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Copper ion sensing with fluorescent electrospun nanofibers

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ABSTRACT

In this work, the use of electrospun nanofibrous materials as highly responsive fluorescence quenchingbased copper sensitive chemosensor is reported. Poly(methyl methacrylate) and ethyl cellulose were used as polymeric support materials. Sensing slides were fabricated by electrospinning technique. Copper sensors based on the change in the fluorescence signal intensity of fluoroionophore; N'-3-(4-(dimethylamino phenly)allylidene)isonicotinohydrazide. The sensor slides exhibited high sensitivities due to the high surface area of the nanofibrous membrane structures. The preliminary results of Stern–Volmer analysis show that the sensitivities of electrospun nanofibrous membranes to detect Cu(II) ions are 6–20-fold higher than those of the continuous thin films. By this way we obtained linear calibration plots for Cu(II) ions in the concentration range of 10^{-12} – 10^{-5} M. The response times of the sensing slides were less than 1 min. Stability of the employed ionophore in the matrix materials was excellent and when stored in the ambient air of the laboratory there was no significant drift in signal intensity after 6 months. Our stability tests are still in progress.

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1. Introduction

Copper is the third most abundant element in the human body and is essential in several biological pathways including electron transport, oxygen metabolism and enzymatic catalysis. Copper is on one side essential for life but on the other hand, depending on the dose, it may be highly toxic for organism. A high level of Cu(II) concentration is not necessary to produce toxicity in the human body. Over time the heavy metal can reach toxic concentration levels. Therefore, monitoring of trace amounts of Cu(II) levels in water and food samples is quite important. In recent years a number of chemosensors detecting Cu(II) ions either in biologically relevant species or in aqueous solutions were proposed [1–11].

In these sensing approaches absorption and/or emission responses of the chromophore molecules resulting from interaction with Cu(II) ions were recorded as analytical signal.

These investigations provided valuable information for chemosensing of copper ions. Sometimes, indicators proposed for detection of Cu(II) exhibited weak responses to other cations within stated tolerance limits [5]. Over the last two decades researchers mainly used the chemosensing agent in embedded form [6–9]. In most of these designs optical chemical sensing of Cu(II) ions is mainly performed with a chromoinophore immobilized in polymer membranes such as polyvinylchloride (PVC) [6–8] or ethyl cellulose (EC) [9] in thin film form. That kind of sensing films display analyte dependent optical properties such as absorbance, fluorescence or reflectance. Mostly, such sensors provided enhanced stability, reversibility and sensitivity with respect to the solution based sensing. However, development of new technologies toward highly sensitive detection techniques is still very important for the researchers working in the field of analytical chemistry. In Table 1 the recently published luminescence methods for Cu(II) determination were compared in terms of their working ranges, Limit of Detections (LODs), sensing agents and matrix materials with the offered work.

Sumner et al., used DsRed and the reference dye Alexa Fluor 488 in a polyacrylamide matrix and produced spherical, ratiometric, 80 nm-sized sensors. They detected copper ions, in the nanomolar (ppb) regime, in biologically relevant samples [12]. Wang et al. compared performance of thin films and nanomaterials in terms of sensitivity, and revealed the advantage of nanoscale membranes over continuous thin films [13]. In spite of these few examples, usage of nanomaterials in optical chemical sensing is very rare. In the light of the above discussion, usage of polymeric nanomaterials is expected to offer good results in comparison to the sensing films prepared with the same composition.

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Table 1

Recently published emission based methods for copper ion sensing.

Ref.	Reagent or matrix	Method	Working range	LOD
[1] [3]	Luminol derivative, alkaline aqueous media CdS quantum dots, chitosan	Emission/410 nm Emission/400–700 nm	0–500 nmol L ⁻¹ 8.0 nmol L ⁻¹ to 3.0 μmol L ⁻¹	0.27 nmol L ⁻¹ 1.2 nmol L ⁻¹
[5]	4,5-Dihydroxy-1,3-benzenedisulfonic acid, Water	Emission/350 nm	5.0×10^{-7} to 1.0×10^{-5} M	$3.83\times 10^{-7}M$
[6]	N,N-Bis(salycilidene)-1,2-phenylenediamine, PVC	Absorption/420 nm	5.01×10^{-8} to $6.32\times10^{-4}molL^{-1}$	$4.7\times10^{-8}\ mol\ L^{-1}$
[7]	4-(1-Phenyl-1-methylcyclobutyl-3-yl)-2-(2-hydroxy-1- naphtylidene) thiazole, PVC	Emission/491 nm	$\begin{array}{l} 5.0\times 10^{-8} \\ to 1.0\times 10^{-4} M \end{array}$	$2.6\times 10^{-8}\ M$
[8]	1-Hydroxy-3,4-dimethyl-9H-thioxanthen-9-one, PVC	Absorption/410-480 nm	1.6×10^{-7} to 1.3×10^{-2} M	$4.0\times 10^{-7}~M$
[9]	Bis(naphtho[2,1- <i>b</i>]furan-2-yl)methanone semicarbazone, PVC and EC	Emission/429 and 424 nm for PVC and EC	1.0×10^{-9} to $3.0\times10^{-4}\text{M}$	4.3 ppb
[10]	Terbium-[1-methyl-4-hydroxy-3-(N-2-ethyl-5- aminothiadiazolyl-)-carbamoyl-quinoline-2-one], Aqueous Buffer	Emission/547 nm	10–300 nmol L ⁻¹	4.3 nmol L ⁻¹
[11]	Functional CdS nanoparticles, Aqueous Buffer	Emission/460 nm	$0.09-27.0 \mu g L^{-1}$	3.2 ng L ⁻¹
[12]	DsRed and the reference dye Alexa Fluor 488, polyacrylamide matrix by a microemulsion polymerization process	Ratiometric Emission/DsRed 583 nm Alexa Fluor 520 nm	Sensitive to Cu(II) ppb levels	Not defined
DPAINH (This work)	N'-3-(4-(dimethylamino phenly) allylidene)isonicotinohydrazide, PMMA and/or EC nanofibers	Emission/535 and 490 nm for PMMA and EC respectively	1.0×10^{-12} to 1.0×10^{-3} M for EC 1.0×10^{-12} to 1.0×10^{-2} M for PMMA	3.8×10^{-14} M for EC 1.4×10^{-13} M for PMMA

In this report, we have successfully combined nanoscale electrospun fiber materials with optical sensing technology for Cu(II) ion detection. We conceive of making electrospun fibers from a relatively novel solvent system containing room temperature ionic liquids (RTILs). Here we have demonstrated that it is possible to electrospun the EC and PMMA based composite fibers in presence of non-volatile RTIL; EMIMBF₄.

The employed RTIL was chosen as polymer electrolyte due to its high ionic conductivity, low vapor pressure, low melting point, high thermal stability, low toxicity and recyclability [14,15]. The successful application of RTILs in electrospinning could enhance the number and types of materials from which the fibers can be made.

On the other hand; electrospinnig has recently gained much attention as a unique technique to fabricate high surface area and highly responsive nanofibrous materials [16-20]. In electrospinning, when a sufficiently large electric voltage is applied to the precursor solution in a capillary, it becomes stretch to form a cone because of coupled effects of the electrostatic repulsion within the charge droplet and attraction to a grounded electrode of opposite polarity. As the strength of the electric field is increased the charge overcomes the surface tension and at a critical voltage fine jet is ejected from the tip of the cone. These jets dry to form very thin polymer fibers, which are collected on a substrate as nanofibers. The diameters of electrospun fibers can range from several micrometers to nanometers depending on the nature of precursor polymer and other experimental conditions; such as temperature, flow rate, voltage, the distance between precursor solution and substrate and humidity. As a result, electrospun nanofibrous membranes can have a large surface area. It is expected that this large amount of functional surface area has the potential to provide unusually high sensitivity and fast response in sensing applications.

In this paper matrix materials of poly(methyl methacrylate) and ethyl cellulose were used together with ionic liquids. Optical nano-fibrous membrane chemical sensors were fabricated by electrospinning technique. The *N*'-3-(4-(dimethylamino phenly)allylidene)isonicotinohydrazide dye (DPAINH) was chosen as the Cu(II) selective fluoroinophore. The electrospun nanofibers were characterized using scanning electron microscopy (SEM) and their average diameters were evaluated.

2. Experimental

The polymers ethyl cellulose (EC) and poly(methyl methacrylate) (PMMA) were from Acros and Aldrich, respectively. The plasticizer, dioctyl phthalate (DOP) was from Aldrich. The ionic liquid, 1-ethyl-3-methylimidazolium tetrafluoroborate (EMIMBF₄) was supplied from Fluka.

Potassium tetrakis-(4-chlorophenyl) borate (PTCPB) was from Aldrich. Absolute ethanol (EtOH), tetrahydrofuran (THF), dichloromethane (DCM), dimethylformamide (DMF) and chloroform (CH₃Cl) were of analytical grade. Solvents for the spectroscopic studies were used without further purification.

Buffer components; acetic acid/acetate, phosphate and metal salts were of reagent grade (Merck and Fluka). Millipore ultra pure water was used throughout the studies. The 1000 ppm Atomic Absorption Spectroscopy (AAS) certified reference standard solution of Cu(II) was from Merck. Other metal cations were prepared from respective metal nitrates, or chlorides and diluted with 5.0×10^{-3} M buffers to demanded pH. All of the experiments were carried out at room temperature; 25 ± 1 °C utilizing reagent grade chemicals.

Schematic structures of the exploited Cu(II) sensitive fluoroinophore; N'-3-(4-(dimethylamino phenly)allylidene)isonicotinohydrazide and EMIMBF₄ were shown in Fig. 1. Dye was prepared in one step by the condensation of isoniazide (1 mmol) and 4-dimethylamino cinnamaldehyde (1 mmol) in the presence of glacial acetic acid in ethanol under reflux conditions according to literature information [21]. The following experimental data identifies chemical structure of the fluorescent copper selective chromoionophore molecule. m.p.: 252 °C IR(KBr): 3302 (NH), 1664 (C=O), 1595 (C=N) ¹H NMR (400 MHz, CDCl₃): δ 3.01 (s, 6H, N(CH₃)₂), 6.67 (d, 2H, *J*=7.2 Hz, ArH), 6.81–6.91 (m, 1H, =CH), 7.36 (d, 2H, *J*=7.4 Hz, ArH), 7.67



Fig. 1. Structures of the Cu²⁺ sensitive fluoroinophore; *N*-3-(4-(dimethylamino phenly)allylidene)isonicotinohydrazide; DPAINH (I) and 1-ethyl-3-methylimidazolium tetrafluoroborate; EMIMBF₄ (II).

(d, 2H, *J* = 7.8 Hz, ArH), 7.75–7.82 (m, 1H, =CH), 7.97–8.01 (m, 1H, N=CH), 8.74 (d, 2H, *J* = 7.6 Hz, ArH), 8.92 (bs, 1H, NH).

2.1. Apparatus and instruments

The homogeneous PMMA or EC solutions were placed in a 10 mL plastic syringe fitted with a metallic needle of 0.4 mm of inner diameter. The syringe is fixed vertically on the syringe pump (Top Syringe Pump Top-5300) and the electrode of the high voltage power supply (Gamma High Voltage ES30) was clamped to the tip of the metallic needle. Schematic structure of the electrospinning apparatus was shown in Fig. 2. Absorption spectra were recorded with a Shimadzu UV-1601 UV-visible spectrophotometer. Steady state fluorescence emission and excitation spectra were measured using Varian Cary Eclipse Spectrofluorometer.

2.2. Preparation of electrospun nanofibers

Electrospinning was used to fabricate PMMA or EC based continuous nanofibers. Experimental conditions were optimized to form bead-free nanofibers varying concentrations of plasticizer, PMMA or EC and RTIL in the precursor polymer solution. The concentration of RTIL was varied from 10% up to 25% (w/w) with respect to the PMMA or EC content. We find that the presence of the RTIL in the polymer solutions facilitates the electrospinning of bead-free nanofibers from the lower polymer concentrations. This result can be attributed to the ionic conductivity and proper viscosity of the RTIL doped precursor polymer solutions.

The resulting composites were prepared by mixing 240 mg of polymer (PMMA or EC), 144 mg of plasticizer (DOP) and 96 mg of RTIL. 1.5 mg of dye were dissolved in CH₃Cl:EtOH (75:25) or THF solvent systems. Then, added into the polymeric precursor solution under magnetic stirring.

The polymer solution was taken in a hypodermic syringe and an electric potential of 25 kV was applied between the needle and the substrate in the form of an aluminum sheet. The distance between the needle and the electrode was 10 cm. The solution flow rate was maintained at 1 mL/h. When high voltage is applied the charged polymer solution overcame the surface tension of the viscous liquid and a stream of polymer jet was produced. The solvent evaporated and very fine fibers were completely coated on the aluminum sheet or deposited as bulk fibers. The surface morphology of the fabricated nanofibers was studied using the scanning electron microscope (SEM) instrument (6060-JEOLJSM). The nanofibers deposited on aluminum substrate were cut into proper size, fixed in the cuvette or flow cell and the excitation or emission spectra were recorded. The electrospun fibers exhibited good adhesion to the aluminum substrate. SEM images of electrospun membranes of the EC and PMMA at various magnifications are shown in Fig. 3. It is observed that the membrane has a 3-D structure with a random fiber orientation that is evenly distributed on the substrate. Average fiber diameters were calculated as 536 ± 76 and 360 ± 82 nm for PMMA and EC based electrospun fibers, respectively. When conditions of the electrospinning process such as solvent composition, viscosity, concentration, temperature, humidity and working distance were kept constant reproducibility in the preparation of electrospun membranes was quite good.

This type of nanostructure provides higher surface area with respect to continuous thin films.

However further enhancement of the surface area may be achieved by changing the experimental conditions of the electrospinning process which results in either smaller diameter fibers or increased porosity at the fiber surface [22].

2.3. Thin film preparation procedures

The thin films were prepared employing the same composition explained for the electrospun nanofibers. The resulting mixtures were spread onto a 125 μ m polyester support (Mylar TM type) with a spreading device. Thickness of the films was measured using Tencor Alpha Step 500 Prophylometer and found to be $5.11 \pm 0.081 \mu$ m (n = 8). Each sensing film was cut to 1.2 cm diameter, fixed in the cuvette or flow cell, and the excitation and/or emission spectra were recorded.

Metal response measurements were carried out with fiber optic probe (2 m long) and solid sample tip accessories constructed on the spectrofluorometer. For instrumental control, data acquisition and processing, the software package of the spectrofluorometer was used. The tip of the bifurcated fiber optic probe was interfaced with a sensing film in a buffer containing homemade 300 μ L flow cell. The flow cell was equipped with a four channel Ismatec Reglo Analog peristaltic pump. Flow rate of the peristaltic pump was kept at 2.4 mL min⁻¹. Analyte solutions or buffers were transported via tygon tubing of 2.06 mm i.d.

Analysis at the sub-nanomolar level is possible only by exploiting ultra pure reagents and careful tracking of blanks. In this work reagent grade chemicals and polyethylene or Teflon containers were used. Stable Cu(II) standard was obtained commercially. Loss of significant amounts of Cu(II) by adsorption onto container walls can cause serious problems for quantification of copper in glass containers. Therefore we treated the polyethylene vessels with distilled deionized ultrapure water to equilibrate for 2 days prior to the solution preparation.



Fig. 2. Schematic structure of the electrospinning apparatus.

3. Results and discussion

3.1. Effect of RTILs on electrospinning process

To provide an electrical conductivity in polymers one must add proper electrically conductive additive into the polymer. As mentioned earlier when the high electric voltage is applied on such an electrically conductive polymer precursor, the solution at the tip of the needle becomes stretched to form a cone because of coupled effects of the electrostatic repulsion within the charged droplet and attraction to a grounded electrode of opposite polarity. As the strength of the electric field is increased, fine jet is sputtered from the tip of the cone. In this report, we have demonstrated that it is possible to electrospun the EC and PMMA composite fibers in presence of non-volatile room temperature ionic liquid; EMIMBF₄. The employed RTIL was chosen as polymer electrolyte due to its ionic conductivity, high thermal and chemical stability, non-volatile, non-flammable and low toxicity characteristics [23].

Room temperature ionic liquids (RTIL), specifically 1-alkyl-3methylimidazolium salts have been effectively used as volatile organic solvent replacements in liquid/liquid separations of metal ions from aqueous solutions [24]. As expected, the exploited RTIL not only enhanced the electrospinning performance but also



Fig. 3. SEM images of electrospun membranes at different magnifications (a and b; PMMA based, c and d; EC based nanofibers).

600

500

400

300

contributed a remarkable ability to extract the Cu(II) ions from the aqueous phase into the polymeric materials. Additionally the EMIMBF₄ enhanced photostability of the embedded chromoionophore within the polymer matrix acting as a sink for acidogenic or basic species from the ambient air of laboratory. This result arises from the intrinsic acidic-basic characteristics of the employed RTIL [25,26].

3.2. Effect of pH

The exploited Cu(II) sensitive fluoroionophore exhibited a cross sensitivity to proton ions between pH 3.0 and 11.0 in solution phase. When embedded in RTIL containing matrices the DPAINH dye became more resistive for protonation.

We also tested the Cu(II) sensing ability of the chemosensor at different pH values, between pH 5.0 and 9.0 in acetic acid/acetate and phosphate buffer solutions. We obtained the highest sensitivity in acetic acid/acetate buffer and for further studies we have chosen this buffer as optimum working condition.

During calibration studies, we exploited 5×10^{-3} M of acetic acid/acetate buffer solutions to avoid pH induced signal fluctuations. Distribution of the Cu(II) related chemical species in the working conditions was checked with chemical equilibrium software program visual MINTEQ at pH 4.0 in presence of acetate ions. The equilibrium related species in the buffer environment were found to be as follows: $acetate_{(aq)}$ 15.2%; H-acetate_{(aq)} 84.8%; $Cu^{2+}_{(aq)}$ 89.8%; Cu-acetate⁺_(aq) 10.1%. This distribution reveals that approximately 90% of the copper is in form of $Cu^{2+}_{(aq)}$ under these circumstances. Due to the solubility considerations acetic acid/acetate buffered solutions of pH 4.0 was used for further studies.

3.3. Photocharacterization of the dye

Photocharacterization of the ionophore was performed in diluted solutions of EtOH, DCM, THF and DMF and in solid matrices of EC and PMMA. Solution phase excitation and emission spectra of the ionophore were shown in Fig. 4(I). The ionophore; DPAINH yielded two distinct emission peaks in the exploited solutions between 300-340 nm and 560-600 nm, depending on the solvent. The exploited Cu(II) sensitive fluoroinophore exhibits strong absorption and bright fluorescence due to the intramolecular charge transfer (ICT) in the excited state. The ICT is continuous along with 9 covalent bonds throughout the molecule from electron donating N,N-dimethylamino group to the nitrogen atoms around carbohydrazide moiety.

Immobilization of the molecule in polymers resulted with dramatic spectral changes. Upon immobilization the emission maxima recorded in diluted solutions around 300 nm were disappeared. Interestingly, the excitation wavelength was shifted to the visible end of the electromagnetic spectrum and the emission efficiency was only appeared at longer wavelengths; around 500 nm (see Table 2 and Fig. 4(II)). When doped in EC and PMMA the Stoke's shifts of DPAINH exhibited an enhancement with respect to the solution phase (see Table 2). Therefore when immobilized, the DPAINH dye could be excited at further wavelengths with respect to the solution phase. This result can be attributed to the restricted vibrational rotational motions in solid states.

3.4. Copper ion sensing studies: Cu(II) uptake into the membrane and fluorescence based response

The optical sensors are mainly composed of a polymer compatible ion-selective indicator dye (chromoionophore), and a lipophilic anionic additive dissolved in polymer matrix material. When doped into polymeric matrices together with the anionic additive;



Fig. 4. (I) Excitation and emission spectra of the fluoroinophore in (a) EtOH, (b) THF, (c) DMF, (d) DCM. (II) (a) in embedded form in EC, (b) in PMMA.

potassium tetrakis-(4-chlorophenyl) borate; the DPAINH dye becomes a Cu(II) sensitive molecular probe. In this system Cu(II) ions are extracted into the electrospun fibers by the enforcement of the anionic additive meanwhile two potassium ions diffuse from the polymer into the aqueous phase via the mechanism of ion exchange. Therefore the charge balance was maintained within the polymer. Following equation reveals the ion exchange mechanism.

$$DPAINH_{(org)} + 2K^{+}_{(org)} + 2TpCIPB^{-} + Cu^{2+} \leftrightarrow DPAINH_{(pink)}Cu^{2+}$$

$$+ 2TpCIPB^{-} + 2K^{+}$$
(1)

Figs. 5 and 6 show the variations in fluorescence spectra of the thin film and electrospun materials upon exposure to Cu(II) ions for PMMA and EC matrices respectively. The emission maxima approximately 20 nm and 10 nm red shifted in form of nanofiber with respect to continuous thin films for PMMA and EC respectively (see Figs. 5 and 6). In all cases, response to Cu(II) ions was in direction of decrease in signal intensity. In form of nanomaterials the relative signal changes were approximately three times and two times better compared to the continuous thin films. In PMMA, the relative signal drops were reported as 20% and 62.5% for thin film and nanofiber forms, respectively. Similarly 40% and 83% relative signal drops were calculated for EC based matrices for thin film and nanomaterials. The insets of Figs. 5 and 6 reveal intensity based linear response ranges of the ionophore to Cu(II) ions in different forms in terms of molar concentration. In PMMA based matrices the working range was shifted from 10^{-12} - 10^{-5} M to 10^{-12} - 10^{-2} M. Similarly in EC based sensing agents the enhancement in working range was more distinct and enlarged from 10^{-9} – 10^{-4} M to 10^{-12} – 10^{-3} M. The detection limits (LOD) were calculated exploiting concentration of the metal ion giving a signal equal to average of the blank

Ι

excitation-emission spectra related characteristics of the DPAINH in diluted solutions of DCM, THF, and DMF and in solid matrices of EC and PMMA.							
mpound	Matrix	λ_{max}^{ex} (Excitation wavelength)	λ_{max1}^{em} (Emission wavelength)	λ_{max2}^{em} (Emission wavelength)	$\Delta \lambda_{\text{ST}}$ (Stoke's shift)		
AINH	EtOH	285	315	560	30		
AINH	THF	275	315	605	40		
AINH	DMF	285	340	565	55		
AINH	DCM	265	305	560-605	40		
AINH	EC	405		490	85		
AINH	PMMA	395		535	140		

signal (n=20) plus three standard deviation and were found to be 3.8×10^{-14} M for EC and 1.4×10^{-13} M for PMMA respectively. The attained LOD values exploiting nano-materials for the offered design is an evident of the enhanced sensitivity (see Table 1).

The intensity drops is known to be due to the quenching of the DPAINH dye by Cu(II) ions.

Quenching can occur by different mechanisms. In collisional quenching, excited state fluorophore is deactivated upon contact with the ion in solution which is called the quencher. However, sometimes the fluorophore can be guenched both by collisions and by complex formation. The intensity related data (I_0/I) exhibiting an upward-concave curvature toward the y-axis is the evidence of the combined quenching both by collisions (dynamic quenching) and by complex formation (static quenching) by the same quencher [27]. In case of dynamic quenching because the collisions between the quencher and the fluorophore effects only the excited state of the molecule, no changes in absorption or excitation spectrum are expected. Contrarily, the formation of ground state complex in static quenching will influence the absorption spectra of the

fluorophore. Therefore, by a careful evaluation of the absorption spectrum, mechanism of the quenching can easily be distinguished.

Fig. 7 shows the absorption spectra of the fluoroionophore in the absence and presence of the quencher. From the absorption spectral data, it can be concluded that the guencher affects only the excited state of the fluorophore which indicates dynamic quenching. When the linear shape of the fluorescence intensity based response and absorption spectroscopy related data were considered together, the quenching mechanism between DPAINH and Cu(II) can be concluded as dynamic quenching.

Sensitivity of the quenching process and consequently sensor dynamics can be manipulated by controlling the quencher diffusion rate to fluorophores encapsulated in the polymer phase via the microstructural properties of the sensing agent. The offered nanostructure was quite beneficial for both the sensitivity and dynamic characteristics of the sensor due to its large specific area, which has high number of active sites for diffusion of Cu(II) ions towards the solid phase with respect to the thin films made up of same material. In these structures the possibility of interaction of the analyte



Fig. 5. (1) Fluorescence response of the dye doped PMMA thin film to Cu(II) ions at pH 4.0. (a) Cu-free buffer, (b) 10⁻¹² M, (c) 10⁻¹¹, (d) 10⁻¹⁰, (e) 10⁻⁹, (f) 10⁻⁸, (g) 10⁻⁷, (h) 10⁻⁶, (i) 10⁻⁵, (j) 10⁻⁴, (k) 10⁻³ M Cu(II). Inset: Calibration plot for the concentration range of 10⁻¹²–10⁻⁵ M Cu(II). (II) Response of the PMMA based nanofiber to Cu(II) ions at pH 4.0. (a) Cu-free buffer, (b) 10^{-12} M, (c) 10^{-11} , (d) 10^{-10} , (e) 10^{-9} , (f) 10^{-8} , (g) 10^{-7} , (h) 10^{-6} , (j) 10^{-4} , (k) 10^{-3} , (l) 10^{-2} , (m) 10^{-1} M Cu(II). Inset: Linearized calibration plot for the concentration range of 10⁻¹²-10⁻² M Cu (II).

Table 2

Co

DF

DF

DP

DP DP

DF



Fig. 6. (I) Fluorescence response of the dye doped EC thin film to Cu(II) ions at pH 4.0. (a) Cu-free buffer, (b) 10^{-12} M, (c) 10^{-11} , (d) 10^{-10} , (e) 10^{-8} , (g) 10^{-7} , (h) 10^{-6} (i) 10^{-5} , (j) 10^{-4} , (k) 10^{-3} M Cu(II). Inset: Calibration plot for the concentration range of $10^{-9}-10^{-4}$ M Cu(II). (II) Response of the EC based nanofiber to Cu(II) ions at pH 4.0. (a) Cu-free buffer, (b) 10^{-12} M, (c) 10^{-11} , (d) 10^{-10} , (e) 10^{-9} , (f) 10^{-8} , (g) 10^{-7} , (h) 10^{-6} , (i) 10^{-5} , (j) 10^{-4} , (k) 10^{-3} M Cu(II). Inset: Linearized calibration plot for the concentration range of $10^{-12}-10^{-3}$ M Cu(II).

ions with the Cu(II) selective chromoionophore molecules is higher than that of in conventional thin films.

3.5. Stern–Volmer analysis

The employed fluoroionophore is sensitive to quenching by Cu(II) ions. In a homogeneous medium, such as in solution, the quantitative measure of fluorescence quenching is described by the Stern–Volmer constant, K_{sv} ,

$$\frac{I_0}{I} = 1 + K_{\rm sv}[Q] \tag{2}$$

In Eq. (2), I_0 and I are the intensities of fluorescence in the absence and in the presence of the quencher, respectively. According to the equation, I_0/I increases directly proportional to the concentration of the quencher. When all other variables are kept constant, the higher the K_{sv} , the lower the concentration of quencher required to quench the fluorescence. In a heterogeneous medium, such as in polymer films, a negative deviation from the linear Stern–Volmer equation occurs at high quencher concentration [28,29]. The data obtained by gathering Stern–Volmer analysis for each electrospun and continuous thin film are shown in Fig. 8(I) and (II). For quencher concentrations in the range of 10^{-12} – 10^{-5} M, linear plots between concentration of quencher and I_0/I are obtained showing an excellent Stern–Volmer relationship.

Stern–Volmer constants (K_{sv}) of the electrospun films, calculated from slopes of the plots were found to be 0.23 (M⁻¹) and 0.07 (M⁻¹) for EC and PMMA nanofibers respectively. These values are 23- and 6-fold greater than that obtained from the continuous thin film sensor slides of EC and PMMA, respectively (see Table 3). The higher K_{sv} values reveal important practical consequences. The sensitivity of the quenching process is enhanced by controlling the

quencher diffusion rate to fluorophores employing electrospinning technique. The Stern–Volmer plots for quenching by Cu(II) in both cases, exhibited excellent linearity indicating that most of the total emission may be accessible to quenching. Additionally the recorded response time for the electrospun nanofibers is less than 1 min ($\tau_{90} = 0.83 \pm 0.16$ min, n = 5). This value is at least five times better than that of the response times measured for continuous thin films ($\tau_{90} = 4.27 \pm 0.8$ min, n = 5).

3.6. Recovery, selectivity and regeneration studies

The applicability of the sensing system for real samples was tested for the tap water.



Fig. 7. Absorption spectra of the fluoroionophore in the absence (a) and presence (b) of the quencher; Cu (II).

Table 3

The Stern–Volmer plot related calibration characteristics of PMMA and EC based electrospun nanofibers and thin films
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Indicator dye	Matrix/form	Linear regression equation	Regression coefficient (R^2)	K _{sv} constant
DPAINH	EC/electrospun nanofiber	y = 0.2323x + 0.733	0.9827	0.2323
DPAINH	PMMA/electrospun nanofiber	y = 0.0695x + 0.99	0.9962	0.0678
DPAINH	EC/thin film	y = 0.0124x + 0.9806	0.6148	0.0124
DPAINH	PMMA/thin film	y = 0.0133x + 0.992	0.9498	0.0133

The content of Cu²⁺ in the diluted certified reference material of Merck (SRM from NIST Cu(NO₃)₂ in HNO₃ 0.5 mol L⁻¹ 1000 mg L⁻¹ Cu CertiPUR[®]) were determined by the prepared electrospun nanofibers. The tap water samples were freshly collected from the laboratory in polyethylene vials, after allowing the water to flow for 5 min. All water samples were filtered through a 0.45 μ m membrane filter and analyzed as soon as possible after sampling. The recovery experiments were carried out adding certified reference material into the tap water. The resulting concentration of the Cu²⁺ was 10⁻⁹ M.

The average recovery performance was found to be $103 \pm 3.7\%$ for five measurements. The recovery test results reveal the capability of the offered sensing design for the determination of copper ions in real samples without considerable error.

In order to examine the cross sensitivities of the DPAINH dye to possible interferents the sensing agents were treated with 10^{-5} M concentrations of Ag⁺, Al³⁺, Ca²⁺, Co²⁺, Cu²⁺, Fe³⁺, Hg²⁺, Mn²⁺, Mg²⁺, Ni²⁺, Pb²⁺, Sn²⁺ and Zn²⁺ cations in acetic acid/acetate buffer solutions at pH 4.0. Fig. 9 reveals that the sensing agent is capable of determining Cu(II) ions with a high selectivity over other ions. The fluorescence was distinctly quenched in the presence of Cu(II) at 535 nm and 490 nm for PMMA and EC based sensing slides respectively. The interference effects of the conventional anions of NO₃⁻, ClO₄⁻, SO₄²⁻, Cl⁻, HCO₃⁻ and HPO₄³⁻ were also tested. Relative signal changes of less than 5% were obtained for most of the tested potential interferents. Only Fe³⁺ and HCO₃⁻ ions exhibited



Fig. 8. Gathered Stern–Volmer plots of the electrospun nano-fibers and continuous thin films in PMMA (I) and in EC (II).



Fig. 9. (I) Response of DPAINH to 10^{-5} M of different metal cations in acetic acid/acetate buffer solutions at pH 4.0. (II) Response of the dye to 10^{-5} M of the major anions in near neutral waters.

remarkable response in terms of signal intensity. However these problems can be overcome tuning the pH of the working conditions.

3.7. Sensor dynamics and analytical figures of merit

The major requirements of an ideal sensor are high sensitivity, good LOD values, wide linear working range, selectivity and fast response time. The working ranges of the sensing agents calculated from emission profiles were broad enough and extends from 10^{-12} to 10^{-3} M and from 10^{-12} to 10^{-2} M for EC and PMMA respectively. The quadratic fit is more convenient for such kind of large working ranges. However we obtained excellent linearity with nanomaterials for both of the matrices within the whole working range. The regression coefficients of 0.9827 and 0.9962 for ethyl cellulose and poly(methyl methacrylate) are evidences of the attained linearity. The measured equilibrium response times of less than 1 min and high analytical signal values upon exposure to Cu(II) ions with electrospun nanofibers are very promising.

4. Conclusion

Electrospinning looks like a promising, simple, and an effective method for fabricating nanomaterials. On the other hand such kind of materials should be coupled with optical chemical sensor devices. With respect to continuous thin films, electrospun films can offer enhanced sensitivity and reactivity in sensing applications. In this work, we performed coupling of electrospun nanomaterials with fluorescence based measurement techniques without scattering and other side effects. We performed to measure the Cu(II) concentrations as low as 10^{-12} M exploiting nanofibrous materials. Our nano-material based sensing approach resulted with large linear working ranges extending to 10^{-12} – 10^{-3} mol L⁻¹ and 10^{-12} – 10^{-2} mol L⁻¹ Cu(II) for EC and PMMA respectively. To our knowledge these are the largest reported working ranges and best LOD values among similar emission based sensing methods. Further efforts will focus on exploring new sensing materials and polymer compositions, controlling the nanoscale size of the electrospun membranes, and optimizing the sensitivities for the detection of a variety of analytes.

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References

- [1] Y. Luo, Y. Li, B. Lv, Z. Zhou, D. Xiao, M.M.F. Choi, Microchim. Acta 164 (2009) 411-417.
- [2] Y. Rahimi, A. Goulding, S. Shrestha, S. Mirpuri, S.K. Deo, Biochem. Biophys. Res. Commun. 370 (2008) 57–61.
- [3] S. Lai, X. Chang, C. Fu, Microchim. Acta 165 (2009) 39-44.
- [4] J. Cody, C.J. Fahrni, Synth. Receptors Sens. 60 (2004) 11099-11107.
- [5] H.S. Kim, H.S. Choi, Talanta 55 (2001) 163-169.
- [6] M.B. Gholivand, P. Niroomandi, A. Yari, M. Joshagani, Anal. Chim. Acta 538 (2005) 225–231.
- [7] N. Aksuner, E. Henden, I. Yilmaz, A. Cukurovali, Sens. Actuators B 134 (2008) 510–515.

- [8] A. Yari, N. Afshari, Sens. Actuators B 119 (2006) 531-537.
- [9] O. Oter, K. Ertekin, C. Kirilmis, M. Koca, Anal. Chim. Acta 584 (2007) 308-314.
- [10] M. Turel, A. Duerkop, A. Yegorova, Y. Scripinets, A. Lobnik, N. Samec, Anal. Chim. Acta 644 (2009) 53–60.
- [11] H.Q. Chen, A.N. Liang, L. Wang, Y. Liu, B.B. Qian, Microchim. Acta 164 (2009) 453–458.
- [12] J.P. Sumner, N.M. Westerberg, A.K. Stoddard, C.A. Fierke, R. Kopelman, Sens. Actuators B 113 (2005) 760–767.
- [13] X. Wang, C. Drew, S.H. Lee, K.J. Senecal, J. Kumar, L.A. Samuelson, Nano Lett. 2 (2002) 1273-1275.
- [14] R. Sheldon, Chem. Commun. 23 (2001) 2399–2407.
- [15] T. Welton, Solvents Synth. Catal. Chem. Rev. 99 (1999) 2071-2083.
- [16] H. Schreuder-Gibson, P. Gibson, K. Senecal, M. Sennett, L. Samuelson, J. Walker, W. Yeomans, D. Ziegler, Z. Huang, D. Wang, S. Yang, J. Wen, Z. Ren, C. Hill, E. Boring, K. Klabunde, T.C. Cheng, R. Yin, Property Enhancements for Military Applications Abstr. Pap. ACS 221st: IEC 288 (2001).
- [17] C. Drew, X. Wang, K. Senecal, S.H. Gibson, J. He, S. Tripathy, L. Samuelson, ANTEC Conf. Soc. Plast. 2 (2000) 1477–1481.
- [18] K. Senecal, D. Ziegler, M. Auerbach, H. Schreuder-Gibson, L. Samuelson, Abstr. Pap. ACS 221st: IEC 358 (2001).
- [19] A.G. Macdiarmid, I.D. Norris, W.E. Jones, M.A. El-sherif, J. Yuan, B. Han, F.K. Ko, Abstr. Pap. ACS 220th IEC 310 (2000).
- [20] D.H. Reneker, I. Chun, Nanotechnology 7 (1996) 216–223.
- [21] N. Sinha, S. Jain, A. Tilekar, R.S. Upadhayaya, N. Kishore, G.H. Jana, S.K. Arora, Bioorgan. Med. Chem. Lett. 15 (2005) 1573-1576.
- [22] J.M. Deitzel, J. Kleinmeyer, D. Harris, N.C. Beck Tan, Polymer 42 (2001)261–272.
 [23] M. Galinski, A. Lewandowski, I. Stepniak, Electrochim. Acta 51 (2006) 5567–5580.
- [24] A.E. Visser, R.P. Swatloski, S.T. Griffin, D.H. Hartman, R.D. Rogers, Sep. Sci. Technol. 36 (2001) 785-804.
- [25] O. Oter, K. Ertekin, D. Topkaya, S. Alp, Anal. Bioanal. Chem. 386 (2006) 1225-1234.
- [26] O. Oter, K. Ertekin, S. Derinkuyu, Talanta 76 (2008) 557–563.
- [27] J.R Lakowicz, Principles of Fluorescence Spectroscopy, 3rd ed., Springer, New York, 2006.
- [28] J.R. Bacon, J.N. Demas, Anal. Chem. 59 (1987) 2780–2785.
- [29] E.R. Carraway, J.N. Demas, B.A. DeGraff, J.R. Bacon, Anal. Chem. 63 (1991) 337-342.