

Glucose diffusivity in cell culture medium used in tissue engineering bioreactor

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ABSTRACT

The diaphragm cell method (DCM) was applied to determine the self-diffusion of glucose in cell culture medium (CCM) and water. For this purpose, a diffusion cell was built. The results show that glucose diffusivity in CCM is significantly smaller than those for water, due to the viscosity and extra components.

INTRODUCTION

Glucose diffusion in tissue engineering fluids is fundamental in tissue engineering. But there is a serious lack of data in the literature for glucose diffusivity in cell culture medium (CCM). Hence, the aim of this study is to measure glucose diffusivities in CCM and water (as reference).

In this study, the glucose diffusion coefficient is measured by the diaphragm cell method (DCM). Self-diffusion experiments are conducted in water and CCM at 27 and 37 ± 1 °C. It is envisaged that the diffusivity values as determined in this work will provide improved tool for designing and modeling nutrient transport in tissue engineering bioreactors.

MATERIALS AND METHODS

1) *Materials*: The solute used was of D-glucose-anhydrous (Fisher Scientific UK Ltd, Loughborough, UK). The cell culture medium (CCM) used was Dulbecco's Modified Eagle Medium (DMEM) (Life Technologies Ltd, Paisley, UK). Polyvinylidene fluoride (PVDF) membranes with pore size of 0.1 µm were used in the diffusion cell, as a diaphragm, which were wetted overnight in deionised water to remove any remaining preservative on the surface [1].

2) *Diffusion cell design*: A diffusion cell (Figure 1) was built to determine the liquid diffusivities of glucose in both CCM and water. The cell consisted of two compartments with 52.5 ml each of which. PVDF membrane was placed in between. Each cell had a stirrer shaft with 40 RPM, controlled by a motor. The whole apparatus was placed in a temperature-controlled box with an attached thermocouple.

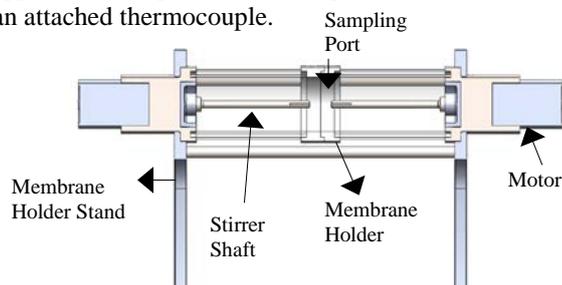


Figure 1. Cell design used in diffusion experiments

3) *Calibration of the diffusion cell*: Prior to starting the diffusion experiments, cell constant, β , was determined, which is necessary for diffusivity determination (1.28×10^9 m²/s) [2]. For this purpose, one of the compartments was filled with ethanol solution while the other with deionized water. The diffusion of ethanol was monitored by taking samples from both the compartments, at intervals of 1 h for

a period of 5 h. The samples were analysed by a UV spectrophotometer (UV Mini 1240, Shimadzu, Japan) at a wavelength of 196.5 nm. The samples were poured back into the compartments almost immediately after analysing, in order to keep the volume constant. The cell constant, β ; was determined by the following equation:

$$D = \frac{1}{\beta t} \ln \left(\frac{C_{i,glucose-CCM/water} - C_{i,CCM/water}}{C_{f,glucose-CCM/water} - C_{f,CCM/water}} \right) \quad (1)$$

where D is the diffusion coefficient of ethanol in water, t is the diffusion time, C is the concentration of the diffusing solute molecule and i and f denote the initial and final, respectively. β was calculated by dividing the slope of the line with the diffusion coefficient of ethanol in water.

4) *Methodology for diffusion experiments*: Both compartments were filled with glucose solution and CCM/water. Both solutions were warmed to 27 or 37 °C in the water bath for at least 60 min. An YSI glucose analyser (YSI 2300 STAT PLUS, YSI UK Ltd, Hampshire, UK) was used to measure the glucose concentration at initial and final conditions. The glucose-in-water diffusion experiments were run for 24 and 22h at 27 and 37 ± 1 °C, respectively, while the glucose-in-CCM diffusion experiments were run for a period of 7–11 h for both temperatures. All diffusion experiments were repeated three times. The corresponding diffusion coefficients were calculated according to Eq. (1) where β is the cell constant determined experimentally. More details has been reported recently by Suhaimi et al [3].

RESULTS AND DISCUSSION

From Table 1, it can be seen that the diffusion coefficient of glucose in CCM is lower at a given temperature due to a higher dynamic viscosity of CCM.

Table 1 Self-diffusivities of glucose in CCM and water.

	Temperature (°C)	Average dynamic viscosity (kg/m/s)	Experimentally Determined D (m ² /s)
CCM	27 ± 1	0.001306489	5.67 ± 0.74 × 10 ⁻¹⁰
	37 ± 1	0.001100855	6.16 ± 1.25 × 10 ⁻¹⁰
Water	27 ± 1	0.000865269	6.98 ± 0.60 × 10 ⁻¹⁰
	37 ± 1	0.000649516	9.58 ± 0.13 × 10 ⁻¹⁰

CONCLUSIONS

The results show the diffusion coefficients of glucose in CCM are significantly reduced at a given temperature due to the larger dynamic viscosity of CCM compared to the ones in water. This may also be due to the multi-component interactions present in CCM and what we obtained is therefore a lumped effect from a number of inter-related phenomena.

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