Carbohydrate Polymers 115 (2015) 723–731

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Controlled release of thiamin in a glassy κ-carrageenan/glucose syrup matrix

Naksit Panyoyai, Anna Bannikova, Darryl M. Small, Stefan Kasapis*

School of Applied Sciences, RMIT University, City Campus, Melbourne 3001, VIC, Australia

ARTICLE INFO

Article history:
Received 4 June 2014
Received in revised form 11 July 2014
Accepted 30 July 2014
Available online 12 August 2014

Keywords:
κ-Carrageenan
Glucose syrup
Thiamin
Glass transition temperature
Diffusion kinetics

ABSTRACT

The work dealt with the diffusional mobility of thiamin embedded in a high-solid matrix of κ-carrageenan with glucose syrup. It utilized thermomechanical analysis in the form of modulated differential scanning calorimetry and small-deformation dynamic oscillation in shear, Fourier transform infrared spectroscopy, wide angle X-ray diffraction, scanning electron microscopy and UV–vis spectrophotometry. The structural properties of the matrix were assessed in a temperature induced rubber-to-glass transformation. A thiamin-dye binding assay was employed to monitor the diffusion process of the vitamin from the high-solid preparation to ethylene glycol. The relationship between mechanical properties of the carbohydrate matrix and vitamin mobility was assessed *via* the application of the combined framework of the free volume theory and the predictions of the reaction rate theory. Results argue that the transport of the micronutrient is governed by the structural relaxation of the high-solid matrix. These were further treated with the concept of Fickian diffusion coefficient to provide the rate of the bioactive compound mobility within the present experimental settings.

1. Introduction

Thiamin (vitamin B1) is the first member of a wide group of B complex vitamins. Staple foods like cereals, grains and rice as well as the various forms of dietary supplements (capsules, tablets, powder and gels) are vehicles for thiamin fortification (Bui, Small, & Coad, 2013). The consistency and retention of thiamin in these materials have been evaluated in relation to temperature, pH, water activity and added counterion (Bell & White, 2000; Bui & Small, 2007; Zhou & Roos, 2012). A recent approach in examining the stability of micronutrients in systems of industrial interest has been focusing on the phenomenon of glass transition that allows modeling of the time–temperature function during processing and subsequent storage (Leśková et al., 2006; Rahman, 2006). This is based on the expectation that in the glassy state various diffusion processes, and rates of physicochemical, enzymatic and biological reactions become extremely slow thus facilitating the preservation of nutrients in foodstuffs (Roos, 1995, 2003, 2010).

Thermoanalytical methods including modulated differential scanning calorimetry (MDSC) and dynamic mechanical analysis (DMA) have become useful techniques in the determination of the glass transition temperature of condensed systems (Gunasekaran & Ak, 2000). Further work using small-deformation dynamic oscillation in shear offered an avenue to define a mechanical or network glass transition temperature with physical significance in biopolymer/co-solute preparations (Kasapis, 2006). It utilized the concept of free volume to follow the relaxation kinetics within the glass transition region as a function of solids content, polymer molecular weight and extent of counterion or thermally induced network formation in biomaterials (Kasapis, 2001, 2008; Kasapis, Mitchell, Abeysekera, & MacNaughtan, 2004).

κ-Carrageenan is the collective name of a family of sulphated galactans comprising an alternating α(1-3)-D-galactose-4-sulphate and β(1-4)-3,6-anhydro-D-galactose (Morris & Chivers, 1983; Michel, Mestdagh & Axelos, 1997). The marine-based polysaccharide is extracted from red algae (*Eucheuma cottonii*) and is used extensively in the food industry as a gelling, thickening, stabilizing and water holding agent, diffusion controller, and texture enhancer (Campo, Kawano, da Silva & Carvalho, 2009; Michel et al., 1997). The rapid gelation of κ-carrageenan involves coil-to-helix transformation and aggregation of the ordered molecules with alkali metal ions (particularly those of potassium), sugars and polyols (Hermansson, Eriksson & Jordansson, 1991; Nishinari & Watase, 1992; Nishinari, Watase, Williams & Phillips, 1990). Recently, the effect of network formation in a high-solid system of κ-carrageenan

* Corresponding author. Tel.: +61 3 992 55244; fax: +61 3 992 55241.
E-mail addresses: stefan.kasapis@rmit.edu.au, stefankasapis@hotmail.com (S. Kasapis).

http://dx.doi.org/10.1016/j.carbpol.2014.07.060
0144-8617/

and glucose syrup on the preservation and diffusion of caffeine as a typical psychoactive stimulant and diuretic in humans has been examined (Jiang & Kasapis, 2011).

Various micronutrients and antioxidants have been widely incorporated in delivery vehicles in the form of capsules, tablets or colloidal gels, which has generated great interest in fundamental approaches that build a foundation for the design and fabrication of food-grade systems targeting specific applications (Fisher & Windhab, 2011; Lesmes & McClements, 2009; Pothakamury & Barbosa-Cánovas, 1995). The current investigation aims to provide basic understanding on the physical mechanisms and transport rates of thiamin entrapped in a condensed matrix of κ-carrageenan/glucose syrup undergoing thermally induced vitrification.

2. Materials and methods

2.1. Materials

Glucose syrup, as the co-solute of this investigation, is a product of Edlyn Foods Pty Ltd (Sydney, Australia) with a dextrose equivalent (DE) value of about 42. The total level of solids in the stock solution is 81% (w/w), and percentages in the preparations of this investigation refer to dry solids. This is a non-crystalline material that converts from a viscous solution at ambient temperature to a rigid and transparent glass at subzero temperatures.

κ-Carrageenan, as the structuring agent of this investigation, is supplied by Sigma-Aldrich Pty Ltd (Sydney, Australia). The polysaccharide is extracted from *E. cottonii* type III and used as the basic material for further purification prior to our experimentation.

Thiamin. The vitamin in its hydrochloride form ($C_{12}H_{17}ClN_4OS{\cdot}HCl$ with a molecular weight of 337.27 g/mol) was obtained from Sigma-Aldrich Pty Ltd (Sydney, Australia). The material was in the form of small white crystals with an analytical grade of more than 99% purity.

Ethylene glycol was purchased at a spectrophotometric grade (purity of about 99.9%) from Sigma-Aldrich Pty Ltd (Sydney, Australia). It is commonly used as an antifreeze fluid for the preservation of biological tissues and organs. Our eye observations and calorimetric analysis at a scan rate of 0.1 °C/min showed that the freezing point of ethylene glycol was below −30 °C. This property facilitates its utilization as a stable liquid phase in the current experimental temperature range of −22 to 26 °C.

Thiamin hydrochloride analytical reagents. The acid dye, Alizarin Brilliant Violet R, was purchased from Jacquard (Healdsburg, USA) in the form of a violet powder. Chloroform was obtained from Sigma-Aldrich Pty Ltd (Sydney, Australia) and potassium dihydrogen phosphate, for the preparation of the phosphate buffer at pH 4.5, was purchased from BDH Chemicals Pty Ltd (Poole, England). Reagents were used without further purification and Millipore type II water was the diluent in all experiments.

2.2. The potassium form of κ-carrageenan

The material of this investigation was converted in the potassium form by ion exchange, as described by Evageliou, Kasapis, and Hember (1998). In doing so, an Amberlite IR-120 exchanging resin from Supelco (Bellefonte, Pennsylvania, USA) was used. Two hundred grams of the resin was first eluted with 0.1 M HCL to bring it to the hydrogen form until the eluent was at pH 1, and then with a solution of 2 M KCl to bring it to the potassium form. The resin was rinsed with millipore water to remove the excess of salt until there was no precipitation in the washings with 0.1 M silver nitrate, and was heated up to 90 °C with Millipore water.

Table 1
Cation and sulphate contents of the potassium κ-carrageenan, as compared to its commercial counterpart.

Ions	Commercial κ-carrageenan (% w/w)	Potassium κ-carrageenan (% w/w)
Potassium	6.5	7.8
Calcium	2.8	0.3
Sodium	0.7	0.1
Magnesium	0.2	0.02
Sulphate	17.8	17.5

Five grams of κ-carrageenan were transferred in a conical flask and dissolved with 1 L of Millipore water followed by heating to 90 °C on a hot plate with stirring. The hot resin was introduced into the polysaccharide flask and stirred for 30 min. κ-Carrageenan in the potassium form was separated from the resin, dialyzed against Millipore water for 24 h at 25 °C and freeze-dried using an Operon freeze dryer (Gimpo, Seoul, Korea).

The purity of the ion exchanged material was analyzed with an atomic absorption spectrometer (Agilent, Santa Clara, California, USA). K^+, Na^+ and Mg^{2+} were identified using an air-acetylene flame whereas Ca^{2+} was determined with a nitrous oxide-acetylene flame. Calibration curves were constructed for each cation, with the absorbance readings versus cation concentration in ppm being expressed finally in percentages (w/w). The amount of sulfate was evaluated gravimetrically (Chan, Mirhosseini, Taip, Ling, & Tan, 2013). The method is based on sulfate hydrolysis with 1 M HCl and reaction of the sulfate groups with barium chloride leading to the formation of a white precipitate of barium sulfate. The sulfate content was calculated by multiplying the weight of barium sulfate with a conversion factor (0.4116) and expressing in percentages (w/w). All measurements were performed in triplicate and results are presented in Table 1.

2.3. Sample preparation

In general, samples were prepared at 85% (w/w) total solids. In doing so, κ-carrageenan in the potassium form was dispersed in Millipore water at 90 °C with constant stirring for 10 min and then the temperature was reduced to 70 °C. Appropriate amounts of glucose syrup were dissolved separately and added carefully to the polymer solution at the same temperature. The total level of solids was higher than the required final concentration but that was adjusted with the addition of thiamin hydrochloride, in a potassium dihydrogen phosphate buffer (50 mM, pH 4.5), to the viscous carbohydrate preparation at 30°C to yield the experimental concentration of 1% thiamin with 2% κ-carrageenan and 82% glucose syrup (w/w).

The above conditions encourage smooth network formation and prevention of thiamin degradation (Imeson, 2000; Pachapurkar & Bell, 2005). Care was taken to dissolve the thiamin in the potassium dihydrogen phosphate buffer using an amber glass bottle. Beakers with final preparations were wrapped in aluminum foil and the top was sealed with a stretchable film to prevent exposure to light. Samples were kept overnight at 4°C to allow sample equilibration and used subsequently to study the controlled release of thiamin in the polymeric matrix. Other samples without thiamin were made in a similar manner to analyze the structural properties of the matrix including single systems of 85% glucose syrup, and 83% glucose syrup with 2% κ-carrageenan (w/w).

2.4. Structural studies

2.4.1. Modulated differential scanning calorimetry

Measurements were performed using Q 2000 (TA Instruments, New Castle, DE). The instrument interfaced a refrigerated

cooling system (RCS 90) to achieve temperatures down to −90 °C, and nitrogen was the purging gas at a flow rate of 50 mL/min. Heat flow signals were calibrated using a traceable indium standard (ΔH_f = 28.3 J/g) and the heat capacity with a sapphire standard. About 10 mg of sample were hermetically sealed and analyzed by cooling from 20 to −90°C at the scan rate of 1 °C/min followed by heating to 20 °C at the same rate. Measurements were performed at modulation amplitude of ±0.53 °C for each period of 40 s, and an empty pan served as a reference. Duplicates of the thermograms were recorded yielding effectively overlapping traces.

2.4.2. Small-deformation rheology

Mechanical properties were studied as a function of temperature and angular frequency with small-deformation dynamic-oscillation measurements in shear. This type of mechanical analysis provides readings of the elastic (storage modulus, G') and viscous (loss modulus, G'') components of the network, with variations of the relative liquid-like and solid-like structure of the material being assessed with the damping factor ($\tan\delta = G''/G'$).

Low amplitude oscillatory measurements were executed using AR-G2, a controlled strain rheometer with a magnetic thrust bearing technology (TA Instruments, New Castle, DE). Samples were placed on a preheated Peltier plate supporting a parallel-plate measuring geometry (10 mm diameter) at 80 °C and exposed edges were covered with silicone oil (50 cS) to minimize moisture loss. High-solid preparations were cooled to −25 °C with an environmental test chamber (ETC) at 1 °C/min using a frequency of 1 rad/s and 0.1% strain that was within the linear viscoelastic region of our materials. That allowed recording of the rubbery plateau, glass transition region and glassy state as a function of temperature. Further, mechanical spectra were recorded from 0.1 to 100 rad/s at temperature intervals of 4° upon subsequent heating.

Fourier transform infrared spectroscopy. Data for single, binary and tertiary samples were obtained with a Perkin Elmer Spectrum 100 using MIRacle^{TM} ZnSe single reflection ATR plate (Perkin Elmer, Norwalk, CT). The absorbance spectra were recorded within the range of 600 to 4000 cm^{-1} at an average scan number of 8 and a resolution of 4 cm^{-1}. That was normalized against the background spectrum of Millipore water at ambient temperature, and each measurement was performed in triplicate.

Wide angle X-ray diffraction. Measurements were carried out using a D8 Advanced Bruker AXS (Karlsruhe, Germany) attached with Cu-$K\alpha$ radiation source (l = 1.54 Å). Diffractograms of freeze-dried samples of κ-carrageenan, glucose syrup, thiamin hydrochloride and their combinations were obtained at 25 °C using an accelerating voltage and current of 40 kV and 40 mA, respectively. Raw data were obtained within a 2θ range of 5° and 90° in the measuring interval of 0.1° and analyzed using DIFFRAC^{plus} Evaluation (Eva), version 10.0 revision 1, which is a Bruker Advanced X-ray Solutions software. As before, experimentation was performed in triplicate.

Scanning electron microscopy. Philips XL30 SEM (Edwards High Vacuum, Sussex, England) was used to investigate the network morphology and phase topology of single preparations and mixtures of our materials. Freeze dried preparations were attached to sample holders followed by gold plating. A high-vacuum mode at an accelerating voltage of 5 and 10 kV, spot size from 4.3 to 5 and observing distance between 9.7 and 12.5 mm were used to obtain micrographs.

2.5. Control release kinetics

Four grams of the high-solid κ-carrageenan/glucose syrup preparation with thiamin hydrochloride were transferred into twelve 25 mL beakers (overall thirty six replicates per experimental temperature) and wrapped with aluminum foil to avoid exposure to light. The thickness and diameter of the sample in the beaker were 5 and 25 mm, respectively. Care was taken to obtain a smooth surface without any bubble formation following sample transfer to the beaker. These materials and 4 mL of ethylene glycol in test tubes were kept overnight at the experimental temperature of interest for equilibration. After that, ethylene glycol was transferred swiftly to the beakers as a separate phase on top of the sample; ethylene glycol is insoluble in the κ-carrageenan/glucose syrup system. Beakers were promptly sealed with stretchable film to prevent solvent evaporation and returned to the required temperature for immediate removal of an aliquot thus setting the zero time of the experimentation. Subsequent aliquots were obtained within 2 h at intervals of one to twenty minutes for the experimental temperature range of −22 to 26 °C every 4 °C.

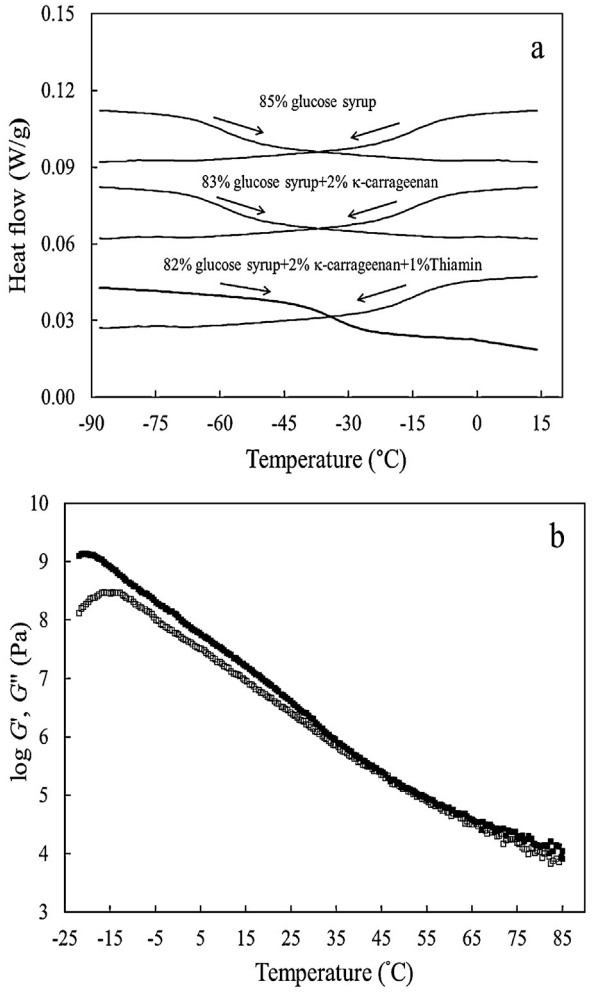

Fig. 1. (a) Variation of heat flow as a function of temperature for 82% glucose syrup plus 2% potassium κ-carrageenan with 1% thiamin hydrochloride (50 mM K^+; pH 4.5), 83% glucose syrup with 2% potassium κ-carrageenan, and 85% glucose syrup alone obtained using MDSC at a scan rate of 1 °C/min; (b) cooling profiles of storage (G', ■) and loss (G'', □) modulus as a function of temperature for 82% glucose syrup plus 2% potassium κ-carrageenan with 1% thiamin hydrochloride (50 mM K^+; pH 4.5) scanned at a rate of 1 °C/min, frequency of 1 rad/s and strain of 0.01%.

Vitamin diffusion from the high-solid matrix to ethylene glycol was estimated in the form of absorbance using a dye-binding assay and a Lamda 35 UV–vis spectrophotometer (Perkin Elmer, Singapore). This is based on the formation of a colored ion-pair complex between thiamin hydrochloride and Alizarin brilliant violet *R* (modified assay from Prasad, Rajasree, Khan & Reddy, 1997). In doing so, 1 mL ethylene glycol containing diffused thiamin was mixed with 2 mL of 50 mM potassium dihydrogen phosphate buffer

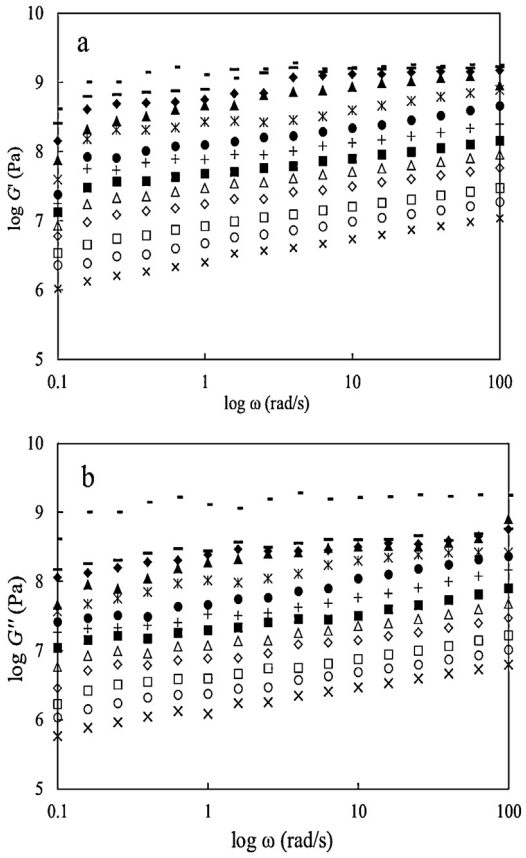

Fig. 2. Frequency variation of G' (a) and G'' (b) for 82% glucose syrup plus 2% potassium κ-carrageenan with 1% thiamin hydrochloride (50 mM K^+; pH 4.5). The bottom curve was taken at 26 °C (×), other curves successively upward were taken at 22 (○), 18 (□), 14 (◇), 10 (△), 6 (■), 2 (+), −2 (●),−6 (⋆),−10 (▲),−14 (♦),−18 (-) and −22 (–) °C.

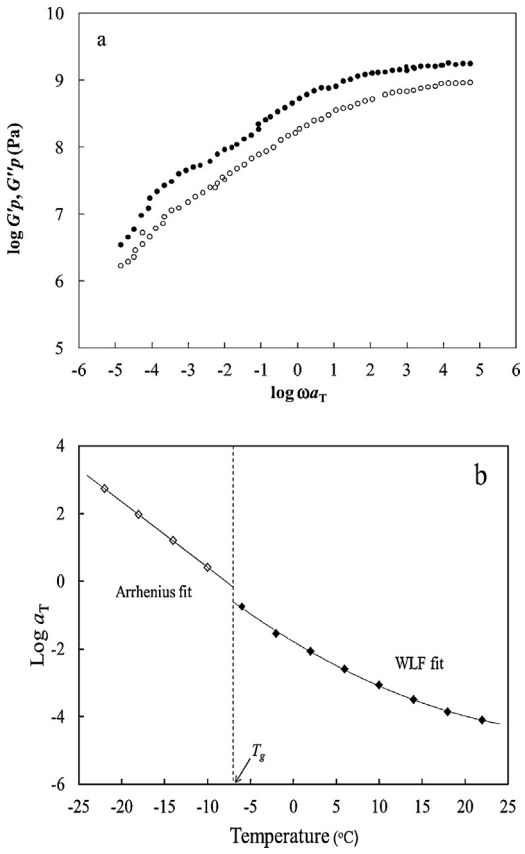

Fig. 3. (a) Master curve of reduced shear modulus (G_p', ●; G_p'', ○) for 82% glucose syrup with 2% potassium κ-carrageenan (50 mM K^+; pH 4.5) plus 1% thiamin hydrochloride as a function of reduced frequency of oscillation (ωa_T) based on the frequency sweeps in Fig. 2 and utilizing a reference temperature of −6 °C, and (b) logarithmic shift factors a_T as a function of temperature for this sample within the glass transition region (closed symbols) and glassy state (open symbols), with the solid line reflecting the WLF and modified Arrhenius fits of the shift factors, and the dash line pinpointing the prediction of network T_g.

to bring the solution to pH 4.5 and prevent vitamin degradation. Then, the preparation was transferred into a 100 mL separating funnel and mixed with 10 mL chloroform, 2 mL of 0.2% Alizarin brilliant violet *R* and Millipore water to a total of 20 mL. The separator was shaken for 2 min and set aside at ambient temperature for 10 min to yield a two-layer liquid mixture.

Some of the bottom layer of blue chloroform was removed into 1 cm glass cell and maximum absorbance was measured at λ_{max} = 575 nm; color intensity remains constant within 4 h of observation at ambient temperature. A calibration curve was constructed by dissolving thiamin hydrochloride in ethylene glycol at concentrations ranging from 0.0001 to 0.006% and applying the same assay for absorbance measurements as for the diffusion studies. Tests were carried out in triplicate and average values are reported.

3. Results and discussion

3.1. Single and mixed κ-carrageenan/glucose syrup/thiamin systems examined calorimetrically

MDSC is a common technique to investigate changes in heat capacity with temperature leading to mechanical, enthalpic and dielectric transformations. In the present study, addition of high levels of glucose syrup prevents ice formation at subzero temperatures, thus allowing observation of molecular relaxations in supersaturated matrices of κ-carrageenan/glucose syrup/thiamin, κ-carrageenan/glucose syrup and glucose syrup alone, with the total solids content for all preparations being fixed at 85% (w/w). Direct measurements of heat capacity at modulated regime were performed in heating and cooling cycles at the relatively low scan rate of 1 °C/min.

Fig. 1a reveals a decrease in the total heat capacity of samples during cooling due to reduction in thermal motion and molecular mobility. The calorimetric glass transition temperature can be pinpointed empirically at the middle of the thermogram. This mid-point T_g for the single glucose syrup and a binary mixture of κ-carrageenan/glucose syrup was recorded to be approximately −37 °C. Addition of thiamin shifted the T_g estimate to a slightly higher temperature of −35 °C. It appears, therefore, that the presence of small amounts of κ-carrageenan and thiamin hydrochloride has no significant effect on the characteristics of the glucose-syrup thermogram. Similar reports on the DSC readings for high-solid carbohydrate preparations were obtained by Kumagai, MacNaughtan, Farhat, and Mitchell (2002) and Jiang and Kasapis (2011). This outcome argues that calorimetric spectra are mainly dominated by the enthalpic relaxation of small molecule co-solute, with the addition of threshold gel concentrations of polymeric materials acting merely as a cross-contaminant in the system (Chaudhary, Small, & Kasapis, 2013).

3.2. Rheological examination of vitrification in high-solid preparations

The calorimetric glass transition temperature describes micromolecular aspects of high-solid preparations, which are complemented by small-deformation oscillatory measurements

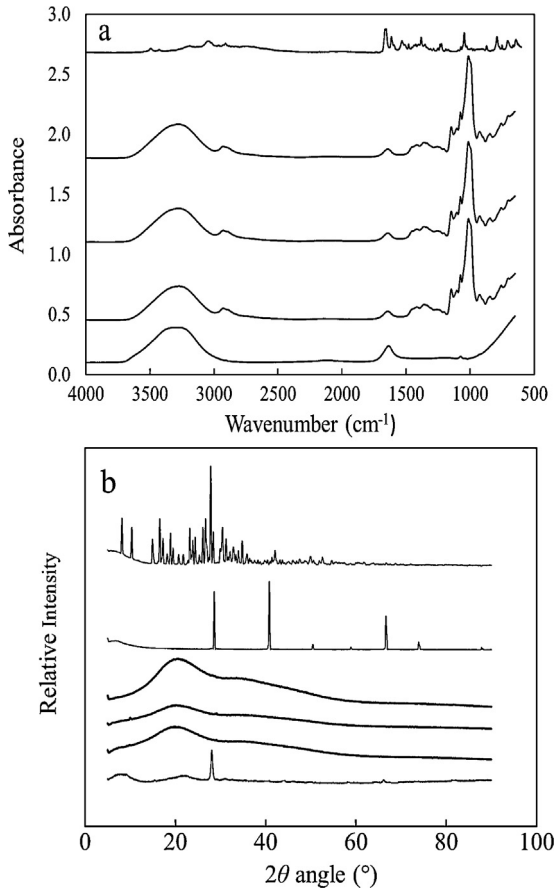

Fig. 4. (a) FTIR spectra and (b) X-ray diffractograms of 2% potassium κ-carrageenan with 0% or 83% glucose syrup, an 85% glucose syrup preparation, 2% potassium κ-carrageenan plus 82% glucose syrup with 1% thiamine hydrochloride, potassium dihydrogen phosphate (for X-ray diffractograms only), and thiamine hydrochloride arranged successively upwards.

identifying macromolecular features of glass transition phenomena. This section examines the viscoelastic nature of 2% κ-carrageenan (50 mM K^+) with 82% glucose syrup and 1% thiamin hydrochloride over a temperature range and scan rate that are comparable to the calorimetric routine in the preceding section and results are reproduced in Fig. 1b. Similar viscoelasticity has been obtained for the system of 2% κ-carrageenan (50 mM K^+) with 83% glucose syrup but is not shown here.

Storage (G') and loss (G'') modulus values cover five-and-a-half orders of magnitude from $10^{3.8}$ to $10^{9.2}$ Pa within the experimental temperature range (Fig. 1b). At the high temperature end, e.g. at 80 °C, the low and relatively flat mechanical traces indicate the consistency of a rubbery plateau. This is followed by the glass transition region (e.g. at 45 °C) where the gap between the two moduli is reduced. Further cooling of the system leads gradually to the glassy state where the modulus values diverge from each other culminating in a sharp drop in the viscous response at the low end of the cooling run, i.e. at temperatures below −10 °C. Throughout the rubber-to-glass transformation, storage modulus remains above the liquid-like counterpart indicating the formation of a coherent network in the presence of 2% κ-carrageenan and 50 mM K^+ counterions (Iijima, Hatakeyama, Takahashi, & Hatakeyama, 2007; Loret, Ribelles, & Lundin, 2009). This κ-carrageenan/glucose syrup network yields a rapid manifestation of the mechanical vitrification, as compared to the calorimetric T_g of about −37 °C in Fig. 1a.

Next, we used the time–temperature superposition (TTS) principle to advance the discussion from the empirical state in Fig. 1b to an understanding that disentangles the temperature and time contributions in glass transition behavior (Nickerson & Paulson, 2005). In doing so, shear modulus measurements were taken within the frequency range of 0.1 to 100 rad/s in fixed intervals of 4 °C covering a temperature range of 26 to −22 °C (Fig. 2a and b). Clearly, mechanical spectra remain relatively flat in the glassy state (e.g. at −18 °C) while exhibiting a steep rise in the glass transition region with increasing oscillatory frequency (e.g. at 22 °C).

Mechanical spectra were superposed horizontally by selecting arbitrarily a reference temperature within the glass transition region (T_o = −6 °C). The technique is based on the premise that moduli recorded at any temperature are equivalent to those at T_o provided that the frequency, ω, is multiplied by a shift factor, a_T. Values of factor a_T were calculated using proprietary software (Orchestrator from TA Instruments) and confirmed manually by performing least square calculations of the horizontal displacement in shear-modulus data required for the construction of the viscoelastic master curves. Fig. 3a illustrates the master or composite curve obtained over a frequency window of ten decades from 10^{-5} to 10^5 rad/s, with the values of G'_p exceeding those of G''_p, as observed in the temperature analog of Fig. 1b.

Generated shift factors were modeled by utilizing the modified Arrhenius equation, which advocates that molecular processes are proportional to (E_a/RT), with E_a being the activation energy of molecular reorientation from one conformational state to another and R being the universal gas constant (Kasapis, 2001):

$$\log\, a_T = \frac{E_a}{2.303R}\left(\frac{1}{T} - \frac{1}{T_o}\right) \tag{1}$$

The approach follows well the temperature dependence of factor, a_T, at temperatures below −10 °C in Fig. 3b to generate a linear gradient with an activation energy value of about 180 kJ/mol that reflects the specific physicochemical fingerprints of this system.

We found that at temperatures higher than −10 °C in Fig. 3b, progress in viscoelasticity is better described by the concept of free volume, which in rheological terms can be given by the mathematical framework of Williams, Landel and Ferry (WLF equation in Ferry, 1980):

$$\log a_T = -\frac{C_1^0\,(T - T_0)}{C_2^0 + T - T_o} = -\frac{\left(B/2.303f_o\right)(T - T_o)}{\left(f_o/\alpha_f\right) + T - T_o} \tag{2}$$

where C_1^0 and C_2^0 are the WLF constants at T_o, f_o is the fractional free volume (the ratio of free to total volume per gram of material), α_f is the thermal expansion coefficient, and B is usually taken as one for simplicity.

Utilization of the WLF equation yields good fits for the temperature dependence of molecular processes at the upper range of experimental temperatures. It also pinpoints, in conjunction with the modified Arrhenius equation, the network glass transition temperature at −7 °C in Fig. 3b, as the threshold of the free volume and reaction rate theories; detailed discussion of the scientific basis for the derivation of a glass transition temperature with physical significance can be found in Kasapis (2008). WLF modeling produces values of C_1^0 (11.20), C_2^0 (50°), α_f (7.6×10^{-4} deg^{-1}) and f_g (0.038 at T_g), which are congruent with earlier estimates for amorphous synthetic polymers and bioglasses (Kasapis, 2008).

3.3. Molecular morphology of condensed carbohydrate/thiamin matrices

Thermomechanical work in the preceding sections was augmented with physicochemical and microscopy studies. FTIR was utilized first to identify potential interactions between the three constituents of these preparations. Fig. 4a illustrates spectra for κ-carrageenan indicating a broad —OH stretching absorption band at wavenumbers between 3650 and 3100 cm^{-1}. Glucose syrup, on

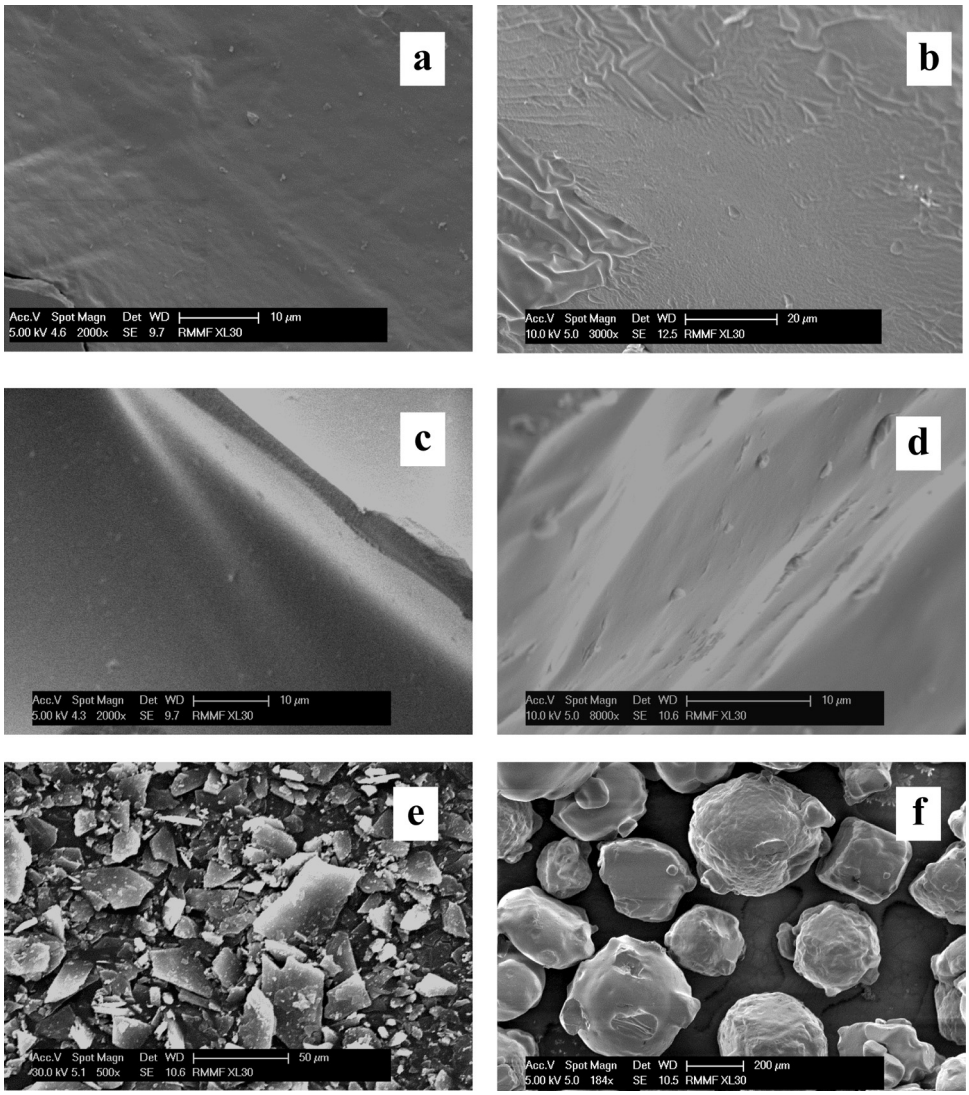

Fig. 5. SEM micrographs of (a) 2% potassium κ-carrageenan, (b) a binary mixture of 2% potassium κ-carrageenan with 83% glucose syrup, (c) 85% glucose syrup, (d) 2% potassium κ-carrageenan plus 82% glucose syrup with 1% thiamine hydrochloride, (e) thiamin hydrochloride crystals, and (f) potassium dihydrogen phosphate crystals.

the other hand, produces peaks of C—H stretching along with a major OH-group at 3645 to 2845 cm^{-1}, C═O stretching vibrations of aldehyde at 1624 cm^{-1}, C—O stretching and bending at 1493 to 1173 cm^{-1} and the C—O—C glycosidic linkage at 1172 to 875 cm^{-1} (Almrhag et al., 2012). Clearly, molecular FTIR spectra argue that the addition of thiamin does not affect the structural integrity and physical interactions within the high-solid κ-carrageenan/glucose syrup mixture.

FTIR studies were complemented with WAXD in an effort to observe possible alterations in the microsized order of the high-solid samples. Diffraction analysis in Fig. 4b indicates the specific site-binding of κ-carrageenan in the potassium form due to the cation exchange process implemented in this work. A typical non-crystalline aggregate supporting irregular junction zones is reflected in the diffractogram with one sharp peak ($2\theta = 28.06^{\circ}$), as reported by Martins et al. (2012). The thiamin and potassium dihydrogen sulphate crystals, positioned on the top of this presentation, exhibit multiple sharp peaks between 5 and 70°. In contrast, single glucose syrup and glucose syrup/κ-carrageenan systems, with or without thiamin, record a broad pattern centering at approximately 20° and shouldering at 35°, which is characteristic of the processing conditions used to manufacture the ingredients. The amorphous nature of the glucose-syrup containing samples is in agreement with the thermomechanical studies in Fig. 1a and b that detected vitrification phenomena in these systems.

Conventional scanning electron microscopy was used to obtain tangible evidence of the network characteristics in these systems. Lyophilized single preparations of κ-carrageenan, in the potassium form and in the presence of added 50 mM K^+, produce aqueous gels made of rather broad and loose aggregates (Fig. 5a). Polysaccharide preparations with co-solute in Fig. 5b and d exhibit an amorphous morphology, which is similar to the futureless background observed in Fig. 5c for single glucose–syrup fluids (Al-Amri, Al-Adawi, Al-Marhoobi, & Kasapis, 2005). Anhydrous thiamin and potassium dihydrogen sulphate crystals are also presented in Fig. 5e and f to make a contrast with the smooth three-dimensional structures in the hydrated preparations.

3.4. Experimental observations of thiamin's diffusional mobility in the high-solid carbohydrate matrix using UV–vis spectroscopy

Colloidal control release is a novel technique for the delivery of micronutrients (e.g. vitamins and minerals) in foods and nutraceuticals in relation to providing an overall quality index for the delivery of bioactivity in processed materials (Bell & White, 2000; Bui & Small, 2007; Bui et al., 2013; Zhou & Roos, 2012).

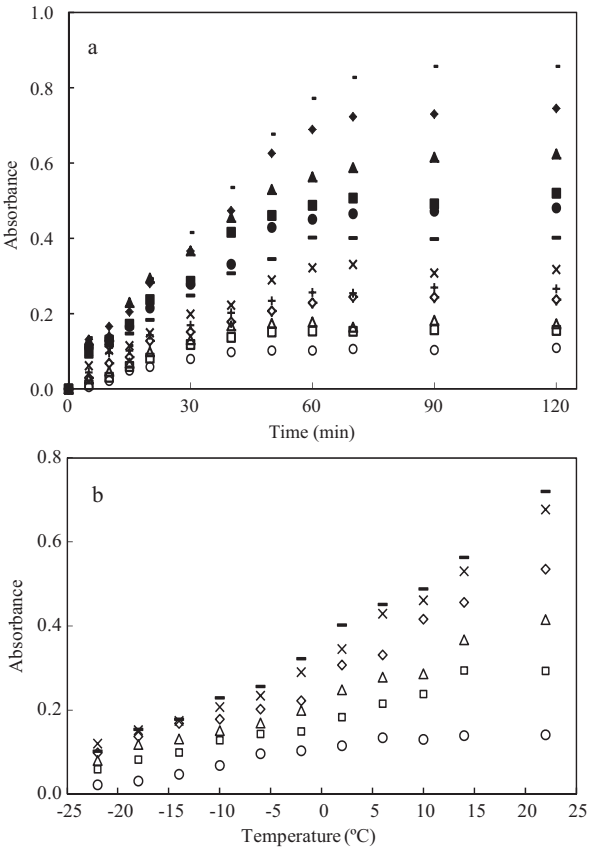

Fig. 6. Absorbance of 1% thiamin hydrochloride diffused from 82% glucose syrup with 2% potassium κ-carrageenan (50 mM K^+; pH 4.5) to ethylene glycol: (a) as a function of the time of observation at −22 (o), −18 (□), −14 (Δ), −10 (◊), −6 (+), −2 (×), 2 (−), 6 (●), 10 (■), 14 (▲), 18 (♦) and 22 (−)°C, and (b) as a function of experimental temperature for the periods of 10 (o), 20 (□), 30 (Δ), 40 (◊), 50 (×) and 60 (−) min, obtained at 578 nm.

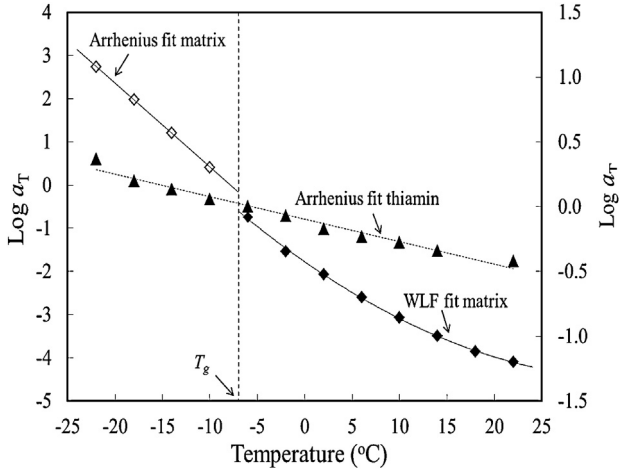

Fig. 7. Logarithmic shift factors, a_T, as a function of temperature within the glassy state (◊), and glass transition region (♦) for 82% glucose syrup plus 2% potassium κ-carrageenan with 1% thiamin (left *y*-axis), and for the kinetic data of 1% thiamin hydrochloride being diffused from the carbohydrate matrix to ethylene glycol (▲, right *y*-axis); reference temperature for both systems is −6 °C, with the dash line pinpointing the prediction of the network T_g.

It requires knowledge of the micronutrient's release kinetics in relation to the changing consistency of the high-solid structuring matrix in the time–temperature equivalence (Patel & Velikov, 2011; Pothakamury & Barbosa-Cánovas, 1995).

The present work elucidates the interplay between structural properties of a condensed matrix and diffusional mobility of an entrapped vitamin. Diffusion data, as a function of time and temperature of observation, were gathered with UV–vis spectroscopy, and the assay is based on the development of a colored ion-pair complex of thiamin hydrochloride and alizarin brilliant violet R in chloroform (Prasad et al., 1997). A bluish violet complex absorbed at λ_{max} of 578 nm yielding a highly linear relationship ($R^2 = 0.9992$) with the thiamin hydrochloride concentration in the ethylene glycol medium within the range of 0–0.74 Å (20 °C), which is in accordance with the Beer–Lambert law; calibration curve is not shown, but its equation of Absorbance = 121.340 × thiamin concentration (%) can be used for estimation of the vitamin amount released in the experimental time–temperature domain.

Observations on thiamin release from the κ-carrageenan/glucose syrup gel to ethylene glycol were carried out for 120 min within the temperature range of −22 to 22 °C, which according to Fig. 3b covers the glass transition region. It is evident in Fig. 6a that the absorbance of thiamin increases eight fold in this experimental timeframe but reaches an asymptotic equilibrium within the first 60 min of measurement. Increasing the temperature from subzero to ambient raises the intensity of micronutrient release, an outcome that argues for a considerable effect of the changing consistency in the polymeric matrix on the diffusion process. Fig. 6b illustrates these results by replotting them as a function of temperature for selected recording times. It is apparent that there is a slowdown in the rate of micronutrient mobility at subzero temperatures during the passage from the glass transition region to the glassy state, as argued from thermomechanical work in this system.

3.5. Modeling the kinetics of thiamin diffusion in the condensed carbohydrate matrix

Rationalization of the kinetic data describing thiamin's diffusional mobility was carried out by considering a linear relationship between absorbance and time of observation in the first 60 min in Fig. 6a. This section of the spectrum can be treated as a zero-order kinetic reaction with the gradient being constant at $k = dx/dt$. The recently introduced spectroscopic shift factor ($\log k_o/k$ in Jiang and Kasapis (2011)) was then utilized throughout the experimental temperature range, with k_o being the rate constant at the reference temperature of −6 °C. The outcome model is plotted in Fig. 7, where a direct comparison is afforded between kinetics of vitamin transport and relaxation of carbohydrate matrix.

The modified Arrhenius mathematical expression, i.e. Eq. (1) with two temperature terms provided a highly linear correlation ($R^2 = 0.980$) and a constant value of the activation energy (31.6 kJ/mol) for the diffusion of thiamin within the glassy matrix. In contrast, the activation energy of the carbohydrate matrix in this and earlier work is considerably higher, i.e. between 180 and 250 kJ/mol (Kasapis, 2001). This outcome argues that although the mobility of thiamin is restrained within the tertiary mixture, its mode of transport is distinct from the structural relaxation of the glassy matrix.

The next step of this work includes a correlation between the physics of the molecular processes taking place in Fig. 7 and the transport rate determining the diffusion of thiamin in the medium of this investigation. In doing so, we employed the concept of Fickian diffusion, which is based on the following power law equation (Pothakamury & Barbosa-Cánovas, 1995; Ritger & Peppas, 1987):

$$\frac{M_t}{M_\infty} = kt^n \quad (3)$$

where, M_t/M_∞ is the fractional release of a bioactive compound over the release time, t, k is a constant characteristic of the

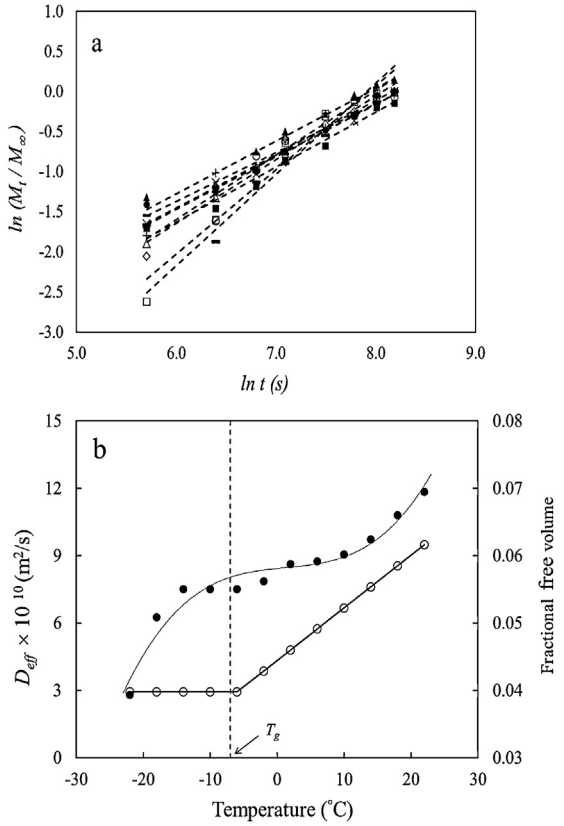

Fig. 8. (a) Plot of $\ln(M_t/M_\infty)$ versus $\ln t$ (s) to evaluate the diffusion exponent, n, and gel characteristic constant, k, for the high-solid matrix of potassium κ-carrageenan plus glucose syrup at −26 (o), −18 (□), −14 (Δ), −10 (◊), −6 (+), −2 (×), 2 (−), 6 (●), 10 (■), 14 (▲), and 22 (−)°C within an hour of observation arranged successfully upwards, and (b) effective diffusion coefficient of thiamin from the carbohydrate matrix to ethylene glycol as a function of temperature within an hour of observation (●, left y-axis), and fractional free volume of the high-solid matrix in the glass transition region and glassy state (o, right y-axis).

bioactive compound-polymer system, and n is the diffusion exponent for the release mechanism. Literature reports that normal Fickian diffusion is characterized by an n-value of 0.5, while Case II diffusion is defined by an n-value of 1.0, which makes the range of n-values between 0.5 and 1.0 the non-Fickian or anomalous diffusion.

Absorbance values within and at 60 min (M_t and M_∞, respectively) in Fig. 6a were used to produce the relevant parameters from the gradient and intercept of the plot $\ln M_t/M_\infty$ versus $\ln t$ (s) in Eq. (3). Thus, values of diffusion exponent and gel characteristic constant throughout the experimental temperature range were derived from Fig. 8a and are depicted in Table 2. Diffusion exponent estimates were found to lie between 0.61 and 0.87, hence arguing for an anomalous and rather rapid diffusion of the mainly hydrophilic thiamine molecule within the largely hydrophilic κ-carrageenan/glucose syrup matrix.

The above school of thought provides further insights in the form of a diffusion coefficient that can be identified in Fick's second law, as follows (Busk & Labuza, 1979):

$$\frac{\delta C}{\delta t} = D\frac{\delta^2 C}{x^2} \tag{4}$$

where C is the concentration of the diffusant molecule, t is the time of diffusion, D_{eff} is the effective diffusion coefficient, and x is the relative distance travelled.

Valid application of Fick's second law requires that the molecular sieve of the matrix maintains its physicochemical morphology

Table 2
Diffusion exponent (n) and gel characteristic constant (k) for the release of thiamin hydrochloride from the high-solid carbohydrate matrix.

Temperature (°C)	Diffusion exponent (n)	Gel characteristic constant (k) × 10^{-3}
−22	0.87	1.03
−18	0.86	0.98
−14	0.86	1.16
−10	0.80	1.64
−6	0.67	4.07
−2	0.65	4.52
2	0.64	4.86
6	0.61	6.53
10	0.69	5.34
14	0.66	3.59
22	0.83	0.93

throughout the diffusion process, which is the outcome argued in the spectroscopic observations in Fig. 4a and b. Further conditions of compliance require that the sample is a finite slab with negligible edge effects and the micronutrient release takes place in a single dimension within the geometry of the matrix. Under the above specified conditions, the theoretical Eq. (4) of Fick's second law can be solved in the form of a trigonometric series (Siepmann & Siepmann, 2008):

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2\pi^2} \quad \exp\left[\frac{-D_{\text{eff}}(2n+1)^2\pi^2 t}{L^2}\right] \tag{5}$$

where M_t and M_∞ denote the absolute accumulative amounts of compound release at time (t) and infinity (∞), n is a dummy variable in the algorithmic solution, D_{eff} is the effective diffusion coefficient of the compound within the matrix, and L is the slab's thickness.

Moisture transfer studies in breakfast cereals has shown that only the first term in this series equation is significant, which, in the absence of the higher terms, yields the following relationship (Tutuncu & Labuza, 1996):

$$\ln\left(\frac{M_\infty - M_t}{M_\infty - M_i}\right) = \ln\frac{8}{\pi^2} - \frac{D_{eff}\pi^2 t}{4L^2} \tag{6}$$

where M_i, M_t, and M_∞ denote the original absorbance reading in Fig. 6a for each experimental temperature, during experimentation and infinity/equilibrium, respectively, and L is the thickness of the slab.

Application of Eq. (6) to the absorbance data of thiamine release yields Fig. 8b, where the values of diffusion coefficient as a function of temperature are visualized. These show a considerable drop from about 12×10^{-10} m^2/s at 22 °C, i.e. within the glass transition region to 3×10^{-10} m^2/s at −22 °C, i.e. at the glassy state. Variation in the D_{eff} values follows closely progress in the fractional free volume of the carbohydrate supporting phase also plotted in Fig. 8b. Thus, the fractional free volume falls with controlled cooling from 0.062 to 0.039 at the mechanical glass transition temperature of −7 °C.

Clearly, the diffusional mobility of thiamin is controlled by the reduction in free space within the glassy state of the condensed matrix of κ-carrageenan with glucose syrup. Holes between packing irregularities of the carbohydrate gel allow diffusion of the micronutrient whose transport rate, as modeled by non-Fickian kinetics, can be related to the network glass transition temperature of the tertiary system. Estimates of thiamin's diffusion coefficient and polymeric free volume, reproduced in Fig. 8b, show a strong synchronization between physics and kinetic rate in the thermally induced process of vitrification.

4. Conclusions

It has long been known that bioactive compounds can be delivered in specific applications following preservation within systems of rubbery or glassy consistency. However, limited information is available on the effect of environmental conditions on the physics of vitrification in carbohydrate matrices, used as carriers of bioactivity, which determine the transport rates of diffusant molecules. The present work combines thermomechanical analysis and UV–vis spectroscopy to investigate the controlled release of thiamin embedded in a gelled κ-carrageenan/glucose syrup sample. Care was taken that the structural behavior of the constituents in the composite gel reflects the properties of single preparations thus being able to obtain the network glass transition of the mixture. The diffusion processes of the vitamin from the core to the surface of the carbohydrate matrix were followed using the spectroscopic shift factor, and were then modeled with the concept of non-Fickian kinetics. A similar pattern of behavior has been obtained for the diffusion of the hydrophilic molecule of Vitamin C in the congruent polarity of high-methoxy pectin with polydextrose being the supporting matrix (Panyoyai, Bannikova, Small, & Kasapis, 2014). In both cases, the diffusion rate of bioactive compound is moderated in accordance with the structural relaxation of the glassy matrix.

Acknowledgements

Scholarship awarded to Naksit Panyoyai by the Cooperation Office in The Civil Service Commission of the Thai Government is duly acknowledged.

References

Al-Amri, I. S., Al-Adawi, K. M., Al-Marhoobi, I. M., & Kasapis, S. (2005). Direct imaging of the changing polysaccharide network at high levels of co-solute. *Carbohydrate Polymers*, *61*, 379–382.

Almrhag, O., George, P., Bannikova, A., Katopo, L., Chaudhary, D., & Kasapis, S. (2012). Analysis on the effectiveness of co-solute on the network integrity of high methoxy pectin. *Food Chemistry*, *135*, 1455–1462.

Bell, L. N., & White, K. L. (2000). Thiamin stability in solids as affected by the glass transition. *Journal of Food Science*, *65*, 498–501.

Bui, L. L. T., & Small, D. M. (2007). The influence of formulation and processing on stability of thiamin in three styles of Asian noodles. *Food Chemistry*, *102*, 1394–1399.

Bui, L. T. T., Small, D. M., & Coad, R. (2013). The stability of water-soluble vitamins and issues in the fortification of foods. In V. R. Preedy, V. R. Srirajaskanthan, & V. B. Patel (Eds.), *Handbook of food fortification and health: From concepts to public health applications volume 1* (pp. 199–211). New York, NY: Humana Press, Springer.

Busk, J. R. G. C., & Labuza, T. P. (1979). A dye diffusion technique to evaluate gel properties. *Journal of Food Science*, *44*, 1369–1372.

Campo, V. L., Kawano, D. F., da Silva, D. B., Jr., & Carvalho, I. (2009). Carrageenan: Biological, chemical modification and structural analysis—A review. *Carbohydrate Polymers*, *77*, 167–180.

Chan, S. W., Mirhosseini, H., Taip, F. S., Ling, T. C., & Tan, C. P. (2013). Comparative study on the physicochemical properties of κ-carrageenan extracted from *Kappaphycus alvarezii* (doty) doty ex Silva in Tawau, Sabah, Malaysia and commercial κ-carrageenans. *Food Hydrocolloids*, *30*, 581–588.

Chaudhary, V., Small, D. M., & Kasapis, S. (2013). Structural studies on matrices of deaclated gellan with polydextrose. *Food Chemistry*, *137*, 37–44.

Evageliou, V., Kasapis, S., & Hember, M. W. N. (1998). Vitrification of κ-carrageenan in the presence of high levels of glucose syrup. *Polymer*, *39*, 3909–3917.

Ferry, J. D. (1980). *Dependence of viscoelastic behavior on temperature and pressure, in viscoelastic properties of polymers*. New York, NY: John Wiley.

Fisher, P., & Windhab, E. J. (2011). Rheology of food materials. *Current Opinion in Colloid and Interface Science*, *16*, 36–40.

Gunasekaran, S., & Ak, M. M. (2000). Dynamic oscillatory shear testing of foods-selected applications. *Trends in Food Science and Technology*, *11*, 115–127.

Hermansson, A. M., Eriksson, E., & Jordansson, E. (1991). Effects of potassium, sodium and calcium on the microstructure and rheological behaviour of kappa-carrageenan gels. *Carbohydrate Polymers*, *16*, 297–302.

Iijima, M., Hatakeyama, T., Takahashi, M., & Hatakeyama, H. (2007). Effect of thermal history on kappa-carrageenan hydrogelation by differential scanning calorimetry. *Thermochimica Acta*, *452*, 53–58.

Imeson, A. P. (2000). Carrageenan. In G. O. Phillips, & P. A. Williams (Eds.), *Handbook of hydrocolloids*. Abington, MA: CRC Press.

Jiang, B., & Kasapis, S. (2011). Kinetics of a bioactive compound (caffeine) mobility at the vicinity of the mechanical glass transition temperature induced by gelling polysaccharide. *Journal of Agricultural and Food Chemistry*, *59*, 11825–11832.

Kasapis, S., Mitchell, J., Abeysekera, R., & MacNaughtan, W. (2004). Rubber-to-glass transitions in high sugar/biopolymer mixtures. *Trends in Food Science and Technology*, *15*, 298–304.

Kasapis, S. (2001). The use of Arrhenius and WLF kinetics to rationalise the rubber-to-glass transition in high sugar κ-carrageenan systems. *Food Hydrocolloids*, *15*, 239–245.

Kasapis, S. (2006). Definition and applications of the network glass transition temperature. *Food Hydrocolloids*, *20*, 218–228.

Kasapis, S. (2008). Recent advances and future challenges in the explanation and exploitation of the network glass transition of high sugar/biopolymer mixtures. *Critical Reviews in Food Science and Nutrition*, *48*, 185–203.

Kumagai, H., MacNaughtan, W., Farhat, I. A., & Mitchell, J. R. (2002). The influence of carrageenan on molecular mobility in low moisture amorphous sugars. *Carbohydrate Polymers*, *48*, 341–349.

Lešková, E., Kubíková, J., Kováčiková, E., Košická, M., Porubská, J., & Holčíková, K. (2006). Vitamin losses: Retention during heat treatment and continual changes expressed by mathematical models. *Journal of Food Composition and Analysis*, *19*, 252–276.

Lesmes, U., & McClements, D. J. (2009). Structure-function relationships to guide rational design and fabrication of particulate food delivery systems. *Trends in Food Science and Technology*, *20*, 448–457.

Loret, C., Ribelles, P., & Lundin, L. (2009). Mechanical properties of κ-carrageenan in high concentration of sugar solutions. *Food Hydrocolloids*, *23*, 823–832.

Martins, J. T., Cerqueira, M. A., Bourbon, A., Pinheiro, A. G., Souza, B. W. S., & Vicente, A. A. (2012). Synergistic effects between κ-carrageenan and locust bean gum on physicochemical properties of edible films made thereof. *Food Hydrocolloids*, *29*, 280–289.

Michel, A-S., Mestdagh, M. M., & Axelos, M. A. V. (1997). Physico-chemical properties of carrageenan gels in presence of various cations. *International Journal of Biological Macromolecules*, *21*, 195–200.

Morris, V. J., & Chilvers, G. R. (1983). Rheological studies of specific cation forms of kappa carrageenan gels. *Carbohydrate Polymers*, *3*, 129–141.

Nickerson, M. T., & Paulson, A. T. (2005). Time–temperature studies of κ-carrageenan gelation in the presence of high levels of co-solutes. *Carbohydrate Polymers*, *61*(2005), 231–237.

Nishinari, K., & Watase, M. (1992). Effects of sugars and polyols on the gel–sol transition of kappa-carrageenan gels. *Thermochimica Acta*, *206*, 149–162.

Nishinari, K., Watase, M., Williams, P. A., & Phillips, G. O. (1990). κ-Carrageenan gels: Effect of sucrose, glucose, urea and guadinine hydrochloride on the rheological and thermal properties. *Journal of Agricultural and Food Chemistry*, *38*, 1188–1193.

Pachapurkar, D., & Bell, L. N. (2005). Kinetics of thiamin degradation in solutions under ambient storage conditions. *Journal of Food Science*, *70*, C423–C426.

Panyoyai, N., Bannikova, A., Small, D. M., & Kasapis, S. (2014). Diffusion kinetics of ascorbic acid in a glassy matrix of high-methoxy pectin with polydextrose. *Food Hydrocolloids*, http://dx.doi.org/10.1016/j.foodhyd.2014.07.01 (in press)

Patel, A. R., & Velikov, K. P. (2011). Colloidal delivery system in foods: A general comparison with oral drug delivery. *LWT-Food Science and Technology*, *44*, 1958–1964.

Prasad, P. S. S., Rajasree, K. P., Khan, K. A., & Reddy, M. N. (1997). Colorimetric determination of Thiamine hydrochloride using Alizarin brilliant violet R. *Indian Journal of Pharmaceutical Sciences*, *59*, 194–196.

Pothakamury, U. R., & Barbosa-Cánovas, G. V. (1995). Fundamental aspects of controlled release in foods. *Trends in Food Science and Technology*, *6*, 397–406.

Rahman, M. S. (2006). State diagram of foods: Its potential use in food processing and product stability. *Trends in Food Science and Technology*, *17*, 129–141.

Ritger, P. L., & Peppas, N. A. (1987). A simple equation for description of solute release I. Fickian and non-Fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs. *Journal of Controlled Release*, *5*, 23–36.

Roos, Y. H. (1995). Characterization of food polymer using state diagrams. *Journal of Food Engineering*, *24*, 339–360.

Roos, Y. H. (2003). Thermal analysis, state transitions and food quality. *Journal of Thermal Analysis and Calorimetry*, *71*, 197–203.

Roos, Y. H. (2010). Glass transition temperature and its relevance in food processing. *Annual Review of Food Science and Technology*, *1*, 469–496.

Siepmann, J., & Siepmann, F. (2008). Mathematical modeling of drug delivery. *International Journal of Pharmaceutics*, *364*, 328–343.

Tutuncu, M. A., & Labuza, T. P. (1996). Effect of geometry on the effective moisture transfer diffusion coefficient. *Journal of Food Engineering*, *30*, 433–447.

Zhou, Y., & Roos, Y. H. (2012). Stability and plasticizing and crystallisation effects of vitamins in amorphous sugar systems. *Journal of Agricultural and Food Chemistry*, *60*, 1075–1083.