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Investigation of the Gelation Mechanism between Amino Acid Surfactant Based Microemulsion and Kappa-Carrageenan Gel Network

(Kajian tentang Mekanisme Penggelan antara Mikroemulsi Berdasarkan Surfaktan Amino-Asid dan Rangkaian Gel Kappa-Carrageenan)

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ABSTRACT

Kappa-carrageenan is one form of necessary hydrocolloid. Hydrocolloids are macromolecular materials, which swell upon absorption of water; in some cases, forming a stiff gel in the presence of additives. This property is very important to suspend nanocarriers into gel network, which provide them long time stability at a varying temperature range. In this work, we prepared microemulsion and trapped these particles inside the kappa-carrageenan gel network. The microemulsion was composed of sodium N-lauroylsarcosinate hydrate (SNLS), oleic acid and deionized water. The purpose of this study was to immobilize them into the gel network, giving longer shelf life at a range of temperatures for oral drug delivery. Morphological properties were investigated by transmission electron microscope (TEM), dynamic light scattering (DLS) and Fourier transform infrared (FTIR) spectra. The TEM results showed that microemulsions are trapped in the gel network, and the diameter of the microemulsions are below 100 nm, which is comparable with the DLS results. The important functional groups of kappa-carrageenan and microemulsion were shown from the FTIR result of the complex microemulsion gel. These results confirmed the interaction between SNLS based microemulsion and kappa-carrageenan gel.

Keywords: Gel; kappa-carrageenan; microemulsion

ABSTRAK

Kappa-carrageenan *adalah sejenis hidrokoloid. Hidrokoloid merupakan bahan makromolekul yang mengembang apabila menyerap air; dalam kes tertentu boleh membentuk gel yang tegar dengan penambahan bahan aditif. Sifat ini sangat penting bagi mengapungkan nanopembawa ke dalam rangkaian gel dan memberikan kestabilan jangka panjang pada julat suhu yang luas. Dalam kajian ini, kami menyediakan mikroemulsi yang diperangkap ke dalam rangkaian gel* kappa-carrageenan. *Mikroemulsi mempunyai komposisi daripada sodium –N-lauroylsarcosinat hidrat (SNLS), asid oleik dan air nyahion. Tujuan kajian ini adalah untuk memegunkan mikroemulsi ke dalam rangkaian gel bagi memberikan masa simpan yang lama pada julat suhu penghantaran dadah secara oral. Sifat morfologi telah ditentukan dengan menggunakan mikroskop elektron transmisi (TEM), serakan cahaya dinamik (DLS) dan spektrum inframerah penjelmaan Fourier (FTIR). Hasil TEM menunjukkan bahawa mikroemulsi terperangkap dalam rangkaian gel dengan diameter mikroemulsi kurang daripada 100 nm tekal dengan ukuran DLS. Kumpulan berfungsi utama* kappa-carrageenan *dan mikroemulsi juga dikenal pasti melalui pencirian spektroskopi-FTIR dalam kompleks gel mikroemulsi. Hasil kajian membuktikan bahawa interaksi yang kuat wujud antara mikroemulsi berdasarkan SNLS dan* kappa-carrageenan *gel.*

Kata kunci: Gel; kappa-carrageenan*; mikroemulsi*

INTRODUCTION

There is a pressing need to use poly-electrolytes in nano-level to create new biocompatible nanocarriers. These materials in gel form play an important role as suspending medium and thus provide comparatively long term stability to those nanoparticles. The complex product can be easily adapted in the biological environment due to their biocompatible property. A large number of hydrocolloids can produce such bio-poly-electrolytes.

This work deals on the use of one form of poly-electrolyte 'kappa-carrageenan', to prepare a microemulsion gel. Kappa-carrageenan in its natural origin is a sulfated galactent, mostly used for the industrial applications due to their unique physico-chemical properties. It is a high molecular weight polysaccharide with 15 to 40% of ester-sulfate content (Fennema 2002), formed by alternate units of D-galactose and 3.6 anhydro-galactose (3.6-AG) joined by α-1, 3 and β-1, 4 -glycosidic linkage. On the other hand, microemulsions are fine oil-in-water or water-in-oil droplets or biphasic dispersions, having droplet size ranging from 20–300 nm. These size ranges enable them as excellent drug delivery vehicle. By immobilizing them into the carbohydrate gel network, these types of carrier can be used as an oral drug delivery vehicle.

For biological application, amino acid based bio surfactants are considered to be more suitable due to their bio-origin, less-toxicity, biodegradability, cheaper rate and above all, their excellent physicochemical properties. These molecular properties enable them to self assemble into fascinating structures (Akter et al. 2013; Infante et al. 2004). An amino acid based surfactant, Sodium N-lauroylsarcosinate hydrate (SNLS), was used to prepare microemulsion. It is reported that SNLS has very rich morphologies to self assemble into different aggregate structures. The carboxylate and amide functional groups are highly active sites for hydrogen bonding formation. Besides, chemical richness, it represents superior skin permeability (Karande et al. 2007). The SNLS based nanocarrier was found to be stable at very low pH and physiological temperature (Ghosh & Dey 2011). These are very important characteristics for targeted drug delivery. The already established fascinating properties of SNLS based systems motivated us to prepare microemulsion gel to use in drug delivery field.

Our objective was to prepare a stable drug delivery vehicle. Before using in clinical purpose, a systematic study should be done to confirm the morphology of the nanocarriers. With this intention, we examine the mechanism of immobilization of microemulsion inside the kappa-carrageenan gel network. Formation of microemulsion was confirmed by visual inspection of the sample, using cross polarizing filters, transmission electron microscope (TEM), dynamic light scattering (DLS) and Fourier transform infrared spectra (FTIR). All of these characterizations confirmed the presence of microemulsion droplets trapped inside the gel network.

Experimental Details

MATERIALS

Kappa-carrageenan was purchased from Fluka-chimica (USA). Sodium N-lauroylsarcosinate hydrate was purchased from Toshima chemical industry (TCI), Japan. Potassium chloride (KCl) and Oleic acid was purchased from Fluka (Buchs, Switzerland). All samples were prepared with deionized water (Milli-Q filtered, 18.2 MΩ/cm^2 resistivity).

PREPARATION OF MICROEMULSION

SNLS was used as surfactant and oleic acid was used as oil phase. Phase diagram was determined at 25ºC by stepwise addition of water to the mixture of oil and surfactant. After each addition, the samples were mixed with gentle hand shaking and then centrifuged. The occurrence of phase separation was observed and the single phase sample was selected for the preparation of kappa-carrageenan based microemulsions.

PHASE DIAGRAM DETERMINATION

Approximately 10 samples of each 3 mL were prepared to cover a narrow range of compositions in the surfactant rich corner in the ternary phase diagram. The phase separation of this region was determined by visual appearance of the sample. The phase, which was stable for several months, was selected for the preparation of microemulsion gel. In this experiment the point at 90 wt. % water with SNLS: oleic acid wt. % ratio 4:1 was selected as the stable point for microemulsion formation. No attempts were made to identify the other regions of the phase diagram in detail and these have been described in terms of their visual appearance. The phase diagram is shown in Figure 1.

The total concentration of each sample was divided into two parts, i.e. 50% (3 mL) of the sample was prepared as gel and 50% (3 mL) as microemulsion and mixed together with sonication at 80ºC. After preparation, each sample was checked through cross polarizers. No birefringence was observed between cross polarizers. The prepared microemulsion sample was transparent and isotropic. For direct evidence, the samples were characterized by TEM following negative staining method.

PREPARATION OF KAPPA-CARRAGEENAN BASED MICROEMULSION GEL

Carrageenan (0.2 g/L) was dissolved in 30 mM KCl solution and heated above 80ºC for 2 h to dissolve the carrageenan completely. Finally, both the microemulsion and kappa-carrageenan gel ware mixed in a bath sonicator for 10 min above 80ºC to get the microemulsions trapped in gel network.

TRANSMISSION ELECTRON MICROSCOPIC (TEM) CHARACTERIZATION

Observation of the kappa-carrageenan gel and microemulsion gel was examined using TEM (Philips-CM12). To preserve their structure, gels were placed on the carbon grid very carefully and left for partial drying to allow the gel structures to adhere onto the carbon grid. The excess gel was removed by filter paper from the opposite direction. A drop of 3% uranyl acetate solution was added to the grid and left for 10 s. Again, the excess solution was removed washing the grid thrice. Each time the liquid was absorbed with filter paper, the sample was dried in the air. The sample was then characterized under an electron microscope at an accelerating voltage of 100 kV.

MEASUREMENT OF THE PARTICLES

The size distribution on microemulsion droplets was determined with a Zetasizer Nano ZS (Malvern Instruments, Herrenberg, Germany) after equilibration. Z-average particle sizes were measured at the scattering angle of 90º at 25°C using DLS combined with Malvern's DTS software (v.5.02). The Fourier transform infrared (FTIR) spectra were recorded using a Perkin Elmer Spectrum BX FTIR system over the region of 4000-400 cm^{-1} of the sample solution. Prior to the assay, liquid samples were prepared by placing a few drops of the solution between two sodium chloride (NaCl) salt plates.

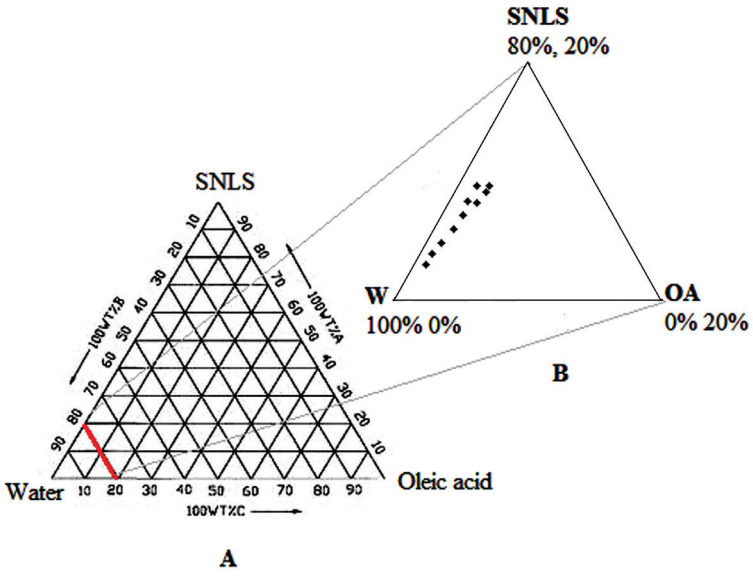

FIGURE 1. Ternary phase diagram of a SNLS/oleic acid/water system, (a) represents the phase diagram of the whole system with 100% of each component and (b) represents the partial phase diagram of the system

RESULTS AND DISCUSSION

MICROSCOPY

The structures of k-carrageenan gel and microemulsion gel were examined by macroscopic appearance, cross polarizing filters, TEM, DLS and FTIR. Schematic representation of the mechanism of gel formation is shown in Figure 2(a), 2(b), 2(c) and their inverted conditions are shown in Figure 2(d), 2(e), 2(f). Owing to the coil like structure, the sample before mixing and after mixing at high temperature remain in the liquid phase which can flow like water. It is well known that in the presence of monovalent cations gelation of carrageenan causes in a two-step mechanism: Firstly, the pairs of random coils associate into double helices and secondly these helices aggregated into larger crystalline clusters (Anderson et al. 1968; Rees et al. 1969).

Gelation property of the compounds was tested by subsequent heating and cooling method. Upon cooling at room temperature and inversion of the vial, if the solution did not flow out of the vial, the compound was considered to be a gelator of the corresponding solvent. This result indicated the already established results of kappa-carrageenan gelation, which results in due to the formation of contact ion pair between K^+ and sulfate groups (Braudo et al. 1991). Moreover, the absence of phase separation between kappa-carrageenan gel and microemulsion complex indicates that microemulsion droplets suspended within gel matrix (Bourriot et al. 1999). The inverted solution of the kappa-carrageenan gel and microemulsion gel is shown in Figure 2(g).

In TEM characterization, gel network was observed at room temperature; the bundle of helical structures with diameters in the range of 200 nm could be observed in each side of the tetrahedral network of the gel. Kappa-carrageenan is a long thin stranded polymer with each strand being approximately 20 nm in diameter (Spagnuolo et al. 2005); thus each junction zone may consist of at least 5-10 double helices. The double helices aggregate into continuous three dimensional networks in the presence of K+ ion, called gelation (Morris et al. 1980).

Until now, no reports represented the microscopic image of individual helices of kappa-carrageenan gel network. This indistinctness was attributed to the poor contrast or resolution of microscope (Borhstrom et al. 1996; Piculell 1998). According to Borgstrom et al. (1996), the contrast in the image depends either on the sulfate group in the carrageenan backbone or the density difference between the gel network and the continuous phase. To confirm the interaction of carrageenan with any system, besides microscope some other evidences are needed. Based on the microscopic evidence image and DLS data, Spagnuolo et al. (2005) reported interaction of kappa-carrageenan with casein micelles.

In this experiment, after mixing microemulsion with kappa-gel at 80°C, this gel transforms into a liquid state (coil confirmation). These coils rearrange and accommodate with the microemulsion droplets and found new equilibrium states in helical conformation after cooling down at room temperature. The single phase macroscopic appearance, micrograph of TEM, DLS data (Figure 3) and FTIR spectroscopic results indicated that there was an interaction between SNLS and a bio-electrolyte will be replaced by kappa-carrageenan. Although the treatment of the samples for TEM with uranyl acetate might have a possibility to cross-link the microemulsion droplets and kappa-carrageenan gel and might have resulted in bridging between neighboring microemulsions via gel network. This possibility was ruled out by the evidence of DLS data

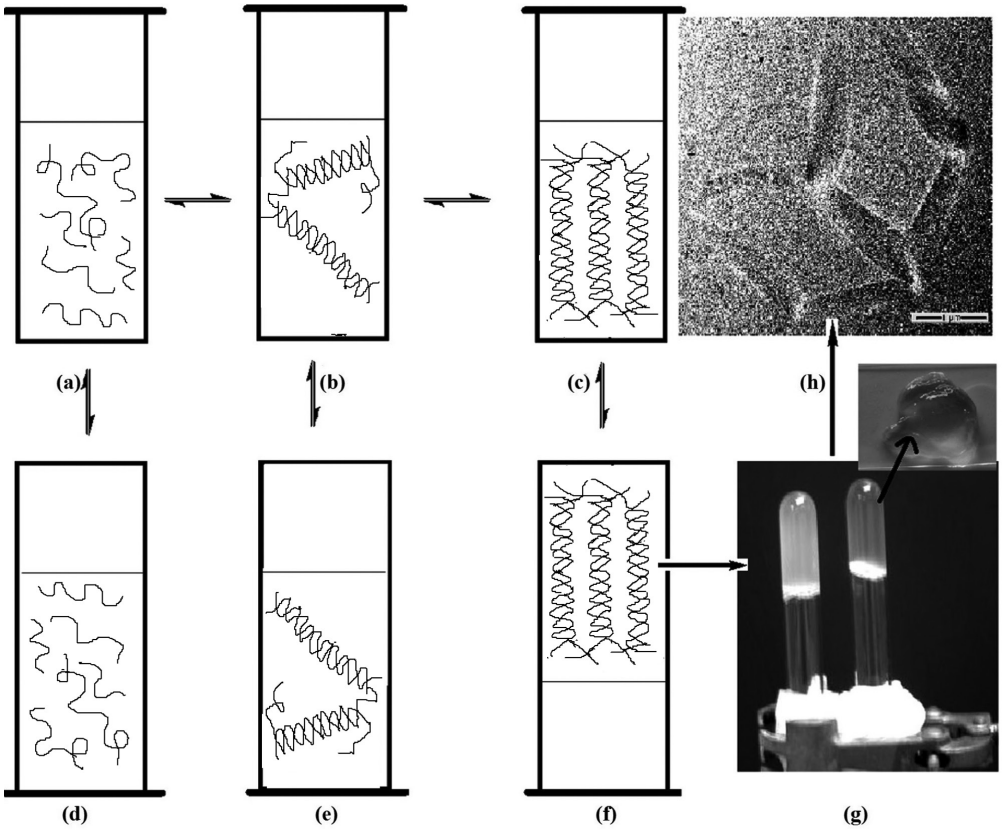

FIGURE 2. Schematic representation of helical structure of twisted kappa-carrageenan linear chain (a) represents the coil conformation of linear chains in hot water, (b) helical formation in hot solution, (c) aggregated helical structure upon cooling, (d), (e), (f) represents the inverted samples of (a), (b), (c), (g) visual observation of kappa-carrageenan gel and microemulsion gel in upside down condition and (h) TEM image of gel network

that showed an increase in droplet diameter. A significant increase in droplet size was observed than the initial droplet size (35 nm). Moreover, key functional groups of both SNLS and kappa-carrageenan of microemulsion appeared in the FTIR spectra. Therefore, we can conclude that the ribbon-like micro structures seen (Figure 3(b)) around the surface of the microemulsion were k-carrageenan gel network. This is well known that kappa-carrageenan demonstrates twisted ribbon confirmation due to the torsion effect forced to the molecule through the 3, 6 anhydro-D-galactose bridges. The symmetry is 3:1 and the periods are 24.6 and 26.0A° for the kappa and the iota form of carrageenan, respectively (Anderson et al. 1969; Rees 1969).

FTIR SPECTRA OF KAPPA-CARRAGEENAN AND ITS DERIVATIVES

The evidence of interaction between polyelectrolyte and microemulsion was studied by FTIR spectroscopy. A spectrum of kappa-carrageenan in the range of 4000-400 cm^{-1} is presented in Figure 4. The broad band at 3400-3200 cm^{-1} is due to hydrogen bonded stretching vibration of OH group with a shoulder centered at 2958 cm^{-1} (Figure 4(a)). The two bands from 4000-2000 cm^{-1} are common to all polysaccharide standards and seaweed samples (Gómez-Ordóñez et al. 2011). The characteristic strong band at 1260-1210 cm^{-1} is an evidence of the presence of sulphate ester (S=O) on the protruding side chain.

The bands at 1158 cm^{-1} and 1030-1010 cm^{-1} assigned to C-O and C-C stretching vibrations of pyranose ring common to all polysaccharides. In the anomeric (950-700 cm^{-1}) region kappa-carrageenan shows several bands. The strong band at 922 cm^{-1} assigned to the presence of 3, 6-anhydro-galactose residue. Bands at 770, 736 and 696 cm^{-1} are assigned to the skeleton bending of pyranose ring. The bands at 848 cm^{-1} corresponds to galactose-4-sulphate (Abad et al. 2003; Coimbra et al. 1998; Synytsya et al. 2010; Tapia et al. 2004; Volery et al. 2004).

IR spectrum of Kappa-carrageenan gel also showed a broad asymmetric band in the 850 cm^{-1} region due to the presence of D-galactose-4-sulphate (Figure 4(b)). Furthermore, additional absorption spectrum also observed in the region of 930 cm^{-1} indicating the presence of 3, 6-anhydrogalactose. The band at 1233-1206 cm^{-1} with a shoulder at 1047 cm^{-1}, corresponds to the ester sulfate stretching of kappa-carrageenan in the former case and the latter represents the presence of glycosidic linkage.

Both microemulsion and microemulsion gel present a peak around 3500 cm^{-1}. This is assigned to the typical OH stretching vibration of the carboxyl acid functional group of oleic acid. Two bands at 2924 and 2854 cm^{-1} and

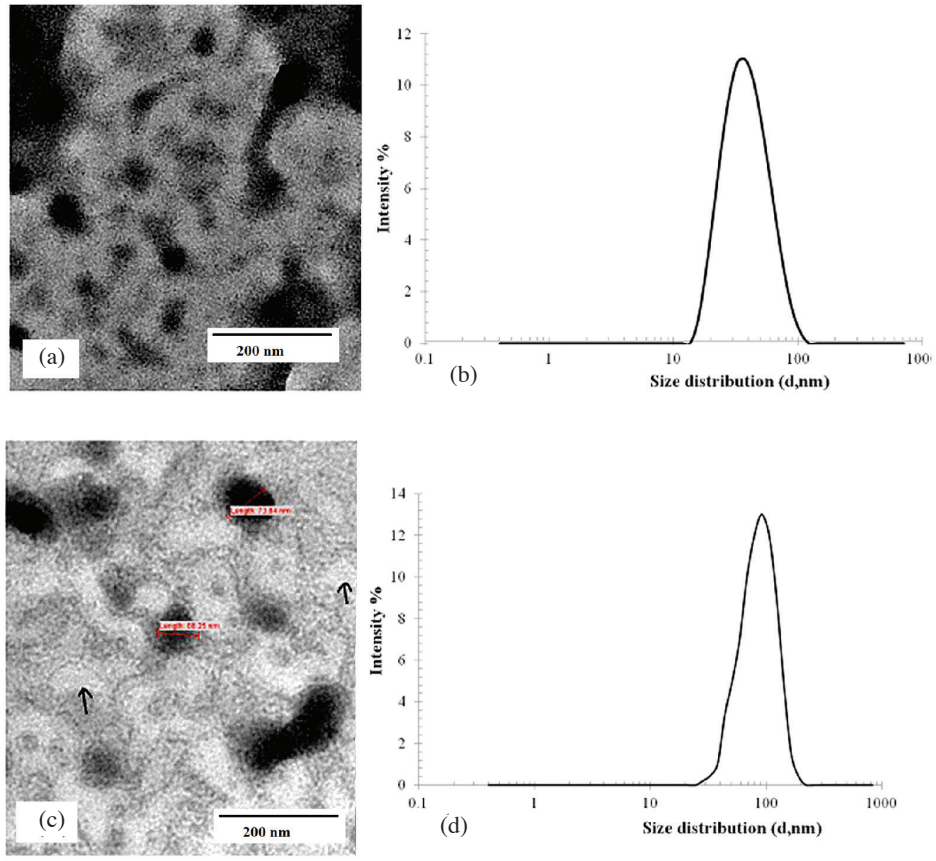

FIGURE 3. TEM image microemulsions; (a) without kappa-carrageenan gel and (b) with gel in immobilized state. The arrow of (b) indicates that a collapsed-ordered gel network in the presence of surfactant mesophases (c) and (d) represents the size distribution of microemulsion droplets without and with kappa-gel

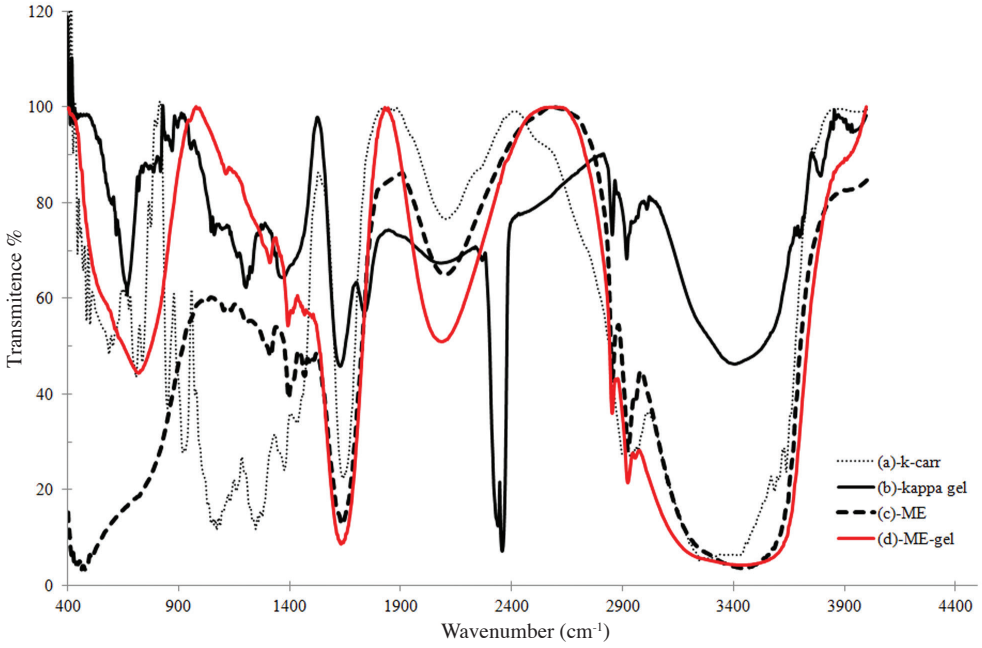

FIGURE 4. FTIR spectrums of (a) kappa-carrageenan, (b) kappa-carrageenan gel, (c) microemulsion (ME) and (d) kappa-carrageenan based microemulsion gel

a very weak band at 2957 cm^{-1} appeared as shoulder on the OH stretching vibration. These bands can be attributed to the asymmetric and symmetric stretch of CH_2 and asymmetric stretch of CH_3, respectively. The elimination of these bands from SNLS and oleic acid and recurrence as weak band with decreasing frequency in microemulsion phase can be attributed to the closely packed acylation of the hydrocarbon chains (Wu et al. 2004).

In comparison to kappa-carrageenan, the characteristic peak at 4000-2000 cm^{-1} of carbohydrate appears at the same position with slight broadening in microemulsion gel. This broadening at 3400 cm^{-1} is an evidence of stretching vibration of OH and hydrogen bonding between OH of oleic acid, amino acid based headgroup of surfactant and also with water. The absorption band appeared at 1632 cm^{-1} in both microemulsion and microemulsion gel, a group frequency 1680-1630 cm^{-1} is assigned to amide groups (Coats 2000). A broad and moderately strong peak appear at 944-650 cm^{-1} centered 730 cm^{-1}, in where the 3, 6 anhydro galactose and the pyranose ring bands superimposed on the skeletal vibration of SNLS.

It was also observed that gelling properties increased with the incorporation of the microemulsion. This can be assigned to the shorter distance between the charges of the carrageenan helices. The TEM image of gel and microemulsion gel exhibit size variation, such as gel domain become smaller in the latter case. It was reported that k-carrageenan gel collapsed in the presence of surfactant molecules and formed an ordered structure through hydrophobic and other interactions (electrostatic, hydrogen bonds and Van der Waals) (Kolesov et al. 2008; Rees 1982). In this case, strong electronegative atom Oxygen (O) and Nitrogen (N) of the compound plays the crucial role. Oxygen with two lone pair electrons and N with one lone pair electron contribute strong hydrogen bonding among helical network, water and microemulsion. The absorption peak of FTIR also shows broadening; indicating that hydrogen bonding occurs between SNLS headgroup and surrounding hydrogen molecules of anomeric ring. Moreover, amide peak did not change after gelation, but this peak shifted from the original peak of SNLS, where split peak was observed at 1640 and 1648 cm^{-1} (Akter et al. 2011). After complex formation with oleic acid, this split peak of SNLS disappeared and another peak with decreased frequency appeared at 1634 cm^{-1}. This shifting can be attributed to the interaction between C=O of SNLS and OH of oleic acid.

Conclusion

In this study, the mixture of kappa-carrageenan gel with microemulsion was investigated to facilitate the oral drug delivery with these nanocarriers. It was shown that adding gel to a microemulsion solution results in a stiff gel that can hold its own weight upon tube inversion. In fact, gel point generally reached when the largest molecular cluster diverges to the infinite size (Flory 1941; Stockmayer 1943). This is known as the threshold value of the chemical conversion. Our results evidenced that k-carrageenan in the helical form interacts with the surface of the microemulsion; perhaps the gelation process interrupts the mobility of self-assembled structures by hydrogen bonding, which has strong electronegative atoms in the periphery of the monolayer. Based on these results, it can be concluded that k-carrageenan microemulsion can be used as a carrier for the drug in the future.

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