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Core-shell-structured molecularly imprinted polymer on upconverting nanoparticles for selective and sensitive fluorescent sensing of sulfamethazine

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A core-shell structured molecularly imprinted polymer on upconverting nanoparticles (UCNPs@MIP) was synthesized for the fluorescent (FL) sensing of sulfamethazine (SMZ). Hexagonal UCNPs were synthesized by the solvothermal method, then coated with a thin silica shell and modified with vinyl groups. Finally, surface polymerization was initiated among the vinyl groups, the functional monomers and cross-linking agents by the initiator. The MIP synthesized by this procedure was anchored on the surface of UCNPs, possessed better site accessibility and lower transfer resistance for the target molecule compared to bulk imprinted materials. The obtained UCNPs@MIP showed good binding capacity, fast response, high selectivity and specificity to the SMZ. The FL intensity of the UCNPs@MIP decreased sensitively with the increasing concentration of SMZ in the range of 50-700 ng mL^{-1}, the detection limit was 34 ng mL^{-1} (S/N=3). The UCNPs@MIP was successfully applied to the detection of SMZ in chicken samples. Thanks to the unique near-infrared (NIR) excitation nature of UCNPs, the chicken meat only needed some simple extraction procedures before FL detection, no complex purifications were required. The average recoveries ranged from 96.01% to 98.90%, with relative standard deviations (RSDs) below 4.5%. This work offered a novel sensing system that combined the advantages of upconverting nanotechnology and molecularly imprinted technology.

1. Introduction

Sulfonamides are a class of broad-spectrum antibiotics which are widely used not only in human, but also in livestock and aquaculture to protect from infection and to promote growth. [1-4] Their inexpensiveness and high efficiency always lead to overuse, especially in animal cultivation industry. [5] Toxicology has proven that excessive sulfonamide residual in animal origin foodstuffs will lead to antibacterial resistance and even cellular canceration. [6-8] Because of these healthy risks, European Union and China have set regulations that the total residues of all sulfonamide in animal origin foodstuffs must not exceed 100 ng g^{-1}. [9,10] The existing analytical methods for the determination of sulfonamide include capillary electrophoresis, [11,12] high-performance liquid chromatography (HPLC), [13,14] gas chromatography-mass spectrometry (GC-MS), [15] liquid chromatography-mass spectrometry (LC-MS), [16] these conventional methods based on large instruments have the merits of sensitive and accurate, but inevitably very expensive and complicated in pretreatment. Therefore, immunoassay, [17] electrochemical [18] and fluorescent sensors [19] were developed as alternative determination methods which have drawn a lot of attention.

Upconverting nanoparticles (UCNPs) are a class of lanthanide-doped nanocrystals which can convert NIR excitation into visible light through a nonradioactive process. [20-22] UCNPs have become a hot point in recent years due to their out-standing photo-physical properties such as tunable fluorescent wavelength, sharp full width at half maxima, large anti-Stokes shifts, high photostability, considerably low excitation power, deep tissue penetration and little autofluorescent background in biological samples. [23-25] These advantages render UCNPs more promising fluorophores compared to commonly used quantum dots (QD) and organic dyes, especially in the field of biological imaging and optical sensing. [26,27]

Molecularly imprinted polymer (MIP), so called "man-made antibody", were proven to be effective to capture target analyte by selective binding site. [28-30] The MIP have advantages such as stable, recyclable, robust and resistant to harsh chemical conditions. [31,32] Recently, some sulfonamides templated MIP have been synthesized. Gao et al. [33] reported a core-shell-shell imprinted polymer based on silica-coated magnetite as solid-phase extraction sorbent, to combine with LC-MS/MS for the determination sulfonamide in milk samples. Kong et al. [34] reported a core–shell magnetic molecularly imprinted polymer for separation and enrichment of sulfamethazine in poultry feed samples. Here, we studied the fluorescent sensing of sulfamethazine based on UCNPs and MIP. In the sensing system, the MIP were anchored on the surface of fluorescent core, possessing more favorable binding kinetics compared to those prepared from bulk polymerizations. The UCNPs@MIP was proven to detect SMZ in acetonitrile with high selectivity. The sensor was further applied to the determination of SMZ in chicken meat with simple pretreatment.

2. Experimental

2.1 Chemicals.

$YCl_3 \cdot 6H_2O$ (99.99%), $YbCl_3 \cdot 6H_2O$ (99.99%), $ErCl_3 \cdot 6H_2O$ (99.9%), 1-octadecylene (ODE), NaOH, Na_2SO_4, NH_4F were purchased from Xiya Co., Ltd. Ethanol, methanol, oleic acid (OA), cyclohexane, ammonia (25%), acetonitrile, toluene, acetic acid (HAc), isopropanol were from Tianjin Fuyu Co., Ltd. IGEPAL CO-520, Tetraethoxysilane (TEOS) were from Sigma Aldrich. Methylacrylic acid (MAA) was form Acros. [3-(methacryloyloxy)propyl]trimethoxysilane (MPS), ethylene glycol dimethacrylate (EGDMA), 2,2'-azobisisobutyronitrile (AIBN), sulfamethazine (SMZ), sulfadiazine (SDZ), sulfamethoxazole (SMO), sulfadimethoxine (SDM), sulfathiazole (STZ) were purchased from J&K chemical Ltd.

2.2 Instruments

The morphologies of the sensing materials were examined by a TECNAI $G^2$20 S-TWIN Transmission Electron Microscope at an acceleration voltage of 20-200 kV. Fourier transform infrared spectra were measured on a FTS6000 spectrometer (Bio-rad, America) with the KBr pellet technique. The UV-Vis absorption spectra were recorded on a TU-1901 UV-Vis spectrophotometer (Beijing Purkinje General Instrument Co., Ltd.). The upconversion fluorescent spectra and intensities were recorded on an F-4500 spectrophotometer (Hitachi, Japan) equipped with an external 980 nm diode continuous-wave (CW) laser (Beijing Hi-Tech Optoelectronics Co., Ltd.). The excitation power was 230 mW, the excitation and emission silt were fixed at 1 nm. The emission intensity at 541 nm was taken for quantification throughout the work.

2.3 Preparation of vinyl modified UCNPs

2.3.1 Synthesis of OA-UCNPs

The hexagonal $NaYF_4$: Yb^{3+}, Er^{3+} was synthesized by the solvothermal method according to Liu et al. [35] $YCl_3 \cdot 7H_2O$ (0.78 mmol), $YbCl_3 \cdot 7H_20$ (0.20 mmol), $ErCl_3 \cdot 7H_2O$ (0.02 mmol) were mixed with 9 mL oleic acid and 15 mL ODE in a 50 mL three-necked flask. The solution was heated to 120 ℃ under vacuum for 1 h to form a homogenous yellow solution, then cooled down to room temperature. 10 mL of methanol solution containing NaOH (2.5 mmol) and NH_4F (4 mmol) were slowly dropped to the flask, followed by stirring at 30 ℃ for 1.5 h. Subsequently, the solution was heated to 120 ℃ with condensation pipes to remove methanol. Then the solution was quickly heated to 320 ℃ under nitrogen atmosphere for 1.5 h. After cooling, the OA-UCNPs were obtained by centrifugation and washed with ethanol for three times.

2.3.2 Synthesis of UCNPs@SiO2 and the modification with vinyl groups.

70 mg UCNPs were dispersed in 80 mL cyclohexane, 4 mL IGEPAL CO-520 were added with sonication to form reversed micro-emulsion, then 640 μL $NH_3 \cdot H_2O$ and 80 μL TEOS were dropped respectively. The solution was stirred vigorously for 3 h in dark. UCNPs@SiO_2 were obtained by centrifugation and washed with ethanol. Procedures to graft vinyl groups on the UCNPs@SiO_2: 100 mg UCNPs@SiO_2 were dispersed in 100 mL ethanol, then 50 μL acetic acid and 1 mL MPS were added, the solution was refluxed at 60℃ for 6 h.

2.4 Synthesis of core-shell UCNPs@MIP and UCNPs@NIP

SMZ (0.2 mmol) and MAA (1.2 mmol) were mixed with 37 mL acetonitrile and stirred at 30 ℃ for 12 h to form template–monomer complex. 50 mg vinyl modified UCNPs@SiO_2 were dispersed in 13 mL toluene in a 100 mL flask, followed by addition of the template-monomer solution. Then 2 mmol EGDMA and 20 mg AIBN were added to the flask. Then the mixture was purged with N_2 for 30 min, sealed with nitrogen, heated and stirred with reflux, first at 50 ℃ for 4 h, then at 65 ℃ for 24 h, and finally at 85 ℃ for 30 min. At last the products were separated by centrifugation and washed with methanol for three times. NIPs were synthesized by the same procedure in

absence of the template molecular SMZ. The template was removed by wash with methanol: HAc (9:1, v/v), until no template was examined in the supernatant.

2.5 Binding experiment of the UCNPs@MIP and UCNPs@NIP

10 mg UCNPs@MIP or UCNPs@NIP was incubated in 2 mL SMZ acetonitrile solution of different concentrations (10, 20, 40, 60, 80, 100, 120 μg mL^{-1}) and shaken at 25℃ for 3 h. The supernatant was separated by centrifugation, and tested by UV-Vis spectrophotometer to determine the remaining content of SMZ. Three parallel experiments were conducted to get the standard deviation of adsorption at each concentration.

2.6 FL measurement of SMZ

Examination solution was prepared by disperse UCNPs@MIP (UCNPs@NIP) in acetonitrile at a concentration of 0.5 mg mL^{-1}. Then 30 μL of the examine solution was spiked to 2 mL standard SMZ acetonitrile solution at concentrations of 0, 50, 100, 150, 200, 300, 400, 500, 600, 700 ng mL^{-1}. After shaken for 1 h, the fluorescent signal was scanned by the Hitachi F-4500 fluorescence spectrometry equipped with an external 980 nm laser. Each measurement was performed for 3 times and the FL intensity at 541 nm was recorded.

2.7 Selectivity test

SDZ, SMO, SDM and STZ were used as analogues of SMZ. Each analogues with different concentration (0, 300, 500 ng mL^{-1}) was tested using the examination solution of UCNPs@MIP (UCNPs@NIP) by the same analytical procedure mentioned above.

2.8 Detection of SMZ in chicken sample

Chicken meat were purchased from local market. They were cut into pieces and homogenized by a grinder. 5 g of the homogenized sample was accurately weighted in a tube, the spiking amount of SMZ were 40, 120, 240 ng g^{-1}, respectively. Then 20 g Na_2SO_4 and 20 mL acetonitrile were added, the mixture was sonicated and shaken by vortex for 5min. The supernatant was collected and the residues were washed with 20 mL acetonitrile again by the same procedure. The supernatant was combined in a round flask, followed by the addition of 2 mL isopropanol. Afterwards, the extracting solution was evaporated to dryness by a vacuum rotary evaporation at 50 ℃, then re-dissolved by 1 mL acetonitrile and transferred to a PE tube. Another 1 mL acetonitrile was used to wash the round flask and transferred to the PE tube too. The concentration of the SMZ acetonitrile solution in the PE tube was determined by the fluorescence spectrometry.

3 Results and discussion

3.1 Preparation and Characterization of the imprinted UCNPs

The multi-step synthetic procedures were illustrated in Fig.1. An ultrathin silica shell was coated on the UCNPs core by controlling the hydrolysis time of TEOS. According to Wolcott et al., [36] the coating of silica shell on the fluorescent UCNPs

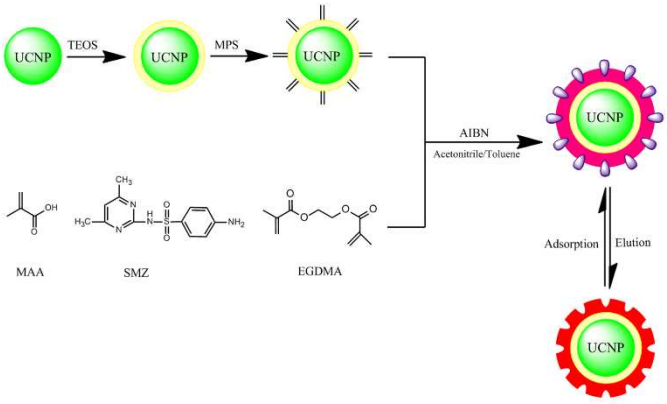

Fig 1. Schematic illustration of the synthetic procedure of UCNPs@MIP

could not only improve the stability and biocompatibility, but also favor their dispersion in aqua solution. Then vinyl groups which could participate in polymerization were subsequently grafted onto the silica shell. Before polymerization, SMZ was prearranged with MAA the monomer in acetonitrile to form template-monomer complex. The intermolecular interaction between SMZ and MAA was confirmed by UV-Vis spectra (Fig S1). The UV absorption peak of SMZ at 217 nm slightly shifted to 225 nm with the increasing concentration of MAA. The red shift of the absorption peak suggested the formation of hydrogen bond between SMZ and MAA. According to literature, excessive amount of monomer had steric hindrance effect to the formation of template-monomer complex. [37] So we choose SMZ:MAA=1:6 as the optimal ratio for the prearrangement. At last, polymerization between SMZ, MAA, EGDMA was initiated by AIBN in the mixture of acetonitrile and toluene.

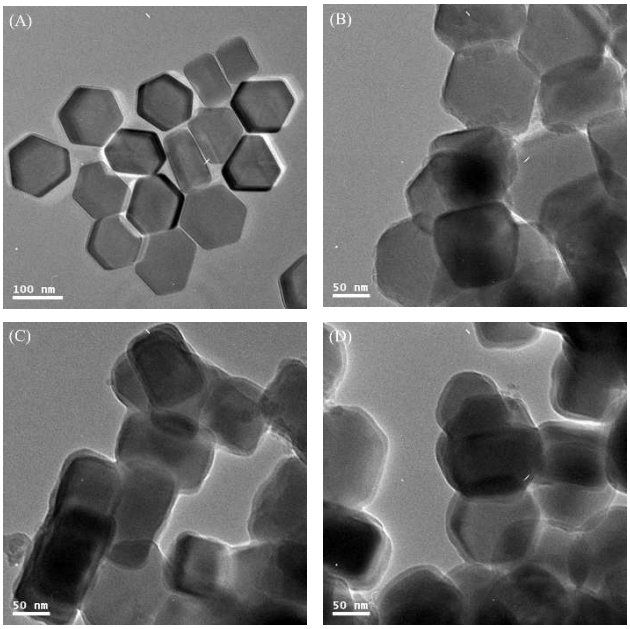

Fig 2. TEM images of the OA-UCNPs in cyclohexane (A), UCNPs@SiO_2 in water (B), UCNPs@MIP (C) and UCNPs@NIP (D) in acetonitrile.

The size and shape of the synthesized materials were characterized by Transmission electron microscope (TEM). As shown in Fig 2A, the UCNPs were hexagonal in shape with a diameter of ca.100 nm. The UCNPs displayed extensive green fluorescence with the excitation of 980 nm laser. The silica shell coated on the UCNPs was very thin as designed, which could only be distinguished by the rough surface of the nanoparticles in Fig 2B. The MIP shell was uniformly coated on the UCNPs@SiO_2 with a thickness of ca. 8 nm (Fig 2C). The morphology of UCNPs@NIP was the similar with that of MIP (Fig.2D). The thin MIP shell have the advantage of completely removal of templates, excellent site accessibility and low mass-transfer resistance, thus enabled the effective quenching of FL emission.[38,39] XRD patterns (Fig S3) show the crystalline structure of the UCNPs was maintained during the modification processes.

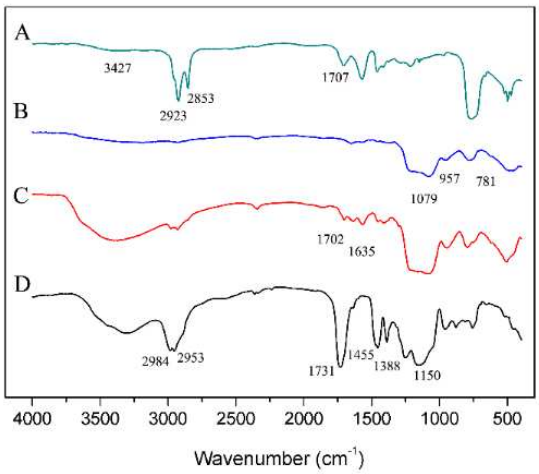

Fig 3.FT-IR spectra of OA-UCNPs (A), UCNPs@SiO_2 (B), vinyl modified-UCNPs@SiO_2 (C) and UCNPs@MIP (D).

FT-IR was used to study the surface modification procedures. The OA-UCNPs exhibited a broad adsorption band at 3427 cm^{-1} which was assigned to the vibration of OH on the carboxyl group. The absorption peaks associated with the asymmetric and symmetric stretching of the -CH_2- in the alkyl chain could be found at 2923 cm^{-1} and 2853cm^{-1}, respectively. In addition, the absorption peak at 1707 cm^{-1} was attributed to the vibration of C=O group (Fig 3A). After the modification of silica shell, a broad band in the region around 1079 cm^{-1} (Fig b-d) could be distinguished, which was assigned to the symmetric stretching vibration of Si-O bond. In addition, Si-O bending vibration peak at 781 cm^{-1} and Si-O-H vibration peak at 957 cm^{-1} further indicated the UCNPs were indeed coated with silica. In Fig 3C, the characteristic peak at 1702 cm^{-1} and 1635 cm^{-1} represented the stretching vibration of C=O and C=C groups of the MPS, respectively. The curve D in Fig 3 contained many characteristic absorption peaks related to MIP shell. The bands at 2953 cm^{-1} and 2984 cm^{-1} were associated to the vibrations of CH_3 and CH_2 groups in EGDMA , the strong absorption at 1731 cm^{-1} and 1150 cm^{-1} were assigned to the stretching vibration of C=O and C-O-C, the peaks at 1388 cm^{-1} and 1455 cm^{-1} were assigned to the bending vibration of C–H from methyl groups. On the other hand, the TGA curves (Fig S4) further indicated the coating of MIP. The UCNPs@MIP had a rapid weight loss (19%) from 300 ℃-600 ℃, which could be attributed to the decomposition of the organic MIP.

3.2 Binding properties of the imprinted UCNPs

In order to evaluate the binding capacity of the imprinted polymer, the static adsorption experiments were conducted. Acetonitrile was chosen as the working solvent throughout the adsorption experiment. 10 mg UCNPs@MIP or UCNPs@NIP were added into SMZ of different concentrations (from 10 to 120 μg mL^{-1}), the static adsorption capacity Q (mg g^{-1}) was calculated by the equation

$$Q=(C_0-C)V/W$$

where C_0 and C were the concentration (μg mL^{-1}) of the SMZ in the supernatant before and after adsorption for 3h, respectively. V (L) was the volume of tested solution and W (g) was the weight of the absorbent used in each concentration. As shown in Fig 4A, the adsorption capacity of the UCNPs@MIP and UCNPs@NIP increased along with increasing the SMZ concentration when below 120 μg mL^{-1}. When the concentration of SMZ was120 μg mL^{-1}, the maximum adsorptions were obtained, they are 1.7 mg g^{-1} and 0.5 mg g^{-1} for the MIP and NIP, respectively.

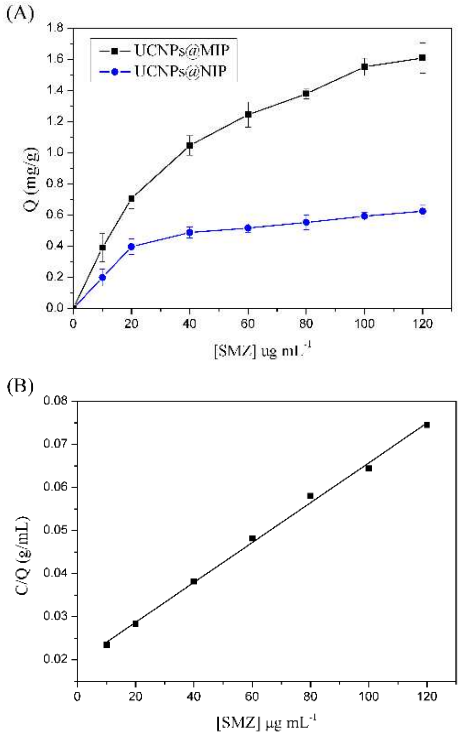

Fig 4. Adsorption isotherms of the UCNPs@MIP and UCNPs@NIP at 25℃ (A); Langmuir plot of UCNPs@MIP towards SMZ (B).

The maximum adsorption capacities decrease slightly during ten circles (Fig S6), which indicating high chemical stability and excellent regeneration properties of the UCNPs@MIP.

The data were further processed with Langmuir adsorption isotherm model. Fig 4B shows the Langmuir plot of UCNPs@MIP towards SMZ. The linear regression equation is:

$$[SMZ]/Q=0.01945+0.00047[SMZ]$$

From the slope of the regression line, the theoretical maximum adsorption capacity (Q_{max}) is 2.1 mg g^{-1}.

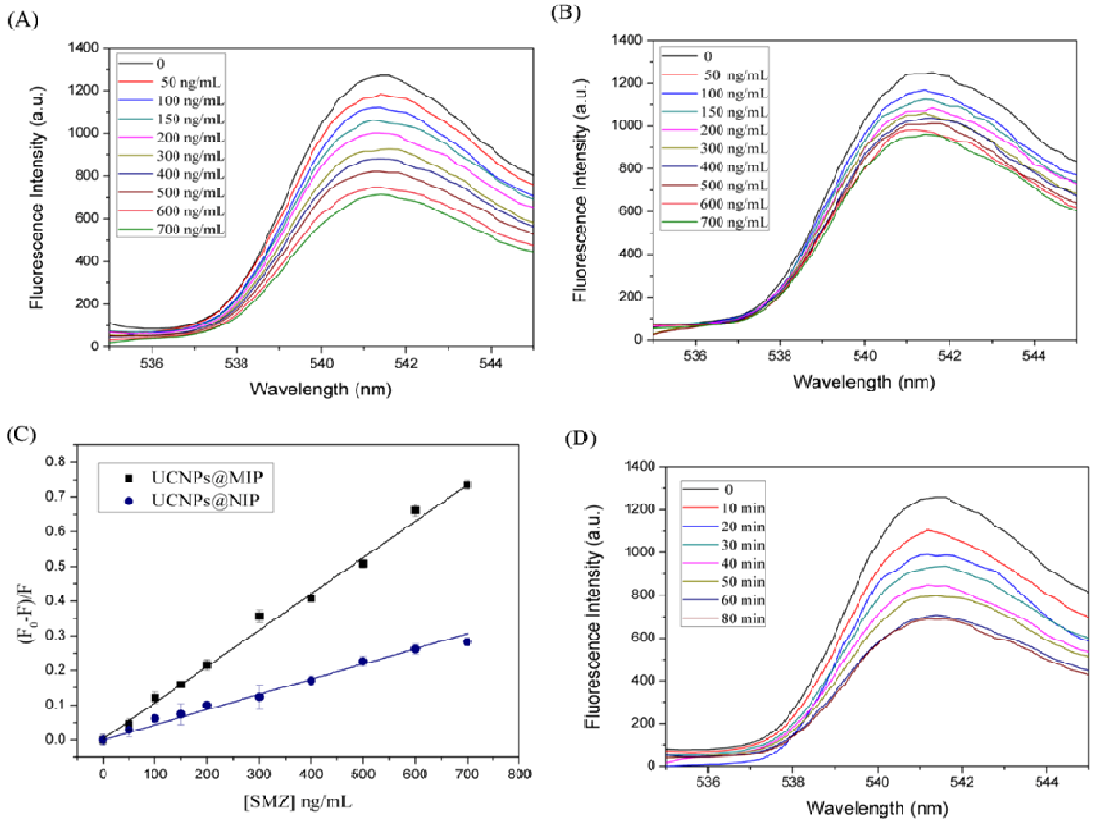

Fig 5. Fluorescence spectra of UCNPs@MIP (A) and UCNPs@NIP (B) at various SMZ concentrations. (C) Stern–Volmer plot of UCNPs@MIP and UCNPs@NIP. (D) Fluorescence spectra of the UCNPs@MIP in the presence of SMZ (1 μg mL^{-1}) after incubating for different times.

3.3 FL measurement of SMZ and the calibration curve.

The FL intensity of the UCNPs@MIP decreased sensitively with the increasing concentration of SMZ in acetonitrile (Fig 5A). The quenching mechanism might be charge transfer from the UCNPs to SMZ. [40,41] As shown in Fig S2, there was no overlap between the characteristic absorption band of SMZ and the FL emission peak of UCNPs (541 nm), so the fluorescence resonance energy transfer (FRET) was excluded. On the other hand, the absorption peak of SMZ was close to the band gap of UCNPs, as shown by the UV-Vis absorption spectra of UCNPs@MIP (Fig S2), and the charges could transfer from UCNPs to the bounded SMZ. The ultra-thin silica shell and the thin MIP shell were helpful to the close proximity of UCNPs and the bounded SMZ, which enabled the effective charge transfer and a maximum quenching. The FL quenching in this system can be described as following:

$$F_0/F=1+K_{sv}[C]$$

where F_0 and F are the FL intensities of UCNPs@MIP in absence and presence of the analyte SMZ, respectively. [C] is the concentration of analyte and K_{sv} is the Stern–Volmer quenching constant. It was shown in Fig 5A and B that both UCNPs@MIP and UCNPs@NIP showed FL response to the analyte SMZ. The linear Stern–Volmer relationships were observed in the concentration range of 50-700 ng mL^{-1}. The linear equation of UCNPs@MIP was

$$F_0/F-1=0.00105[C]+0.00265$$

and the correlation coefficient was $R^2=0.9965$. The detection limit was calculated to be 34 ng mL^{-1} (S/N=3). The ratio of the K_{sv} values of the UCNPs@MIP and UCNPs@NIP (linear slop of in Fig 5C): $K_{sv,MIP}/K_{sv,NIP}$ was defined as imprinting factor (IF), which was used to evaluate the specific

recognition of the materials. The IF of our FL sensor was 2.39, which indicated the successful preparation of molecularly imprinted polymer with high specific recognition. In addition, the incubation time was tested (Fig 5D). The quenching of FL intensity reached equilibrium after incubation for 60 min, indicating the maximum adsorption was obtained within 1 h. The performance of our FL sensor was compared to other previously reported analytical method, and the data were summarized in supporting information (Table S1).

3. 4 Selectivity Study

When target molecules were removed by extraction, imprinted binding cavities were left in the MIP shell which were crucial to the selectively rebinding of the analyte. So control experiments were carried out to the structural analogues: SDZ, SMO, SDM and STZ (structures shown in Fig 6B) to evaluate the selectivity of our FL sensor. Fig 6A depicts the K_{sv} of UCNPs@MIP to SMZ was much higher than that of the structural analogues, because the recognition of template was based on the size, shape, location of hydrogen bond of the binding cavity. The K_{sv} of UCNPs@NIP to SMZ and the analogues were the similar, indicating there was no selective binding cavities in NIP, and the molecules were retained on the NIP shell through a non-specific process.

3.5 Application of the UCNPs@MIP in detection of SMZ in chicken sample.

The as-prepared UCNPs@MIP was further conducted for the detection of SMZ in chicken meat samples. Spiking levels were set at 40, 120, 240 ng g^{-1} (the national standard limitation of SMZ in meat was 100 ng g^{-1}). The extraction and enrichment procedures were conducted according to the National Standards of the People's Republic of China GB/T 20759—2006, except for the omissions of purification procedures. It is well known that the fluorescence of UCNPs was excited by near-infrared (NIR) light, and NIR is proven to be a more suitable stimulating source compared to UV and visible light because the autofluorescence of biosamples aroused by NIR is much lower. So the sensing of meat samples can be carried out only through some simple extraction procedures. The results of recoveries and relative standard deviations (RSDs) are summarized in table 1, the amount of SMZ determined by FL measurement were in good agreement with the spiking levels. All the three spiking levels showed good recoveries from 96.01% to 98.9%, indicating the UCNPs@MIP have potential applicability for the detection of SMZ in meat samples.

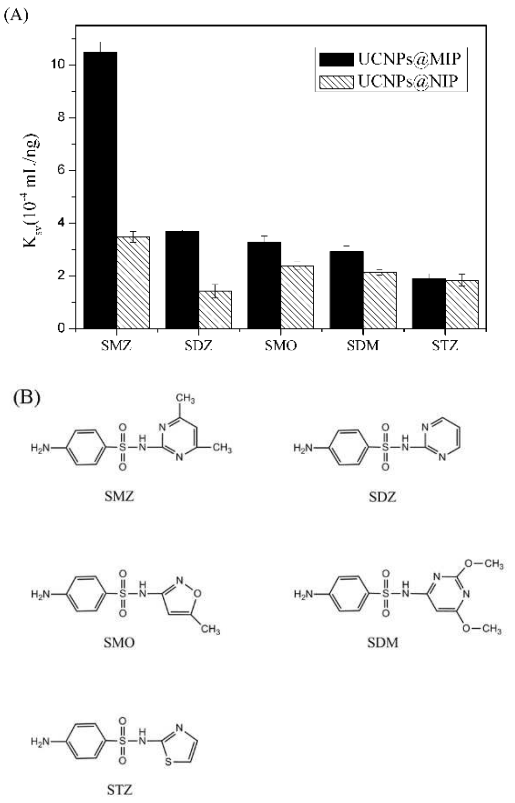

Fig 6. (A) Stern–Volmer quenching constants of UCNPs@MIP and UCNPs@NIP toward the analogues of SMZ (SDZ, SMO, SDM, STZ). (B)The chemical structure of each analogues.

Table 1 Determination of SMZ in chicken samples and the recovery test (n=3).

	Spiked (ng g^{-1})	Measured[a] (ng g^{-1})	Recovery (%)[a]	RSD (n=3)
Chicken Sample	40	39.56	98.9	2.1
	120	117.05	97.5	4.5
	240	230.44	96.01	2.8

[a] Mean values of three measurements.

4. Conclusions

In summary, a core-shell UCNPs@MIP FL sensor was synthesized and investigated for the selective and sensitive detection of SMZ. The sensor was designed to be with uniform morphology, appropriate thickness of MIP shell and stable FL emission. To our knowledge, this is the first study focused on core-shell structured UCNPs@MIP used for the sensing of SMZ. The UCNPs@MIP showed excellent recognition specificity, fast response, wide linear range and a low detection limit. Moreover, the UCNPs@MIP was successfully used for the determination of SMZ in chicken

samples. UCNPs can overcome interferences from bio samples, so no more purifications were needed. The as-prepared sensor for fast FL detection of SMZ would provide promising possibilities for the imprinting and FL sensing of other molecules in future.

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Notes and references

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† Electronic Supplementary Information (ESI) available: UV-vis spectra of SMZ with different amount of MAA, SEM, XRD, TGA characterizations, UV absorption spectra of SMZ and UCNP@MIP, reusability of UCNPs@MIP, method comparisons.

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