**Capillary Electrophoresis Analysis of Metal/Metalloid Oxide
Nanoparticles in Water: Method Development for the
Enhancement of UV Detection Sensitivity**

by

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Graphical Abstract

Abstract

Increasing production and applications of metal/metalloid oxide nanoparticles (NPs) have greatly raised the demand for new analytical techniques capable for trace quantification in water to assess their environmental impacts and health risks. A new analytical method was developed for the sensitive detection of silica (SiO_2), titania (TiO_2) and zinc oxide (ZnO) as model metal/metalloid oxide NPs. This method was based on the formation of molecular layers and polymeric coatings on the NPs directly in water to selectively add chromophores to their surface for enhanced ultraviolet (UV) light absorption in capillary electrophoresis (CE) analysis. One unique advantage is the ability to identify nanoparticles by observing a stronger peak and/or a shifted migration time.

Controlled polymerization of 2-hydroxypropyl methacrylate (HPMA) on SiO_2 NPs added a coating of poly-2-hydroxypropyl methacrylate (PHPMA) that increased their UV detection sensitivity by 6 ± 1 folds initially. A second coating with polydopamine produced a larger size of PHPMA- SiO_2 NPs, as confirmed by dynamic light scattering (DLS) and transmission electron microscopy, further enhancing their UV detection sensitivity by 12 ± 2 folds. Chitosan coating and HPMA binding on SiO_2 NPs produced a significant enhancement of UV detection sensitivity by 50 ± 1 folds. This method was selective for SiO_2 in the presence of TiO_2 NPs in 10 mM Na_2HPO_4 , a background electrolyte used for CE analysis.

Selective enhancement of UV detection sensitivity of TiO_2 in the presence of alumina (Al_2O_3), SiO_2 , and ZnO NPs in 100 mM Tris was achieved using deoxyribonucleic acid (DNA) and polyethylene glycol (PEG). Single-stranded DNA (ssDNA) exhibited better performance than double-stranded DNA in enhancing the

sensitivity of UV detection. PEG coating of ssDNA-TiO₂ NPs further enhanced the UV detection sensitivity in CE analysis, by providing electrosteric stabilization, up to 13±3 folds for the determination of TiO₂ NPs.

A monolayer adsorption of dithiothreitol (DTT) and cysteine (Cys) onto ZnO NPs in 10 mM Na₂HPO₄ improved their detection sensitivity by 28±1 and 25±1 folds, respectively. The selectivity of DTT and Cys towards ZnO was validated in the presence of Al₂O₃, ceria (CeO₂), SiO₂ and TiO₂ NPs as no changes were observed in the CE-UV peak area of either adsorbates or the NPs. Similar evidence was provided using DLS by determining the hydrodynamic diameters of NPs in the presence of adsorbates. Cys improved the colloidal stability of ZnO NPs by breaking down the aggregates, as evidenced by a reduction of their hydrodynamic diameter.

The new approach provides a simple, rapid and efficient CE-based method towards the detection of SiO₂, TiO₂ and ZnO NPs selectively with enhanced sensitivity. The buffer composition was found to influence the selectivity of various molecular and polymeric coatings. Large enhancement factors have been obtained, improving the UV detection limits of NPs. This work demonstrates the feasibility of a new method for the direct detection of NPs in environmental waters. The lack of certified reference materials, particularly for the mass concentration of NPs, is hindering the validation of the developed method. The present UV detection limits are still not adequate for the quantitative determination of trace NPs in environmental waters. Further improvement will be required by coupling CE to laser-induced fluorescence and/or inductively coupled plasma- mass spectrometry.

Preface

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Chapter 3

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Chapter 6

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These articles were wholly reproduced for formatting and clarity of presentation. The student performed all work presented in the articles. Writing was collaborative between the co-authors.

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In the Name of God, the All-Merciful, the All-Compassionate

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Table of Contents

Graphical Abstract	ii
Abstract	iii
Preface	v
Acknowledgments	vi
List of Tables	xiii
List of Figures	xiv
List of Abbreviations	xvii
 Chapter 1: Introduction	 1
1.1 Nanoparticles and their applications	1
1.2 Environmental and health impacts of nanoparticles.....	3
1.3 Environmental fate of metal/metalloid oxide nanoparticles	6
1.4 Influence of water chemistry on the behaviour of nanoparticles	7
1.4.1 Van der Waals attraction	7
1.4.2 Electrostatic interaction	8
1.4.3 DLVO Theory	9
1.4.4 Stabilization mechanisms	9
1.5 Analytical strategies for the quantification of metal/metalloid oxide nanoparticles ..	11
1.5.1 Sample pretreatment	13
1.5.2 Separation, fractionation and preconcentration methods	13
1.5.3 Screening methods for the detection of metal/metalloid oxide NPs	16
1.6 Online and coupled techniques	18
1.6.1 Hydrodynamic and size exclusion chromatography	18
1.6.2 Field flow fractionation	19
1.6.3 Capillary electrophoresis	21
1.6.3.1 Principles of capillary electrophoresis	22
1.6.3.2 Physicochemical characterization of nanoparticles by capillary electrophoresis	25

1.6.3.3 Recent advances in the development of capillary electrophoresis.....	27
1.7 Efforts towards method validation and reference materials	29
1.8 Research goal	30
1.9 Hypothesis	31
Chapter 2: Materials and Instrumentation	33
2.1 Materials	33
2.2 Apparatuses and analytical methods	33
2.3 Dynamic light scattering	34
2.4 Transmission electron microscopy	34
2.5 Fourier transform infrared spectroscopy	35
Chapter 3: Polymer Coatings for Sensitive Analysis of Colloidal Silica	36
3.1 Abstract	36
3.2 Introduction	37
3.3 Methods	38
3.3.1 Polymerization of HPMA	38
3.3.2 PHPMA growth on colloidal SiO ₂ nanoparticles	38
3.3.3 Effect of AIBN on PHPMA growth	38
3.3.4 Polymerization of HPMA with polyvinyl alcohol or bisphenol A	39
3.3.5 Polydopamine growth on SiO ₂ @ PHPMA	39
3.4 Results and Discussion	39
3.4.1 Silica nanoparticles	39
3.4.2 CE-UV characterization of PHPMA particles	41
3.4.3 PHPMA growth on SiO ₂	41
3.4.4 Effect of AIBN on PHPMA growth	44
3.4.5 Polymerization of HPMA with PVA or BPA	45

3.4.6 PDA growth on SiO ₂ @PHPMA particles in water	46
3.4.7 Dynamic light scattering	49
3.4.8 Transmission electron microscopy	51
3.5 Conclusion	52
3.6 Connection to chapter 4	53

Chapter 4: Hydroxypropyl Methacrylate Interaction and Chitosan Coating for Enhanced UV Detection Sensitivity of Colloidal Nanoparticles in Capillary Electrophoresis Analysis 54

4.1 Abstract	54
4.2 Introduction	55
4.3 Methods	56
4.3.1 HPMA binding interaction with colloidal SiO ₂ or TiO ₂ nanoparticles	56
4.3.2 HPMA binding interaction with polymeric nanoparticles	56
4.4 Results and discussion	57
4.4.1 CE-UV characterization of silica and titania nanoparticles	57
4.4.2. HPMA binding interaction with silica and titania nanoparticles	59
4.4.3 Dynamic light scattering analysis of silica and titania nanoparticles	60
4.4.4. Selectivity of HPMA binding with SiO ₂ and TiO ₂ nanoparticles	61
4.4.5. Coating of silica and titania nanoparticles with chitosan	62
4.4.6. HPMA binding with chitosan-coated SiO ₂ nanoparticles	64
4.5 Conclusions	68
4.6 Connection to chapter 5	68

Chapter 5: Electrosteric Stabilization of Colloidal TiO₂ Nanoparticles with DNA and Polyethylene Glycol for Selective Enhancement of UV Detection Sensitivity in Capillary Electrophoresis Analysis..... 70

5.1 Abstract	70
5.2 Introduction	71
5.3 Methods	72

5.3.1 Triton X-100 adsorption onto TiO ₂ nanoparticles	72
5.3.2 DNA binding to TiO ₂ nanoparticles	72
5.3.3 PEG coating on TiO ₂ and DNA-TiO ₂ nanoparticles	72
5.4 Results and discussion	72
5.4.1 Triton X-100 adsorption onto TiO ₂ nanoparticles	72
5.4.2 Double-stranded DNA binding to TiO ₂ nanoparticles	77
5.4.3 PEG coating of TiO ₂ and dsDNA-TiO ₂ nanoparticles	80
5.4.4 Single-stranded DNA adsorption onto TiO ₂ nanoparticles	84
5.4.5 PEG coating of ssDNA-TiO ₂ nanoparticles	86
5.5 Conclusions	90
5.6 Connection to chapter 6	91
Chapter 6: Selective Enhancement of UV Detection Sensitivity for ZnO Nanoparticles in Capillary Electrophoresis Analysis using Dithiothreitol and L-Cysteine	93
6.1 Abstract.....	93
6.2 Introduction	94
6.3 Methods	95
6.3.1 DDT and Cys adsorption onto ZnO nanoparticles	95
6.3.2 DDT and Cys adsorption onto Al ₂ O ₃ , CeO ₂ , SiO ₂ and TiO ₂ nanoparticles	96
6.4 Results and Discussion	96
6.4.1 Stability of DTT and Cys solutions	96
6.4.2 DDT or Cys adsorption onto ZnO nanoparticles	97
6.4.3 Adsorption kinetics	102
6.4.4 Adsorption isotherms	107
6.4.5 Effect of ZnO dose on DTT and Cys adsorption	110
6.4.6 DDT and Cys adsorption onto Al ₂ O ₃ , CeO ₂ , SiO ₂ and TiO ₂ nanoparticles ..	111
6.4.7 Dynamic light scattering	112

6.5 Conclusion	113
6.6 Connection to chapter 7	114
Chapter 7: Conclusions	115
7.1 General conclusions and outlook	115
7.2 Contributions to knowledge	119
References	120
Appendix	143

List of Tables

Table 3.1	CE-UV peak areas of HPMA, PHPMA and SiO ₂ @PHPMA under different conditions of HPMA polymerization. All peak areas are expressed in arbitrary units of mV.s.....	46
Table 3.2	DLS measurement of the hydrodynamic diameters of original and polymer-coated SiO ₂ particles.....	50
Table 4.1	DLS analysis to determine the hydrodynamic diameters of different particles, followed by calculation of the HPMA thickness.....	64
Table 5.1	Enhancement factors obtained after addition of various organic/bioorganic compounds onto TiO ₂ , SiO ₂ , Al ₂ O ₃ and ZnO nanoparticles as well as the LOD and LOQ for stabilized TiO ₂ nanoparticles.....	89
Table 5.2	Hydrodynamic diameters of TiO ₂ , SiO ₂ , Al ₂ O ₃ and ZnO nanoparticles measured by DLS before and after addition of various organic and bioorganic adsorbates.....	90
Table 6.1	Kinetic parameters for DTT adsorption onto ZnO nanoparticles.....	105
Table 6.2	Isotherm models parameters for the adsorption of DTT and Cys onto ZnO nanoparticles.....	109
Table 6.3	Hydrodynamic diameters of Al ₂ O ₃ , CeO ₂ , SiO ₂ , TiO ₂ and ZnO nanoparticles measured by DLS before and after addition of DTT or Cys.....	113

List of Figures

Figure 3.1	CE-UV characterization of SiO ₂ nanoparticles (at 20 g/L) in LUDOX® AM colloid.....	40
Figure 3.2	CE-UV characterization of (a) MO, (b) HPMa (and PHPMA) after 22 h of polymerization.....	41
Figure 3.3	Schematic representation of the polymerization process with/without SiO ₂	42
Figure 3.4	CE-UV analysis of PHPMA and SiO ₂ @PHPMA particles after 22 h of polymerization using AIBN at 3 wt. %.....	42
Figure 3.5	SiO ₂ @PHPMA peak area obtained from different SiO ₂ concentrations (g/L).....	43
Figure 3.6	CE-UV analysis of PHPMA and SiO ₂ @PHPMA particles after 22 h of polymerization using AIBN at 1 wt. %.....	44
Figure 3.7	Schematic representation of PDA growth on PHPMA and SiO ₂ @PHPMA at room temperature (RT).....	47
Figure 3.8	CE-UV analysis of DA, HPMa, PHPMA@PDA, SiO ₂ @PHPMA@PDA and SiO ₂ @PDA particles.....	48
Figure 3.9	Dynamic light scattering measurements of (a) lognormal distribution of SiO ₂ nanoparticles in LUDOX® AM colloid, (b) lognormal distribution of PHPMA in DDW after 22 h of polymerization at 60°C.....	49
Figure 3.10	Transmission electron micrographs of (a) SiO ₂ @PHPMA, and (b) SiO ₂ @PHPMA@PDA.....	51
Figure 4.1	CE-UV characterization of (a) SiO ₂ (20 g/L) and (b) TiO ₂ (20 g/L) nanoparticles. SDS was present at a concentration of 2 mg/mL.....	58
Figure 4.2	CE-UV characterization of SiO ₂ (20 g/L) nanoparticles with 3.0 mL of HPMa. SDS was present at a concentration of 2 mg/mL.....	59
Figure 4.3	Dynamic light scattering measurements to determine the lognormal distribution of (a) SiO ₂ nanoparticles and (b) HPMa-bound SiO ₂ nanoparticles.....	61
Figure 4.4	Schematic representation of chitosan coating on SiO ₂ in the presence of TiO ₂	62

Figure 4.5	CE-UV characterization of HPMa-bound chitosan-coated SiO ₂ particles..	66
Figure 4.6	HPMa-bound chitosan-coated SiO ₂ peak areas obtained for different SiO ₂ concentrations.....	66
Figure 4.7	Schematic representation of selective binding of HPMa to chitosan-coated SiO ₂ in the presence of TiO ₂	67
Figure 5.1	Enhancement of UV detection sensitivity in CE analysis at 280 nm after adding TX-100 to TiO ₂ nanoparticles suspended in 100 mM Tris.....	74
Figure 5.2	FTIR spectra of TiO ₂ , TX-100- TiO ₂ and TX-100.....	75
Figure 5.3	Enhancement of UV detection sensitivity in CE analysis after adding dsDNA to TiO ₂ nanoparticles suspended in 100 mM Tris.....	78
Figure 5.4	FTIR spectrum of TiO ₂ and dsDNA-TiO ₂ nanoparticles.....	79
Figure 5.5	CE analysis, with UV detection at 260 nm, of (a) TiO ₂ nanoparticles, (b) dsDNA (c) PEG@dsDNA and (d) TiO ₂ nanoparticles after dsDNA adsorption and PEG coating.....	81
Figure 5.6	CE analysis, with UV detection at 325 nm, of (a) TiO ₂ nanoparticles, (b) PEG@dsDNA, and (c) TiO ₂ nanoparticles after dsDNA adsorption and PEG coating. Only TiO ₂ can absorb the UV light; neither dsDNA nor PEG can.....	82
Figure 5.7	FTIR spectra of TiO ₂ , PEG, PEG@TiO ₂ and PEG@dsDNA-TiO ₂	83
Figure 5.8	CE analysis, with UV detection at 260 nm, of (a) TiO ₂ nanoparticles, (b) ssDNA and (c) TiO ₂ nanoparticles after ssDNA adsorption.....	84
Figure 5.9	Enhancement of UV detection sensitivity in CE analysis at 260 nm after adding ssDNA to TiO ₂ nanoparticles suspended in 100 mM Tris.....	86
Figure 5.10	CE analysis, with UV detection at 260 nm, of (a) PEG@ssDNA and (b) TiO ₂ nanoparticles after ssDNA adsorption and PEG coating.....	87
Figure 5.11	FTIR spectra of ssDNA, TiO ₂ , ssDNA-TiO ₂ , PEG and PEG@ssDNA-TiO ₂	88
Figure 6.1	Stability of DTT and Cys in 10 mM Na ₂ HPO ₄ and 100 mM Tris by capillary electrophoresis with UV detection at 220 nm.....	97

Figure 6.2	CE analysis, with UV detection at 220 nm, of (a) ZnO nanoparticles, (b) ZnO nanoparticles after DTT adsorption and (c) ZnO nanoparticles after Cys adsorption.....	97
Figure 6.3	Enhancement of UV detection sensitivity in CE analysis after adding DTT or Cys to ZnO nanoparticles suspended in 10 mM Na ₂ HPO ₄	99
Figure 6.4	Schematic illustration of the possible different modes of DTT adsorption onto ZnO surface; (a) the cross-linking mode, (b) the horizontally aligned mode, and (c) the vertically aligned mode.....	99
Figure 6.5	FTIR spectra of ZnO, DTT-ZnO and DTT.....	100
Figure 6.6	FTIR spectra of ZnO, Cys-ZnO and Cys.....	102
Figure 6.7	Effect of contact time on the kinetics of DTT adsorption at two different concentrations onto ZnO nanoparticles.....	103
Figure 6.8	Pseudo-first-order kinetic, pseudo-second-order kinetic and intraparticle diffusion models for DTT adsorption onto ZnO nanoparticles.....	106
Figure 6.9	Langmuir, Freundlich and Temkin isotherm models for DTT adsorption onto ZnO nanoparticles.....	110
Figure 6.10	Effect of ZnO dose on % adsorption of DTT and Cys.....	111

List of Abbreviations

Alumina	Al ₂ O ₃
2,2'-azobis-2-isobutyronitrile	AIBN
Background electrolyte	BGE
Bisphenol A	BPA
Capillary electrophoresis	CE
Ceria	CeO ₂
Certified reference materials	CRMs
Cysteine	Cys
Deoxyribonucleic acid	DNA
Dithiothreitol	DTT
Dopamine	DA
Double-stranded deoxyribonucleic acid	dsDNA
Dynamic light scattering	DLS
Electric double layer	EDL
Electroosmotic flow	EOF
Engineered nanoparticles	ENPs
Fourier transform infrared	FTIR
Graphene quantum dots	GQDs
2-hydroxypropyl methacrylate	HPMA
Laser induced fluorescence	LIF
Limit of detection	LOD
Limit of quantification	LOQ
Mesityl oxide	MO
Natural organic matter	NOM
Nanoparticles	NPs
Polydopamine	PDA

Polyethylene glycol	PEG
Poly-2-hydroxypropyl methacrylate	PHPMA
Polyvinyl alcohol	PVA
Silica	SiO ₂
Single-stranded deoxyribonucleic acid	ssDNA
Single particle inductively coupled plasma mass spectrometry	SP-ICP-MS
Sodium dodecyl sulfate	SDS
Titania	TiO ₂
Transmission electron microscopy	TEM
Tris (hydroxymethyl) aminomethane	Tris
Triton X-100	TX-100
Ultraviolet	UV
Zinc oxide	ZnO

Chapter 1: Introduction

1.1 Nanoparticles and their applications

Nanoparticles (NPs) are ultrafine objects having at least one dimension ranging from 1 to 100 nm. [1] Generally, NPs can be of natural origin, accidentally produced or chemically engineered. [2] Due to their novel optical, electrical and chemical properties, engineered NPs (ENPs) have been commonly used in a wide array of applications covering a broad variety of fields in science and technology. [3] ENPs can be categorized as either carbon-based NPs or metal-containing NPs including zero-valent metals, metal/metalloid oxides, metal sulphides and semiconducting quantum dots. [4] Metal and metal oxide NPs can be synthesized by chemical or green methods [5] and have mainly found applications that take advantage of their sorptive, catalytic, or optical properties. [6] A promising new generation of photoresist materials uses metal oxide NPs to pattern 20-nm features for extreme ultraviolet lithography. [7] Polyethylene-metal oxide NP composites have recently emerged as an excellent material for the insulation of extruded high voltage direct current (HVDC) cables. [8] Flat-panel solar thermal collectors currently use metal oxide NPs in liquid-based nanofluids showing a thriving enhancement of the thermal absorption efficiency. [9] Transition metal oxide NPs have arisen as a potential nano-modifier to enhance the thermal decomposition of rocket propellants. [10] Mixed metal oxide NPs have attracted great interests for many energy-related applications such as water splitting, fuel cells, and battery technology. [11]

The prevalent representative of metal oxide NPs is titania (TiO_2), which is globally produced in different crystalline structures: rutile, anatase and brookite. [12]

Due to its photocatalytic properties and UV light absorptivity, TiO_2 finds numerous applications including utilization in consumer products such as sunscreens [13], cosmetics [14], plastics [15], electronics [16], sports equipment [17], clothing [18], household cleaning solutions [19], and paints. [20] Other fundamental applications [21] include agriculture, environmental remediation of pollutants in wastewater treatment, air purification, solar energy conversion, tunnel lighting, sound insulation, offset printing, medical imaging, and drug delivery. Zinc oxide (ZnO) is widely used in the form of NPs, which exist in three crystal structures: hexagonal wurtzite, cubic zinc-blende, and a rarely-observed cubic rock-salt (NaCl -type) [22]. The unique properties of ZnO NPs have paved their way to diverse applications in photocatalysis, solar cells, sensors, piezoelectric and photodiode devices, sunscreen formulations, antireflection coatings, and biomedicines. [23-26] ZnO quantum dots have received a great deal of attention from the scientific community because of their utilization in various photodetector technologies [27,28] and their controllable exciton energy by changing the confinement conditions. [29] Alumina (Al_2O_3), with fifteen distinct crystallographic phases including alpha, delta, gamma and theta obtained as a function of annealing temperature [30], has been extensively used for high performance ceramics, cosmetic fillers, packing supplies, polishing materials, semiconductors, paints, resins, wear-resistant supports, waterproof materials, catalysts, and catalyst carriers. [31] Ceria (CeO_2) is most commonly applied as a chemically stable catalyst for fuel oxidation and cracking of heavy petroleum residue to yield lighter fractions. [32,33] Silica (SiO_2) is the most prevailing metalloid oxide NPs. It has been widely used in various pharmaceutical products, cosmetics, printer toners, food products, paints, surgical tools,

medical equipment, textiles, and as a potential vehicle for drug delivery. [34]

The 2020 production estimate of nanomaterials is 1 million to 6 million tons, ranging from metals and metal/metalloid oxides to carbon nanotubes and quantum dots. [35] Metal/metalloid oxide NPs have proportionally the greatest potential to enter the environment [36] via direct discharge into wastes/wastewater effluents [37] and accidental release to the aquatic environment. [38] The capture of NPs from water effluents through adhesion to clearing sludge varies in efficiency from 70% to 94%. [39]

1.2 Environmental and health impacts of nanoparticles

Great concerns regarding the possible environmental and health risks have arisen with increasing production of metal/metalloid oxide NPs to meet the growing demands for numerous applications. Those NPs could find their way into the environment where their fate and behaviour are highly uncertain. The toxicity of NPs arises from a number of biophysicochemical factors, including their ability to penetrate the blood-brain barrier, tissues and cells. Their large surface area-to-volume ratio increases oxidative stress and results in undesirable interactions with biological macromolecules. [40,41] At a large environmental system, the small size of NPs enables them to translocate from their deposition site and enter to water sources. [42,43] Hence, there is a growing concern regarding the potential adverse effects of NPs on drinking water, juvenile fish, and terrestrial plants. [44-46] Industrial, commercial and agricultural applications of metal or metal/metalloid oxide NPs could result in their accumulation in soil, threatening higher terrestrial plants. Such NPs may have negative impacts on the growth and yield of food crops. [47] The physiological and biochemical responses of plants to stress imposed by

metal/metalloid oxide NPs have been recently reviewed. [48] Various levels of toxicity have been observed based on bioluminescence activity, seed germination, and bacterial gene mutation in a wide range of organisms. [49] TiO₂ NPs showed toxicity towards *R. subcapitata* (EC₅₀ = 6.8 mg Ti/L) due to aggregation and entrapment of algal cells into TiO₂ aggregates. [50] Exposures to high doses of ZnO NPs (1000 mg kg⁻¹ feed) for 10 days resulted in Zn distribution to the liver of fish. [51] Biochemical disturbances associated with oxidative stress in the liver point to the ability of ZnO NPs to interfere with cytochrome P450 metabolic processes. Co-exposure to perfluorooctane sulfonate (widely distributed in the environment) and ZnO NPs can cause more serious thyroid-disrupting effects in zebrafish than exposure to the sulfonate alone. [52] CeO₂ and TiO₂ NPs have not shown cytotoxic effects in rats at concentrations ≤50 µg/mL; however, they could cause subtle concentration-related changes in spontaneous and/or gamma-aminobutyric acid (GABA) receptor-mediated neuronal activity *in vitro* at times when cytotoxicity is absent. [53] ZnO NPs, for instance, induced a strong decrease in the mitochondrial activity, major perturbations in phagocytosis and an increased damage of the methylglyoxal-associated DNA in mice macrophages. [54,55] Moreover, they induced toxicity in RAW 264.7 cell line, leading to the generation of reactive oxygen species, excitation of inflammation, and cell death. [56] Moreover, Al₂O₃, CeO₂, TiO₂ and ZnO NPs induced changes to some specific hematological and biochemical blood parameters in mice. [57] The release of Zn²⁺ and Ni²⁺ from ZnO and NiO NPs motivated the allergic reaction to inhaled ovalbumin in mice. [58] A global upsurge in antibiotic resistance has led to research in metal oxide-based antimicrobial therapy. [59] Silver and ZnO NPs with antimicrobial action are present in consumer products. The bacterial

toxicity of metal oxide NPs resulted from the action of the NPs themselves, the released ions, or a combination of both mechanisms depending on the composition of the NPs. [60] Anatase TiO_2 NPs induced oxidative DNA damage, lipid peroxidation, and micronuclei formation in human bronchial epithelial cells, even in the absence of photoactivation. [61] Long-term exposure of lung alveolar epithelial cells to low levels of TiO_2 NPs caused oxidative damage to DNA and intracellular accumulation. [62] Al_2O_3 NPs were capable of triggering up-regulation of genes related to cell death in a human A549 lung adenocarcinoma cell line. [63] CuO NPs (5–15 $\mu\text{g/mL}$) exerted cytotoxicity in A549 lung cells in a dose-dependent manner. [64] Co_3O_4 NPs decreased the cellular viability of human lymphocytes along with increasing cell membrane damage followed by Fe_2O_3 , SiO_2 and Al_2O_3 NPs after 24 h of exposure. [65]

The most common exposure routes for humans are via skin contact, inhalation, and oral uptake. [66-70] Sunscreen formulations are normally composed of ZnO (5% w/w) and TiO_2 (25% w/w). Topically applied ZnO could not penetrate into the viable epidermis; however, Zn^{2+} found in the blood and urine after topical application of ZnO probably arose from the acid catalyzed dissolution of ZnO on the skin surface and the permeation of released Zn^{2+} across the stratum corneum, into the viable epidermis, and then to the systemic circulation, from which they may be excreted in the urine. [71] Fe_2O_3 , Fe_3O_4 , MnFe_2O_4 , and CrOOH NPs are representative of those found in the welder's lungs causing the development of peribronchiolar, perivascular and alveolar fibrosis. [72] NPs can potentially induce toxicity in the lung via activating the main mitogen-activated protein kinases (MAPKs) and the nuclear factor $\text{NF}\kappa\text{B}$ to release inflammatory cytokines. [73,74] Inhaled CdO NPs translocated to other organs after

accumulation in the lungs causing serious damage of cells and tissues. [75,76] The ubiquitous use of metal/metalloid oxide NPs in semiconductor fabrication raised alerts to protect the workers' health and safety. [77] A combination of filter-based air sampling and direct-reading instruments have been used to identify, characterize, and quantify the exposure potential to airborne Al_2O_3 and amorphous SiO_2 NPs associated with the chemical-mechanical wafer polishing process. Oral uptake is probably the most prevalent route for metal/metalloid oxide NPs to enter the body from the high amounts of NPs present in food due to the large absorption area of the gastrointestinal tract. [78] These NPs can penetrate biological barriers at a high translocation rate and may distribute differently in the body according to their size and surface functionality. Humans are increasingly exposed through their diet to silver and ZnO NPs used in the food industry. Silver NPs were found to cause adverse effects on the gut microbiota at lower concentrations than that damaging the enterocytes; the opposite was found to be true for ZnO NPs. [79]

1.3 Environmental fate of metal/metalloid oxide nanoparticles

The environmental distribution of metal oxide NPs over air, water, soil and sediment was evaluated using the SimpleBox4nano model through which Monte Carlo simulations were performed on the environmental fate, concentrations and speciation of CeO_2 , TiO_2 and ZnO NPs. The largest amount of metal oxide NPs was predicted to be dominant in the water compartment [80] followed by sedimentation via homo- or hetero-agglomeration, which is highly affected by variations in concentration, ionic strength, pH, temperature and natural colloids. [81] Additionally, surface properties of NPs may be altered by losing their surface coating or developing an additional coating of natural

organic matter (NOM), which is an organic material present in environmental waters including both humic and non-humic fractions. [82] Moreover, metal/metalloid oxide NPs may undergo chemical transformations particularly oxidation, sulfidation, and dissolution due to their reactivity towards other contaminants in water. [83] Chemical transformation of metal/metalloid oxide NPs was exemplified by the complexation of ZnO NPs with carbonate or phosphate in solution, which can reduce ZnO dissolution due to the formation of insoluble carbonate and phosphate species on the NP surfaces. [84] The effects of various aquatic contaminants on the transformation, transport kinetics, and toxicity of metal/metalloid oxide NPs have been reviewed. [85] TiO₂ NPs, for example, showed a high affinity for Cd²⁺ which significantly affected their environmental fate and transformation. [86]

1.4 Influence of water chemistry on the behaviour of nanoparticles

The stability, dissolution, and transformation of NPs in aquatic media determine their environmental fate, bioavailability, and toxicity. One fundamental aspect in colloid science is the stabilization mechanism of NPs in different media. Generally, NPs are unstable and tend to agglomerate/aggregate at short interparticle distances due to attraction via van der Waals or electrostatic forces, which can be counteracted by repulsive forces resulting in electrostatic, steric or electrosteric stabilization. [87]

1.4.1 Van der Waals attraction

Intermolecular forces are commonly explained by the Lennard-Jones potential that describes the potential energy of interaction ($w(D)$) between two molecules at a distance r [88]:

$$w(D) = \frac{B}{r^{12}} - \frac{C}{r^6} \quad (1-1)$$

where B is the Born repulsion constant and C is the attractive van der Waals constant.

The van der Waals attraction energy ($w_a(D)$) between two particles with radii R_1 and R_2 can be obtained by adding all forces between the particles yielding the following final equation for two spherical NPs:

$$w_a(D) = -\frac{\pi^2 \rho_1 \rho_2}{6} C \left[\frac{2 R_1 R_2}{c^2 - (R_1 + R_2)^2} + \frac{2 R_1 R_2}{c^2 - (R_1 - R_2)^2} + \ln \left(\frac{c^2 - (R_1 + R_2)^2}{c^2 - (R_1 - R_2)^2} \right) \right] \quad (1-2)$$

where ρ is the number density of the particle, c is the center to center distance between the two particles, and D is the surface to surface distance between the particles ($D = c - (R_1 + R_2)$).

For two identical particles with $R = R_1 = R_2$ and $D \ll R$ (particles are in close proximity) the $w_a(D)$ becomes;

$$w_a(D) = -\frac{\pi^2 \rho_1 \rho_2 C R}{12D} = -\frac{AR}{12D} \quad (1-3)$$

where A is the Hamaker constant.

1.4.2 Electrostatic interaction

The van der Waals attractions discussed above could promote agglomeration/aggregation of suspended NPs. Stabilization of NPs requires forces opposing the van der Waals attractions. The particle surface charge could result in repulsive interparticle forces. In solution, solvated ions surround the NPs and shield their surface charge forming an electric double layer (EDL), which includes a Stern inner layer and a diffuse outer layer. The ions in the Stern layer are strongly bound whereas the ones in the diffuse layer are loosely bound. Within the diffuse layer a boundary, known as the slipping plane, exists. The potential at this boundary is called the zeta potential (ζ potential), which gives an indication of the colloid stability. NPs with ζ -potential values greater than +30 mV or less than -30 mV are considered electrostatically stable. [89] The

thickness of the EDL is called the Debye length λ (κ^{-1}), which is the inverse of the Debye parameter (κ). [90]

EDL repulsion forces ($W_r D$) can be described using the Derjaguin's equation [91] which assumes a constant surface potential (ψ_δ) and a particle radius much larger than the thickness of the EDL.

$$w_r(D) = 2\pi\epsilon_0\epsilon R\psi_\delta^2 e^{-\kappa D} \quad (1-4)$$

where ϵ_0 is the permittivity of the vacuum and ϵ is the dielectric constant of the solution.

1.4.3 DLVO Theory

The DLVO theory is named after Derjaguin, Landau, Verwey and Overbeek. [91,92] It describes the total force between colloidal NPs by combining the attractive van der Waals and the repulsive EDL forces.

$$w_{total}(D) = w_a(D) + w_r(D) = -\frac{AR}{12D} + 2\pi\epsilon_0\epsilon R\psi_\delta^2 e^{-\kappa D} \quad (1-5)$$

Several factors affect the colloidal stability of NPs including the ionic strength (ion type and concentration), the surface potential and the particle size. The van der Waals attraction is relatively independent of ion concentration, but the repulsive EDL force strongly depends on the concentration of ions, which form the Stern and diffuse layers.

1.4.4 Stabilization mechanisms

Stability of colloidal NPs can be attained by various mechanisms [93];

1. Electrostatic stabilization is the mechanism in which the attractive van der Waals forces are counterbalanced by the repulsive EDL forces. When the repulsion is sufficiently high, it prevents the NPs from agglomeration/aggregation. Surfactants

such as Tween 80, Triton X-100, sodium dodecyl sulfate (SDS) and sodium dodecylbenzenesulfonate are often used as electrostatic stabilizers.

2. Steric stabilization is promoted by the adsorption of large molecules (e.g. polymers) onto the surface of NPs, providing a protective layer surrounding each NP. It operates via three different modes. Firstly, the adsorbed layer causes steric hindrance that can prevent the NPs from getting close to each other and entering into the range of attractive forces. Secondly, when two NPs approach each other, their adsorbed layers overlap. The concentration of adsorbed molecules in the overlap region causes an increase in the osmotic pressure, which is relieved by the influx of water. This influx of water pushes the two NPs apart preventing their agglomeration/aggregation. Thirdly, the freedom of molecules in the overlap region is restricted producing a negative entropy change and thereby a positive change in the Gibbs free energy, which is unfavorable. Spontaneous entropic (steric) repulsion ensues to resist the negative change in entropy and thereby enabling colloidal stability of NPs.
3. Electrosteric stabilization is a combination of electrostatic and steric stabilization mechanisms. Therefore, both electrostatic repulsion and steric restriction prevent NPs from agglomeration/aggregation.

The stability of NPs is further influenced by the water chemistry. The aqueous pH determines surface potential and thereby the colloid status of NPs. Aggregation is triggered when the pH equals the point of zero charge of the NPs. pH also influences the dissolution of NPs. ZnO, for example, has limited dissolution between pH 8 and 12, but increased with acidity and alkalinity. [94] The increase of ionic strength, especially in

seawaters, enhanced the aggregation and sedimentation of NPs. [95] NOM adsorbed onto CeO₂, TiO₂ and ZnO NPs enhanced their stability in environmental waters. [96] High concentration of Ca²⁺ (>6 mM) could screen the charge on alginate, reducing the repulsion between alginate-CuO NPs to enhance their aggregation. [97] On the contrary, a high concentration of SO₄²⁻ (>10 mg/L) enhanced the stability of ZnO NPs. [98] Phosphate anions enhanced the stability of CeO₂ NPs and suppressed their dissolution. [99] Zirconium phosphate improved the stability of TiO₂ NPs in water due to electrosteric stabilization. [100]

Although a number of instrumental techniques are available for the characterization and detection of metal/metalloid oxide NPs, their applications can be impractical when analyzing complex samples at environmentally relevant concentrations (ng/L). [101] Apparently, the chemical and physical processes synchronically affect the occurrence, fate and toxicity of metal/metalloid oxide NPs and greatly constrain the development of innovative approaches to detect, characterize and quantify them in environmental waters. [102-104]

1.5 Analytical strategies for the quantification of metal/metalloid oxide nanoparticles

Quantitative analysis of metal/metalloid oxide NPs is crucial to determine their path from the original source to various environmental compartments and their possible entry into living creatures. Quantification delivers a concentration based on mass, volume or particle number depending on the analysis method. Qualitative analysis of metal/metalloid oxide NPs uncovers their chemical composition to distinguish each type

of NPs. Particle size determination is of high importance for data interpretation. Other parameters of high relevance include the particle shape, surface area, surface charge, surface functionality, and colloidal stability. A variety of complementary analytical techniques must be applied for the full analysis of metal/metalloid oxide NPs. These techniques can be sorted into five main categories:

1. Quantitative techniques to deliver mass- or particle number-based concentrations;
2. Qualitative techniques to identify the chemical composition;
3. Characterization techniques to measure the particle size;
4. Imaging techniques to determine the size and shape of individual NPs;
5. Surface characterization techniques to determine surface area, charge and coating.

The general analytical procedure most often starts with sample pretreatment followed by separation, fractionation, pre-concentration, size determination and quantification of metal/metalloid oxide NPs. Each step will be reviewed in regard to its utilization in environmental water analysis.

1.5.1 Sample pretreatment

The first challenging step in trace analysis is a proper sample pretreatment that avoids destabilization, aggregation/agglomeration, precipitation and contamination during sample transport and storage. Also, the vessels used for sample collection and storage should be carefully selected to eliminate the possibility of NPs adsorption on the vessel wall and prevent photo-induced electron transfer process. Obviously, different analytical techniques involve different sample pretreatment procedures. Therefore, it is essential to understand the influence of each sample treatment step such as temperature, dilution, pH, or ionic strength on the stability of NPs. Freeze-drying and reconstitution of aqueous suspensions of SiO₂ NPs have been verified to provide dispersed suspensions with reproducible sizes using trehalose during the freeze-drying process. Trehalose was capable of replacing water molecules due to hydrogen bonding between silanol groups and trehalose, forming a stable layer around the NPs preventing their aggregation. [105] Dilution [106], addition of stabilizing agents [107], or keeping samples at very low temperatures using liquid nitrogen [108] were successfully applied for the stabilization of metal/metalloid oxide NPs in environmental water samples. TiO₂ NPs in wastewater treatment plants effluents were isolated via filtration followed by rotary evaporation, dialysis, and lyophilization via freeze-drying for subsequent characterization and quantification. [109]

1.5.2 Separation, fractionation and preconcentration methods

Several separation methods have been used to eliminate matrix interferences and preconcentrate metal/metalloid oxide NPs. They include ultrafiltration, dialysis, ultracentrifugation, liquid extraction, solid phase extraction, and cloud point extraction.

Ultrafiltration uses membranes to separate NPs based on their sizes, enabling fractionation as well as preconcentration, speciation (separation from corresponding ions), and purification of NPs. Regenerated cellulose membrane was effectively utilized to remove proteins and lysozyme from metal/metalloid oxide dispersions by selecting the proper membrane properties (molecular weight cut-off (MWCO), hydrophilicity, surface charge) and ultrafiltration conditions (pH, flux). [110] Ultrafiltration membranes with MWCO in the range of 1–100 kDa (from ca. 1 nm) are available. Hence, free ionic species can be isolated from NPs unless they form complexes or show affinity to the membrane surfaces, affecting their removal. [111] In general, ultrafiltration was identified as the best practice for the separation of dissolved species from NPs among various separation techniques. [112]

Dialysis works on the basis of diffusion across a membrane due to a concentration gradient and osmotic pressure. It has been extensively used to study the release of metal ions from metals and metal oxide NPs in model solutions. [113] Dialysis takes long times to reach equilibrium since it is based on diffusion. Therefore, ultrafiltration is preferred to speed up the separation process.

Ultracentrifugation separates NPs based on their mass density and allows fractionation by controlling the centrifugal force and time. However, the size distribution of NPs may be altered due to aggregation when applying a high centrifugal force. Dissolution of NPs could also occur at low concentrations as opposed to high ones. [114] Analytical ultracentrifugation with a multi-wavelength detector showed promise in determining the shape, size distribution and optical properties of NPs. However, high concentrations of NPs were required. [115]

Liquid phase extraction has also been reported for the separation of metal/metalloid oxide NPs from environmental water samples. Surface modification of citrate-stabilized silver and TiO₂ NPs with mercaptoundecanoic acid followed by interaction with octadecylamine, resulted in an efficient transfer of the coated NPs into cyclohexane. Extraction efficiencies of 78% and 73% were obtained for 1 mg/L of citrate-stabilized silver and TiO₂ NPs, respectively. [116] Another method based on a microscopic oil–water interface trapping mechanism was reported for the separation of nanomaterials from contaminated waters. It worked with nearly 100% efficiency for separating 1D and 2D nanomaterials; however, it failed to separate spherical NPs. [117] Lauryl gallate (LG) showed strong adsorption on Ag and MnO₂ NPs facilitating their liquid extraction into the *n*-butanol phase. The adsorption mechanism involved the complexation of metal atoms on the surface of each NP with the phenolic OH groups of LG. [118]

Solid phase extraction, on the contrary, is based on different mechanisms such as covalent or non-covalent binding, complexation, and electrostatic interactions. It is mostly coupled with analytical instrumentations to enhance their detection performance. For example, an anion exchange resin was used to efficiently and selectively retain metal NPs after coating with mercaptosuccinic acid via a noncovalent reversible interaction. [119] Also, a strong metal binding resin (Chelex 100) was used to eliminate dissolved metal ions prior to size characterization and quantification of ZnO NPs by single particle inductively coupled plasma mass spectrometry (SP-ICP-MS). [120]

Cloud point extraction involves the formation of micellar aggregates in which NPs are incorporated for their subsequent separation from the aqueous phase by mild

heating. Cloud point extraction coupled with ICP-MS was successfully applied for the analysis of ZnO NPs in water and wastewater samples. Extraction efficiency of 87% was obtained using Triton X-114 for 40 nm ZnO NPs. [121] Pre-treatment of environmental water samples with H₂O₂ and a suitable complexing agent prior to cloud point extraction was required to improve the extraction efficiency of CuO NPs by eliminating the effect of NOM and the corresponding ionic species of NPs present in the water sample. Similar extraction efficiencies were observed for differently coated CuO NPs. [122] A mixture of Triton X-114 and Triton X-100 (30 wt.%) further improved the extraction efficiency of CuO NPs.

Beside these methods, there are a number of electrophoretic and chromatographic separation techniques coupled online with various detection systems to enable the acquisition of additional information (discussed in section 1.6).

1.5.3 Screening methods for the detection of metal/metalloid oxide NPs

Several screening methods have been proposed for the detection of metal/metalloid oxide NPs. These methods are capable of recognizing the presence of particular metal/metalloid oxide NPs, providing preliminary or screening tests that are of critical value. They, however, could not serve to identify, quantify or characterize the NPs.

A protein-based biosensor was reported for the detection of negatively charged NPs in environmental waters. In particular, a green fluorescent protein (GFP) with a poly-lysine tag was engineered to facilitate its electrostatic interaction with gold, Fe₂O₃, CeO₂, and ZnO NPs stabilized with a poly-acrylic acid (PAA) coating. The interaction between the positively charged GFP and the PAA coating on the negatively charged NPs

resulted in visually observable turbidity changes that were quantified using a portable spectrometer. [123] This method, however; lacks selectivity, as it could not distinguish different types of NPs. Also, the protein biosensor could potentially interact with naturally occurring negatively charged colloids or substrates compromising the assay sensitivity. A simple method used epoxy silane to prepare fluorescent-labelled SiO₂ NPs directly in aqueous solutions. The amount of fluorescent label per particle remained constant regardless of the size of NPs, which facilitated the measurement of number-based concentrations.[124]

A simple colorimetric assay was developed for the detection of NPs including gold, silver, CeO₂, SiO₂, and vanadium dioxide (VO₂) having multiple capping agents (tannic acid, polyvinylpyrrolidone, branched polyethylenimine, polyethylene glycol) in complex matrices. It is based on the catalytic electron transfer mechanism between an organic dye, methylene blue, and a reducing agent, sodium borohydride, in the presence of NPs serving as a catalyst. This method was sufficiently sensitive (ppb levels) to measure concentrations typically used in toxicological studies. [125] However, it was not selective for a specific type of NPs and could give false positive results in complex matrices. Other parameters, such as composition, size, concentration and redox potential, need to be considered to promote the development of dye-reducing agent pairs for use in the detection of NPs.

Raman spectroscopic analysis was used to assess the interactions between TiO₂ NPs and flavonoids (i.e. apigenin, luteolin, fisetin, kaempferol, quercetin, myricetin, and baicalin) since TiO₂ NPs are commonly found in food additives and have been associated with potential adverse health effects. [126] Phenolic groups in flavonoids played a key

role in their interaction with TiO₂ NPs. Differences in the number and positions of phenolic groups contributed to distinctive spectral peaks for the different complexes.

Other contemporary NPs quantification methods such as laser induced break down detection [127], small angle neutron scattering [128], and fluorescent correlation spectroscopy [129] rely upon sophisticated instrumentation and a skilled analyst, making such approaches impractical for regular environmental monitoring.

1.6 Online and coupled techniques

1.6.1 Hydrodynamic and size exclusion chromatography

Hydrodynamic chromatography (HDC) is a solution-phase separation method that can be performed in an open tube (capillary) or in a column packed with nonporous microbeads. [130] Size exclusion chromatography (SEC) is another size fractionation technique that occurs via columns packed with a porous material. Elution order in HDC is the same as in SEC, larger particles being eluted ahead of smaller ones. However, the mechanisms of retention of these two techniques are different. In HDC, retention is due to preferential sampling of the flow streamlines, whereas in SEC it is based on particles preference for certain pore volumes. Secondary interaction of NPs with the stationary phase could compromise the resolving power of the chromatographic separation. Therefore, surfactants may be needed to avoid the entrapment of NPs and the blockage of pores, at the expense of possible alteration of the hydrodynamic diameter of the NPs. [131]

HDC coupled with ICP-MS was evaluated for the analysis of NPs with different coatings and shapes using a commercially available HDC column. Retention behaviour

was slightly affected by the type of the coating material and strongly influenced by the particle shape. However, particle composition had no influence on the retention behaviour. SP-ICP-MS was able to discriminate between spherical and non-spherical particles as long as the size distribution is large enough for profile analysis and the particles are similarly shaped. Complications due to NPs geometry may arise and need to be considered throughout the measurement of unknown NPs to minimize erroneous results. [132]

SEC-ICP-MS was demonstrated as a promising technique for speciation analysis of metal oxide NPs including NiO, CoO, ZnO, CuO and CeO₂ and their corresponding ions using a 1000 Å pore size silica column. The composition of the mobile phase prevented the dissolution of NPs during the SEC separation. Addition of SDS into the mobile phase was crucial to prevent the adsorption of metal oxide NPs and the ions onto the silica column. The high recovery of ions from the SEC column ensured their accurate quantification by ICP-MS. Metal oxide NPs with sizes larger than the column pore size were completely filtered off by the column. Consequently, the quantification of metal oxide NPs was obtained by subtracting the ion content from the total metal content (which was determined with ICP-MS after digestion without SEC separation). The obtained detection limits were as low as 0.02–0.39 µg/L for both ions and metal oxide NPs. [133]

1.6.2 Field flow fractionation

Field flow fractionation (FFF) is a family of separation techniques that are classified according to the nature of the force field. For instance, in flow-FFF (F4), a perpendicular secondary flow is responsible for separating NPs according to their

hydrodynamic diameter, whereas in sedimentation-FFF (SdFFF), NPs are separated as a function of volume and density by applying a centrifugal force. F4, in its two variants (symmetric and asymmetric (AF4)), is commonly used to separate natural and engineered NPs. AF4 and SdFFF are the only FFF techniques that have been coupled online to ICP-MS. AF4 is highly efficient for small particles while SdFFF is best for particles larger than 50 nm, which could readily sediment. [134]

AF4 coupled with ICP-MS technique was evaluated for the separation and detection of CeO₂ NPs. Size characterization of CeO₂ NPs was challenging due to the scarcity of certified reference materials and the instability of CeO₂ suspensions. The estimated size of CeO₂ NPs was in good agreement with those obtained with different techniques (TEM, XRD and DLS). Direct quantification of CeO₂ NPs by ICP-MS using the corresponding ionic standard led to low recoveries (<80%) with a detection limit of 0.9 µg/L. [135] Characterization and quantification of SiO₂ NPs in aqueous suspensions using AF4-ICP-MS allowed for the separation of different sizes of SiO₂ NPs (20, 40, 60, 80, 100 and 150 nm) and provided detection limits between 0.16 and 0.30 mg/L for smaller and larger particles, respectively. The pre-channel mass calibration approach with SiO₂ NPs addressed the common quantification problem associated with losses of NPs during the separation process allowing the simultaneous characterization and quantification of SiO₂ NPs. [136] The challenge of characterizing SiO₂ NPs using SP-ICP-MS has been addressed to reduce or eliminate interfering signals generated due to abundant molecular interferences including dinitrogen ions. Conventional approaches using a helium collision gas or a reactive ammonia gas are sufficient for larger SiO₂ NPs, but are limited by either the inherent random collisions or side reactions that reduce

silicon ion sensitivity. A new approach using microsecond dwell times in SP-ICP-MS allowed for the detection and characterization of SiO₂ NPs without the need for these cell gases. When using shorter dwell times, the particle signal is greater than the constant dinitrogen signal. It was demonstrated that the accurate detection and characterization of SiO₂ NPs using SP-ICP-MS is dependent on achieving a balance between reducing the contribution of the background interference and preserving the intensity of the particle signal. [137]

SdFFF coupled with ICP-MS/MS technique was evaluated for the potential separation and detection of TiO₂ NPs in environmental waters. ICP-MS/MS technique (using the mass shift mode of NH₃) allowed for the removal of different interferences that obscured the reliable detection and quantification of titanium. FL-70 was used as a stabilizer for colloidal TiO₂ NPs. The ionic strength of the medium showed a strong impact on the analysis. Analysis of lake water, with a relatively low ionic strength, resulted in the detection of TiO₂ NPs in the size range between 75 and 400 nm. However, seawater containing high salt concentrations resulted in a pronounced aggregation with the subsequent sedimentation of TiO₂ NPs. The achieved detection limit in different matrix solutions were below 10 ng/L. [138]

1.6.3 Capillary electrophoresis

Capillary electrophoresis (CE) research has come a long way since the technique was discovered in the 1980's. It has been applied to separate macromolecules as well as NPs using various separation modes to attain rapid analysis and high resolution of complex mixtures under a uniform electroosmotic flow (EOF). [139,140] It has been used

for the physicochemical characterization of NPs in terms of electrophoretic mobility, size, charge distribution, ζ -potential and surface functionality. [141]

1.6.3.1 Principles of capillary electrophoresis

In CE analysis, different analytes migrate through the capillary at different speeds as they have different electrophoretic mobilities. CE provides unparalleled resolution in comparison to chromatography by using an open tubular column to eliminate multiple paths. There is no stationary phase in capillary electrophoresis, thereby eliminating the mass transfer term in the Van Deemter equation (1-6), which comes from the time needed for the analyte to equilibrate between the mobile and stationary phases. Longitudinal diffusion is the only source of peak broadening in CE analysis. Significantly, the plate height is reduced, the peak efficiency is increased, and the resolution between analytes is improved:

$$H = A + \frac{B}{u} + Cu \quad (1-6)$$

where H is the plate height, u is the linear flow rate, and A , B and C are constants for a given capillary.

When placing an analyte with charge q (coulombs) in an electric field E (V/m), the force on it is qE (newton). The retarding frictional force $f v_{ep}$ also influences the movement of the analyte in the background electrolyte (BGE) solution. When the frictional force equals the accelerating force, the analyte rapidly attains a constant velocity ($f v_{ep} = qE$).

$$\text{Electrophoretic velocity: } v_{ep} = \frac{q}{f} E = \mu_{ep} E \quad (1-7)$$

where μ_{ep} is the electrophoretic mobility, which is a proportionality constant between the

ion velocity and the electric field strength. It is proportional to the charge of the ion and inversely proportional to the friction coefficient (f) given by Stoke's law:

$$f = 6\pi\eta r \quad (1-8)$$

where η is the viscosity of the solution, and r is the hydrodynamic radius of analyte.

Above pH 2, the inside wall of a fused silica capillary is covered with silanol groups (Si-OH) that are negatively charged (SiO⁻). The electric double layer at the wall of the capillary is composed of a fixed negative layer and excess cations nearby. The tightly adsorbed cations partially neutralize the negative charge on the wall. The mobile cations in the diffuse part of the double layer neutralize the remaining negative charges. When an electric field is applied, cations move toward the cathode and anions move toward the anode. A net momentum toward the cathode is produced by the excess cations in the diffuse part of the double layer. These cations drive a pumping action called electroosmosis and eventually create a uniform EOF of the entire BGE solution toward the cathode.

Electroosmotic mobility (μ_{eo}) is the constant of proportionality between the v_{eo} and E :

$$v_{eo} = \mu_{eo}E \quad (1-9)$$

Experimentally,

$$v_{eo} = \frac{L_d}{t_{neutral}} \quad (1-10)$$

where L_d is the effective length of the capillary (to the detector) and $t_{neutral}$ is the migration time of a neutral marker.

$$\mu_{eo} = \frac{v_{eo}}{E} = \frac{\left(\frac{L_d}{t_{neutral}}\right)}{\left(\frac{V}{L_t}\right)} \quad (1-11)$$

where L_t is the total length of the capillary and V is the applied voltage

Disturbance of the uniform EOF can cause peak broadening. Joule heating produced by the flow of ions in the capillary causes the solution viscosity to decrease and disturbs the uniform profile of the EOF. Therefore, the capillary inner diameter should be sufficiently small ranging from 20 to 50 μm to rapidly dissipate the generated heat.

The apparent (observed) mobility, μ_{app} , of an analyte is the sum of the μ_{eo} of the BGE solution and the μ_{ep} of the analyte.

$$\text{Apparent mobility: } \mu_{app} = \mu_{eo} + \mu_{ep} \quad (1-12)$$

Positively charged analytes move along in the EOF direction. Therefore, μ_{eo} and μ_{ep} have the same sign, contributing a greater value to μ_{app} than μ_{eo} ($\mu_{app} = \mu_{eo} + \mu_{ep}$). On the contrary, negatively charged analytes move in the opposite direction of the EOF. Thus, μ_{eo} and μ_{ep} have opposite signs, contributing a lesser value to μ_{app} than μ_{eo} ($\mu_{app} = \mu_{eo} - \mu_{ep}$). For a neutral analyte, its apparent mobility equals the electroosmotic mobility of the solution ($\mu_{app} = \mu_{eo}$).

The apparent mobility of an analyte is the net velocity of the analyte, v_{net} , divided by the electric field, E .

$$\mu_{app} = \frac{v_{net}}{E} = \frac{\left(\frac{L_d}{t}\right)}{\left(\frac{V}{L_t}\right)} \quad (1-13)$$

where t is the migration time of the analyte.

The amount of the sample (Q) introduced to the capillary during electrokinetic injection can be calculated from the μ_{app} as follows [142]:

$$Q = \frac{\pi r^2 V_i t_i C \mu_{app}}{L_t} \quad (1-14)$$

where C is the sample concentration, V_i is the injection voltage, and t_i is the injection duration.

The electrophoretic mobility, μ_{ep} , is the difference between the μ_{app} and the μ_{eo} . Variance from run to run should not have an effect on the electrophoretic mobility of NPs as it is measured in relation to a neutral marker, unless there are time-dependent or non-equilibrium interactions of NPs with the capillary wall. [143]

$$\mu_{ep} = \left(\frac{L_d L_t}{Vt} \right) - \left(\frac{L_d L_t}{Vt_{neutral}} \right) \quad (1-15)$$

1.6.3.2 Physicochemical characterization of nanoparticles by capillary electrophoresis

For spherical NPs, by assumption, q is proportional to the surface area of NPs ($4\pi r^2$). Therefore, μ_{ep} of a NP could be given by Equation (1-16), where K is a constant of proportionality that is related to the viscosity of the solution. [144,145]

$$\mu_{ep} = \frac{q}{6\pi\eta r} \propto \frac{4\pi r^2}{6\pi\eta r} = Kr \quad (1-16)$$

Equation (1-16) indicates that the μ_{ep} is proportional to r . This relationship suggests that negatively charged particles with larger radii travel at a faster rate toward the anode (or capillary inlet). Since they move in the opposite direction to the EOF, μ_{eo} and μ_{ep} have opposite signs and thereby smaller values of μ_{app} would be obtained. The EOF travels at a much greater magnitude and thereby all particles are flushed toward the cathode at different rates depending on their sizes. Accordingly, CE can be used to determine the μ_{ep} of NPs from their migration times.

The linear relationship between μ_{ep} and r allows the determination of the NP radius from a standard calibration curve. The obtained r values were very similar to the

ones determined by TEM. [146,145] A broad CE peak shape reflects the size distribution of NPs, which can be obtained by Gaussian fitting of the CE peak. [147] The composition and concentration of the BGE strongly influence the dispersion stability of NPs and thereby affecting their peak shape. [148,149]

The motion of particles inside the capillary is affected by four forces [150];

1. The driving force exerted by the external electric field;
2. Stokes viscous drag;
3. The electrophoretic retardation force resulting from the motion of counterions in the diffuse part of the electric double layer in a direction opposite to that of NPs;
4. Relaxation effect: the ionic atmosphere surrounding each NP lags behind it imposing an additional drag to its motion.

These forces are taken into consideration when calculating the electrical potential (ζ -potential) at the plane of shear from the μ_{ep} . This was first done by Wiersema *et al* [151] and further extended to higher value of ζ -potential by O'Brien and White. [152] Few years later, Oshimah presented the following approximate equation, which is valid for 1:1 electrolytes and ζ -potential values ≤ 100 mV [153];

$$\mu = \frac{2\varepsilon_r\varepsilon_0\zeta}{3\eta} \left[f_1(\kappa R_h) - \left(\frac{ze\zeta}{kT} \right)^2 f_3(\kappa R_h) - \frac{m_+ + m_-}{2} \left(\frac{ze\zeta}{kT} \right)^2 f_4(\kappa R_h) \right] \quad (1-17)$$

where ε_r is the relative electric permittivity, ε_0 is the electric permittivity of vacuum, κ is the Debye–Hückel parameter, R_h is the hydrodynamic radius, k is Boltzmann constant, e is the elementary electric charge, T is the absolute temperature, and f_1, f_3 , and f_4 are a function of κR_h and are given by:

$$f_1(\kappa R_h) = 1 + \frac{1}{2 \left[1 + \frac{2.5}{\{\kappa R_h(1 + 2e^{-\kappa R_h})\}} \right]^3} \quad (1-18)$$

$$f_3(\kappa R_h) = \frac{\kappa R_h(\kappa R_h + 1.3e^{-0.18\kappa R_h} + 2.5)}{2(\kappa R_h + 1.2e^{-7.4\kappa R_h} + 4.8)^3}$$

$$f_4(\kappa R_h) = \frac{9\kappa R_h(\kappa R_h + 5.2e^{-3.9\kappa R_h} + 5.6)}{8(\kappa R_h + 1.55e^{-0.32\kappa R_h} + 6.02)^3}$$

The dimensionless ionic drag coefficients (m_+ and m_-) are calculated from the limiting conductances, Λ_+^0 for the cation and Λ_-^0 for the anion, of the background electrolyte solution:

$$m_{\pm} = \frac{2\varepsilon_r\varepsilon_0kTN_A}{3\eta z\Lambda_{\pm}^0} \quad (1-19)$$

where N_A is the Avogadro number and z is the charge of electrolyte ions (symmetrical electrolyte).

When assuming a spherical geometry of NPs and a uniform distribution of charges on the sphere surface, the electric charge density, σ , at the plane of shear for a given ζ -potential and R_h can be calculated as follows [154]:

$$\sigma = \frac{Q}{4\pi R_h^2} = \frac{\varepsilon_r\varepsilon_0kT}{ez} \kappa \left[2 \sinh\left(\frac{e\zeta}{2kT}\right) + \frac{4}{\kappa R_h} \tanh\left(\frac{e\zeta}{4kT}\right) \right] \quad (1-20)$$

The effective charge number (z_{eff}) can be calculated from σ and R_h of NPs by:

$$z_{eff} = \frac{4\pi\sigma R_h^2}{e} \quad (1-21)$$

1.6.3.3 Recent advances in the development of capillary electrophoresis

Several strategies have been developed to improve the sensitivity of detection including metal-enhanced quantum dot fluorescence [155], different stacking strategies for on-line sample pre-concentration [156], online coupling to sensitive detection systems such as dark-field microscopy [157], evaporative light scattering [158], and ICP-MS.

[149] Two CE modes (micellar electrokinetic chromatography (MEKC) [159] and capillary zone electrophoresis (CZE) [149], coupled to ICP-MS were utilized for the separation, identification, size characterization and speciation of different metal NPs (Au, Ag, Pt, and Pd) in various matrices. The type and concentration of the surfactant introduced to the running buffer greatly influenced the resolution capability while the applied voltage and pH values of the buffer largely affected the migration times by varying the EOF. Also, the addition of complexing agents helped to maintain dissolved metal ions, particularly Ag^+ , in solution making elemental speciation possible. For environmental matrix effect, the NOM in a river water sample had no pronounced effect on the migration behavior of gold NPs. This behavior was explained by the strong adsorption of the surfactant onto the surface of gold NPs, which effectively reduced the influence of NOM. [145] CE-ICP-MS techniques seem promising not only for a reliable characterization of NPs but also for rapid screening of NPs in environmental matrices. A minor limitation is the need for calibrants having a similar surface chemistry to that of the NPs. This is generally not possible for environmental samples in which NPs could develop highly distinct coatings. [160]

CE was also coupled to Taylor dispersion analysis (TDA), which has been used to determine the average diffusion coefficient by analyzing peak shape after elution of an analyte from a broad capillary. CE-TDA seems to be a promising technique for the characterization of NPs in complex matrices since it combines the remarkable separation performance of CE to the size-based characterization of TDA. [161]

1.7 Efforts towards method validation and reference materials

Despite the continuous development of various analytical techniques for the characterization and quantification of NPs, validation of such techniques is still challenging due to the lack of certified reference materials (CRMs). There are only four suspensions of NPs (Au, Ag, Si, SiO₂) available as CRMs. The certified value is the size of the NPs while the concentration is only indicative. Also, all available CRMs – with the exception of Si NPs in toluene – are aqueous suspensions and no reference matrices are available to match CE running buffers. Stability of NPs in complex matrices cannot be assured owing to their physicochemical properties that promote their chemical transformation, dissolution, aggregation, and surface reactions. A new CRM for quality control of the size of NPs has been developed by the Institute for Reference Materials and Measurements of the European Commission's Joint Research Centre. The material, ERM-FD102, consists of an aqueous suspension of a mixture of SiO₂ NPs of distinct particle sizes. [162] The characterization relied on an inter-laboratory comparison study in which a variety of techniques were used for particle size analysis including dynamic light scattering (DLS), centrifugal liquid sedimentation (CLS), scanning and transmission electron microscopy (SEM and TEM), atomic force microscopy (AFM), particle tracking analysis (PTA) and AF4. Final validation of new analytical methods and procedures requires real-world reference matrices, which are not yet available. For now, inter-laboratory comparison tests of environmental samples and available CRMs are highly recommended but awaiting.

1.8 Research goal

Analysis of metal/metalloid oxide NPs in environmental waters is currently a subject of intense research. Several analytical techniques have been proposed or applied for the quantitative determination of metal/metalloid oxide NPs in environmental waters despite their limitations. Several techniques require a long, tedious and time- consuming sample preparation that may disturb the original state of NPs in environmental waters. Also, highly trained personnel are required to operate such instruments and analyze the data. The objective of this research project is to develop a simple, rapid, cost effective and highly efficient method for the quantitative determination of metal/metalloid oxide NPs in environmental waters.

The main goal of the work focuses on the enhancement of the ultraviolet (UV) detection sensitivity for different types of metal/metalloid oxide NPs in CE analysis. The first objective is to develop a CE method, by choosing good background electrolytes and optimizing their concentrations, for the efficient detection and separation of metal/metalloid oxide NPs in water. The second objective is to demonstrate improved performance of the CE-UV technique for NPs analysis based on the following advantages:

- I. UV detection at a fixed window position along the capillary, with different wavelengths available to optimize the measurement sensitivity and selectivity, reducing the requirements for sample pretreatment;
- II. Electrokinetic preconcentration of NPs by simply injecting a sufficient volume of the sample suspension as required for low concentrations;
- III. Partitioning provides the ability to use substrate-product combinations that cannot be used in conventional systems.

CE has been employed for screening analysis of NPs due to its superior performance in the separation of differently sized/charged NPs over other separation techniques. [86] However, the major limitation is the relatively poor detection limits of CE-UV for the analysis of NPs. Hence, various molecular and polymeric coating strategies were developed to enhance the colloidal stability and the CE-UV detection sensitivity of metal/metalloid oxide NPs in aqueous suspensions. The binding/coating layer aimed to introduce chromophores to the surface of NPs, thus increasing their UV absorbance to enhance the CE-UV peak area for a significant improvement of their detection limit.

1.9 Hypothesis

The scientific question we had was: Could UV detection be made 10-fold more sensitive when chromophores are added on the surface of metal/metalloid oxide NPs in aqueous suspensions for direct CE analysis? Our hypothesis was that molecular layers and polymeric coatings could be selectively formed on different types of NPs to produce stronger peaks at shifted migration times for both improved detection limits and unmistakable identification. Two predictions that could be tested by experimenting were: (a) if we add molecular adsorbates or form polymeric coatings onto the NPs, then CE-UV analysis would produce standard calibration curves with steeper slopes to indicate enhancement of the analytical sensitivity hopefully by a factor of 10 or more; (b) if we study the scientific literature to find out what was already known on the topic, then the selectivity of molecular adsorbates and polymeric coatings would help identify the chemical composition of unknown NPs present in environmental waters. Even if insufficient information was available from previous publications, the scientific question

could still be tackled through the general knowledge we had learned in chemistry and the experience we had gained from research on molecularly imprinted polymers.

Chapter 2: Materials and Instrumentation

2.1 Materials

2,2'-azobis-2-isobutyronitrile (AIBN) was bought from Pfaltz & Bauer (Waterbury, CT, USA). Alumina (Al_2O_3 , <50 nm) nanopowder, bisphenol A (BPA), ceria (CeO_2) nanopowder, chitosan (medium molecular weight of 190,000-310,000, deacetylation degree of 75-85%), dithiothreitol, dopamine hydrochloride (DA.HCl, $\geq 99.5\%$), dsDNA sodium salt from salmon testes, 2-hydroxypropyl methacrylate (HPMA, 97 %), L-cysteine, mesityl oxide (MO, $\geq 90\%$), polyethylene glycol (PEG, average M_n 6000), polyvinyl alcohol (PVA, with an average molecular weight of 10,000), sodium dodecyl sulfate (SDS, $\geq 99\%$), ssDNA from salmon testes and LUDOX® colloidal silica (SiO_2 , 30% wt. suspension in H_2O , with a surface area of 198-250 m^2/g), titania nanopowder (TiO_2 , 21 nm), triton X-100, Tris (hydroxymethyl) aminomethane and zinc oxide (ZnO , <50 nm) nanopowder were all purchased from Sigma-Aldrich (Oakville, ON, Canada). Sodium phosphate dibasic was obtained from Fisher Scientific (Fair Lawn, NJ, USA).

2.2 Apparatuses and analytical methods

CE-UV analyses were performed on a modular system built in our laboratory, which includes a Spellman CZE1000R high-voltage power supply (Hauppauge, New York, USA). Fused-silica capillary (51 μm i.d., 356 μm o.d.) was obtained from Polymicro Technologies (Phoenix, AZ, USA). The capillary total and effective lengths were 53.5 cm and 46.1 cm, respectively. The background electrolyte (BGE) was composed of 10 mM Na_2HPO_4 or 100 mM Tris in deionized distilled water (DDW) to attain pH 7.5 ± 0.2 or pH 9.5 ± 0.5 , respectively. All CE analyses were run at an applied

voltage of 20 kV, with the capillary inlet 2 mm away and below the electrode tip to improve both precision and baseline stability. A Bischoff Lambda 1010 (Leonberg, Germany) UV detector was set up at a wavelength of 190, 200, 220, 260 or 325 nm to monitor NPs, monomer(s), polymer(s) and the various adsorbates. Electrokinetic injection at 17 kV for 12 s was employed to load the sample into the capillary for CE analysis. An independent run of MO as a neutral marker was carried out to determine the ionic charges of NPs, monomer, polymer and the various adsorbates. A PeakSimple chromatography data system (SRI model 203, Torrance, CA, USA) was used to acquire the detector output signal.

2.3 Dynamic light scattering

The average hydrodynamic diameters of NPs/surface-modified NPs were measured by dynamic light scattering (DLS) using a Brookhaven Instruments nanoDLS particle size analyzer (Holtsville, NY, USA), equipped with a 637-nm laser (variable laser power up to 35 mW) and operating at an angle of 90°. Suspensions of NPs were prepared in 10 mM KNO₃ solution and measured in ten replicates of 10 s each for higher accuracy.

2.4 Transmission electron microscopy

Dilute aqueous suspensions of SiO₂, SiO₂@PHPMA, and SiO₂@PHPMA@PDA NPs were placed on a sample holder to dry for transmission electron microscopy (TEM) examination at an accelerating voltage of 120-200 kV using an FEI Tecnai G2 transmission electron microscope (Hillsboro, OR, USA). The diameters of these NPs were compared to determine the thickness of different coatings.

2.5 Fourier transform infrared spectroscopy

Infrared spectroscopy measurements were obtained using an ABB FTIR spectrometer (Bomem MB Series, Quebec, Canada). Disc samples were prepared by grinding 2 mg of NPs/surface-modified NPs with 200 mg of spectrophotometric-grade KBr.

Chapter 3: Polymer Coatings for Sensitive Analysis of Colloidal Silica

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3.1 Abstract

A new analytical approach has been developed for the sensitive detection of trace NPs in water using silica (SiO_2) as model inorganic NPs. Our approach is based on coating the NPs with a polymer to make them larger in size for better UV light absorption. These polymer-coated NPs can be separated from the monomer and polymer by CE due to differences in their ionic charge, size, and surface functionality. Controlled polymerization of 2-hydroxypropyl methacrylate (HPMA) on SiO_2 NPs increased their UV detection sensitivity by 6 ± 1 fold. A second coating with polydopamine produced an extra 2-fold increase of the UV detection sensitivity. With both polyhydroxypropyl methacrylate and polydopamine coatings, a significant total enhancement of 12 ± 2 fold in detection sensitivity was attained. Alternatively, addition of bisphenol A or polyvinyl alcohol to the PHPMA- SiO_2 NPs resulted in 8 ± 1 fold increase of detection sensitivity due to additional absorption of the UV detector light.

3.2 Introduction

Colloidal SiO₂ NPs are more and more often used in various biomedical applications [163-165], and they are found in fresh water resources over a large range of concentrations. [166] Therefore, analytical techniques with high sensitivity are much sought after for the detection and quantification of SiO₂ NPs. Measurement of the magnitude, frequency and duration of exposure to NPs is a critical first step in risk assessment. Unfortunately, it is difficult to build risk assessment scenarios for NPs due to the limited availability of sensitive methods for their detection and quantification.

Efforts have been focused in our lab to develop polymer growth on NPs as a new method for their trace analysis in water. The coating material was chosen on the basis of several experimental considerations including simplicity of polymerization in aqueous solution, ability to interact with specific NPs, uniform dispersion of the coated NPs in water, strong UV absorptivity, and good electrophoretic mobility for separation by CE with UV detection. Characterization of NPs by CE had been successfully developed in our research lab. [167] Their UV detection limits remained inadequate for general applications in environmental science and engineering studies.

In this chapter, aqueous polymerization of 2-hydroxypropyl methacrylate (HPMA) was investigated on SiO₂ for better detection sensitivity in CE-UV analysis. Attempts were made to decrease the rate of HPMA polymerization by adding polyvinyl alcohol (PVA) [168], thus increasing the PHPMA coating thickness on SiO₂ NPs. Also, bisphenol A (BPA) was added to increase the UV absorbance of SiO₂@PHPMA for extra detection sensitivity. Finally, an adhesive coating of polydopamine (PDA) was put on top of SiO₂@PHPMA to further enhance the detection sensitivity.

3.3 Methods

3.3.1 Polymerization of HPMA

Poly (2-hydroxypropyl methacrylate) (PHPMA) was prepared following a procedure developed by Ali *et al.* [169] with some modification. In a glass vial, for free radical polymerization, HPMA (0.007 mol) was first dissolved in DDW (25 mL). SDS (10 wt. % of HPMA) and AIBN (3 wt. % of HPMA) were then added, followed by pure nitrogen gas bubbling for 5 min to remove dissolved oxygen molecules that could destroy the free radicals generated by the thermal decomposition of AIBN. Finally the vial was sealed and placed in a 60°C thermostatted water bath for 22 h without further mixing or shaking. The polymerization mixture turned cloudy, yielding PHPMA submicron particles with a white color.

3.3.2 PHPMA growth on colloidal SiO₂ nanoparticles

HPMA (0.007 mol) was first dissolved in DDW (25 mL) containing colloidal SiO₂ NPs (5-20 g/L). Sonication for 30 min was allowed to facilitate their hydrogen bonding interaction. Next, SDS (10 wt. % of HPMA) and AIBN (3 wt. % of HPMA) were added, followed by deoxygenation. Finally, the vial was placed in a 60°C thermostatted water bath for 22 h to produce PHPMA and PHPMA-coated SiO₂ NPs (SiO₂@ PHPMA).

3.3.3 Effect of AIBN on PHPMA growth

The amount of AIBN on PHPMA growth on SiO₂ NPs was investigated by using 1, 2 and 3 wt. %. The % conversion to PHPMA was determined by CE analysis for any residual HPMA.

3.3.4 Polymerization of HPMA with polyvinyl alcohol or bisphenol A

PVA (0.1 g) or BPA (0.005 g) was added to the HPMA-polymerization mixture to investigate any enhancement of SiO₂ detection sensitivity.

3.3.5 Polydopamine growth on SiO₂@ PHPMA

Aqueous solution of DA (200 μ L of 25 g/L) was added into SiO₂@PHPMA aqueous suspension. The mixture was left alone at ambient temperature (23 $\pm$ 2°C) to allow for PDA growth on the particles, as monitored by CE-UV analysis daily for one week.

3.4 Results and Discussion

3.4.1 Silica nanoparticles

LUDOX® AM colloidal SiO₂ NPs [170] were diluted by the BGE consisting of 10 mM Na₂HPO₄ for CE-UV analysis using electrokinetic injection. As shown in Fig. 3.1, 20 g/L of SiO₂ NPs exhibited low UV absorbance at the UV detection wavelength of 190 nm, producing a small peak at the migration time of 7.3 $\pm$ 0.2 min.

Figure 3.1 CE-UV characterization of SiO₂ nanoparticles (at 20 g/L) in LUDOX® AM colloid.

The SiO₂ NPs appeared after the neutral marker, mesityl oxide (MO), indicating their negative ionic charges in the BGE due to the adsorption of HPO₄²⁻ anions onto their surface, yielding a negative electrophoretic mobility value. The standard calibration curve exhibited a linear coefficient of determination ($R^2 = 0.98$) between their CE-UV peak area and concentration in the working range up to 20 g/L. The limit of detection (LOD at 3σ) and the limit of quantification (LOQ at 10σ) were determined to be 3 g/L and 9 g/L, which are inadequate for many environmental studies.

3.4.2 CE-UV characterization of PHPMA particles

Figure 3.2 CE-UV characterization of (a) MO, (b) HPMA (and PHPMA) after 22 h of polymerization.

CE-UV analysis was next performed on the HPMA polymerization mixture after 22 h at 60°C. As the neutral compound (MO), illustrated in Fig. 3.2a, the residual HPMA migrated at nearly the electroosmotic flow velocity to appear at a time close to 3.7 ± 0.1 min for the neutral marker as shown in Fig. 3.2b. The late migration time of 5.6 ± 0.1 min for PHPMA indicated its negative ionic charge in the BGE, possibly due to the adsorption of SDS anions on the hydrophobic polymer surface.

3.4.3 PHPMA growth on SiO₂

HPMA polymerization can be done in aqueous solutions, enabling its utilization to encapsulate NPs in water samples to improve their CE-UV detection. Polymerization of HPMA at 60°C was conducted for 22 hours in the presence of SiO₂ NPs over a range

of concentrations from 5-20 g/L. A schematic of the polymerization process is shown in Fig. 3.3.

Figure 3.3 Schematic representation of the polymerization process with/without SiO₂.

The resultant SiO₂@PHPMA particles were analyzed by CE-UV to determine their detection sensitivity.

Figure 3.4 CE-UV analysis of PHPMA and SiO₂@PHPMA particles after 22 h of polymerization using AIBN at 3 wt. %.

As shown in Fig. 3.4, a new peak for SiO₂@PHPMA particles was detected at a migration time of 6.8±0.1 min. These particles exhibited larger peak height and peak area than the original SiO₂ NPs (see Fig. 3.1). Apparently, SiO₂ NPs were coated by PHPMA to have their Si-O⁻ groups buried under the surface, resulting in a faster migration than SiO₂ NPs (at 7.3±0.2 min) and good separation from PHPMA (at 5.6±0.1 min). Upon varying the concentration of SiO₂ NPs in the pre-polymerization mixture, the resultant SiO₂@PHPMA peak area changed accordingly as shown in Fig. 3.5, which provides very convincing proof of the SiO₂@PHPMA formation.

Figure 3.5 SiO₂ and SiO₂@PHPMA peak areas obtained at different SiO₂ concentrations (g/L).

The LOD and LOQ for SiO₂@PHPMA particles were determined to be 0.6 g/L and 1.8 g/L. Consequently a 5-fold better CE-UV detection sensitivity was attained for SiO₂ NPs after PHPMA coating. Hence, the approach seemed promising towards their sensitive detection in water.

3.4.4 Effect of AIBN on PHPMA growth

Polymerization of HPMA to form a layer of PHPMA on SiO₂ NPs was slightly increased from 94% to 96% when the amount of AIBN (the initiator) was reduced from 3 to 1 wt. %. At 3 wt. %, a larger concentration of reactive radicals would be generated leading to early termination of the polymerization process and production of a polymer of low molecular weight. [171,172] On the contrary, at 1 wt. %, a chain carrier will be produced from the reaction of a free radical with a monomer unit and propagation will occur continuously with other monomer units present, resulting in a higher conversion of HPMA to PHPMA. Thereby, larger SiO₂@PHPMA particles were produced. 33 % increase in SiO₂@PHPMA peak area was attained and the amount of residual SiO₂ NPs was slightly reduced as shown in Fig. 3.6. Thus, PHPMA growth using AIBN at 1 wt. % resulted in 6±1 fold enhancement of SiO₂ detection sensitivity.

Figure 3.6 CE-UV analysis of PHPMA and SiO₂@PHPMA particles after 22 h of polymerization using AIBN at 1 wt. %.

3.4.5 Polymerization of HPMA with PVA or BPA

As shown in Table 3.1, the PHPMA peak areas attained in the presence of SiO₂ NPs under different conditions of polymerization were significantly larger than those obtained in the absence of SiO₂. The difference in PHPMA peak areas between the presence and absence of SiO₂ NPs was also significantly larger than the peak area of SiO₂@PHPMA. One speculation for this significant difference is the catalytic effect of SiO₂ NPs that could facilitate the HPMA polymerization and thus produce more PHPMA particles. Another speculation is the formation of a thick coating of PHPMA to cover up all the negative charges present on the SiO₂ surface, thus producing SiO₂@PHPMA particles with effectively the same migration time as PHPMA particles. Nonetheless, the SiO₂@PHPMA peak area and height were significantly larger than that of the original SiO₂ NPs. These SiO₂@PHPMA particles might have only a monolayer of PHPMA that could not cover up all the negative charges on SiO₂ and thus they migrated after the PHPMA peak. Chu *et al* had previously used PVA to decrease the rate of HPMA polymerization, thereby producing larger particles. [173] In our study, PVA increased the PHPMA peak area, which indicates the formation of larger PHPMA particles as confirmed by DLS analysis below. Addition of BPA also resulted in the formation of larger PHPMA particles. Consequently, PVA or BPA addition to the HPMA-polymerization mixture containing SiO₂ NPs resulted in 8±1 fold increase of SiO₂ detection sensitivity probably due to additional absorption of the UV detector light.

Table 3.1 CE-UV peak areas of HPMA, PHPMA and SiO₂@PHPMA under different conditions of HPMA polymerization. All peak areas are expressed in arbitrary units of mV.s.

Condition of HPMA polymerization	Peak area of residual HPMA	Peak area of PHPMA	Peak area of SiO ₂ @PHPMA	% Polymerization of HPMA to form PHPMA
Without SiO ₂ (AIBN at 3 wt. %)	32±1	57±1	-----	~ 94%
With SiO ₂ (AIBN at 3 wt. %)	31±3	156±1	27±1	~ 94%
Without SiO ₂ (AIBN at 1 wt. %)	29±1	86±1	-----	~ 95%
With SiO ₂ (AIBN at 1 wt. %)	25±1	221±10	40±3	~ 96%
Without SiO ₂ + PVA	55±1	153±1	-----	~ 90%
With SiO ₂ + PVA	48±1	261±4	42±2	~ 91%
Without SiO ₂ + BPA	98±1	102±2	-----	~ 82%
With SiO ₂ + BPA	185±1	258±6	47±1	~ 66%

3.4.6 PDA growth on SiO₂@PHPMA particles in water

DA, as derived from the catecholamine and phenethylamine families, is a strong absorber of UV light. DA undergoes oxidation followed by intramolecular cyclization via Michael addition prior to polymerization. It can easily self-oxidize to PDA in basic aqueous solutions without the need for oxidants other than dissolved oxygen in water. Therefore, PDA growth on SiO₂@PHPMA particles in aqueous suspension over one

week was investigated for further enhancement of detection sensitivity. A schematic representation of PDA growth on PHPMA and $\text{SiO}_2\text{@PHPMA}$ is shown in Fig. 3.7.

Figure 3.7 Schematic representation of PDA growth on PHPMA and $\text{SiO}_2\text{@PHPMA}$ at room temperature (RT).

The resultant $\text{SiO}_2\text{@PHPMA@PDA}$ particles were analyzed by CE-UV daily. Fig. 3.8 shows the CE-UV electropherogram of DA, HPMA, PHPMA@PDA , $\text{SiO}_2\text{@PHPMA@PDA}$ and $\text{SiO}_2\text{@PDA}$ particles. DA (at 3.2 ± 0.1 min) is positively charged in the BGE as it migrated before the neutral marker. Hence, electrostatic attraction between DA and PHPMA, $\text{SiO}_2\text{@PHPMA}$ or residual SiO_2 particles were expected. Due to the adsorption of SDS anions on the hydrophobic polymer surface, PDA acquired a slight negative charge and migrated behind the neutral marker (as observed in a separate CE-UV analysis). After seven days of polymerization, the suspension turned black as a result of PDA formation. Both the PHPMA@PDA peak at 7.0 ± 0.1 min and $\text{SiO}_2\text{@PHPMA@PDA}$ peak at 8.1 ± 0.1 min increased in height and area upon PDA growth. Apparently, PHPMA and $\text{SiO}_2\text{@PHPMA}$ particles were coated with a

thin layer of PDA to be acquiring extra negative charges on their surfaces, rendering them slower in migration. This thin layer of PDA coating produced an extra 2-fold enhancement of the CE-UV detection sensitivity for $\text{SiO}_2\text{@PHPMA}$ NPs. Thus, with both PHPMA and PDA coatings, a total of 12 ± 2 fold enhancement in detection sensitivity was attained for SiO_2 NPs in the original sample.

Figure 3.8 CE-UV analysis of DA, HPMA, PHPMA@PDA, $\text{SiO}_2\text{@PHPMA@PDA}$ and $\text{SiO}_2\text{@PDA}$ particles.

3.4.7 Dynamic light scattering

Figure 3.9 Dynamic light scattering measurements of (a) lognormal distribution of SiO₂ nanoparticles in LUDOX® AM colloid, (b) lognormal distribution of PHPMA in DDW after 22 h of polymerization at 60°C

Aqueous suspensions of original and polymer-coated SiO₂ particles were analyzed by DLS to determine their hydrodynamic diameters to gain some insight of their growth. The hydrodynamic diameter is the diameter of a sphere (made of material with the same density) that diffuses at the same velocity as the particle being measured. It represents the actual particle diameter plus the layer of hydrated polymers surrounding the particle as it moves under the influence of Brownian motion. Velocity of the Brownian motion is defined by the translational diffusion coefficient (D), which can be converted into a particle size using the Stokes-Einstein equation;

$$d_h = \frac{\kappa T}{6 \pi \eta D} \quad (3.1)$$

where d_h is the hydrodynamic diameter, κ is the Boltzmann's constant, T is the temperature, η is the medium's viscosity, and D is the diffusion coefficient.

As shown in Fig. 3.9a and 3.9b, mean diameters of 53 ± 3 nm and 77 ± 5 nm were obtained for SiO₂ NPs and PHPMA particles, respectively. Larger particle diameters were exhibited by SiO₂@PHPMA, SiO₂@PHPMA@PDA, and SiO₂@PHPMA formed in the presence of BPA and PVA as summarized in Table 3.2. These results provide strong evidence that SiO₂ NPs in water can be made larger in size by polymer growth under simple experimental conditions to offer more sensitive detection by CE-UV analysis, with the option of electrophoretic separation from organic compounds possibly found in water.

Table 3.2 DLS measurement of the hydrodynamic diameters of original and polymer-coated SiO₂ particles.

Particles	Hydrodynamic diameter (nm)
SiO ₂	53 ± 3
PHPMA (AIBN at 3 wt. %)	64 ± 3
PHPMA (AIBN at 1 wt. %)	77 ± 5
BPA-PHPMA	78 ± 3
SiO ₂ @PHPMA (AIBN at 3 wt. %)	89 ± 2
PVA-PHPMA	91 ± 3
SiO ₂ @PHPMA (AIBN at 1 wt. %)	101 ± 1
SiO ₂ @BPA-PHPMA	114 ± 2
SiO ₂ @PVA-PHPMA	129 ± 4
SiO ₂ @PHPMA@PDA	140 ± 4

Polymer growth on NPs formed larger particles with an increased signal response relative to original NP sizes. Treating a particle as a circle helps to understand this size dependent increase in peak intensity [174]; a particle with a larger diameter, and therefore

larger area, scatters/absorbs more light than a particle with a smaller diameter, as confirmed in the present work. Following this line of thought, and taking into account Beer's Law, which states that the absorbance of a sample is proportional to its concentration, the apparent concentration of particles in a given volume of a sample is dependent on the number of particles as well as the diameter (and thereby the area) of those particles. Hence, the apparent concentration, C_{app} , is the product of the cross-sectional area of a particle (A_{circle}) and its concentration, C , according to:

$$C_{app} \left(\frac{nm^2}{mL} \right) = A_{circle} \left(\frac{nm^2}{particles} \right) \times C \left(\frac{particles}{mL} \right) \quad (3.2)$$

3.4.8 Transmission electron microscopy

Figure 3.10 Transmission electron micrographs of (a) $SiO_2@PHPMA$, and (b) $SiO_2@PHPMA@PDA$.

A mean diameter of 14.6 ± 2.9 nm was found for SiO_2 NPs. As shown in Fig. 3.10a and 3.10b, mean diameters of 18.6 ± 2.2 nm and 20.1 ± 1.6 nm were obtained for $SiO_2@PHPMA$ and $SiO_2@PHPMA@PDA$, respectively. The thickness of PHPMA coating on the surface of SiO_2 NPs was approximately 2.0 ± 0.4 nm whereas PDA coating

on top of PHPMA was about 0.8 ± 0.3 nm. The diameters of these dry particles, as determined by TEM, seem to be much smaller than the corresponding hydrodynamic diameters obtained by DLS measurement for particles in aqueous suspensions. Particles in liquid media develop a hydration layer around their surfaces, which impacts on their movement under the influence of Brownian motion. As this hydration layer is not present in TEM analysis, smaller diameters were obtained.

3.5 Conclusion

This work has demonstrated a new analytical approach to enhance the sensitivity for CE-UV detection of colloidal SiO₂ NPs in water by coating with PHPMA alone or with the assistance of BPA, PVA or PDA. The problem of inadequate sensitivity was broken down into smaller attempts toward technically simple and operationally cost effective solutions. Experimental results have demonstrated the feasibility of this approach that can potentially be applied for the determination of other NPs in water analysis. Controlled growth of thicker polymer coatings is underway in our lab to attain higher sensitivity needed for the detection of lower concentrations of NPs. Our ultimate objective is the implementation of this new approach in toxicology research regarding NPs in water, to gain better understanding of their long-term impact on environmental sustainability and public health.

3.6 Connection to chapter 4

The current study brought up some new challenges including the partial coating of SiO₂ NPs, the modest enhancement factors, and the long polymerization time. These challenges are tackled in **Chapter 4** by studying SiO₂ interaction with HPMA and developing a coating of chitosan on the NPs to obtain a greater enhancement of UV detection sensitivity. In addition, the selectivity towards SiO₂ is investigated in the presence of TiO₂ NPs.

Chapter 4: Hydroxypropyl Methacrylate Interaction and Chitosan Coating for Enhanced UV Detection Sensitivity of Colloidal Nanoparticles in Capillary Electrophoresis Analysis

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4.1 Abstract

The binding interactions between SiO₂, TiO₂ and polymeric NPs (e.g. chitosan) with HPMA were investigated for enhancing the UV detection sensitivity of these NPs in CE analysis. HPMA interacted with colloidal SiO₂ NPs, 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 NPs in an aqueous suspension. Chitosan coating was also selective for SiO₂ NPs producing a significantly larger hydrodynamic diameter to further enhance the sensitivity of their UV detection after HPMA binding. The analytical technique, which involves coating SiO₂ NPs with chitosan first and binding with HPMA next, has allowed us to achieve a significant enhancement of 50±1 fold in detection sensitivity.

4.2 Introduction

Different chemistry-based encapsulation processes of NPs have been developed and showed promising results. [175] The growth of NPs in solution is complicated by several factors including changes in pH as well as interactions with ions and surfactants. [176] Either covalent attachment of end-functionalized polymers to the surface or in situ polymerization of monomers using immobilized initiator seems to be versatile. [177-178] Our 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 CE due to differences in electrophoretic mobility, depending on their electronic charge, size, and surface functionality. A first coating of SiO₂ NPs with PHPMA was successfully developed in our laboratory to enhance the UV detection sensitivity by 6 ± 1 fold during CE analysis. [179] A second coating with PDA produced an extra 2-fold increase of the UV detection sensitivity to attain a total enhancement of 12 ± 2 fold.

The goal of this chapter was to explore other coating materials that could further improve the CE-UV detection sensitivity for metal/metalloid oxide NPs (TiO₂ and SiO₂) in aqueous suspensions. Investigation started with the binding interaction between these NPs and HPMA, followed by coating with chitosan to produce larger diameters for enhanced UV detection sensitivities. Their hydrodynamic diameters were measured by DLS and their ionic charges were determined by CE.

4.3 Methods

4.3.1 HPMA binding interaction with colloidal SiO₂ or TiO₂ nanoparticles

To investigate the binding interaction between HPMA and colloidal SiO₂ or TiO₂ NPs, HPMA (3 mL) was first dissolved in DDW (25 mL) containing colloidal SiO₂ or TiO₂ NPs (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 NPs. Percent binding of HPMA to SiO₂ or TiO₂ NPs was calculated as [180]:

$$\% \text{ binding of HPMA} = \left[\frac{(\text{HPMA peak area before binding} - \text{HPMA peak area after binding})}{\text{HPMA peak area before binding}} \right] \times 100$$

4.3.2 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.* [181] The mixture was stirred magnetically for 3 h to obtain a homogeneous suspension. Then, the chitosan suspension (1 mL) was added to 10 mM Na₂HPO₄ BGE (30 mL) containing colloidal SiO₂ or TiO₂ NPs 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.

4.4 Results and discussion

4.4.1 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. [182] In our work, an aliquot of LUDOX® AM colloidal SiO_2 NPs was spiked into the BGE consisting of 10 mM Na_2HPO_4 that was loaded into the capillary by electrokinetic injection for CE-UV analysis. As shown in Fig. 4.1a, 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. SiO_2 NPs appeared after the neutral marker, indicating their negative ionic charges in the BGE.

Figure 4.1 CE-UV characterization of (a) SiO₂ (20 g/L) and (b) TiO₂ (20 g/L) nanoparticles. SDS was present at a concentration of 2 mg/mL.

On the contrary, 20 g/L of TiO₂ 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 Fig. 4.1b. Similar to SiO₂, TiO₂ NPs migrated after the neutral marker, which indicates their negative ionic charges in the BGE.

4.4.2. HPMA binding interaction with silica and titania nanoparticles

Figure 4.2 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.

Binding interaction of HPMA with SiO₂ NPs was conducted at 60°C for 22 hours and the resultant SiO₂-HPMA particles were analyzed by CE-UV. As shown in Fig .4.2, a new peak for HPMA-bound SiO₂ 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 SiO₂ NPs (see Fig. 4.1a). Apparently, the SiO₂ NPs bound to HPMA to have their Si-O⁻ groups buried under the surface, resulting in a faster migration than SiO₂ NPs (at 8.7 ± 0.1 min) with good separation from HPMA (at 4.0 ± 0.1 min). HPMA binding to SiO₂ NPs was determined to be $19 \pm 3\%$. Binding interaction of HPMA with TiO₂ 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 changes were observed for HPMA peak area or the TiO₂ peak area and migration time. Correspondingly, HPMA binding to TiO₂ NPs was determined to be 0±5%.

4.4.3 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 Fig. 4.3a and 4.3b, the mean diameters of 53±3 nm and 83±13 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. 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.

Figure 4.3 Dynamic light scattering measurements to determine the lognormal distribution of (a) SiO₂ nanoparticles and (b) HPMA-bound SiO₂ nanoparticles.

4.4.4. 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 the CE-UV peak area or migration time. This chemical method could allow us to analyze an aqueous sample containing the two kinds of NPs, simply by adding HPMA and repeating the CE-UV analysis to observe any peak changes. A sample injection time of 3 s was used to maximize the resolution between different kinds of NPs. Initially, SiO₂ NPs (20 g/L) produced a small peak (at 9.4±0.1 min) with substantial overlap with the TiO₂ NPs (1 g/L) peak (at 8.6±0.1 min). After 3 mL of HPMA were added, the HPMA-bound SiO₂ NPs moved ahead to appear as a sharp peak (at 8.4±0.1 min) that could be readily quantified on top of the TiO₂ NPs peak (still at 8.6±0.1 min). Thus the chemical method was proven to be capable of identifying SiO₂

NPs and quantifying them with enhanced sensitivity.

4.4.5. Coating of silica and titania nanoparticles with chitosan

Chitosan is a natural polysaccharide consisting of repeated glucosamine and N-acetyl-glucosamine units. It is multi-functional having hydroxyl, amino, and acetyl amino groups. Although the main chain is hydrophilic, chitosan shows a slight degree of hydrophobic behavior due to the presence of N-acetyl groups. The intra and inter-molecular hydrogen bonds (due to the presence of OH and NH₂ groups along the chitosan backbone) and hydrophobic interactions contribute to chitosan self-assembly into nano-aggregates. There are two types of chitosan self-assembly; the first one happening in aqueous solution wherein chitosan can self-assemble into nano-aggregates easily by direct dissolution with slight stirring. The other type occurs at the liquid/solid interface, wherein templates are involved to trigger the self-assembly of chitosan. Coating of SiO₂ NPs with chitosan, as represented in the schematic shown in Fig. 4.4, was next investigated.

Figure 4.4 Schematic representation of chitosan coating on SiO₂ in the presence of TiO₂.

A significant increase in hydrodynamic diameter, from 53 ± 3 nm for SiO_2 particles to 513 ± 30 nm for chitosan-coated SiO_2 particles (at 20 g/L) was observed, as shown in Table 4.1. The hydrodynamic diameter of chitosan-coated SiO_2 particles was also larger than that of chitosan (470 ± 9 nm). Electrostatic and hydrogen bonding interactions between chitosan amino groups and silanol groups enabled the encapsulation process. [183] On the contrary, DLS measurement of the hydrodynamic diameter of chitosan in the presence and absence of TiO_2 NPs showed no significant difference, indicating no possible coating of TiO_2 NPs occurred.

Analysis of chitosan-coated SiO_2 particles by CE-UV failed badly because chitosan interacted with the capillary inner wall via electrostatic attraction, reducing the electroosmotic flow (EOF) significantly.

Table 4.1 DLS analysis to determine the hydrodynamic diameters of different particles, followed by calculation of the HPMA thickness.

Particles	Hydrodynamic Diameter (nm)	HPMA Thickness (nm)	Coating Thickness (nm)	Polydispersity Index (PDI)
SiO ₂	53 ± 3	---	---	0.357
HPMA-bound SiO ₂	83±13	15	---	0.356
TiO ₂	155±6	---	---	0.319
HPMA–TiO ₂ mixture	154±5	0	---	0.323
Chitosan	470±9	---	---	0.322
HPMA-bound chitosan (HPMA at 0.05% to 0.1% v/v)	411±10 to 509±12	-30 to +20	---	0.132-0.280
Chitosan-coated SiO ₂	513±30	---	230	0.343
HPMA-bound chitosan-coated SiO ₂ (HPMA at 0.05% to 0.1% v/v)	417±17 to 440±12	-48 to -37	---	0.322-0.338
Chitosan-TiO ₂ mixture	477±11	---	---	0.300
HPMA-bound chitosan- TiO ₂ mixture (HPMA at 0.05% to 0.1% v/v)	416±11 to 514±8	-31 to +19	---	0.122-0.288

4.4.6. HPMA binding with chitosan-coated silica nanoparticles

Afterwards, the interaction of HPMA with chitosan-coated SiO₂ particles was investigated in the hope of covering up chitosan surface to prevent its interaction with the capillary inner wall. 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. [184]. Further addition of HPMA, however, changed the hydrodynamic diameter to 440 ± 12 nm. Similarly, HPMA (at 0.05% v/v) interacted with chitosan NPs decreasing their hydrodynamic diameter from 470 ± 9 nm to 411 ± 10 nm. However, further addition of HPMA (at 0.1% v/v) increased their hydrodynamic diameter to 509 ± 12 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. Fig. 4.5 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 Fig. 4.6. A significant total enhancement of 50 ± 1 fold in detection sensitivity was attained by coating SiO₂ NPs with chitosan followed by HPMA binding. This high enhancement factor could be attributed to the significant increase in SiO₂ size as a result of the template triggered self-assembly of chitosan. This result is better than the enhancement of 12 ± 2 fold previously attained by using both PHPMA and PDA coatings. In addition, no chemical initiator is needed for this technique, which does not involve in-situ polymerization.

Figure 4.5 CE-UV characterization of HPMa-bound chitosan-coated SiO₂ particles.

Figure 4.6 HPMa-bound chitosan-coated SiO₂ peak areas obtained for different SiO₂ concentrations.

However, CE-UV analysis of HPMa-bound chitosan in the presence of TiO₂ NPs showed no sign of interaction, as no change in their peak areas was observed. TiO₂ NPs strongly adsorb phosphate anions [185] present in the 10 mM Na₂HPO₄ medium, possibly forming a layer surrounding the NPs that prevented their interaction with

HPMA-bound chitosan. These results confirmed the obtained DLS data and demonstrated the selectivity of the method towards SiO_2 in the presence of TiO_2 as illustrated in Fig. 4.7.

Figure 4.7 Schematic representation of selective binding of HPMA to chitosan-coated SiO_2 in the presence of TiO_2 .

Apparently, SiO_2 NPs can be made larger in size by interaction with HPMA alone and even larger after coating with chitosan under very simple experimental conditions offering more sensitive detection of SiO_2 NPs in water by CE-UV analysis.

4.5 Conclusions

A simple and inexpensive method for the detection of NPs, which are a burden in aquatic ecosystem, has been successfully developed. HPMA and chitosan interacted with colloidal SiO₂ NPs to produce larger sizes for more sensitive detection by CE-UV. They, however, did not interact with TiO₂ NPs in 10 mM Na₂HPO₄, resulting in no change of the CE-UV detection sensitivity of TiO₂ NPs. This chemical technique allows the analysis of an aqueous sample containing SiO₂ and TiO₂ NPs, both qualitatively and quantitatively, simply by adding chitosan and HPMA to observe any CE-UV peak enhancement. HPMA interaction is time-saving as less than 22 hours can be used to reach a binding equilibrium with the NPs. Chitosan coating on SiO₂ NPs produced a significant increase in their hydrodynamic diameter to 513±30 nm. Subsequent HPMA binding facilitated the CE-UV analysis without causing disturbance of the EOF. A total enhancement of 50±1 fold in detection sensitivity was attained by coating SiO₂ NPs with chitosan followed by HPMA binding. The new detection limit is estimated at 0.06 g/L for SiO₂. Only NPs, 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 the in-situ polymerization to attain coating with PHPMA.

4.6 Connection to chapter 5

A promising enhancement of 50±1 fold in UV detection sensitivity along with the complete coating of SiO₂ NPs using chitosan and HPMA overcame the encountered challenges. The method applied in this chapter was selective towards SiO₂ in the presence of TiO₂. Nonetheless, the LOD is not quite satisfactory for environmental water analysis

of NPs. Hence, deoxyribonucleic acid binding followed by polyethylene glycol coating of Al_2O_3 , SiO_2 , TiO_2 , and ZnO NPs are ensued in **Chapter 5** to optimize the LOD of NPs in CE-UV analysis.

Chapter 5: Electrosteric Stabilization of Colloidal TiO₂ Nanoparticles with DNA and Polyethylene Glycol for Selective Enhancement of UV Detection Sensitivity in Capillary Electrophoresis Analysis

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5.1 Abstract

A new approach to selectively enhance the UV detection sensitivity of TiO₂, albeit in the presence of SiO₂, Al₂O₃ and ZnO, NPs in CE analysis was developed. Interactions of Triton X-100 (TX-100), polyethylene glycol (PEG), deoxyribonucleic acid (DNA) with TiO₂ NPs produced larger CE-UV peaks at various enhancement factors. Single-stranded DNA (ssDNA) was a more effective adsorbate than double-stranded DNA (dsDNA) due to its flexible molecular structure that participated in a stronger interaction with TiO₂ NPs via its sugar-phosphate backbone. Disaggregation of TiO₂ NPs upon DNA binding due to electrosteric stabilization was validated using dynamic light scattering. PEG coating of DNA- TiO₂ NPs further enhanced the UV detection sensitivity in CE analysis by providing extra electrosteric stabilization. This analytical technique, which involves binding of TiO₂ NPs with DNA followed by coating with PEG, has allowed us to achieve progressively an enhancement factor up to 13±3 fold in analytical sensitivity for the accurate determination of TiO₂ NPs.

5.2 Introduction

Strong interactions between human DNA and TiO₂ NPs were evidenced by both a hyperchromic effect in UV–visible spectroscopy and a marked decrease in fluorescence spectral characteristics. [186] A direct chemical interaction between TiO₂ NPs and DNA through the phosphate group has been reported. [187,188]

In this chapter, we investigated the binding interaction between TiO₂ NPs and DNA for potential enhancement of their UV detection sensitivity in CE analysis. Additional enhancement was explored by further interaction with PEG. Previous investigations had found that the molecular size of PEG could affect the thermal stability of DNA triplex and duplexes of varying nucleotide lengths. [189,190] Another study had demonstrated that the hydrocarbon chains of PEG promoted hydrophobic interactions with DNA nucleobases to enhance the formation of nanosized DNA. [191] Our work attempted to disaggregate TiO₂ NPs for quantitative UV detection at a specific migration time in a qualitative CE analysis. Their selective interaction with DNA, followed by coating with PEG, attains one order-of-magnitude enhancement in the analytical sensitivity of CE-UV determination. Disaggregation via electrosteric stabilization also ensures higher accuracy in the determination of TiO₂ as individual NPs, rather than aggregates or agglomerates.

5.3 Methods

5.3.1 Triton X-100 adsorption onto TiO₂ nanoparticles

Triton X-100 (2% v/v) was added to TiO₂ NPs suspended in 100 mM Tris at different concentrations (0.05 – 2.5 mg/mL). The mixtures were sonicated for 30 min before analysis by CE with UV detection at 280 nm. Triton X-100 adsorption onto SiO₂, Al₂O₃ and ZnO NPs was also examined.

5.3.2 DNA binding to TiO₂ nanoparticles

Double- or single-stranded DNA from salmon testes was used. The concentrated ssDNA solution was boiled for 10 minutes and then cooled on ice for at least 5 minutes prior to use in order to reduce the chances of reannealing. TiO₂ NPs were added to a DNA solution (0.5 mg/mL) in 100 mM Tris with increasing concentrations (0.05 –12.5 mg/mL). Each mixture was sonicated for 30 min before analysis by CE with UV detection at 260 nm. Further examination of DNA binding to SiO₂, Al₂O₃ and ZnO NPs was also investigated.

5.3.3 PEG coating on TiO₂ and DNA-TiO₂ nanoparticles

PEG was added to TiO₂, SiO₂, Al₂O₃ and ZnO as well as DNA-TiO₂ suspensions (at 2:1 w/w ratio). Each mixture was stirred for 2 hours before analysis by CE with UV detection at 260 nm.

5.4 Results and discussion

5.4.1 Triton X-100 adsorption onto TiO₂ nanoparticles

Triton X-100 (TX-100) is a non-ionic surfactant consisting of a hydrophilic polyethylene oxide chain and a hydrophobic phenyl group. It is well established in bioanalytical chemistry that TX-100 adsorption onto NPs often results in enhanced

colloid stability. [192] As the phenyl group strongly absorbs UV light at 280 nm, this surfactant was selected to investigate whether it could enhance the UV detection sensitivity of TiO_2 in CE analysis. TX-100 in 100 mM Tris gave a sharp peak at a migration time of 2.8 min, just after the neutral marker MO, indicating its slightly negative charge in the BGE. TiO_2 NPs in 100 mM Tris were separated into two or three peaks appearing around the migration time of 4.2 min. Multiple peaks of TiO_2 NPs could be attributed to the effect of the co- and counter-ion in the buffer solution, influencing the peak shape of TiO_2 . Another doable explanation is that TiO_2 sample contains a population of NPs of various sizes as confirmed by the multimodal size distribution of TiO_2 obtained by DLS measurement. CE can separate TiO_2 NPs to several peaks based on their different sizes. Hence, those peak areas were added together to construct a plot of total peak area versus concentration of TiO_2 NPs. As shown in Fig. 5.1, a linear calibration curve was obtained using 280 nm for UV detection. The limit of detection (LOD) and the limit of quantification (LOQ) of TiO_2 NPs were determined to be 0.06 mg/mL and 0.18 mg/mL, respectively. CE-UV analysis of the TX-100- TiO_2 NPs showed an increase in the total peak area after TX-100 adsorption.

Figure 5.1 Enhancement of UV detection sensitivity in CE analysis at 280 nm after adding TX-100 to TiO₂ nanoparticles suspended in 100 mM Tris.

Triton X-100 had traditionally been used to fully disperse TiO₂ NPs due to its strong adsorption, which is independent of the ionic strength. [193,194] With a steeper slope of ~ 256 , the enhancement factor was approximately 4.6 ± 0.1 fold using 280 nm for UV detection of TX-100-TiO₂ NPs.

Figure 5.2 FTIR spectra of TiO_2 , TX-100- TiO_2 and TX-100.

Strong adsorption of TX-100 onto TiO_2 NPs was confirmed by the FTIR spectra shown in Fig. 5.2. Many TX-100 peaks from between 948 and 1580 cm^{-1} appear in the TX-100- TiO_2 spectrum. Notably, the $\nu(\text{C-O})$ stretching vibration of TX-100 at 1113 cm^{-1} shifted to a lower wavenumber upon adsorption onto a TiO_2 surface. In addition, the TX-100- TiO_2 spectrum showed bands at 2869-2949 and $1349\text{-}1466\text{ cm}^{-1}$ corresponding to the symmetric/asymmetric stretching and bending vibrations, respectively, of the $-\text{CH}_2$ groups in TX-100.

The average hydrodynamic diameters of TiO_2 , SiO_2 , Al_2O_3 and ZnO NPs, as determined by DLS, were 447 ± 22 , 76 ± 3 , 591 ± 27 and $558\pm 21\text{ nm}$, respectively. The diameters of these dry NPs, as determined by TEM, seem to be much smaller than the corresponding hydrodynamic diameters obtained by DLS measurement for NPs in

aqueous suspensions. This is not totally surprising due to different principles behind each technique used. DLS measures Brownian motion of particles and calculate the size distribution to yield the mean hydrodynamic diameter that includes the diameter of the particle plus the hydration layers surrounding it, providing sizes greater than that observed for dry particles under vacuum in a TEM analysis. Also, variations in sample preparation may in principle introduce some incongruity when comparing results obtained from various techniques. [195] A wide range of primary particle sizes of TiO₂ measured by TEM (5,16, 50, and 100 nm) had previously been evaluated by DLS. All particles tested were highly aggregated in aqueous solutions giving sizes above 1500 nm. [196] Several factors highly contribute to the aggregation of NPs including composition, concentration, size, surface coating, zeta potential, sonication time, temperature, dispersant pH, presence of serum, salt and/or surfactant. [197] All these parameters may provide a reasonable explanation for the discrepancy between the average size (and the size distribution) of NPs given by TEM versus DLS.

The average hydrodynamic diameters of TiO₂, SiO₂, Al₂O₃ and ZnO NPs after TX-100 addition were 330±16, 79±4, 595±28 and 552±22 nm, respectively. Apparently, TX-100 helped to electrostatically stabilize the colloidal TiO₂ NPs by increasing the number of surface charges. [198] This strengthens the repulsion forces between the NPs and hampers their aggregation (to form dimers, trimers, tetramers and pentamers), thus resulting in a decrease of the average hydrodynamic diameter for the TX-100-TiO₂ NPs.

In comparison, TX-100 did not seem to adsorb onto SiO₂ NPs as no change in SiO₂ peak area or height was observed by CE-UV analysis. Also, no significant change

was observed in the peak areas for Al₂O₃ or ZnO NPs and only a slight increase in their peak heights was noticeable. DLS measurements validated the CE-UV results, showing no significant changes in the hydrodynamic diameters of SiO₂, Al₂O₃ and ZnO NPs after TX-100 addition. This indicated that TX-100 adsorption could not take place.

The present work demonstrated, for the first time to our knowledge, a new benefit of sensitivity enhancement by 4.6 ± 0.1 fold using the λ_{max} of 280 nm for TX-100 detection to determine TX-100-TiO₂ NPs. This enhanced sensitivity could be used as a reference point for us to pursue further enhancement via the binding interaction of TiO₂ NPs with DNA and PEG.

5.4.2 Double-stranded DNA binding to TiO₂ nanoparticles

DNA is a long double-stranded biopolymer with each strand comprising a unique combination of four nucleotides to carry genetic information. Each nucleotide consists of a purine or pyrimidine base (adenine, guanine, thymine, or cytosine) associated with a deoxyribose sugar molecule and a phosphate group. Sperm cells from salmon testes are a good source for non-mammalian DNA. Salmon sperm DNA can be used to study the physicochemical interactions of DNA with various binding agents, intercalation agents, modification agents, detection agents and compaction agents, biomolecules as well as NPs. An adsorption test of dsDNA (0.5 mg/mL) was performed with increasing concentrations of TiO₂ NPs (0.05 – 12.5 mg/mL) in 100 mM Tris. CE-UV analysis using 260 nm for UV detection showed two or three peaks for TiO₂ NPs around 4.2 min and one sharp peak for dsDNA at 6.2 min indicating their negative ionic charge in the BGE. The LOD and the LOQ of TiO₂ NPs using 260 nm for UV detection were determined to be 0.01 mg/mL and

0.03 mg/mL, respectively. Adsorption of dsDNA produced increases in the peak area and height of TiO₂ NPs as well as the migration time from 4.2 min to 4.6 min. As shown in Fig. 5.3, the peak areas were enhanced for all the concentrations of TiO₂ NPs studied up to 12.5 mg/mL. As a result of dsDNA adsorption, the sensitivity was enhanced by 1.6 ± 0.1 fold using the λ_{max} of 260 nm for dsDNA detection and dsDNA-TiO₂ NPs determination. Thus, dsDNA was proven to be a good adsorbate for sensitivity enhancement in the determination of TiO₂ NPs by the CE-UV method. UV detection of TiO₂ NPs was attempted at two other wavelengths (325 and 190 nm) but the slopes were actually gentler. In comparison, dsDNA did not seem to adsorb onto SiO₂ NPs as no change in SiO₂ peak area or height was observed by CE-UV analysis. A change in Al₂O₃ peak shape was seen, after dsDNA addition, from a sharp peak to a very small one probably due to the change in the surrounding environment. Upon addition of dsDNA to ZnO suspension and with continuous sonication for about 30 min, ZnO NPs clustered together and some precipitated down the vial and the sample was no longer suitable for CE-UV analysis.

Figure 5.3 Enhancement of UV detection sensitivity in CE analysis after adding dsDNA to TiO₂ nanoparticles suspended in 100 mM Tris.

Apparently, dsDNA helped to electrosterically stabilize the colloidal TiO₂ NPs, reducing their hydrodynamic diameter down to 365±9 nm. In comparison, dsDNA addition showed no significant change in the hydrodynamic diameters of SiO₂ and Al₂O₃ NPs. However, dsDNA addition to ZnO NPs resulted in an increase in their hydrodynamic diameter to 1185±193 nm, indicating the formation of clusters of larger aggregates. Binding of dsDNA onto TiO₂ NPs was confirmed by the FTIR spectra shown in Fig. 5.4

Figure 5.4 FTIR spectrum of TiO₂ and dsDNA-TiO₂ nanoparticles

Binding interaction took place via the phosphate backbone of dsDNA as indicated by a shift of the phosphate peaks from 967 to 982 cm⁻¹ and from 1234 to 1283 cm⁻¹. In addition, the dsDNA-TiO₂ spectrum showed bands at 2854-2923 and 1400-1500 cm⁻¹ corresponding to the symmetric/asymmetric stretching and bending vibrations of the -CH₂

groups, respectively. The peak at 1044 cm^{-1} corresponded to the C-O-C bond of deoxyribose [199], and 1019 cm^{-1} was assigned as a C-O-C stretching band from the carbohydrate component of dsDNA. [200]

5.4.3 PEG coating of TiO₂ and dsDNA-TiO₂ nanoparticles

PEG is a coiled polymer of repeating ethylene ether units. It has been reported that PEG improves the stability of TiO₂ in aqueous suspensions by increasing the steric distance along with improving the NPs hydrophilicity via hydrogen bonding formation between the ether repeating units and water molecules. [201,202] The polymer slightly absorbs UV light, exhibiting low detection sensitivity at wavelengths longer than 200 nm. [203] In the present work PEG coating resulted in a significant increase of the TiO₂ peak area and height while no change in the TiO₂ migration time was observed, which suggests that only a thin coating of PEG was formed. An enhancement factor of 3.0 ± 1.0 was determined by comparing the TiO₂ peak areas before and after PEG coating. One plausible reason is that PEG coating improved the stability of TiO₂ NPs, preventing their aggregation (as confirmed by DLS analysis showing a hydrodynamic diameter of 255 ± 7 nm) and thereby maximizing the absorption of UV light. In comparison, PEG addition to SiO₂, Al₂O₃ and ZnO suspensions showed no change in the peak area or height of the NPs in CE-UV analyses as well as no significant changes to their hydrodynamic diameters, indicating that PEG coating could not take place.

PEG addition to the dsDNA-TiO₂ suspensions resulted in a total of 5.5 ± 1.5 fold enhancement of CE-UV detection sensitivity of TiO₂ as shown in Fig. 5.5d.

Figure 5.5 CE analysis, with UV detection at 260 nm, of (a) TiO_2 nanoparticles, (b) dsDNA (c) PEG@dsDNA and (d) TiO_2 nanoparticles after dsDNA adsorption and PEG coating.

CE-UV analyses of PEG@dsDNA and PEG@dsDNA-TiO₂ were also attempted at 325 nm. At this wavelength, no peak can be seen for PEG@dsDNA, however; the PEG@dsDNA-TiO₂ peak can be detected as evidenced in Fig. 5.6.

Figure 5.6 CE analysis, with UV detection at 325 nm, of (a) TiO₂ nanoparticles, (b) PEG@dsDNA, and (c) TiO₂ nanoparticles after dsDNA adsorption and PEG coating. Only TiO₂ can absorb the UV light; neither dsDNA nor PEG can.

This proves that the peak at 4.6 min assigned for PEG@dsDNA-TiO₂ actually contains TiO₂ NPs.

Figure 5.7 FTIR spectra of TiO₂, PEG, PEG@TiO₂ and PEG@dsDNA-TiO₂.

FTIR spectroscopy confirmed the formation of a thin PEG coating on the dsDNA-TiO₂ NPs. As evidenced in Fig. 5.7, the C-O stretching vibrations shifted to lower wavenumbers (at 1024 and 1040 cm⁻¹) relative to PEG. The symmetric/asymmetric stretching and bending vibrations of the -CH₂ groups indicated that PEG coating was successfully formed.

Adsorption of PEG and dsDNA resulted in a decrease in the average hydrodynamic diameter of TiO₂ NPs, as presented in Table 5.2, due to an improved colloidal stability of TiO₂ by electrosteric stabilization using the two anionic polyelectrolytes as a dispersant. [204-206] These findings are in agreement with the results obtained by Pacia *et al.* who reported a decrease in the hydrodynamic diameters of TiO₂ after chemisorption of various organic ligands. [207]

5.4.4 Single-stranded DNA adsorption onto TiO₂ nanoparticles

Adsorption of ssDNA onto TiO₂ NPs was next investigated for comparison with dsDNA. An adsorption test of ssDNA (0.5 mg/mL) was performed with increasing concentrations of TiO₂ NPs (0.05 – 5.0 mg/mL) in 100 mM Tris. Fig. 5.8b showed ssDNA as a sharp peak at 7.3 min due to its more negative ionic charge in the BGE than dsDNA. Adsorption of ssDNA produced increases in the peak area and height of TiO₂ NPs as well as the migration time from 4.6 min to 5.6 min. TiO₂ and ssDNA peaks were actually separated as they had different migration times. Thereby, no possibility of overlapping between these peaks could occur.

Figure 5.8 CE analysis, with UV detection at 260 nm, of (a) TiO₂ nanoparticles, (b) ssDNA and (c) TiO₂ nanoparticles after ssDNA adsorption.

As shown in Fig. 5.9, the peak areas were enhanced for all the concentrations of TiO₂ NPs studied up to 5 mg/mL. Higher concentrations caused the ssDNA peak to

broaden and overlap with the TiO₂ peaks. As a result of DNA adsorption, the UV detection sensitivity was enhanced by 7.0 ± 0.2 fold using the λ_{max} of 260 nm. Apparently ssDNA exhibited more adsorption onto TiO₂ NPs than dsDNA. This might be explained by the fact that ssDNA is much more flexible to wrap around the NPs in large numbers. Also, the sugar-phosphate backbone of ssDNA could participate in stronger interaction with TiO₂ NPs, thus maximizing the binding affinity. On the contrary dsDNA is rigid, difficult to bend and has to break hydrogen bonds with base partners to get free bases, hampering the strong adsorption onto the NPs. [208-210] Hence, ssDNA was proven to be a better adsorbate than dsDNA for sensitivity enhancement in the determination of TiO₂ NPs by the CE-UV method. Similar to dsDNA, ssDNA did not seem to adsorb onto SiO₂ NPs as no change in SiO₂ peak area or height was observed by CE-UV analysis. A change in Al₂O₃ peak shape was seen after ssDNA addition, comparable to the effect of dsDNA. No significant change was observed in the peak area or height for ZnO NPs after ssDNA addition. DLS measurements validated the CE-UV results, showing no significant changes to the hydrodynamic diameters of SiO₂, Al₂O₃ and ZnO NPs after ssDNA addition, as summarized in Table 5.2.

Figure 5.9 Enhancement of UV detection sensitivity in CE analysis at 260 nm after adding ssDNA to TiO₂ nanoparticles suspended in 100 mM Tris.

5.4.5 PEG coating of ssDNA-TiO₂ nanoparticles

Last, PEG was added to ssDNA-TiO₂ NPs at a PEG:TiO₂ ratio of 2:1. Coating of the ssDNA-TiO₂ NPs with PEG resulted in a total of 13±3 fold enhancement of the CE-UV detection sensitivity of TiO₂ NPs as shown in Fig. 5.10b. PEG has no UV absorbance at 260 nm and did not show any peak during its CE-UV analysis. This eliminated any interference that could arise due to overlapping of consecutive TiO₂ and ssDNA peaks.

Figure 5.10 CE analysis, with UV detection at 260 nm, of (a) PEG@ssDNA and (b) TiO₂ nanoparticles after ssDNA adsorption and PEG coating.

FTIR spectra shown in Fig. 5.11, exhibited a shift to lower wavenumbers of the C-O stretching vibrations (at 1024 and 1041 cm⁻¹) relative to PEG. The symmetric/asymmetric stretching and bending vibrations of -CH₂ groups also verified the successful coating of ssDNA-TiO₂ NPs with PEG.

Figure 5.11 FTIR spectra of ssDNA, TiO₂, ssDNA-TiO₂, PEG and PEG@ssDNA-TiO₂.

All the enhancement factors obtained from adsorption of various organic and bioorganic compounds onto TiO₂, SiO₂, Al₂O₃ and ZnO NPs are summarized in Table 5.1. It becomes apparent that a simple method has been successfully developed for the detection of TiO₂ NPs. It involves binding TiO₂ NPs with DNA followed by coating with PEG to attain a total enhancement of 13 ± 3 fold in detection sensitivity in CE-UV analysis. We propose that the progressive enhancement factors, specifically 7.0 ± 0.2 for ssDNA and 13 ± 3 for ssDNA+PEG, are reporting on the total surface area of disaggregated NPs as well as the chemical functionality of the nanoparticle surfaces. The new analytical method is rapid, as only 3 hours are needed to reach a binding and coating equilibrium with the NPs. No chemical initiator or elevated temperature is required. It is

worth noting that the method is selective and does not work for colloidal SiO₂, Al₂O₃ or ZnO NPs.

Table 5.1 Enhancement factors obtained after addition of various organic/bioorganic compounds onto TiO₂, SiO₂, Al₂O₃ and ZnO nanoparticles as well as the LOD and LOQ for stabilized TiO₂ nanoparticles.

Organic/Bioorganic Compound	UV Detection Wavelength (nm)	Enhancement Factor for TiO₂ Nanoparticles	LOD for TiO₂ (mg/mL)	LOQ for TiO₂ (mg/mL)	Enhancement Factor for SiO₂, Al₂O₃ and ZnO Nanoparticles
TX-100	280	4.6± 0.1	0.013	0.039	0.00± 0.1
dsDNA	260	1.6± 0.1	0.006	0.018	0.00± 0.1
PEG	260	3.0± 1.0	0.003	0.009	0.00± 0.1
dsDNA + PEG	260	5.5± 1.5	0.002	0.006	0.00± 0.1
ssDNA	260	7.0± 0.2	0.001	0.003	0.00± 0.1
ssDNA + PEG	260	13± 3	0.0008	0.0024	0.00± 0.1

Binding of TiO₂ NPs with ssDNA produced a decrease in hydrodynamic diameter to 339±8 nm, as measured by DLS. Electronegative charges of DNA could play an important role on the size of TiO₂ NPs. With more electronegative charges on its surface than dsDNA (as verified by CE-UV analysis), ssDNA proved to be a more effective adsorbate in controlling the aggregation of TiO₂ NPs to give a smaller hydrodynamic diameter. These findings are in agreement with the results obtained by Dharanivasan *et al.* who found that ssDNA has superior capability to control gold NPs aggregation than dsDNA. [211] Further reduction of hydrodynamic diameter down to 323±6 was observed after PEG coating due to extra steric stabilization. These results, as summarized in Table 5.2, supported the observed enhancement of UV detection sensitivity of TiO₂ NPs in CE

analysis.

Table 5.2 Hydrodynamic diameters of TiO₂, SiO₂, Al₂O₃ and ZnO nanoparticles measured by DLS before and after addition of various organic and bioorganic adsorbates.

	Hydrodynamic Diameter (nm)			
Organic/ bioorganic Adsorbate	TiO ₂	SiO ₂	Al ₂ O ₃	ZnO
-----	447±22	76±3	591±27	558±21
TX-100	330±16	79±4	595±28	552±22
PEG	255±7	74±2	586±25	555±40
dsDNA	365±9	77±3	588±35	1185±193
PEG@dsDNA	351±6	-----	-----	-----
ssDNA	339±8	78±4	588±38	558±27
PEG@ssDNA	323±6	-----	-----	-----

5.5 Conclusions

The total concentration of TiO₂ NPs in aqueous suspensions is conventionally measured by inductively coupled plasma–mass spectrometry after acidification and microwave-assisted digestion. [212] However, no distinction between soluble and insoluble fractions of engineered NPs can be attained. To the best of our knowledge, this is the first work that attempts to determine TiO₂ as disaggregated NPs by UV detection at a specific migration time in CE analysis. Their selective interaction with DNA, followed by coating with PEG, attains an enhancement of many fold in the analytical sensitivity of CE-UV determination. The reaction medium seemed to be the most influencing factors controlling the selectivity of various adsorbates towards TiO₂ NPs. Tris was chosen as

the BGE instead of phosphate since the latter could strongly adsorb onto TiO₂ surface [213,214] preventing any further interaction with another adsorbate. On the other hand, SiO₂ adsorbed Tris molecules that provided a dense coverage of hydroxyl groups surrounding it. [215] Additionally, ZnO strongly adsorbed Tris molecules that inhibited its growth. [216] Moreover, Al₂O₃ could strongly adsorb Tris, forming Al-O-C bonds [217] and disabling any further adsorption onto its surface. Therefore, SiO₂, ZnO and Al₂O₃ were apparently inert to ssDNA and PEG due to their strong adsorption of Tris. We propose that the progressive enhancement factors, specifically 7.0 ± 0.2 for ssDNA and 13 ± 3 for ssDNA+PEG, are reporting on the total surface area of disaggregated NPs, as well as the chemical functionality of the NP surfaces. Now that the feasibility is demonstrated for this approach, further exploration with various biochemical probes (including dyes for laser induced fluorescence detection of DNA-stabilized TiO₂ NPs) to further enhance sensitivity should be exciting. [218] Analytical separation from other NPs, if needed for complex environmental water samples, can be further improved by adding a gel matrix inside the capillary for electrophoresis under the size filtration mechanism. [219] Interferences occurring in the presence of dissolved organic matter (DOM) can be overcome by pretreatment of environmental water samples with hydrogen peroxide (H₂O₂), in which inorganic oxide NPs are chemically inert. [86]

5.6 Connection to chapter 6

In an effort to improve the LOD of SiO₂ NPs utilizing DNA and PEG, a new route to enhance the CE-UV detection sensitivity of TiO₂ NPs was revealed. ssDNA+PEG resulted in a progressive enhancement factor of 13 ± 3 fold showing a superior performance over dsDNA+PEG in improving the UV detection sensitivity of

TiO₂. Accordingly, in **Chapter 6**, the search is directed towards investigating the adsorption of dithiothreitol and L-cysteine onto Al₂O₃, CeO₂, SiO₂, TiO₂ and ZnO NPs for selective enhancement of their UV detection sensitivity.

Chapter 6: Selective detection of ZnO nanoparticles in aqueous suspension by capillary electrophoresis analysis using dithiothreitol and L-cysteine adsorbates

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6.1 Abstract

The UV detection sensitivity of ZnO NPs in CE analysis was selectively enhanced, by 28 ± 1 or 25 ± 1 fold, after adsorption of dithiothreitol (DTT) or cysteine (Cys) in 10 mM sodium phosphate buffer. Adsorption equilibrium was reached within 90 min for DTT but only 10 min for Cys. The adsorption process was best modeled by the Langmuir isotherm, indicating the formation of a monolayer of DTT or Cys onto the surface of ZnO NPs. The selectivity of DTT and Cys towards ZnO NPs was tested using Al_2O_3 , CeO_2 , SiO_2 and TiO_2 NPs. No changes in the CE-UV peak area of either adsorbates or NPs were observed, indicating a lack of adsorption. Dynamic light scattering (DLS) provided similar evidence of the selectivity of both adsorbates towards ZnO. Cys also improved the colloidal stability of ZnO NPs by breaking down the aggregates, as evidenced by a reduction of their average hydrodynamic diameter. This new analytical approach provides a simple and rapid methodology to detect ZnO NPs selectively by CE-UV analysis with enhanced sensitivity.

6.2 Introduction

The innovation of nanotechnologies, in a truly scientific revolution, has deeply transformed many aspects of our lives. Engineered NPs are commonly used in groundbreaking applications. The global market value of nano-enabled products is expected to rise to \$4.4 trillion by 2018 as declared by the National Science Foundation. [220] As per global annual production, ZnO NPs are ranked the third with 550 tons, after SiO₂ with 5550 tons and TiO₂ with 3000 tons. [221] Massive production of ZnO NPs poses a high risk to environmental ecology and human health.

Comprehensive understanding of environmental nanotoxicity is still a major challenge due to the limitations of analytical methods. SP-ICPMS is one of the very few existing techniques that can characterize and detect ZnO NPs. However, significant dissolution of ZnO largely increases the background levels of dissolved Zn²⁺ to the point where measurement of NPs at very low concentrations is not likely possible. [222] Online combination of UV-visible, fluorescence and ICP-MS detectors to hydrodynamic chromatography has been utilized for the detection of ZnO NPs. However this approach is not quantitative since larger particles or agglomerates have to be removed before analysis. [223]

In this chapter, we investigated the binding interaction between ZnO NPs and dithiothreitol (DTT) or L-cysteine (Cys) for potential enhancement of their UV detection sensitivity in CE analysis. DTT is a strong reducing agent often used to reduce protein disulfides. It has been used to probe dissolved oxygen in the presence of carbon NPs. [224] As a strong chelator of zinc [225], DTT was expected to adsorb onto ZnO NPs to

form a monolayer via its dithiol functionality. Cys is an amino acid having carboxylic, amino, and thiol functional groups. Therefore, it can potentially interact via the different functional groups with ZnO NPs. Sandmann *et al.* reported that Cys acted as a source for sulfur resulting in the formation of a ZnS shell around the ZnO core and helped to stabilize the core-shell NPs. [226] Our goal was to use DTT and Cys to both improve the dispersion of ZnO NPs in aqueous suspensions and enhance their CE-UV detection sensitivity.

6.3 Methods

6.3.1 DTT and Cys adsorption onto ZnO nanoparticles

Adsorption experiments were carried out to optimize various parameters including the interaction medium, contact time and ZnO dose. The wavelength of maximum light absorption of each adsorbate (DTT or Cys) was determined by UV-visible spectroscopy. The adsorbate concentration before and after adsorption onto ZnO NPs was determined using CE-UV at a wavelength of 220 nm. The % adsorption of each adsorbate was calculated as:

$$\% \text{ Adsorption} = \frac{(C_0 - C_e)}{C_0} \times 100 \quad (6-1)$$

where C_0 is the initial adsorbate concentration (mg L^{-1}) and C_e is the equilibrium adsorbate concentration (mg L^{-1}). The adsorption capacity, q_e (mg g^{-1}), was calculated as:

$$q_e = \frac{(C_0 - C_e)V}{m} \quad (6-2)$$

where V is the volume of adsorbate solution (L) and m is the mass of ZnO NPs (g).

The stability of adsorbate solution (0.45 mg mL^{-1} DTT or Cys) in 100 mM Tris at pH 9.5 ± 0.5 and 10 mM Na_2HPO_4 at pH 7.5 ± 0.2 over time was investigated. Kinetic studies were performed by adding ZnO NPs (0.05 mg mL^{-1}) into adsorbate solutions ($0.15\text{-}0.45 \text{ mg mL}^{-1}$) in 10 mM Na_2HPO_4 and analyzing the mixtures by CE-UV at

different time intervals (15-120 min). Adsorption isotherms were studied by mixing ZnO NPs (0.05 mg mL^{-1}) with various concentrations of adsorbate solution ($0.15\text{-}0.45 \text{ mg mL}^{-1}$) in $10 \text{ mM Na}_2\text{HPO}_4$ and analyzing the mixtures by CE-UV at the optimal contact time. The effect of ZnO dose ($0.02\text{-}0.2 \text{ mg mL}^{-1}$) on adsorption was investigated using standard adsorbate solutions (0.45 mg mL^{-1}).

6.3.2 DTT and Cys adsorption onto Al_2O_3 , CeO_2 , SiO_2 and TiO_2 nanoparticles

DTT or Cys (0.45 mg mL^{-1}) was added to Al_2O_3 , CeO_2 , SiO_2 and TiO_2 suspensions (1 mg mL^{-1}) in $10 \text{ mM Na}_2\text{HPO}_4$ to test its selectivity towards ZnO NPs. Suspensions containing DTT were stirred for 2 hours and those containing Cys were stirred for 10 min before analysis by CE-UV at 220 nm to determine DTT, Cys, and the NPs.

6.4 Results and Discussion

6.4.1 Stability of DTT and Cys solutions

CE-UV analysis of DTT solution in 100 mM Tris (at $\text{pH } 9.5 \pm 0.5$) showed a peak at a migration time of 4.2 min (after MO, the neutral marker), indicating its negative charge. As time progressed, a decrease in DTT peak area was noticeable as shown in Fig. 6.1. A new peak was also seen at 3.6 min with increasing peak area over time due to the oxidation of DTT by deprotonation of the two thiol groups ($\text{pK}_a = 9.2$ and 10.1) to form a disulfide bond, which may not facilitate the adsorption of DTT onto the NPs. Hence, DTT in 100 mM Tris lacked chemical stability required for quantitative analysis of the NPs. On the contrary, DTT in $10 \text{ mM Na}_2\text{HPO}_4$ (at $\text{pH } 7.5 \pm 0.2$) exhibited a migration time of 3.7 min (slightly after MO), indicating its low negative charge. No change in the DTT peak area was noticeable over time. Similarly Cys exhibited a migration time of 4.2 min indicating its higher negative charge than DTT. It was also stable over time as no

change in its peak area was observed. Therefore, 10 mM Na₂HPO₄ was chosen as the BGE for all subsequent experiments.

Figure 6.1 Stability of DTT and Cys in 10 mM Na₂HPO₄ and 100 mM Tris by capillary electrophoresis with UV detection at 220 nm.

6.4.2 DTT or Cys adsorption onto ZnO nanoparticles

Figure 6.2 CE analysis, with UV detection at 220 nm, of (a) ZnO nanoparticles, (b) ZnO nanoparticles after DTT adsorption and (c) ZnO nanoparticles after Cys adsorption.

CE-UV analysis of ZnO suspension in 10 mM Na₂HPO₄ (pH 7.5±0.1) showed a small peak at a migration time of 6.6 min (after MO), as shown in Fig. 6.2a, indicating their strong negative charge in this BGE due to the adsorption of HPO₄²⁻ anions onto their surface. A linear calibration curve was obtained using 220 nm for UV detection of ZnO NPs as shown in Fig. 6.3 (at bottom). The limit of detection (LOD) and the limit of quantification (LOQ) were determined to be 0.005 mg mL⁻¹ and 0.015 mg mL⁻¹ respectively. DTT (0.45 mg mL⁻¹) adsorption onto ZnO NPs (0.02-0.14 mg mL⁻¹) in 10 mM Na₂HPO₄ was next investigated for enhancing their UV absorptivity. As shown in Fig. 6.2b, DTT adsorption increased the peak height and area of ZnO NPs; their migration time also decreased down to 5.7 min. Peak areas were enhanced for all the concentrations of ZnO suspension studied as a result of DTT adsorption as shown in Fig. 6.3 (at top). The sensitivity was enhanced by 28±1 fold, calculated as the ratio of the two trend line slopes, using 220 nm for the determination of DTT-ZnO NPs too. Thus, DTT was proven to be an excellent adsorbate for sensitivity enhancement in the determination of ZnO NPs by the CE-UV method. The new LOD and LOQ were determined to be 0.2 µg mL⁻¹ and 0.6 µg mL⁻¹, respectively.

Figure 6.3 Enhancement of UV detection sensitivity in CE analysis after adding DTT or Cys (0.45 mg mL⁻¹) to ZnO nanoparticles suspended in 10 mM Na₂HPO₄.

DTT, with its two thiol functional groups, could adsorb on the surface of ZnO NPs with high affinity. Three possible mechanisms of adsorption are speculated: (a) the cross-linking mode that bridges two ZnO NPs by forming Zn-S bonds, (b) the horizontally aligned mode that permits the formation of a dithiolate-Zn bond, and (c) the vertically aligned mode that forms a single Zn-S bond per DTT molecule as shown in Fig. 6.4.

Figure 6.4 Schematic illustration of the possible different modes of DTT adsorption onto ZnO surface; (a) the cross-linking mode, (b) the horizontally aligned mode, and (c) the vertically aligned mode.

Figure 6.5 FTIR spectra of ZnO, DTT-ZnO and DTT.

FTIR spectroscopy was performed to investigate the adsorption of DTT onto ZnO NPs. As shown in Fig. 6.5, the characteristic absorption peak of -SH at 2567 cm^{-1} disappeared in the FTIR spectrum of DTT-ZnO, indicating that DTT had been successfully adsorbed onto the surface of ZnO via the thiolate group. Moreover, the C-O stretching vibration at 1058 cm^{-1} , as well as the symmetric/asymmetric stretching and bending vibrations of the -CH_2 groups at $2859\text{-}2923$ and $1390\text{-}1426\text{ cm}^{-1}$ respectively, appeared in the FTIR spectrum of DTT-ZnO.

Cys (0.45 mg mL^{-1}) adsorption onto ZnO NPs ($0.02\text{-}0.14\text{ mg mL}^{-1}$) in $10\text{ mM Na}_2\text{HPO}_4$ was next investigated using 220 nm for the determination of Cys-ZnO NPs. As shown in Fig. 6.2c, Cys adsorption increased the peak height and area of ZnO NPs as well as their migration time (from 6.6 min) to 7.6 min indicating their very strong negative charge in this BGE. The difference in the peak shapes between DTT-ZnO and Cys-ZnO could be attributed to the variation in the size distribution of ZnO NPs by deagglomeration after DTT and Cys adsorption. As shown in Fig. 6.3 (at middle), the

peak areas of all concentrations of ZnO suspension were enhanced as a result of Cys adsorption and the enhancement factor was calculated to be 25 ± 1 fold. Thus, Cys was proven to be a very good adsorbate for sensitivity enhancement in the determination of ZnO NPs by the CE-UV method. The LOD and LOQ were determined to be $0.3 \mu\text{g mL}^{-1}$ and $0.9 \mu\text{g mL}^{-1}$, respectively.

The white suspension of ZnO NPs became transparent after Cys addition. One proposed mechanism of interaction is that cysteine is dissociatively adsorbed onto ZnO NPs converting the ZnO surface to ZnS with the release of oxygen. The excess Cys molecules are adsorbed onto the ZnS surface stabilizing the NPs. [226] Apparently, Cys improved the dispersion of ZnO NPs in the aqueous suspension (breaking down aggregation by repulsion of negative charges) and enhanced their CE-UV detection sensitivity.

FTIR spectroscopic analysis confirmed the adsorption of Cys onto ZnO NPs. As shown in Fig. 6.6, the -SH vibration peak at 2551 cm^{-1} disappeared in the FTIR spectrum of Cys-ZnO, indicating that Cys had been successfully adsorbed onto the surface of ZnO. Moreover, the C-O and C-N stretching vibration at $1051\text{-}1092 \text{ cm}^{-1}$, -COO^- stretching vibration at 1409 cm^{-1} as well as the symmetric/asymmetric stretching vibrations of -CH at $2861\text{-}2924 \text{ cm}^{-1}$ appeared in the FTIR spectrum of Cys-ZnO.

Figure 6.6 FTIR spectra of ZnO, Cys-ZnO and Cys.

6.4.3 Adsorption kinetics

To study the adsorption kinetics, ZnO (0.05 mg mL^{-1}) was added into DTT solutions (0.15 and 0.35 mg mL^{-1}) in $10 \text{ mM Na}_2\text{HPO}_4$ and the mixtures were analyzed by CE-UV at regular time intervals up to 120 min. As shown in Fig. 6.7, % DTT adsorption exhibited a fast initial increase followed by a leveling off as time proceeded. The adsorption equilibrium was reached within 90 min for the two DTT concentrations studied.

Figure 6.7 Effect of contact time on the kinetics of DTT adsorption at two different concentrations onto ZnO nanoparticles.

Pseudo-first-order, pseudo-second-order and intraparticle diffusion models were applied for characterizing the kinetics for DTT adsorption to provide valuable insights into the mechanism of reaction. The Lagergren's pseudo-first order rate equation had previously been used to describe liquid-solid adsorption systems. [227] Its linear form is expressed as:

$$\log (q_e - q_t) = \log q_e - \left(\frac{k_1}{2.303} \right) t \quad (6-3)$$

where q_e and q_t (mg g^{-1}) are the adsorption capacities at equilibrium and at time t , respectively. k_1 (min^{-1}) is the pseudo-first order rate constant; their values are presented in Table 6.1. The coefficient of determination (R^2) values for the pseudo-first-order rate equation were the same and equal to 0.81. The calculated values of q_e (obtained by the pseudo-first-order model) were much larger than the experimental value.

The kinetics data were next analyzed by the pseudo-second order kinetic model. The linear form of this model is generally expressed, as proposed by Ho and McKay [228]

$$\text{as: } \frac{t}{q_t} = \left(\frac{1}{k_2 q_e^2} \right) + \frac{t}{q_e} \quad (6-4)$$

where k_2 ($\text{g mg}^{-1} \text{ min}^{-1}$) is the pseudo-second-order rate constant.

The values of q_e and k_2 were calculated from the slope and intercept of the plot shown in Fig. 6.8 and are summarized in Table 6.1. The R^2 values for the pseudo-second-order rate equation were the same and equal to 0.99. The calculated values of q_e obtained by the pseudo-second-order model were 2000 and 3333 mg g^{-1} .

The kinetics data were last analyzed by Weber-Morris intraparticle diffusion model [229]:

$$q_t = k_{id} t^{0.5} + C \quad (6-5)$$

where C is the intercept, k_{id} is the intraparticle diffusion rate constant ($\text{mg g}^{-1} \text{ min}^{0.5}$), which is the slope of the linear plot of q_t versus $t^{0.5}$. In this model, the equilibrium changes as a function of $(Dt/r^2)^{0.5}$, where D is the diffusion coefficient ($\text{nm}^2 \text{ s}^{-1}$) and r is the particle radius (nm).

The R^2 values for the intraparticle diffusion equation were 0.97 and 0.78. The calculated values of q_e obtained were 1852 and 2707 mg g^{-1} . In accordance with the Weber-Morris model, the intercept C should cross the origin if the diffusion mechanism is controlled by intraparticle diffusion. However both intercepts did not cross the origin; therefore intraparticle diffusion was not the rate-limiting step.

Table 6.1 Kinetic parameters for DTT adsorption onto ZnO nanoparticles

Kinetics model	Linear Equation	Parameters	Values at 0.15 mg mL ⁻¹ DTT	Values at 0.35 mg mL ⁻¹ DTT
Pseudo-first order	$\log (q_e - q_t) = \log q_e - \left(\frac{k_1}{2.303}\right) t$	k_1 (min ⁻¹)	0.079	0.191
		q_e (mg g ⁻¹)	10000	165959
		R ²	0.81	0.81
Pseudo-second order	$\frac{t}{q_t} = \left(\frac{1}{k_2 q_e^2}\right) + \frac{t}{q_e}$	k_2 (g mg ⁻¹ min ⁻¹)	0.00001	0.00003
		q_e (mg g ⁻¹)	3333	2000
		R ²	0.99	0.99
Intraparticle diffusion	$q_t = k_{id} t^{0.5} + C$	k_{id} (mg g ⁻¹ min ^{-0.5})	189.25	110
		q_e (mg g ⁻¹)	2707	1852
		R ²	0.97	0.78

The R² values of different kinetic models in Table 6.1 were compared to select the best model. When the R² value is close to 1, it means that the experimental data and the model-predicted values are in agreement. It is quite clear from the results reported that the R² value for pseudo-second-order kinetics (0.99) is the highest obtained. Thus, it can be concluded that the pseudo-second-order model is best fitted to the experimental kinetic data. The adsorption kinetics is governed by the rate of surface adsorption, which is the transition of adsorbate molecules from the free state in solution to the adsorbed state on the NP surfaces.

Figure 6.8 Pseudo-first-order kinetic, pseudo-second-order kinetic and intraparticle diffusion models for DTT adsorption onto ZnO nanoparticles.

Cys adsorption onto ZnO NPs was very rapid as the equilibrium was reached within 10 min and therefore kinetic study was not performed.

6.4.4 Adsorption isotherms

Adsorption isotherms help to elucidate the adsorption behavior in liquid-solid systems and are represented as the amount of adsorbate molecules per unit adsorbent mass as a function of equilibrium concentration at a constant temperature. The fitting of equilibrium data to different isotherm models (including Langmuir, Freundlich and Temkin) were applied to study the adsorption behavior of DTT and Cys onto ZnO NPs. A previous investigation had found that ZnO NPs adsorbed cationic Victoria blue B dye from aqueous solution by the Langmuir and Temkin isotherm models. [230] The Langmuir model describes the saturated monolayer adsorption on homogenous adsorption sites [231,232], as represented by:

$$\frac{C_e}{q_e} = \left(\frac{1}{q_{max} b} \right) + \frac{C_e}{q_{max}} \quad (6-6)$$

where C_e is the equilibrium concentration of the adsorbate in solution (mg L^{-1}), q_e is the amount adsorbed per unit mass of adsorbent (mg g^{-1}), q_{max} is the maximum adsorption capacity (mg g^{-1}), and b (L mg^{-1}) is the Langmuir isotherm constant which relates to the energy of adsorption. All Langmuir parameters and correlation coefficient are presented in Table 6.2. The characteristics of the Langmuir isotherm can be explained by the dimensionless constant, which is called the separation factor (R_L) expressed as [233]:

$$R_L = \frac{1}{1 + bC_0} \quad (6-7)$$

The value of R_L indicates if the isotherm is either favorable ($0 < R_L < 1$) or unfavorable ($R_L > 1$).

The adsorption data were next analyzed by the Freundlich isotherm model, which assumes a multilayer adsorption on heterogeneous adsorption sites. The linear form of the Freundlich equation is expressed as [234]:

$$\log q_e = \log k_f + \frac{1}{n} \log C_e \quad (6-8)$$

where k_f (mg g⁻¹) is the adsorption capacity and $\frac{1}{n}$ is the adsorption intensity. The value of $\frac{1}{n}$ indicates if the isotherm is favorable ($0 < \frac{1}{n} < 1$), unfavorable ($\frac{1}{n} > 1$) or irreversible ($\frac{1}{n} = 0$). The values of $\frac{1}{n}$ and k_f obtained from the slope and intercept of the plot of $\log q_e$ vs $\log C_e$ are presented in Table 6.2

The adsorption data were last analyzed by the Temkin isotherm model, which assumes a monolayer adsorption on heterogeneous adsorption sites, expressed as [235]:

$$q_e = B \ln k_t + B \ln C_e \quad (6-9)$$

where B and k_t are the Temkin isotherm constant (J mol⁻¹) and equilibrium binding constant (L mg⁻¹), respectively. The parameters of Temkin isotherm obtained from the linear plot of q_e vs $\ln C_e$ are presented in Table 6.2.

The R² values suggest that the experimental data are best represented by the Langmuir isotherm model, indicating the formation of a monolayer of DTT or Cys onto the surface of each ZnO NP. The value of $q_{\max}$ calculated from the Langmuir isotherm is higher for Cys than that of DTT. This might be affected by the orientation of each adsorbate on the ZnO NP surface. Cys has only one probable adsorption mode on the ZnO surface, which is the vertically aligned mode. This would intuitively allow more Cys molecules to be adsorbed. On the other hand, DTT adsorption is more likely to have a mixed conformation (i.e., a combination of the three possible modes of adsorption; vertically aligned, horizontally aligned and cross-linking modes)[236] on the ZnO surface.

Table 6.2 Isotherm models parameters for the adsorption of DTT and Cys onto ZnO nanoparticles

Adsorbate	Langmuir				Freundlich			Temkin		
	q_{max} (mg g ⁻¹)	b (L mg ⁻¹)	R_L	R ²	k_f (mg g ⁻¹)	$\frac{1}{n}$	R ²	k_t (L mg ⁻¹)	B (J mol ⁻¹)	R ²
DTT	2500	0.19	0.03	0.99	1938	0.06	0.68	620983	139.7	0.67
Cys	3333	0.14	0.05	0.99	2139	0.08	0.98	2590	253.6	0.98

Figure 6.9 Langmuir, Freundlich and Temkin isotherm models for DTT adsorption onto ZnO nanoparticles.

6.4.5 Effect of ZnO dose on DTT and Cys adsorption

The effect of ZnO dose (0.02–0.2 mg mL⁻¹) on the adsorption of DTT and Cys (0.45 mg mL⁻¹) was next investigated. As shown in Fig. 6.10, a linear increase of % adsorption of DTT and Cys was achieved with increasing ZnO dose. DTT adsorption was completed using 0.14 mg mL⁻¹ of ZnO NPs with 99% of 2.9 mM DTT adsorption while 3.7 mM Cys adsorption reached 98% using 0.20 mg mL⁻¹ of ZnO NPs.

Figure 6.10 Effect of ZnO dose on % adsorption of DTT and Cys.

6.4.6 DTT and Cys adsorption onto Al₂O₃, CeO₂, SiO₂ and TiO₂ nanoparticles

DTT or Cys (0.45 mg mL⁻¹) was added into other types of NPs, namely Al₂O₃, CeO₂, SiO₂ and TiO₂, (1 mg mL⁻¹), suspended in 10 mM Na₂HPO₄ for comparison. The CE-UV analysis results showed no changes in the peak area of either adsorbates or NPs, indicating no occurrence of adsorption. These results confirm the selectivity of DTT and Cys towards ZnO, without any potential interference by the presence of Al₂O₃, CeO₂, SiO₂ and TiO₂ NPs, in aqueous suspension. A plausible explanation of the selectivity of DTT and Cys towards ZnO is the strong affinity of thiols to zinc [237] compared with aluminum, cerium, silicon and titanium. In contrast to TiO₂, ZnO exhibited a stronger adsorption for thiol-functionalized dyes than carboxylic acid-functionalized dyes via the formation of Zn–S bonds. [238] Also, Al₂O₃ and TiO₂ strongly adsorbed phosphate anions [239,240] present in the 10 mM Na₂HPO₄ medium, possibly forming a layer

surrounding the NPs that prevented their interaction with DTT and Cys. The redox property of CeO₂ was blocked by phosphate anions that occupied the oxygen vacancies at the surface forming CePO₄ due to the strong association between cerium and phosphate. Blocking surface sites with phosphate had previously been reported to avert the CeO₂ toxicity. [241] This high affinity for phosphate most likely prevented DTT and Cys from adsorbing onto the CeO₂ NP surfaces. Moreover, SiO₂ showed no affinity for thiols [242,243], hence facilitating neither DTT nor Cys adsorption onto the NP surfaces.

6.4.7 Dynamic light scattering

Aqueous suspensions of the original and DTT-adsorbed ZnO NPs were analyzed by DLS to determine their hydrodynamic diameters with the hope of gaining more insight regarding their size increase after DTT addition. The hydrodynamic diameter represents the actual particle diameter plus the surrounding layer of hydrated adsorbates when the particle undergoes Brownian motion. As shown in Table 6.3, mean diameters of 355 ± 17 nm and 404 ± 22 nm were obtained for ZnO and DTT-ZnO NPs, respectively. Apparently DTT formed a layer (hydrodynamically 25 nm thick) surrounding each agglomerate of ZnO NPs. These results provide strong evidence that ZnO NPs in water can adsorb DTT to develop a strongly light-absorbing layer surrounding the NPs under very simple experimental conditions to offer more sensitive detection by CE-UV. On the other hand, Cys adsorption onto ZnO NPs reduced their average hydrodynamic diameter down to 198 ± 6 nm. Cys adsorption onto ZnO increased its migration time to 7.6 min due to more negative charges on its surface. The higher negative charges improved the colloidal stability of the resultant Cys-ZnO nanoparticles. DLS analysis showed no significant change in the hydrodynamic diameters of Al₂O₃, CeO₂, SiO₂ and TiO₂ NPs after DTT or

Cys addition, as summarized in Table 6.3. This confirms the above CE-UV analysis results (in Section 6.4.6) that indicated no DTT or Cys adsorption onto these NPs.

Table 6.3 Hydrodynamic diameters of Al₂O₃, CeO₂, SiO₂, TiO₂ and ZnO nanoparticles measured by DLS before and after addition of DTT or Cys.

	Hydrodynamic Diameter of Original Nanoparticles (nm)	Hydrodynamic Diameter of Nanoparticles After DTT Addition (nm)	Hydrodynamic Diameter of Nanoparticles After Cys Addition (nm)
Al ₂ O ₃	425±18	430±17	421±15
CeO ₂	264±7	262±12	260±4
SiO ₂	95±2	91±4	96±10
TiO ₂	450±24	448±22	440±30
ZnO	355±17	404±22	198±6

6.5 Conclusion

A simple and rapid method has been developed for the detection of ZnO NPs in aqueous suspensions by CE-UV analysis. Modeling by the Langmuir isotherm confirmed the adsorption in a monolayer of DTT or Cys molecules around the NPs to enhance their colloidal stability and UV light absorptivity. Adsorption equilibrium was reached within 90 min for DTT but only 10 min for Cys. Cys adsorption onto ZnO NPs prevented their aggregation. After centrifugation of the transparent Cys-ZnO suspension, no precipitation of NPs occurred. This greatly helped to separate Cys-ZnO NPs from any precipitated Al₂O₃, CeO₂ and TiO₂ NPs. DTT and Cys adsorption onto ZnO NPs enhanced the UV detection sensitivity of ZnO NPs by 28±1 and 25±1 fold, respectively. Importantly, any

observed changes in CE migration time and UV peak area could identify the analyte to be NPs but not solute species. More significantly, the developed method is selective towards ZnO NPs even in the presence of Al₂O₃, CeO₂, SiO₂ and TiO₂ NPs in the water sample. Potential matrix interference can be minimized by sample dialysis to remove UV-absorbing solutes prior to the addition of DTT or Cys for CE-UV analysis.

6.6 Connection to chapter 7

DTT and Cys showed a rapid and selective adsorption onto ZnO in the presence of Al₂O₃, CeO₂, SiO₂ and TiO₂ NPs enhancing its UV detection sensitivity by 28 ± 1 and 25 ± 1 fold, respectively. Reaching this milestone, the ongoing research is concluded with accomplishments summarized in **Chapter 7**.

Chapter 7: Conclusions

7.1 General conclusions and outlook

The projects described in this thesis all concern the quantitative determination of metal/metalloid oxide NPs in aqueous solutions using CE-UV analysis. CE has recently emerged as a well-suited technique for the analysis of environmental samples due to its separation capability. [86] During NPs analysis, it is of critical importance to consider their stability and tendency to aggregate in solution as a consequence of their intrinsic characteristics such as size and surface chemistry in combination with the complex aqueous matrices found in realistic environmental conditions.

A rapid, simple, cost- effective and highly efficient CE-based method for the analysis of NPs was developed. Herein, various polymeric and molecular coatings were investigated to assess their feasibility of enhancing the UV detection of different metal/metalloid oxide NPs including SiO₂, TiO₂ and ZnO, optimizing parameters such as the compositions and concentration of the BGE, the injection time, the applied voltage and the use of surfactants. This method allowed for the separation of various components in the analysis mixture including the monomer and polymer as well as bare and coated NPs. Also, UV detection at different wavelengths enabled optimizing the measurement sensitivity and selectivity. Moreover, changes observed in CE migration time and UV peak area could identify the analyte to be NPs but not solute species. The obtained results suggest the viability of the method for the direct detection of NPs in environmental waters in hopes to assess their potential risks for humans and the environment. However, the obtained detection limits are still not adequate for the quantitative determination of NPs in environmental waters, despite the many folds enhancements in UV detection.

Also, the lack of CRMs, particularly for the mass concentration of NPs, hindered the validation of the developed method.

The work presented in chapter 3 concerned the CE-UV characterization and detection of colloidal SiO₂ NPs in water. The challenge of inadequate sensitivity was tackled using technically simple and operationally cost effective solutions. PHPMA and PDA coatings resulted in a moderate enhancement of the UV detection sensitivity of SiO₂ NPs of about 12±2 fold, demonstrating the feasibility of the method towards the quantitative determination of NPs in water. However, the partial coating of SiO₂ NPs, the modest enhancement factors and the long polymerization time suggested the need for thicker polymer coatings to attain higher sensitivity preferably in shorter times.

These challenges were tackled in chapter 4 by studying SiO₂ interaction with HPMA and chitosan, each of which were able to produce larger sizes of SiO₂ for more sensitive detection by CE-UV. On the contrary, no interactions with TiO₂ NPs in 10 mM Na₂HPO₄ could take place, resulting in no change of their CE-UV detection sensitivity. Chitosan coating was more efficient producing a significant increase in SiO₂ hydrodynamic diameter. However, CE-UV analysis of chitosan or chitosan-coated SiO₂ failed due to the adsorption of chitosan onto the capillary wall affecting the EOF. Subsequent HPMA binding facilitated the CE-UV analysis without any disturbance. A total enhancement of 50±1 fold in SiO₂ detection sensitivity was gained after chitosan coating and HPMA binding. The applied method is simple, rapid and most importantly selective for SiO₂ in the presence of TiO₂ NPs in 10 mM Na₂HPO₄. Also, it is more environmentally friendly as no chemical initiator was required, unlike the in-situ polymerization of HPMA. Nonetheless, the promising enhancement of 50±1 fold in

detection sensitivity is still not sufficient for the quantitative determination of NPs at environmentally relevant concentrations.

Further investigation of the binding interaction of other polymers including DNA and PEG as well as the UV absorbing surfactant, TX-100, onto Al_2O_3 , SiO_2 , TiO_2 and ZnO NPs was ensued in chapter 5. The equilibrium was reached within 3 hours, improving the speed of analysis. All adsorbates were selective for TiO_2 NPs in 100 mM Tris, enabling the enhancement of many folds in UV detection sensitivity in CE analysis. The reaction medium was the most influencing factor controlling the selectivity of various adsorbates towards TiO_2 NPs. ssDNA exhibited more adsorption onto TiO_2 NPs among the other adsorbates. ssDNA+PEG produced a progressive enhancement of 13 ± 3 fold in UV detection sensitivity of TiO_2 NPs, reporting on the total surface area of disaggregated NPs, as well as their chemical functionality.

Thiol molecular coatings including DTT and Cys, investigated in chapter 6, were found to be selective for ZnO in the presence of Al_2O_3 , CeO_2 , SiO_2 and TiO_2 NPs in 10 mM Na_2HPO_4 . Adsorption equilibrium was reached within 90 min for DTT but only 10 min for Cys, further improving the speed of analysis. A monolayer of DTT or Cys molecules around ZnO NPs enhanced their colloidal stability and UV light absorptivity. Cys adsorption onto ZnO NPs enabled their long-term colloidal stability. Centrifugation of the transparent Cys- ZnO suspension showed no precipitation of the NPs, allowing their separation from any precipitated Al_2O_3 , CeO_2 and TiO_2 NPs. Promising enhancement factors of 28 ± 1 and 25 ± 1 fold were attained after DTT and Cys adsorption onto ZnO NPs, respectively.

In-capillary stacking can greatly help to concentrate water samples providing more than 10 fold improvement in detector response. It is based on a sudden change in the analyte electrophoretic velocity that can be brought about by different magnitudes of the electric field in the water sample versus the BGE. Environmental water samples can be treated prior to CE analysis by dialysis to reduce their conductivity for in-capillary stacking. Concentric dialysis tubes of ultra-high MWCOs can be used to fractionate NPs on the basis of size and to remove unwanted microparticles. Now that the feasibility is demonstrated for this approach, exploring various fluorescent probes to further enhance the sensitivity should be exciting. Introducing a fluorescent label to SiO₂ NPs directly in aqueous solutions by employing a cationic inorganic dye, tris(2,2'-bipyridyl)dichlororuthenium(II) hexahydrate, Ru(bpy)₃, for laser induced fluorescence (LIF) detection in CE analysis can be explored.

DAPI (4',6-diamidino-2-phenylindole), a DNA-specific probe, could form a fluorescent complex by attaching in the minor groove of A-T rich sequences of DNA, enabling its visualization and quantitation. DAPI can be investigated for the detection of DNA-stabilized TiO₂ NPs using CE-LIF.

Graphene quantum dots (GQDs), the zero-dimensional form of graphene with diameters below 100 nm, offer a promising potential in divers applications due to their tunable electronic and optoelectronic properties directly associated with quantum confinement and chemical functionalization. GQDs can be easily prepared in aqueous solutions using citric acid via hydrothermal process at a high temperature. GQDs can be simultaneously functionalized with Cys in a basic environment through an amidation reaction.^[244] Cys-GQDs can be explored as a fluorescent probe for the sensitive

detection of ZnO NPs in water using CE-LIF.

With the successful application of these proposed strategies in improving the detection sensitivity of NPs, the method must be validated using CRMs to assure its applicability for the quantitative determination of NPs in real-world water samples.

7.2 Contributions to knowledge

The main goal of this research was to investigate whether UV detection can be made more sensitive for the quantitative determination of metal/metalloid oxide NPs in aqueous suspensions for direct CE analysis. Molecular layers and polymeric coatings could selectively form on different types of NPs to produce stronger peaks at shifted migration times for enhanced colloidal stability and detection limits as well as unmistakable identification. While this research provides a number of important insights, it devoted significant efforts on optimizing the CE-UV analysis of NPs. The developed method is exciting because it bridges the well-known advantages of CE analysis and the achievable improvement of UV detection in a simple, rapid and cost effective technique. UV detection sensitivities can in principle be further enhanced by multiple molecular layers and polymeric coatings, being limited only by the onset of NPs precipitation. The selectivity and identity knowledge can promote the direct detection of NPs in environmental waters to gain prominent understanding of their behavior, fate and toxicity.

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Appendix- UV-vis Spectra

Figure A1: UV-vis spectrum of SiO₂.

Figure A2: UV-vis spectrum of TiO₂.

Figure A3: UV-vis spectrum of ZnO.