NANOTECHNOLOGY FOR DISTANCE DIAGNOSTICS

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Abstract

The notion of theragnostic brings together such terms as telemedicine, bionanosensors, and personalized medicine. Synergy in the mainstream areas can bring new approaches and provide a fresh perspective on long-established areas. The present research aims to prepare technological equipment for evaluating and measuring functionalized nanomembranes with analyte. Due to the functionalization of nanomaterials, it is possible to capture selectively the analyte or the targeted group. It can be, for example, viruses, bacteria, dangerous chemicals, body fluids, or miRNA. Electronic and optical methods have been preselected for the biomarkers binding evaluation. Methods were selected as technically and scientifically proven. Optical methods have a leading place in "in vitro" diagnostics. In the long-term horizon, it can be used as a Nano Chip biosensor (Including Nano Receptor, Nano Biosensor, and microelectronics).

Keywords: Diagnostics, Nanotechnology, Biosensors, Early-stage cancer detection, Luminescence, Electric impedance, EIS

1. INTRODUCTION

Nanomaterials are crucial in developing nanobiosensors, personalized medicine, and telemedicine. In nanobiosensors, nanomaterials offer unique properties that enable highly sensitive and specific detection of biomolecules. These nanomaterials, such as nanoparticles, nanowires, and nanotubes, can be functionalized with specific receptors or probes to selectively bind to target molecules, allowing for detecting diseases or biomarkers with high precision.

Telemedicine, which involves remote healthcare services, can also significantly benefit from nanomaterials. Nanosensors integrated into wearable devices or implantable sensors can continuously monitor vital signs and

transmit real-time data to healthcare providers. This allows for remote patient monitoring, early detection of health issues, and timely intervention, even from a distance. However, it is essential to note that developing and implementing nanomaterials in these fields requires careful consideration of safety and ethical concerns. Extensive research and regulatory measures are necessary to ensure nanomaterials' safe and responsible use in nanobiosensors, personalized medicine, and telemedicine applications. Diagnostic devices for the evaluation of nanomembranes and nanosensors have the potential to be widely used, not only in the medical sector. They can be realized at an acceptable price, with acceptable technical and diagnostic parameters, in a small device size. In personalized medicine, nanomaterials have the potential to revolutionize diagnostics and therapeutics. Nanoparticles can be engineered to deliver drugs directly to specific cells or tissues, increasing the efficacy and reducing side effects. Additionally, nanomaterial-based biosensors can provide real-time monitoring of patient health parameters, enabling personalized treatment plans based on individual needs. Overall, nanomaterials hold immense potential in advancing the fields of nanobiosensors, personalized medicine, and telemedicine, offering improved diagnostics, targeted therapies, and remote healthcare services for enhanced patient care.

2. METHODOLOGY

MilliQ water was used for the preparation of all aqueous solutions (Milli-Q® IQ 7005 Water Purification System, Millipore, Billerica, MA, USA), Biotin (B4501, Sigma-Aldrich, CAS: 58-85-5,) and Biotin-Fluorescein (Pierce™ Biotin-Fluorescein Conjugate, Thermo Scientific™, CAS: 2420-94-2). All reagents and chemicals used in experiments were of analytical grade. For performing this research, we selected methods that are easy to implement quickly and have the potential to be scaled up for future use. The methods may also depend on the behaviour of the analyse or detection substances. Therefore, two ways were chosen in a diversified manner. To achieve and compare results, we choose physical evaluation:

I. Electrical impedance - measured with electro impedance spectrography (“EIS”) [3] [4].

II. Optical methods in the spectral area - by optical luminescence “OL”.

Because we expected unknowns in the measurement system and nanomembrane evaluation, we applied variations in methods and variations in test bed design (setup). Then, this approach helps distinguish with unknowns. We expect the most significant occurrence of unknowns and influences with the EIS method, which is illustrated in **Figure 1**.

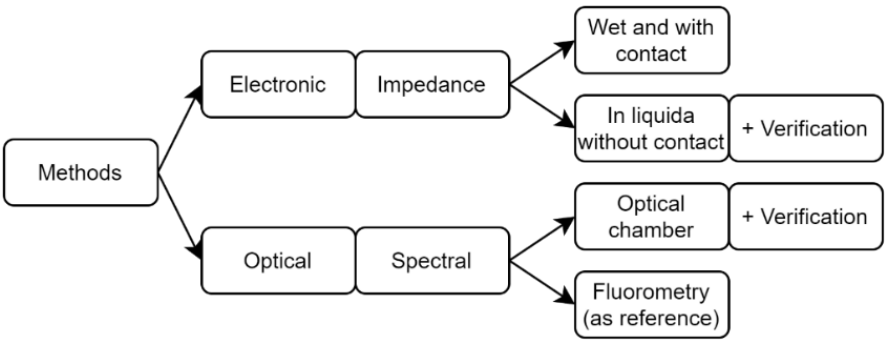


Figure 1 Methodology and evaluation

2.1. Electrical Impedance

Electronic impedance is a frequently used method in electronics and analytical chemistry. In addition to the primary method, the electrochemical impedance spectroscopy (“EIS”) [3] method is used. Because of the

frequency sweep with EIS, it allows monitoring of the impedance behavior in a wide area. This makes it possible to convert the curves into amplitude, phase, frequency, and complex waveforms (**).

We built a test bench based on electrotechnical measurement devices that work identically with chemical-analytics EIS devices, which was proven in [3]. The test bed for EIS and membrane was made as the following configuration:

a) For the wetted membrane, the horizontal bed was pressed with specified force into or between electrodes. The expected behaviour is in the area of R, L, and C impedance components and in the Nyquist chart to identify proper theoretical models.

b) Liquid chamber for "in liquid" measurements. This method differs in force - there is no force on the sample applied. And differs in distance sample from electrodes. Distance is higher (in mm). Thus Helmholtz dual layer is not in effect, bulk transfer is in effect instead [1]. This configuration and EIS method can be verified by water impedance measurement. Impedance behaviour Z(f) of pure electrolytes can be compared with the theoretical impedance model of water. Due to the planned future use of the water chamber, it was also equipped with other features for rising, self-testing, cleaning, et cetera.

The expected behavior is in impedance changes in the swept frequency band Z(f). The individual Z impedance is formed from resistive ("R") and reactance (“X”) components. These reactance X components are identified as capacitive "X_C" or inductive "X_L" reactance. Complex numbers [Re, Im] are used for mathematical processing. Furthermore, with the help of conversion, it is possible to obtain the indicated curves and charts (**), from which it is then possible to identify the theoretical model of the replacement electronic circuit.

2.2. Optical Spectral Response

The optical luminescence (“OL”) event was chosen because it has a response in the spectral region and also in luminosity. The compact optical chamber (test bed) was created by 3D modeling and afterward manufactured by FFF 3D printing. The optical chamber was tailored to the size of samples (like in EIS methods) and electronic modules used for detection. The optical chamber and components can be easily verified with a fiber spectrometer or a spectrofluorometer.

The chamber can be miniaturized for future use to create an accessible, inexpensive mobile device with the possibility of commercialization. Currently, our experiments focus on the preparation of bionanosensors for the highly sensitive detection of bioactive substances (such as specific proteins or miRNAs) to open the possibility for the diagnosis of diseases at an early stage.

3. EXPERIMENTAL PART

3.1. Electrical Impedance Experiments

We conducted experiments with EIS on 2 testbeds (in **Figure 1**). As a membrane, we used a functionalized nanomembrane with Biotin, where we evaluated different exposition times in the Avidity membrane in solution to create an Avidin-Biotin complex. The aim was to obtain a calibration curve at several different concentrations. Spectrophotometer USB2000-XR-ER by Ocean Optics was used.

Furthermore, the EIS aimed to determine the behaviour and artefacts in the Z (f). Based on the measurement data conversion into complex numbers, it was possible to make a Nyquist diagram, which further helps with easy identification of the order and complexity of the theoretical electronic model. To continue the research, we consider using the Wartburg impedance model and Helmholtz’s double layer [1] area.

During the measurement with the liquid chamber (b), the EIS for the water-electrolyte was also validated, and the same results were achieved with the theoretical impedance curve of water.

As an EIS device, we used the electronic measurement device RLC meter, also used by other researchers [4] as an alternative to an EIS device. We used an RLC meter by Keysight, E4980A, with a frequency sweep up to 2MHz. They are controlled by PC software designed in NI LabView. This software helps set up the device, sweep the frequency, and acquire measured values. Then values are stored, plotted, and calculated to different charts (impedance Z(f), phase φ(f), complex [Re(Z), Im(Z)] as Nyquist chart).

3.2. Optical Spectral Response Experiments

We conducted optical measurements on the functionalized membrane samples. We target the properties of the luminescent substance (fluorescein, quinine, marked biotin) excited with one single spectral source that can be easily distinguished or filtered.

The test bed was equipped with 5x UV excitation sources (from UV-B to UV-A, 270 - 420 nm in 5 steps). For measuring luminosity, a sensor was used. Optical components were used to improve sensitivity and remove excitation from measurement. The sensor was selected and set for the best sensitivity in the area of expected radiation emission (430 - 560 nm). Results were compared with a fiber spectrophotometer, USB2000-XR-ER, by Ocean Optics.

For the fluorescent measurements, we used a solution of Biotin-labeled with fluorescein isothiocyanate fluorophore (Biotin-FITC), a green derivative of fluorescein whose excitation is at 498 nm and emission at 517 nm. Different concentrations of Biotin-labeled with FITC fluorophore were prepared in deionized water (ddH2O) (**Table 1**). The fluorescent measurements were performed using a spectrofluorometer (Horiba FluoroMax+4).

4. RESULTS

We faced instability problems and membrane damage during the first measurements during handling. We developed membrane frames for the measurement, which hold the membrane, protect it, and help ensure good repeatability. Subsequently, the measuring test beds were adapted to this membrane attachment system, and a small ecosystem was thus created to evaluate nanomembranes.

4.1. Electrical Impedance Results

In measurement on horizontal bed (a), we encountered a few problems, which we continuously identified and eliminated through the development and modifications of the test bed. However, we were successful during the measurement and managed to capture the impedance behavior.

We achieved precise results during the measurement in the liquid chamber(b). We were the first to verify distilled water, which should have shown a known impedance (theoretical) behavior. A measurement was obtained where the "water plateau" and the identical course of the impedance curve can be identified. Next, we performed measurements with the samples. Here, the impedance manifested itself mainly in the capacitive component.

4.2. Optical Spectral Response Results

During the measurement with the optical chamber, we made several measurements. First, a dark experiment was performed to assess the noise level. The following experiment was completed without a sample but with excitation to determine how much radiation enters the optical sensor. Final measurements were made with different amounts (µg, mg) of luminophores and luminescent substances, and the sensor's response was monitored.

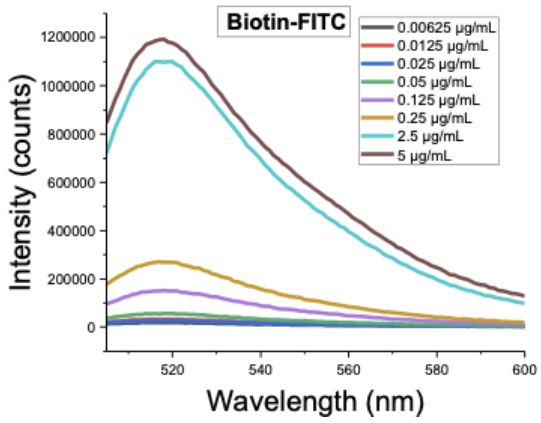
With an extra experiment with CaF2(Eu) it was found that it is also possible to evaluate the fluorescence in time.

During the measurement with Fluorospectrometer, the excitation wavelength was set at 491 nm, while the emission at 505 nm, setting the slit at 2 nm. The measured fluorescence intensities are shown in **Graph 1**.

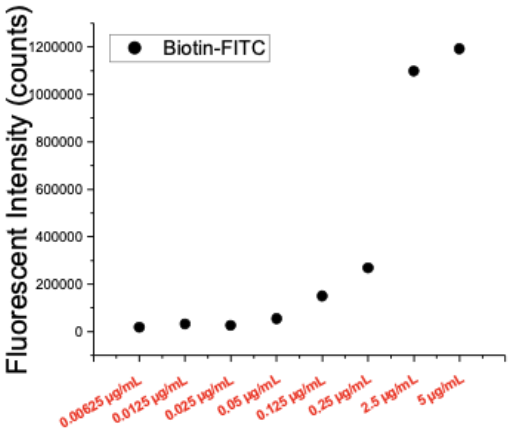
Fluorescent intensities peak at 518 related to Biotin-FITC are shown in the Scattered plot **Graph 2**.

Table 1 Fluorescent Intensity at 518 nm peak of different concentrations of Biotin-FITC.

Biotin-FITC (µg/mL)	Fluorescence intensity at 518 nm (counts)
	1192240
2.5	1097440
0.25	268490
0.125	150280
0.05	55450
0.025	26420
0.0125	32810
0.00625	18980



Graph 1 Fluorescent Intensities of different concentrations of Biotin-FITC.



Graph 2 Scattered Plot showing the fluorescent intensities (at 518 nm) related to the different concentrations of Biotin-FITC concentrations

5. DISCUSSION AND CONCLUSION

We achieved verification of the optical and impedance detection. In the future, we plan to test more samples and different behavior can be assumed, where one of the methods may be more favorable. As an unplanned result of the work, we achieved a significant improvement in the handling and stability of the membrane.

5.1. Electrical Impedance Results

In the test of setup horizontal bed (a) we observed time changes in resistance and capacitance, but more strongly in resistance. This led us to hypothesis of escape of Cu^{+} ions to electrolyte [5] that changes conductivity in time. This was expected, because we didn't use complete noble metal electrode [2], but only thin plated Cu+Au (<1um). Usage of complete noble metal electrode, or thick layer of noble metal most likely solve the problem. Electrode surface then can be checked on electron-microscopy.

With the liquid chamber (b) we firstly tested distilled water and was verified theoretical behaviour of EIS of water. This was verified on presence of plateau region on frequency plot, then half circle plot on Nyquist plot that verifies that.

On both horizontal bed (a) and liquid chamber (b), we observed Z(f), resistance, and capacitance behaviour of samples. Frequency plot and Nyquist diagram leads to different impedance models for horizontal bed (a) and liquid chamber(b). In case of liquid chamber (b) there was characterized also Wartburg impedance

component. Measurements are planned for the next run, with a more stabilized environment, electrolyte, and construction of electrodes [2].

5.2. Optical Response Results

We performed measurements on a compact optical chamber. We observed that high-quality optical elements are necessary because they influence measurements by passing small optical energy amounts from excitation to the sensor. We also observed that the reflection inside the chamber needs spec. we were coating to suppress thought. Conversely, the sensor sensitivity can be improved by a concentration mirror. With the first optical chamber, we can detect up to mMol/L units. With the chamber using the fiber spectrophotometer as a sensor, we achieve sensitivity up to nMol/L units. We performed measurements on a spectrofluorometer and obtained data (in Graphs **1 and 2**). Using polynomial 2nd order interpolation, the curve reaches fit R^2 = 0.99, which we rate as excellent and demonstrative.

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REFERENCES

[1] OPEKAR, F.; BAREK, J., STULIK, K. *Elektroanalytická chemie*, UK, Praha, 2005.

[2] SOMMER, L. *Základy teorie analytické chemie I*. VUT, Brno, 1990.

[3] TATARKOVIC, M.; BRONCOVA, G.; KRONDAK, M. *Laboratorní přístroje a postupy - Elektroimpedanční spektroskopie a její využití v chemické analýze*. Chemické listy 106, VSCHT and UJV, Praha, 2012.

[4] GROSSI, M.; DI LECCE, G.; TOSCHI, T.G.; RICCO, B. Fast and Accurate Determination of Olive Oil Acidity by Electrochemical Impedance Spectroscopy. *IEEE Sensors Journal*. 2014, pp.2947-2954.

[5] SUN, F.; PENG X.; BAI, X.; CHEN, Z.; XIE, B.; HAN, P. *EIS analysis of the electrochemical characteristics of the metal–water interface… RSC Adv.*. 2022, 12, pp. 16979-16990.