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Using capillary electrophoresis to characterize polymeric particles

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ABSTRACT

Capillary electrophoresis (CE) was used for the characterization of a variety of polymeric micron and sub-micron particles based on size, surface functionality, and binding properties. First, a robust capillary zone electrophoresis (CZE) method was developed for the baseline separation and quantitation of commercially available polystyrene particles with various surface modifications (including amino, carboxylate, and sulfate functional groups) and various sizes (0.2, 0.5, 1.0, and 3.0 μm). The separation of DNA-templated polyacrylamide particles from untemplated particles (as used for the Ion Torrent Personal Genome Machine) was demonstrated. Finally, using the 29-base thrombin aptamer and thrombin protein as a model system, a study was undertaken to determine dissociation constants for the aptamer and protein in free solution and when the aptamer was conjugated to a particle, with the goal of better understanding how the use of solid substrates, like particles, affects selection and binding processes. Dissociation constants were determined and were found to be approximately 5-fold higher for the aptamer conjugated to a particle relative to that in free solution.

1. Introduction

Materials with sub-micrometer and nanometer dimensions have found increased use in the scientific community over the past decade. Specifically, metallic, semiconductor, organic, inorganic, and polymeric particles have been used for a variety of environmental and clinical applications [1,2] ranging from microbial monitoring and detection to the purification of biomolecules [1]. Beyond these applications, particles have been used for drug delivery, bioimaging, and as MRI contrast agents [3].They are excellent sensing agents and have been used in a variety of assays to detect proteins, pathogens, oligonucleotides, antigens, and biomarkers for cancer [3,4]. For example, due to their easy modification with a variety of surface functionalities, particles have been conjugated with antibodies and used as sensors for specific antigens [3,4]. Particles have found further utility in separation science as separation mediators and pseudo-stationary phases [2,5].

However, it still remains to regulate and understand their synthesis so that particle size can be highly controlled. Often, many synthetic approaches are used for a single type of particle, so determination of particle size and size distribution are important for assessing synthetic schemes [6]. Further, some particles have size-dependent properties, so it is important to have methods that can distinguish particles by size in order to ensure optimal function of the particles for their specified application [6]. Beyond these quality control matters, it is important to develop sensitive techniques for the detection and speciation of particles in human tissues and fluids or in environmental matrices due to their increased use in a variety of commercial products and their potential toxicity to a variety of organisms [7].

Additionally, next generation sequencing (NGS) platforms—which are increasingly used for a range of applications including selection of novel aptamers [8,9]– rely on automated templating technologies to conjugate micrometer-sized particles with the desired oligonucleotide sequence to create what is called a 'templated bead library'. For the Ion OneTouch™ 2 System (Life Technologies, Carlsbad, CA) used in this work, templating efficiencies are typically less than 50%, so it is necessary to remove non-templated particles from the sample prior to chip loading through an enrichment procedure. Library enrichment generally results in a sample with >90% templated particles. Currently, luminescence-based assays, like those developed using the Qubit®

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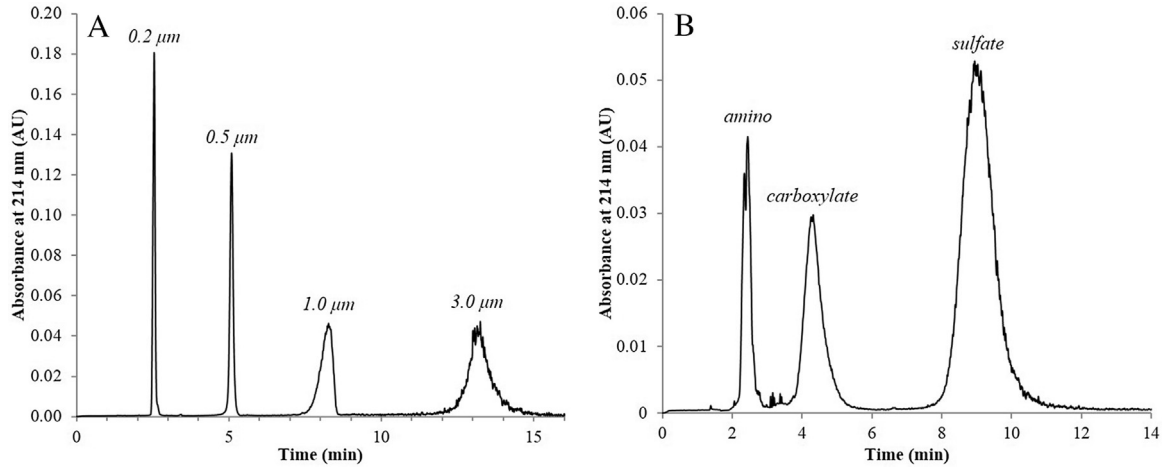

Fig. 1. Electropherograms demonstrating the separation of polystyrene particles based on A) size (0.2, 0.5, 1.0, and 3.0 μm carboxylate particles) and B) surface functionality (1.0 μm amino, carboxylate, and sulfate particles). The separation buffer was 5 mM Tris − 50 mM Gly (pH 7.75) and the separation voltage was 30 kV.

Fluorometer (in this work, version 2.0, Life Technologies, Carlsbad, CA), are used to assess templating efficiencies [10]. The emergence of these new technologies necessitates the continued development of methods for the reliable characterization of particles based on their surface functionality.

Since many aptamer and drug discovery platforms rely on the use of solid substrates including particles [11], it is also important to understand the behavior of particles conjugated with aptamers, proteins, or small synthetic molecules in binding and selection assays. Typically, binding conditions employed for aptamer and drug selection include interaction of the aptamer and target in free solution (homogeneous binding) or immobilization of the aptamer or the target on a solid substrate while the other component remains in free solution (heterogeneous binding) [12]. One of the primary disadvantages of heterogeneous binding conditions is that the immobilized component has decreased mobility, which may limit its ability to interact with the other component in solution phase. Aptamers typically fold into complex secondary structures, which allow them to interact with their protein targets [12]. However, if one end of the aptamer is tethered to a solid support, it is reasonable to speculate that the efficiency of binding will be adversely affected due to the compromised ability of the aptamer to fold. Thus, analyses are needed to compare these binding environments in order to understand the potential limitations of heterogeneous binding assays.

The goal of this work was to demonstrate the utility of capillary electrophoresis (CE) for three particle applications; namely, for the characterization of functionalized polystyrene particles based on their diameter and surface functionality; for the separation of templated and untemplated particles used for NGS; and for the determination of dissociation constants of DNA-conjugated particles and their protein targets. CE has been previously used to study functionalized particles ranging in size from 20 nm up to 2 μm [13–21]; however, baseline resolution was not achieved in all cases and detection of micrometer-sized particles often resulted in broad peaks [14–16]. CE focusing and stacking techniques have also been demonstrated for the analysis of particles, including isotachophoresis (ITP) [22], the addition of surfactants, like sodium dodecyl sulfate [23,24], or the use of reversed electrode polarity stacking mode (REPSM) [24]. Additionally, affinity CE methods employing DNA-nanoparticle conjugates have been employed for genotyping experiments [25].

Here we describe the development of a capillary zone electrophoresis (CZE) method for the characterization and separation of commercially available polystyrene particles with various surface modifications (including amino, carboxylate, and sulfate functional groups) and various sizes (0.2, 0.5, 1.0, and 3.0 μm). These methods were then subsequently applied to the separation of DNA-templated polyacrylamide particles from untemplated particles as a quality control measure for automated bead templating processes (as necessitated by NGS platforms). Further application involved determination of dissociation constants for the 29-base thrombin aptamer and thrombin protein when the aptamer was either in free solution or conjugated to a particle. Dissociation constants were calculated by way of established NECEEM (nonequilibrium capillary electrophoresis of equilibrium mixtures) theory [26–29]. These latter two applications (involving the characterization of DNA-functionalized particles) involved a novel detection scheme, whereby the particles were labeled with a noncovalent, DNA intercalating dye, SYBR Gold, (see Experimental Section) and visualized using laser induced fluorescence (LIF)

detection.

2. Materials and methods

2.1. Reagents, solutions, and samples

Polybead® Functionalized Microspheres were purchased from Polysciences, Inc. (Warrington, PA) and included amino particles with a diameter of 1.0 μm, carboxylate particles with diameters of 0.2, 0.5, 1.0, and 3.0 μm, and sulfate particles with a diameter of 1.0 μm. Particle concentrations of these commercial particle samples, as-received, varied according to particle size, as follows: 0.2 μm particle size, provided in solution as 5.68×10^{12} particles/mL; 0.5 μm particles as 3.64×10^{11} particles/mL; 1.0 μm particles as 4.55×10^{10} particles/mL; and 3.0 μm particles as 1.68×10^{9} particles/mL. Particle size distributions, as reported by the manufacturer, may be found in Table S-1.

Tris (hydroxyl methyl) aminomethane (Tris) and glycine (Gly) were obtained from Sigma (St. Louis, MO) and used as-received. SYBR® Gold Nucleic Acid Gel Stain was purchased from Life Technologies, Inc. (Carlsbad, CA) and stored at −20 °C. The 29mer thrombin aptamer (5′–CCT CTC TAT GGG CAG TCG GTG AT**A GTC CGT GGT AGG GCA GGT TGG GGT GAC T**TA AGG AGT TCA ATA TCT GAG TCG GAG ACA CGC AGG GAT GAG ATG G–3′) and the 29mer nonsense DNA sample (5′–CCT CTC TAT GGG CAG TCG GTG AT**A GTC ACC CCA ACC TGC CCT ACC ACG GAC T**TA AGG AGT TCA ATA TCT GAG TCG GAG ACA CGC AGG GAT GAG ATG G–3′) were purchased from Integrated DNA Technologies (Coralville, IA). Human-α-thrombin