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Characterization of sugar diffusion coefficients in alginate membranes

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The diffusivity of several monosaccharides and disaccharides in calcium alginate gels was determined using a specially designed diaphragm cell. The diffusion coefficients of the tested sugars are 4 to 18% smaller in alginate gel than in water and, with the exception of fructose, this difference increases with increasing sugar molecular weight. Also the position of the carbonyl group seems to be determined in the value of the diffusion coefficient – ketoses have lower diffusion coefficients than aldoses.

Introduction

Among the numerous techniques for immobilization, entrapment in polysaccharide gels is commonly used for immobilization of whole microbial, plant or animal cells (Westrin and Axelsson, 1991; Groboillot *et al.*, 1994). By immobilizing cells, high density cell cultures can be obtained, leading to faster reaction rates and, at the same time, to product stream with a smaller cell concentration or even without cells, simplifying downstream processing. Furthermore, the activity of immobilized cells is kept at a higher value for a larger period of time than in cell free systems (Groboillot *et al.*, 1994).

Calcium alginate is one of the support material most frequently used. Being of low cost and easy to use, the success of alginate as an immobilisation support is also due to the gentle environment that is provided to the entrapped material. However several disadvantages are known, namely its low stability, in the presence of several substances such as citrate and phosphate, and its high porosity, which limits its application to the immobilisation of large particles such as high molecular weight compounds and whole cells (Smidsrod and Skjak-Braek, 1990).

Materials and methods

Sodium alginate was obtained from Riedel-de-Haen. Fructose, glucose, lactose, maltose, sucrose and xylose were obtained from Merck (Darmstadt, Germany).

Sugars concentration was measured according to Miller (1959), after hydrolysis when necessary (sucrose analysis).

Calcium alginate membranes were prepared as discribed elsewhere (Teixeira *et al.*, 1994). In all experiments, sodium alginate concentration was 3% (w/v), membranes were of defined thickness and diameter and were hardened for 3 h in a 2% (w/v) $CaCl_2$ bath.

The diffusion cell (Figure 1) is made of Perspex and consist of two chambers, with a volume of 120 ml, divided by the gel plate. Both chambers were agitated to eliminate external mass transfer limitations.

Diffusion coefficients were determined using the lag-time analysis (Teixeira *et al.*, 1994). The diffusion mass transfer process that occurs through the alginate membrane can be described by the Fick's law. Assuming that the two chambers are well mixed, that both chambers are sufficiently large so that changes in concentration are negligible and that initially solute is only present in the bottom chamber, Ficks law can be solved to:

$$Q_t = \frac{ADC_1}{l}\left(t - \frac{l^2}{6D}\right) \qquad (1)$$

where Q_t, A, D, C_1, l and t represent, the total amount of solute transferred through the membrane until time t, membrane area, diffusion coefficient, solute concentration in the upper chamber, membrane thickness and time, respectively.

The intercept of the linear part of the curve obtained by plotting Q *vs.* time is the so-called 'lag time'. Diffusion coefficients are then calculated from the lag time and the membrane thickness.

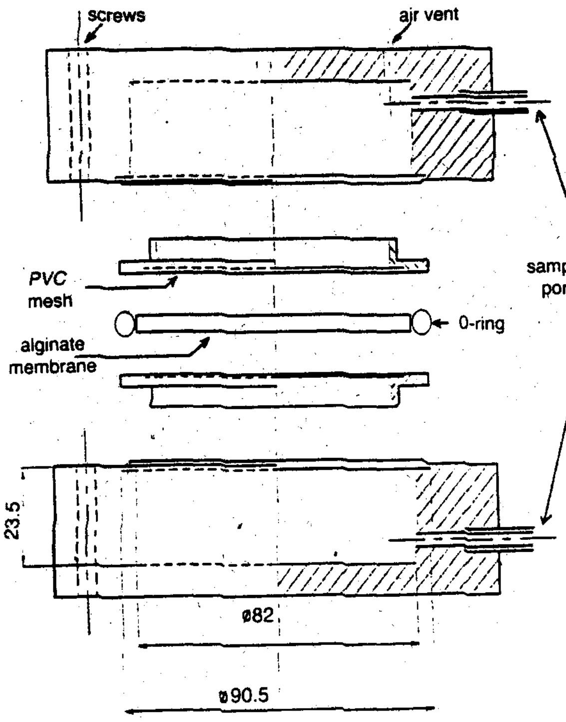

Figure 1 Diffusion cell, all dimensions are in millimeters (from Teixeira et al., 1994).

Experiments were performed at 30°C with an initial sugar concentration of 10 gl^{-1} in the lower chamber.

Results and discussion

The diffusion coefficient, D_a, in 3% (w/v) calcium alginate membranes were determined for a series of single solutes and compared with its value in water, D_w. Solutes were selected with the aim of characterizing the effect of solute molecular weight and position of the carbonyl group in the diffusion values of carbohydrates in alginate menbranes and its deviation from the diffusivity in water.

Table 1 shows the values of D_a, D_w and the ratio D_a/D_w at 30°C for the six carbohydrates examined in this study. With the exception of xylose, experimentally determined diffusivity values were 4% to 18% lower than the corresponding diffusivity in water. This small reduction in diffusivity is in agreement with data reported in literature (Oyaas *et al.*, 1995) an confirms that diffusional mass transfer limitations are negligible in alginate membranes.

Also, these results do indicate that the decrease in diffusivity depends on the sugar being considered.

Table 1 Sugars diffusivities in alginate membranes (D_a), in water (D_w) and its ratio

Sugar	D_a (cm^2s^{-1})	D_w (cm^2s^{-1})[a]	D_a/D_w
Fructose	$6.00 \times 10^{-6} \pm 0.05$	6.84×10^{-6} [b]	0.88
Glucose	$6.70 \times 10^{-6} \pm 0.01$	7.0×10^{-6}	0.96
Xylose	$9.01 \times 10^{-6} \pm 0.05$	7.63×10^{-6} [b]	1.18
Lactose	$4.66 \times 10^{-6} \pm 0.04$	$5.0–5.6 \times 10^{-6}$	0.93–0.83
Maltose	$4.60 \times 10^{-6} \pm 0.05$	5.6×10^{-6}	0.82
Sucrose	$4.67 \times 10^{-6} \pm 0.03$	$5.23–5.56 \times 10^{-6}$	0.89–0.84

[a] Data from Bruins (1926) – International Critical Tables (Oyaas et al., 1995.)
[b] Data from Wilke-Chang equation (Wilke and Chang, 1955).

Although being both hexoses, fructose and glucose present significative differences when considering deviation from diffusivity in water. This may be explained by the different position of the carbonyl groups – fructose is a keto-hexose and glucose an aldo-hexose. This structural difference may be responsible for different interactions with the alginate membrane, with the consequent modification in the diffusion process. The importance of interactive mechanisms between solutes and gel has already being reported by Furui and Yamashita (1985) and Teixeira *et al.* (1994).

With the exception of fructose, it was also observed that the diffusivity of carbohydrates in gels decrease with increasing molecular weight of the sugar. Diffusion coefficients of all the tested disacharides are of the same magnitude and also present similar deviation from the diffusivity value in water. As reported by other authors, the ratio D_a/D_w for glucose is close to one.

For xylose the ratio D_a/D_w is larger than one, what is unexpected and can be explained by the way D_w was estimated. Anyway, the main conclusion remains – xylose, the carbohydrate with the smallest molecular weight is the one that presents the larger diffusivity in alginate.

Conclusions

Diffusion coefficient in 3% calcium alginate membranes were determined for a series of single solutes and compared with its value in water. For the tested carbohydrates it was observed that the diffusivity in alginate and its deviation from the diffusivity in water depends on the molecular weight of the solute and the position of the carbonyl group.

Lower diffusivities and higher deviations from diffusivities in water are obtained for higher molecular weight carbohydrates, while ketoses are more affected by the alginate matrix.

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