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Preparation, characterisation and application of europium(III) chelate-dyed polystyrene–acrylic acid nanoparticle labels

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ABSTRACT

Preparation, characterisation and application of europium(III) chelate-incorporated polystyrene–acrylic acid (AAc) nanoparticle labels featuring diverse AAc proportions is described. Emulsion copolymerisation of styrene and AAc was used to synthesise uniform-sized nanoparticles, approximately 50 nm in diameter. The structural, fluorescence and functional properties of the nanoparticles were characterised to obtain the optimal polymer composition of particulate labels. The AAc content had only a delicate effect on the fluorescence of the chelate and structural characteristics of the particles. The fluorescence spectra or lifetime of the incorporated europium(III) chelate were not notably affected, and all the particles were analogous in size, had monomodal size distributions and good colloidal stability. However, the AAc content affected strongly on the stability of the incorporated dye and functionality of the labels. Nanoparticles having up to 5.4 mass% of AAc were stable and applicable in high-sensitivity assays, where low detection limit and variation were achieved. The nanoparticles possessing 7.2 or 11.5 mass% of AAc lost remarkably the dye content during storage influencing their usability as labels in assays. Overall, the characterisation and employment results achieved evidenced the importance of the structural composition of nanoparticles, moreover, the knowledge of the effects of structural changes is utilisable in the development of improved nanoparticle labels and assay applications.

1. Introduction

Various luminescent micro- and nanosized particles functionalised using bioconjugation have been applied as labels in diverse diagnostic applications. New particulate label technologies often possess characteristics that allow a reduction of background signal and an increase of specific signal due to higher specific activity. Typically, the employment of new particulate label technologies enables assays with improved performance properties in the sense of sensitivity and methodology. Illustrative representatives of the relatively new particulate label technologies are semiconductor quantum dots possessing a wide excitation range and size- and material-dependent emission spectra [1,2], up-converting

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materials featuring background reduction, that is based on a spectral up-conversion generated by a two-photon excitation [3], and lanthanide chelate-dyed nanospheres having the long-lifetime fluorescence properties of the corresponding lanthanide ions [4–6].

Recently; synthesis, characterisation and employment of various lanthanide chelate-incorporated submicron sized particle labels have been presented. Functionalised europium oxide nanoparticles have been prepared employing microwave-assisted surface chemistry and applied as labels in a competitive high-throughput immunoassay for atrazine [7]. Uniform-sized 50-nm silica-based terbium(III) and europium(III) nanoparticle labels were synthesised using a covalent binding–copolymerisation method and used in assays for prostate-specific antigen and hepatitis B surface antigen, respectively [8,9]. Emulsion copolymerisation of styrene and acrylic acid has been used to synthesise 50-nm particulate labels that were dyed using four different lanthanide chelates. The labels were also successfully applied in a heterogeneous high-sensitivity immunoassay for prostate-specific antigen [10]. Characteristically the lanthanide chelate-incorporated nanoparticle labels have featured relatively simple preparation and assay procedures and high-quality performance in various proof-of-concept assays presented [11–14].

In the present paper, the preparation of monomodal, ~50-nm europium(III) chelate-embedded polystyrene nanoparticle labels containing different AAc content is described. The AAc content was varied to produce characteristically and functionally diverse nanoparticle labels featuring different surface ζ-potential values; and, based on the characterisation results, the structural composition of the particulates was optimised. The polymerised nanoparticles were dyed with chelated europium(III) and the structural and fluorescence properties of the nanoparticles were characterised. The nanoparticle labels were bioconjugated with monoclonal antibodies (Mab) and the functionality of the Mab-coated nanoparticles in immunoassay was investigated performing a heterogeneous sandwich-type assay for prostate-specific antigen (PSA) utilising the long-lifetime fluorescence of europium(III) nanoparticles and time-resolved fluorometry.

2. Experimental

2.1. Materials

Monomers, styrene (>99%; Merck, Darmstadt, Germany) and AAc (>99%; Fluka, Milwaukee, WI), were distilled under reduced pressure. EuCl3 (Alfa Aesar, Ward Hill, MA), 4,4,4-trifluoro-1-(2-naphthyl)-1,3-butanedione (2-NTA, 99%; Acros Organics; Geel Belgium), and tri-n-octylphosphine oxide (TOPO, 99%; Sigma–Aldrich, St. Louis, MO) were used as received. 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC) and N-hydroxysulfosuccinimide sodium salt (sulfo-NHS) were bought from Fluka, low-fluorescence MaxiSorp microtitration wells from Nunc A/S (Roskilde, Denmark), PSA calibrators and Delfia® anti-mouse IgG microtitration strips from PerkinElmer Life and Analytical Sciences, Inc., (Boston, MA) and Assay Buffer from Innotrac Diagnostics (Turku, Finland). PSA-specific Mabs 5A10 and 5E4 were produced as described [15,16].

2.2. Synthesis and characterisation of nanoparticles

Emulsion copolymerisation of styrene (7 mmol) and AAc (0, 0.22, 0.43, 0.58, or 0.97 mmol) was carried out in a sealed round-bottom flask equipped with a magnetic stirrer and an oil bath to control the reaction temperature. Sodium dodecylsulfate (0.44 mmol; >99%, Merck), was dissolved in 9 mL of water in the reaction flask after which the monomers were added and the flask was sealed with a septum. The mixture was bubbled with nitrogen to remove radical polymerisation inhibiting oxygen and stirred at room temperature for 20 min. The nitrogen in- and outlets were removed and the flask was placed into the oil bath at 65 °C. Polymerisation was initiated after 20 min by injecting 1 mL of aqueous potassium peroxodisulphate (0.074 mmol; >99 %, Merck) to the reaction mixture. After 5 h of reaction, the flask was unsealed and the product was cooled to room temperature. The product was filtered through a filter paper (Whatman 2 V, pore size 8 μm, Whatman Plc., Brentford, U.K.) and purified by dialysing for 7 days against distilled water, which was refreshed daily. Regenerated cellulose tubular membrane CelluSep 4 (MWCO 12000–14000 g mol^{-1}, Membrane Filtration Products Inc., Seguin, TX), was used as a dialysis membrane. The dialysed aqueous particle dispersion was extracted several times with n-hexane (>99%; Rathburn, Walkerburn, U.K.) and stored as such at 4 °C.

Fourier transform infrared (FT-IR) spectra of the nanoparticles were measured from freeze-dried particle samples using a Spectrum One FT-IR spectrometer (PerkinElmer Life and Analytical Sciences, Boston, MA). The mean hydrodynamic diameter and the ζ-potential of particles in 0.1 g L^{-1} aqueous solution, pH 9.0, were obtained using a Malvern Zetasizer 3000HS (Malvern Instruments Ltd., Worcestershire, U.K.). Polymer concentration of aqueous particle dispersion was obtained by drying a weighed sample of aqueous dispersion to equilibrium weight in vacuum.

2.3. Dyeing and characterisation of nanoparticle labels

The polystyrene nanoparticles were dyed by incubating 300 μL, 2.8 mass%, of nanoparticles in 1650 μL of 10 mM carbonate buffer, pH 9.5, containing 1.8 mM EuCl3, 5.5 mM 2-NTA, and 5.5 mM TOPO. The embedding of lanthanide chelates was performed at room temperature overnight. The nanoparticles were dialysed against distilled water using spectra/por poly(vinylidene difluoride) membrane (MWCO 300 kDa, Spectrum Laboratories, Rancho Dominiquez, CA) overnight to remove loose chelates present in solution. During the optimisation process of the dyeing both dialysis and centrifugal (Nanosep® 300k Omega centrifugal device, Pall Life Sciences, Ann Arbor, MI; 6000 rcf, +23 °C, 5 min) separation steps were carried out and found useful. However, large particle quantities became difficult to centrifuge through the filter and dialysis was preferred over centrifugation in the final procedure. The number of particles was calculated by comparing scattering signal of the prepared particles to the calibrator solution, which contained a known concentration of commercial nanoparticles, using Coulter N4 Plus submicron particle