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A fundamental approach for the estimation of the mechanical glass transition temperature in gelatin

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Abstract

The paper constitutes an attempt to overcome the empiricism prevalent in the estimation of the glass transition temperature (T_g) of gelatin networks using rheological techniques. In doing so, it presents a study of the viscoelastic properties of a well-characterised gelatin sample covering the structural properties from the rubbery region to the glassy state. The pattern of oscillatory behaviour on shear is given by a master curve produced by shifting data obtained at different temperatures along the logarithmic time scale. Data reduction does not hold for all temperatures thus giving rise to thermorheological complexity. Within the temperature range at which molecular processes are represented by a simple distribution of relaxation times, a fundamental argument is developed to pinpoint the mechanical T_g. This should improve confidence in measured glassy properties over the empirical indicators found in the literature. As a demonstration, the glass transition temperature of gelatin at "zero moisture" obtained using the proposed framework of analysis is contrasted with earlier attempts to identify the mechanical T_g of gelatin solids.

Keywords: Mechanical T_g; Gelatin; Time–temperature superposition

1. Introduction

Until a relatively short time ago, the molecular mechanisms responsible for the structural properties of biomaterials that exhibit a "glassy" consistency were only poorly understood [1]. At first, experiments in this field were concerned mainly with obtaining a broad survey of the phenomena involved [2]. The advent of a firm theoretical picture on molecular dynamics underlined the process of the "rubber-to-glass transition" in synthetic materials [3]. This paved the way for a more intensive experimental investigation in order to provide a comprehensive picture of vitrification in small organic molecules, biopolymers and their mixtures.

Differential scanning calorimetry (DSC) has been used mainly to measure as a function of temperature the difference in energy inputs into a substance and its reference, thus assisting in the build up of a database of functional properties in a plethora of model systems. Prime examples of this type of work include the temperature band of vitrification of aqueous carbohydrate solutions in terms of the onset (T_{g_1}), middle (T_{g_2}), and completion (T_{g_3}) of changes in the heat flow trace [4]. Starch hydrolysates are essential constituents of foods and pharmaceuticals, and it was ascertained that, critically, there is no effect of molecular weight on the DSC T_g at conditions above the molecular weight of coil overlap and entanglement [5].

The importance of the glassy state was discussed in relation to the physicochemical state of various industrial processes, and the aging of products leading to structural collapse, chemical reactions and enzymic activity [6]. Other studies outlined the difficulty of modelling the distribution of relaxation processes in phase separated/complex mixtures of drug matrices, and introduced the concept of mobility transition temperatures of residual water below T_g [7]. Clearly, the strength of this field is that it brings together activities so

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diverse in their approach and interests (foods and pharmaceuticals) under the unifying umbrella of the glass phenomenon.

During the past several years, there has also been an increasing interest in the viscoelastic properties of bioglasses following the advent of advanced measuring devices (transducers) and microcomputers [8]. To date, reproducible experiments have been restricted to behaviour under "small to moderate loadings" but experimental temperatures stretch far below the glass temperature of these materials. Two notable attempts have been made to provide a protocol of identifying and rationalising general glassy behaviour in biomaterials. It is now clear that the first attempt can be no more than qualitatively correct since it fails to identify a theoretical basis for relating molecular mobility to specific aspects of pictorial rheology [9,10]. Shortcomings of this approach will be discussed in the following sections of the paper.

The second attempt, due to Ferry [11], was restricted to the case of the response of a synthetic polymer network to a sinusoidal force within the linear viscoelastic region. This method has not been extended, apparently because of the relative complexity of the formalism of the time–time superposition, and the separation of the variables of time and temperature in the mechanical properties of biomaterials [12]. Recently, we treated the case of polysaccharides at normal levels of industrial use (up to 2%) in the presence of high levels of sucrose and/or glucose syrup (60–90%) to a sinusoidal driving force on shear. Isochronal heating or cooling unveiled three distinct phenomena: the rubbery plateau, the glass transition region (α relaxation), and the glassy state [13]. A theoretical foundation of data analysis was pursued by separating the mechanical properties of linear viscoelasticity into a basic function of time alone and temperature alone. In this paper, we show that the equations of the free volume and reaction-rate theories can predict the observed shape and superposition properties found by means of sinusoidal vibration methods. This should offer a fundamental basis, as compared to existing indices, for the determination of the glass transition temperature in high-solid gelatin networks.

2. Experimental

2.1. Materials

The gelatin sample came in the form of a fine powder and it was prepared especially for research from Sanofi Bio-Industries, Baupte, Carentan, France. It was the first extract from a single batch of cowhide produced by alkaline hydrolysis of collagen (type B). Compared to the acidic process on pigskin, cowhides require the longer and more drastic lime treatment before extraction, as the skins are much older. In this case, the treatment may last several months and the long soak converts many of the basic side chains into acidic groups thus reducing considerably the resultant gelatin's isoelectric point (pI = 4.5 in our case) due to the amidolysis of asparagine and glutamine amino acid residues. Table 1 reproduces analytical characteristics of the sample, which were determined by the manufacturer. The isoelectric point was measured by completely deionising the sample on a bed of ion-exchanged resin and then measuring the pH of the eluant. Gel permeation chromatography was used to identify the number average molecular weight (M_n) of the sample and the percent weight of 10 molecular mass classes. Pullulan with a number average molecular weight ranging from 6 to 788 kD was used as a standard. The Bloom value is proportional to the elastic modulus of the gelatin gel, and it decreases with decreasing M_n.

Table 1
Data on the physicochemical characterisation of the gelatin sample

Sample	Gelatin
Bloom (g)[a]	259
Isoelectric point (pI)	4.5
% Moisture (wwb)	8.5
Calcium (ppm)	80
Sulphate (%)	<0.1
Chloride (%)	0.16
Phosphate (ppm)	53
M_n[b]	98,700
Molecular weight > 10^6 kD	8.6
Molecular weight > 540 kD	9.5
Tetra + penta	8.7
Gamma	8.8
Beta	17.2
Alpha	29.3
Subunit 1	8.0
Subunit 2	5.0
Subunit 3	2.0
Subunit 4	2.9

[a] Bloom is the weight in grams required to push a piston of strictly defined shape 4 mm into a gelatin gel matured for 16–18 h at 10 °C.

[b] The alpha, beta and gamma fractions of gelatin are well-characterised and monodisperse with characteristic masses. The latter two are, respectively, a dimer and a trimer of the alpha fraction. The tetra and penta are higher order but less well-defined fractions. The low molecular weight side of the GPC spectrum is divided arbitrarily into four different fractions of subunits. The percent weight of the GPC spectrum in each of the 10 molecular mass classes is quoted.

2.2. Methods

2.2.1. Sample preparation

The hydration temperature of the protein did not exceed 65 °C and low-solid solutions (up to 40%) were readily prepared within 30 min. The water activity of gelatin was used as a reliable indicator of the moisture content in high-solid gels based on sorption-isotherm data available in the literature [14]. Following the isopiestic method, known quantity of 40% gelatin samples (about 25 g each) were placed in separate open weighing bottles and stored in air-sealed glass jars that had reached to very-low equilibrium relative humidity with a saturated salt solution of lithium chloride (BDH, Laboratory Supplies, Poole, England). The relative humidity value of the solution was about 11% [15]. A test tube containing thymol was placed inside the jars to prevent mold growth on the gelatin sample during storage. The low relative humidity