

Lab Report

“Glucose and Insulin Diffusion through Polymer Membranes”

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1 Introduction

Diabetes mellitus is a disease affecting almost 10 % of the world's population, but can currently not be cured [1]. In type 1 diabetes beta-cells of the pancreas producing insulin become completely destroyed due to autoimmune processes [2]. Contrary, in type 2 diabetes cells develop a resistance against insulin, leading to exhausted beta-cells [2]. Usually, an increasing blood glucose level stimulates insulin secretion in the pancreatic beta-cells of the Langerhans islets [3]. Glucose represents the main energy source for most of the cells in the human body. To uptake glucose, that is unable to diffuse into cells, glucose transporters (GLUT) are expressed on their surfaces, where insulin stimulates the active transport of glucose from the blood through the cell membrane [4]. If the beta-cells are damaged, and no insulin is secreted, body cells cannot uptake a sufficient amount of glucose anymore, and the blood sugar raises unnaturally having a dangerous overall influence on the human organism [5].

The idea of minimally invasive inserted beta-cell implants replacing the function of damaged beta-cells in the human body is a promising approach to treat diabetes mellitus in future. With the technique of macroencapsulation, several beta-cells become embedded into a polymer membrane, preventing implanted beta-cells from host immune attack. In this practical, different polymer membranes were investigated regarding their time-dependent diffusion properties of glucose and insulin.

2 Methods

A glucose (40 μM) and an insulin solution (200 $\mu\text{U/ml}$), each in one reactor, were separated by a polymer membrane. For evaluation of the membrane's diffusion properties, regarding these two types of molecules, samples of both solutions were taken at different time points each, listed in Table 1. The insulin solution was observed for its glucose concentration (conc.) with a Glucose-Glo™ Assay by Promega, and the glucose solution was investigated for its insulin conc. with an Insulin ELISA test by TECAN. Both tests were conducted as recommended by the manufacturer each.

Table 1: Timepoints for taking glucose and insulin samples from the respective solutions

Sample	1	2	3	4	5	6	7	8
Time [min]	0	1	2	5	10	20	30	60

Furthermore, standard solutions of glucose and insulin were prepared. Their concentrations are listed in Table 2.

Table 2: Concentrations of standard solutions of glucose and insulin

Sample	1	2	3	4	5	6	7	8
Glucose [μM]	0	1	2	5	10	20	30	50
Insulin [$\mu\text{U/ml}$]	0	6.25	12.5	25	50	100		

2.1 Glucose-Glo™ Assay

The glucose detection is based on a luminescence signal. In presence of the enzyme Glucose dehydrogenase, D-Glucose, contained in the sample, is oxidated into D-glucono-1,5-lactone. As a side reaction, NAD^+ is converted into NADH, that's presence is necessary for the transformation of Pro-luciferin into Luciferin by the Reductase. With Luciferase and ATP, Luciferin is made visible by luminescence. In this practical, a so-called glucose detection reagent was mixed, consisting of Glucose dehydrogenase, NAD^+ , Reductase, Pro-luciferin and Luciferin detection solution. The detection reagent was added in a ratio of 1:1 to the sample, leading to a luminescence signal proportional to the amount of glucose contained in the sample. The signal increases until all glucose in a sample is transformed. Then, a steady signal can be determined.

2.2 Insulin ELISA

For quantifying the insulin conc. an ELISA test kit, based on the sandwich principle, was used. The insulin contained in the sample added into the well-plate bound to monoclonal antibodies against insulin that already adhered in the well-plate. Additionally, an enzyme conjugate, that functions as antibody against insulin, was added. Since the detection principle of this ELISA was based on light absorbance of a Biotin-Streptavidin complex (BSC), the antibody added was conjugated with Biotin. After incubation, the unbound antibodies were washed off, and the wells were incubated with the streptavidin peroxidase enzyme complex that bound to the biotin present in the solution. The unbound streptavidin complexes were washed off, and a substrate that was converted by BSC was added. As the number of bound BSC is proportional to the amount of insulin in a sample, the intensity of color due to the enzymatic reaction of the substrate is also proportional to the amount of insulin in the sample.

3 Results

3.1 Glucose Measurement

Figure 1 shows the luminescence measured for the glucose standard solution. The calibration curve is only depicted until a conc. of $5 \mu\text{M}$, since the incubation time with

the glucose detection reagent for the standards of higher conc. was significantly less (60 min) than for the first ones (100 min). Therefore, these luminescence values are much lower, and would also not fit to the samples that had incubated about 100 min. Based on the glucose calibration curve above, the determined glucose conc. of the samples A (blue) and B (orange) were plotted against the time points when the samples were taken from the respective solutions (see Figure 2). Both curves show a diffusion kinetic of limited growth. For sample B, the measured concentrations were slightly higher. However, the values from sample B taken at 2, 5 and 10 min are slightly deviating from the curve's limited growth form. The plotted values of sample B represent the calculated mean of a triplicate, whereas sample A is calculated of a duplicate, respectively the last two values are based on a single measurement due to different incubation times.

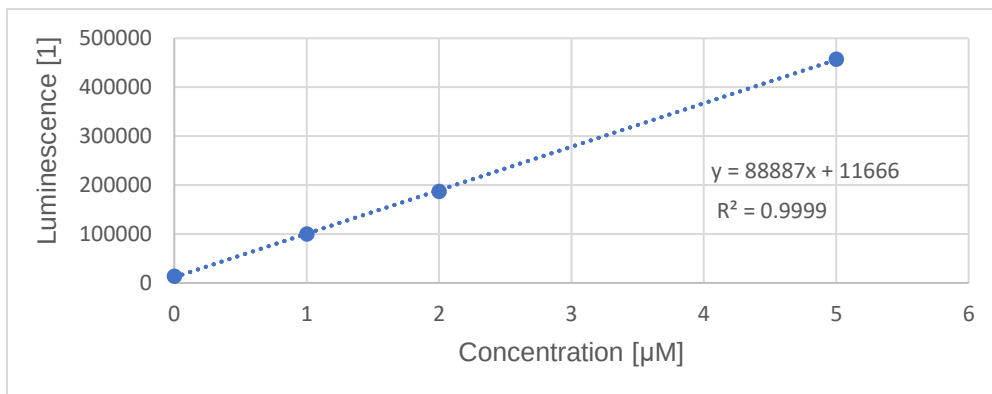


Figure 1: Calibration curve determined by luminescence measurement of glucose standard solution

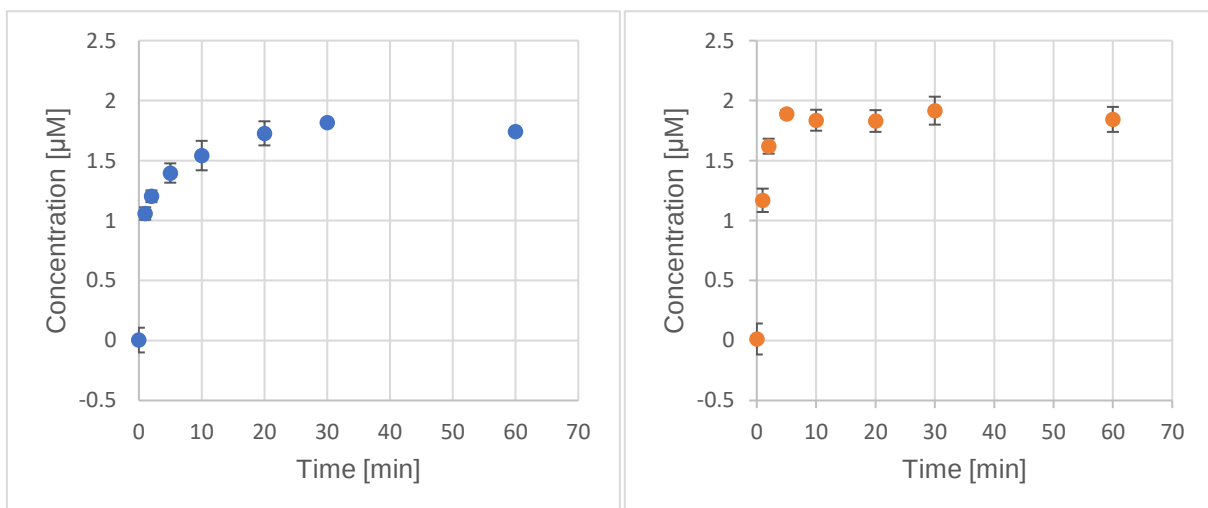


Figure 2: Glucose concentration of samples A (blue) and B (orange) plotted against the time when taken from the respective solution

In Table 3 it can be observed that the timepoint for 80 % total glucose diffusion is much lower for sample B than for sample A.

Table 3: Total glucose diffusion through membrane and timepoint for 80 % diffusion of glucose

Sample	A	B
Total Diffusion [μM]	1.8	1.9
Timepoint for 80 % Diffusion [min]	6	1.7

3.2 Insulin Measurement

Figure 3 shows the absorption measured for the insulin standard solution at 450 nm, after subtracting the absorption of the background (measured at 620 nm). Based on this calibration curve, the determined insulin concentrations of the samples A and B were plotted against the time points when the samples were taken from the respective solutions. The diffusion kinetics of the two insulin samples are depicted in Figure 4.

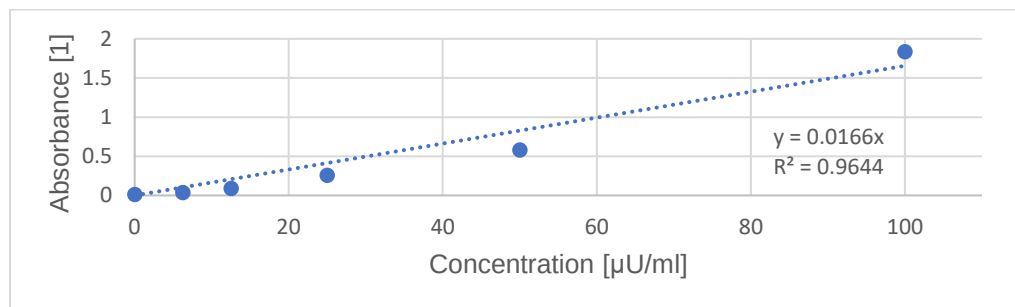


Figure 3: Calibration curve determined by absorption measurement of insulin standard solution

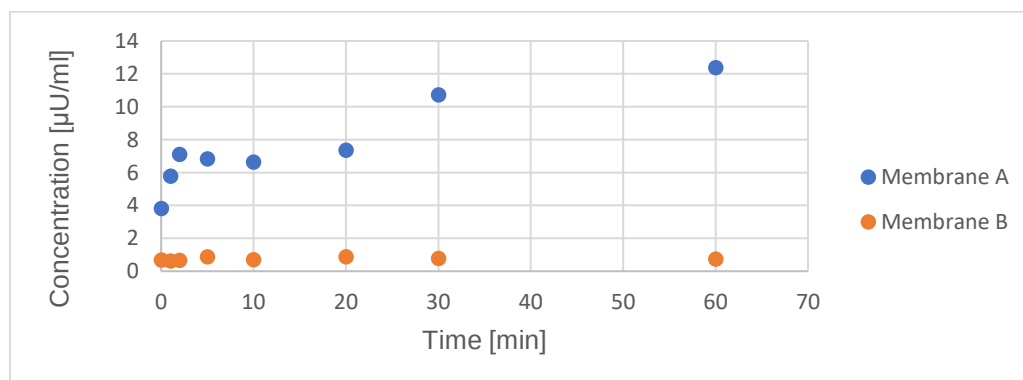


Figure 4: Insulin concentration of samples A and B plotted against the time when taken from the respective solution

The curves obtained for sample A and B are based on a single measurement at each point, and not on a triplicate. As depicted in Figure 4, the insulin conc. in sample A increases over time, but not homogeneously. In sample B, only minimal conc. changes can be detected. In Table 4 the total insulin diffusion as well as the timepoint for 80 % of diffusion can be observed for sample A. As the diffusion values of sample B range around one value, no timepoint for 80 % of diffusion can be determined.

Table 4: Total insulin diffusion and timepoint for 80 % diffusion of insulin

Sample	A	B
Total Diffusion [$\mu\text{U/ml}$]	12.4	0.7
Timepoint for 80 % Diffusion [min]	27.5	-

4 Discussion

This practical was conducted with the aim to observe diffusion properties of glucose and insulin through polymer membranes, to evaluate whether the investigated membranes would be suitable for macroencapsulation of beta-cells. Membrane A and B allowed glucose diffusion until a conc. limit of $1.8 \mu\text{M}$. As the conc. of the glucose sample was $40 \mu\text{M}$, it seems that both membranes are enabling a diffusion of approx. 5 %. The three deviating measurement values of sample B might be explained by inconsistent pipetting. As these three values seem much higher than expected, the 80 % diffusion value of sample B, indicating a faster diffusion than sample A, might be falsified. Membrane A allowed 6 % of insulin to diffuse through ($12 \mu\text{U/ml}$ of $200 \mu\text{U/ml}$) without reaching a plateau. However, it has to be considered that the insulin conc. in the sample amounted already $4 \mu\text{U/ml}$ at timepoint 0 min. Membrane B seems to be non-permeable for insulin. It has to be considered that the insulin measurement is based on one measurement value for both membranes, what easily falsifies the results. For Membrane A, the time needed for 80 % diffusion of glucose is significantly less than for insulin, indicating a faster diffusion kinetics of glucose through this membrane.

Generally, a beta-cell implant should allow glucose to enter, and insulin to diffuse out of the cell. Moreover, the membrane should enable in/out diffusion of further important molecules as e.g. glucagon out, or nutrients in, as well as functioning as barrier as e.g. against immune cells. In this practical only a conclusion about the diffused conc. was drawn. This setup is not suitable to evaluate whether a low conc. is resulting from either the membrane being non-permeable, or adsorbing the molecules. For this, additionally the time-dependent conc. of glucose and insulin in their initial reactors have to be measured. In this practical membrane A turned out as potentially suitable for usage in beta-cell implants, and should be further observed for other properties mentioned above, as well as for its biocompatibility and viability, and the fact that after 30 min a plateau of $1.8 \mu\text{M}$ was reached for glucose diffusion. Membrane B is not suitable at all due to its non-permeable properties regarding insulin.

5 Literature

1. Saeedi, P., et al., Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Research and Clinical Practice, 2019. **157**: p. 107843.
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I hereby declare that my elaboration was made independently and is not a copy of an existing protocol.

Date: May, 18th 2022

Signature: