sustainability

MDPI

Article

Development of New Alkylated Carrageenan Derivatives: Physicochemical, Rheological, and Emulsification Properties Assessment

Selma Toumi [1], Madiha Melha Yahoum [2], Sonia Lefnaoui [3], Abdelkader Hadjsadok [4], Asma Nour El Houda Sid [5], Amel Hind Hassein-Bey [6], Abdeltif Amrane [7,*], Jie Zhang [8], Amin Aymen Assadi [7,9] and Lotfi Mouni [10]

1 Faculty of Sciences, University of Medea, Nouveau Pole Urbain, Medea 26000, Algeria
2 Materials and Environmental Laboratory (LME), University of Medea, Nouveau Pole Urbain, Medea 26000, Algeria
3 Laboratory of Experimental Biology and Pharmacology, University of Medea, Nouveau Pole Urbain, Medea 26000, Algeria
4 Functional Analysis of Chemical Processes Laboratory, Chemical Engineering Department, Saad Dahlab University, BP 270, Blida 09000, Algeria
5 Department of Chemical Engineering, Faculty of Process Engineering, University of Salah Boubnider Constantine 3, Constantine 25000, Algeria
6 Laboratory of Biomaterials and Transport Phenomena (LBMTP), University of Medea, Medea 26000, Algeria
7 Ecole Nationale Supérieure de Chimie de Rennes, Centre National de la Recherche Scientifique (CNRS), ISCR—UMR 6226, Université de Rennes, F-35000 Rennes, France
8 School of Engineering, Merz Court, Newcastle University, Newcastle upon Tyne NE1 7RU, UK
9 College of Engineering, Imam Mohammad Ibn Saud Islamic University, IMSIU, Riyadh 11432, Saudi Arabia
10 Laboratory of Management and Valorization of Natural Resources and Quality Assurance, SNVST Faculty, Akli Mohand Oulhadj University, Bouira 10000, Algeria
* Correspondence: abdeltif.amrane@univ-rennes1.fr

Citation: Toumi, S.; Yahoum, M.M.; Lefnaoui, S.; Hadjsadok, A.; Sid, A.N.E.H.; Hassein-Bey, A.H.; Amrane, A.; Zhang, J.; Assadi, A.A.; Mouni, L. Development of New Alkylated Carrageenan Derivatives: Physicochemical, Rheological, and Emulsification Properties Assessment. *Sustainability* **2023**, *15*, 6473. https://doi.org/10.3390/su15086473

Academic Editor: Levente Csóka

Received: 25 February 2023
Revised: 1 April 2023
Accepted: 7 April 2023
Published: 11 April 2023

Abstract: In this research, amphiphilic derivatives of kappa carrageenan (KC) were synthesized by hydrophobic modification with an alkyl halide (1-Octyl chloride). Three hydrophobic polymers with different degrees of substitution (DS) were obtained by the Williamson etherification reaction in an alkaline medium. The effect of the molar ratio (R = reagent/polymer) on the DS was investigated at different ratios (1, 2, and 3). The KC derivatives (KCRs) were characterized by different techniques such as FT-IR, ^{1}H-NMR, X-ray Diffraction, Scanning electron microscopy, and a rheological assessment. The FT-IR and ^{1}HNMR analyses confirmed the binding of the hydrophobic groups onto the KC molecule. The degrees of substitution calculated by ^{1}H-NMR demonstrated that the derivative KCR3 (0.68) presented a higher degree of substitution compared to KCR1(0.45) and KCR2 (0.53). The XRD and SEM analyses revealed that the alkaline etherification conditions did not alter the morphological and crystallographic properties, as well as the rheological behavior of the obtained derivatives. The amphiphilic character of the KCRs was investigated using a conductivity method which revealed that the molecular aggregation occurred above the critical aggregation concentration (CAC). Decreasing CAC values of 0.15% (KCR1), 0.11% (KCR2) and 0.08% (KCR3)with the degree of substitution (DS) were found. Furthermore, KCR's derivatives greatly improved the stability of oil/water emulsions as the droplet size decreased with increasing DS. The derivative (KCR3) with higher DS, showed a greater amphiphilic character, and improved emulsifying power.

Keywords: κ-carrageenan; hydrophobic modification; critical aggregation concentration; emulsion stability; rheology

1. Introduction

Polysaccharides are currently used in food and pharmaceutical technology, serving as crucial components for thickening, binding, texturing, and stabilizing, and also as

emulsifying, suspending, and gelling agents [1–3]. The specific functional properties of these polysaccharides depend on their molecular characteristics, including their molecular weight, conformation, hydrophobicity, and electrical charge [4]. Polysaccharides are abundant in marine microorganisms such as bacteria, microalgae, and algae. In addition, the carbohydrate polymers most commonly found in commercially exploited marine species are alginates, agar, and carrageenan.

Carrageenans, from carrageenophyte red seaweed sources, feature a repeating disaccharide sequence consisting of β-D-galactopyranose residues linked glycosidically through positions 1 and 3 (G-units), and α-galactopyranose residues linked glycosidically through positions 1 and 4 (DA-units) [3,4]. As versatile food additives, red seaweed polysaccharides can bind water, promote gel formation, and serve as thickening agents [5], further advantages lie in the improvement of palatability and appearance [3].

In recent years, carrageenans have gained particularly high attention in the pharmaceutical industry due to their ability to inhibit virus attachment, including the human papillomavirus, dengue virus, and herpes virus [6,7]. Furthermore, carrageenans are utilized in various drug delivery systems, including as matrixes for controlling drug release [7,8], microcapsules [7], and microspheres [7,9].

The most commonly utilized type of carrageenan in the food and pharmaceutical industry as a thickening or gelling agent is κ-carrageenan, which is extracted from *Kappaphycus alvarezii*, also known as *Eucheuma cottonii* [10]. Despite its widespread use, native κ-carrageenan presents several drawbacks and limitations that restrict its use in various industries [5]. Indeed, the κ-Carrageenan gels are hard and brittle [11], and exhibit significant syneresis, or separation of liquid from the gel, when subjected to mechanical deformation or stored for extended periods, leading to instability and altered rheological and mechanical properties [12]. Moreover, the hydrophilic character of kappa carrageenan (KC) makes KC challenging to utilize in dispersed systems such as emulsions and suspensions [13].

To overcome this limitation, it is essential to understand the relationship between the functional and molecular properties of KC to modify its chemical structure and obtain new desired structural properties. Molecular modifications of κ-Carrageenan chains, such as carboxymethylation [14], phosphorylation [15], thiolation [16], oversulfation [15,17] acetylation [15], oxidation [18,19], and cationisation [20], are reportedly related to changes, enhancements, or development in the physicochemical and rheological properties of KC. For example, Ellis, Mills, and Norton (2019) [21] reported that in-situ interaction improves the surface activity of kappa carrageenan.

This investigation focuses on the study of the effect of the degree of substitution on the physicochemical and surface-active proprieties of κ-carrageenan, which can be utilized to create and stabilize oil-in-water emulsions. This study is complementary to our previous work which reported the study of the influence of heating method (conventional against microwave) on the proprieties of alkylated κ-carrageenan derivatives at a constant reagent/polymer ratio (R) [22]. However, in the present work, three reagent/polymer ratios (R) were varied, namely 1.2 and 3. Amphiphilic derivatives of κ-carrageenan were generated via an etherification reaction between the hydroxyl groups of κ-carrageenan and octyl chloride using conventional heating, as depicted in Figure 1. The hydrophobic modification was carried out using a Williamson synthesis, which is an organic reaction occurring between an alkoxide and an alkyl halide conducing to the formation of ethers. Three derivatives were obtained and subjected to major techniques of physicochemical analysis to demonstrate the effect of the increased degree of substitution on the structural, rheological, and emulsifying properties. The obtained derivatives are intended to be used as polymeric surfactants, mainly in the pharmaceutical and food industry, but also in many other fields.

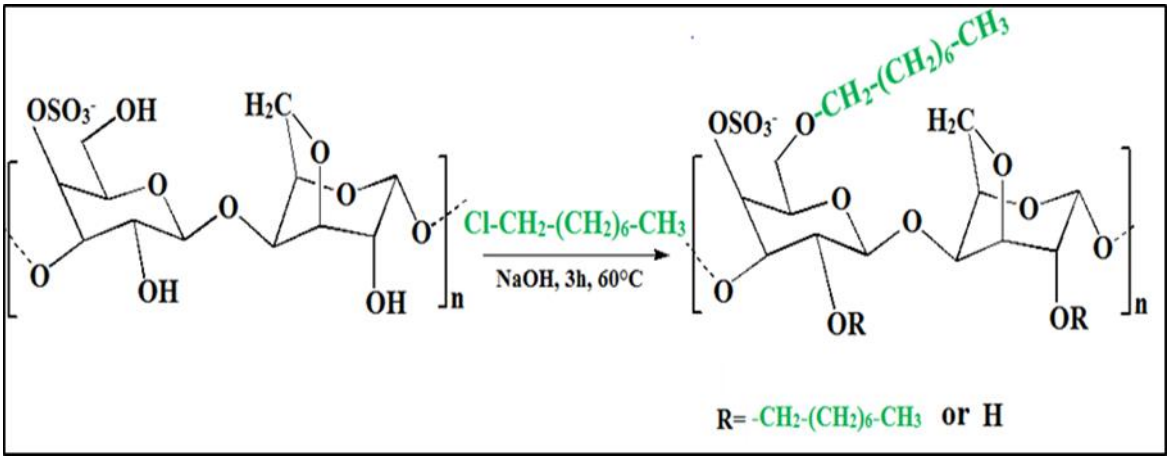

Figure 1. The alkylation reaction of kappa carrageenan with 1-Octyl Chloride.

2. Materials and Methods

2.1. Materials

κ-Carrageenan (KC) extracted from red seaweed (*Rhodophyceae*) was purchased from Azelis (Algiers, Algeria); Black seed oil (BSO) was supplied by Abu El Kassim industries (Algiers, Algeria); 1-Octyl Chloride ($C_8H_{17}Cl$), Sodium Hydroxide (NaOH), Acetone, and Acetic Acid (CH_3COOH) were procured from Sigma Aldrich (Hamburg, Germany).

2.2. Hydrophobic Modification of Kappa Carrageenan (KC)

The etherification reaction of kappa carrageenan (KC) was similar to that described by Toumi et al. [22], and Lefnaoui and Moulai-Mostefa 2014 [8], with minor modifications. Initially, 5 g of native KC powder was dispersed in 100 mL of distilled water and stirred for 40 min at room temperature. Subsequently, 5 mL of 16 N sodium hydroxide solution (NaOH) was added gradually, with constant stirring, at a rate of 1 mL per 15 min at room temperature. Dropwise addition of 1-octyl chloride was made to the reaction medium, followed by stirring at 60 ± 0.5 °C for 3 h to obtain the alkylated kappa carrageenan (KCR) derivatives. The neutralization of KCRs was carried out using acetic acid and the product was poured into acetone. Finally, the product was recovered through filtration and dried in an oven at 70 °C overnight, followed by powdering in a glass mortar. Three alkylated kappa carrageenan (KCR) derivatives KCR1, KCR2, and KCR3 correspond, respectively, to the reagent/polymer ratios (R) equal to 1.2 and 3.

2.3. Physicochemical Characterization

2.3.1. Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR spectra of kappa carrageenan and its KCR derivatives were obtained using a Bruker Tensor II spectrometer equipped with a single reflection diamond ATR accessory. A small quantity of each sample (approximately 10 mg) was placed onto the diamond measuring device of the spectrometer and the spectra of the samples were recorded (10 scans, at a resolution of 4 cm^{-1}) within the 4000 to 400 cm^{-1} range.

2.3.2. ^{1}H-NMR Analysis and Degree of Substitution (DS)

A 400 Hz Bruker ^{1}H NMR spectrometer was utilized to obtain the proton nuclear magnetic resonance (^{1}H NMR) spectra at 65 °C. The powder biopolymer was dissolved in D_2O (deuterium oxide) at 25 °C. Subsequently, MestReNova software (Version 2.9) was used to analyze the ^{1}H-NMR spectra of all samples.

The calculation of the degree of substitution (DS) for the derivatives was conducted using ^{1}H-NMR analysis, according to the following Equation (1) [22].

$$DS = \frac{ACH_3 + ACH_2}{(2(n-1)+3) \times AH_{anomeric}} \quad (1)$$

In the given formula, A represents the area under the picks corresponding to CH_3, CH_2 groups, and the anomeric proton (H). Meanwhile, n refers to the carbon atom number in the alkyl chain, which in this case is 8.

2.3.3. Scanning Electron Microscopy (SEM)

Scanning electron micrographs (SEM) of KC and KCRs were taken using scanning electron microscopy (FEI Quanta 250) at 25 °C. The accelerating voltage used ranged from 10 kV to 12.5 kV, with SEM images taken at various magnification levels including 1000×, 6000×, and 16,000×. The analysis was carried out using xT microscopy software (Version 4.1.0.1864).

2.3.4. X-ray Diffraction Analysis

X-ray diffraction analysis of KC and its hydrophobically modified derivatives was realized in a (Bruker D2 PHASER) diffractometer using copper radiation at the differential angle ranging from 15° to 80° (2θ). The DOC (degree of crystallinity) was determined using Match3 software (version 3.12, 2021).

2.3.5. Viscosity Analysis

Viscosity analyses were performed using an Anton Paar rheometer. Aqueous solutions of κ-Carrageenan (KC) and its KCRs derivatives at the concentrations of 0.75 and 1%, (*w*/*v*) were prepared and subjected to rheological measurements at a temperature of 25 °C using a plate-plate measurement device (60 mm diameter, 1 mm gap). Flow curves were determined by the variation in the apparent viscosity as a function of shear rate over the range of 0.001 to 1000 (1/s). The kappa carrageenan (KC) and its derivatives' average molecular weights (MW) were determined by measuring the intrinsic viscosity using an Anton Paar rheometer according to the procedure described by Toumi et al. [22].

2.3.6. Critical Aggregation Concentration (CAC)

The Critical Aggregation Concentration (CAC) of KCR's derivatives was determined by the conductimetric method. First, solutions of kappa carrageenan and its KCR derivatives were prepared and the conductivity was measured using a J.P. Selecta conductivity meter. These measurements were performed three times. The CAC was determined from the point where there was a break in the slope of the plot.

2.3.7. Emulsifying Study

Surfactant-free direct emulsions (O/W) were prepared with the addition of kappa carrageenan (KC) and its hydrophobically modified derivatives (KCRs) as stabilizers following the protocol outlined by Shahin et al. [23]. To begin the emulsification process, 1% (*w*/*v*) aqueous solutions of KC native and KCRs were prepared by dissolving a specific quantity of the biopolymer (KC and KCRs) (Table 1) in distilled water. Next, the aqueous and oil phases were heated separately to 60 °C, where the oil phase was composed of black seed oil (BSO). The pre-emulsion was created by gradually adding the oil phase to the aqueous phase while stirring until a homogenous mixture was formed. The resulting oil-in-water emulsions were homogenized for 5 min using an ultra-turrax at 22,000 rpm, and then left at room temperature for 24 h.

Table 1. Composition of the formulated emulsions.

Composition (%)	F1	F2	F3	F4
BSO	20	20	20	20
KC	1	-	-	-
KCR1	-	1	-	-
KCR2	-	-	1	-
KCR3	-	-	-	1
Water	79	79	79	79

The O/W emulsions obtained were subjected to an accelerated stability test performed by centrifugation method [23], where a sample of 10 mL of each emulsion was submitted to centrifugation at 2000, 4000, and then 6000 rpm for 10 min using a Hettich EBA 20 centrifuge. The creaming index (H) was determined using the Equation (2) below:

$$\mathrm{H}\ (\%) = \frac{Hs}{He}\ 100 \quad (2)$$

The value of H, which represents the creaming index, was obtained by dividing the height of the transparent serum formed at the bottom of the tube (*Hs*) by the total height of the emulsion (*He*).

Thereafter, a long-term stability test was conducted to observe the emulsions' macroscopic appearance immediately after preparation, and then again after 24 h up to 30 days later. The color, homogeneity, and consistency of the emulsions were visually assessed. The emulsion microstructures were studied using an optical microscope (Micros MCX 100) equipped with a camera (Infinity lite). Photomicrographs of various emulsions were recorded and the droplet size distribution was measured using ImageJ 152 software (Version 1.52a).

2.3.8. Statistical Assessment

The mean ± standard deviation error (SD) values were used to present the obtained results. The statistical analysis was performed using Tukey's one-way comparison tests (ANOVA) at a 95% confidence interval ($p < 0.05$) with the aid of OriginPro 9 software (Version 9.00.00 SR2 b87).

3. Results and Discussion

3.1. FTIR Analysis

The infrared spectra of kappa carrageenan (KC) and its modified derivatives are presented in Figure 2. The KC spectrum showed bands at 1224.12 cm^{-1} and 844.14 cm^{-1}, corresponding to ester sulfate groups (O=S=O symmetric vibration) and C_4-O-S stretching vibration, respectively [24]. In addition, the 3,6-anhydro-D-galactose residue's C-O-C band at 924.38 cm^{-1} and the C-O bridge stretching pick at 1148.70 cm^{-1} were observed [24].

Signals at 1036.50 cm^{-1}, 2917.34 cm^{-1}, and 3387.56 cm^{-1}, respectively, were assigned to C-O stretching, C-H bands, and O-H stretching; these results are similar to those found by Fan et al. [24]. The structural water deformation band was associated with the strong pick at 1628.14 cm^{-1} [25]. The FT-IR spectra of KCR's samples showed a reduction in the intensity of hydroxyl groups (-OH) at 3362.99 cm^{-1}, confirming that the -OH groups of kappa carrageenan were successfully replaced by octyl chains [26].

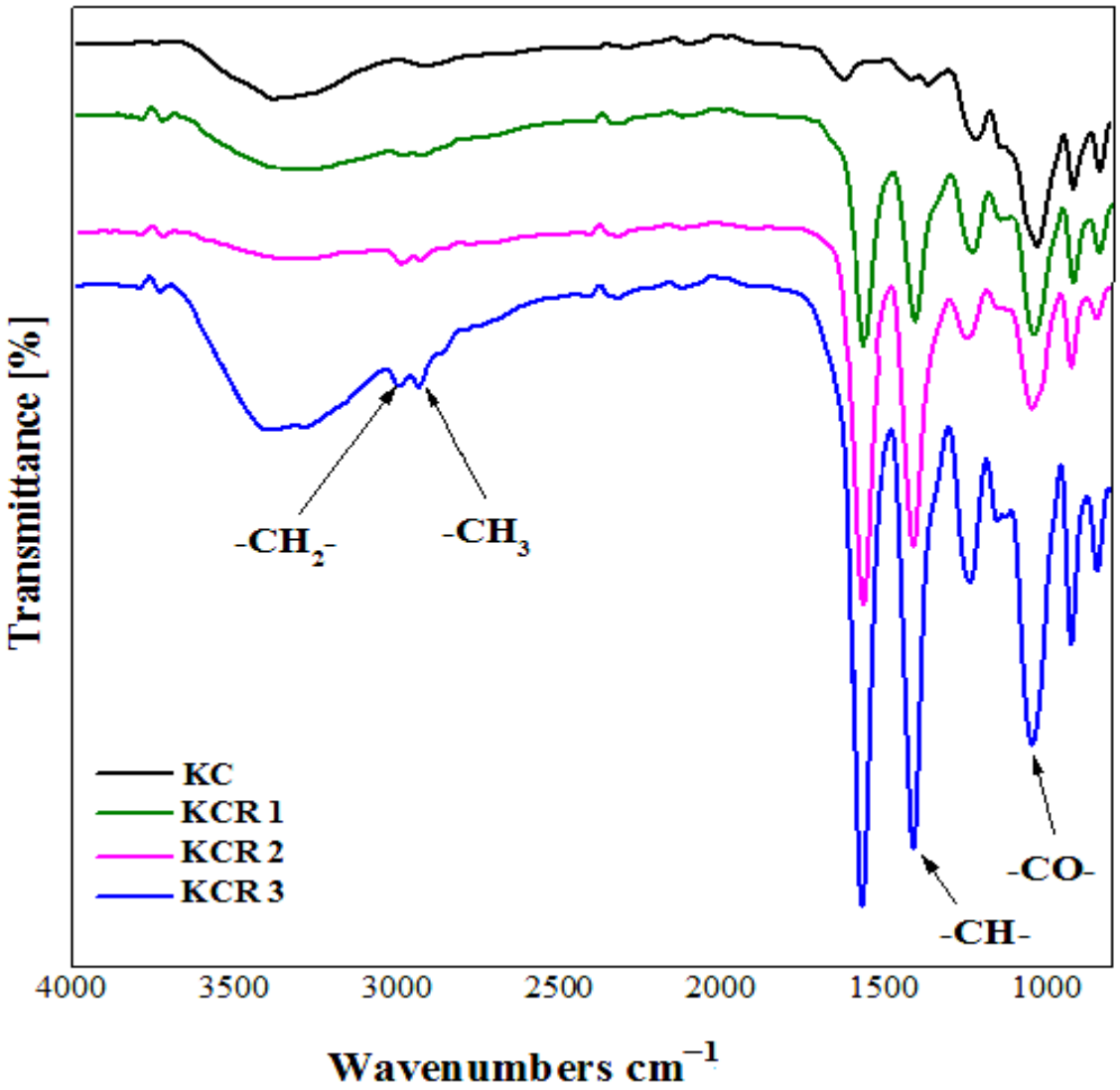

Figure 2. FTIR spectra of kappa carrageenan and its derivative KCRs.

Additionally, new peaks were also found at 1414.73 cm^{-1} corresponding to the symmetric vibration of -CH banding of alkyl chains, as well as other peaks at 2857.87 and 2925.54 cm^{-1} that were attributable to the methyl ($-CH_3$) and methylene ($-CH_2-$) stretching, respectively [27]. For the ether function (-C-O-C), at 1043.69 cm^{-1} [22], it was noted that this peak was stronger in the KCRs spectra. It was also observed that the derivative KCR3 displayed a greater intensity than KCR2 and KCR1. Finally, these findings confirmed the successful grafting of the alkyl chains onto the backbone of native kappa carrageenan and that KCR3 is the most hydrophobically substituted derivative.

3.2. ^{1}H-NMR Analysis

The effectiveness of hydrophobic modification of kappa carrageenan was confirmed by the ^{1}H-NMR spectroscopy method. The ^{1}H-NMR spectra of native and modified kappa carrageenan dissolved in deuterium oxide are illustrated in Figure 3. The anomeric proton of kappa carrageenan was identified at 5.09 ppm, while the significant peak detected at 4.7 ppm corresponds to the anomeric proton that was overlapped by the deuterium oxide (D_2O) solvent [28].

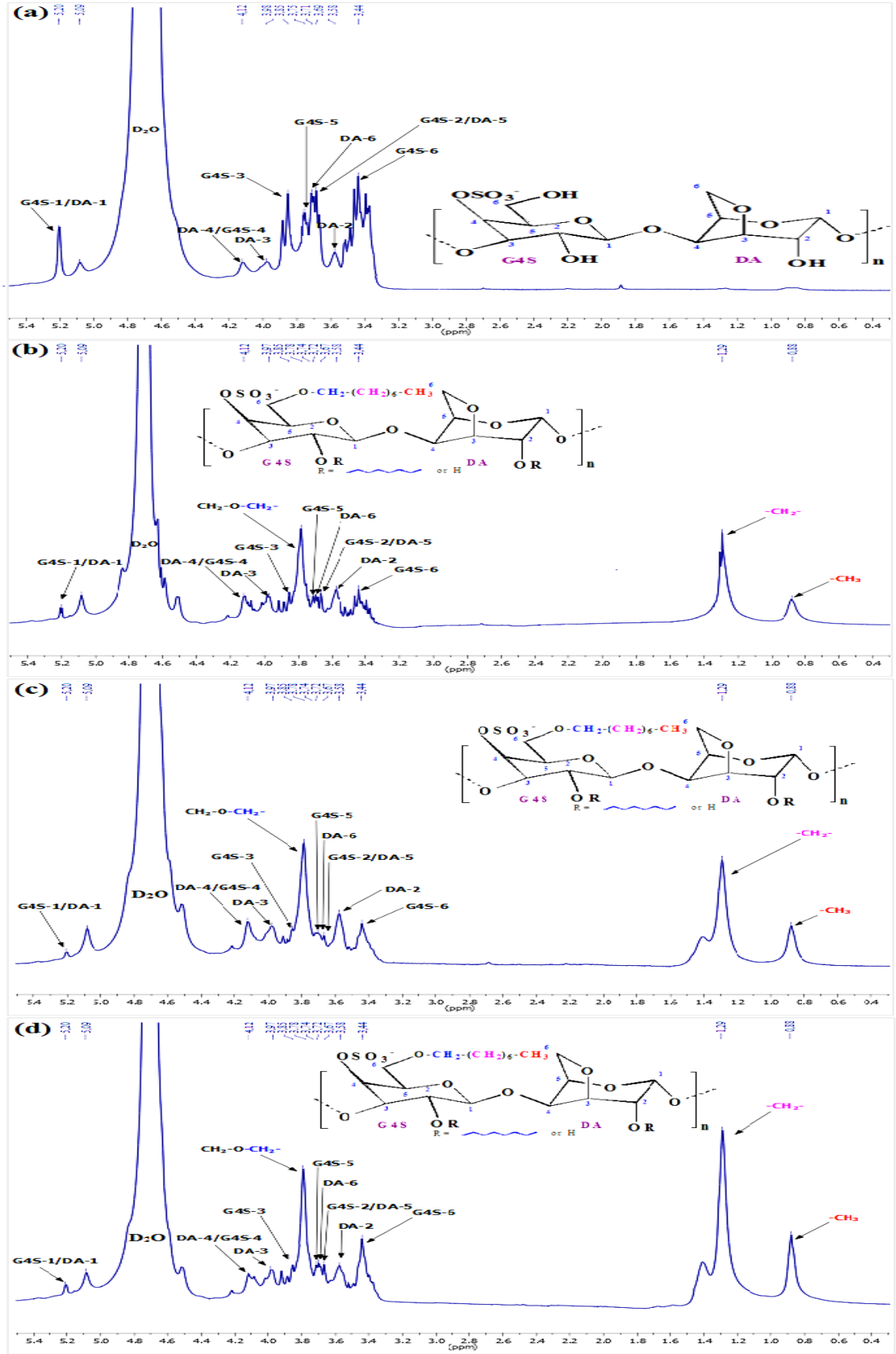

Figure 3. ^{1}HNMR spectra of kappa carrageenan (**a**), KCR1 (**b**), KCR2 (**c**), and (**c**) KCR3 (**d**).

The spectrum depicted in Figure 3a represents the unmodified kappa carrageenan and showed the presence of peaks referring to the (G-units) and (DA-units), which are the repeated and alternated units of 3-linked β-D-galactopyranose and 4-linked 3,6-anhydro-α-D-galactopyranose, respectively. The chemical shifts of kappa carrageenans are listed in Table 2.

In the spectra of KCRs, including KCR1 (Figure 3b), KCR2 (Figure 3c), and KCR3 (Figure 3d), new signals were observed. The peaks at 0.88 ppm and 1.29 ppm were assigned to the terminal methyl (CH_3) group [22] and methylene (CH_2) protons of the octyl chain, respectively [27]. In addition, a new apparent peak was detected at 3.78 ppm in the three ^{1}H-NMR spectra of the KC derivatives, which were attributed to the methylene protons linked to the ether function (-CH_2-O-R), indicating that the native kappa carrageenan was successfully etherified [22,29,30].

Finally, the degree of substitution (DS) values are presented in Figure 4, indicating that KCR3 had a higher DS value (0.68) compared to KCR2 (DS = 0.53) and KCR1 (DS = 0.45). These results support the findings obtained from the FTIR analysis.

Table 2. Chemical shifts (δ ppm) of kappa-carrageenan (KC).

Peaks	Chemical Shifts (δ ppm)	References
G4S-1	5.20	[31,32]
G4S-2	3.69	[31,32]
G4S-3	3.85	[31,32]
G4S-4	4.12	[31,32]
G4S-5	3.75	[31,33]
G4S-6	3.44	[31,33]
DA-1	5.20	[22,31–33]
DA-2	3.58	[22,31–33]
DA-3	3.98	[22,31–33]
DA-4	4.12	[22,31–33]
DA-5	3.69	[22,31–33]
DA-6	3.71	[22,31–33]

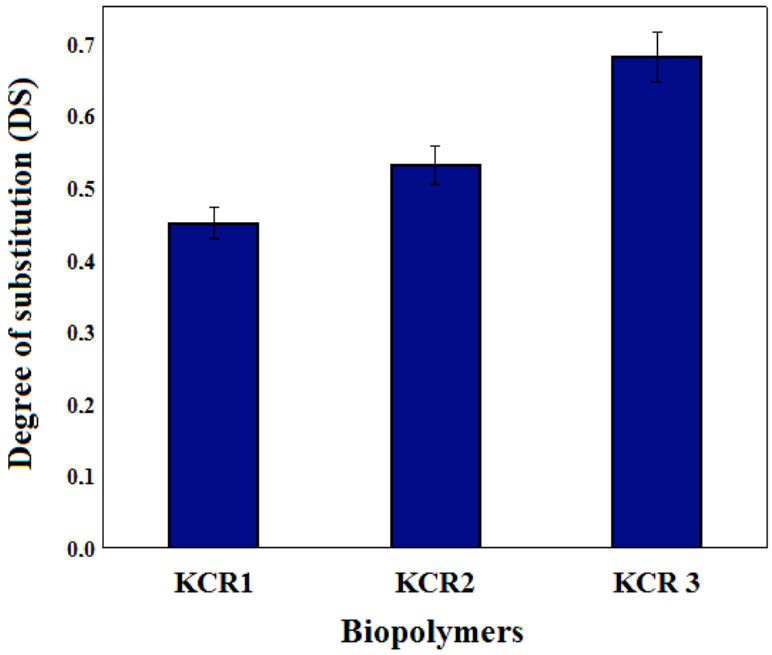

Figure 4. Variation of the degree of substitution (DS) of kappa carrageenan derivatives.

3.3. Scanning Electron Microscopy (SEM)

The SEM images of native kappa carrageenan and its etherified derivatives are displayed in Figure 5. Before grafting hydrophobic moieties on the KC's backbone, the surface morphology of native kappa carrageenan (Figure 5(a1–a3)) exhibits a granular and laminated structure with smaller granule size compared to the KCR derivatives [22,34–36]. On the other hand, the SEM image of KCRs in Figure 5 showed a few alveolate holes on the granules' surfaces, with a slight difference between the three derivatives. Additionally, KCR microstructures showed a smoother, less porous, and crystalline appearance when compared to κ-carrageenan.

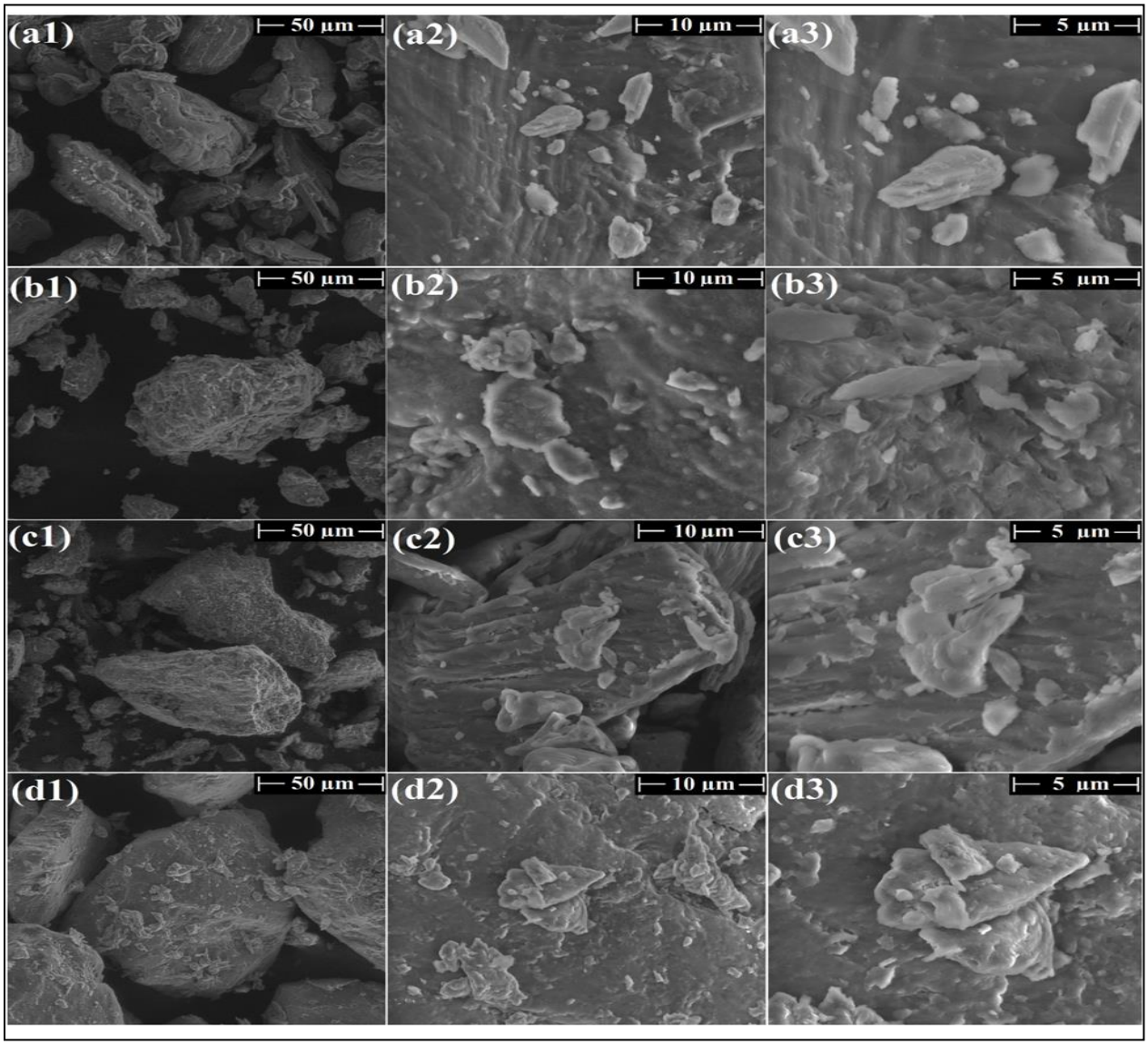

Figure 5. SEM micrographs of KC (**a1–a3**), KCR1 (**b1–b3**), KCR2 (**c1–c3**), and KCR3 (**d1–d3**) at different magnifications. From left to right: 50 µm, 10 µm, 5 µm, and 1000×, 6000×, 16,000×.

However, compared to the KCR2 (Figure 5(c1–c3)) and KCR1 (Figure 5(b1–b3)), the KCR3 presented slightly higher grain size (Figure 5(d1–d3)). Moreover, the photomicrographs indicated that there were no significant changes between the native KC structure and the granular structure of KCRs. It was also confirmed that the grafting of hydrophobic fragments onto the kappa carrageenan skeleton by etherification did not lead to any denaturation of the KC molecule's morphology. Similar results were found by other authors,

who reported that the hydrophobic modification did not affect the morphological structure of the modified polysaccharide [29].

3.4. X-ray Diffraction Analysis (XRD)

The XRD results presented in Figure 6 indicate that both native KC and modified KC samples possessed a semi-crystalline structure, with diffraction peaks appearing at 2θ = 18.41° and 28.26° being present in all samples [36]. However, the KCRs diffractogram showed less intense peaks compared to the KC sample diffractogram, indicating that they had different degrees of crystallinity (DOC). The DOC values of KC, KCR1, KCR2, and KCR3 were 8.01%, 3.50%, 4.63%, and 5.49%, respectively. These findings demonstrated that the physical state of the samples was not significantly altered after alkylation and that the molecular structure of KC did not undergo a denaturation effect since the grafting of the new hydrophobic alkyl moieties occurred primarily in the amorphous regions [37]. This result is in complete agreement with Jacquier et al. [38], who reported that the etherification of kappa carrageenan with octyl chloride did not cause any degradation of the polymer. Similar findings have been asserted by different authors, such as Sara et al. [29] for hydrophobically modified xanthan gum, and also by Chen et al. [39], who reported that the hydroxybutylation reaction took place in the amorphous area and had very little effect on the crystallinity of waxy corn starch. However, Sav et al. [40] affirmed that hydrophobic modification slightly influenced the crystalline nature of the octenyl succinate anhydride derivative of fenugreek gum.

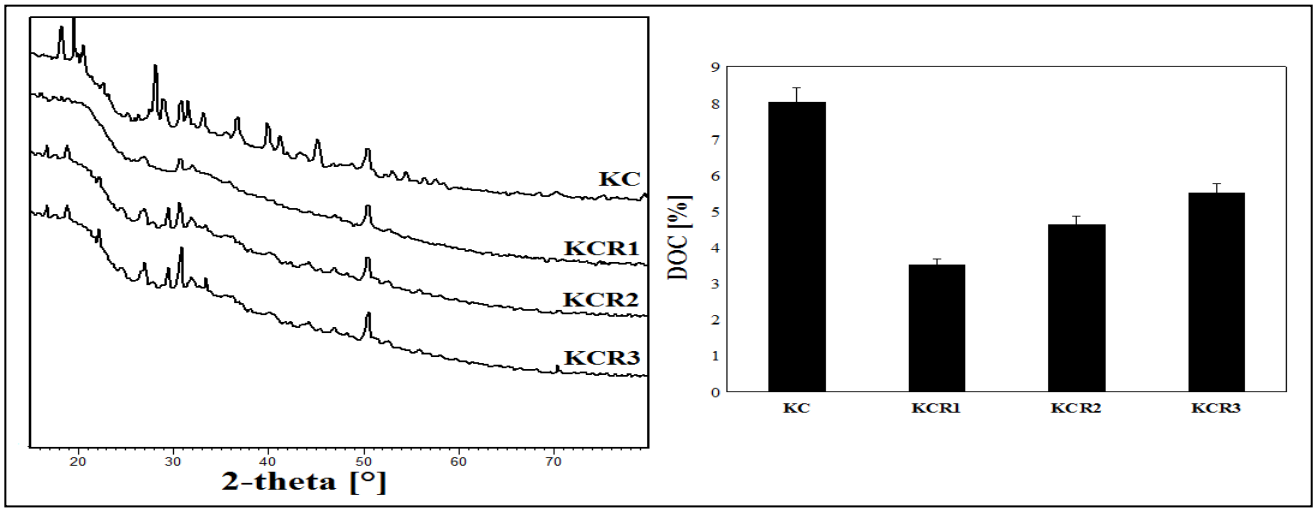

Figure 6. X-ray diffractograms and degree of crystallinity (DOC) of kappa carrageenan and its KCRs derivatives.

3.5. Viscosity Analysis

The flow behavior of kappa carrageenan (KC) and its derivatives at 0.75 and 1% concentrations was examined and plotted in Figure 7. The results demonstrated that the biopolymers exhibited non-Newtonian properties, with shear-thinning pseudoplastic behavior, which meant that the viscosity decreased with an increase in the shear rate [22].

In addition, the viscosity of KC derivatives was lower than that of KC due to the lower molecular weight of the derivatives compared to native kappa carrageenan. The average molecular weights of KC, KCR1, KCR2, and KCR3 were 2.23×10^5 g·mol^{-1}, 0.94×10^5 g·mol^{-1}, 1.13×10^5 g·mol^{-1}, and 1.21×10^5 g·mol^{-1}, respectively. The decrease in molecular weight was attributed to the slight depolymerization of the kappa carrageenan polymeric chains under the alkaline treatment of the etherification process. Previous studies also reported that chemical modification in alkaline conditions caused a decrease in the molecular weight of kappa carrageenan [8,31,41].

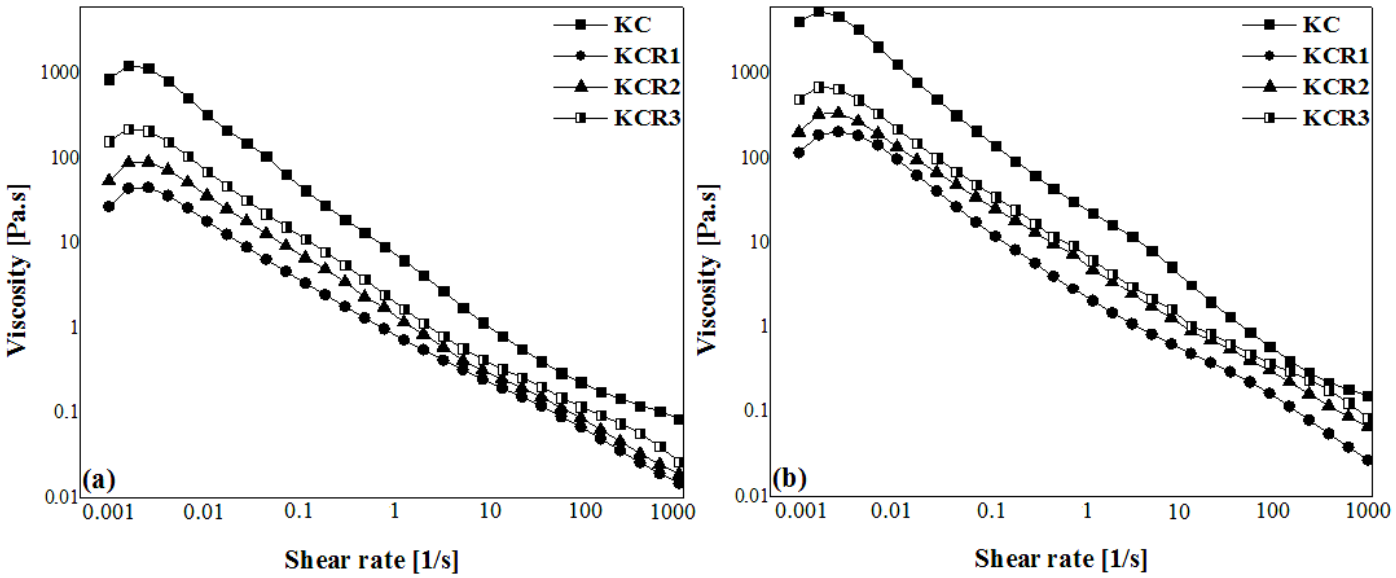

Figure 7. Flow curves of native kappa carrageenan, and its derivative KCRs at concentrations of 0.75% (**a**) and 1% (**b**).

Thus, the 1% (w/v) biopolymer (KC and KCRs) solutions showed higher viscosity values than the 0.75% (w/v) solutions. Moreover, the viscosity of KC derivatives increases with increasing DS, which can be attributed to the increase in hydrophobic associations through the attraction between the grafted chains, which can be intramolecular and intermolecular [21].

3.6. Critical Aggregation Concentration (CAC)

Figure 8 presents the electrical conductivity variation of KC derivatives at various concentrations, at a temperature of 25 °C. The results indicated that electrical conductivity increased as the concentration of KCRs increased due to the presence of free counter-ions in the solutions. The graphs showed two linear regions with different slopes. These two regions are separated by a point corresponding to the critical aggregation concentration (CAC). At lower concentrations, the conductivity increased linearly until the critical aggregation concentration (CAC) was reached, which marked the beginning of micelle formation. Beyond this concentration, the conductivity continued to increase, but at a slower rate. The CAC values for KCR1, KCR2, and KCR3 samples were 0.15%, 0.11%, and 0.08% w/v, respectively. As the degree of substitution (DS) increased, the CAC values decreased [42], suggesting the amphiphilic character of the hydrophobically modified KCRs. This behavior can be explained by the surfactant-like action of KCRs in aqueous solutions [43].

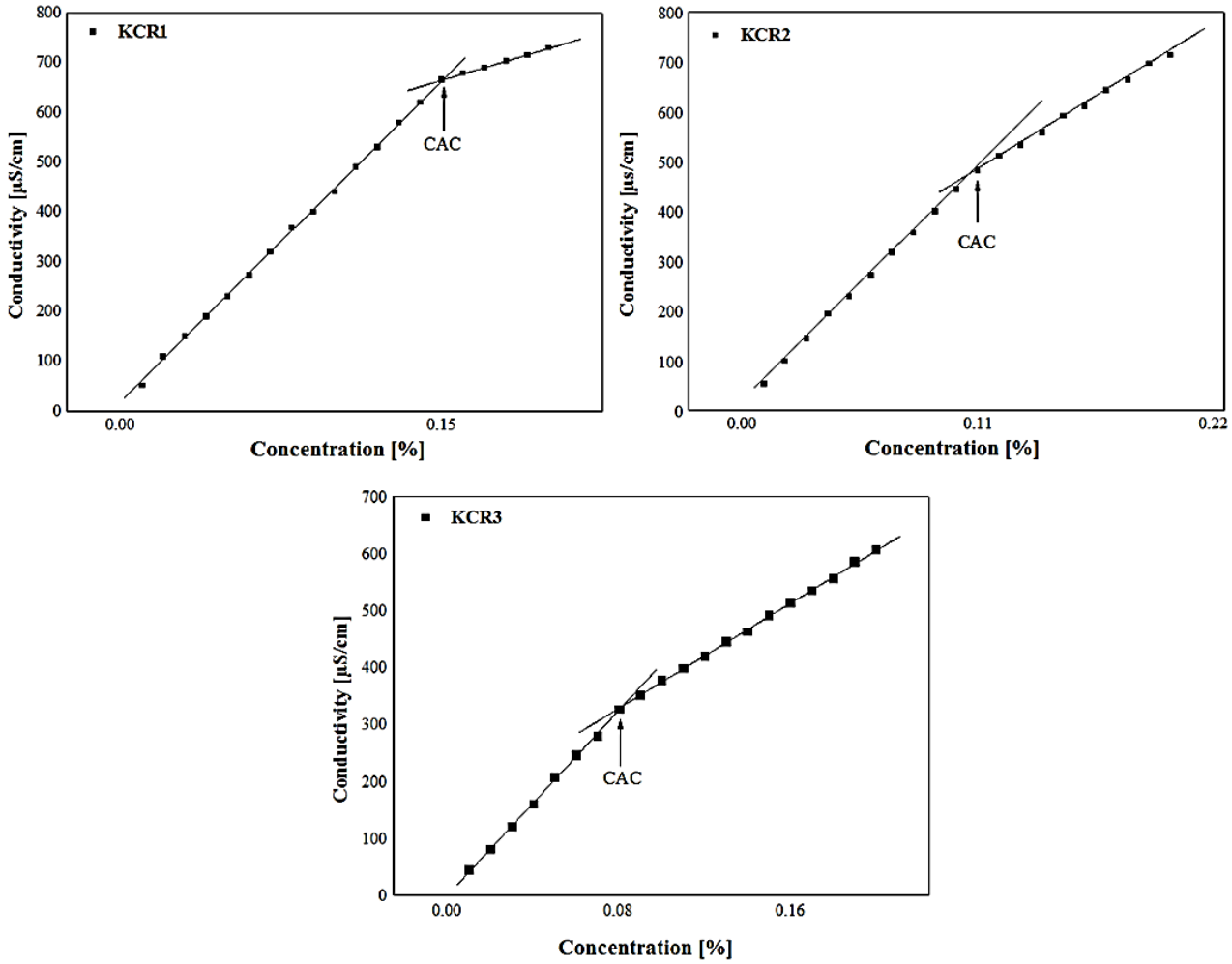

Figure 8. Variation of conductivity of KCRs in aqueous solutions.

3.7. Emulsifying Properties

3.7.1. Accelerated and Long-Term Stability Testing (Creaming Index)

The visual properties of the formulations were assessed after preparation (first day), and during 30 days of storage for the stability test (Figure 9). All the emulsions exhibited a uniform creamy appearance and exhibited a yellow (KC stabilized formulations) to pale-yellow color (KCR stabilized emulsions). The stability test results after one day and one month of storage are illustrated in Figure 9. It was observed that F1, which was based on KC, showed oily droplets present on the surface which is a result of phase separation that became even more evident after 30 days, by the appearance of a well-distinguished layer of oil on the surface of the emulsion. In contrast, the emulsions containing KCRs demonstrated better stability, as they maintained a homogeneous and viscous consistency with a pale-yellow color even after 30 days of storage.

Figure 10 displays the results of the centrifugation accelerated stability tests at the varied centrifugation speeds of 2000 rpm, 4000, and 6000 rpm, immediately after emulsions preparation (Figure 10a), and after 30 days of storage (Figure 10b), where it is observed that the maximal creaming index values Hmax are obtained at 6000 rpm. The results indicate that the emulsions stabilized by KCRs present the lowest values of Hmax compared to those based on kappa-carrageenan (KC). The formula F4, stabilized with KCR3 derivative, showed lower Hmax values both on the 1st day ($5 \pm 0.25\%$) or after 30 days ($18 \pm 0.9\%$) of storage, indicating higher stability compared to KCR2 that presents Hmax values of (19 ± 0.95)% and (25 ± 1.25)% respectively on 1st and 30th day of storage and KCR1 derivative which the Hmax values were found (42 ± 2.1)% at 1st day and (51 ± 2.55)% after

one month. These results confirmed the amphiphilic character of the KCR derivatives and also demonstrated that their emulsion stabilizing capacity is improved with an increased degree of substitution.

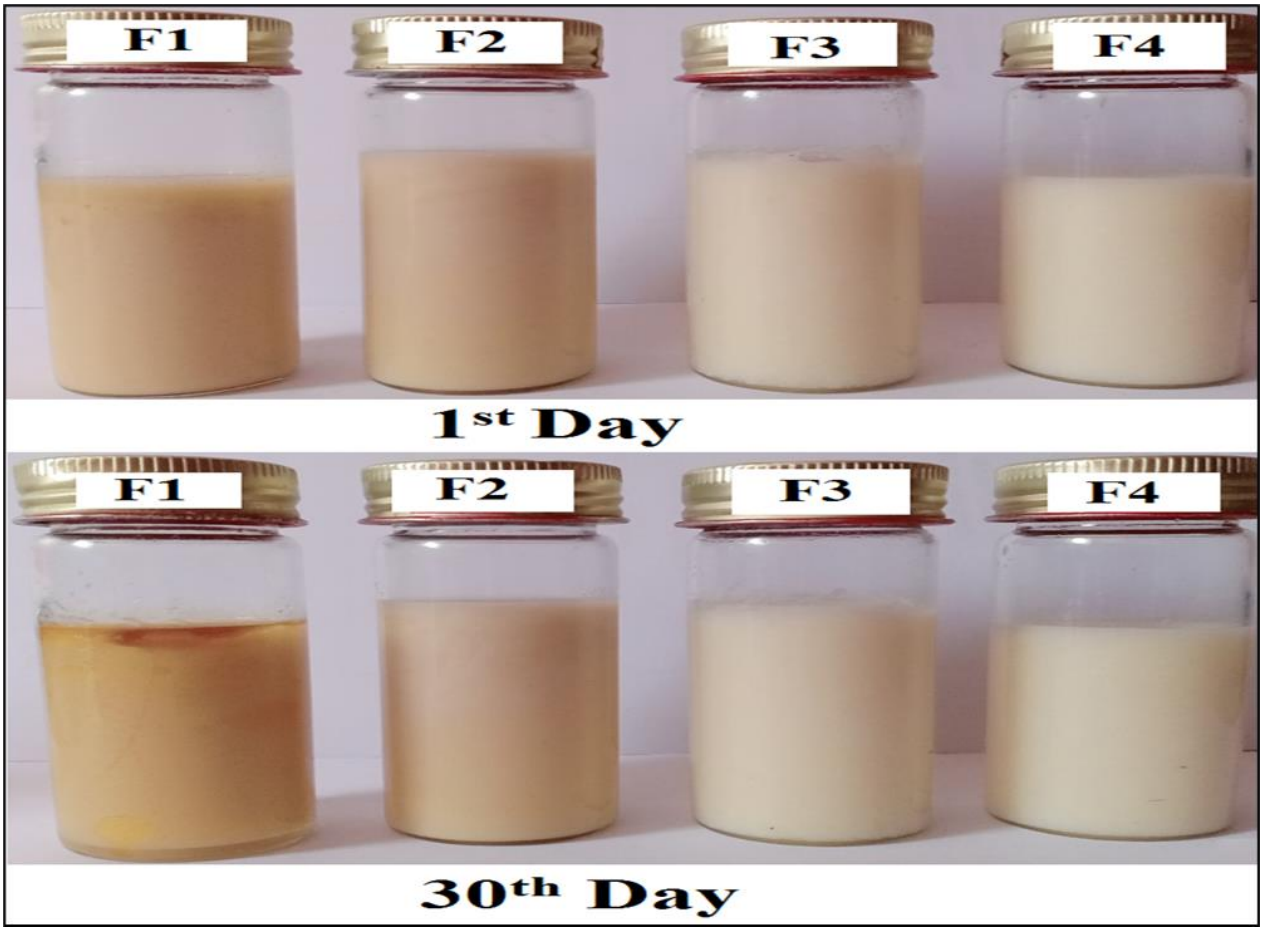

Figure 9. Photography of the emulsions F1, F2, F3, and F4 based on 1% of KC, KCR1, KCR2, and KCR3, respectively, on the first and the thirtieth days of the formulation.

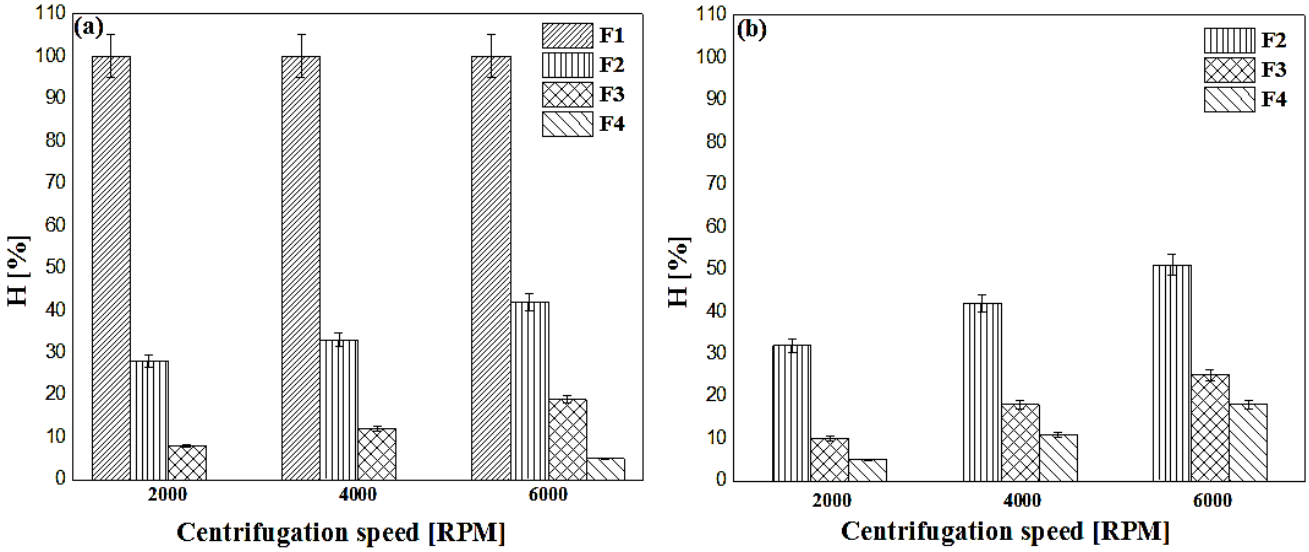

Figure 10. Variation of the creaming index of the emulsions as a function of centrifugation speed at the 1st (**a**) and 30th (**b**) day of storage.

3.7.2. Microscopic Aspect

Optical microscopy was employed to investigate the microstructure of the emulsions one day after preparation and 30 days after storage. The photomicrographs and particle size distribution of the emulsions stabilized by KC and KCRs are shown in Figure 11.

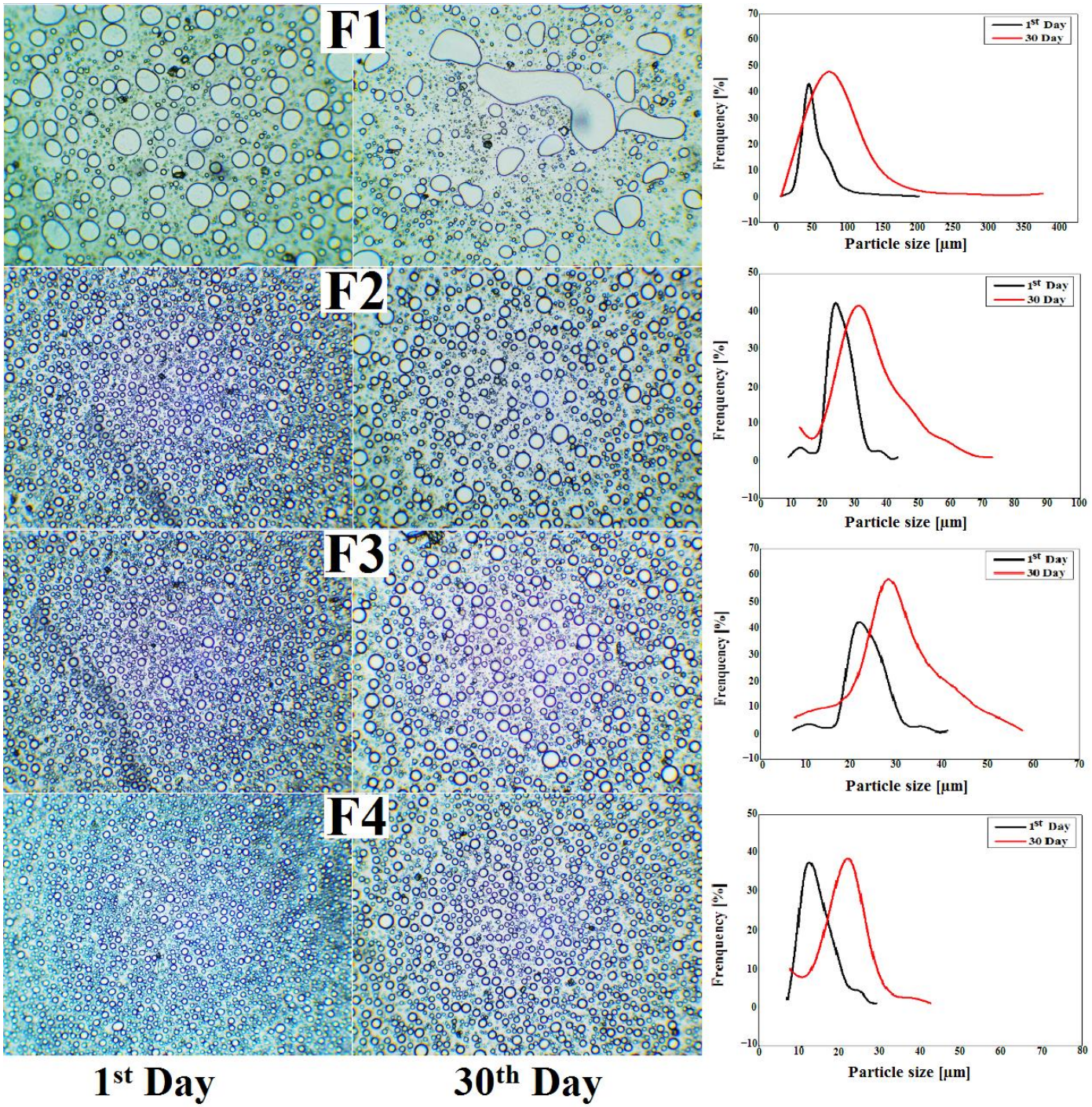

Figure 11. Photomicrographs and particle size distribution of the prepared formulations on the 1st day and the 30th day. Micrographs with a magnification of 10×: Emulsions F1, F2, F3, and F4 stabilized by KC, KCR1, KCR2, and KCR3 derivatives, respectively, at 1%.

The results indicate that the formula F1 based on kappa carrageenan shows no stabilizing power. The oil droplets in these emulsions showed an irregular evolution, resulting in complete coalescence after 30 days, which leads to phase separation. This can be attributed

to the fact that KC lacks surface activity and therefore cannot adsorb at the oil-water interface. Moreover, the average size of the globules in these emulsions increased considerably from 43.6 ± 0.47 µm to 72.42 ± 0.83 µm after one month due to the coalescence phenomenon.

During the storage period, the emulsions based on KCRs, namely F2, F3, and F4, retained their homogeneous appearance without any signs of instability or phase separation. The KCRs derivatives demonstrated superior stabilization ability by forming micellar aggregates at the oil/water interface and creating a three-dimensional network around the globules due to the intermolecular associations between the hydrophobic groups. These intermolecular associations enhanced the stabilizing capacity of the derivatives, preventing coalescence and improving the stability of the emulsions.

After one month (30 days) of the storage period, the stability of F2 formulation, based on the KCR1 derivative, was inferior compared to F3 formulation based on KCR2 and F4 emulsion stabilized with KCR3. The larger oil droplets and aggregated globules found in F2 formulation could be attributed to the lower degree of substitution of KCR1, where the average particle size of F2 varied from 23.52 ± 0.29 µm to 31.13 ± 0.38 µm for the first and 30th day of storage, respectively.

On the other hand, F3 and F4 emulsions exhibited the creation of uniformly distributed, round oil droplets with a consistent particle size distribution. F3 exhibited an average globule size ranging from 21.54 ± 0.62 µm to 28.69 ± 0.18 µm during the first and 30th day of storage, while F4 presented an average droplet size of 12.51 ± 0.05 µm that slightly increased to 22.58 ± 0.10 µm after 30 days. These findings are due to the higher degree of substitution of KCR2 and KCR3, which imparted them with better emulsifying properties

Finally, the grafting of hydrophobic fragments onto the kappa carrageenan backbone resulted in the development of amphiphilic properties in the obtained derivatives (KCRs). The amphiphilic character of these derivatives was observed to be effective in stabilizing direct emulsions, as a substitute for synthetic surfactants. The findings indicated that these biopolymers possess emulsifying power, which is improved with an increase in the degree of substitution. This suggests that by reducing the average size of dispersed oil droplets, increasingly stable emulsions can be obtained [44].

4. Conclusions

In this work, the hydrophobic functionalization of kappa carrageenan (KC) was successfully achieved with the grafting of alkyl chains onto its hydrophilic backbone. The resultant derivatives (KCRs) were evaluated for their physicochemical features and amphiphilic character. The integration of octyl groups into the native kappa carrageenan structure was verified using FT-IR and ^{1}H-NMR analyses.

The findings from the physicochemical analysis showed that the hydrophobic modification of κ-carrageenan did not cause any significant changes in its morphological or rheological properties. Moreover, the emulsifying study demonstrated the amphiphilic character of the new derivatives and the increased stabilizing effect with the degree of the substitution where the KCR3 derivative with the higher degree of substitution (DS), displayed superior emulsifying power and greater amphiphilic character.

Therefore, this present work has led to the development of new amphiphilic derivatives endowed with very interesting properties allowing them to be used as excipients in the pharmaceutical field but also as an alternative material to synthetic surfactants in the cosmetics and food industry.

Author Contributions: Conceptualization, S.T., S.L. and M.M.Y.; methodology, S.T., M.M.Y., S.L. and A.H.; software, S.T. and S.L.; validation and supervision, S.L. and A.A.; investigation, S.T., S.L. and A.H.; writing—original draft preparation, S.T., M.M.Y. and S.L.; writing—review and editing, S.T., S.L. and M.M.Y.; visualization, A.N.E.H.S., A.H.H.-B., A.A., J.Z., A.A.A. and L.M.; resources, A.N.E.H.S., A.H.H.-B., A.A., J.Z., A.A.A. and L.M.; project administration, S.L. and A.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lefnaoui, S.; Moulai-Mostefa, N. Investigation and Optimization of Formulation Factors of a Hydrogel Network Based on Kappa Carrageenan–Pregelatinized Starch Blend Using an Experimental Design. *Colloids Surf. A Physicochem. Eng. Asp.* **2014**, *458*, 117–125. [CrossRef]
2. Bai, L.; Liu, F.; Xu, X.; Huan, S.; Gu, J.; McClements, D.J. Impact of Polysaccharide Molecular Characteristics on Viscosity Enhancement and Depletion Flocculation. *J. Food Eng.* **2017**, *207*, 35–45. [CrossRef]
3. Stephen, A.M.; Phillips, G.O. *Food Polysaccharides and Their Applications*; CRC Press: Boca Raton, FL, USA, 2016; ISBN 0-429-11616-0.
4. Krylova, N.V.; Kravchenko, A.O.; Iunikhina, O.V.; Pott, A.B.; Likhatskaya, G.N.; Volod'ko, A.V.; Zaporozhets, T.S.; Shchelkanov, M.Y.; Yermak, I.M. Influence of the Structural Features of Carrageenans from Red Algae of the Far Eastern Seas on Their Antiviral Properties. *Mar. Drugs* **2022**, *20*, 60. [CrossRef] [PubMed]
5. Mihaila, S.M.; Gaharwar, A.K.; Reis, R.L.; Marques, A.P.; Gomes, M.E.; Khademhosseini, A. Photocrosslinkable Kappa-carrageenan Hydrogels for Tissue Engineering Applications. *Adv. Healthc. Mater.* **2013**, *2*, 895–907. [CrossRef]
6. Jiang, J.-L.; Zhang, W.-Z.; Ni, W.-X.; Shao, J.-W. Insight on Structure-Property Relationships of Carrageenan and Its Chemically Modified Derivatives: A Review. *Carbohydr. Polym.* **2021**, *257*, 117642. [CrossRef] [PubMed]
7. Li, L.; Ni, R.; Shao, Y.; Mao, S. Carrageenan and Its Applications in Drug Delivery. *Carbohydr. Polym.* **2014**, *103*, 1–11. [CrossRef]
8. Lefnaoui, S.; Moulai-Mostefa, N. Polyelectrolyte Complex Based on Carboxymethyl-Kappa-Carrageenan and Eudragit RL 30D as Prospective Carriers for Sustained Drug Delivery. *Chem. Eng. Res. Des.* **2015**, *97*, 165–174. [CrossRef]
9. Hezaveh, H.; Muhamad, I.I. The Effect of Nanoparticles on Gastrointestinal Release from Modified κ-Carrageenan Nanocomposite Hydrogels. *Carbohydr. Polym.* **2012**, *89*, 138–145. [CrossRef] [PubMed]
10. Rhein-Knudsen, N.; Ale, M.T.; Meyer, A.S. Seaweed Hydrocolloid Production: An Update on Enzyme Assisted Extraction and Modification Technologies. *Mar. Drugs* **2015**, *13*, 3340–3359. [CrossRef]
11. Falshaw, R.; Bixler, H.J.; Johndro, K. Structure and Performance of Commercial Kappa-2 Carrageenan Extracts: I. Structure Analysis. *Food Hydrocoll.* **2001**, *15*, 441–452. [CrossRef]
12. Meena, R.; Lehnen, R.; Schmitt, U.; Saake, B. Effect of Oat Spelt and Beech Xylan on the Gelling Properties of Kappa-Carrageenan Hydrogels. *Carbohydr. Polym.* **2011**, *85*, 529–540. [CrossRef]
13. Bonnaud, M.; Weiss, J.; McClements, D.J. Interaction of a Food-Grade Cationic Surfactant (Lauric Arginate) with Food-Grade Biopolymers (Pectin, Carrageenan, Xanthan, Alginate, Dextran, and Chitosan). *J. Agric. Food Chem.* **2010**, *58*, 9770–9777. [CrossRef] [PubMed]
14. Madruga, L.Y.; Sabino, R.M.; Santos, E.C.; Popat, K.C.; Balaban, R.D.C.; Kipper, M.J. Carboxymethyl-Kappa-Carrageenan: A Study of Biocompatibility, Antioxidant and Antibacterial Activities. *Int. J. Biol. Macromol.* **2020**, *152*, 483–491. [CrossRef] [PubMed]
15. Yuan, H.; Zhang, W.; Li, X.; Lü, X.; Li, N.; Gao, X.; Song, J. Preparation and in Vitro Antioxidant Activity of κ-Carrageenan Oligosaccharides and Their Oversulfated, Acetylated, and Phosphorylated Derivatives. *Carbohydr. Res.* **2005**, *340*, 685–692. [CrossRef]
16. Suchaoin, W.; Bonengel, S.; Hussain, S.; Huck, C.W.; Ma, B.N.; Bernkop-Schnürch, A. Synthesis and in Vitro Evaluation of Thiolated Carrageenan. *J. Pharm. Sci.* **2015**, *104*, 2523–2530. [CrossRef] [PubMed]
17. Opoku, G.; Qiu, X.; Doctor, V. Effect of Oversulfation on the Chemical and Biological Properties of Kappa Carrageenan. *Carbohydr. Polym.* **2006**, *65*, 134–138. [CrossRef]
18. Zhu, M.; Ge, L.; Lyu, Y.; Zi, Y.; Li, X.; Li, D.; Mu, C. Preparation, Characterization and Antibacterial Activity of Oxidized κ-Carrageenan. *Carbohydr. Polym.* **2017**, *174*, 1051–1058. [CrossRef]
19. Cosenza, V.A.; Navarro, D.A.; Pujol, C.A.; Damonte, E.B.; Stortz, C.A. Partial and Total C-6 Oxidation of Gelling Carrageenans. Modulation of the Antiviral Activity with the Anionic Character. *Carbohydr. Polym.* **2015**, *128*, 199–206. [CrossRef] [PubMed]
20. Barahona, T.; Prado, H.J.; Bonelli, P.R.; Cukierman, A.L.; Fissore, E.L.; Gerschenson, L.N.; Matulewicz, M.C. Cationization of Kappa-and Iota-Carrageenan–Characterization and Properties of Amphoteric Polysaccharides. *Carbohydr. Polym.* **2015**, *126*, 70–77. [CrossRef]
21. Ellis, A.L.; Mills, T.B.; Norton, I.T.; Norton-Welch, A.B. The Hydrophobic Modification of Kappa Carrageenan Microgel Particles for the Stabilisation of Foams. *J. Colloid Interface Sci.* **2019**, *538*, 165–173. [CrossRef]
22. Toumi, S.; Yahoum, M.M.; Lefnaoui, S.; Hadjsadok, A. Synthesis, Characterization and Potential Application of Hydrophobically Modified Carrageenan Derivatives as Pharmaceutical Excipients. *Carbohydr. Polym.* **2021**, *251*, 116997. [CrossRef] [PubMed]
23. Shahin, M.; Hady, S.A.; Hammad, M.; Mortada, N. Development of Stable O/W Emulsions of Three Different Oils. *Int. J. Pharm. Stud. Res.* **2011**, *2*, 45–51.

24. Fan, L.; Peng, K.; Li, M.; Wang, L.; Wang, T. Preparation and Properties of Carboxymethyl κ-Carrageenan/Alginate Blend Fibers. *J. Biomater. Sci. Polym. Ed.* **2013**, *24*, 1099–1111. [CrossRef] [PubMed]
25. Tranquilan-Aranilla, C.; Nagasawa, N.; Bayquen, A.; Rosa, A.D. Synthesis and Characterization of Carboxymethyl Derivatives of Kappa-Carrageenan. *Carbohydr. Polym.* **2012**, *87*, 1810–1816. [CrossRef]
26. Fang, J.M.; Fowler, P.A.; Sayers, C.; Williams, P.A. The Chemical Modification of a Range of Starches under Aqueous Reaction Conditions. *Carbohydr. Polym.* **2004**, *55*, 283–289. [CrossRef]
27. Han, L.; Ratcliffe, I.; Williams, P.A. Synthesis, Characterisation and Physicochemical Properties of Hydrophobically Modified Inulin Using Long-Chain Fatty Acyl Chlorides. *Carbohydr. Polym.* **2017**, *178*, 141–146. [CrossRef]
28. van de Velde, F.; Rollema, H.S.; Grinberg, N.V.; Burova, T.V.; Grinberg, V.Y.; Tromp, R.H. Coil–Helix Transition of I-carrageenan as a Function of Chain Regularity. *Biopolym. Orig. Res. Biomol.* **2002**, *65*, 299–312. [CrossRef]
29. Sara, H.; Yahoum, M.M.; Lefnaoui, S.; Abdelkader, H.; Moulai-Mostefa, N. New Alkylated Xanthan Gum as Amphiphilic Derivatives: Synthesis, Physicochemical and Rheological Studies. *J. Mol. Struct.* **2020**, *1207*, 127768. [CrossRef]
30. Yahoum, M.M.; Moulai-Mostefa, N.; Le Cerf, D. Synthesis, Physicochemical, Structural and Rheological Characterizations of Carboxymethyl Xanthan Derivatives. *Carbohydr. Polym.* **2016**, *154*, 267–275. [CrossRef]
31. Toumi, S.; Yahoum, M.M.; Lefnaoui, S.; Hadjsadok, A. Synthesis and Physicochemical Evaluation of Octenylsuccinated Kappa-Carrageenan: Conventional versus Microwave Heating. *Carbohydr. Polym.* **2022**, *286*, 119310. [CrossRef]
32. Liew, J.W.Y.; Loh, K.S.; Ahmad, A.; Lim, K.L.; Wan Daud, W.R. Synthesis and Characterization of Modified κ-Carrageenan for Enhanced Proton Conductivity as Polymer Electrolyte Membrane. *PLoS ONE* **2017**, *12*, e0185313. [CrossRef]
33. Youssouf, L.; Lallemand, L.; Giraud, P.; Soulé, F.; Bhaw-Luximon, A.; Meilhac, O.; D'hellencourt, C.L.; Jhurry, D.; Couprie, J. Ultrasound-Assisted Extraction and Structural Characterization by NMR of Alginates and Carrageenans from Seaweeds. *Carbohydr. Polym.* **2017**, *166*, 55–63. [CrossRef] [PubMed]
34. Rodriguez, S.A.; Weese, E.; Nakamatsu, J.; Torres, F. Development of Biopolymer Nanocomposites Based on Polysaccharides Obtained from Red Algae Chondracanthus Chamissoi Reinforced with Chitin Whiskers and Montmorillonite. *Polym.-Plast. Technol. Eng.* **2016**, *55*, 1557–1564. [CrossRef]
35. Hosseini, S.M.; Hosseini, H.; Mohammadifar, M.A.; German, J.B.; Mortazavian, A.M.; Mohammadi, A.; Shojaee-Aliabadi, S.; Khaksar, R. Preparation and Characterization of Alginate and Alginate-Resistant Starch Microparticles Containing Nisin. *Carbohydr. Polym.* **2014**, *103*, 573–580. [CrossRef] [PubMed]
36. Giri, T.K.; Pradhan, M.; Tripathi, D.K. Synthesis of Graft Copolymer of Kappa-Carrageenan Using Microwave Energy and Studies of Swelling Capacity, Flocculation Properties, and Preliminary Acute Toxicity. *Turk. J. Chem.* **2016**, *40*, 283–295. [CrossRef]
37. Gahruie, H.H.; Eskandari, M.H.; Van der Meeren, P.; Hosseini, S.M.H. Study on Hydrophobic Modification of Basil Seed Gum-Based (BSG) Films by Octenyl Succinate Anhydride (OSA). *Carbohydr. Polym.* **2019**, *219*, 155–161. [CrossRef]
38. Jacquier, J.-C.; MacArtain, P.; Dawson, K.A. Hydrophobically Modified Calcium-Induced κ-Carrageenan Gels. *Trends Colloid Interface Sci. XV* **2001**, *118*, 127–131.
39. Chen, Y.; Hao, Y.; Li, S.; Luo, Z.; Gao, Q. Preparation of Hydroxybutyl Starch with a High Degree of Substitution and Its Application in Temperature-Sensitive Hydrogels. *Food Chem.* **2021**, *355*, 129472. [CrossRef]
40. Sav, A.K.; Fule, R.A.; Ali, M.T.; Amin, P. Synthesis and Evaluation of Octenyl Succinate Anhydride Derivative of Fenugreek Gum as Extended Release Polymer. *J. Pharm. Investig.* **2013**, *43*, 417–429. [CrossRef]
41. Xu, J.; Liu, W.; Yao, W.; Pang, X.; Yin, D.; Gao, X. Carboxymethylation of a Polysaccharide Extracted from Ganoderma Lucidum Enhances Its Antioxidant Activities in Vitro. *Carbohydr. Polym.* **2009**, *78*, 227–234. [CrossRef]
42. Yang, S.R.; Jeong, J.H.; Park, K.; Kim, J.-D. Self-Aggregates of Hydrophobically Modified Poly (2-Hydroxyethyl Aspartamide) in Aqueous Solution. *Colloid Polym. Sci.* **2003**, *281*, 852–861. [CrossRef]
43. Muhammad, M.T.; Khan, M.N. Study of Electrolytic Effect on the Interaction between Anionic Surfactant and Methylene Blue Using Spectrophotometric and Conductivity Methods. *J. Mol. Liq.* **2017**, *234*, 309–314. [CrossRef]
44. Li, X.-M.; Zhu, J.; Pan, Y.; Meng, R.; Zhang, B.; Chen, H.-Q. Fabrication and Characterization of Pickering Emulsions Stabilized by Octenyl Succinic Anhydride-Modified Gliadin Nanoparticle. *Food Hydrocoll.* **2019**, *90*, 19–27. [CrossRef]