



Rapid and selective luminescent sensing of allergenic gluten by highly phosphorescent switch-on probe

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ABSTRACT

First time the luminescent switch-on probe using novel water-soluble cyclometallated iridium complex (Ir-dc) has been developed for sensitive and selective detection of gluten in the presence of several interfering elements. Linear concentration range of gluten is obtained from 5 to 200 $\mu\text{g/mL}$ with a limit of detection 2.6 $\mu\text{g/mL}$. The Ir-dc complex responded to the broad pH range which is advantageous for the detection of gluten in various food samples. Additionally, It has been successfully employed for the detection of gluten in commercial food samples of wheat flour and oats with highest recovery values, indicating applicability of Ir-dc for practical usage.

1. Introduction

Food allergy is a medical condition in which hypersensitivity of the immune system occurs due to the consumption of food with certain allergenic elements [1]. These allergenic food elements are necessary to be detected in our consumed food otherwise it can pose to serious health hazards or clinical implications to sensitized individual. Gluten is one of the constituent which creates allergy to 1–2% of the world population and still many of us are not aware of having an allergy for gluten [2]. The official limits described in the Codex Draft Revise Standard (2000) are 20 ppm (20 $\mu\text{g/mL}$) for foodstuffs naturally gluten-free and 200 ppm (200 $\mu\text{g/mL}$) for foodstuffs rendered gluten-free [3]. The main sources of gluten are wheat, rye, barley and some varieties of oats [4]. For sensitive individuals intake of gluten causes allergic situation resulting in clinical complications and Coeliac disease (CD, an autoimmune disorder) [5]. Gluten contains mostly glutenin and potentially toxic gliadin (about 10%). Gliadin can lead to the gastrointestinal and non gastrointestinal symptoms [6]. Thus only available treatment for CD patients is lifelong adherence to gluten-free diet. Otherwise, untreated patients can develop other chronic or malignant diseases like intestinal lymphoma, esophageal carcinoma, osteoporosis, etc [7,8]. For these reasons, analysis methods to ensure gluten-free diet are very important in reducing mortality and the occurrence of CD-associated Conventional Enzyme-linked immune sorbent assay (ELISA) [9], mass spectrometry (MS) [10] or real-time PCR [11] based detection systems have usually been performed in a centralized lab, requiring considerable time and sample/reagent consumption and expensive detection instruments. ELISA and based on large instruments are costly

and the reaction is time consuming. Alongside high end available analytical spectrophotometric methods or instrumental analysis for detection of a sample of interests require complicated and time consuming sample pre-treatment [12–14], making the search for alternative analytical method for the development of chemosensor with high sensitivity and selectivity is highly desirable. (Scheme 1)

In contrast to other detection methods of gluten, cyclometallated iridium (III) complexes are regarded as excellent phosphorescent chemosensors due to their high photoluminescence efficiencies, relatively long emission lifetimes and excellent colour tuning capability through ligand structural control, hence they have found applications in many different research areas. In particular, they could provide phosphorescent detection of some analytes [15–17]. Among them synthesis of water soluble complex by coordinating appropriate ligand is a crucial task for a synthetic chemist. Some research groups have shown to increase water solubility by embedding carboxyl, sulfonate groups and exchange of counter ions of complexes to increase the water solubility of dyes [18,19]. Previously we have demonstrated such an approach of water solubility for multiple iridium complexes and those have been successfully employed as luminescent sensor for detection of several analytes [20–22]

We report herein the synthesis and application of a novel readily water soluble highly gluten selective luminescent cyclometallated iridium complex (Ir-dc, Fig. 1), $[\text{Ir}(\text{pq-COOH})_2\text{dcbpy}]$ (pq-COOH = 2-phenylquinoline-4-carboxylic acid, dcbpy = 2,2'-Bipyridine-4,4'-dicarboxylic acid) was synthesized by incorporation of carboxyl group on ancillary as well as main ligand (Fig. S1). Our obtained results showed that the complex could detect gluten at $\mu\text{g/mL}$ level with high accuracy.

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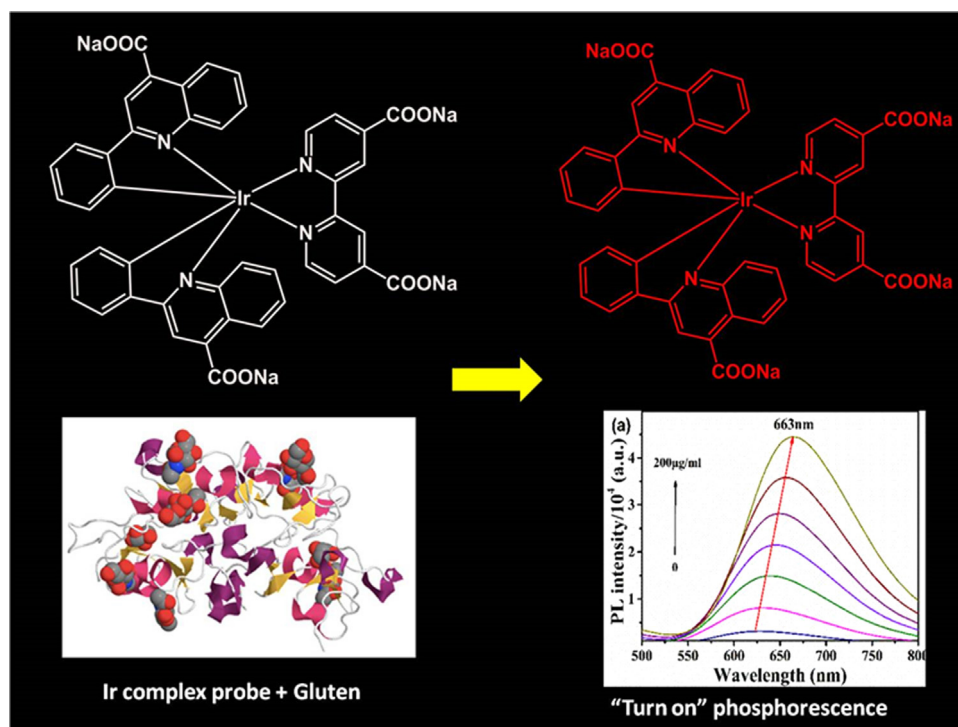
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Scheme 1. Schematic illustration of the strategy for the detection of gluten.

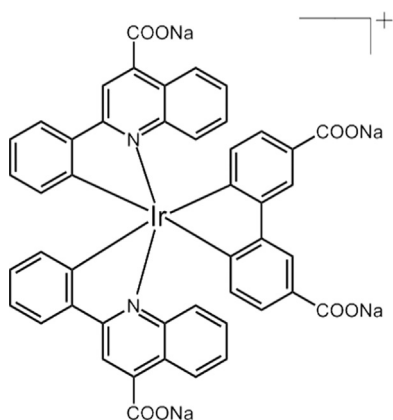


Fig. 1. Chemical structure of cyclometallated iridium (III) complex, Ir-dc.

The chemosensor has also been successfully applied to the commercial wheat and oats samples. To best of our knowledge this is the first ever approach for detection of gluten by any cyclometallated iridium complex.

2. Experimental sections

2.1. Materials and reagent

$\text{IrCl}_3 \cdot 3\text{H}_2\text{O}$, 2-phenylquinoline-4-carboxylic acid (pq-COOH), 2,2'-bipyridine-4,4'-dicarboxylic acid (dcbpy) were purchased from Sigma and used without further purification. Proteins and enzymes (gluten, HSA, BSA, casein, pepsin, lysozyme, ovalbumin), amino acids (L-tryptophan (Try), L-glycine (Gly), L-alanine (Ala), L-proline (Pro), L-phenylalanine (Phe), L-serine (Ser), L-cysteine (Cys), L-glutamic acid (Glu), L-tyrosine (Tro), L-glutamine (Gln), L-leucine (Leu), L-aspartic acid (Asp), L-lysine (Lys), L-histidine (His), L-valine (val), L-isoleucine (ile), L-arginine (Arg), glutathione (GSH), glutathiol (GSSG) and protein unfolding agents (urea, guanidine hydrochloride (GdHCl) were

purchased from Shanghai Chemical Company. Common inorganic ions solution were prepared by dissolving either nitrate or sodium salts in deionized water (18.2 MΩ cm), generated from a Millipore Milli-Q water purification system. This Milli-Q water was used in entire study. Britton-Robinson buffer solution (BR, 0.04 M) was used to maintain the pH of all testing sample solutions. Wheat flour, Quaker oats and gluten-free wheat flour samples were purchased from a local supermarket (Wuxi, China).

2.2. Apparatus

^1H -NMR spectra were measured with a Bruker Avance 400 MHz spectrometer using TMS as internal standard. ESI-MS spectra were measured on a Bruker MicroTOF instrument with electrospray ionization (ESI) mode of detection. Phosphorescence spectra and intensities were recorded on Edinburgh Instruments' FS5 spectrofluorimeter equipped with a xenon discharge lamp using 1 cm quartz cells. Phosphorescence lifetime measurements were performed with Steady State and Transient State Fluorescence Spectrometer (FluoroLog 3-22-TCSPC, Horiba). All pH measurements were carried out using PHS-3D pH meter (Shanghai Precision Scientific Instruments Co. Ltd., China). UV-vis absorption spectral measurements were carried out on Shimadzu UV-2700 spectrophotometer.

2.3. Quantum yield measurement

Optically dilute method was used to calculate luminescence quantum yield (QY) at room temperature with an aerated aqueous solution of $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$ (QY = 0.028) as the standard solution. The QY of the synthesized complex was calculated from Eq. (1):

$$\varphi_f^i = \varphi_s^i \times F_s^i n_i^2 / F_f^i n_s^2 \quad (1)$$

Where φ_f^i and φ_s^i are the QY of the sample and that of the standard respectively, the subscript f is used because in most cases one is dealing with phosphorescence. F^i and F^s are the integrated area of sample and standard spectra, respectively. f_x is the absorption factor the refractive indices of the sample and reference solution are and n_i and n_s are the

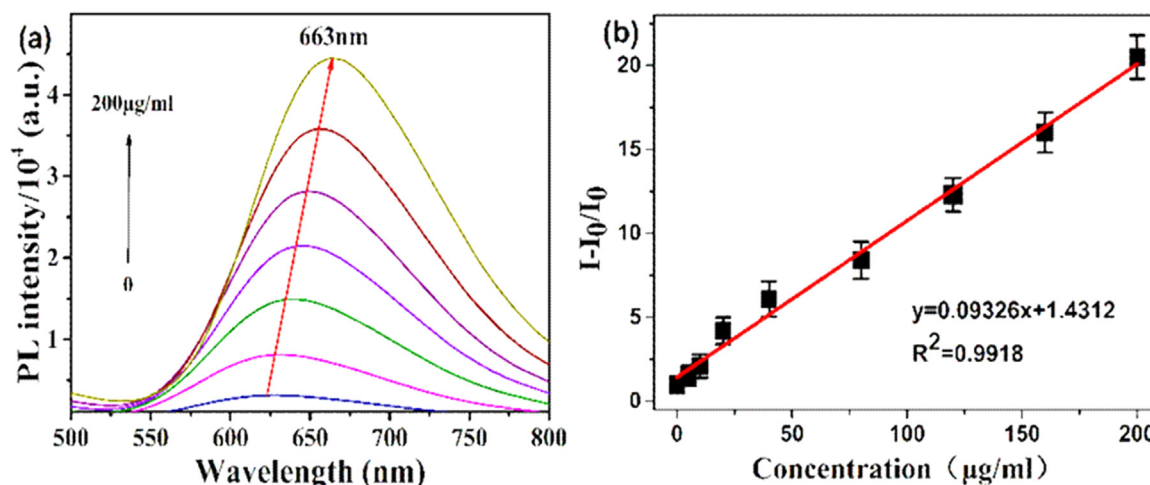


Fig. 2. (a) The phosphorescent spectra of 10 μM Ir-dc in the presence of different concentration of gluten (5–200 μg/mL) in PBS solution at pH = 7.0. (b) The linear relationship of the phosphorescence intensity with gluten concentration of 5–200 μg/mL (Error bars, SD, n = 3), λ_{exc} = 345 nm.

refractive indices of the sample and reference solutions respectively [23].

2.4. Synthesis of complex Ir-dc

The synthesis of Ir-dc was carried out as illustrated in Fig. S1. In a typical reaction a mixture of water and 2-ethoxyethanol (1:3, v/v) was added to a flask containing $\text{IrCl}_3 \cdot 3\text{H}_2\text{O}$ (1.0 mmol, 298 mg) and pq-COOH (2.2 mmol, 548 mg) and then reaction solution was refluxed for 24 h. After cooling the reaction mixture, the obtained solid precipitate was filtered and washed to give the crude iridium (III) chlorobridged dimer $[\text{Ir}(\text{pq-COOH})_2(\mu\text{Cl})]_2$ as an intermediate. The solution of $[\text{Ir}(\text{pq-COOH})_2(\mu\text{Cl})]_2$ (1.0 eq) and dcbpy (2.2 eq) in MeOH was heated to reflux for 8 h under N_2 . The solution was evaporated to dryness under reduced pressure and purified via a silica column with MeCN/EtOH (1:2) as the eluent. Orange coloured solid was obtained as a final product (Ir-dc complex) after drying in the vacuum overnight (yield: 20%).

3. Results and discussion

3.1. Characterization of complex Ir-dc

The novel water-soluble iridium complex was synthesized by incorporation of four carboxyl groups in the main and ancillary ligand simultaneously to increase water solubility. The synthetic route is shown in Fig. S1. The structure of the designed complex was confirmed by MS, ^1H NMR spectroscopy (Fig. S2). MS (ESI-TOF) [m/z]: Calc. for $\text{C}_{45}\text{H}_{28}\text{IrN}_4\text{O}_8$: 945.99. Found: 945.89. As expected the obtained iridium complex, Ir-dc exhibits good water solubility, a concentration up to 10 mM can be readily dissolved in aqueous solution, which would be especially suitable for biological applications and real samples. Our laboratory continuously has focused on the novel luminescent and electrochemiluminescent materials and their unique application as sensor [24–27].

The absorption spectrum and the phosphorescence emission spectrum of Ir-dc in PBS solution were measured (pH = 7.0), which bears the weak emission intensity of Ir-dc in PBS solution (pH = 7.0) (Fig. S3). The phosphorescence emission appears at 634 nm and excitation appears at 345 nm at room temperature with a quantum yield of emission is 0.19. The measured excited-state lifetime for Ir-dc is 840 ns, confirming the phosphorescence nature of light emission. Hence, Ir-dc opens a new possibility for the construction of a sensitive “turn on” phosphorescence probe for detection.

3.2. Detection of gluten

Gluten is the dough-forming protein fraction of the wheat flour; gluten plays multiple critical functions in many biological processes. Gluten contains hundreds of protein components which are present either as monomers or linked by inter chain disulphide bonds, as oligo- and polymers [28]. They are unique in terms of their amino acid compositions, which are characterized by high contents of glutamine and proline and by low contents of amino acids with charged side groups. The molecular weights (MWs) of native proteins range from around 30,000 to more than 10 million. Therefore without going into structural modification and folding of gluten protein while its detection we mostly focused on direct application of gluten detection by developed selective phosphorescence probe.

Hence, the assay of gluten is of great significance in clinical applications. Based on the above phenomenon, a “turn on” type phosphorescence probe was developed for the detection of gluten. As shown in Fig. 2, the emission intensities increased linearly with the increase of gluten concentration in the range of 5–200 μg/mL with a limit of detection as low as 2.6 μg/mL based on the definition by IUPAC ($\text{CDL} = 3 \text{ Sd}/m$, where Sd is the standard deviation of the blank solution and m is the slope of the calibration curve). The red shift in the fluorescence spectra upon addition of increasing gluten concentration is attributed to the microenvironmental changes in the solution surrounding Ir-dc and possible interaction of Ir-dc complex with the gluten. Compared to this and other sandwich assays [29], our approach presented here relies on the direct detection of gluten, eliminating the necessity of enzymatic amplification, which brings improvement in analysis time and costs.

In order to emphasize the effect of pH on the emission intensity of the Ir-dc complex we have performed pH titration as a function of emission intensity. Our observation showed that the pH has less influence on the overall “turn-on” phenomenon of Ir-dc by presence of gluten. The detection of gluten by Ir-dc complex approach is disposable, upon one time detection of the gluten the probe has to replace it with the fresh Ir-dc complex for the next measurement.

3.3. Interference study

Interference experiments were also performed to rule out the influence of foreign substances including common inorganic ions and amino acids on the phosphorescence intensity of Ir-dc-gluten (Fig. 3). It was found that up to 100-fold above mentioned species have no significant effect on the determination of gluten, proving that Ir-dc could be used as a gluten-selective optical chemosensor.

To validate ubiquity of above mentioned specific recognition, the

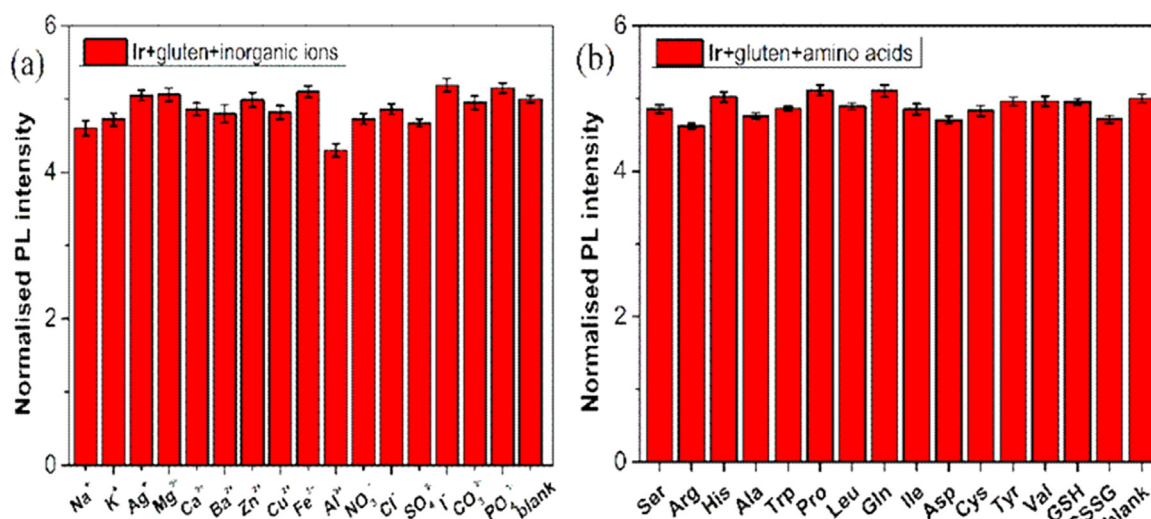


Fig. 3. Phosphorescence responses of Ir to gluten (blank, 10 μ M) in the presence of (a) different metal ions: Na⁺, K⁺, Ag⁺, Zn²⁺, Mg²⁺, Ca²⁺, Ba²⁺, Al³⁺, Cl⁻, I⁻, NO₃⁻, SO₄²⁻, CO₃²⁻, PO₄³⁻ (10 mM), Cu²⁺, Fe³⁺ (1 mM) and (b) amino acids (10 mM). Amino acids including Ser, Arg, His, Ala, Trp, Pro, Leu, Gln, Ile, Asp, Cys, Tyr, Val, GSH, GSSG. λ_{ex} = 345 nm (Error bars, SD, n = 3).

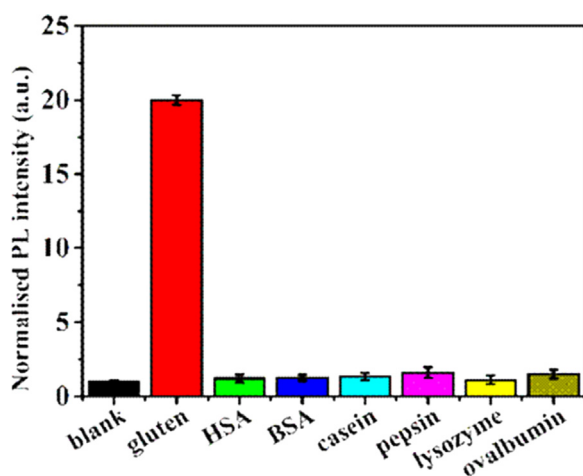


Fig. 4. Comparison of the phosphorescence intensity of Ir-dc (10 μ M) in the presence of other proteins (1 mg/mL). Excitation is performed at 345 nm.

phosphorescence responses of Ir-dc to proteins including BSA, HSA, ovalbumin, lysozyme, pepsin and casein were also compared. As demonstrated in Fig. 4, these phosphorescence probe only have response to gluten not other proteins, maybe can attributed to its special folding structure and network [30]. The covalent structure of the gluten network is superimposed by non-covalent bonds (hydrogen bonds, ionic bonds, hydrophobic bonds) [28]. Compared to other hydrophobic proteins (like HSA/BSA are basically hydrophilic with several hydrophobic pockets inside), the surface of gluten is mostly composed by hydrophobic amino acids, which can more easily interact with Ir-dc by hydrophobic interaction.

3.4. Real samples detection

Our method can be more easily performed and has improved accuracy when applied to real samples. Our method was also applied for detection of gluten in Sample-A (gluten containing wheat flour) and Sample-B (oats). The 1% (v/w) solutions of Sample-A and Sample-B were prepared in PBS (pH 4.80). The required amount of gluten

Table 1

Concentration and Recovery values of gluten from gluten-containing and gluten-free wheat flour using Ir-dc phosphorescence “turn on” probe. In all cases, n = 3 replicate measurements were performed.

Phosphorescence “turn on” probe			
Spiking (μ g/mL)	Final (μ g/mL)	Found concentration (μ g/mL)	Recovery %
Sample-A gluten-containing wheat flour			
0	51.5	51.5 $\pm$ 2.2	N/A
15	66.5	66.0 $\pm$ 2.0	99.2
30	81.5	81.1 $\pm$ 1.8	99.6
45	96.5	97.5 $\pm$ 2.8	101.0
Sample-B oats			
0	19.1	19.1 $\pm$ 2.2	N/A
10	29.1	28.6 $\pm$ 2.1	98.3
20	39.1	39.9 $\pm$ 1.8	102.1
30	49.1	50.1 $\pm$ 2.6	102.0
Sample-C gluten-free wheat flour			
0	1.6	1.6 $\pm$ 0.4	N/A
5	6.6	6.4 $\pm$ 0.4	97.0
10	11.6	11.4 $\pm$ 0.3	98.3
15	16.6	16.8 $\pm$ 0.5	101.2

standard in different concentrations was spiked into the solution of real food sample, and then the gluten was analysed under the same conditions. The phosphorescence signal of solution of control sample prepared by PBS (pH 7.4) was also checked at each gluten concentration. Results from the analysis of wheat flour and oats samples are reported in Table S1, the table showed excellent results using phosphorescence “turn on” probe Ir-dc by luminescent approaches, and presented good difference between the different amount gluten in different real food samples.

Additionally, to test the applicability of the developed gluten sensor for the analysis of real food gluten-free samples [31] the phosphorescent assay was tested for sample-C (gluten-free wheat flour) and sample-A (gluten-containing wheat flour) (Table 1). Significant difference on gluten levels were detected in gluten-free and gluten-containing samples proving efficacy of our developed sensor. The results showed the gluten amount of gluten-containing samples were about thirty-two times greater than those of gluten-free samples. In particular, the sensor could distinguish between gluten-free flour and gluten-containing wheat flour, making the sensor well adapted for gluten sensing.

Table 2
Comparison of different methods for gluten detection.

methods	Linear range	Detection limit	Reference
DPV-pencil graphite electrode	20–100 µg/mL	7.11 µg/mL	[32]
DNA aptamers	0.075 – 100 µM	0.5 µg/mL	[33]
Long-Wavelength fluorescence Immunoassay	0.5 – 5 µg/mL	0.8 µg/mL	[34]
Gold nanoparticles-light scattering	1.5–15 µg/mL	0.8 µg/mL	[35]
Electrochemical genosensors	—	0.9 µg/mL	[36]
LC-ESI-MS/MS	2–18 mg/kg	—	[37]
Iridium complex Phosphorescent probe	5–200 µg/mL	2.6 µg/mL	Present work

4. Conclusions

In conclusion, this is a first ever approach for the phosphorescence detection of gluten and successfully performed in real samples as well using novel transition metal Ir-dc complex. The phosphorescent “turn-on” probe in combination with water-soluble cyclometallated iridium complex could show important advantages over the existing methods in the literature such as being easy to use, less time consuming and cheaper detection protocol of gluten in real samples. In comparison to earlier reports on gluten detection protocols, the phosphorescent “turn-on” probe detection of gluten was performed herein more practical way compared to time-consuming methods such as immunoassay, or without using any ligands such as aptamers and DNA probes (Table 2). The linear concentration range of 5–200 µg/mL is obtained with the limit of detection 2.6 µg/mL, which is in accordance with the official limits described in the Codex Draft Revise Standard. This phosphorescent “turn-on” detection protocol provides a fast, inexpensive, direct, sensitive and selective phosphorescent “turn-on” probe determination of gluten without using any external separation, or any expensive biomolecules, like antibody, DNA or aptamers.

Furthermore the practical applicability of Ir-dc gluten probe is proved by detecting content of the gluten in the commercial food samples like wheat flour and oats with highest recoveries.

As bottom-line, our initiated approach could be developed further to evaluate the purity of gluten-free products especially to ensure a safe diet for allergic patients.

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Conflicts of interest

There are no conflicts to declare.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2018.08.013.

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