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Additional Information

Water-assisted synthesis of stable and multicolored CsPbX₃@SiO₂ core-shell nanoparticles as fluorescent probes for biosensing

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ABSTRACT

Colloidal lead halide perovskite nanocrystals are highly luminescent materials with great promise as fluorescent probes in biosensing as long as their intrinsic instability in aqueous media is effectively addressed. In this study, we successfully prepared stable and multicolored CsPbX₃@SiO₂ (X = Cl/Br, Br and I) core-shell nanoparticles through a simple method based on the water-induced transformation of Cs₄PbX₆ to CsPbX₃, combined with sol-gel procedures. We observed that the concentration of Cs₄PbX₆ precursor plays a crucial role in the formation of isolated nanospheres with uniform silica coating and in controlling the number of core-free particles. Furthermore, our research expands this approach to other halide compositions, resulting in multicolor core-shell nanoparticles with emission wavelengths ranging from 490-700 nm, average sizes below 30 nm, and photoluminescence quantum yields close to 60%. Unlike previous reports, the silica coating boosts the photoluminescence quantum yields compared to uncoated counterparts and provides increased structural stability for more than four days. Moreover, a controlled thermal treatment confers water stability to the as-synthesized nanoparticles. To establish the feasibility of the developed materials as fluorescent probes, we successfully demonstrated their specific recognition of a humanized antibody (omalizumab) used in treating patients with severe allergic asthma. This work paves the way to develop *in vitro* tests using CsPbX₃@SiO₂ core-shell nanoparticles as a fluorogenic probe.

KEYWORDS

Perovskite nanoparticles, silica shell, color gamut, water stability, biosensing

1. INTRODUCTION

Colloidal nanocrystals (NCs) of CsPbX₃ metal halide perovskites (MHPs) have gathered significant attention in multiphoton-imaging applications and lighting materials. These NCs possess remarkable properties, including an ultrahigh photoluminescence (PL) quantum yield (~99%),¹ tunable emission properties with a wide color gamut², and unprecedented multiphoton-photon-excited up-conversion fluorescence³ Moreover, their ease of synthesis and cost-effectiveness further contribute to their appealing.⁴ As a result, these NCs have emerged as promising luminescent tags for imaging, biosensing, and clinical diagnosis. However, two main challenges need to be addressed to establish their viability as luminescent probes. First, the synthesis of water-stable NPs is essential to ensure their stability and functionality in aqueous media. Secondly, it is crucial to fabricate nano-sized monodisperse particles that remain structurally intact under various chemical environments and processing conditions, enabling precise biomolecule conjugation at a single particle level.^{5,6}

To overcome this matter, the surface of the NCs needs to be protected with materials that chemically prevent the water from reaching the NC surface. A wide range of materials including metal oxides, polymers, MOFs, metal chalcogenides, and perovskite derivatives have been used as shells to protect the surface of perovskite NCs.⁷ Among various encapsulants, SiO₂ has received significant interest because of its transparency, biocompatibility, and chemical stability. However, coating SiO₂ shells on MHP NCs is challenging because the hydrolysis reaction requires some amount of water that degrades the NCs. Several attempts have been made to coat the SiO₂ on MHP NCs reducing or eliminating the amount of water, yielding multiple NC-embedded SiO₂ matrices.⁸⁻¹⁶ Another approach that is often used is the in situ crystallization of perovskite NCs inside mesoporous silica nanoparticles by intercalating salt precursors and thermal treatment. The studies showed that mesoporous encapsulation enhances the stability of MHPs and prevents the halide ion exchange when NCs of two different halides are mixed.^{17,18}

In an alternative promising strategy for encapsulating metal halide perovskites, several research groups have exploited the transformative properties of metal halides, specifically the transition from 0D Cs₄PbBr₆ to 3D CsPbBr₃ transformation features to synthesize core-shell nanoparticles and nanocomposites. This transformation can be achieved by creating a Pb-enriched environment¹⁹ or a Cs-deficient situation where the CsPbX₃ phase is more stable. For example, in nonpolar-water interphase, CsX ions are stripped from the Cs₄PbX₆ due to their ionic nature and high solubility in water,²⁰ resulting in luminescent CsPbBr₃ NCs. Other approaches involve the use of Cs⁺-adsorbent materials like Prussian blue²¹ and thermal annealing as a physical strategy to induce phase conversion.²² In addition to these approaches, Udayabhaskararao *et al.* described spontaneous transformation from CsPbX₃ into Cs₄PbX₆ NCs (and *vice versa*) by adjusting the oleylamine: oleic acid ratio.²³ Recently, Baranov *et al.* reported that poly(maleic anhydride-1-*alt*-octadecene) (PMAO) triggered phase transformation²⁴ where the polymer destabilizes the Cs₄PbX₆ NC surface by removal of olefin ligands, inducing CsPbBr₃ NCs formation.

The route of transformation from 0D to 3D results in the production of CsPbBr₃ NCs that exhibit improved stability in polar media²⁰ This issue presents an opportunity to apply the systematic sol-gel process to grow a silica (SiO₂) shell on the NC surface, thereby enhancing their stability to moisture. Some studies have successfully combined transformation-triggering conditions with a sol-gel process. For instance, Hu *et al.* encapsulated CsPbX₃ (X = Cl/Br, Br, Br/I and I) into a Janus-type SiO₂ heterostructure of 18 nm and PLQY of 80%. To obtain entirely coated NPs, Li *et*

al. incorporated a pre-silanization step of Cs₄PbBr₆ NCs with partially hydrolyzed TMOS (PH-TMOS).²⁵ This method introduces silanization seeds on MHP NCs surface before phase conversion and encapsulation, rendering CsPbBr₃@SiO₂ core-shell NPs of 60 nm and PLQY of 65%. In an alternative approach, Park *et al.* use nitric acid to accelerate the hydrolysis of tetraethyl orthosilicate (TEOS).²⁶ However, instead of obtaining isolated colloidal NPs, they obtained composites. On this research line, Rossi *et al.* exploited a transformation route triggered by maleic anhydride, synthesizing CsPbBr₃@SiO₂ NPs of 22-33 nm with PLQY of 1-4%.²⁷

In this study, we have successfully synthesized multi-colored CsPbX₃@SiO₂ NPs (X = Cl/Br, Br and I) core-shell NPs with remarkable and tunable emission wavelength, which offers excellent potential as a multimodal fluorescent tag for imaging and biosensing, in health or biotechnological applications. Our work is based on the water-triggered phase transformation of 0D Cs₄PbBr₆ NCs to CsPbBr₃ in the presence of TMOS. Moreover, long-term stable and water-dispersible perovskite NPs were also obtained under a controlled thermal treatment. Finally, to demonstrate the suitability of these materials as fluorescent labels, as-synthesized NPs were conjugated to a humanized antibody, and the specific recognition for its paratope was proven in a direct immunoassay as a proof of concept.

2. EXPERIMENTAL SECTION

2.1. Materials

Cesium carbonate (Cs₂CO₃, 99.9%, Sigma Aldrich), lead bromide (PbBr₂, ≥98%, Sigma Aldrich), lead iodide (PbI₂, 98.5%, Alfa Aesar), lead chloride (PbCl₂, 99%, Alfa Aesar), 1-octadecene (ODE, 90% tech, Sigma Aldrich), oleic acid (OA, tech. 90%, Alfa Aesar), oleylamine (OAm, approximate C18-content 80-90%, Sigma Aldrich) tetramethyl orthosilicate (TMOS, 99%, ACROS Organics), toluene and hexane.

2.2. Synthesis of Cs₄PbX₆ NCs (X = Br, I and mixed composition of Cl/Br)

Cs₄PbX₆ NCs were prepared via the hot-injection method described by Akkerman with some modifications.¹⁹ Briefly, a 100 mL 3-neck flask containing 0.2 mmol PbX₂ (0.1 mmol of each lead halide for the mixed composition), 5 mL ODE and 0.2 mL OA was heated under mild stirring and vacuum at 120 °C for 30 min. Then, 1.5 mL OAm was injected under an inert atmosphere until the dissolution became clear, indicating PbX₂ was dissolved entirely, and the flask was cooled down to room temperature.

The flask was heated again under vacuum and mild stirring and reached the optimal temperature (60 °C for PbI₂, 80 °C for PbBr₂, and 100 °C for the mixture PbCl₂ + PbBr₂), it was left for 15 min more. After injection of 0.5 mL Cs-OA (400 mg Cs₂CO₃ previously dissolved into 8 mL OA under vacuum at 100 °C), the dissolution turned turbid within 15-30 s, and finally, the flask was cooled down to room temperature. The total volume was distributed into four microtubes and centrifuged at 4.000 rpm for 5 min. The sediment was dispersed in ~ 5 mL of anhydrous hexane. The concentration of the stock solution was determined by weighing mass residues after solvent removal during the purification step: 25.74 ± 3.38 mg/mL for Cs₄PbBr₆, 35.19 ± 1.07 mg/mL for Cs₄PbI₆ and 28.75 ± 1.54 mg/mL for Cs₄Pb(Cl_xBr_{6-x}).

2.3. Synthesis of CsPbX₃@SiO₂ core-shells (X = Br, I and mixed composition of Cl/Br)

To prepare core-shell NPs, 2 µL of partially hydrolyzed TMOS (prepared by adding 2 mL toluene, 12 µL milli-Q H₂O filtered by 0.45 µm and 198 µL TMOS into a glass vial and stirred at 500 rpm for 18 h) were added to 2 mL of a solution of Cs₄PbX₆ NCs. After stirring at 500 rpm for 5 h, 10

μL , TMOS was added under magnetic stirring at 900 rpm for 1 min. After, 300 μL milli-Q H_2O was added under magnetic stirring at 1200 rpm for 2 min. The vial remained undisturbed overnight. Core-shell NPs were purified by centrifugation at 9.000 rpm for 5 min.

To obtain water-resistant core-shell NPs, 5 mL of the $\text{NCs}@SiO_2$ NPs were placed in a porcelain plate and calcinated at 565°C for 10 min with a mean heating rate of $1^\circ\text{C}/\text{min}$ in the muffle furnace under an air atmosphere. After cooling to room temperature, the sample was dispersed in water and ultrasonicated for 30 min before TEM preparation.

2.4. Immunoassay

(i) *Preparation of core-shell NPs derivatized with amine groups ($\text{MHP}@SiO_2\text{-NH}_2$)*. 10.5 mg of $\text{CsPbX}_3@SiO_2$ NPs and 6 μL of (3-aminopropyl)triethoxysilane were added to 1 mL of anhydrous toluene and stirred for 5 h. Functionalized NPs were purified by centrifugation at 9.000 rpm and resuspended first in ethanol and then in 3.75 mL phosphate saline buffer. (ii) *Preparation of NP- αlgE perovskite-Omalizumab conjugates*. 25 μL of Omalizumab (humanized antibody used in asthma treatment) at 1.5 mg/mL were added to $\text{MHP}@SiO_2\text{-NH}_2$ NPs and incubated overnight at 4°C . Conjugated NPs were centrifugated, and the resultant sediment was dispersed in 250 μL of 10 mM sodium phosphate buffer, 150 mM NaCl, 0.05% Tween 20%, pH 7. (PBS-T). (iii) *Direct immunoassay*. A black polystyrene 96-well plate was coated overnight at 4°C with $5\text{ mg}\cdot\text{L}^{-1}$ of specific paratope receptor (100 $\mu\text{L}/\text{well}$) in 50 mM sodium carbonate/bicarbonate buffer, pH 9.6. Ovalbumin was used ($5\text{ mg}\cdot\text{L}^{-1}$) as a negative control. The plate was washed four times with PBS-T. Then, 100 $\mu\text{L}/\text{well}$ of the NP- αlgE conjugates were added and incubated for 1 h at room temperature. After washing the plate six times with PBS-T, PL spectra were measured using the EnSpire Multimode Plate Reader (PerkinElmer).

2.5. Characterization methods.

UV-vis absorption spectra were recorded using a UV-visible spectrophotometer (Agilent 8453, Agilent Technologies). The photoluminescence spectra were obtained with a spectrofluorometer (PTI QMA4, Horiba) with an excitation wavelength of 355 nm for all spectra. PLQY was determined by using Quinine Sulfate in H_2SO_4 0.5 M ($\Phi = 0.45$) as a standard for $\text{CsPb}(\text{Cl}_x\text{Br}_{3-x})$ and CsPbBr_3 NPs with an excitation wavelength of 310 nm. TEM images were taken by a transmission electron microscope at 120 kV (JEM-1400, Flash) and a field emission transmission electron microscope at 200 kV with an X-Ray detector (JEM 2100F, JEOL).

3. RESULTS AND DISCUSSION

For the preparation of core-shell NPs, Cs_4PbX_6 NCs ($X = \text{Cl}/\text{Br}$, Br, and I) were synthesized according to the procedure described by Akkerman.¹⁹ The synthesis yielded NPs that present the characteristic absorption peaks at 305, 314, and 367 nm (Figure S1) and exhibit hexagonal or rhombohedral morphology with mean particle sizes of 18.97 ± 2.84 nm, 14.83 ± 1.62 nm, and 17.59 ± 2.99 nm, respectively (Figure S2).

Cs_4PbX_6 NCs were first silanized using partially hydrolyzed TMOS (PH TMOS). At this step, silanol groups of TMOS, attached to the Cs_4PbX_6 NCs surface, replace the original hydrophobic olefin precursor and introduce growth sites for condensation of the SiO_2 network. In the second step, silane-derivatized NCs were treated with TMOS aqueous solution, which promotes 0D to 3D phase transition via CsX stripping and shell formation around the transformed NCs via

condensation of TMOS.²⁵ A schematic representation of the formation mechanism of core-shell NPs from Cs₄PbX₆ NCs is illustrated in Figure 1.

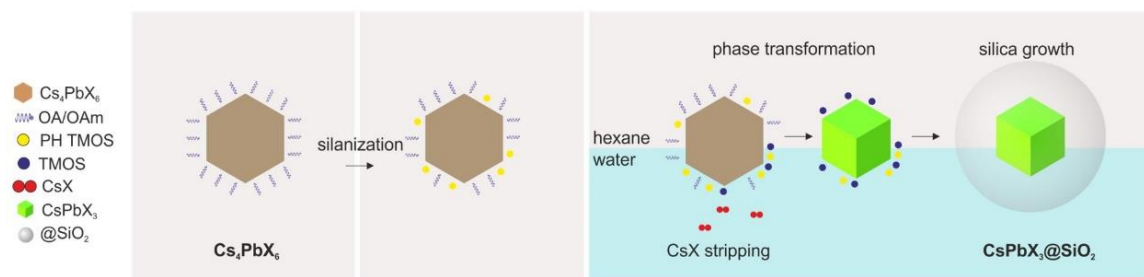


Figure 1. Scheme of the core-shell NPs formation mechanism.

To optimize the Cs₄PbX₆ NCs concentration for the synthesis of CsPbX₃@SiO₂ NPs, serial dilutions were tested, starting from the following concentrations of the purified NCs: 14.4, 25.7 and 17.6 mg/mL for Cs₄Pb(Cl_xBr_{6-x}), Cs₄PbBr₆ and Cs₄PbI₆, respectively. Figure S3 summarizes the transmission electron microscopy (TEM) results for three representative concentrations per composition, and Table S1 provides structural features of the resulting core-shell NPs.

It should be observed that, independently of the halide composition, high starting material concentrations produce a heterogeneous population of NPs, containing uncoated crystals of different sizes and shapes and silica spheres with or without a nanocrystal core. It may be associated with the more extensive formation of CsPbBr₃ NCs, which reduces the coating efficiency of the silane, leading to the evolution of the uncoated NPs into other perovskite structures.²⁸ At an intermediate concentration, uniform-size monodispersed NPs are prevalent, while at low concentrations, the presence of core-free silica nanoparticles increases. It could be explained because decreasing CsPbBr₃ NCs would facilitate homogeneous nucleation, resulting in the prevalence of core-free NPs.

Figure S3 shows how remarkable is the reduction of core size when comparing the intermediate and the lowest concentration (from 11 to 3 nm in CsPb(Cl_xBr_{3-x})@SiO₂, from 4 to 2 nm in CsPbBr₃@SiO₂ and from 2 nm to almost undetectable or coreless nanospheres CsPbI₃@SiO₂). This behavior is due to the increase in the water: Cs₄PbX₆ ratio, which shifts the equilibrium of the CsX stripping reaction (Cs₄PbBr₆ → CsPbBr₃ + 3CsX), producing smaller core particles.

Regarding the emission properties, the right panel of Figure S3 shows PL intensity and maximum wavelength for different tested serial dilutions. It must be noticed that the emission peak presents similar features for the intermediate and lower concentration, while a slight blue shift is observed for the highest one. This shift could be attributed to the heterogeneous NPs population obtained under the synthesis conditions.

Attending to the particle uniformity and PL emission intensity, we chose 7.2 mg/mL, 3.2 mg/mL and 2.2 mg/mL Cs₄PbX₆ (X = Cl/Br, Br and I) as the optimal concentrations for preparing CsPbX₃@SiO₂ NPs. Figure 2 depicts the absorption/emission spectra and TEM images of the resulting nanoparticles. Table 1 gathers information about the emission peak, full width at half maximum (fwhm), PLQY, mean particle diameter, core size, and the number of cores per particle, including NPs features of the most representative works in the literature. From this data, it is noteworthy that all samples showed relatively sharp peaks with the full width at half-maximum in the 13 to 34 nm range. Our developed CsPbX₃@SiO₂ core-shell NPs present the tiniest particle

sizes reported in the literature by water-assisted synthesis methodologies while preserving emission quantum yield. Also, the methods described are extended to other halide compositions of NPs, obtaining multi-colored NPs with a color gamut from blue to red. Concerning PLQY, coated NPs exhibit an enhancement of emission efficiency of 44% and 32% regarding uncoated CsPb(Cl_xBr_{3-x}) and CsPbBr₃ counterparts. These results highlight the beneficial role of silica in shallow defects passivation of CsPbX₃ NCs formed via water-induced transformation of Cs₄PbX₆ NCs. It should be considered that, according to the literature, NPs of comparable size to ours are poor emitters.²⁷ Paying attention to our data, it is worth mentioning that we synthesized core-shell NPs that cover all the practical needs to be exploited as a fluorescent label for biosensing, such as tunable emission properties through the visible region, sharp emission peaks, and reduced particle size (20-30 nm). Although works exist that describe core-shell NPs with significant PLQY and within those sizes, they are devoted to Br compositions, do not report evidence on the formation of core-shell NPs of other halide compositions, and do not provide size dispersion analysis.²⁹⁻³¹

Table 1. Properties of the as-prepared CsPbX₃@SiO₂ NPs (X = Cl/Br, Br, and I) at the selected concentrations: 7.2, 3.2 and 2.2 mg/mL, respectively.

<i>Composition</i>	<i>Emission peak (nm)</i>	<i>Fwhm (nm)</i>	<i>PLQY</i>	<i>Particle size (nm)</i>	<i>Core size (nm)</i>	<i>Cores/Particle (nm)</i>	<i>Ref.</i>
<i>Water-triggered Cs₄PbBr₆ to CsPbBr₃ phase transition</i>							
<i>CsPb(Cl_xBr_{3-x})@SiO₂</i>	492	26	18%	30.06 ± 2.98	11.62 ± 2.1	1	This work
<i>CsPbBr₃@SiO₂</i>	506	22	57%	22.15 ± 1.94	4.26 ± 0.9	1	
<i>CsPbI₃@SiO₂</i>	689	33	N.R*	33.89 ± 4.82	2.05 ± 0.46	5	
<i>CsPbBr₃@SiO₂</i>	521	16.4	65%	60	12.25	1	²⁵
<i>CsPbBr₃@SiO₂</i>	507	20	8%	17	10	1	²⁷
<i>Ligand assisted reprecipitation</i>							
<i>CsPbBr₃@SiO₂</i>	501	22	88	26	10.5	1	²⁹
<i>Hot injection</i>							
<i>CsPbBr₃@SiO₂</i>	519	16	87	NR	NR	1	³⁰

*Not reported. PLQY for CsPbI₃ was not determined due to the low sample stability.

Concerning the core dimensions' size-shrinkage of CsPbX₃ compared to their precursor NCs, it is explained by the CsX-stripping mechanism.^{20,24,25,32} Cs₄PbX₆ NCs are CsX-rich perovskite structures; therefore, upon water treatment, stripping of CsX occurs because of the ionic nature of Cs₄PbX₆ and the very high solubility of CsX in water, which lead to the decomposition of

Cs_4PbX_6 and the formation of CsPbX_3 NCs. During the process, the rhombohedral Cs_4PbX_6 NCs are converted to simple cubic structured CsPbX_3 NCs, and it could be confirmed by the shrinkage of particle size.²⁰ In our work, the reported size reduction (32%, 75%, and 88% for X = Cl/Br, Br, and I) is more significant than the one described in the literature. This fact might be caused by the harsher conditions in our method, which ends up etching, to a greater extent, the 3D nanocrystals surface. On the other hand, it also appears to exist a correlation between the halide nature and the size and number of cores per particle. We attributed this behavior to the relative extrinsic stability of ternary cesium halide perovskites towards water and oxidant environment, which decreases in the sequence $\text{CsPbCl}_3 > \text{CsPbBr}_3 > \text{CsPbI}_3$. This behavior may be associated with substituting larger I, by Br or Cl, leading to the reduction of lattice constants and transition to the cubic phase, a more compact and stable structure than the tetragonal pseudo-cubic phase.^{33,34} Hence, the degradation of the as-formed NCs is accelerated in iodine species, leading to crystal decomposition, core size reduction before silica shell formation, and simultaneous encapsulation of several cores per particle.

Table S2 and Figure S4 show the coexistence and ratio of core-shell to empty SiO_2 nanoparticles for each composition. The results are 29.1% in $\text{CsPbCl}_x\text{Br}_{3-x}@\text{SiO}_2$, 78.2% in $\text{CsPbBr}_3@\text{SiO}_2$, and 6.8% in $\text{CsPbI}_3@\text{SiO}_2$. The presence of core-free silica nanoparticles in the samples could be explained according to LaMer theory^{35,36}, which claims that to ensure coated perovskite NCs without core-free SiO_2 particles, the monomer concentration must fulfill the condition $C < C_{\text{homo}}$ throughout the reaction process, where C_{homo} is the homogeneous nucleation TMOS concentration threshold. In our method to prevent perovskite degradation, the concentration of TMOS is large, thus co-existing core-shell and core-free nanoparticles in the final product. Future research is mandatory to achieve effective coating without core-free silica NPs as long as they lead to a loss of the effective fluorescence signal. This matter could be addressed by: i) The fractionated drop method which can always meet the above-mentioned condition, in which fresh TMOS is added after the previous TMOS is consumed. ii) Replacing TMOS with TEOS, which presents slower condensation rates, thus reducing the homogeneous nucleation threshold.

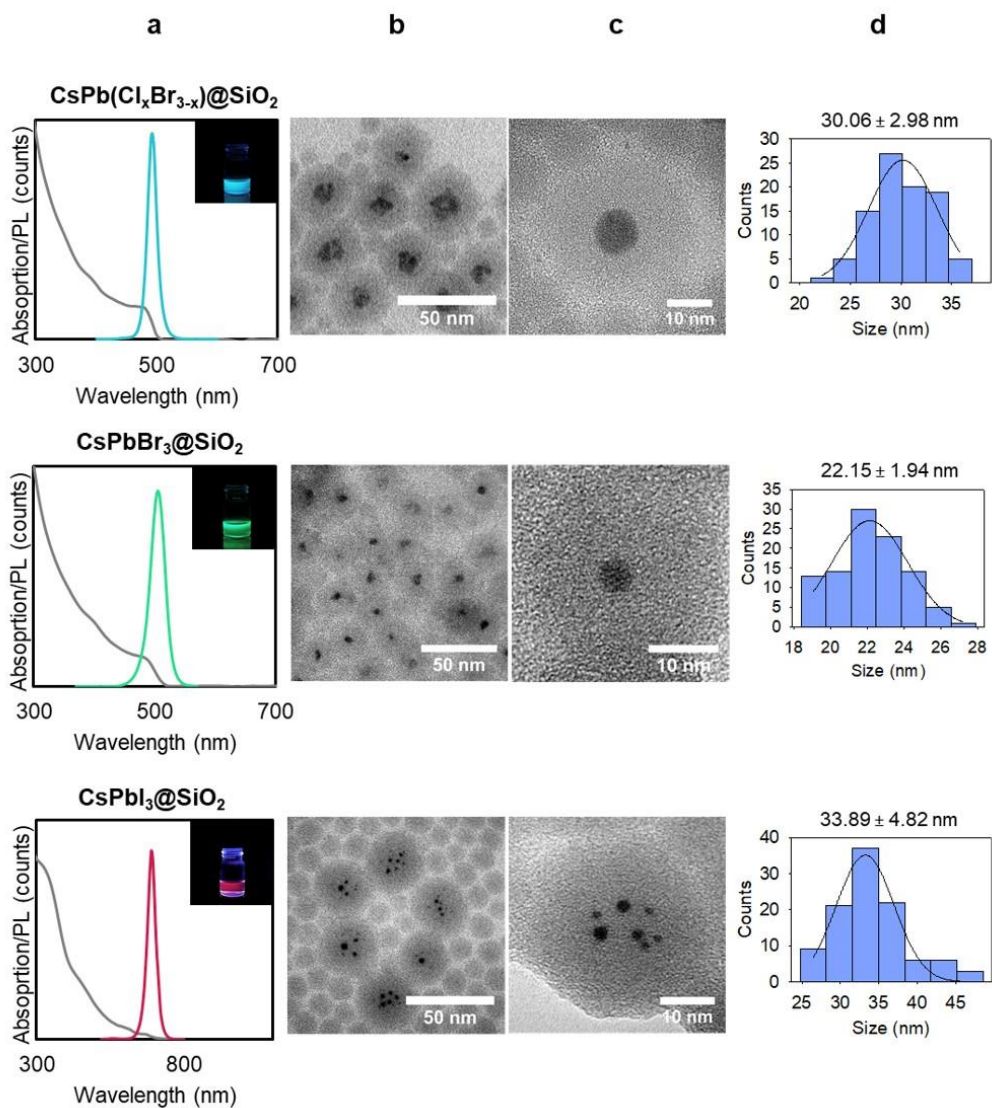


Figure 2. Optical and structural characterization of CsPbX₃@SiO₂ NPs. (a) UV-Vis absorption and PL spectra recorded at an excitation wavelength of 355 nm (b) low and (c) high magnification TEM images and (d) histogram showing the particle size distribution.

A set of coated and uncoated NPs samples with comparable concentration (i.e., equal absorbance value at 310 nm) were prepared, and their PL was recorded for fresh samples and after four days (storage RH 60%) to evaluate structural stability. PL spectra and pictures of samples under UV-light are presented in Figure 3. Remarkably, PL intensity diminishes faster over time in the uncoated NPs. CsPb(Cl_xBr_{3-x}) NCs suffer a decay of 75%, and the emission peak is blue-shifted from 488 to 458 nm after four days. It could be associated with reduction of Br amount in CsPbX₃ that blue-shifts the emission peak. In the case of CsPb(Cl_xBr_{3-x})@SiO₂ NPs, it barely moves 7 nm and boosts its PL intensity up to 60%; this increase in emission yields over time could be attributed to halide diffusion and rearrangement on nanoparticles confined perovskite NCs, reducing nonradiative losses. CsPbBr₃ NCs' photoluminescence was quenched after one day, while CsPbBr₃@SiO₂ maintained strong green emission after four days. These results indicate that the SiO₂ coating confers structural stability at a certain degree against moisture, except for the iodine samples, which both quickly suffer from degradation. This latter could be attributed to the iodine-based perovskites' poor phase and chemical stability, making it difficult to obtain stable NCs in the red region, also called the "perovskite red wall."³⁷

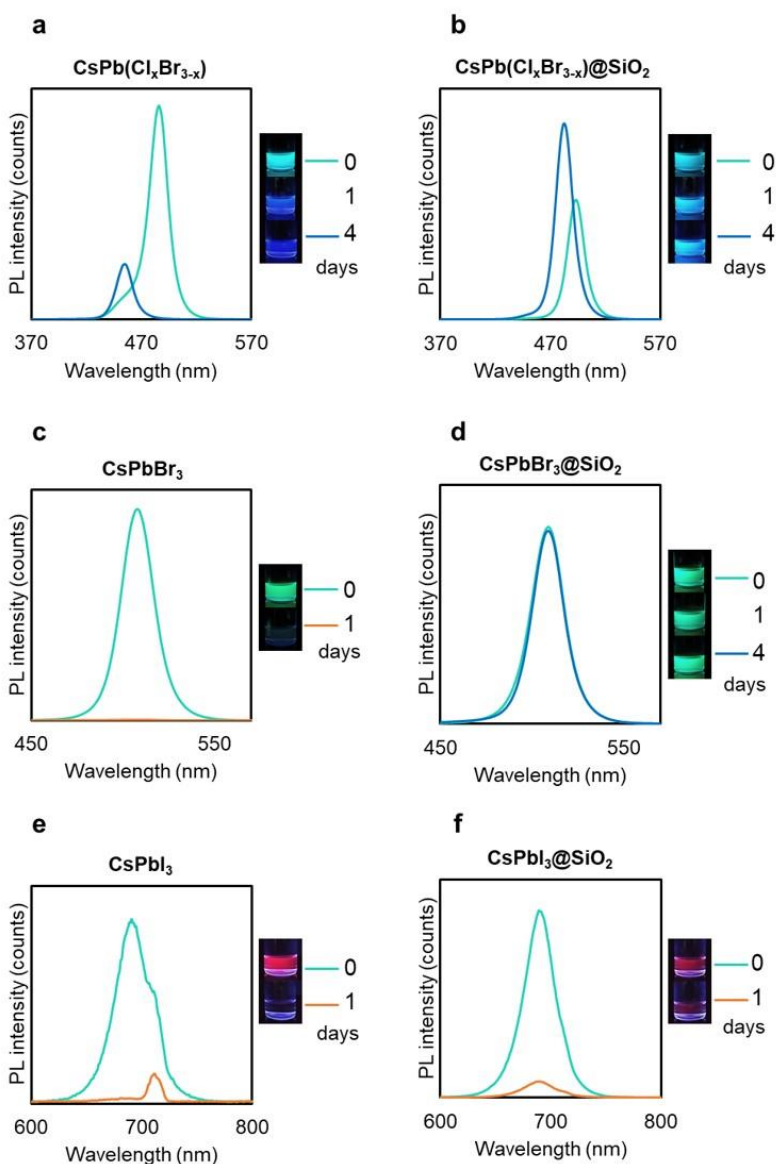


Figure 3. Emission spectra recorded at an excitation wavelength of 355 nm for fresh (orange) and samples stored at RH 60% (blue) of non-encapsulated CsPbX₃ NCs (a, c and e) and CsPbX₃@SiO₂ NPs (b, d, and f) prepared at an equal concentration.

Finally, despite the structural stability conferred by the SiO₂ shell coating, it cannot withstand the diffusion of water molecules. Consequently, the developed core-shell NPs suffer fast degradation in water. Intending to obtain water-resistant NPs, we performed a straightforward approach based on strategical silica pore collapse under thermal treatment.³⁸ For this purpose, core-shell NPs were thermally treated at 565 °C. Under these conditions, the pores of the silica shell transform into a compact network. In parallel, the sublimated perovskite NCs are retained inside the silica structure. In the cooling down process, CsPbX₃ NCs are formed again, conferring bright photoluminescence and complete stability against water media to the resulting nanoparticles.

The thermally treated particles maintain their stability in water for four days (Figure 4). Elemental EDS analysis of calcinated samples is depicted in Figure S4. The EDS patterns showed an atomic ratio of O to Si of 2. After extracting the silica contribution from elemental composition analysis we observed that mixed halide CsPb(Br_{0.7}Cl_{0.3})₃ contains 70% bromine and 30% chloride. Besides that elemental atomic ratio (Cs: Pb:X) of small nanocores trapped inside are (1:0.5:0.5) and (1:1.1:0.3) for CsPbBr₃ and CsPb(Br_{0.7}Cl_{0.3})₃, respectively. The deviation from stoichiometry can be attributed to the poor reliability for EDS quantification when the particles are too small and the thickness of the shell is remarkable. According to the literature, our described NPs exhibit more extended stability in pure water solvent (See Table S1 in the supporting information section). In this context, it should be remarked that conventionally fluorescent probes are presented as lyophilized nanoconjugates in buffer-free media, and immunoassay takes time lower than 1 hour, thus making those NPs suitable for their use as fluorescent probes.

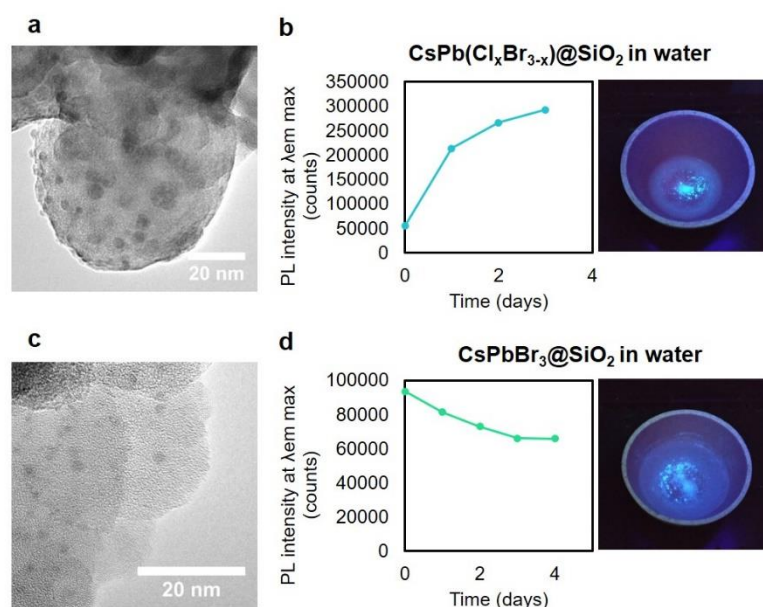


Figure 4. TEM images of the calcinated (a) CsPb(Cl_x/Br_{3-x})@SiO₂ and (c) CsPbBr₃@SiO₂ NPs and PL intensity at maximum emission versus time in water (b and d).

Furthermore, as proof of concept, we tested the as-synthesized NPs as fluorogenic labels for the *in vitro* detection of a humanized antibody (Figure 5a). For this purpose, core-shell NPs were conjugated by direct adsorption with omalizumab (NP-αIgE), a therapeutic antibody used for

treating severe asthma. A multi-well black polystyrene plate was coated with a specific receptor for the paratope of the humanized antibody ($5 \text{ mg}\cdot\text{L}^{-1}$). Then, the coated wells were incubated for 1 h at $25 \text{ }^\circ\text{C}$ with the fluorogenic conjugate, which selectively recognized the receptor. Finally, photoluminescence spectra were recorded using the EnSpire Multimode Plate Reader (PerkinElmer). As a negative control, wells were coated with ovalbumin protein at $5 \text{ mg}\cdot\text{L}^{-1}$ (for further experimental details, see the experimental section).

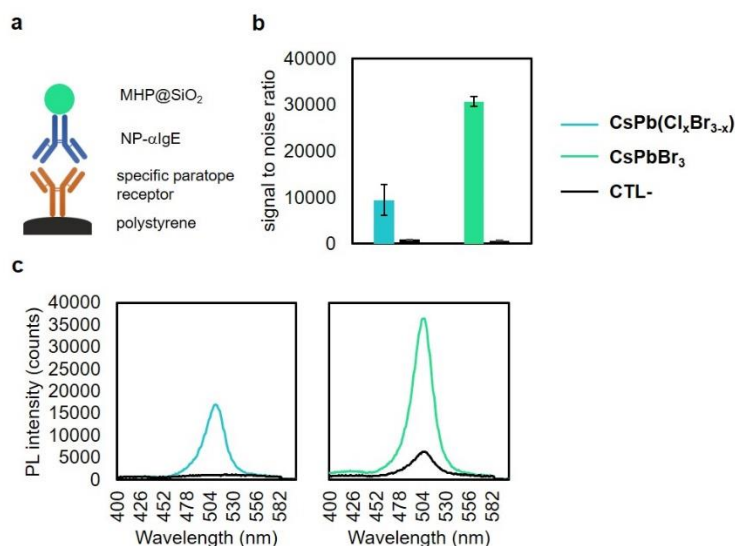


Figure 5. (a) Schematic illustration of the fluorogenic recognition event. (b) Signal to noise ratio, and (c) photoluminescence spectra of the test (colored line) and control assay (black line).

Figures 5b and c show the signal-to-noise ratio and photoluminescence spectra for polystyrene wells incubated with NP- α IgE conjugates. Our results confirm the successful chemical biorecognition of allergen-specific humanized antibodies, achieving a signal-to-noise ratio of ~ 10000 and 30000 for CsPb(Cl_xBr_{3-x}) and CsPbBr₃, respectively. Besides, the control experiment showed negligible photoluminescence signal, revealing the excellent selectivity of the assay. Remarkably, CsPb(Cl_xBr_{3-x}) presents a similar emission wavelength to CsPbBr₃ ($\sim 505 \text{ nm}$). This fact can be attributed to the poor amount of Cl in the hybrid structure after thermal treatment, as shown in Figure S4. We are expanding the methodology to other halide compositions to develop multiplexed immunoassays. Hence, the findings presented herein provide initial evidence of the potential of perovskite nanoparticles as a new fluorogenic probe in immunochemistry. These results pave the way for developing immunoassay tests. As for concerns regarding the toxicity associated with the presence of lead in perovskite compositions, it is essential to note that there is a wide range of *in vitro* applications in biosensing (luminescent microarrays, flow cytometry, paper-based immunoassays, etc.) and bioimaging (immunostaining, cell histology, *in vitro* cell tracking, etc.) where lead toxicity is not a limiting factor. Additionally, it is worth mentioning that while this work serves as a proof of concept for the suitability of these materials as fluorescent labels, ongoing research is actively exploring lead-free perovskite NCs with reduced toxicity. Thus, we anticipate that this methodology can soon be extended to lead-free perovskite NPs.

4. CONCLUSIONS

In summary, we have developed a simple method for synthesizing CsPbX₃ core-shell nanoparticles. This novel approach involves the transformation of water-triggered 0D Cs₄PbBr₆ to CsPbX₃, followed by encapsulation inside a silica shell using alkoxy silane. Our findings indicate

that the initial concentration of Cs_4PbX_6 plays a crucial role in achieving a uniform distribution of monodisperse perovskite core-shell NPs and controlling the number of core-free NPs. By optimizing our method, we successfully obtained core-shell nanoparticles with emissions ranging from 480-690 nm. These nanoparticles exhibited small particle sizes (20-30 nm), narrow size distribution cores (2-11 nm), and sharp emission peaks (fwhm of 20-30 nm). Moreover, the core-shell nanoparticles demonstrated structural stability and maintained their luminescent properties under RH of 60% for up to four days. Although previous reports described perovskite core-shell NPs obtained by similar methodologies,^{25,27} it is important to highlight that our work goes one step ahead and extends this method to other halide compositions, achieving NPs with a color gamut covering most of the visible region. In addition, according to the literature, the size reduction presents a deleterious effect on PLQY, and CsPbBr_3 NPs described in the literature prepared by exploiting transformative features with sizes below 30 nm are poor emitters. Their PLQY is almost totally quenched upon the coating with SiO_2 . Conversely, our NPs present PLQY of 18% and 57% for $\text{CsPb}(\text{Cl}_x\text{Br}_{3-x})@\text{SiO}_2$ and $\text{CsPbBr}_3@\text{SiO}_2$ versus 10% and 43% of the uncoated counterparts, meaning that the coating yields a recovery factor of 80% and 32%. This data points out the dual functionality of silica shell that reduces nonradiative losses via shallow defects passivation of CsPbX_3 NCs and prevents their structural degradation.

Furthermore, this size reduction has significant implications for the practical application in biosensing, as their dimensions are comparable to functional biological units (protein, enzymes, nucleic acids), allowing for a single tag per biomolecule ratio.⁶ Additionally, by employing controlled post-synthetic thermal treatment, we achieved water-resistant NPs through strategic silica pore condensation of the silica shell pores into a compact shell structure. Remarkably, our developed NPs meet the requirements of size, color gamut, and emission yields, along with their mild synthesis conditions, positioning them as promising labels with the potential to outperform organic dyes, fluorescent proteins, and chalcogenide QDs. The water-stable NPs were successfully conjugated with a humanized antibody to evaluate their viability as fluorescent reporters, demonstrating specific recognition for its paratope.

Although further research is required to synthesize water-stable labels of different halide compositions, we believe that MHP NPs will enable the development of sensitive, multicolor, and multiplexed assays, opening exciting possibilities for their applications in life sciences.

CONFLICTS OF INTEREST

There are no conflicts to declare.

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ASSOCIATED CONTENT

Supporting information. Optical and structural characterization of Cs_4PbX_6 NCs, TEM microscopy and EDS analysis of core-shell NPs, description of optical and structural features of core-shell NPs obtained at different synthesis conditions.

AUTHORS CONTRIBUTION

C.C designed the methodology, performed the experiments, and wrote the original draft. W.T. carried out thermal treatment studies to obtain water-stable nanoparticles. P.Q.C and S.M design the immunoassay and provide the immunoreagents. V.G.P conceptualized the investigation and co-wrote, reviewed, and edited the manuscript. M.J.B. and A.M. supervised this study and acquired financial support for the project leading to this publication. All the authors proofread and approved the final manuscript for submission.

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