GENERATION OF MOLECULARLY IMPRINTED ELECTROSPUN NANOFIBERS FOR ADSORPTION OF 4-NITROPHENOL WITH QUARTZ CRYSTAL MICROBALANCE METHOD

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ABSTRACT
In this study, it has targeted determination of 4-Nitrophenol (4-NP), which is a toxic and carcinogenic compound at high selectivity. For this purpose, firstly molecularly imprinted nanoparticles was synthesis by mini-emulsion polymerization by using 4-NF as template. Then nanofiber systems containing 4-NP imprinted nanoparticles have been developed by electro spinning technology. 4-NP imprinted nanoparticles containing nanofiber systems is coated onto Quartz Crystal Microbalance (QCM) electrode. The electrode was used for determination of 4-NF from aqueous solution. Scatchard, Langmuir, Freundlich and Temkin adsorption models were applied to describe the adsorption isotherms.

Keywords: 4-Nitrophenol, QuartzCrystalMicrobalance, MolecularlyImprintedPolymers, Electrospinning

1. INTRODUCTION
Polymer based nanomaterials, due to their chemical and mechanical properties can be used in a number of different applications such as adsorption of various metal and organic chemicals [1], catalyst (Günter Wulff, 2011), chromatography (Svec & Kurganov, 2008), development of sensors and electrodes (Chen, Wen, & Teng, 2003). Also polymeric nanomaterials offer a wide opportunity of modulating their adsorption capacity through the control of both morphology and chemical composition (Kang, Chen, Zhang, Liu, & Gu, 2008; Khajeh, Laurent, & Dastafkan, 2013) Among the different synthetic strategies, molecular imprinting is probably the most straightforward for the purpose of producing nano and micro structured materials that have predesigned molecular recognition capabilities(Conrad et al., 2003; H. C. Huang, Lin, Joseph, & Lee, 2004; Yang et al., 2004)
The technique of molecular imprinting creates specific molecular recognition sites in solid materials by using template molecules. In this process, a complex of a functional monomer with a template is copolymerized with a cross-linking agent and removal of the template from the polymerized matrix generates the binding sites that are compatible to the template (Kim & Chang, 2011). Three different methods to prepare MIPs have been reported, depending on the bond that is established between the template molecule and the polymer (covalent, noncovalent, and semicovalent approaches) are reported (Turriel & Martín-Esteban, 2010). A noncovalent approach has been used in this work, because relatively weak noncovalent intermolecular interactions, between the template molecule 4-NP and the functional monomers served to form the molecular assemblies. To use MIPs in applications such as sensing or affinity separation, it is often required to immobilize the polymer onto solid surfaces. Several research studies have been carried out by combining the amazing properties of MIPs and nanofibers to obtain molecularly imprinted polymer nanofibers (MIP-NFs)(Zaidi, 2015)(Li, 2012). Furthermore, nanofiber sorbents not only possesses a great specific surface area, which allows a more efficient adsorption, but also the possibility to remove them by solution more easily(Piperno, Tse, Bui, Haupt, & Gheber, 2011).

The electrospinning process produces continuous polymer nanofibers with diameters ranging from a few nanometers to several micrometers (usually between 50 and 500 nm) through the action of an external electric field imposed on a polymer solution or melt(Chronakis, Milosevic, Frenot, & Ye, 2006; Z.-M. Huang, Zhang, Kotaki, & Ramakrishna, 2003; Subbiah, Bhat, Tock, Parameswaran, & Ramkumar, 2005). Electrospun nanofibers have very high surface area, mechanical strength, and flexibility. When collected on a plain target plate, they can easily form defined three-dimensional mats with well-controlled pore sizes. This makes electrospun nanofiber mats ideal candidates for industrial filtration purposes. It is highly desirable for selective molecular recognition sites to be able to be introduced into nanofiber materials as they may provide highly efficient compound separation involving only a simple filtration step(Gibson, Schreuder-Gibson, & Rivin, 2001). In this study, we report on the entrapping of MIP nanoparticles in electrospun polymer nanofibers, thus creating a nonwoven mat of sensing sites with a high surface area and accessibility.

2. EXPERIMENTAL

2.1. Materials

4-NP, methacrylic acid (MAA), Ethylene glycol dimethacrylate (EDMA), hexadecane, 2,2’-azobis(2-methylpropionitrile) (AIBN), polyvinyl alcohol (PVA, MN ~ 72.000), potassium bromide (FTIR grade) and sodium dodecyl sulphate (SDS) were supplied by SIGMA-ALDRICH (Milwaukee, WI, USA). All glassware was extensively washed with diluted HNO₃ before use. All other chemicals were of analytical grade purity and purchased from Merck AG (Darmstadt, Germany). All water used in the experiments was purified using a Barnstead (Dubuque, IA) ROpure LP® reverse osmosis unit with a high flow cellulose acetate membrane (Barnstead D2731) followed by a Barnstead D3804 NANO pure® organic/colloid removal and ion exchange packed-bed system.

2.2. Apparatus

House-made electrospun system was built and used for fiber production. A custom-made high voltage DC converter (EMCO 4300) was biased with a DC power supply. FTIR spectroscopy was used in the 4000-400 cm⁻¹ range for the characterization of functional monomer, pre-organized complex, and imprinted beads in the solid state (FTIR 100 series, Perkin Elmer, USA). The size of the particles was determined by dynamic light scattering on a Malvern Instruments Zeta Sizer. The imprinted beads and nanofibers were characterized
by field emission gun scanning electron microscope (FESEM, ZEISS Ultraplus), SONOPULS HD 2070, BANDELIN, (Germany) model homogenizer and MPW-251, MPW Med-Instruments (Poland) centrifuge were used for homogenization and centrifuge processes.

2.3. Synthesis of 4-NP imprinted nanoparticles

MIP nanoparticles were prepared with mini emulsion polymerization method which used by Belmont et. al. (Belmont et al., 2007). Therefore, the organic phase that contained 1.72 mmol of MAA, 6.9 mmol of EDMA, 80 μL of hexadecane and 30 mg of AIBN was prepared and then ultrasonicated for 1 min. The aqueous phase was prepared by dissolving of 0.5 mmol 4-NP and 38.5 mg of SDS in 18 mL of water. The organic phase was slowly added to the aqueous phase. In order to obtain mini-emulsion, the mixture was homogenized at 25.000 rpm by a homogenizer (SONOPULS HD 2070, BANDELIN, Germany). Polymer mixture was stirred and held in 70°C water bath for 18 hours to obtain nanoparticles. The 4-Np imprinted nanoparticles were washed three times 5h against water and water/ethylalcohol mixtures in order to remove unreacted monomers, surfactant and initiator. To removing imprinted molecule, the mixture was washed with methanol: acetic acid (4:1) solution three times for two hours.

FT-IR, Zeta-sizer and SEM analyzes were performed to nanoparticles characterization. Particle size was measured by Malvern Instrument Zeta-sizer device. 30 μg/ml water suspension was prepared for this purpose. The imprinted 4-NP and nonimprinted MIP nanoparticles were examined and imaged by Scanning Electron Microscope. 4-NP imprinted and nonimprinted MIP nanoparticles were mixed with 98 mg potassium bromide and grounded until turn into fine powder form then pressed to pellet for FTIR measurement.

2.4. The coating of QCM sensors with 4-NP imprinted polymer encapsulated nanofibers and their characterization.

Chronakis et al.’s method was used for preparation of 4-NP imprinted polymer encapsulated nanofibers (Yoshimatsu, Ye, Lindberg, & Chronakis, 2008). 3 ml Dichloromethane (DCM), 3 ml trifluoroacetic acid (TFA) were mixed then 0,5 gr poly ethylene terephthalate (PET), 0,05 gr polyvinyl alcohol (PVA), NaCl were added to this mixture and stirred for two hours. The prepared mixture was filled in plastic syringe and placed to syringe pump (KDS-200, Focus Co., Ltd., USA) for electrospinning. The positive side of the high voltage power supply was attached to the syringe’s 0,8 mm diameter metal needle tip. The solution feeding rate was adjusted to 1,5 mL/h. Gold coated quartz crystals were used as collector electrode and placed horizontally which has a 130 mm distance away from the needle tip. Before electrospinning, the QCM crystal surface was cleaned in piranha solution (H₂SO₄/30% H₂O₂ (3:1, v/v)) for 5 min, rinsed thoroughly with Milli-Q water, and dried with a stream of nitrogengas. The quartz portion of the QCM electrode was covered with aluminum folio to prevent unwanted interaction with nanofibers. System voltage was adjusted as 10kV in room temperature. The fibers were deposited on the quartz crystal (Figure 2.1a) and left to dry after deposition to remove trace amount of solvent. 4-NP nonimprinted polymer encapsulated nanofibers preparation steps were remained same. For this purpose 4-NP nonimprinted particles were added to the PET-PVA mixture and stirred for one hour to obtain homogenous mixture. The same parameters and conditions were applied during electrospinning.
Figure 2.1. (a) The schematic view of nano fiber creation on QCM electrode with electrospinning process and (b) formation of Taylor cone

2.5. The sensor measurements of nanofibers coated QCM electrode

The QCM sensors consist of a disk-shaped, AT-cut piezoelectric quartz crystal, coated with gold thin layer on both sides and operate at a frequency of 5 MHz. The resonance frequencies were measured by using a QCM digital controller (MAXTEK RQCM, Research Quartz Crystal Microbalance Monitor). For this purpose electrode was attached to the holder, rinsed with water via peristaltic pump at room temperature for stabilization then measured constant resonance frequency ($F_0$). 0.5 ppm 4-NP solution was passed on electrode and sensor frequency observed until stable value ($F_1$). The frequency shift of all 4-NP concentrations (0.5-100 ppm) were calculated from $\Delta F = F_0 - F_1$ equation. The electrodes were rinsed with H$_2$O and 0.1 mM glisin/10 mM HCl mixture until sensor frequency come close to $F_0$ value to remove template after every concentration measurement step. The binding value of nano sensor to 4-NP molecules is statistically calculated from three values of blank solution.
3. RESULTS
3.1. 4-NP imprinted nanoparticles characterization
4-NP imprinted nanoparticles were prepared by two-phase emulsion polymerization method. In this technique, polymerization process performed by passing from stable oily phase to water phase. The FTIR characterization of 4-NP imprinted and nonimprinted polymers were carried out by FTIR spectrometer. The dried polymer mixed with KBr and shaped to tablet form to obtain FTIR spectrum. The FTIR spectrum of 4-NP imprinted and nonimprinted polymers can be shown in Figure 3.1A. As seen from FTIR spectra specific bands of the polymeric structure have been detected. In the FTIR spectrum characteristic peaks of 4-NP imprinted nanoparticles are carboxylic acid O-H band at 3568 cm\(^{-1}\), C-H stretching at 2980 cm\(^{-1}\) and carbonyl peak (C=O) at 1728 cm\(^{-1}\). When FTIR spectrums of imprinted and nonimprinted polymers are compared, the peak is observed at 1637 cm\(^{-1}\) in 4-NP imprinted polymers. It is believed that this peak should be come from benzene ring’s C=C bending band. For Zeta-Sizer measurements 4-NP imprinted nanoparticles were suspended in deionized water with 30 µg/ml ratio to measure particle size. Zeta-Sizer is benefit from light scattering techniques and is capable of hydrodynamic sizing of nanoparticles, determining of zeta potential and molecular weight. Zeta sizer analysis result can be shown in Figure 3.1B. The obtained nanoparticles size is found about 200 nm. 4-NP imprinted nanoparticle’s size and morphology were revealed with SEM analysis. Nanoparticles were dried and coated with gold before SEM surface analysis. SEM view of 4-NP imprinted nanoparticles is given in Figure 3.1C.
Figure 3.1. (A) The FTIR spectrum of 4-NP imprinted (a) and nonimprinted polymers (B) Zeta-Size analysis result of 4-NP imprinted nanoparticles (C) SEM photograph of 4-NP imprinted nanoparticles

3.2. 4-NP imprinted nanoparticle encapsulated nanofibers characterization

4-NP imprinted nanoparticle encapsulated nanofiber’s surfaces were also characterized with SEM. 4-NP imprinted nanoparticle encapsulated nanofibers were dried, coated with gold before SEM images were taken. The SEM view of nanofibers that were not containing 4-NP imprintedp articles can be shown in Figure 3.2- a. 1 mg 4-NP imprinted nanoparticles were added into the polymer mixture and nanofiber creation process was repeated. The obtained nanofiber’s SEM view is given in Figure 3.2-b.
3.3.4-NP imprinted polymer encapsulated nanoparticle coated QCM sensor’s binding interactions.

When target molecules bind to the 4-NP imprinted nanofibers, the crystal’s mass changes (Δm) that effects to frequency. The relation between Δm and frequency shift (ΔF) is expressed with following equation.

\[ ΔF = CxΔm \]

Where,
- ΔF : frequency shift
- Δm: the amount of matter that binds on quartz
- C: \(56.6 \text{ Hzcm}^2 \mu \text{g}^{-1}\) for 5 mHz crystal

The 4-NP imprinted polymer is expected to bind the 4-NP sensing. The frequency of the sensor increased after pumping the 4-NP solution. These frequency changes strongly indicated that the 4-NP molecules were bounded to the imprinted polymer on the quartz crystal. 4-NP solutions with different concentration between 0.5 to 100 ppm were pumped to 4-NP imprinted electrode (Figure 3.3). LOD and LOQ values were calculated as 0.395 nM and 1.2 mM respectively.

![Figure 3.2. SEM images of nanofibers (a) PET/PVA (b) 4-Nitrophenol imprinted nanoparticle/PET/PVA](image)

![Figure 3.3. Change in ΔF and time of 4-NP imprinted particle encapsulated nanofiber coated sensor](image)
The binding interactions between imprinted nanofibers and 4-NP template is obtained from Scatchard analysis method. Following equation is used in Scatchard analysis method (Atay, 2016).

\[
\frac{\Delta m}{[C]} = -\frac{\Delta m}{K_D} + \frac{\Delta m_{\text{max}}}{K_D}
\]

Where,
\(\Delta m\): The amount of matter that binds on quartz,
\(C\): Free 4-NP concentration
\(K_D\): Equilibrium constant in reverse direction
\(\Delta m_{\text{max}}\): Maximum mass increasing in QCM sensor unit area

Using with this equation, the binding constant and number of ligand changing sites are found as \(K_A\) \(2 \times 10^6\) M\(^{-1}\) and \(Q_{\text{max}}\) 11,76 respectively. The founded \(K_A\) value indicates the binding site’s has strong affinity. \(K_A\) value of 4-NP imprinted QCM sensor proves that the developed system is nearly as selective as biological sensors (Diltemiz, 2010), (Amanda, 2004).

![Figure 3.4](image)

Figure 3.4. 4-NP imprinted particle encapsulated nanofiber coated QCM sensor’s Scatchardcurve

\[
\frac{\Delta m}{C} = -2 \times 10^6 \Delta m + 11.76
\]

In this equation;
\(K_L\): Langmuir constant for a homogeny adsorbent
\(C\): 4-NP free concentration
\(\Delta m_{\text{max}}\): Maximum mass increasing in unit area of QCM sensor
\(\Delta m\): Mass increasing amount in unit area of QCM sensor

The binding interactions-equations between imprinted polymer encapsulated nanofibers and 4-NP are also examined with Langmuir, Freundlich and Temkin analysis method. 4-NP imprinted polymer’s Langmuir curve is obtained by following equation and \(K_L\) and \(\Delta m_{\text{max}}\) values are found from equation as \(2,33 \times 10^5\) M and \(5,8 \times 10^{-5}\) respectively. The founded \(K_L\) value indicates the binding site’s has strong affinity to target molecule 4-NP.

\[
\Delta m = \frac{\Delta m_{\text{max}}}{K_L} \times C + C
\]
Figure 3.5. 4-NP imprinted polymer’s Langmuir curve

4-NP imprinted polymer’s Freundlich curve is plotted from following equation (Atay, 2016). KF and n values are found from equation as $5.39 \times 10^{-12}$ M and 66.6 respectively.

$$\ell n \Delta m = \frac{1}{n} \ell n C + \ell n K_F$$

Where,
- $K_F$: Freundlich constant for heterogenic adsorbent
- $C$: 4-NP free concentration
- $\Delta m$: Mass increasing amount in unit area of QCM sensor
- $n$: Relation between adsorption force magnitude and energy distribution of adsorbent area
Figure 3.6. 4-NP imprinted polymer’s Freundlich curve

4-NP imprinted polymer’s Temkin curve is obtained by following equation:

$$\Delta m = B \ln K_T + B \ln C_e$$

Where,

$K_T$: Binding constant of Temkin isotherm (L/gr)

$C_e$: 4-NP free concentration

$\Delta m$: Mass increasing amount in unit area of QCM sensor

$B$: Adsorption constant related with heat (J/mol)

Figure 3.7. 4-NP imprinted polymer’s Temkin curve
Table 3.1. The comparison of 4-NP imprinted QCM sensor’s Langmuir, Freundlich and Temkin analysis.

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CONCLUSION

4-NP imprinted polymer encapsulated nanofibers were created firstly to remove 4-NP in this study. PVA and PET polymers that are capable of water bending are used in this stage. As can be understood from SEM images nanofibers are created in randomly distributed, uniform morphology and dense forms. Accessibility to target molecule and appearance of large part of the nanoparticle surface is accomplished by trapping of MIP particles with larger diameter than PET/PVA fibers to the fiber interspaces. The binding amount of 4-NP imprinted nanofibers to 4-NP molecule, calculated from 3 statistical data of blank solution. LOD and LOQ values are found as 0.027 ppm and 0.081 ppm respectively. QCM nanosensor is developed to determining 4-NP with high selectivity. 4-NP imprinted nanoparticles encapsulated nanofibers are coated on QCM electrode. The binding interactions between 4-NP imprinted polymer encapsulated nanofibers and 4-NP are examined with Scatchard, Langmuir, Freundlich and Temkin analysis methods. The binding constant ($K_A$) for binding 4-NP molecules to 4-NP imprinted particle-encapsulated nanofibers coated QCM sensor is found as 2x10^6 M^{-1}. This value indicates that the binding sites have strong affinity. Linear character of plotted curve shows that adsorption process is more suitable to matched with Langmuir. Moreover when comparing Langmuir, Freundlich and Temkin curves, Langmuir curve has highest correlation coefficient. This situation indicates single layer and homogenous adsorption exists on QCM sensor. The binding amount of nanosensor to 4-NP molecule, calculated from 3 statistical data of blank solution. LOD and LOQ values are found as 0.395 nm and 1.2 mM respectively. The calculated values of observability and determinity limits are given in table 5.1. The writers of this study couldn’t find a study about determining of 4-NP using with QCM in literature. Therefore 4-NP determining with using QCM technique could be accomplished firstly reported in literature with this study.

REFERENCES


