Hydroxypropyl Methacrylate Interaction and Chitosan Coating for Enhanced UV Detection Sensitivity of Colloidal Nanoparticles in Capillary Electrophoresis Analysis

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Abstract

The binding interactions between silica (SiO₂), titania (TiO₂) or polymeric nanoparticles with hydroxypropyl methacrylate (HPMA) were investigated for enhancing the ultraviolet (UV) detection sensitivity of these nanoparticles in capillary electrophoresis (CE) analysis. HPMA interacted with colloidal SiO₂ nanoparticles, producing a larger CE-UV peak at a slightly shorter migration time. An increase in particle size with HPMA binding was validated using dynamic light scattering. The interaction was selective as HPMA did not interact with TiO₂ nanoparticles in aqueous suspension. Chitosan coating of SiO₂ or TiO₂ nanoparticles produced significantly larger hydrodynamic diameters to further enhance the sensitivity of their UV detection. The analytical technique, which involves coating SiO₂ nanoparticles with chitosan first and binding with HPMA next, is novel. It has allowed us to achieve a significant enhancement of 50 folds in detection sensitivity.

Keywords: Capillary electrophoresis; Chitosan; Colloid; Dynamic light scattering; Hydroxypropyl methacrylate; Nanoparticles; Polymer; Silica; Titania

Introduction

The growing use of inorganic oxide nanoparticles (NPs) [1-3], due to their novel properties and high biochemical reactivity [4], has led to unintended release of significant amounts of these NPs into the water cycle through industrial and household wastewater effluents [5-7]. The removal of NPs from water effluents varies in efficiency from 70% to 94% [8]. Their toxicity, especially to humans [9-11], arises from a number of biophysicochemical factors including their aggregation, chemical composition, concentration, dissolution, exposure routes, particle size, nanostructure, quantum effects, and self-assembly [12,13]. Uncertainties on their persistence in the water cycle have raised many concerns and provide the impetus to develop analytical techniques with high sensitivity to detect and quantify these NPs. Contemporary NPs quantification methods (such as laser induced break down detection [14], small angle neutron scattering [15], and fluorescent correlation spectroscopy [16]) rely upon sophisticated instrumentation and a skilled analyst, making such approaches impractical for regular environmental monitoring.

Different chemistry-based encapsulation processes have been developed and showed promising results [17]. The growth of NPs in solution is complicated by several factors including changes in pH as well as interactions with ions and surfactants [18]. Either covalent attachment of end-functionalized polymers to the surface or in situ polymerization of monomers using immobilized initiator seems to be versatile [19] and [20]. Our novel approach is based on the controlled coating of NPs with a thick layer of polymer to grow them into a larger size for strong UV light absorption. The polymer coating also helps with the stabilization of NPs in aqueous suspension. These grown NPs can be separated by capillary electrophoresis (CE) due to differences in electrophoretic mobility, depending on their electronic charge, size, and surface functionality. A first coating of silica (SiO₂) NPs with polyhydroxypropyl methacrylate (PHPMA) was successfully developed in our laboratory to enhance the ultraviolet (UV) detection sensitivity by 6 folds during CE analysis [21]. A second coating with polydopamine (PDA) produced an extra 2-fold increase of the UV detection sensitivity to attain a total enhancement of 12 folds in detection sensitivity.

The goal of this study was to explore other coating materials that could further improve the CE-UV detection sensitivity for inorganic oxide nanoparticles (SiO₂ and TiO₂) in aqueous suspension. Investigation started with the binding interaction between these nanoparticles and HPMA, followed by coating with chitosan to produce larger diameters for enhanced UV detection sensitivities. Their hydrodynamic diameters were measured by dynamic light scattering and their ionic charge states were determined by capillary electrophoresis.

Material and Methods

Instrumentation

CE-UV analyses were performed on a laboratory built system, which includes a Spellman CZE1000R high-voltage power supply (Hauppauge, New York, USA). Fused-silica capillary (51 mm i.d., 356 mm o.d.) was obtained from Polymicro Technologies (Phoenix, AZ, USA). The background electrolyte (BGE) was composed of 10 mM Na₂HPO₄ in deionized distilled water (DDW) to attain pH 7.5 ± 0.2. Electrokinetic injection at 17 kV for 1.2 s, 3 s or 12 s was employed to load the sample into the capillary for CE analysis. All CE analyses were run at an applied voltage of 20 kV. A Bischoff Lambda 1010 (Leibnig, Germany) UV detector was set to a wavelength of 190 nm to determine the migration times of nanoparticles. A PeakSimple chromatography

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Received April 08, 2015; Accepted April 28, 2015; Published May 05, 2015


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data system (SRI model 203, Torrance, CA, USA) was used to acquire the detector output signal. The separated chemical compounds and/or nanoparticles were recorded in the electropherogram as peaks (represented by a blue line) with different migration times. Their peak areas above the baseline (represented by a pink line) were determined using a numerical integration algorithm.

The average diameters of SiO$_2$, TiO$_2$, and polymeric nanoparticles, before and after HPMA-binding or chitosan-coating, were measured by dynamic light scattering (DLS) using a Brookhaven Instruments nanoDLS particle size analyzer (Holtsville, NY, USA). Ten replicate measurements of 10 s each were performed to ensure high accuracy.

Reagents and materials

Chitosan (medium molecular weight of 190,000-310,000, deacetylation degree of 75-85%), 2-hydroxypropyl methacrylate (HPMA, 97%), mesityl oxide (MO, ≥ 90%), sodium dodecyl sulfate (SDS, ≥ 99%), LUDOX® colloidal silica (SiO$_2$, 30% wt suspension in H$_2$O, with a surface area of 198-250 m$^2$/g) and titanium oxide nanopowder (TiO$_2$, 21 nm) were all obtained from Sigma-Aldrich (Oakville, ON, Canada). 2,2'-azobis-2-isobutyronitrile (AIBN) was bought from Pfaltz & Bauer (Waterbury, CT, USA).

HPMA binding interaction with colloidal SiO$_2$ or TiO$_2$ nanoparticles

To investigate the binding interaction between HPMA and colloidal SiO$_2$ or TiO$_2$ nanoparticles, HPMA (3 ml) was first dissolved in DDW (25 ml) containing colloidal SiO$_2$ or TiO$_2$ nanoparticles (20 mg/ml). Next, SDS (1.7 wt% of HPMA) was added and then the mixture was sonicated for 5 min to ensure homogeneity. Finally, the vial was placed in a 60°C thermostatted water bath for 22 h to facilitate the binding interaction between HPMA and colloidal SiO$_2$ or TiO$_2$ nanoparticles. Percent binding of HPMA to SiO$_2$ or TiO$_2$ NPs was calculated as 

\[
\text{Percent binding of HPMA to SiO}_2 = \left(\frac{\text{HPMA peak area before binding} - \text{HPMA peak area after binding}}{\text{HPMA peak area before binding}}\right) \times 100\% \quad [22].
\]

HPMA binding interaction with polymeric nanoparticles

A suspension of chitosan (1 wt%) in 1% acetic acid was prepared following a procedure reported by Shuai et al. [23]. The mixture was stirred magnetically for 3 h to obtain a homogeneous suspension. Then the chitosan suspension (1 ml) was added to 10 mM Na$_2$HP0$_4$ BGE (30 ml) containing colloidal SiO$_2$ or TiO$_2$ nanoparticles at varying concentrations (0-20 g/L), followed by magnetic stirring for 22 h. Addition of SDS (0.06 g) helped disperse the chitosan-coated particles to prevent precipitation. After addition of HPMA (0.03%, 0.05% or 0.1% v/v) to the chitosan-coated particles, the vial was placed in a 60°C thermostatted water bath for 22 h under continuous magnetic stirring.

Results and Discussion

CE-UV characterization of silica and titania nanoparticles

Growing interest in the development of nanocomposites consisting of TiO$_2$ and SiO$_2$ NPs has led to the release of these NPs in significant amounts to the water cycle, threatening both humans and aquatic ecosystems. Cytotoxicity arises from the oxidative damage of these NPs owing to the production of reactive oxygen species. Moreover, TiO$_2$ NPs can further damage cells due to photocatalysis-enhanced oxidation upon exposure to light or UV radiation. Wastewater treatment plants and filters are often poorly suited to efficiently remove these NPs [24]. LUDOX® AM colloidal SiO$_2$ nanoparticles [25] do not settle and cannot be separated out by ordinary filtering or centrifuging like those in a suspension. In our work, an aliquot was spiked into the BGE that was loaded into the capillary by electrokinetic injection for CE-UV analysis. As shown in Figure 1a, 20 g/L of SiO$_2$ NPs exhibited low UV absorbance at the detection wavelength of 190 nm, producing a small peak at the migration time of 8.7 ± 0.1 min. The SiO$_2$ NPs appeared after the neutral marker, indicating their negative ionic charges (probably as SiO$_2^{-}$) in the BGE and hence yielding a negative electrophoretic mobility value.

On the contrary, 20 g/L of TiO$_2$ NPs showed high UV absorbance at 190 nm, producing a large peak at the migration time of 8.4 ± 0.1 min, as shown in Figure 1b. Similar to SiO$_2$ NPs, TiO$_2$ NPs migrated after the neutral marker, which indicates their negative ionic charges in the BGE and therefore yielding a negative electrophoretic mobility value.

HPMA binding interaction with silica and titania nanoparticles

Binding interaction of HPMA with SiO$_2$ NPs was conducted at 60°C for 22 hours. The resultant SiO$_2$-HPMA particles were analyzed by CE-UV to determine their electrophoretic mobility. As shown in Figure 2, a new peak for HPMA-bound SiO$_2$ particles was detected at a migration time of 8.2 ± 0.1 min. These particles exhibited larger peak height and peak area than the original SiO$_2$ NPs (Figure 1a). Apparently, the SiO$_2$ NPs bound to HPMA to have their Si-O- groups buried under the surface, thus resulting in faster migration than SiO$_2$ NPs (at 8.7 ± 0.1 min) with good separation from HPMA (at 4.0 ± 0.1 min). HPMA binding to SiO$_2$ NPs was determined to be 19 ± 3%. Binding interaction of HPMA with TiO$_2$ NPs was next conducted at 60°C for...
22 hours. CE-UV analysis of the mixture showed no sign of interaction between HPMA and TiO₂ NPs as no significant change was observed for the TiO₂ peak shape or its migration time. Correspondingly, HPMA binding to TiO₂ NPs was determined to be 0 ± 5%. The oxygen atom of HPMA carbonyl group could interact with the Si atom of SiO₂ but not with the Ti atom of TiO₂. The difference between their interactions was likely due to the chemical properties of SiO₂ and TiO₂ NPs. Inorganic chemistry is highly dependent on the element; Si is a semiconductor and Ti is a metal.

Dynamic light scattering analysis of silica and titania nanoparticles

SiO₂ and TiO₂ NPs in aqueous suspension, as well as their mixtures with HPMA, were analyzed by DLS to determine their hydrodynamic diameters. In DLS analysis, multiple scattering (where light scattered from one particle is scattered from a second particle before reaching the detector) occurs to give the size distribution as particles undergo Brownian motion caused by thermally induced collisions between the suspended particles and solvent molecules. As shown in Figures 3a and 3b, the mean diameters of 53 ± 3 nm and 134 ± 5 nm were obtained for SiO₂ NPs and HPMA-bound SiO₂ particles, respectively. These results provide strong evidence that SiO₂ NPs became larger in size after interaction with HPMA under very simple experimental conditions. The interaction offers more sensitive detection of SiO₂ NPs (now in the form of HPMA-bound SiO₂ particles) by CE-UV, with optional electrophoretic separation from organic compounds possibly found in water. Mean diameters of 155 ± 6 nm and 154 ± 5 nm were obtained for TiO₂ NPs and their mixture with HPMA, respectively. DLS analysis confirmed that no interaction between TiO₂ NPs and HPMA took place as no significant change in the hydrodynamic diameter was observed.

Selectivity of HPMA binding with SiO₂ and TiO₂ nanoparticles

HPMA selectively interacted with colloidal SiO₂ NPs to produce a larger CE-UV peak at a slightly shorter migration time. It did not interact with TiO₂ NPs in aqueous suspension, resulting in no change of CE-UV peak area or migration time. This chemical method could allow us to analyze an aqueous sample containing the two kinds of nanoparticles, simply by adding HPMA and repeating the CE-UV analysis to observe any peak changes. A sample injection time of 3 min was sufficient to achieve the size distribution as particles undergo Brownian motion caused by thermally induced collisions between the suspended particles and solvent molecules. As shown in Figures 3a and 3b, the mean diameters of 53 ± 3 nm and 134 ± 5 nm were obtained for SiO₂ NPs and HPMA-bound SiO₂ particles, respectively. These results provide strong evidence that SiO₂ NPs became larger in size after interaction with HPMA under very simple experimental conditions. The interaction offers more sensitive detection of SiO₂ NPs (now in the form of HPMA-bound SiO₂ particles) by CE-UV, with optional electrophoretic separation from organic compounds possibly found in water. Mean diameters of 155 ± 6 nm and 154 ± 5 nm were obtained for TiO₂ NPs and their mixture with HPMA, respectively. DLS analysis confirmed that no interaction between TiO₂ NPs and HPMA took place as no significant change in the hydrodynamic diameter was observed.

Coating of silica and titania nanoparticles with chitosan

Coating of SiO₂ NPs with chitosan was next investigated. A significant increase in hydrodynamic diameter, from 53 ± 3 nm for SiO₂ particles to 513 ± 30 nm for chitosan-coated SiO₂ particles (at 20 g/L) was observed, as shown in table 1. Electrostatic and hydrogen bonding interactions between chitosan amide groups and silanol groups enabled the encapsulation process [26]. The uniform suspension of chitosan-coated SiO₂ particles did not precipitate upon dilution with a 10 mM KN0₃ solution in water during the sample preparation for DLS analysis [27]. Analysis of chitosan-coated SiO₂ particles by CE-UV failed badly because chitosan interacted with the capillary inner wall via electrostatic attraction, reducing the electroosmotic flow (EOF) down to zero. Similarly, coating of TiO₂ NPs with chitosan produced chitosan-coated TiO₂ NPs with a hydrodynamic diameter of 477 ± 11 nm, which is significantly larger than the 155 ± 6 nm for TiO₂ NPs.

HPMA binding with chitosan-coated nanoparticles

Afterwards, the interaction of HPMA with chitosan-coated SiO₂ particles was investigated. Initially the hydrodynamic diameter of the chitosan-coated SiO₂ particles decreased to 417 ± 17 nm. One plausible reason for the reduction in the hydrodynamic diameter of chitosan-coated SiO₂ particles upon HPMA binding is that HPMA disturbed the natural agglomeration tendency of chitosan [28]. The hypothesis is that chitosan initially formed a loose coating (by surface adsorption onto SiO₂ or TiO₂) that condensed upon addition of HPMA. Further addition of HPMA, however, changed the hydrodynamic diameter to 440 ± 12 nm. Similarly, HPMA (at 0.05% v/v) interacted with chitosan-coated TiO₂ NPs decreasing their hydrodynamic diameter from 477 ± 11 nm to 416 ± 11 nm. However, further addition of HPMA (at 0.1% v/v) increased their hydrodynamic diameter to 514 ± 8 nm.

A significant increase in peak area was observed after HPMA (0.03% v/v) bound to chitosan-coated SiO₂ particles. More importantly, after HPMA binding, CE-UV analysis could be performed with no disturbance of the EOF. Figure 4 shows the improved detection of HPMA-bound chitosan-coated SiO₂ particles. Upon varying the SiO₂ concentration in chitosan suspension, the resultant HPMA-bound chitosan-coated SiO₂ peak area changed accordingly as shown in Figure 4. A significant total enhancement of 50 folds in detection sensitivity was attained by coating SiO₂ NPs with chitosan followed by

[Figure 2: CE-UV characterization of SiO₂ (20 g/L) nanoparticles with 3.0 ml of HPMA. SDS was present at a concentration of 2 mg/mL.]

[Figure 3: Dynamic light scattering measurements to determine the lognormal distribution of (a) SiO₂ nanoparticles and (b) HPMA-bound SiO₂ nanoparticles.]

[Figure 4: Improved detection of HPMA-bound SiO₂ nanoparticles after HPMA binding, CE-UV analysis could be performed with no disturbance of the EOF.]

[Figure 5: A significant total enhancement of 50 folds in detection sensitivity was attained by coating SiO₂ NPs with chitosan followed by HPMA binding.]

Table 1: DLS analysis to determine the hydrodynamic diameters of different particles, followed by calculation of the HPMA thickness.

<table>
<thead>
<tr>
<th>Particles</th>
<th>Hydrodynamic Diameter (nm)</th>
<th>HPMA Thickness (nm)</th>
<th>Coating Thickness (nm)</th>
<th>Polydispersity Index (PDI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiO₂</td>
<td>53 ± 3</td>
<td>---</td>
<td>---</td>
<td>0.357</td>
</tr>
<tr>
<td>HPMA-bound SiO₂</td>
<td>63 ± 13</td>
<td>30</td>
<td>---</td>
<td>0.356</td>
</tr>
<tr>
<td>TiO₂</td>
<td>155 ± 6</td>
<td>---</td>
<td>---</td>
<td>0.319</td>
</tr>
<tr>
<td>HPMA – TiO₂ mixture</td>
<td>154 ± 5</td>
<td>0</td>
<td>---</td>
<td>0.323</td>
</tr>
<tr>
<td>Chitosan-coated SiO₂</td>
<td>513 ± 30</td>
<td>---</td>
<td>460</td>
<td>0.343</td>
</tr>
<tr>
<td>HPMA-bound chitosan-coated SiO₂</td>
<td>417 ± 17 to 440 ± 12</td>
<td>-96 to -73</td>
<td>---</td>
<td>0.322-0.338</td>
</tr>
<tr>
<td>Chitosan-coated TiO₂ (SDS)</td>
<td>477 ± 11</td>
<td>---</td>
<td>322</td>
<td>0.300</td>
</tr>
<tr>
<td>HPMA-bound chitosan-coated TiO₂</td>
<td>416 ± 11 to 514 ± 8</td>
<td>-61 to +37</td>
<td>---</td>
<td>0.122-0.288</td>
</tr>
</tbody>
</table>

Figure 4: CE-UV characterization of HPMA-bound chitosan-coated SiO₂ particles.

Figure 5: HPMA-bound chitosan-coated SiO₂ peak areas obtained for different SiO₂ concentrations.

HPMA binding. This result is better than the enhancement of 10-14 folds previously attained by using both PHPMA and PDA coatings. In addition, no chemical initiator is needed for this novel technique which does not involve in-situ polymerization.

Similarly, HPMA-bound chitosan-coated TiO₂ NPs were easily analyzed by CE-UV in conjunction with improvement of the CE-UV detection of chitosan-coated TiO₂ NPs as shown. All these results provide clear and convincing proof that SiO₂ NPs can be made larger in size by interaction with HPMA alone and even larger after coating with chitosan under very simple experimental conditions. The larger sizes offer more sensitive detection of SiO₂ NPs in a water sample by CE-UV analysis.

Conclusions

A simple and inexpensive method for the detection of nanoparticles, which are a burden in aquatic ecosystem, has been successfully developed. Hydroxypropyl methacrylate interacted with colloidal SiO₂ nanoparticles to produce a larger size for more sensitive detection by CE-UV. It did not interact with TiO₂ nanoparticles in aqueous suspension, resulting in no change of CE-UV detection sensitivity. It interacted with chitosan-coated SiO₂ or TiO₂ nanoparticles, resulting in a significant enhancement of their CE-UV detection. This chemical technique allows us to analyze an aqueous sample containing different kinds of nanoparticles, both qualitatively and quantitatively, simply by adding hydroxypropyl methacrylate to observe any CE-UV peak enhancements. HPMA interaction is time-saving as less than 22 hours can be used to reach a binding equilibrium with the nanoparticles. Coating of SiO₂ and TiO₂ NPs with chitosan produced a significant increase in hydrodynamic diameter to 513 ± 30 nm for chitosan-coated SiO₂ particles and 477 ± 11 nm for chitosan-coated TiO₂ nanoparticles. Subsequent HPMA binding facilitated CE-UV analysis without causing disturbance of the EOF. A total enhancement of 50 folds in detection sensitivity was attained by coating SiO₂ nanoparticles with chitosan followed by HPMA binding. The new detection limit is estimated at 0.06 g/L of SiO₂. Only nanoparticles, but not organic compounds, would change in area and migration time after binding to HPMA or coating with chitosan. No chemical initiator is required, unlike what we reported previously using in-situ polymerization to attain coating with PHPMA. In our future work, the ligand-directed formation of colloidal polymers through chemical interactions of surface ligands between adjacent nanoparticles [29] can further enhance the CE-UV detection sensitivity of SiO₂, TiO₂, and other kinds of NPs.

Acknowledgements

Financial support from NSERC Canada is gratefully acknowledged. S. Alsudir thanks the Saudi Ministry of Higher Education for her scholarship.

References


