SYNTHESIS AND CHARACTERIZATION OF HARD KAPPA CARRAGEENAN CAPSULE THROUGH GLYOXAL AND GLYOXYLIC ACID CROSSLINKING

INTAN LYANA BINTI AHMAD

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Faculty of Chemical & Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

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ABSTRACT

This research is about the synthesis and characterization studies of the hydrogel for hard kappa carrageenan capsule through glyoxal and glyoxylic acid crosslinking. In this work, kappa carrageenan hydrogel were prepared by chemical crosslinking using glyoxal and glyoxylic acid as the crosslinking agent and hydrochloric acid (HCl) as the catalyst. The purpose of this study is to select the best crosslinker material to develop hard kappa carrageenan capsule that can improve its propertis through molecular recognition approach by using crosslink technique. Aqueous kappa carrageenan (3% w/v) was reacted with a certain amount of glyoxal and glyoxylic acid (25%) respectively and HCl (0.1N) at 40°C. After 30 minutes of reaction, the homogenous solution was cooled at room temperature producing gel form. The gel was soaked in the ethanol to remove unreacted residue or chemicals. The obtained hydrogel was air dried at room temperature to constant weight. The FTIR spectra, DSC, TGA and the value of swelling ratio of obtaining hydrogel showed that kappa carrageenan could be crosslinked using glyoxal or glyoxylic acid. Based on the FTIR, there were some functional group detected showed that the crosslinker and kappa carrageenan was successsful crosslinked. There were no significant differences in the swelling degree of hydrogel synthesized in the distilled water and all buffer solution. The crosslinking with a certain amount of glyoxal and HCl may be produce hydrogel with a lower swelling degree. The kappa carrageenan hydrogel was found to be pH sensitive, indicating a high potential to be used in drg delivery polymer system.

ABSTRAK

Kajian ini adalah mengenai sintesis dan pencirian kajian hidrogel untuk kapsul kappa carragenan keras melalui glyoxal dan asid glyoxylic silang. Dalam karya ini, kappa carrageenan hidrogel telah disediakan oleh silang kimia menggunakan glyoxal dan asid glyoxylic sebagai ejen silang dan asid hydroklorik (HCl) sebagai pemangkin. Tujuan kajian ini adalah untuk memilih bahan crosslinker terbaik untuk membangunkan kappa carrageenan yang boleh meningkatkan propertis melalui pendekatan pengiktirafan molekul dengan menggunakan teknik sambung silang. Akueus kappa carrageenan (3% w/v) telah bertindak balas dengan sejumlah glyoxal dan asid glyoxylic (25%) masing-masing dan HCl (0.1N) pada 40°C. Selepas 30 minit tindak balas, campuran kappa carrageenan dan crosslinker telah disejukkan pada suhu bilik menghasilkan bentuk gel. Gel telah direndam dalam ethanol untuk mengeluarkan sisa yang tidak bertindak balas atau bahan kimia. Hidrogel yang telah siap dikeringkan melalui udara pada suhu bilik untuk menghasilkan berat yang sama. Hasil keputusan daripada FTIR spectra, DSC, TGA dan nilai nisbah ‘swelling’ daripada hidrogel menunjukkan kappa carrageenan boleh disambung silang menggunankan glyoxal atau asid glyoxylic. Berdasarkan FTIR, terdapat beberapa kumpulan berfungsi dikesan menunjukkan bahawa crosslinker dan kappa carrageenan berjaya disambung silang. Tidak ada perbezaan yang signifikan dalam tahap ‘swelling’ hidrogel di sintesis di dalam air suling dan semua ‘buffer solution’. Teknik silang dengan sejumlah glyoxal dan HCl boleh menghasilkan hidrogel dengan nisbah ‘swelling’ yang lebih rendah. Oleh itu, hidrogel kappa carrageenan didapati pH sensitif, menunjukkan potensi yang tinggi untuk digunakan dalam sistem polimer penghansilan kapsul ubat.

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1 INTRODUCTION

1.1 Introduction

In 2013, the worldwide population of Muslims is about 1.6 billion. The population keeps increasing by 1.8% per annum. At present, Muslim consumer demand for halal pharmaceutical product which is a paramount importance. Nevertheless, the limited amount of halal pharmaceutical medicine available in the current market is because of the consumption of non-halal capsule (Moawia M., 2010). Most of the capsule is manufactured using the animal gelatine. The main animal gelatine source's origin from pig or unslaughtered animal, skin, bone, meat, fat, waste, used cooking oils of animals (Sahilah et al., 2012). In this work, carrageenan from plant-based material was studied to produce the hard gel to replace the gelatine as a capsule.

In the designing of pharmaceutical drugs, gelatine is widely used as a shell or capsule because of "melt in mouth" quality. Gelatine is attained by partial hydrolysis of collagen molecule derived from skin, white connective tissue and bones of animals (Morrison et al., 1999). Within the last decade, the most abundant sources of gelatine are from pig skin (46%), bovine hide (29.4%), pork and cattle bones (23.1%) and fish (<1.5%) (Gómez-Guillén et al., 2009). This has increased the awareness of religious (particularly Muslim) and vegetarian consumers to avoid the prohibited gelatine material (Karim and Bhat, 2008). Thus, the alternative materials such as carrageenan, alginate and agar have been introduced to replace gelatine (Murano, 1998). Carrageenan is one of the most potential and interest material due to the similar properties to gelatine, such as thermo reversible, elasticity and syneresis.

Carrageenan is classified as a polysaccharide consists of a group of sulphated galactans. It can be extracted from several different species of seaweed. For example, red algae (Rhodophyta) produce galactans (e.g. carrageenans and agars) and brown algae (Phaeophyceae) produce uronates (alginates) (De Ruiter and Rudolph, 1997). East coast of Sabah, Malaysia, is abundant of *Eucheuma cottonii* and has been largely cultivated for the last three decades (Lee, 2008). This seaweed quality is unique, as it contains almost pure kappa carrageenan and less than 10% of iota carrageenan (Lee,

2008). At present, carrageenan is commercially used in pharmaceutical, food and cosmetic industry as viscosity builder, gelling agent and stabilizer. Carrageenan present in three different isomers, which are kappa (κ), lambda (λ) and iota (ι). In common, seaweed cannot produce the pure kappa carrageenan, but will produce the mixture or range of hybrid structures. There are several other carrageenan isomers such as xi, Θ, β, μ and υ (Falshaw R, 2001). The precursors of μ and υ can be transformed into κ and ι respectively, when exposed to alkali treatment through the formation of the 3, 6-anhydro-galactose bridge (Rudolph, 2000).

The gelation property of carrageenan is dependent on the salts and the ionic strength of the medium. Potassium is identified to support the gelation of kappa carrageenan (Morris ER; Rees DA, 1982). The rheological properties of the gelling carrageenan of kappa carrageenan are its forms gels that are hard, strong and brittle at the molecular level. Hydrogen bonding occurred during the crosslinking process between kappa carrageenan and crosslinker (glyoxal or glyoxylic acid) to form a rigid structure that may be important in designing for hard kappa carrageenan capsule. Crosslinked kappa carrageenan trough hydrogen bond leads to a strong network formation of carrageenan capsule. This work will focus to conduct a Crosslink process for commercial kappa carrageenan to find the best crosslinker material (glyoxal or glyoxylic acid) which able to provide the best encapsulate on properties.

1.2 Problem Statement

This research is being conducted to identify whether the crosslinker used such as glyoxal and glyoxylic acid is ideal for the production of the hard kappa carrageenan capsules. This is because, the crosslinker is quite affordable compared to the other crosslinker (e.g. Genipin). Based on the previous research, genipin has been used as a crosslinker because of the low toxicity contained in it, but the issue is the current market for genipin's price is very expensive. Based on the sigmaaldrich.com, the current price for genipin, glyoxal and glyoxylic acid is RM502.54/ 25 mg, RM1.05/ 25 mg and RM0.02/ 25 mg respectively. So, it can be seen that the price of genipin is very expensive among others. It can be conclude that the production of capsules based on

plant will be expensive too. As alternatives, both crosslinker glyoxal and glyoxylic acid will be used and test to the kappa carrageenan in this study.

1.3 Research Objective

The main objective of this study is to find the best crosslinker material between glyoxal and glyoxylic acid which able to provide the best encapsulate properties through molecular recognition approach by using crosslinking technique. The capability of intermolecular interaction to form between the crosslinking agent and kappa carrageenan such as hydrogen bonding will be investigated.

1.4 Scope of Study

The scope of this study is to identify the best crosslinker material between glyoxal and glyoxylic acid in order to produce hard kappa carrageenan capsules. The selection of crosslinking agents is based on the low toxicity because high toxicity is not suitable in food application and poisoned. At first, kappa carrageenan (3% w/v) will be reacted with a certain amount of glyoxal and glyoxylic acid (25%) respectively with 0.1N HCl as catalyst at 40°C. Once the preparation of hydrogel is done, the kappa carrageenan hydrogel will be characterized for their physicochemical properties and molecular characteristic using FTIR, DSC and TGA. In addition, different solvents are used for the swelling test study such as distilled water, buffer solution pH 1.0, 7.0 and 13.0. Nevertheless, concentration, temperature and also the amount of crosslinking agent will be varied.

1.5 Expected Outcome

In this research, it is expected that the molecular structure of crosslinked kappa carrageenan hydrogel with glyoxal and glyoxylic acid, respectively can be successfully identified by using FTIR spectroscopy analysis. Besides, the value of the swelling degree will shows the best crosslinker in this study. The obtained results have shown

that the glyoxal is the best crosslinker compare to glyoxylic acid even the price of glyoxylic acid more cheap compare to glyoxal. It can be proven when glyoxal react with kappa carrageenan and produce a lower swelling degree comparable to glyoxylic acid. Other than that, glyoxal crosslinked kappa carrageenan also form more hydrogen bonding based on FTIR analysis.

1.6 Significance of Study

This study will focus on investigating and identifying the best crosslinker to produce hard kappa carrageenan capsules. This research will give the benefit of the development of economics in drug delivery system. This situation will be seen as there has awareness among Muslim people and vegetarian nowadays that desired vegan hard capsule rather than gelatine capsule. So, this research will provide a new alternative for replace gelatine as hard capsule by using kappa carrageenan as one of the plant based material that suitable in drug delivery system. In addition, glyoxal and glyoxylic acid has been chosen as the crosslinker to produce strong hydrogen bonding with kappa carrageenan.

1.7 Summary

In conclusion, this study is about synthesis and characterization of hydrogel for hard kappa carrageenan capsule through glyoxal and glyoxylic Acid Crosslinking. Thus, this research will investigate and identify the best crosslinker material between glyoxal and glyoxylic acid which able to provide to provide the best encapsulate properties through molecular recognition approach by using crosslinking technique.

2 LITERATURE REVIEW

2.1 Overview

This paper presents a synthesis and characterization of hydrogel study for hard kappa carrageenan capsule. Hydrogel were prepared by using crosslinking technique and glyoxal and glyoxylic acid as the crosslinker. The hydrogel characterization was performed by using some instrumentation in the FKKSA laboratory. FTIR was used in order to characterize the molecular structure of the hydrogel. Besides, thermal characterization such as melting point and weight loss of the hydrogel were performed by using DSC and TGA. Last but not least, swelling degree was conducted using different solvents such as distilled water and buffer solution (pH 1.0, pH 7.0 and pH 13.0) to select the best crosslinker material between glyoxal and glyoxylic acid.

2.2 Introduction

People nowadays realize about the importance of Halal products in market particularly, Muslim and vegetarian. World Muslim and vegetarian population are increasing annually. They desire for Halal and plant-based pharmaceutical drug capsule. Gelatine is widely used for capsule in pharmaceutical industry. Kappa carrageenan has been touted as a viable alternative for the replacement of gelatine in production of capsules. It is evaluated as the most potential material which exhibits the similar properties to gelatine in the drug design process. It can be extracted from the commercial red seaweed *Eucheuma cottonii* which is plenty in coastal of Sabah, Malaysia. To alleviate this issue, researchers were developing new alternatives plant-based material capsules using suitable cross-linkers as an agent (i.e. genipin, glutaraldehyde, among others) for the crosslinking method. In this work, glyoxal and glyoxylic acid has been chosen as the cross-linker agent due to the similarity in chemical structure to genipin, less toxicity, less expensive and suitable for capsules design. Therefore, kappa carrageenan will be crosslinked through the formation of hydrogen bonding to manipulate the physicochemical properties.

2.3 Capsule

2.3.1 Definition

Capsule in the English language is derived from the Latin word *capsula* which means a small box or container. The word occurs in many scientific disciplines ranging from anatomy, as an enclosing membrane and botany as a descriptive for fruit to astrophysics, as a space vehicle. In pharmacy, capsule is the most versatile of all dosage forms. Capsules are pharmaceutically elegant dosage forms offering and improved drug stability, because the content is tightly enclosed by the capsule shell and thus protected from oxygen, moisture and light and also from physiological fluids until the drug is released (Bussemer and Bodmeier, 2003).

2.3.2 Types of Capsule

There are two types of capsules, ‘hard’ and ‘soft’. The hard capsule is also called ‘two pieces’ as it consists of two pieces in the form of small cylinders closed at one end, the shorter piece is called the ‘cap’ which fits over the open end of the loner piece called the ‘body’. The soft gelatine capsule is also called as ‘one piece’. Capsules are available in many sizes to provide dosing flexibility. Unpleasant drug tastes and odours can be masked by the tasteless gelatine shell. The administration of liquid and solid drugs enclosed in hard gelatine capsules is one of the most frequently utilized dosage forms.

2.3.2.1 Hard Capsule

The hard two-piece capsule was first patented in 1846 and has been manufactured on an industrial scale since 1870s (Jones, 1987). Hard capsule is usually filled with solid materials, but some drugs require a liquid formulation for solubility or bioavailability reasons (Savio et al., 1998). The liquids may often be absorbed onto inert carrier powders to form dry powders suitable for capsule filling. There are many advantages of hard capsules usually called hard gelatine capsules such as tasteless and odourless, swallowing is easy, flexibility in formulating, uniquely suitable for blinded clinical trials and also useful for extemporaneous compounding by the pharmacist.

Instead of all the advantages, there are also some disadvantages consume hard capsules such as tend to be more expensive to produce than tablets and it is not suitable for highly soluble salts.

Figure 2-1: Hard gelatine capsule

Source: Capsuland.com

2.3.2.2 Soft Capsule

Soft capsules are single unit dosage forms, consisting of a liquid or semi-solid fill enveloped by one piece sealed elastic outer shell. The amount of drug or extract together with adjuvant is enclosed within a globular, oval or other shape of a soft shell (Habiba and Munyendo, 2011). The soft gel can contain the active ingredient in solution, suspension or emulsion which will inherently lead to better absorption of the active ingredient as compared with delivery in a tablet or as a powder (Bussemer and Bodmeier, 2003). The advantages of the soft capsule in a pharmaceutical industry are easy to use where easy to swallow, no taste, unit dose delivery and versatile which is accommodated a wide variety of compounds filled as a semi-solid, liquid, gel or paste and also a wide variety of colours, shape and sizes (Bhatt, 2007). Soft capsules have some disadvantages such as requiring special manufacturing equipment, stability concerns with highly water soluble compounds susceptible to hydrolysis and lastly limited choices of the excipients or carriers compatible with gelatine (Bhatt, 2007).

Figure 2-2: Soft gelatine capsule

Source: Capsuland.com

2.4 Gelatine

The name gelatine is derived from the Latin word *gelata* which describes its most characteristic properties such as gel formation in water. Gelatine is obtained by thermal denaturation or physical and chemical degradation of collagen, the most widespread protein in the body occurred in most connective tissue as the skin, tendon and bone (Bigi et al., 2002). It is derived from the parent protein collagen by processes that break up the secondary and higher structures with varying degrees of hydrolysis of the polypeptide backbone. Besides, it also can be produced from both mammalian and porcine sources but the physical properties of this gelatine are different (Philips and Williams, 2011).

Figure 2-3: Gelatine

Source: gelatine.org

2.4.1 Functional Properties of Gelatine

Gelatine is a protein product produced by partial hydrolysis of collagen extracted from skin, cartilage, ligaments and others. There are two types of gelatine with different characteristics including type-A, acid treated collagen (isoelectric point at pH 6-9) and type-B, an alkaline treated (isoelectric point at pH 5) where acid treatment is suitable for less fully crosslinked collagen commonly found in pig or fish skins, whereas alkaline treatment is appropriate for the more complex collagens found bovine hides (Mehraj and Soottawat, 2011). Efficiency of gelatine extraction depends on the method, in which collagens are pre-treated. The natural molecular bonds between individual collagen strands are broken down into a form that rearranges more easily. Gelatine melts when heated and solidifies when cooled again. Together with water it forms a semi-solid colloidal gel (Bhatt, 2007).

On a commercial scale, gelatine is made from by-products of the meat and leather industry, mainly pork skins, pork and cattle bones or split cattle hides. Contrary to popular belief, horns and hooves are not commonly used. The raw materials are prepared by different curing, acid and alkali processes which are employed to extract the dried collagen hydrolysate (Bhatt, 2007). Gelatine is a denatured fibrous protein derived from collagen by partial thermal hydrolysis. Furthermore, different types of gelatine have different thermal and rheological properties such as melting and gelling temperatures and bloom strength (Mehraj and Soottawat, 2011). There are also some unique properties of gelatine which are 'melt-in-the-mouth' property, thermal reversible, versatile or multifunction hydrocolloid, tailor made application and easy to use (Karim and Rajeev, 2008).

2.4.2 Functional Uses of Gelatine

Gelatine is one widely used raw materials in foods, pharmaceutical, medical, cosmetic products and photographic industries (Yudi et al., 2007). It was extracted from bones, fat, meat waste, used cooking fats and oils of animals (Sahilah et al., 2012). It is a hydrocolloid products which are special and unique, serving multiple functions with a wide range of applications in various industries including food ingredient as gelling, foaming agent, thickener, plasticizer, emulsifier, foaming agent, moisture retention,

improve texture and binding agent. All the characteristics have made gelatine widely used in dairy and bakery products (Karim and Rajeev, 2008). In fitness product, gelatine has been used due to its easily digestible, low in calories and contains no cholesterol. On the other hand, in the pharmaceutical industry, gelatine is used as hard and soft capsules, sugar coated pills, tablets, serum substitute and vitamin encapsulation. The use of gelatine in pharmaceutical is inevitable because it helps to protect the medicines against harmful influences such as light and oxygen. The soft capsules for instance are mainly used for powders. Besides, gelatine capsules have been developed and used in pharmaceutics since the early 19th century and the technology has remained essentially unchanged (Zhang et al., 2013).

2.4.3 Rationale for Developing Gelatine Alternatives

Religious and vegetarian lifestyle choices may prohibit consumer groups from eating all products food that containing gelatine, an animal-based ingredient. The main sources of gelatine are generally bovine and porcine skins and bones (Mehraj and Soottawat, 2011). The global demand for gelatine has been increasing over the years. Recent reports indicate that the annual world production of gelatine is nearly 326,000 tons in 2007, of which 46% were from pig skin, 29.4% of bovine hides, 23.1% from bones and 1.5% from other parts (Karim and Rajeev, 2008). Production of gelatine from pig skins is not acceptable for Judaism and it is also 'haram' for Muslims. Gelatine from cattle is acceptable only if it has been prepared according to religious requirements. Thus, to cater to this market segment, the development of gelatine alternatives is highly desirable to food processors as the global market for foods certified halal is growing very rapidly. Furthermore, the world's population projected 30% of the Muslims by 2025 (Karim and Rajeev, 2008). Many gelatine alternatives proposed for the food industry are polysaccharides which gel based on cation induced junction zones and which do not have the defined melt-set characteristics of gelatine such as gellan, alginate or carrageenan-based gels. The polysaccharides based gelatine alternatives generally have less flexible molecular backbones, leading to higher viscosities than gelatine (Morrison et al., 1999). In this study, carrageenan is one of the type polysaccharides will be studied in order to produce hard kappa carrageenan capsules.

2.5 Carrageenan

Carrageenan is a water soluble hydrocolloid derived from red seaweed. It is found in the cell wall of the seaweed from which it is derived and its content can vary from species to species and seasonally. It is harvested from various regions of the of the world including the northern part of the US, Philipines, Indonesia, Chile, Argentina, Morocco, France and the latest found in East Coastal of Sabah. The most common species of carrageenan used commercially are *Chondros Crispus, Gigartina* and *Eucheuma cottonii*.

Red and brown seaweed provides many different types of hydrocolloids for the food industry. Brown seaweed species provide alginate food gums where the red seaweed family, *Rhodophycae*, provides the polysaccharides agar, carrageenan and furcellaran. The main species of the *Rhodophycae* family include *Eucheuma cottonii* and *Eucheuma spinosum* which carrageenan gum is extracted from. *Eucheuma cottonii* produces kappa carrageenan, *Eucheuma spinosum* yields iota carrageenan and *Chondrus crispus* yields both kappa and lambda carrageenan, although the same plant does not produce both types. Individual plants that grow together will produce both types carrageenan (McCandles, 1973).

Figure 2-4: Red seaweed

Source: www.seaweed.ie

Carrageenan represents one of the major texturising ingredients in the food industry especially. They are natural ingredients, which are used for decades in food applications. It is a generic name for a family of polysaccharides, obtained by extraction from certain species of red seaweeds which is *Rhodophyta*. Since natural carrageenan mixtures of different sulphated polysaccharides, their composition differs from batch to batch. Therefore, the quantitative analysis of carrageenan batches is of greatest importance for both ingredient suppliers and food industries to deliver a constant consumer product and to develop new applications based on their unique intrinsic properties (Van de Velde et al., 2002).

2.5.1 Structure of Carrageenan

Carrageenan has a linear back bone of repeating galactose units with different proportions and locations of ester sulphated groups and 3,6-anhydrogalactose (anhydrogalactose bridges). Carrageenan is one of the two food gums that are naturally sulphated, furcellan being the other. Varying compositions provide different rheological behaviour, ranging from viscous thickeners to thermally reversible gels, which range in texture from soft and elastic to firm and brittle. There are three isomers of carrageenan which are kappa (κ), iota (ι) and lambda (λ) (Anon, 1988).

Besides, the other three isomer forms found in carrageenan are mu (μ), theta (Θ) and nu (υ) which are precursors to kappa and iota forms and a successor to lambda respectively (Rees, 1963). The number of ester sulphated groups and bridge present on the backbone distinguishes kappa, iota and lambda carrageenan from one another. These variations allow different rheological properties to be attained as well as influencing hydration, gel strength, texture, syneresis, synergism, melting and setting temperatures. The differences among these carrageenan types are due to the type of seaweed, as well as the processing and blending processes. The sulphate groups make the carrageenan molecule more water soluble whereas the anhydro bridges inhibit water solubility because of its natural hydrophobic properties (Hoefler, 2001). Kappa carrageenan has one sulphated group and one anhydro bridges for every two galactose molecules. Iota carrageenan has a similar structure however it possesses an additional support groups, making iota carrageenan more water soluble then kappa. Lambda, which is the most

water soluble of the three consist of three sulphate groups and no anhydro bridges for every two galactose molecules. For simplicity of categorizing carrageenan, it is described as extracts from *Rhodopyceae* which contain an ester sulphate content of 20% or above and are alternatively α-(1,3) and β-91-4) glycosidally linked (Anon, 1988).

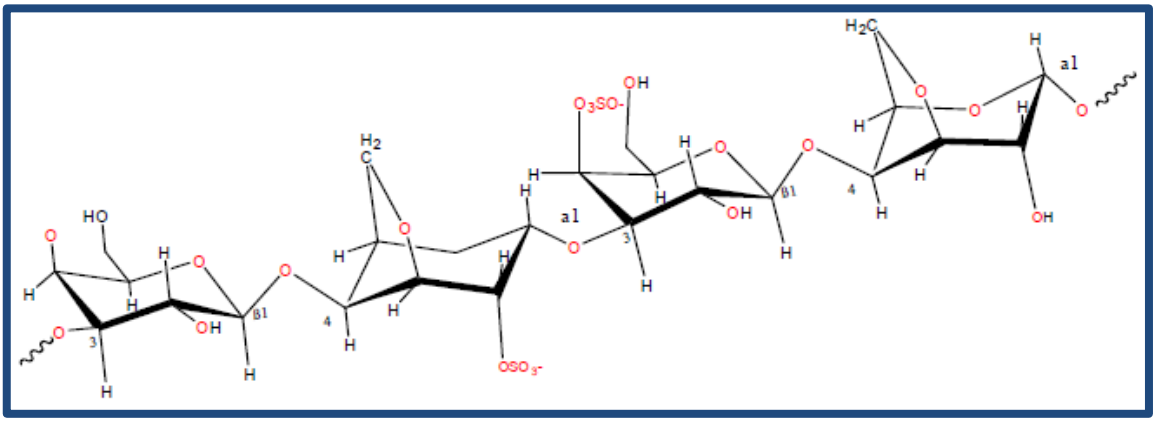

Figure 2-5: Structure of Carrageenan

Source: Farnoosh Kianfar, 2011

2.5.2 Types of Carrageenan

There are three forms of carrageenan which are kappa, iota and lambda. Each of carrageenan types are determined by the number and position of the sulphate groups on each sugar and the presence or absence of the 3,6-anhydro group. The 3,6-anhydro group promotes α helix formation which is important for gelling (Lamond, 2004). This is a result of increased flexibility that promotes a random coil structure. The conformation of the glycosidic bond will changes to equatorial (Therkelsen, 1993).

2.5.2.1 Kappa Carrageenan

Kappa carrageenan is one of the three most important commercial forms of carrageenan and is widely used in biomedical, food and non-food applications (Charito et al., 2012). Kappa carrageenan is linear polysaccharides sulphated galactan extracted from red seaweed (Rhodophyta) such as Kappahycus alvarezii (known as *Eucheuma cottonii* contains almost pure kappa carrageenan, with less than 10% iota carrageenan

(Lee et al., 2008). This natural polymers comprise of repeating units of (1,3)-D-galactopyranose and (1,4)-3,6-anhydro-α-D-galactopyranose with sulphate groups in the certain amount and position (Campo et al., 2009). It has approximately 25% ester sulphates and 34% anhydro bridges with iota having approximately 32% ester sulphated and 30% anhydro bridges (Moraino, 1977). Lambda has the highest amount of ester sulphated at 35% with little or no anhydro bridges. On the other hand, kappa carrageenan when used with potassium cation forms a brittle gel (Imeson, 2000). This gel is subject to syneresis which causes gelling shrinkage due to the loss of fluid. Because of this, kappa carrageenan exhibits very poor potential good freeze-thaw stability. Different combinations of kappa and iota carrageenan can be made in order to have a range of gelling texture which in turn can exhibit good freeze-thaw stability as well as moisture binding (Imeson, 2000).

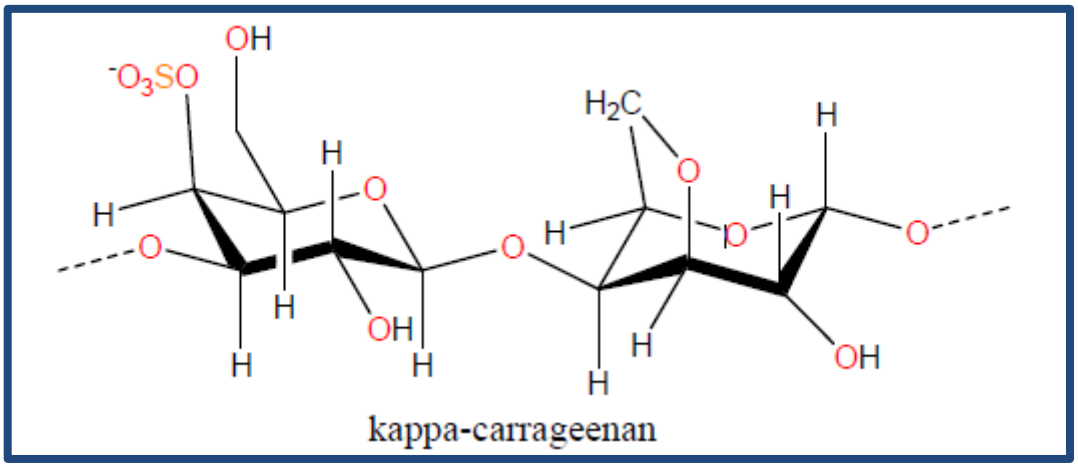

Figure 2-6: Structure of Kappa Carrageenan

Source: William, 2011

2.5.2.2 Iota Carrageenan

Iota carrageenan, also a right handed double helix of parallel chains, contains two sulphate groups per repeat dimer, located one on each of the sugar units. Iota carrageenan forms strong, elastic, thermo reversible gels with limited syneresis. Calcium forms ionic bridges between iota carrageenan chains, yielding gels with increased gelling and melting temperature (Whistler, 1997).

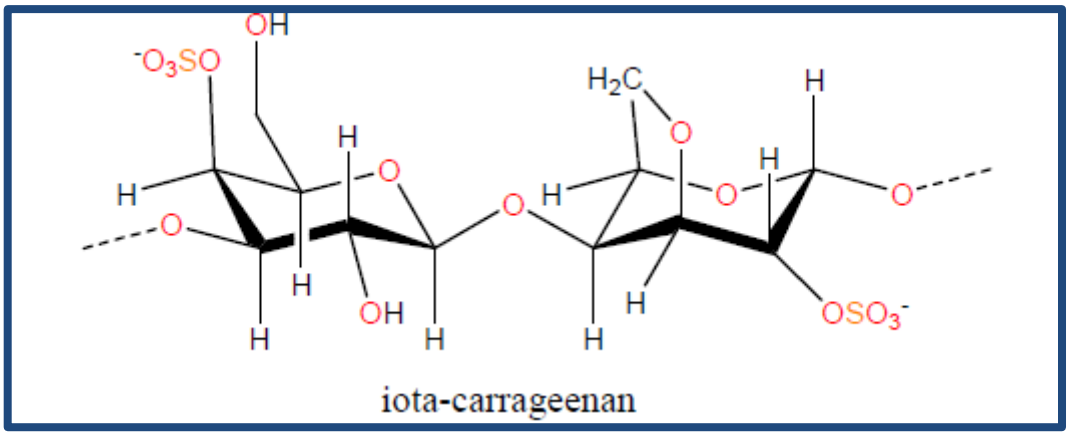

Figure 2-7: Structure of Iota Carrageenan

Source: William, 2011

2.5.2.3 Lambda Carrageenan

Non-gelling lambda carrageenan contains three sulphated groups with repeating dimer units of D-galactose-2-sulphate-D-galactose-2,6-disulphate. It does not contain the 3,6-anhydro group necessary to form the double helix. Lambda carrageenan does not form gels but is widely used as a viscosifier in many food applications (Whistler, 1993).

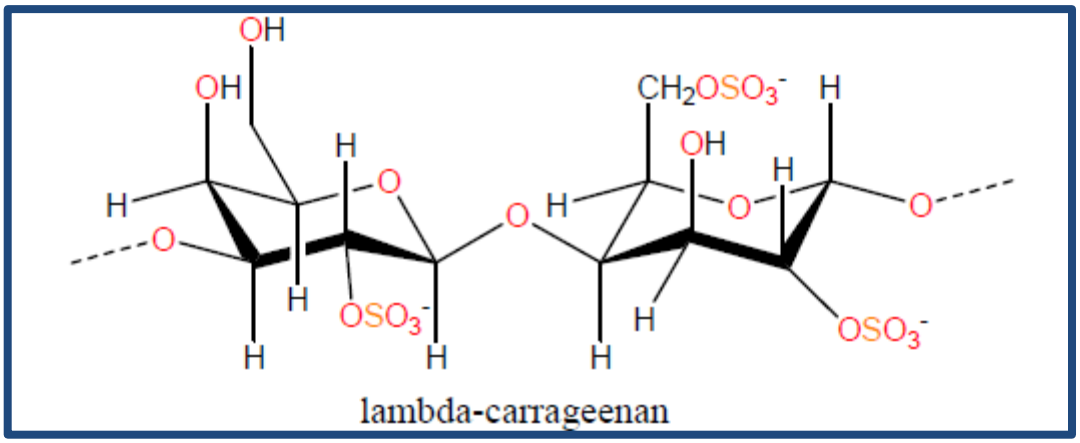

Figure 2-8: Structure of Lambda Carrageenan

Source: William, 2012

2.5.3 Properties of Carrageenan

Each type of carrageenan has its own physical properties. All carrageenan types are hot water soluble however not all of them are soluble in cold water. Only lambda is fully soluble in cold water in addition to Na^+ salts of kappa and iota. Lambda carrageenan produces a viscous solution which exhibits pseudoplastic characteristics when it is pumped or stirred. Usually, it is used for thickening in order to provide body to a product. When heating a carrageenan solution, the required temperature of hydration depends on factors such as concentration of carrageenan, the cation associated with it as well as the cation present in the food system. In most food products, full hydration of kappa and iota achieves temperatures above 70°C (Moirano, 1977).

Carrageenan is most stable at neutral and alkaline pH even at increased temperature. However, if a pH drops 4.5, carrageenan solutions will lose viscosity and gel strength. This occurs because of auto hydrolysis which is due to the acid form of carrageenan cleaving the glycosidic linkages (Moirano, 1977; Hoffmann, 1996). At elevated temperatures and low cation concentrations, this process occurs even quicker. An exception to this is when kappa and iota carrageenan are used in low pH food systems when gelling is induced prior to a decrease in pH. Once the carrageenan is in a gelled state, the secondary and tertiary structures protect the glycosidic linkages from cleavage as well as the cation being unavailable (Moirano, 1977; Imeson, 2000).

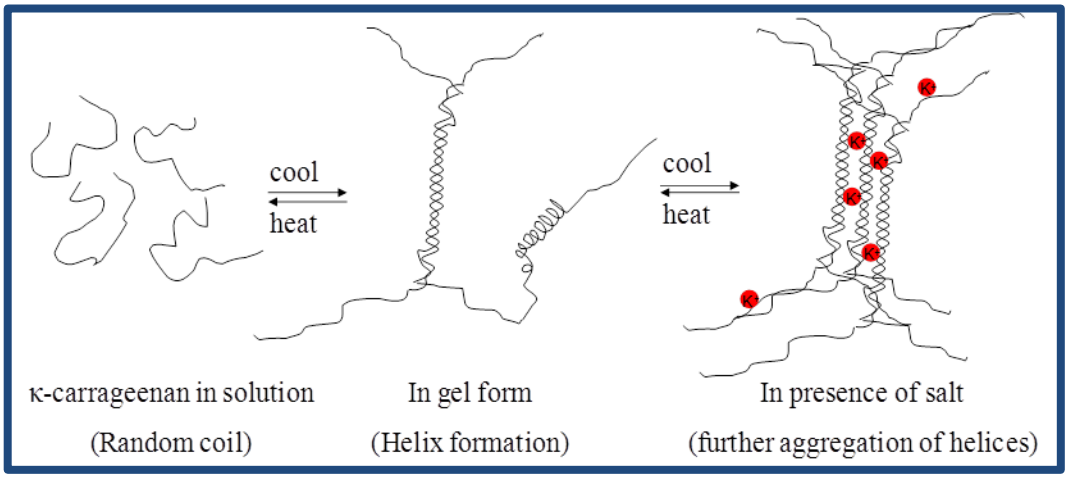

Figure 2-9: Double helix formation of Carrageenan upon cooling

Source: Peng Wu, 2012

Kappa and iota carrageenan is capable of forming a range of gel textures upon cooling of a hot solution. The gel textures depend on the cation used to induce gelling. The gels are thermally reversible as they are melt 5-20°C above the gelling temperature and re-gel upon cooling. It is believed the ability of gelling is due to double helix formation (Anderson, 1969; Glicksman, 1979; Hoefler, 2001). Kappa carrageenan selects potassium ions to stabilize the junction zones within the characteristically firm, brittle gel. Potassium ions counter sulphate charges without strictly hindering close approach and double-helix formation as shows in **Figure 2.9** (Peng Wu, 2012). When the temperature is above the melting point of the gel, random coils are present because thermal agitation overcomes the ability to form helices. Upon cooling, double helices form junction zone which produces a three dimensional network. Further cooling induces aggregation of these junction zones by hydrogen bonding of adjacent double helixes (Rees, 1969; Moraino, 1977; Hoefler, 2001). Sulphation of carrageenan can explain some of the gelling properties. The sulphated group in lambda carrageenan acts as a 'wedging group' which prevents the double helix from forming. However the sulphated on the anhydro bridges of iota projects outward and does not interfere with double helix formation. This is also true for kappa carrageenan through the sulphate group is located on the 1,3-linked galactoside (Moraino, 1977). The higher degree of anhydro bridges the better the gelling properties (Moraino, 1977) as it increased the capability of forming double helices.

The type of cation used to induce gelling is extremely important in the gelling properties of carrageenan. The most common cation used in food applications are K^+, Ca^+ and NH_4^+. These cations influence the hydration temperature as well as the setting and melting temperatures. Carrageenan exhibits hysteresis which means there is a difference between the gelling and re-melting temperature of carrageenan. The melting temperatures are always higher than the gelling temperatures due to the need of extra energy to disrupt the existing gel network. The number of sulphate group, anhydro bridges and final texture is presented below in the **Table 2.1**.