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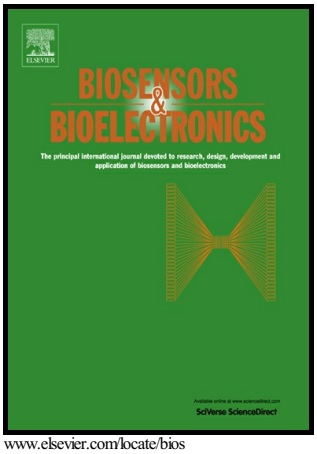

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A convenient purification method for silver nanoclusters and its applications in fluorescent pH sensors for bacterial monitoring

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Abstract

Herein, we report a convenient approach to purify water-soluble dihydrolipoic acid (DHLA)-capped Ag nanoclusters (Ag NCs) by pH-induced precipitation under acidic conditions. The fluorescence of Ag NCs could be completely recovered by re-dispersing the precipitate into a basic solution using DHLA and $NaBH_4$ as stabilizing ligands and etching reagent. DHLA-Ag NCs-doped agarose hydrogels have been prepared to monitor pH with a wide range from 8.0-4.0. When pH decreased, the fluorescence of the hydrogels under a UV lamp decreased and completely disappeared after pH 5. The DHLA-Ag NCs-doped agarose hydrogels biosensor showed low cytotoxicity and long stability. Accordingly, a fluorescent pH sensor for bacterial monitoring has been employed based on the "OFF–ON" signal switch of the Ag NCs-agarose hydrogel.

Keywords: Ag nanoclusters; fluorescent pH sensor; Bacterial monitoring; Fluorescence sensor; Signal off-on

1. Introduction

Over the past decades, pH sensors have received much attention because of the significance of pH detection in various scientific studies, especially in the physiological and metabolic process. pH is about 7.4 for the normal intracellular, 6.5 for the extracellular matrix of cancer cells, and 4.0 to 5.0 for acidic organelles. (Wang et al., 2014b. Mura et al., 2013. Tannock and Rotin, 1989). Moreover, the growth of some microbial populations produces pH changes in their environment (Uria et al., 2016). Consequently, there has been a significant focus on developing new non-invasive and accurate diagnostic techniques to measure pH in a very wide range *in vivo* and *in vitro* with high spatiotemporal resolution (Zhu et al., 2013). Fluorescent pH probes have many benefits such as high selectivity, sensitivity, convenience and rapid operation (Ding and Tian, 2015). They can be roughly divided into three categories: organic dye (Sun et al. 2014), semiconductor quantum dots (QDs) (Jin et al. 2010) and metal nanomaterials (Chen et al., 2014). Considering the toxicity of the organic matter and QDs, it is of great interest for

[1] H. Xiong and H. Zheng contributed equally to this work.

the development of reliable and facile protocols to prepare photoluminescent pH sensors with metal nano-materials. Moreover, to enable optimal spatial resolution in case of bacteria with their typical diameters between 2 and 10 μm, it is mandatory to design nanosized sensors rather than using conventional imaging sensor layers (Wang et al. 2013). Metal nanoclusters with ultra-small sizes near the Fermi wavelength of electrons have gained particular research interest for their unique properties, such as strong fluorescence. The emissive metal clusters have potential applications in single-molecule microscopy, biological labelling, and optical sensing (Shang and Dong, 2008). Based on the intrinsic fluorescence of nano-clusters, we tried to construct a simple and novel biocompatible detection platform for real-time tracking of pH in the bacterial metabolism and growth. Hydrogels were selected in our work because they have negligible background colour and fluorescence emissions, large loading capacities and controllable shapes (Yuan et al. 2014, Dave et al. 2010).

Until now, extensive studies have concentrated on the synthesis of luminescent and water-soluble metal nanoclusters using various types of templates or stabilizing agents such as peptides (Yuan et al., 2014), proteins (Wang et al., 2014a), DNA (Copp et al., 2014), polymers(Shang and Dong, 2008), dendrimers (Tanaka et al., 2011) and thiols (Wu et al., 2009). Experimentally, largely excessive templates were required to stabilize nanoclusters or reduce metal salts to form template-protected metal clusters. The large amount of remaining free templates in the cluster solution may react with the analytes, which will disturb the detection (Li et al., 2011), and the fluorescent inner-filter effect also decreases the fluorescence of the clusters (Shang and Dong, 2009). Dialysis, ultracentrifugation and chromatographic techniques are three main methods to remove molecules from systems. With these methods, it is difficult to isolate the highly soluble clusters of extremely small size from templates that have notably similar molecular weights. Thus, developing a convenient separation method to effectively remove the free templates from the cluster solution was necessary. Recently, Guan et al removed free bovine serum albumin (BSA) from a notably basic cluster solution by co-precipitating the protein-protected gold clusters with zinc hydroxide (Guan et al., 2014). After washing with water, the co-precipitated clusters were dialyzed with PBS buffer to remove the remaining Zn^{2+} ions. The addition of foreign ions made the purification process of gold clusters complicated.

In this study, highly fluorescent Ag nanoclusters (Ag NCs) have been prepared using dihydrolipoic acid (DHLA) as the stabilizing ligands from a previous report (Shang et al., 2012). We have developed a simple method to remove free DHLA from clusters by precipitating the clusters in acid. After Ag NCs are re-dispersed into an alkaline medium, the fluorescence of Ag NCs can be completely recovered by adding $NaBH_4$. In addition, the constructed detection platforms based on Ag NCs-agarose hydrogels may be used as an “OFF–ON” signal switch to monitor the pH levels in water samples or the metabolic activity of some microorganisms in the growth culture medium.

2. Materials and methods

2.1.Reagents and materials

Lipoic acid, Silver nitrate (AgNO3), sodium borohydride ($NaBH_4$) and Luria-Bertani medium (LB) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Agarose was obtained from Aladdin Reagent (Shanghai, China). The rest of the chemical reagents were commercially available and at least analytical grade. The normal rat

kidney (NRK-52E) cell line was obtained from the American Type Culture Collection. All aqueous solutions were prepared using ultrapure water (Mill-Q, Millipore).

2.2 Instrumentation

The size and morphology of Ag nanoclusters were characterized by a Tecnai G20 (FEI Co., Holland) transmission electron microscope with an acceleration voltage of 200 kV. The UV-vis absorption spectra were acquired using a UV-3600 UV-vis spectrometer (Shimadzu, Japan). The fluorescence measurements were performed with a RF-5301 PC fluorometry (Shimadzu, Japan). The pH value of the solutions was determined using a 320-S acidity meter (Mettler-Toledo, Switzerland). All hydrogels were imaged using a ChemiDoc MP (Bio-Rad) imager.

2.3 Synthesis of Ag nanoclusters

In a typical protocol, 60.8 mg of lipoic acid were solubilized in 15 mL of sodium hydroxide solution (0.36 mmol), and 0.02 mmol of $AgNO_3$ aqueous solution was subsequently added. After stirring for 5 min, 0.096 mmol of freshly prepared $NaBH_4$ aqueous solution was slowly added to the mixture under rapid stirring. The reaction was stopped after stirring overnight, and the solution was stored at 4 °C in the dark until it was used.

2.4 The preparation of agarose hydrogel

100 mg of agarose was completely dissolved in 10 mL of distilled water under microwave heating. When the solution cooled to 80 °C, 5 mL of DHLA-Ag NCs solution (24 mg/L, which was analysed by Inductively Coupled Plasma–Atomic Emission Spectrometry technique) was added with rapid stirring for 1 min. Subsequently, 1 ml of the mixture was poured into a 24 well plate and maintained static for 30 min to form the hydrogel. The agarose-Luria-Bertani (agarose-LB) hydrogels were prepared in the same way as described above but with the co-dissolved of 200 mg of LB broth and 100 mg of agarose power in water.

2.5 Cell culture and treatment

The NRK-52E cells were grown in Dulbecco's modified Eagle's medium (DMEM)/F12 (Gibco) containing 10% FBS (Gibco) at 37°C in an atmosphere containing 5% CO_2. Viability of NRK-52E cells was determined by cell counting kit-8 (CCK-8) assay. Cells were spread at the concentration of 10^5/ml in 96-well plates. The culture medium was exchanged one day before starting the experiment. Particles were diluted 1:10, 1:50, 1:100, 1:200 with culture medium. After 24 h incubating, 10 μl of CCK-8 was added to each well and further incubated at 37 ℃ for 3 h. The absorbance of CCK-8 was detected at 450 nm by a microplate reader. Triton-X (0.5%) was added to the positive control cells 0.5 h before the end of the incubation.

2.6 Cultivation

Wild-type *Escherichia coli* (*E. coli*), type K-12 (MG1655), was sperad loosely on the agarose-LB hydrogels in a 24 well plate via a glass rod. The bacterium was grown at 37 °C in an ordinary cell culture incubator for 12 h.

3. Results and discussion

3.1 Characterization of DHLA-stabilized Ag NCs

The present approach to prepare Ag NCs is notably simple. Initially, the mixture solution of $AgNO_3$ and DHLA was colourless, and the UV/Vis spectrum showed no absorption. As time increased, an obvious colour change of the reaction solution from colourless to turbid yellow and subsequently clear reddish was observed. Under a UV lamp, the solution exhibited bright red emission (Inset of Fig. 1A). The formation of Ag NCs was characterized based on the UV-vis absorption spectrum, fluorescence emission spectra, TEM and Zetasizer Nano ZS. DHLA-Ag NCs in the aqueous solution exhibited three absorption bands centred at 330 nm, 427 nm and 498 nm (Fig. 1A), which are similar to previous reports (Adhikari and Banerjee, 2010). Upon excitation at 332, 432 or 500 nm, Ag NCs displayed an intense emission band with a maximum at approximately 659 nm (Fig. 1B). With the change in excitation wavelength, no change in emission maximum was observed, which illustrates that the emission is a luminescence from the relaxed states of ultra-small Ag NCs. TEM images of the cluster shows entities of approximately 2 nm in diameter (Fig. 1C). No larger Ag particles or aggregations were observed, which demonstrates the fabrication of well-controlled nanoclusters. The hydrodynamic diameter of the DHLA-Ag NCs solution, which was determined using a Zetasizer Nano ZS, was also approximately 2 nm (Fig. 1D). These synthesized clusters were stable for over 2 months with no precipitation, which demonstrates the potential applications of fluorescent Ag NCs in fluorescent sensors.

3.2. Effect of different pH values on fluorescence of DHLA-stabilized Ag NCs

The prepared DHLA-stabilized Ag NCs in this study were pH sensitive. The fluorescence emission spectra of DHLA-Ag NCs with different pH values are displayed in Fig. 2A. The fluorescence intensity of Ag NCs decreased at a lower pH and linearly changed in the pH range of 6-9. The intensity reached the maximum value at pH 9.0 and decreased to approximately one-third at a pH of 6.0 (Inset of Fig. 2A). In the experiment process, we found that grey precipitates were formed by adding HNO_3 to the DHLA-Ag NCs solution with stirring. The precipitates began to generate at pH 8.36 and were visible to the naked eye at approximately pH 4.0-5.0; then, the maximum fluorescence emission at 659 nm simultaneously disappeared. The corresponding photographs of the Ag NCs solution at different pH values under visible-light (upper) and UV-light irradiation (lower) are shown in Fig. 2B. Strong emission at high pH and no emission in lower pH conditions show that DHLA-Ag NCs can be used as an "OFF–ON" signal switch. The reason for the fluorescence quenching of DHLA-Ag NCs at lower pH values may be the formation of reversible aggregates. The results suggest that DHLA-Ag NCs may serve as effective fluorescent pH-prober candidates.

3.3. The purification of DHLA-stabilized Ag NCs

A convenient separation method to effectively remove raw material from the cluster solution without affecting the fluorescent ratio is useful to develop. In this study, we have developed a simple method to remove redundant DHLA from the cluster solution via the precipitation of Ag NCs in an acid solution. The DHLA-protected Ag NCs immediately precipitated after the addition of HNO_3 to pH 4. After centrifugation to remove the supernatant, the

precipitate was washed with distilled water three times and re-dispersed into a basic solution; the turbid solution became clear, and the lost fluorescence intensity was partly recovered (67%) after continuous stirring. The corresponding photographs of the Ag NCs solution upon acid-base regulation under visible-light and UV-light irradiation are shown in Fig. 2C. In comparison, no emission wave was detected for the supernatant (Fig. S1), which indicates the complete precipitation of Ag NCs. In a control experiment, adding HNO_3 into the DHLA solution in the absence of clusters did not generate the precipitations in identical conditions, which suggests the possibility of successfully removing free DHLA from the cluster in an acidic condition. The possible mechanisms about the precipitation and re-dispersing of Ag NCs were as follows: Lipoic acid is slightly soluble in water, but dissolves in NaOH solution. The dissolved lipoic acid could reduce by the freshly prepared $NaBH_4$ to form soluble DHLA. DHLA contains two free thiol groups per molecule, which are very sensitive to the surfaces of Ag nanoclusters (Adhikari and Banerjee, 2010). The two thiol groups and carboxylate moiety present in the DHLA molecule could stabilize the fluorescent Ag nanoclusters very well. Once solution was tuned to acidic, DHLA could not keep the chemical nature of the surface ligands in acidic medium, which made it lose its protective effect on Ag NCs and the Ag NCs quickly aggregated to form larger size of Ag nanoparticles (Ag NPs) (Fig. 2D), resulted in the fluorescence quenching. Stable chemical natures of the surface ligands were formed again by adjusting pH to alkaline, and then template features were recovered. The good coordination ability between Ag and thiol groups protected the Ag NCs stable in aqueous solution. A possible reason for the uncompleted recovered of the fluorescence intensity is that aggregates of clusters could not totally disperse into small nanometre size after the change of pH.

As stated above, the fluorescence intensity of Ag NCs can be partly recovered by re-dispersing the precipitate into a basic solution. We wondered whether the fluorescence of Ag NCs could be completely recovered, and a further study attempted to solve the problem. Certain amounts of $NaBH_4$ were added into the re-dispersion solution under continuous stirring at room temperature. Figures S2- S4 of the Supporting Information show the effects of pH, $NaBH_4$ content and reaction time on the fluorescence intensity. The results show that the fluorescence intensity only recovered in basic condition but decreased in the highly alkaline solution. pH 10.0 was suitable for the reaction (Fig. S2). With the increase in $NaBH_4$ content, the fluorescence first increased and subsequently decreased until plateau (Fig. S3). Under the optimal conditions, the solution produced more intense fluorescence with increasing stirring time in air, and the fluorescence completely recovered after 6 h (Fig. S4). No significantly change has been observed over two months, indicating the good stability of DHLA-capped Ag NCs. After the clusters precipitated under acidic condition to remove free DHLA from the cluster solution, the precipitated clusters were washed with water and dissolved in PBS, BR, CH_3COOH-CH_3COONa or Tris-HCl buffer with pH 10.0 (Fig. S5); then, $NaBH_4$ was added. The fluorescence completely recovered after stirring for a certain amount of time, which suggests that the clusters were stable in a series of buffer solutions and could be applied in various environments.

3.4 Analytical performance of Ag NCs biosensor for pH determination in buffer and bacterial growth

Because of the remarkable response of DHLA-Ag NCs to pH, we used them for fluorimetric detection of pH based on the “OFF–ON” signal switch of the fluorescence. For this purpose, we prepared DHLA-Ag NCs-doped

agarose hydrogels. Agarose was selected as the hydrogel because the material has high loading capacity and low background fluorescence. The accumulative properties of hydrogels enable their use to detect the pH (Yuan et al., 2014). Because the heating process during the hydrogel preparation may affect the optical properties of the Ag NCs, DHLA-Ag NCs solution was added after the solution cooled to 80 °C with rapid stirring. Because of the good dispersibility in water, DHLA-Ag NCs can be evenly distributed in the agarose layer. The prepared transparent hydrogel emitted strong fluorescence under UV irradiation, which suggests that the DHLA-Ag NCs and agarose were successfully combined. The hydrogels were immersed into PBS with various pH values. When the pH decreased from 8 to 4, the fluorescence of the hydrogels under a UV lamp decreased and completely disappeared after pH 5 (upper of Fig. 3A). The wide pH change range of 4.0 to 8.0 for the fluorescence sensors could response to various physiological pH environments. And the response range to pH was much wider than many other fluorescence nano-probes, such as gold nanocluster fluorescence biosensor (pH 6.0-7.8)(Ding and Tian, 2015), quantum dot-based sensor (pH 6-8) (Jin et al., 2010) and Ag NPs/polymer nanohybrids sensor (pH 1-5) (Ma et al., 2015). No obviously change was observed for agarose hydrogels only (lower of Fig.3A).The excellent "OFF–ON" signal of these DHLA-Ag NCs-doped agarose hydrogels suggests that the hydrogels are applicable for convenient pH detection under practical conditions. And the hydrogels are stable in buffer solutions for at least 1 month if kept at 4°C in the dark (Fig. S6).

The difference of luminescence emitted by the probe at pH 6 and 7 is small but sufficient to visualize bacterial growth. Cytotoxicity tests using NRK cells showed that these Ag NCs had a low cytotoxicity (Fig. S7). It suggested that the nano-sensors do not interfere in the growth or metabolism of bacteria. To demonstrate the microbiological practicability, different concentrations of *E. coli* were spread on the pH-sensitive agarose-LB hydrogels in a 24 well plate for 12 h. Notably, before the addition of DHLA-Ag NCs, the agarose-LB solution was steam sterilized at 120 °C at 1.1 bar for 30 min. Fig.3B shows that the pH-sensitive agarose-LB hydrogels displays a homogeneous red fluorescence at the absence of *E. coli*. After growth of and metabolization by *E. coli*, significant acidifications of the plate have occurred. The intensity of the red luminescence is reduced indicating that the protons produced by the bacteria during growth have caused a substantial change in the local pH value (Wang et al., 2013). And the metabolism of a large concentration of bacteria lowers more local pH values. The yellow region shown in the picture is a grown colony. Different concentrations of *E. coli* were also cultivated on the LB solid medium (no DHLA-Ag NCs). No obviously change was observed for those plates (bottom of Fig.3B), warranting DHLA-Ag NCs was necessary probe for pH response and fluorescence imaging.

4. Conclusions

In conclusion, this work demonstrates an effective separation method to isolate DHLA -protected Ag NCs from free DHLA in the as-synthesized cluster solution. The as-prepared emissive nanoclusters exhibit good pH-dependent emission behaviour. By combining DHLA-Ag NCs with hydrogels, DHLA-Ag NCs-doped agarose hydrogels were constructed as a pH detection platform. Dramatic fluorescence quenching was observed with pH below 5, which suggests that this system is a competent pH "OFF–ON" signal switch in a wide application range, including chemical and biological sensing. The change of pH value during the bacterial growth and metabolism could be monitored indicating the microbiological practicability. We envision that the hydrogels will enable different species of bacteria to be cultivated and monitored.

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References

Adhikari, B., Banerjee, A., 2010, Chem.Mater. 22, 4364-4371.

Chen, P.C., Ma, J.Y., Chen, L.Y., Lin,G.L., Shih, C.C., Lin, T.Y., Chang, H.T. 2014, Nanoscale, 6, 3503-3507.

Copp, S. M., Bogdanov, P., Debord, M., Singh, A., Gwinn, E.h. 2014, Adv. Mater. 26, 5839-5845.

Dave, N., Chan, M. Y., Huang, P.-J. J., Smith, B. D., Liu, J. 2010, J. Am. Chem. Soc. 132, 12668-12673.

Ding, C., Tian, Y. , 2015, Biosens. Bioelectron. 65, 183-190.

Guan, G., Zhang, S., Cai, Y., Liu, S., Bharathi, M. S., Low, M., Yu, Y., Xie, J., Zheng, Y., Zhang, Y., Han. M. 2014, Chem. Commun., 50, 5703-5705.

Jin, T., Sasaki, A., Kinjo, M., and Miyazaki, J. 2010, Chem. Commun., 46, 2408-2410.

Li, H.-W., Ai, K., Wu,Y. 2011, Chem. Commun., 47, 9852-9854.

Ma, H., Li, A., Xu, Y., Zhang, W., Liu, J. 2015, European Polymer Journal 72, 212–221.

Mura, S., Nicolas, J., Couvreur, P. 2013, Nat.Mater. 12, 991-1003.

Shang, L., Dong, S., 2008, Chem. Commun. 9, 1088-1090.

Shang, L., Dong, S. 2009, Anal. Chem., 81, 1465-1470.

Shang, L., Dörlich, R. M., Trouillet, V., Bruns, M. Ulrich Nienhaus,, G. 2012, Nano Res. 5, 531-542.

Sun, R., Liu, X., Xun, Z., Lu, J., Xu, Y., Ge, J. 2014, Sens Actuators B 201, 426-432.

Tanaka, S., Miyazaki, J., Tiwari, D. K., Jin, T., Inouye,Y. 2011, Angew. Chem., Int. Ed., 50, 431-435.

Tannock, I.F., Rotin, D. 1989, Cancer. Res. 49, 4373-4384.

Uria, N., Abramova, N., Bratov, A., Muñoz-Pascual, F., Baldrich E. 2016, Talanta 147, 364–369.

Wang, C., Wang, C., Xu, L., Cheng, H., Lin, Q., Zhang, C. 2014a, Nanoscale, 6, 1775-1781.

Wang, X., Meier, R. J., Wolfbeis, O. S. 2013, Angew. Chem. Int. Ed. 52, 406-409.

Wang, Y., Shim, M.S., Levinson, N.S., Hsing-Wen, S., Xia, Y. 2014b, Adv. Funct. Mater. 24, 4206-4220.

Wu, Z., Lanni, E., Chen, W., Bier, M. E., Ly' D., Jin, R. 2009, J. Am.Chem. Soc., 131, 16672-16674.

Yuan, Z., Cai, N., Du, Y., He, Y., Yeung, E. S., 2014, Anal. Chem. 86, 419-426.

Yuan, X., Setyawati, M. I., Leong, D. T., Xie, J. 2014, Nano Research 7, 301-307.

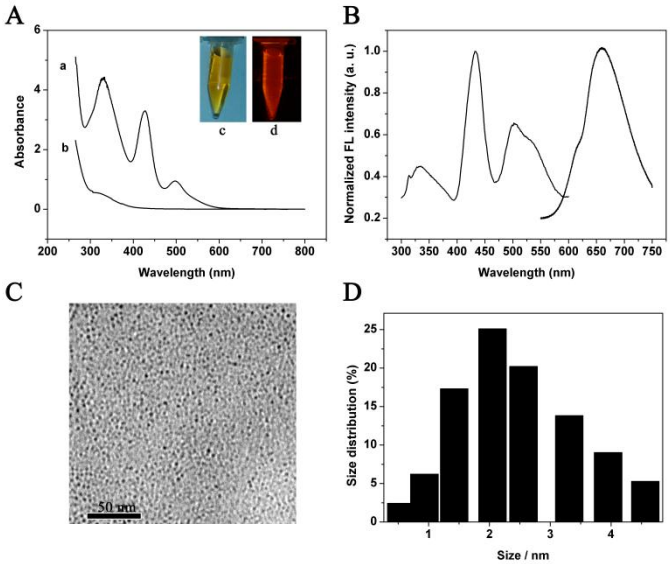
Zhu, L., Smith, P. P., Boyes. S. G. 2013, J. Polym Sci Pol Phys. 51, 1062–1067

Figure 1. Characterization of DHLA-stabilized AgNCs. (A) UV-vis absorption of (a) DHLA-AgNCs and (b) DHLA. Inset: photographs of the AgNCs under visible-light (c) and 365-nm UV-light irradiation (d). (B) Normalized fluorescence excitation (left) and emission (right) spectra of the AgNCs. (C) TEM image of the AgNCs (scale bar: 50 nm). (D) Size distributions of the AgNC solution based on statistics.

Figure 2. (A) Fluorescence emission spectra of AgNCs at different pH values: 4, 5, 6, 7, 8, 9, 10, and 11 (excitation at 432 nm). Inset: Calibration curve of the fluorescence intensity of the AgNCs versus the pH values in the range of 4-10 (excitation at 432 nm, emission at 659 nm). (B) Photographs of the AgNC solution at different pH values under visible-light (upper) and UV-light irradiation (lower). (C) Photographs of the the purified process of DHLA-AgNCs (from left to right, DHLA-AgNCs, the floccules under acidic conditions, the supernatant after removing the precipitate and the re-dispersed AgNCs under basic media) under visible-light (upper) and 365-nm UV-light irradiation (lower). (D) TEM image of precipitate (Ag NPs, scale bar: 100 nm).

Figure 3. (A) Fluorimetric responses for pH using DHLA-AgNC-doped agarose hydrogels (upper) and agarose hydrogels (lower) under UV-light irradiation. **(B)** Fluorimetric monitoring of bacterial growth and metabolism using DHLA-AgNC-doped agarose-LB hydrogels (upper) and LB solid medium (lower) under UV-light irradiation.

Figures:



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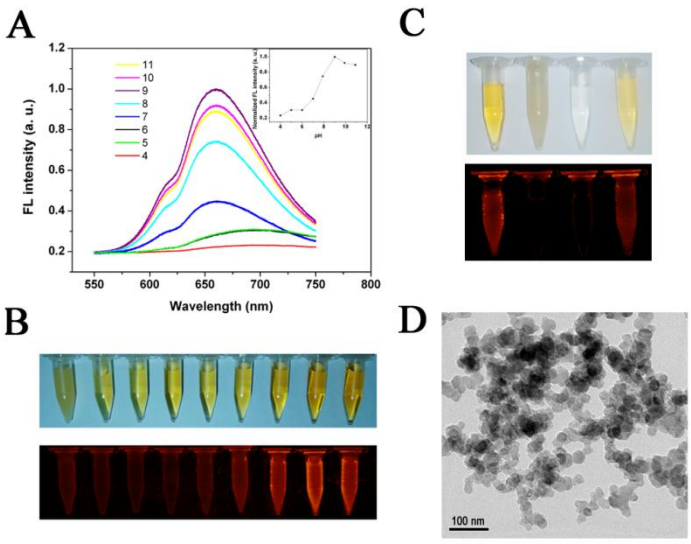

Figure 2.

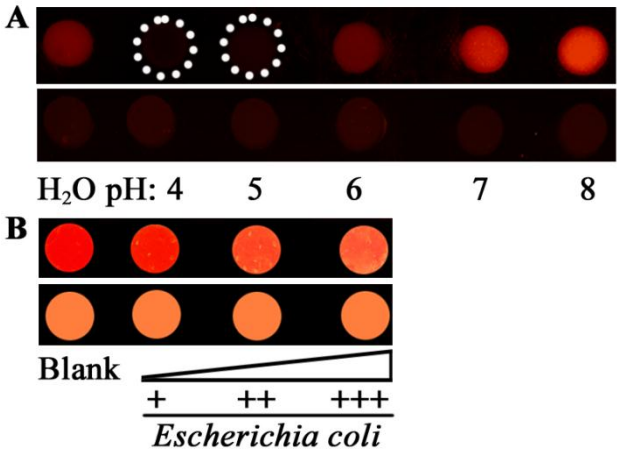

Figure 3.

Highlights

- We proposed a simple colorimetric method to detect pH for bacterial monitoring.
- The method is a signal off-on fluorescent strategy.
- We reported a convenient approach to purify AgNC by pH-induced precipitation.