Integrated carbon nanotube fibre–quartz tuning fork biosensor

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Abstract. We report on a novel label-free biosensor for in situ measurements in liquid. It is comprised of a porous carbon nanotube (CNT) fibre attached to one prong of a quartz tuning fork (QTF) resonator. Only the protruding CNT fibre is immersed into a liquid, while the QTF is kept above the liquid to avoid short circuit of its electrodes. The low density and large surface area of the CNT fibre assure sufficient sensitivity without affecting the performance of the QTF significantly. Efficiency of the sensor was demonstrated experimentally by comparison of the adsorption rate of bovine serum albumin to the CNT fibre at two different pH values; differences in adsorption rates were clearly distinguishable.

Key words: biosensing, adsorption, instrumentation, protein, quartz tuning fork, bovine serum albumin.

1. INTRODUCTION

Quartz tuning forks (QTFs) have previously been proposed [1] as cost-effective and simple alternatives to conventional label-free mass-sensing quartz crystal microbalances (QCM) [2]. As in the case of QCM, the resonant frequency (F_{res}) of an oscillating QTF depends on the adsorbed mass, yielding comparable sensitivity [1]. However, QTF has certain advantages, such as a markedly lower cost, more stable resonant frequency, and simpler electronics due to the considerably lower working frequency (up to a hundred kHz for QTF and from one to several dozens of MHz for QCM). Moreover, lower frequency is preferable when working in liquid because viscosity and other properties of the liquid can cause increased measurement uncertainties under high frequency excitation [3]. In addition, QTFs have been shown to attain superior sensitivity compared with that of QCM when measuring high-molecular weight polymer solutions [3], which strongly encourages the development of QTF biosensors.

Application of QTFs in biosensing was proposed and experimentally demonstrated by Su et al., who studied the adsorption of human IgG to an anti-human IgG-modified QTF [4]. They also compared the performance of a mechanically excited QTF (excitation by external piezo-element) with the performance of a QTF operating in self-excitation mode (excitation by an AC voltage) and found that the latter was more sensitive, yielding a detection limit of approximately 5 µg mL^{-1}.

The main limitation of QTF-based biological sensing is that in situ measurements in water or other liquids with high dielectric permeability cannot be performed due to the arrangement of the QTF's electrodes.

A QTF is also used as a sensor in scanning near-field optical microscopy (SNOM) [5], where a thin optical fibre is attached to one of the QTF's prongs along its length. Such a configuration enables SNOM measurements to be performed in water. Indeed, an externally excited QTF–SNOM sensor operating in pure water was successfully demonstrated by Rensen et al. [6]. Furthermore, Lee et al. [7] showed an analogous system to be viable in buffer solutions. Instead of immersing the entire QTF in the solution, they inserted only the optical fibre, while the resonator was kept above the liquid. A similar approach, i.e. immersing only the scanning probe, was used in self-excitation mode by Kim et al. [8]. The authors concluded that their technique is readily applic-

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able to imaging soft biomaterials. To our knowledge, this approach has not been attempted in the case of a QTF biosensor, where the requirements for the probe are different and more demanding than those for SNOM sensors. The probe must be lightweight, stiff, and have a large surface area to provide sufficient sensitivity without affecting the performance of the QTF.

In this work, we demonstrate a novel label-free biosensor for in situ measurements in aqueous solutions. The biosensor is comprised of a carbon nanotube (CNT) fibre attached to one prong of a QTF. The CNT fibre was chosen because of its porous structure, low density, and high stiffness [9]. Moreover, CNTs can be easily functionalized [10], which is necessary for designing specific biorecognition assays. The performance of the sensor was demonstrated experimentally by monitoring the adsorption rate of bovine serum albumin (BSA) to the CNT fibre at two different pH values. According to Valenti et al., BSA is adsorbed directly onto CNTs, with the adsorption rate depending on the pH [11]. For the BSA adsorption experiments, pH values corresponding to maximal (pH 4.8) and minimal (pH 7) adsorption rates were selected.

2. MATERIALS AND METHOD

BSA was purchased from PAA Laboratories GmbH. BSA solutions ($0.1 \, mg \, mL^{-1}$, pH 4.8 and pH 7) were prepared in phosphate buffers.

Multiwall carbon nanotubes (O.D. × I.D. × L = 20–40 nm × 5–10 nm × 0.5–50 μm) were purchased from Sigma-Aldrich. Fibres were prepared by dielectrophoresis [12]. Briefly, the tip of a sharp tungsten wire was immersed into a droplet of a CNT suspension in water and a fibre of desired length was drawn under an AC voltage. Our set-up enables preparation of fibres up to 100 mm in length. By changing the concentration of the CNT suspension, the drawing speed, and the voltage, it is possible to vary the diameter of the fibres from submicron to 400 μm. A typical CNT fibre used in our BSA adsorption experiments is shown in Fig. 1.

To assure comparability of individual sensors, a precursor fibre several centimetres long was drawn and then cut into pieces of equal length to ensure uniformity of diameter and other parameters within the set of different sensors.

Biosensors were made by gluing the CNT fibre to one prong of the QTF (F_{res} = 32768 Hz; Clock quartzes TC-38) using an epoxy glue (Eccobond 286, Emerson & Cuming).

The sensor was connected to a circuit consisting of an AGILENT 33120A 15 MHz Function/Arbitrary Waveform Generator and a METRIX 3354 oscilloscope. METRIX software was used to record and analyse the data on a PC.
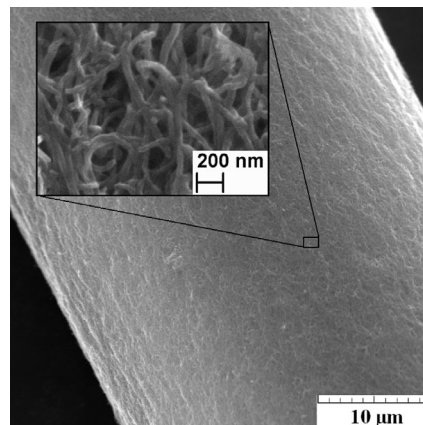

Fig. 1. SEM micrograph of a CNT fibre.

The principle of the operation of such a sensor consists in immersing only the CNT fibre into the solution, while keeping the QTF above the liquid (Fig. 2). In this case, the CNT fibre acts as an extension of the QTF's prong. Changes in the fibre's mass result in an immediate shift of the QTF's resonant frequency. During measurements, oscillations of the QTF are excited electrically by applying AC voltage from a generator, and frequency response is constantly monitored on an oscilloscope, thus providing real-time information on the adsorption rate. If previously calibrated, the sensor can measure the exact mass of the adsorbed substance.

With the CNT fibre immersed in a liquid QTF has a q-factor over 100 (Fig. 3). It is at least one order of magnitude higher than in case of immersing the whole QTF in liquid [3].

The BSA adsorption measurements included several steps. First, the sensor was conditioned by immersing the fibre into a pure buffer solution for several minutes
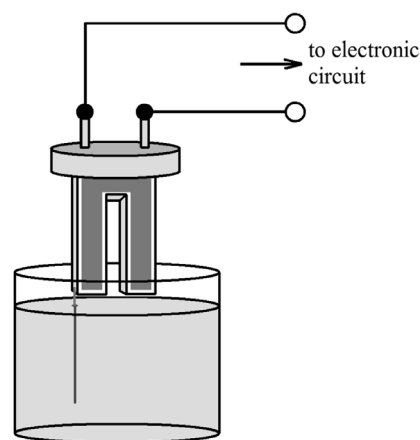

Fig. 2. Schematic representation of the experimental set-up: CNT fibre is immersed into an analyte solution, while QTF is kept above the liquid.
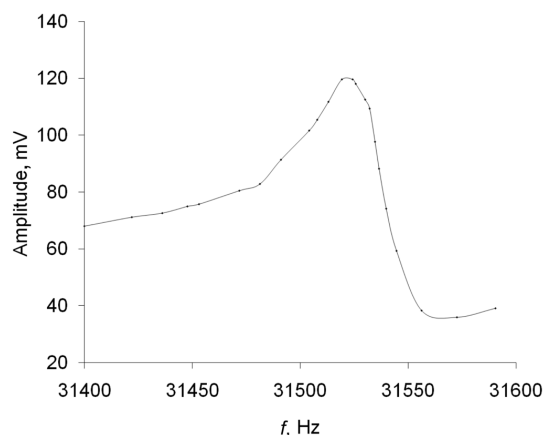
Fig. 3. Frequency response of the QTF sensor with the CNT fibre immersed in liquid. Driving voltage is 10 V.

until the slow diffusion of the liquid into its pores was completed. Diffusion of the solvent resulted in a decrease in the resonant frequency. Conditioning was considered finished when the resonant frequency stabilized. Then, the fibre was immersed in the BSA solution, and the shift in resonant frequency corresponding to the adsorption of BSA was monitored. Finally, the fibre was immersed into the pure buffer again to determine whether the adsorption process was reversible.

All experiments were performed at room temperature ($22 \pm 2\,°C$).

3. RESULTS AND DISCUSSION

Figure 4 shows the typical frequency shifts for the 20 mm CNT fibre in $0.1\ mg\ mL^{-1}$ BSA solution at two different pH values. It is evident that at pH 4.8, the adsorption rate of BSA is significantly higher than at pH 7. The total frequency shift within 25 min was 4.5 Hz for pH 7 and 20 Hz for pH 4.8. The saturation time was approximately 10 min. After BSA adsorption measurements, the fibre was transferred to a pure buffer
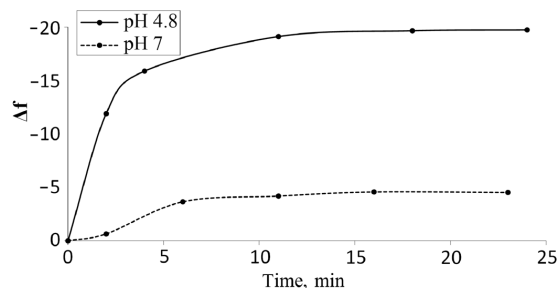

Fig. 4. Typical resonant frequency shifts for 20 mm CNT fibres immersed into $0.1\ mg\ mL^{-1}$ BSA solution at two characteristic pH values.

solution. No recovery of the initial F_{res} was observed, indicating that the BSA adsorption process was irreversible. These results are in good agreement with the findings by Valenti et al. [11], who studied the adsorption of BSA to CNTs by reflectometry. They also found the adsorption of BSA to CNTs to be irreversible, and reported an approximately twofold difference in the adsorption rates for pH 4.8 and pH 7. The saturation time was also of the same order. According to their explanation, the pH dependence of BSA adsorption is related to the conformations of the protein molecules and intermolecular electrostatic interactions. At the isoelectric point for BSA (IEP, pH 4.8), the compact structure and absence of lateral electrostatic interactions facilitate the adsorption of BSA to CNTs. On the other hand, the lower adsorption observed at a pH far from the IEP (BSA molecules are positively charged at pH values lower than 4.8 and negatively charged at higher pH values) is mainly caused by increased intermolecular repulsion.

We found the performance of the sensor to be strongly dependent on the characteristics of the CNT fibre. The fibre must have a porous structure to provide sufficient area for analyte adsorption. The pores have to be large enough to enable the BSA molecules to travel in. The diameter of the fibre should be optimal: too thin or too thick fibres do not provide sufficient sensitivity, as thinner fibres are not stiff enough to transfer the vibration energy to the QTF, while in case of thicker fibres there is a high damping of oscillations due to the fluid resistance. The optimal length and cross-section of the fibre were found to be of the order of 20 mm and 30 μm, respectively. Shorter fibres provided lower sensitivity, while for longer fibres, no increase in sensitivity was observed. Apparently the oscillation does not propagate in the fibre further than 20 mm and the additional length of the longer fibre does not participate in sensing.

As the aim of our measurements was only to compare BSA adsorption rates at different pH values, no mass calibration of the sensors was performed and, hence, no information on the amount of the adsorbed BSA was extracted.

It should be noted that we have also tried to insulate the QTF electrodes to perform the measurements directly in buffer solutions, as was suggested by Su et al. [4]. We have tested different coating methods, including silanization, TiO_2 atomic layer deposition, and sol-gel and polymer coatings. However, due to the arrangement of its electrodes, the QTF acts as a capacitor, meaning that if the surrounding medium has a high dielectric permeability, there will be unavoidable capacitive losses, regardless of the presence of the coating.

4. CONCLUSIONS

The performance of a novel in situ biosensing device comprised of a carbon nanotube (CNT) fibre attached to a quartz tuning fork was experimentally demonstrated by monitoring the adsorption of bovine serum albumin at two different pH values corresponding to minimal (pH 7) and maximal (pH 4.8) BSA adsorption rates. Total frequency shifts of 4.5 Hz for pH 7 and 20 Hz for pH 4.8 were registered. These results are in good agreement with measurements made by reflectometry [11]. It is possible that the sensor can be developed to target specific biological molecules by proper chemical treatment of the CNT fibre. Thus, this new sensor may augment the variety of available label-free in situ biosensing devices.

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Helihargi tüüpi kvartsresonaatoriga integreeritud süsiniknanotorufiibril baseeruv biosensor

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Artiklis on välja pakutud uudse märgisevaba biosensori kontseptsioon mõõtmisteks *in situ* vedelikukeskkonnas. Sensori tööelemendiks on poorne süsiniknanotorufiiber, mis on kinnitatud kahvlikujulise kvartsresonaatori ühe haru külge. Mõõtmise teostamisel sisestatakse uuritavasse lahusesse ainult fiiber, kvartsresonaator jääb lahuse kohale (see on vajalik, vältimaks lühist kvartsresonaatori elektroodidel, mis paratamatult kaasneks selle sisestamisel kõrge dielektrilise läbitavusega keskkonda). Lahusest fiibrile adsorbeeruva analüüdi detekteerimise teevad võimalikuks süsiniknanotorufiibri suur poorsus (eripind) ja madal tihedus, st fiibri algmassiga võrreldes on fiibrile lisanduva massi osakaal suhteliselt suur. Sensori töö demonstreerimiseks eksperimentaalselt mõõdeti veise seerumi albumiini adsorptsiooni fiibrile kahel erineval lahuse pH-väärtusel. Maksimaalse adsorptsiooni määr, millele vastab kvartsresonaatori resonantssageduse muut, oli selgelt eristatav: vastavalt 4,5 Hz pH 7 ja 20 Hz pH 4,8 juures. Saadud tulemused on heas kooskõlas teiste autorite reflektomeetria abil saadud andmetega [Valenti et al., *J. Colloid Interface Sci.*, 2007, **307**, 349–356]. Seadme edasine arendus on suunatud sensori spetsiifilisuse saavutamisele läbi fiibri keemilise eeltöötluse. Oma tehnilise lihtsuse ja soodsa hinna tõttu on kirjeldatud uudne sensor paljutõotavaks täienduseks märgisevabade biosensorite nimistus.