Abstract—Small concentrations of zinc metal nanoparticles (Zn NP) have been shown to significantly enhance the sense of smell in rats [1]. Olfactory enhancement is important to many U.S. agencies where particular interests lie in canine detection for illicit drugs and explosives (DHS and DARPA); the perfume and food industry (DOC); and health-related conditions such as Alzheimer’s disease and anosmia (CDC and NIH). Hence, the engineered synthesis and characterization of odor enhancing agents is of great potential importance. Physiological data provides evidence that Zn NPs (nominal diameter 1 nm - 2 nm) enhance the olfactory response in rats, however chemical characterization of these NPs is still necessary to fully understand the enhancement mechanism. Amino acids play a vital role in the signal transduction of neurotransmitters during an olfactory-induced response [2]. Vital information about NP-induced olfactory enhancements may be obtained by assessing interactions between NPs and underivatized amino acids, using capillary electrophoresis (CE) and ultraviolet-visible (UV-Vis) spectroscopy. Although the small size of these odor-enhancing Zn NPs present great challenges during analytical measurements, the size and concentration of these Zn NPs were estimated using small angle x-ray scattering (SAXS), dynamic light scattering (DLS) and field emission-scanning electron microscopy (FE-SEM), and found to be approximately 15 nm, in diameter. Inductively coupled plasma-mass spectroscopy (ICP-MS) measurements revealed the presence of Fe (II) and Ni (II) in Zn NP dispersions, which may cause interferences with Zn (II) concentration and dissolution measurements. Zn NP and amino acid interactions, analyzed by UV-Vis spectroscopy, showed that interactions were concentration dependent; higher concentrations of amino acids interacted with more Zn NPs, therefore further investigations of the interactions between NPs and biomolecules is necessary. This work has potential for the development of innovative technologies applicable to the advancement of canine detection, health and performance sciences.

Keywords— Nanocharacterization; Nanometrology; Zinc Nanoparticles; Olfaction.

I. INTRODUCTION

Although physiological data provides substantial evidence that zinc metal nanoparticles (Zn NP), of nominal diameter 1 nm - 2 nm, enhance the olfactory response of rats to odors, the chemical characteristics of these NPs have not been fully understood.

Analytical measurements of small Zn NPs present many challenges particularly due to the fact that small, spherical NPs are estimated to contain only about 300 atoms [3]. This limited number of atoms reaching an analytical detector provides a significant analytical challenge to detection. There is a growing need to develop NP characterization methods, as well as to determine their fate in biological systems. Although NP fate remains largely unknown, studies provide some evidence that interactions between NPs and biological systems can be both harmless [4] and toxic [5].

NP size, size distribution, dissolution and concentration measurements are important characteristics in determining a NP’s reactive nature. These measurements can be obtained using field emission-scanning electron microscopy (FE-SEM), dynamic light scattering (DLS) and small angle x-ray scattering (SAXS). Inductively coupled – mass spectrometry (ICP-MS) measurements can also provide information about the dissolution of the NP as well as the identification of metal ion species. Interactions between NPs and biomolecules will be explored on a small scale in this study; by monitoring changes in the chemical profiles of the Zn NPs as well as changes in the chemical profiles of amino acids (AA). Capillary electrophoresis (CE) and ultraviolet-visible spectroscopy (UV) will be used to determine the initial and post-interaction chemical properties of both analytes.

CE coupled to a UV detector is a useful tool for measuring interactions between NPs and AAs because it provides both an electrophoretic separation component of the analyte (CE) and qualitative as well as quantitative identification (UV) of the separated analytes. The separation of AAs by CE is typically obtained using derivatized AAs [6] to enhance UV detection, however, in this study derivatization is not necessary, because a subset of AAs (L-Tryptophan and L-Tyrosine), both of which are chromophores and strong UV absorbers, were used. Metallic nanoparticles have unique light absorbing properties that are distinct from the bulk metal. This property, called surface plasmon resonance (SPR), is caused by their increased surface area and consists of a surface propagation of electromagnetic waves caused by oscillating electrons along the NP surface [7]. Thus, many metallic NPs are UV detectable. The analysis of mixtures of AAs and NPs both by CE and UV is therefore a promising technique for NP fate assessments.

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II. MATERIALS

L-Tryptophan (L-Trp) was purchased from SigmaUltra (St. Louis, MO) and L-Tyrosine (L-Tyr) was purchased from CalBIOChem (La Jolla, CA). All buffers (sodium phosphate, sodium acetate and sodium borate) were purchased from Fluka Analytical (St. Louis, MO). Non-functionalized Zn NPs, suspended in water, were obtained from Auburn University School of Veterinary Medicine and consisted of either small zinc nanoparticles (Zn sNP) or big zinc nanoparticles (Zn bNP).

III. METHODS

A. Nanoparticle Characterization

NP size and morphology measurements were made using a Hitachi S-4700-II FE-SEM, by depositing and air-drying droplets of Zn NPs onto silicon wafers. A Wyatt Technologies DLS and Field Flow Fractionation System and a ChemMatCARS Ultra SAXS instrument were used to obtain NP sizes.

B. Capillary Electrophoresis

Sodium acetate, sodium phosphate and sodium borate buffers solutions were used in CE measurements (Beckman P/ACE 5510 CE) and were adjusted to different pH (ranging from 2-10) with 0.1 mmol/L NaOH and 0.1 mmol/L acids corresponding to the buffers. Initial CE runs were measured using all buffers at different pH in conjunction with other separation parameters (voltage, detection wavelength, run time) to determine optimal separation conditions. CE measurements of Zn sNPs, were obtained using optimal conditions for AA determined during CE-separation experiments.

C. Amino acid and nanoparticle interaction

High and low concentrations of each AA were prepared in water (high L-Trp - 0.01 mmol/L; low L-Trp - 0.001 mmol/L; high L-Trp - 0.5 mmol/L; low L-Tyr; 0.25 mmol/L). For stand-alone UV measurements, an Agilent Technologies Cary Series double beam UV-Vis Spectrophotometer was used. AAs and Zn NPs were analyzed both individually, and in a combined fashion, to monitor changes in either the AA peaks or Zn NP peaks after interaction. For this, Zn bNPs were exposed to each AA concentration in a 1:1 volume ratio, for 24 h. An Agilent 7500cs ICP-MS provided concentration, dissolution, and the presence of other metal species within the Zn NP suspensions.

IV. RESULTS

FE-SEM images revealed a clear distinction in size between the two Zn NP particles (fig. 1). NP sizes were also estimated by DLS and SAXS (table 1), and found to be different from their initially identified sizes. Interferences from the water matrix gave high variation in size measurements for Zn sNPs, since they can be easily obstructed by the presence of minimal amounts of untargeted species present in the matrix. Zn bNPs, however, measured by DLS, had similar sizes between scans, indicating less susceptibility to interference.

CE measurements of both AAs studied provided information for parameter selection including optimal separation buffer, pH, separation voltage, and detection wavelength. These parameters were used to analyze Zn sNPs independently, in preparation for CE measurements in which both AAs and NPs would be combined. Results (not shown) reveal that Zn sNPs were undetectable by CE-UV. This is likely due to their small size. Subsequent interaction measurements were performed using AAs and Zn bNPs and analyzed by stand-alone UV-Vis spectroscopy. Results (fig. 2.) indicate that both L-Trp and L-Tyr participate in Zn bNP interactions, and that the interaction is concentration dependent. Zn NP peaks appeared very broad (280-365nm) and were consistent with previously reported UV spectra [8].

Both high concentrations of AAs interacted with more Zn bNPs, as a decrease was seen in their Zn bNP peaks relative to the control sample containing no AA. The Zn bNPs in low concentrations of AAs showed a similar decrease, however it was not as pronounced.
The concentration-dependent reduction in the Zn bNPs peak was seen regardless of the AA used in the study. This suggests that some Zn bNPs may remain unaltered at lower AA concentrations.

Results from ICP-MS measurements reveal that Zn sNPs are difficult to characterize, based on increasing interferences in signal from other species (Fe (II) and Ni (II)), at such a small size. Concentration and dissolution measurements also confirmed the presence of interfering metal ionic species, since Zn concentrations exceeded theoretical estimates due to signal contributions from other interfering ionic species.

**CONCLUSION**

The interaction of NPs with small biomolecules is an important area of study, as technology continues to incorporate the use of NPs in commercial and medical products. The findings of this study indicate that there is an important interaction between Zn NPs and AAs. Subsequent analyses may provide further insight pertaining to the chemical mechanism of this interaction and may also distinguish between specific and nonspecific binding. All measurements obtained in this study, particularly by UV-Vis spectroscopy, will be used to determine suitable conditions for additional CE measurements of NP-AA interactions. Finally, complete content and organizational editing before formatting. Please take note of the following items when proofreading spelling and grammar:

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**REFERENCES**

[1] N. Viswaprakash, J.C. Dennis, L. Gloria, O. Pustovyy, E.M. Josephson, P. Kanju, E.E. Morrison, V. Vodyanoy, “Enhancement of Odorant-Induced Responses in Olfactory Receptor Neurons by Zinc Nanoparticles,” Chem Senses 2009, 34, 547-557.

[2] T.T. Win-Shwe, M. Mitsumura, S. Yamamoto, A. Fukushima, T. Funabashi, T. Kobayashi, H. Fujinami, “Changes in neurotransmitter levels and proinflammatory cytokine mRNA expressions in the mice olfactory bulb following nanoparticle exposure,” Toxicol Appl Pharm 2008, 226: 2, 192-198.

[3] A.Y. Olenin, G.V. Lisichkin, “Metal nanoparticles in condensed media: preparation and the bulk and surface structural dynamics Russian Chemical Reviews 2011, 80 (7) 605-630.

[4] B.C. Nelson, E.J. Petersen, B.J. Marquis, D.H. Atha, J.T. Elliott, D. Cleveland, S.S Watson, I-H. Tseng, A. Dillon, M. Theodore, J. Jackman, “NIST gold nanoparticle reference materials do not induce oxidative DNA damage,” Nanotoxicology 2013, 7 (1) 21-29.

[5] E.J. Petersen, R. Vytas, S.S. Watson, D.L. Stanley, S.A. Rabb, Nelson,B.C, “DNA Damaging Potential of Photoactivated P25 Titanium Dioxide Nanoparticles,” Chem. Res. Toxicol., 2014, 27 (10), 1877-1884.

[6] P. Bohn, “Amino acids and peptides: capillary electrophoresis,” *Encyclopedia of Separation Science.* Elsevier Science Ltd. 2000 Ed. I. Wilson, 2038-2047.

[7] P. Muharney, “Surface Plasmon Spectroscopy of Nanosized Metal Particles,” Langmuir 1996 12, 788.

[8] H. Ceylan, C. Ozgit-Akgun, T.S. Erkal, I. Donmez, R. Garifullin, A.B. Tekinay, H. Usta, N. Biyikli, M.O. Guler, Size-controlled conformal nanofabrication of biotemplated three-dimensional TiO2 and ZnO nanonetworks. Nature Scientific Reports 2013 3 id. 2306.