



Abstract

Electrochemical Diffusion Study in Hydrogels [†]

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Abstract: In this study, poly(ethylene glycol) dimethacrylate (PEG-DMA)-based hydrogels were investigated with respect to the diffusion properties of methylene blue (MB) and MB conjugated proteins (MB-BSA and MB-IgG). Electrochemical sensors were used to monitor the diffusion process via the redox-active MB-label. All tested molecules showed good mobility in the hydrogel. Also, the release of MB-BSA could be demonstrated after drying the hydrogel containing MB-BSA, which is a promising result for the development of hydrogel-based reagent reservoirs for biosensing.

Keywords: hydrogels; electrochemical sensors; diffusion study; methylene blue

1. Introduction

Hydrogels are used in numerous areas to transport [1] or store reagents [2]. The development of printable hydrogels opens up a wide range of applications [3], such as reagent delivery in microfluidics for point-of-care (PoC) diagnostics [4]. In this study, printable PEG-DMA-based hydrogel reservoirs are investigated. Figure 1 depicts the workflow of the study. It starts with the modification of the sensor with a hydrogel (Figures 1A and S1A). The hydrogel is then overlaid with a MB or MB protein conjugate solution (Figure 1B). As proteins, BSA and mouse IgG were chosen because they are good representatives for bioanalytical assays [4]. The measurement is immediately started after adding the MB(-conjugate) and the diffusion is monitored using differential pulse voltammetry (DPV) (Figure 1C). To understand the diffusion properties, experiments were carried out using (i) sensors without hydrogel, (ii) sensors coated with a 10 kDa PEG-DMA hydrogel, (iii) sensors coated with different-molecular-weight (MW) PEG-DMA hydrogels, and (iv) wet and dry hydrogels for testing the diffusion of MB-BSA.



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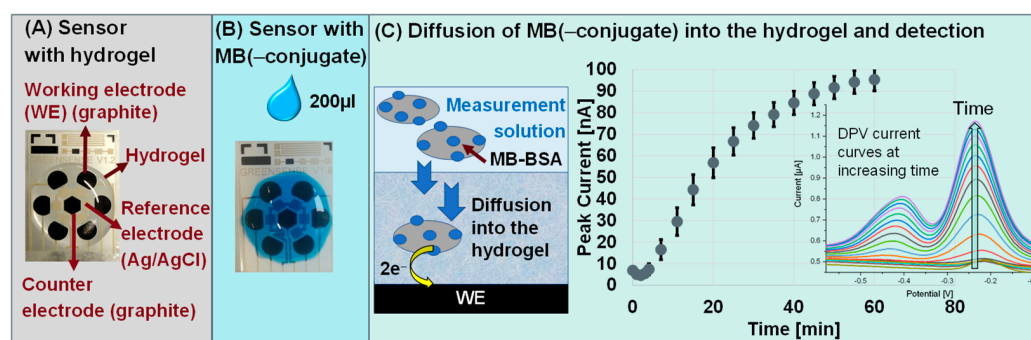


Figure 1. Sensors covered with hydrogel (A) were overlaid with an MB(-conjugate) solution (B) for electrochemical diffusion monitoring via DPV (C).

2. Materials and Methods

The hydrogel inks were fabricated by mixing 30 μL of 166 mg/mL PEG-DMA in water with 60 μL di(ethylene glycol) vinyl ether, 8 μL of water, and 2 μL of a lithium phenyl (2,4,6-trimethylbenzoyl) phosphinate (LAP) solution (10 mg LPA per 100 μL in a 1:1 mixture of ethanol and ultrapure water). In total, 80 μL of the ink was applied on the sensor (Figure 1A), UV-crosslinked at 365 nm (1 J/cm²), and washed two times with physiological PBS buffer (1 \times 10 min, 1 \times 15 min). The hydrogel layers were tested in wet (Figure 1A) and dry state. MB, MB-BSA, and MB-IgG were used for the study. The MB conjugation was performed in-house (see Supplementary Materials). For the DPV measurements, a PalmSens MUX8 R2 potentiostat was used (Figure S3). The measurements were carried out up to 160 min, and the DPV peak currents, I_{peak} , were recorded.

3. Discussion

First, different concentrations of MB(-conjugates) were measured on sensors without a hydrogel layer. It was found that when the I_{peak} values for MB-BSA and MB-IgG conjugates are normalized by dividing them by the degree of labelling (DOL), comparable results are obtained for both conjugates. This indicates that conjugated MB molecules equally contribute to the current output (Figure S5). Second, sensors modified with a 10 kDa PEG-DMA hydrogel were measured with different concentrations of MB(-conjugates). The corresponding I_{peak} values were analyzed at 60 min. It was found that the I_{peak} values were reduced up to 92% for MB (Figure S4), 73% for MB-BSA, and 23% for MB-IgG. Furthermore, the normalization of the I_{peak} by division with the DOL did not lead to comparable results for MB-BSA and MB-IgG, which indicates different transport properties of BSA and IgG in the hydrogel (Figure S6). However, the results demonstrate the good migration property of MB(-conjugates) through the ~ 2 mm thick wet hydrogel. Third, hydrogels with different MW PEG-DMAs (1, 2, 3.4, and 10 kDa) were compared regarding the diffusion properties for the MB(-conjugates). The I_{peak} values were normalized with respect to the MB content in μM to compare the time resolved results of MB(-conjugates). Higher peak currents were observed with increasing MWs of PEG-DMA for MB and MB-BSA (Figures S6 and S7). Fourth, the MB-BSA diffusion into and out of a wet (Figure 2A) and a dry hydrogel (Figure 2B) was investigated. It was found that MB-BSA can diffuse even if the MB-BSA-loaded hydrogel is dried for 12 h (Figure 2B). These results are promising for the fabrication of hydrogel reservoirs for reagent delivery in PoC diagnostics.

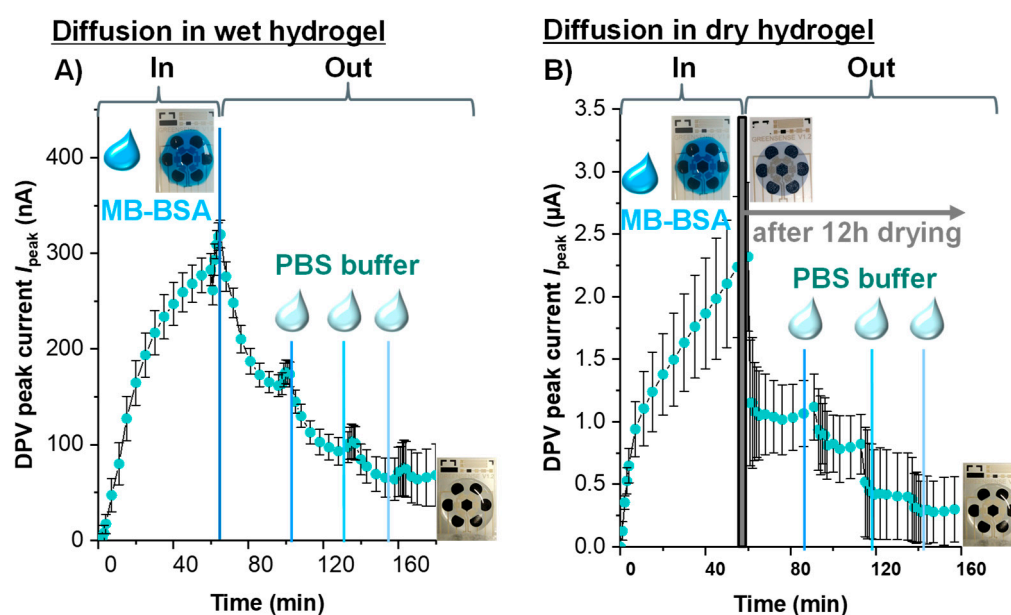


Figure 2. Diffusion study of MB-BSA for wet (A) and dry hydrogel (B).

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/proceedings2024097118/s1>, Figure S1: (A) Graphite sensor, (B) Screen-printed sheet, (C) Peak currents measured on nine sensors of the screen-printed sheet; Table S1: Values of dry weight, swollen weight, water content, and swelling ratio of the hydrogel layers; Table S2: Settings for the lyophilization; Figure S2: SEM images of vacuum dried and lyophilized hydrogel structures; Figure S3: Measurement setup: (1) PalmSens multiplexer MUX8-R2 (PSTrace software 5.7), (2) connector, (3) sensor, (4) computer; Figure S4: Detection of MB with and without hydrogel coating; Figure S5: (A) Concentration dependent measurement of MB-BSA and MB-IgG, (B) normalization of the DPV peak current I_{peak} with the DOL of 5.8 for MB-BSA and of 3.8 for MB-IgG leads to a better comparability of the signals; Figure S6: Normalized DPV peak currents (nA/ μM MB) of MB, MB-BSA, and MB-IgG measurements on 1, 2, 3.4 and 10 kDa PEG-DMA hydrogel modified sensors over 60 min. Curves were fitted with a Langmuir fitting function; Figure S7: Maximum equilibration current $I_{\text{peak_max}}$ of MB, MB-BSA, and MB-IgG for different molecular weight PEG-DMA hydrogels.

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Conflicts of Interest: The authors declare no conflict of interest.

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