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INTERACCIONES ENTRE κ-CARRAGENINA Y DISTINTOS TENSIOACTIVOS PARA PRODUCIR EMULSIONES EN GEL

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RESUMEN

El conocimiento de las interacciones que ocurren entre biopolímeros y tensioactivos en los alimentos es importante para mejorar la estabilidad de los productos donde se utilizan estas moléculas y para desarrollar sistemas con características específicas de encapsulación y liberación de componentes. En este trabajo se utilizaron distintas técnicas analíticas para estudiar las interacciones entre κ-carragenina, un polisacárido aniónico, y tensioactivos de distinta naturaleza química de uso alimentario.

Primero, se determinaron las principales propiedades fisicoquímicas de los componentes para entender su comportamiento en sistemas más complejos. Después se estudiaron las interacciones entre la κ-carrageenan y los diferentes tensioactivos. Los materiales incluyeron el polisacárido, dos tensioactivos solubles en agua; estearoil lactilato de sodio (ELS) y Tween 20, y uno soluble en aceite; lecitina de soya, cuyo uso es permitido en la industria de alimentos. El ELS es un tensioactivo aniónico, el Tween 20 es no-iónico y la lecitina de soya es zwitteriónico. Posteriormente, se estudiaron las interacciones de sistemas acuosos de κ-carragenina en presencia de los tensioactivos ELS o Tween 20. La adición de ELS a los sistemas de κ-carragenina modificó su conformación mediante diferentes mecanismos. El principal es la interferencia del ELS sobre la agregación de las moléculas de κ-carragenina. Además, se observaron cambios en las interacciones polímero-disolvente como consecuencia de la existencia de interacciones electrostáticas de repulsión entre ambos componentes. Por otro lado, la adición de Tween 20 no mostró algún efecto significativo sobre los sistemas de κ-carragenina.

Finalmente, se prepararon y analizaron emulsiones en gel, es decir, sistemas en los cuales las gotas de aceite están dispersas y embebidas en el interior de una red tridimensional formada por el polisacárido. Las emulsiones se prepararon con aceite comestible de maíz, κ-carragenina y los tensioactivos ELS, Tween 20 y lecitina, por separado y en diferentes concentraciones. Las condiciones de preparación permitieron obtener geles con temperaturas de fusión entre 30 y 40 °C, las cuales son cercanas al procesamiento oral en seres humanos. Esta condición permitiría tener mayor control sobre la liberación de sabores y aromas en aplicaciones de este tipo.

La naturaleza y la concentración de los tensioactivos son factores importantes que determinan las características finales de la emulsión; en este caso térmicas, reológicas y de estructura, debido a sus diferentes interacciones con la κ-carragenina.

ABSTRACT

Knowledge of the interactions that occur between biopolymers and surfactants in food is important to improve the stability of the products where they are used and for the development of systems with specific characteristics of component release and encapsulation. In this work, several analytical techniques were used to study the interactions between κ-carrageenan, an anionic polysaccharide, and surfactants of different chemical nature approved for food use.

First, the main physicochemical properties of the components were determined to understand their behavior in more complex systems. Subsequently, the interactions of κ-carrageenan systems in the presence of surfactants were studied. Materials included the polysaccharide, two water-soluble surfactants; sodium stearoyl lactylate (SSL) and Tween 20, and an oil-soluble surfactant; soy lecithin, with all of them being permitted in the food industry. SSL is an anionic surfactant, Tween 20 is a non-ionic surfactant and soy lecithin is a zwitterionic surfactant. The interactions of aqueous κ-carrageenan in the presence of ELS or Tween 20 were studied. The addition of SSL to κ-carrageenan systems modified its conformation by different mechanisms. The main one is interference of SSL molecules on the conformation of κ-carrageenan chains. In addition, changes in polymer-solvent interactions because of the presence of electrostatic repulsions between both components were observed. On the other hand, the addition of Tween 20 did not show a significant effect on the κ-carrageenan systems.

Finally, gel emulsions were prepared and analyzed. In these systems the oil droplets are dispersed and embedded in the three-dimensional network formed by the polysaccharide. Emulsions were prepared with edible corn oil, κ-carrageenan and the surfactants SSL, Tween 20 and lecithin, separately, in different concentrations. The preparation conditions allowed obtaining systems with melting temperatures between 30 and 40 °C, which are close to oral processing in human beings. This condition would allow to have greater control over the release of flavors and aromas in applications of this type.

The nature and concentration of the surfactants are important factors that determine the final characteristics of the emulsion; in this case thermal, rheological and structure, due to their different interactions with κ-carrageenan.

INTRODUCCIÓN GENERAL

Existe un creciente interés en la Industria Alimentaria en el desarrollo de sistemas a base de coloides para encapsular, proteger y liberar compuestos lipofílicos presentes en los alimentos o liberar de manera controlada diferentes ingredientes activos. Uno de los enfoques más prometedores para crear partículas coloidales es el uso de biopolímeros: proteínas y polisacáridos, como bloques de construcción (Matalanis, Jones & McClements, 2011) en combinación con tensioactivos los cuales permiten la formación de emulsiones y aumentan su estabilidad (Bouyer, Mekhloufi, Rosilio, Grossiord & Agnely, 2012).

Los biopolímeros más utilizados y estudiados son las proteínas, debido principalmente a que tienen propiedades estabilizantes, gelificantes y emulsionantes, por lo cual se las puede utilizar para manipular el comportamiento reológico y las propiedades de superficie (Chen & Dickinson, 1998; Chen, Dickinson & Edwards, 1999; Dickinson, 2012; Dickinson & Hong, 1995; Dickinson & Chen, 1999; Kelley & McClements, 2003; Kim, Renkema & van Vliet, 2001; Xiong, Aguilera & Kinsella, 1991). Sin embargo, la utilización de polisacáridos como quitosano, almidón, alginato, carboximetilcelulosa y carrageninas ha cobrado auge. Dentro de este grupo de polisacáridos el quitosano es el más estudiado (Chiappisi & Gradzielski, 2015; Grant, Cho & Allen, 2006), debido a que la mayoría de los tensioactivos permitidos en alimentos son aniónicos, i.e., presentan cargas negativas, o no tienen cargas y el quitosano es un polisacárido catiónico. Por otro lado, los estudios de polisacáridos aniónicos como el alginato y las carrageninas se han realizado con tensioactivos catiónicos no permitidos en alimentos como el dodecilsulfato de sodio, el cloruro de dodeciltrimetilamonio, el bromuro de tetradecilamonio, entre otros (Covis, Vives Gaillard, Benoit & Benvegnu, 2015; Kogej, 2008; Mata, Patel, Jain, Ghosh & Bahadur, 2006; Vinceković, Katona, Bujan & Sovilj, 2011).

Cuando se utilizan polisacáridos en mezclas biopolímero-tensioactivo, es importante establecer el entorno y las condiciones de las disoluciones en las cuales las moléculas de polisacárido se pueden asociar con otros polisacáridos o con otras moléculas como proteínas y tensioactivos, entre otros. Esto requiere conocer las propiedades fisicoquímicas del polisacárido involucrado, como, por ejemplo, su temperatura de transición sol-gel, sus propiedades eléctricas, su sensibilidad a iones mono o divalentes específicos, o su susceptibilidad a reacciones químicas o enzimáticas (Matalanis, Jones & Mc Clements, 2011). Las interacciones que pueden presentarse entre estos compuestos dependen en gran medida de las condiciones ambientales del medio acuoso como el pH, la fuerza iónica y la temperatura, por lo que el conocimiento de estos parámetros también es de gran importancia en su estudio.

En los estudios de mezclas biopolímero-tensioactivo se deben considerar, además de las características mencionadas anteriormente, las técnicas analíticas que se utilizarán para dilucidar las interacciones entre ambos componentes. En general, las técnicas más utilizadas son reometría, calorimetría de barrido diferencial o de titulación isotérmica, microscopía confocal, de fuerza atómica o electrónica de transmisión, difracción de rayos X, dispersión dinámica de luz, potencial zeta y medición de la tensión superficial. Habitualmente, dichas técnicas se utilizan en combinación; sin embargo, algunas de ellas se han utilizado de manera individual (Ortiz-Tafoya & Tecante, 2018). La selección de las técnicas depende de los componentes por analizar, ya que en general, se utilizan sistemas modelo, lo que significa que se realizan en disolución acuosa, puesto que los alimentos son sistemas muy complejos para llevar a cabo estudios fundamentales. Las técnicas utilizadas deben ser capaces de identificar diferencias debidas a factores como la concentración y la naturaleza de los componentes. Además, deben poder adaptarse a sistemas líquidos, sólidos o semisólidos, ya que algunos polisacáridos tienden a aumentar la viscosidad del medio o incluso a gelificar. Debido a todo esto, la selección de las técnicas de análisis es de gran importancia en este tipo de estudios.

En este trabajo se estudiaron las interacciones entre κ-carragenina, un polisacárido aniónico, y algunos tensioactivos de uso alimentario como el estearoil lactilato de sodio, el Tween 20 y la lecitina en sistemas modelo, i.e., disolución acuosa, y en aplicación dentro de una emulsión en gel, es decir, las gotas de aceite de la emulsión se encuentran dispersas y embebidas dentro de una red tridimensional formada por las cadenas del polisacárido. Estos sistemas tienen características específicas de fusión, que se logran modificando la concentración de iones añadidos.

El trabajo está organizado en un formato en el que se presentan las publicaciones producto en las que se muestra cómo fueron abordados los diferentes aspectos de la investigación. Asimismo, permite comprender los resultados y su interrelación. Por ello, cada capítulo corresponde a una publicación. En el Capítulo 1 se presenta el estado que guarda el estudio de las interacciones polisacárido-tensioactivos en general. En el Capítulo 2 se muestran las propiedades fisicoquímicas que caracterizan a los diferentes componentes. En el Capítulo 3 se discuten los resultados de la interacción entre la κ-carragenina y los tensioactivos, excepto la lecitina, estudiadas con diferentes técnicas. En el Capítulo 4 se discute la aplicación en la preparación de emulsiones en gel con los distintos surfactantes. Finalmente, se ofrecen las conclusiones generales de toda la investigación.

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OBJETIVOS

GENERAL

Comparar el efecto de tensioactivos de diferente naturaleza química, sobre las propiedades fisicoquímicas de la κ-carragenina en sistemas acuosos simples con dos a cuatro componentes, para establecer las interacciones que ocurren entre estos compuestos.

PARTICULARES

Determinar el efecto de la concentración de tensioactivo sobre las transiciones gel-sol-gel, las propiedades reológicas y la conformación de sistemas elaborados con κ-carragenina en presencia y ausencia de iones potasio, en medio acuoso, para conocer de qué manera interaccionan los componentes.

Establecer el impacto de diferentes tensioactivos sobre las propiedades térmicas, reológicas y de tamaño de gota de emulsiones en gel O/W elaboradas con κ-carragenina, para evaluar su uso potencial como sistemas de encapsulación de otras moléculas.

HIPÓTESIS

Si se adicionan tensioactivos a sistemas acuosos de κ-carragenina, entonces, se modificarán sus propiedades térmicas y reológicas en relación con aquellas del polisacárido sin los agentes de superficie. Sin embargo, las interacciones entre los componentes dependerán de la naturaleza química del tensioactivo y de su concentración.

El Capítulo 1 proporciona información necesaria para comprender las interacciones que se presentan entre polisacáridos y tensioactivos en medios acuosos, principalmente.

En general, la literatura sobre el tema contiene información obtenida en su mayoría, con tensioactivos no aprobados en la industria de alimentos. Sin embargo, estos estudios previos pueden utilizarse para interpretar nueva información obtenida con tensioactivos de naturaleza similar a la descrita en este capitulo, pero utilizados en la elaboración de alimentos.

Es importante resaltar que la mayoría de los estudios realizados con sistemas polisacárido-tensioactivo involucran un gran número de distintas técnicas analíticas, las cuales en su conjunto brindan un panorama general de los fenómenos que se presentan en estos sistemas.

En general, las interacciones que se presentan en los sistemas polisacárido-tensioactivo son interacciones electrostáticas o interacciones hidrofóbicas, principalmente; las cuales dependen de la naturaleza del polisacárido y del tensioactivo.

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CHAPTER 15

Interrelation Between Polysaccharides and Different Surfactant Types

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Abstract: Interactions between polysaccharides and surfactants in aqueous solutions are the topic of this chapter. These components are often found in foods as well as in other systems like cosmetics, paints, detergents, textiles and many more. Therefore, investigation of the interactions between polysaccharides and surfactants has a great practical and fundamental interest. For the sake of clarity, we have divided the discussion into three sections: polysaccharides-nonionic surfactants; polysaccharides- cationic surfactants and polysaccharides-anionic surfactants. The behavior of polysaccharide-surfactant systems depends on many factors, which mainly include: 1) physical properties of both components, like molecular weight, degree of branching, number, and type of charge groups, backbone rigidity, 2) polysaccharide concentrations, 3) surfactant type; ionic or nonionic, polar head, chain length, and concentration. Environmental conditions like pH, ionic strength and presence of salt can partially screen electrostatic interactions, and there are also other factors needed to be considered when working with this type of mixtures. All these factors make it difficult to predict the behavior of polysaccharide-surfactant mixtures. However, in almost all cases, addition of surfactant modifies the behavior of the polysaccharide, regardless of polysaccharide and surfactant, but the modification is particular to the chemical nature of the polysaccharide and surfactant.

Keywords: Alginate, Biopolymers, Carboxymethylcellulose, Carrageenan, Chitosan, Electrostatic interactions, Emulsion, Hydrophobic interactions, Micelles, Polyelectrolyte, Polysaccharide, Surface tension, Surfactant.

INTRODUCTION

Foods are complex systems formed by a great number of components, including but not restricted to, sugars, proteins, polysaccharides, lipids, salts and surfactants.

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These components may interact with each other in different ways depending on process conditions such as temperature and pH, which make it difficult to control the shelf life and characteristics of the product such as texture and stability.

Surfactant is an abbreviation of surface active agent. A surfactant is a chemical compound with a tendency to adsorb at surfaces and interfaces. An interface is a boundary between two immiscible phases, and the term surface indicates that one of the phases is a gas, usually air [1]. The use of surface-active molecules in prepared foods is quite common. Low molecular weight surfactants, *e.g.* mono and diglycerides, phospholipids, are frequently used in combination with macromolecules like proteins and polysaccharides. In general, polysaccharides do not exhibit surface activity, but proteins can adsorb at interfaces and therefore it is important to know the interfacial properties and the dynamic behavior of all these molecules not only to understand the properties and behavior of complex systems but to develop products for specific purposes [2, 3].

Polysaccharides and surfactants are added to prepared foods basically to improve the stability of a food system. Polysaccharides are usually used to manage the rheological properties, while surfactants are used to handle the interfacial properties. In the case of polysaccharides, the main purpose is to get the desired viscoelastic properties. The interaction between polysaccharides and surfactants is studied in a model system (*i.e.* in aqueous solution), as foods are too complex to conduct fundamental studies. Therefore, most of our knowledge comes from a "simple" system. On the other hand, when polysaccharides and surfactants are used in combination, undesirable effects may occur, and the stability of the system can be affected. For example, incompatibility and formation of polysaccharide-surfactant complexes may eventually lead to phase separation. The interactions between polysaccharides and surfactants depend strongly on the properties of the former, on the type of the latter, on their respective concentrations and proportions in the mixture and on the environmental conditions of the aqueous medium, *e.g.* pH, ionic strength and temperature [4].

Surfactants can self-associate into micelles or micelle-like structures, and these aggregates can, in turn, interact with polymers present in the bulk phase. The concentration of surfactant when the interaction occurs is called the critical aggregation concentration (CAC). This concentration is lower than the critical micelle concentration (CMC) which is eventually reached by increasing the concentration of the surface-active molecule at a constant temperature. Polymers and surfactants can interact in different ways. Electrostatic, hydrophobic and dipolar interactions can be present depending on the nature of the polymer and the surfactant. Neutral polysaccharides can interact with anionic and cationic surfactants. However, the ways these interactions occur are different for each type of surfactant. Charged polymers, *i.e.* polyelectrolytes, interact with oppositely charged surfactants by electrostatic attraction and when the surfactant and the polymer bear the same charge, interaction is still possible if the macromolecule is sufficiently hydrophobic. The behavior of surfactants is also different because anionic surfactants show stronger interaction than cationic ones of comparable chain length [5].

Interactions between surfactants and macromolecules are intensely investigated both for food and non-food applications. Most of the previous studies have been focused on protein and surfactant interaction while the interaction between polysaccharide and surfactant not currently approved for food use, has deserved less attention. Understanding the interactions between polysaccharides and surfactants can be crucial to obtain a given target product. In this chapter, the discussion is focused exclusively on the interaction between polysaccharides with nonionic, cationic and anionic surfactants regardless of the specific application, *i.e.* food or non-food. The chapter is expected to provide insight on the advancement and challenges of this interesting and complex subject.

POLYSACCHARIDES AND NONIONIC SURFACTANTS

Chitosan is one of the most studied polysaccharides and its interaction with different surfactants has been the object of numerous investigations. Mixtures of chitosan with nonionic surfactants like sorbitan monolaurate (Span 20), sorbitan monooleate (Span 80) and sorbitan trioleate (Span 85), have numerous applications in the food and cosmetic industries due to their ability to stabilize emulsions [6]. Although the interaction of chitosan with nonionic surfactants is weak, it is possible to prepare the emulsion, solution or cream with mixtures of various compositions of chitosan and sorbitan ester surfactants. However, some differences are observed depending on the chitosan-surfactant concentration and chain length of the surfactant. For example, the size of surfactant droplets dispersed in the continuous phase of chitosan is essentially the same (*ca.* 2.0 μm) in dilute (0.02 mol/dm^3) polysaccharide-surfactant solution. A ten-fold increase in the concentration of both components leads to higher droplet sizes, but the effect depends on the hydrophobicity of the surfactant; it is more dramatic for Span 80 in which aggregation of surfactant molecules leads to droplets approximately ten times larger than in dilute solutions [7]. On the other hand, confocal microscopy shows chitosan-surfactant aggregates formed by a chitosan-rich shell and a chitosan-poor core with highly ordered micrometric supramolecular structures resulting from high surfactant concentrations. These results indicate the affinity of chitosan, arising from its amphiphilic character to surfactants. This characteristic makes this polysaccharide capable of interacting at the interface of various colloidal systems like micelles, vesicles or emulsion droplets [6].

Viscosity measurement has been used to characterize the interaction between chitosan and octaethyleneglycol mono *n*-dodecyl ether ($C_{12}E_8$) in dilute and semidilute solutions [8]. The interaction, though weak, between chitosan and $C_{12}E_8$, has a strong effect on the viscosity of admixture solutions. Different effects were observed. At surfactant concentrations essentially below or near the CMC, the viscosity is constant regardless of aging the solutions for up to nine days. However, even if viscosity is independent of surface concentration, it decreases with aging time as normally observed in individual chitosan solutions. Above the CMC, a sudden drop in viscosity occurs, but this drop depends on the aging time of the solutions [8]. Therefore, there is a time-dependent effect apart from the time dependency of individual chitosan solutions. The rate of viscosity decreases with time for a given constant chitosan concentration depending on surfactant concentration. For dilute solutions, *i.e.* below CMC, the rate is independent of surfactant concentration. For semi-diluted solutions, a steep increase of the rate with concentration is observed and for concentrated solutions, the rate remains again constant or slightly decreases depending on the aging time of the solutions, *e.g.* up to 16 days. This behavior has been explained by changes in chitosan conformation induced by the presence of the surfactant. When surfactant concentration reaches a certain critical value, C_1, micelles are formed, and association with chitosan chains takes place. This cooperative process leads to conformational changes in the chitosan molecules that induce polysaccharide adsorption at the interface to minimize the electrostatic interactions between chitosan chains. Association continues as long as the surfactant concentration is increased and reaches a given value, $C_{2,}$ for which every chitosan molecule is bound to one micelle. If the surfactant concentration is further increased, the rate of viscosity increase becomes essentially constant until a third critical surfactant concentration, C_3, is reached. This condition points the existence of interactions only between micelles. At this concentration, chitosan may be adsorbed onto more than one micelle. Under this condition, the system is composed of spherical micelles enclosed by chitosan segments and linked by chitosan coils that coexist with free chitosan chains and free surfactant micelles. A further association is impeded by steric repulsion within the chitosan layer surrounding the micelle surface [8]. The occurrence of time-dependent effects is explained by the presence of very slow conformational changes.

Surface tension studies of individual chitosan and its mixture with a surface-active triblock copolymer of polyethylene oxide-polypropylene oxide-polyethylene oxide (PEO-PPO-PEO) traded under the commercial name Lutrol® F127 and known generically as poloxamer 407, in water and in acetate buffer solutions have demonstrated that pH, ionic strength, chitosan concentration, surfactant concentration, and temperature, play different and significant roles in self- aggregation and adsorption at the air-solution interface [9]. Chitosan dilute solutions (< 0.001%) in water do not show surface activity at 25 (pH 5.06) and 34 °C (pH 5.21). However, pH (and also ionic strength) has an important role in this behavior as it has an influence on the hydrophilic character of the polysaccharide. At pH 5.0, the amino groups of chitosan molecules are protonated, and there are not large hydrophobic groups capable of adsorbing at the interface. Under this condition, the polysaccharide does not exhibit surface activity. As pH increases (≈ 6.0) amino groups are less protonated, and the hydrophilic character of chitosan becomes stronger. The polysaccharide can self- aggregate, and its hydrophilic segments adsorb at the interface. Under this condition, chitosan exhibits slight, but noticeable, surface activity. On the other side, the surface tension of individual surfactant solutions and chitosan-surfactant mixtures decreases linearly with the natural logarithm of surfactant concentration regardless of pH and temperature as shown schematically in Figure 1. Three zones and two break points have been observed in the surface tension isotherm of chitosan-F127 solutions, which means different adsorption behavior at the interface. It is suggested that before the occurrence of the first break point, *i.e.* zones I and

II, surfactant molecules can be adsorbed at the interface as 1) individual chains without aggregation, 2) dimers or larger aggregates and 3) chains with a modified configuration. The first break provides the critical aggregation concentration (CAC). The second break point, *i.e.* zones II and III, is attributed to the formation of micelles and therefore is the critical micelle concentration (CMC). Chitosan-F127 solutions in water behave in a different way than solutions in acetate buffer. In the case of water solutions, it has been speculated that before the first brake, complex formation occurs between chitosan and F127. It is shown in Fig. (1) that surfactant solution has a steeper slope and higher surface tension than the surfactant-free solution. Therefore, such complexes are less surface active and desorb from the interface. Beyond the CMC, all systems converge to the same surface tension. On the other side, solutions in acetate buffer show steeper slopes, but surface tensions for a given surfactant concentration are lower than chitosan-free solutions. This has been attributed to coadsorption of both components at the interface. The observed effects are the result of multiple interaction processes in which hydrophobic and electrostatic interactions are involved depending on pH, temperature, and ionic strength. As a consequence, the behavior of these systems is complex [9].

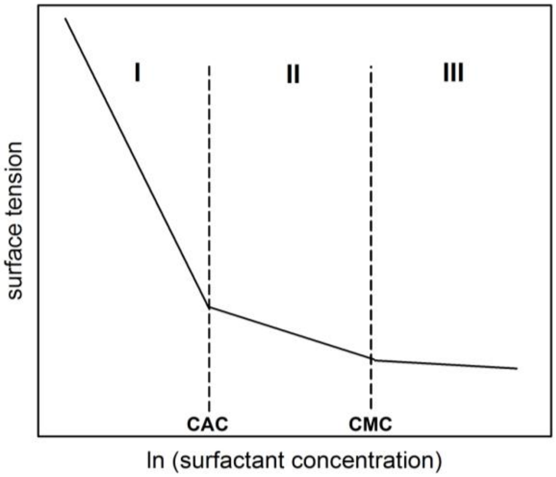

Figure. 1. Surface tension isotherm showing three zones and two break points; CAC and CMC (highly schematic).

The effect of the chemical nature of the head and tail groups of nonionic surfactants on the properties of ethyl cellulose (EC) oleo gels has been studied using differential scanning calorimetry (DSC) and rheometry techniques [10]. The surfactants included sorbitan monostearate (SMS), sorbitan monooleate (SMO), glycerol monostearate (GMS) and glycerol monooleate (GMO). DSC results show a shift to lower sol-gel and gel-sol transition temperatures; the reduction is higher for glycerol-based surfactants, GMS and GMO, than for sorbitan-based surfactants, SMS, and SMO. This behavior is attributed to the plasticizing nature of the head group of glycerol. The addition of surfactants to EC oleo gels results in an increase in gel strength. This behavior can be due to surfactant-EC interactions, and the effect is higher for GMS, probably due to crystallization of the GMS molecules. As a conclusion, the surfactant head group plays an important role in the association between the polysaccharide and the different surfactants [10].

Different effects are observed when various polysaccharides and surfactants are mixed. For example, the presence of Triton X-100 can modify the adsorption behavior of carboxymethylcellulose on the solid surface of alumina particles [10]. The most striking effect is that the amount of polysaccharide adsorbed on the particles surface increases when the surfactant is added, but the thickness of the adsorbed layer become smaller compared with that for carboxymethylcellulose-sodium dodecyl sulfate (SDS), an anionic surfactant, mixtures. This has been attributed to differences in polysaccharide conformation, with anionic Triton X-100 carboxymethylcellulose adopting a closely packed conformation [11]. The mechanical strength, rigidity (G'), gelling and melting temperatures of agar gels and viscosity of agar sols decrease when the nonionic surfactants Triton X-100, Brij 35 (polyoxyethylene 23-lauryl ether), polyethylene 10 lauryl ether ($C_{12}E_{10}$), SC893 and Crexlox 3485 are added to the polysaccharide in concentrations near or above the critical micelle concentration (CMC) [12]. The reason for this behavior is still unclear for sols and gels. However, it is believed that in sols interaction between surfactant and water is stronger than that with the polysaccharide and in gels surfactant micelles might interfere with network formation [12]. Addition of TR 8 (polyoxyethylene 7 isododecyl ether), TR 12 (polyoxyethylene 11 isododecyl ether), Brij(R) 56 (polyoxyethylene 10 cetyl ether) and Brij(R) 58 (polyoxyethylene 20 cetyl ether) to alginate, guar and sodium carboxymethyl guar aqueous solutions results in different effects on the steady shear and linear viscoelastic properties of the mixtures depending on the surfactant type and concentration [13]. In general, marked shear-thinning and strong viscoelasticity is observed and only in the case of guar, phase separation attributed to a strong association between the polysaccharide and the surfactant, *via* hydrogen bonding, is observed for high (3%) surfactant concentrations. These results will be valuable in textile printing applications [13]. The release profiles of the hydrophilic and hydrophobic drugs theophylline and tolbutamide, respectively, from agarose gels containing the surfactants Pluronic® F-68, a polyoxye- thylene-polyoxypropylene block copolymer, and Tween 80, polyoxyethylene (20) sorbitan monooleate, are remarkably affected by the solubility of drugs and the microstructure of the lyophilized hydrogels. The water uptake of the dehydrated hydrogels increases due to the diminution of the surface tension of the water used to rehydrate the gels. Surfactant micelles, which actuate as a structure support of the agarose network, reduce the hysteresis normally observed after rehydrating and increase the swelling of the dehydrated material. The pore architecture and the polysaccharide-surfactant interactions play an important role in the controlled release of those drugs [14]. The inclusion of Triton X-100 in κ-carrageenan solutions increases the adsorption of the polysaccharide on the surface of alumina particles showing a cooperative behavior. The interaction between carrageenan and alumina particles is electrostatic, while hydrogen bonding is responsible for complexation with Triton X-100. Adsorption of carrageenan on alumina particles is exacerbated when the cationic surfactant hexadecyltrimethylammonium bromide and its equimolar mixtures with Triton X-100 are used [15].

POLYSACCHARIDES AND CATIONIC SURFACTANTS

Carrageenans are present in many food and non-food systems and in most of them oppositely-charged surfactants are also present; so, the interaction between both components is expected. Kogej [16] used a potentiometric technique to study the interactions between the sodium forms of kappa, iota and lambda carrageenan and the cationic surfactants N-dodecyl pyridinium chloride (DPC) and N-cetylpy- ridinium chloride (CPC) in aqueous solution with the addition of sodium chloride. Kappa and iota carrageenan were studied under non-gelling conditions. The variation in polysaccharide charge density, *i.e.* type of carrageenan, and surfactant hydrophobicity, for example, CPC has a shorter hydrocarbon length than DPC, resulted in noticeable differences attributed to distinct ways of interaction. The critical aggregation concentration (CAC) was essentially independent of sodium chloride concentration for kappa and iota carrageenan but increased with salt concentration for lambda carrageenan. This behavior was observed for both surfactants, but the CAC values for DPC were approximately two orders of magnitude higher than for CPC. The results rationalized through a binding model providing different cooperativity parameters, point to the existence of a lamellar- type ordering of surfactant resulting from the association with polysaccharide chains. Therefore, aggregation of surfactants in the presence of carrageenans is different from the usual micelle formation, and the cooperative effect is due to the hydrophobic interaction between bound surfactant ions.

The interactions between dodecyl ammonium chloride (DDACl) and the sodium form of κ, ι and λ-carrageenan have been studied using dynamic light scattering (DLS), zeta potential and rheometry [15]. The addition of the cationic surfactant to carrageenan solutions leads to the formation of different soluble or insoluble complexes. The collapse of carrageenan chains is revealed by a decrease in the size and charge of the insoluble complexes in addition to the decrease in solution viscosity with increasing DDACl. DLS shows that at low surfactant concentration the size of the insoluble complex particles was slightly lower than the smaller sizes observed in the surfactant-free solutions. The particle size decreases when surfactant concentration increases. This is attributed to a reduction in the intrachain repulsion in carrageenans. Carrageenan solutions show shear-thinning behavior. The addition of surfactant to carrageenan solutions changes the chain conformation of carrageenan and when the DDACl concentration increases the flow behavior starts to be nearly Newtonian. κ-carrageenan shows a higher effect than ι and λ-carrageenans, suggesting that the structure properties of the complexes are mainly determined by the type of carrageenan chains. All surfactant-free solutions show viscoelastic behavior. Progressive addition of DDACl increases the viscous contribution to viscoelasticity, so the elastic character of carrageenan solutions is slightly reduced. This behavior is more evident in all κ-carrageenan mixtures compared with ι and λ-carrageenan. All these changes depend on carrageenan type and DDACl concentration [17].

Isothermal titration calorimetry (ITC), zeta-potential measurements, DLS, transmission electron microscopy (TEM), atomic force microscopy (AFM), and surface tension measurement have been used to study the interactions between κ, ι, λ-carrageenan, alginate and ulvan, and the cationic surfactant glycine betaine (GB) amide [16]. The variation of the interaction enthalpy with surfactant concentration for κ-carrageenan, alginate, and ulvan showed three regions associated with different phenomena dependent on polysaccharides and surfactant concentrations. This clearly indicates the existence of different interactions between polysaccharide and surfactant. At low cationic surfactant concentrations, electrostatic attractions exist between the negative polar group of the polysaccharide and the positive polar head of GB, and as a result, the polysaccharides bind to the surfactant and form a complex. At intermediate cationic surfactant concentrations, unbound micelles dissociate, and interactions are created between the polysaccharide and micelles or particles of surfactant. At high cationic surfactant concentrations, additional micelles appear in the solution because the surfactant concentrations in the aqueous phase exceed the CMC. Only in the case of ι and λ-carrageenans, it is possible to distinguish two stages, which show that the nature of the interactions of GB with various types of carrageenans is different. In addition, higher cationic surfactant concentrations are required to reach neutral interactions. This result is may be due to the difference in carrageenan chemical structures, *i.e.*, the number of sulfate groups along the polysaccharide backbone [18].

The interaction of carboxymethylcellulose in water and in aqueous salt solutions with the cationic surfactants, dodecyl trimethyl ammonium chloride (DTAC), dodecyl trimethyl ammonium bromide (DTAB), tetradecyl trimethyl ammonium bromide (TTAB), tetradecyl triphenyl phosphonium bromide (TTPB), hexadecyl trimethyl ammonium bromide (CTAB), tetradecyl piridinium bromide (TPB) and dodecyl trimethyl ammonium iodide (DTAI) has been studied [19]. The goal of this investigation was to determine the effect of the size of the head group and chain length of the surfactant, different halide counterion, and ionic strength of the medium on the binding between the polysaccharide and surfactant. The variation of surface tension with surfactant concentration shows that the CAC of the mixtures decreases when chain length increases from C_{12} to C_{14}, although no perceptible changes are noticed from C_{14} to C_{16} and decreases with the increase in the size of the head group because the surfactant is more hydrophobic and there is a reduction in counterion binding. The counterion binding also influences the CMC, micelle shape, and phase equilibria in the order $Cl^{-1} < Br^{-1} < I^{-1}$. The CAC for iodide is the lowest of all the halides. This can be attributed to the highest binding capacity of this counterion and as a consequence better packing of surfactant molecules. Therefore, the order for CAC is $I^{-1} < Br^{-1} < Cl^{-1}$. Moreover, the viscosity of surfactant solutions is higher than that of water. At a polymer concentration Cp, the system is in the semidilute regime, which means that polymer chains are extended and can overlap. In carboxymethylcellulose-surfactant solutions, the polysaccharide chains collapse because they cannot longer be overlapped, and as a result, the viscosity drops to that of water. The addition of different salts is necessary to separate the hydrophobic from the electrostatic effects. Viscosity decreases slightly until salt concentration reaches 0.1 mmol/dm^3 because the electrostatic repulsions between polysaccharide charges are screened, which lead to a collapse of the chains. In the presence of surfactants, polysaccharide chains can collapse due to the electrostatic interactions with the surfactant. The same behavior is observed when salt is added.

Differences are not observed for the different salts used $BaCl_2$, NaCl, and KCl; hence the colligative effect dominates over the hydration behavior of the different ions. Furthermore, the size of the polymer-surfactant complex is reduced because of the increment in salt concentration. This can be attributed to the screening effect of the salt in the electrostatic repulsion between the head groups of the surfactant in the bound micelles. As a result, the repulsion between micelles held onto the polysaccharide backbone is reduced too [19].

POLYSACCHARIDES AND ANIONIC SURFACTANTS

Chitosan can display strong interactions with sulfated anionic surfactants like sodium dodecyl sulfate (SDS), sodium lauryl ether sulfate (SLES) and sodium taurocholate (ST) among others. Water insoluble and structured complexes are formed with these surfactants over a wide range of concentration and mixing ratios. Binding of chitosan to SDS is a cooperative process governed by the hydrophobic interactions with the surfactant alkyl chain. This is also true for other sulfated surfactants. However, strong electrostatic interactions are found between the glucosammonium units of chitosan and the sulfated and sulfonated head groups of surfactants. On the other hand, chitosan also exhibits weak interactions with anionic surfactants like fatty acids. As in the case of sulfated surfactants, chitosan-fatty acids mixtures form water insoluble and structured complexes. The degree of ionization of chitosan and fatty acids and their mutual interaction depend on pH. This yields very complex mixtures. Depending on pH, three regions can be identified: 1) at low pH, chitosan is positively charged and soluble while fatty acids are neutral and insoluble, 2) at intermediate pH, chitosan, and fatty acids are partially charged and soluble and 3) at high pH, chitosan is not charged, and insoluble and fatty acids are ionized. These different states should be considered when preparing chitosan-fatty acids mixtures since pH is a very important process parameter [6].

The behavior of chitosan-fatty acids mixtures at the air-water interface is determined by hydrophobic interactions, but hydrogen bonds are also present. When short chain carboxylic acids (C_n, $n \leq 5$) are added to chitosan solutions, complex formation does not occur due to the absence of hydrophobic interactions between aliphatic chains. However, for long chain carboxylic acids (C_n, $n \geq 16$) emulsions are formed even if these acids are not protonated. Finally, aggregates are observed when intermediate chain carboxylic acids are present [6].

The stability to flocculation and coalescence of an oil-in-water emulsion containing sodium stearoyl-2-lactylate (SSL) as the surface active agent can be improved by the addition of chitosan. This is attributed to the formation of a thicker interface consisting of chitosan-SSL complexes with better surface active properties and to the enhancement of bulk viscosity induced by the presence of the polysaccharide [20, 21].

The interactions between maltodextrin (MD) (DE = 10) and SDS have been studied at neutral pH by isothermal titration calorimetry (ITC), surface tension, DSC and turbidity techniques [22]. ITC and surface tension measurements were used to determine the CMC of SDS. Each one of these two techniques yielded different results; 3.4 mmol/dm^3 with ITC and 2.2 mmol/dm^3 with tensiometry. The existence of interactions between both components, at low surfactant concentrations is revealed by exothermic enthalpy changes in heat flow in buffer solutions. As surfactant concentration increases, binding of both components decreases, because fewer sites in maltodextrin molecules are available for interacting with SDS. In addition, it seems that one SDS molecule is bound to two MD molecules. Surface tension measurements indicate insignificant binding between MD and SDS when surfactant concentration is around 0.05 mmol/dm^3. Above this concentration, more complexes of MD-SDS are formed when MD concentration increases. The phase diagram of MD-SDS mixtures obtained from tensiometry and ITC data shows three concentration zones; 1) at low SDS concentrations MD does not interact with SDS, 2) at intermediate SDS concentrations formation of MD-SDS complexes occur and SDS molecules in the complex are in equilibrium with free surfactant molecules, 3) at high SDS concentrations SDS saturates maltodextrin molecules, and the excess surfactant exists as monomers in the aqueous phase.

Binding of starch to the anionic surfactants SDS, SSL and glycerol monostearate (GMS) has been studied by X-ray diffraction, DSC and rheometry [23, 24]. Amylose forms strong complexes with SSL or GMS. Formation of these complexes is favored by the increased mobility of amylose molecules resulting from swelling of starch granules. However, surfactants may also form complexes with amylose molecules present in the swollen granules. Amylose-surfactant complexes are less flexible than individual amylose molecules. This can retard the separation of amylose and amylopectin during heating of starch suspensions. Thus, the formation of the amylose-surfactant complex should explain the ability of some surfactants to retard firmness and retrogradation. Amylose and SDS also form complexes. This surfactant can also form complexes with amylopectin, but this union is more restricted. The number of SDS molecules bound per 1000 glucose units is approximately, 35 for amylose on a 0.2% (w/w) amylose solution and only 6 to 7 for amylopectin on a 3 and 5% (w/w) amylopectin solution. In this case, the addition of SDS has the same effect than SSL or GMS, *i.e.* firmness and retrogradation are retarded [25].

On the other side, the viscosity of 2-hydroxy-3-trimethylammonium propyl starch solutions, *i.e.* cationic starch, decreases when SDS is added [26]. This reduction in viscosity is attributed to the increasing repulsion between charged segments in the polyelectrolyte. Beyond a certain SDS concentration, viscosity drops rapidly because the addition of enough surfactant causes the starch chains to collapse. Viscosity falls until gel particles are formed. The length of the hydrocarbon chain of the surfactant has an effect on viscosity behavior. Viscosity decreases when the surfactant chain length increases, but the end group of the surfactant does not have a significant impact.

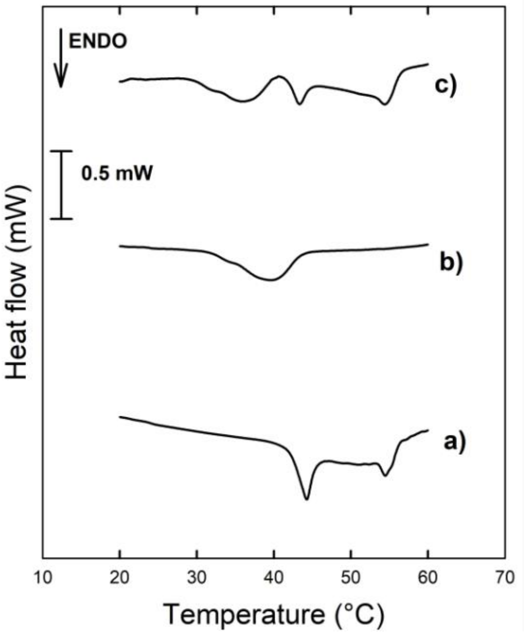

Figure. 2. Heating thermographs of a) 0.5% SSL, b) 0.5% κ-carrageenan in 13 mmol·dm^{-3} KCl and c) 0.5% κ- carrageenan in 13 mmol·dm^{-3} KCl with 0.5% SSL. Cooling thermographs show the same number of peaks at lower temperatures.

The behavior of aqueous semidilute anionic hydroxyethylcellulose (HEC(-)) in the presence of the anionic surfactants SDS or the cationic surfactant CTAB or Gemini(+) was studied by turbidity, small-angle neutron scattering (SANS), rheometry, and DLS measurements [27]. Weak interactions exist between HEC(-) and anionic SDS, while electrostatic attraction between HEC(-) and cationic CTAB or Gemini(+) is present. The key point here is that addition of an oppositely-charged surfactant reinforces the structure of the resulting complexes, flocculation occurs at higher surfactant concentrations and association of the complex leads to the formation of a mixed fragmented network. On the other hand, the addition of a like-charged surfactant promotes the formation of polysaccharide-surfactant micelles.

In our laboratory, we have used DSC and rheometry to study the effect of the nonionic Tween 20 (T20) and the anionic SSL surfactants on the viscoelastic properties and the gel-sol-gel transition of KCl-containing κ-carrageenan gels. The gels were made with 0.5% κ-carrageenan, 1-5 mM T20 or 0.05-0.5% SSL and surfactant concentrations were above the CMC. The storage modulus, G'(ω), of carrageenan-T20 gels is on the average 35% higher than that in surfactant-free gels. On the contrary, G'(ω), of carrageenan-SSL gels is lower by an average of 36% than that in individual carrageenan gels. On the other side, the melting and gelling temperatures of κ-carrageenan-T20 gels are higher with respect to those of surfactant-free gels. As shown in Figure 2 carrageenan-SSL gels show three thermal transitions on melting and gelling; one transition is attributed to κ- carrageenan and the remaining two to the surfactant. The temperatures and enthalpies of melting and gelling of κ-carrageenan gels decrease when the concentration of SSL is increased. The second transition occurs at a constant temperature of about 43 °C on heating and 40 °C on cooling. The third transition decreases further on heating and cooling. The melting and gelling enthalpies in the presence of surfactant show an increase for both transitions. These results suggest that κ-carrageenan gels are strengthened by the presence of the nonionic surfactant possibly by hydrogen bonding with the hydroxyl groups of T20. On the other hand, the negative charges in κ-carrageenan and SSL cause repulsive electrostatic interactions, and this affects the formation of the κ-carrageenan network. As a consequence, the transition temperature and enthalpy decrease upon increasing the SSL concentration.

CONCLUSION

Unlike an individual surfactant, (Figure 3 (top)), in general, the association between polysaccharides and surfactants involves three regions as shown in Figure 3 (bottom). Each region is characterized by the existence of different interactions between both components. In the first region when a small amount of surfactant is added to a polysaccharide solution, surfactant molecules interact with the macromolecule. The interaction depends on the type of surfactant and polysaccharide. Surface tension decreases with the increase in surfactant concentration until a first critical point which is the CAC and corresponds to the onset of micelle formation on the polysaccharide. In the second phase, further addition of surfactant does not change surface tension, because surfactant molecules bind to the polysaccharide. The progressive increment in surfactant concentration saturates the polysaccharide molecules and the second breakpoint appears. The addition of more surfactant after this point causes a decrease in surface tension because the surfactant-polysaccharide complex is desorbed from the surface and surfactant molecules adsorb on it. This point is called the polymer saturation point (PSP) and is close to CMC [28]. Finally, the third region occurs when the surface tension of the polysaccharide-surfactant mixture reaches the surface tension value of the surfactant solution; third critical point. At this point, the surfactant molecules cover the surface, and their concentration is high enough to form free micelles.

On the other hand, the most important interactions between polysaccharides and oppositely-charged surfactants are electrostatic and hydrophobic. Hydrophobic interactions are present when polysaccharides have hydrophobic segments in their structures. If these segments are not present, these kinds of interactions occur due to surfactant-surfactant binding. Hydrophobic interactions are very important in neutral polysaccharides, but hydrogen bonds have an important contribution too.

Important information on polysaccharide-surfactant interactions is currently being provided by a number of techniques, such as tensiometry, rheometry, dynamic light scattering, zeta potential, isothermal titration calorimetry, differential scanning calorimetry, X-ray diffraction, turbidity, small-angle neutron scattering, confocal laser scanning microscopy, electron microscopy and atomic force microscopy. The complexity of the systems justifies the combined use of several of these techniques.

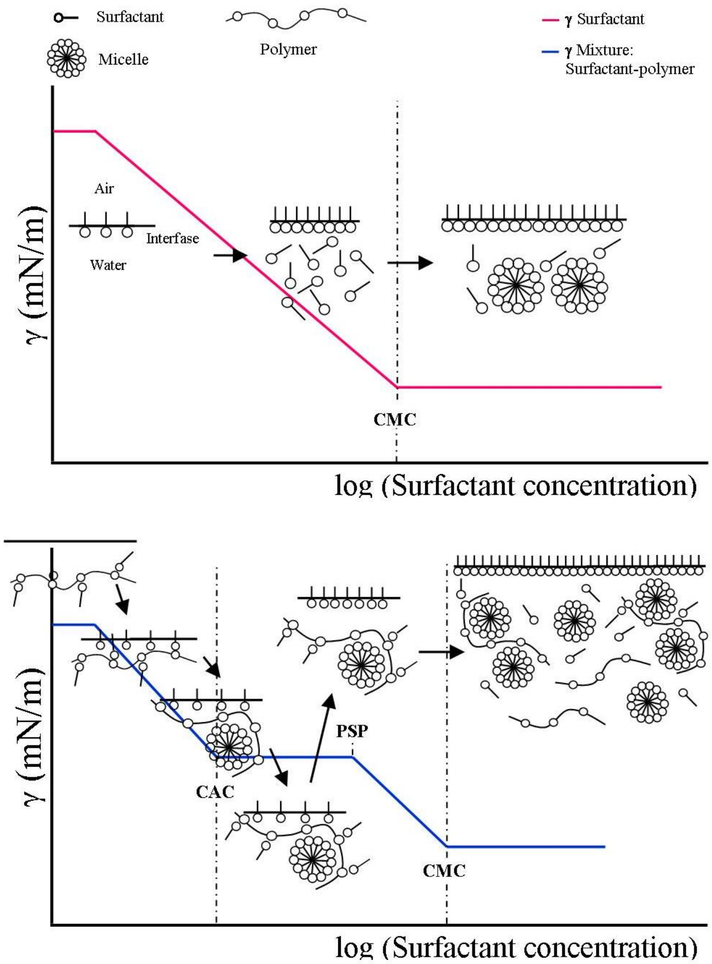

Figure. 3. Simple model of association of surfactant in water (top) and surfactant-polymer in water (bottom).

The process conditions of a system containing polysaccharides affect the association of a polysaccharide with another polysaccharide or non- polysaccharide structure-forming molecules, like proteins or surfactants. Physicochemical properties, *e.g.* helix-coil transition temperature, electrical properties, sensitivity to specific monovalent or multivalent ions, are very important and have to be taken into account in order to have a better understanding of their behavior in multicomponent systems [29].

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En el Capítulo 2 se presentan las principales características fisicoquímicas de los materiales usados en este trabajo.

El conocimiento de características físicas como la masa molar, el contenido de iones mono y divalentes, la temperatura de transición sol-gel, entre otras, y químicas como la estructura, es importante, en la industria y en las instituciones académicas, ya que facilita el desarrollo de nuevos productos y ayuda a crear las condiciones para que los componentes expresen su funcionalidad.

Además, el capítulo contiene información general del polisacárido κ-carragenina y de los tensioactivos seleccionados para la realización del estudio, a saber, estearoil lactilato de sodio (ELS en español y SSL en inglés), Tween 20 y lecitina de soya. Estos tensioactivos fueron seleccionados por ser de naturaleza química diferente. El ELS es un tensioactivo aniónico, el Tween 20 es no-iónico y la lecitina de soya es zwitteriónica, por lo cual se espera que su comportamiento en presencia de κ-carragenina sea diferente.

La principal información que se presenta en esta sección corresponde al contenido de iones, y espectros de infrarrojo de todos los componentes utilizados y en el caso de la κ-carragenina los resultados de resonancia magnética nuclear (RMN) realizados con la finalidad de conocer la pureza del polisacárido. Por otro lado, también se información del comportamiento térmico en calorimetría de barrido diferencial de la κ-carragenina y el ELS así como los valores de tensión superficial del ELS y Tween 20 en medio acuoso.

CAPÍTULO 2

CARACTERIZACIÓN FISICOQUÍMICA DEL ESTEAROIL LACTILATO DE SODIO (ELS), EL MONOLAUREATO DE POLIOXIETILEN SORBITÁN (TWEEN 20), LECITINA Y LA κ-CARRAGENINA

PARTE I. GENERALIDADES

κ-carragenina

La κ-carragenina es un polisacárido de D-galactano sulfatado que se encuentra en algas marinas de la clase Rhodophyta. Su estructura primaria consiste en una unidad repetitiva compuesta por un disacárido de (1→4) α-3,6-anhidro-D-galactosa y (1→3) β–D-galactosa-4-sulfato alternado (Figura 1) unidos por enlaces sulfato. Se utiliza en la industria de alimentos como estabilizante y gelificante. Sus geles son rígidos y frágiles y exhiben sinéresis e histéresis térmica. La gelificación ocurre cuando las disoluciones calientes se enfrían e involucra una transición de hebra aleatoria a doble hélice seguida de agregación de las dobles hélices, en general, en presencia de sales (De Ruiter & Rudolph, 1997).

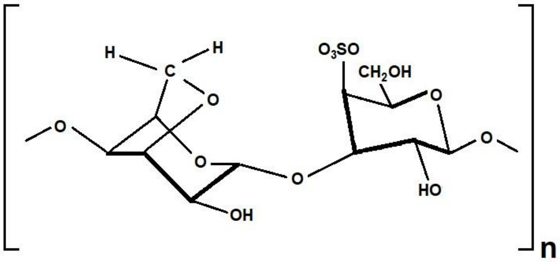

Figura. 1. Unidad repetitiva de la κ-carragenina, (Kara, Arda, Kavzak & Pekcan, 2006).

Se han sugerido varios modelos para explicar el proceso de gelificación de la κ-carragenina. Uno de ellos propone que sus moléculas experimentan una transición conformacional de hebras aleatorias a dobles hélices entrecruzadas la cuales forman racimos solubles o dominios, debido a las cadenas dobladas. Los dominios se agregan en presencia de iones específicos para dar lugar a un gel con estructura tridimensional (Figura 2a).

Otro mecanismo plantea la formación de dímeros helicoidales en lugar de dobles hélices entrelazadas, y adicionalmente señala que el entrecruzamiento en las cadenas no es necesario para formar la estructura del gel, sino que la gelificación se produce como resultado de la cristalización parcial de los dímeros helicoidales provocada por una reducción en la solubilidad del polímero (Figura 2b). Además, se ha demostrado que existe una interacción específica entre iones K^+ y κ-carragenina que disminuye la carga total del polímero, reduciendo así su solubilidad (Day, Phillips & Williams, 1988).

Se ha sugerido que el primer paso de la agregación es la formación de una estructura intermedia, una varilla compuesta por hélices densamente empacadas. Conocidas anteriormente como varillas super-helicoidales. Estas varillas y sus agregados son en última instancia las responsables de la gelificación (Wittgren, Borgström, Piculell & Wahlund, 1998). El paso clave para la gelificación de la κ-carragenina es la transición de hebra aleatoria a doble hélice. Además, la gelificación es muy sensible a la presencia de sales y puede controlarse por la presencia de iones específicos como K^+, Cs^+ y Rb^+, ya que estos incrementan la estabilidad de las hélices e intervienen en su agregación. Dicha agregación ocurre a una temperatura crítica y su valor depende de la concentración y el tipo de catión presente (Rochas & Rinaudo, 1984).

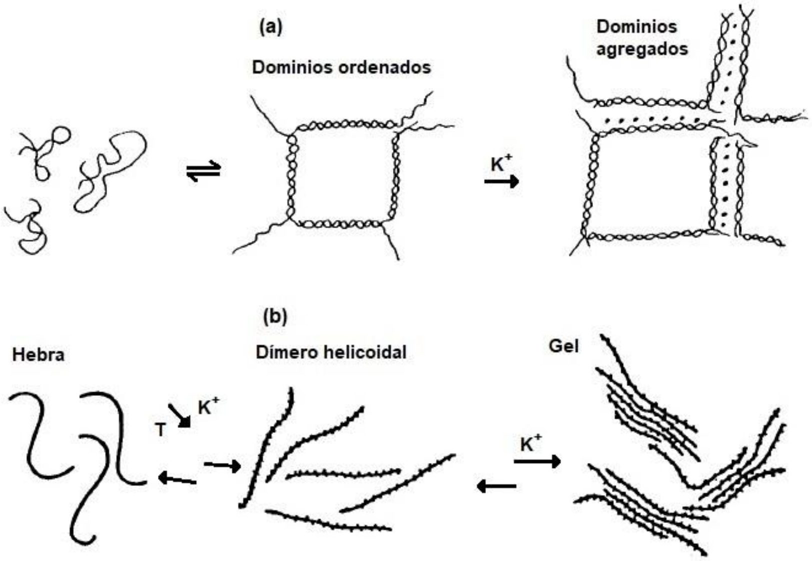

Figura. 2. Modelos de gelificación para κ-carragenina (a) Modelo de dominios de Morris *et al.*, (b) Modelo de separación de fases de Rochas y Rinaudo (Day, Phillips & Williams, 1988).

Estearoil lactilato de sodio

El estearoil lactilato de sodio (ELS) es un tensioactivo aniónico (U.S. FDA: 172.846; E No. E481) con una masa molar de 451.6 $g \cdot mol^{-1}$ sintetizado a partir de ácidos grasos y ácido láctico en presencia de sodio. En la Figura 3 se presenta su estructura generalizada. El ácido láctico contiene un grupo hidroxilo y un carboxilo el cual puede esterificarse a un solo ácido láctico o a un polímero del ácido láctico (Boutte & Skogerson, 2004). Está formado por una cabeza hidrofílica cargada que proviene del ácido láctico y una cadena hidrofóbica hidrocarbonada del ácido esteárico (Grigoriev, Leser, Michel & Miller, 2007).

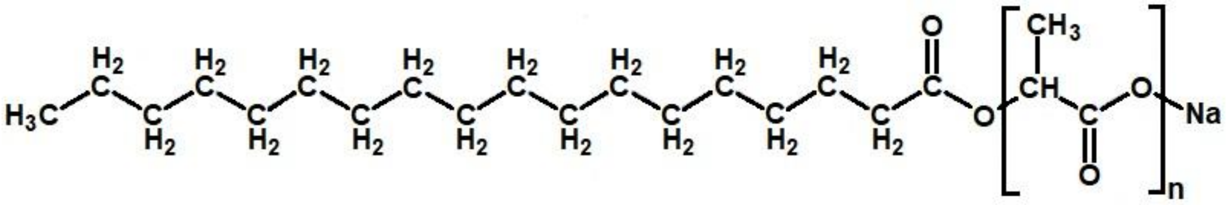

Figura. 3. Estructura química del estearoil lactilato de sodio, n es el número de ácidos lácticos y varía entre 1 y 4, (Meshram & Jadhay, 2012).

Comercialmente el ELS es una mezcla de 50 % de estearoil-1-lactilato (ELS1), 20 % de estearoil-2-lactilato (ELS2) y 5 % de estearoil-3-lactilato (ELS3) (Boutte & Skogerson, 2004). Es ampliamente utilizado en alimentos como emulsionante en productos cárnicos y como agente de batido y suavizante en productos de panadería (Zinoviadou, Moschakis, Kiosseogkou & Biliaderis, 2011). Todos los componentes del ELS presentan diferentes actividades de interfase, por ejemplo, el grupo hidrofílico de cabeza del ELS2 hace que su actividad de superficie sea menor que la del ELS1 (Grigoriev, Leser, Michel & Miller, 2006).

Tween 20

El polisorbato 20 o monolaureato de polioxietilen (20) sorbitano, conocido comercialmente como Tween 20 (U.S. FDA: 172.515; E No. E432) es un tensioactivo no iónico derivado del sorbitol con una masa molar de 1227.5 g·mol^{-1}. Los polisorbatos se fabrican modificando los ésteres de sorbitán con óxido de etileno en una autoclave.

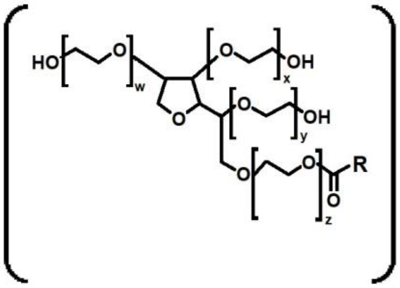

Figura. 4. Estructura generalizada para la serie de polisorbatos. Para Tween 20 R = $C_{11}H_{23}$ y w+x+y+z = 20 (Mahmood & Al-Koofee, 2013).

Los polisorbatos son algunos de los emulsionantes más hidrofílicos permitidos en los alimentos, debido a la larga cadena de polioxietileno. Las moléculas de óxido de etileno están en una sola cadena, lo que da como resultado una estructura fuertemente polarizada (Figura 4). Todos los polisorbatos se consideran hidrofílicos y son solubles en agua. Se utilizan comúnmente en combinación con otros tensioactivos para lograr una mayor estabilización de las emulsiones, debido a que son potentes estabilizadores estéricos. Por ello, estos emulsionantes son importantes en la preparación de emulsiones o concentrados que deben diluirse con agua de dureza desconocida. La interacción de los polisorbatos con el agua ocasiona que la adición de pequeñas cantidades del tensioactivo disminuya de manera notable la tensión superficial (Cottrell & Peij, 2004).

Lecitina de soya

La lecitina (U.S. FDA: 184.1400; E No. E322) es uno de los emulsionantes más utilizados en la industria alimentaria y tiene una masa molar promedio de 643.9 $g \cdot mol^{-1}$ considerando que la fosfatidilcolina es el componente mayoritario. La lecitina no es un material uniforme y estandarizado, sino una mezcla natural de compuestos químicos similares, pero claramente diferenciados que contribuyen al rendimiento general del emulsionante y se clasifican como fosfolípidos o fosfátidos (Figura 5). Se localizan en todos los tejidos orgánicos tanto de plantas como animales, y las concentraciones más altas, además de en la yema de huevo, se encuentran en semillas oleaginosas como la soya, el girasol y otras.

Las especies individuales de fosfolípidos difieren en la naturaleza del alcohol que se encuentra esterificado “Z” (Figura 5). Estas pueden tomar la forma de un amino-alcohol, e.g., colina, etalonamina, un alcohol polivalente, e.g., inositol, glicerol, o un ácido hidroxiamino, e.g., serina. Si Z es un átomo de hidrógeno, entonces se tiene el ácido fostatídico. La solubilidad de los componentes de la lecitina en disolventes orgánicos depende principalmente de sus grupos polares y de la composición de los ácidos grasos. Las lecitinas son sustancias que se “disuelven” en agua con la formación de un alto número de mesofases de cristal líquido altamente ordenadas, este fenómeno es llamado polimorfismo liotrópico. Una de las aplicaciones más tradicionales de las lecitinas es en el chocolate. Sin embargo, también se utiliza en recubrimientos para helados, en caramelos suaves y duros y en la industria de la panificación debido a la versatilidad de estos compuestos (Bueschelberger, 2004).

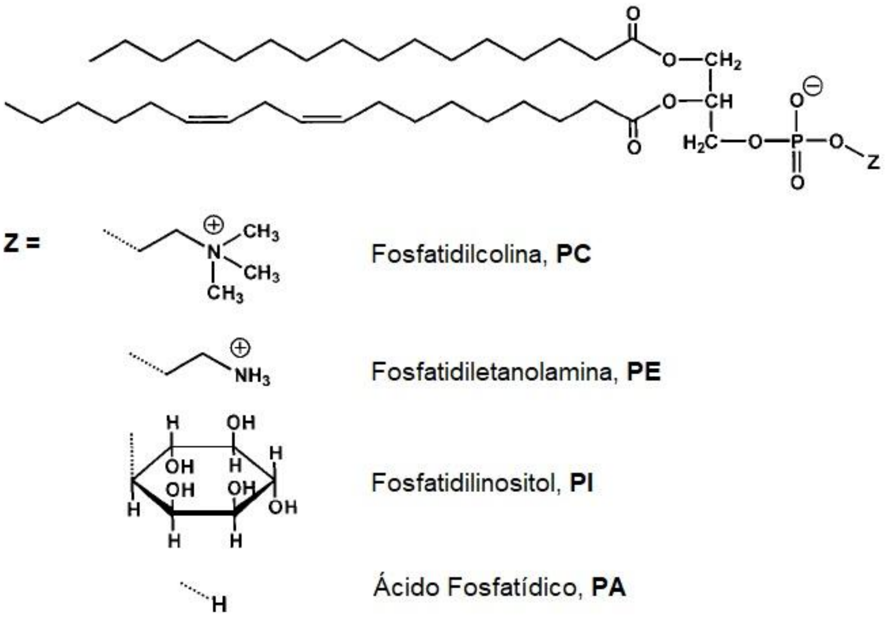

Figura. 5. 1,2-Diacil-sn-glicero-3-fosfolípidos, nomenclatura de acuerdo con el sustituyente "Z" (Bueschelberger, 2004).

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PARTE II. Physicochemical characterization of sodium stearoyl lactylate (SSL), polyoxyethylene sorbitan monolaurate (Tween 20) and κ-carrageenan

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Data article

Physicochemical characterization of sodium stearoyl lactylate (SSL), polyoxyethylene sorbitan monolaurate (Tween 20) and κ-carrageenan

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A B S T R A C T

Surfactant-polymer mixtures are common in food, cosmetic and pharmaceutical products. These components can interact with each other. The interactions depend on the type of polymer and surfactant, the purity of the ingredients, the ionic content and their concentration. Therefore, the data presented here provide valuable information that could be useful for those working with these mixtures in different applications, particularly in blends with polyelectrolytes and their counterions. This article contains experimental data about the physicochemical characterization of sodium stearoyl lactylate (SSL), polyoxyethylene sorbitan monolaurate (Tween 20) and κ-carrageenan. Techniques included atomic absorption, DSC, FTIR-ATR, NMR, and surface tension.

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Specifications Table

Subject area	*Chemistry*
More specific subject area	*Physical chemistry*
Type of data	*Tables and figures*
How data was acquired	*Atomic absorption spectrometer 3110 (Perkin Elmer); Spectrophotometer with an ATR universal accessory (Spectrum 400, Perkin Elmer Cetus Instruments); Microcalorimeter μDSC 7 Evo (Setaram); Microtensiometer EZPiplus (Kibron) and spectrometer AVANCE-III 500 (Bruker)*
Data format	*Analyzed results*
Experimental factors	*Commercial forms were used without further purification*
Experimental features	*Physicochemical properties were determined for κ-carrageenan and two surfactants (SSL and Tween 20) by using atomic absorption, infrared and nuclear magnetic resonance, calorimetry, and measurements of surface tension*
Data source location	*Authors' affiliation*
Data accessibility	*Data are presented in this article*

Value of the data

Intrinsic ions content is a key factor in mixtures of ionic components, like κ-carrageenan and SSL, because interactions between them can be affected by the presence of salts.

DSC analysis, IR and NMR spectra are used to identify the components and to determine their purity, which are relevant characteristics since the presence of distinct species could modify their behavior.

Surface tension is useful to know the adsorption parameter of surfactants and how it changes in a mixture with other components.

1. Data

SSL had sodium as the primary ion and small quantities of other ions, whereas Tween 20 only had traces of ions. κ-carrageenan was found mainly as the potassium salt form since it is the primary ion. The IR spectrum of SSL showed typical carbonyl, ether groups, and aliphatic linear chain bands. Tween 20 showed an OH band, and characteristic carbonyl and ether groups bands. The spectrum of κ-carrageenan showed sulphate ester and glycosidic linkage bands, which are not due to the presence of ι-carrageenan because the NMR spectra show a kappa: iota hydrogen molar ratio of 1:0.073. The melting transition temperature of SSL was 54.6 °C and the recrystallization temperature 40.5 °C. Tween 20 did not show any apparent change over the working temperature range. The thermal behavior of κ-carrageenan depends on the ionic content since its gelation is affected by the presence of K^+ ions. Therefore, the addition of KCl increased the transition temperature and enthalpy compared with κ-carrageenan without the salt. The addition of KCl modified the critical micelle concentration (CMC) of SSL solutions because of the chemical nature of the surfactant. This effect was not observed for Tween 20. The CMC values were higher for SSL due to electrostatic repulsion between its head groups. The same behavior was observed in mixtures of κ-carrageenan with the individual surfactants.

2. Experimental Design, Materials and Methods

2.1. Materials

Materials included food-grade κ-carrageenan (Ingredients Solutions, USA) without further purification, powder sodium stearoyl lactylate (Palsgaard, Juelsminde, Denmark), liquid Tween 20 (Hycel de México S.A. de C.V., Mexico), and potassium chloride (Merck, Germany). Solutions were prepared in deionized water; its resistivity was greater than 50 kΩ·m and its total organic carbon (TOC), less than 30 ppb.

Table 1. Intrinsic ion content (mg·kg^{-1}) of κ-carrageenan, SSL and Tween 20.

Ion	κ-carrageenan	SSL	Tween 20
Na^+	21 514 ± 1727	21 700 ± 460	1 000 ± 30
K^+	62 100 ± 1684	1 200 ± 170	1 100 ± 10
Ca^{2+}	1 301 ± 61	100 ± 10	500 ± 10
Mg^{2+}	< 10	< 10	100 ± 10

2.2. Atomic absorption

The ion concentration of SSL, Tween 20 and κ-carrageenan (Table 1) was determined in an atomic absorption spectrometer 3110 (Perkin Elmer, USA) equipped with a deuterium background corrector, using an air-acetylene flame. Wavelengths employed to identify Na^+, K^+, Ca^{2+}, and Mg^{2+} were 330, 404, 422 and 202 nm, respectively. All samples were previously digested with a mixture 80:20 of nitric to sulfuric acids at 90 °C for 2 h or until the disappearance of the combustion gas of the raw material.

2.3. Fourier transform infrared-attenuated total reflectance (FTIR-ATR)

FTIR-ATR tests were performed in a spectrophotometer with an ATR universal accessory (Spectrum 400, Perkin Elmer Cetus Instruments, USA). The spectra were collected in the range of 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1} with 32 scans per sample. Figure 1 shows the IR spectra for SSL, Tween 20 and κ-carrageenan. Table 2 shows their characteristic absorption bands, allocated according to available sources [1-5].

2.4. Nuclear magnetic resonance (NMR)

^{1}H NMR spectra of κ-carrageenan (Figure 2) were obtained with a spectrometer (AVANCE-III 500, Bruker, USA), operated at a frequency of 500 MHz. Tests were carried out using a BBI-5 mm with z-field gradient at 40 °C and 3-(trimethylsilyl)-2,2,3,3-tetradeuteropropionic acid (TSP) was added as an internal reference. A mass of 5 mg of κ-carrageenan was added to D_2O, to a final concentration of 0.5 %, and the dispersion was heated to dissolve the polysaccharide. This solution was used for NMR analysis. Data were processed with a commercial software (The MestReNova, v12.0.0-20080, Spain). The α-anomeric protons for κ and ι-carrageenan where identified from reported data [6].

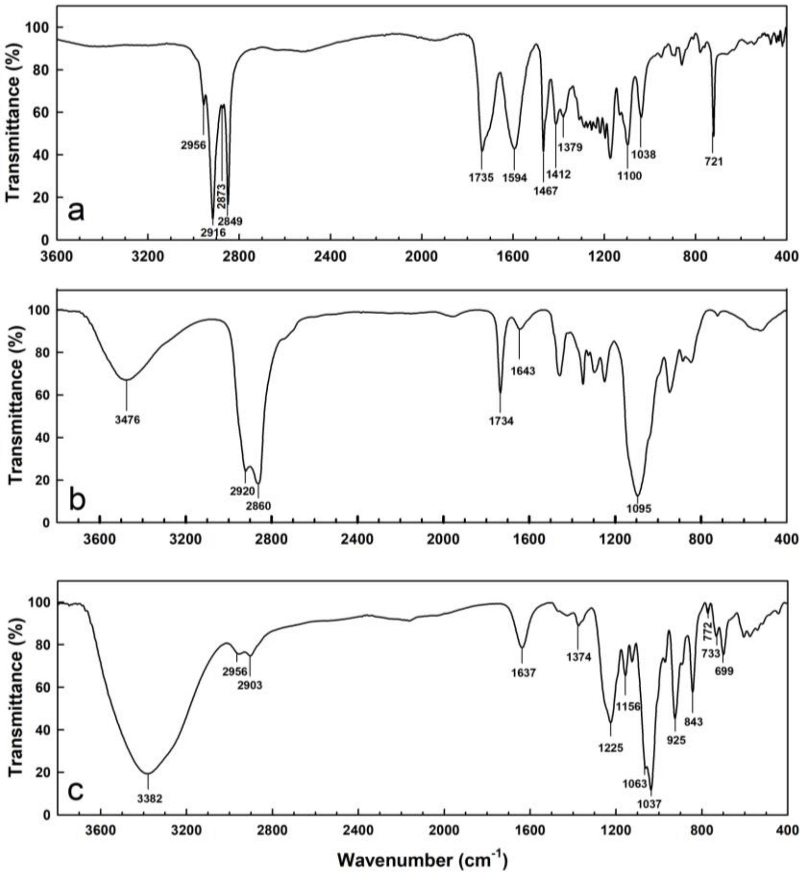

Figure. 1. FTIR-ATR spectra of (a) sodium stearoyl lactylate, (b) Tween 20 and c) κ-carrageenan.

2.5. Differential scanning calorimetry

DSC analysis (Figure 3) was carried out in a microcalorimeter (μDSC 7 Evo, Setaram, France) under N_2 gas atmosphere. SSL was analyzed at a rate of 1.2 °C·min^{-1} from 2 to 80 °C in one heating-cooling cycle using 30 mg of a powder sample. The reference cell was empty. κ-carrageenan was analyzed using a solution with 0.5 % with or without 13 mmol·dm^{-3} KCl, at a rate of 0.8 °C·min^{-1} from 2 to 80 °C in three heating-cooling cycles using 600 mg of sample. The reference cell contained deionized water with or without 13 mmol·dm^{-3} KCl. Calorimetry data were analyzed with commercial software (OriginPro 2016, b9.3.226, USA). Enthalpies were expressed in J·g^{-1} of dry matter. Transition temperatures and enthalpies for SSL and κ-carrageenan are shown in Table 3.

2.6. Surface tension

Surface tension was measured at 25 ± 0.5 °C with a microtensiometer (Kibron EZPiplus, Finland). The instrument was calibrated with deionized water, and 3 mL of sample was used for each test.

Samples of SSL were prepared from dilutions of stock solutions. The latter were prepared by dissolution of the surfactant in hot water (60 °C); KCl was added when it was necessary. Samples of Tween 20 were prepared as SSL samples, but without heating. Samples of surfactant-κ-carrageenan were prepared by mixing surfactant and κ-carrageenan stock solutions with deionized water, after the combination of both components the system was heating at 92 °C to dissolve the κ-carrageenan. Surface tension was measured 18 h after solutions preparation to let them to stabilize.

The CMC was obtained from the variation of surface tension with surfactant concentration as shown in Figure 4. The excess surface concentration, Γ, at constant pressure and temperature, was calculated from the Gibbs adsorption isotherm (Eq. 1) for surfactant and surfactant + KCl solutions [7].

$$\Gamma_{(T,\,P)} = -\,(d\sigma/d\ln x_s)/nRT \qquad (1)$$

where R is the gas constant (8.314 $J \cdot K^{-1} \cdot mol^{-1}$), T absolute temperature (298 K), σ surface tension ($N \cdot m^{-1}$), x_s mole fraction of surfactant; and n is the number of independent components. It was used a molar mass of 451.6 and 1227.5 $g \cdot mol^{-1}$ for SSL and Tween 20, respectively, to calculate the fraction mol. For nonionic surfactants, neutral molecules or ionic surfactants in the presence of excess electrolyte n = 1 and for 1:1 ionic surfactants, assuming electrical neutrality of the interface, n = 2.

Table 2. Major absorption bands of SSL, Tween 20 and κ-carrageenan IR spectra.

Component	Absorption (cm^{-1})	Functional group
SSL	2956/2873 2916/2849	Asymmetric and symmetric methyl and methylene stretch vibrations.
	1735	Carbonyl stretching from R-CO-O-R
	1594	Carbonyl stretching from $R\text{-}CO_2^-$
	1467/1379/721	Methylene/methyl band, methyl band, methylene rocking vibration. Evidence of a long aliphatic linear chain
	1412	Methyl symmetric bend vibration
	1100/1038	C-O stretching vibration
Tween 20	3476	Hydrogen-bonded O-H stretching
	2920/2860	Asymmetric and symmetric methylene stretching vibrations
	1734	Carbonyl group from R-CO-O-R
	1640	Carbonyl stretching
	1095	Stretch vibration of $-CH_2\text{-}0\text{-}CH_2-$
κ-carrageenan	3382	Hydrogen-bonded O-H stretching
	2956/2903	Methine stretch vibrations
	1640	Polymer bound water
	1374	Sulphate stretching vibration
	1225	Ester sulphate group asymmetric stretching
	1156	C-O-C asymmetric stretching
	1063/1037	C-O and C-OH modes and glycosidic linkage
	925	C-O stretching vibrations of 3,6-anhydro-D-galactose
	843	D-Galactose-4-sulphate
	772/733/699	Skeleton bending of pyranose ring

From the excess surface concentration, the area per molecule, A, was calculated according to Eq. 2.

$$A = 1/\Gamma_{(T,\,P)}N \tag{2}$$

where N is Avogrado´s number; $6.022 \cdot 10^{23}$ mol^{-1} [8]. The standard free energy of micellization was determined using Eq. 3 for the nonionic surfactant, and Eq. 4 for the ionic surfactant [9]. The information obtained from equations 1 to 3 is summarized in Table 4.

$$\Delta G_{CMC} = RT \cdot \ln(x_{CMC}) \tag{3}$$

$$\Delta G_{CMC} = (2 - \beta)RT \cdot \ln(x_{CMC}) \tag{4}$$

where, β is the counter-ion dissociation constant and x_{CMC} is the surfactant mole fraction at the CMC. According with Tandros [10], the degree of dissociation for many ionic surfactants are ~ 0.2, so Eq. 4 is as follows:

$$\Delta G_{CMC} = 1.8RT \cdot \ln(x_{CMC}) \tag{5}$$

Data obtained for mixtures of κ-carrageenan with SSL and Tween 20, are presented in Figura 5. Adsorption parameters (Γ, A and ΔG) were not calculated, since the combinations of κ-carrageenan with the surfactants presented three break points in the graph. The first point is the critical aggregation concentration (CAC), and corresponds to the concentration where interaction between polymer and surfactant occurs. The second is known as the polymer saturation point (PSP) and represents the surfactant concentration at which the polymer-surfactant complex is desorbed from the interface. Finally, the third point occurs when the surface tension of the polymer-surfactant complex reaches the surface tension of the surfactant solution [11].

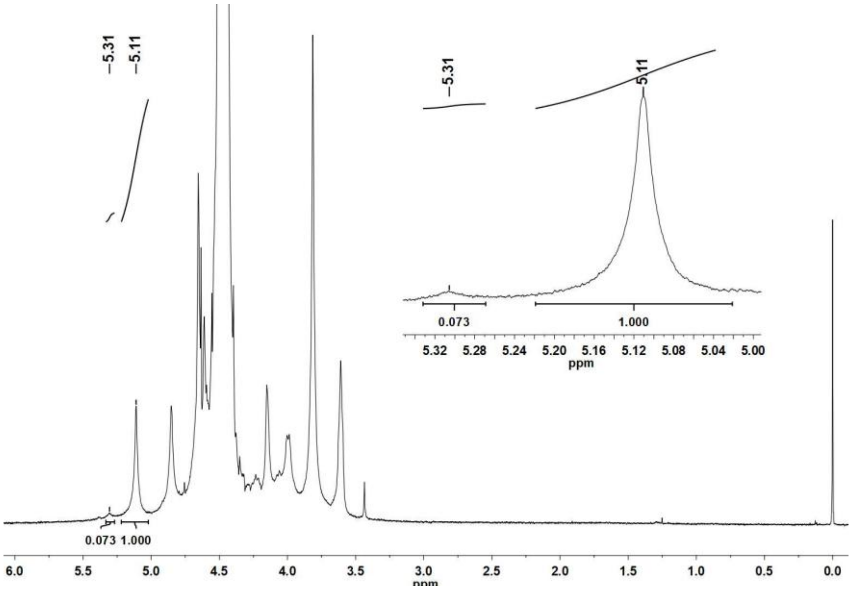

Figure. 2. ^{1}H NMR spectra of κ-carrageenan. The signal at 5.11 ppm is due to the α-anomeric proton of κ-carrageenan, while the signal at 5.31 is for ι-carrageenan [6]. The internal reference (TSP) is at 0 ppm.

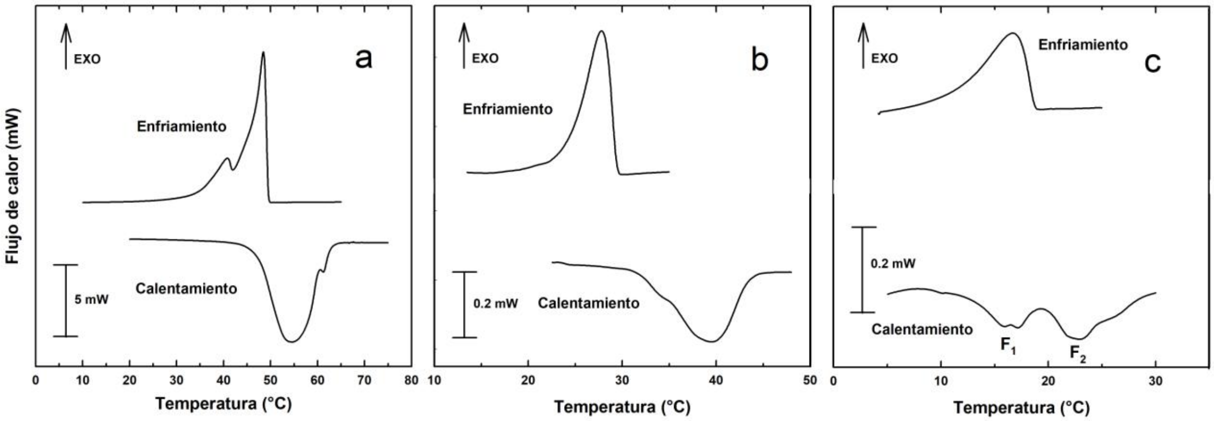

Figure. 3. DSC heating and cooling thermograms for (a) sodium stearoyl lactylate at a rate of 1.2 °C min^{-1}, (b) 0.5 % κ-carrageenan + 13 mmol·dm^{-3} KCl at a rate of 0.8 °C min^{-1} and (c) 0.5 % κ-carrageenan at a rate of 0.8 °C min^{-1}. M_1 = Melting 1 and M_2 = Melting 2.

The CMC value, 0.05 wt%, for SSL was different from those, 0.08-0.10 wt% reported by others [12]. This discrepancy can be explained by impurities present in the commercial sample. Another factor could be the SSL1 and SSL2 proportion, both molecules are the main components of the sample, and have different interfacial activity [13].

2.7. Statistical analysis

Means and standard deviations were calculated with Microsoft® Office Excel® 2007.

Table 3. Transition temperatures and enthalpies for SSL and κ-carrageenan in the presence and absence of KCl.

Component		Temperature (°C)	Enthalpy (J·g^{-1})
SSL	Melting	54.6 ± 0.01	124 ± 0.15
	Gelling	40.5 ± 0.02	101 ± 0.17
κ-carrageenan	Melting 1	15.88 ± 0.02	5.46 ± 0.17
	Melting 2	23.02 ± 0.01	10.10 ± 0.07
	Gelling	16.72 ± 0.01	19.28 ± 0.09
κ-carrageenan + 13 mmol·dm^{-3} KCl	Melting	39.82 ± 0.06	36.08 ± 0.29
	Gelling	28.02 ± 0.02	33.92 ± 0.37

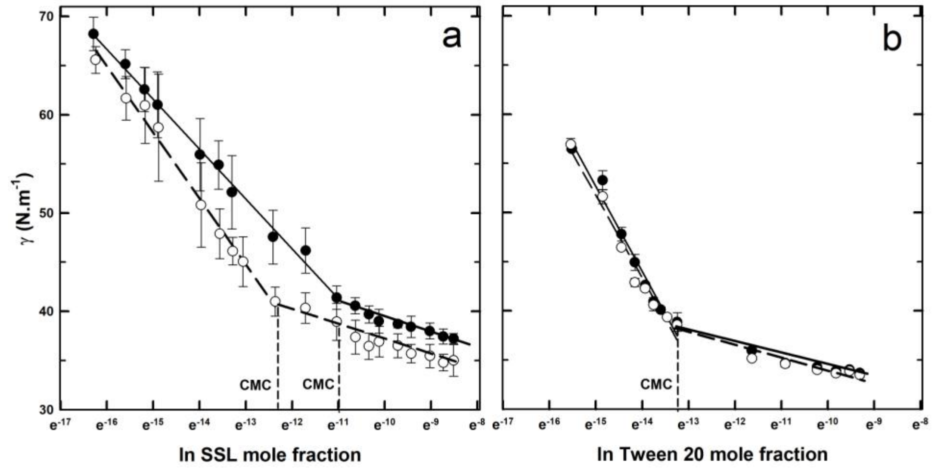

Figure. 4. Influence of (a) sodium stearoyl lactylate and (b) Tween 20 concentration on the surface tension of water without (full circles) and with KCl (empty circles) at 25 °C. CMC = Critical micelle concentration.

Table 4. Critical micelle concentration (CMC), excess surface concentration (Γ), molecular area (A) and free energy of micellization of SSL and Tween 20 without and with KCl calculated from their mole fraction.

	KCl (mmol dm^{-3})	CMC x 10^6 (X_S)	$\Gamma \cdot 10^6$ (mmol·m^{-2})	A (nm^2)	ΔG_{CMC} (kJ·mol^{-1})
SSL	0	16.1	1.023	1.624	-49.25
	13	4.26	2.692	0.617	-55-18
Tween 20	0	1.77	3.487	0.476	-32.83
	13	1.77	3.384	0.491	-32.84

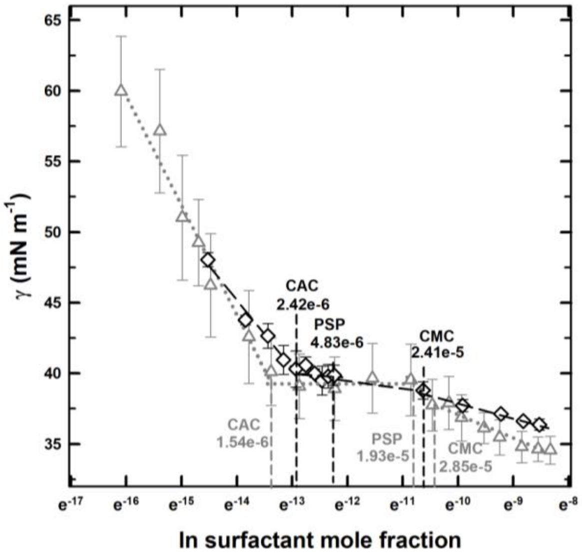

Figure. 5. Influence of sodium stearoyl lactylate (gray triangles) and Tween 20 (black diamonds) concentration on the surface tension of 0.5 % of κ-carrageenan solutions at 25 °C. CAC = Critical aggregation concentration, PSP = Polymer saturation point, CMC = Critical micelle concentration. Units are mmol·dm^{-3}.

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Transparency document. Supporting information

Transparency data associated with this article can be found in the online version at https://doi.org/10.1016/j.dib.2018.05.064.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.dib.2018.05.064.

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PARTE III. RESULTADOS LECITINA DE SOYA

La lecitina tiene un alto contenido de potasio, ya que este ion se encuentra presente en la soya que es de donde se extrae. Los demás iones se encuentran en bajas cantidades, como se muestra en la Tabla 1.

Tabla 1. Contenido iónico intrínseco (mg·kg^{-1}) de lecitina.

Ión	Na^+	K^+	Ca^{2+}	Mg^{2+}
	822 ± 21	7 168 ± 329	781 ± 35	42 ± 1

El espectro infrarrojo de la lecitina (Fig. 1) muestra las siguientes bandas:

A 3317 cm^{-1} (ancha) que corresponde al grupo O-H y muestra claramente la presencia de ésteres de alcohol.

Las bandas correspondientes a la vibración simétrica y antisimétrica del CH_2, antisimétrica del CH_3 y de tijeras del CH_2 en 2853, 2923, 2956 y 1464 cm^{-1}, respectivamente, que corresponden a las regiones hidrofóbicas de cola.

La banda en 1742 cm^{-1} (fuerte) representa el estiramiento del grupo carbonilo para ésteres, grupo localizado entre las cadenas hidrofóbicas y el grupo hidrofílico de la molécula de lecitina, indicando la presencia de fosfatidiletanolamina y fosfatidilserina de lecitina.

Las vibraciones de estiramiento del enlace C-O se encuentran en 1378 y 1228 cm^{-1}.

La banda en 1163 cm^{-1} corresponde al estiramiento asimétrico del grupo C-O de ésteres, alcoholes terciarios y aminas alifáticas (N-H).

Las vibraciones infrarrojas activas de PO_2^- y P-O-C altamente solapadas en la región de 1200 a 970 cm^{-1}, con una absorbancia máxima en 1059 cm^{-1}.

La banda en 972 cm^{-1} indica la presencia de fosfolípidos que contienen colina; en 518 cm^{-1} representa la flexión del grupo fosfato (O-P-O) y en 466 cm^{-1} se revela la deformación del grupo fosfato, O-P-O

Todos estos datos corresponden a los reportados por varios autores (Kuligowski, et al., 2008; Shah, Gaitonde & Ganesh, 2018 y Tantipolphan, Rades, McQuillan & Medlicott, 2007).

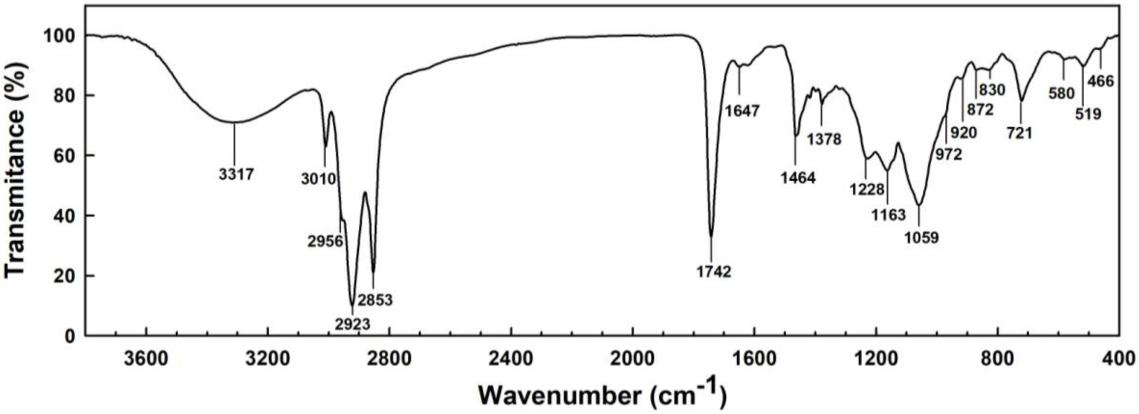

Fig. 1. Espectro FTIR-ATR de lecitina de soya.

Conclusiones

Los resultados de absorción atómica y RMN muestran que la forma comercial de κ-carragenina se encuentra principalmente como sal de potasio y que tiene un bajo contenido de ι-carragenina, por lo que el comportamiento de los sistemas donde se utilice podrá ser atribuido principalmente a la κ-carragenina.

Por su parte los resultados obtenidos para los tensioactivos, ELS, Tween 20 y lecitina de soya, indican que en general tienen bajo contenido de iones y presentan los grupos característicos de sus estructuras. Además, se cuenta con información acerca del comportamiento interfacial de los tensioactivos solubles en agua; ELS y Tween 20.

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Este capítulo muestra principalmente, las interacciones que se presentan entre la κ-carragenina y el estearoil lactilato de sodio. Ambos componentes tienen grupos cargados negativamente en su estructura. Esto ocasiona modificaciones en las propiedades examinadas.

El comportamiento reológico muestra una disminución, principalmente del carácter elástico, representado por G' con el incremento del tensioactivo en los sistemas. Por su parte, las propiedades térmicas, también exhiben variaciones no proporcionales con la concentración del tensioactivo; es decir, se observa una disminución tanto de las entalpías como de las temperaturas de transición. En el caso de la masa molar y del radio de giro se aprecia un incremento con el aumento de la concentración del tensioactivo. Finalmente, la microestructura de los sistemas cambia con la presencia del tensioactivo. Todos estos cambios observados al adicionar el tensioactivo a las disoluciones de κ-carragenina sugieren que las moléculas de ELS obtaculizan la formación de la red tridimensional de κ-carragenina. Aunado a esto es posible que existan interacciones electrostáticas repulsivas ya que las cargas negativas de ambos compuestos, no se encuentran completamente apantalladas. Finalmente, el cambio en las interacciones polisacárido-disolvente también pueden contribuir a los cambios observados en las diferentes técnicas utilizadas.

Por otro lado, se presentan también los resultados obtenidos para las mezclas κ-carragenina y Tween 20. En este caso no se presentan cambios significativos en la red tridimensional que forma el polisacárido. Entre estos compuestos se pueden presentar interacciones débiles de tipo puentes de hidrógeno, puesto que ambos contienen grupos hidroxilo en su estructura; sin embargo, el efecto de estas interacciones no es evidente ya que la cantidad de estas interacciones no es suficiente para presentar cambios.

CAPÍTULO 3

INTERACCIONES ENTRE κ-CARRAGENINA Y ESTEAROIL LACTILATO DE SODIO (ELS) O TWEEN 20

PARTE I.

Carbohydrate Polymers 193 (2018) 289-297

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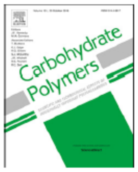

Thermal, conformational and rheological properties of κ-carrageenan sodium stearoyl lactylate gels and solutions

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ABSTRACT

Polysaccharide-surfactant blends are widely used in foods. However, their possible mutual interactions have not been extensively studied. The purpose of this work was to examine how the anionic surfactant sodium stearoyl lactylate (SSL) affects different properties of κ-carrageenan solutions and gels. Rheometry, differential scanning calorimetry, asymmetrical flow field-flow fractionation coupled with multiangle laser light scattering, among others, were used to determine the flow and viscoelastic behavior, thermal transitions, and conformation changes, respectively. Interference caused by SSL is postulated as the primary factor to explain the variations in the conformation of κ-carrageenan in gels and solutions. However, electrostatic repulsions between κ-carrageenan and SSL can also be involved. These latter interactions are more important for high SSL concentrations (13 mmol dm^{-3}) without addition of KCl, because of the higher net negative charge density of the system. SSL significantly modifies the properties of κ-carrageenan in aqueous media.

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1. Introduction

Polysaccharides and surfactants are used in dairy, meat and bakery products, among others, to stabilize emulsions and foams. Interactions between surfactants, proteins, and polysaccharides are of paramount importance in food and non-food applications. Research has primarily focused on mixtures of proteins and surfactants and to a lesser extent on blends of polysaccharides and non-food surfactants. Mixtures of chitosan with nonionic and anionic surfactants have been amply studied. Interactions between chitosan and nonionic surfactants like sorbitan monolaurate, sorbitan monooleate, sorbitan trioleate, octaethyleneglycol and mono n-dodecyl ether are weak and depend on the chitosan-surfactant concentration and chain length of surfactant (Chiappisi & Gradzielski, 2015; Grant, Cho, & Allen, 2006). Interactions between chitosan and anionic sulfated surfactants like sodium dodecyl sulfate, sodium lauryl ether sulfate, and sodium taurocholate are governed by hydrophobic interactions, although electrostatic interactions can also be present. Hydrogen bonds between chitosan and fatty acids can also exist, but hydrophobic interactions are more significant in the final behavior of these mixtures because of the contribution of the hydrocarbon chain of the acids. The degree of ionization of chitosan and fatty acids changes with pH, and therefore these interactions are also pH dependent (Chiappisi & Gradzielski, 2015).

Mixtures of carrageenans with different surfactants have been studied to a lesser extent. κ, ι, and λ carrageenans mixed with cationic surfactants like N-dodecyl pyridinium chloride, N-cetylpyridinium chloride, and dodecyl ammonium chloride have been investigated. The type of carrageenan and surfactant concentration determine the behavior of the polysaccharide-surfactant complexes (Kogej, 2008), but it is mainly governed by electrostatic repulsions between oppositely charged species (Vinceković, Katona, Bujan, & Sovilj, 2011). However, the role of the physical characteristics of the polysaccharides in general, like molar mass, the degree of branching, charge density, the rigidity of the chain, and polar group and chain length of surfactants, should be examined. It is also essential to know the impact of the sol-gel-sol transition temperatures and the sensitivity of the polysaccharide to mono or divalent ions. All this knowledge would facilitate the development of new and more stable products and create the conditions for the components to express their functionalities (Bouyer, Mekhloufi, Rosilio, Grossiord, & Agnely, 2012).

κ-carrageenan is a sulfated D-galactan polysaccharide. Its primary structure consists of a repeating disaccharide of alternate (1 → 3) β− D-galactose-4-sulfate and (1 → 4) α-3,6-anhydro-D-galactose. It is used in the food industry as a stabilizer and gelling agent. Its gels are stiff, brittle, exhibit syneresis and thermal hysteresis. Gelation occurs when hot solutions are cooled and involves a coil-helix transition followed by helix aggregation (De Ruiter & Rudolph, 1997). The coil to double helix transition is the key step for gelation to occur. Moreover, gelation is very sensitive to salts and can be controlled by the presence of specific cations like K^+, Cs^+, and Rb^+, since they increase the double helix stability and mediate helix-helix aggregation. Double helix aggregation occurs at a critical temperature, and its value depends on concentration and type of cation present (Rochas & Rinaudo, 1984).

Sodium stearoyl lactylate (SSL) is an anionic surfactant synthesized from fatty acids and lactic acid in the presence of sodium (Boutte & Skogerson, 2004). It is formed by a hydrophilic charged head coming from ionic lactic acid and a long hydrophobic hydrocarbon tail from stearic acid (Grigoriev, Leser, Michel, & Miller, 2007). Commercial SSL is mainly a mixture of 50% stearoyl-1-lactylate (SSL1), 20% stearoyl-2lactylate (SSL2), and 5% stearoyl-3-lactylate (SSL3) (Boutte & Skogerson, 2004). SSL is widely used in the food industry as an emulsifier in meat products, and as a whipping agent and softener in bakery products (Zinoviadou, Moschakis, Kiosseoglou, & Biliaderis, 2011). All the components of SSL exhibit different interfacial activities; as an example, the larger hydrophilic head group of SSL2 makes it less surface active than SSL1 (Grigoriev, Leser, Michel, & Miller, 2006).

The goal of this work was to determine how SSL, a surfactant approved for food use, affects the conformation and physicochemical properties of κ-carrageenan. The information obtained will serve to understand the behavior of polysaccharide-surfactant mixtures to use it in the development of more complex systems.

2. Materials and methods

Food-grade κ-carrageenan (Ingredients Solutions, USA) was used without further purification. Its molar mass (see Section 2.4) was 308 366 g mol^{-1}. Its potassium, sodium, calcium and magnesium contents determined by atomic absorption spectroscopy were 62 100, 21 513, 1301 and 5.2 ppm, respectively. KCl (Merck, Mexico) and NaCl (AppliChem, Germany) were reagent grade. Food-grade sodium stearoyl lactylate (SSL, CAS: 25383-99-7; EC: 246-929-7; Palsgaard, Denmark) was used with potassium, sodium, calcium and magnesium contents of 1230, 21 710, 118 and 1.4 ppm, respectively; and a measured critical micellar concentration (CMC) of 0.893 mmol dm^{-3}. Deionized water was used in all experiments.

2.1. Preparation of κ-carrageenan solutions and gels

κ-carrageenan was dispersed in deionized water under stirring for 30 min at room temperature. After complete dispersion, the surfactant was added to obtain concentrations of 0–1.0% (g/100 g), and the mixture was stirred for 15 min more. Next, the mixture was heated to 92 °C for 10 min. The hot solutions were cooled to 50 °C. Evaporative losses were compensated by the addition of deionized water. In systems with KCl, the salt was added when the temperature reached 92 °C. In this case, gels were formed on cooling.

2.2. Differential scanning calorimetry (DSC)

The thermal transitions of 0.5% κ-carrageenan gels and solutions were determined using a microcalorimeter (μDSC 7 Evo, Setaram, France). A mass of 600 ± 10 mg of sample was weighed into a Hastelloy cell. The reference cell contained an equivalent mass of sample solvent. Both cells were equilibrated for 30 min and then heated from 5 to 80 °C, held at 80 °C for 10 min, cooled at 5 °C, and kept at this temperature for 10 min, in three heating-cooling cycles. The heating and cooling rates were 0.8 °C min^{-1}. Melting and gelation temperatures and enthalpies were determined with commercial software (OriginPro 9.1, OriginLab Corporation, USA). Enthalpies were expressed in J g^{-1} of dry matter.

2.3. Zeta potential

Zeta potentials of κ-carrageenan solutions and their blends with SSL were measured at 20 °C with a Zeta Meter 3.0+ (Zeta-Meter, Inc., USA) equipped with a microprocessor unit. κ-carrageenan solutions were diluted to 0.1% (w/w). Blends with surfactant were diluted to 0.01% (w/w) SSL. pH was measured at 20 °C with a potentiometer (Thermo Scientific Orion, model 420A, Cole-Parmer, USA). The sample was placed in a cell, and electrophoretic mobility of charged particles was measured by tracking their displacement in an electric field with a stereoscopic microscope. Zeta potential (mV) was calculated by the microprocessor unit. Average values obtained by tracking at least twenty different particles are reported.

2.4. Asymmetrical flow field-flow fractionation coupled with multiangle laser light scattering (AF4-MALLS)

Molar mass, size, and conformation were analyzed by AF4-MALLS. The AF4 channel flow, cross flow, sample injection and focus flow were controlled with a Wyatt Eclipse AF4 flow chassis (Wyatt Technology Corporation, Santa Barbara, USA), a pump and an autosampler (ThermoFisher Scientific; Waltham, MA, USA). The sample was introduced at 25 °C into a 275 mm long trapezoidal channel equipped with a spacer of 350 μm thickness and a regenerated cellulose membrane with a nominal cut-off of 10 kDa (Millipore, Bedford, MA, USA). The on-line detectors included a MALLS unit (Dawn® HELEOS™) fitted with a K5 flow cell and a He-Ne laser (λ = 658 nm), and a refractometer (Optilab T-rEX) operating at the same wavelength (Wyatt Technology Corporation). κ-carrageenan concentrations were 0.15 and 0.05% for samples without and with SSL, respectively. All samples were filtered through a 0.45 μm membrane (Millipore) before injection. Injection volumes were 65 and 100 μL of κ-carrageenan solutions or mixtures with the surfactant, respectively. The carrier, 100 mmol dm^{-3} NaCl (Millipore), was filtered through a 0.1 μm membrane (Millipore) and eluted initially at 1 mL min^{-1} in the channel. κ-carrageenan samples were eluted following a procedure adapted from Bourgoin, Zablackis, & Poli (2008). The cross-flow was set to 0.8 mL min^{-1} and the sample was injected at 0.20 mL min^{-1} for 300 s. After injection, the sample was left to relax and focus on 120 s. The carrier flow was set to 1 mL min^{-1} for elution, and the cross flow decreased from 0.8 mL min^{-1} to 0.05 mL min^{-1} for 40 min, maintained at this value for 3 min, and finally kept at 0 mL min^{-1} for 2 min. Elution recovery rates were calculated from the ratio of the mass eluted from the channel, given by the integration of the differential refractive index signal, and the injected mass. The latter was obtained from the exact concentration in the injected samples determined by desiccation of 5 g of sample at 100 °C for 24 h.

The number-average molar mass, $\overline{\mathrm{M_n}}$, the weight-average molar mass, $\overline{\mathrm{M_w}}$, the dispersity, $\overline{\mathrm{M_w}}/\overline{\mathrm{M_n}}$, and the z-average radius of gyration (nm), $\overline{\mathrm{R}}_{\mathrm{G}}$, were obtained with ASTRA® software (Wyatt Technology Corporation; version 6.1.2.84 for PC) as previously described for other polysaccharides (Rolland-Sabaté, Colonna, Mendez-Montealvo, & Planchot, 2007; Rolland-Sabaté, Guilois, Jaillais, & Colonna, 2011). The refractive index increment, dn/dc, for glucans was 0.146 mL·g-1. The molar mass and radius of gyration of the ith slice, Mi, and RGi, respectively, were obtained for each slice of the chromatogram peak using the Berry extrapolation with a first order polynomial fit of the light scattered to zero angle.

2.5. Atomic force microscopy (AFM)

κ-carrageenan gels and its mixtures with 0.5 and 1.0% SSL were prepared as described in 2.1. Before cooling, they were diluted with hot (85 °C) deionized water to a final concentration of 20 μg mL^{-1}. A 0.04% (w/w) SSL solution was prepared in water at 55 °C under mechanical stirring and was not diluted. Aliquots of 5 μL of each sample were deposited on mica (diameter 20 mm), then onto a magnetic disc, dried at room temperature and relative humidity for at least 30 min, and observed. Each sample was scanned over an area of 10 μm^2 at a rate of 1 Hz and 256 lines in an Innova Scanning Probe microscope (Veeco Intruments Inc., USA) equipped with a Nanodrive 8.02 controller. The tapping mode was employed using a scanning probe (RTESPA-CP; Bruker, USA) with a constant force and a resonance frequency of 40 N m^{-1} and 300 kHz, respectively. Imaging was made at room conditions. AFM data were processed with the Nanoscope Analysis 1.40 software (Bruker, USA) and edited with the Gwyddion 2.49 software (Czech Republic).

2.6. Rheometry

Rheological properties were determined using a rotational rheometer (ARES-RFS III, TA Instruments, USA). Flow curves of κ-carrageenan solutions without KCl were obtained from 0.01–100 s^{-1} at 25 °C in a cone-plate fixture, 50 mm in diameter, cone angle 2.3° (0.04 rad) and 40.6 μm gap. Viscoelastic properties of gels with KCl and solutions were determined under low-amplitude oscillatory shear from 0.1–100 rad s^{-1} at 25 °C using serrated parallel plates, 25 mm in diameter, 3 mm gap. The zone of linear viscoelasticity was established from strain sweeps at 6.28 rad·s^{-1}. Solutions were stored at 4 °C at least 18 h before the rheological measurements. The dependence of the dynamic moduli G’ and G” with angular frequency, ω, was determined by power-law relationships of the type G’ $\alpha\omega^{\alpha}$ and G” $\alpha\omega^{\beta}$.

2.7. Statistical analysis

All measurements were made in triplicate. A one-way ANOVA was done with commercial software (Microsoft® Office Excel® 2007, 12.0.6750.5000). Differences between treatment means were obtained by Fisher’s least significant difference (LSD) procedure, using Eq. (1).

$$LSD = t\sqrt{\frac{2MS_E}{n}} \quad (1)$$

In this equation, t is the Student's t-distribution percentile based on the error degrees of freedom at $\alpha = 0.05$ level of significance, n is the number of scores in each treatment sample, and MS_E is the mean squared error for the ANOVA.

3. Results and discussion

3.1. DSC thermal behavior

3.1.1. Gelation and melting in the presence of SSL and KCl

DSC experiments allowed obtaining the transition temperatures, melting enthalpy, ΔH_m, and the absolute value of gelation enthalpy, $|\Delta H_g|$, of κ-carrageenan chains in the presence and absence of potassium ions. All these parameters decreased with the increase in SSL concentration as shown in Fig. 1. The transition temperatures (T_m = 39.8 °C; T_g = 28.0 °C), and enthalpies (ΔH_m = 36.1 J g^{-1}; ΔH_g = 33.9 J g^{-1}) of κ-carrageenan gels were similar to those previously reported (Núñez-Santiago & Tecante, 2007). The melting and gelation enthalpies were similar, showing that comparable energy was required for double helix aggregation and melting of the aggregates. Thermal hysteresis, i.e., $T_m > T_g$, similar to that observed by Gekko, Mugishima, & Koga (1987) was present. They observed a difference of 10 °C between melting and gelation transitions in mixtures of κ-carrageenan with 25 mmol dm^{-3} KCl. This result was attributed to the cooperative nature of gelation, i.e., the formation of the first helix facilitates the development of new helices. Thermal hysteresis has been associated with helices aggregation above a total ionic concentration, C*, of 7 mmol dm^{-3}. Below this concentration, this effect is not observed because only a coil-helix transition takes place (Núñez-Santiago & Tecante, 2007).

The addition of SSL decreased the transition temperatures of gels as shown in Figure 1a. However, melting exhibited three different zones; 1, 2, and 3, for concentration ranges of 0–5.3, 5.3–11, and 11–27 mmol dm^{-3}, respectively. The gelation temperatures had a single-slope linear decrease for 0–27 mmol dm^{-3} SSL. The linear correlations are given by Eqs. (2)–(5) with C_{SSL} units as shown in Fig. 1, the slope in °C dm^3 mol^{-1}, and the ordinate in °C.

Melting

Zone 1 $T_{m_1} = -443C_{SSL} + 39.8$ r = 0.9834 (2)

Zone 2 $T_{m_2} = -174C_{SSL} + 38.5$ r = 0.9941 (3)

Zone 3 $T_{m_3} = -93.5C_{SSL} + 37.4$ r = 0.9547 (4)

Gelling

Zone 1 $T_{g_1} = -123C_{SSL} + 28.0$ r = 0.9989 (5)

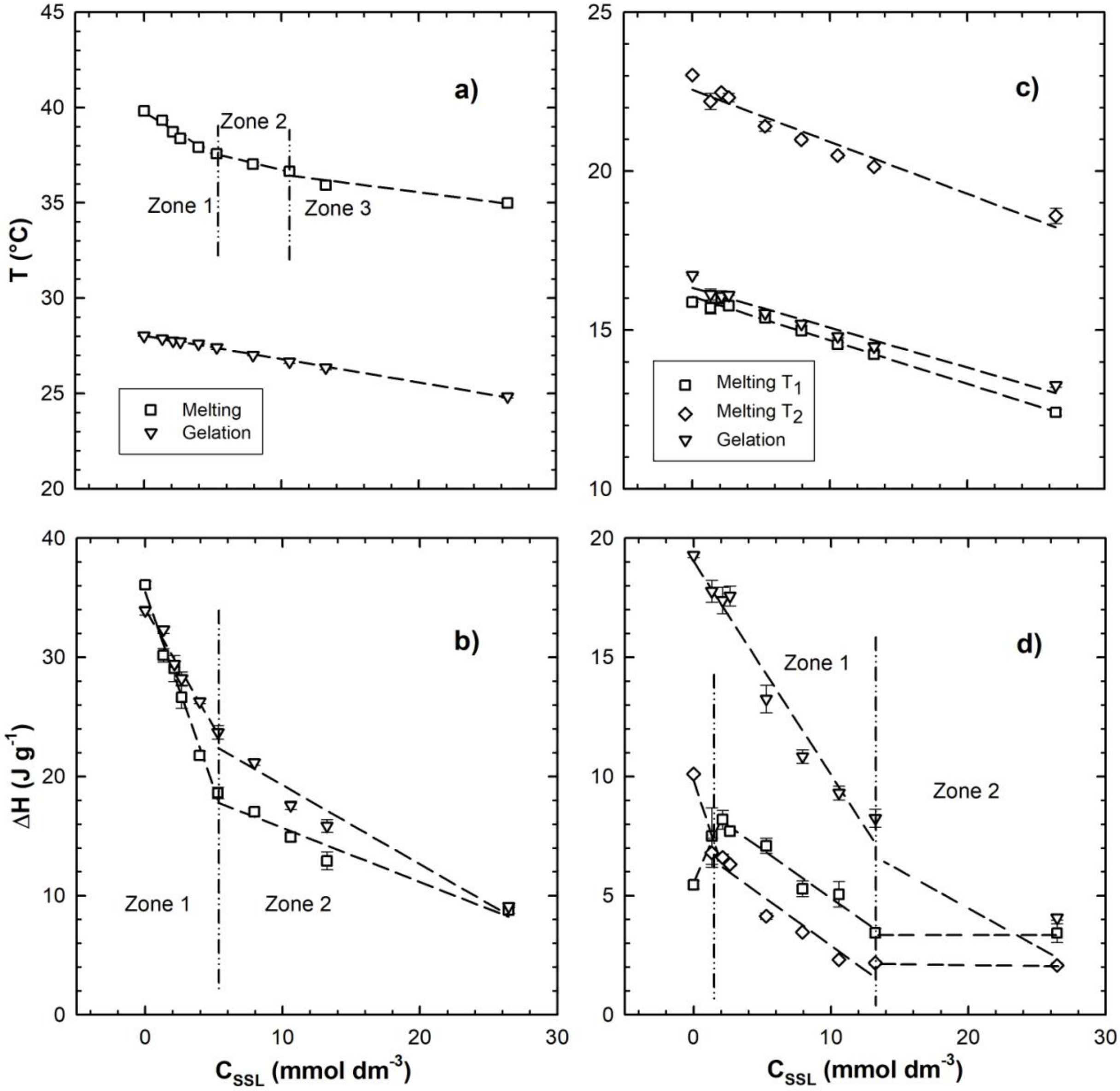

Figure. 1. Variation with SSL concentration of transition temperatures and enthalpies of 0.5 % (w/w) κ-carrageenan gels with 13 mmol·dm^{-3} KCl, (a), (b), respectively, and without KCl, (c), (d), respectively. Melting (squares and diamonds), gelation (inverted triangles).

The rate of decline of transition enthalpies was linear but depended on the range of surfactant concentration. ΔH_m decreased seven times faster in the range of 0–5.3 mmol dm^{-3} (Zone 1) than in the range of 5.3–27 mmol dm^{-3} (Zone 2) (Figure 1b). On the other hand, $|\Delta Hg|$ dropped three times faster in Zone 1 than in Zone 2. The linear relationships between transition enthalpy and surfactant concentration are given by Eqs. (6)–(9) with C_{SSL} in mmol dm^{-3}, the slope in J dm^3 g^{-1} mol^{-1}, and the ordinate in J g^{-1}.

Melting

Zone 1 $\Delta H = -3291 C_{SSL} + 35.5$ r = 0.9940 (6)

Zone 2 $\Delta H = -453 C_{SSL} + 20.2$ r = 0.9735 (7)

Gelling

Zone 1 $\Delta H = -1987 C_{SSL} + 34.1$ r = 0.9903 (8)

Zone 2 $\Delta H = -663 C_{SSL} + 25.9$ r = 0.9763 (9)

These variations show that the surfactant had a significant impact on the melting process, and the behavior shown in Figure 1a and b can be interpreted as the result of a lower degree of association between κ-carrageenan molecules. This action suggests the existence of a less dense aggregation leading to the destabilization of the κ-carrageenan gel network.

The melting process is noticeably affected by the surfactant, and significant changes occur below 5.3 mmol dm^{-3}. The differences between melting and gelation temperatures can be explained by the aggregation of doubles helices. It is evident that gelation in the presence of KCl is less affected by SSL, perhaps due to the cooperative nature of carrageenan gelation but also to the mobility of the SSL molecules at the gelation temperature which improves the formation of the network. Besides, the addition of surfactant decreases progressively the thermal hysteresis which means less aggregation of helices. It has been shown that the gradual addition of glycerol, erythritol, xylitol, sorbitol, maltitol and carbohydrates like ribose, xylose, mannose, galactose, fructose, maltose and sucrose to κ-carrageenan and 25 mmol dm^{-3} KCl, increased gelation temperatures but decreased the absolute values of ΔH_g (Gekko et al., 1987). The authors attributed this unusual behavior to polymer-solvent interactions because in general, absolute values of ΔH_g increase with the increment in KCl and other salts concentration. Changes in polymer-solvent interactions modify electrostatic, hydrophobic and hydrogen bond interactions of the κ-carrageenan network, and therefore its physicochemical properties (Gekko et al., 1987).

3.1.2. Behavior in the presence of SSL without KCl

The thermal behavior of κ-carrageenan, without added KCl, in the presence of SSL was similar to that of gels. There was a reduction in transition temperatures and enthalpies as in gels as shown in Fig. 1c and d, respectively. However, two transition peaks, (not shown), were observed on melting, in all the mixtures. The first peak, designated here as Melting T_1, can be attributed to disaggregation of double helices and the second, Melting T_2, to the unwinding of double helices to separate coils. Double helices exist as aggregates when the ionic concentration, C_T, of K^+ counterions is higher than the critical concentration C*. For κcarrageenan, C* = 7.1 mmol dm^{-3} (Rochas & Rinaudo, 1984). C_T for the solutions analyzed here was calculated from the concentration of K^+ ions, obtained by atomic absorption, in the samples of κ-carrageenan and SSL. The values are between 14.35 and 14.67 mmol dm^{-3} without SSL and with the maximum SSL concentration, respectively, due to the intrinsic potassium content of the samples. On the other hand, for an ionic concentration of 5 mmol dm^{-3} KCl, the amount of helical dimmers and single helices was estimated to be 35 and 65%, respectively (Rochas and Landry, 1987). Considering our atomic absorption data, the ionic concentration in a 0.5% (w/w) κ-carrageenan solution is 7.9 mmol dm^{-3} K^{+}. These facts mean that in all the κ-carrageenan solutions, aggregates and double helices can coexist.

The presence of these conformations, i.e., double helices and their aggregates, explains the two transitions on heating. Moreover, as indicated in Section 2, the commercial sample of κ-carrageenan used in this work was not purified. The appearance of two transition peaks could also be due to the possible presence of ι-carrageenan as a minor component. However, NMR analysis (data not shown) showed 93% κcarrageenan and 7% ι-carrageenan in the sample. Therefore, the contribution of ι-carrageenan was considered marginal, since in the solutions its concentration is lower than 0.04%.

The effect of SSL concentration on the transition temperatures followed the same general trend for melting and gelation. In the entire concentration range, 0–27 mol dm^{-3}, there was a single-slope decrease. The corresponding equations with the same units as (2–5) are:

Melting T$_1$ $T_{m_1} = -137C_{SSL} + 16.0$ r = 0.9938 (10)
Melting T$_2$ $T_{m_2} = -164C_{SSL} + 22.6$ r = 0.9740 (11)
Gelling $T_g = -125C_{SSL} + 16.3$ r = 0.9808 (12)

The addition of SSL had a more significant effect on gelation because ΔH$_g$ decreased twice faster than each ΔH$_m$. Changes in the enthalpies of gelation and melting transitions did not occur over the same SSL concentration range; 0–13 mmol dm^{-3} for gelation, 2.1–13 mmol dm^{-3} for melting (Figure 1d, Zone 1 and before). Below 2.1 mmol dm^{-3}, Figure 1d, before zone 1, the behavior of enthalpy for both melting processes was different. For Melting T$_1$, enthalpy increased, but for Melting T$_2$ there was a reduction. Eqs. (13)–(15) show the relationships between enthalpy and SSL concentration with the same units as in Eqs. (6)–(9):

Melting T$_1$ $\Delta H = -407C_{SSL} + 9.0$ r = 0.9849 (13)
Melting T$_2$ $\Delta H = -417C_{SSL} + 7.1$ r = 0.9612 (14)
Gelling $\Delta H = -896C_{SSL} + 19.3$ r = 0.9831 (15)

From all these results, modification of the conformation of κ-carrageenan in gels and solutions by SSL is evident regardless of the differences in thermal behavior between these systems. In this way, the SSL changes the spatial arrangement of κ-carrageenan. This phenomenon can be explained by the occurrence of hindrance caused by SSL in both systems. The aggregation of κ-carrageenan double helices is hindered by SSL, which explains the decrease of the melting enthalpy; but, over a specific surfactant concentration, surfactant molecules can aggregate causing a drop of melting enthalpy to a lesser extent than below such level.

Another possibility to explain the behavior of κ-carrageenan in the presence of SSL is the existence of electrostatic repulsion between both components as they are negatively charged. This effect occurs in solutions and gels because negative charges are not entirely screened by the positive charges of the counterions (Figure 2).

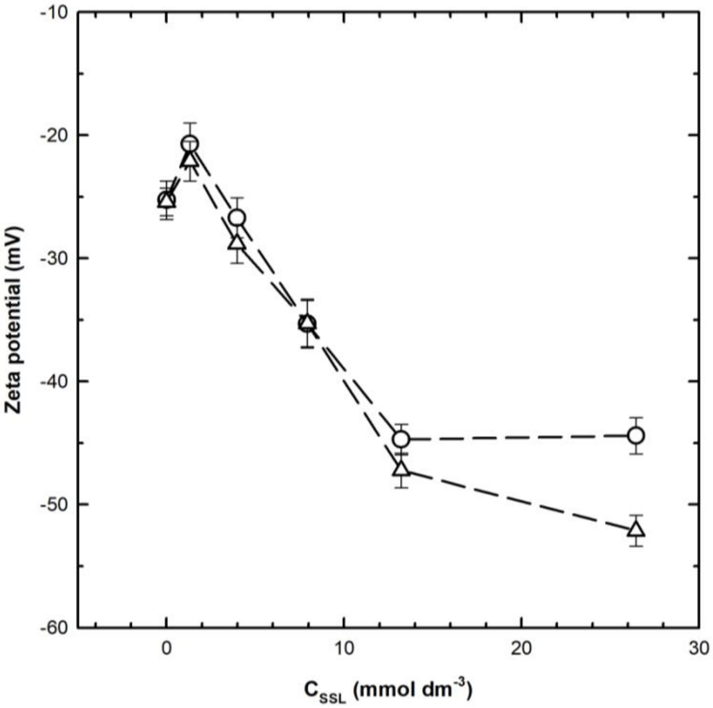

Figure. 2. Variation of the zeta potential of 0.5% (w/w) κ-carrageenan solutions with different SSL concentrations at 20 °C, without KCl (triangles) and with 13 mmol dm^{-3} KCl (circles).

Negative charges are in excess, either with or without KCl, and the addition of SSL increases the negative charge density. However, this behavior tends to be stable for high SSL concentrations (13 mmol dm^{-3}), probably due to aggregation of SSL molecules into micelle-like structures. The overall negative charge density is higher without KCl than with the salt, because of the screening effect of potassium ions. The combination of hindrance and electrostatic repulsion could explain the behavior differences between solutions and gels. In both systems, a decrease of enthalpies and transition temperatures occurs. However, in the solutions, the decline is continuous throughout the range of concentrations, while in the gels it happens above 5.3 mmol dm^{-3} SSL.

3.2. Size, molar mass and conformation in solution

Table 1 shows the size, molar mass, and dispersity of κ-carrageenan and its mixtures with SSL, using NaCl as an eluant. The molar mass of $3.08 \cdot 10^5$ g mol^{-1} found for κ-carrageenan without surfactant agrees with those reported on other samples in 100 mmol dm^{-3} NaCl (Wittgren, Borgström, Piculell, & Wahlund, 1998; Bourgoin et al., 2008). The molar mass and the radius of gyration increase clearly with SSL concentration. These results suggest that a change in the aggregation state of κ-carrageenan macromolecules occurs, as they appear more prominent in the mixtures with SSL, especially for the highest surfactant concentration.

Table 1. Elution recovery, weight average molar mass ($\overline{M_w}$), z-average radius of gyration ($\overline{R}_G$), dispersitiy ($\overline{M_w}/\overline{M_n}$), and Flory exponent (ν) of κ-carrageenan solutions obtained by AF4-MALLS.

κ-carrageenan (%)	SSL (%)	Elution recovery (%)	$\overline{\mathbf{M_w}} \cdot 10^{-5}$ (g·mol^{-1})	$\overline{\mathbf{R_G}}$ (nm)	$\overline{\mathbf{M_w}}/\overline{\mathbf{M_n}}$	ν
0.15	0.0	83	3.08 ± 0.16	56 ± 2.1	1.4 ± 0.01	0.60 ± 0.02
0.05	0.004	89	4.64 ± 0.13	75 ± 4.4	1.3 ± 0.18	0.50 ± 0.02
0.05	0.017	67	12.92 ± 1.03	157 ± 20.3	2.9 ± 0.74	0.48 ± 0.02

This phenomenon is probably due to polymer-surfactant interactions. These data show the same general trend than the one observed in separate mixtures of bovine serum albumin (BSA) with sodium dodecyl sulfate (SDS), dodecyl trimethylammonium bromide (DTAB) and polyoxyethylene 8 lauryl ether (C12E8), which are anionic, cationic and nonionic surfactants, respectively. That is to say, the increase in the hydrodynamic radius of BSA compared to the hydrodynamic radii of the BSA-surfactants complexes, when the surfactant concentration increased. Such behavior has been attributed to cooperative binding of surfactants, which means that binding of one ligand enhances the affinity of the protein for subsequent ligands (Valstar, Almgren, Brown & Vasilescu, 2000). Light scattering has also been used to study the behavior of individual blends of a capsular anionic bacterial polysaccharide with the cationic surfactants benzyl dimethyl-n-hexadecyl ammonium chloride (BDHAC), cetyltrimethylammonium bromide (CTAB), cetyl pyridinium chloride (CPC) and dodecyl pyridinium chloride (DPC). The variation of the hydrodynamic radius with surfactant concentration exhibited a maximum. This behavior was attributed to aggregation derived from polysaccharide-surfactant complexation by electrostatic attraction between both components. Beyond the maximum, the decrease in hydrodynamic radius was explained by coiling up of the polysaccharide resulting from its charge neutralization (Dasgupta, Nath, Manna, Mitra, & Panda, 2014).

The dispersities of κ-carrageenan without surfactant and with 0.004% SSL were similar (≈1.4). However, for the maximum SSL level, a higher dispersity was obtained, indicating a broader size distribution, also shown in the cumulative molar mass distribution (Figure 3), which highlights a considerable proportion of high molar masses in the solution with 0.017% SSL.

This result could be due to macromolecule association caused by polymer-surfactant interactions. It is also in line with the lower elution recovery observed for κ-carrageenan with the maximum SSL level (67% instead of 83 and 89% for κ-carrageenan without SSL and with the minimum SSL level, respectively), which could correspond to the retention of the major macromolecular structures in the AF4 channel.

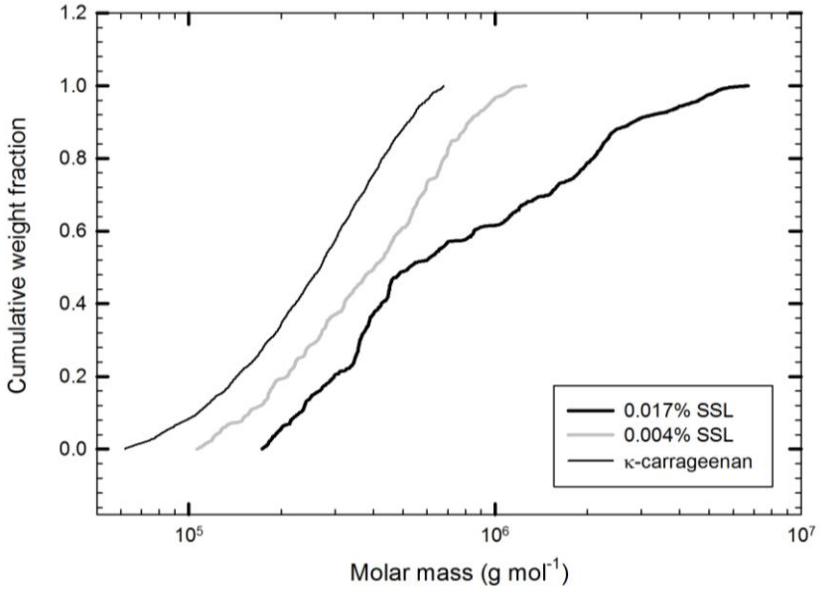

Figure. 3. Cumulative molar mass distributions obtained by AF4-MALLS of 0.5 % (w/w) κ-carrageenan solutions without SSL (continuous line), with 0.004 and 0.017 % SSL (dashed and dotted line, respectively).

Table 1 also includes values of the Flory exponent (ν), calculated by linear regression of the log-log plot of R_{Gi} versus M_i, according to $R_{Gi} = K\ M_i^{\nu}$, where K is a constant. For a swollen linear polymer, ν is around 3/5. The exponent is used to determine whether the polymer is in a suitable solvent. In such case, polymer-solvent interactions are more favorable than polymer-polymer interactions (Rubinstein & Colby, 2003). In κ-carrageenan solutions without SSL, polysaccharide chains are in a suitable solvent (ν = 0.60). The addition of surfactant decreased the value of ν to about 0.50 for both conditions. For this value of the Flory exponent, theta conditions exist, i.e., the polysaccharide is on the border between good and poor solvent. In the theta state, the solvent has a minimal solvation effect on the polymer molecules, i.e., the polymer is becoming insoluble (Su, 2013).

These results suggest a change in polymer-solvent interactions probably due to electrostatic interactions between κ-carrageenan and SSL, which can cause compaction of polymer chains and therefore a modification in their conformation.

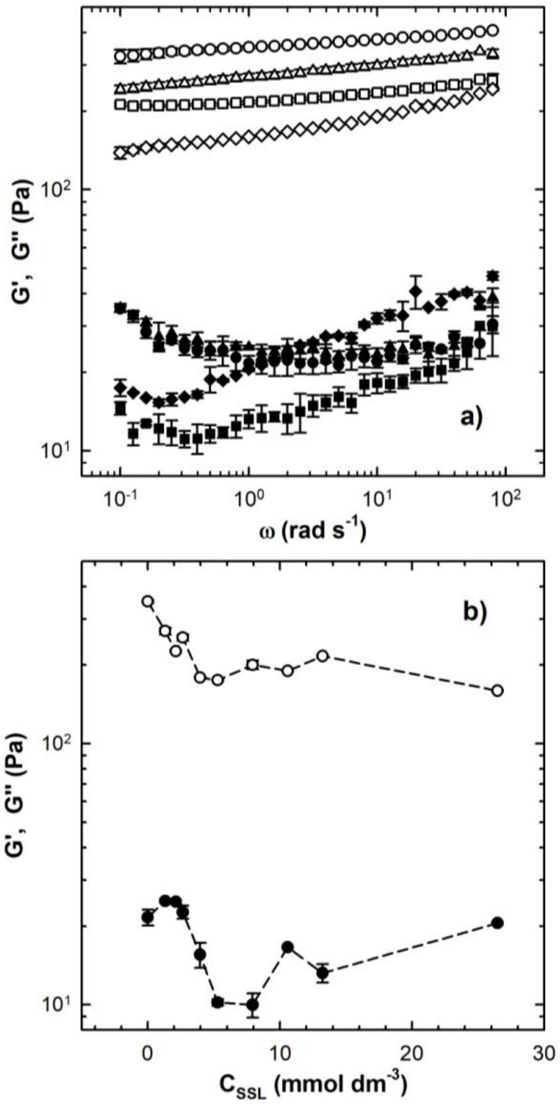

Figure. 4. a) Variation of G' (open symbols) and G" (filled symbols) with angular frequency (ω) of 0.5% (w/w) κ-carrageenan gels with 13 mmol dm^{-3} KCl, and without SSL (circles), 1.3 mmol dm^{-3} (triangles), 13 mmol dm^{-3} (squares), and 27 mmol dm^{-3} (diamonds) at 25 °C; b) Variation of G' and G" with SSL concentration at ω =1 rad s^{-1} and 25 °C.

3.3. Rheological behavior

3.3.1. Gels

Frequency sweeps for κ-carrageenan gels with different SSL concentrations are shown in Figure 4a. The slopes of G' and G" were found to increase with SSL concentration; $\alpha = 0.032$-0.078 with $r > 0.9669$, and $\beta = 0.125$–0.167 with $r > 0.9109$, respectively. This variation means that both dynamic moduli were more frequency dependent as SSL concentration increased. Therefore, the presence of SSL intensified the viscous character of κ-carrageenan gels, showing a less organized network. This effect is also evidenced by the disappearance of the plateau in G’ at low frequency for the highest SSL concentration.

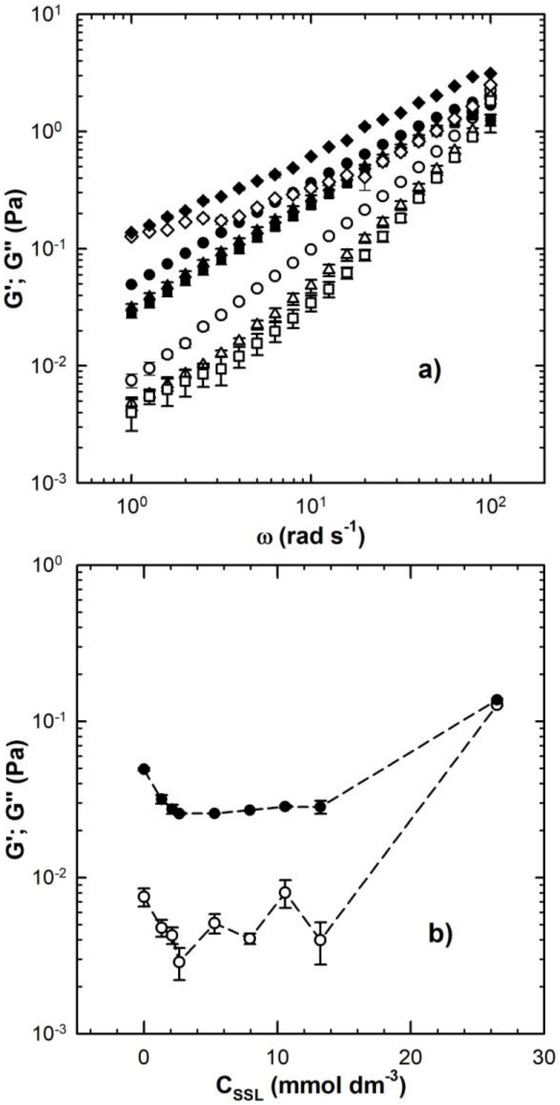

Figure. 5. a) Variation of G’ (open symbols) and G” (filled symbols) with frequency of 0.5% (w/w) κ-carrageenan solutions without SSL (circles), 1.3 mmol dm^{-3} SSL (triangles), 13 mmol dm^{-3} SSL (squares), and 27 mmol dm^{-3} SSL (diamonds) at 25 °C, and b) Variation of G’ and G” with SSL concentration at ω =1 rad s^{-1} and 25 °C.

Also, the elastic character of the gels decreased, i.e., the loss angle, δ, increased from 3.7 to 8.4° for 27 mmol dm−3 SSL. These features can be observed in Figure 4b. For concentrations below 4 mmol dm−3, G’ at 1 rad s−1 decreased as SSL concentration increased. Above this concentration, changes were not significant. G” exhibited the same behavior for κ-carrageenan without SSL and with 1.3 mmol dm−3. However, for 13 and 27 mmol dm−3 SSL, G” increased with the frequency which means an accentuation of the viscous behavior of the systems.

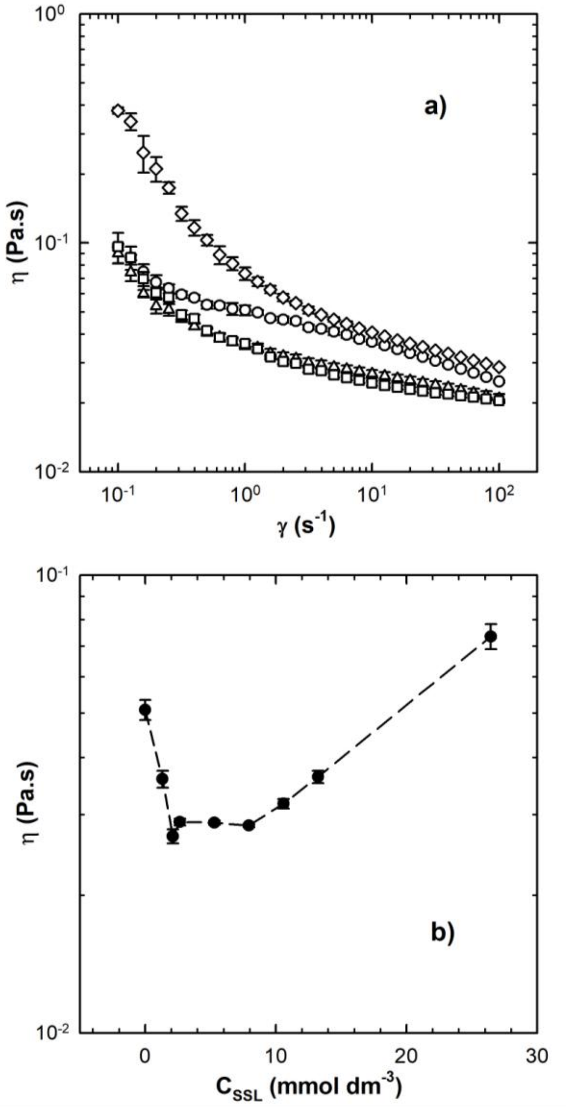

Figure. 6. a) Viscosity flow curves of 0.5% (w/w) κ-carrageenan solutions without SSL (circles), 1.3 mmol dm^{-3} SSL (triangles), 13 mmol dm^{-3} SSL (squares) and 27 mmol dm^{-3} SSL (diamonds) at 25 °C, and b) Variation of viscosity with SSL concentration at $\dot{\gamma}$ = 1 s^{-1} and 25 °C.

3.3.2. Solutions

The κ-carrageenan solutions exhibited a typical macromolecular behavior (Figure 5a), i.e., G" was greater than G' and both moduli were highly dependent on frequency (Doublier, Garnier & Cuvelier, 2016). As in the gels, G' and G" decreased as SSL concentration increased, but for the highest level, 27 mmol dm^{-3}, both moduli increased. The evolution of the apparent viscosity of κ-carrageenan solutions, Figure 6a, was like that of the dynamic moduli. All solutions exhibited shear-thinning behavior, i.e., the apparent viscosity decreased as shear rate increased. It is well known that the viscosity of polyelectrolyte solutions decreases with the increment of salt concentration because there is a compaction of the polymer chain due to the screening of the electrostatic repulsions between charges along the chain by the counterions (Wyatt, Gunther, & Liberatore, 2011).

This effect explains the reduction of the apparent viscosity with the increment of SSL concentration in agreement with the light scattering results. Increases in G', G" and η for the κ-carrageenan solution with 27 mmol dm^{-3} SSL are due to the surfactant concentration. Indeed, the viscosity of the SSL solution with the same concentration is 282 mPa·s at 25 °C and $\dot{\gamma} = 1\ s^{-1}$, although κ-carrageenan had a lower viscosity from 51 mPa·s.

The rheological results coincide with thermal effects, since the addition of SSL to κ-carrageenan gels and solutions modifies the polysaccharide behavior. The presence of at least two zones with different responses is observed with both techniques. Thus, two effects are proposed for these differences. In the first zone, electrostatic repulsions would predominate, while in the second zone hindrance caused by SSL micellization could also be present.

3.4. Microstructure of gels

The AFM microstructures of SSL, κ-carrageenan gels and its mixtures are shown in Figure 7. Spherical vesicles can be observed in the SSL solution (Figure 5a). These structures are similar but smaller than those observed by differential interference contrast light microscopy in 0.1% SSL (Bezelgues, Serieye, Crosset-Perrotin & Leser, 2008). The lower surfactant concentration used in our study could explain the differences. The black areas in the image are air holes left by water evaporated before the observations. This phenomenon also explains the proximity between the structures. The fibrous structure of κ-carrageenan with 13 mmol dm^{-3} KCl is shown in Figure 7b. This salt concentration causes incipient side-by-side aggregation like that observed in κ-carrageenan solutions without KCl, but with a higher κ-carrageenan concentration (25 μg mL^{-1}) (Sokolova et al., 2013). Despite this difference, a network was formed, and gelation occurred, which is consistent with the rheological data shown in 3.3.1. The addition of SSL leads to segregation between both components (Figure 7c), may be caused by the electrostatic repulsions. A higher SSL concentration (Figure 7d) originated structures resembling to those obtained without surfactant. However, areas with segregation were still observed. It is evident that the addition of SSL to κ-carrageenan modifies its space arrangement, since surfactant alters the formation of the κ-carrageenan network. SSL concentration is a crucial factor for these modifications to occur, and the interactions between SSL and κ-carrageenan depend on the amount of SSL. When SSL concentration in the mixture was 13 mmol dm^{-3}, a noticeable segregation occurred. However, when the level increased to 27 mmol dm^{-3}, this phenomenon was reduced. This behavior agrees with the thermal and rheological behaviors. On the other hand, the cationic surfactant cetyl(trimethyl) ammonium bromide (CTAB) gives rise to a denser cross-linking of the double helices leading to a denser κcarrageenan network. The interaction between CTAB and κ-carrageenan stabilizes the helices of the polysaccharide even in the absence of K^+ ions (Kolesov et al., 2008). The behavior of κ-carrageenan with SSL and CTAB is different because the former is negatively charged, while the latter has positive charges.

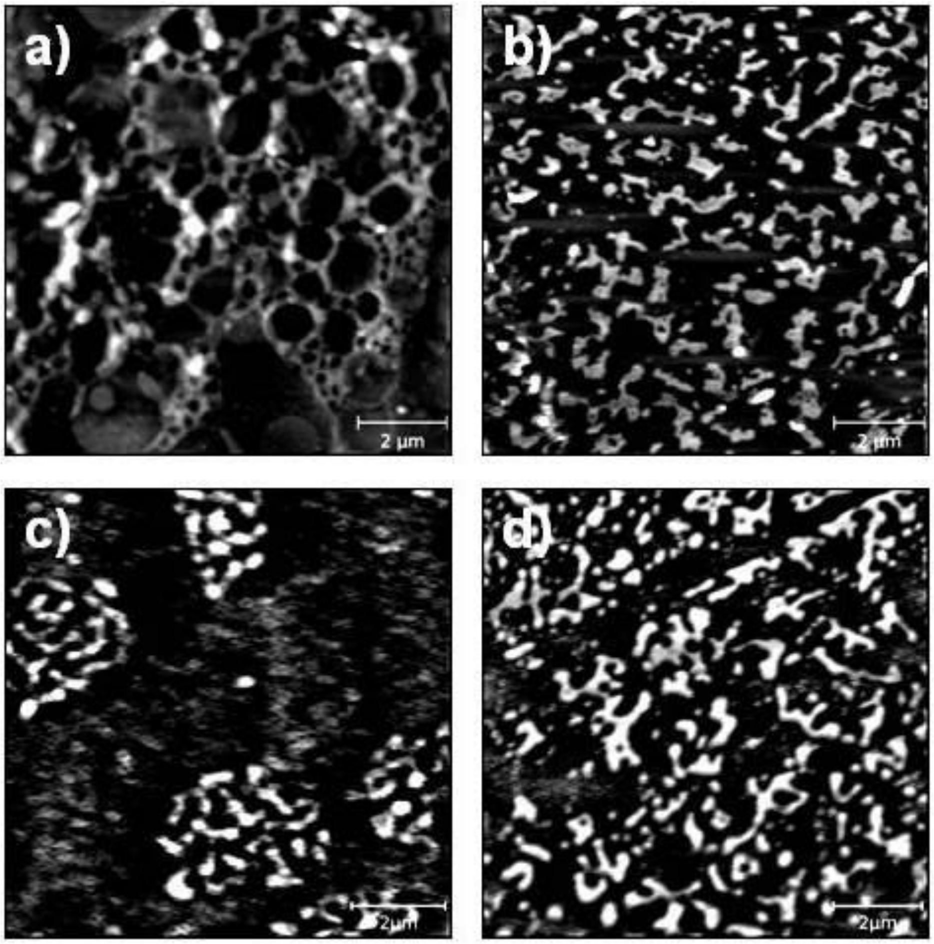

Figure. 7. AFM height profiles of a) 1.1 mmol dm^{-3} SSL solution; 0.5% κ-carrageenan solutions: b) with 13 mmol dm^{-3} KCl, c) with 13 mmol dm^{-3} SSL, and d) with 27 mmol dm^{-3} SSL. All κ-carrageenan solutions were diluted to 20 µgmL^{-1} before cooling. Size 10 × 10 µm.

3. Conclusions

The addition of SSL to κ-carrageenan gels and solutions modified the conformation of the polysaccharide. The mixtures presented widespread but more compact conformations than the polysaccharide without surfactant. This alteration is due to hindrance of SSL molecules on κ-carrageenan chains and changes in polysaccharide-solvent interactions caused by electrostatic interactions between both components, which affect the thermal, rheological and conformational parameters. In general, a destabilization of κ-carrageenan conformation is suggested. This phenomenon explains the results obtained in this work.

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PARTE II. κ-carragenina y Tween 20

Metodología

Materiales

Se utilizó κ-carragenina grado alimenticio, Tween 20 líquido y cloruro de potasio. Todas las disoluciones se prepararon con agua desionizada.

Preparaciones de geles de κ-carragenina

La κ-carragenina se dispersó en agua desionizada mediante agitación constante durante 30 min a temperatura ambiente en cantidad suficiente para obtener una concentración final de 5 $mg\cdot g^{-1}$. Después de la dispersión completa del polisacárido se adicionó el Tween 20 para obtener concentraciones de entre 0 a 5 $mmol\cdot dm^{-3}$, y la mezcla se agitó durante 15 min más. Posteriormente, la mezcla se calentó hasta 92 °C y se mantuvo a esta temperatura por 10 min. Las disoluciones calientes se enfriaron a 50 °C y la pérdida de agua se compensó adicionando agua desionizada. En los sistemas con KCl, la sal se adicionó cuando la temperatura de la mezcla alcanzó 92 °C.

DSC

Se siguió la misma metodología que la descrita en la primera parte de este capítulo.

Reometría

Se siguió la misma metodología que la descrita en la primera parte de este capítulo.

Resultados

Comportamiento térmico (DSC)

El comportamiento térmico de los sistemas con Tween 20 y κ-carragenina en presencia de KCl se presenta en la Tabla 1. Las temperaturas de fusión y de gelificación se mantienen constantes después de adicionar el tensioactivo. Se aprecian cambios ligeros en las entalpías de transición de gelificación y fusión, sin embargo, no existen diferencias significativas entre los valores obtenidos.

El Tween 20 es un tensioactivo no iónico con grupos hidroxilo dentro de su estructura que puede ocasionar la presencia de interacciones con la κ-carragenina por medio de puentes de hidrógeno. Sin embargo, dichas interacciones son escasas, por lo tanto, los cambios en el comportamiento térmico son esencialmente despreciables.

Tabla 1. Propiedades térmicas de transición de κ-carragenina con 8.5 mmol·dm^{-3} KCl y diferentes concentraciones de Tween 20.

	FUSIÓN		GELIFICACIÓN	
Tween 20 (mmol·dm^{-3})	T (°C)	ΔH (J·g^{-1})	T (°C)	ΔH (J·g^{-1})
0	35.35 ± 0.15	33.99 ± 0.15	25.23 ± 0.08	33.59 ± 0.35
1	35.48 ± 0.53	35.15 ± 1.30	25.25 ± 0.06	34.39 ± 0.24
5	35.94 ± 0.33	35.04 ± 0.23	25.67 ± 0.19	33.22 ± 0.34

Comportamiento reológico

En el caso del comportamiento reológico de los sistemas con Tween 20, se aprecia un ligero aumento de los módulos dinámicos G’ y G” al adicionar el tensioactivo a la κ-carragenina (Figura 1). Sin embargo, el comportamiento viscoelástico general se mantiene sin cambios, es decir, los valores de tan δ no muestran diferencias significativas. En general, el Tween 20 no tiene un efecto significativo sobre el comportamiento de la κ-carragenina en presencia de KCl.

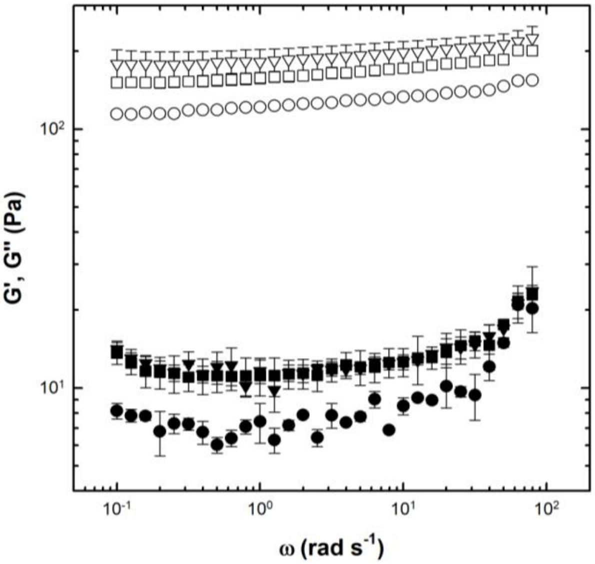

Figura. 1. Variación de G’ (símbolos vacíos) y G’’ (símbolos llenos) con la frecuencia angular (ω) de sistemas con 5 mg·g^{-1} de κ-carragenina sin Tween 20 (círculos), 1 mmol·dm^{-3} (triángulos) y 5 mmol·dm^{-3} (cuadrados) de Tween 20 a 25 °C.

Conclusiones

La adición de Tween 20 a los sistemas de κ-carragenina con KCl, no presenta efectos significativos en ninguna de las concentraciones analizadas.

En el Capítulo 4 se presentan los resultados obtenidos para los sistemas de emulsiones en gel de κ-carragenina. Se utilizaron tres diferentes tensioactivos en distintas concentraciones con la finalidad de conocer el efecto de estos factores en el comportamiento reológico, calorimétrico y en el tamaño de partícula de las emulsiones en gel.

Se encontró que cuando la concentración de los tensioactivos es mayor, el tamaño medio de las gotas de aceite es menor en comparación con concentraciones bajas de tensioactivo, lo que también genera una mayor homogeneidad de las gotas. Además, la naturaleza de los tensiactivos también ocasiona diferencias en los sistemas. La lecitina es el tensioactivo que modifica de manera más significativa la red de κ-carragenina, seguida del ELS y finalmente el Tween 20.

Como consencuencia de las diferentes concentraciones y tensioactivos utilizados en la preparación de las emulsiones en gel, estas tienen temperaturas de fusión cercanas a la corporal de un ser humano (entre 30-37 °C). Esta característica de los sistemas favorece que se utilicen como vehículos para la liberación de componentes solubles en aceite biológicamente activos.

CAPÍTULO 4

PAPEL DE DIFERENTES TENSIOACTIVOS SOBRE LAS RESPUESTAS TÉRMICA Y REOLÓGICA DE EMULSIONES EN GEL O/W DE κ-CARRAGENINA

Role of different surfactants on the thermal and rheological responses of O/W emulsion gels of κ-carrageenan

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Abstract

BACKGROUND: The purpose of this work was to determine the role of the surfactants, Tween 20, lecithin, and sodium stearoyl lactylate (SSL) and their concentration on the viscoelastic and thermal properties, and the oil droplet size distribution (ODSD) of emulsion gels produced with κ-carrageenan. Rheometry, differential scanning calorimetry, and fluorescence microscopy were used to determine the viscoelasticity, thermal parameters, size distribution, and morphology of emulsion gels, respectively.

RESULTS: Substantial changes were observed in the melting and gelation enthalpies of emulsion gels depending on the type and concentration of surfactant. The mean diameters of oil drops decreased on increasing surfactant concentration and were more homogeneously distributed in the gel matrix. Lecithin had a more significant effect on the κ-carrageenan network, followed by SSL and Tween 20.

CONCLUSIONS: The interference of oil and surfactant molecules and electrostatic repulsion interactions are proposed as the primary mechanisms responsible for modification of the κ-carrageenan network.

Keywords: κ-DSC, κ-carrageenan, emulsion, gel, surfactant, rheology.

INTRODUCTION

Emulsions are essential systems in the cosmetic, pharmaceutical, chemical, and food industries. They are mainly used to stabilize food products, but also to encapsulate, protect or release lipophilic compounds present in food [1]. The primary challenge in handling emulsions is their instability due to chemical, physical and thermodynamic incompatibility factors. The addition of surface-active molecules or thickeners is a customary practice to increase emulsion stability. However, despite the use of these additives, emulsions tend to phase separate to minimize their interfacial contact area and free energy. This instability is manifested by various mechanisms like flocculation, coalescence, cremation, sedimentation, and Ostwald ripening [2].

The instability of emulsions can be circumvented by embedding the oil drops in a gelled continuous phase. These systems are known as emulsion-filled gels or emulsion gels. Many food products can be considered emulsion-filled gels; for example, fresh cheese, yogurt, sausages, dairy desserts and starch puddings [3].

Most of the emulsion-filled gels are prepared using proteins as the emulsifier and gelling agent, e.g., whey protein isolate (WPI) or gelatin [3-6]. However, gellan [7], inulin [8], κ-carrageenan [3-4, 9] and alginate [6] have been used as well. The mechanical response, i.e., texture, of these systems via small and large deformation rheometry tests is their most studied characteristic.

The rheological behavior depends on the emulsion and the gelled continuous phase. The latter is responsible to a high degree for the viscoelastic characteristics of these materials at low deformations [10]. However, the presence of oil droplets in the emulsion can change the rheological properties of the emulsion gel in diverse ways because depending on their nature, the droplets are less, equally or more deformable than the gel matrix [4, 7].

On the other hand, the rheological behavior of emulsion gels in large deformation depends mainly on the gel matrix but is affected by the oil droplets in it, since they induce stress concentration after deformation [3].

Sodium stearoyl lactylate (SSL) is an anionic surfactant (CAS: 25383-99-7; E481) synthesized from fatty acids and lactic acid in the presence of sodium [11]. SSL is formed by a hydrophilic charged head coming from ionic lactic acid and a long hydrophobic hydrocarbon tail from stearic acid [12]. The presence of lactylate in the molecular structure of SSL allows it to interact with charged groups via electrostatic interactions and ionic bonds [13].

Polysorbate 20, more commonly known as Tween 20 (CAS: 9005-64-5; E432) is a non-ionic surfactant derived from sorbitol. It is one of the most hydrophilic emulsifiers allowed in food, and has high water solubility. It is commonly used in combination with other surfactants to achieve greater stabilization of emulsions because it is a strong steric stabilizer [14].

Soy lecithin (CAS: 8002-43-5; E322) is one of the most used surfactants in the food industry. Phosphatidylcholine is its primary component. Soy lecithin is a natural mixture of similar chemical compounds classified as phospholipids. It is soluble in organic solvents, but the solubility depends on polar head groups and fatty acid composition. It has low water solubility, and its primary uses are in baking, chocolate, coatings, soft and hard caramels and chewing gums [15].

κ-carrageenan (CAS: 11114-20-8; E407) is a sulfated D-galactan polysaccharide. Its primary structure is based on a repeating disaccharide consisting of alternate (1→3) β–D-galactose-4-sulfate and (1→4) α-3,6-anhydro-D-galactose. This polysaccharide is used in the food industry as a stabilizer and gelling agent. κ-carrageenan gels are stiff, brittle, exhibit syneresis and thermal hysteresis [16]. Gelation of κ-carrageenan is carried out in two stages: Coils ↔ helix formation

↔ aggregation [17]. The coil to double helix transition is the most crucial step for gelation to occur. Also, gelation is sensitive to salts and can be controlled by the presence of specific cations like K$^+$, Cs$^+$, and Rb$^+$, since they increase the double helix stability and mediate helix-helix aggregation [18].

In a previous report the interaction between polysaccharides and nonionic, and ionic surfactants has been reviewed [19]. Also the effect of SSL on the thermal transitions and other physicochemical properties of k-carrageenan solutions and gels has been studied [20].

The goal of this work was to extend these investigations to examine the influence of Tween 20, lecithin and SSL in several concentrations on the rheological and thermal properties, and the oil drop size distribution (ODSD), and morphology of the gelled O/W emulsions whose melting is close to human body temperature, i.e., 37 °C. The underlying premise is that the different chemical nature these surfactants and the oil phase will modify the sol-gel-sol transition of the polysaccharide differently under gelling conditions.

MATERIALS AND METHODS

Materials

Food-grade κ-carrageenan (Ingredients Solutions, USA) without further treatment. Reagent-grade potassium chloride (Merck, Mexico), food-grade powder sodium stearoyl lactylate (Palsgaard, Denmark), liquid Tween 20 (Hycel de Mexico S.A. de C.V., Mexico), and liquid soy lecithin (Archer Daniels Midland Co., USA). Technical-grade Nile red, 9-diethylamino-5-benzo[α]phenoxazine-5-one, (Sigma Chemicals, USA), corn oil (México) and deionized water. Table 1 shows the molar mass, ionic content and critical micelle concentration for Tween 20, lecithin. SSL, and κ-carrageenan, taken from a previous report [21] and other sources [12, 14, 15, 22].

Table 1. Molar mass, ionic content and critical micelle concentration (CMC) of κ-carrageenan, SSL, lecithin and Tween 20.

	κ-carrageenan	SSL	Tween 20	Lecithin
Na$^+$ (mg·kg^{-1})§	21 514 ± 1727	21 700 ± 460	1 000 ± 30	822 ± 21
K$^+$ (mg·kg^{-1})§	62 100 ± 1684	1 200 ± 170	1 100 ± 10	7 168 ± 329
Ca^{2+} (mg·kg^{-1})§	1 301 ± 61	100 ± 10	500 ± 10	781 ± 35
Mg^{2+} (mg·kg^{-1})§	< 10	< 10	100 ± 10	42 ± 1
Molar mass (g. mol^{-1})	308 366§	451.6$^{\#}$	1227.5$^{\#}$	643.9$^{\#}$
CMC (mmol·dm^{-3})^{a}	---	0.893§	0.098§	0.2 – 15$^{\&}$

§Taken from [21]; $^{\#}$Taken from [12, 14, 15]; $^{\&}$Taken from [22]

Preparation of κ-carrageenan emulsion gels

The necessary amount of κ-carrageenan for concentration of 5.0 mg·g^{-1} was dispersed in deionized water under stirring for 30 min at around 25 °C. The mixture was heated to 92 °C, and the amount of KCl necessary for a concentration of 11 mmol·dm^{-3} was added. The resulting solution was kept at 92 °C for 10 min. The hot solutions were let to cool to 50 °C. Deionized water was added to compensate for evaporative losses.

The corn oil was heated to 50 °C, and the required amount of surfactant was added to obtain the final concentration according to the composition and stirred until complete dissolution.

O/W emulsion gels were prepared with oil-solution mass proportions of 20:80, 35:65 and 50:50, respectively. The aqueous phase contained κ-carrageenan, KCl, and 1.0, 2.5, and 5.0 mg·g^{-1} of each surfactant. The emulsions were prepared by mixing and subsequent homogenization of the aqueous and oil phases using a high-speed blender (IKA Ultra-Turrax T25 digital). This operation was carried out at 50 ± 1 °C and increasing speeds of 8000, 10000 and 12000 rpm for 30 s each, for a total time of 1.5 min.

Differential scanning calorimetry (DSC)

DSC analysis was carried out in a microcalorimeter (μDSC 7 Evo, Setaram, France) under N_2 gas atmosphere. Emulsion gels were analyzed at a rate of 1.2 °C·min^{-1} from 2-80-2 °C in three heating-cooling cycles using 600 ± 10 mg of sample. The reference cell (Hastelloy C276) contained solutions with the same salt concentration than the samples. Emulsions were stored at 4 °C at least 18 h before measurements. Calorimetry data were analyzed with commercial software (OriginPro 9.1, OriginLab Corporation, USA). Enthalpies were expressed in J·g^{-1} of dry matter.

Rheometry

Rheological properties were determined using a rotational rheometer (ARES-RFS III, TA Instruments, USA). Viscoelastic properties of emulsion gels were determined under small-amplitude oscillatory shear from 0.1–100 rad·s^{-1} at 20 °C in serrated parallel plates, 25 mm in diameter, 3 mm gap. The zone of linear viscoelasticity was determined from strain sweeps at 6.28 rad·s^{-1}, emulsions were stored at 4 °C at least 18 h before the rheological measurements to allow complete setting of k-carrageenan. The dependence of G' and G'' with angular frequency, ω, is expressed by a power law relationship of the type $G' \alpha \omega^n$ and $G'' \alpha \omega^m$.

Oil drop size distribution (ODSD)

Fluorescence microscopy was used to determine the ODSD. Aliquots of 20 μL of each sample were deposited onto a microscope slide. Next, the sample was covered with a coverslip and observed in a microscope (Olympus BX45, Japan) with a reflected fluorescence system with excitation wavelengths between 510-550 nm. Corn oil in the emulsions was stained with 0.2 mg·mL^{-1} Nile red solution in ethanol [22]. The lipophilic stain was observed at excitation-emission wavelengths of 485 and 525 nm, respectively. The final stain concentration in the O/W emulsion was 0.013 mmol·dm^{-3}. The images were captured, and the diameter of the oil droplets determined with Image-Pro® Plus software (version 4.5.1.22). In all cases at least eight hundred drops were examined.

The average oil droplet size and its distribution was expressed by the Sauter mean diameter, d_{32}, and the De Brouckere mean diameter, d_{43}, given by equations (1) and (2)

$$d_{32} = \frac{\sum_{i=1} \mathrm{n}_i \mathrm{d}_i^3}{\sum_{i=1} \mathrm{n}_i \mathrm{d}_i^2} \qquad (1)$$

$$d_{43} = \frac{\sum_{i=1} \mathrm{n}_i \mathrm{d}_i^4}{\sum_{i=1} n_i d_i^3} = \sum_{i=1} \phi_i d_i \qquad (2)$$

In these equations, d_i is the diameter of the oil droplets in the *i*th size class, n_i is the number of droplets in each size class, and ϕ_i is the volume fraction of the disperse phase. d_{32} is an area-volume mean diameter related to the average surface area of droplets exposed in the continuous phase per unit volume of the emulsion. It can be interpreted as the diameter of a sphere that has the same volume/surface area ratio as the oil droplet. d_{43} is a volume-length mean diameter that expresses the average size of a polydisperse emulsion. This quantity is more sensitive to flocculation phenomena because its value is more susceptible to the presence of large particles in the emulsion than d_{32}.

The mean diameter can also be expressed as a function of the “moment of a distribution” (eq. 3).

$$S_n = \int_0^\infty x^n F(x) dx \approx \sum_{i=1} n_i x_i^n \qquad (3)$$

Here, S_n is the nth moment of the distribution and *x* is the average droplet size. In turn, the moment distribution was used to calculate the relative standard deviation (c_n) from equation (4).

$$c_n = \left(\frac{S_n S_{n+2}}{S_{n+1}^2} - 1\right)^{1/2} \qquad (4)$$

When n = 2, $c_n = c_2$, and it is possible to represent the width of the ODSD. In general, c_2 is between 0.1 and 1.3 for very narrow and wide distributions, respectively. For food emulsions it takes values between 0.5 and 1.

Considering n = 2 and $x_i = d_i$, equations (3) and (4) become, respectively

$$S_2 \approx \sum_{i=1} n_i d_i^2 = \frac{A_N}{\pi} \qquad (5)$$

$$c_2 = \left(\frac{S_2 S_4}{S_3^2} - 1\right)^{1/2} \qquad (6)$$

In equation (6)

$$S_3 \approx \sum_{i=1} n_i d_i^3 = \frac{6V_N}{\pi} = \frac{6\phi}{\pi} \qquad (7)$$

$$S_4 \approx \sum_{i=1} n_i d_i^4 \qquad (8)$$

In these equations A_N, and V_N are the total surface area and volume of the oil droplets per unit emulsion volume, and ϕ is the volume fraction of the disperse phase [1].

Statistical analysis

All measurements were made in triplicate. Droplet size distribution was determined with commercial software (Microsoft® Office Excel® 2007, 12.0.6750.5000). Differences between treatment means were obtained by Fisher's least significant difference (LSD) procedure [23], using equation (10)

$$LSD = t\sqrt{\frac{2MS_E}{n}} \qquad (10)$$

In this equation t is the Student's t-distribution percentile based on the error degrees of freedom at $\alpha = 0.05$ level of significance, n is the number of scores in each treatment sample, and MS_E is the mean squared error for the ANOVA.

RESULTS AND DISCUSSION

Thermal behavior from DSC

Effect of surfactant type and concentration

DSC thermal parameters for gels are presented in Table 2. Values on the first row correspond to surfactant-free and oil-free 5 $mg \cdot g^{-1}$ κ-carrageenan gels with 11 $mmol \cdot dm^{-3}$ KCl. The melting and gelation temperatures, *ca*. 38 and 27 °C, respectively, and enthalpies, 39 and 37 J/g, respectively are close to published values [24]. The transition temperatures diverge with $T_m > T_g$ because κ-carrageenan helices aggregate into a continuous network above a specific total ionic concentration ($C^* = 7\ mmol \cdot dm^{-3}$). . This thermal hysteresis of about 12 °C occurred for most of the emulsions examined. ΔH_m and ΔH_g are significantly different ($p < 0.05$), yet they are close enough to consider that the energy required for κ-carrageenan aggregates to form and disrupt is essentially the same.

The second row in Table 2 shows the values for surfactant-free emulsion gels with 20 % corn oil. The difference between T_m and T_g regarding the oil-free gels was not more significant than 2 %, with $T_m > T_g$. The difference between ΔH_m was of the same order of magnitude, i.e., *ca* 2 %. However, ΔH_g differed by about 10 %, with the oil-free gels having the grater one. This difference could be due to the decrease in the amount of k-carrageenan and KCl produced by partial replacement of the aqueous phase was always constant. This condition from now on referred to as the replacement effect, will be further treated in the next section. So, ΔH_g could be more sensitive to this substitution. Despite this behavior, the thermal behavior of oil-containing gels was mostly the same than the individual κ-carrageenan ones. It is worth noting, that emulsion creaming occurred faster than κ-carrageenan gelation and consequently the surfactant-free systems were not stable. No further consideration was given to non-gelled emulsions because this work was focused on the behavior of the gelled systems and not on the stability of the former.

Table 2. Melting (m) and gelling (g) properties of 5.0 mg·g^{-1} κ-carrageenan, 11 mmol·dm^{-3} KCl emulsion gels with different concentrations of surfactants (mg·g^{-1}) and 20:80 oil-aqueous phase ratio, respectively.

		Melting		Gelation	
Surfactant	Concentration	T_m (°C)	ΔH_m (J·g^{-1})	T_g (°C)	$\|\Delta H_g\|$ (J·g^{-1})
No*	0	38.47 ± 0.19	39.25 ± 0.23	26.52 ± 0.01	37.35 ± 0.17
No**	0	37.95 ± 0.21	38.45 ± 1.37	25.99 ± 0.01	33.36 ± 0.18
	1.0	38.85^{a} ± 0.10	32.37^{a} ± 0.30	26.58^{a} ± 0.12	33.16^{a} ± 0.23
Tween 20	2.5	38.62^{a} ± 0.22	24.58^{b} ± 1.19	26.56^{a} ± 0.07	24.51^{bd} ± 1.31
	5.0	38.81^{a} ± 0.19	15.74^{c} ± 0.97	26.44^{a} ± 0.03	17.71^{c} ± 0.05
	1.0	38.20^{a} ± 0.57	28.18^{a} ± 3.24	26.01^{b} ± 0.08	28.84d^{f} ± 2.17
Lecithin	2.5	38.52^{a} ± 0.13	23.33^{b} ± 0.82	26.51^{ac} ± 0.04	26.63^{bd} ± 1.79
	5.0	38.44^{a} ± 0.30	15.36^{c} ± 0.82	26.76^{c} ± 0.10	18.35^{e} ± 0.21
	1.0	37.26^{b} ± 0.11	30.64^{a} ± 1.70	26.01^{b} ± 0.08	29.18^{f} ± 1.73
SSL	2.5	36.59^{c} ± 0.16	17.61^{d} ± 1.25	25.42^{d} ± 0.32	32.08^{g} ± 0.57
	5.0	30.87^{d} ± 0.26	27.82^{e} ± 0.78	25.22^{d} ± 0.08	35.58^{h} ± 0.18

*surfactant, and oil-free; **surfactant-free with 20 % oil.

Averages with different letters in the same column indicate statistically significant differences ($p<0.05$).

Basically, in emulsion gels with Tween 20 or lecithin, increasing surfactant concentration decreased ΔH_m and ΔH_g (Figure 1). The corresponding temperatures were not significantly modified ($p > 0.05$) regardless of the concentration and type of added surfactant; $T_m \approx 38$ °C, and $T_g \approx 26$ °C. Thermal hysteresis with $T_m > T_g$ of about 12 °C was also observed. On the other hand, the behavior of emulsion gels with SSL was different. T_m and T_g decreased with the increase in surfactant concentration (Table 2), with T_m being more sensitive, implying that the mobility of κ-carrageenan molecules occurred at lower temperatures regarding Tween 20 and lecithin. Also, ΔH_m and ΔH_g exhibited a different behavior. ΔH_g increased around 22 % from 29.18 ± 1.73 to 35.58 ± 0.18 J/g for the 20:80 proportion when SSL increased from 1.0 to 5.0 mg·g^{-1} (Table 2). Increases of 2.6 and 3.8 times were observed for 35:65 and 50:50 proportions when SSL concentration increased from 2.5 to 5.0 mg·g^{-1} as illustrated in Figure 1. On the other hand, ΔH_m initially decreased and then increased upon increasing SSL concentration for the different proportions (Table 2 and Figure 1).

ΔH_m, and ΔH_g were lower than those for the individual κ-carrageenan gels. This behavior indicates the existence of fewer or weaker interactions between polysaccharide chains because a combined effect of surfactant presence and the replacement effect mentioned previously. It can be postulated that surfactants interfere with the formation of the κ-carrageenan network, while the addition of oil leaves the systems with less mass of polysaccharide and potassium counterions. It is worth noting that the amount of intrinsic counterions (Table 1) is not enough for κ-carrageenan to gel. For the lowest surfactant concentration, 1.0 mg·g^{-1}, the addition of Tween

20 resulted in a smaller decrease in ΔH_m and ΔH_g than SSL or lecithin regarding oil-containing, surfactant-free gels (Table 2). This behavior is probably due to the interaction between hydroxyl groups of Tween 20 and κ-carrageenan via hydrogen bonds. Electrostatic interactions are ruled out because this surfactant is nonionic. The addition of SSL caused a more significant decrease in enthalpies than Tween 20, but lower than lecithin, always regarding oil-containing, surfactant-free emulsion gels (Table 2). SSL interferes κ-carrageenan conformation depending on its concentration [20]. Finally, lecithin resulted in the most significant decrease in transition enthalpies (Figure 1). This effect might be the result of destabilization mechanisms like those observed with SSL. The pKa of the anionic phosphate groups on lecithin is 1.5 [25-27]. Therefore, above this value, the molecule is negatively charged and can be considered an anionic surfactant like SSL. Also, lecithin has a higher molar mass, 643.9 $g \cdot mol^{-1}$, than SSL; 451.6 $g \cdot mol^{-1}$. Consequently, these two factors point to a stronger effect on κ-carrageenan conformation than that caused by Tween 20 and SSL.

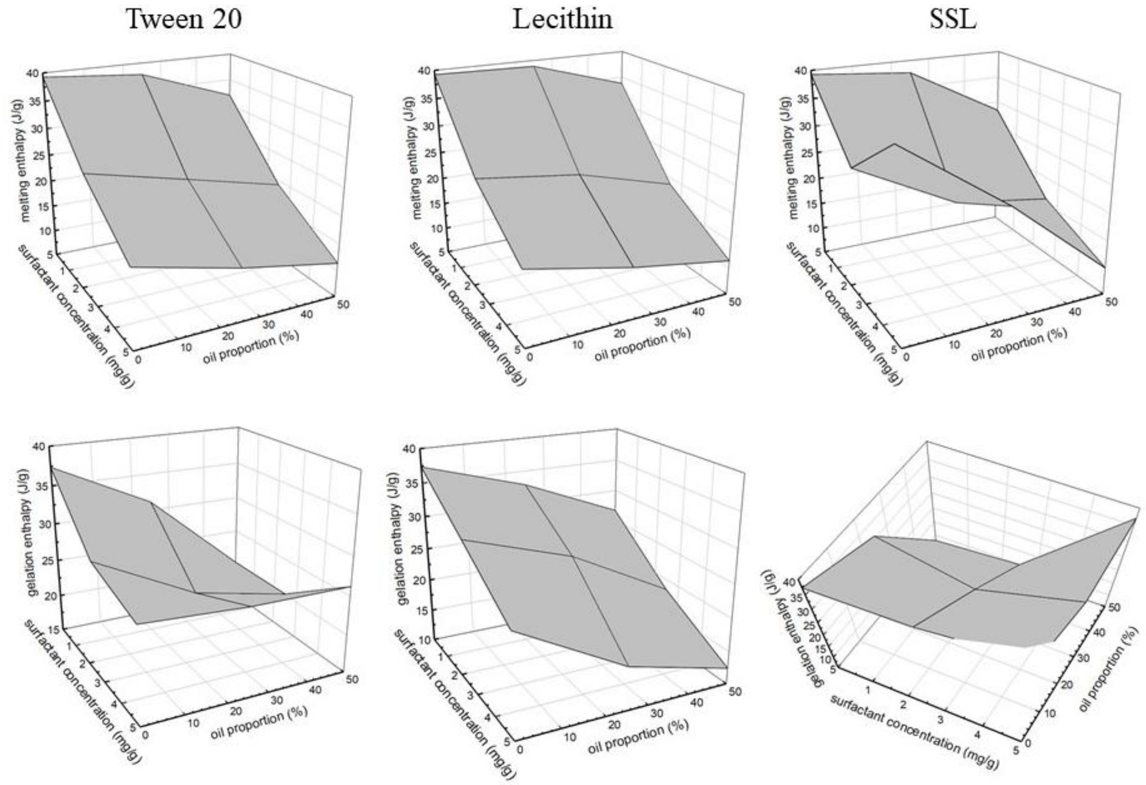

Figure. 1. Variation of ΔHm (top) and ΔHg (bottom) of emulsion gels with Tween 20 (left), lecithin (middle) and SSL (right) concentration and oil proportion.

Effect of oil-water ratio

The values of T_m and T_g shown in Table 2 for Tween 20 and lecithin remained practically constant regardless of oil and surfactant concentrations. However, ΔH_m and ΔH_g were sensitive to changes in oil and surfactant concentrations (Figure 1). The replacement effect on both enthalpies was weaker than the addition of surfactant. For example, considering ΔH_m for Tween 20 and 20 % oil (Table 2, Figure 1) a drop of 24.1 % from 1.0 to 2.5 $mg \cdot g^{-1}$ and 36.0 % from 2.5 to 5.0 $mg \cdot g^{-1}$ occurred. The overall drop from 1.0 to 5.0 $mg \cdot g^{-1}$ was 51.4 %, i.e., more than half the enthalpy for 1.0 $mg \cdot g^{-1}$.

On the other hand, for a constant surfactant concentration and oil proportions from 20 to 50 %, ΔH_m dropped 22.0 and 27.8 % for 2.5 and 5.0 $mg \cdot g^{-1}$ Tween 20, respectively (Figure 1). Therefore, ΔH_m was more sensitive to changes in surfactant concentration than in oil proportion. Similar behavior was observed for ΔH_m of lecithin (Figure 1). The replacement effect explains why less energy is required to melt the gels.

On the other hand, ΔH_g of emulsion gels with 5.0 $mg \cdot g^{-1}$ Tween 20 increased from 12.56 ± 0.12 to 15.38 ± 0.27 $J \cdot g^{-1}$ when the oil proportions changed from 35 to 50 % (Figure 1). This behavior suggests the formation of a higher number of interactions between polysaccharide chains. The increase in oil proportion led to a larger oil-water interface, with more surfactant needed to cover it. Consequently, the possible polysaccharide-surfactant interactions decrease and interchain interactions between κ-carrageenan molecules are favored. This behavior was not observed in ΔH_m, suggesting the presence of irreversible new links.

As it happened with Tween 20, when lecithin was added to emulsion gels, T_m and T_g remained constant with values practically the same as those shown in Table 2, while ΔH_m and ΔH_g decreased as the oil content increased (Figure 1). Here again, this behavior is mainly attributed to the replacement effect.

Emulsion gels with SSL are also shown in Figure 1. The behavior of this surfactant was different from the other two. T_m and T_g for 2.5 $mg \cdot g^{-1}$ surfactant, remained constant (Table 2) regardless of the oil content. However, when the surfactant concentration increased to 5.0 $mg \cdot g^{-1}$, T_m increased about 5 °C when oil content increased, while T_g decreased by 3 °C. ΔH_m and ΔH_g for emulsion gels with 2.5 $mg \cdot g^{-1}$ surfactant decreased due to the change in oil-aqueous phase proportion, as observed with Tween 20 and lecithin.

On the other hand, emulsion gels with 5.0 $mg \cdot g^{-1}$ surfactant, ΔH_m also decreased, but ΔH_g initially decreased and then increased. This behavior is probably due to the presence of new interactions which suggests some lower degree of interference in the conformation of κ-carrageenan by the surfactant because a higher amount of the latter is required at the interface. As in the case of emulsion gels with Tween 20, $\Delta H_m < \Delta H_g$ which indicate that some of the physical links are not reversible and therefore their rupture was not appreciated.

Finally, the T_m of emulsion gels were around 38-39 ºC, for Tween 20 and lecithin, and 28-36 ºC for SSL. These values are close to the human body temperature (37 °C), and therefore most of the gels are expected to melt in the mouth. Also, emulsion gels have two significant advantages. First, fatty raw materials could be protected against oxidation or loss by volatilization because they are encapsulated in a three-dimensional network that also prevents phase separation. Second, flavor and aroma release can be controlled by temperature, directly modifying KCl and polysaccharide concentrations.

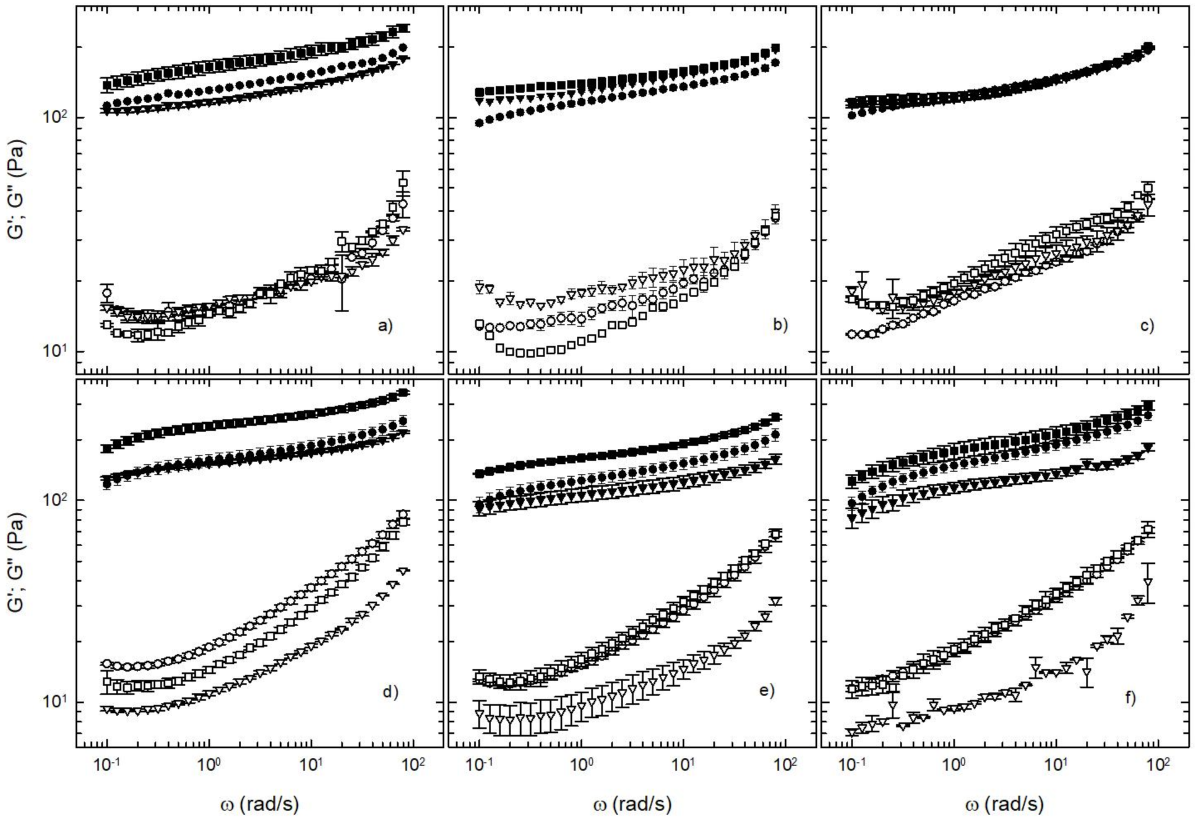

Figure 2. Variation of G’ (filled symbols) and G” (void symbols) with angular frequency at 20 °C of emulsion gels with 20:80 (top), 50:50 (bottom) oil proportions, and SSL (circles), lecithin (triangles down), and Tween 20 (squares). 1.0 (a, d), 2.5 (b, e), and 5.0 mg·g^{-1} (c, f) surfactant.

Rheological behavior

The rheological behavior of emulsion gels with 20 and 50 % oil and different surfactant concentrations is shown in Figure 2. Moduli depended on the frequency with G’ > G” for both proportions. Emulsion gels had low-rigidity because of the low concentration of κ-carrageenan.

In emulsion gels with 20 % oil, G’ was in the range of 100 to 200 Pa, while G” extended from 10 to 50 Pa (Fig. 2 top). In general, the increase in surfactant concentration resulted in practically the same G’ and closer G” regardless of the surfactant type. The variation of tan δ with frequency (data not shown) indicates that the elastic contribution to viscoelasticity decreased 44 % at a higher surfactant concentration, e.g., for $\omega = 1$ rad·s^{-1}, tan $\delta = 0.088$ ($\delta = 5^{o}$), and 0.161 ($\delta = 9^{o}$), for 1.0 and 5.0 mg·g^{-1} surfactant, respectively. Furthermore, when SSL or lecithin concentration increased, the frequency dependence of G’ and G” was accentuated; $n = 0.071$-0.085 ($r > 0.9611$) and $m = 0.104$-0.183 ($r > 0.8819$), confirming the decrease in the elastic character. In general, the viscoelastic behavior was not significantly affected ($p > 0.05$) by the chemical nature or concentration of the surfactants.

In emulsion gels with 50 % oil, G’ and G” also depended on frequency (Figure 2, bottom). In this case $n = 0.066$-0.128 ($r > 0.9831$) and $m = 0.184$-0.280 ($r > 0.9336$), respectively. This difference regarding 20 % oil is again attributed to the replacement effect. Therefore, the contribution of the elastic character to the viscoelasticity of these emulsion gels was weaker compared with those with less oil. Also, G’ and G” were higher for 50 % oil. This behavior might be explained by the fact that the oil viscosity is higher than that of water.

In general, Tween 20 and SSL emulsion gels had higher G’ than those with lecithin. This result agrees with DSC data. Lecithin had a more significant effect on ΔH_m and ΔH_g of emulsion gels suggesting a possible relationship with the number of interactions formed or disrupted. The reason for this effect is that lecithin bears negative charges and a higher molar mass than SSL. These characteristics affect the conformation of κ-carrageenan.

Oil droplet size distributions

Figure 3 shows the ODSD of 20:80 proportion emulsion gels and different surfactant concentrations. All the distributions are broad, with smaller oil drop sizes for higher surfactant concentrations. The peak values of the distributions for emulsion gels with 1.0 $mg\cdot g^{-1}$ surfactants were 96.6 μm for SSL, 58.3 and 95.1 μm for lecithin, and 42.5 and 152 μm for Tween 20. On the other hand, for emulsion gels with 5.0 $mg\cdot g^{-1}$, the corresponding values are 11.2, 21.6 and 12.8 μm for SSL, lecithin and Tween 20, respectively.

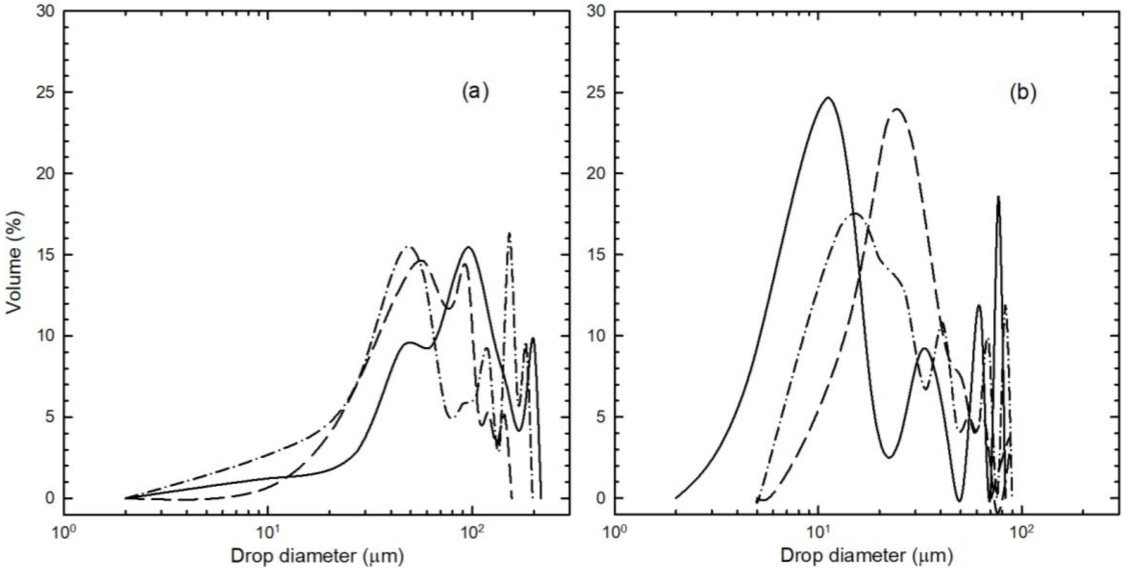

Figure. 3. ODSD of emulsion gels (O/W; 20:80) with κ–carrageenan, KCl and SSL (continuous line), lecithin (dashed line) or Tween 20 (dashed-dotted line). For a) 1.0; and b) 5.0 $mg\cdot g^{-1}$ surfactant concentration.

The behavior of each ODSD is shown in Table 3 in which the corresponding mean diameters are also included. The decrease of d_{32} and d_{43} with the increase in surfactant concentration was observed for the three surfactants. This change can be attributed to greater surfactant adsorption at the interface, which enhances the interfacial stability [28]. Table 3 also presents the relative standard deviation, which is within the typical values reported for food emulsions [1].

The behavior of the surfactants was different according to their concentration. For emulsion gels with 1.0 mg·g^{-1} surfactant, lecithin produced a smaller oil droplet size and a narrower ODSD than SSL and Tween 20, whereas SSL produced the greatest oil droplet size.

In the case of 5.0 mg·g^{-1} surfactant, lecithin presented a narrower ODSD than SSL and Tween 20, as it happened for the low concentration. However, SSL showed the smallest oil droplet size. Likewise, SSL was the surfactant with the most significant size reduction; 71.2 %, followed by Tween 20 and lecithin with 61.1, and 53.7 %, respectively.

Table 3. Mean droplet diameters and relative standard deviation (c_2) for emulsion gels (O/W; 20:80) with 5.0 mg·g^{-1} κ-carrageenan, 11 mmol·dm^{-3} KCl, and different concentrations of surfactants.

	Surfactant (1.0 mg/g)			Surfactant (5.0 mg/g)		
Mean droplet diameter (μm)	SSL	Lecithin	Tween 20	SSL	Lecithin	Tween 20
d_{32}	81.8	59.3	65.2	18.9	27.4	25.4
d_{43}	111	74.8	100	38.2	34.7	39.0
$c_2^{\&}$	0.595	0.512	0.822	1.011	0.516	0.731

&dimensionless

In general, lecithin stabilized the emulsions better than SSL or Tween 20, when the surfactant concentration was 1.0 mg·g^{-1}, but when the concentration increased to 5.0 mg·g^{-1}, SSL adsorbed better at the interface. These results are consistent with the physicochemical characteristics of the surfactants. Tween 20 is nonionic and very soluble in water, but it has the highest molar mass of the three surfactants used here. Lecithin is zwitterionic, but above pH 1.5 it is negatively charged and has low water solubility. SSL is anionic and soluble in hot water. These differences make their adsorption at the interface different and therefore their ability to form emulsions too.

Microstructure

Figure 4 shows the morphology of emulsion gels. In general, the same behavior described in the preceding section was observed, i.e., the oil droplet size decreased when the surfactant concentration increased. In these images, it is also possible to notice that the droplet size was more homogeneous when the surfactant concentration was 5.0 mg·g^{-1}. When the surfactant concentration was 1.0 mg·g^{-1}, surfaces covered with a higher amount of oil, red zones, were observed, suggesting that under these conditions phase separation might occur more easily during κ-carrageenan gelation. However, when the concentration increased, the distribution of the oil throughout the emulsions was more homogeneous.

The spheroidal shape and larger size of some oil drops instead of the expected smaller spherical one could be attributed to early flocculation and coalescence. This phenomenon was observed in all the systems, was independent of surfactant concentration, and occurred during κ-carrageenan gelation, basically because mechanical agitation was stopped to avoid gel destruction. However, beyond the time necessary for gelation to occur, phase separation never happened in the emulsion gels even after two months of refrigeration storage (*ca*. 4 °C) in closed glass containers.

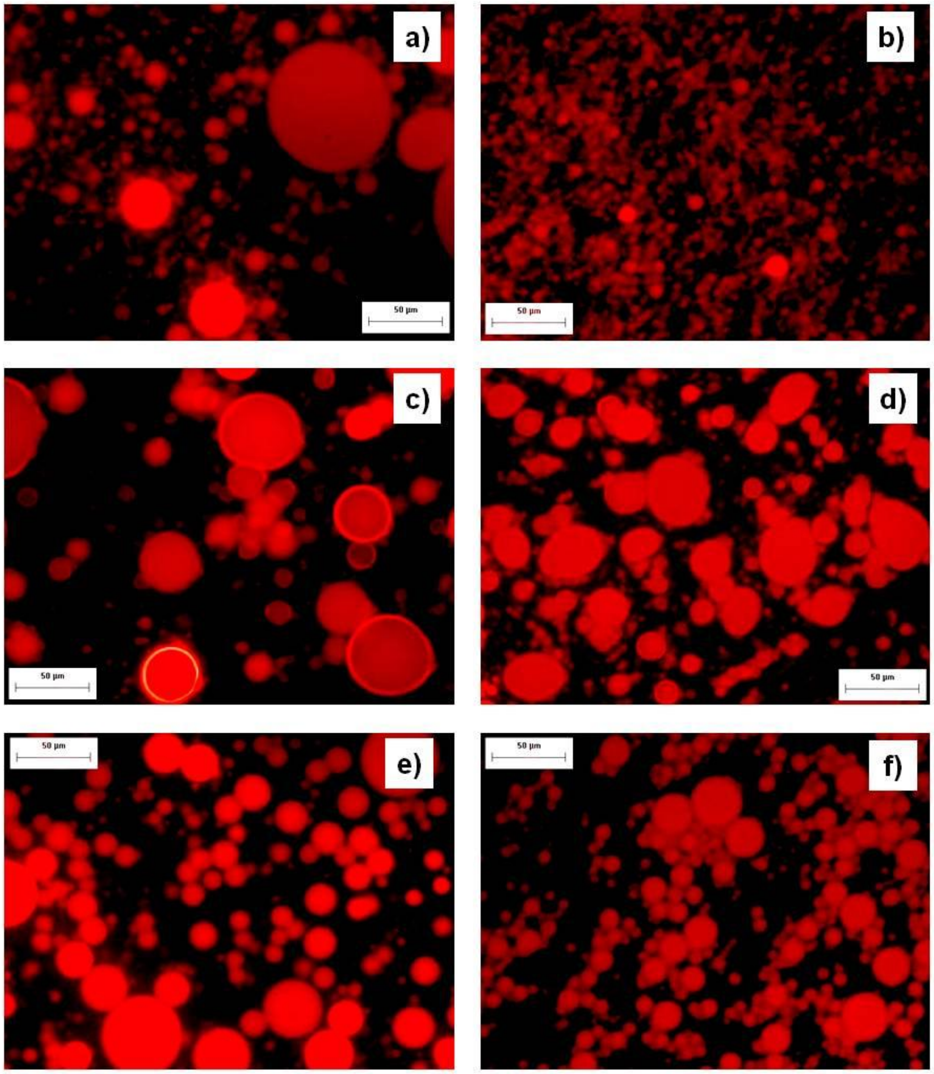

Figure. 4. Fluorescence microscopy images of emulsion gels (O/W; 20:80) with κ-carrageenan, KCl, SSL a) 1.0 and b) 5.0 mg·g^{-1}; lecithin c) 1.0 and d) 5.0 mg·g^{-1} or Tween 20 e) 1.0 and f) 5.0 mg·g^{-1}. Oil droplets stained with Nile red to a final concentration of 0.013 mmol·dm^{-3}.

Emulsions with lecithin (Figures 4c and d) showed halos with higher fluorescence on the periphery of the drops. This effect is due to the phospholipids in lecithin as fluorescence of Nile red increases in the presence of this surfactant [29].

The black zones observed in the images correspond to the solvent and κ-carrageenan. So, the interaction between κ-carrageenan chains is more favorable when the surfactant concentration is lower since there are zones with a high κ-carrageenan concentration and others with oil. These results agree with DSC data presented here. On the other hand, for a surfactant concentration of

5.0 mg·g^{-1}, the presence of more molecules interferes the formation of the κ-carrageenan network and the excess surfactant can interact with κ-carrageenan to produce the same effect.

Conclusions

The chemical nature and concentration of the surfactants examined here are key factors with a direct effect on the ODSD and drop diameters. These changes alter the κ-carrageenan network, which is evident by the remarkable changes in the thermal properties and less significant modifications in the rheological properties of the polysaccharide. Also, T_m of the emulsion gels could make them attractive as carriers to release biologically active oil-soluble components at the human body temperature.

Acknowledgments

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CONCLUSIONES FINALES

La presencia de tensoactivos con diferente naturaleza química en sistemas elaborados con κ-carragenina modifica la estructura de este polisácarido. El mecanismo principal propuesto es la interferencia de las moléculas de los tensioactivos entre las cadenas de κ-carragenina. Sin embargo, también se observaron cambios en las interacciones polímero-disolvente, como consecuencia de interacciones electrostáticas de repulsión entre el polímero y los tensioactivos con carga (ESL y lecitina de soya).

El incremento en la concentración de ELS en los sistemas acuosos de κ-carragenina, disminuye las temperaturas de transición tanto de fusión como de gelificiación, en ausencia y presencia de iones potasio. Siendo mayor el efecto en los sistemas sin KCl adicionado. Este mismo comportamiento se observa en las propiedades reológicas.

Por su parte, la adición de Tween 20 no presenta un efecto significativo sobre los sistemas de κ-carragenina.

La naturaleza y concentración de los tensioactivos adicionados en emulsiones en gel de κ-carragenina modifican el comportamiento térmico y reológico de estos sistemas, teniendo un mayor efecto sobre las propiedades térmicas.

Lo anterior como consecuencia de la modificación de la estructura de las cadenas de κ-carragenina, debido a la presencia de los tensioactivos y las gotas de aceite, los cuales interfieren en las moléculas del polisacárido