CHARACTERIZATION OF UPCONVERSION NANOPARTICLES BY FLUORESCENCE SPECTROMETRY AND CAPILLARY ELECTROPHORESIS

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Abstract: Upconversion nanoparticles (UCNPs) are a novel class of luminescent tags for applications in life and material sciences. Unlike traditional fluorophores, UCNPs exhibit emission of shorter wavelength under near-infrared excitation (typically 980 nm). In this work, we have examined these unique photophysical properties by fluorescence spectrometry and capillary electrophoresis. UCNPs co-doped with Yb(III) and Er(III) were characterized using laboratory-made fluorescence spectrometer. We have exploited and evaluated two excitation sources and the dependence of the fluorescence of UCNPs on the relative excitation power. Moreover, capillary electrophoresis with laser-induced fluorescence (CE-LIF) detection was for the first time used for characterization of the nanoparticles. It was proved that CE-LIF is a valuable method to be used for investigation of upconversion luminiscence and monitoring of the interactions of UCNPs with other molecules of interest.

Key Words: upconversion nanoparticles, UCNP, fluorescence spectrometry, capillary electrophoresis

INTRODUCTION

Lanthanide-doped photon upconversion nanoparticles (UCNPs) have been in the focus of research interest due to their unique photophysical properties. The anti-Stokes shifted luminescence is a result of a sequential photon absorption (Nadort et al. 2016, Zhu et al. 2017). Most upconversion materials rely on a crystalline host such as metal fluoride (most commonly $NaYF_4$, CaF_2), oxide (Y_2O_3), or phosphate (YPO_4), and are co-doped with a single Ln^{3+} ion or a combination of Ln^{3+} such as Er^{3+}, Yb^{3+}, Tm^{3+} Ho^{3+}, and Gd^{3+} (Gai et al. 2014).

Advantageous features of UCNPs are basically no background due to anti-Stokes shifted emission (Zhu et al. 2017), low toxicity (Zhou et al. 2015), no photobleaching or photobrightening (Zheng et al. 2015, Zhou et al. 2015), applicability for long-term imaging (Wu et al. 2015), and particularly well suitability for deep tissue imaging (Wu et al. 2015, Xu et al. 2013, Yang 2014, Zhou et al. 2012). Therefore, UCNPs can in many ways overcome limitations of traditional fluorescent reporters, such as organic dyes or semiconductor nanocrystals (quantum dots).

Due to their superior properties, a broad field of applications of UCNPs can be found. Recent progress enabled an increasing number of (bio)analytical (Hlavacek et al. 2016, Hlavacek et al. 2017, Chatterjee et al. 2010), diagnostic (Yang 2014), and sensing applications (Hao et al. 2013, Shi et al. 2015), as well as photovoltaic (Ramasamy et al. 2014) and security applications (Meruga et al. 2014).

Despite above mentioned advantages, there is no commercially available system with ability to measure upconversion properties, which limits further expansion of the applications of UCNPs. To measure fluorescence emission properties, one needs to utilize custom-made spectrometers. This analytical tool, however, needs to be standardized. As mentioned in literature, different applications have specific requirements regarding laser power density (Kaiser et al. 2017).

In addition to the fluorescent spectrometry and gel electrophoresis (Hlavacek et al. 2014, Sedlmeier et al. 2016), capillary electrophoresis is a useful tool to characterize nanoparticles. As reviewed in work of (Stanisavljevic et al. 2014), CE is an indispensable method for examination of bioconjugation to targeting ligands (e.g., folic acid, RGD peptide) or monitoring of their interactions with other molecules of interest.

In this study, we have characterized carboxyl-silica-coated UCNPs by laboratory-made spectrometer. We have examined two excitation sources and the dependence of the fluorescence of the UCNPs on the excitation power. In addition, CE was involved to characterize the proposed nanoparticles. To the best of our knowledge, this is the first time that CE was used for characterization of UCNPs.

MATERIAL AND METHODS

Synthesis of the upconversion nanoparticles

UCNPs were synthesized by high-temperature coprecipitation method according to a protocol described elsewhere (Hlavacek et al. 2014, Wang et al. 2010). Subsequently, carboxyl-silica-coated UCNPs (COOH-UCNPs) were prepared by a reverse microemulsion method: UCNPs (418 mg) were diluted in cyclohexane to a final volume of 36.8 ml. This dispersion was mixed with 4600 mg of Igepal CO-520 and 251 µl of tetraethyl orthosilicate (TEOS) and stirred intensively for 10 min. A mixture of 279 µl 25% (w/v) of aqueous ammonium hydroxide and 279 µl of water was added to form a microemulsion that was slowly stirred overnight. Then, 126 µl of TEOS were added and the microemulsion was again stirred for 240 min. After adding 251 µl of 25% (w/v) sodium carboxyethylsilanetriol in water, the microemulsion was first sonicated for 15 min and then stirred for 60 min. The COOH-UCNPs were extracted with 5000 µl of dimethylformamide and washed four times with 20 ml of acetone, and five times with 6000 µl of water.

Laboratory-made spectrometer

A laboratory-made detection device for optical characterization of the nanoparticles was built using modular spectrometer components purchased by Ocean Optics (Dunedin, FL, USA) and external light sources, laser diode (980 nm, 300 mW) or LED diode (980 nm, 3 mW), obtained from Roithner Lasertechnik (Vienna, Austria). Spectra of UCNPs under excitation with different relative power of the diode were acquired (scans to average 2, integration time 1000 ms) with use of OCEANview 1.6.3 software and postprocessed in MATLAB.

Capillary electrophoresis with IR light source

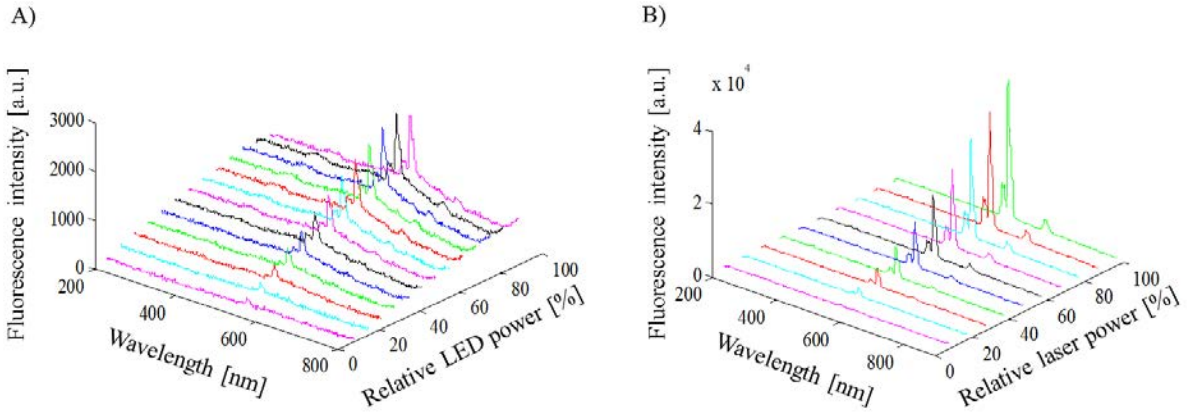
Capillary electrophoresis Beckman Coulter PACE/MDQ was coupled with the external 980 nm laser diode connected via optical fibre. The separation was performed in uncoated fused silica capillary with internal dimeter of 75 µm, total length of 64 cm and effective length of 54 cm. 20 mM borate buffer (pH 9) was used as a separation electrolyte and separation voltage of 25 kV was applied.

RESULTS AND DISCUSSION

Comparison of excitation sources

UCNPs spectra were measured using LED light source set to 100–700 mA (Figure 1A). Laser diode was set to 10 values of power from 200 mA (1.75 V) to 670 mA (3 V) (Figure 1B). As expected, increasing excitation power leads to higher fluorescence yields. Based on the results obtained, the laser diode was used in subsequent CE experiments taking into account the extremely low sample volume injected into CE (nl).

Figure 1 Emission spectra of UCNPs – comparison of the excitation sources and their settings. A) LED diode, B) laser diode

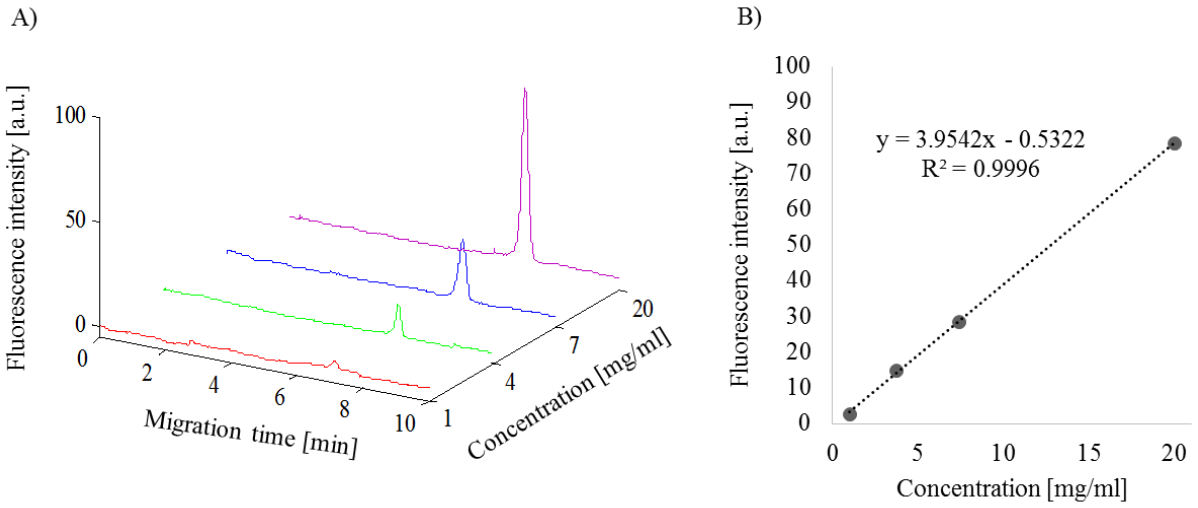


CE-LIF characterization

According to the literature, CE-LIF is an excellent tool for characterization of nanoparticles as well as for monitoring of their interaction with other biomolecules (e.g. antibodies).

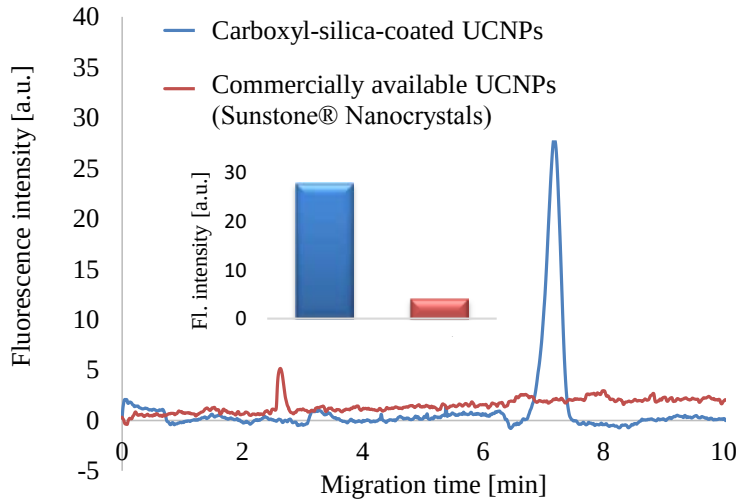
As shown in Figure 2A, UCNPs exhibited under given CE conditions a single peak with migration time of 6.7 minutes. The peak height was linearly dependent on nanoparticle concentration. The coefficient of determination R^2 = 0.9996 (Figure 2B) was reached and obtained limit of detection (LOD = 3 × SD/slope) was 0.26 mg/ml. The absolute amount of the injected nanoparticles was calculated to be 9.1 ng (injected volume was 35 nl).

Figure 2 CE characterization of UCNPs@Yb,Er. A) electropherograms of different concentrations of UCNPs, B) calibration curve obtained from the peak heights



The potential improvement of LOD would involve increasing the excitation power used, however the main limitations are in the imperfect coupling of the laser diode light into the optical fibre used in the CE instrument. Despite these limitations, it was possible to compare the behaviour of the prepared UCNPs and commercially obtained nanoparticles. As shown in Figure 3, the fluorescence intensity of the prepared nanoparticles was significantly higher (5.6×). Both particle types were carboxylated and therefore negatively charged in pH of the separation electrolyte (pH 9). The fact that migration time of the commercial nanoparticles was shorter (2.4 minutes) corresponds to the smaller size declared by the supplier (60 nm) in comparison to the size of prepared nanoparticles (105 nm).

Figure 3 CE characterization. Carboxyl-silica-coated UCNPS@Yb,Er (blue) and commercially available UCNPs (red), both in concentration 7 mg/ml (diluted by water)



CONCLUSION

Upconversion nanoparticles belong to a group of novel nanomaterials exhibiting fluorescence with large anti-Stokes shift. In this work, laboratory-made spectrometer was built. Different excitation sources and power were used for spectra acquisition. Additionally, UCNPs were characterized by capillary electrophoresis. In comparison to commercially available UCNPs, prepared nanoparticles exhibited 5.6-times higher fluorescence intensity.

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