Bioconjugated silica-coated nanoparticles for bioseparation and bioanalysis

Joshua E. Smith, Lin Wang, Weihong Tan

We have discussed the applications of silica nanoparticles doped with either magnetic materials or fluorescent dye molecules. Both the Stöber and water-in-oil microemulsion systems were used for the nanoparticle's preparation. These nanoparticles are uniform and can be made with a large size range from a few nanometers to hundreds of nanometers. The silica surface displays an easily modifiable chemistry with a variety of biomolecules, and can be reproducibly synthesized. Silica nanoparticles have been successfully used in bioseparation of nucleic acids, peptides and cells. Mass spectroscopy has been used for peptide analysis, and dye doped nanoparticles have been used as ultrasensitive labels in bioanalysis. The nanoparticles demonstrated great potential in separating and analyzing biological molecules.

Keywords: Nanoscience and nanotechnology; Nanoparticles; Magnetic nanoparticles; DNA separation; Bioanalysis using bionanotechnology

Joshua E. Smith, Lin Wang, Weihong Tan*
Center for Research at the Bio/nano Interface, Department of Chemistry and Shands Cancer Center, UF Genetics Institute and McKnight Brain Institute, University of Florida Gainesville, FL 32611-7200, USA

*Corresponding author.
Tel./Fax: +1 352 846 2410;
E-mail: tan@chem.ufl.edu

1. Introduction

Nanotechnology deals with the creation of materials typically in the sub-100-nm size range and has taken precedence in several fields, including biotechnology, biomedical sciences, and material sciences and engineering [1–10]. One major advantage of using nanomaterials versus conventional ones in these fields is that the nanomaterials bring unique chemical and physical properties to enable new and advanced functions. In biotechnology and biomedical sciences, there is strong dependence on having proper understanding of biochemical processes; some of the nanomaterials developed include nanoparticles, and nanoprobes along with others can be used to obtain much deeper understanding of biological processes [1–12].

The property of the nanomaterials differ them from bulk materials in that the nanomaterials have high surface-to-volume ratio and other size-dependent qualities. The composition of these materials determines their compatibility and suitability for the relevant applications. Some of the materials used to create nanoparticle probes include semiconductor nanocrystals (quantum dots) [13–16], gold [17–24], polystyrene latex [25–29], magnetic [30] and dye doped [31,32] nanoparticles.

There is a great need for new tools and new methods in biomedical studies. For example, complex biological samples typically need a great deal of sample preparation before analysis, including purification, enrichment and preconcentration. Sample-preparation methods include solid-phase extraction (SPE) procedures that have multiple steps for analyte adsorption and extraction. While these procedures allow for sample clean-up and preconcentration, they do not remove analytes selectively [33]. However, another method has used magnetic nanoparticles for selective extraction and enrichment of various types of cells [34].

Currently, there is a lack of efficient biotechnologies for selective collection of trace amounts of bioanalytes from complex biological matrices [35]. Because of this, a great deal of research has been done utilizing nanomaterials to overcome the inadequacies of separation techniques commonly used for bioseparation and bioanalysis.

Dye-doped nanoparticles possess high signal amplification, excellent photostability and easy surface modification [36]. Magnetite-doped silica nanoparticles display superparamagnetism and an easily modifiable surface [35]. Silica is used because it is chemically and physically

0165-9936/$ - see front matter © 2006 Elsevier Ltd. All rights reserved. doi:10.1016/j.trac.2006.03.008

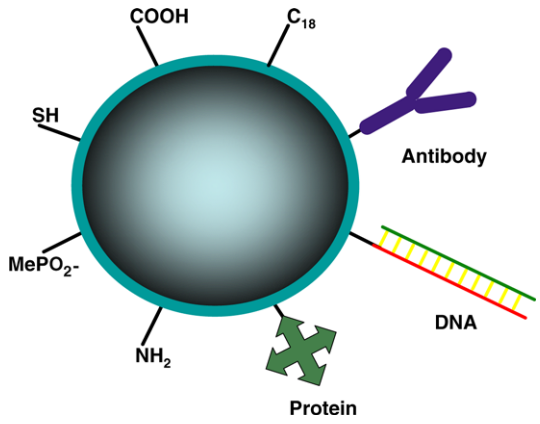

Figure 1. Schematic representation of functionalized nanoparticles used for biological analysis.

inert; it protects the inner core of the particle from the natural defenses of the body or other environments [37–39]. Silica has well-established chemistries that allow surface modification with various functional groups (e.g., amine, thiol, carboxyl and methacrylate). These nanoparticles can be further modified with biomolecules for biological applications (Fig. 1). This review focuses mainly on the separation of oligonucleotides, peptides and whole cells using silica-coated magnetic nanoparticles and, briefly, on the use of silica-based fluorescent nanoparticles for biomolecule detection. These two types of nanoparticles have unique properties and have been used effectively in bioanalysis and biotechnology fields.

2. Nanoparticle formation and characterization

There are two major approaches to form nanoparticles, as follows:

- The ''top-down'' approach starts with bulk materials and ends in the formation of nanomaterials through a series of degradation processes. The limitations are the size, the shape and the poor reproducibility of the materials formed.
- In the ''bottom-up'' approach, the materials are formed by self-assembly with an atom-by-atom or molecule-by-molecule motif creating complex structures. For this approach, the limitations are incomplete understanding of the synthesis processes, including how to control them to obtain desirable materials and the types of structures created. Two ''bottom-up'' methods are generally used to create silica nanoparticles: the Stöber [40–51]; and, the reverse-micelle [31,32,45,52–54]. Both are used to make pure silica, dye-doped silica, and silica-coated magnetic nanoparticles.

The Stöber method has been used to prepare spherical silica particles with different sizes. This method uses a bulk ethanol solution in the presence of ammonia, and silica particles form by the hydrolysis of silane precursors (e.g., tetraethylorthosilicate (TEOS)). The Stöber process is relatively simple to perform and can be carried out in only a few hours. It has been modified to incorporate organic dye molecules, such as rhodamine 6G (R6G), tetramethylrhodamine (TMR) and fluorescein dye molecules [31,32,41,42] within the silica nanoparticles. Magnetic nanoparticles made of Fe_3O_4 and Fe_2O_3 have also been incorporated in silica particles using this method [33]. However, the nanoparticles formed via this method are typically large and non-uniform [41] (Fig. 2A).

An alternative method to form these particles is to use the reverse-micelle system, also known as the water-in-oil (W/O) microemulsion system, which has three primary components: water, oil, and, a surfactant [56–58]. These components form a single-phase microemulsion system that is both isotropic and thermodynamically

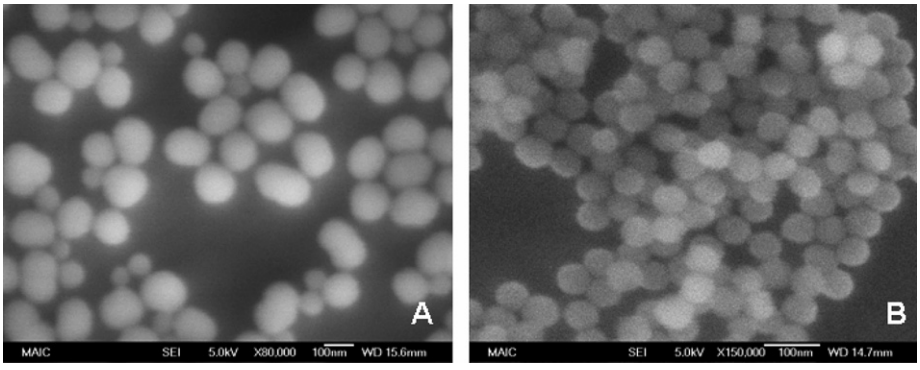

Figure 2. SEM images: (A) silica-based nanoparticles prepared by the Stöber process; and, (B) silica nanoparticles prepared by the microemulsion process.