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Synthesis of trypsin-protected CsPbCl₃ fluorescent nanocrystals for hydroxyl radical sensing

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Abstract

Water-dispersible perovskite nanocrystals (PNCs) show promising applications in recognizing ionic and molecular species because of their excellent optical properties. However, lead halide PNCs have some limitations when they are used as probes for molecular species sensing in aqueous media. Here, we introduce trypsin (Try) as a bioligand for the synthesis of cesium lead chloride (CsPbCl₃) PNCs with high water stability. The as-fabricated Try-CsPbCl₃ PNCs show $\lambda_{Em/Ex}$ at 433/370 nm with a quantum yield of 17.26%. The fluorescence emission spectral characteristics of Try-CsPbCl₃ PNCs demonstrated that water-stable Try-CsPbCl₃ PNCs acted as a promising fluorescent probe for the detection of hydroxyl radical ($^{\bullet}$ OH) via turn-off mechanism. The Try-CsPbCl₃ PNCs-based turn-off fluorescence approach displayed good selectivity for hydroxyl radical in water, showing a wider linear range (0.01–5 μ M) with a remarkable detection limit of 3.10 nM for hydroxyl radical. The Try-CsPbCl₃ PNCs were demonstrated to be a facile probe for sensing $^{\bullet}$ OH in water samples, which signifies that Try-CsPbCl₃ PNCs exhibited broad applications for hydroxyl radical sensing in real samples.

Keywords Try-CsPbCl₃ PNCs · Hydroxyl radical · Fluorescence spectrometry · HR-TEM

Introduction

In recent years, lead halide perovskite nanocrystals (PNCs) have emerged as outstanding materials in fabricating solar cells, sensors, light-emitting diodes, and optoelectronic devices because of their remarkable characteristics including superior quantum yield (QY), narrow full width at half-maximum, impressive charge transport capabilities, high photocatalytic properties, and tunable emission wavelength in complete visible and near-infrared region [1–5]. Interestingly, it was noticed that perovskite oxides are quite stable in water, whereas lead halide PNCs are highly unstable in water and easily degraded by exposing them to heat, light, and moisture [6–8], resulting in limiting their promising applications in multidisciplinary research.

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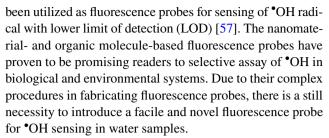
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To improve lead halide PNCs water stability, several researchers have introduced different strategies to fabricate lead halide PNCs including lead chloride PNCs with high water stability. For example, perflorocompounds were used as ligands for the preparation of water-stable CsPbBr₃@Cs₄PbBr₆ PNCs [9]. Furthermore, various polymers (poly(l-lactide) polypropylene glycol, polysulfone [10], NH₂-PEG-COOH [11], NH₂ group terminated hyperbranched polymer [12], polymethyl methacrylate [13], polystyrene [14], polystyrene-cetyltrimethylammonium bromide [15], and polyethylene glycol [16]), organic molecules (ethylenediaminetetraacetic acid [17], bolaamphiphilic ligand (NKE-12) [18], adamantane-1-amine [19], (4,4'-bipyridine and 2,2'-bipyridine) [20], glycyrrhizic acid [21], 4-bromobutyric acid-oleylamine [22], oleylamine-oleic acid [23], and oleic acid-3-bromopropionic acid [24]), and inorganic salts and compounds (cesium trifluoroacetate [25], MAPbBr₃@ lead laurate [26], mesoporous silica [27], Al₂O₃ [28], metal-organic frameworks [29], ZrO₂ [30], and TiO₂ [31]), respectively. The above approaches successfully produced high stability of lead halide PNCs without losing their fluorescence properties, suggesting encapsulation of lead halide PNCs with suitable ligand chemistry offers several



features such as water stability, good QY, and superior optical properties. Furthermore, it is a very challenging task to design water- and air-stable lead chloride PNCs without the use of complicated synthetic approaches as well as ligand chemistry. Since hydroxyl radical is selectively damaged, the protein structures include trypsin [32]. Furthermore, free radicals including hydroxyl radicals were effectively inactivated trypsin by the oxidation of trypsin residues to N-formylkynurenine and other residues [33]. In order to alter the surface chemistry and fluorescence properties of CsPbCl₃ PNCs, trypsin (Try) was explored as a bioligand for the preparation of water-stable CsPbCl₃ PNCs in the sensing of hydroxyl radical.

Reactive oxygen species (ROS) are produced by mitochondria in the cells, exhibiting a highly reactive nature [34]. The ROS (oxygen-containing radicals-superoxide, peroxyl, hydroxyl (${}^{\bullet}OH$), and hydroperoxyl) and non-radical agents, ozone, HOCl, and H₂O₂, which are easily converted into ROS, play a key role in numerous biochemical pathways in the cells [35]. It was observed that certain levels of ROS efficiently enhance cellular functions (migration, proliferation, and differentiation) [36]. However, ROS have potentially induced oxidative stress and cell damage, which yield cell death [37]. Among ROS, hydroxyl radical is recognized as one of the highly reactive ROS, exhibiting a lifetime in the nanoseconds [38, 39]. Usually, hydroxyl radical is produced in in vivo via oxygen molecule oxidation to superoxide, higher levels of OH cause oxidative damage to various bio-macromolecules (nucleic acids, lipids, carbohydrates, and proteins), demonstrating that significant attention must be paid to monitoring of OH in cells and various environments. Importantly, OH plays a key role in initiating numerous free radical-induced reactions (oxidation of many organic and inorganic chemical species via electron transfer reactions and addition reactions), favoring to boost-up advanced oxidation process, which improves the removal of organic and inorganic pollutants from environmental water and wastewater treatment plants [40, 41]. It was also observed that the levels of OH can enhance the biotreatability of agro- and pharmaceutical and industrial wastewaters, thereby minimizing concentrations of toxic organic and inorganic species via degradation process [42, 43]. These reports illustrated that OH is widely present in aquatic regions, exhibiting an essential role in biochemical cycles. In order to identify OH radical, several analytical techniques such as electrochemical, electron spin resonance, fluorescence, and UV-visible spectroscopic and high-performance liquid chromatographic techniques have been applied to detect ROS including OH radical [44–52]. Importantly, several fluorescent probes including terbium complexes [38], Coumarin-Neutral Red [47], copper and molybdenum nanoclusters [53, 54], Ag-Au nanocages [55], carbon dot-based hydrogel [56], and dihydroquinolines have



In this work, we report a simple and selective hydroxyl radical (*OH) sensing strategy using aqueous-stable trypsin (Try) encapsulated cesium lead chloride (CsPbCl₃) PNCs as a nanoprobe (Scheme 1). The as-synthesized Try-CsPbCl₃ PNCs were stable in aqueous medium and displayed spherical shape morphology with a mean size of 2.5 ± 0.5 nm. Further, Try-CsPbCl₃ PNCs exhibited blue fluorescence under UV lamp (365 nm), showing $\lambda_{Em/Ex}$ at 433/370 nm, which offers a QY of 17.26%. Noticeably, the emission intensity of Try-CsPbCl₃ PNCs was quenched by hydroxyl radical, leading to the development of a fluorescence turnoff approach for OH assay with an LOD of 3.10 nM. The developed sensing strategy was used to detect OH in water samples, demonstrating Try-CsPbCl₃ PNCs-based fluorescence method could be an effective tool for monitoring OH in real samples.

Experimental section

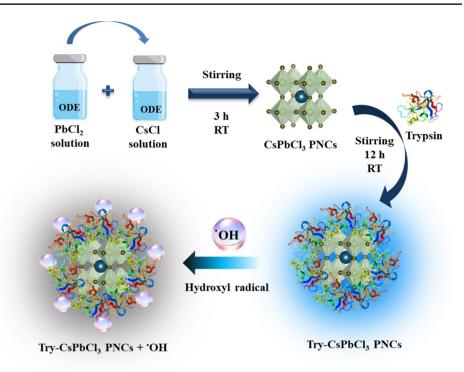
Materials and instruments

Lead (II) chloride (PbCl₂, 98%) and cesium chloride (CsCl, 99.99%) were produced from SRL and BLD pharm, respectively. Trypsin was obtained from Enzyme Bioscience Pvt. Ltd. (Surat, Gujarat, India) as a gift sample. 1-Octadecene (ODE) and potassium superoxide (KO₂) were obtained from Sigma-Aldrich (St. Louis, MO, USA); N-bromo succinimide (NBS), sodium hypochlorite (NaOCl), and hydrogen peroxide (H₂O₂) were purchased from SRL, FINAR, and SDFCL Chemicals, respectively. Deionized water was used for the preparation of solutions and sensing experiments, and analytical-grade chemicals were utilized without any further purification.

The as-prepared Try-CsPbCl₃ PNCs size and morphology were investigated using field emission transmission electron microscopy (FETEM) (JEOL-200, Tokyo, Japan). Fluorescence (emission and excitation) spectra of Try-CsPbCl₃ PNCs were examined by a Cary Eclipse fluorescence spectrometer (Agilent Technologies, Santa Clara, CA, USA). UV/Vis absorption spectra were recorded with a Maya Pro 2000 spectrophotometer (Ocean Optics, Orlando, FL, USA). Infrared spectra of Try-CsPbCl₃ PNCs were examined with an ALPHA II Fourier transform infrared (FT-IR) spectrometer (Bruker,



Scheme 1 Schematic illustration for Try-CsPbCl₃ PNCs synthesis and sensing of OH



Billerica, MA, USA). X-ray diffraction (XRD) spectrum of Try-CsPbCl₃ PNCs was recorded using a D8-Advance Instrument (Bruker AXS). X-ray photoelectron spectroscopy (XPS) (K-alpha+, Thermo Fisher Scientific, Waltham, MA, USA) was performed for the confirmation of the elemental composition of Try-CsPbCl₃ PNCs. Hydrodynamic diameter and zeta potentials of Try-CsPbCl₃ PNCs were obtained using a NanoZS90 nanoparticle size potential analyzer (Malvern, UK).

Synthesis of try-CsPbCl₃ PNCs

Blue fluorescence water-stable CsPbCl₃ PNCs were prepared with a slight modification [58, 59] using Try as a ligand via a simple reaction. Firstly, 100 mM (33.6 mg) of PbCl₂ and 100 mM (55.6 mg) of CsCl precursors were dispersed in 2 mL of ODE separately and stirred for 30 min at room temperature. Then, the preparation of CsPbCl₃ PNCs was initiated by mixing both solutions in 10 mL of reaction flask. The mixture was stirred at room temperature for 3 h. The formed CsPbCl₃ PNCs were capped with Try by adding Try (32 mg) into CsPbCl₃ solution (4.0 mL) and then stirred for 12 h, triggering the formation of blue fluorescent Try-CsPbCl₃ PNCs. The formed Try-CsPbCl₃ PNCs were washed with hexane and then dispersed in water for sensing applications.

Fluorescence sensing of 'OH using try-CsPbCl₃ PNCs as a turn-off probe

In the sensing study, the following reactive species solutions were prepared as follows, hydroxyl radical (OH) solution was prepared via the Fenton reaction (OH and OH were produced by the reaction between Fe^{2+} and H_2O_2 , superoxide anion (${}^{\bullet}O_{\overline{2}}$) was prepared by using 0.711 mg of KO_2 in 10 mL DMSO, and other species (MnO_4^- , $Cr_2O_7^{2-}$, HPO₄²⁻, S₂O₈²⁻, and NBS) were generated by dissolving their salts in water. For OH sensing, the as-prepared Try-CsPbCl₃ PNCs were used to detect OH radical, and the fluorescence spectra of Try-CsPbCl₃ PNCs (1 mL, 2.45 mg/ mL) were investigated with different concentrations of OH radical (0.5 mL). Briefly, 1.0 mM of OH radical was generated by mixing Fe^{2+} ion (1 mM) with H_2O_2 (10%) at a volume ratio of 1:1. Different concentrations of *OH radical (0.01-500 µM, 0.5 mL) were treated separately with 1 mL of Try-CsPbCl₃ PNCs (2.45 mg/mL), and then their emission spectra were recorded, leading to establish good calibration graph between the ratio of I_0/I at 433 nm and concentrations of OH radical. To ensure the selectivity of Try-CsPbCl₃ PNCs, different chemical species (MnO₄⁻, $\operatorname{Cr_2O_7^{2-}}$, $\operatorname{HPO_4^{2-}}$, $\operatorname{S_2O_8^{2-}}$, $\operatorname{O_2^{\bullet-}}$, and NBS) were added separately into Try-CsPbCl₃ PNCs solutions and examined their impact on the emission spectral intensities of Try-CsPbCl₃ PNCs. The fluorescence emission spectra of Try-CsPbCl₃



217 Page 4 of 11 Microchim Acta (2025) 192:217

PNCs were recorded with λ_{Ex} at 370 nm for ${}^{\bullet}OH$ radical sensing and selectivity tests. The selectivity of Try-CsPbCl₃ PNCs toward ${}^{\bullet}OH$ radical was evaluated by investigating the emission spectra of Try-CsPbCl₃ PNCs in the presence of various biomolecules (cysteine, arginine, tryptophan, and alanine, 500 μ M), cations (Na⁺, Ca²⁺, Mg²⁺, Zn²⁺, Cu²⁺, and Fe³⁺, 500 μ M), and anions (Cl⁻, I⁻, Br⁻, PO₄³⁻, and SO₄²⁻, 500 μ M) with and without addition of ${}^{\bullet}OH$ radical.

Fluorescence detection of 'OH radical in water samples

To apply potential application of the probe, tap and river waters from Tapi River, Surat, Gujarat, India, were used in the present study. The water samples were filtered through a microfilter and then treated the sample with different concentrations (0.01, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, and 25.0 μM) of OH radical and then introduced 1 mL of Try-CsPbCl₃ PNCs and vortexed for 2 min. The fluorescence emission intensities of Try-CsPbCl₃ PNCs at 433 nm were examined, and the spectral studies were repeated three times and represented the statistical data as mean ± relative standard deviation (RSD).

Results and discussion

Synthesis and characterization of Try-CsPbCl₃ PNCs

The synthesis pathway for the preparation of Try-CsPbCl₃ PNCs and their application for OH radical sensing in aqueous medium were shown in Scheme 1. Initially, the influence of Try concentration (2-10 mg/mL) was studied on the fluorescence spectra of CsPbCl₃ PNCs using PbCl₂ (100 mM) and CsCl (100 mM) as precursors (Figure S1). Upon increasing Try concentration from 2.0 to 8.0 mg/mL, the intensity of fluorescence emission spectra of CsPbCl₃ PNCs was increased; however, the emission intensity was decreased by using 10 mg/mL of Try due to the excessive Try molecules on Try functionalized CsPbCl₃ PNCs, which leads to form Try-CsPbCl₃ PNCs nanoaggregates that will result to quench the emission peak of CsPbCl₃ PNCs. These results suggest that 8.0 mg/mL of Try effectively enveloped CsPbCl₃ PNCs, thereby improving their dispersion ability in water with good fluorescence intensity. Similarly, we also investigated the optimum reaction time for the preparation of Try-CsPbCl₃ PNCs (Figure S2). It was clearly observed that the emission intensity of Try-CsPbCl₃ PNCs at 433 nm was increased with increasing reaction time from 3 to 12 h; after that, the emission peak intensity was decreased, confirming the 12 h was found to optimum reaction time for the fabrication of Try-CsPbCl₃ PNCs. In order to confirm the origin of emission spectra, absorption and emission spectra of Try and Try-CsPbCl₃ PNCs were examined and shown in Figure S3. The spectral results demonstrated that Try did not show any emission peak; however, Try-encapsulated CsPbCl₃ PNCs displayed a characteristic emission peak at 433 nm (Figure S3a). Interestingly, pure Try and Try-CsPbCl₃ PNCs exhibited different absorption spectral characteristics (Figure S3b), indicating the formation of Try-CsPbCl₃ PNCs. In order to investigate the role of halide ion in improving fluorescence properties and stability of Try-CsPbX₃ PNCs, we studied the water dispersion abilities and fluorescence properties of Try-CsPbBr₃ and Try-CsPbI₃ PNCs (Figure S4). It can be noticed that the aqueous solution of Try-CsPbBr₃ and Try-CsPbI₃ PNCs exhibited λ_{Em} at 521 and 580 nm when excited at 440 and 482 nm, respectively. It can be noticed that the emission spectra of both Try-CsPbBr₃ and Try-CsPbI₃ PNCs were blue shifted to 482, 498, 410, and 433 nm with a noticeable decrease in their intensities when the emission spectra measured after PNCs synthesis at different time intervals 3 h and 6 h. Furthermore, the emission spectra of three synthesized PNCs, i.e., Try-CsPbCl₃, Try-CsPbBr3, and Try-CsPbI3 PNCs were investigated by applying the same excitation wavelength ($\lambda_{ex} = 370 \text{ nm}$) at three different times (10 min, 3 and 6 h) (Figure S5). The as-prepared Try-CsPbCl₃ PNCs exhibited intense emission peak spectra at three-time intervals, whereas Try-CsPbBr₃ PNCs showed a weak emission peak at 521 nm at 10 min as compared to Try-CsPbCl₃ PNCs. Noticeably, the emission spectra of Try-CsPbBr₃ PNCs were noticed with very weak intensity at 3 and 6 h time intervals, whereas Try-CsPbI₃ PNCs did not show any noticeable emission intensities at three-time intervals by applying excitation wavelength of 370 nm. These spectral results confirmed that the synthesized Try-CsPbCl₃ PNCs exhibited good water resistance with high emission intensity by applying λ_{ex} at 370 nm. Importantly, Try-CsPbCl₃ PNCs showed remarkable stability in the aqueous medium even after 6 days (Figure S3 and S6), indicating that the aqueous stability of Try-CsPbCl₃ PNCs is high as compared to Try-CsPbBr₃ and Try-CsPbI₃ PNCs, which promotes to use Try-CsPbCl₃ PNCs as a fluorescent probe.

After optimizing the reaction conditions, we examined the spectral characteristics, size, morphology, zeta potential, and elemental composition of Try-CsPbCl₃ PNCs. The as-fabricated Try-CsPbCl₃ PNCs displayed λ_{max} at 377 nm, whereas the emission/excitation peaks exhibited at 433/370 nm (Fig. 1). The obtained spectral characteristics of Try-CsPbCl₃ PNCs were well matched with the reported method for CsPbCl₃ PNCs dispersed in organic solvent (hexane) [60]. To further investigate the role of Try to encapsulate CsPbCl₃ PNCs, we studied the absorption and emission spectra of Try-capped CsPbCl₃ PNCs, unmodified CsPbCl₃ PNCs (dispersed in toluene and water), and pure Try (Figure S3 and S7). It can be noticed that Try capped



Microchim Acta (2025) 192:217 Page 5 of 11 217

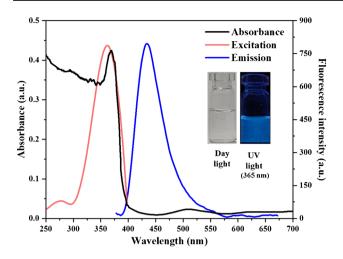


Fig. 1 Absorption (λ_{max} = 377 nm), fluorescence excitation, and emission (λ_{Ex} = 370 nm; λ_{Em} = 433 nm) spectra of Try-CsPbCl₃ PNCs (Inset: Digital images of Try-CsPbCl₃ PNCs in daylight and under UV light at 365 nm)

CsPbCl₃ PNCs (water), pure Try (water), and unmodified CsPbCl₃ PNCs (toluene) exhibited λ_{max} at 377, 301, and 355 nm, respectively, confirming that they have different absorbance values. Importantly, the characteristic emission peak of unmodified CsPbCl₃ PNCs exhibited good intensity when they dispersed in toluene; however, the emission spectrum of unmodified CsPbCl₃ PNCs does not appear when they dispersed in water, revealing that unmodified CsPbCl₃ PNCs exhibited poor water stability. These absorption and emission spectral data demonstrated that the water stability of CsPbCl₃ PNCs was greatly improved by the encapsulation of CsPbCl₃ PNCs with Try. Upon irradiation of Try-CsPbCl₃ PNCs solution with 365 nm of UV light, blue fluorescence was noticed, which confirms the formation of Try-CsPbCl₃ PNCs (Inset of Fig. 1). Furthermore, excitation-dependent emission spectral characteristics of Try-CsPbCl₃ PNCs were studied and shown in Figure S8. The emission spectral profiles of the as-prepared Try-CsPbCl₃ PNCs at different excitation wavelengths (300–400 nm) displayed the almost nonvariant nature in the emission spectra; however, the emission peak intensity was increased with increasing λ_{Ex} from 300 to 370 nm; after that, the intensity was decreased. The maximum emission intensity was noticed at $\lambda_{Em} = 433$ nm upon excitation of Try-CsPbCl₃ PNCs at $\lambda_{Ex} = 370$ nm. In addition, the fluorescence QY of Try-CsPbCl₃ PNCs was 17.26%, and the lifetime of Try-CsPbCl₃ PNCs was $\tau = 1.64$ ns (Figure S9). In order to apply Try-CsPbCl₃ PNCs as a promising fluorescence probe, it is essential to investigate the stability of Try-CsPbCl₃ PNCs. The stability of fabricated Try-CsPbCl₃ PNCs in water was examined by monitoring the emission spectral profiles at different time intervals (Figure S6). The emission spectral profiles of Try-CsPbCl₃ PNCs exhibited almost negligible changes up to 8 days; after that, the emission peak intensity was decreased, which confirms that Try-CsPbCl₃ PNCs displayed good stability to use as a fluorescence probe for sensing applications.

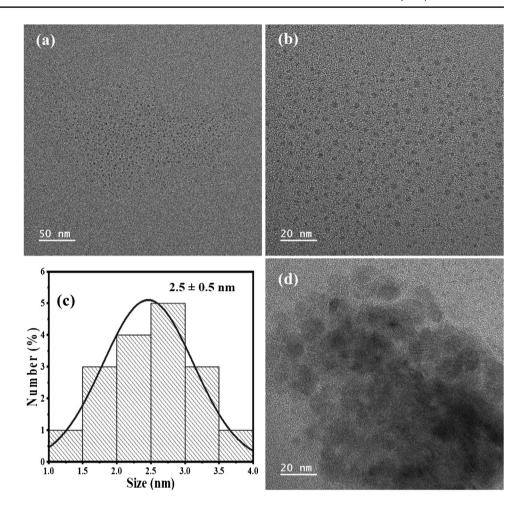
The FT-IR spectral profiles of pure Try and Try-CsPbCl₃ PNCs were studied, and the data were depicted in Figure S10. The FT-IR spectrum of Try displayed a strong broad band in the range of 3600-3000 cm⁻¹, which confirms to O-H and -N-H bonds stretching vibrations. The bands at 1650 and 1523 cm⁻¹ are ascribed to the amide I and II bands of Try, respectively. Similarly, the stretching vibrations of COO⁻, C-N stretching, and N-H bending were noticed at 1438, 1253, and 1519 cm⁻¹, respectively. The characteristic FT-IR spectral profiles of Try were completely changed due to the encapsulation of CsPbCl₃ PNCs. It can be observed that a noticeable decrease in the intensity of broadband in the range of 3600-3000 cm⁻¹ for -OH and -NH₂ groups stretching and a drastic shift in the characteristic amide bands confirm the backbone structural deformation in Try due to the formation of Try encapsulated CsPbCl₃ PNCs. Size and morphological analyses of the as-synthesized Try-CsPbCl₃ PNCs were further confirmed by using FE-TEM and dynamic light scattering (DLS) (Fig. 2 and Figure S11a). The as-prepared Try-CsPbCl₃ PNCs are nearly spherical shape with a mean size of 2.5 ± 0.5 nm, suggesting that Try-CsPbCl₃ PNCs are highly monodispersity, as confirmed from histogram (Fig. 2a-c). The hydrodynamic diameter of Try-CsPbCl₃ PNCs was 10.34 nm, displaying a higher size as compared to FETEM data due to the measurement of Try-CsPbCl₃ PNCs with water molecules. The as-fabricated Try-CsPbCl₃ PNCs exhibited a negative charge (-19.51 mV) (Figure S12a), which was confirmed by measuring zeta potential.

The elemental spectral profile of Try-CsPbCl₃ PNCs was further investigated by XPS, and the XPS survey spectrum of Try-CsPbCl₃ PNCs was shown in Fig. 3a, indicating the as-synthesized Try-CsPbCl₃ PNCs contains C, O, Cs, Pb, and Cl elements. Figure 3b shows the Cs 3d high resolution (HR) spectrum, displaying two peaks at 726.7 eV and 740.7 eV are correspond to the Cs $3d_{5/2}$ and Cs $3d_{3/2}$, respectively, confirming the presence of Cs⁺ ion in Try-CsPbCl₃ PNCs. It can be observed that HR-XPS spectrum of Pb 4f exhibited two peaks at 144.7 and 139.6 eV, which are ascribed to the Pb 4f_{5/2} and Pb 4f_{7/2} of Try-CsPbCl₃ PNCs, respectively (Fig. 3c). Similarly, the HR spectrum of Cl 2p displayed two peaks at 200.3 and 201.8 eV, indexing to the Cl $2p_{3/2}$ and Cl $2p_{1/2}$ binding energy levels, respectively (Fig. 3d). Furthermore, the XRD pattern of Try-CsPbCl₃ PNCs is shown in Figure S13, exhibiting the diffraction peaks at $2\theta = 15.9$, 22.4, 32.2, 35.7, 43.12, 46.3, and 54.5 correspond to 100, 110, 200, 211, 220, 310, and 222 lattice planes of Try-CsPbCl₃ PNCs, which is well agreed with the XRD pattern of CsPbCl₃ PNCs [61, 62]. The as-fabricated



217 Page 6 of 11 Microchim Acta (2025) 192:217

Fig. 2 HR-TEM pictures of Try-CsPbCl₃ PNCs with scale bars of a 50 nm and b 20 nm. c The size distribution of Try-CsPbCl₃ PNCs. d HR-TEM picture of Try-CsPbCl₃ PNCs with OH



Try-CsPbCl₃ PNCs are in crystalline nature with high monodispersity. All the above data strongly support the formation of Try-CsPbCl₃ PNCs with monodispersity and good spectral characteristics, which explore them as potential probes for *OH sensing.

Fluorescence sensing of 'OH radical

To examine the fluorescence detection capability of Try-CsPbCl₃ PNCs toward *OH radical, several oxidizing and ROS (MnO₄⁻, Cr₂O₇²⁻, HPO₄²⁻, *OH, S₂O₈²⁻, O₂ *-, and NBS, 1 mM, 0.5 mL) were mixed with 1 mL of Try-CsPbCl₃ PNCs, separately, followed by vertexing the samples for a few minutes. The fluorescence emission spectra of the samples were evaluated (Fig. 4). As can be seen, the emission spectra of Try-CsPbCl₃ PNCs in a significant decrease in the fluorescence emission intensity of Try-CsPbCl₃ PNCs was noticed in the presence of *OH radical, while no significant fluorescence quenching was noticed in the presence of other oxidizing and ROS. These emission spectral profiles demonstrated that the as-prepared Try-CsPbCl₃ PNCs could be utilized as a probe for fluorescence analysis of *OH radical in aqueous media. Moreover, the fluorescence color of

Try-CsPbCl₃ PNCs solution with the addition of the above species was monitored under 365 nm of UV light, indicating the blue fluorescence of Try-CsPbCl₃ PNCs was almost non-fluorescent nature (Inset of Fig. 4), which signifies that Try-CsPbCl₃ PNCs act as a turn-off fluorescent probe for OH radical sensing. The fluorescence emission signals of Try-CsPbCl₃ PNCs were evaluated in the presence of phosphate-buffered saline (PBS) with pH from 2.0 to 12.0 with and without OH radical (Figure S14a), demonstrating that the addition of PBS pH (2.0–12.0) into Try-CsPbCl₃ PNCs did not affect the fluorescence spectra of Try-CsPbCl₃ PNCs. However, the maximum fluorescence emission quenching was observed with *OH radical at PBS of pHs 10 and 12 (Figure S14b), and PBS of pH 10 was selected as an optimum pH for sensing of OH radical using Try-CsPbCl₃ PNCs as a probe.

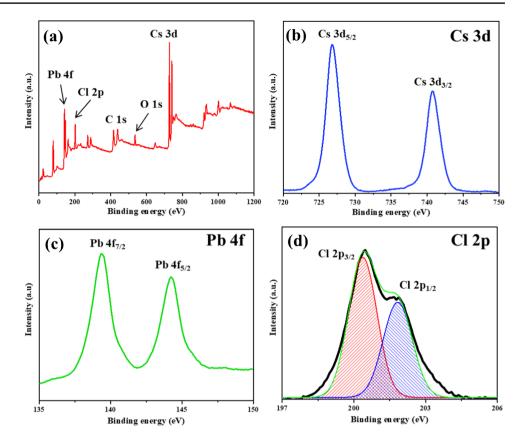
Fluorescence sensing mechanism

In order to evaluate the fluorescence sensing mechanism of OH radical using Try-CsPbCl₃ PNCs as a probe, several analytical techniques (TEM, DLS, zeta potential, and lifetime) were examined. As can be seen in the FETEM image



Microchim Acta (2025) 192:217 Page 7 of 11 217

Fig. 3 XPS characterizations of Try-CsPbCl₃ PNCs: a survey spectrum, HR spectra of b Cs 3d, c Pb 4f, and d Cl 2p



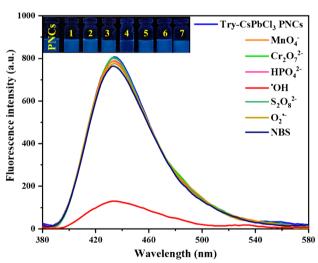


Fig. 4 Fluorescence emission spectra of Try-CsPbCl₃ PNCs after adding different ROS (MnO₄ $^-$, Cr₂O₇ 2 $^-$, HPO₄ 2 $^-$, $^{\bullet}$ OH, S₂O₈ 2 $^-$, O₂ $^{\bullet}$ $^-$, and NBS). Inset picture of Try-CsPbCl₃ PNCs after the addition of various ROS (1. MnO₄ $^-$, 2. Cr₂O₇ 2 $^-$, 3. HPO₄ 2 $^-$, 4. $^{\bullet}$ OH, 5. S₂O₈ 2 $^-$, 6. O₂ $^{\bullet}$ $^-$, and 7. NBS) under UV light (365 nm)

of Try-CsPbCl₃ PNCs with the addition of *OH radical (Fig. 2d), the morphology and size of Try-CsPbCl₃ PNCs were drastically changed by the addition of *OH radical, indicating the deformation of Try-CsPbCl₃ PNCs by *OH radical, which leads to form Try-CsPbCl₃ nanoaggregates.

Similarly, the hydrodynamic diameter of Try-CsPbCl₃ PNCs was significantly increased to 21.5 nm by introducing OH radical (Figure S11b), leading to destabilization of Try-CsPbCl₃ PNCs, which resulted in quenching the fluorescence of Try-CsPbCl₃ PNCs. The zeta potential of Try-CsPbCl₃ PNCs was – 19.51 mV; however, it was increased to – 28.23 mV (Figure S12b). Interestingly, the negligible change (from 1.64 to 1.51 ns) in the lifetime of Try-CsPbCl₃ PNCs was observed with the addition of OH radical, indicating the static quenching mechanism (Figure S9). The characteristic FT-IR spectral profiles of Try-CsPbCl₃ PNCs were significantly changed upon the addition of OH radical (Figure S15), suggesting the structural changes in the Try-CsPbCl₃ PNCs by OH radical. Importantly, FT-IR spectral data are commonly used to examine the variations in the secondary structure of proteins and protein-chemical species interactions [32, 33]. As shown in Figure S15, the amide I and II spectral characteristics of Try were observed in the range of 1600–1700 and of 1500–1600 cm⁻¹, respectively. By comparing FT-IR spectrum of pure Try to that of Try-CsPbCl₃ PNCs with OH radical, the peak intensity and peak position of the amide I (1600–1700 cm⁻¹) and II (1500–1600 cm⁻¹) bands were changed, revealing the drastic changes in the secondary structure of Try upon the addition of OH radical. To further evaluate the inner filter effect (IFE), the fluorescence (excitation and emission) spectra of Try-CsPbCl₃ PNCs and the absorption spectrum of OH



217 Page 8 of 11 Microchim Acta (2025) 192:217

radical were studied (Figure S16), revealing the overlapping of absorption spectrum of *OH radical with excitation spectrum of Try-CsPbCl₃ PNCs, which confirms the IFE. Thus, the as-prepared Try-CsPbCl₃ PNCs act as a turn-off fluorescence probe for the detection of *OH radical.

Sensitivity

Under the optimal conditions, the variations in the fluorescence intensity of Try-CsPbCl₃ PNCs were investigated by adding different concentrations of OH radical (0.01–500 μM). As can be noticed in Fig. 5, the fluorescence emission intensity of Try-CsPbCl₃ PNCs centered at 433 nm was gradually quenched along with increasing concentration of OH radical. Then, the emission intensity of Try-CsPbCl₃ PNCs was quenched at 77% when OH radical at 500 µM. Figure S17 displayed the constructed plot of the ratio I_0/I (where I_0 and I represent the emission intensity of Try-CsPbCl₃ PNCs in the absence and presence of OH radical, respectively) against OH radical concentration (0.01-500 µM). Furthermore, with OH radical concentration in the range of 0.01–5.0 µM, the fluorescence quenching efficiency showed the linear fitting equation of y = 0.2596x + 1.2061 ($R^2 = 0.9795$) (Inset of Figure S17). The LOD was 3.10 nM ($3\sigma/s$, where "s" is the slope of the calibration curve and "\sigma" is the standard deviation of the blank) for OH radical. The analytical characteristics of the developed Try-CsPbCl₃ PNCs-based fluorescence approach were compared with other reported methods [53, 63–70] for OH radical sensing (Table S1), revealing the developed probe exhibited superior and comparable analytical performance with other analytical techniques for OH

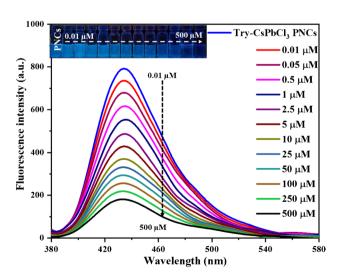
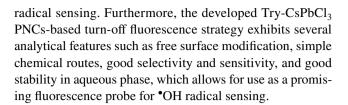


Fig. 5 Fluorescence spectra of Try-CsPbCl $_3$ PNCs in the existence of various concentrations of $^{\bullet}$ OH (0.01–500 μ M). Inset picture demonstrates the variations in the blue fluorescence of Try-CsPbCl $_3$ PNCs with the addition of $^{\bullet}$ OH (0.01–500 μ M) under UV light at 365 nm



Selectivity

The sensing selectivity of Try-CsPbCl₃ PNCs toward OH radical was investigated in the presence of various metal ions, ROS, and biomolecules phosphate buffer (20 mM, pH 7.4). As shown in Figure S18, the fluorescence behavior of Try-CsPbCl₃ PNCs in the presence of biomolecules (cysteine, arginine, tryptophan, and alanine, 500 μM), cations (Na⁺, Ca²⁺, Mg²⁺, Zn²⁺, Cu²⁺, and Fe³⁺, 500 μM) and anions (Cl⁻, I⁻, Br⁻, PO₄³⁻, and SO₄²⁻, 500 μM) is almost same and did not show any significant changes. As anticipated, Try-CsPbCl₃ PNCs exhibited a remarkable fluorescence quenching only with OH radical even in the existence of the other interfering chemical species, revealing that the as-prepared Try-CsPbCl₃ PNCs stand out as a highly selective turn-off fluorescence probe for OH radical sensing.

Analysis of 'OH radical in water samples

To evaluate the practical application of Try-CsPbCl₃ PNCs in monitoring OH radical in real samples, the collected water (tap and river) samples were filtered and subsequently added different concentrations of OH radical (0.01, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, and 25.0 μM). Then, OH radical treated water samples were added into Try-CsPbCl₂ PNCs, and their concentrations were estimated by the aforesaid procedure. From Table S2, it can be noticed that the recovery rates of OH radical in water samples were 98.80–101.40% with a relative standard deviation of < 2.0%. In order to estimate the accuracy and precision of the developed method for the analysis of OH radical in spiked water samples, inter- and intra-day accuracy and precision of Try-CsPbCl₃ PNCsbased fluorescence turn-off approach was performed, and the data were depicted in Table S3. The data showed that the percentage recoveries were in the ranges of 98.80–99.76% and 98.57-99.64% for tap and river waters. Moreover, interand intra-day accuracy and precision of the method are in the ranges of 0.38-1.20% and 0.5 and 1.13% for tap water and 0.42–1.29% and 0.41–0.76% for river water, respectively. The data in Table S2-S3 represent that the Try-CsPbCl₃ PNCs-based fluorescence method is a facile, more precision and accurate analytical approach for the detection of OH radical in real samples, demonstrating that Try-CsPbCl₃ PNCs could be used as the potential fluorescence turn-off probe for the detection of OH radical in real samples. Although the developed Try-CsPbCl₃ PNCs-based



Microchim Acta (2025) 192:217 Page 9 of 11 217

fluorescence method showed good selectivity and sensitivity towards *OH radical sensing in real samples, however the synthesis of Try-CsPbCl₃ PNCs requires lead salts, which is a toxic nature. Furthermore, the developed method had a limitation to detect *OH radical in living cells. Overall, the as-prepared Try-CsPbCl₃ PNCs have good water stability for the development of a facile fluorescent probe for sensing *OH radicals in water samples with high selectivity.

Conclusions

In summary, a simple analytical tool was developed for sensing OH radicals using water-dispersible Try-CsPbCl₂ PNCs as a turn-off fluorescence probe. The as-fabricated Try-CsPbCl₃ PNCs exhibited blue fluorescence under UV irradiation ($\lambda \sim 365$ nm) and displayed $\lambda_{Em/Ex}\!=\!433/370$ nm. The developed Try-CsPbCl₃ PNCs-based fluorescence approach has a highly selective and sensitive response toward OH radical with a good linearity in the concentration range of 0.01-5.0 µM, which achieves the LOD of 3.10 nM. Importantly, Try-CsPbCl₃ PNCs exhibit superior selectivity for OH radical with virtually no fluorescence quenching by other interfering chemical species (ROS, metal ions, anions, and biomolecules). Furthermore, Try-CsPbCl₃ PNCs-based analytical approach was successfully applied to detect OH radical in water samples. Thus, the as-synthesized Try-CsPbCl₃ PNCs could be successfully integrated with fluorescence spectrometry for the detection of *OH radical in real samples.

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Data availability Data will be available from corresponding author on demand.

Declarations

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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217 Page 10 of 11 Microchim Acta (2025) 192:217

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Microchim Acta (2025) 192:217 Page 11 of 11 217

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