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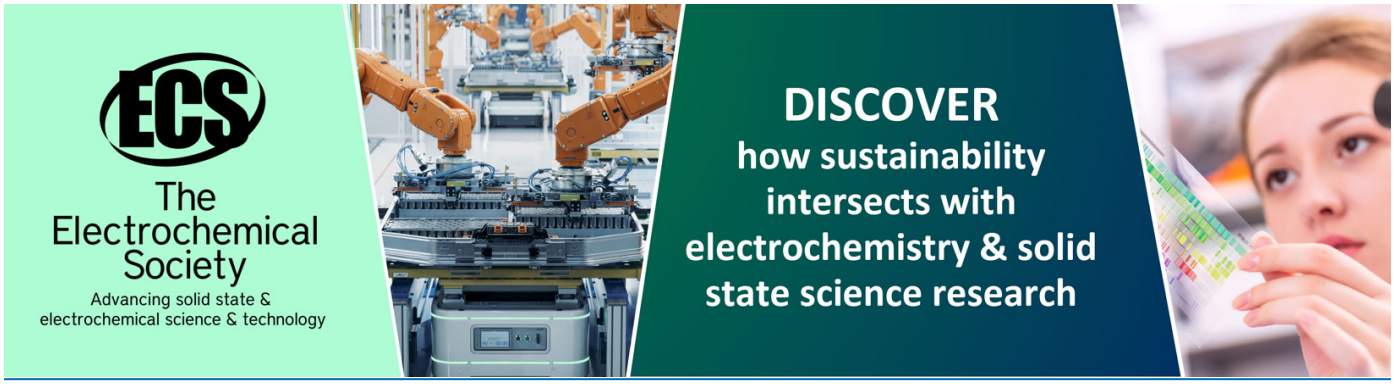

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PALS: A unique probe for the molecular organisation of biopolymer matrices

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Abstract. This short review aims to illustrate the versatility of Positron Annihilation Lifetime Spectroscopy (PALS) when utilized for the characterization of biopolymers (e.g.: starch, fractionated maltooligomers, gelatin and cellulose derivatives) commonly used for the formulation of pharmaceutical encapsulants. By showing examples from a number of recent PALS studies, we illustrate that this technique can be used to probe the changes in thermodynamic state and molecular packing for a wide range of biopolymer matrices as a function of temperature, matrix composition and water content. This provides a basis for establishing composition-structure-property relationships for these materials, which would eventually enable the rational control of their macroscopic properties and the design of optimal encapsulating matrices and intelligent drug delivery systems.

1. Introduction

Biopolymers are of utmost importance for the pharmaceutical industry where there they are widely used in dense matrices at low water contents for the formulation of pharmaceutical excipients and encapsulation matrices for labile bioactive ingredients (e.g. drugs, vitamins and essential nutrients) [1, 2]. Such pharmaceutical encapsulants are often based on lipids (e.g.: wax, palm fat), proteins (e.g.: gelatin, milk proteins), polysaccharides (e.g: starch) and oligosaccharides (hydrolyzed starch, lactose), as well as cellulose and its derivatives (methylcellulose, hydroxypropylmethyl cellulose). These materials have the ability of forming films and matrices with adjustable morphologies, which have high barrier properties for gases [3] and organic molecules [4]. One of the key issues in the design of optimal encapsulants is the molecular mobility (diffusion/permeation) which governs the barrier properties of these matrices [2]. It is now widely recognised that the molecular mobility is determined by the thermodynamic state [2], as well as the local free volume which exists between molecules in soft matter due to irregular packing, density fluctuations and topological constraints [5, 6]. This free volume consists of a large number of sub-nanometer sized free volume elements ("holes") and a number of theories have been developed to describe phenomena such as self diffusion and the diffusion of guest molecules in terms of the size and size distributions of these free volume elements [6, 7]. The importance of quantifying the free volume in these systems, therefore, cannot be overestimated, since it provides a promising route towards a better understanding of the material properties and the eventual rational design of bio-encapsulants, rather than the current largely trial and error approach practised within the industry.


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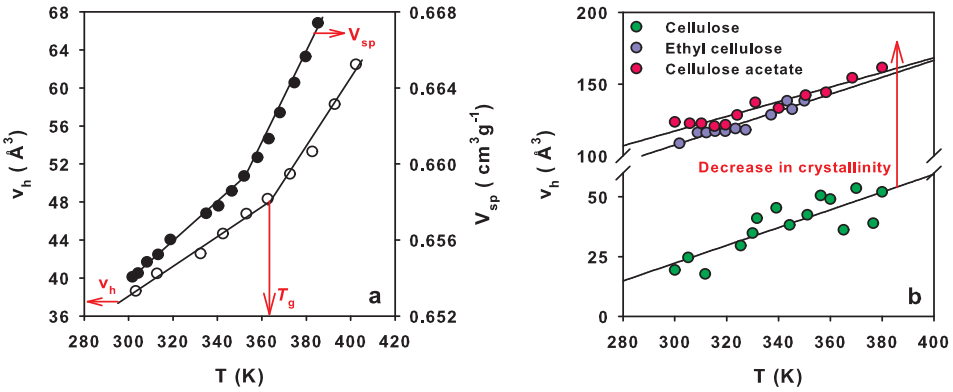

Figure 1. **(a)** Temperature dependence of the average molecular hole size, v_h, and specific volume, V_{sp}, measured for an amorphous maltooligomer matrix equilibrated at a well defined water activity, $a_w = 0.22$ [11][1]. **(b)** Temperature dependence of the average molecular hole size measured for partially crystalline cellulose and cellulose derivative matrices. For both cellulose derivatives the degree of substitution is ~ 2.5 [20, 21][2].

Positron annihilation lifetime spectroscopy (PALS) is a unique technique which is capable of measuring the size and size distribution of the free volume elements directly on the sub-nanometre length scale [8, 9]. Over the last decade, this technique has been successfully used to study the phase transitions and changes in molecular packing in a wide range of biopolymers with varying degree of order. Experiments using PALS have shown that the molecular organisation in these materials is strongly influenced by factors such as temperature [10, 11], molecular weight distribution [11, 24], matrix composition (addition of low molecular weight diluents) [12–15] and water content [10, 12, 14]. Furthermore, it was shown that these measurements on the molecular length scale can be successfully correlated with the macroscopic properties of these materials (e.g. density, mechanical strength, permeation properties) [11, 13], proving PALS is an invaluable tool for the material characterization of these systems. Here, we illustrate the versatility of this technique by presenting a short review of recent studies on biopolymer systems commonly used for the formulation of pharmaceutical excipients and encapsulants.

2. Molecular organisation in biopolmers - Opening it up at the nanolevel

Biopolymers in dense matrices can occur in states of varying degree of order, from perfectly crystalline, to partially ordered and amorphous. In all cases, the molecular packing in these materials is strongly influenced by temperature, as shown in Fig. 1. For amorphous systems, the changes in molecular hole size (and specific volume) with temperature often show two linear branches, as illustrated in Fig. 1a which shows the temperature dependence of the average hole (and specific) volume measured for a completely amorphous maltooligomer matrix. The point of intersection of the two branches can be identified as the glass transition temperature, T_g, in agreement with Differential Scanning Calorimetry measurements [11]. For crystalline and partially ordered systems, however, the average molecular hole size generally shows a linear expansion with temperature, as illustrated in Fig. 1b for cellulose and cellulose derivative matrices [20]. It is interesting to note that the formation of cellulose derivatives leads to the marked loss of crystalline structure, accompanied by a significant increase in average molecular hole size, as shown in Fig. 1b [20, 21]. It has been previously shown that the molecular organisation and degree of crystallinity in cellulose based materials is strongly influenced by the type of chemical substitution, as well as the degree of substitution per glucose unit [20, 21]. These two parameters are thus often used to fine-tune the molecular architecture of cellulose derivatives when used for the formulation of pharmaceutical excipients.

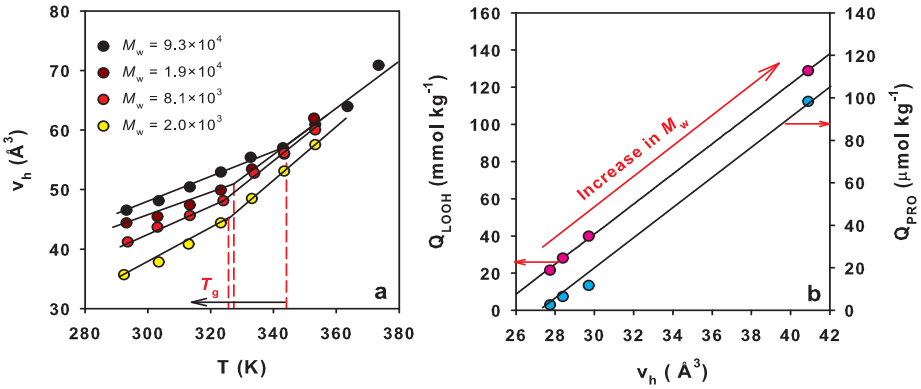

Figure 2. **(a)** Average hole volume as a function of temperature for maltooligomer matrices with different molecular weight distributions equilibrated at a well defined water activity, $a_w = 0.54$. [11][3] **(b)** Relation between the average molecular hole size of encapsulating maltooligomer matrices with different molecular weight distributions and the concentration of primary (hydroxiperoxide, Q_{LOOH}) and secondary (propanal, Q_{PRO}) oxidation products of the encapsulated lipid [23][4].

2.1 The effect of molecular weight distribution

Another method of effectively altering the molecular packing, and hence the physical properties of biopolymer matrices is by changing their molecular weight profile. For example, it has been shown that for relatively low molecular weights both, the molecular packing and T_g are strongly dependent on the average molecular weight of a system, as illustrated in Fig. 2a for a set of maltooligomer matrices [11]. It is clear that the density of the molecular packing increases with decreasing molecular weight and this densification effect is most pronounced in the glassy state, where we measure the largest reductions in v_h. Furthermore, this decrease in average molecular hole size has been shown to relate to improvements in the barrier properties of glassy maltooligomer matrices. Drush *et.al* successfully demonstrated that the oxidative stability of a labile lipid encapsulated in glassy fractionated carbohydrate oligomer matrices improved significantly if lower molecular weight fractions were used [23]. A linear relationship was found between the average molecular hole size of the encapsulating matrix and the concentration of primary (hydroxiperoxide) and secondary (propanal) oxidation products of the encapsulated lipid [23], as shown in Fig. 2b. Even small reductions in v_h (achieved by correct molecular weight selection of the encapsulating carbohydrate blend) were shown to significantly improve the oxidative stability of the encapsulated lipid, suggesting that oxygen diffusivity is one of the key determinants of autoxidation of bioactives encapsulated in glassy biopolymer matrices [23].

2.2 The effect of low molecular weight additives

Although the physical properties of biopolymer encapsulants can be controlled by careful selection of their molecular weight distribution, for practical applications these improvements are often achieved more easily by the addition of low molecular weight diluents [2]. Additive selection is normally based on the compatibility with the base biopolymer (in order to prevent phase separation), as well as permanence inside the matrix to prevent changes in material properties due to the time dependent migration of the diluent. The most common additives for the formulation of pharmaceutical encapaulants include polyols (e.g. glycerol, sorbitol), saccharides (e.g. maltose, sucrose and trehalose) and polyethers (e.g. poly(ethylene glycol)) [1]. The effect of these additives on the molecular organisation and physical properties of the matrices are system specific. Some diluents have been shown to act as plasticisers, whilst others act as packing enhancers for the biopolymer matrices. For example, poly(ethylene glycol) has been

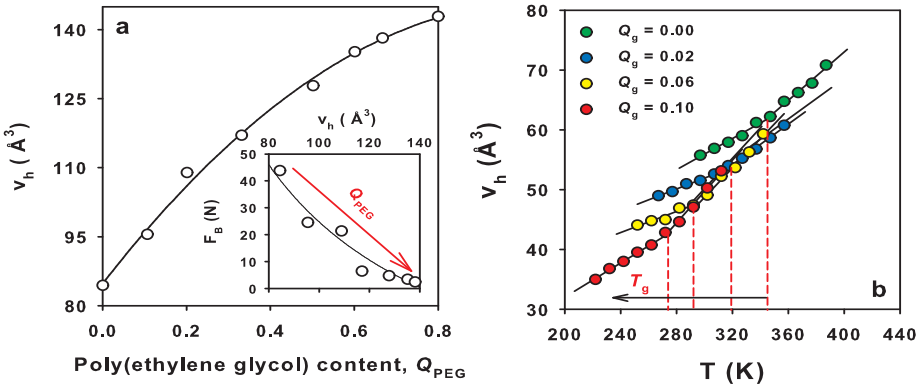

Figure 3. **(a)** The plasticising effect of poly(ethylene glycol) on methylcellulose, accompanied by an increase in the average hole size and the strength (*Inset*: decrease in force of breaking, F_b) of the matrices [22][5]. **(b)** Temperature dependence of the average molecular hole size for maltooligomer matrices with different compositions (glycerol contents, Q_g) equilibrated at a well defined water activity, $a_w = 0.54$. Glycerol is a packing enhancer for the maltooligomer matrices, causing systematic reductions in the average molecular hole size (both, in the glassy and rubbery states), accompanied by a reduction in T_g of the matrices [14][6].

shown to have a plasticising effect on methylcellulose films, as depicted in Fig. 3a, accompanied by an increase in the average hole size and the strength (decrease in force of breaking) of the films [22]. Conversely, low molecular weight additives such as glycerol [14,15] and maltose [12,13] have been shown to act as packing enhancers for a number of biopolymer systems. As illustrated in Fig. 3b, the addition of glycerol to amorphous maltooligomers causes a decrease in the average molecular hole size both, in the glassy and the rubbery states, accompanied by a reduction in T_g. In the glassy state, a non-linear decrease in v_h has been observed as a function of increasing glycerol content, indicative of a non-ideal packing behaviour [14]. The rapid initial decrease in v_h may be associated with the reduction in molecular frustration (due to the rapid initial depression of T_g) upon the addition of the smaller glycerol molecules to the carbohydrate matrices [14]. Apart from acting as a packing enhancer, glycerol has also been shown to enhance the biostabilisation performance of encapsulating matrices and alter their water vapour sorption behaviour [14].

2.3 The effect of water

Indeed, one important property of biopolymers is their ability to absorb water vapour from the atmosphere (i.e. they are highly hygroscopic) [2]. Water is a highly efficient plasticiser for biopolymer matrices and the glass transition temperature of these systems decreases strongly as function of increasing water content [2]. Furthermore, the diffusional mobility of small molecules (e.g. water, gases and volatile organic compounds) in biopolymer matrices has been shown to increase rapidly with increasing water content, both, in the glassy and the rubbery states [3,27]. A number of recent PALS studies have also elucidated that the sorption of water causes complex changes in the molecular organisation of biopolymer matrices [11–15, 19]. To illustrate this point, in Fig. 4, we present examples of the changes in average hole size measured upon the sorption of water vapour for three biopolymer systems. Although in all cases water has a strong plasticising effect on the biopolymer matrices, the observed changes in v_h as probed by Ps are system-specific. In Fig. 4a we show the changes in average hole size as a function of water content for a starch/sucrose blend at various temperatures [17]. The observed changes in v_h with increasing level of hydration appear to be somewhat similar to the changes in v_h as a function of increasing temperature, commonly observed for biopolymer matrices (for example,

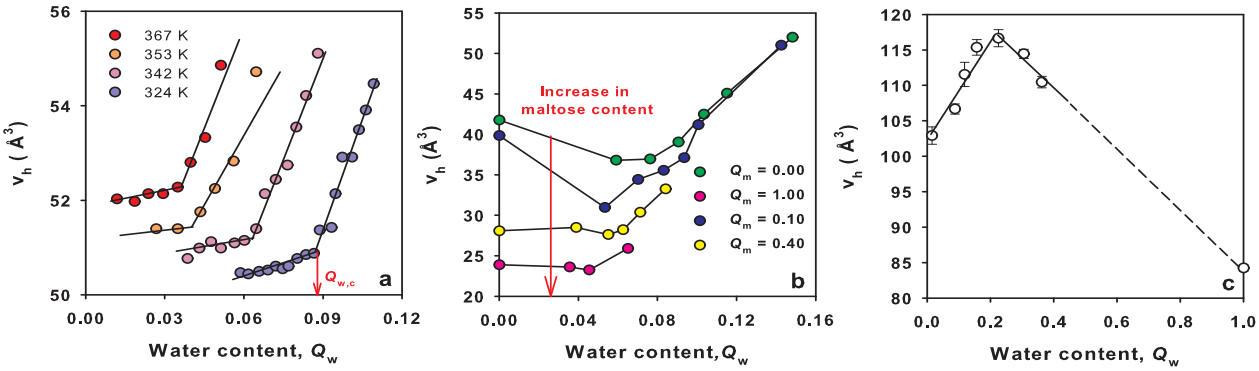

Figure 4. The plasticising effect of water on biopolymer matrices. The changes in average molecular hole size as a function of increasing water content measured for **(a)** starch/sucrose blends at different temperatures [17][7], **(b)** a series of fractionated maltopolymer/maltose blends [13][8] and **(c)** HPMC [19][9] matrices measured at $T = 298$ K.

as shown in Fig. 1a). At low water contents we observe a smooth linear increase in v_h up to a critical water content, $Q_{w,c}$, followed by at significantly sharper linear increase in v_h at higher water contents. At the $Q_{w,c}$ the starch/sucrose matrices undergo a transition from the glassy to the rubbery state at a constant temperature, illustrating the superposition of the effects of temperature and water content on the thermodynamic state of the matrices. As expected, the $Q_{w,c}$ values decrease with increasing temperature. Interestingly, this behaviour is not reflected in the changes in molecular packing observed as a function of increasing level of hydration for bidisperse fractionated maltopolymer/maltose matrices, as shown in Fig. 4b [12, 13]. Starting from the anhydrous state, initially a decrease in v_h is measured upon sorption of water. At such low water contents, the water molecules are present in a highly dispersed state and are closely associated with the hydroxyl residues on the carbohydrate chains (confirmed by clustering analysis [11, 14]) so they partially "occupy" the molecular free volume which exists between the carbohydrate chains, causing the observed reduction in v_h. The observed decrease in v_h can also result from the reduction in molecular frustration of the system upon the addition of the small, polar water molecules. As the water content increases further ($Q_w \sim 0.05$, but depending on the maltose content, Q_m), whilst the matrices are still in the glassy state, we observe an increase in v_h as a function of water content. It has been shown that water molecules have a significant diffusional mobility even in the glassy state [26, 27], thus allowing them to plasticise their local environment, causing an increase in v_h [13]. Upon further hydration ($Q_w \sim 0.10$, but depending on the matrix composition), the matrices become rubbery and the differences in v_h between the various matrix compositions become smaller. Furthermore, clustering analysis has shown that in the rubbery state the preferential homo-coordination between water molecules becomes significant [11, 14], leading to the formation of small clusters of water around the carbohydrate chains. Clustering of water molecules at high levels of hydration has also been observed in other biopolymer systems, such as hydroxypropyl methylcellulose [19] and bovine gelatin [15]. In Fig, 4c we show the changes in average hole size measured for hydroxypropyl methylcellulose (HPMC) as a function of increasing water content. Initially we observe a pronounced increase in v_h as the HPMC matrices become plasticised by water [19]. The average molecular hole volume reaches maximum size at a water content of about $Q_w = 0.23$, after which it begins to decrease steadily upon further sorption of water. The average hole sizes measured for the plasticised HPMC matrices are larger than the average hole size measured for pure bulk water ($v_h = 81.6$ $\mathring{A}^3$ at $T = 298$ K). This indicates that the decrease in v_h as a function of increasing water content

may be attributed to the formation of small clusters of water in the matrices. This would, in turn, result in the overall *o*-Ps lifetime measured for the sample being a sum of the weighted contributions of an *o*-Ps in the plasticised HPMC matrix and the *o*-Ps in bulk water [19]. Since *o*-Ps probes the local molecular organisation at the sub-nanometer length scale, water clusters of a size less than one nanometer would be probed by *o*-Ps as bulk water.

3. Final Remarks

In this short review we have illustrated that PALS is an invaluable tool for probing the molecular organisation/free volume in a wide range of biopolymers commonly used for the formulation of pharmaceutical encapsulants. Factors such as temperature, composition and the amount of water absorbed by the matrices strongly influence their molecular packing and thermodynamic state. Water was shown to act as a strong plasticiser, whereby causing a reduction in T_g, generally accompanied by an increase in v_h of the biopolymer matrices, provided clustering of water molecules does not occur. Furthermore, it was shown that the molecular organisation and physical properties of these materials can be improved by careful selection of their molecular weight distribution and through the addition of low molecular weight additives (e.g. glycerol, maltose).

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