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$NH_4Y_3F_{10}$ 多孔纳米晶的制备及其在 MALDI-TOF-MS 中的应用

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摘要: 利用水热法合成出 $NH_4Y_3F_{10}$ 多孔纳米晶。由于 Y^{3+}离子的激发态能量可以转移给具有较高振动能的有机分子,因此这些多孔纳米晶可以作为基质辅助激光解析电离飞行时间质谱的基体材料,用于检测小分子和聚乙二醇。通过与商品化的基体材料(CHCA、DHB)对比,证明 $NH_4Y_3F_{10}$ 多孔纳米晶是一种性能优异的基体材料。这种新型基体材料已经成功应用于有机分子、小肽、C_{60}、缺氧诱导因子(HIFs)和聚乙二醇的分子量的检测,显示出这种基体材料具有广泛的应用前景。

关键词: $NH_4Y_3F_{10}$; 基体; 基质辅助激光解析电离飞行时间质谱; 小分子; 聚乙二醇
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Synthesis of Mesoporous $NH_4Y_3F_{10}$ Nanocrystals and Its Application in MALDI-TOF-MS

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Abstract: Mesoporous $NH_4Y_3F_{10}$ nanocrystals were fabricated to serve as a background-free matrix for analysis of small molecules and polyethylene glycols (PEGs) by matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). The excited state of yttrium ions can transfer energy to high-energy vibrations of organic molecules, which provides the potential technological application in MALDI-TOF-MS analysis of small molecules and PEGs. The advantage of this matrix in comparison with α-cyano-4-hydroxycinnamic acid (CHCA) and 2,5-dihydroxybenzoic acid (DHB) was demonstrated by MALDI-TOF-MS analysis of a peptide mixture. The efficiency of the mesoporous $NH_4Y_3F_{10}$ nanocrystals as a novel matrix of low molecular weight compounds was verified by analysis of small peptide, organic compounds, C_{60} and hypoxia-inducible factors (HIFs). This matrix is also successfully used for analysis of PEGs (PEG 8000 and PEG 15000), suggesting a potential for monitoring reactions and for synthetic polymers quality control.

Key words: $NH_4Y_3F_{10}$; matrix; MALDI-TOF-MS; small molecules; PEG

Rare-earth compounds attract a great deal of attention due to the potential technological applications in MALDI matrix [1], opticals [2-4], biolabels [5-8] and catalysts[9] based on their unique properties arising from the transitions of 4*f* electrons. As a rare-earth ions host matrix, rare-earth fluorides have many advantageous features which the conventional oxygen-based systems (e.g. oxides and inorganic salts) do not have, such as low vibrational energies, good optical transparency over a wide wavelength range, and the subsequent

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minimization of the quenching of the excited state of the rare-earth ions[10-13]. Among various rare-earth fluorides, MF-LnF_3 (M=alkaline metal, NH_4^+ and Ln=rare earth) has been widely studied due to its potential applications in biological labels [8], solid-state lasers and display panels [14]. $NH_4Ln_3F_{10}$ is a typical ionic family that consists of a large number of compounds. To obtain $NH_4Ln_3F_{10}$ crystals with different shapes and sizes, many wet chemistry approaches have been developed. For instance, $NH_4Ln_3F_{10}$ (Ln=Dy, Ho, Y, Er, Tm) has been synthesized using a hydrothermal method [15]. Cubic $NH_4Gd_3F_{10}$, $NH_4Tb_3F_{10}$, $NH_4Dy_3F_{10}$, $NH_4Ho_3F_{10}$, $NH_4Er_3F_{10}$ and $NH_4Tm_3F_{10}$ could be obtained by precipitation from a solution containing ammonium ions [16-17]. $NH_4Y_3F_{10}$ sub-microcrystals were prepared using a solution method[18-19]. Herein, we present a hydrothermal route to the synthesis of mesoporous $NH_4Y_3F_{10}$ nanocrystals by reaction of aqueous Y^{3+} with ammonium fluoride in the presence of EDTA. Since $NH_4Y_3F_{10}$ have a high UV absorbance and can be used as UV absorbents. These mesoporous $NH_4Y_3F_{10}$ nanocrystals can be employed as a background-free matrix for analysis of small molecules and polyethylene glycols (PEGs) by matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). The performance of mesoporous $NH_4Y_3F_{10}$ nanocrystals matrix was demonstrated by determining a series of analytes, including peptide, organic compounds, C_{60}, hypoxia-inducible factors (HIFs) and PEGs. Mass spectra of analytes were observed without the interference of background ions, revealing the matrix of mesoporous $NH_4Y_3F_{10}$ nanocrystals to be background free.

1 Experimental

1.1 Materials

Acetonitrile (ACN), 2-phenylbenzimidazole, 2-nitrobenzaldehyde, trifluoroacetic acid (TFA), matrix of CHCA and DHB were purchased from Sigma (St. Louis, MO). Peptides of Val-Gly-Gly, Pro-Glu, Met-Phe, Trp-Leu, and Val-Tyr-Val were obtained from Serva (Feinbiochemica, Heidelberg, Germany). Citric acid, propranolol, Y_2O_3, ethylenediaminetetraacetic acid (EDTA) and NH_4F were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). C_{60} was obtained from Yongxin Technology Co., Ltd. Hypoxia-inducible factors (HIFs) was obtained from GenScript USA Inc. PEG 8000 and PEG 15000 were obtained from American Polymer Standards Corp. (Mentor, OH). Other reagents were analytical grade and purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). The water used throughout the experiment was prepared from a Milli-Q water purification system (Millipore, Milford, MA).

1.2 Synthesis of mesoporous $NH_4Y_3F_{10}$ nanocrystals

In a typical synthesis, EDTA solution was obtained by dissolving 500 mg EDTA in 6.5 mL of 1 mol·L^{-1} ammonium hydroxide solution. NH_4F solution was obtained by dissolving 200 mg NH_4F in 7 mL water/ethanol (2/5, *V*/*V*). Y_2O_3 (0.5 mmol) was completely dissolved in 6.8 mL of 0.74 mol·L^{-1} HNO_3 to form $Y(NO_3)_3$ solution. Then, the EDTA solution and 10 mL ethanol were added to the obtained $Y(NO_3)_3$ solution in sequence. After 5 min ultrasonic bath, the NH_4F solution was introduced. Subsequently, the mixed system was sonolyzed for 5 min to ensure homogeneous dispersion of all reagents in the solutions and transferred into a Teflon-lined autoclave. After the autoclave was tightly sealed and heated at 110 ℃ for 12 h, the system was allowed to cool to room temperature naturally. The as-obtained white precipitate was collected, washed with distilled water and absolute ethanol several times, and finally dried at 110 ℃ in air for 0.5 h.

1.3 Preparation of analyte solution

Peptides of Val-Gly-Gly, Met-Phe, Trp-Leu, and Val-Tyr-Val were all dissolved in water (0.1% TFA) at the concentration of 5 mmol·L^{-1} as storage solutions, separately, the mixture solution of the 4 peptides were obtained by mixing storage solutions and diluting with water (0.1% TFA) to the concentration of 1.0 mmol·L^{-1} each. The storage solutions for citric acid, propranolol, 2-phenylbenzimidazole, 2-nitrobenzaldehyde and HIFs were prepared by dissolving them in water (0.1% TFA) at the concentration of 0.1 mg·mL^{-1}, separately. The storage solutions for C_{60} were obtained by dissolving

them in toluene (0.1% TFA) at the concentration of 1.0 mg·mL^{-1}, separately. The storage solutions for PEG 8000 and PEG 15000 were obtained by dissolving them in water (0.5% TFA) at the concentration of 2.0 mg·mL^{-1}, separately. All storage solutions were refrigerated at around 4 ℃ for future use.

1.4 Sample preparation for MALDI-TOF-MS

Matrix of CHCA was prepared as a saturated solution in TA (0.1% TFA in water/acetonitrile, 2/1, *V*/*V*). Matrix of DHB was obtained by dissolving DHB in TA at 20 mg·mL^{-1}. The matrix solution of mesoporous $NH_4Y_3F_{10}$ nanocrystals was prepared by dispersing them in acetonitrile with sonication for 2 min at the concentration of 0.5 mg·mL^{-1}. Solution of analyte (1 μL) was pipetted onto the sample target and left in air at room temperature for 5 to 10 min to form a thin layer of analyte by evaporating water. Then 1 μL solution of matrix was pipetted onto the layer of analyte and left in air for 5 to 10 min for the evaporation of the solvent and for further analysis by MALDI-TOF-MS.

1.5 Characterization

XRD patterns of the products were recorded on a Shimadzu XRD-6000 X-ray diffractometer with Cu *Kα* radiation (λ=0.154 06 nm) at a scanning rate of 0.1°·s^{-1} in the 2*θ* range from 20° to 80°. The morphologies of the products were characterized by a JEM-200CX transmission electron microscope and a JEM 2010 high-resolution transmission electron microscope. Mass spectra were performed on Bruker Autoflex with a nitrogen laser (λ=337 nm) at −20~20 kV.

2 Results and discussion

2.1 Structure and morphology

The synthesis of mesoporous $NH_4Y_3F_{10}$ nanocrystals has been achieved in water/ethanol solution containing $Y(NO_3)_3$ and NH_4F by thermal treatment in the presence of EDTA for 6 h. Fig.1a shows the XRD pattern of the as-prepared sample. All the positions of the peaks can be readily indexed to cubic $NH_4Y_3F_{10}$ (ICSD 93690). No other impurity peaks are detected in the XRD pattern, indicating high purity of our products. Well-resolved diffraction peaks reveal good crystallinity of the $NH_4Y_3F_{10}$ specimens.

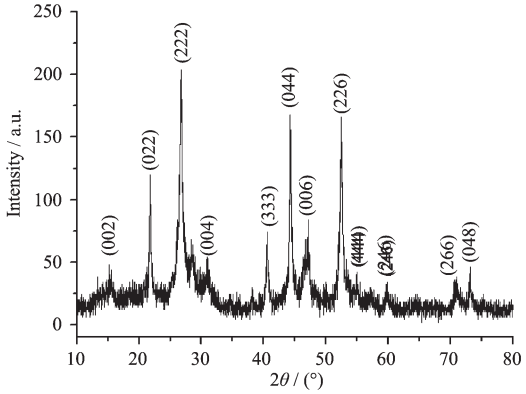
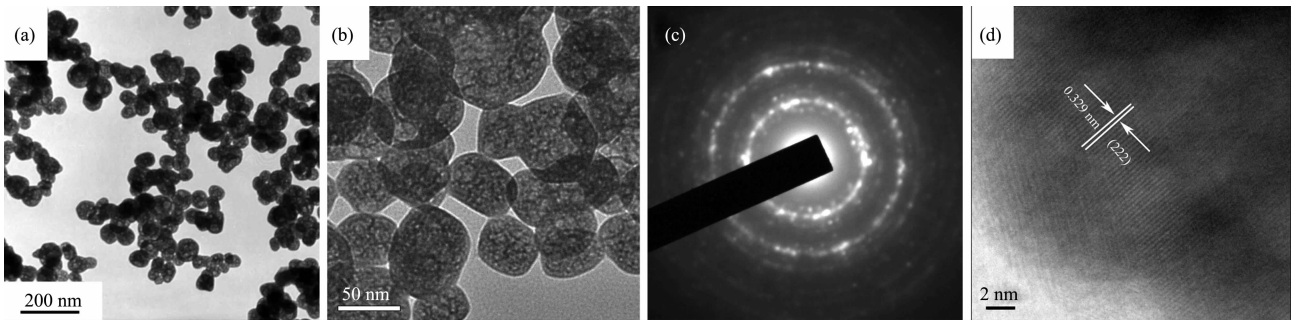


Fig.1 XRD pattern of mesoporous mesoporous $NH_4Y_3F_{10}$ nanocrystals

The morphology of the as-obtained product was investigated by transmission electron microscopy (TEM). Fig.2a shows a typical TEM image of the product obtained at 110 ℃ for 6 h, indicating that the sample is composed of ellipsoidal particles. The enlargement shows that these ellipsoidal nanocrystals have obvious porous structures (Fig.2b). The



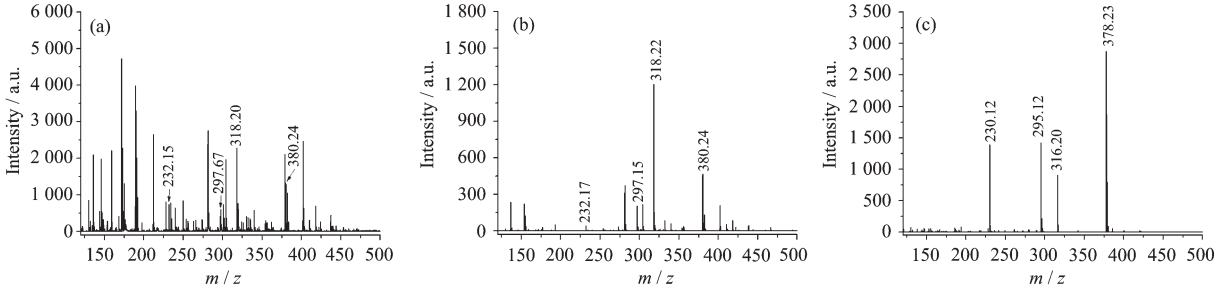
(a) and (b) TEM images; (c) a selected area electron diffraction pattern (SAED); (d) high-resolution TEM (HRTEM) image of mesoporous $NH_4Y_3F_{10}$ nanocrystals

Fig.2 morphology characterization of mesoporous $NH_4Y_3F_{10}$ nanocrystals

corresponding selected area electron diffraction (SAED) pattern in Fig.2c indicates that the $NH_4Y_3F_{10}$ ellipsoidal nanocrystals are polycrystalline and the most distinct four concentric diffraction rings can be indexed to (022), (222), (333) and (006) planes from the center, sequentially. The high-resolution transmission electron microscopy (HRTEM) image of a single $NH_4Y_3F_{10}$ nanocrystals (Fig.2d), indicates that the distance between the adjacent lattice planes is 0.329 nm, is ascribed to (222) crystal planes of the cubic phase $NH_4Y_3F_{10}$.

2.2 MALDI matrix

$NH_4Y_3F_{10}$ mesoporous nanocrystals can be employed as matrix for MALDI-TOF-MS analysis of small molecules. In order to evaluate the performance of $NH_4Y_3F_{10}$ mesoporous nanocrystals as a background-free ionization element, a peptide mixture containing Val-Gly-Gly, Met-Phe, Trp-Leu and Val-Tyr-Val, were chosen as models. We compared the MALDI-TOF mass spectra obtained from the conventional matrixes of CHCA, DHB and $NH_4Y_3F_{10}$ mesoporous nanocrystals. As shown in Fig.3, all the analytes were detected as $[M+H]^+$ (Val-Gly-Gly, 232; Met-Phe, 297; Trp-Lea, 318 and Val-Tyr-Val, 380), but strong interference of backgrounds caused by matrix of CHCA (Fig.3a) and DHB (Fig.3b) makes it difficult to identify analytes. Moreover, the suppression effects among analyte molecules result in the poor reproducibility and resolution of peaks for analytes. It is clear that the mass spectrum of $NH_4Y_3F_{10}$ mesoporous nanocrystals with the peptide mixture were obtained in the negative-ion reflection mode. Four prominent peaks without the interference of background ions for Val-Gly-Gly (230, $[M-H]^-$), Met-Phe (295, $[M-H]^-$), Trp-Lea (316, $[M-H]^-$), and Val-Tyr-Val (378, $[M-H]^-$) were observed as displayed in Fig.3c, suggesting that $NH_4Y_3F_{10}$ mesoporous nanocrystals should be an excellent matrix for MALDI-TOF-MS analysis of small molecules.



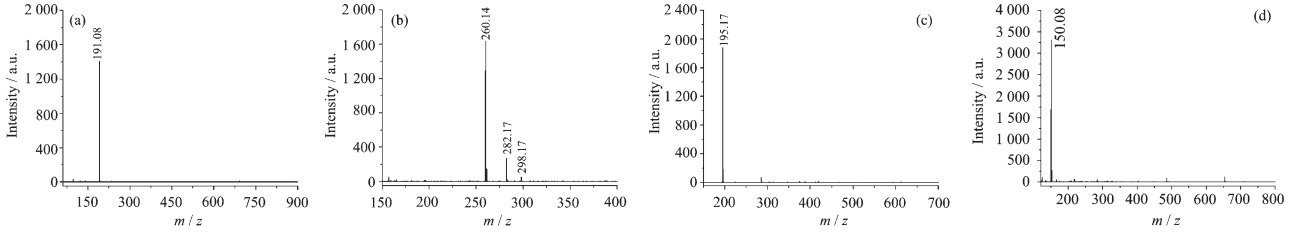
(a) CHCA matrix, detected in the positive-ion reflection mode; (b) DHB matrix, detected in the positive-ion reflection mode; (c) $NH_4Y_3F_{10}$ mesoporous Nanocrystals, detected in the negative-ion reflection mode

Fig.3 Mass spectra of a peptide mixture containing Val-Gly-Gly, Met-Phe, Trp-Leu, and Val-Tyr-Val

To demonstrate the general applicability of the matrix of $NH_4Y_3F_{10}$ mesoporous nanocrystals, we analyzed 4 small-molecule compounds with distinct chemical structures, including citric acid, propranolol, 2-phenylbenzimidazole, 2-nitrobenzaldehyde (0.1 mg · mL^{-1}). The matrix of $NH_4Y_3F_{10}$ mesoporous nanocrystals generated very clean spectra that show the presence of only the small-molecule compound-related peaks for each analyte, including the cationic parent, sodium adduct, potassium adduct, and anion parent, as shown in Fig.4.

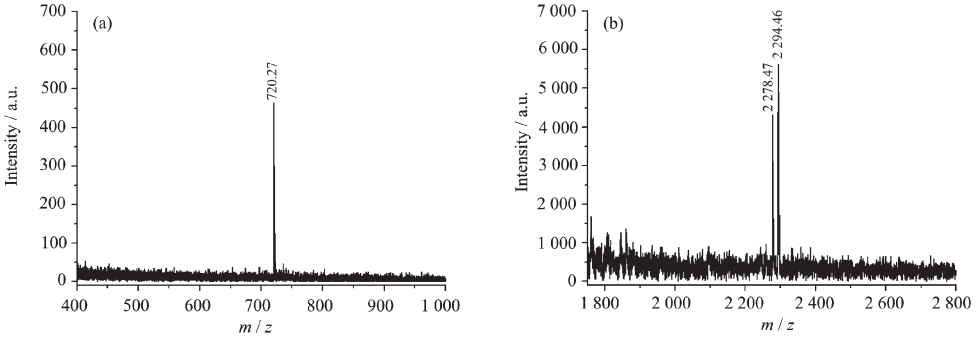
$NH_4Y_3F_{10}$ mesoporous nanocrystals can also be employed as matrix for MALDI-TOF-MS analysis of larger molecules. Fig.5 shows the mass spectrum of C_{60} and Hypoxia-inducible factors (HIFs) with matrix of $NH_4Y_3F_{10}$ mesoporous nanocrystals. The peak of m/z at 720 Da could be easily indexed to $[M-e]^+$ cationic parent of C_{60} (Fig.5a), and peaks of m/z at 2278 and 2294 Da could be attributed to $[M+H]^+$ and $[M+O+H]^+$ adduct ions (Fig.5b).

MALDI-TOF-MS has an advantage in the high-speed determination of structural information, molecular weight averages, and purity of macromolecules. We characterized PEG 8000 and PEG 15000 with $NH_4Y_3F_{10}$ mesoporous nanocrystals to explore the application of this matrix in the detection of synthetic polymers. Fig.6 shows the mass spectra of



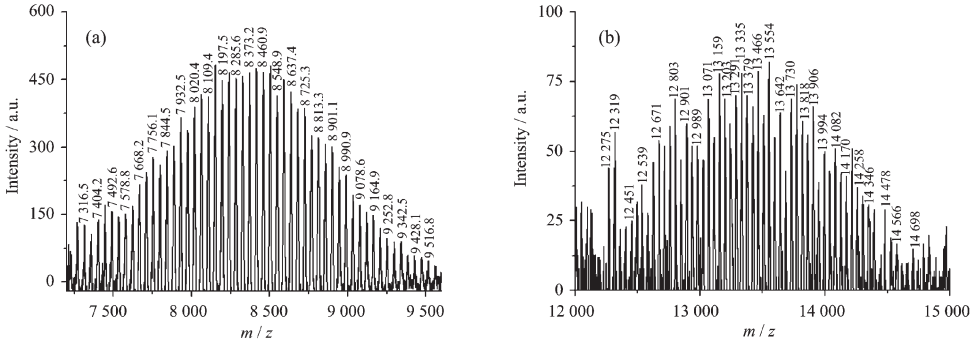
(a) citric acid (191, $[M-H]^-$); (b) propranolol (260, $[M+H]^+$; 282, $[M+Na]^+$; 298, $[M+K]^+$); (c) 2-phenylbenzimidazole (195, $[M+H]^+$); (d) 2-nitrobenzaldehyde (150, $[MH]^-$)

Fig.4　Mass spectra of citric acid, propranolol, 2-phenylbenzimidazole and 2-nitrobenzaldehyde with matrix of $NH_4Y_3F_{10}$ mesoporous nanocrystals



(a) C_{60}(720, $[M-e]^+$); (b) HIFs (2278, $[M+H]^+$; 2294, $[M+O+H]^+$)

Fig.5　Mass spectra of C_{60} and HIFs with matrix of $NH_4Y_3F_{10}$ mesoporous nanocrystals



(a) PEG 8000, 7 129~9 561 Da; (b) PEG 15000, 12 000~15 000 Da

Fig.6　Mass spectra of PEG 8 000 and PEG 15 000 with matrix of $NH_4Y_3F_{10}$ mesoporous nanocrystals

PEG 8000 and PEG 15000 with matrix of $NH_4Y_3F_{10}$ mesoporous nanocrystals, indicating that the upper limit of detectable mass range was ~15000 Da. It is noted that the mass ranges of PEG 8 000 and PEG 15 000 are at 7129~9561 Da and 12 000~15 000 Da, respectively.

3　Conclusion

In summary, mesoporous $NH_4Y_3F_{10}$ nanocrystals were fabricated via a straightforward method. These mesoporous $NH_4Y_3F_{10}$ nanocrystals were employed as an ionization platform, in which the $NH_4Y_3F_{10}$ nanocrystals matrix functions as the energy receptacle for UV laser radiation and the energy transporter for the desorption/ionization of analytes with the minimization of interference signals caused by matrix ion. The suitability of the mesoporous $NH_4Y_3F_{10}$ nanocrystals matrix was demonstrated by MALDI-TOF-MS analysis of a series of analytes, including organic compounds,

C_{60}, HIFs, PEGs and a peptide mixture.

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