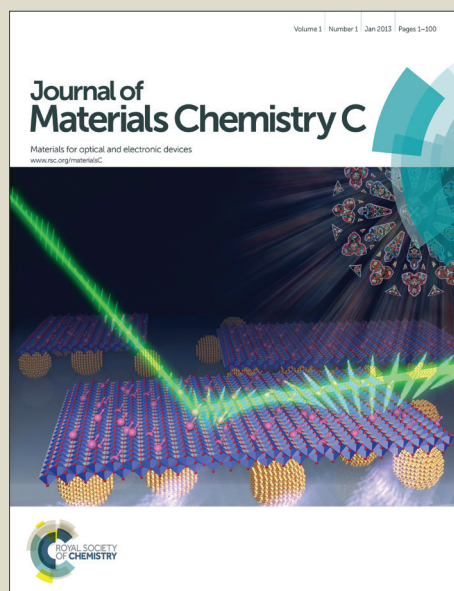


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ARTICLE

7 Highly luminescent covalently bonded double 8 hydroxide nanoparticle-fluorescent dye nanohybrids 9

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12 Incorporation of fluorescein dye molecules into layered double hydroxide (LDH) nanoparticles to form
13 nanocomposites has great potential for optical and biological applications. Herein, we report a novel
14 highly luminescent covalently bonded LDH-sodium fluorescein dye nanohybrid. This nanocomposite
15 shows a significantly higher quantum yield (QY) of 55.1% than the nanohybrids prepared from the
16 previously reported anion exchange (3.0%) and coprecipitation (12.4%) approaches. Our LDH-sodium
17 fluorescein nanohybrid is superior to the pure fluorescent dye in a number of perspectives. Firstly, the
18 nanohybrid exhibits an excellent linearity between the fluorescence intensity and the concentration in a
19 certain range. Secondly, the nanohybrid remains fluorescent even in dry powder form. Thirdly, the
20 nanohybrid can self-assemble to a transparent and free-standing film which is fluorescent under UV,
21 which will lead to important applications in optical devices.
22

23 Introduction

24 Layered double hydroxide (LDH) is an unusual class of
25 layered materials composed of bivalent and trivalent metallic
26 cations (M^{2+} and M^{3+}) in layers and negative charged anions
27 occupying the gallery in between.¹⁻³ The LDH family can be
28 expressed as $[M^{2+}_{1-x}M^{3+}_x(OH)_2][A^{n-}]_{x/n} \cdot zH_2O$, where M^{2+}
29 may be Mg^{2+} , Zn^{2+} , or Ni^{2+} and M^{3+} may be Al^{3+} , Ga^{3+} , Fe^{3+} , or
30 Mn^{3+} ; A^{n-} as a charge compensating anion may be CO_3^{2-} , Cl^- ,
31 etc.³ Since A^{n-} functions as non-framework anions, other types
32 of negatively charged ions can easily enter the gallery between
33 the LDH layers by ion exchange. Owing to the powerful anion
34 exchange capacity, positive surface charge and excellent
35 biocompatibility, LDH nanoparticles have been widely used as
36 carriers of negatively charged genes such as DNA and siRNA
37 for intracellular delivery and other biomedical applications.⁴⁻¹⁰
38 Negatively charged fluorescent dyes can also be directly
39 loaded into the LDH layers by anion exchange. However, with
40 these strategies, dye molecules uncontrollably fill in the
41 gallery or attach to the surface of the LDH nanoparticles with
42 very high local concentration, which leads to close spacing
43 between molecules and a corresponding concentration
44 quenching. Therefore, the resultant hybrid nanomaterials retain
45 very low or even no fluorescence.¹¹ To solve this problem,
46 Musumeci *et al.*¹² reported LDH nanoparticles loaded with dye
47 molecules by co-precipitation. In this method, the dye
48 molecules were added to the reaction mixture during LDH
49 fabrication. This approach generates LDH nanoparticles with
50 much higher fluorescence intensity compared with those made
51 via ion-exchange. In addition, several other efforts have also
52 been made to circumvent dye aggregation in matrices.¹³⁻¹⁵
53 Sasai *et al.*¹³ and Bujdak *et al.*¹⁶ introduced a method using
54 various surfactants to modify the microenvironment
55 physicochemical properties of the interlayer regions. With this

56 principle, Shi *et al.*¹¹ reported that many properties including
57 fluorescence quantum efficiency may be tuned by co-
58 intercalating sodium fluorescein and surfactants with different
59 alkyl chain lengths into the LDH nanoparticle gallery by the
60 anion-exchange. The surfactant molecules reduced the
61 fluorescence quenching by inhibiting nonradiative processes
62 and influencing on the orientational order and aggregation
63 characteristics of the dye molecules.¹¹ Herein we show a novel
64 approach of covalent bonding fluorescein sodium salt dye
65 molecules into LDH nanoparticles so that certain spacing
66 between fluorophores can be maintained. The resulting
67 nanocomposites possess much higher fluorescence quantum
68 efficiency than those made from anion exchange and co-
69 precipitation approaches. In addition, the nanohybrids also
70 show a number of advantages over free dye molecules.

72 Experimental

73 **Preparation of nanoparticles:** 80 mL of 0.15 M NaOH
74 (International Laboratory, USA) solution with 4 mmol of SDS
75 (Acros) was mixed with 20 mL of solution containing 2.0
76 mmol of $MgCl_2$ (International Laboratory, USA) and 1.0 mmol
77 of $AlCl_3$ (Sigma, USA) under vigorous stirring. The container
78 was sealed and the solution was stirred for 10 minutes. Next,
79 the solution was centrifuged and washed once with water. The
80 obtained slurry was dispersed in 80 mL of water and stirred at
81 80 °C for 24 hours in an airtight container under a N_2
82 atmosphere. After heat treatment, the LDH-SDS particles were
83 separated by centrifugation at 4000 rpm for 5 minutes and
84 dried under vacuum. Pristine LDH nanoparticles were
85 prepared according to the method described by Xu *et al.*¹²
86 Briefly, 40 mL of 0.15 M NaOH (International Laboratory,
87 USA) solution was mixed with 10 mL of solution containing
88 2.0 mmol of $MgCl_2$ (International Laboratory, USA) and 1.0

1 mmol of AlCl_3 (International Laboratory, USA) under vigorous stirring. The container was sealed and the solution was stirred for 10 minutes. Next, the solution was centrifuged and washed once with water. The obtained slurry was dispersed in 40 ml of water and hydrothermally treated at 80 °C for 4 hours in an airtight container.

Preparation of fluorescein sodium salt conjugated nanoparticles: 2 mg of fluorescein sodium salt (International Laboratory, USA) was mixed with 2 mL of (3-aminopropyl) triethoxysilane (International Laboratory, USA) for 48 hours in the presence of *N*-(3-Dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC) (Acros, USA) as a catalyst. 100 mg of LDH-SDS nanoparticles were further dried in vacuum for 30 minutes before the next stage of the reaction. 7.5 mL of methylene chloride (Acros, USA) was mixed with 0.4625 g *N*-cetyl-*N,N,N*-trimethylammonium (CTAB) (Acros, USA) at 40 °C to make CTAB fully dissolvable in methylene chloride. Then, the prepared fluorescein sodium salt conjugated APTES and methylene chloride with CTAB solution were simultaneously added into a glass vial containing 100 mg of LDH-SDS nanoparticles. Subsequently, the solution was ultrasonicated for 30 minutes at around 40–45 °C. After sonication, the solution was continuously stirred for 24 hours at 40–50 °C. Finally, the prepared nanoparticles were collected by centrifugation and washed with methylene chloride.

Fabrication of films: Pristine LDH solution was fabricated by the method reported by Xu et al.² Briefly, 40 mL of 0.15 M NaOH (International Laboratory, USA) solution was mixed with 10 mL of solution containing 2.0 mmol of MgCl_2 (International Laboratory, USA) and 1.0 mmol of AlCl_3 (International Laboratory, USA) under vigorous stirring. The container was sealed and the solution was stirred for 10 minutes. Next, the solution was centrifuged and washed once with water. The obtained slurry was dispersed in 40 mL of water and hydrothermally treated at 80 °C for 4 hours in an airtight container. The concentration of LDH is about 0.4 wt%, determined by weighing the LDH mass collected from suspension. 10mg of the fabricated nanocomposite was mixed with 10mL of pristine LDH solution and 5 ml of Millipore water with strong sonication for 30 minutes. The nanoparticle suspension was centrifuged at 500 rpm to precipitate non-dispersed nanoparticles and the supernatant solution was dried in a 60 °C oven to form a transparent film.

Characterization: The fabricated film was observed by scanning electron microscopy (FEG-SEM JEOL JSM-6335 F). Zeta potentials were measured by dynamic light scattering using a Zetasizer (Malvern). Transmission electron microscopy was performed by Philips CM20 instrument. The photoluminescence spectra and UV-Vis absorption spectra were measured using an Edinburgh Instrument FLS920P spectrometer with a 450 W xenon lamp as the steady-state excitation source and a Varian Cary 50 UV-visible spectrophotometer, respectively. Powder X-ray diffraction patterns were collected with a Smartlab instrument using Cu-K_α radiation. FTIR spectra were measured by a Perkin-Elmer 2000 FTIR spectrometer.

Results and discussion

LDH nanoparticles were prepared by a co-precipitation method.¹⁷ Sodium dodecyl sulfate (SDS) was used to expand

the gallery of the LDH nanoparticles. The product of this step is referred to as LDH-SDS. X-ray diffraction (XRD) patterns of the nanoparticles are presented in Fig. 1a. The (003) diffraction peak of pristine $\text{Mg}_2\text{-Al-CL}$ LDH at $2\theta=11.4^\circ$ indicates a basal spacing of 7.7 Å between LDH layers. After SDS was incorporated into the layers, the peak shifts to 3.3° , from which the interlayer space was calculated to be 26.8 Å, a value very close to the 26.5 Å reported in a previous study.¹⁷ It is apparent that the interlayer distance was dramatically increased, because of the attachment of SDS molecules. In the second step, we covalently attached fluorescein sodium salt molecules to (3-Aminopropyl) triethoxysilane (APTES) in presence of *N*-(3-Dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC) as a catalyst.¹⁸ The product is denoted as APTES-Dye. In the third step, the APTES-Dye was incorporated with the LDH-SDS nanoparticles by standing for 24 hours in methylene chloride with *N*-cetyl-*N,N,N*-trimethylammonium (CTAB) with ultrasonication for the initial 30 minutes. This final product is termed as LDH-Co-Dye. The role of CTAB is to react with SDS by forming salt to extract it from the LDH gallery.^{17,18} Fig. 1b shows Fourier transform infrared spectroscopy (FTIR) data for four samples including pristine LDH nanoparticles, SDS molecules, LDH-SDS nanoparticles and LDH-Co-Dye nanoparticles. C-H stretching modes at 2963, 2932 and 2842 cm^{-1} are observed from the spectrum of the LDH-SDS sample. These modes are not present in the spectrum of the LDH nanoparticles, indicating successful attachment of the SDS to the LDH nanoparticles. The bonds of the $-\text{SO}_4$ group at 826 and 1062 cm^{-1} are also observed in the spectrum of the LDH-SDS sample, which also suggests that the SDS molecules are attached to LDH nanoparticles. Significant diminution of these peaks in the spectrum of LDH-Co-Dye nanoparticles indicates the successful removal of SDS after the covalent bonding of fluorescein sodium salt molecules to the LDH nanoparticles. The broad band at 1000–1200 cm^{-1} in the spectrum of the LDH-Co-Dye nanoparticles can be ascribed to the Si-O-Si asymmetric stretching vibration, which confirms the attachment of APTES-Dye to the LDH nanoparticles.^{18,19} Fig. 2a shows the fabricated LDH-Co-Dye nanocomposite particles are about 50 nm in size and with morphology similar with our previously reported folic acid conjugated self-assembly LDH nanoparticles.¹⁸ The zeta potential of the fabricated LDH-Co-Dye nanoparticles was measured to be positively charged (+48.7 mV), which is very similar to that of the pristine LDH, indicating that the covalent bonding does not affect the positive charge of the LDH nanoparticles.

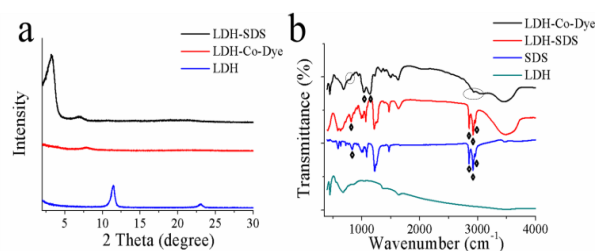


Fig. 1 a) Powder X-ray diffraction patterns of LDH-SDS nanoparticles, fluorescein conjugated LDH nanoparticles (LDH-Co-Dye) and pristine LDH nanoparticles. b) Fourier transform infrared spectroscopy of fluorescein conjugated LDH nanoparticles (LDH-Co-Dye), LDH-SDS nanoparticles, SDS molecules, and pristine LDH nanoparticles.

To confirm that the high quantum efficiency of fluorescein sodium salt can be retained by covalent bonding of the dye molecules to the LDH layers, we studied the optical properties of the as-prepared LDH-Co-Dye hybrid nanoparticles and compared them with the LDH-Dye nanohybrids made by conventional ion-exchange (LDH-Dye (Ex)) or co-precipitation (LDH-Dye (CoP)).¹² To simply compare the fluorescence efficiency, we excited the three samples at 489 nm, at which the absorbance of LDH-Dye (CoP) and LDH-Co-Dye nanoparticles is the same. The emission spectra of the samples are shown in Fig. 2b. The results show that, at the same absorbance, the fluorescence intensity of the LDH-Co-Dye nanoparticles is as high as four times of that of the LDH-Dye (CoP) nanoparticles. In great contrast, the LDH-Dye (Ex) nanoparticles prepared by the ion-exchange approach display negligible emission, indicative of serious fluorescence quenching. These contrasting results indicate that covalently bonding dye molecules to LDH interlayers can effectively maintain their fluorescence efficiency.

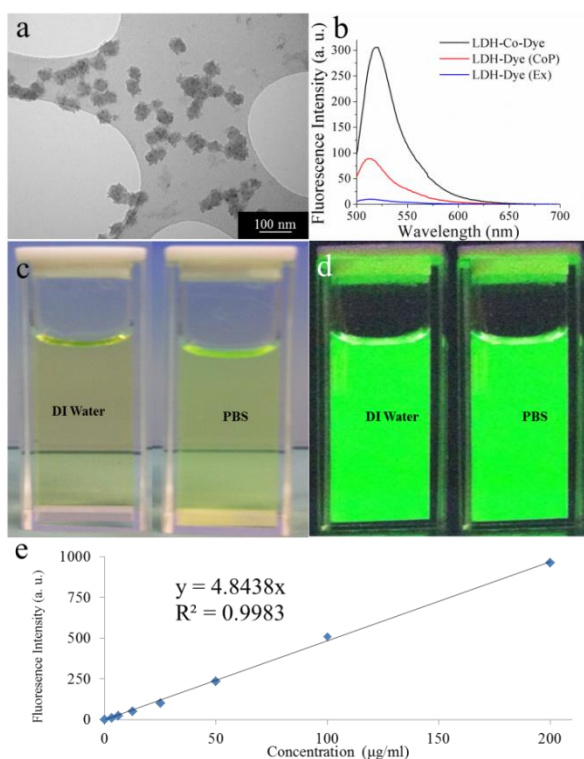


Fig. 2 a) TEM image of well dispersed LDH-Co-Dye nanoparticles. b) Emission spectra of fluorescein covalently conjugated LDH nanoparticles (LDH-Co-Dye), and LDH-dye hybrid prepared by ion-exchange (LDH-Dye (Ex)) or co-precipitation (LDH-Dye (CoP)). c-d) The digital images of the LDH-Co-Dye nanoparticles in DI water and PBS solution under c) day light and d) UV illumination. e) Fluorescence emission intensity of fluorescein covalently conjugated LDH nanoparticles (LDH-Co-Dye) as function of nanoparticle concentration.

We also determined the quantum yields (QYs) of the hybrid nanoparticles by absolute measurement with integrated sphere, and compared them with those of the free fluorescein sodium salt and the LDH nanohybrids made from ion-exchange or co-precipitation, as shown in Table 1. Our LDH-Co-Dye hybrid nanoparticles show a significantly higher QY of 55.1% than both LDH-Dye (CoP, 12.4%) and LDH-Dye (Ex, 3.0%) nanoparticles. The LDH-Co-Dye hybrid nanoparticles can be well-dispersed in DI water and phosphate

buffered saline (PBS). As shown in Fig. 2c-d, the nanoparticle solutions in both DI water and PBS are clearly transparent and remain highly fluorescent under UV illumination, indicating that LDH-Co-Dye hybrid nanoparticles are promising for bioimaging applications.

Table 1 Fluorescence quantum yield of free dye, fluorescein covalently conjugated LDH nanoparticles (LDH-Co-Dye), and LDH-dye hybrid prepared by ion-exchange (LDH-Dye (Ex)) or co-precipitation (LDH-Dye (CoP)).

	Free Dye	LDH-Co-Dye	LDH-Dye (CoP)	LDH-Dye (Ex)
QY(%)	70	55.1	12.4	3.0

The QY of the LDH-Co-Dye is lower than that of the free dye in solution at low concentration and this may suggest that the spacing between the covalently bonded dye molecules should be further increased. However, our LDH-Co-Dye nanoparticles nonetheless have a number of advantages over the free dye:

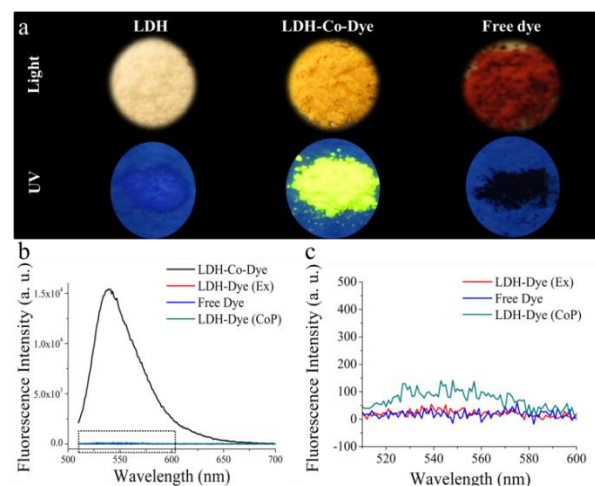


Fig. 3 a) Digital images of pristine LDH, fluorescein covalently conjugated LDH nanoparticles (LDH-Co-Dye), and free dye under day light and UV illumination. b) Solid-state emission spectra of free dye, fluorescein covalently conjugated LDH nanoparticles (LDH-Co-Dye), and LDH-dye hybrids prepared by ion-exchange (LDH-Dye (Ex)) or co-precipitation (LDH-Dye (CoP)). c) Magnified solid-state photoemission spectra of the indicated area in b).

Firstly, we studied their fluorescence emission intensity as a function of concentration ($\lambda_{\text{ex}}=495$ nm, $\lambda_{\text{em}}=520$ nm) (Fig. 2e). At concentrations from 3.125 to 200 $\mu\text{g/mL}$, the nanocomposite fluorescence exhibited an excellent linearity with concentration. The fluorescence intensity of free fluorescein sodium salt molecules does not follow this trend, with the fluorescence efficiency changing far more rapidly with concentration, which makes quantitative analysis challenging.

Secondly, the fluorescent LDH-Co-Dye nanoparticles are also fluorescent in dry powder form. To demonstrate this, pristine LDH and LDH-Co-Dye nanoparticles were dried as powders and images were taken under room and UV light. Free dye powder was used as a reference for comparison. The images (Fig. 3a) show that the pristine LDH nanoparticles and free fluorescent dye powder are not fluorescent under UV while the LDH-Co-Dye nanoparticles are strongly luminescent. Fig. 3b-c shows the solid state fluorescence spectra of different powders. It can be seen that the free dye molecules and the LDH-Dye (Ex) and the LDH-Dye (CoP)

nanoparticles are completely quenched in dry form, while the LDH-Co-Dye nanoparticles retain their strong emission.

There is a growing interest in the area of LDH chemistry to fabricate LDH crystallites into well-organized two-dimensional structures (films or membranes) for practical applications.²⁰⁻²² After validating our hypothesis that covalent bonding can significantly mitigate the fluorescence quenching of dye molecules in LDH matrices, we investigated methods to assemble these nanoparticles as self-supporting films. Fig. 4a shows images of a self-supporting transparent film (top) and the same film under UV exposure (bottom). This type of self-supporting films can be made with dimensions of centimeters, which is similar to the LDH film previously reported by Wang *et al.*,²² but our films show very strong photoluminescence under UV exposure and can still be transparent to some extent. We characterized the structure of the self-supporting film by scanning electron microscopy (SEM), as shown in Fig. 4b-d. Interestingly, we found that the film consist of two layers, with the top layer full of self-assembled cubic blocks of 1-2 μm (Fig. 4c) while the base layer containing densely packed nanoparticles (Fig. 4d). Such a self-assembly of LDH nanoparticles to cubic blocks has not been reported before. The mechanism needs further investigation. The bottom layer is composed of highly oriented stacked nanoparticles, similar to that previously reported highly oriented stacking structure of self-standing LDH films.²² Such a structure is important for the transparency of the films because of its capacity of greatly reduced light scattering.²²

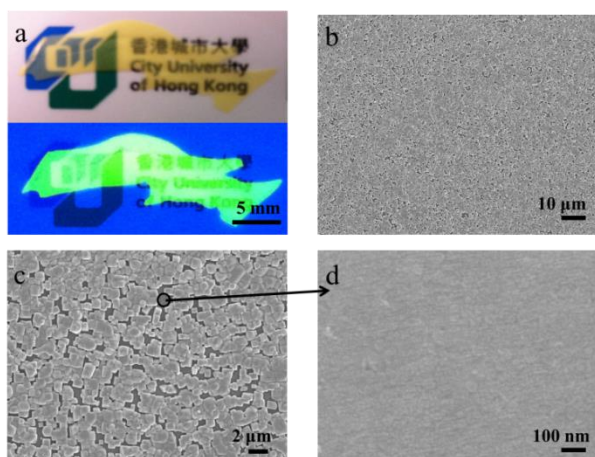


Fig. 4 a) Digital image of transparent LDH-Co-Dye film under UV excitation (upper part) and day light (bottom part). b-c) SEM images of the film. d) Magnified SEM image of bottom layer of the film as pointed in c).

Conclusions

In summary, we demonstrated that covalently bonding fluorescent dye molecules to LDH nanoparticles can be helpful of suppressing concentration quenching of their fluorescence. The LDH-Co-Dye nanoparticles possess much higher emission QYs than those of other hybrid nanoparticles which were produced by previously reported approaches including ion-exchange and co-precipitation. LDH-Co-Dye hybrid nanoparticles can be well dispersed in water and PBS and the fluorescence intensity linearly increases with concentration in a certain range, which is an excellent characteristic for quantitative analysis. Attractively, the nanoparticles even remain strong fluorescence in powder form, and can be used to

form highly fluorescent and transparent free-standing films, all of which cannot be realized by free dye molecules or with previously reported LDH hybrid nanoparticles derived by ion-exchange and co-precipitation.

Notes and references

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