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Oxygen solubility and permeability of carbohydrates

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Abstract—The saturated oxygen concentration in a series of aqueous solutions of sorbitol (up to 35% w/w) and maltitol (up to 50% w/w) was measured using colorimetric reagent vials based on Rhodazine D. The results indicate that the solubility of oxygen in low-water carbohydrates is considerably lower than its solubility in pure water. It was concluded that the low-oxygen solubility is a major factor contributing to the barrier properties of low-water content carbohydrates used in the encapsulation of flavours, lipids, peptides and other oxidisable species.

Keywords: Oxygen solubility; Oxygen permeability; Encapsulation; Barrier; Lipid oxidation

1. Introduction

Polysaccharides and low-molecular weight carbohydrates, including starch and maltodextrins, are used as barrier materials or matrices for the preservation and encapsulation of active ingredients, including flavours and pharmaceuticals. An important aspect of preservation is the chemical stability of the active ingredient to oxidation and the effect of the barrier or matrix on oxygen transport. The permeability of a matrix to oxygen is dependent on the solubility, S, of oxygen in the matrix and its diffusivity, D, through it.[1] It is important to understand the physical origins of the permeability of these materials in order to devise strategies for its control. For example, solubility can be addressed by formulating the matrix in such a way as to minimise this solubility, while diffusivity can be modified by reducing molecular mobility in the matrix through controlling plasticisation or storage temperature. Both are potentially viable strategies for reducing the oxygen permeability.

For an initially oxygen-free plane sheet, the oxygen flux, J, at a time, t, is given by:[1]

$$J(t) = \frac{Pp}{l}\left[1 + 2\sum_{n=1}^{\infty}(-1)^n \exp\left(\frac{-D\pi^2 n^2 t}{l^2}\right)\right] \quad (1)$$

where l is the film thickness and p the oxygen pressure, and P the permeability. The solubility, S, can be calculated from the relationship, $S = PD^{-1}$. For synthetic barrier materials, the importance of both parameters, D and S, in affecting oxygen flux is recognised. The importance of solubility is demonstrated by calculating the oxygen flux through a 200 μm film. At room temperature, the synthetic polymer, poly(ethylene naphthalate),[1] has an oxygen diffusivity of 1.5×10^{-13} m^2 s^{-1} and an oxygen solubility of 0.127 cm^3 (STP) cm^{-3} atm^{-1}, giving an oxygen flux through the film of ~8.3 cm^3 (STP) cm m^{-2} day^{-1} atm^{-1}. If the solubility of oxygen in the film was the same as the solubility of oxygen in pure water, 0.007 cm^3 (STP) cm^{-3} atm^{-1}, then the oxygen flux through the film becomes ~0.3 cm^3 (STP) cm m^{-2} day^{-1} atm^{-1}. The marked effect on predicted oxygen flux is due to the relatively poor solubility of oxygen in the hydrogen-bonded solvent compared to the more lipophilic polymer.

There have been a number of studies on the permeability of films of the starch polysaccharides amylose and amylopectin, to oxygen.[2–4] There is a general agreement that starch films are potentially good barriers to oxygen transport. Increasing the water content of the

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film increases oxygen permeability. The increased permeability may have at least two physicochemical origins. Water is a potent plasticiser of low-water content amorphous carbohydrates including starch.[5] The plasticisation results in an increase in molecular mobility of the matrix, and as a consequence the expectation that diffusive processes within the matrix would become more rapid. Alternatively, increasing the water content of the film might increase the solubility of oxygen in the film leading to the increased permeability. To determine which effect dominates, there is a need for data on the diffusivity and solubility of oxygen in carbohydrate/water mixtures. This contribution examines the solubility of oxygen in some simple carbohydrate/water mixtures.

2. Results and discussion

Wilkin et al.[6] have made a comparison of methods for the determination of dissolved oxygen in environmental waters and found that for low levels of dissolved oxygen (<1 ppm) the colorimetric test based on Rhodazine D gave a good correlation with the Winkler titration method, whereas oxygen selective electrodes are unreliable in this concentration range. The Rhodazine D method is recommended for the determination of low levels of dissolved oxygen in boiler feed waters and has also been used in the analysis of ground waters.[7] The colorimetric test kits using Rhodazine D are supplied in partially evacuated glass vials with a pre-scored tip; breaking the tip whilst inverted in the liquid to be sampled allows a pre-determined quantity of sample to be drawn into the vial and mix with the reagent solution. We initially chose this method as a rapid and convenient assay for the oxygen content of glassy carbohydrates. This was to be achieved by dissolution of a sample of the glassy material in deoxygenated water and determination of the dissolved oxygen content of the solution. While the contents of the reagent vials are known,[7] the details of the chemistry, including the effect of reducing sugars on the accuracy of the test, are not in the public domain. It was thought prudent therefore to use non-reducing sugars or sugar alcohols for the determination and consequently sorbitol and maltitol were chosen as the materials for study. An assay for the reducing sugar content of the commercially obtained carbohydrates (Nelson–Somogyi test[8]) gave values of $0.056 \pm 0.005\%$ for sorbitol and $0.045 \pm 0.001\%$ for maltitol based on glucose (measurements performed in triplicate).

A custom-designed apparatus (Fig. 1) was made for the oxygen measurements, with a ground-glass neck, stopcock sidearm and a screw-threaded sidearm with a PTFE and silicone-gasketed drilled cap to allow the 13 mm o.d. reagent vial to be held inverted in the apparatus with the pre-scored tip resting on a raised glass ridge, such that the flask contents can be sampled without exposure to atmospheric oxygen. Our initial experiments on solid (glassy) materials highlighted a number of problems with the method: the results obtained gave low values but appeared to be independent of sample treatment. This raised questions about how rapidly the samples could reach equilibrium with atmospheric oxygen. As methods of preparation of glassy carbohydrates (freeze-drying or melt-quenching) will also tend to remove dissolved gases, this was an important issue. These and other difficulties with the use of glassy samples led us to approach the problem from a different direction. A series of solutions of the sugar alcohols, from pure water to close to the limit of solubility, were prepared

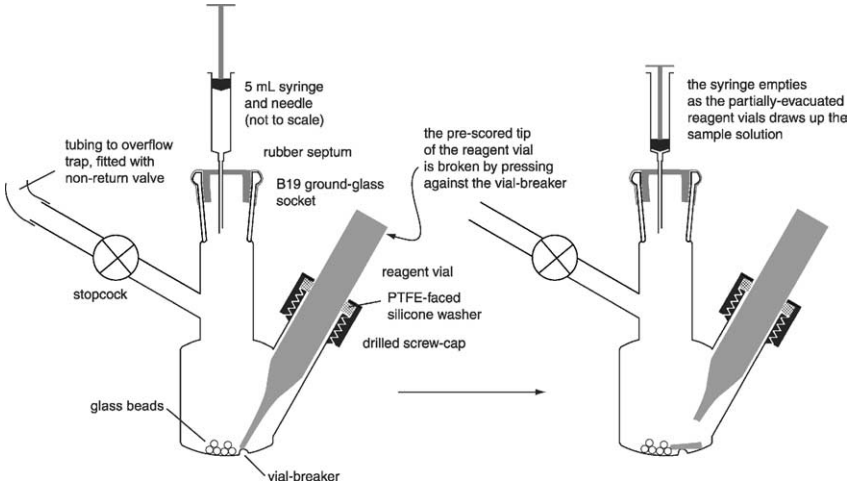

Figure 1. Apparatus and sampling procedure: The custom-designed measurement flask is based on a Schlenk tube with an additional screw-threaded sidearm and ridge for holding and breaking the tip of the reagent vial, respectively. The carbohydrate solutions were analysed by introducing 1 mL of the sample into a flask completely filled with deoxygenated water, the displacement being taken up by the 5 mL syringe. Breaking the reagent vial caused a pre-determined amount of the solution to be drawn into the vial, the contents of the syringe emptying as the sample was drawn up.

for equilibration at constant temperature and subsequent measurement of the saturated oxygen content in order to extrapolate the oxygen concentration at low-water content.

It was found in experiments with oxygen-saturated water that the presence of a headspace of argon in the flask during filling and sample injection gave lower than expected results, presumably due to exchange of oxygen with the headspace. This was confirmed by a comparison of the results obtained with different fill volumes of the measurement flask. A method was therefore developed for analysing liquids without headspace by injection into a completely filled flask. An additional reservoir of deoxygenated water in a syringe was used to account for the volume change when the reagent vial was broken.

Samples were prepared using oxygen-saturated water and the sugar alcohols. These were held at 20 °C in glass tubes for at least 2 weeks before measurement to ensure equilibration with air. The results are presented in Figure 2a. The solubility of oxygen, plotted against the percentage of sugar alcohol by mass, declines steeply as the concentration of carbohydrate increases and would intercept the *x*-axis at around 70% sugar if linearly extrapolated. This behaviour is broadly in line with observations of the oxygen solubility in solutions of other carbohydrates, obtained over a narrower concentration range.[9–11] Linear extrapolation clearly cannot sufficiently describe the solubility results, since there must be a finite solubility over the entire concentration range, even if it is very small at low-water contents. An empirical fit to the data was obtained using a simple exponential function, but a fit could only be obtained when the data (single determinations on sorbitol solutions) were plotted against mole fraction of the sugar alcohol (Fig. 2b). This equation was used to calculate the broken line in Figure 2a. While this function extrapolates beyond the limit of the data, and therefore can only be seen as a guide to behaviour at very low-water content, it does indicate that the solubility of oxygen in carbohydrates with water contents consistent with the glassy state is likely to be several orders of magnitude lower than the solubility in pure water.

In conclusion, the results presented here support the hypothesis that the water content is a critical determinant of the oxygen solubility in carbohydrate water mixtures. Oxygen flux through a low-water content carbohydrate barrier is therefore likely to be much lower than the figure calculated in the introduction and suggests that the poor solubility of oxygen is a major contributor to the barrier properties of carbohydrates against oxidation of encapsulated materials.

3. Experimental

3.1. General methods

Ultra-pure water (resistivity = 18.2 MΩ cm) was prepared using an Elgastat Maxima water purifier. Air-saturated water was prepared by passing a stream of air through a glass frit into a flask of ultra-pure water, cooled in an ice-bath. The aerated water was stored in a refrigerator before use. Sorbitol was obtained from Fluka and maltitol and glucose were purchased from Sigma. The reducing sugar content of the sugar alcohols was determined by the Nelson–Somogyi method[8] using glucose solutions as standards. Dissolved oxygen reagent in partially evacuated Vacu-vials, 0–800 ppb dissolved oxygen (CHEMetrics Inc.) were obtained from Galgo (UK) Ltd., St Albans, UK. Spectroscopic measurements were performed on a Perkin Elmer

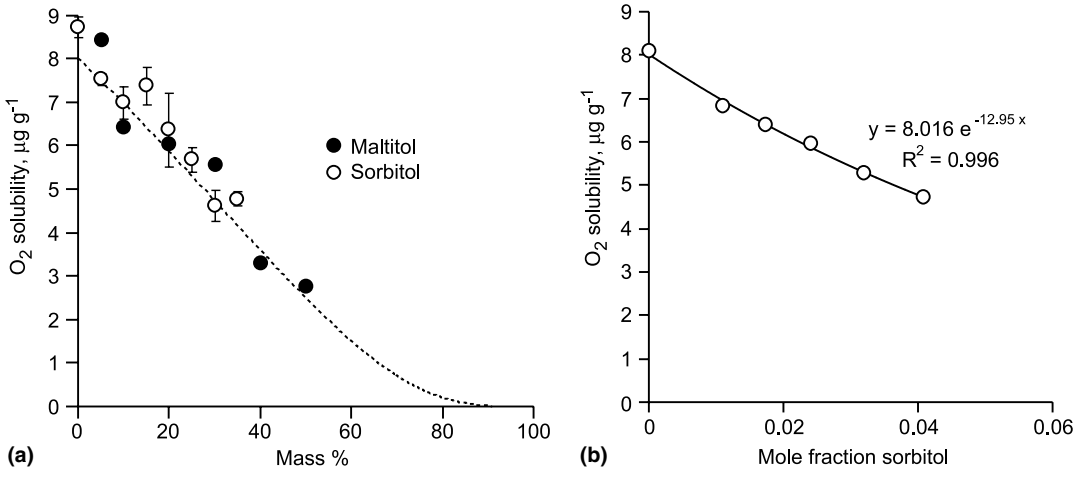

Figure 2. (a) Oxygen solubility for maltitol (single determinations) and sorbitol (average of measurements performed in triplicate) solutions. Error bars represent 1 SD. The dashed line is the extrapolation of the function fitted to the data in (b). (b) Data for single determinations of the solubility of oxygen in sorbitol solutions (single determinations) plotted against mole fraction and the empirical fit to a simple exponential function.

Lambda 15 spectrophotometer in absorbance mode. The dissolved oxygen calibration table was supplied by CHEMetrics Inc. from the values obtained for a set of calibration standards for the Rhodazine D system.

3.2. Preparation and equilibration of the sugar alcohol solutions

Samples were prepared by weighing the sugar alcohol (5–35% w/w in 5% w/w steps for sorbitol, 10–50% w/w in 10% w/w steps for maltitol) and air-saturated water into glass tubes containing a small glass-coated magnetic follower. A control sample, containing air-saturated water alone, was also prepared. Immediately after weighing, tubes were stood in ice and stoppered with a rubber septum into which was placed a syringe needle to allow pressure equalisation and exchange of gases with the atmosphere, while minimising evaporation. Tubes were then transferred to a rack in air at ambient pressure in a constant temperature environment at 20 °C. The tube contents were stirred until homogeneous before being allowed to stand for at least 2 weeks to reach equilibrium. Samples were transferred to a thermostatted water-bath, also at 20 °C, before measurement.

3.3. Determination of density and solids content

Samples of 250 μL from each of the solutions were withdrawn using a positive displacement pipette into preweighed glass vials with perforated stoppers. The vials were weighed again before transfer to a vacuum oven where the solutions were vacuum-dried over phosphorus pentoxide at 60 °C. The density of pure water at 20 °C was used to calculate the exact volume dispensed using the water (control) samples as reference; this figure was then used to calculate the densities of the solutions. The vials were re-weighed after drying to determine the solids content of the solutions. All determinations were performed in triplicate.

3.4. Preparation of deoxygenated water

Ultra-pure water was heated to boiling in a three-necked 2 L round-bottomed flask fitted with a condenser, gas inlet tube and rubber septum, while passing a vigorous stream of argon through the liquid under reflux conditions. The top of the condenser was fitted with a non-return valve attached by a piece of flexible tubing. After several hours of heating, the flask contents were allowed to cool and the water tested using the dissolved oxygen test apparatus. The heating cycle was continued until a negative oxygen test was found. Deoxygenated water was stored under a slow stream of argon until use.

3.5. Measurement of oxygen solubility in the carbohydrate solutions

The dissolved oxygen content of the carbohydrate solutions was determined using CHEMetrics Vacu-vials, containing Rhodazine D reagent. Measurements were performed using the custom-made apparatus shown in Figure 1. The steps carried out in making an individual measurement were: (i) assembly and weighing of the apparatus; (ii) flushing the apparatus with inert gas (argon) to remove atmospheric sources of oxygen; (iii) filling the apparatus completely with deoxygenated water, displacing all of the headspace; (iv) injecting the sample (weighing before and after sample addition) and finally (v) breaking the reagent vial tip, allowing the flask contents to be drawn into the vial and measuring the absorbance of the indicator dye in the reagent vial to determine the concentration of oxygen in the solution. Since the apparatus and assay are both somewhat unusual, these individual steps are described in more detail below.

The apparatus was assembled by fixing a reagent vial in the screw-capped sidearm with its pre-scored tip resting on the vial-breaker (Fig. 1). Twelve small glass beads (to aid mixing) were placed into the flask before sealing with a rubber septum. A 5 mL disposable syringe and needle was located at the neck of the flask by insertion of the needle through the rubber septum. The syringe was to allow for a volume change when the partially evacuated reagent was broken. The empty flask, including septum, beads, vial and syringe, was weighed at this stage.

The argon flush was performed after connecting the apparatus, described above, to the reservoir flask containing the deoxygenated water by a cannula (double-ended needle) passed through the rubber septa of both flasks. The end of the cannula in the reservoir flask was held in the headspace above the water. The open stopcock of the measurement flask was connected by flexible tubing to a trap, fitted with a non-return valve. To start the argon flush, the water reservoir flask though which argon was passed was pressurised by restricting the outlet from the condenser, allowing argon to flow through the cannula. A flushing time of at least 10 min was employed. Half-way through the flushing time, the syringe was allowed to partially fill and empty three times to displace air trapped in the syringe.

Filling with deoxygenated water was initiated immediately after the argon flush by lowering the inlet of the cannula below the surface of the water in the reservoir flask. As the water level in the measurement flask rose, the flask was tilted to allow the headspace (argon) to be displaced through the stopcock sidearm. When the water level rose above the tip of the syringe needle, water was slowly drawn up into the 5 mL syringe and expelled to displace gas from the syringe. The final bub-

bles were displaced by tilting and tapping the flask to allow them to leave by the stopcock sidearm. When all the headspace of the flask was displaced with water, the stopcock was closed and the 5 mL syringe was allowed to fill to the 5 mL mark in the case of a blank (no sample) or to the 4 mL mark if a sample was to be added. The cannula was removed and the flask disconnected from the trap.

Before injecting a sample, the apparatus was carefully dried and weighed. In the case of blanks (deoxygenated water only), the next stage was carried out immediately. In the case of samples, either sugar alcohol solution or oxygen-saturated water, equilibrated in air at 20 °C, was drawn-up into a 2 mL syringe fitted with a needle. Syringes were overfilled in order to avoid the transfer of small bubbles. Air was removed from the syringe by inversion and expulsion of a small quantity of the liquid before approximately 1 mL was injected through the septum of the measurement flask, allowing the 5 mL syringe to take up the displacement. The flask was then reweighed and inverted several times to allow the glass beads to mix the contents.

The tip of the reagent vial was broken against the raised base of the flask by pressing on the base of the vial, allowing it to pivot on the cap and washer. Breaking the vial allowed the partial vacuum within the vial to draw a pre-determined volume of the solution from the flask, mixing with the reagents in the vial. As the liquid was drawn up into the vial, the contents of the 5 mL syringe were emptied into the flask in response to the change in pressure. The apparatus was disassembled after opening the stopcock to break the seal. The reagent vial was withdrawn from the apparatus and inverted several times to mix the contents. The outside was then dried and the absorbance at 555 nm measured in a spectrophotometer.

Results were determined with successive measurements in triplicate or as continuous run of single measurements. The blank value determined for that batch of samples was subtracted and the concentration of oxygen calculated from the calibration, assuming that the water in the syringe did not significantly exchange with the water in the flask before the reagent vial was broken.

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