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Dependence of yield of nuclear track-biosensors on track radius and analyte concentration

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ABSTRACT

In swift heavy ion track-based polymeric biosensor foils with incorporated enzymes one exploits the correlation between the analyte concentration and the sensor current, via the enrichment of charged enzymatic reaction products in the track's confinement. Here we study the influence of the etched track radius on the biosensor's efficiency. These sensors are analyte-specific only if both the track radii and the analyte concentration exceed certain threshold values of ~15 nm and $\sim 10^{-6}$ M (for glucose sensing), respectively. Below these limits the sensor signal stems un-specifically from any charge carrier. In its proper working regime, the inner track walls are smoothly covered by enzymes and the efficiency is practically radius independent. Theory shows that the measured current should be slightly sub-proportional to the analyte concentration; the measurements roughly reconfirm this. Narrower tracks (~5–15 nm radius) with reduced enzyme coverage lead to decreasing efficiency. Tiny signals visible when the tracks are etched to effective radii between 0 and ~5 nm are tentatively ascribed to enzymes bonded to surface-near nano-cracks in the polymer foil, resulting from its degradation due to aging, rather than to the tracks. Precondition for this study was the accurate determination of the etched track radii, which is possible only by a nanofluidic approach. This holds to some extent even for enzyme-covered tracks, though in this case most of the wall charges are compensated by enzyme bonding.

1. Introduction: Going to the sensor's limits

Since a few decades latent and etched ion tracks in thin polymer foils [1,2] are applied for biosensing [3]. From the different measuring strategies, we favour the "Product Enhancement" strategy [3] most. Here, enzymes are fixed to the walls of transparent etched ion tracks in a polymer foil. Whenever analyte molecules enter, the enzymes digest them towards the corresponding characteristic reaction products. If the product's formation speed is higher than their escape speed from the tracks, they will enrich within the tracks [4]. This is given whenever the analyte concentration is not too low and whenever the etched track's aspect ratio l/r (l = track length, r = track radius) is sufficiently large. The latter case is fulfilled for conventional etched ion tracks in polymers such as polyethylene terephthalate (PET), which have typical aspect ratios (i.e., length/diameter ratios) of up to ~1000 or so. The higher the product accumulation within the tracks is,[1] the larger is the biosensing signal [4]. Basic pre-condition for this sensing technique is that both the analyte and the enzymatic reaction products differ in their charge states[2] from each other, so that changes in the track conductivity reveal the presence of the analyte.

Hitherto, this technique has been applied by us for sensing of glucose (via the enzyme glucose oxidase, GOx) [5], urea (via the enzyme urease) [6] and phenols (via the enzyme laccase) [7]. The "Product Enhancement" strategy is advantageous due to the ease of production (no facility for single ion track production necessary), a wide tolerance for sensor operation (concerning the etched track fluences and radii), relatively large sensor currents (due to the simultaneous use of up to $\sim 10^9$ tracks/cm^2 in a foil), its environmental friendliness (only

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[1] The product accumulation can be enhanced b, g., long track lengths, narrow track diameters, a high ratio between the analyte's to the product's diffusion coefficients or by the closure of one track opening [4].

[2] See, e.g., the case of glucose sensing with glucose oxidase, where the analyte glucose is neutral and the product gluconic acid is ionized; hence the enzymatic reaction leads to an increase in the charge state of the liquid in the etched in tracks.

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polymers and enzymes are required), its low cost (only ~ng of enzymes are used), its possibility for manyfold sensor re-use (at least some ~30 times), the robustness of the sensors against thermal denaturation (storing them outside the fridge for several days hardly has any negative influence on their performance) and the prolonged sensor lifetimes (up to ~3 months or more are possible if stored in a fridge).

In the view of the success of this strategy we thought it worthwhile to test its utmost limits of applicability. We were especially interested in finding out what happens when steadily reducing both the analyte concentration and the etched track diameter (hence also rising the track aspect ratio). Two questions arose in this connection, namely a) how does nanofluidics influence the sensor's behaviour and b) does the enzyme's reaction kinetics influence the sensor's detection sensitivity?

2. Experimental

2.1. Details of sensor preparation

For our biosensor experiments, we applied PET (Melinex and Hostaphan) and polycarbonate (Makrofol KG) foils (denoted here as foils #6, #8 and #15 for consistency with recent track etching studies in Ref. [8]). Though these foils are already 20, 4 and 6 years old, respectively, they all still maintain good plasticity and etchability and therefore are feasible for biosensor production. These foils were irradiated by energetic heavy ions to create straight parallel radiation-damaged regions ('latent ion tracks') therein. For this publication, we mostly used foil #8 with the following specifications: thickness 19 μm, irradiated by 170 MeV Xe ions up to a fluence of 1×10^7 cm^{-2}. The less frequently used foil #6 is 12 μm thick and was irradiated by 250 MeV Kr ions up to a fluence of $4 \times 10^6\,cm^{-2}$, Finally, foil #15 is 8 μm thick and was irradiated by 985 MeV Au ions up to a fluence of $2 \times 10^8\,cm^{-2}$. The samples, cut to typical sizes of $\sim 1 \times 1\,cm^2$, were etched with 4 M NaOH at room temperature (~22C) in a specially designed etching chamber (which was made after the example of Ref. [9]). The etching procedure was constantly monitored by conductometry, applying for this purpose a power supply (inner resistance: $R_{int} = 1\,M\Omega$) which delivered a sinusoidal voltage of ~2 V at ~50 mHz. The transmitted currents were measured with an oscilloscope (inner resistance $R_o = 1\,M\Omega$) with a measuring point resistance R_s which could take values of 0 and 9 MΩ, depending on the measuring task [8].

During the etching the radiation-damaged material along the latent ion tracks was preferentially dissolved and removed, thus transforming the latent ion tracks into "etched tracks". As in this transition region we observe a gradual opening and merging of the radiation-induced free volume zones towards narrow nanopores, these tiny structures are by no means all of perfectly conical or cylindrical shape, but instead exhibit individual statistical variations. This means that the track radii r derived here must be considered as "effective radii" only, i.e., they are mean values, averaged over the whole track lengths, and they do not give any clue for the accurate track shapes. For this work, we prepared etched tracks of cylindrical (in the track core region for radii < 10 nm) or funnel-type shapes (for larger tracks) [2,8,10]).

After etching to appropriate track diameters (as measured after replacing the etchant by ultrapure water and calculating the radius from the transmitted current), the foils were washed with activation buffer (0.1 M MES (2-[morpholino]ethanesulfonic acid), 0.5 M NaCl, pH 6.0) and immersed in a solution of 2 mM 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC) and 5 mM *N*-hydroxysulfosuccinimide (S-NHS) for 15 min. The activated foils were then washed with coupling buffer (0.1 M sodium phosphate, 0.15 M NaCl, pH 7.2) and immersed into a solution of 10 mg/mL glucose oxidase (GOx). After 2 h, the foils were rinsed extensively with phosphate buffer to eliminate the non-bonded GOX and stored at 4 °C in phosphate buffer until use. For reliable comparison of the sensors with each other, we treated all of them in exactly the same way, independent of their individual etched track size. Immediately before starting with the measurements, the biosensor foils were accommodated to the new ambient, by either washing them typically 10 times or so with ultrapure water (supplier: Millipore Corp. (Merck), conductivity $18.2\,M\Omega^{-1}\,cm^{-1}$) until their conductivity remained constant, or by allowing them to release their buffer ions for at least ~40 min in ultrapure water, with roughly the same result, Fig. 1. Thereafter the sensors were ready for use with the utmost possible measuring sensitivity.

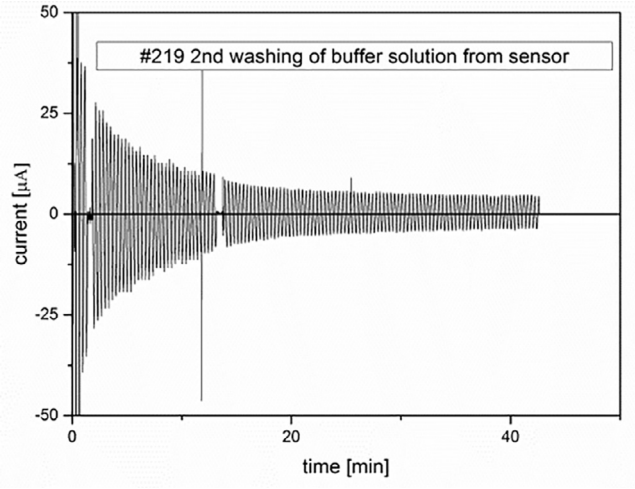

Fig. 1. Accommodation of biosensor #219, (made of foil #6, side B [8]) from storage in buffer solution to the measurement in ultrapure water, as described by the transmitted current as a function of accommodation time. In this case, we both allowed the buffer ions to diffuse out from the sensor foil into the ultrapure water ambient by just waiting, and also changed the ambient solution two times (see the interruptions). No remarkable difference shows up during either waiting for an hour or so, or during changing the washing water multiple times. Sinusoidal alternating voltage of 5 V, 49.5 mHz applied; measurement with oscillscope; no shunt used.

2.2. Derivation of the etched track radii from measured transmitted test currents

Until recently, it is convenient to calculate the etched track radii from test currents transmitted through them by conventional macroscopic conductometry [2,10,11]. However, meanwhile it is known that macroscopic flow conditions break down in nanopores and have to be replaced by a nanofluidic approach (see eg., the excellent recent review article by Bocquet et al. [12], or others [13–15]). As to our knowledge, the nanofluidic conversion from conductometrically determined test currents across etched tracks to nanopore radii has not yet been explicitly reported,[3] we care for it here. To follow this procedure, the knowledge of both the ionic conductivity in the nanopore K_{Bulk} and the density of the charges $K_{Surface}$ on the nanopore surfaces is essential.

Scaling Bocquet's numerical example of a representative monovalent nanofluidic system [12] down to our *as-etched* and subsequently water-filled tracks (i.e., before any enzyme deposition starts), then $K_{Bulk} \sim 5.5 \times 10^{-8}\,Scm^{-1}$ (corresponding to the impurities in ultrapure water, estimated to be in the order of $C_{Bulk} \sim 10^{-7}$ M) and $K_{Surface} \approx 0.1\,e/nm^2 = 1.6\,\mu C/cm^2$ are applicable. Then, the system's characteristic nanofluidic lengths are: the Bjerrum length: $l_B = 0.7$ nm (for whatever K_{Bulk} and $K_{Surface}$), the Debye length: $\lambda_D \sim 0.9$ μm and the Dukhin length: $l_{DU} \sim 1.6$ mm. Scaling, however, these results down to enzyme-covered *biosensor* tracks (where surface charges are largely neutralized due to enzyme bonding), we should rather assume $C_{Bulk} \sim \{10^{-7}$ to $10^{-6}\}$ M (hence $K_{Bulk} < 5.5x10^{-7}\,Scm^{-1}$) and $K_{Surface}$

[3] Possibly this stems from the researcher's (perhaps intentional) selection of high salt concentrations, specific pH values, and low voltages for track radius calculations, which reduces the nanofluidic influence considerably and avoids the otherwise necessary lengthy calculations.