Dual-Mode Encoded Magnetic Composite Microsphere Based on Fluorescence Reporters and Raman Probes as Covert Tag for Anticounterfeiting Applications

Ruimin Li, Yuting Zhang, Jing Tan, Jiaxun Wan, Jia Guo, and Changchun Wang*

State Key Laboratory of Molecular Engineering of Polymers, and Department of Macromolecular Science, Laboratory of Advanced Materials, Fudan University, Shanghai 200433, China

ABSTRACT: Utilizing fluorescence reporters and SERS probes as the security labels, a series of dual-mode encoded magnetic composite microspheres with micrometer size was designed and prepared for anticounterfeiting applications. At first, the micro-meter-sized melamine formaldehyde microspheres with different fluorescence molecules (FMF) were prepared by precipitation polymerization, and then the magnette composite microspheres (FMF/MNPs) were fabricated by direct immobilization of magnetic nanoparticles (MNPs) onto the surface of FMF microspheres. After deposition of Ag nanoparticles (Ag-NPs) onto FMF/MNPs microspheres, the SERS probes were absorbed onto the surface of Ag-NPs, and then a protection layer of silica was coated on the composite microspheres by Stöber method. The combination of different fluorescence reporters and SERS probes greatly increased the encoding complexity and volume for high-level anticounterfeiting. The structure of the dual-encoded FMF/MNPs/Ag-NPs/SiO₂ composite microspheres was characterized by FESEM, TEM, FLS(fluorescence spectrometer), XRD, VSM, UV−vis and EDS. The embedded magnetic nanoparticles enable the composite microspheres to be quickly isolated from the marked latex paint by magnet at the concentration of as low as 1 ppm, and the covert tag information can be read out even from one composite microsphere. In addition, the covert security information in the marked coating film can be also read out in situ and the existence of the composite microspheres does not influence the visible appearance of the coating film. All the above outstanding properties will make these dual-mode encoded composite microspheres as advanced security tags for next-generation anticounterfeiting applications.

KEYWORDS: dual-encoded, anticounterfeiting, fluorescence reporters, Raman probes, SERS, fingerprint characteristics

1. INTRODUCTION

Nowadays, the proliferation of counterfeiting and adulteration is a big challenge in our world.1,2 Fuels, medicine, coatings and other chemicals are routinely counterfeited, which seriously infringe the legal rights and cause significant financial damage of the copyright owners.3−6 For instance, coating products adulterated with harmful chemicals or cheaper raw materials have become more frequent in recent years, which probably cause severe damage to consumers’ health and hinder the development of the coating industry. Hence, development of anticounterfeiting materials with plenty of detectable covert information is essential for this critical issue.

During the last decades, a wide variety of security and anticounterfeiting technologies has been developed. For example, the technologies on the basis of watermarks,1 fluorescent nanostructure,11,12 thermo-paint layer13 and magnetically assembled Fe₃O₄@SiO₂ colloids14–16 exhibit unique physical features for anticounterfeiting. They are made of stimuli-responsive molecules and a very evident change in their physical or optical properties can be triggered by external stimuli, such as light, heat and magnetic field etc. However, with the application of these technologies, some of the anticounterfeiting strategies have been known to the counterfeiters because the mechanisms are predictable and deterministic. Another example is the “covert security” technology as next-generation anticounterfeiting strategy, which involves the code information inside of security labels as invisible tags and can be only exclusively identified by specific analytical instruments and procedures. For example, the digital encoded polymers,17 biomimetic microfingerprints,18 DNA/silica encapsulates19 are utilized as anticounterfeiting elements to offer an increased level of protection against counterfeiting on the basis of the additional code information. As a high-performance security label against counterfeiting, it should be invisible,
robust and harmless. In addition, it should not affect the properties and appearance of products, and it is cheap and easily detected.

Surface plasmon associated with noble metal nanoparticles can generate strong enhancement of local electromagnetic fields, and lead to intense SERS signals from Raman probe molecules in the vicinity of nanostructures.\(^{20-24}\) Besides, SERS is currently the only way capable of simultaneously detecting a single molecule and providing its fingerprint chemical information,\(^{25}\) which holds great potential as next-generation covert security labels. Besides, fluorescent microspheres doped with different fluorescence molecules are also good candidates for security labels against counterfeiting, because the fluorescent microspheres can be labeled with various fluorescence molecules and can be easily detected by fluorescence spectrometry or fluorescence microscope. However, it should be noted that the fluorescent microspheres alone hold relatively lower security level due to the nonfingerprint characteristic spectra for limited encoding volume. Then the fluorescence and SERS dual-mode encoded protocols have been employed in bioimaging,\(^{26}\) immunoassay,\(^{27}\) and high-throughput biodetection.\(^{28}\) SERS spectra are employed as high-level security technique with the remarkable advantages of richness of spectroscopic information and high sensitivity toward analytes. Meanwhile, fluorescence is regarded as a fast and effective indicator. The dual-mode encoded tags showed great potential for biomedical applications. However, as far as we know, no paper based on this concept has been reported in anticounterfeiting applications.

Herein, we design and fabricate a new kind of dual-mode encoded magnetic composite microspheres (FMF/MNPs/Ag-NPs/SiO\(_2\)) utilizing fluorescence reporters and SERS probes as high performance security labels for anticounterfeiting applications. Different from the reported systems for biomedical applications,\(^{26-28}\) our dual-mode encoded tags are micrometer size rather than nanometer size, each micro-meter-sized composite microsphere has very strong signal intensity and relative large volume, which has excellent resistance ability against dilution for low concentration detection. Besides, we introduced magnetic nanoparticles to the dual-mode encoded system for the first time, making them effectively isolated and enriched by an external magnet even at extremely low concentration (1 ppm). The combination of different fluorescence reporters and Raman probes enables the dual-mode encoded composite microspheres with large encoding volume and unique fingerprint characteristics for high-level anticounterfeiting security.

### 2. Experimental Section

#### 2.1. Materials
Melamine (99%, \(\text{C}_3\text{H}_6\text{N}_6\)), 7-hydroxycoumarin (7-HCM), fluorescein isothiocyanate (90%, FITC), phloxine B (280%, PHB), ferric chloride hexahydrate (99%, \(\text{FeCl}_3\cdot6\text{H}_2\text{O}\)), ferrous sulfate hexahydrate (99%, \(\text{FeSO}_4\cdot6\text{H}_2\text{O}\)), sodium citrate dehydrate (99%, \(\text{C}_6\text{H}_5\text{Na}_3\text{O}_7\cdot6\text{H}_2\text{O}\)), sodium citrate dehydrate (99%, \(\text{C}_6\text{H}_5\text{Na}_3\text{O}_7\cdot6\text{H}_2\text{O}\)), sodium citrate dehydrate (99%, \(\text{C}_6\text{H}_5\text{Na}_3\text{O}_7\cdot6\text{H}_2\text{O}\)), sodium citrate dehydrate (99%, \(\text{C}_6\text{H}_5\text{Na}_3\text{O}_7\cdot6\text{H}_2\text{O}\)), sodium citrate dehydrate (99%, \(\text{C}_6\text{H}_5\text{Na}_3\text{O}_7\cdot6\text{H}_2\text{O}\)), sodium citrate dehydrate (99%, \(\text{C}_6\text{H}_5\text{Na}_3\text{O}_7\cdot6\text{H}_2\text{O}\)), sodium citrate dehydrate (99%, \(\text{C}_6\text{H}_5\text{Na}_3\text{O}_7\cdot6\text{H}_2\text{O}\)), sodium citrate dehydrate (99%, \(\text{C}_6\text{H}_5\text{Na}_3\text{O}_7\cdot6\text{H}_2\text{O}\)), sodium citrate dehydrate (99%, \(\text{C}_6\text{H}_5\text{Na}_3\text{O}_7\cdot6\text{H}_2\text{O}\)), sodium citrate dehydrate (99%, \(\text{C}_6\text{H}_5\text{Na}_3\text{O}_7\cdot6\text{H}_2\text{O}\)), sodium citrate dehydrate (99%, \(\text{C}_6\text{H}_5\text{Na}_3\text{O}_7\cdot6\text{H}_2\text{O}\)), sodium citrate dehydrate (99%, \(\text{C}_6\text{H}_5\text{Na}_3\text{O}_7\cdot6\text{H}_2\text{O}\)), sodium citrate dehydrate (99%, \(\text{C}_6\text{H}_5\text{Na}_3\text{O}_7\cdot6\text{H}_2\text{O}\)) were utilized as model molecules in the vicinity of nanostructures. 18.0 M \(\text{HCl}\) was used as the hydrochloric acid and 10 mL of aqueous solution containing 2 mg of 7-HCM, 0.2 mg of FITC and 1 mg of PHB were added into the reaction mixture for catalyzing the polycondensation reaction and the fluorescence staining of the microspheres, respectively. After 2 h, the suspension was naturally cooled to room temperature. Finally, the supernatant was removed by centrifugation and the resulting FMF microspheres were washed by water and ethanol for 3 times respectively, and then dried under vacuum at 40 °C for 4 h. In all experiments, the ratio between melamine and formaldehyde was kept constant.

#### 2.2. Preparation of FMF Microspheres
Precipitation polymerization was used to prepare the fluorescent melamine formaldehyde (FMF) microspheres. For a typical synthesis, 0.625 g of melamine was thoroughly dissolved in 60 mL of deionized water under vigorous stirring at 70 °C. Next, 2.45 mL of aqueous formaldehyde solution (containing 0.9 g of formaldehyde) was added to the solution, and the heating and stirring were continued for 30 min. Then 10 μL of formic acid and 10 mL of aqueous solution containing 2 mg of 7-HCM, 0.2 mg of FITC and 1 mg of PHB were added into the reaction mixture for catalyzing the polycondensation reaction and the fluorescence staining of the microspheres, respectively. After 2 h, the suspension was naturally cooled to room temperature. Finally, the supernatant was removed by centrifugation and the resulting FMF microspheres were washed by water and ethanol for 3 times respectively, and then dried under vacuum at 40 °C for 4 h. In all experiments, the ratio between melamine and formaldehyde was kept constant.

#### 2.3. Preparation of FMF/MNPs Composite Microspheres
We utilized electrostatic interaction between FMF microspheres and magnetic nanoparticles to fabricate the FMF/MNPs composite microspheres. First, the magnetic nanoparticles (MNPs) were prepared by chemical coprecipitation method. Typically, 0.01 mol of \(\text{FeSO}_4\cdot6\text{H}_2\text{O}\) and 0.02 mol of \(\text{FeCl}_3\cdot6\text{H}_2\text{O}\) were mixed with 50 mL of deionized water in a 100 mL three-necked flask. The dispersion was ultrasonicated for 3 min to ensure homogeneity. Next, 25 mL of 10 M \(\text{NaOH}\) solution was added into the dispersion under \(\text{N}_2\) atmosphere for 1 h at room temperature, the dispersion color changed into black immediately upon the addition of \(\text{NaOH}\) solution. Then the reaction was performed for another 2.5 h at 90 °C. After that, the brownish black product was washed with \(\text{HCl}\) aqueous solution several times until the product was pH neutral, then washed with water for 3 times and isolated by an external magnet. The product was then added into 50 mL of sodium citrate solution (0.3 M) and stirred for 30 min at 90 °C under \(\text{N}_2\) atmosphere. After it was cooled to room temperature, the product was separated by an applied magnet and washed with excess amount of acetone. Finally, the purified product was dispersed in 40 mL of water and stirred vigorously at 80 °C for removal of remnant acetone. Second, The FMF microspheres and the magnette nanoparticles were mixed based on a certain scale in 30 mL of ethanol solution, and then incubated in a shaker with vigorous shaking at 25 °C for 4 h. The product was isolated by an external magnet, and those composite microspheres with weak magnetic response were removed.

#### 2.4. Preparation of FMF/MNPs/Ag-NPs Microspheres through in Situ Deposition Method
A typical route of the deposition of Ag nanoparticles (i.e., Ag-NPs) onto FMF/MNPs composite microspheres was carried out as follows: 40 mg of FMF/MNPs microspheres was dispersed in 60 mL of \(\text{AgNO}_3\) ethanol solution and then a certain amount of butylamine was added. The mixture was treated by ultrasonication for 2 min and stirred for 2 h with vigorous stirring at 50 °C. In all experiments, to ensure the complete reduction of \(\text{AgNO}_3\), the amount of butylamine was excessive and the ratio between \(\text{AgNO}_3\) and butylamine was kept constant (1 mg: 0.5 μL). The MF/MNPs/Ag-NPs composite microspheres were rinsed with ethanol under sonication and then isolated by an external magnet.

#### 2.5. Immobilization of Raman Reporter Molecules onto FMF/MNPs/Ag-NPs Microspheres
In this section, 4-amino- thiophenol (4-ATP), 4-chlorothiophenol (4-CTP), 4-mercaptophenol (4-HTP), 4-nitrophenol (4-NTP), 4-methoxy-a-toluene thiol (MATT), 5,5’-dithiobis(2-nitrobenzoic acid) (DTNB) and 3,5-bis(trifluoromethyl)benzenethiol (3-FMBT) were utilized as model Raman probe molecules. Typically, 10 mg of FMF/MNPs/Ag-NPs composite microspheres was dispersed in 20 mL of ethanol solution containing 0.1 M 4-CTP and 0.1 M 4-ATP for 2 h of incubation with gentle shaking under room temperature. After that, the composite microspheres were separated from the solution by centrifugation. The dispersion was centrifuged twice with ethanol to remove excess 4-CTP and 4-ATP and then dried under vacuum at 50 °C for 4 h.
Scheme 1. Preparation Route of FMF/MNPs/Ag-NPs/SiO₂ Composite Microspheres

![Scheme 1](image)

Figure 1. FESEM images of the MF microspheres synthesized with various mass ratios of formic acid: (a) 0.5 μL; (b) 1 μL; (c) 2 μL; (d) 5 μL; (e) 8 μL; (f) 10 μL; (g) 25 μL; (h) 50 μL. Melamine 0.625 g; formaldehyde 0.9 g; reaction temperature 70 °C; reaction time 2 h. All scale bars are 5 μm.

2.6. Silica Coating for Preparation of FMF/MNPs/Ag-NPs/SiO₂ Composite Microspheres. To protect the microspheres from the environmental disruption, a thin silica layer was coated on the surface of the FMF/MNPs/Ag-NPs composite microspheres based on the modified Stöber method as reported. For a typical synthesis, 10 mg of composite microspheres was added into the mixture of 30 mL of ethanol, 9 mL of water, and 1 mL of 28 wt % ammonia hydroxide. Then the dispersion was homogenized by ultrasonic vibration. Finally, under vigorous mechanical stirring, 50 mg of TEOS was slowly added into the dispersion and after continuous stirring for 5 h, silica layer was formed on the surface of the composite microspheres.

2.7. Characterization. Field-emission scanning electron microscopy (FESEM) was performed on an Ultra 55, Zeiss, which was operated at an accelerating voltage of 3 kV. Samples were dispersed in an appropriate concentration and cast onto mica plates at room temperature and sputter-coated with gold. Energy dispersive spectrometry (EDS) measurement was performed on Oxford Instrument X-MAX 50, work distance (WD) = 8.5 mm, accelerating voltage was kept 12 kV, and the scan times were kept constant as 5 times. Hydrodynamic diameter distribution measurement was conducted on a Nano ZS Zetasizer (model ZEN3600, Malvern Instruments) using a He–Ne laser at a wavelength of 632.8 nm. The fluorescence photomicrographs were obtained from OLYMPUS inverted fluorescence microscope (model U-TV0.63XCF). The fluorescence spectra were obtained from the steady state/life time fluorescence spectrometer (model 920). Thermogravimetric analysis (TGA) measurements were performed on a Pyris 1 TGA instrument with N₂ atmosphere and a constant heating rate of 20 °C/min. All measurements were taken under a constant flow of air of 40 mL/min. Powder X-ray diffraction (XRD) patterns were obtained using a X’Pert Pro (Panalytical, The Netherlands) powder diffractometer with Cu Kα radiation at λ = 0.154 nm operating at 40 kV and 40 mA. Fourier transform infrared (FT-IR) spectra of different samples were collected on a Magna-550 (Nicolet, USA) spectrometer using KBr pellets. The magnetic properties were carried out on a Model 6000 physical property measurement system (Quantum Design, USA) at 300 K. A 46.5 × 46.5 × 18 mm permanent NdFeB magnet (model: N50) with 5000 GS magnetic force was utilized in separating the superparamagnetic hybrid microspheres from solution. Transmission electron microscopy (TEM) images were obtained on a transmission electron microscope (H-600 Hitachi, Japan) at an accelerating voltage of 75 kV. Raman spectra were recorded on a HORIBA spectrometer (model XploRA) with 638 nm laser excitation and 1800 cm⁻¹ grating. The data acquisition time was usually 10 s, and peak intensities of the samples were normalized with respect to that of the silicon wafer at 520.7 cm⁻¹. The laser spot focused on a single composite microsphere randomly each time to obtain the Raman spectra.

3. RESULTS AND DISCUSSION

3.1. Preparation of Fluorescent Melamine Formaldehyde (FMF) Microspheres. To prepare functional FMF/MNPs/Ag-NPs/SiO₂ composite microspheres (Scheme 1), we need to prepare the monodispersed melamine formaldehyde (MF) and fluorescent melamine formaldehyde (FMF) microspheres at first. The formation of MF microspheres consists of two steps (Scheme S1). First, the prepolymerization of melamine and formaldehyde leads to hydroxymethylation, meanwhile, the hydrogen atoms in –NH₂ groups of the melamine are substituted by methylol groups (–CH₂OH) under heating. Second, the cross-linking structure based on the methylolmelamines is formed under the catalysis of formic acid, and the cross-linking reaction involves the formation of both methylene-ether and methylene bridges. As a result, a dense polymer network is formed, and the polymer network tends to form a spherical structure to reduce the surface energy. Usually, the morphologies and sizes of the microspheres are critical for their practical applications, so we studied the effects of the fabricating parameters on the morphologies and sizes of the MF microspheres. The molar ratio between the melamine and formaldehyde was fixed at 1:6 in all reaction systems. When the reaction time ranged from 0.5 to 2 h, the size of MF microspheres increased from 0.4 to 2.5 μm. With the reaction time extending further, the size of the microspheres increased only slightly, indicating that the monomer ran out and the reaction reached its limit after 2 h. It was also found that the formic acid, which served as the catalyst, has a significant effect on the morphologies and sizes of MF microspheres as shown in Figure 1. When the dosage of formic acid increased from 0.5 to 50 μL, the sizes of MF microspheres could be well controlled to vary from 4.20 to 0.56 μm and the microspheres were uniform in morphology (Figure S1). When the dosage of formic acid
was below 0.5 μL, the sizes of the MF microspheres were no longer uniform, because the catalytic sites are less than the nuclei numbers when the catalyst is insufficient. Although a sufficient amount of catalyst can increase the nucleus number and form a large amount of preliminary particles, the small-sized microspheres can be obtained with the increase of the catalyst. The corresponding size and size distribution were characterized by dynamic light scattering (DLS) (Figure S2 and Table S1), all the microspheres are very uniform and the polydispersity indexes are lower than 0.3.

Following the above studies, we introduced fluorescence molecules (7-HCM, FITC and PHB) inside of the MF microspheres during precipitation polymerization to prepare fluorescent melamine formaldehyde (FMF) microspheres, which also can avoid the quenching of the subsequently deposited Ag-NPs. When little amounts (0.2−2 mg) of fluorescence molecules were added into the reaction system during precipitation polymerization, a strong fluorescence signal of the microspheres could be observed by using the inverted fluorescence microscope (Figure 2). We also found that the feeding amount of 7-HCM and PHB should be higher than that of FITC to guarantee the comparable signals, so we gradually changed the feeding amount of the two former dyes until we got the clear fluorescence images of 7-HCM, FITC and PHB (Figure 2b−d). Meanwhile, we utilized fluorescence spectrometer to detect the fluorescence emission spectra of the FMF microspheres qualitatively (Figure S3). It is worth noting that the relative intensity of the individual fluorescence peaks can be tuned by adjusting the addition ratio of 7-HCM, FITC and PHB. As the precipitation polymerization of fabricating the FMF microspheres is a homogeneous nucleation process, the dissolved fluorescence molecules can be easily encapsulated into the microspheres without disrupting the morphologies and sizes of the microspheres. The size distribution of the FMF microspheres prepared with 5 μL formic acid was the narrowest (PDI = 0.02) and the hydrodynamic diameter was about 2.5 μm, which could be clearly identified and individually manipulated under a conventional optical microscope, thus this sample was chosen for further modification and used as the isolated SERS-active substrate. To be noted, we can utilize multiple fluorescence molecules, whose fluorescence emission peaks are not overlapped and can be distinguished easily by a fluorescence microscope, to increase the fluorescence encoding volume.

### 3.2. Preparation of FMF/MNPs Microspheres.

In this part, a simple but straightforward way was developed for construction of polymer/magnetite composite microspheres, involving direct immobilization of magnetic nanoparticles (MNPs) onto the surface of FMF microspheres. Such interfacial immobilization method was based on the strong affinity between MNPs and FMF microspheres. The precipitation polymerization between melamine and formaldehyde introduced abundant −NH₂ groups on the surface of the FMF microspheres (Figure S4), making it positively charged (+42.1 ± 1.4 mV). Citrate-acid-stabilized MNPs were prepared from coprecipitation as reported in our groups and owned a negative charge as much as −39.1 ± 1.1 mV, which is desirable for electrostatic interaction with positively charged FMF microspheres. Hence, the carboxyl-capped MNPs were added into the ethanol solution of the FMF microspheres in a certain mass ratio, followed by vigorous shaking for 2 h to promote electrostatic interaction and formed raspberry-structured FMF/MNPs composite microspheres eventually. The mass ratio of FMF microspheres to MNPs varied from 9:1 to 1:6, and we investigated that the influence of the mass ratio affected the interfacial immobilization of MNPs onto the FMF microspheres accordingly. Figure 3 shows the FESEM and EDS images of FMF/MNPs microspheres prepared at different mass ratios of MNPs to FMF microspheres. As the feeding amount of MNPs increased, increasing number of MNPs could be found on the surface of the FMF microspheres.

The TGA curves of FMF/MNPs microspheres were also measured to characterize the adsorption quantity of MNPs onto FMF microspheres (Figure S5). The FMF/MNPs microspheres exhibited three steps of the decomposition process, where the weight losses appeared from 100 to 400 °C, 400 to 500 °C and above 500 °C. The first stage was caused by evaporation of the low molecular weight products in the microspheres, and the second decomposed stage between 400 to 500 °C was resulted from the carbonization and oxidation of FMF resin and triazine ring at high temperature. The third thermal-decomposition stage started at 500 °C, which involved further decomposition of ether bonds of the cross-linked polymer and oxygenolysis of the FMF resin. The MNPs will be remained after the ignition, so from the TGA curves we can determine that the adsorption quantity of MNPs onto the FMF microspheres was from 5 wt % (FMF:MNPs = 9:1) to 72 wt % (FMF:MNPs = 1:6), indicating the strong electrostatic interaction between FMF microspheres and MNPs, the adsorbed amount of MNPs can be easily modulated through the feeding ratio. After that, we tested the magnetic response performance of the series of FMF/MNPs microspheres. We found that it took about 2 min for the FMF/MNPs (9:1) microspheres to complete separation from the solution, but for the other groups, about 0.5 min is enough for a thorough magnetic separation. Considering the deposition of MNPs will occupy a certain area of the FMF microsphere’s surface, which will influence the further deposition of Ag-NPs on the microspheres due to the Ag-NPs have strong interaction with

![Figure 2. Photographs of FMF microspheres doped with 7-HCM, FITC and PHB simultaneously with the mass ratio of 10:1:5: (a) optical image of FMF microspheres; (b) fluorescence image of FMF microspheres in blue channel (fluorescence from 7-HCM); (c) fluorescence image of FMF microspheres in green channel (fluorescence from FITC); (d) fluorescence image of FMF microspheres in red channel (fluorescence from PHB). All scale bars are 10 μm.](image)
FMF microspheres,\textsuperscript{36} thus too many MNPs adsorbing onto the FMF microspheres may hinder the deposition of Ag-NPs afterward. We choose the FMF/MNPs microspheres with 3:1 ratio to continue the subsequent fabrication of the composite microspheres in consideration of its fast magnetic separation and the suitable adsorption level of the MNPs.

3.3. Preparation of FMF/MNPs/Ag-NPs/SiO\textsubscript{2} Composite Microspheres. The deposition of Ag nanoparticles (Ag-NPs) onto FMF/MNPs microspheres was carried out by reduction of AgNO\textsubscript{3} with butylamine. The mass ratios of AgNO\textsubscript{3} to FMF/MNPs microspheres were varied from 0.5:1 to 20:1. Figure 4 shows the FESEM and EDS images of FMF/MNPs/Ag-NPs microspheres prepared at different mass ratios of AgNO\textsubscript{3} to FMF/MNPs microspheres, the efficiency of silver deposition was also determined (Table S2). To be noted, the MNPs on the FMF microspheres may interfere the observation of Ag nanoparticles, so the EDS detection of Ag element is indispensable as a supplement to observe the distribution of Ag nanoparticles.

Without AgNO\textsubscript{3}, no Ag nanoparticles other than the FMF/MNPs microspheres were observed on the basis of the FESEM and EDS image (Figure 4a,\textsuperscript{a'}). As the feeding amount of AgNO\textsubscript{3} increased, more and more Ag nanoparticles can be observed on the surface of the FMF/MNPs microspheres according to the Ag element mapping images (Figure 4b,\textsuperscript{b'}–f,\textsuperscript{f'}). The surface was poorly coated with Ag nanoparticles when the mass ratios were below 2:1. When the mass ratios were in the range of 2:1 to 10:1, Ag nanoparticles could be uniformly

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image.png}
\caption{FESEM images of FMF/MNPs microspheres with different mass ratios between FMF and MNPs: (a) 9:1; (b) 6:1; (c) 3:1; (d) 1:1; (e) 1:3; (f) 1:6. Fe element mapping images are displayed in panels a’–f’ corresponding to panels a–f. All scale bars are 5 µm.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image.png}
\caption{FESEM images of FMF/MNPs/Ag-NPs microspheres synthesized with different mass ratios of AgNO\textsubscript{3} to FMF/MNPs microspheres: (a) 0:1; (b) 0.5:1; (c) 2:1; (d) 5:1; (e) 10:1; (f) 20:1. Ag element mapping images are displayed in panels a’–f’ corresponding to panels a–f. All scale bars are 5 µm.}
\end{figure}
coated onto FMF/MNPs microspheres. With further increase of feeding amount of AgNO₃, there were many large chunks of Ag-NPs formed and accumulated around the FMF/MNPs microspheres (Figure 4f), which may lead to poor reproducibility and reliability for SERS experiments. All the experimental results hinted that the coverage and size of Ag nanoparticles could be well tuned by controlling the mass ratio of AgNO₃ to FMF/MNPs microspheres while keeping all other parameters fixed.

We also measured the UV−vis spectra of MF/MNPs/Ag-NPs microspheres prepared with different mass ratios of AgNO₃ to MF/MNPs (Figure S6a). Because the fluorescence dyes may disturb the UV−vis measurement of Ag-NPs because of the fluorescence background, we replaced the FMF/MNPs with MF/MNPs for studying the deposition of Ag-NPs. Before taking the UV−vis spectra, the MF/MNPs/Ag-NPs microspheres were collected using an external magnet and then dispersed into ethanol with gentle sonication. Upon the deposition of the Ag-NPs, the absorbance increased around 450 nm due to the plasmon resonance excitation. Obviously, the plasmon resonance peaks became red-shifted gradually, and the surface plasmon absorption bands of Ag-NPs deposited onto the MF/MNPs microspheres broadened as the amount of AgNO₃ increased. The probable reason is that the plasmon coupling between the Ag-NPs, as well as the interface structure between the Ag-NPs and the MNPs, may contribute to the red-shift and the broadening of the absorption band.37

The deposition of Ag-NPs onto MF/MNPs microspheres can be further confirmed by XRD (Figure S6b). A broad peak, present at 20−30°, originated from the amorphous phase of the FMF microspheres. For FMF/MNPs microspheres, five XRD peaks were clearly observed at 2θ values of 30.4, 35.5, 43.2, 57.1 and 62.8° (Δ), which could be indexed to the magnetite structures of Fe₃O₄ (JCPDS 75-1609). The peaks of Ag-NPs also could be clearly observed at 2θ values of 38.1, 44.3, 64.4 and 77.4° (□), corresponding to the reflection of the (111), (200), (220) and (331) crystalline planes of the cubic Ag (JCPDS 04-0783), respectively. Besides, there is little difference of the XRD peaks between the FMF/MNPs/Ag-NPs and the FMF/MNPs/Ag-NPs/SiO₂ microspheres, because the amorphous structure of the SiO₂ coating has not any peak in XRD detection. Meanwhile, the XRD peaks of the FMF/MNPs microspheres became weak but still visible after the deposition of Ag-NPs and SiO₂ coating.

The FESEM images of the FMF/MNPs/Ag-NPs microspheres with the mass ratio of 10:1 were also obtained (Figure S7), the Ag-NPs and MNPs on the microspheres are well-stacked. On the basis of the previous research in our group,38 we utilized the FMF/MNPs/Ag-NPs microspheres (10:1) to continue the subsequent modification with Raman reporter molecules and SiO₂ coating. After treated with Raman probes, the FMF/MNPs/Ag-NPs microspheres were coated with a thin layer of SiO₂ (Figure S8), which was achieved through hydrolysis of TEOS, and the thin SiO₂ layer with average thickness of 18 nm will protect the functional composite microspheres from the environmental disruption of weak base and acid. We have demonstrated the good short-time stability (in extreme acidic condition) and the long-term stability (stored in latex paint for over 5 months) of the composite microspheres (Figure S9 and Figure S10). The FESEM and elemental mapping images of the FMF/MNPs/Ag-NPs/SiO₂ composite microspheres is shown in Figure 5. The elemental mapping images of Figure Sb–d shows the distribution of Fe, Ag and Si elements in the FMF/MNPs/Ag-NPs/SiO₂ composite microspheres, respectively. The Ag-NPs and SiO₂ were uniformly distributed on the composite microspheres, indicating the successful deposition of Ag-NPs and SiO₂ layers. Meanwhile, the Fe element distributes more sparsely in the composite microspheres compared with Ag and Si elements. In our system, the amount of the adsorbed MNPs is enough to guarantee the fast separation of the composite microspheres.

In addition, we investigated the magnetic performances of the FMF/MNPs microspheres before and after the deposition of Ag-NPs and SiO₂ layer with a vibrating sample magnetometer at 300 K (Figure S11), the three curves present minor hysteresis loops and the magnetic saturation values of FMF/MNPs, FMF/MNPs/Ag-NPs and FMF/MNPs/Ag-NPs/SiO₂ microspheres are 11.4, 8.8 and 5.8 emu/g, respectively. It can be regarded as another evidence for the successful deposition of Ag-NPs and SiO₂ coating onto the FMF/MNPs microspheres, the decrease of the magnetic saturation value is in proportion with the reduced amount of the MNPs, as well as the increase amount of Ag-NPs and SiO₂ (Table S3). The quick magnetic response of the FMF/MNPs/Ag-NPs/SiO₂ composite microspheres is ideal for magnetic separation and enrichment.

3.4. Evaluation of the SERS Signal Intensity of Individual Composite Microsphere. In this part, seven SERS probes (NTP, MATT, HTP, DTNB, CTP, ATP and 3-FMBT) were used to evaluate the SERS activity of the single FMF/MNPs/Ag-NPs/SiO₂ microsphere, all the SERS spectra are the average of three isolated FMF/MNPs/Ag-NPs/SiO₂ microspheres in three randomly chosen areas. The optical microscopic photograph of CTP-treated FMF/MNPs/Ag-NPs/SiO₂ microspheres showed that the individual microsphere could be clearly identified (Figure S12a), and the laser beam could be easily focused on each single FMF/MNPs/Ag-NPs/SiO₂ microsphere. The SERS spectrum of each Raman probe is notably different from each other and the spectrum according to its characteristic Raman shifts is easy to be distinguished (Figure S12b). This result indicated that the individual composite microsphere possessed a very high SERS activity and had great potential as an isolated SERS-active substrate.
Furthermore, we can also immobilize multiple Raman probes simultaneously onto one microsphere in order to achieve larger SERS encoding volume. The selected Raman probes had their own unique Raman spectra, the Raman characteristic shifts did not overlap with each other. Figure 6a shows the gradual change of the Raman spectra from single Raman probe to...

Figure 6. Raman spectra of the FMF/MNPs/Ag-NPs/SiO₂ microsphere immobilized with (a) different kinds of Raman probes, (b) two kinds of Raman probes and (c) three and four kinds of Raman probes; (d) Raman spectra of the isolated CTP-immobilized microsphere randomly chosen from 15 areas.

Figure 7. Fluorescence spectra, Raman spectra and the binary coding patterns of the dual-mode encoded microsphere utilizing 7-HCM, FITC and PHB as fluorescent reporters, and ATP, CTP, DTNB and HTP as Raman reporters.
multiple Raman probes immobilized on the FMF/MNPs/Ag-NPs/SiO₂ composite microsphere. For instance, compared with the Raman spectra of ATP-immobilized microsphere, the corresponding characteristic Raman shifts of CTP could be observed whereas the characteristic shifts of ATP remained after addition of CTP probe to the composite microsphere. Furthermore, we could observe the gradual increase of different Raman characteristic shifts when further addition of DTNB and HTP onto the microsphere, all the previous characteristic shifts were remained, which did not overlap with the new shifts. 

Meanwhile, as for the Raman spectra of the FMF/MNPs/Ag-NPs/SiO₂ microsphere immobilized simultaneously with two types of Raman probes (Figure 6b), it is obvious that the characteristic shifts can be easily distinguished with each other. For example, for the ATP/CTP and MATT/DTNB immobilized microsphere, the former one has the characteristic Raman shifts of 1440 (ATP), 336 (CTP) and 536 cm⁻¹ (CTP), and the latter one’s characteristic shifts locate at 1147 (DTNB), 665 (MATT) and 729 cm⁻¹ (MATT). If the fluorescence encoding is fixed, we can recognize the Raman signals alone to identify the composite microsphere. When multiple Raman probes are immobilized onto the composite microsphere, as shown in Figure 6c, it is still obvious to distinguish the characteristic Raman shifts from 3 or even 4 different Raman probes, thus we can increase the Raman encoding capability with combination of multiple Raman probes. In addition, we can further enlarge the dual-encoded volume by combining the fluorescence codes, which is much efficient to enlarge the encoding volume in comparison with the “layer coding” technology.39

Furthermore, to explore the reproducibility and homogeneity of the SERS signals, SERS spectra of the CTP-treated composite microsphere (Figure 6d, the treated concentration of CTP is 10⁻³ mg/mL) were measured in 15 randomly chosen areas. As shown in Figure 6d, for the CTP-treated FMF/MNPs/Ag-NPs/SiO₂ composite microsphere, really good reproducibility and homogeneity of the corresponding SERS signals were observed, which is beneficial for the single microsphere detection and identification.

3.5. Dual-Mode Encoded Microsphere as Covert Tag for Latex Paint. Utilizing the two encoding elements, fluorescence reporters and Raman probes, we fabricated a large amount of dual-mode encoded composite microspheres with different fluorescence reporters (7-HCM, FITC and PHB) and Raman probes (ATP, CTP, DTNB and HTP) in a certain combination order. To date, the Raman probe is numerous for Raman coding. Furthermore, the combination of fluorescence and SERS make it possible to increase greatly the encoded volume of the composite microsphere, and we can even have combinations of different fluorescence reporters and numerous Raman probes to achieve high encoded capacity. However, the spectra analyses are complicated and it’ll take heavy workload for artificial identification. To solve the problem, we used a binary coding method for better and simpler storing of the encoded volume. Typically, we utilized three fluorescence reporters (7-HCM, FITC and PHB) and four Raman probes (ATP, CTP, DTNB and HTP) to establish the binary coding pattern. As shown in Figure 7, each kind of encoded microsphere is in one to one correspondence with the binary coding pattern. After detection of the fluorescence and Raman spectrum, we are able to change directly the complicated spectra into simple binary code based on the database that we established, and we can easily recognize the codes by scanning the “barcode” of the binary sequence.40 Because the database is on the basis of the combinations of numerous characteristic spectra, it will contain large coding capacity, which is expected to find real application in the commercial products like coatings, oils and inks with high-level anticounterfeiting security.

Herein, we not only can blend a certain amount of the dual-encoded microspheres into one specified product as the identity code hidden in the product but also can mix the microspheres with different batches of the products thus increase the anticounterfeiting secure level; it also can satisfy the demand of the simple on-site detection (Figure S13). Thanks to the MNPs embedded in the composite microsphere, it is feasible to separate and enrich the FMF/MNPs/Ag-NPs/SiO₂ composite microspheres from the encoded system by magnet. In our experiment, we added extremely low amount of the microspheres into the coating product as the covert tag and then isolated them from marked system using an external magnet, and further detected the fluorescence and Raman signals of the isolated microsphere to identify the specific signals according to the binary database.

For a typical application, we mixed 1 ppm dual-encoded composite microsphere in the latex paint in a 50 mL centrifuge tube. After vigorous magnetic stirring for 1 h, we added a defined amount of water into the tube and mix evenly to decrease the viscosity of the system. After that, we put an external magnet at the bottom of the tube with gentle shaking for about 10 min to isolate and enrich the hybrid microspheres, and then we removed the supernatant and dropped the microspheres onto the glass slide and detected the fluorescence and Raman signals of the isolated microsphere. As shown in Figure 8, we could simply read out the corresponding binary code according to the established database (Figure 8a, inset). The experimental results showed that the trace amount (1 ppm) of the microsphere was enough for labeling the latex paint as covert tag, i.e., even 1 g of FMF/MNPs/Ag-NPs/SiO₂ composite microsphere...
composite microspheres was added into 1 ton of latex paint, the microspheres also could be isolated and enriched magnetically for reading out the binary coding information and identifying the product information.

Another real demand is to detect directly the coating film and read out the hidden information. To realize this objective, we added 1 mg of FMF/MNPs/Ag-NPs/SiO₂ composite microspheres labeled with two fluorescence reporters (FITC and PHB) and one Raman probe (CTP) into 2.5 g of latex paint. After homogeneous mixing, the labeled paint was applied on a glass slide to form a uniform coating film. As shown in Figure 9a–c, strong fluorescence signals of FITC and PHB of the microspheres could be detected in the coating film with the help of the fluorescence microscope, the composite microspheres were well distributed in the coatings film. Besides, we also can utilize Raman mapping method to detect the microsphere distribution in the coating film. As shown in Figure 9d–f, the red spots are the reflection of the characteristic Raman signals. Meanwhile, we almost cannot distinguished the appearance between the doped and undoped coating films in visible color as shown the inset in Figure 9a, which indicated the doped dual-mode encoded microspheres did not influence the visible appearance of the latex paint film. It needs to be noted that the dual-mode encoded covert security technology not only can be applied in coating products but also can have broad applications in multiple products like oil, inks etc. From the above experiment results, we can conclude that the advanced security performance can be obtained with combination of the Raman probes into the fluorescent composite microspheres for anticounterfeiting applications.

4. CONCLUSION

In conclusion, we have successfully fabricated a new kind of dual-mode encoded FMF/MNPs/Ag-NPs/SiO₂ composite microspheres that exhibited excellent covert encoding ability as well as quick magnetic separation. The synthetic procedure involved the preparation of FMF microspheres, the deposition of MNPs and Ag-NPs and the immobilization of Raman probes. To improve the stability of the composite microspheres, a thin silica layer was coated on the surface of the FMF/MNPs/Ag-NPs composite microspheres. The coverage level of MNPs and Ag-NPs on FMF microspheres were easily tuned by varying the feeding amount of MNPs and AgNO₃, respectively. The composite microspheres were encoded by combination of various fluorescence reporters and Raman probes, and the binary database was established to simplify the analysis procedure. What's more, the dual-mode encoded magnetic composite microspheres in the marked latex paint could be easily retrieved and identified. We successfully separated and enriched the composite microspheres from the latex paint by as low as 1 ppm, which was sufficient to perform authenticity tests for the coating product. Meanwhile, the covert security information on the composite microspheres in the marked coating film had been read out in situ and the existence of the composite microspheres did not influence its appearance. This dual-mode encoded technology is efficient, easily detected and cost-saving, which will greatly broaden its application in the anticounterfeiting area.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.6b02359.

Optical microscope images and hydrodynamic diameter distribution of the MF microspheres synthesized with various mass ratios of formic acid; fluorescence emission spectra of the FMF microspheres loaded with 7-HCM, FITC and PHB; FTIR spectrum of MF microspheres; TGA curves of the FMF/MNPs microspheres with different feeding mass ratios; UV–vis spectra and XRD patterns of FMF/MNPs/Ag-NPs microspheres prepared with different mass ratios between AgNO₃ and FMF/MNPs microspheres; FESEM images of FMF/MNPs/Ag-NPs composite microspheres with the mass ratio of 10:1 (AgNO₃:FMF/MNPs); TEM images of FMF/MNPs/Ag-NPs and FMF/MNPs/Ag-NPs/SiO₂ composite microspheres; changes of UV–vis absorbance spectra of FMF/MNPs/Ag-NPs/SiO₂ and FMF/MNPs/Ag-NPs
with time treated with 1 M HNO₃ aqueous solution; optical microscope image and fluorescence photographs of FMF/MNPs/Ag-NPs/SiO₂ composite microspheres (stored in latex paint for over 5 months); magnetic hysteresis curves of FMF/MNPs, FMF/MNPs/Ag-NPs and FMF/MNPs/Ag-NPs/SiO₂ microspheres at 300 K; optical photograph of the CTP-treated FMF/MNPs/Ag-NPs/SiO₂ microspheres on a glass slide under a confocal Raman scope; SERS spectra for the individual composite microsphere treated with seven different SERS probes; photographs of the samples lighted by normal flashlight and UV lamp with central wavelength of 365 nm (PDF).

## AUTHOR INFORMATION

**Corresponding Author**
*C. Wang. E-mail: ccwang@fudan.edu.cn.*

**Notes**
The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

This work was supported by National Science and Technology Key Project of China (Grant No. 2012AA020204), National Science Foundation of Shanghai (Grant No. 13JC1400500).

## REFERENCES


