A nanostructural investigation of the effect of glycerol on the molecular packing, thermodynamic state and water sorption behaviour of glassy gelatin films

P Díaz-Calderón[1], M Roussenova[2], J Enrione[3*] and M A Alam[2]

1 Department of Food Science and Technology, Universidad de Santiago de Chile, Av. Ecuador 3343, Estación Central, Santiago, Chile.
2 H. H. Wills Physics Laboratory, Tyndall Avenue, Bristol, BS8 1TL, United Kingdom.
3 Facultad de Medicina, Escuela Administración de Servicios, Universidad de los Andes, San Carlos de Apoquindo 2200, Las Condes, Santiago, Chile.

[*] *Corresponding author email*: jenrione@uandes.cl

Abstract. This work explores the effect of glycerol, a low molecular weight polyol, on the molecular packing, thermodynamic state and water sorption of low water content gelatin films. For this purpose, bovine gelatin films with different glycerol contents (0-10% wt.) were equilibrated at a range of relative humidities (RH=11-44%, *T*=298K). Our PALS measurements show that over the concentration range studied, glycerol acts as a packing enhancer (causing a non-linear decrease in the average molecular hole size), whilst reducing the glass transition temperature of the gelatin films. Glycerol also alters the water sorption behaviour of the glassy gelatin films, (whereby reducing the amount of water absorbed at well defined relative humidities), highlighting the importance of molecular packing for the sorption of water vapour in the glassy state.

1. Introduction

Gelatin is a hydrocolloid widely used in the food, pharmaceutical and biomedical industries [1, 2]. It is obtained from collagen, the principal component of fibrous connective tissue by hydrolytic degradation using either acids or alkali [3]. Gelatin is one of the most versatile biopolymers and its traditional uses are based on its well known gelling capability and its properties as a viscosity enhancer [2]. Gelatin also has the ability of forming strong, clear films which are readily soluble in water, making it an ideal raw material for the manufacture of hard and soft pharmaceutical capsules, as well as microcapsules for the food industry [4]. Such capsules are commonly used to encapsulate labile bioactive compounds (e.g. drugs, probiotics, flavors, fatty acids, etc), protect them from undesirable external influences (such as oxygen and water) and achieve their targeted release when required [5]. Unfortunately, low water content glassy gelatin capsules have the disadvantage of high brittleness which may lead to their undesired failure during handling and storage, allowing the loss of the bioactive or the migration of surrounding molecules (e.g. oxygen and water) [6]. Furthermore, gelatin is highly hygroscopic (it can readily absorb water vapor from the atmosphere [7]) which can cause significant modifications in the barrier properties of gelatin capsules, leading to the oxidation or premature release of the encapsulated bioactive compound. In order to overcome these problems,


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gelatin films are commonly modified by the inclusion of small amounts of low molecular weight polyols [6] such as glycerol. Glycerol has been widely used in the pharmaceutical and food industries to alter the mechanical, water sorption and permeation (towards gases and water vapour) properties of biopolymers in order to design optimal encapsulating matrices [7, 8].

One of the main issues in the design of encapsulating gelatin matrices with optimal barrier properties is the molecular mobility through the matrix, which in the glassy state is governed by the density of the molecular packing of the gelatin oligomer chains [4]. In amorphous and partially ordered soft matter, the irregular arrangement of the constituent molecules results in a certain amount of local free volume. This local free volume consists of a large number of sub-nanometer sized free volume elements and it plays an important role in phenomena such as self-diffusion, the diffusion of guest molecules and the glass transition [9, 10].

The objective of this work is to assess the effect of glycerol on the molecular packing, thermodynamic state and water vapour sorption properties of low water content gelatin films by using Positron Annihilation Lifetime Spectroscopy (PALS) in conjunction with a number of complementary techniques.

2. Materials and Methods

Films of bovine gelatin (M_w=2.84 x 10^5 Da, Rousselot, Brazil) and glycerol (purity > 99.99%, Merck, Germany) were prepared by cold casting from suspension. A suspension of 7% w/v gelatin was prepared with various amounts of glycerol, Q_g, expressed as mass fractions on an anhydrous basis $Q_g = m_g/(m_{gel} + m_g)$ (where m_g and m_{gel} denote the mass of glycerol and gelatin, respectively). Films with compositions of Q_g = 0.0, 0.02, 0.06 and 0.10 were measured in this study. The films were equilibrated at different relative humidities (RH=11-44%) at T = 298K in desiccators containing saturated salt solutions [11]. The sorption of water was followed gravimetrically until equilibrium was achieved (generally within 25 days). Further details of the method used to determine the water content of the films are given in Ref.[12]. The glass transition temperatures, T_g, of the films were measured by Differential Scanning Calorimetry (Diamond DSC, Perkin Elmer, USA) performing two temperature scans between 233-450K at a heating rate of 10K/min. The changes in T_g as a function of glycerol content were modeled using the semi-empirical Gordon-Taylor equation [13]. See reference [12] for further details. Positron Annihilation Lifetime Spectroscopy (PALS) experiments were performed

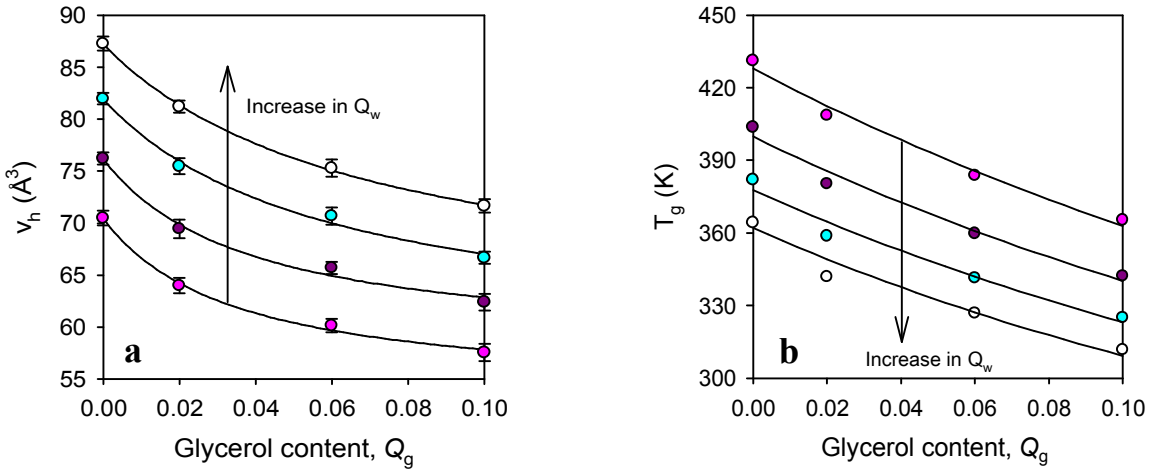

Figure 1. Average hole volume, v_h, (T=298 K) **(a)** and glass transition temperature, T_g, **(b)** as a function of increasing glycerol content for glassy gelatin films with a well defined water contents: Q_w = 0.02 (pink series), Q_w = 0.04 (purple series), Q_w = 0.06 (cyan series) and Q_w = 0.08 (white series). The data presented in this figure were derived by interpolation of the data series presented in Figures 6a and 5, respectively in Ref. [12].

using a fast-fast coincidence system. ^{22}Na was used as a positron source which was prepared by depositing aqueous ^{22}NaCl between two 7.5 μm sheets of Kapton foil. Spectra were collected over a period of two hours to generate at least 2.5 million events per spectrum and spectra were decomposed by three components using the routine Life Time (version 9.1) [14]. Specific details of the spectra analysis can be found in references [12] and [15].

3. Results and Discussion

The effect of glycerol on the molecular packing of the glassy gelatin films is presented in Figure 1a. It can be seen that over the concentration range studied, glycerol acts as a packing enhancer for the glassy gelatin films. For films with well defined water contents, glycerol causes a non-linear decrease in the average molecular hole size which is indicative of non-ideal packing behavior in the glassy state [15-17]. This effect has been previously observed in glassy maltooligomer matrices upon the addition of low molecular weight diluents, such as maltose and glycerol [15, 17]. The initial rapid decrease in v_h may be related to the reduction in the molecular frustration of the system, accompanied by a sharp reduction in the glass transition temperature, T_g, of the gelatin films, as is shown in Figure 1b. For films with well defined water contents, we observe a non-linear reduction in T_g as a function of increasing glycerol content, which was fitted well to the semi-empirical Gordon-Taylor equation [13] (yielding a Gordon-Taylor coefficient for glycerol, $k_g = 3.6 \pm 0.3$, in agreement with previous studies [17, 18]). Figure 1 (a and b) also illustrates the plasticizing effect of water on the gelatin films, causing an increase in the average molecular hole size accompanied by a reduction in T_g. Water has been shown to be a strong plasticizer for biopolymer matrices due to its small molecular size and its propensity to form hydrogen bonds (see Refs. [15-17] for a more detailed discussion).

The presence of glycerol in the glassy gelatin films also has an effect on their water sorption behavior as illustrated in Figure 2. It can be seen that at all relative humidities, the presence of glycerol reduces the amount of water absorbed by the glassy gelatin films. For example, at RH=11% the water content

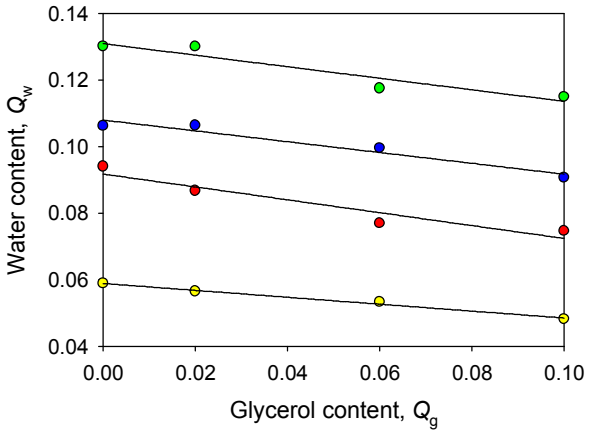

Figure 2. Water content as a function of increasing glycerol content for glassy gelatin films at well defined relative humidities at T=298K: RH=11% (yellow series), RH=22% (red series), RH=33% (blue series) and RH=44% (green series). All linear regression coefficients are R^2>0.95 and the errors in Q_w are the same size as, or smaller than the symbols shown.

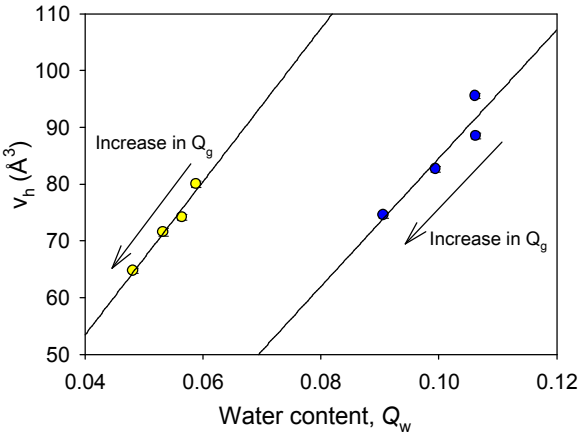

Figure 3. Relation between average molecular hole size and the amount of water absorbed by gelatin films (T=298K) with different compositions equilibrated at well defined relative humidities, RH=11% (yellow series), RH=33% (blue series). All linear regression coefficients are R^2>0.94 the errors in v_h are the same size as, or smaller than the symbols shown.

Q_w=0.059 for the pure gelatin films and Q_w=0.048 for gelatin films containing 10 wt. % glycerol (i.e. a 19% reduction in water content). In fact, there is a positive linear relationship between the average molecular hole size and the amount of water absorbed by films with different compositions at well defined relative humidities (Figure 3), further illustrating the effect of glycerol as a packing enhancer. This effect has been previously reported for maltooligomer-maltose [15] and maltooligomer-glycerol [17] systems and it highlights the importance of molecular packing for the sorption of water by biopolymers in the glassy state. It is worth mentioning that one would expect to see the opposite effect for gelatin films in the rubbery state, i.e. an increase in the amount of water absorbed by the films at a well defined relative humidity as a function of increasing glycerol content [17, 18]. This is due to the fact that in the rubbery state, water sorption is related to the entropy of mixing of the constituent molecules [18].

4. Final Remarks

Our study shows that the molecular organisation, thermodynamic state and water sorption properties of low water content gelatin films can be modified by the addition of low molecular weight polyols such as glycerol. Glycerol acts as a packing enhancer, causing a reduction in the average molecular hole size of the gelatin films, whilst reducing their glass transition temperature and ability to absorb water in the glassy state. By establishing composition-structure relationships for this biopolymer we present a new step towards enabling the rational control of its material properties leading to the eventual design of optimal pharmaceutical encapsulants.

Acknowledgements

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