Sensors and Actuators B 195 (2014) 8–14

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

A sensing microfibre mat produced by electrospinning for the turn-on luminescence determination of Hg^{2+} in water samples

F.J. Orriach-Fernández[a], A.L. Medina-Castillo[b], J.E. Díaz-Gómez[b], A. Muñoz de la Peña[c], J.F. Fernández-Sánchez[a,*], A. Fernández-Gutiérrez[a]

[a] Department of Analytical Chemistry, University of Granada, Avd. Fuentenueva s/n, 18071 Granada, Spain
[b] NanoMyP, Nanomateriales y Polimeros S.L., Spin-Off company of the UGR, BIC Building, Avd. Innovacion 1, E-18016 Granada, Spain
[c] Department of Analytical Chemistry, University of Extremadura, 06006 Badajoz, Spain

ARTICLE INFO

Article history:
Received 28 October 2013
Received in revised form 1 January 2014
Accepted 2 January 2014
Available online 13 January 2014

Keywords:
Optical sensor
Hg^{2+}
Water analysis
Electrospinning
Rhodamine derivative

ABSTRACT

A novel turn-on fluorescent microfibre mat with high selectivity towards Hg^{2+} has been prepared for determining and quantifying Hg^{2+} in water samples. It is based on the use of electrospinning for encapsulating a spirocyclic Rhodamine 6G phenyl-thiosemicarbazide derivative (called FC1) into polymeric microfibres and the well-known spirolactam (non fluorescent) to ring opened amine (fluorescent) equilibrium of Rhodamine derivatives as recognition mechanism. The fluorescence intensity was proportional to Hg^{2+} concentration in a linear range from 0.4 to 4.0 μM with a detection limit (S/N = 3) of 0.1 μM.

1. Introduction

Mercury is considered as one of the most hazardous pollutants [1]. It is widely distributed in air, water and soils through different processes [2,3], and it is present in the environment in several different forms [4]. Inorganic mercury can cause a wide range of diseases such as digestive, heart, kidney and especially neurological disorders [4]. Therefore, it is important to monitor Hg^{2+} levels in aquatic ecosystems as a potential source of contamination [5].

Recently, the usual methods for determining Hg^{2+} and the necessity to develop chemical sensors for determining and quantifying Hg^{2+} in water samples have been pointed out by our research group [6]. The use of optical sensors offers potential advantages over electrochemical ones such as, electrical isolation, reduced noise interference, the possibility of miniaturisation and remote sensing [7]. In addition, they are often inexpensive and can be mass-produced as disposable materials [8]. Among other methods, the fluorescence-based sensors represent a simple but sensitive technique for fast controlling Hg^{2+} in many samples [6,9] and turn-on responses are preferred due to the ubiquitous nature of fluorescence quenching, enhanced sensitivity and practical utility [10].

Although the number of examples of molecular probes reported for Hg^{2+} in solution is high, there are only few examples of probes immobilised in solid supports that concern Hg^{2+} detection. Hu et al. [11] proposed a double hydrophilic block co-polymer, composed of poly(ethylene oxide)-b-poly(N-isopropylacrylamide-co-RhBHA) bearing Rhodamine B-based Hg^{2+} reactive moieties, as multifunctional chemosensors to pH, temperature and Hg^{2+} ions. This optical sensor showed a detection limit of 1.6 ng mL^{-1} but the running temperature is high (40 °C), obstructing its easy applicability to real samples. Liu et al. [12] proposed novel fluorescent silica nanoparticles, formed by quantum dot SiO_2-Rhodamine nanoparticles, with high selectivity towards Hg^{2+} and with a detection limit of 0.52 ng mL^{-1} which is excellent for analysing Hg^{2+} in drinking water, but it is a complex and expensive system from an industrial point of view.

To solve these inconveniences, Orriach-Fernandez et al. [6] proposed the use of a spirocyclic phenilthiosemicarbazide Rhodamine 6G derivative (called FC1) immobilised into poly(2-hydroxyethyl methacrylate-co-methyl methacrylate), providing a highly selective chemosensor for Hg^{2+} ion in water. It works at room temperature and the production is simple and cheaper, but its sensitivity is not so good (LOD of 0.3 μM). In this report the authors proposed the use of pre-concentration steps, which are time-consuming, or the use of nanotechnology for improving it.

Electrospinning is a highly versatile method to process solutions or melts, mainly of polymers, into continuous fibres with diameters

---

* Corresponding author. Tel.: +34 958240451; fax: +34 958243328.
E-mail address: jffernan@ugr.es (J.F. Fernández-Sánchez).

0925-4005/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.snb.2014.01.010

ranging from few nanometers to few micrometers. Electrospinning fundamentals are reviewed in several extensive articles [13,14] and books [15,16]. The resulting fibres are characterised by their large surface areas and they are being successfully incorporated in multitude of different areas [17] such as: sensors [18–21], filters and selective removal, tissue engineering, batteries, smart textiles, catalysis, self-cleaning, composites, cosmetics and pharmaceuticals, but always in applications where high value products are needed. The effort for commercialising fibre based products is supported by many companies which have come out with different strategies for the up-scaling of the electrospinning technique [15,16].

In this work, we proposed the encapsulation of the FC1 chemodosimeter into microfibre nonwoven mats produced by electrospinning in order to improve its sensitivity, allowing the development of an optical sensing mat that meets the discharge limit for industrial wastewater according to the U.S. EPA standard [22] and the China SA standard [23].

2. Materials and methods

2.1. Chemicals and reagents

Polymeric hydrophilic blends Polym-B1, Polym-B2 and Polym-B3 with average molecular weights higher than 400 kDa and with different % of hydroxyl monomers in the polymeric chains: Polym-B1 < Polym-B2 < Polym-B3 (nanoMyP®, Granada, Spain, www.nanomyp.com; 99%), DMF (Panreac, Barcelona, Spain; HPLC-grade), glycerol (Scharlau, Barcelona, Spain; 86–88%), and potassium tetrakis(4-chlorophenyl)borate (KTpClPB, Fluka, Madrid, Spain; 98%) were used as received. KTpClPB is an alkaline lipophilic salt, which was incorporated into the membrane for reasons of electroneutrality.

For the characterisation of the sensing films, the following salts were used as received: $HgCl_2$ (Sigma, Madrid, Spain; >99.5%), Ag_2SO_4 (Sigma, Madrid, Spain; >99%), $Pb(NO_3)_2$ (Panreac, Barcelona, Spain; 97%), $Cd(NO_3)_2 \cdot 4H_2O$ (Sigma, Madrid, Spain; <99%) and CH_3HgCl (Fluka, Madrid, Spain; <99%).

FC1 was synthesised following the procedure described by Bohoyo et al. [24], based in a combination of the procedure of Yang et al. [25] and Dujols et al. [26], following the detailed described two steps protocol [6,27]. Briefly, in a first step, to 0.4 g of Rhodamine 6G dissolved in 15 mL of ethanol, an excess of hydrazine hydrate (0.5 mL) was added and then the reaction solution was refluxed until the pink colour disappeared. After that, the cooled reaction solution was poured into distilled water and extracted with ethyl acetate (6 × 25 mL). The combined extracts were dried with anhydrous sodium sulphate, filtered, and then evaporated. The solvent yielded 0.48 g (92%) of Rhodamine 6G hydrazide. In a second step, the Rhodamine 6G hydrazide (200 mg, 0.47 mmol) in DMF (1.5 mL) was added to a solution of phenyl isothiocyanate (0.1 mL, 0.65 mmol) in DMF (1.5 mL). The reaction mixture was stirred for 6 h at room temperature. After the solvent was evaporated under reduced pressure, the crude product was column chromatographed on silica gel (elution with hexane/EtOAc/CH_2Cl_2 = 4:1:1) to give 166 mg (67%) of FC1.

2.2. Instrumentation and apparatus

The fluorescence characterisation was developed with a Fluorescence spectrometer Varian Cary Eclipse (Agilent Technologies Inc., Loveland, CO, USA) coupled with an optical fibre accessory and a fibre optic probe (Agilent Technologies Inc., Loveland, CO, USA).

A homemade cell was designed to couple the tip of the optical fibre with the sensing film. It is equipped with a support with a metrical screw to regulate the distance between the solid phase and the optical fibre which transports and collects the light from the spectrometer to the sample and vice versa. The optimal distance between optical fibre and the sensing mat was 1 mm. Electronic Supporting Information (ESI) shows pictures of the measuring system and the homemade cell (see ESI, Fig. ESI-1). The fibre optical probe consists on a perpendicular end. It is well-known that it is not the best optical architecture to be used in fluorescence [28] but this is just a preliminary set-up to analyse the sensing nanofibre mat. Therefore, its analytical figures of merit could be improved by optimising the optical architecture of the sensor.

Commercial electrospinning equipment, Electrospinner 2.2.D-500 (Yflow S.L., Malaga, Spain), was employed to fabricate the nonwoven mats.

MicropH2000 Crison pHmeter (Crison Instruments, Alella, Spain) was used to adjust the pH of the employed solutions.

2.3. Fabrication and characterisation of Hg(II)-sensitive microfibre mats

Table 1 shows the cocktail solutions used for producing the Hg^{2+}-sensitive fibre mats. They were loaded into 5 ml Teflon syringes (Norm-Ject® HSW) and extruded through a stainless steel capillary tube with outer and inner diameters of 1.5 mm and 1.1 mm, respectively. Table 2 shows the working parameters of the electrospinning process. The flow rates and voltages were selected in order to allow the collection of dry fibres in nonwoven mats. In all cases, the process was run to collect 20 cm diameter rounded mats of microfibres onto a flat static collector. Then, they were dried in a vacuum oven at room temperature during 12 hours to remove any residual solvent. ESI shows pictures of the processed mats (see Fig. ESI-2).

Table 1
Composition of the cocktails for obtaining the nonwoven mats.

Name	Polymeric Blend (g)		Glycerol (mg)	FC1 (mg)	KTpClPB (mg)	DMF (g)
NWM-B1	B1	1.12	0	9.5	18.5	6.3
NWM-B2	B2	1.12	0	9.5	18.5	6.3
NWM-B3	B3	1.12	0	9.5	18.5	6.3
NWM-G1	B3	1.12	0	9.5	18.5	6.3
NWM-G2	B3	1.12	57	9.5	18.5	6.3
NWM-G3	B3	1.12	100	9.5	18.5	6.3
NWM-G4	B3	1.12	156	9.5	18.5	6.3
NWM-F1	B3	1.12	76	4.8	18.5	6.3
NWM-F2	B3	1.12	76	7.2	18.5	6.3
NWM-F3	B3	1.12	76	9.5	18.5	6.3
NWM-F4	B3	1.12	76	14.5	18.5	6.3
NWM-K1	B3	1.12	76	9.5	13.2	6.3
NWM-K2	B3	1.12	76	9.5	18.5	6.3
NWM-K3	B3	1.12	76	9.5	22.8	6.3