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Rate of fatty acid transport in glassy biopolymers: A free volume based predictive approach

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ABSTRACT

Metastable properties of biopolymer networks affect significantly the diffusion kinetics of bioactive compounds. That was shown to be the case in high solid samples of protein and polysaccharide supporting a homogeneous distribution of polyunsaturated fatty acids. Thermomechanical behaviour of these matrices was characterised in relation to their glass transition temperature (T_g). A free volume theory of diffusion was considered to treat transport phenomena of fatty acids within glassy polymers. It was found that at $T > T_g$ the effective diffusion coefficient of microconstituent transport would increase in accordance with the free volume of the polymer matrix. Fitting experimental diffusivity data in glassy polymers to a free volume based theory generates a two-parameter equation that calculates the extent of molecular interaction between macromolecule and microconstituent. Gradual substitution of polymer with small-molecule co-solute, glucose syrup in this case, induces a plasticising effect that profoundly affects the level of interaction, hence the diffusion of fatty acids in the condensed biomaterial.

This article has been written in celebration of the scientific work of Professor Glyn O. Phillips on the occasion of his 90th birthday in 2017. He is a Founding Father of research in structure-function relationships of industrial hydrocolloids and Founder of the prestigious journal *Food Hydrocolloids*. We wish him many happy returns in leading the field of hydrocolloid research.

In the area of high-solid systems, biopolymer mobility at the vicinity of the glass transition temperature is related to complex molecular phenomena. Within the glass transition region, amorphous viscoelastic materials see a dramatic reduction in free volume with rapid cooling, which can be monitored as a broad variation in heat capacity or steady shear viscosity (Perez, 1990; Roudaut & Champion, 2011). The dramatic decrease in viscosity is accompanied by reduced thermal vibration leading to limited translational mobility that promotes physicochemical stability (Le Meste, Champion, Roudaut, Blond, & Simatos, 2002; Roudaut, Maglione, van Dusschoten, & Le Meste, 1990). Devitrification can be achieved by increasing the temperature above T_g, with the frozen-in molecules starting to resonate leading to a structural relaxation of the condensed matrix (Roudaut, Simatos, Champion, Countreras-Lopez, & Le Meste, 2004).

Mechanical properties of the polymeric material within the glass transition region can be followed with the free volume theory, as quantified by the Williams-Landel-Ferry (WLF) equation (Ferry, 1991; Kasapis, 2009, pp. 225–260). The approach is followed presently for several matrices based on natural polymers, i.e. high methoxy pectin/glucose syrup, κ-carrageenan/polydextrose, and whey protein/glucose syrup at various combinations: 100:0, 80:20, 70:30, 60:40, 40:60 and 0:100 (w/w).

We prepared polysaccharide matrices that were composed of 3% (w/w) high-methoxy pectin (HMP) with 81% (w/w) glucose syrup and 2% (w/w) κ-carrageenan with 83% (w/w) polydextrose. To each formulation, 1% (w/w) of fatty acid, i.e. oleic acid and α-linolenic acid were added, respectively. Polysaccharide powder was dissolved in Milli-Q water at about 90 °C, followed by cooling to 50 °C for the addition of co-solute. Temperature was decreased even further to 40 °C prior to fatty acid addition. A 2 M HCl solution was added to the HMP/glucose syrup mixture to obtain pH 3, which is needed for gelation, and 50 mM KCl solution was utilised for gelation of the κ-carrageenan/polydextrose sample. These were concentrated under vacuum to achieve 85% (w/w) total solids.

Whey protein/glucose syrup (wp/gs) matrices were prepared by dissolving whey protein isolate at ambient temperature for 2 h in Milli-Q water, diluting the glucose syrup solution in Milli-Q water and mixing them up at appropriate levels to create a system of 30% total solids. That was stirred for 15 min prior to addition of 1% (w/

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w) fatty acid. Mixture was stirred for another 30 min, homogenised using Ultra-Turrax T25 at 3000 rpm for 3 min and condensed under vacuum to obtain an overall level of solids of 80% (w/w).

All systems were subjected to small deformation dynamic oscillation in-shear to determine the mechanical transformation from "rubbery-to-glassy consistency". In doing so, frequency sweeps from 0.1 to 100 rad/s were recorded and data were superposed horizontally at an arbitrarily chosen reference temperature (T_o) within the glass transition region. This is the so-called time-temperature superposition principle that produces the master curve of viscoelasticity and a series of shift factors (a_T) due to the horizontal superposition of mechanical spectra to generate the master curve (Kasapis, 2006; Oroian, Amariei, Escriche, & Gutt, 2013).

Reduced solid-like (G') and liquid-like (G'') shear modulus in the form of shift factor were modeled with the WLF equation (Ferry, 1980):

$$\log a_T = -\frac{C_1^0(T - T_o)}{C_2^0 + (T - T_o)} \quad (1)$$

$$C_1^0 = \frac{B}{2.303 f_0} \quad \text{and} \quad C_2^0 = \frac{f_0}{\alpha_f} \quad (2)$$

where, C_1^0 and C_2^0 (deg) are the WLF constants, f_o is the fractional free volume of the material at T_o, α_f is the thermal expansion coefficient (deg^{-1}) above T_g, and B is usually taken as one (Hoare & Kohane, 2008).

Table 1 summarises data from this school of thought for the polysaccharide or whey protein matrices plus co-solute for total solid levels of 80 or 85% (w/w). For each system, the mechanical glass transition temperature is estimated alongside the free volume at T_g, i.e. f_g. In the case of whey protein/gose syrup preparations, free volume at −16 °C increases from 0.032 to 0.058 without and with the co-solute. That should be attributed to the plasticising effect of glucose syrup leading to higher estimates of free volume and lower values of the glass transition temperature, i.e. from −16 to −39 °C for the 100:0 and 0:100 formulations, respectively.

Besides the structural observations within the glass transition region, properties of biomaterials within the glassy state, i.e. at $T < T_g$, are of interest. Here, structural relaxation of polymeric networks and molecular motion of chain segments are retarded considerably to produce stable systems over a prolonged storage period (Kasapis, 2012; Zhang & Wang, 1987a). Within the glassy state, shift factors derived from the superposition of frequency sweeps do not obey the free volume theory, and instead configurational rearrangements from one state to another follow the modified Arrhenius equation that includes a set of two experimental temperatures (Kasapis, 2008):

$$\log a_T = \frac{E_a}{2.303R}\left(\frac{1}{T} - \frac{1}{T_o}\right) \quad (3)$$

where, E_a is the activation energy for the extremely slow molecular flow and R is the gas constant of 8.31 J K^{-1} mol^{-1}.

Activation energy estimates for the glassy state of all systems are given in Table 1. Clearly, the compactness of the globular protein molecule (Bocque, Voirin, Lapinte, Cailol, & Robin, 2016) accounts for the high values of E_a (335 kJ/mol in 100:0). This is in contrast to the lower estimates for polysaccharide chains that dissolve in the high solid environment of glucose syrup and polydextrose, for example, 233 kJ/mol for the κ-carrageenan preparation. Inclusion of co-solute in the whey protein matrix reduces the activation energy required for configurational rearrangements in the glassy state to 206 kJ/mol in the 40:60 mixture. Again, the plasticising effect of glucose syrup on the polymer network is demonstrated, as reported earlier for the values of free volume.

Introduction of polyunsaturated fatty acids (1% w/w) to the biopolymer/co-solute matrix generates a new dimension of molecular mobility, which relates to the diffusion of the microconstituent from the glassy polymer to a liquid medium. The latter was chosen to be an organic liquid (ethanol, dichloromethane or ethyl acetate) that is immiscible with the matrix in order to ensure stationary boundaries during experimentation. The rate of diffusion was monitored as a function of time and temperature and analysed for total lipids using a chromophoric method (sulfo-phospho-vanillin assay).

UV−vis data of fatty acid release as a function of time for each experimental temperature were found to follow a linear relationship, with the gradient yielding the rate constant of a zero order kinetic ($k = dx/dt$). In an analogous manner to the superposition of frequency sweeps yielding the mechanical shift factor, absorbance data at a series of assayable temperatures were superposed. That facilitated calculation of the spectroscopic shift factor, as follows (Kasapis & Shrinivas, 2010):

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

where, k_o is the rate constant of fatty acid diffusion at the reference temperature. T_o. Plotting the spectroscopic shift factors within the glass transition region creates a good linear relationship indicating an Arrhenius type mechanism of fatty acid diffusion for our environmental conditions. Energy of activation for the diffusion of fatty acids in Table 1 ($E_{a,FA}$) is much lower than for the polymeric counterparts ($E_{a,M}$), an outcome which argues that the molecular mobility of the microconstituent is decoupled from the structural

Table 1
Parameters characterising structural properties of polymer matrices and diffusional mobility of fatty acids.

Matrix	HMP/GS	κ-Car/PD	WP/GS					
			100:0	80:20	70:30	60:40	40:60	0:100
Fatty acid	Oleic acid	α-linolenic acid	Linoleic acid					
Total solids (%)	85	85	80	80	80	80	80	80
T_g (°C)	−15	−8	−16	−18	−22	−26	−35	−39
f_g	0.040	0.042	0.040	0.034	0.035	0.037	0.040	0.040
f at −16 °C	0.041	0.042	0.032	0.035	0.039	0.044	0.055	0.058
$E_{a,M}$ (kJ/mol)	251	233	335	296	275	230	206	193
$E_{a,FA}$ (kJ/mol)	24	20	55	52	49	46	42	41
Diffusion constant (n)	0.34	0.35	0.85	0.83	0.69	0.68	0.65	0.60
Diffusion coefficient ($\times 10^{-10}$ m^2/s) at −16 °C	22.6	7.36	1.63	1.70	1.86	2.04	2.31	2.50
Coupling parameter, ξ ($\times 10^{-3}$)	5.99	4.25	5.80	5.99	6.43	6.69	6.95	7.55

HMP stands for high-methoxy pectin; *κ-Car* is κ-Carrageenan; *WP* is whey protein; *GS* is glucose syrup; *PD* is polydextrose; f and f_g are the fractional free volumes at T and T_g, respectively; $E_{a,M}$ and $E_{a,FA}$ are the activation energies of polymer matrix and fatty acid, respectively.

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