5 Polysaccharide-based Drug Carriers

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Abstract
Many challenges arise during the development of new drug carrier systems and paramount among them are safety, solubility and controlled release requirements. Although synthetic polymers are effective, the possibility of side effects imposes restrictions on their acceptable use and dose limits. Thus, there is a clear need for a new drug carrier system that is safe to handle and free from side effects, and in this regard food-grade polysaccharides stand tall as worthy alternatives. Organized polysaccharide networks in particular and the available water pockets are effective in encapsulating and protecting the drug molecules as well as releasing them in a sustained manner. Overall, human compatible carbohydrate polymers possessing stable architectures will indeed cause a paradigm shift in the design of effective drug delivery systems.

Introduction

Drug discovery and development involve highly challenging, laborious and expensive protocols. There are numerous new drug molecules arising from these high throughput-screening processes. However, the majority fail to become potential drugs due to poor pharmacokinetics. A formulation scientist continuously aims to optimize the delivery with defined dose levels, chosen rate, selected time intervals and targeted sites. In this regard, drug carriers are critical for developing effective pharmaceutical products, since their main functionality is to overcome the natural barriers presented by the human body to the assimilation of active compounds. The carrier must meet certain important requirements such as safety, solubility and stability, and – most vital – the delivery profile (Langer, 1990). Synthetic and biodegradable polymers serve this purpose to a great extent but the possibility of side effects, toxicity and associated higher costs impose restrictions on their acceptable dosage formulations (Langer and Tirrell, 2004). There is thus a great need for a new carrier system that is safe to handle, less expensive and with virtually no side effects. There are several reports with biomacromolecules as possible carriers (Takakura and Hashida, 1996; Goldberg and Gomez-Orellana, 2003; Juliano, 2007) but many of them do not possess a stable molecular structure and organized network. Carriers with stable architecture generally have advantages over those with non-rigid structures for retaining the active structural state of the encapsulated molecules and delivering effectively at the intended site. Exploitation of other biopolymer systems, preferably GRAS (Generally Recognized As Safe) materials

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that are abundant, low-in-cost and compatible with human digestibility (as well being able to maintain the ordered molecular and packing structures) would be the best scientific and industrial approach to circumvent this quandary (Fig. 5.1); and polysaccharides stand out as favourable choices (Smelcerovic *et al.*, 2008). Polysaccharides are ubiquitous biopolymers that are used extensively in food and pharmaceutical applications as thickeners, viscosifiers and gelling agents (Stephen, 1995; Rinaudo *et al.*, 2004).

Strategy

The proposed research is based on our hypothesis that drug molecules (DMs) could be effectively embedded into the ordered networks of crystalline polysaccharide matrices, protected from external stressors (e.g. heat, moisture and pH, to name a few) and released in a controlled manner. This hypothesis is based on the following three observations: (i) although polysaccharides are mostly amorphous, ordered structures could be accomplished by preparing crystalline and well-oriented fibres under suitable experimental conditions (Chandrasekaran, 1997); (ii) in the crystalline state, polysaccharides adopt well-organized helical structures coupled with well-orchestrated networks stabilized by intra- and inter-helical hydrogen bonds. These interactions are further stabilized via ordered water molecules and cations, in some cases (Rees, 1981); and (iii) in the polysaccharide networks, especially in anionic systems, there are 15–20 Å-wide voids often filled with water molecules (Janaswamy and Chandrasekaran, 2005). Such water pockets, along with well-ordered arrangements of polysaccharide helices, are amenable for embedding DMs. Thus, the main thrust of this research is to utilize ordered polysaccharide fibre matrices for encapsulating and delivering DMs (Fig. 5.2). Natural systems such as cellulose, chitin and starch possess prearranged and steady networks during biosynthesis. On the other hand, several GRAS polysaccharides that are utilized in food and pharmaceutical applications do not possess organized networks; however, under suitable experimental conditions extended networks composed of sturdy molecular and packing structures could be prepared. For example, iota-carrageenan, gellan and locust bean

Fig. 5.1. Cartoon highlighting the research idea of encapsulating drug molecules in the oriented polysaccharide fibres that possess network structures. The outcome provides an elegant and cost-effective approach for developing drug carriers based on inexpensive, non-toxic and GRAS (Generally Recognized As Safe) polysaccharide excipients.

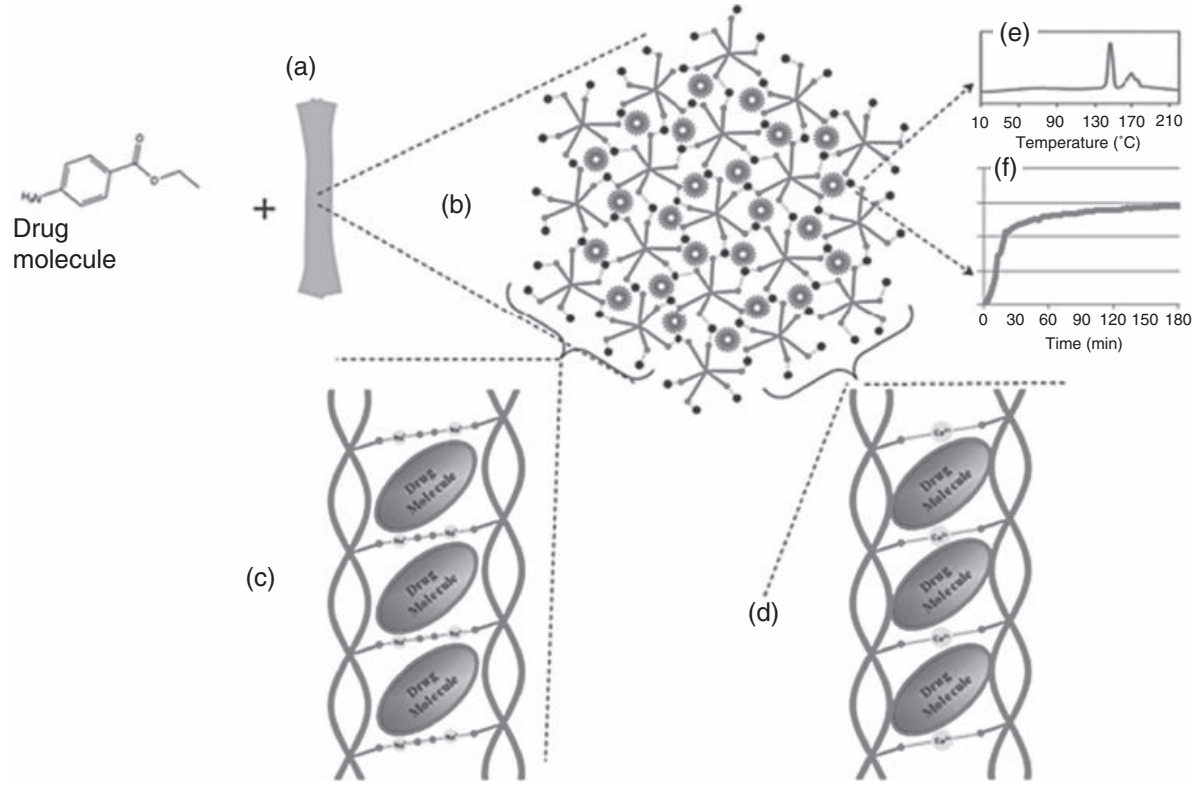

Fig. 5.2. Schematic encapsulation of drugs in the polysaccharide fibre: (a) The drug molecule is encapsulated in a well-oriented and crystalline polysaccharide fibre. (b) Cross-section of complex assembly containing ordered drugs (small star) in the polysaccharide, e.g. iota-carrageenan network, viewed down the helix-axis. The larger stars represent carrageenan double helix and small black circles correspond to cations. (c) Drugs are securely trapped between a pair of helices, viewed normal to the helix-axis, and gain the required protection. (d) Compared to sodium ions, adjacent helices are drawn closer in the presence of calcium ions, resulting in much stronger interactions. The encapsulated drugs (e) are thermally protected and (f) released in a controlled manner by/from the network.

gum fibres, to name a few, could yield highly ordered and robust molecular and packing structures (Chandrasekaran, 1997; Janaswamy and Chandrasekaran, 2001, 2002, 2006, 2008). Overall, the significant outcome of this research lies in providing a scientifically elegant and nature-based approach for the design and development of innovative drug delivery systems.

Iota-carrageenan–Ibuprofen/ Griseofulvin Complexes

Iota-carrageenan (IC) is an important member of the carrageenan family of 15 sulfated polysaccharides extracted from marine algae (Stortz and Cerezo, 2000). Carrageenans have long been used in food and pharmaceutical applications as thickeners, viscosifiers, gelling agents and stabilizers. The chemical structure of IC is composed of a disaccharide repeat of →3)-β-D-Gal*p*-4-SO_3^-(1→4)-3,6-anhydro-α-D-Gal*p*-2-SO_3^-(1→ where Gal*p* is galactopyranose. X-ray structural analysis of sodium/calcium salt forms of IC fibres suggests that it has a rigid core structure with flexible peripheral sulfate groups (Janaswamy and Chandrasekaran, 2001, 2002, 2006, 2008). The IC network is quite stable, with well-organized cation-mediated inter-helical interactions. Interestingly, there are pockets of free space of about 15–20 Å between the double helices that could be exploited for entrapping DMs (Fig. 5.2) through van der Waals, ionic and hydrogen bonding interactions.

Complex confirmation

The first example is about the encapsulation of ibuprofen and griseofulvin in the sodium IC

fibres (Janaswamy *et al.*, 2013). The encapsulation experiments were performed at room temperature by dissolving the DMs (~0.1%) in isopropyl alcohol. X-ray fibre diffraction is the only available methodology to structurally characterize the polysaccharide fibres and hence the complexes have been subjected to this analysis. Figure 5.3 compares the diffraction patterns of IC and its complexes. In general, in any fibre diffraction pattern the first reflection on the meridian – an imaginary line perpendicular to the reflection layers that passes through the centre in a north–south direction – suggests the helix fold. In the case of IC the meridional reflection is seen on the 3rd layer line, suggesting a threefold helical arrangement. In the complexes the meridional reflection is also on the 3rd layer line, signifying an intact IC helical structure. This observation is indicative of the fact that DMs enter the voids in the carrageenan lattice and are held through van der Waals and hydrogen bonding interactions with little to no influence on the carrageenan molecular structure. However, there are significant variations in the intensity distribution and positions of the reflections on the individual layer lines, portraying alterations in the packing arrangement upon encapsulation. The calculated unit cell dimensions indicate that the *c* values (that is, the layer line spacing) are relatively unchanged, meaning that the molecular structure of iota-carrageenan (IC) is rigid but the variable basal net dimensions of 53.25, 24.57 and 21.32 Å for IC, IC:ibuprofen and IC:griseofulvin, respectively, imply the packing alterations. Overall, the results highlight the remarkable combination of rigidity and flexibility of the IC network towards encapsulating the DMs. While the rigidity of the IC molecular structure confers a highly structured configuration, its flexibility (due to peripheral sulfate groups) illustrates the ability of the IC network to adjust the cavity size to the dimensions of DMs, leading to complex formation.

Thermal stability upon encapsulation

The exothermic peak (180°C) in the IC fibres is due to crystallization of the amorphous phase and the endothermic event (187°C) corresponds to the melting of junction zones (Fig. 5.4). The ibuprofen melts at around 75°C; however, upon encapsulation, melting of neither ibuprofen nor IC is observed. Instead, crystallization at 170°C is noticed, around 10°C less than the IC. Thus, the ibuprofen molecules present in the complex fibre are responsible for the crystallization of the amorphous phase at a lower temperature. Absence of ibuprofen melting in the complex is mainly due to lack of inter-molecule interactions between the ibuprofen molecules, as the neighbours in the newly formed lattice are separated by the IC helices. The griseofulvin, which melts at around 220°C, displays the glass transition temperature change by about 8°C after encapsulating in IC. The crystallization and melting temperatures of the complex are 180°C and 187°C respectively. Overall, in the case of low melting-point DMs the IC network protects them from heat, and most probably from other stresses too, and in the case of higher melting-point DMs the solubility increases in addition to the required protection.

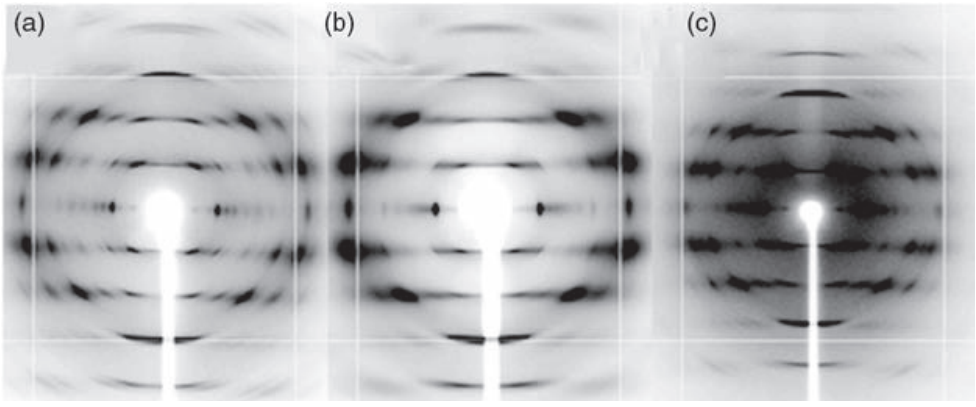

Fig. 5.3. X-ray fibre diffraction patterns of (a) iota-carrageenan and its complexes with (b) ibuprofen and (c) griseofulvin.

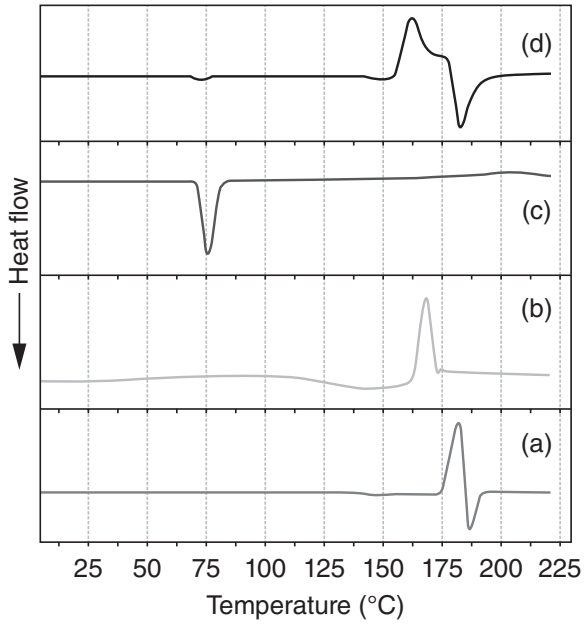

Fig. 5.4. Melting profiles of (a) iota-carrageenan fibre; (b) iota-carrageenan:ibuprofen complex; (c) ibuprofen powder; and (d) a physical mixture of iota-carrageenan fibres and ibuprofen powder.

Control release of encapsulated molecules

The griseofulvin release amount versus time is shown in Fig. 5.5. The complex displays faster release within the first 30 min and after 2 h a plateau is reached. It appears that around 1.8 µg of griseofulvin is released per 1 mg of IC. It is believed that different DMs will have variable loading and release profiles.

Iota-carrageenan–Curcumin Complexes

Complex formation and curcumin release

Experiments have been undertaken at 4°C, 25°C, 35°C and 45°C (Janaswamy and Youngren, 2012). The IC and 4°C and 25°C complexes yield a trigonal unit cell with dimensions $a = b = 24.1(2)$, $c = 13.1(1)$ Å; $a = b = 24.8(1)$, $c = 13.0(1)$ Å and $a = b = 24.5(1)$, $c = 13.2(1)$ Å, respectively. On the other hand, 35°C and 45°C samples correspond to an orthorhombic net with dimensions $a = 21.2(1)$, $b = 26.8(1)$, $c = 13.1(1)$ Å; and $a = 21.1(1)$, $b = 27.2(2)$, $c = 13.1(1)$ Å, respectively. The changes in the basal net dimensions unequivocally suggest a complex formation. The encapsulated curcumin (Fig. 5.6) releases very fast, within the first 20 min, and slowly later to reach saturation at around 3 h. The complexation temperature has an effect on the total loading amounts. The 25°C complex has a maximum of 0.15 µg followed by 0.9, 0.12 and 0.9 µg/mg for 4°C, 35°C and 45°C, respectively. At 25°C the trigonal unit cell accommodates three IC helices laterally spaced at 14.1 Å apart. A more compact arrangement of four double helices separated by 12.6 Å occurs upon the orthorhombic lattice transformation at 45°C. Thus, the reduction in the inter-helical space leads to less encapsulation of curcumin. Although the inter-helical spacing in the 4°C complex is the highest at 14.3 Å, slower kinetics or insufficient experiment time could have been the cause of its inability to hold sufficient curcumin.

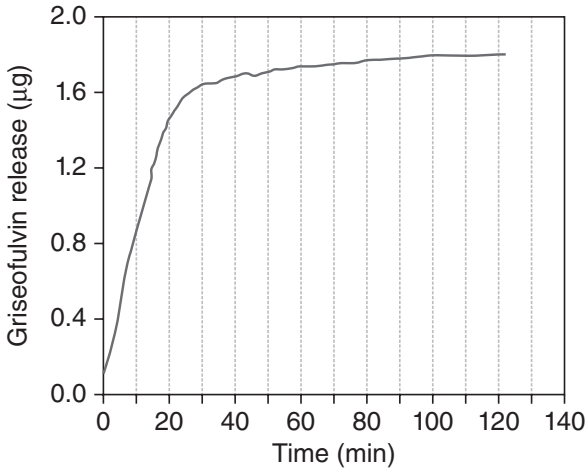

Fig. 5.5. The nature of release of griseofulvin from the iota-carrageenan:griseofulvin complex.

Thermal protection of curcumin from the carrageenan network

Curcumin melts around 175°C (Fig. 5.7) while IC fibres display crystallization at 125°C followed by melting at 140°C and 160°C. On the other hand, thermal behaviour of the 4°C complex is very interesting: upon encapsulation melting from neither curcumin nor IC is noticed, instead crystallization around 145°C and 170°C has been observed. As seen from the diffraction patterns,

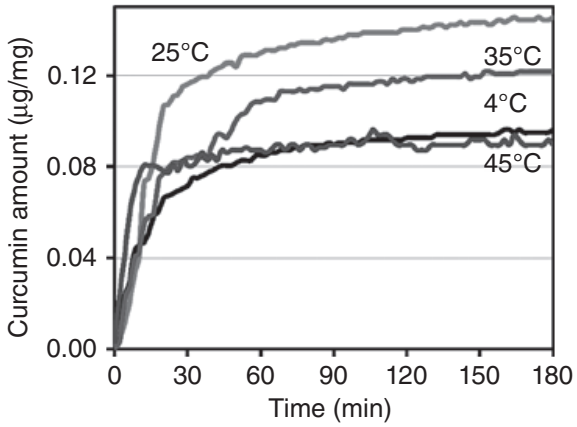

Fig. 5.6. The amount of curcumin released from the iota-carrageenan:curcumin complex.

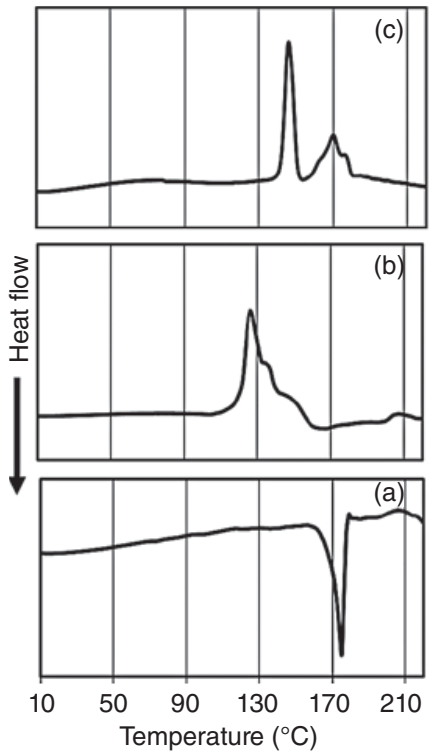

Fig. 5.7. DSC profiles (exothermic up) of (a) curcumin powder, (b) iota-carrageenan fibers, and (c) iota-carrageenan:curcumin complex prepared at 4°C.

the complex prepared at 4°C is comparatively less crystalline, suggesting the presence of a more amorphous network. It appears that most of the amorphous carrageenan chains crystallize at higher temperatures, but the important point to note is that the presence of curcumin molecules pushes the crystallization of carrageenan helices to higher temperatures. The absence of curcumin melting suggests that the IC network is able to provide the thermal protection. Thus, it is believed that the active structural state of curcumin will be preserved for longer durations and the bioavailability will be enhanced in actual biological applications.

Iota-carrageenan–Eugenol Complexes

The research strategy was to encapsulate eugenol molecules in the organized networks of monovalent (Na^+) and divalent (Ca^{2+}) salt forms of IC to understand the influence of cations on the loading amounts and release profiles (Polowsky and Janaswamy, 2015). The diffraction patterns and the melting profiles (not shown) clearly indicate the encapsulation and thermal protection of eugenol in and from the IC network. Figure 5.8 depicts the time-dependent release profiles of eugenol from Na and Ca IC fibre complexes prepared at 25°C. The results clearly showcase the intrinsic ability of cation type in modulating the encapsulated amounts and the associated release patterns. In the case of Na IC, major release occurs during the initial 20 min that later slows down and saturates. Around 3.2 µg of eugenol per mg of IC has been loaded and measured at 2.5 h. On the other hand, Ca IC fibres do not release any eugenol for about 18 min but later a gradual release is noticed that slowly peaks at around 36 min with subsequent saturation. Surprisingly, the load is only 1.6 µg/mg, 50% less than the Na IC.

The reason behind distinct encapsulated amounts and release rates between the Na IC and Ca IC complexes is altered inter-helical interactions of IC in the presence of Na and Ca ions. The three-dimensional structure analysis of Na IC (Janaswamy and Chandrasekaran, 2001) reveals that a pair of helices is connected by hydrogen bonding (···) interactions such as 4-S···*W*/Na···4-S, 2-S···*W*···*W*···*W*···4-S, 2-S···Na···2-S, 2-S···Na···4-S, 2-S···Na···*W*···2-S, 4-S···*W*···*W*···4-S, 4-S···Na···*W*···*W*···*W*···4-S, 2-S···*W*···Na···4-S, 4-S···Na···*W*···Na···4-S and 2-S···*W*···*W*···*W*···4-S; wherein 4-S, 2-S and *W* represent 4-sulfate group on β-D-Gal, 2-sulfate group on α-D-Gal and water molecule, respectively. In the case of Ca IC, direct interactions such as 2-S···Ca···2-S, 2-S···Ca···4-S and 4-S···Ca···4-S are made possible by the divalent Ca ions (Janaswamy and

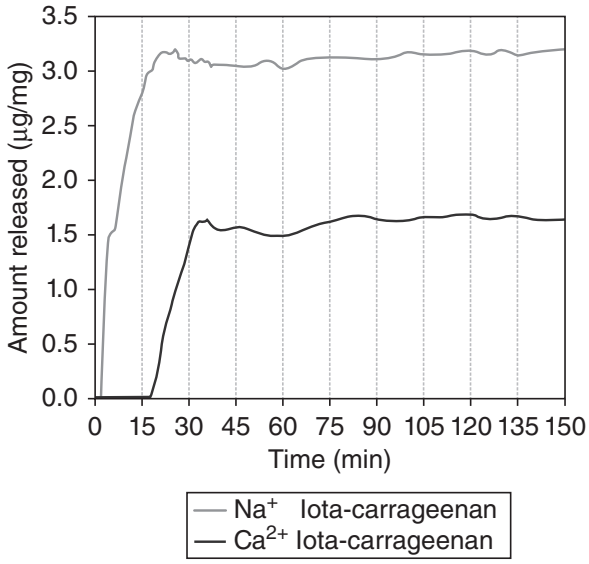

Fig. 5.8. The release rate of eugenol from the iota-carrageenan:eugenol complex prepared at 25°C.

Chandrasekaran, 2002). Thus, each Ca ion not only balances the charge on two sulfate groups of adjacent helices but also draws them closer and tighter; whereas a Na ion merely binds to a sulfate moiety on one helix and hence the inter-helical interactions are comparatively weaker and further mediated by space fillers such as water molecules. Thus, the resulting Na IC network is more flexible than that of Ca IC. These network variations reflect significantly on the encapsulated amounts and subsequent release profiles.

The IC:eugenol release behaviour compares well with those of IC:curcumin (Janaswamy and Youngren, 2012) and IC:griseofulvin (Janaswamy *et al.*, 2013). While the former accommodates around 3.2 µg/mg the latter two encompass the far smaller amounts of 0.15 and 1.8 µg/mg, respectively. Thus, it appears that the guest molecule's chemical structure, size and available functional groups, along with its associative kinetics with the polysaccharide helices, appear to dictate the embedding amounts as well as the release characteristics.

Conclusions

Polysaccharides exhibit a wide variety of unique chemical structures and physiological functions. They are capable of significantly altering the texture, gelation and viscosity of aqueous-based solutions, and a wide range of products could be developed using polysaccharides as functional ingredients. They form the bulk of many foods consumed by humans and play a central role in human health as well; for example, their regular consumption is accompanied by a reduction in chronic diseases such as diabetes, cardiovascular disease and cancer.

The present research is about utilizing the structural organization of polysaccharide helices in oriented fibres, and the innate water pockets present in the polysaccharide network, to encapsulate active molecules and protect them from external influences such as temperature, to maintain their functional state until delivered at the target site. The outcome will be equally applicable to protect and deliver vitamins, antioxidants and flavour compounds, for example, using several routinely used polysaccharides such as kappa-carrageenan, lambda-carrageenan and xanthan. In general, polysaccharides are inexpensive and many of their uses in food and non-food applications have already been approved by the US Food and Drug Administration. Obtaining further authorization for large-scale production of polysaccharide-based delivery systems is, therefore, quite feasible and would certainly aid in capitalizing heavily used and low-cost biomaterials as value-added products.

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References

Chandrasekaran, R. (1997) Molecular architecture of polysaccharide helices in oriented fibers. *Advances in Carbohydrate Chemistry and Biochemistry* 52, 311–439.

Goldberg, M. and Gomez-Orellana, I. (2003) Challenges for the oral delivery of macromolecules. *Nature Reviews: Drug Discovery* 2, 289–295.

Janaswamy, S. and Chandrasekaran, R. (2001) Three-dimensional structure of the sodium salt of iota-carrageenan. *Carbohydrate Research* 335, 181–194.

Janaswamy, S. and Chandrasekaran, R. (2002) Effect of calcium ions on the organization of iota-carrageenan helices: an X-ray investigation. *Carbohydrate Research* 337, 523–535.

Janaswamy, S. and Chandrasekaran, R. (2005) Cation-induced polymorphism in iota-carrageenan. *Carbohydrate Polymers* 60, 499–505.

Janaswamy, S. and Chandrasekaran, R. (2006) Sodium iota-carrageenan: a paradigm of polymorphism and pseudopolymorphism. *Macromolecules* 39, 3345–3349.

Janaswamy, S. and Chandrasekaran, R. (2008) Heterogeneity in iota-carrageenan molecular structure: insights for polymorph II→III transition in the presence of calcium ions. *Carbohydrate Research* 343, 364–373.

Janaswamy, S., Gill, L., Campanella, O. and Pinal, R. (2013) Organized polysaccharide fibers as stable drug carriers. *Carbohydrate Polymers* 94, 209–215.

Janaswamy, S. and Youngren, R.S. (2012) Hydrocolloid-based nutraceutical delivery systems. *Food & Function* 3, 503–507.

Juliano, R. (2007) Cellular delivery of therapeutic macromolecules: challenges to macromolecular drug delivery. *Biochemical Society Transactions* 35, 41–43.

Langer, R. (1990) New methods of drug delivery. *Science* 249, 1527–1533.

Langer, R. and Tirrell, D.A. (2004) Designing materials for biology and medicine. *Nature* 428, 487–492.

Polowsky, P.J. and Janaswamy, S. (2015) Hydrocolloid-based nutraceutical delivery systems: Effect of counter-ions on the encapsulation and release. *Food Hydrocolloids* 43, 658–663.

Rees, D.A. (1981) Polysaccharide shapes and their interactions – some recent advances. *Pure and Applied Chemistry* 53, 1–14.

Rinaudo, M., Auzely, R. and Mazeau, K. (2004) Polysaccharides. In: *Encyclopedia of Polymer Science and Technology*. Wiley, New York, pp. 200–261.

Smelcerovic, A., Knezevic-Jugovic, Z. and Petronijevic, Z. (2008) Microbial polysaccharides and their derivatives as current and prospective pharmaceuticals. *Current Pharmaceutical Design* 14, 3168–3195.

Stephen, A.M. (ed.) (1995) *Food Polysaccharides and Their Applications*. Marcel Dekker Inc., New York.

Stortz, C.A. and Cerezo, A.S. (2000) Novel findings in carrageenans, agaroids and “hybrid” red seaweed galactans. *Current Topics in Phytochemistry* 4, 121–134.

Takakura, Y. and Hashida, M. (1996) Macromolecular carrier systems for targeted drug delivery: Pharmacokinetic considerations on biodistribution. *Pharmaceutical Research* 13, 820–831.