PROPERTIES OF ESTERIFIED κ-CARRAGEENAN AND ESTERIFIED κ-CARRAGEENAN-ZIRCONIA COMPOSITE MATERIAL

By

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LIST OF ABBREVIATIONS

FTIR	Fourier transform infra-red
^{1}H-NMR	Nuclear magnetic resonances for proton
DSC	Differential scanning calorimetric
DTG	Differential thermogravimetric
GPC	Gel permeation chromatography
DS	Degree of substitution

LIST OF SYMBOLS

M_n	Number average molecular weight
M_w	Weight average molecular weight
MP	Viscosity average molecular weight
T_g	Glass transition temperature
T_{max}	Maximum degradation temperature
Cp	Heat capacity at constant pressure
T_m	Melting temperature

SIFAT ESTER κ-KARAGEENAN DAN BAHAN KOMPOSIT ESTER κ-KARAGEENAN-ZIRKONIA

ABSTRAK

Suatu kaedah baru untuk pengesteran κ-karageenan tanpa penggunaan air sebagai pelarut telah dihasilkan. Dalam kaedah ini, piridina telah digunakan sebagai pelarut dan mangkin dalam sintesis ini. Kesan suhu dan jumlah asil klorida terhadap proses esterifikasi telah dikaji secara sistematik. Pencirian menggunakan FTIR dan NMR menunjukkan pembentukan ester pada semua sampel. Suhu peralihan kaca, T_g, bagi ester κ-karageenan telah dikaji menggunakan DSC dan keputusan menunjukkan kesemua ester κ-karageenan yang disintesis mempunyai suhu peralihan kaca yang lebih rendah daripada κ-karageenan. Dengan menggunakan spektrum NMR, darjah penukargantian kumpulan asid didapati paling tinggi pada suhu 90 ^oC berbanding dengan suhu tindak balas lain. 0.175 mol juga dikenalpasti sebagai amaun asil klorida yang optima bagi menghasilkan ester karageenan dengan darjah penukargantian yang tinggi. Semua ester karageenan menunjukkan keterlarutan yang baik dalam toluena tetapi tidak dalam air. Penulenan lanjut ester κ-karageenan dengan menggunakan natrium bikarbonat dan toluena menghasilkan ester κ-karageenan yang lebih tulen. Ester κ-karageenan yang disintesis tanpa penulenan lanjut telah digunakan dalam penyediaan komposit menggunakan teknik sonikator. Komposit yang terhasil tidak larut dalam air dan toluena serta menunjukkan ciri-ciri termal yang lebih baik berbanding dengan ester κ-karageenan.

PROPERTIES OF ESTERIFIED κ-CARRAGEENAN AND ESTERIFIED κ-CARRAGEENAN-ZIRCONIA COMPOSITE MATERIAL

ABSTRACT

A new method which does not involve the use of water as solvent for esterification of carrageenan, was developed to synthesize κ-carrageenan esters. Pyridine was used as solvent and catalyst in this synthesis. The effect of temperature and amount of decanoyl chloride to the esterification process was studied systematically in this work. Characterisation by Fourier Transforms Infra-Red Spectrometer (FTIR) and Nuclear Magnetic Resonances Spectrometer (NMR) techniques revealed the formation of ester bond in all samples. The glass transition temperatures, T_g of κ-carrageenan esters which were studied using Differential Scanning Calorimetry (DSC) and the result revealed that all the κ-carrageenan esters synthesized have lower glass transition temperature than the κ-carrageenan. By using ^{1}H-NMR spectra, the degree of substitution of acyl group was determined to be highest at 90 ^{o}C reaction temperature. The optimum amount of acyl chloride used to prepared κ-carrageenan ester with the highest degree of substitution was determined to be 0.175 mol. All κ-carrageenan esters show good solubility in toluene but not in water. Further purification of the esters using sodium bicarbonate and toluene yield a purer κ-carrageenan ester. κ-carrageenan ester synthesized without further purification was used in the preparation of κ-carrageenan ester and zirconia composite using the sonication technique. The composite which is insoluble in water and toluene, showed better thermal properties as compared to κ-carrageenan esters.

1. Introduction

Natural anionic polysaccharides (carrageenan) are mainly obtained through water or alkaline extraction from red marine algae. Common grade of commercially available carrageenan are refined carrageenan and semi-refined carrageenan. The differences between the two carrageenans are the method used to obtain the carrageenan and their purification process. Refined carrageenan sometimes is also known as filtered carrageenan. It is obtained through alkaline extraction which is followed by filtration and precipitation using alcohol or potassium chloride. Potassium chloride is used in the precipitation process of κ-carrageenan, a type of carrageenan with one sulphate group at its galactopyranose monomer. It is so because κ-carrageenan readily forms gel in the presence of potassium ion (Towle, 1976).

Meanwhile, semi-refined carrageenan which is also known as seaweed flour can be obtained through a more cost effective process. The freshly harvested seaweed is subjected to hot potassium hydroxide solution. The potassium ion combines with carrageenan to form gel in the seaweed and this prevent it from dissolving in hot solution while protein, carbohydrate and salt is extracted out from the seaweed. The seaweed is then dried and grinded to powder. Thus, semi-refined carrageenan contains high content of cellulose (McHugh, 2003).

Malaysia has been involved in the cultivation of seaweed since the 70s in Semporna. Recently, Malaysian government has been actively promoting the cultivation of seaweed as it is one of the most important food farming commodities for the country in 2010 Budget. Universiti Malaysia Sabah is assisting the local seaweed industry to make Malaysia as one of the global seaweed producer in the near future by introducing the mini estate concept (Majaham, 2010). Currently, Sabah being the main producer of seaweed in the country has seaweed estates located at Semporna, Lahad Datu, Kudat and Kunak. Sabah is focusing on the cultivation of *Kappaphycus alvarezii* and *Euchema spinosu*m species of seaweed which are the main sources for κ-carrageenan.

This research work is an effort to enhance the commercial value of seaweed, as κ-carrageenan was chosen as the starting material for this work. All carrageenan fractions were water soluble but insoluble in organic solvents (Campo *et al*., 2009). Carrageenans can be categorized into different types according to the number and position of its sulphate group. The three main types are (i) kappa (κ), (ii) iota (ι) and (iii) lambda (λ), Figure 1.1. κ-carrageenan has approximately 25% sulphate content with a sulphate group for every two anhydroglucose units.

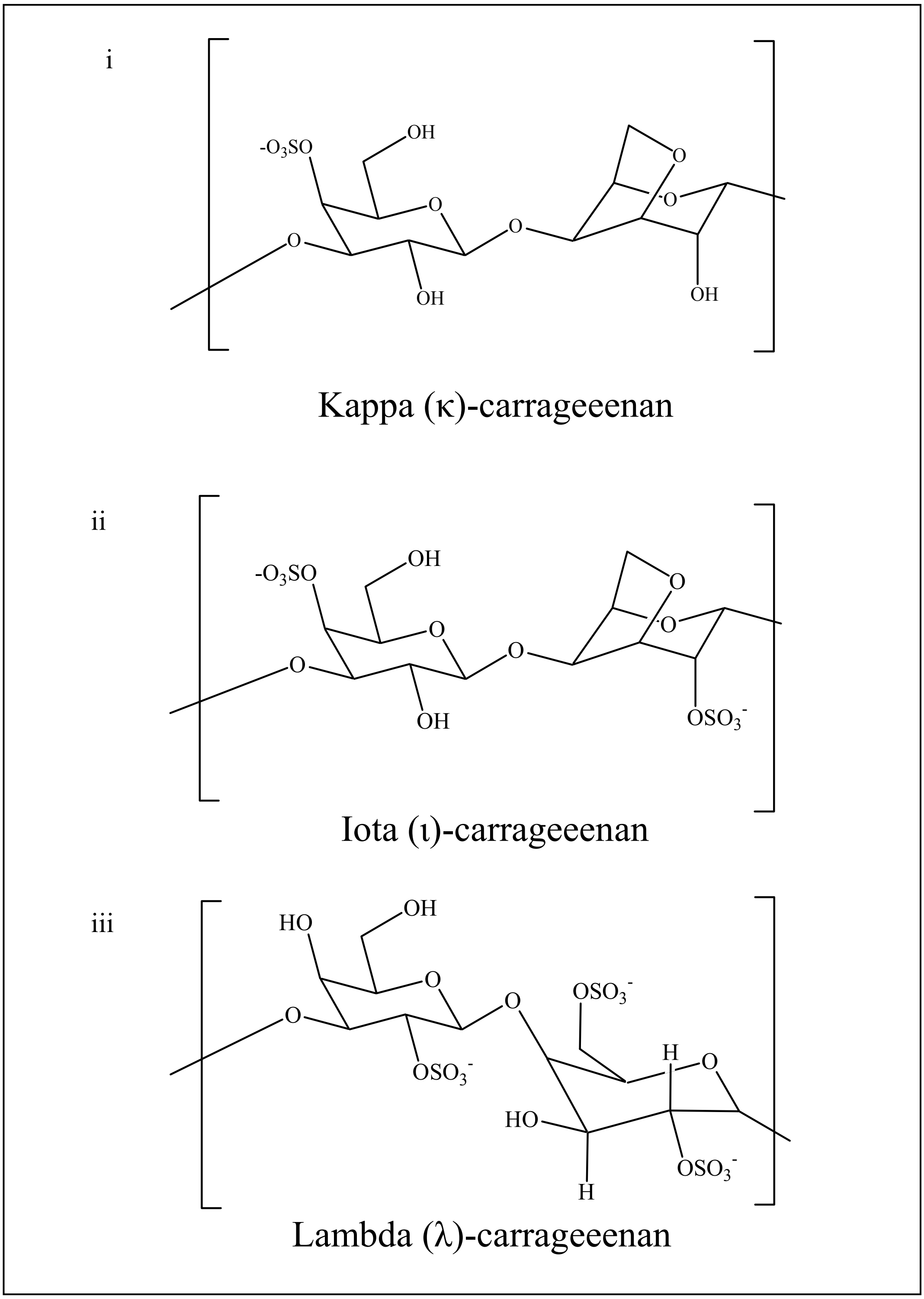

Figure 1.1: The chemical structures of (i) kappa (κ), (ii) iota (ι) and (iii) lambda (λ) carrageenan.

Limited natural resources and serious environment problems have lead to the development of biodegradable material as a substitute for non-biodegradable petrochemical-based counterpart (Siracusa *et al*., 2008). Carrageenan can be a good source of biodegradable materials. Chemical structures, characteristics and application of carrageenans has been widely studied by many researchers (Abad *et al*., 2011, Freile-Pelegrín *et al*., 2011, Prasad and Kadokawa, 2010). Carrageenan has found its applications in many non-food products, such as pharmaceutical, cosmetics, printing and textile formulation besides its traditional application in food industry (Imeson, 2000).

There have been reports on the chemical modifications such as esterification, depolymerization, copolymerization and etheration to modify physicochemical of carrageenan. These modified carrageenans have also enhanced the application of carrageenans in non-food products (Pourjavadi *et al*., 2004, Pourjavadi *et al*., 2007, Sagbas *et al*., 2012, Fan *et al*., 2011, Yuan *et al*., 2005).

Attempts to increase the anti-oxidation activity of κ-carrageenan were carried out by addition of acetyl, sulfate and phosphate groups to κ-carrageenan. The relationship between chemical structure and properties of modified carrageenan against antioxidant activity were studied *in vitro*. It was found that oversulfated and acetylated derivatives, which scavange superoxide radicals exhibited significant anti-oxidation activities. The phosphorylated with low degree of substitution of acyl derivatives, which scavange hydroxyl radicals, and the phosphorylated derivatives, which scavenge 1,1-diphenyl-2-picrylhydrazy (DPPH) radicals also exhibited significant anti-oxidation activities in the systems examined (Yuan *et al*., 2005).

Carrageenan itself is also known to exhibit anti-HIV properties whereby anti-HIV activities can be increased by polymerization and sulfation while the anti-coagulant activities of carrageenan were preserved (Yamada *et al*., 1997). Further research has lead to the preparation of O-acylated low-molecular-weight carrageenans with potent anti-HIV activity and low anticoagulant effect through depolymerization and esterification process (Yamada *et al*., 2000). During their preparation, carrageenan was depolymerized before being acylated and sulfated. Acylated carrageenan exhibits higher anti-HIV activities as compared to the highly sulfated carrageenan.

Recently, research works done on carboxymethylated κ-carrageenan, as drug carrier and wound healing matrix with anticoagulant and antimicrobial properties have shown some success (Fan *et al*., 2011). The introduction of carboxymethyl groups into κ-carrageenan through alkaline activated etheration promotes the anti-coagulant activity, anti-bacterial activity, moisture absorbability and retention capacity.

Besides chemical modification, carrageenan has been used in preparation of composite material as well. New composite material composed of carrageenan and polymeric ionic liquids were prepared by *in situ* polymerization of ionic liquids having vinyl benzyl and acrylate group. These materials showed good mechanical and electrical conductivities properties comparable to semiconductor (Prasad and Kadokawa, 2010). The proposed incorporation of polymeric material such as natural polymer can also assist in reducing the demand on synthetic resource.

Inorganic-organic composite of carrageenan can be prepared through sol-gel method by impregnation of carrageenan gel with silica sol. Silica-pillaring helps to maintain the texture of carrageenan hydrogel during supercritical drying. The composite which was stabilized by silica nanoparticles has a void fraction higher than 90% which would be an added advantage for it to be used in drug-delivery system (Boissière *et al*., 2006).

Porous carrageenan with calcium phosphate nanocomposite scaffolds is yet another example of a novel material for the application of carrageenan. It was prepared by co-precipitation of calcium phosphate into carrageenan and followed by thermally induced gelification and lyophilization (Daniel-da-Silva et al., 2007).

Hence, chemical modifications and preparation of composite materials can be an excellent route to improve the biological, mechanical and physical properties of carrageenan.

1.1 Esterification process

Generally, esterification is the condensation reaction which involves acid and alcohol to yield esters. The acid involved may be an inorganic acid or an organic acid. During the reaction, at least one hydroxyl group from acid will be replaced by alkoxy group. Acetylation, sulfation and phosphorylation are examples of esterification process commonly conducted on carrageenans (Yamada *et al*., 2000, Yamada *et al.*, 1997, Yuan *et al*., 2005).

In the present work, esterification was the main route of chemical modification. Common esterification catalyst such as sulfuric acid and hydrochloride are very efficient for producing organic ester. However, it is not practical to employ these catalysts as there will be side reactions such as hydrolysis of carrageenan glucosidic backbone (Mullen and Pacsu, 1942, Yuan *et al.*, 2005). Hydrolysis which results in splitting of one of the glucosidic backbone linkage to form two carrageenan molecules of the same weight from a carrageenan with 1000 glucosidic linkage is only accounted for 0.1 percent of the total glucosidic linkage but the effect on the molecular weight is great. In addition, acidified water as the by-product of common esterification process by acid catalyst may also induce the lost of sulphate ester group in carrageenan.

In this present work, κ-carrageenan was esterified using modified Pascu method which employed pyridine as solvent and catalyst. κ-carrageenan was pretreated with pyridine at a minimum temperature of 60 ^{o}C to have a good dispersion of κ-carrageenan in pyridine. Figure 1.2, shows the proposed general scheme of reactions for the esterification of κ-carrageenan.

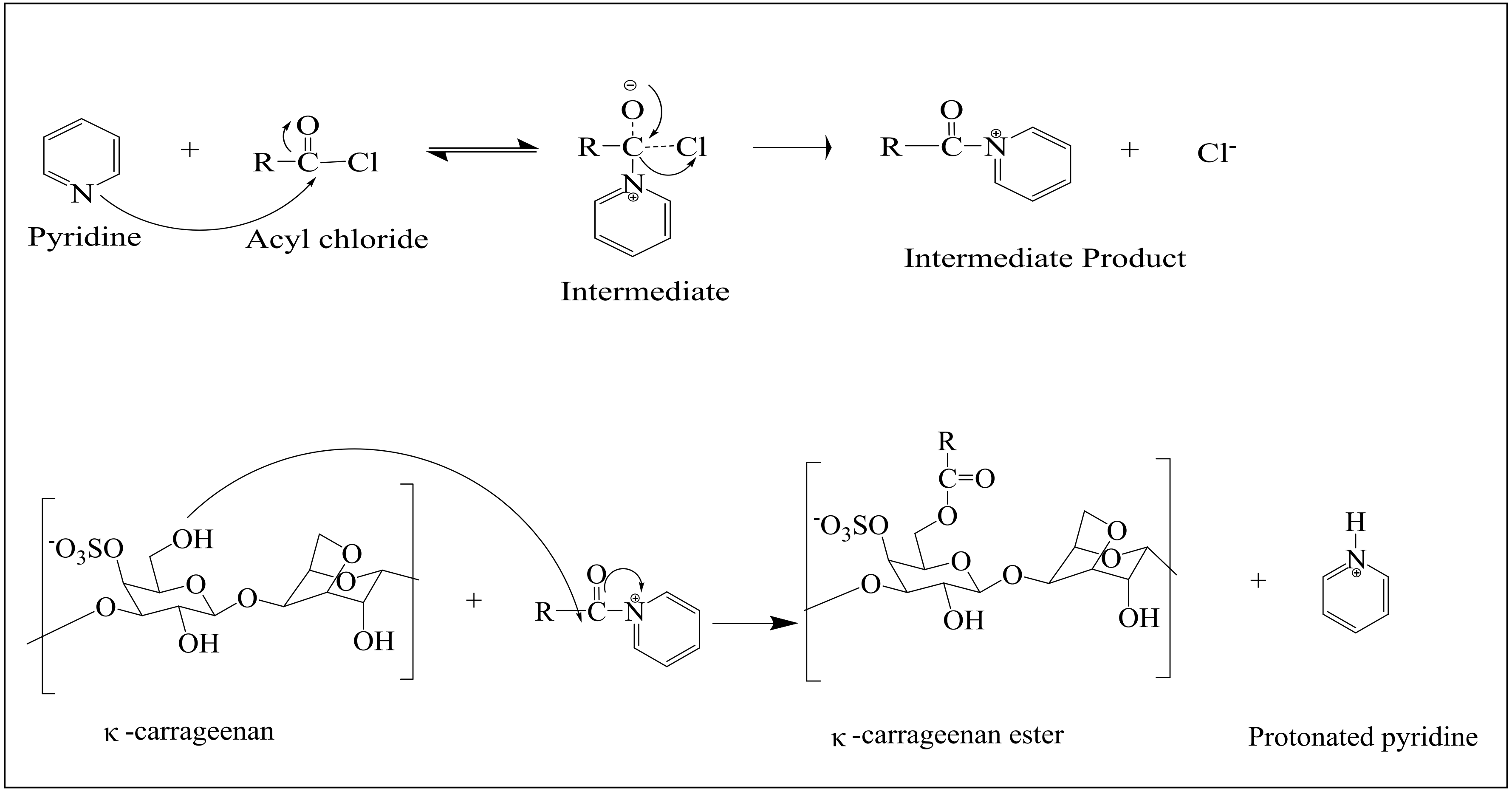

Figure 1.2: The proposed scheme for the esterification process.

During the esterification process, acyl chloride was reacted with pyridine to form an activated intermediate product by donation of lone pair electron from pyridine to the carbonyl group of acyl chloride followed by leaving of chloride ion (Bender, 1960). The hydroxyl group of κ-carrageenan then reacted with activated intermediate product to form κ-carrageenan ester. This was an aqueous free route to synthesized ester. Hydrochloric acid which was the by-product of the reaction was formed under the non-optimum condition of the synthesis. The hydrochloric acid was removed through continuous flow of nitrogen gas during the reaction while the pyridinium salt was removed through washing with ethanol.

Previously, there have been several attempts to form acetylate carrageenan (Jiang *et al*., 2007, Yamada *et al*., 2000, Yuan *et al*., 2005). In all these work, the carrageenan was subjected to depolymerization first, before undergoing acetylation. In Jiang (2007) and Yamada (2005) works, depolymerized carrageenan were converted to tetrabutylammonium salt before dissolving it in dimethylformamide for acylation process. Acid anhydride was employed as reactant while tributylamine and 4-dimethylaminopyridine acted as base catalyst.

1.2 Sol-gel Process

Polycarbohydrate normally has decomposition point lower than 270 ^{o}C (Raemy and Schweizer, 1983). However, the common route for preparation of inorganic composite required high temperature for the reaction to proceed. Thus, sol-gel process has been employed in the preparation of organic-inorganic composite material..

In sol-gel process, the reactants undergo chemical reactions that convert them from dispersions of colloidal particles in liquid which is known as sols to solid particles. The colloid particles form a continuous amorphous network through reaction within each others during the sol-gel process. The spaces whereby the solvent and reactant are entrapped in the continuous amorphous network may become pores of submicron dimensions as a result of drying (Niederberger and Pinna, 2009).

There are two general routes for sol-gel process described in the literatures. Those are aqueous and non-aqueous synthesis route (Niederberger and Pinna, 2009). Aqueous synthesis route consists of conversion of precursor solution into an inorganic solid via inorganic polymerization reactions induced by water. In non-aqueous sol gel synthesis, precursor solution conversion to gel takes place in organic solvent without the presence of water.

Sol-gel process is initiated by the hydrolysis of inorganic precursors followed by condensation, gelation, drying and densification. For the aqueous sol-gel synthesis, during hydrolysis, the alkoxide groups of metal alkoxide are substituted by hydroxyl group of water through nucleophilic substitution process to form metal hydroxide with the release of alcohol. Two hydroxylated metal species will then condense to become metal oxide with the formation of M-O-M bonds (M=metal) and the release of water. Meanwhile, the reaction of a hydroxide and an alkoxide will also form metal oxide but under the release of an alcohol (Niederberger and Pinna, 2009) . Following are the reaction steps that usually associated with sol-gel reaction.

Hydrolysis process, $\equiv M - OR + H_2O \rightarrow \equiv M - OH + ROH$ (1)

Condensation process, $\equiv M - OH + HO{-}M \equiv \rightarrow \equiv M - O{-} M \equiv + H_2O$ (2)

$\equiv M - OR + HO{-}M \equiv \rightarrow \equiv M - O{-} M \equiv + ROH$ (3)

1.3 Metal alkoxides

Metal alkoxides are metal-organic compound which have organic ligands with oxygen attached to metal. They are not organometallic compounds as they do not have direct metal-carbon bonds. Metal alkoxides are popular precursor for sol-gel reaction because they react readily with water through a reaction known as hydrolysis. The hydroxyl ion becomes attached to the metal atom as shown in the following reaction:

$$M(OR)_4 + H_2O \rightarrow HO - M(OR)_3 + ROH \qquad (9)$$

M represents metal and OR represents a proton or other ligand (if R is an alkyl, then OR is an alkoxyl group) and ROH is an alcohol. Hydrolysis may go to completion depending on the amount of water and catalyst (Brinker and Scherer, 1990). In hydrolysis reaction, the less electronegative ligands are first to be removed and in a faster rate compared to the more electronegative ligands. The detail of the aqueous sol-gel synthesis process using metal alkoxide was explained in Section 1.2.

When dealing with metal alkoxide, extra care has to be taken to minimize the exposure of metal alkoxide to atmosphere moisture due to its sensitivity to water. Studies on metal alkoxides and their physical properties such as volatility have been carried out by previous worker (Guglielmi and Carturan, 1988).

Metal alkoxide as one of the key parameters in sol-gel process besides gelling time and drying condition was studied systematically in order to develop materials of demanded properties (Ward and Ko, 1995).

1.4 Organic-inorganic composites material

Organic-inorganic composites material has homogeneous combination of inorganic and organic moieties in a single-phase. In organic-inorganic composites material, the interface between two different materials is a crucial feature for their durability and mechanical properties. Synthesis of this material employed the concept of molecular level mixing between two different materials (Chujo, 1996). Generally, organic-inorganic composite material is synthesized through sol-gel process. There are two major classes of organic-inorganic material:

Class I. The material consists of organic molecules or polymers which are embedded in an inorganic matrix. During the synthesis of this material, hydrolysis and condensation of inorganic compound occurred in which inorganic network is formed in the presence of organic compound. It can also be synthesized through the polymerization of organic polymer in porous inorganic hosts. Only weak bonds exist between inorganic and organic phase.

Class II. The material consists of inorganic and organic components that are bonded together through covalent bonding. The precursor to synthesize this material contain hydrolytically stable bond between the element and organic moieties (Schottner, 2001).

Organic-inorganic composites material is widely studied in order to alter its mechanical, electrical and optical properties for various applications. The presence of organic polymer in the synthesis of organic–inorganic composite material was reported to reduce volume shrinkage and cracking during sol-gel process (Deng *et al.*, 1998). Meanwhile, the presence of metal oxide in the organic-inorganic composites material improves the mechanical and optical properties such as surface hardness, modulus, tensile strength, transparency and reflective index of the material (Yano *et al*., 1998) .

Polyacrylates-silica composites material was successfully synthesized through sol-gel reactions of tetraethyl orthosilicate and 2-hydroxyethyl methacrylate along with polymerization of acrylate monomer. The method used in the synthesis was simple and fast while producing crack-free, transparent and monolithic composites material. The volume shrinkage during sol-gel process was significantly reduced to about 6-20%. The composites material synthesized has better thermal stability than its buck polymer (Wei *et al*., 1996).

Several works have also been done by our previous research groups on the synthesis of organic-inorganic composite materials through sol-gel method at School of Chemical Sciences, USM (Goh, 2006; Lok, 2001; Periaman, 2000; Jamarun, 2000). The current research works differ from our previous research group's works in the type of organic polymeric material used. The organic polymeric materials that they used in their works were mainly epoxidized natural rubber (ENR) and liquid natural rubber (LNR) while the current research work was on κ-carrageenan ester.

1.5 Objectives of the project

1. To study the synthesis of κ-carrageenan ester using modified Pascu method in order to obtain κ-carrageenan esters with high molecular weight.
2. To establish the optimum condition for the esterification process by observing the effect of temperature and amount of reactant used systematically.
3. To study the chemical and thermal properties of κ-carrageenan ester. A better understanding of κ-carrageenan ester may help in utilizing it in the development of inorganic-organic composite material.
4. To prepare organic-inorganic hybrid material from the κ-carrageenan ester synthesized.
5. To establish the thermal and chemical characteristics of κ-carrageenan ester composite synthesized.

2.Experimental

2.1 Chemicals

All chemical except toluene was used as received. The chemicals used throughout the project are listed as follows:

2.1(a) Chemicals obtained from Sigma-Aldrich, United States:

- κ -carrageenan, 99.9%
- Decanoyl chloride, 98%
- Pyridine, 99.8%
- Zirconium (IV) isopropoxide
- Deuterated toluene, 99.6 atom %D

2.1(b) Chemicals obtained from Qrec, Malaysia:

- Toluene, 99.5%
- Chloroform, 99.5%
- Ethanol, 95%

2.1(c) Chemicals obtained from Merck, Germany:

- Sodium hydrogen carbonate
- Deuterated chloroform, deuterated degree min 99.96%

2.2 Experimental Procedures

2.2.1 Esterification of κ-carrageenan

The esterification process were performed in accordance with the procedure developed by Pascu and Mullen with some modification (Mullen and Pacsu, 1942). 35 mL of pyridine in round bottom flask was heated to corresponding temperature (60 °C, 70 °C, 80 °C and 90 °C) before addition of 3.02 g κ-carrageenan. The mixture was then stirred for 30 min under N_2 atmosphere at the same temperature. Appropriate amount of decanoyl chloride (0.05 mol, 0.125 mol, 0.150 mol, 0.175 mol and 0.200 mol) was then added to the reactant dropwise and the solution was stirred thoroughly under reflux condition and nitrogen atmosphere for 6 hours.

After the reaction, the product was leave to cool down to room temperature. Excess ethanol was added to the mixture to obtain carrageenan ester. The precipitate was washed a few times with ethanol. The precipitate was grinded in ethanol and filtered during the washing process. The grinding and filtering step was repeated until the ethanol used to rinse the precipitate was clear in colour. The precipitate was left to dry in fume cupboard. After drying, the yield was kept in a desiccator and covered with aluminium foil. The general process for the esterification of carrageenan in this work was shown in Figure 2.1.

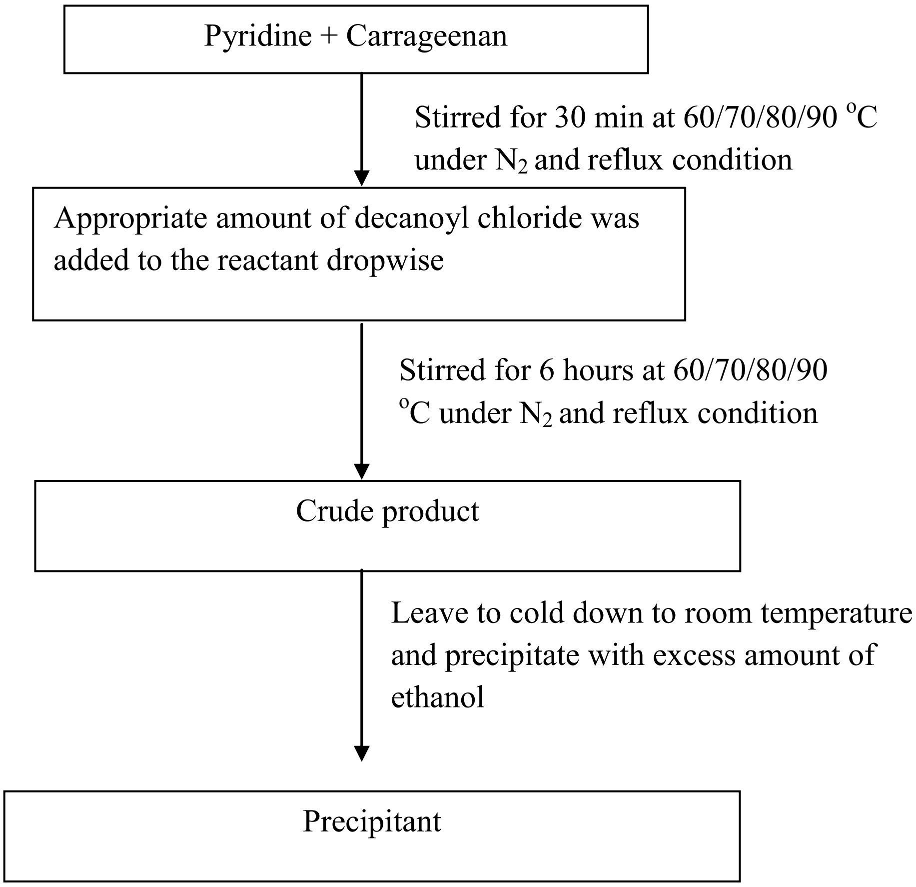

Figure 2.1: Synthetic routes to the formation of κ-carrageenan esters.

The variation of reaction condition for the synthesis of κ-carrageenan esters are given in Table 2.1 and Table 2.2

Table 2.1: Variation of temperature used to synthesize κ-carrageenan esters.

Sample	Temperature, °C	κ-carrageenan, g	Decanoyl Chloride, mol
1a	60	3.02	0.050
1b	70	3.02	0.050
1c*	80	3.02	0.050
1d	90	3.02	0.050

Table 2.2: Variation decanoyl chloride amount used to synthesize κ-carrageenan esters.

Sample	Temperature, °C	κ-carrageenan, g	Decanoyl Chloride, mol
2a*	80	3.02	0.050
2b	80	3.02	0.125
2c	80	3.02	0.150
2d	80	3.02	0.175
2e	80	3.02	0.200

Note: Samples 1c and 2a are similar. The amount of κ-carrageenan used was determined by the mechanical characteristics of the apparatus. 30mL of 8.07 w/w% κ-carrageenan in pyridine solution was the maximum amount that can be handled in the glass apparatus while providing a good dispersion.

2.2.2 Purification of κ-carrageenan esters

κ-carrageenan esters synthesized were subjected to further purification process to obtain a purer product. The κ-carrageenan was purified using 2 different methods based on the solubility properties of κ-carrageenan and κ-carrageenan esters which are widely different.

2.2.2.1 Purification of κ-carrageenan esters using $NaHCO_3$

The κ-carrageenan esters were added to ice-cooled 0.125 M $NaHCO_3$ and were stirred for 1 hour. The unreacted κ-carrageenan and other impurities were then removed by vacuum filtration. Excess distilled water was used to rinse the κ-carrageenan ester which is the residue from the filtration. The ester was then been rinsed with ethanol and left to dry in fume cupboard. The filtrate was collected and freeze-dried for further analysis.

2.2.2.2 Purification of κ-carrageenan esters using toluene

The κ-carrageenan esters were added to toluene and were stirred overnight to dissolve it. The unreacted κ-carrageenan was then removed by vacuum filtration. In this case, the filtrate which was the solution of κ-carrageenan ester was left to dry the fume cupboard.

2.2.3 Synthesis of κ-carrageenan ester composite with zirconium (IV) isopropoxide.

The κ-carrageenan esters was dissolved in toluene by stirring and heating. It was then filtered with cotton wool to remove the non-dissolved κ-carrageenan esters. The butanol and zirconium (IV) isopropoxide was added to the κ-carrageenan ester solution and immediately moved to ultrasound for 1 hour at room temperature. The solution was then poured into a petri dish and left to form film under room condition. The compositions of composite film that were prepared are given in Table 2.3

Table 2.3: Composition of composite films that were prepared.

Sample	κ-carrageenan, g	Toluene, mL	Butanol, mol	Zirconium (IV) propoxide, mol
3a	0.175 g	3.800	0.019 mol	1.28 x 10^{-4} mol
3b	0.175 g	3.800	0.019 mol	2.58 x 10^{-4} mol
3c	0.175 g	3.800	0.019 mol	5.16 x 10^{-4} mol

2.3 Characterisations Instruments

All samples were characterized using various methods as listed below:

I. Fourier Transform Infrared Spectroscopy, FT-IR:

The change in chemical structure of κ-carrageenan was qualitatively analyzed using Perkins Elmer System 2000. Potassium bromide (KBr) pellets were prepared by grinding the sample together with potassium bromide powder in the ratio of 1:100 and then, analyzed in range of frequency from 400 cm^{-1} to 4000 cm^{-1}. The samples were dried and kept in the desicator overnight before the analysis.

II. Nuclear Magnetic Resonance Spectroscopy, NMR:

For ^{1}H-NMR analysis of κ-carrageenan, sample was prepared with the concentration of 0.5-1.0 % in D_2O. It was analyzed using Bruker Avance 500 spectrometer at 500 MHz and at probe temperature of 24°C. Meanwhile, κ-carrageenan esters were dissolved in d-chloroform and d- toluene depending on the solubility of sample at 0.5-1.0% before analyzed using Bruker DPX 400 at 400 MHz. For solid state ^{13}C-NMR analysis, Bruker DPX400 Mas II was used. Sample was packed into rotor of 17 mm length and 4 mm diameter using compression tool and caps with Kel-F cap before analyzed at 100 MHz at 24 °C. Tetramethysilane was used as internal standard for calibration of chemical shift of ^{1}H for liquid NMR analysis meanwhile adamantane was used as internal standard for solid state NMR analysis.

III. Gel Permeation Chromatography, GPC:

The weight-average-weight molecular weight (Mw) and number average weight molecular weight (Mn) of κ-carrageenan were obtained through gel permeation chromatography (GPC) at 60 °C and 0.1 M $NaNO_3$ was used as the eluting agent for this analysis. Meanwhile, κ-carrageenan esters were dissolved and analyzed using chloroform as the eluting agent. The model Water 1525 with binary HPLC pump and Detected Refractive Index Detector (Water 2414) was used in this analysis.

IV. Thermogravimetric analysis, TGA:

Thermogravimetric analysis was carried out to quantify the weight loss of sample against the change of temperature. The samples were heated under nitrogen atmosphere from room temperature to 900 °C at the rate of 20 °C min^{-1} with exception of composite materials samples. These samples were heated at the rate of 5 °C min^{-1}. Slower heating rate was used for composite materials samples to get more accurate and clear spectrum that showed different decomposition steps.

V. Differential Scanning Calorimetric analysis, DSC:

All samples were analyzed using calorimeter (Pyris 1 DSC) from Perkin Elmer. The analyses were done from -50 °C to 170 °C with 20 mL min^{-1} of nitrogen flow to determine the glass transition temperature of all sample except for the κ-carrageenan. For κ-carrageenan, the analysis was done from -50 °C to 210 °C . The heating was hold for 1 min at to 170 °C and 210 °C for κ-carrageenan ester and κ-carrageenan respectively and cooled to -50 °C at 100 °C min^{-1} before the second heating. The rate of heating for DSC analysis was 20 °C min^{-1} and the amount of samples used for this analysis was about 10 mg.

2.3.1 Solubility Test for κ-carrageenan and κ-carrageenan Ester.

The solubility of κ-carrageenan and κ-carrageenan ester in water, chloroform and toluene were tested by stirring 0.050 g of sample in 10 mL of solvent for 3 hours under mild heating. However, only the maximum amount of κ-carrageenan esters which were able to dissolve in toluene was determined due to the limiting amount of sample. 0.400 g of κ-carrageenan ester samples were stirred in 10 mL toluene under mild heating for three hours. It was then leave to cool to room temperature before filtered using sintered glass filter equipped with vacuum pump. The residues of sample were dried in the oven overnight before weighting. The solubility of carrageenan esters in toluene determined was expressed in wt/wt %.

<u>Calculations for the solubility of κ-carrageenan esters in toluene are as followed</u>:

Calculation for the solubility of carrageenan esters was carried out to have a brief knowledge about the maximum concentration of polymer solution able to be prepared using different carrageenan ester samples. Almost similar method for solubility determination was also been carried out by earlier worker for starch acetate (Shogren and Biresaw, 2007).

Weight of κ-carrageenan ester dissolved, (g)

$$\text{Initial weight of } \kappa\text{-carrageenan ester (g)} - \text{weight of residue(g)} \qquad (10)$$

Solubility of κ-carrageenan ester , wt/wt

$$= \frac{\text{Weight of } \kappa\text{-carrageenan ester dissolved (g)}}{\text{Weight of } \kappa\text{-carrageenan ester dissolved(g)} + \text{Weight of toluene used (g)}} \times 100\% \qquad (11)$$