Paper 2

by Vilia Paramita

Release mechanism of omega-3 fatty acid in κ-carrageenan/polydextrose undergoing glass transition

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ABSTRACT

A high-solid matrix of κ-carrageenan with polydextrose was developed to entrap α-linolenic acid, which is an omega-3 bioactive compound. Physicochemical analysis of this system utilised modulated DSC, dynamic oscillation in shear, ESEM, FTIR and WAX diffraction. The carbohydrate matrix was conditioned through an extensive temperature range to induce changes in molecular morphology and identify the network glass transition temperature. Thermally induced variation in phase morphology was employed to rationalise transportation patterns of the bioactive compound within the high-solid preparation. Thus, experimental observations using UV–vis spectroscopy modelled diffusion kinetics to document the mobility arresting effect of the vitrifying matrix on the micro-constituent. Within the glass transition region, results argue that free volume theory is the molecular process governing structural relaxation. Further, Less Fickian diffusion follows well the rate of molecular transport of α-linolenic acid as a function of time and temperature of observation in the condensed matrix.

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1. Introduction

α-Linolenic (ALA, C18:3, $n-3$) is a long chain polyunsaturated fatty acid with double bonds at 9, 12 and 15-carbon from the carboxylic acid end. This fatty acid is known as a precursor of longer chain omega-3 fatty acids including the eicosapentaenoic (EPA, C20:5, $n-3$) and docosahexaenoic (DHA, C22:6, $n-3$) acids. These lipids are considered to be essential to wellbeing, since they impart many health benefits and can only be acquired through the diet (Jacobsen, 2011). Consequently, attempts have been made to increase the level of α-linolenic acid in the diet by incorporating it in processed foods. The challenge remains, however, to efficiently design protective matrices in order to prolong bioavailability by limiting oxidation, due to the polyunsaturated nature of the fatty acid, in novel formulations (Ma, Floros, & Zieger, 2011).

Oxidation of omega-3 fatty acids leads to undesirable changes in sensory perception and nutritional profile. Thus, protective and controlled delivery of these essential fatty acids in processed foods attempts to significantly improve preservation status. Commonly, efforts focus on protocols of empirical utility that protect fatty acids against oxidative substances through encapsulation in biopolymer shells or physicochemical binding with antioxidants (Karmas, Buera, & Karel, 1992). Entrapment of essential fatty acid in condensed systems, i.e. above seventy percent solids in formulations, is increasing of considerable interest (Karel et al., 1994). The novelty behind this approach lies in the phenomenon of glassy consistency that is common in dehydrated preparations being able to curb physicochemical, biological and enzymatic reactions (Le Meste, Champion, Roudaut, Blond, & Simatos, 2002).

Informed manipulation of structural functionality based on type and concentration of biopolymer, physicochemical environment, e.g. pH and/or ionic strength, and the addition of co-solute in the form of maltodextrins, sugar and their substitutes, engineers a temperature range of interest where molecular rearrangements are retarded (Farhat, Mousia, & Mitchell, 2003). Thus, the concept of glass transition temperature, T_g, has been evolved from an empirical index of convenience to a fundamental parameter that rationalises the preservation of bio- and technofunctionality in systems on the basis of an amorphous vitreous transition (Rieger, 2001). This has been used in following the glass-to-rubber transformation in a plethora of biomaterials (Gunning, Parker, & Ring, 2000) and, more recently, in the elucidation of diffusional kinetics of bioactive compounds in high-solid matrices; the examples of caffeine and vitamin B1 among others are cited here (Jiang & Kasapis, 2011; Panyoyai, Bannikova, Small, & Kasapis, 2015).

The current investigation aims to extend fundamental understanding on the physics and kinetic rates of molecular mobility in condensed carbohydrate matrices. It takes advantage of earlier work on structure formation based on associations between potassium ions and κ-carrageenan helices according to the domain

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Frequency vibrations of κ-carrageenan, in the presence or absence of α-linolenic acid, are intensified as polydextrose is incorporated into the system. Spectra of the two mixtures follow that of 85% polydextrose, showing peaks at 1200–900 cm^{-1} of C—O—C and C—O ring vibrations being typical of carbohydrate systems. Additional bands at 1500–1100 cm^{-1} correspond to C—C stretch, C—H bend and C—O stretch of alcohol, carboxylic acids, or ester groups (Probst et al., 2013).

α-Linolenic acid shows the characteristic spectrum of most oils with C—H, C=O, C—C, and C—O bands in regions of 3000–2850, 1710–1665, 1500–1400 and 1320–1000 cm^{-1}. Further, the spectra between 1000 and 700 cm^{-1} are important suggesting =C—H bands that correspond to the degree of unsaturation of the fatty acid (Guillén & Cabo, 1997). Lack of shift or presence of extra peaks in the molecular vibrations of Fig. 4b indicates the absence of substantial physicochemical interactions amongst the three components, i.e. κ-carrageenan, polydextrose and α-linolenic acid, of this work.

Scanning electron microscopy provides tangible evidence that native κ-carrageenan powder (Fig. 5a) forms bulky flocks with uneven surfaces, whereas freeze-dried aqueous preparations of 2% κ-carrageenan show cross-sections that are fragmented but with a rather smooth morphology (Fig. 5b). Polydextrose powder exhibits
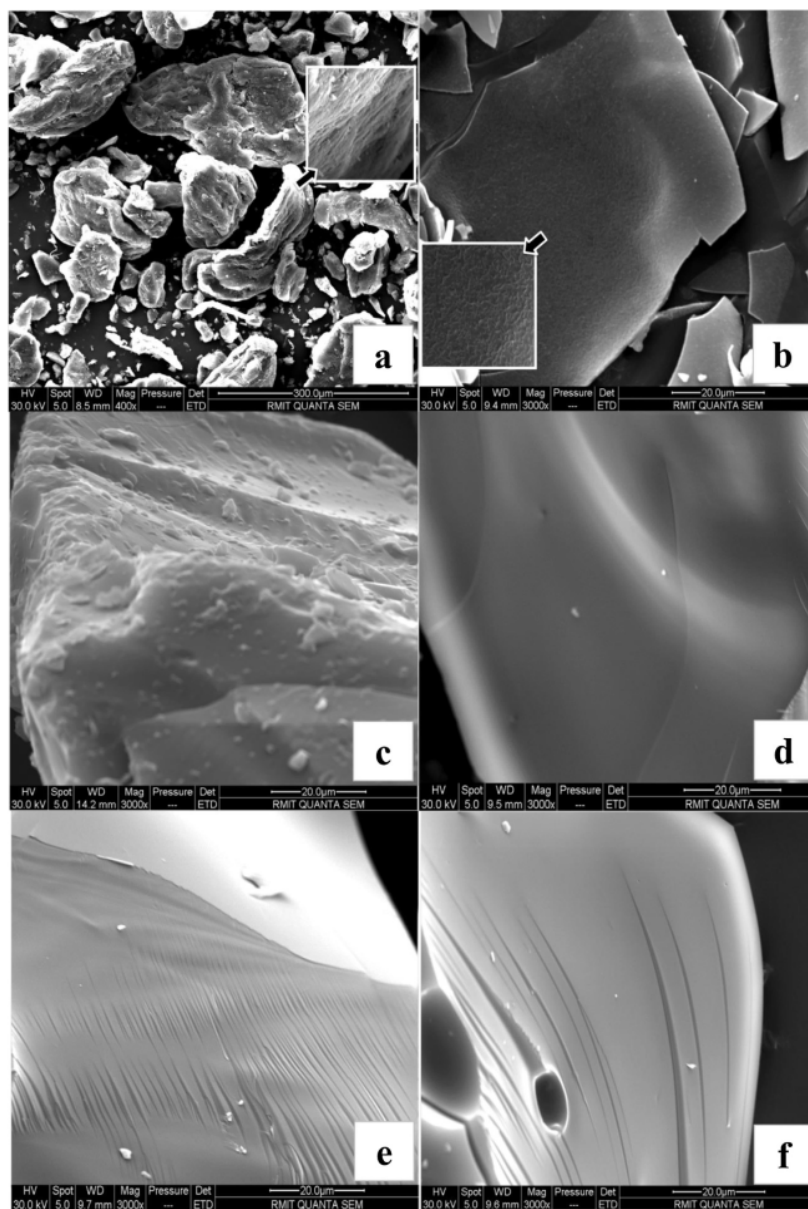

Fig. 5. SEM micrographs for freeze-dried samples of (a) κ-carrageenan powder, (b) 2% κ-carrageenan, (c) polydextrose powder, (d) 85% polydextrose, (e) 2% κ-carrageenan with 83% polydextrose and (f) 2% κ-carrageenan with 82% polydextrose and 1% α-linolenic acid.

large agglomerates with a relatively uneven surface (Fig. 5c), which contrast strongly with the featureless characteristics of the micrograph depicting condensed preparations at 85% solids (Fig. 5d). Composite matrices comprising the polysaccharide, the co-solute in the absence (Fig. 5e) or presence (Fig. 5f) of the fatty acid exhibit coherent structures that flow in amorphous streams, arguing for efficient entrapment of the micro-constituent within the dense carbohydrate matrix.

3.3. Release kinetics of α-linolenic acid in the κ-carrageenan/polydextrose system

The early work of Shimada, Roos, and Karel (1991) focused on oxygen permeability in an amorphous lactose matrix in relation to oxidation of methyl linoleate. The aim of the present study is to identify the physics and quantify the kinetics of fatty acid release within a glassy system. Release of the micro-constituent from the condensed κ-carrageenan/polydextrose matrix to dichloromethane was followed with the sulfo-phospho-vanillin method. The assay is based on the hydrolysis of linolenic acid by sulphuric acid producing a carbonium ion that develops a pink adduct upon reacting with phospho-vanillin reagent. Colour is recorded as total fatty acid diffused to dichloromethane at $\lambda_{max} = 525$ nm. The relationship between fatty acid concentration and absorbance returns a very acceptable fit that follows the Beer–Lambert Law from 0 to 0.998 AU (data not shown here).

Rate of fatty acid diffusion is presented in Fig. 6a extending for 3.5 h over the temperature range of −19 to 22 °C, which encompasses much of the glass transition region and the glassy state. Increasing time and temperature of observation results in higher release rates of the micro-constituent from the carbohydrate sample to dichloromethane. In particular, release is rapid in the first 60 min and approaches an asymptotic equilibrium at longer times of observation. The magnitude of increase varies from 0.30 AU at −19 °C to 0.95 AU at 22 °C over the timeframe of 3.5 h. Data is replotted as a function of temperature in Fig. 6b to demonstrate the drop in the values of absorbance within the glassy state of the κ-carrageenan/polydextrose matrix. Statistical analysis of data demonstrates that time and temperature significantly influence the rate of diffusion of the fatty acid in Fig. 6a ($p < 0.05$ from two-ways ANOVA test), and temperature intervals of four degree centigrade in Fig. 6b were also significant in changing molecular mobility ($p < 0.05$ from Tukey's post hoc analysis).

Absorbance-time data in Fig. 6a exhibited a very acceptable linearity ($r^2 = 0.962 \pm 0.020$) for all temperatures tested in the first 60 min and their gradient was employed to calculate the rate constant considering a zero-order reaction rate ($k = dx/dt$). Based on this, the concept of spectroscopic shift factor was developed for each experimental temperature, as follows (Kasapis & Shrinivas, 2010):

$$\log a_T = \log \frac{k_o}{k} \qquad (4)$$

where k_o is the rate constant at the reference temperature of −7 °C (as for the earlier studies of rheological simplicity in Fig. 3b).

Fig. 7 contrasts the molecular dynamics, as seen in shift-factor variation in relation to experimental temperature, for the polymeric sample and bioactive compound. The latter produces a good quality linear relationship that allows utilisation of the modified Arrhenius equation to estimate the energy of activation ($E_a = 20$ kJ/mol). This is contrasted with the corresponding value for the macromolecular system within the glassy state yielding an E_a value of 233 kJ/mol. Clearly, the energy barrier for structural relaxation of the carbohydrate molecules is much higher than for the diffusional mobility of the fatty acid. The two molecular processes exhibit distinct temperature profiles but it is the vitrification state
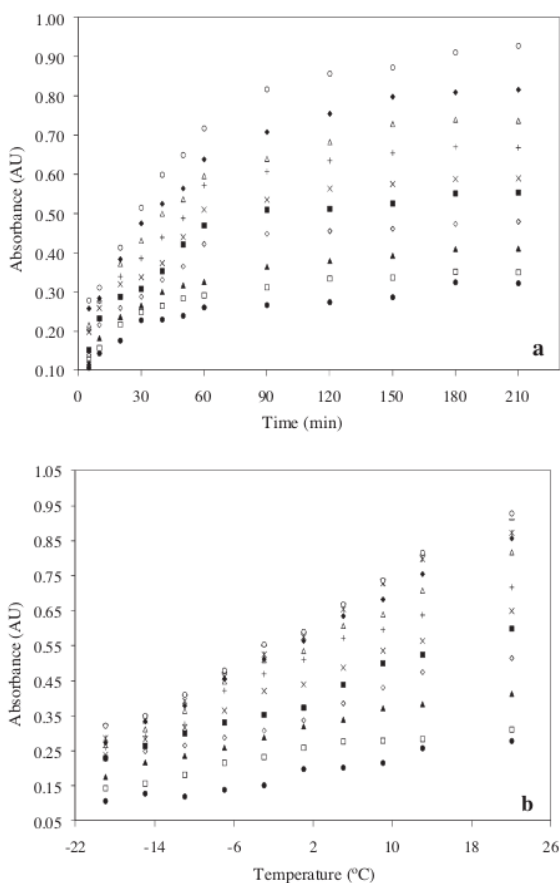

Fig. 6. (a) Absorbance of 1% α-linolenic acid diffused to dichloromethane from 2% κ-carrageenan with 82% polydextrose (50 mM added KCl) as a function of time of observation at −19 (●), −15 (□), −11 (▲), −7 (◇), −3 (■), 1 (×), 5 (+), 9 (△), 13 (◆) and 20 (○) °C obtained at 525 nm; (b) Absorbance of 1% α-linolenic acid diffused to dichloromethane from the same carbohydrate sample as a function of temperature at 5 (●), 10 (□), 20 (▲), 30 (◇), 40 (■), 50 (×), 60 (+), 90 (△), 120 (◆), 150 (*), 180 (−) and 210 (○) min obtained at the same wavelength.
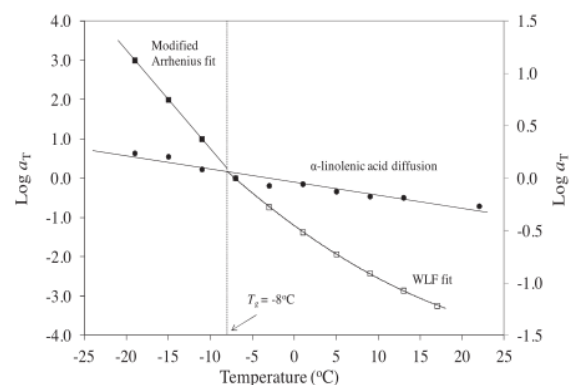

Fig. 7. Logarithmic shift factor as a function of temperature for 1% α-linolenic acid diffused to dichloromethane from the 2% κ-carrageenan with 82% polydextrose matrix at the reference temperature of −7 °C (trace on the right y-axis), with the structural relaxation of the carbohydrate matrix also shown within the glassy state and glass transition region (trace on the left y-axis).
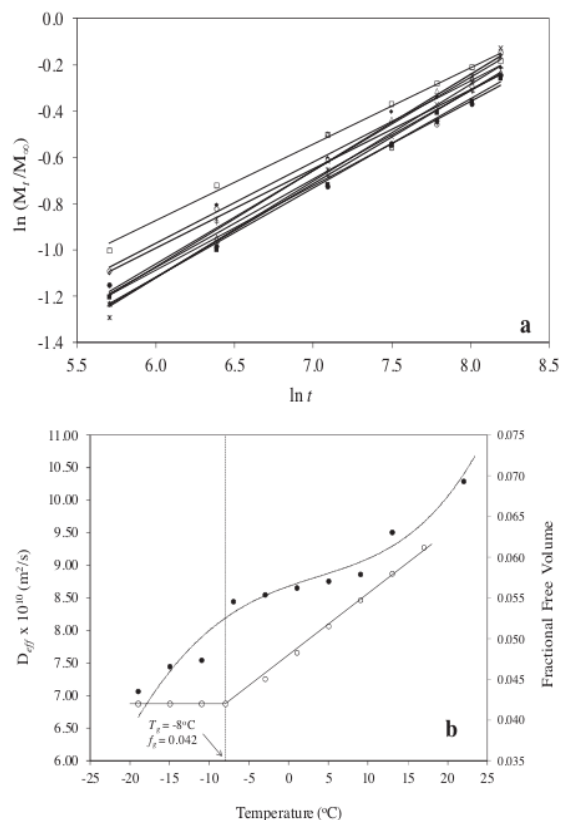
Table 2
Variation of the kinetic diffusion exponent (n) and gel characteristic constant (k) for α-linolenic acid in κ-carrageenan and polydextrose.

Temperature (°C)	Kinetic diffusion exponent, n	Gel characteristic constant, k	Diffusion mechanism
22	0.35	5.67E−04	Less Fickian
13	0.34	7.54E−04	Less Fickian
9	0.34	7.96E−04	Less Fickian
5	0.33	9.79E−04	Less Fickian
1	0.31	1.68E−03	Less Fickian
−3	0.34	8.31E−04	Less Fickian
−7	0.32	1.27E−03	Less Fickian
−11	0.32	1.24E−03	Less Fickian
−15	0.26	4.27E−03	Less Fickian
−19	0.28	2.73E−03	Less Fickian

Fig. 8. (a) Variation of absorbance with different times of observation at −19 (◆), −15 (□), −11 (△), −7 (×), −3 (*), 1 (○), 5 (+), 9 (−), 13 (●) and 22 (■) °C; (b) Fractional free volume of 2% κ-carrageenan with 82% polydextrose and 1% α-linolenic acid (○, trace on the right y-axis) and relative diffusion coefficient of the fatty acid from the same carbohydrate matrix within 60 min of observation (●, trace on the left y-axis).

and free volume of the κ-carrageenan/polydextrose matrix that dictates the kinetic rates of α-linolenic acid transportation.

Next, we delved deeper into the mobility of the fatty acid by considering a Power Law equation that was first developed from Fick's Law to monitor the sorption/desorption of water molecules in breakfast cereals (Ritger & Peppas, 1987; Singh & Chauhan, 2009):

$$\frac{M_t}{M_\infty} = kt^n \tag{5}$$

where n is the kinetic diffusion exponent, M_t/M_∞ is the extent of α-linolenic acid release at experimental and equilibrium time, k is a constant characteristic of the bioactive compound–polymer system and t is given in seconds.

Plotting the natural logarithms of M_t/M_∞ versus time, from data in the first 60 min of Fig. 6a, produces the required values of n and k for the various experimental temperatures in Fig. 8a ($r^2 = 0.981 \pm 0.011$), which are reproduced in Table 2. The kinetic diffusion exponent in this investigation was found to vary from 0.26 to 0.35. Based on Fick's law, the ideal transport mechanism has an n-value equal to 0.5, whereas the rapid Case II transport is characterised by an n-value of 1.0. This makes the range of n-values between 0.5 and 1.0 a non-Fickian or anomalous diffusion (Bajpai, Bajpai, & Shukla, 2001). The low diffusion exponent of this investigation, i.e. Less Fickian with values below 0.5, reflects a low mobility due to the dense state of the vitrified polymeric matrix

and the amphiphilic nature of α-linolenic acid that may interact in part with κ-carrageenan and/or polydextrose.

Fick's second law can be further utilised to estimate the diffusion coefficient, D_{eff}, of a small molecule within a solid-like macromolecular system (Busk & Labuza, 1979; Crank, 1975):

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} \tag{6}$$

where c is the concentration of the diffusant molecule, x is the relative distance travelled and t is time in seconds. Siepmann and Peppas (2011) integrated Eq. (6) for samples of slab shape, where diffusion occurs through the slab's surface without any edge effects, to produce the following error function series:

$$\frac{M_t}{M_\infty} = 4\left(\frac{D_{eff}t}{L^2}\right)^{1/2}\left[\left(\frac{1}{\pi^{1/2}}\right) + 2\sum_{n=1}^{\infty}(-1)^n ierfc\frac{nL}{2\sqrt{D_{eff}t}}\right] \tag{7}$$

where the term $ierfc\ x$ represents the integrated complementary error function of x. Earlier work in various food systems demonstrated that only the first term in this series equation is of consequence, which yields the below relationship (Tutuncu & Labuza, 1996):

$$\frac{M_\infty - M_t}{M_\infty - M_i} = 4\left(\frac{D_{eff}t}{\pi L^2}\right)^{1/2} \tag{8}$$

where, M_i, M_t and M_∞ denote the absolute amounts of the diffusant compound released at times zero, during experimentation and infinity/equilibrium, respectively, and L is the thickness of the slab.

Fig. 8b reproduces values of the diffusion coefficient over the temperature range that leads to carbohydrate vitrification. This is further compared with the fractional free volume of the matrix producing a discontinuity at the mechanical glass transition temperature of −8 °C. As the fractional free volume increases from 0.042 in the glassy state to 0.061 at ambient temperature, values of diffusion coefficient also increase from 8.5×10^{-10} to 10.3×10^{-10} m^2/s. A sudden drop in D_{eff} estimates down to 7.1×10^{-10} m^2/s is recorded at temperatures below the network T_g. Clearly, vacant spaces amongst the adjacent macromolecules of κ-carrageenan and polydextrose govern the nature and mobility rate of α-linolenic acid in the vitrified matrix.

Phenomenological estimations of the diffusion coefficient follow an approach that has proved to be a mainstay of utility in recording and then modelling experimental data to unveil the kinetics of drug delivery (Siepmann & Siepmann, 2008). Furthermore, interest in this work lies in proposing a relationship between free volume of the polymeric matrix and diffusion kinetics of the bioactive compound in the vitrified mixture, as shown in Fig. 8b. Computer simulations have also been employed in an attempt to predict the techno-functionality of self-assembling colloidal materials (de las Heras, Tavares, & Telo da Gama, 2012).

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