

Article

Selective Detection of Folic Acid Using a Water-Stable Fluorescent CsPbBr₃/Cs₄PbBr₆ Perovskite Nanocrystal Probe

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Abstract: A water-stable cesium lead bromide (CsPbBr₃/Cs₄PbBr₆) perovskite nanocrystal (PNC) was synthesized and studied as a fluorescence probe for the selective detection of folic acid (FA). The as-prepared PNCs emitted strong green fluorescence at 525 nm, and their structure was systematically characterized by X-ray powder diffraction, X-ray photoelectron spectroscopy, and transmission electron microscopy. The interaction between the PNCs and small biological molecules was investigated and the results indicated that the fluorescence of the PNCs could be selectively quenched by FA. The quenching rate has a linear relationship with the concentration of FA in the concentration range from 10 to 800 μM, with a correlation coefficient R^2 of 0.9841, and a limit of detection (LOD, 3σ) of 1.69 μM. The mechanism of the interaction between the PNCs and FA was discussed, and the reliability of the method for real sample detection was also verified by the standard addition method. The method proposed here, using a fluorescence PNCs probe, provided a simple alternative strategy for detecting FA that will play an important role in biochemical analysis.

Keywords: water-stable; perovskite nanocrystals; selective detection; folic acid



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1. Introduction

All-inorganic cesium lead halide perovskite nanocrystals (CsPbX₃ PNCs, X = Cl, Br or I), a new class of luminescent materials [1,2], exhibit excellent optical properties, such as high photoluminescence quantum yield, half-peak width, full-width, and a wide color gamut [3], while possessing unique carrier separation properties, high charge carrier mobility and long carrier diffusion length [4–8]. However, conventional CsPbX₃ PNCs are highly sensitive to moisture and other polar solvents, leading to degradation [9–11], which makes it difficult to develop analytical methods for the detection of targets in aqueous solutions. Therefore, the synthesis of water-stable PNCs and their application in the detection of targets in aqueous solutions have attracted extensive attention [12,13].

To improve the water stability of PNCs, polymers [14–16], inert oxides (silica or alumina) [17–19], metal–organic framework materials [20–22], and carbon materials [23] have been widely used as coatings or stabilizers. For example, Ma et al. promoted the stripping and fragmentation of MnO₂ nanosheets by utilizing the reducibility of cysteine and intramolecular hydrogen bonding, providing a protective microenvironment [24]. However, the as-prepared products tend to agglomerate when in solution, due to the bulk formation of coating materials on the surface of PNCs. Directly preparing water-stable CsPbX₃ PNCs using suitable ligands has attracted much research attention. For example, Li et al. synthesized water-stable CsPbBr₃/Cs₄PbBr₆ PNCs using a fluorocarbon agent (FCA) [3,25]. The FCA that they used has a hydrophobic structure and promotes colloidal dispersion, which enables the direct synthesis of PNCs in aqueous solutions. Hu et al. utilized water-stable L-lysine to directly synthesize Lys-PNCs in water, and the synthesized

Lys-PNCs exhibited enhanced water resistance [26]. The reports of these highly water-stable perovskite synthesis methods lay the foundation for the application of perovskites in aqueous solutions.

FA is commonly taken by pregnant women to treat and prevent macrocytic anemia [27]. Insufficient levels of folate can disrupt cell division, leading to defects in fetal development. FA deficiency also leads to elevated serum homocysteine levels [28], which, in turn, leads to cardiovascular diseases such as coronary heart disease and stroke [29]. Traditional methods for the determination of FA include capillary electrophoresis [30,31], chromatography [32–34], electrochemical sensors [35], chemiluminescence [36–38], and fluorescence [39–42]. However, some methods still suffer from certain limitations, such as being time-consuming, low sensitivity, and high cost, along with the need for complex sample pretreatment. Therefore, it is necessary to develop a simple and effective method to detect FA. The method proposed in this paper, using a fluorescence PNCs probe, provides a simple alternative strategy for detecting FA.

In this work, we improved the reported aqueous emulsion method for the synthesis of water-stable cesium lead bromide PNCs using CsBr, PbBr₂, N-6-trifluoroacetyl-L-lysine, and oleylamine (OLA) as the raw materials. The synthesized nanocrystals exhibited strong green fluorescence at 525 nm and had good water stability. The structure of the PNCs was systematically characterized by X-ray powder diffraction, X-ray photoelectron spectroscopy, and transmission electron microscopy. We also found that folic acid (FA) could selectively quench the fluorescence of PNCs in an aqueous solution. The quenching rate has a linear relationship with the concentration of FA, in the concentration range from 10 to 800 µM. Based on this phenomenon, a fast, efficient, selective, and visual detection method for FA was established, using the PNCs as a fluorescent probe.

2. Experimental Section

2.1. Materials and Instruments

CsBr (AR), PbBr₂ (AR), N-6-trifluoroacetyl-L-lysine (98%), oleylamine (OLA) (90%), and small biomolecules were purchased from Adamas-beta China Shanghai Sinopharm Reagent Chemical Reagent Co., Ltd. Shanghai, China. HBr (40%) was sourced from Tianjin Fengchuan Chemical Reagent Technology Co., Ltd. Tianjin, China. DMF (99.5%) was purchased from Shanghai Titan Scientific Co., Ltd. Tianjin, China. All chemicals were used as received, without further purification. Ultra-pure water (18.25 MΩ) was prepared by the Milli-Q ultrapure water preparation system.

Fluorescence and absorption spectra were measured using an F-7000 spectrofluorometer (Hitachi, Tokyo, Japan) and a UV-2700 spectrophotometer (Shimadzu, Tokyo, Japan), respectively. X-ray photoelectron spectroscopy (XPS) data were collected on a K-Alpha X-ray photoelectron spectrometer (American Thermo Fisher Scientific, Waltham, MA, USA). Transmission electron microscope (TEM) images and HAADF were taken on FEI Talos F200s (American Thermo Fisher Scientific, Waltham, MA, USA). X-ray diffraction (XRD) measurements were carried out using an Ultima IV diffractometer, with monochromatized Cu Kα radiation at 40 kV and 40 mA, and were recorded (Rigaku Corporation, Tokyo, Japan).

2.2. Synthesis of the CsPbBr₃/Cs₄PbBr₆ PNCs

The CsPbBr₃/Cs₄PbBr₆ PNCs were synthesized using the aqueous emulsion method, with appropriate modifications of the reported method [3,25]. First, 0.5 M CsBr, 0.5 M PbBr₂, and 0.05 M N-6-trifluoroacetyl-L-lysine were prepared in 40% HBr solution. Take 1.0 mL of DMF into a 5.0 mL tube, add 125.0 µL of CsBr and 125.0 µL of PbBr₂ solution, in turn, mix well, and, after the temperature has cooled to room temperature, add 125.0 µL of N-6-trifluoroacetyl-L-lysine. After mixing evenly, the mixture was maintained at −18 °C and left to react for 2.5 h to obtain a precursor solution. Then, 250 µL of OLA was added to the precursor solution, mixed well, and diluted to 40 mL with ultrapure water. After sonication for 20 min, the mixture was centrifuged at 6000 rpm for 5 min, and the resulting supernatant of CsPbBr₃/Cs₄PbBr₆ PNCs was stored in a refrigerator at 4 °C for further use.

2.3. Detection of Folic Acid by $\text{CsPbBr}_3/\text{Cs}_4\text{PbBr}_6$ PNCs

In a typical process, the resulting supernatant is diluted twice with ultra-pure water, then 200 μL of $\text{CsPbBr}_3/\text{Cs}_4\text{PbBr}_6$ PNCs solution is added to a 1.5 mL tube and a series of different concentrations of FA are added to the tube containing $\text{CsPbBr}_3/\text{Cs}_4\text{PbBr}_6$ PNCs solution. It is then filled with ultra-pure water to 1.0 mL and mixed well. After thorough mixing and standing for 5 min, the mixture was transferred to fluorescence measurements. For verifying the selectivity of FA, other small biomolecules including cysteine (Cys), dopamine (DA), folic acid (FA), glucose (Glu), glutathione (GSH), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp), and ascorbic acid (Vc) were utilized under the same experimental conditions. The concentrations of FA and other small biomolecules were always the same. In order to explore the feasibility of FA detection in actual samples, human urine collected from healthy people was selected for testing and the standard addition method was used for verification. The test conditions were the same as in the detection of FA described above.

3. Results and Discussion

3.1. Characterization of $\text{CsPbBr}_3/\text{Cs}_4\text{PbBr}_6$ PNCs

As shown in Figure 1, the $\text{CsPbBr}_3/\text{Cs}_4\text{PbBr}_6$ PNCs showed strong fluorescence emission, with a maximum emission wavelength of 525 nm; a narrow half-peak width can be observed in the figure. The UV-Vis absorption showed that the PNCs had a weak absorption peak at 510 nm, and the peak shape and peak position were consistent with the $\text{CsPbBr}_3/\text{Cs}_4\text{PbBr}_6$ PNCs reported in the literature [3]. The PNCs solution showed strong green fluorescence under UV light irradiation with a wavelength of 365 nm (see inset in Figure 1). At the same time, we also measured the fluorescence lifetime of the PNCs, as shown in Figure S5 of the Supplementary Materials, the fluorescence lifetime of the PNCs was 8.28 ns, established by fitting the fluorescence lifetime decay curve.

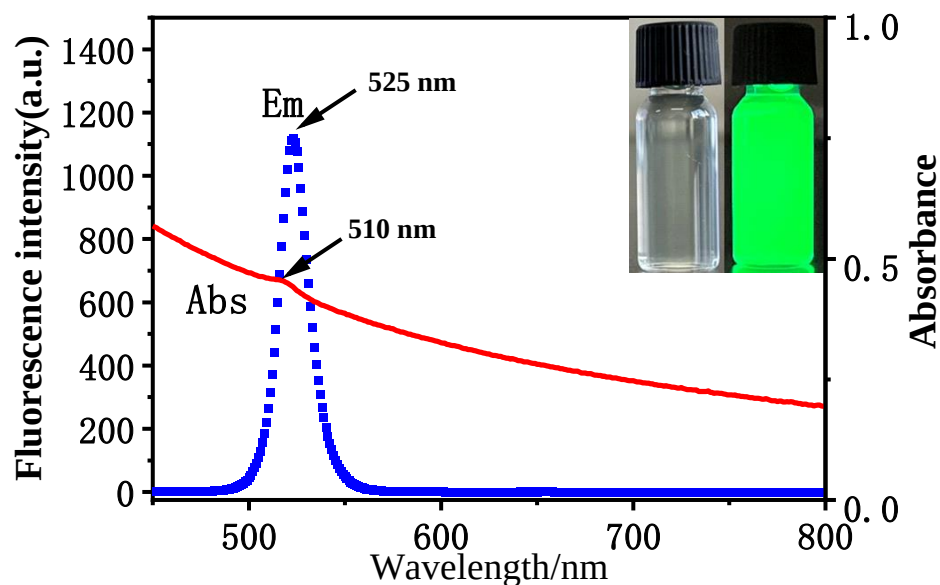


Figure 1. Fluorescence and UV absorption spectra of $\text{CsPbBr}_3/\text{Cs}_4\text{PbBr}_6$ PNCs. Inset: photos of the $\text{CsPbBr}_3/\text{Cs}_4\text{PbBr}_6$ PNCs under UV irradiation and natural light. Experimental conditions: synthetic precursors: CsBr (45.4 mM), PbBr_2 (45.4 mM), N-6-trifluoroacetyl-L-lysine (4.54 mM), reaction time: 2.5 h, reaction temperature: -18°C . Synthesis of $\text{CsPbBr}_3/\text{Cs}_4\text{PbBr}_6$ PNCs: OLA (250 μL), precursor (1.375 mL). Reaction time: 20 min. Reaction temperature: 25°C .

The as-synthesized PNCs were observed in the transmission electron microscopy (TEM) images (Figure 2A) as regular cube-like shapes, with different sizes and length distributions, ranging from 30 to 130 nm. Clear lattice fringes can be observed on the cubes in Figure 2B, and a clear and distinct lattice can be observed via high-resolution transmission

electron microscopy (HRTEM) magnification (Figure 2C), with a lattice spacing of 0.42 nm. It was characterized by X-ray powder diffraction; the results are shown in Figure 2D, at 12.6° , 20.1° , 25.4° , 27.5° , 30.2° , 30.9° , 34.1° , and 38.9° (shown in green in the figure). The diffraction peaks that appeared correspond to the 012, 113, 024, 131, 223, 006, 134, and 324 crystal planes of Cs_4PbBr_6 (PDF card 73-2478). The diffraction peaks at 18.3° , 22.01° , 35.5° , 37.4° , 42.05° , and 54.14° (marked with red stars in the figure) correspond to the 020, 111, 132, 041, 140, and 055 crystal planes of CsPbBr_3 (PDF card 54-0752). It has a typical cubic phase (111), which is consistent with that reported in the literature [3].

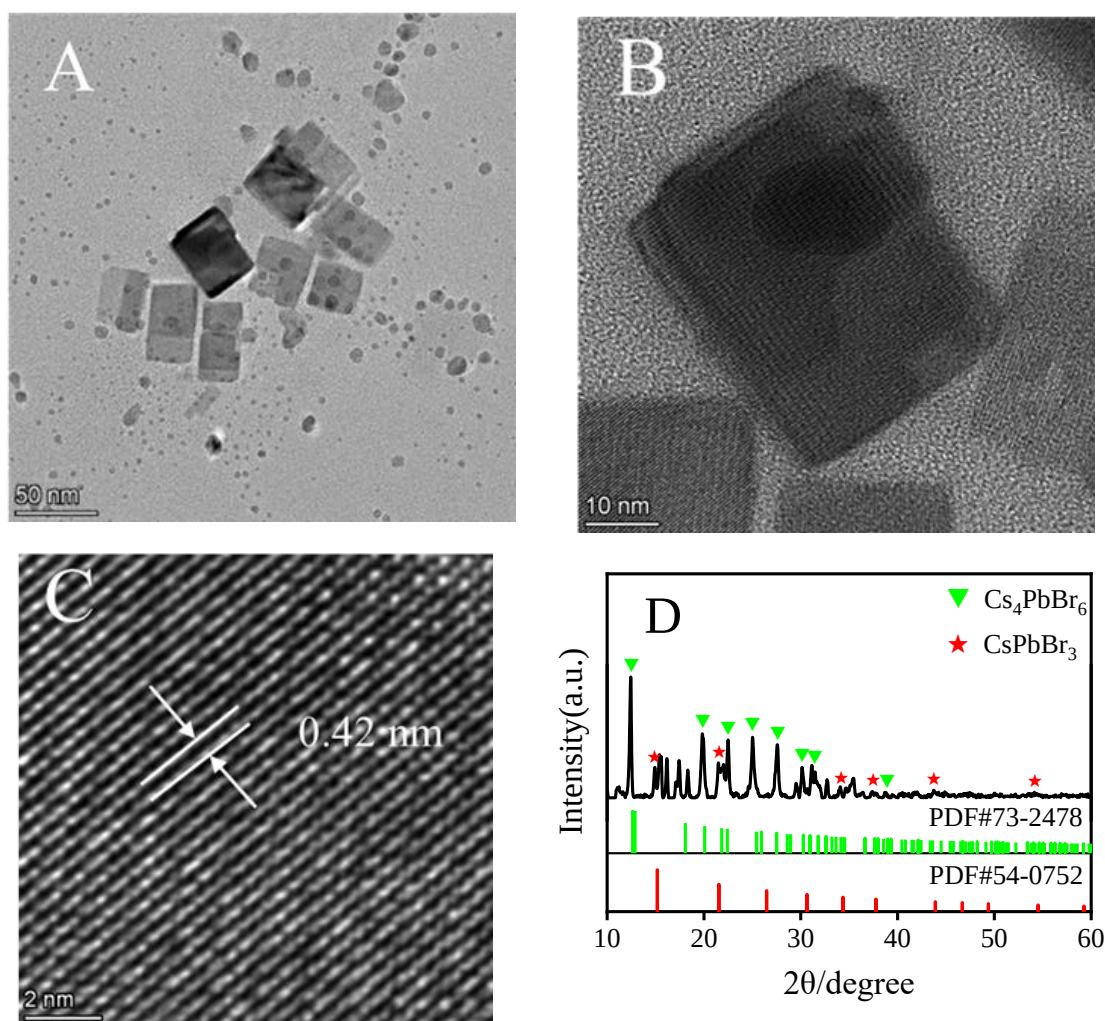


Figure 2. TEM images of $\text{CsPbBr}_3/\text{Cs}_4\text{PbBr}_6$ PNCs (A–C), X-ray powder diffraction patterns of $\text{CsPbBr}_3/\text{Cs}_4\text{PbBr}_6$ PNCs (D). Experimental conditions: synthetic precursors: CsBr (45.4 mM), PbBr_2 (45.4 mM), N-6-trifluoroacetyl-L-lysine (4.54 mM), reaction time: 2.5 h, reaction temperature: -18°C . Synthesis of $\text{CsPbBr}_3/\text{Cs}_4\text{PbBr}_6$ PNCs: OLA (250 μL), precursor (1.375 mL). Reaction time: 20 min, reaction temperature: 25°C .

In order to determine the elements contained in the PNCs and the valence states of each element, X-ray electron spectroscopy (XPS) was used to characterize them, and the results are shown in Figure 3. The characteristic peak at the binding energy of 723.69 eV in Figure 3B is fitted to Cs 3d5, and the characteristic peak at the binding energy of 737.57 eV is fitted to Cs 3d3; the characteristic peak at the binding energy of 138.44 eV in Figure 3C is fitted to Pb 4f7. The characteristic peak at the binding energy of 143.31 eV was fitted to Pb 4f5; the characteristic peak at the binding energy of 68.33 eV in Figure 3D was fitted to Br 3d5, and the characteristic peak at the binding energy of 69.40 eV was fitted to Br

3d3. The resulting binding energies for each element are consistent with those reported previously [43,44].

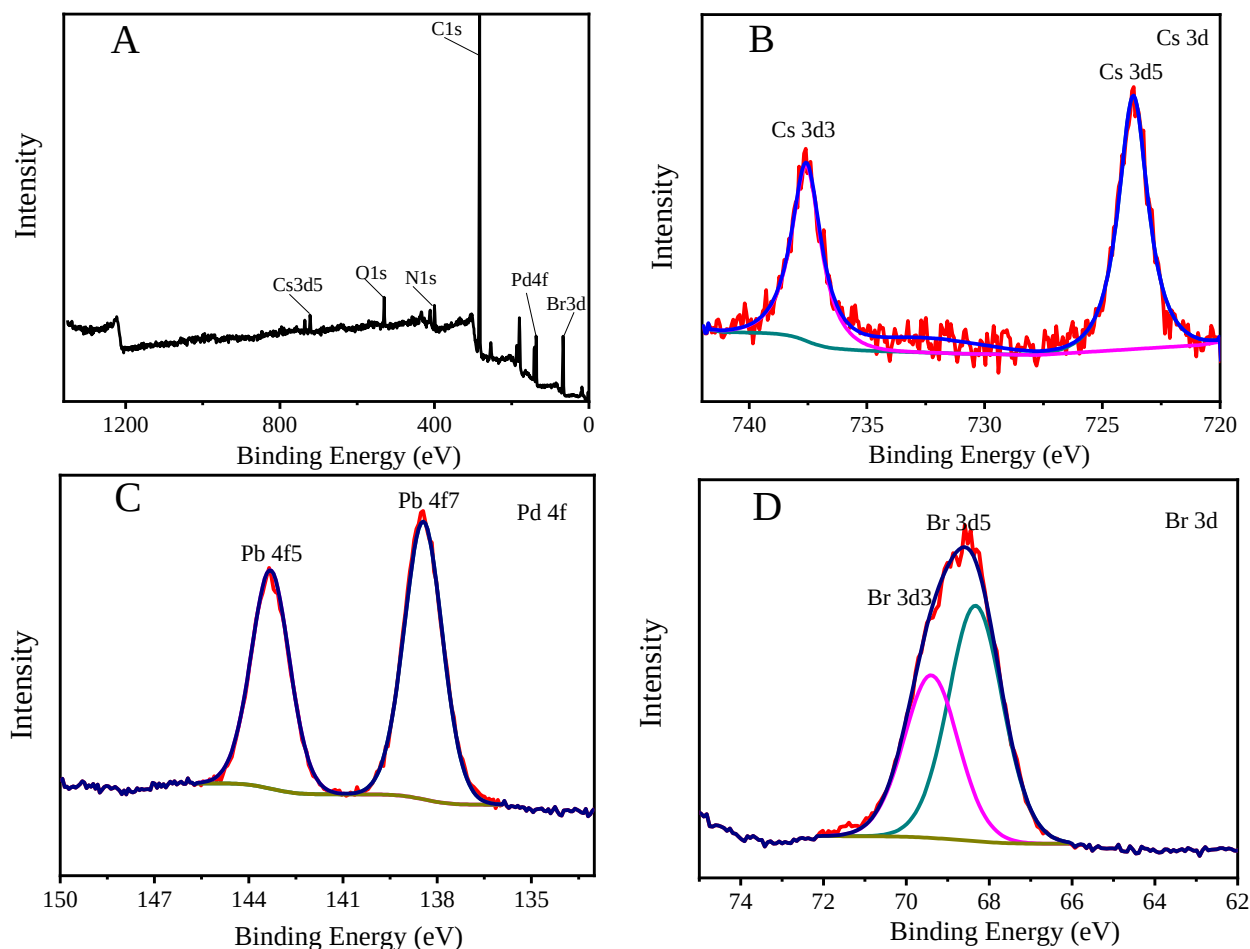


Figure 3. X-ray photoelectron spectra of CsPbBr₃/Cs₄PbBr₆ PNCs (A–D). Experimental conditions: synthetic precursors: CsBr (45.4 mM), PbBr₂ (45.4 mM), N-6-trifluoroacetyl-L-lysine (4.54 mM), reaction time: 2.5 h, reaction temperature: −18 °C. Synthesis of CsPbBr₃/Cs₄PbBr₆ PNCs: OLA (250 μL), precursor (1.375 mL). Reaction time: 20 min, reaction temperature: 25 °C.

3.2. Optimization of Synthesis Conditions for CsPbBr₃/Cs₄PbBr₆ PNCs

The synthesis of the PNCs was carried out in two steps: the synthesis of the precursor solution and the reaction of the precursor solution and the ligand solution to obtain an aqueous solution of the PNCs. During this process, the reaction temperature and time of the precursor synthesis, the volume ratio of the precursor and the ligand, and the reaction time of the precursor and the ligand were studied. The reaction temperature of the precursor is important for the formation of the precursor, and it can be seen in Figure S1 of the Supplementary Materials that the fluorescence of the PNCs is rapidly enhanced as the reaction temperature of the precursor decreases, which is because the low temperature helps the perovskite ore precipitation [3]. Therefore, −18 °C was chosen as the optimum precursor reaction temperature. The effect of the precursor reaction time on the fluorescence intensity of PNCs is shown in Figure S2A,B of the Supplementary Materials. With the increase in reaction time, the fluorescence intensity of the PNCs gradually increased and reached a maximum after 2.5 h. When the reaction time was longer than 2.5 h, the fluorescence intensity gradually decreased. The volume ratio of the precursor solution and the ligand (OLA) also affected the synthesis of PNCs, and the results are shown in Figure S3 of the Supplementary Materials. When the volume ratio of the precursor to the ligand was 11:1–11:6, the fluorescence intensity of the PNCs gradually

increased with the increase in OLA volume, and the fluorescence intensity reached the maximum when the ratio was 11:2. Conversely, when the volume ratio of the precursor solution to the ligand was greater than 11:2, the fluorescence intensity gradually decreased, because the strong scattering of the ligand will affect the luminescence performance of the nanocrystals with the increase in the ligand concentration [45]. The effect of reaction time on the fluorescence intensity of the PNCs is shown in Figure S4 in the Supplementary Materials. The results show that the time required for the ligand to bind to the precursor is very short, and PCNs with strong fluorescence can be formed in 5 min. In order to ensure better fluorescence properties in the synthesized PNCs, the reaction time of the precursor and ligand was selected as 20 min.

3.3. Stability of $\text{CsPbBr}_3/\text{Cs}_4\text{PbBr}_6$ PNCs

The reaction time and the temperature of the medium are important factors that affect the fluorescence intensity. Temperature and standing time stability were investigated in this experiment, and the results are shown in Figure 4. It can be seen that with the gradual increase in temperature, the fluorescence intensity of $\text{CsPbBr}_3/\text{Cs}_4\text{PbBr}_6$ PNCs gradually decreases, indicating that the fluorescence intensity of PNCs is closely related to temperature and will weaken its fluorescence intensity at high temperatures. This is because the Ostwald ripening occurred during the crystal growth process with the increase in temperature, resulting in a larger crystal size [46]. Therefore, we stored the prepared $\text{CsPbBr}_3/\text{Cs}_4\text{PbBr}_6$ PNCs in a low-temperature environment. At the same time, the fluorescence intensity of the PNCs did not change much within 21.5 h of placement. As long as it was used within this time range, the fluorescence intensity of the PNCs remained stable.

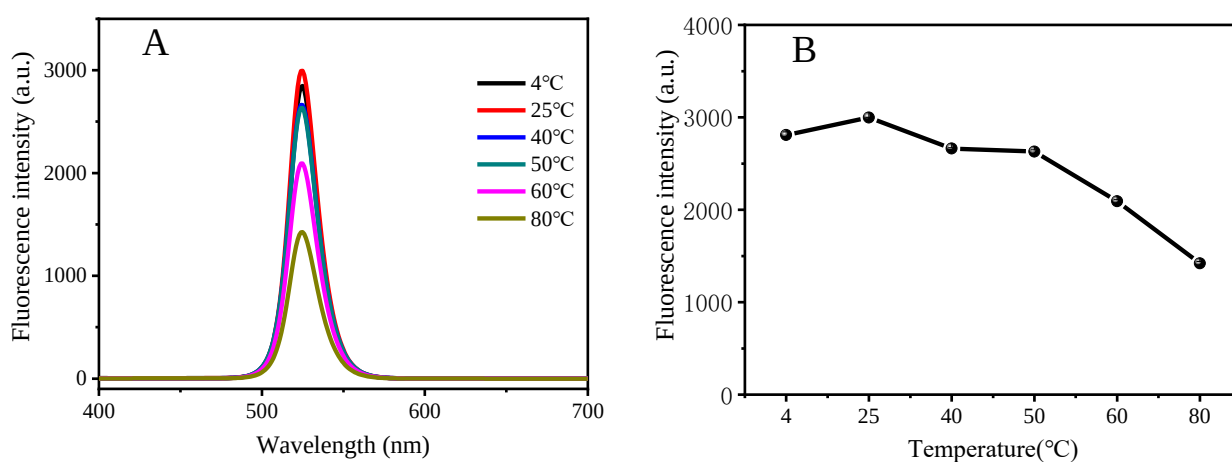


Figure 4. Cont.

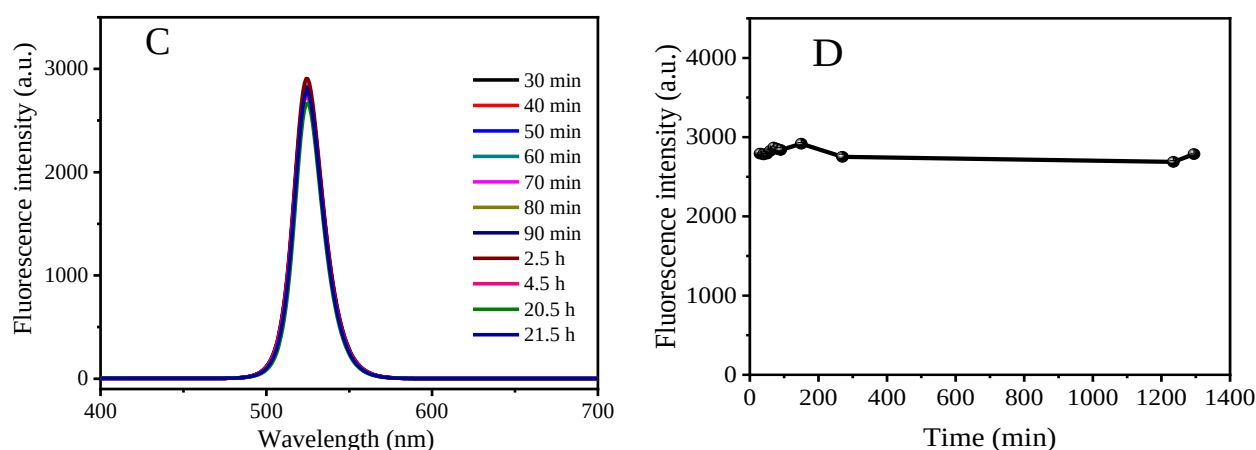


Figure 4. Variation of fluorescence intensity of CsPbBr₃/Cs₄PbBr₆ PNCs, with temperature (A,B) and time (C,D). Experimental conditions: synthetic precursors: CsBr (45.4 mM), PbBr₂ (45.4 mM), N-6-trifluoroacetyl-L-lysine (4.54 mM), reaction time: 2.5 h, reaction temperature: -18°C . Synthesis of CsPbBr₃/Cs₄PbBr₆ PNCs: OLA (250 μL), precursor (1.375 mL). Reaction time: 20 min, reaction temperature: 25°C .

3.4. Analytical Parameter of FA Detection

Various common small biomolecules, including cysteine (Cys), dopamine (DA), folic acid (FA), glucose (Glu), glutathione (GSH), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp), and ascorbic acid (Vc) were added to the PNCs for studying the selectivity of the fluorescence probe. The results shown in Figure 5A,B indicated that FA can significantly quench the fluorescence of the PNCs, while other small biomolecules have little effect on the fluorescence of the PNCs. We also investigated the interference in terms of the detection of FA in the presence of other small biomolecules. As shown in Figure 5C, the detection of FA using the PNCs was not disturbed in the presence of 2000 μM of other biomolecules.

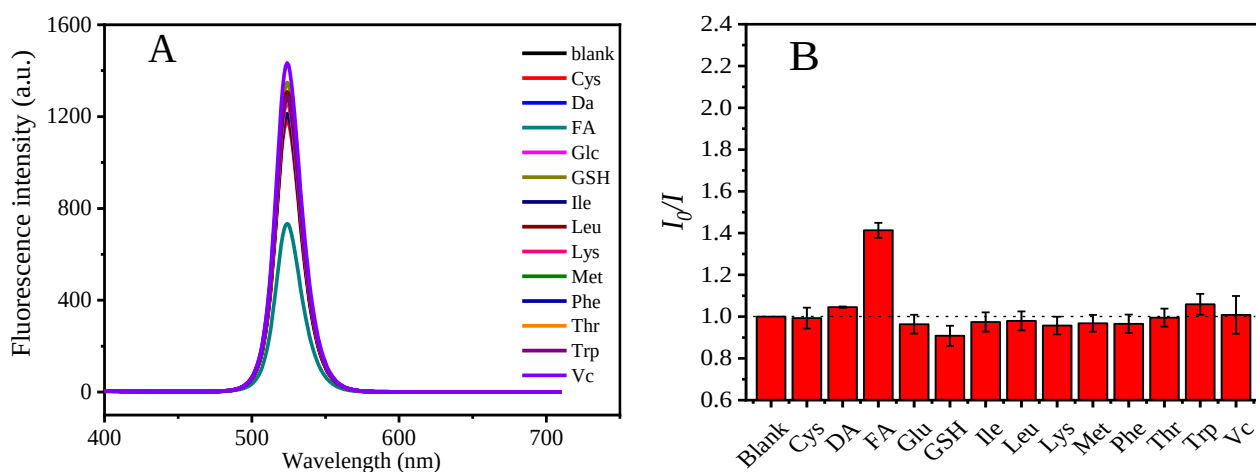


Figure 5. Cont.

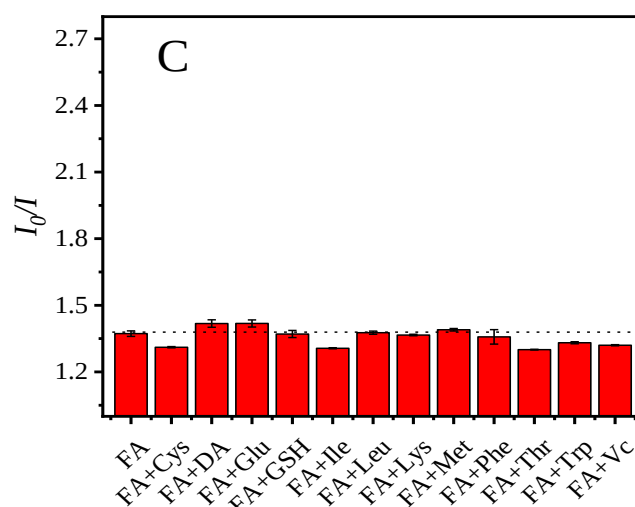


Figure 5. Selective detection of biological small molecules by CsPbBr₃/Cs₄PbBr₆ PNCs (A,B) and interference experiments (C). Experimental conditions: the concentration of CsPbBr₃/Cs₄PbBr₆ PNCs is 0.156 mmol, in terms of Cs atoms, the concentration of all small molecules is 200 μ M (selectivity experiment), the concentration of FA is 200 μ M, the concentration of other small molecules is 2000 μ M (interference experiment). Reaction temperature: 25 $^{\circ}$ C, response time: 5 min.

Since the fluorescence of the PNCs can be quenched by FA selectively, a method for the quantitative detection of FA using the PNCs as a fluorescent probe could be established. Before the quantitative detection of FA, the reaction time and temperature need to be studied. As shown in Figure 6, the fluorescence quenching rate (I_0/I) of the PNCs was not greatly affected by temperature, and the reaction temperature was fixed at 25 $^{\circ}$ C. The interaction between the PNCs and FA could be completed within 5 min and would not change in the following 1 h.

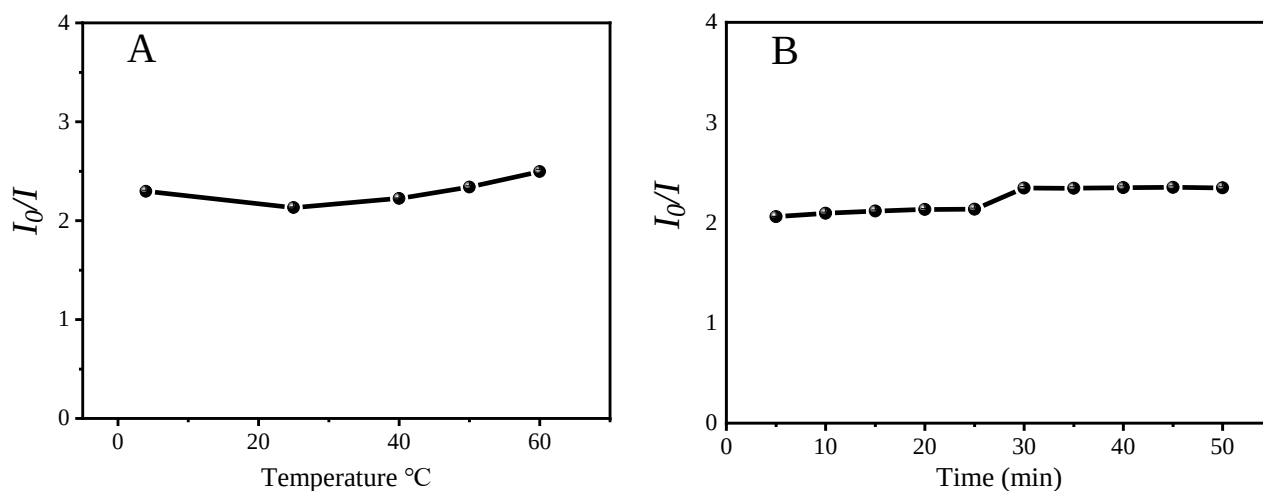


Figure 6. Optimization of temperature (A) and time (B) for the detection of FA by CsPbBr₃/Cs₄PbBr₆ PNCs. Experimental conditions: the concentration of CsPbBr₃/Cs₄PbBr₆ PNCs is 0.156 mmol, in terms of Cs atoms, the concentration of FA is 200 μ M; (A) reaction temperature: 4–60 $^{\circ}$ C; reaction time: 15 min; (B) reaction temperature: 25 $^{\circ}$ C, reaction time: 5–50 min.

For the detection of FA, the linear relationship between the fluorescence quenching rate and the FA concentration of the PNCs was investigated, as shown in Figure 7A,B; they have a good linear relationship, and the calibration curve is $I_0/I = 0.01145_{\text{cFA}} + 0.5822$. The correlation coefficient, R^2 , was 0.9841, and the limit of detection (LOD) was 1.695 μ M in the range of a FA concentration of 10–800 μ M. In addition, our method can also be used for the

rapid real-time detection of FA by the naked eye. As shown in Figure 7C, the fluorescence intensity gradually decreased from left to right with the increase in FA concentration under the 365 nm ultraviolet light.

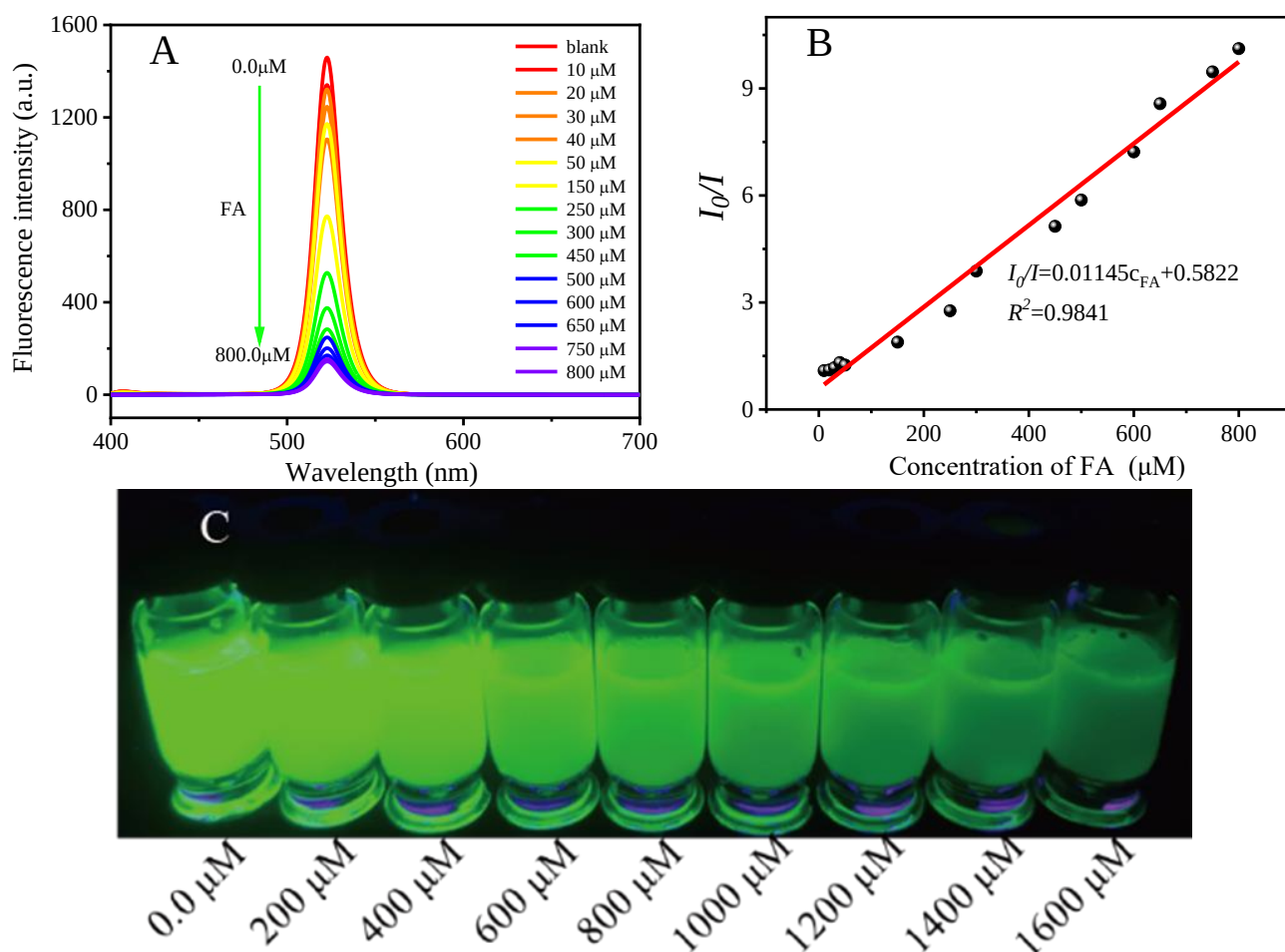


Figure 7. Quantitative detection (A,B) and visual detection (C) of folate acid by CsPbBr₃/Cs₄PbBr₆ PNCs. Fluorescence quenching ratio (I_0/I) (B) and fluorescence spectrum (A) as a function of FA concentration. Experimental conditions: the concentration of CsPbBr₃/Cs₄PbBr₆ PNCs is 0.156 mmol, in terms of Cs atoms, and the concentration of FA is 10–1600 μM . Reaction temperature: 25 $^{\circ}\text{C}$, response time: 5 min.

Recently, several assays have been developed to detect FA. A comparison of this analytical method with other reported folate assays is shown in Table 1, and the concentration range and LOD are comparable to or better than most of the reported methods [34,47–52]. In addition, fluorescence analysis is simple and time-saving, compared to other methods.

Table 1. The comparison of the proposed method with other methods of FA determination.

Method	System	Linear Range (μM)	LOD (μM)	Ref.
HPLC	Pterin-6-carboxylic acid	0.11~2.3	0.017	[33]
Electrochemical analysis	MAAP-Gd(III)@EDMA	0~100	0.0080	[36]
Electrochemical analysis	ZIF-67/AgNWs@SPCE	0.10~10	0.030	[37]
Fluorescence	Eu-MOF	1.0~12.5	0.30	[38]
Fluorescence	D-Trp@AuNCs	6.3~100	5.8	[39]
Fluorescence	Mn ²⁺ – doped CdTe/ZnS QDs	0.10~50	0.20	[41]
Fluorescence	CsPbBr ₃ /Cs ₄ PbBr ₆ PNCs	10~800	1.695	This work

3.5. Fluorescence Quenching Mechanism of the $\text{CsPbBr}_3/\text{Cs}_4\text{PbBr}_6$ PNCs by Folic Acid

The mechanism for the fluorescence quenching of the PNCs by FA is discussed. As shown in Figure 8A,B, the high concentration of FA has a weak fluorescence and the mixing of FA and PNCs would quench their fluorescence. The UV absorption spectrum of FA overlaps with the excitation spectrum of the PNCs, so the inner filter effect may be one of the possible factors leading to the fluorescence quenching of the PNCs. However, later study found more evidence for the quenching of fluorescence. As shown in Figure 8C, the zeta potential analysis showed that FA and the PNCs have opposite charges (-23.4 and 79.0 mV, respectively) and the mixing of FA and the PNCs would reduce the distance between each other. The fluorescence decay of the PNCs decreased significantly in the presence of FA (Figure 8D), and the changes in fluorescence lifetime (shown in Table 2) indicated that the quenching process is that of dynamic quenching, which may be caused by the collision between the two via electrostatic interaction. The inner filter effect of PNCs and FA is not the only cause for the quenching of fluorescence because the fluorescence lifetime does not change in terms of the inner filter effect [53]. To further study the electrostatic interaction of FA and the PNCs, similar molecules (including methotrexate, pemetrexed, aspartic acid, and histidine) containing polar groups (such as OH, $-\text{COOH}$, and $-\text{NH}_2$) were used to interact with the PNCs. The zeta potential test results showed that methotrexate, pemetrexed, and histidine have negative charges on their surfaces and can interact with the PNCs electrostatically. However, the fluorescence of the PNCs did not change after interaction with these molecules (Supplementary Materials, Figure S7). Thus, electrostatic interaction is not a key factor for fluorescence quenching. Another possible reason is that the amino group on the surface of the PNCs interacts with the carboxyl group on the surface of folic acid to form a complex. The energy in the conduction band of the PNCs is transferred to the LUMO orbital of the complex, eventually leading to fluorescence quenching [54]. However, other similar molecules do not affect the fluorescence of the PNCs because the LUMO orbital energy is higher than the energy of the perovskite conduction band after the formation of the complex.

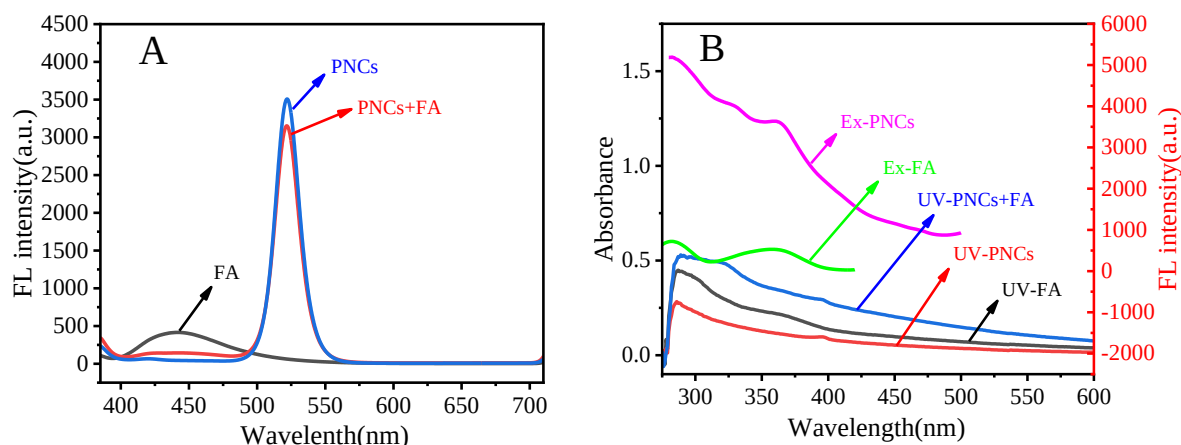


Figure 8. Cont.

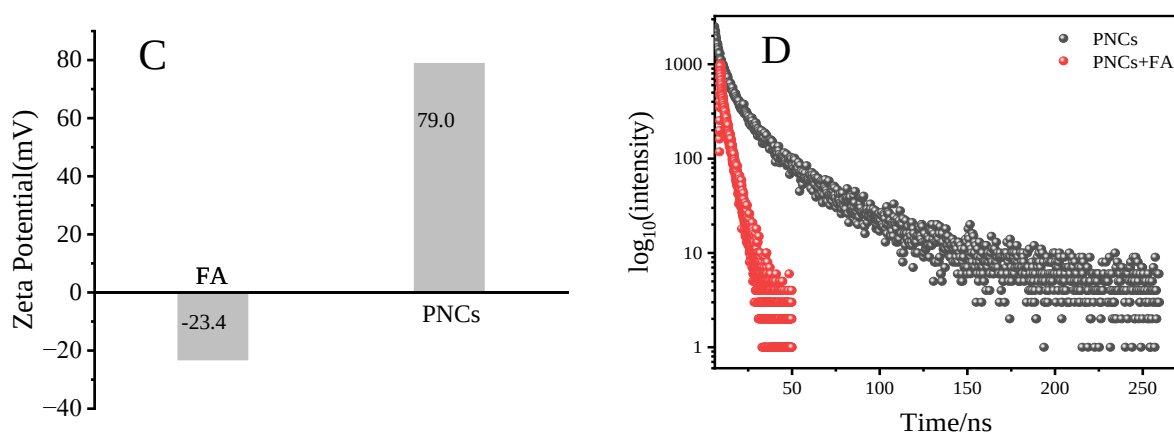


Figure 8. The fluorescence emission of CsPbBr₃/Cs₄PbBr₆ PNCs, FA, and CsPbBr₃/Cs₄PbBr₆ after adding FA spectra (A); the fluorescence excitation spectra of CsPbBr₃/Cs₄PbBr₆ PNCs and FA; UV-Vis absorption spectra of CsPbBr₃/Cs₄PbBr₆ PNCs, FA, CsPbBr₃/Cs₄PbBr₆ PNCs after adding FA (B); the Zeta potential of FA and CsPbBr₃/Cs₄PbBr₆ (C) and fluorescence lifetime decay curves of CsPbBr₃/Cs₄PbBr₆ in the presence and absence of FA (D). Experimental conditions: the concentration of CsPbBr₃/Cs₄PbBr₆ PNCs (0.156 mmol, in terms of Cs atoms), the concentration of FA was 20 μ M. The reaction temperature was 25 $^{\circ}$ C; the response time was 5 min.

Table 2. PL decay parameters of the CsPbBr₃/Cs₄PbBr₆ PNCs and CsPbBr₃/Cs₄PbBr₆ PNCs/FA mixture.

	τ_1 (ns)	τ_2 (ns)	τ_{average} (ns)
CsPbBr ₃ /Cs ₄ PbBr ₆ PNCs	2.81	17.55	8.28
CsPbBr ₃ /Cs ₄ PbBr ₆ PNCs + FA	18.19	3.43	3.97

3.6. Real Sample Detection

In order to further investigate the practicability and accuracy of the method, this experiment further adopted the standard addition method to detect an actual sample (urine). As shown in Table 3, the results show that the detection recovery rate of FA by this method is 99.04–106.1%. Therefore, we believe that the analytical method for the detection of FA using the PNCs as fluorescent probes is a highly selective and accurate method.

Table 3. Detection of FA in the actual samples.

Samples	Added (μ L)	Found (μ M)	Recovery (%)	RSD ($n = 3$, %)
1	0	N.D ^b	—	—
2	100.0	99.04	99.04	0.2520
3	350.0	339.4	96.98	0.1220
4	450.0	411.7	91.48	0.1960
5	600.0	657.1	109.5	0.2000

N.D^b means below the detection limit (not detected).

4. Conclusions

In summary, we have developed a method for the sensitive and selective detection of FA, based on fluorescence quenching. FA can effectively quench the fluorescence of the PNCs. After optimizing the synthesis conditions of the PNCs and the experimental conditions for the detection of FA, the fluorescence intensity of the PNCs was linearly correlated with the concentration of FA in the range of 10–800 μ M, and the LOD was 1.695 μ M. The proposed method was used to detect the folate measured in urine, with recoveries of between 99.04% and 106.1%. These satisfactory results demonstrate that this

novel sensing system greatly expands the application range of PNCs in the field of fluorescence detection. The mechanism of the FA and the PNCs interaction was also discussed. The results indicated the fluorescence quenching is caused by the electrostatic interaction between FA and PNCs, which reduced the distance between each one, transferring and consuming the exciting energy in this process.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/chemosensors11010019/s1>, Figure S1: Effect of the precursor reaction temperature on the fluorescence intensity of CsPbBr₃/Cs₄PbBr₆ PNCs; Figure S2: The effect of precursor reaction time on the fluorescence intensity of CsPbBr₃/Cs₄PbBr₆ PNCs (A and B); Figure S3: Effect of the volume ratio of the precursor to the ligand on the fluorescence intensity of CsPbBr₃/Cs₄PbBr₆ PNCs (A and B); Figure S4: The effect of the reaction time of precursor and ligand on the fluorescence intensity of CsPbBr₃/Cs₄PbBr₆ PNCs (A and B); Figure S5: Fluorescence lifetime map of CsPbBr₃/Cs₄PbBr₆ PNCs; Figure S6: Optimization of temperature (A) and time (B) for detection of FA by CsPbBr₃/Cs₄PbBr₆ PNCs. Experimental conditions: the concentration of CsPbBr₃/Cs₄PbBr₆ PNCs (0.156 mmol, in terms of Cs atoms), the concentration of FA is 200 μM; (A) reaction temperature: 4–60 °C; response time: 15 min; (B) reaction temperature: 25 °C, response time: 5–50 min; Figure S7: Zeta potential of different molecules and the PNCs (A); Effects of four similar molecules on the fluorescence intensity of the PNCs (B). Experimental conditions: The concentration of the four molecules was 200 μM.

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