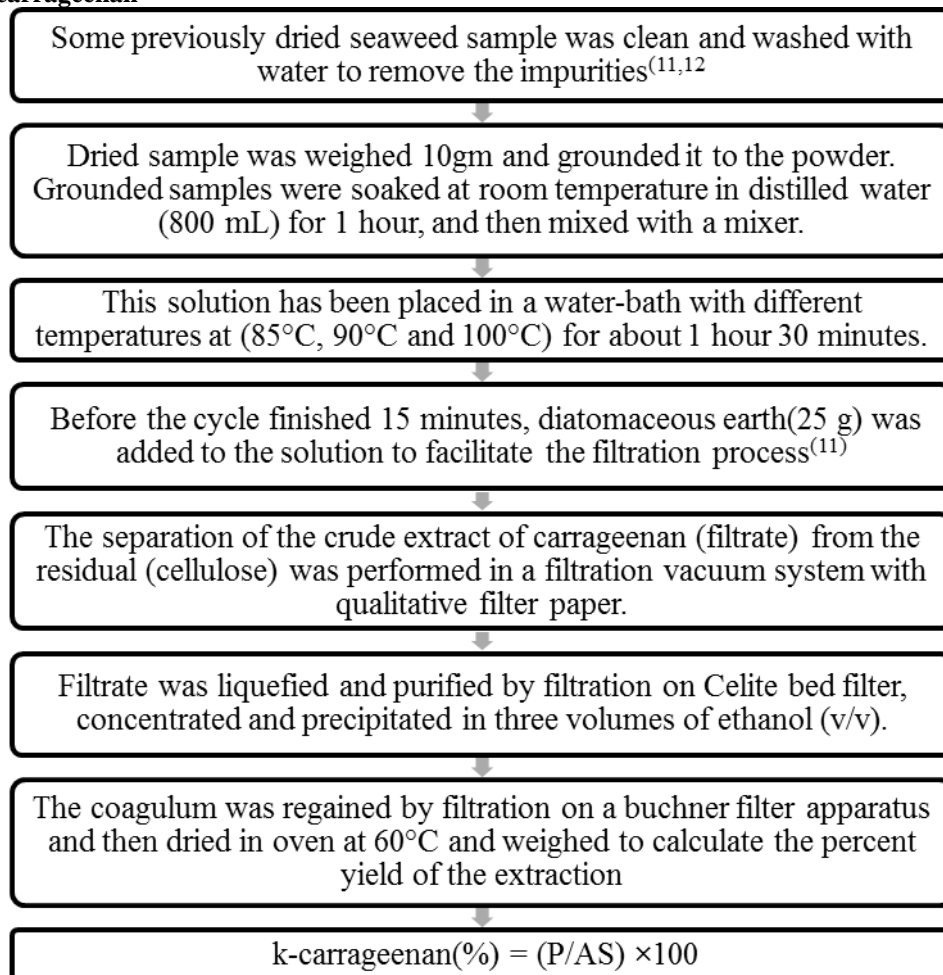
chemical properties

- All form of carrageenan can be easily dissolved in hot water but only lambda form of carrageenan and sodium salts of kappa and iota are cold water soluble.^[15]
- The form of carrageenan, pH, temperature, medium ionic strength and presence of cations are factors that affect carrageenan's aqueous solubility.^[29]
- As concentration increase viscosity also increase and as temperature increase viscosity decreases. At below pH 4.3 carrageenan completely loss its functionality.^[2,15]
- All three form of carrageenan are called differently but their base structure remains the same; which mainly consist of D-galactopyranose units bound together with alternating α -1,3 and β ,1,4 linkages.^[36]
- Kappa-carrageenan has only one sulfate ester group, while iota-and lambda carrageenan have two and three sulfates per dimer respectively.
- Molecular weight of kappa carrageenan extract is between 400–560kDa and the molecular weight of

Euchemia seaweed is 615kDa which is slightly higher than kappa carrageenan.^[18,15]

MATERIALS AND METHODS

Samples of the red seaweed algae were collected, clean and washed with water for elimination of impurities and then transported in cold to the laboratory. The cleaned plants were left to dry under the sun for two days, covered and then sealed in plastic bags.

Extraction of carrageenan**Fig 2: Extraction of carrageenan.**

Where, k-carrageenan% is the percent yield of crude carrageenan extract, P is the amount of extracted carrageenan in grams and AS is the amount of seaweed (10g dry weight) used for extraction.

Analysis of carrageenan extract**1. Determination of sulphate content**

Sulphate content of carrageenan extracted from seaweed was analyzed by turbidometric method. Initially, barium-agarose reagent was freshly prepared by mixing 0.02% agarose and 0.5% barium chloride (BaCl₂). Then 1 mL sample of carrageenan extract was applied to 1.2 mL of 8% trichloroacetic acid (TCA) and then 600 µL of barium-agarose reagent was added to the mixture, followed by 30 minutes of incubation at room temperature. The absorbance, after incubation was measured at 500 nm against a blank solution. Sodium sulphate was used as standard.^[12,20]

2. Galactose content determination

The galactose content of carrageenan was measured using anthrone reagent via colorimetric methods, and galactose was used as a reference. Anthrone reagent stock solution was prepared by 200 mg of anthrone reagent dissolving in 100 mL of 83.6% sulphuric acid

and processed for further use at 4°C. 1 mL solution of carrageenan with 10 mL of anthrone reagent was moved to the boiling tube and heat for 11 minutes in a boiling water bath. Then the tube was quickly cooled up in an ice bath. The absorbance was detected at 630 nm.^[20]

3. Determination of anhydrogalactose content

Resorcinol reagent about 130 mg dissolving in 100 mL of ethanol and used to prepare a stock solution. Then, 10mL of the stock solution were added to 100mL of 12M hydrochloric acid. This solution was freshly prepared and stored it in a brown bottle for further used. 2mL carrageenan solution with freshly prepared 10mL of resorcinol reagent was moved to the boiling tube and kept for 5 min in the ice bath. Then tube was heated at 80°C for 10 min and cooled in an ice bath. The absorbance was recorded at 500 nm. Variation in anhydrogalactose content of carrageenan influence the structural characteristics and rheological properties of carrageenan.^[20,26]

4. Determination of viscosity

Carrageenan exhibit different gelling or viscosity improving properties in aqueous solution.^[25] Carrageenan solutions viscosity depends on the type,

concentration, temperature and molecular weight of carrageenan.^[19] The viscosity increases exponentially with a higher concentration of solution and/or molecular weight.^[2] The viscosity of Carrageenan increases when a hot solution of carrageenan is cooled.^[7] To prevent the gelation effects, the measurements of viscosity of carrageenan solutions should be calculated at sufficiently high temperatures (75°C). The crude carrageenan viscosity was determined by the transferring 7.5 g of the sample into 450 ml of water with agitation for 10 to 20 min. By adding water and heating in a water bath with continuous stirring, the final weight was reduced to 500 g until a temperature of 80 ° C was reached. A

Brookfield viscometer's bob and guard was adjusted to around in water at about 75°C. The bob and guard had a spindle (19 mm in diameter and about 65 mm in length) attached to the viscometer. The height of the bob in the sample solution was adjusted and readings were taken after six revolutions at 30 rpm. The viscosity of carrageenan is usually between 5 to 800cps when it is measured at 75°C.

Biological activity of carrageenan

Carrageenan possesses some biological activity including anticoagulant, immunomodulatory, antithrombotic, antiviral and antitumor activities etc.^[1,31,32]

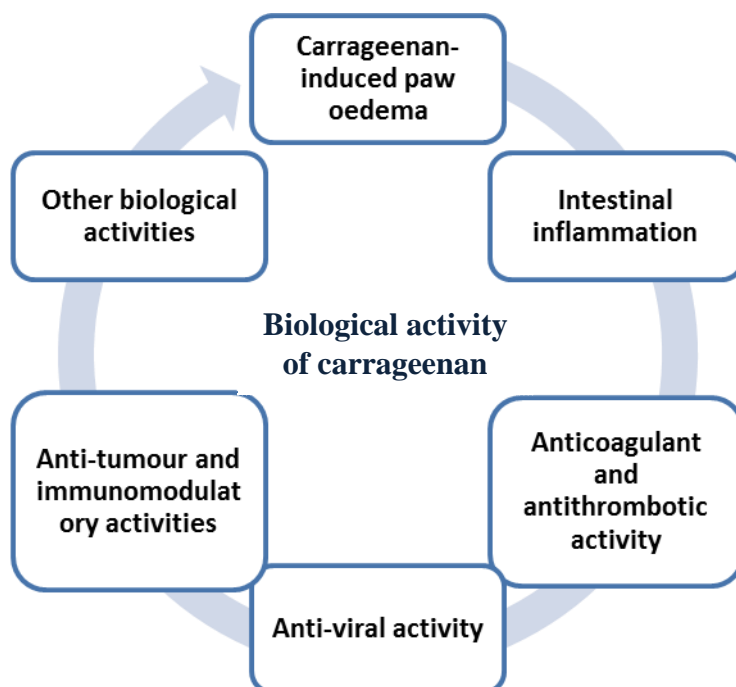


Fig 3: Biological activity of carrageenan.

1. Carrageenan-induced paw oedema

Carrageenan-induced rat paw oedema and it is the commonly used test for determined anti-inflammatory activity and is a simple, routine animal model for pain assessment at the inflammation site without injury or damage to the inflamed paw. Mouse paw oedema has been progressively used to test the activity of new anti-inflammatory drugs and to examine the mechanisms involved in inflammation.^[1,2] There are about 400 papers in the literature which report the use of mouse paw oedema. A freshly prepared 1–3% carrageenan solution in saline is commonly used as an intraplantar injection in 50–150 µl doses. Higher concentrations have been used for the modelling of specific pathophysiological conditions. There are various number of mediators involved in inflammation. Histamine, serotonin, and bradykinin are the first detectable early-stage inflammation mediators caused by carrageenan; prostaglandins (PGs) are implicated in increased vascular permeability and are detectable in the late-stage inflammation. Local and/or systemic inflammation is associated with enhanced levels of the pro-inflammatory

cytokines TNF-, IL-1, and IL-6. Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin or aspirin have not inhibited the initial phase of oedema, has been attributed to the release of histamine, 5-hydroxytryptamine (5-HT) and bradykinin. The later phase of swelling was not only associated with elevated prostaglandin development, but was more recently attributed to induction of inducible cyclooxygenase (COX-2) in the hind paw. It can be blocked by the NSAIDs. Another essential mediator involved in acute inflammation is the nitric oxide (NO) released under pathological conditions by three separate isoforms of nitric oxide synthase (NOS): endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS). Carrageenan stimulates production and release of NO at the injured site. Aminoguanidine hemisulfate (AG), a perfusion of an inducible NOS inhibitor, suppressed the NO release for 2.5–8 hrs after carrageenan injection. Neurectomy totally block the NO release for up to 3 hours after carrageenan injection and partially suppressed release of NO for 4.5–8 h. The production and release of NO by these NOSs is thought to contribute

to the oedema and hyperalgesia caused by tissue injury and inflammation.

2. Intestinal inflammation

Easy method to induce ulcer formation in the guinea pig's large intestine requiring only the addition of a degraded carrageenan to the drinking water. The method can be used as an experimental model for the study of different aspects of ulcerative lesion pathology in this section of the dietary tract. Freshly prepared, 5 per cent degraded carrageenan concentration has been added for guinea pigs to the drinking water. No more than 2 g / kg of contaminated carrageenan in the drinking fluid for 20 to 45 days results in ulcerative lesions consistent with clinical and physiological changes closely resembling ulcerative colitis in humans in some respects.^[1,2]

Clinically, weight loss, loose stools and supernatant or visible blood were found in the faeces. Pathologically, ulceration was found in all parts of the large bowel, extensive lesions occurring in the rectum. Minutely, the likenesses incorporate central mucosal hemorrhages and cell penetrates, oedema, sepulcher abscesses, unpredictable dilatation of tombs with loss of mucin-discharging cells and degeneration of the covering epithelium, ulceration including predominantly the mucosa, just as ulcerations in different phases of movement and recuperating. The ulcerative sores, in any case, seem to start in the caecum and expand distally toward the rectum.

3. Anticoagulant and antithrombotic activity

The framework for blood clotting includes intrinsic and extrinsic pathways, with various factors involved in the mechanisms.^[2] In cases of irregular vascular conditions and damage to non-endothelial surfaces at sites of vascular injury, blood coagulation is facilitated by coagulation factors to stop the bleeding through the damaged vessel wall. As endogenous or exogenous anticoagulants interfere with coagulation factors by inactivating them or restricting their activity, blood coagulation can be prolonged or stopped. The primary basis of carrageenan's anticoagulant activity appears to be an anti-thrombic property.^[1] Carrageenan exhibited greater anti-thrombic activity because of its sulfate content. Mechanisms of carrageenan anticoagulant activity include inhibition of thrombin. Originally, amidolytic experiments suggested that the anti-thrombin activity was mediated by AT-III (anti-thrombin-III), the main mechanism by which heparin works. Carrageenan tend to inhibit thrombin amidolysis directly and via AT-III in these studies; however, only AT-III potentiated Xa was observed. Some critical qualities of the polyanionic polymers can influence these interactions i.e., sulphation, size, ionic substitution pattern and polymer rigidity. Subsequent studies with AT-III reduced plasma, however, demonstrated residual anti-thrombin activity in the presence of carrageenans. It has been showed that carrageenan potentiates thrombin inactivation through 'anti-thrombin BM'. Therefore these findings will

suggest either an anti-thrombin potential via heparin co-factor II (HC-II) and/ or a direct anti-thrombin effect.

4. Anti-viral activity

Carrageenan is a potent inhibitor of several involved viruses, including human pathogens such as human immunodeficiency virus, herpes simplex virus (HSV), human cytomegalovirus, human rhinovirus, and others.^[1,2] Carrageenan initially acts by preventing virions from binding or entry into cells. This result is consistent with the fact that heparin sulfate-like carrageenan is a catalyst for the attachment of HPV cells. Carrageenan was found to be active against a number of common types of sexually transmitted HPV that can cause cervical and genital warts. Carrageenan is also used in *in vitro* and murin modeling systems against other viruses, including herpes simplex viruses and some HIV-1 strains. However, *in vitro* IC50 values are about a thousand times higher for herpes simplex virus carrageenan inhibition and HIV-1 infectivity than the IC50s observed for genital HPV inhibition *in vitro* carrageenan.

5. Anti-tumour and immunomodulatory activities

Various studies showed that carrageenans have *in vitro* antiproliferative activity in cancer cell lines as well as inhibitory tumor growth activity in mice.^[1,2] They also have antimetastatic activity by blocking the interactions between cancer cells and the basement membrane, inhibiting tumour cell proliferation and adhesion of tumour cell to various substrates, but their exact mechanisms of action are not yet fully understood. A significant reduction in *in vivo* carcinogenesis may be caused by oral administration of several seaweeds.

6. Other biological activities

All carrageenan have been studied for antioxidant activity. Red marine algae in rodents and mice leucocyte primes are considered a potent inflammatory agent to develop tumor necrosis factor- α (TNF- α) in response to bacterial lipopolysaccharide.^[2] In addition, some types of carrageenans induce potent activation of the macrophage while some carrageenan seem to inhibit macrophage functions. The sustaining of Fischer 344 betrays consumes less calories containing 15% kappa/lambda-carrageenan from *G. radula* brought about a cholesterol-bringing down impact. Comparative impacts of the incorporation of carrageenan in the eating regimen may bring about decreased blood cholesterol and lipid levels in human subjects.

Toxicity of carrageenan

Carrageenan toxicity was found to be dependent on molecular weight and not sulphate content. Carrageenan can be just as low molecular weight, carrageenan "degraded," or high molecular weight or carrageenan "undesgraded." The United States Adopted Names Council (USAN) defines "degraded carrageenan" as "poligeenan" with an average molecular weight of 10,000–20,000 Da, obtained by acid hydrolysis rather

than alkali. Polioegeenan has no nutritional use. Studies showed that polioegeenan was associated with inflammatory and proliferative GIT lesions including tumors at high doses in laboratory animals unlike carrageenan. The International Cancer Research Agency (IARC) has also provided evidence of polygeen carcinogenicity in laboratory animals, and classifies degraded carrageen as group 2B "Carcinogenic potential for humans." Degraded carrageenan is widely used to cause inflammation in studies with the immune system. In 1969 it was shown that degraded carrageenan caused cecal and colonic ulceration in guinea pigs. Several studies have since been published which show that exposure to degraded carrageenan causes bleeding and colon ulceration in some laboratory animals. The FDA proposed regulations that commercial-grade carrageenan could not have a molecular weight of less than 100 kDa as a result of these findings, although these regulations were never revised and the proposal was withdrawn in 1979. The average molecular weight of carrageenan that is available commercially is between 453 and 652 kDa.^[1,40] In 2001, Tobacman of the University of Iowa conducted a study of these 45 studies and concluded that exposure to degraded and/or undegraded carrageenan was associated with intestinal lesions such as ulcers and neoplasms in various animal models, including monkey, guinea pig, ferret, mouse, rat and rabbit. Animal studies that have been published since 1997 and have not been included in Tobacman's review showed conflicting results. Although some studies have confirmed that carrageenan causes or encourages inflammation of the gastrointestinal tract, ulcerations and/or neoplasms in animal models. Carrageenan should not be used in infant formulas intended for children under the age of 13 months based on a concern over the narrow margin of exposure between the amount of carrageenan ingested by infant formula and the lowest doses reported in laboratory rats and mice to cause inflammatory responses.^[1,41] So also, the European Commission Logical Advisory group on Nourishment (presently the European Sanitation Authority) finished up in 2003 that there is no proof of unfavorable impacts in people from presentation to nourishment grade carrageenan, yet informed against use with respect to carrageenan in baby equation because of an absence of data in regards to conceivable assimilation of carrageenan in the juvenile gut and impacts of carrageenan on the youthful insusceptible framework. While the committee acknowledged that there was insufficient evidence to lower the ADI or otherwise restrict carrageenan intake levels, it proposed that a cap should be established that no more than 5% of carrageenan of food grade would fall below 50 kDa. In comparison, the US FDA requires carrageen in infant formula because it believes that the health benefit (preventing fat isolation and thus offering standardized nutrition) outweighs the potential risks. The carrageenan health has been widely evaluated by the Scientific Community on Food (SCF) and the International Food Additives Council (IFAC). Contrary to Tobacman's results, all of those experts accept that

carrageenan is safe for food use. Additionally, the positive effects of carrageenan on human health are starting to be explored.^[1]

Application of carrageenan

Carrageenan is one of the most abundant polysaccharides in nature to be used as a gelling, stabilizing and thickening agent in prepared foods and cosmetics due to its biocompatibility, biodegradability, high water retention capability and mechanical strength of gels.^[1,15,33]

1. Industrial application of carrageenan

The immobilisation of both enzymes and whole cell systems is of great importance to enhance the stability, function and reusability of these biocatalysts.^[2] Carrageenan is an appropriate supportive material for the immobilization of whole cells, as demonstrated by several applications in different industrial processes. The acceptance of carrageenan as a food-grade additive and the ease of the protocol on immobilisation have facilitated its use in the food industries. The mild conditions of immobilization and reaction applied to carrageenan immobilization of entire cells facilitate their application for pharmaceutical compounds and fine chemicals in highly selective (enantio) production processes.

2. Application in food and dairy industries

For simplicity, carrageenan gum's food applications were divided into dairy based and water based applications.^[1,29] Carrageenan is used as a food additive in a wide variety of processed foods, including dairy products, water-based foods, meat products, drinks, condiments, baby formulas and pet foods.^[13] Carrageenan can serve as a bulking agent, gelling agent, carrier, emulsifier, glazing agent, stabilizer, moisturizer or thickener.^[19,31] Carrageenan is added to processed foods because, by interacting with other food substances, it can bind water, encourage gel formation, thicken, stabilize and improve palatability and appearance. (e.g. proteins, starch, sodium or calcium phosphates, galactomannan, carboxymethyl-cellulose). In certain applications, carrageenan has likewise been added to handled meat as fat substitute to improve dampness maintenance and reestablishes delicacy in low-fat prepared meat, for example, burger.^[36] Carrageenan improves texture, thickness and solubility when it comes to milk products. In milk gels (such as custards, flans and fluffy fillings), whipped cream, yogurt and milkshakes, and 0.03 per cent in frozen desserts and liquid milk products, carrageenan will effectively avoid separation and preserve texture in dairy products.^[1] A new application of carrageenan in the food industry is its use as a protective coating on freshly cut packaged foods, whereby carrageenan acts as a gas barrier, improving the cut surfaces of the fruit and reducing breathing, thereby decreasing discoloration and preserving texture throughout the shelf life. Recent studies have demonstrated that carrageenan is effective in preventing

coloring, preserving texture over shelf life, and providing antibacterial protection when used on sliced lychee bananas and mangoes as an edible fruit coating.

Table 1: Different utilizations of carrageenan in food and dairy item.

Sr.No.	Product	Purpose for addition/action in product
1	Ice cream	Prevent separation caused by adding gums to monitor the growth of texture and ice crystals, the stabiliser and the emulsifier
2	Coffee creamers, condensed and evaporated milk	Avoid separation of fat
3	Whipping cream	Maintain "lightness"
4	Low calorie jellies	Replace pectin and sugar with carrageenan in order to help
5	Drink mixtures (powdered lemonade, punched fruit etc.)	Give texture if reconstituted in cold water
6	Canned pet food	Bind meat back together, make gravy / jelly around meat parts
7	Beer	Clare by precipitating of proteins
8	Flans, custards, cream fillings	Stabilise gelling agent
9	Cheese	Stabilizer, binding property with moisture, increased sliceability
10	Low-fat/low-sodium processed meat and poultry	Improves juiciness and tenderness; functions like fat and preserves moisture through cooking; helps to bind meat products during cooking
11	Flavoured milk and milkshake	Holds suspended chocolate or any other flavouring

3. Pharmaceutical applications

Carrageenan has numerous therapeutic properties, such as anticoagulant, antiviral, antithrombotic, antitumor, hypolipidemic effects, immunomodulatory function, and antioxidant activity. The use of carrageenan in the pharmaceutical sector was based mostly on these pharmaceutical aspects. Antibiotic medication and chlorotetracycline creation, semisynthetic anti-microbial generation, D-aspartic corrosive creation and modern effluents cleaner all are at present accessible and potential utilizations of carrageenan.^[2,36]

2.1. Tetracycline and chlorotetracycline production

Tetracyclines are most important class of antibiotics and conventional fermentation is the method normally employed for their industrial production.^[2] k-carrageenan is used as an immobilizer for *Streptomyces aureofaciens* and *Pseudomonas dacunhae* cells to improve tetracycline and chlorotetracycline (antibiotic) and D-aspartic acid and L-alanine production in drug production.^[1,2,36]

2.2. Development of semi-synthetic antibiotics

Semi-synthetic antibiotics are prepared by combining a β -lactam nucleus with the so-called side chain, such as phenylacetic acid, d-phenylglycine or d-p-hydroxyphenylglycine. 6-Aminopenicillanic acid (6-APA) is obtained by fermentation of the enzymatic hydrolysis of penicillin G. The suitability of γ -carrageenan as a 6-APA production support was tested with *E. Coli* cells with activity in penicillin-amidase. The cells were immobilized with 90 percent efficiency and could be used for 20 repeated cycles with 60 percent of the initial penicillin-amidase activity maintained. The glutaraldehyde was applied to the carrageenan gel beads. D-p-hydroxyphenylglycine is most important precursors among the side chains, as it is used for

amoxicillin and cefadroxil synthesis. Recombined *E. Coli* cells that expressed both dihydropyrimidinase and carbamoylase were immobilized in k-carrageenan and could convert d / l-hydroxyphenylhydantoin to d-p-hydroxyphenylglycine. A conversion of 93 percent was obtained in a single-step reaction, while a value of 20 percent was observed with the *Agrobacterium radiobacter* strain, which contained the original cloned dihydropyrimidinase gene into *E. coli*.^[1,2]

2.3 D-Aspartic acid production

A variety of D-amino acids in drug production have been shown to be major intermediates. D-Aspartic acid can be used as a synthetic penicillin base.^[1,2] When d / l-aspartic acid is used as a substrate for *P. dacunhae* cells' l-aspartate -decarboxylase, l-aspartic acid is converted to l-alanine but d-aspartic acid remains unchanged due to the biocatalyst's high stereospecificity. This allows for the simultaneous synthesis of d-aspartic acid and l-alanine using carrageenan-immobilised *P. dacunhae* cells. D / l-aspartic acid is formed chemically from the ammonia and fumaric acid. D-Aspartic acid is crystallized by reactor effluent acidification and reconstituted by centrifugation. Upon crystallization, l-Alanine is also recovered by the addition of ammonia to the resulting liquor followed by concentration and refrigeration. Since 1988, this method has been developed for the continuous development of d-aspartic acid and l-alanine using immobilized *P. dacunhae* cells in k-carrageenan. Chibata first developed industrial application of immobilized whole cells in 1973 with the use of k-carrageenan gels for l-aspartic acid preparation. The industrial production of several other compounds for use in food and medical applications (l-malic acid, l-alanine, l-tryptophan, 1,5-dimethyl-2-piperidone) was based on the same principles.

2.4. Cleaning of industrial effluents

An efficient integrated nitrogen removal system was developed by co-immobilizing *Nitrosomonas europaea* and *Pseudomonas* sp. in κ-carrageenan, taking advantage of the oxygen gradient within the trapping beads.^[1,2] The immobilization of *M. aurum* in κ-carrageenan and its utilization in an air-bubble fermenter brought about an improvement of its morpholine-debasing limit. The carrageenan gel and immobilised microbial cell approach was used for the degradation of 4-chlorophenol. Aerobic and anaerobic microbial species were also co-immobilised into gel-beads of κ-carrageenan/gelatine. These immobilisations catalyze degradation of 2,4,6-trichlorophenol under air-limited conditions. Degradation of the pesticide pentachlorophenol in contaminated soil was attempted using *Pseudomonas* sp. UG30 cells in κ-carrageenan immobile.^[1,2]

4. Other applications of carrageenan

In the terminology of food chemists, carrageenan is commonly named as an emulsifier, colloid, stabilizer or gum. Many of the products we now take for granted – especially soy milk, chocolate and other flavored milk, dairy products, infant formulas, and nutritional supplement beverages rely on carrageenan for its consistent consistency.^[2] Without this ingredient they could not be made, packaged and stored for long periods of time. Carrageenans are utilized to gel, thicken, or suspend; in this way they are utilized in emulsion adjustment, for syneresis control, and for bodying, official and scattering.^[2] The main uses are in foods, particularly in the dairy sector. Carrageenan is unusual in its ability to hold cocoa at very low chocolate milk concentrations (approx. 300 ppm); no other gum has been found to equal this. A very fragile structure of the milk gel, undetectable for pouring or drinking the milk, is thought to keep the cocoa in suspension. Carrageenans act as a "binding agent" in toothpastes to impart the desired rheological properties to the paste and to provide "sheen" cosmetic consistency.^[2] Toothpastes are ingredients that interact in complicated and poorly understood ways and often the carrageenan has to be specifically formulated to achieve optimal performance in a specific formulation. In the food industry the use of carrageenan is often discussed in terms of its safety. Worldwide authorities such as JECFA, the Scientific Community on Food (SCF), and the International Food Additives Council (IFAC) have made extensive assessments of carrageenan safety. All such authorities accept that carrageenan is safe for food used.

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