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Design of a Sensory Polymer for the Detection of Zn(II) for the Diagnosis of Chronic Wounds †

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Abstract: Wound repair is a complex process that involves many responses like the regulation of the inflammation or the degradation of the extracellular matrix. In these responses, Metalloproteases (MMP) have an important role, since that, an increase in their enzyme activity could cause a chronic wound. Metalloproteases have a catalytic and structural Zn(II), so the concentration of Zn(II) could be correlated with the activity of MMP, and finally, this enzyme activity also could be correlated with the state of chronic wounds. Then, this study is based on the design and synthesis of new material for the detection of Zn(II) in biological samples for the diagnosis of chronic wounds.

Keywords: chronic wounds; sensory polymer; Zn(II) detection

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1. Introduction

Wound repair is a complex process formed for various phases (formation of fibrin clot or inflammatory response, among others). Metalloproteases (MMP) are enzymes that play a relevant role in this process since their functions are; regulate inflammation and degrade the extracellular matrix (ECM). This degradation allows the beginning of the cellular migration process and the formation of a new ECM.

Moreover, new studies show that an increase in enzyme activity of MMP could cause a chronic wound. Therefore, the control and knowledge of their enzyme activity have a great interest since it can help diagnose and treat these types of wounds [1].

On the other hand, Zn(II) is present as a structural and catalytic component of MMP, so the concentration of this ion could be correlated with the activity of MMP, and finally, this activity is correlated with the state of chronic wounds [2].

Hence, this study is based on the following objectives: (1) Chronic wound evaluation by medical personnel, (2) determination of Zn(II) in chronic wound through ICP-MS as a reference method, and (3) determination of Zn(II) in chronic wounds with an alternative, simpler, and more direct method.

The first two objectives will confirm the relationship between Zn(II) concentration and chronic wound severity, and the third objective is the main line of our study. It is oriented to developing an alternative method for Zn(II) detection "by the easy way", using fluorometric sensory polymers.

The last objective will be carried out through polymer science, specifically with sensory polymers. This kind of material has obtained good results with simple procedures; thus, we proposed an inexpensive and rapid sensory material, which causes a change of fluorescent that can be measured both visually (naked eye) and smartphone boosted.

Our sensory motif is based on quinoline structure, that is usually used to detect Zn(II), as we have seen in previous studies of the group [3]. Moreover, this method is cheap, simple, and the measurements can be easily carried out by unskilled personnel.

2. Methods

2.1. Sensory Monomer Synthesis

The sensory monomer derived from 8-nitroquinoline was prepared according to the synthesis that appears in Figure 1.

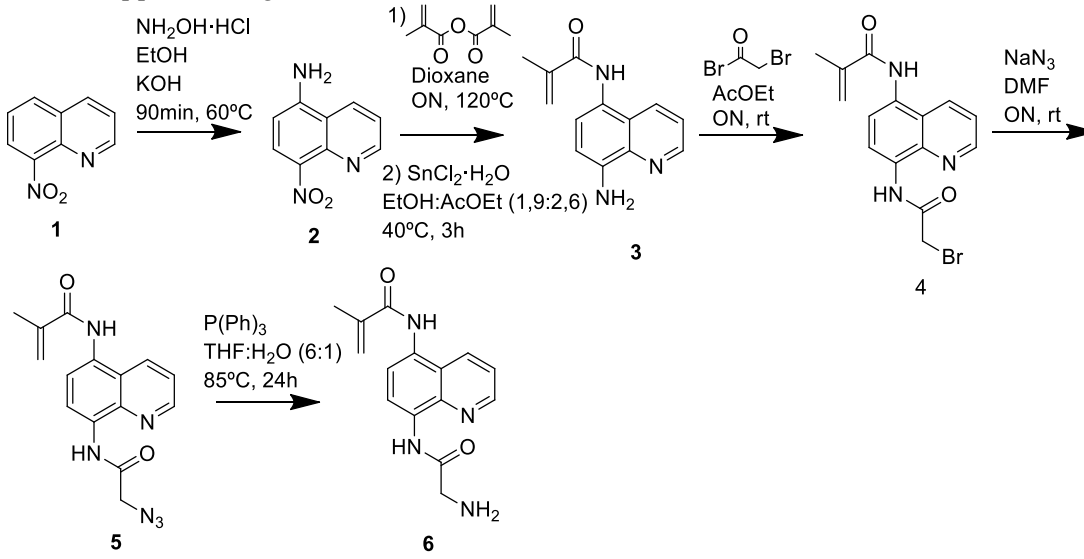

Figure 1. Synthesis of the sensory monomer.

2.1.1. 8-nitroquinolin-5-amine (2)

8-nitroquinoline **(1)** (6 g, 34.45 mmol) and hydroxylamine (15 g, 216 mmol) were dissolved in ethanol (180 mL) at 60 °C. Then, 200 mL of a solution of potassic hydroxide (20% in ethanol) was added dropwise with stirring. Once the addition is complete, stirring is maintained at 60 °C for 90 min. Next, it's cooled to room temperature and allowed to cool at 4 °C for a few hours. Then, it's poured into 1 L of water and the yellowish solid was filtered and washed with water. Yield: 65% (4.22 g, 22.31 mmol). ^{1}H-RMN (300 Hz, DMSO-d^{6}): 8,96(1H, dd, J=4.2, 1.6 Hz), 8.69 (1H, dd, *J* = 8.6, 1.6 Hz), 8.19 (1H, d, *J* = 8.8 Hz), 7.53 (1H, dd, *J* = 8.6, 4.2 Hz), 7.38 (2H, s), 6.66 (1H, d, *J* = 8.8 Hz). ^{13}C NMR (75 MHz, DMSO-*d*$_{6}$) δ 152.81, 152.21, 142.27, 134.70, 132,12, 130,20, 120.55, 116.36, 105,06.

2.1.2. N-(8-aminoquinolin-5-yl)methacrylamide (3)

20 mL of methacrylic anhydride was added to a solution of 8-nitroquinolin-5-amine **(2)** (2 g, 10.57 mmol) in 20 mL of 1,4-dioxane. The mixture was stirred overnight at 120 °C. After cooling to room temperature, the mixture was added dropwise in hexane (400 mL), and the formed oil and supernatant were removed. Then, a solution of tin chloride monohydrated (8.8 g, 31.60 mmol) in 60 mL of the mixture of solvents (THF-H_2O (6:1)) was added, and the mixture was stirred for 3 h at 40 °C. The solvent was removed, and the solution was neutralized with a saturated sodium bicarbonate solution. The solid was filtered, dried, and collected and then, it was purified in a soxhlet apparatus using acetone as solvent. Finally, the solvent was concentrated under pressure to obtain a brown solid. Yield: 35% (834 mg, 3.67 mmol). ^{1}H NMR (300 MHz, DMSO-*d*$_{6}$) δ 9.68 (s, 1H), 8.75 (s, 1H), 8.07 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.48 (dd, *J* = 8.5, 4.1 Hz, 1H), 7.22 (d, *J* = 8.1 Hz, 1H), 6.86 (d, *J* = 8.1 Hz, 1H), 5.96 (s, 3H), 5.51 (s, 1H), 2.01 (s, 3H). ^{13}C NMR (75 MHz, DMSO-*d*$_{6}$) δ 169,13, 147.33, 144.52, 140.54, 137.72, 132.46, 126,45, 125.83, 121.67, 121.15, 120.39, 108.02, 19.43.

2.1.3. N-(8-(2-bromoacetamido)quinolin-5-yl)methacrylamide (4)

N-(8-aminoquinolin-5-yl)methacrylamide **(3)** (2 g, 8.80 mmol)**,** was dissolved in ethyl acetate (67 mL), and bromoacetyl bromide (1.4 mL, 16.07 mmol) was added dropwise. The mixture was stirred overnight at room temperature. The greenish solid was filtered and washed with ethyl acetate. Yield: 95% (2.85 g, 8.18 mmol). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.71 (s, 1H), 10.11 (s, 1H), 9.04 (dd, *J* = 4.4, 1.6 Hz, 1H), 8.59 – 8.37 (m, 2H), 7.76 (dd, *J* = 8.6, 4.4 Hz, 1H), 7.63 (d, *J* = 8.3 Hz, 1H), 6.03 (s, 1H), 5.60 (s, 1H), 4.43 (s, 2H), 2.02 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.09, 165.87, 161.37, 149.02, 140.06, 138.07, 135.22, 131.69, 130.26, 126,04, 124.91, 122.34, 121.41, 31.16, 19.30.

2.1.4. N-(8-(2-azidoacetamido)quinolin-5-yl)methacrylamide (5)

N-(8-(2-bromoacetamido)quinolin-5-yl)methacrylamide **(4)** (712 mg, 2.04 mmol) was dissolved in DMF (18 mL), and sodium azide (263 mg, 4.05 mmol) was added. The mixture was stirred overnight at room temperature. 50 mL of water was added, and the mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with water (3 × 50mL), dried, and the solvent was removed under vacuum. (350 mg, 1.13 mmol) of the brownish solid was obtained. Yield: 55% ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.51 (s, 1H), 10.00 (s, 1H), 8.98 (s, 1H), 8.66 (d, *J* = 8.3 Hz, 1H), 8.34 (dd, *J* = 8.5, 1.6 Hz, 1H), 7.78 – 7.50 (m, 2H), 6.02 (s, 1H), 5.60 (s, 1H), 4.43 (s, 2H), 2.04 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.05, 166.96, 162.76, 149.45, 140.27, 138.83, 133.35, 132.59, 129.29, 124.80, 122.28, 121.04, 116.88, 52.44, 19.27.

2.1.5. N-(8-(2-aminoacetamido)quinolin-5-yl)methacrylamide (6)

To a solution of N-(8-(2-azidoacetamido)quinolin-5-yl)methacrylamide (5) (0,450 mg, 1.45 mmol) in THF-water (6:1) (9 mL) , triphenylphosphine (1 g, 3.81 mmol) was added at room temperature. The mixture was then stirred overnight at 85 °C, and the solvent was removed under reduced pressure. The final residue was purified by column chromatography on SiO2 using ethyl acetate-ethanol (5:1) as the eluant to afford (6) as a yellowish solid (170 mg, 0.60 mmol). Yield: 41%. ^{1}H NMR (300 MHz, Chloroform-*d*) δ 11.27 (s, 1H), 8.87 (d, *J* = 4.1 Hz, 1H), 8.79 (d, *J* = 8.3 Hz, 1H), 8.15 (d, *J* = 8.5 Hz, 1H), 7.75 (s, 1H), 7.62 (d, *J* = 8.3 Hz, 1H), 7.47 (dd, *J* = 8.5, 4.2 Hz, 1H), 5.95 (s, 1H), 5.55 (s, 1H), 3.64 (s, 2H), 2.14 (s, 3H). ^{13}C NMR (75 MHz, Chloroform-*d*) δ 171.50, 148.40, 140.07, 138.84, 134,85, 132.84, 131,93, 131.37, 128,42, 128,91, 126.86, 120.67, 115.80, 46,11, 18.89.

2.2. Polymer Synthesis

2.2.1. Hydrophilic Film

The material was obtained by radical polymerization of the different monomers: Vinilpirrolidone (**VP**) as a hydrophilic monomer, methyl methacrylate (**MMA**) as the hydrophobic monomer, and (**5**) as the sensory monomer (Figure 1). The radical polymerization was carried out in silanized glass mold (100 μm thick) in an oxygen-free atmosphere at 60 °C overnight, using 0,65% mol of AIBN. The molar ratio of the monomers shows in Figure 2.

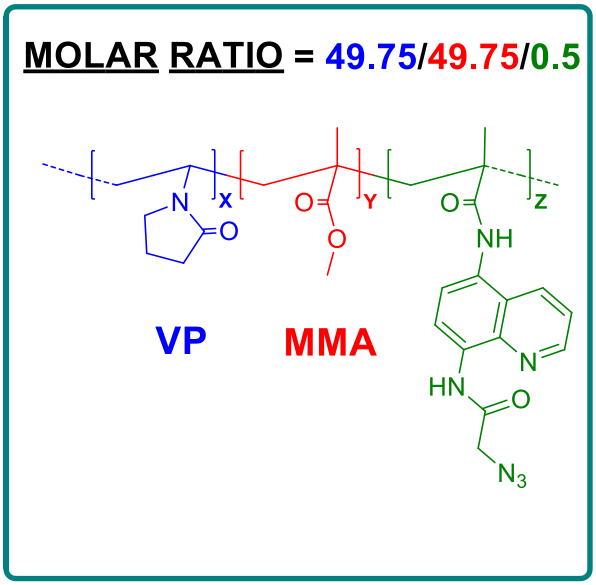

Figure 2. Structure and molar ratio of hydrophilic film.

2.2.2. Linear Polymer

The linear polymer was prepared by radical polymerization of **VP, MMA,** and sensory monomer **5** in a 49.5/49.5/1 molar ratio, respectively. The sensory monomer, VP, and MMA were dissolved in dimethylformamide (DMF) and the solution was added to a round bottom pressure flask. Radical thermal initiator AIBN was added and the solution sonicated for 5 min, then was heated at 60ºC overnight.

3. Results and discussion

3.1. Sensing Study

Compounds **(5)** and **(6)** have similar chemical structures, and it was expected that the sensory response in the presence of cation Zn(II) is the same. Thus, sensing studies were carried out with both compounds.

First, 0.45 mL of a solution of **(5)** in dimethylacetamide **(DMA)** ($5 \cdot 10^{-3}$ M), 0.5 mL of buffer solution (pH = 4.66), and 50 µL of cation solution (0.1 M), were mixed in a test tube. This process was carried out with 29 cations (K(I), Sm(III), Al(III), Ag(I), Nd(III), Pb(II), Na(I), Sr(II), Ni(II), Hg(II), Rb(I), Dy(III), Li(I), Cd(II), Fe(III), Ce(III), Zr(IV), Mg(II), La(III), Cs(I), Mn(II), Ca(II), Cr(III), Ba(II), Zn(II), Co(II), $NH4(I)^{+}$, Cu(II), Cr(VI)). All the tests showed a background fluorescence, but the only cation which increases the fluorescence was Zn(II), as shows in Figure 3.

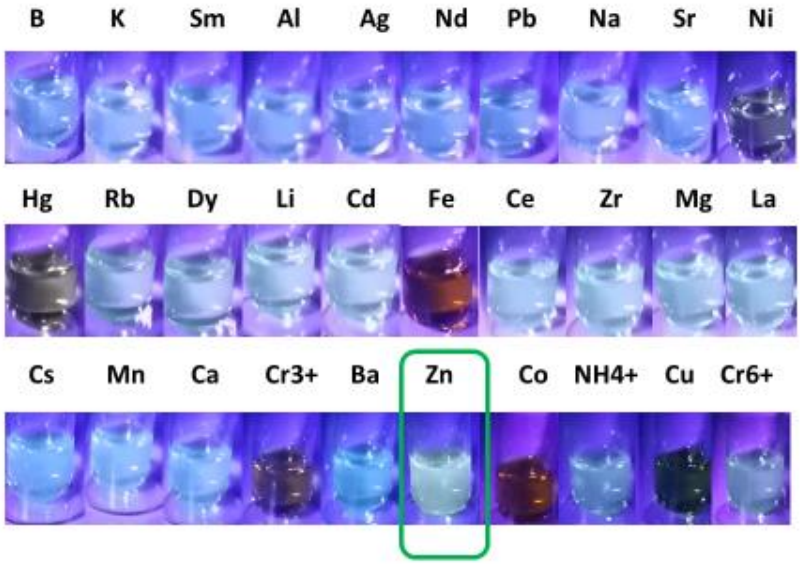

Figure 3. Sensing study with 29 cations**.** Fluorescence of a buffered solution of **5** (pH: 4.66, volume: 1 mL which corresponded to 0.45 mL of a solution of **5** in **DMA** ($5 \cdot 10^{-3}$M), 0.5mL of buffer solution, and 50 µL of cation solution (0.1 M)),.

Secondly, the interference study with **(6)** was carried out following the same experimental procedure. Figure 4 shows the interference study with 29 cations.

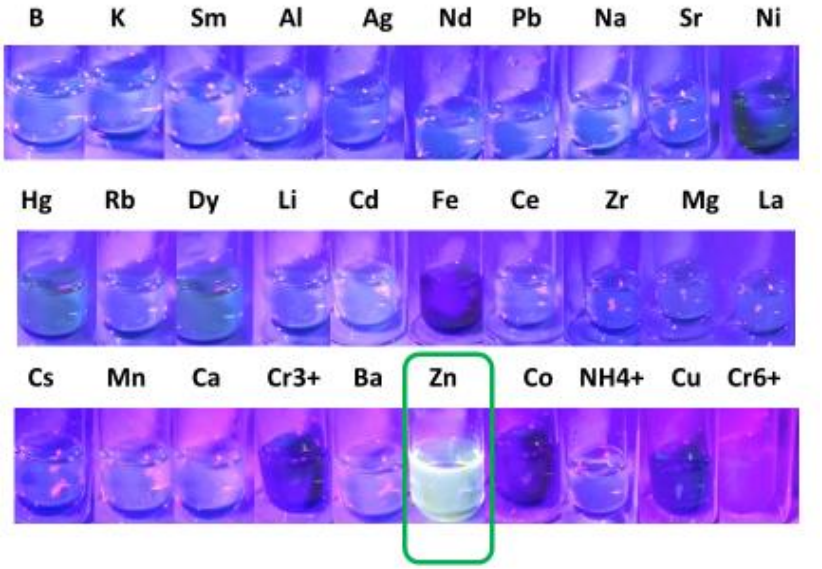

Figure 4. Sensing study with 29 cations**.** Fluorescence of a buffered solution of **6** (pH: 4.66, volume: 1 mL which corresponded to 0.45 mL of a solution of **6** in **DMA** ($5 \cdot 10^{-3}$M), 0.5 mL of buffer solution, and 50 µL of cation solution (0.1 M)).

In this case, no background fluorescence was observed, and the OFF-ON fluorescence process in the presence of cation Zn(II) was so much accentuated. Therefore, compound **(6)** is a better candidate for the preparation of a sensory polymer than compound **(5)**.

3.2. Future Prospects

We are working in the next steps, specifically, we are doing the interference study with linear polymers from sensory monomers **5** and **6**. Then, we will be able to measure biological samples from common and chronic wounds with polymeric films to confirm the relationship between Zn(II) concentration and chronic wound severity.

4. Conclusions

We have designed a new sensory material for the determination of Zn(II) in biological samples, that could be correlated with the enzyme activity of MMP and therefore, with the state of chronic wounds.

We have obtained the sensory material related to objective 3, and we are working on the study of chronic wound samples containing zinc, namely, objectives 1 & 2.

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References

1. Xue, M.; Le, N.T.V.; Jackson, C.J. Targeting matrix metalloproteases to improve cu-taneous wound healing. *Expert Opin. Ther. Targets* **2006**, *10*, 143–155. DOI: 10.1517/14728222.10.1.143
2. Adjepong, D.; Jahangir, S.; Malik, B.H. The Effect of Zinc on Post-neurosurgical Wound Healing: A Review. *Cureus* **2020**, *12*, 1–7. DOI: 10.7759/cureus.6770
3. Guembe-García, M.; Vallejos, S.; Carreira-Barral, I.; Ibeas, S.; García, F.C.; San-taolalla-García, V.; Moradillo-Renuncio, N.; García, J.M. Zn(II) detection in biological samples with a smart sensory polymer. *React. Funct. Polym.* **2020**, *154*. DOI: 10.1016/j.reactfunctpolym.2020.104685