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Short Communication

Smart sensory polymer for straightforward Zn(II) detection in pet food samples

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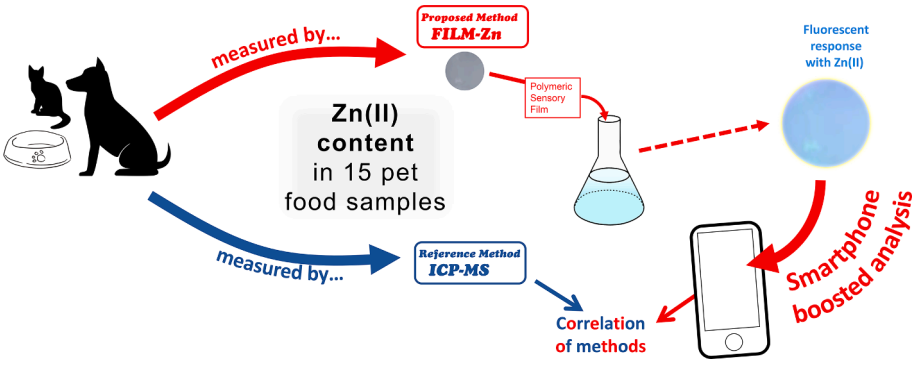
HIGHLIGHTS

- Fluorescence sensory polymer film toward Zn(II).
- Dipping the films in water with Zn(II) turn-on its fluorescence.
- A picture taken to the sensory polymer allows for the quantification of Zn(II).
- The Zn(II) content of commercial pet food was measured.
- The use of the sensory film and proposed methodology saves time and costs.

GRAPHICAL ABSTRACT



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ABSTRACT

We report on an innovative method to measure the Zn(II) concentration in commercial pet food samples, both wet and dry food. It is based on a colorimetric sensory polymer prepared from commercial monomers and 0.5 % of a synthetic monomer having a quinoline sensory core (*N*-(8-(2-azidoacetamido)quinolin-5-yl)methacrylamide). We obtained the sensory polymer as crosslinked films by thermally initiated bulk radical polymerization of the monomers of 100 μm thickness, which we punched into Ø6 mm sensory discs. The immersion of the discs in water solutions containing Zn(II) turned the fluorescence on, allowing for the titration of this cation using the G parameter of a digital picture taken to the discs. The limits of detection and quantification were 29 and 87 μg/L, respectively. Furthermore, we measured the concentration of Zn(II) even in the presence of other cations, detecting no significant interferences. Thus, in a further step, we obtained the concentration of Zn (II) from 15 commercial pet food samples, ranging from 19 to 198 mg/kg, following a simple extraction procedure and contacting the extractant with our sensory discs. These results were contrasted with that obtained by ICP-MS as a reference method.

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1. Introduction

Zn(II) cation is an essential trace element for all living beings and is part of countless proteins where it acts as a catalytic, structural, or regulatory cofactor [1–3]. Despite its important role in life, it can be toxic in high concentrations, like all metals [4,5]. Excess of zinc in diet avoids the absorption of other essential elements, such as copper or iron, due to mere competition between cations [6]. Also, the assimilation of zinc by living organisms is inversely proportional to the consumed amount, so the zinc ingested in excess is excreted. And this is the leading environmental problem with zinc since manures contain high zinc concentrations that seep through soils into groundwater [7].

Generally speaking, environmental zinc contamination has three primary sources: leaching from galvanized steel (~30 %), industrial point sources (10 %), and others (60 %), including agriculture and stockbreeding [7]. Animals ingest high amounts of Zn(II) as salts (zinc sulphate monohydrate, zinc chelate of amino acids hydrate, or zinc oxide) added in high quantities in the feed of all animals, especially on farms, as it favours the rapid growth of animals. This represents the main route of Zn(II) intake by humans through the food chain.

Given the concern about the environmental effect and the effect on people's health, countries such as China [8] and the European Union [7] have already established the maximum amount of zinc in animal feed at 150–250 mg/kg and 100–150 mg/kg, respectively. In addition, the most recent studies have shown that soil contamination with zinc leads to bacterial resistance to antibiotics, is phytotoxic, and drastically decreases microbiota activity [9]. However, the most significant risk is not associated with soil contamination but with contamination of groundwater, drainage, and zinc runoff from farmland to surface water [7].

Analysing a target's concentration in a given medium is an expensive and time-consuming procedure and is a task generally carried out by specialized personnel [10–12]. This is one of the big problems when talking about regulatory control compliance. In other words, more straightforward zinc detection methods are still necessary [13,14], since traditional methods are difficult to apply *in situ*; they always require advanced equipment, and a specialist must carry them out.

In this study, we have designed and prepared a polymeric film for detecting Zn(II) in aqueous media that meets all these requirements. Thus, the equipment for the measurement is only a smartphone, and the procedure is as simple as dipping the sensory polymeric film, waiting, and photographing [15–17]. Furthermore, the new material and measuring method have been tested in real conditions, with water and pet food samples. The results have been compared and contrasted with a highly selective and sensitive method such as Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

2. Experimental

2.1. Materials

All materials and solvents were commercially available and used as received unless otherwise indicated. The following materials and solvents were used: 1-vinyl-2-pyrrolidone (**VP**) (99 %, Aldrich), methyl methacrylate (**MMA**) (99 %, Aldrich), ethylene glycol dimethacrylate (**E**) (97.5 %, Aldrich), pH 4.66 Buffer (VWR), acetone (99 %, VWR), potassium hydroxide (99 %,VWR-Prolabo), methacrylic anhydride (94 %, Alfa Aesar), hydrochloric acid (37 %, VWR-Prolabo), trichloroacetic acid (≥99.99 %, Sigma-Aldrich), 8-nitroquinoline (98 %, Alfa Aesar), hydroxylamine hydrochloride (97 %, TCI), ethanol (≥99.9 %, VWR), methanol (≥99.8 %, VWR), 1,4-dioxane (≥99.9 %, VWR), ethyl acetate (≥99.9 %, VWR), bromoacetyl bromide (98 %, Alfa Aesar), sodium azide (99 %, Alfa Aesar), dimethylsulfoxide-d_6 (99.9 %, VWR), dimethylformamide (99.9 %, Supelco).

Azo-bis-isobutyronitrile (AIBN, Aldrich, 98 %) was recrystallized twice from methanol to provide the highest quality polymerization initiator.

Selectivity and interference study was performed with the following metallic salts: zinc(II) nitrate hexahydrate (98 %, Sigma-Aldrich), iron (III) nitrate nonahydrate (99 %, Sigma-Aldrich), manganese(II) nitrate hexahydrate (98+%, Alfa Aesar), cobalt(II) nitrate hexahydrate (≥99 %, Labkem), calcium(II) nitrate tetrahydrate (≥99 %, Sigma-Aldrich), mercury(II) nitrate (98 %, Alfa Aesar), cadmium(II) nitrate tetrahydrate (98.5 %, Alfa Aesar), potassium(I) nitrate (99+%, Sigma-Aldrich), lead(II) nitrate (≥99 %, Fluka), iron(II) sulphate heptahydrate (99 %, Sigma-Aldrich), magnesium(II) nitrate hexahydrate (≥99 %, Labkem), copper(II) sulphate pentahydrate (98 %, Sigma-Aldrich), nickel(II) nitrate hexahydrate (98.5 %, Sigma-Aldrich), sodium(I) nitrate (99 %, LabKem), cesium(I) nitrate (≥99 %, Fluka), barium(II) chloride dihydrate (99 %, Labkem), ammonium nitrate (≥98 %, Sigma-Aldrich), chromium(III) nitrate (98.5 %, Alfa Aesar), rubidium(I) nitrate (99.95 %, Sigma-Aldrich), dysprosium(III) nitrate (99.9 %, Alfa Aesar), lithium (I) chloride (≥99 %, Sigma-Aldrich), cerium(III) chloride tetrahydrate (≥99.99 %, Sigma-Aldrich), zirconium(IV) chloride (98 %, Alfa Aesar), lanthanum(III) nitrate hexahydrate (99.9 %, Alfa Aesar), samarium(III) nitrate (99.9 %, Alfa Aesar), aluminium(III) nitrate (≥98.9 %, Sigma-Aldrich), silver(I) nitrate (≥99.9 %, Sigma-Aldrich), neodymium(III) nitrate (99.9 %, Alfa Aesar), strontium(II) nitrate (99 %, Sigma-Aldrich).

2.2. Instrumentation and methods

^{1}H and $^{13}C\{^{1}H\}$ NMR spectra (Advance III HD spectrometer, Bruker Corporation, Billerica, Massachusetts, USA) were recorded at 300.17 MHz for ^{1}H and 75.38 MHz for ^{13}C using deuterated dimethyl sulfoxide (DMSO-d_6) as solvent. The experiments were acquired at 298 K with the standard pulse sequences from the Bruker library and processed with MestReNova software (v. 12.0, Mestrelab Research SL, Santiago de Compostela, Spain). The chemical shifts (δ) are reported in ppm relative to the solvent resonance as the internal standard (DMSO-d_6 = 2.51 ppm).

The powder X-ray diffraction (PXRD) patterns were obtained using a diffractometer (D8 Discover Davinci design, Bruker Corporation, Billerica, Massachusetts, USA) operating at 40 kV, using Cu(Kα) as the radiation source, a scan step size of 0.02°, and a scan step time of 2 s. X-ray diffraction studies were conducted at 200 K on a Bruker D8 VENTURE diffractometer.

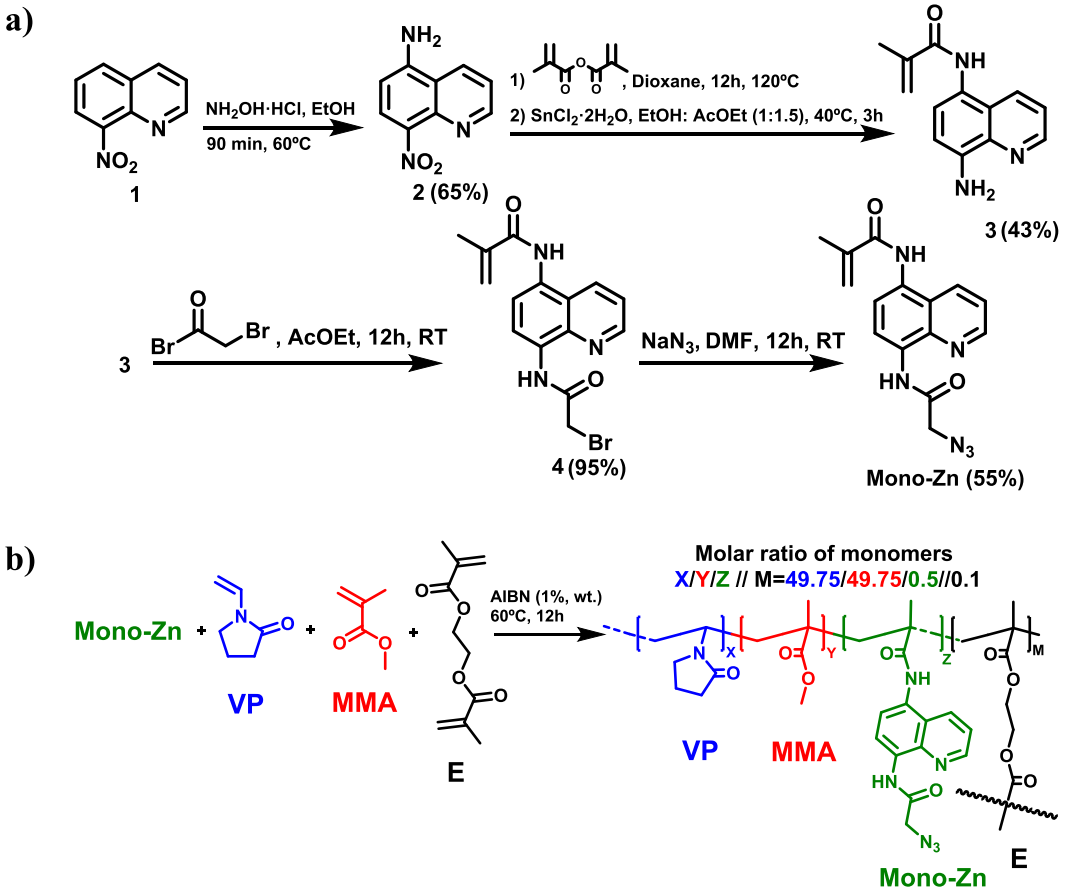
The polymers thermal and mechanical characterization was performed by: a) thermogravimetric analysis (Q50 TGA analyser, TA Instruments, New Castle, DE, USA) with 10–15 mg of sample under synthetic air and nitrogen atmosphere at 10 °C min^{-1}; b) differential scanning calorimetry, with 10–15 mg of the sample under a nitrogen atmosphere at a heating rate of 10 °C min^{-1} (Q200 DSC analyser, TA Instruments, New Castle, DE, USA), and c) Young's modulus analysis were calculated in tensile test of dried Film-Zn strips (5 × 9.44 × 0.122 mm) performed at a rate of 1 mm min^{-1} (Shimadzu EZ Test Compact Table-Top Universal Tester, Shimadzu, Kyoto, Japan).

Infrared spectra (FTIR) were recorded with an infrared spectrometer (FT/IR-4200, Jasco, Tokyo, Japan) with an ATR-PRO410-S single reflection accessory.

Solution fluorescence spectra were recorded using a F-7000 Hitachi Fluorescence spectrophotometer (Hitachi, Tokyo, Japan). Solution measurements for the interaction study were carried out in a conventional cuvette with no special procedures. A rectangular 10 mm cuvette was used for the fluorescence measurements, measuring all data at 25 °C ± 0.1 °C.

The weight percentage of water taken up by the films upon soaking in pure water at 20 °C until reaching equilibrium (water-swelling percentage, WSP) was obtained from the weight of a dry sample film (ω_d) and its water-swelled weight (ω_s) using the following expression: $WSP = 100 \times [(\omega_s - \omega_d)/\omega_d]$.

High-resolution electron-impact mass spectrometry (EI-HRMS) was carried out on a Micromass AutoSpect (Waters mass, Micromass Holdings ltd., Cary, North Carolina), using ionisation energy of 70 eV, and a



Scheme 1. Synthetic route for: a) the sensory monomer **Mono-Zn**; and b) the sensory polymer **Film-Zn**.

mass resolving power: >10,000. Inductively coupled plasma mass spectrometry (ICP-MS) measurements were recorded on an Agilent 7500 ICP-MS spectrometer (Agilent, Santa Clara, USA).

Digital photographs were taken with a Huawei p30 pro (Huawei, Shenzhen, China) in a dark room (distance to the object = 25 cm) under UV radiation (365 nm lamp, 45° inclination). G parameter of digital photographs was extracted using the smartphone app "Colorimetric Titration" [18,19].

2.3. Synthesis of the sensory monomer *Mono-Zn*

We prepared the sensory monomer **Mono-Zn** following conventional organic reactions starting from 8-nitroquinoline. The preparation route is depicted in Scheme 1, and the detailed description of the synthesis steps and the characterisation of intermediates and **Mono-Zn** can be found in the Supporting Information (**SI-Section S1**).

2.4. Preparation of the sensory polymer synthesis *Film-Zn*

We prepared the sensory film **Film-Zn** by bulk thermally initiated bulk radical polymerization [20]. Thus, two commercial monomers (**VP** and **MMA**), a crosslinker (ethylene glycol dimethacrylate, **E**), and the synthesised sensory monomer (**Mono-Zn**) were mixed in a molar ratio of 49.75/49.75/0.5/0.1 (VP/MMA/Mono-Zn/E). Additionally, we added AIBN (1 %, wt.) radical thermal initiator. Then, we injected the mixture into a mould (100 um thickness) comprised of two sealed silanised glasses and heated it overnight at 60 °C, where the polymerization took place under an oxygen-free atmosphere. Finally, we washed the resultant material with water and acetone and punched it into Ø6 mm discs. The FTIR, TGA, DSC and PRXD patterns can be found in the **SI-Section S2**.

2.5. Interference study

We carried out a preliminary experiment for testing the fluorimetric response of the synthesized sensory monomer with 29 different cations by preparing solutions of **Mono-Zn** (2.6×10^{-3} M) and a cation (5×10^{-3} M) in an aqueous:organic medium. All vials were photographed together (**SI-Section S3,** Fig. S6) under 365 nm light irradiation.

In a second step, we conducted a more profound study with the identified potential interferents. Thus, for each interferent, we prepared the following tubes containing: a) Ø6mm disc of **Film-Zn**, 1 mL of 10 mg/L Zn(II) solution buffered at pH 4.66; and b) Ø6mm disc of **Film-Zn**, 1 mL of 10 mg/L Zn(II) and 10 mg/L cation solution buffered at pH 4.66. The films were dipped for 2 h, washed in triplicate with water (10 min each), and photographed under 365 nm light irradiation to obtain the G parameter.

2.6. Titration of *Mono-Zn* with Zn(II)

This study was carried out by adding $Zn(NO_3)_2{\cdot}6H_2O$ to a **Mono-Zn** solution (1.02×10^{-4} M) in buffered organic-aqueous media (pH 4.66 buffer:DMA at 50:50 ratio), in concentrations ranging from 0.2 ppm to 72 mg/L. The fluorescence spectra were recorded at 25 °C ± 0.1 °C using the following conditions: excitation slit = 10 nm; emission slit = 10 nm; excitation wavelength = 380 nm; scan speed = 1200 nm/min; step = 0.5 nm.

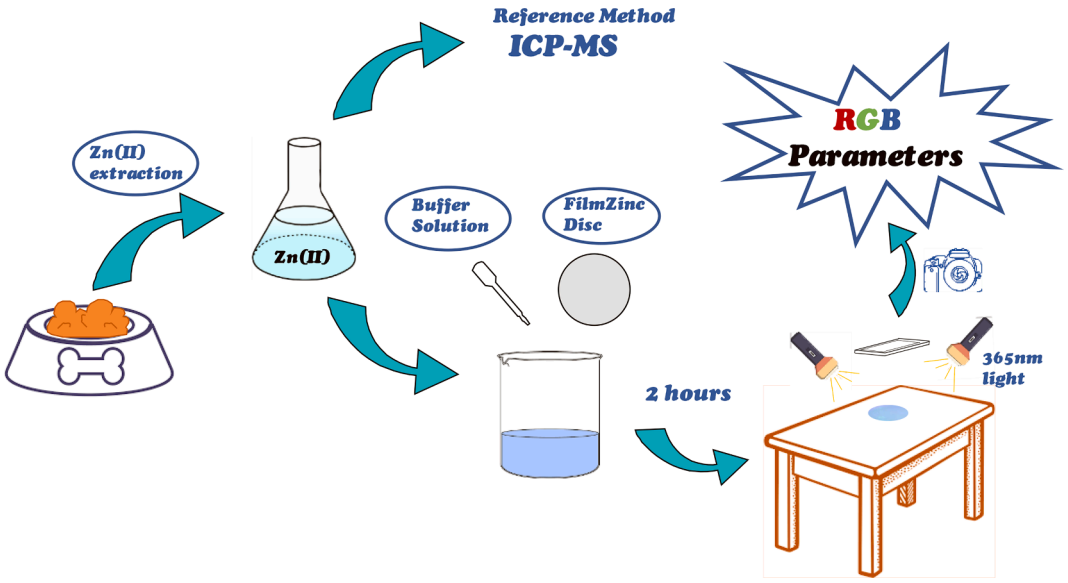


Fig. 1. Representation of the procedures for the determination of Zn(II) content in pet food by a conventional method (ICP-MS) and by using the sensory polymer **Film-Zn**.

2.7. Titration of *Film-Zn* with Zn(II)

We dipped Ø6mm **Film-Zn** discs for 2 h in fully aqueous solutions with different Zn(II) concentrations (from 0.5 to 15 mg/L) buffered at pH 4.66. After that, the discs were extracted from solutions and photographed with the smartphone. Finally, we carried out the experiments in triplicate.

2.8. Detection and quantification of Zn(II) from pet food

We analysed 15 dry and wet commercial pet food from the retail market (the product/brand is shown in the **SI-Section S4**) by the following procedure (we follow the extraction procedure described by Wang et al. with some modifications [21]): a) firstly, we mixed 2 g of wet or 1 g of dry food with 40 mL of trichloroacetic acid (TCA, 1 %); b) secondly, the mixture was magnetically stirred for 5 min, centrifuged for 10 min at 11,000 rpm, and filtered off; c) finally, we measured the Zn(II) content of the extractant by two ways: by a reference method (quantitative ICP-MS, data are means of 5 replicates for each pet food) and using our sensory film **Film-Zn** (data are means of 2 replicates for each pet food).

For measuring the Zn(II) concentration with **Film-Zn**, we followed the following procedure: 1 mL of the extract was diluted with 1 mL of pH 4.66 acetate buffer, and then one disc of **Film-Zn** (Ø6mm) was dipped in the solution for 2 h. Afterward, the disc was washed in triplicate with distilled water (10 min each) and photographed with the smartphone. Fig. 1 shows this procedure schematically. G parameter was extracted from the digital photographs, and the results were correlated with the obtained ones from the quantitative ICP-MS measurements.

2.9. Limits of detection (LOD) and quantification (LOQ)

The limit of detection (LOD) and the limit of quantification (LOQ) of our sensory system was calculated by the following equations: LOD = 3.3 × SD/s and LOQ = 10 × SD/s, respectively, where "SD" is the standard deviation of the blank sample and "s" is the slope of a calibration curve in the region of low analyte content (below 1 mg/L) (more information in **SI-Section S5**) [22,23].

3. Results and discussion

We report in this work on film-shaped sensory materials useful for the selective detection and quantification of Zn(II) in water solution and on the application of this sensor to the quantification of Zn(II) in pet food samples. To explain the advantage and potential of detecting Zn(II) using our sensor, we will briefly describe the synthetic procedure for the preparation of the sensory films, the interaction of Zn(II) with the sensory material that gives rise to the *turn-on* of the fluorescence of the sensory films, responsible of the sensor phenomenon, and, finally, the titration of water solutions of Zn(II) and the exploitation of the sensory materials for the determination of Zn(II) concentration in dry and wet pet commercial samples.

3.1. Design of the material

For this kind of application, aimed at simple and direct use by non-skilled personnel, we opted for a manageable and resistant material appropriate for careless handling. For this a hydrophobic co-monomer providing rigidity to the material is needed when dealing with polyacrylic materials. Accordingly, methylmethacrylate (**MMA**) was used. However, this material must detect Zn(II) in water, and therefore a hydrophilic monomer is also necessary, which allows the target species (in this case, Zn(II)) to enter the three-dimensional polymeric network by diffusion, such as 1-vinyl-2-pyrrolidone (**VP**). Therefore, the molar ratio between the hydrophilic and the hydrophobic monomer (in our case 1) is key to obtaining a manageable material being, at the same time, able to swell in water. In addition, the material must be prepared with the sensory monomer **Mono-Zn**. As it is a synthetic monomer (not commercially available), the minimum ratio to other co-monomers was used to obtain an adequate visual response (0.5 % mol).

The films were designed as thermostable materials crosslinked with ethyleneglycol dimethacrylate (Scheme 1**b**, the nominal crosslinking ratio was 1,000, i.e., the ratio of feed moles of co-monomers to the crosslinking agent), which gives rise to a water swelling percentage of 71 %, and a Young's modulus of 974 MPa. As a result, the films had a glass transition temperature of 138 °C and thermal resistance, in terms of 5 % and 10 % weight loss, of 224 and 343 °C, respectively.

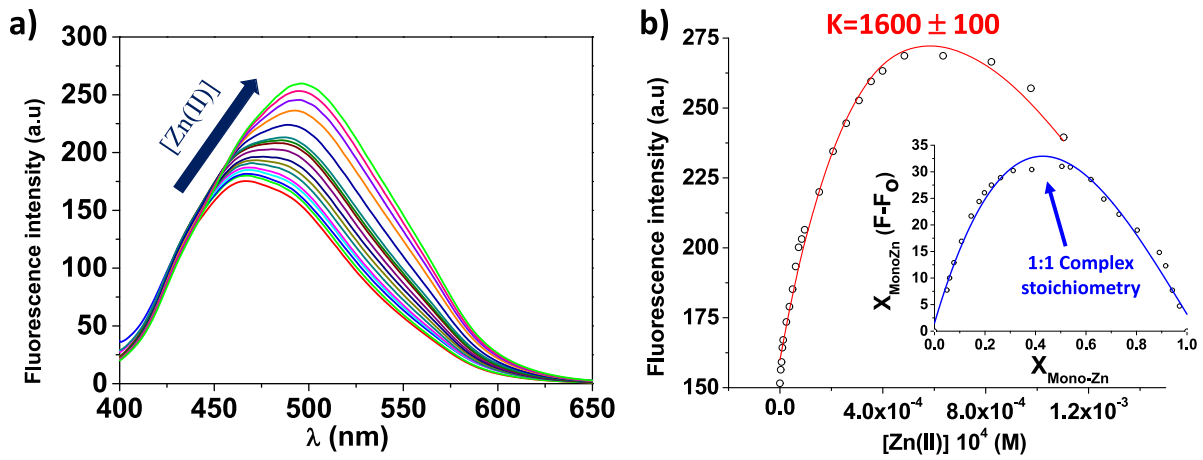


Fig. 2. a) Titration of **Mono-Zn** with Zn(II) in buffered organic-aqueous media (pH 4.66:DMA at 50:50 ratio). The concentration of Zn(II) in the cuvette was increased from 0.2 to 72 mg/L (from 3.1 × 10^{-6} M to 1.1 × 10^{-3} M), and the initial concentration of **Mono-Zn** was 32 mg/L (1.02 × 10^{-4} M). **b)** (In red) Representation of the fluorescence intensity at 497.6 nm versus Zn(II) molarity. The fitted curve was used for the calculation of the complex formation constant; (in blue) Job's Plot diagram, which represents X_{MonoZn}(F-Fo) versus the molar fraction of **Mono-Zn.** Measuring conditions: excitation slit = 10 nm; emission slit = 10 nm; excitation wavelength = 380 nm; scan speed = 1,200 nm/min.

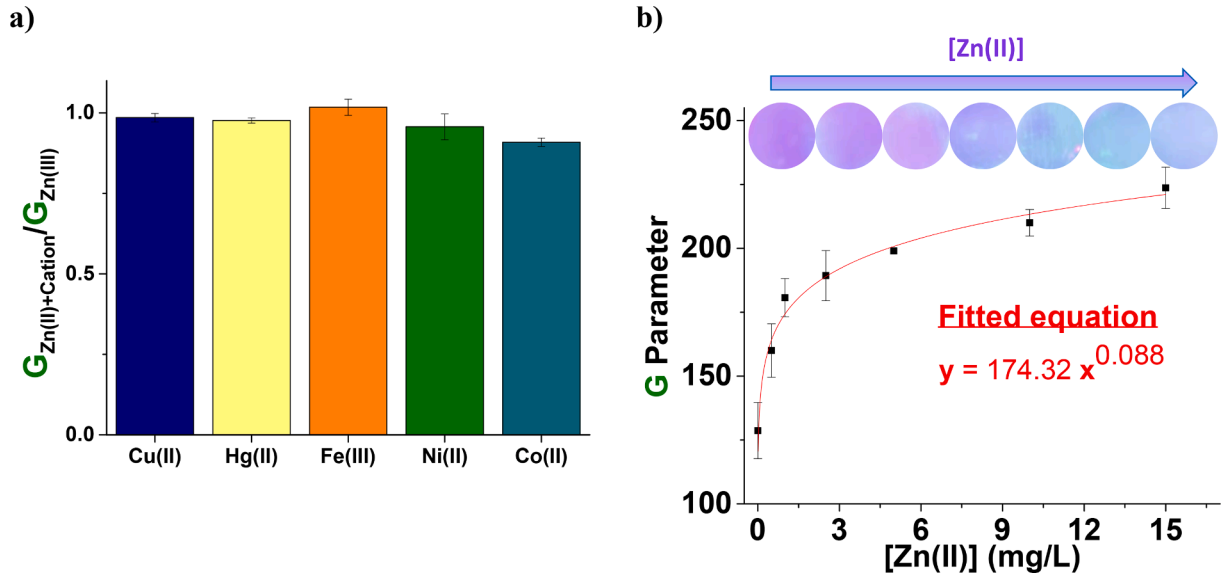


Fig. 3. a) Interference study with metal cations that produce quenching of the fluorescence of the system, i.e., Cu(II), Hg(II), Fe(III), Ni(II), Co(II). Experimental procedure: a Ø6mm disc of **Film-Zn** was dipped in 1 mL of 10 mg/L Zn(II) solution buffered at pH 4.66, another disc in 1 mL of 10 mg/L Zn(II) and 10 mg/L cation solution buffered at pH 4.66. The films were dipped for 2 h and photographed under 365 nm light irradiation to obtain the G parameter. A value of $G_{Zn(II)+Cation}$ / $G_{Zn(II)}$ equal to 1 means no interference. **b)** Graphical representation of the G parameter (mean ± standard error of 3 replicates) from the photographed discs *vs* Zn(II) concentration (fully aqueous solutions buffered at pH 4.66 with Zn(II) concentrations ranging from 0.5 to 15 mg/L).

*3.2. Study of the interaction of the sensory monomer **Mono-Zn** with Zn (II)*

We initially envisaged that the interaction of Zn(II) with **Mono-Zn** would probably follow the previously described interaction of these cations with 1-(quinoline-8-yl)urea [24], which would give rise to a complex **Mono-Zn**:Zn(II) with a 1:1 stoichiometry. Thus, we analysed the complexation behaviour following the fluorescence *turn-on* associated with the formation of the complex **Mono-Zn**:Zn(II), represented in Fig. 2**a** as an increase and displacement of a fluorescence band centred at 497 nm. From the representation of fluorescence intensity against the concentration of Zn(II), we obtained the complex formation constant, 1.6 × 10^3 M, and the Job's plot, which confirmed the 1:1 stoichiometry for the complex (Fig. 2**b**). Additionally, **SI-Section S3** includes the three-dimensional fluorescence graphics, the species distribution diagram, and the graphic representing the contribution of each species to the total fluorescence of the system.

To proceed with the preparation of the sensory material, once described the efficient interaction of the sensory monomer with Zn(II), we first had to ensure the effectiveness of this interaction in terms of lack or controllable interferences. Therefore, we started with an overall study of **Mono-Zn** comparing the fluorescence response with 29 different cations, and we observed an only OFF-ON fluorescence process with Zn (II), but 4 ON-OFF fluorescence processes with Cu(II), Hg(II), Fe(III), Ni (II) and Cd(II). The image with all the studied cations can be found in **SI-Section S5** (**Fig. S6**), where the interference effect can also be visually observed under 365 nm light irradiation.

Thus, after identifying potential interferents, we carried out the analysis with the sensory film **Film-Zn** and the above-mentioned cations in more realistic concentrations. Fig. 3**a** shows the normalised G

Table 1
Figure of merits of **Film-Zn**-based methodology in comparison with the most relevant method for the detection of Zn(II).

Detection method	Quantification in animal feed samples	Equipment required	LOD	Ref.
ICP-Mass	No	ICP-Mass spectrometer	2.0 μg/g	[30]
	Yes	ICP-Mass spectrometer	–	[31]
AAS (Atomic Absorption Spectrometry)	No	Atomic absorption spectrometer	0.01 μg/g	[32]
	Yes	Atomic absorption spectrometer	0.05 μg/L	[33]
Continuous wavelength transforms	No	UV/Vis spectrophotometer	0.9 μg/L	[34]
Fluorimetric probes in solution	Yes	Fluorimeter	1 μg/L	[35]
	No	Fluorimeter	0.2 μg/L	[36]
Colorimetric probes in solution	Yes	UV/Vis spectrophotometer	0.004 μg/mL	[21]
Poly (azomethine-urethane) as fluorescent probe	No	Fluorimeter	19.6 mg/L	[37]
Fluorometric film (**Film-Zn**)	Yes	Smartphone	29 μg/L	This Work

parameter ($G_{Zn(II)+Cation}/G_{Zn(II)}$) extracted from the digital photographs. The results point out the validity of our sensor in applications devoted to detecting and quantifying Zn(II) in commercial pet food. Additionally, Ni(II) and Co(II) are not relevant in pet food because they are usually in lower concentrations than Zn(II) [25–28]; Ni(II) can be even toxic for dogs [29], and these cations have not been indicated as additives in any commercial product.

Once we studied the sensory material's interaction with Zn(II) and disregarded the possible interferents, we carried out studies with **Film-Zn** in aqueous solutions with different Zn(II) concentrations, and a proof of concept with pet food samples, as reported on below.

3.3. Titration of *Film-Zn* in aqueous samples of Zn(II) with a smartphone

The titration curve was carried out by correlating the G parameter of the digital image with the concentration of Zn(II), as shown in Fig. 3**b**. G parameter was chosen because after analysing all the coordinates of the colour space (R, G and B), we realised that it was the most variant parameter and that it followed a clear trend with the concentration of Zn (II) in the solutions.

Additionally, we performed a second experiment in the linearity zone at low concentrations of Zn(II) (from 0.15 to 0.75 mg/L). This assay allows the calculation of the detection and quantification limits of the system (LOD and LOQ). In our case, these limits are low enough (LOD = 29 μg/L; LOQ = 87 μg/L) to point out the validity of the prepared material for the application we have addressed, i.e., the determination of Zn (II) in commercial pet food. Table 1 shows a comparative study with the most used methodologies for determining zinc in terms of LOD, equipment required for the measurement, and validation for the proposed application.

Table 2
Zn(II) concentration of measured pet foods obtained by the reference method (ICP-MS) and the proposed method, based on the use of Film-Zn and a smartphone. Zn(II) concentration data from reference and **Film-Zn** methods are means of ± standard errors of 5 and 2 replicates, respectively.

Sample N°	Zn(II) concentration (mg/kg)		
	Indicated by manufacturer*	ICP-MS	Film-Zn
1	134	133.1 ± 0.7	112.5 ± 4.9
2	54	182.9 ± 2.6	182.8 ± 6.2
3	135	198.7 ± 5.2	197.5 ± 28.3
4	80	123.6 ± 0.6	137.2 ± 27.1
5	148	132.4 ± 0.9	118.7 ± 25.9
6	42	162.6 ± 0.4	182.8 ± 13.6
7	108	135.0 ± 26.0	130.6 ± 4.1
8	82	149.1 ± 3.1	155.2 ± 17.7
9	20	37.1 ± 0.1	28.5 ± 1.8
10	154	90.4 ± 0.4	117.4 ± 4.9
11	120	113.1 ± 1.3	107.6 ± 2.5
12	86	134.9 ± 17.4	127.3 ± 2.5
13	106	143.4 ± 1.3	116.2 ± 8.6
14	36	40.5 ± 0.2	40.2 ± 2.5
15	9	14.5 ± 0.1	19.3 ± 3.7

* Zn(II) content added by the manufacturer as an additive (it does not take into account the Zn(II) contained in the ingredients used in the manufacturing).

3.4. Proof of Concept. Zn(II) detection in real samples

Once addressed the validity of our sensory system for the *in vitro* evaluation of Zn(II), even in the presence of other cations, our next step was evaluating the performance of the polymer sensor in determining the concentration of Zn(II) in commercial samples.

After we measured the Zn(II) concentration in 15 commercial pet foods (see brand names in the **SI-Section S4**) by two different methods, as we depicted in the experimental section, we represented the concentration obtained by the reference method (ICP-MS) against G parameter (Fig. 4). The fitted line in this representation should be

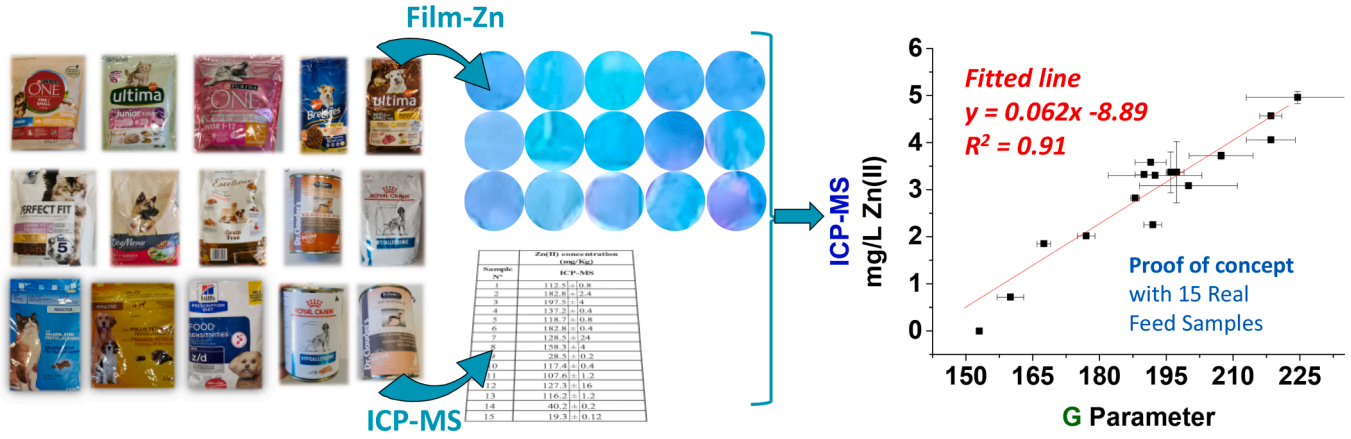


Fig. 4. Graphical abstract of the proof of concept, including the representation of the Zn(II) concentration obtained by the reference method (ICP-MS, data are means ± standard error of 5 replicates) against the G parameter obtained from the photographs (**Film-Zn**, data are means ± standard error of 2 replicates).

visualised as a 15-point calibration line, which from now on, an end-user can utilise in the following way: 1) perform the extraction of Zn(II) from a pet food problem sample; 2) insert a Ø6mm disc of **Film-Zn** for 2 h; 3) take a picture of the disk and extract the G parameter with the "Colorimetric titration" app; 4) enter the value G as the "x" in the fitted line and obtain the concentration of Zn(II).

Therefore, we have inserted the G data obtained for each pet food in this fitted equation (y = 0.062x – 8.89), and analysed the results obtained in comparison with the reference method. The data shown in Table 2 have been statistically analysed (**SI-Section S6**), and we can affirm that there are no significant differences between both methods.

In short, our method is a more innovative and costless alternative to any reference method, e.g., ICP-MS, since the analysis can be carried out *in situ* and without the concurrence of specialised personnel or scientific equipment, just a smartphone.

4. Conclusions

The use of smart materials in different fields is opening new technological opportunities. Among them are the new analysis systems using sensory polymers, systems that in colorimetric sensory polymers allow the detection and quantification of chemical species of interest with the naked eye, and in a fine way using common tools, such as mobile phones. In addition, this analysis can be carried out *in situ*, very cheaply, and by non-specialised personnel, which leads to the democratisation of analytical systems. In this framework, we have prepared a sensory system based on polymer sensors prepared in film form for visual evaluation and through a mobile application of Zn(II) in water and animal feed. Furthermore, we have verified the reliability and usability of this system through the alternative evaluation of the Zn(II) concentration with a proven technique, such as ICP-MS. The concentrations determined by both methods are coincident, in the range of mg/kg.

CRediT authorship contribution statement

José Carlos Guirado-Moreno: Investigation, Conceptualization, Writing – original draft. **Lara González-Ceballos:** Investigation, Writing – original draft. **Israel Carreira-Barral:** Investigation, Writing – original draft. **Saturnino Ibeas:** Conceptualization, Writing – review & editing, Visualization. **Miguel A. Fernández-Muiño:** Conceptualization, Methodology. **M. Teresa Sancho:** Investigation, Methodology, Writing – review & editing. **José M. García:** Writing – review & editing, Visualization. **Saúl Vallejos:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Visualization.

Open Data

Open Data is available at https://riubu.ubu.es/handle/10259/5684 under the name "Dataset of the work Smart sensory polymer for straightforward Zn(II) detection in pet food samples".

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data were used for the research described in the article.

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Appendix A. Supplementary material

Synthesis and characterisation of **Mono-Zn** (including X-ray crystallographic data, CCDC 2180092); characterisation of the sensory polymer **Film-Zn**; fluorescence study; proof of concept; limit of detection (LOD) and limit of quantification (LOQ); statistical analysis. Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa.2022.121820.

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