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Hydrocolloid-based nutraceutical delivery systems

Srinivas Janaswamy^{a,*} and Susanne R. Youngren^b

^aWhistler Center for Carbohydrate Research, Department of Food Science, 745 Agriculture Mall Drive, Purdue University, West Lafayette, IN 47907-2009, USA

^bCollege of Pharmacy, 575 Stadium Mall Drive, Purdue University, West Lafayette, IN 47907-2091, USA

Abstract

Nutraceuticals are important due to their inherent health benefits. However, utilization and consumption are limited by their poor water solubility and instability at normal processing and storage conditions. Herein, we propose an elegant and novel approach for the delivery of nutraceuticals in their active form using hydrocolloid matrices that are inexpensive and non-toxic with GRAS status. Iota-carrageen and curcumin have been chosen as models of hydrocolloid and nutraceutical compound, respectively. The iota-carrageenan network maintains a stable organization after encapsulating curcumin molecules, protects them from melting and then releases in a sustained manner. These findings lay a strong foundation for developing value-added functional foods and medicinal foods.

Introduction

Modern lifestyles and dietary habits in association with sparsely consumed nutraceuticals are some of the contributing factors for the proliferation of chronic diseases such as diabetes, obesity, cardio-vascular disease and cancer.¹ Functional foods and food supplements enriched with nutraceutical compounds (NCs) are effective in preventing these diseases and gaining health benefits.^{2, 3} The intrinsic low aqueous solubility of NCs coupled with their instability at conditions encountered during storage and processing – temperature, oxygen and light – as well as in the gastro-intestinal (GI) tract – pH, enzymes and other nutrients – significantly limits their activity and envisioned benefits.^{4, 5} Importantly, efficacy of NC enriched products greatly relies on preserving the bioavailability of active NC, and towards this end carriers play vital role in protecting and delivering NCs. There are two important characteristics that an efficient carrier should possess: (1) preserve the active structural form of NC until the time of delivery, and (2) effectively deliver the preserved form to the physiological target. Biomedical and pharmaceutical sectors utilize synthetic polymers as delivery vehicles, especially for drug molecules, by entrapment within a polymer network.^{6–9} Despite success, these polymers cannot be used in food applications as they are not Generally Recognized As Safe (GRAS) for regular consumption by healthy individuals. On the other hand, protein hydrogels¹⁰ are found to be viable and a wide range of multi-component matrices utilizing food proteins have surfaced. Though they seem passable, dearth of knowledge about protein-protein and protein-nutraceutical interactions at the molecular-level and their impact on functional properties impedes the design and development of ideal NC carriers. Further, in order to retain the active structural form of the embedded molecules at the target site, carriers with stable architecture are generally more advantageous than those with non-rigid structures. Exploitation of other biopolymer systems

*Corresponding author, Tel: (765) 494-4914, Fax: (765) 494-7953, janaswam@purdue.edu.

that are abundant, low-in-cost, compatible with human digestibility and able to maintain ordered molecular structures would be the best scientific and industrial approach to circumvent this quandary. Here we show that hydrocolloids stand out as worthy alternatives for encapsulating and protecting nutraceuticals.

Hydrocolloids are ubiquitous biopolymers utilized extensively in food, non-food, pharmaceutical and medical applications as thickeners, viscosifiers and gelling agents.^{11, 12} They possess different chemical groups and exhibit a gamut of secondary and tertiary structures. We hypothesized that NCs could be effectively embedded and protected in the crystalline network of hydrocolloids. Our hypothesis is strongly supported by the following three observations: (1) though hydrocolloids are mostly amorphous in nature, structural ordering can be achieved by preparing crystalline and well oriented fibers, (2) in the ordered state, hydrocolloids adopt stable helical structures with well-orchestrated networks stabilized by inter-helical hydrogen bonds mediated via water molecules and cations,¹³ and (3) in the hydrocolloid network, especially in anionic hydrocolloids, there could be 8–15 Å wide voids often filled with water molecules.¹⁴ These water pockets are amenable for embedding and protecting NCs (Fig. 1). Toward this goal, we have chosen curcumin and iota-carrageenan as model NC and hydrocolloid, respectively. Curcumin, a plant phenol, makes up to 5% of the dietary spice turmeric and has anti-cancer, anti-inflammatory and anti-obese properties.¹⁵ Curcumin's insolubility at neutral, acidic and alkaline conditions, as well as its photosensitive nature curtails general utility and associated functionality. Iota-carrageenan belongs to a family of 15 sulfated polysaccharides extracted from marine algae, and long been used in food and pharmaceutical applications.¹⁶ The chemical structure of iota-carrageenan is a disaccharide repeat of $\rightarrow 3)\text{-}\beta\text{-D-Gal}p\text{-4-SO}_3^-(1\rightarrow 4)\text{-3,6-anhydro-}\alpha\text{-D-Gal}p\text{-2-SO}_3^-(1\rightarrow$ where Gal p is galactopyranose. The three-dimensional structures of sodium¹⁷ and calcium¹⁸ salt forms reveal that this anionic biopolymer has a rigid double helical core with flexible peripheral sulfate groups. Its assembly is stabilized by well-organized cation-mediated inter-helical interactions and has the ability to expand and/or contract depending on cation type used to balance the charge on the backbone.¹⁴

Materials and Methods

Preparation of iota-carrageenan fibers

The sodium salt of iota-carrageenan solution was prepared by dissolving 10mg of hydrocolloid in 1 ml of deionized distilled water. The solution was thoroughly heated at 90°C for 30 minutes with intermediate vortexing, and later cooled to room temperature. A droplet of 20μL was placed in between two glass rods in a fiber puller. Relative humidity (RH) inside the fiber puller was maintained at 66%. Once the droplet was partially dried (after around 2 – 3 hrs) it was stretched to about 2 – 3 mm in length in regular intervals. Fiber was allowed to dry further for 12 hours, removed from the fiber puller and stored in a desiccator at 66% RH for further analysis.

Preparation of iota-carrageenan:curcumin complexes

1 mg of curcumin was dissolved in 0.9 mL of isopropanol, and later 0.1 mL of distilled water was added. Excess water was to loosen the fiber network during complexing. Stretched iota-carrageenan fibers were immersed in the isopropanol:curcumin:water mixture for 3 days at temperatures 4, 25, 35, and 45°C. The resulting fibers were then stored in a desiccator at 66% RH for further analysis.

Diffraction patterns and Unit cell dimensions

Synchrotron X-ray diffraction data were obtained at 14-BMC beamline, BioCARS, Argonne National Laboratory (ANL), Chicago, IL. The wavelength of the X-ray beam was set to

0.979 Å and the exposure lasted for 5 seconds. Calcite powder (3.035 Å characteristic spacing) was used for internal calibration. FibreFix²⁸ version 1.3.1 from CCP13 was used to estimate the pattern center, detector to fiber distance, tilt, rotation and unit cell dimensions as well as Miller indices (*h*, *k*, *l*) of each reflection.

Melting behavior of iota-carrageenan:curcumin complexes

Modulated Differential Scanning Calorimetry (mDSC) was performed on pure curcumin power, iota-carrageenan fibers and iota-carrageenan:curcumin complex prepared at 4 °C using the TA DSC Q 2000. The equipment was calibrated with an Indium disk. Around 1.0±0.1 mg were analyzed under a nitrogen gas flow of 50 ml/min. A modulation of ±0.68°C for every minute was used and the temperature was ramped from 0–230 °C at a constant rate of 3°C per minute.

Curcumin release from iota-carrageenan:curcumin complexes

Concentration testing was performed using a Beckman Coulter DU 730 Life Science UV/Vis spectrophotometer with disposable UV cuvettes. Initially, wavelength scan was performed in the range 200 to 600 nm for determining the optimum wavelength of absorbance (430 nm) for curcumin. Subsequently, calibration curve was generated by dissolving known amounts of curcumin in isopropyl alcohol. Distilled deionized water was used as the solvent for estimating the curcumin content in the complex. Around 0.8 mg of curcumin:iota-carrageenan fibers were placed at the bottom of the cuvette and 600 µL of distilled deionized water was added. The spectrometer was zeroed before the measurements with cuvette containing only water. The kinetic/time program was used to take continuous measurements in two minute increments for a total of 3 hours. Curcumin amounts in the complexes were estimated using the calibration curve with the known absorbances.

Results and Discussion

Structural characterization

Our strategy is to encapsulate curcumin in the well-oriented and crystalline fibers of sodium iota-carrageenan. Isopropyl alcohol was used as solvent as curcumin is water insoluble. The complexation experiments were performed at four selected temperatures: 4, 25, 35 and 45 °C so as to understand the effect of temperature on the loaded amounts. Fiber diffraction is the only available tool to structurally characterize the helix forming hydrocolloids in the solid-state; hence, we analyzed iota-carrageenan and iota-carrageenan:curcumin (IC) complexes using X-ray fiber diffraction principles. In the case of iota-carrageenan, the meridional reflection on the third layer line (Fig. 2a) suggests 3-fold helix symmetry for the molecular structure. All the observed Bragg reflections index on a trigonal unit cell with dimensions $a = 24.1(1)$, $c = 13.1(1)$ Å, and agree well with the reported values.¹⁷ Also, the occurrence of meridional reflection on the third layer line (Fig. 2b–e) confirms that the 3-fold molecular structure of iota-carrageenan is intact in all the IC complexes. This observation is expected as curcumin molecules enter the water pockets in the carrageenan lattice. Nevertheless, there are significant variations in the intensity distributions and positions of reflections on individual layer lines, indicating alterations in the packing arrangements. The Bragg reflections from the 4 and 25 °C complexes yield trigonal unit cells with dimensions; $a = 24.8(1)$, $c = 13.0(1)$ Å, and $a = 24.5(1)$, $c = 13.2(1)$ Å, respectively, and those from 35 and 45 °C prefer orthorhombic nets $a = 21.2(1)$, $b = 26.8(1)$, $c = 13.1(1)$ Å, and $a = 21.1(1)$, $b = 27.2(1)$, $c = 13.1(1)$ Å, respectively. The changes in the basal net dimensions attest to complex formation.

We have not performed any experiments about the effect of temperature on the unit cell constants of sodium iota-carrageenan. However, our preliminary analysis on guanidinium

iota-carrageenan (unpublished results) shows that unit cell values are unperturbed in the 5 to 70 °C range. We anticipate this trend to hold good for other cations including sodium, calcium, iron and so on. Hence, we believe that the changes observed in the unit cell dimensions of IC are originating from the temperature induced variations in the interaction sequences of curcumin and iota-carrageenan during encapsulation process.

Thermal protection by the hydrocolloid network

Thermograms from the 4 °C complex clearly indicate that the curcumin molecules encapsulated in the iota-carrageenan lattice are heat stable. Curcumin melts around 175 °C (Fig. 3a) while iota-carrageenan fibers show crystallization at 125 °C followed by melting at 140 and 160 °C (Fig. 3b). Generally, hydrocolloid fibers contain both amorphous and crystalline domains. Under favorable conditions, as in gels, ordered segments known as junction-zones, joined by random coils of hydrocolloid chains are formed. The art of fiber preparation preferentially relies in bringing these junction zones together to be aligned along the fiber axis to the highest possible extent; hence, hydrocolloid fibers are semi-crystalline. Consequently, the resolution of their x-ray diffraction reflections seldom reaches beyond 2.5 Å unlike inorganic or small organic compounds wherein atomic level resolution is quite common. In the iota-carrageenan fibers, the exothermic transition observed at 125 °C (Fig. 3b) during DSC heating is due crystallization of amorphous regions, and the two endothermic events at 140 and 160 °C correspond to melting of junction zones. On the other hand, the thermal behavior of IC is different; only crystallization peaks exist around 145 and 170 °C, but no melting peaks anywhere (Fig. 3c). The diffuse and fewer reflections in the x-ray diffraction pattern (Fig. 2b) imply that the network is less crystalline in the 4 °C complex than in iota-carrageenan (Fig 2a). The exothermic peak at 145 °C in the complex, about 20 °C above that of iota-carrageenan, suggests that the curcumin molecules present in the fiber are responsible for the crystallization of amorphous phase at higher temperatures. Further, at 4 °C complexation temperature, curcumin appears to destabilize the junction zones and structural ordering (Fig. 2b); the second exothermic peak at 170 °C seems to arise from re-crystallization of these disentangled junction zones. The absence of endothermic transitions in the complex indicates that due to weak inter-helical interactions in the junction zones, the corresponding melting enthalpies are too small to measure. The possibility of melting beyond 230 °C is unthinkable as fibers decompose at this temperature.

Generally, melting is a cooperative process of molecular interactions during heating; endothermic events from encapsulated curcumin can only occur when ample numbers of curcumin molecules are held together in the water-pockets as well as there are synergistic interactions among neighboring pockets. In our experiment, the number of curcumin molecules entrapped in each water-pocket is not known – one curcumin molecule is likely; nevertheless, three-dimensional structure analysis will reveal the details. However, as successive curcumin pockets are separated laterally by carrageenan helices (Fig. 1b) and vertically by two sugar units (chemical repeat of iota-carrageenan, Fig. 1c) direct curcumin-curcumin interactions are too weak to promote associations that are necessary to trigger their melting during DSC heating. On the whole, absence of curcumin melting in the complex is compelling evidence that curcumin molecules are encased in the carrageenan network. We strongly believe that the active structural state of curcumin will be preserved such that its bioavailability is enhanced in biological applications.

Control release of curcumin from the hydrocolloid matrix

The time-dependent release profiles of curcumin from IC complexes show (Fig. 4) faster release within the first 20 minutes followed by a slower release rate reaching saturation around 3 hrs. Further, complexation temperature has an effect on the loading amounts. The 25 °C complex has maximum of 0.15 µg of curcumin per mg of iota-carrageenan, measured

at 3 hrs, followed by 0.12 and 0.9 $\mu\text{g}/\text{mg}$ for 35 and 45 $^{\circ}\text{C}$ complexes, respectively. The 4 $^{\circ}\text{C}$ sample is an exception with 0.9 $\mu\text{g}/\text{mg}$ of curcumin. The trigonal unit cell of the 25 $^{\circ}\text{C}$ complex accommodates three iota-carrageenan double helices laterally spaced at 14.1 $\text{\AA}$ apart. However, transformation of the packing to an orthorhombic lattice results in a more compact arrangement of four double helices and in the 45 $^{\circ}\text{C}$ sample the closest pairs are at 12.6 $\text{\AA}$ distance. Consequently, the available free space shrinks ensuing less encapsulated curcumin. Though the inter-helical spacing in the 4 $^{\circ}\text{C}$ complex is the highest at 14.3 $\text{\AA}$, its inability to hold enough amounts might be due to slower kinetics or insufficient experiment time or disruption of junction zones by curcumin molecules.

In this study, we used sodium salt form of iota-carrageenan to encapsulate curcumin molecules. However, in the presence of other charge balancing cations such as K^{+} , Ca^{2+} and Fe^{3+} , the nature of interactions among the iota-carrageenan helices and size of water pockets (Fig. 1) will differ. Such a scenario, in combination with the complexation temperatures, provides an elegant opportunity to tailor the loading and releasing attributes of curcumin. In our opinion, results obtained from the selected model systems iota-carrageenan and curcumin will equally hold good for other: (1) heavily used food hydrocolloids: kappa- and lambda-carrageenan, and xanthan, and (2) curcumin like molecules as well as those of vitamins, antioxidants, flavor compounds and drug molecules. Iota-, kappa- and lambda-carrageenan possess two, one and three sulfate groups per disaccharide repeat, respectively, and xanthan has one carboxylate group per pentasaccharide repeat. These test beds coupled with various charge balancing cations, complexation temperatures and pH offer stimulating opportunities for developing NC carriers suitable for specific applications. Further research is necessary to validate this hypothesis.

There are several mechanisms adopted for delivering NCs, namely protein hydrogels¹⁰, microparticles,^{22, 23} liquid crystals,²⁴ self-assembled particles based on lipids,²⁵ micro- and nano-emulsions,²⁶ and starch.²⁷ However, the novelty of our approach is to utilize the well-organized structural arrangement of hydrocolloid networks and their innate water pockets to encapsulate NCs, protect from external influences and deliver. The proposed methodology is elegant and innovative, and to our knowledge this is the first report. Hydrocolloids form the bulk of many foods consumed by humans and hold a central role in human health as well, *e.g.* regular consumption of complex carbohydrates shows reduction of chronic diseases such as diabetes and cardiovascular disease.^{19–21} As they are inexpensive, utilization of hydrocolloid fibers for nutraceuticals protection and delivery will not only be significant in developing health promoting and disease preventing functional foods, but also sets the stage towards capitalizing on heavily used and low-in-cost biomaterials as value-added products.

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Fig. 1. Schematic encapsulation of nutraceutical compounds (NCs) in the hydrocolloid matrix. **(a)** The NC curcumin is encapsulated in a well-oriented and crystalline fiber of iota-carrageenan. **(b)** The cartoon highlights the cross-section of complex assembly containing ordered curcumin molecules (red star) in the iota-carrageenan network, viewed down the helix-axis. The larger stars with short and large arcs (blue, green and red) represent iota-carrageenan double helix, and pink circles correspond to cations. **(c)** Curcumin molecules securely trapped between a pair of helices, viewed normal to the helix-axis, gain the required protection. The filled circles are cations (black), water molecule (green) and sulfate groups (red and blue).

Fig. 2.

X-ray diffraction patterns from well oriented and polycrystalline fibers. (a) Iota-carrageenan, and iota-carrageenan:curcumin complexes prepared at (b) 4; (c) 25; (d) 35 and (e) 45 °C. The fibers are almost perpendicular to the incident X-ray beam. The first reflection on the meridian (meridian is an imaginary line in the North-South direction passing through the center) suggests the helix fold.

Fig. 3. Modulated Differential Scanning Calorimetry (mDSC) profiles. mDSC data confirms the thermal protection of the curcumin molecules from the iota-carrageenan network after encapsulation. (a) Curcumin powder, (b) iota-carrageenan fibers and (c) iota:curcumin complex.

Fig. 4. Release rate of curcumin, measured at room temperature, from the iota-carrageenan:curcumin complex. The 25 °C complex (red curve) has maximum load of curcumin within the iota-carrageenan network; followed by green, black and blue profiles from the complexes prepared at 35, 4 and 45 °C, respectively.