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The level of reactive oxygen species (ROS) is tightly regulated in biological systems as overproduction can lead to oxidative stress and result in a number of diseases. Due to its negative effects, antioxidants are studied and used against ROS in medicine, pharmaceuticals and therapeutics. Because of issues with the uptake and delivery of natural antioxidants, nanoparticle (NP) antioxidants are promising due to their higher bioavailability, solubility and stability. Given the importance of oxidative stress and the potential for certain NPs to modify redox balance, it is important to build a rapid, reproducible, robust assay for analysis of NP chemical behaviors. Many studies have addressed the impact of surface chemistry on single NP antioxidant capacity (AOC), yet few have compared different NP AOCs in relation to their intrinsic properties. In this study, we investigated the AOC of silver, gold and eight lanthanide metal oxide NPs (AgNP, AuNP and LnOxNPs) using Trolox equivalent antioxidant capacity assay. We found that all of the NPs tested exhibited AOC, and AuNP AOC was more pronounced than AgNP when ions were not accounted for in the reaction. When ions released from the surface were removed by the addition of chelators into the dispersion, the AOC of AgNP was higher than AuNP. For the LnOxNPs, the AOC exhibited periodicity based on their elemental properties. LnOxNPs AOC was negatively correlated to the amount of unpaired electrons in 4f orbital and lanthanum crystal ionic radius. This study supports that we can predict AOC of various NPs based on their intrinsic atomic properties. Moreover, it provides a potential mechanism to simulate and explain the effect of surface chemistry on AOC of NPs, which will be valuable for nanomedicine and the nanoscience industry.

Studies on Effect of Stabilizers, Chelators and Inherent Periodicity on Nanoparticle Antioxidant Activity

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CHAPTER 1 INTRODUCTION

1.1 Reactive Oxygen Species

1.1.1 General Occurrence of ROS

A free radical can be defined as any molecular species that includes one or more unpaired electrons in the outmost atomic orbital.[1] Due to the presence of unpaired electron(s), a free radical can either donate an electron to or accept an electron from other molecules and become highly reactive, therefore behaving as oxidants or reductants. Reactive oxygen species (ROS) is a free radical involving an oxygen atom.[2] Among various ROS produced in the biological process, the common and also most important ones are hydroxyl radical, superoxide anion radicals, hydrogen peroxide, singlet oxygen, hypochlorite, nitric oxide radical, and peroxynitrite radical. ROS are formed either from internal sources such as normal essential metabolism in the human body and other organisms, or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals.[3] The consequence of enzymatic and non-enzymatic reactions in the metabolic processes lead to continuous ROS formation in the cells. Enzymatic reactions, which serve as a source of ROS, include those involved in the respiratory chain, in phagocytosis, in prostaglandin synthesis, and in the cytochrome P-450 system.[4] Non-enzymatic reactions, another source of ROS, includes reactions of oxygen with organic compounds as well as those initiated by ionizing reactions.[5] Some internally generated sources of ROS are mitochondria xanthine oxidase, peroxisomes, inflammation, phagocytosis, arachidonate pathways, exercise, ischemia/reperfusion, and injury.[6] The redox system of cells plays an important role in maintaining cellular homeostasis by scavenging ROS via variety of oxidases which complete the ROS elimination process.[7] For instance, superoxide as an ROS can be produced by adding an electron to oxygen. Then it can be degraded by superoxide dismutase to form H_2O_2 and O_2, which finishes the process of generation and elimination of ROS.[8]

1.1.2 Adverse Effects of ROS

The generation of ROS, a type of free radical most often produced *in vivo*,[9,10] is tightly regulated in biological systems as overproduction can lead to oxidative stress and result in a number of disease states including oxidative damage,[11] inflammatory reactions,[12,13] necrotic cell death,[14,15] and DNA damage.[16] The production of ROS has been observed in the oxidative metamorphism of food products as well as in the pathogenesis of several human diseases such as atherosclerosis, diabetes mellitus, chronic inflammation, neurodegenerative disorders and certain types of cancer.[17-19] Based on the negative effects of ROS on environment and human, the protective function of antioxidants against ROS and its adverse

oxidative-induced reactions have received growing attention recently, especially within biological, medical, nutritional, and agrochemical fields.[2]

1.1.3 Nanoparticle Generation of ROS

With the advance of nanotechnology, large amount of NPs are released to the environment, including water, soil and air system. These NPs could enter into biosystem through multiple ways including membrane penetrating, respiration and active uptake. After that, NPs could accumulate in the body and cells to form ROS and induce oxidative toxic effects. NPs such as titanium dioxide (TiO_2NP), zinc oxide (ZnONP), copper oxide (CuO), fullerenes and also some quantum dots could also generate external ROS and have toxic effect to organism.[20-24]

1.2 Introduction to Antioxidant

1.2.1 Definition and History

An antioxidant is a molecule that inhibits the oxidation of other molecules. It was originally used to refer specifically to a chemical that inhibited the consumption of oxygen. In the late 19th and early 20th century, extensive studies were devoted to the uses of antioxidants in important industrial processes, such as the prevention of metal corrosion, the vulcanization of rubber, and the polymerization of fuels in the fouling of internal combustion engines.[25] Early research on the role of antioxidants in biology focused on their use in preventing the oxidation of unsaturated fats, which is the cause of rancidity.[26] Antioxidants are compounds that form exogenously (natural or synthetic) or endogenously and act in multiple ways to remove O_2, scavenge ROS or their precursors, inhibits ROS formation and bind metal ions needed for catalysis of ROS generation.[27] To reduce ROS efficiently, the antioxidant must be a stable enough to scavenge and neutralize ROS by donating electrons.[1] Antioxidants can delay or inhibit cellular damage mainly through their ROS scavenging property.[28] Low-molecular-weight antioxidants are involved directly in the conversion of ROS to more stable species and result in chain reaction termination before vital molecules are damaged.[29]

1.2.2 Natural Antioxidants

Generally, traditional antioxidants can be summarized into two categories: non-enzymatic and enzymatic. Non-enzymatic antioxidants including glutathione, melatonin, ubiquinol and uric acid, are formed in the normal essential metabolic processes in the body.[30] Other lighter antioxidants such as vitamins including the principle micronutrient (vitamins) antioxidants are vitamin E (α-tocopherol), vitamin C (ascorbic acid), and β-carotene.[31] These antioxidants cannot be synthesized independently by

the body and must be supplied in the diet or through supplements or medicine. The primary enzymatic antioxidants include superoxide dismutase (SOD), catalase and glutathione system. This network of antioxidant enzymes react with ROS and in order to protect cells from oxidative damage.[32] This protection pathway results from multiple antioxidant enzymes, the first step is catalytic function of superoxide dismutases and then the hydrogen peroxide is eliminated by catalases and various peroxidases.[33]

1.2.3 Non-enzymatic Antioxidants

1.2.3.1 Ascorbic Acid (Vitamin C)

Ascorbic acid is commonly known as "vitamin C", a monosaccharide antioxidant found in both animals and plants. However, humans must obtain that from the diet.[34] Ascorbic acid plays an important role for the conversion of the procollagen to collagen by oxidizing proline residues to hydroxyproline.[35] In cells, it is maintained in its reduced form by reaction with glutathione, which can be catalyzed by protein disulfide isomerases and glutaredoxins.[35] As an antioxidant, it can reduce ROS such as hydrogen peroxide.[36] Additionally, it acts as a substrate for ascorbate peroxidase, an antioxidant enzyme compared to its direct antioxidant effects.[37]

1.2.3.2 Tocopherols and Tocotrienols (Vitamin E)

Vitamin E is the collective name for a set of eight related tocopherols and tocotrienols, a fat-soluble antioxidants.[38] α-tocopherol, the most important of these lipid-soluble antioxidant scavenges lipid radicals in the lipid peroxidation and protects membranes from oxidation.[39] This removes the excess ROS intermediates and prevents the sequence propagation reactions. Oxidized α-tocopheroxyl radicals produced from this reaction can be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol, or ubiquinol.[40]

1.2.3.3 Glutathione

Glutathione is a cysteine-containing peptide existing in most forms of aerobic life.[41] It is a very important cellular antioxidant because of its high concentration in humans and its central role in maintaining the redox state of cell.[42] Different from ascorbic acid, it can be synthesized in the human body. The antioxidant activity of glutathione comes from the thiol group, a reducing agent that can be either reversibly oxidized or reduced. In cells, the enzyme glutathione reductase stabilizes glutathione in its reduced form making it readily available to reduce other metabolites, enzyme systems and other oxidants.[43]

1.2.3.4 Melatonin

Melatonin, or N-acetyl-5-methoxytryptamine, is a naturally occurring hormone in animals and other living organisms, including algae.[44] It is a powerful antioxidant that can easily get through cell membranes and the blood-brain barrier.[45] Unlike other antioxidants, melatonin interacts with ROS and once oxidized, it forms stable end-products and stops redox cycling. Therefore, melatonin has been referred to as a terminal (or suicidal) antioxidant.[46]

1.2.3.5 Uric Acid

More than 50% of the antioxidant capacity of blood plasma is from uric acid.[1] In fact, uric acid may have substituted for ascorbate in human evolution.[47] However, like ascorbate, uric acid can also mediate the production of reactive oxygen species and oxidative stress by donating electrons.[47] In addition, urate can act as a chelator to scavenge metal ions such as iron and copper to prevent them catalyzing hydroxyl radicals via Fenton reaction.[48]

1.2.4 Enzymatic Antioxidants

1.2.4.1 Superoxide Dismutase

Superoxide dismutases (SODs) are a class of closely related enzymes that catalyze the breakdown of the superoxide anions into oxygen and hydrogen peroxide.[49,50] SOD enzymes are present in almost all aerobic cells and in extracellular fluids.[51] There are three major families of superoxide dismutase, depending on the metal cofactor: Cu/Zn (which binds both copper and zinc), Fe and Mn types (which bind either iron or manganese), and finally the Ni type which binds nickel.[52]

1.2.4.2 Catalase

Catalase is used to catalyze the reaction that hydrogen peroxide decomposed to oxygen and water molecules. As a common enzyme, it exist in almost all living organisms exposed to oxygen.[53] Hydrogen peroxide is an important intermediate but overproduction is a harmful by-product of normal metabolism. Therefore, it must be rapidly decomposed into other, less dangerous substances in order to prevent oxidative damage. From this reason, catalase is used frequently by cells to catalytically decompose hydrogen peroxide into less reactive gaseous oxygen and water molecules.[54] All known animals use catalase in every organ, with particularly high concentrations occurring in the liver.[55]

1.2.4.3 Glutathione System

The glutathione system includes glutathione, glutathione reductase, glutathione peroxidases, and glutathione S-transferases. This system is found in animals, plants, and microorganisms.[56] Four selenium-

cofactors were included in glutathione peroxidase to catalyze the breakdown of hydrogen peroxide and organic hydroperoxides. At least four different glutathione peroxidase isozymes are known in animals.[57] Glutathione peroxidase 1 is the most abundant and is a very efficient scavenger of hydrogen peroxide, while glutathione peroxidase 4 is most active with lipid hydroperoxides. The glutathione S-transferases show high activity with lipid peroxides. These enzymes are at particularly high levels in the liver and serve a major role in detoxification metabolism.[58]

1.2.5 Disadvantages of Natural Antioxidants

Most of the natural antioxidants and antioxidant systems mentioned above come from natural product extraction or chemical synthesis, and have been accepted and widely applied as conventional therapies in medical science, food science, pharmacy and clinic. However, the disadvantages are many of these natural antioxidants are their poor bioavailable and low efficiency[59] due to instability,[60] inefficient permeability,[61] extracellular degradation,[62] and poor solubility.[59,63] In addition, the antioxidant capability of enzyme antioxidants depends on body temperature. All these issue will limit further use and development of natural antioxidants. Therefore, novel antioxidants are being explored to overcome the disadvantages of natural antioxidants.

1.2.6 Nanoparticle Antioxidants

Nanoparticles (NPs) can be defined as particles between 1 and 100 nanometers in all the three dimensions in size. The physicochemical properties of many NPs change are different from their corresponding bulk materials. The reason is typically that NPs have a greater specific area (surface area per mass) than bulk materials which allows them to have more reactive surface area. Based on the high reactivity and catalytic activity of NPs, new inorganic antioxidants constituted as NPs are being investigated for applications in public health and pharmacy. NP antioxidants open a new era of antioxidant therapies for disease prevention and treatment in the realm of oxidative damage caused by ROS. NP antioxidants exhibit the ability to scavenge and reduce concentrations of ROS, and finally act as antioxidants to prevent oxidative damage.[64-66] Furthermore, NP antioxidants can compete even better than some natural antioxidants. For instance, a number of studies showed some vacancy antioxidant NPs metal oxide such as cerium and yttrium oxide act by mimicking the antioxidant capacity of enzyme antioxidants like catalase or superoxide.[67,68] The antioxidant capacity of gold decorated diamond NP was twice that of glutathione.[69] NP antioxidants have been demonstrated to complement and overcome the limitation of natural antioxidants. For instance, gold NPs carrying glutathione can penetrate cell membranes to deliver and release drug molecules in cells.[61] Low bioavailability and solubility of raw taxifolin limited its biomedical applications. Recently, Zu et al. used raw taxifolin to prepare taxifolin NP and significantly

improved its bioavailability, solubility as well as antioxidant capacity.[70] Nanoparticle capsules (PEG-decanyl polymetric, wheat gliadin and PLGA NP) can be used to entrap antioxidants (vitamin E) to improve their antioxidant efficiency by controlling the release rate and extracellular degradation of the antioxidant.[71-73]

To summarize, NP antioxidant are more promising than natural antioxidant because of their

- Higher bioavailability
- Higher cell penetration efficiency
- Higher stability
- Higher solubility
- Lower extracellular degradation
- Higher antioxidant capacity

Some NP antioxidants can possess intrinsic antioxidant activity. In addition, NP can act as antioxidant delivery systems to be functionalized with natural antioxidants. On the basis of antioxidant functionality, NP antioxidants can be generally classified in two groups, inorganic NP with intrinsic antioxidant properties as free radical scavengers and functionalized and composite inorganic NP antioxidants.[71]

1.2.7 Inorganic NP with Intrinsic Antioxidant Properties

Inorganic nanoparticles made of silver, gold and platinum or metal oxides (eg. zinc oxide, iron oxide, nickel oxide, ceria, yttria and silica) have intrinsic antioxidant capacities, which reduce ROS and protects cells. Their antioxidant behavior arise from their physicochemical properties such as redox and catalytic activity, high surface-volume ratio and electronic configuration. Some NP antioxidants such as silver, zinc oxide, ceria and iron oxide NPs produce ROS and oxidative toxicity *in vivo*. The main reason is the free ions released from the surface of the NPs that can act as catalysts to produce ROS and cause toxicity in living systems.

1.2.7.1 Silver Nanoparticle (AgNP)

Mani et al. found that AgNP could be an antioxidant by offering effective protection from free radicals.[75] Ranjbar et. al also investigated the possible antioxidant protective role of AgNP biomarkers in rats. The result showed that AgNP with 500mg/kg induced activities of superoxide dismutase and decreased total antioxidant capacity. And the antioxidant capacities of AgNP was dose dependent.[76] This research also showed that AgNP antioxidant protected against oxidative liver injury was only in low doses.[77]

1.2.7.2 Gold Nanoparticle (AuNP)

The antioxidant activity of AuNP results from redox properties in scavenging free radicals, quenching singlet and triplet oxygen or decomposing peroxides.[64] AuNP is found to inhibit oxidative stress mediated diabetic progression during hyperglycemia in mice, and in this experiment, no toxic effects were observed which indicats the therapeutic potential of AuNP in diabetic treatment.[78] Du et. al used danshensu-gum Arabic to stabilize AuNP. The results showed that the AuNP could alleviate celloxidative damage, Thus, AuNP may provide potential opportunities for the application in nanomedicine.[79]

1.2.7.3 Platinum Nanoparticle (PtNP)

As another noble metal, PtNP stabilized with polyacrylate act as antioxidants and have been found to be effective in alleviating smoke-induced respiratory inflammation. It is associated with the capability of PtNP to quench ROS.[80] PtNP was also shown to eliminate ROS in a dose dependent manner indicating that it is a more potent dismutase/catalase mimetic than EUK-8, a synthetic superoxide dismutase and catalase mimetic. PtNP were also shown to prolong the worm lifespan, regardless of thermotolerance or dietary restriction.[81]

1.2.7.4 Ceria Nanoparticle

Ceria NP is composited with mixture of cerium oxide (Ce_2O_3) and cerium dioxide (CeO_2), its antioxidant activity comes from the presence of highly mobile lattice oxygen at its surface and a large diffusion coefficient of the oxygen vacancy which facilities the conversion between tetravalent and trivalent cerium, allowing oxygen to be released or stored in its cubic structure.[82-84] There have since been a number of studies that reported the antioxidant behaviors of ceria NP. Among all the metal oxide antioxidant NPs, ceria NP has been the most explored one. Ceria NP has been shown to act as a catalyst that mimics SOD, the result showed that it has higher catalytic rate constant when compared to SOD.[67] Another study found that a reduced trivalent cerium was related to its catalase mimetic activity.[85] In addition, the hydroxyl radical scavenging activity of ceria was demonstrated using methyl violet. This activity depended on the size range of the particles and was correlated with reduced trivalent state at the surface of the ceria NP.[86] The reduced cerium state was suggested as a reason for nitric oxide radical scavenging capability ceria NP.[87] Besides using simple ceria NP, some NPs such as titanium dioxide and zirconium dioxide doped with or mixed with ceria displayed greater antioxidant activity.[88] This is due to the fact that ceria could modify local oxygen environment and facilitate the transfer of oxygen atom from bulk material to the surface as ceria is mixed or doped with other rare-earth or other transitional metal oxide.[88,89]

1.2.7.5 Other Nanoparticle Metal Oxide

Other metal oxide NPs such as ZnO, CuO, NiO, Y_2O_3 and iron oxide NPs have antioxidant capabilities. ZnONP scavenge ROS and may protect cells from deleterious oxidative stress.[90] This was due to the fact that ZnONP had the capability of forming excitonic pair electrons and holes.[90] Das et. al evaluated the antioxidant capacity of CuONP using 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) assay[91] and found that CuONP could donate electrons to free radicals located at nitrogen atom in DPPH and its antioxidant capacity was up to 85% in 1 h.[91] The same assay was also used to evaluate the antioxidant capacity of NiONP evaluation through *in vitro* testing which showed that NiONP could scavenge ROS.[92] Moreover, other metal oxide NPs such as Y_2O_3NP and iron oxide NP have been shown to have the antioxidant capability which could mimic superoxide dismutase or catalase.[93,94]

1.2.8 Functionalized and Composite Inorganic NP Antioxidants

NP functionalized with antioxidants are another of group of NP antioxidants. The attachment of naturally occurring or synthetic antioxidants onto the surface of NPs could enhance its antioxidant activity, bioavailability, solubility, or target to delivery. For instance, a significant increase in antioxidant activity was found in AuNP embedded 3,6-dihydroxyflavone with other native dietary nutrients.[95] AuNP functionalized with Trolox, a water soluble Vitamin E analogue, showed that the antioxidant activity of AuNPfunctionalized with Trolox was about eight times higher than that Trolox alone in a DPPH assay.[96] AuNP functionalized with glutathione were more bioavailable and permeable through cell memberanes than bare AuNP.[61]

1.3 Antioxidant Assays

Given the importance of oxidative stress and the potential for certain NPs to alter ROS balance, it is important to have a rapid, reproducible, robust, systematic assay for the qualitative and quantitative analysis of NP chemical behaviors. A lot of scientific research has been devoted to establishing an assay based on redox properties of NPs that may prove useful in predicting toxic properties. Assays such as the Oxygen Radical Absorbance Capacity, Terephthalic acid, 1', 1'-Diphenylpicryl-hydrazyle, and 5, 5-dimethyl-1-pyrroline-N-oxide assay have been employed to investigate the relationship between redox activity and toxic properties of NPs to some extent. Yet, each method utilized to date has specific limitations that preclude its use as a rapid, systematic assay for studying nanoparticle redox activity.

1.3.1 Oxygen Radical Absorbance Capacity (ORAC)

The Oxygen Radical Absorbance Capacity (ORAC) method, developed initially by Cao and colleagues, was originally developed to assess the antioxidative properties of organisms.[97] This method was based on the fluorescence decay of the probe along time under the addition of 2, 2'-Azobis(2-amidinopropane) dihydrochloride (AAPH), which constantly generates free radical from the thermal decomposition process in the solution.[2] The fluorescence decay is inhibited in the presence of antioxidant. The antioxidant capacity is assessed from the net area under the curve (AUCantioxidant - AUCblank)[2] Recently, it was used to examine the antioxidant capacities of nanomaterials such as selenium nanoparticles (nanoSe0)-ascorbic acid (Vc) sol and nanSe0/Vc/selenocystine (SeCys) sol-gel compounds.[98] However, the ORAC assay is sensitive to temperature so that it must be held constant throughout the study. While incubation of the assay buffer at 37°C prior to adding the AAPH decreased the intra-assay variability[99], the wells at the edge of the microplate could not be used due to slight temperature differences which influence the reaction rate.[100] Moreover, reaction time was about 1h which decreased the efficiency of assay although it had been partially solved by development of high-throughput methodologies that could be adapted for the ORAC Assay.[101] Finally, the calculations for antioxidant capacity, area under the curve (AUC), are not simple either.

1.3.2 Terephthalic Acid (TPA)

Terephthalic acid (TPA) has been used as a fluorescence probe to capture hydroxyl free radicals (·OH). TPA (nonfluorescent) scavenges ·OH to yield 2-hydroxyterephthalic acid (HTPA) and displays fluorescence[102] and can be used to examine the ability of NPs to produce ·OH. This methodology has been used to evaluate the antioxidant capacity of palladium oxide nanoparticle modified electrodes.[103] The same assay was also used to evaluate the quantum yield of hydroxyl radical production during TiO_2 photocatalysis.[104] A major limitation of TPA is that it is poorly soluble in water and alcohols. It is better to prepare its sodium salts and dissolve in alkaline solutions as a fluorescent probe. But as pH of the solution increases, more H_2O_2 tends to photodecompose to O_2 and H_2O instead of generating ·OH [Eq. (1), (2)].[105-107] Moreover, oxidizing species hydrogen anion (HO_2^-) is also formed in alkaline medium, which will react with both ·OH and H_2O_2 and continue to scavenge ·OH [Eq. (3), (4)].[107] This will cause the ability of NPs to cleave H_2O_2 and produce ·OH to be underestimated.

$$H_2O_2 \xrightarrow{h\upsilon} \cdot OH + \cdot OH \qquad PH = 1\sim3 \qquad (1)$$

$$2H_2O_2 \xrightarrow{h\upsilon} O_2 + 2H_2O \qquad PH > 3 \qquad (2)$$

$$H_2O_2 + HO_2^- \longrightarrow \cdot OH + O_2 + H_2O \qquad PH > 3 \qquad (3)$$

$$\cdot OH + HO_2^- \longrightarrow O_2^- + H_2O \qquad PH > 3 \qquad (4)$$

1.3.3 1,1'- Diphenylpicryl-hydrazyle (DPPH) Assay

1,1'- Diphenylpicryl-hydrazyle (DPPH) is a free radical scavenger which has an unpaired electron at one atom of the nitrogen bridge.[108] In the DPPH assay, a deep violet color radical (DPPH·) reduced by an antioxidant decolorizes the solution to colorless or pale yellow.[109] The antioxidant capacity is estimated by measuring the absorbance decrease between the start and end of the reaction or by electron spin resonance (ESR).[110] It has been applied to test the antioxidant property of the nickel oxide particles in an *in vitro* system.[92] However, the problem is that DPPH is insoluble to water, it is only applied in hydrophobic environment. DPPH radical is very stable, the stability is due to the resonance structure and steric hindrance of the three benzene rings so that the activity of unpaired electron on the nitrogen atom is decreased. However, steric hindrance is also a major determinant of the reaction. Small antioxidant can more easily get into the radical site and thus show higher antioxidant capacity. In contrast, some large antioxidant which should react rapidly with free radicals may react slowly or even be inert to DPPH radical.[111] Another limitation of DPPH assay is that it is time consuming (20min~6h).[110]

1.3.4 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) Assay

5,5-dimethyl-1-pyrroline-N-oxide (DMPO) has been used as a spin trap agent for testing free radicals through electron paramagnetic resonance (EPR), also called ESR. It can trap ·OH, which can only exist very short time to produce DMPO-OH, a relatively stable radical. DMPO-OH has its own ESR signal, the intensity of ESR decreases with the addition of antioxidants. DMPO was used to assess antioxidant-potential of gold-chitosan nanocomposites.[112] As with DMPO assay, non-radical reactions may cause an ESR signal response very similar to DMPO-OH.[113] Measurement of ·OH would be overestimated by radical adducts of the former spin traps which require a careful analysis to recognize the response of ·OH produced by ESR,[114] ESR spectrometers are not common in laboratories and the measurements require low temperature. These limitations inhibit the accurate evaluation of antioxidant capacity across many laboratories.

1.3.5 TEAC Assay

In this article, a Trolox Equivalent Antioxidant Capacity assay (TEAC), based on $ABTS^{2-}$ /H_2O_2/HRP (Horseradish Peroxidase) decoloration method, was used to assess the antioxidant capacity of 6 lanthanide metal oxide nanomaterials. It was first described by Miller et al (1993).[115] In this assay, ·OH was generated from the reaction between H_2O_2 and HRP then oxidised colorless $ABTS^{2-}$ to a long-lived green-blue radical anion ($ABTS^{\bullet-}$),[116] After addition of NP antioxidants. The decrease in absorbance is used for measuring the antioxidant capacity of NPs. The benefit of this assay over the previously applied assays is that it is very simple and sensitive, and $ABTS^{\bullet-}$ scavenger used is stable at a broad range of pH,

hence it can be used to study pH effects on activity.[117] In addition, the TEAC assay can be applied in estimation of both hydrophilic and lipophilic samples compared to the DPPH assay and the reaction time is short (1-30 min)[2] and can be conducted at room temperature. Because this assay has been widely used and applied infood science,[118-120] it is amenable to the assessment of complex matrixes, such as nanoparticle dispersions.

1.4 Chelators

Ions released from NP are usually accompanied by electron transfer process between NP surfaces and the solution, which is high related to the redox potential of NP. Therefore without considering the dissolution rate of NP, the assays just described cannot accurately quantify the inherent AOC of the NP. In simple media (pure water or buffer solution), ions released from NPs adsorb onto the NP surface and inhibit further dissolution of ions, potentially altering the AOC of the NP.[121] When NPs release ions into more complex solutions that include biological entities, these ions can be consumed by local biomolecules, changing the dissolution rates and altering NP AOCs. Therefore, there is a need to eliminate the ions as confounding factors in the assay in order to better approximate the inherent AOC of NPs in solution.

There are several methods that can be used to remove dissolved ions from nanoparticle dispersions. Membrane filtration is an effective method at removing ions but it is costly to perform due to the price of filters which must be replaced routinely.[122] Chemical precipitation is a commonly used method to remove unwanted ions. However, this method is not amenable for NP dispersions because the precipitatant can coat and passivate the NP surface thereby altering both dissolution and AOC.[123] Chelators, such as ethylenediamine tetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA), are widely used in aqueous environments due to their strong ability to sequester metal ions.[124] Chelators can bind most metal ions to form stable and soluble complexes.[125,126] Moreover, sorption of the entire complex of metal ions bound to EDTA or DTPA onto the NP surface is low allowing for minimal impact to the NP surface.[125] While dissolution rates can be enhanced by chelators, high levels of chelators can be maintained to continually sequester released ions. In addition, chelators not only remove ions released from the NP surface but also provide stability to NP suspensions through electrostatic repulsion. Finally, the low cost of chelators makes them a more advantageous method to remove ions from a NP suspension than chemical precipitation or membrane filtration.[126]

CHAPTER 2

Commonly used nanoparticle stabilizers and chelators alter the antioxidant activity of gold and silver nanoparticles

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ABSTRACT

The ability of silver and gold nanoparticles (Ag/AuNPs) to readily take part in reduction/oxidation (redox) reactions makes them of great interest in designing therapeutics to scavenge free radicals and quench high levels of reactive oxygen in biological systems. In the present study, the antioxidant capacity of Ag/AuNPs was investigated using a modified Trolox equivalent antioxidant capacity (TEAC) assay. In this assay, NPs acting as antioxidants were added to a pre-formed radical solution and assessed for quenching of the radicals spectrophotometrically. Two commonly used NP stabilizers (citrate and phosphate buffer saline, PBS) and two different chelators [ethylenediaminetetraacetic acid (EDTA), diethylenetriamine pentaacetic acid (DTPA)] were used to examine their impact on the antioxidant capacity (AOC) of Ag/AuNPs. The AOC exhibited by the AuNP was more pronounced than AgNP when ions were not accounted for in the reaction. When the released Ag ions were removed by the addition of chelators into the dispersion, the AOC of the AgNP was higher than the AuNP. While the AOC of Ag/AuNPs was enhanced by both EDTA and DTPA, ion chelation by DTPA increased the AOC of the NPs to a greater extent than EDTA. A comparison of the stabilizer impacts revealed that citrate inhibited the antioxidant activity significantly more than PBS likely due to its inherent reducing activity. Here, we discuss the impacts of stabilizers and chelators on the antioxidant activity of Ag/AuNPs and propose a mechanism by which they alter NP redox reactions.

Key words ABTS, TEAC, gold, silver, nanoparticle, chelators, antioxidant capacity.

2.1 Introduction

Nanoparticle (NP) antioxidants may offer significant advantages over traditional antioxidant therapies for disease prevention and treatment. The high surface area available to take part in redox reactions and the ability to precisely engineer the materials at a biologically relevant scale may enable them to act as more effective therapeutics.[1] Oxidative stress, a disease caused by overproduction of reactive oxygen species (ROS), a type of free radical produced *in vivo*[2,3], can lead to toxicity and result in oxidative damage,[4] inflammatory reactions,[5,6] necrotic cell death,[7,8] and DNA damage.[9] Due to the disadvantages of natural antioxidants in clinical trials, it is promising to develop and evaluate novel engineered NP for their usefulness in the design of antioxidant therapeutics.[10-13]

Among the NP antioxidants presently being considered, silver and gold nanoparticles (Ag/AuNPs) have been the most extensively studied and used because of their multidimensional uses in the biomedical field [14-19]. In recent years, a few studies investigated their antioxidant capacities (AOC) utilizing various antioxidant assays to estimate their ability of scavenging free radicals *in vitro*.[20-22] Dauthal et.al used 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) **(**$ABTS^{2-}$) and 2,2-diphenyl-1-picrylhydrazyl (DPPH), the chemicals for stabilizing free radicals, as probes to investigate the AOC of both Ag/AuNPs. The study reported that AuNP had higher AOC than AgNP.[20] When the antioxidant activity of AgNP was investigated using the DPPH assay, the results showed that coated silver had antioxidative potential similar to that of standard Trolox at concentration higher than 100 μg/mL.[21] In addition, Mani et al. found that AgNP could be a neoadjuvant antioxidant offering effective protection from free radicals, and AOC was quantitatively measured using DPPH assay.[22]

Given the importance of Ag/AuNPs as prospective therapeutics, several data gaps need to be filled. Although the aforementioned studies reported on differences between the AOC of AuNP and AgNP, the reasons for those observed differences were not formally studied, such as the influence of the constituent atomic properties, surface chemistry and ion dissolution rate. Since the valence state of the ions released from NPs are directly related to the number of electrons transferred, we hypothesized that the valence of ions released from NPs could be used to predict their theoretical AOC. Differences in AOC between Au and AgNP could also be attributed to their surface chemistry once dispersed in solution.[23,24] Common stabilizers used on AgNP such as citrate and PBS allow conjugation with ionic ligands to create electric repulsion, and thus, stabilization of the NPs.[25] However, it was unclear if the interaction between

stabilizers and the surface of NP could influence their AOC. Therefore, in this study, AgNP stabilized by citrate and PBS were investigated to better understand the influence of stabilizers on AgNP. We hypothesized that different stabilizers could affect the ion dissolution of the NP and alter NP AOC.[26] In simple media (pure water or buffer solution), ions released from NPs would adsorb onto the NP surface and inhibit further dissolution of ions, potentially altering NP AOC.[27] When NP releases ions into more complex solutions that include biological entities, these ions can be consumed by local biomolecules, changing the dissolution rates and altering NP AOC.[28] Therefore, there is a need to eliminate the ions as confounding factors in the assay in order to better approximate the inherent AOC of NP in solution.

There are several methods that can be used to remove dissolved ions from NP dispersions. Membrane filtration is an effective method at removing ions but it is costly to perform due to the price of filters which must be replaced routinely.[29] Chemical precipitation is a commonly used method to remove unwanted ions. However, this method is not amenable for NP dispersions because the precipitant can coat and passivate the NP surface thereby altering both dissolution and AOC.[30] Chelators, such as ethylenediamine tetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA), are widely used in aqueous environments due to their strong ability to sequester metal ions.[31] Chelators can bind most metal ions to form stable and soluble complexes.[32,33] Moreover, sorption of the entire complex of metal ions bound to EDTA or DTPA onto the NP surface is low allowing for minimal impact to the NP surface.[32] Finally, the low cost of chelators makes them a more advantageous method to remove ions from a NP suspension than chemical precipitation or membrane filtration.[33] The time of interaction between NP and chelator was considered. Two methods including normal and preincubation method were introduced to measure the effect of interaction time between NP and chelator on their AOC. Therefore, in this study, we hypothesized that the addition of chelators would reduce the concentration of ions around the surface of NP, inducing further ion releasing from Ag/AuNPs. Thus, we predict that the AOC of Ag/AuNPs would be enhanced by the addition of chelators.

The AOC of Ag/AuNPs with various chelators was assessed using a Trolox equivalent antioxidant capacity (TEAC) assay since it was low cost and sensitive to free radical compared to the DPPH assay.[20,34] It depends on measuring $ABTS^{2-}$ oxidation into a persistent radical form, ABTS radical by electron transfer reaction.[35] Our modified method focuses on the scavenging activity of the NPs on preformed ABTS radicals (ABTS radical), rather than the inhibition of ABTS radical formation.[36] This method provides insight into the action of experimental analytes on persistent radicals without competitive formation of artifacts by measuring the ability of Ag/AuNPs to quench ABTS radical alone rather than ABTS radical and free radicals competitively.[36] The AOC was determined by measuring the

ability of Ag/AuNPs to quench $ABTS^{\bullet-}$ alone. In order to avoid the confounding factor of ions released from the NPs, we used different chelators to remove ions as they are released from the NPs.

2.2 Result and Discussion

2.2.1 Comparison of AuNP and AgNP

A TEAC assay was used to examine the AOC of AuNP stabilized with sodium citrate and AgNP stabilized with phosphate buffered saline solution (PBS) or sodium citrate. These stabilizing agents adsorb onto the NP surface, causing an increase of the electric repulsion between particles, providing increased solution stability. Once on the NP surface, stabilizers such as PBS and citrate are held in place by covalent bonding or electrostatic interactions.[37,38] The calibration curve developed to convert $ABTS^{\bullet-}$ formation to AOC in the form of Trolox equivelants had a high linear relationship (R-sqr = 0.9998).[39] Total AOCs measured as the time-dependent scavenging capacity for Ag/AuNPs are shown in **Fig. 1**. All samples exhibited a significant increase in antioxidant activity compared to the blank $ABTS^{\bullet-}$ solution. In the first 2 minutes, as Ag/AuNPs were added into the solution, the concentration of surface ions was too low to inhibit ion released from NP. This resulted in the fast quenching reactions observed during this time, which later slowed due to the ionic absorption on the surface of the NPs (**Fig. 2**).[25] The total AOC of citrate stabilized AuNP increased by 20% in 10 minutes to 18.1±0.4 μM Trolox, which was higher than both AgNP stabilized by phosphate buffer saline solution (AgNP-PBS, 13.7±0.3 μM Trolox) and AgNP stabilized by sodium citrate (AgNP-Citrate, 11.0±0.5 μM Trolox) as previous reported.[20] The reactions showed 1st order kinetics with respect to $ABTS^{\bullet-}$ reduction which was similar to previous studies.[40-43] Since the $ABTS^{\bullet-}$ concentration throughout the experiment was larger than the NP concentration, this 1st order behavior also indicated passivation of the NP surface and prevention of further metal oxidation.

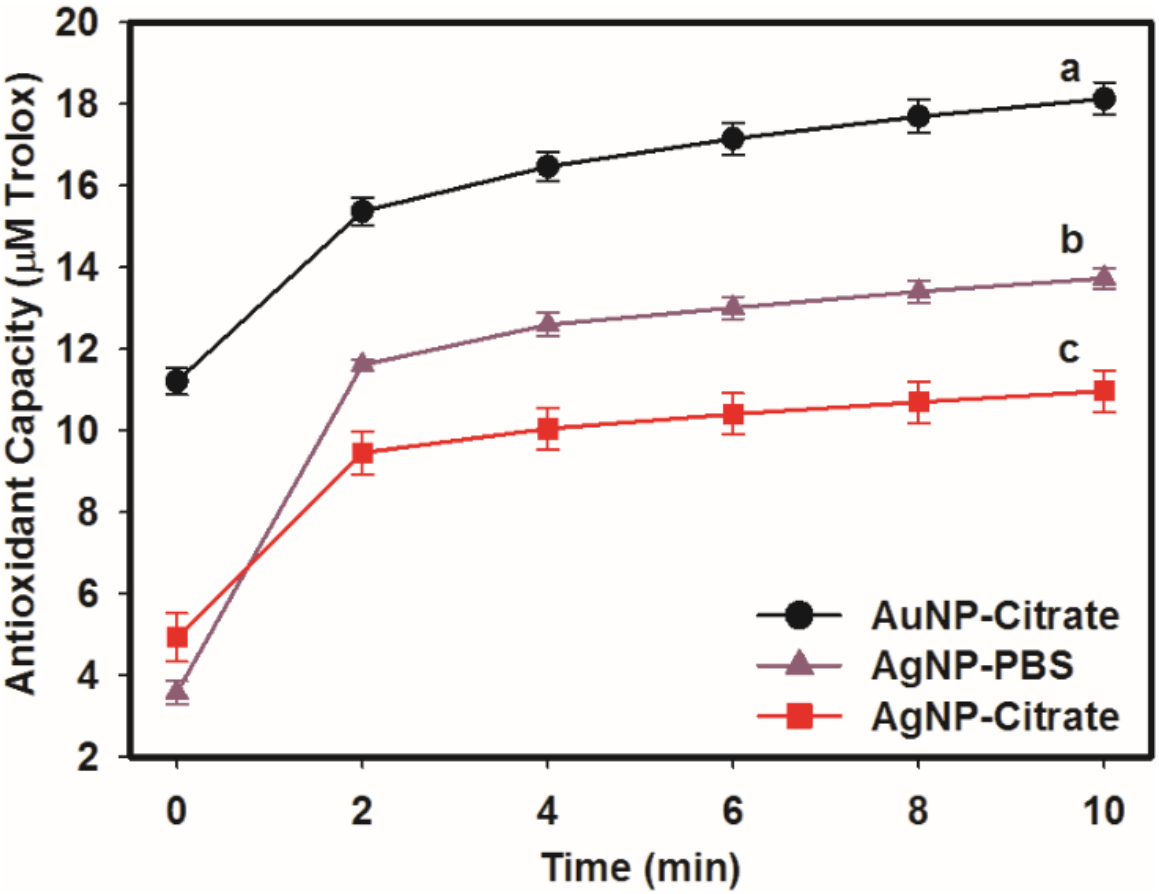

Figure 1. Antioxidant capacity (AOC reported as Trolox equivalents) of Ag/AuNPs. Data are presented as the mean ± SEM. Different letters indicate significant differences in AOC between two nanoparticles at 10 min ($P < 0.05$).

The reaction kinetics of the native metal species were modeled by exponential regression to find the overall reaction rate constant. The reaction rate was markedly lower for AuNP (0.42 minute^{-1}) than both AgNP stabilized with PBS and citrate (0.82 minute^{-1} and 0.71 minute^{-1}, respectively), indicating a lower dissolution rate. However, the higher AOC of AuNP may be explained by the higher valence of the metal product, the AuNP with the Au^{3+} ionic state being prevalent in solution had a higher AOC than the AgNP that release predominantly Ag^{1+} ionic species. In other words, 1 unit of AuNP is needed to donate 3 electrons to produce 1 unit gold ion, whereas 1 unit of AgNP can donate less than 3 electrons to produce 1 unit silver ion. Thus, in the process of producing the same concentration of ions, AgNP donate less electrons than AuNP. Our results indicate that the AOC of the metal was dominated by the metal's final stable valency as we predicted.

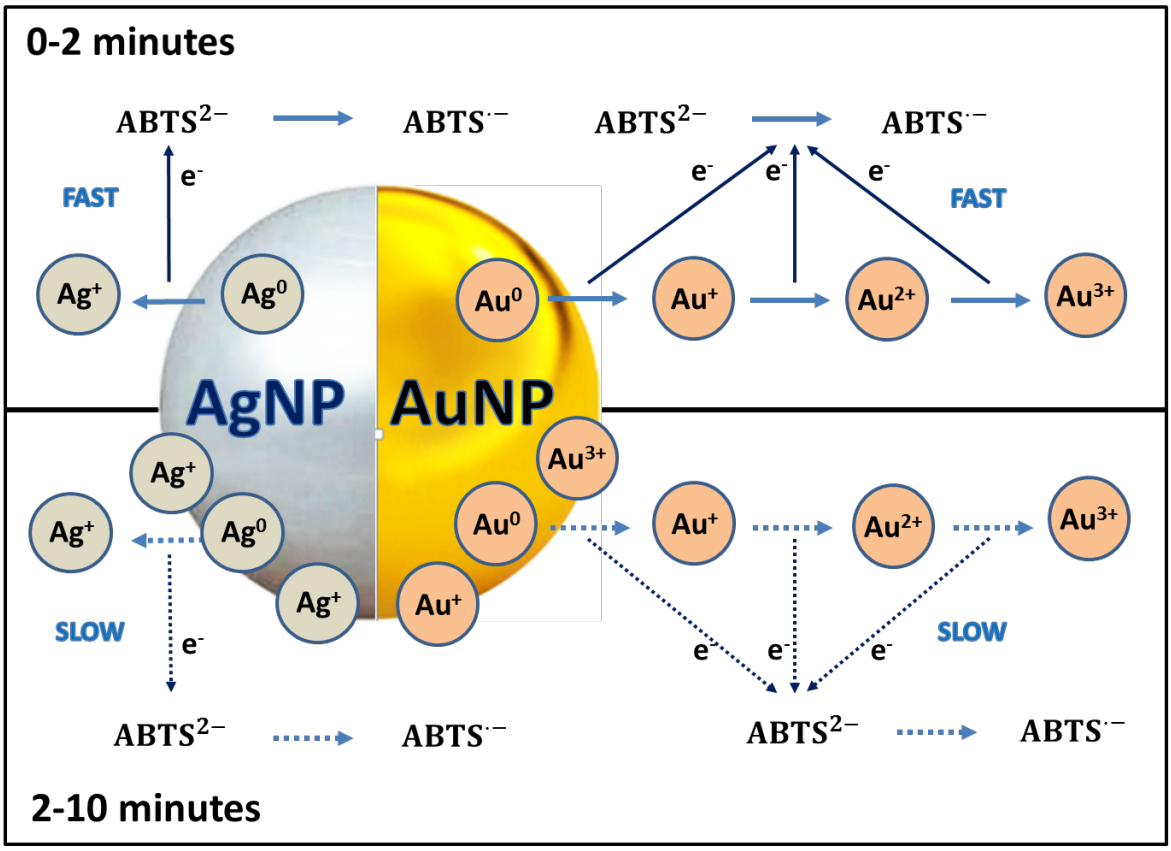

Figure 2. Free radical quenching reaction process and mechanism of Ag/AuNPs during 0-2 minutes and 2-10 minutes.

2.2.2 Impact of stabilizers on AOC of NP

Citrate, which differs from PBS as a universal reducing agent,[2,7] did not significantly alter AOC compared to blank $ABTS^{\bullet-}$ solution (data not shown) likely due to its insensitivity to $ABTS^{\bullet-}$. **Fig. 3** shows that the AOC of AgNP-Citrate was less than AgNP-PBS just as we predicted. The reason is that silver ions can be reduced to zero-valent silver by citrate and results in an offset against the oxidation of AgNP.[44,45] The presence of citrate in the assay reduced the AOC (10.0±0.4 μM Trolox) and reaction rate of AgNP-PBS, see **Fig. 3**, further demonstrating the inhibiting capacity of citrate on released silver ions.

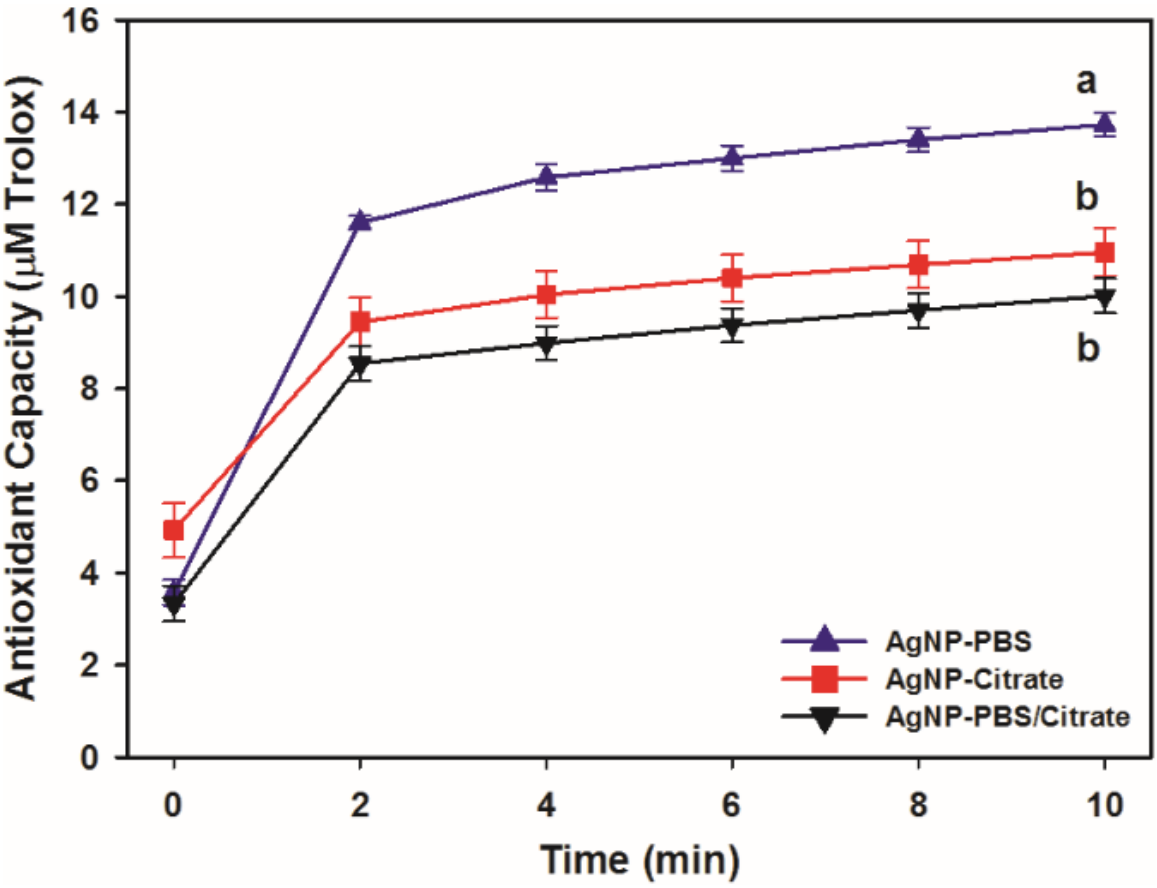

Figure 3. AOC of AgNP capped with PBS, citrate, and Ag-PBS with citrate treatment. Data are presented as the mean±SEM. Significant differences in AOC between each group at 10 min are indicated in the figure as a and b.

2.2.3 The Inherent AOC of Chelators

Two chelators (DTPA, EDTA) were investigated because of their prevalence in NP applications and in aqueous environments for metal chelation.[46-49] DTPA, which has more tertiary amine groups than EDTA, was observed to have significantly greater AOC than EDTA (DTPA, 27.9±0.5 μM Trolox; EDTA, 15.1±0.6 μM Trolox). This is similar to previous reports using the $ABTS^{2-}$ decoloration assay.[50] Balcerczyk et.al. previously demonstrated that the AOC of nitrilotriacetic acid (NTA), EDTA, and DTPA was proportional to the number of amines (1,2, and 3 respectively).[49] Taken together, these results suggested that with similar structure, the AOC could be driven by the oxidation of amino groups (**Fig. 4**). In addition, decay in the presence of DTPA and EDTA was nearly constant, indicating that neither chelator was likely to form complexes with $ABTS^{\bullet-}$ and hinder the electron transfer process.

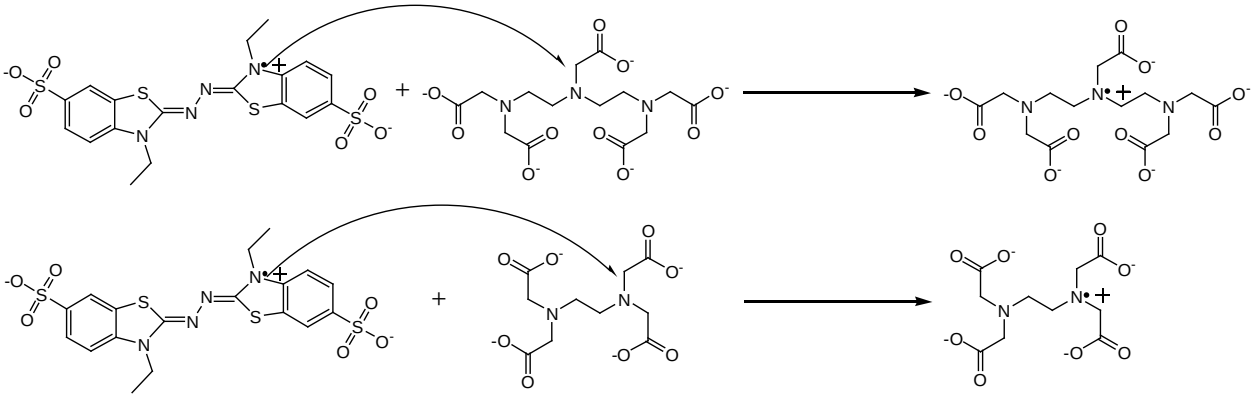

Figure 4. Proposed mechanism for radical transfer from $ABTS^{\bullet-}$ to DTPA, EDTA in ascending order. ABTS was omitted from the products for brevity.

2.2.4 AOC of AuNP/chelators with Long Term Interaction

Preincubation method was used to examine the influence of long term interaction between AuNP and chelators. The impact of preincubating AuNP with the DTPA on measured AOC (AuNP/DTPA) was shown in **Fig. 5A**. The AOC of AuNP/DTPA (19.9±0.3 μM Trolox) increased by 10% relative to the AuNP alone (18.1±0.4 μM). The likely explanation for this is that DTPA can sequester the released ions and facilitate oxidation of the NP surface, thus enhancing the perceived NP AOC. However, **Fig. 5B** shows the influence of EDTA on the AOC of AuNP in which no significant difference was observed between AuNP and AuNP/EDTA until 10 minutes in the reaction at which time the presence of EDTA led to a significant increase the AOC of AuNP by 8% compared to the NP alone (19.6±0.2 μM Trolox). The similar result that EDTA enhanced the dissolution rate of gold was also found in previous study.[51] This may be explained by the fact that gold ions can form a stable complex with DTPA but not EDTA under the conditions of the TEAC assay. After adding AuNP to the solution, the surface of AuNP is oxidized by the ABTS radical and releases Au^{+}, which is at once stabilized by soft sulfur donor on $ABTS^{2-}$ and then further oxidized by ABTS radical to Au^{3+}, and chelated by DTPA to form more stable complexes (**Fig. 6A**). In this process, DTPA likely plays a role in stabilizing Au^{2+} long enough to make it be further oxidized to Au^{3+}. However, EDTA's Au^{2+} complexes may not be as stable as DTPA, potentially allowing for a reversible reaction between EDTA and $ABTS^{2-}$ complexes of Au^{2+} **(Fig. 6B)**. Comparing **Figs. 4A** and **B**, the impact of DTPA and EDTA on the AOC of preincubated AuNP suggests that with a significant period of mixing, the effect of DTPA in enhancing gold oxidation was significantly better than EDTA prior to 6 min. Since DTPA has more coordinating centers, it may be more effective and fully sequestering high valent ionic gold, and therefore preventing reduction to lower oxidation states compared to EDTA.[53] However, no difference was observed between AuNP/DTPA and AuNP/EDTA after 6 minutes. The probable reason is the dissolution of AuNP is inhibited by the complexation of gold ion

and EDTA, or the dissolution rate of AuNP is negligible during the experiment, few gold is present after 6 minutes, meaning that the AOC would mostly come from free EDTA in solution.

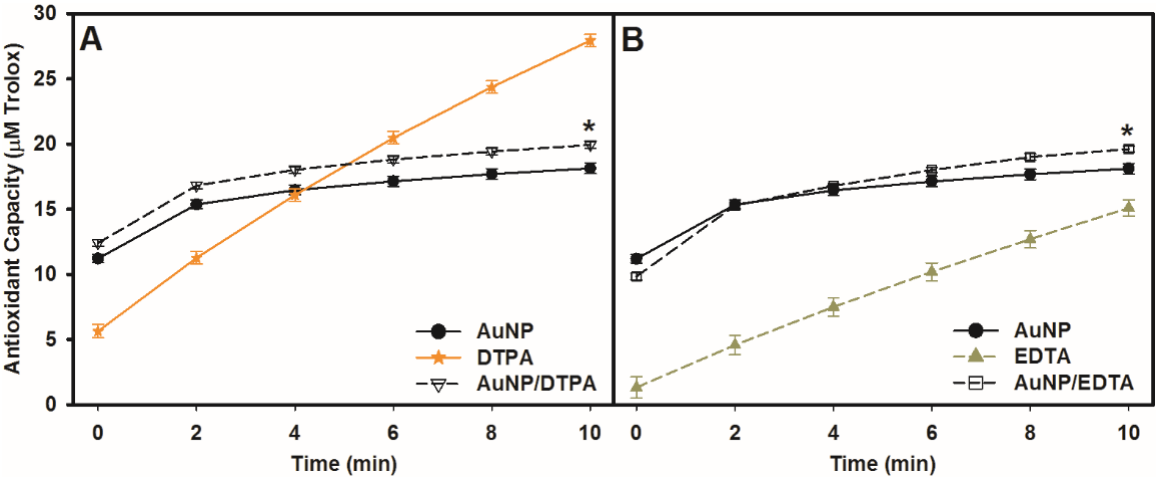

Figure 5. Antioxidant capacity (AOC reported as Trolox equivalents) of AuNP **(A)** with (open triangles) and without (closed circles) incubation with the chelator DTPA (stars) and **(B)** with (open squares) and without (closed circles) EDTA (triangle). AuNP/DTPA and AuNP/EDTA represent preincubation of the AuNP with DTPA/EDTA for 24 hours before adding them together to solution. Data are presented as the mean ± SEM. * represents a significant difference in AOC between AuNP and AuNP with DTPA/EDTA at 10 min.

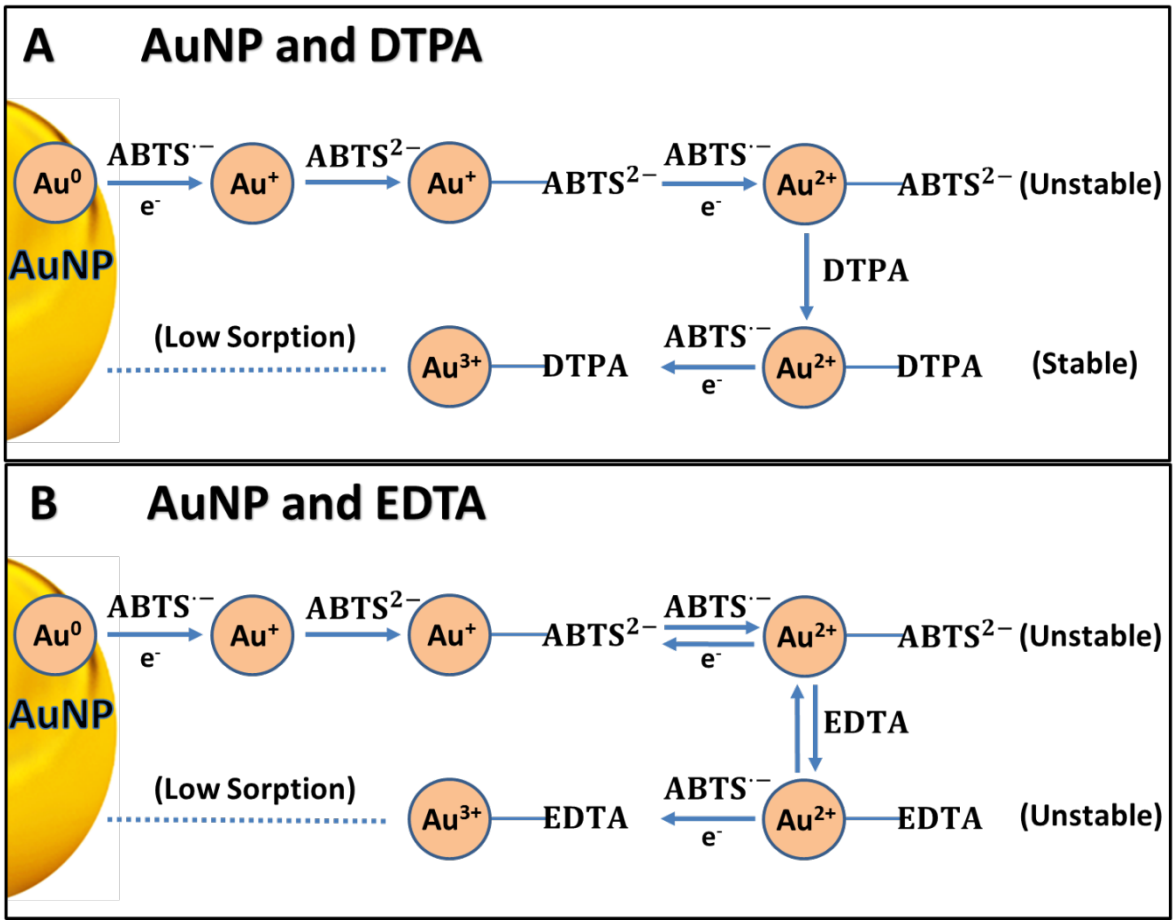

Figure 6. Reaction process and proposed mechanism of antioxidant activity of AuNP preincubated with **(A)** DTPA and **(B)** EDTA.

2.2.5 AOC of AgNP/chelators with Long Term Interaction

The AOC of AgNP capped with either citrate or PBS, was assessed for the impact of chelators (DTPA/EDTA) on their AOCs under preincubation method. The results in Fig. 7 suggest that the addition of chelators significantly increased the AOC of AgNP, which was also supported in previous studies that EDTA could enhance the dissolution rate of AgNP.[53,54] In **Fig. 7A**, the difference between AgNP-PBS and AgNP-Citrate was minimal with the addition of DTPA (54.3±1.1 µM Trolox for AgNP-PBS and 51.0±0.8 µM Trolox for AgNP-Citrate), indicating that the NP stabilizers effect is largely negligible compared to the strong chelating effect of DTPA. However, in **Fig. 7B**, EDTA was found to enhance the AOC of AgNP-PBS much more than that of AgNP-Citrate (29.4±0.0 µM Trolox for AgNP-PBS and 25.3±0.2 µM Trolox for AgNP-Citrate), suggesting that the reducing effect of citrate on dissolution rate of AgNP becomes significant in the presence of EDTA. Overall, the effect of EDTA on the AgNP AOC was less

pronounced than that of DTPA just as it was for the AuNP, likely due to the lower stability constant of silver ion complexed with EDTA (7.32) than DTPA (8.70).[56]

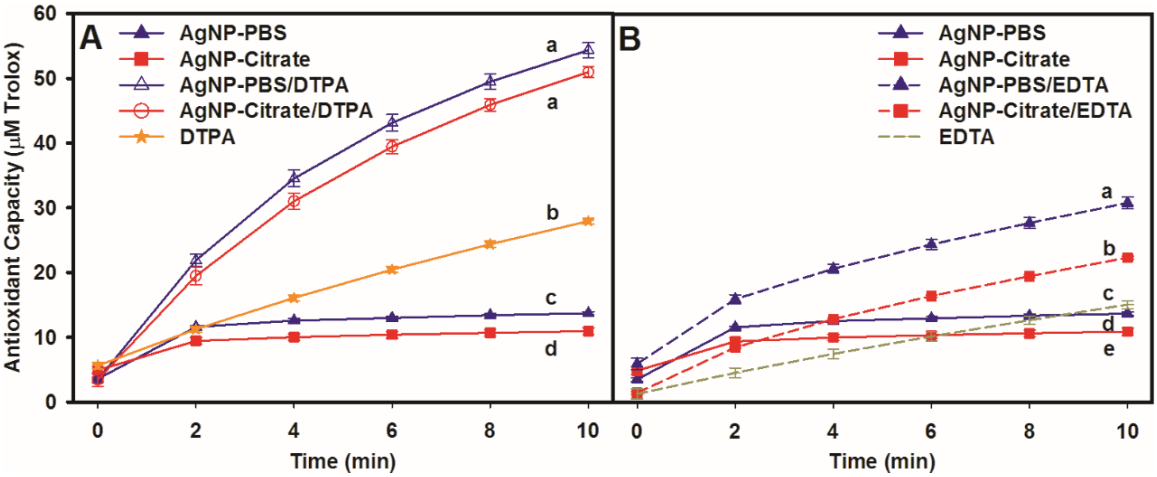

Figure 7. (A) Antioxidant capacity (AOC) of AgNP with PBS or citrate capping agents, with and without DTPA. **(B)** AOC of AgNP with PBS or citrate capping agents, with and without EDTA. Data are presented as the mean±SEM. Different letters indicate significant difference in AOC between each group at 10 min.

The impact of chelators on the AOC of AgNP was greater than AuNP possibly due to the fact that dissolution rate of AgNP is much faster than AuNP, allowing reduction to occur at a higher rate. When AuNP reacted with chelators, a significant reduction of the AOC of chelators was observed, see **Fig. 5**. One possible explanation for this may be that AuNP consumes more chelating groups (carboxyl and tertiary amine) than AgNP due to its higher valence releasing ion and reduces the AOC of chelators. In addition, as the dissolution rate of AuNP is low, the increasing AOC from AuNP is not enough to offset the reduction of AOC from the chelators completely. In contrast, the increasing AOC from AgNP is enough to offset the reduction of AOC from chelators due to its high dissolution rate, see **Fig. 5**.

2.2.6 Long Term vs. Short Term

A normal method of adding chelators just prior to NPs in the solution (AuNP-chelator, AgNP-chelator) was used to assess the short term impact of chelators on NP AOC and compare with long term preincubation method. **Fig. 8** showed the results of the preincubation versus normal method. For AuNP and DTPA with normal method, a higher AOC (25.4±0.1 μM Trolox) appeared compared to the preincubation method (19.9±0.3 μM Trolox). Normal method with EDTA; however, showed no effect on AuNP (19.4±0.5 μM Trolox) compared to the preincubation method (19.6±0.2 μM Trolox). The same

results were also observed in the samples of AgNP with chelators except AgNP-PBS with EDTA as shown in **Fig. 8**. It was notable that AgNP had a higher AOC with DTPA using the normal method compared to the preincubation method regardless of the capping agent. In the experiment, ABTS radical was consumed completely by 6 minutes (80.5±0.0 µM Trolox). Incubation with EDTA using the normal method increased the AOC of AgNP-Citrate compared to the preincubation method (25.3±0.2 µM Trolox for normal method; 22.4±0.2 µM Trolox for preincubation). However, no differences in incubation methods was observed from AgNP-PBS and EDTA.

2.2.7 Summary

As **Fig. 9** shows, in preincubation method, NP interact with chelator for longer time than normal method, thus lead to the higher concentration of chelators with more anion ligands bonding to the surface of NP, so chelators knocked off citrate and recapped on the surface instead. The high concentration of chelators inhibits dissolution rate and results in lower AOC. However, in normal method, the low concentration of chelators only played a role of chelating and removing surface ion released from NP, which enhanced the AOC of NP. Similar responses were reported for assessing the solubility of AgNP stabilized with oleic acid.[56] We hypothesized this mechanism could explain the similar result of AuNP & DTPA, AgNP-Citrate & DTPA, and AgNP-PBS & DTPA. The reason for the insignificant result of AuNP & EDTA and AgNP-PBS & EDTA is not clear, one plausible explanation may be due to the fact that the interaction of EDTA and AuNP is weaker than EDTA and AgNP.[57] It is more difficult for gold to bind with the carboxyl group in EDTA since gold ion is softer Lewis acid compared to silver.[58] Similarity, PBS can bond with AgNP surface better than citrate since phosphate is softer Lewis base compared to the carboxyl group in citrate, which makes it easy to bond with soft Lewis acid AgNP surface.[57,59]

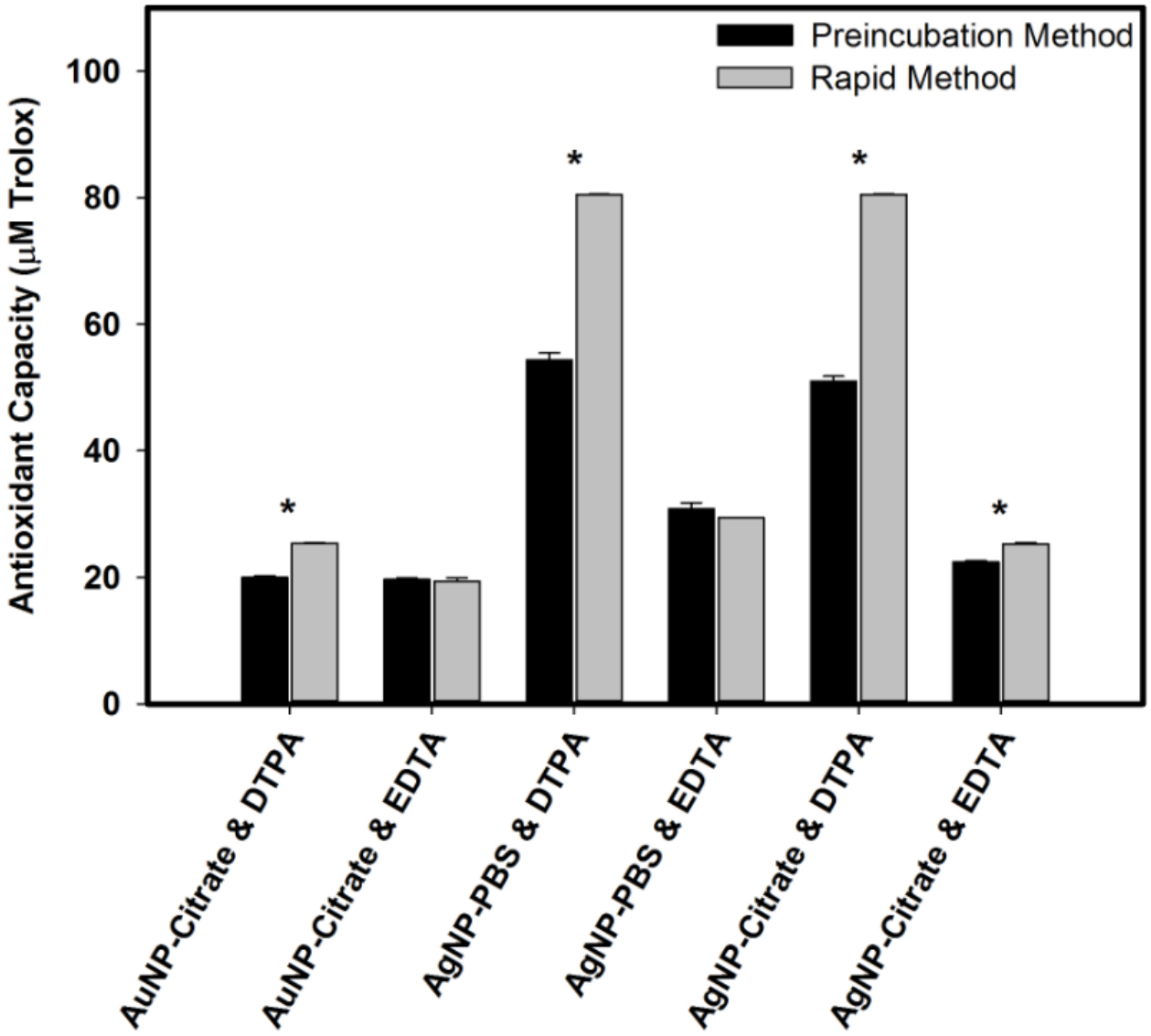

Figure 8. The AOC of nanoparticles using either the normal or preincubation method of Ag/AuNPs and chelators (Ag/AuNPs & chelators). Data are presented as the mean±SEM. * represents a significant difference in AOC between normal and preincubation method.

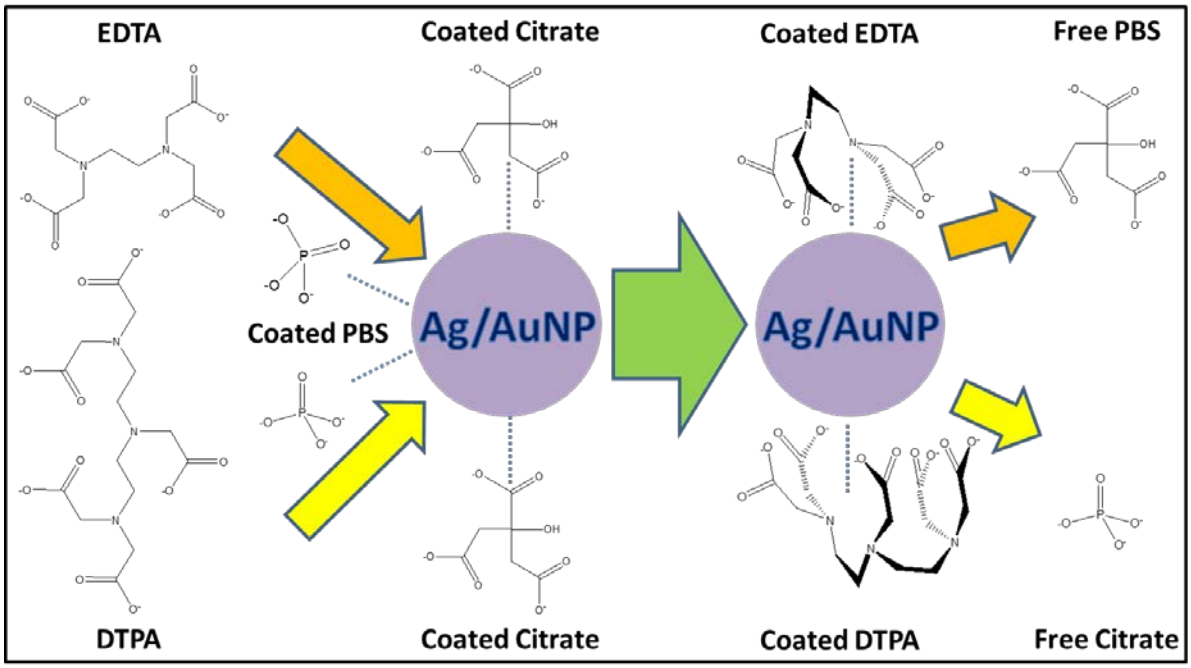

Figure 9. A proposed mechanism of anion exchange processes on Ag/AuNPs with stabilizers and chelators.

2.3 Conclusion

In this study, we mainly investigated i) differences in AOC between AuNP and AgNP, ii) the impact of stabilizers on AOC of Ag/AuNPs and iii) the impact of chelators on AOC of Ag/AuNPs. All of the results are shown in **Fig. 10**. The results show that AOC of AuNP is higher than AgNP. In addition, citrate as a stabilizer could inhibit AOC of AgNP more than PBS. Moreover, the influence of DTPA is more pronounce than EDTA on NP AOC. Given the same mass concentration, the maximum AOC of NP depends on the valence of predominant ion released from them which is influenced by both the redox property of stabilizer and the interaction of stabilizer with the NP surface. Chelators are an effective method to remove the surface ions from NPs in order to better estimate the inherent AOC of NPs. This study contributes to our understanding of the antioxidant activity of Ag/AuNPs which could be beneficial for additional promising biomedical applications. In addition, the interaction of Ag/AuNPs and chelators could also be further applied to investigate the interaction of NPs and chemicals with chelating structure including amino groups in fields such as phytochelatin, hemoglobin and chlorophyll. This would be benefit to study the interaction between NP and biological system including those chemicals.

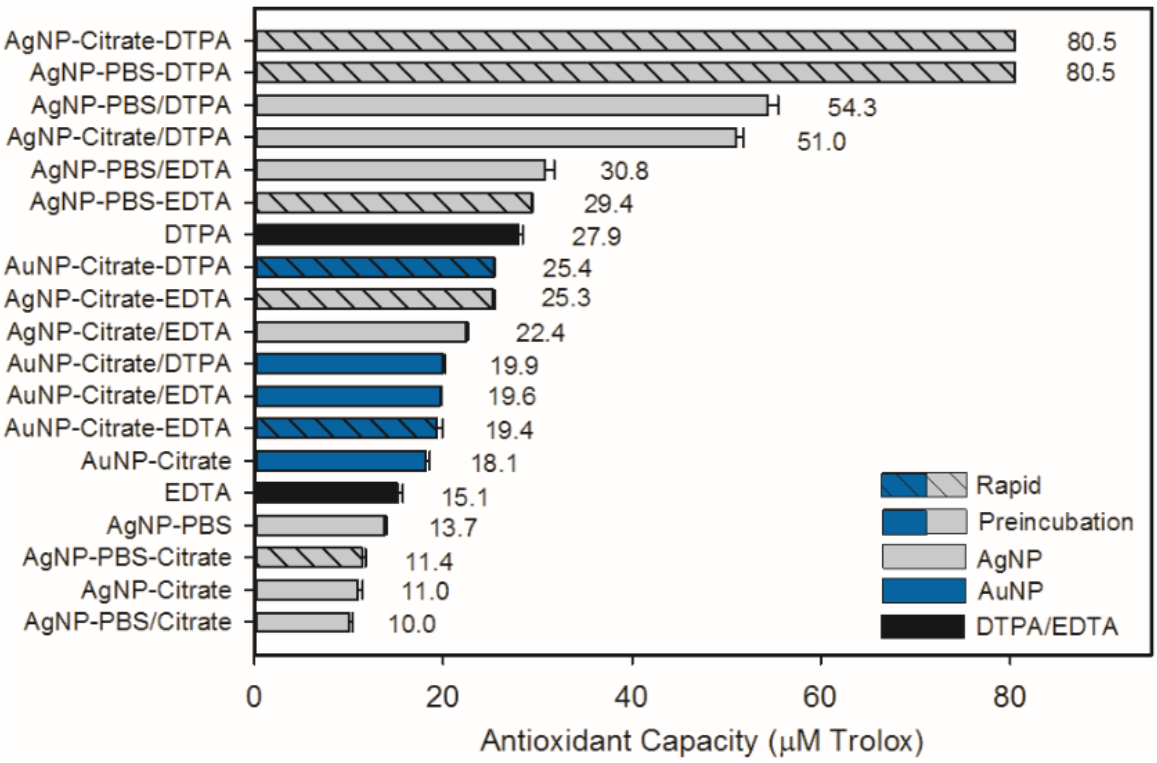

Figure 10. The AOC of Ag/AuNPs, chelators (DTPA/EDTA) and the interaction of Ag/AuNPs and chelators and stabilizer (DTPA/EDTA/citrate). Preincubation is represented by hashmarks, open bars represent normal method. Data are presented as the mean±SEM.

2.4 Methods

2.4.1 Chemicals

All chemicals were reagent grade or higher. Reagents purchased from Sigma Aldrich (St. Louis, MO) include: $ABTS^{2-}$ (2, 2'-azinobis-(3-ethylbenzthiazoline-6-sulfonate acid), 98%) obtained as diammonium salt, HRP (Horseradish peroxidase) obtained as lyophilized powder, EDTA (ethylenediaminetetraacetic acid disodium salt dihydrate, 99%-101%), and DTPA (diethylenetriamine pentaacetic acid, 98+%). Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) was purchased from Calbiochem (Darmstadt, Germany). Nanoparticles were purchased from nanoComposix (San Diego, CA): AgNP (Silver, 90nm, Biopure, 0.86 mg/ml) as dispersions in with PBS (phosphate buffer saline) capping agent, citrate -AgNP (silver, 100nm, Biopure, 1.0 mg/ml) as dispersion with citrate stabilization, AuNP (gold, 100 nm, 0.05 mg/ml). H_2O_2 (hydrogen peroxide, 30%, v/v) and citric acid (Monohydrate, granular, 99.7%) were obtained from Mallinckrodt Chemicals (Phillipsburg, NJ). Copper chelating resin (50% slurry in 20% ethanol/water) was purchased from G-Biosciences (Maryland Heights, MO). Na_2HPO_4 (sodium phosphate, dibasic anhydrous, granular, 99.1%) and sodium hydroxide (Pellet,

clinical requirements) were purchased from Macron Chemicals (Center Valley, PA), and NaH_2PO_4 (Sodium phosphate, monobasic anhydrous, 99%) was from Fisher Scientific (Fair Lawn, NJ). UW (ultrafine water) was purchased from Fisher Scientific (St. Clara, CA).

2.4.2 Measurement of Total Antioxidant Capacity

1 mM Trolox was prepared by dissolving Trolox powder into UW. All buffer solutions used in this assay were first purified with metal affinity chromatography using a copper chelating resin in order to remove residual iron and copper impurities. Phosphate buffer saline (PBS) was made by mixing 50mM Na_2HPO_4 and NaH_2PO_4 stock solutions at a ratio of 21:4 by volume. The final solution volume was 1L with a pH of 7.45. HRP was used to cleave H_2O_2 and produce hydroxyl radicals (·OH), which then oxidized $ABTS^{2-}$ to the blue/green colored ABTS radical. After complete conversion to the radical form, an antioxidant agent was added to reduce to $ABTS^{2-}$ by donating electrons. The reaction rate was measured over time as the change in absorbance at 734 nm wavelength. A calibration curve was made for 1 mM $ABTS^{2-}$ with 4 U/ml HRP (one enzyme unit (U) is defined as the amount of the enzyme that catalyzes the conversion of 1 micromole of substrate per minute) and a series of H_2O_2 concentrations (0 ~ 131 μM). A 1 ml final $ABTS^{2-}/H_2O_2$/HRP working solution (solution) was comprised of 1 mM $ABTS^{2-}$, 91 μM H_2O_2, 4 U/ml HRP and 50 mM PBS (pH = 7.45) which had an absorbance around 1.0. Temperature was held at 26±1℃ and reaction time was 10 minutes. For the AOC assay, solution without NP served as a control. A calibration curve of a series concentration of Trolox (0 ~ 75 μM) vs. $ABTS^{\bullet-}$ absorbance was made to determine the relationship between absorbance change and an equivalent concentration of Trolox.

Table 2. Matrix of test solvents used to dilute NP stocks for testing by modified TEAC assay.

Solute-Stabilizer	Solvents		
	Water	Chelator	
AgNP-PBS	UW	EDTA	DTPA
AgNP-Citrate	UW	EDTA	DTPA
AuNP-Citrate	UW	EDTA	DTPA

Stock 2mM solutions of DTPA, EDTA and citrate (as citric acid and sodium hydroxide) were prepared in UW. Nanoparticle stock solutions were prepared at 40 mg/L using UW, EDTA, DTPA or Citrate as described in **Table 1.** In the first set of experiments we added chelators to the solution 10 seconds prior to the NPs (normal method), and the second was to mix the NPs with the chelators for 24 hours before testing them in the solution (preincubation method). The total reaction solution volume was

1 ml. The concentration of NPs in solution was 1.6 ppm and 0.08 mM for both chelators and citrate. A SpectraMax M2 spectrophotometer (Molecular Devices, Sunnyvale, CA) was used to test the decrease in absorption at 734 nm for 10 minutes in order to measure the AOC of NPs. All experiments were performed in triplicate.

2.4.3 Statistical Analysis

Data are expressed as mean ± standard error of the mean (SEM). Statistical differences between two samples were tested using one-way ANOVA using SigmaPlot 12.5 (San Jose, CA) with significance determined at $P \leq 0.05$.

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References

1. Kovacic, P.; Somanathan, R. Biomechanisms of Nanoparticles (Toxicants, Antioxidants and Therapeutics): Electron Transfer and Reactive Oxygen Species. Journal of Nanoscience and Nanotechnology 2010, 10, 7919-7930.

2. Halliwell, B. Free Radicals and Antioxidants: A Personal View. Nutrition reviews 1994, 52, 253 - 265.

3. Myrtill Simkó, A. G.; Ulrich Fiedeler, M. N. Nanoparticles, Free Radicals and Oxidative Stress. 2011.

4. Mark K. Shigenaga; Hagen, T. M.; Ames, B. N. Oxidative Damage and Mitochondrial Decay in Aging. P Natl Acad Sci USA 1994, 91, 10771 - 10778.

5. Salvatore Cuzzocrea; Michelle C McDonald; Helder Mota Filipe; Giuseppina Costantino; Emanuela Mazzon; Sabrina Santagati; Achille P Caputi; Thiemermann, C. Effects of Tempol, A Membrane-Permeable Radical Scavenger, in a Rodent Model of Carrageenan-Induced Pleurisy. European journal of pharmacology 2000, 390, 209 - 222.

6. Leiro, J. Effects of Cis-Resveratrol on Inflammatory Murine Macrophages: Antioxidant Activity and Down-Regulation of Inflammatory Genes. Journal of Leukocyte Biology 2004, 75, 1156-1165.

7. Takeda, M. S., I; Kobayashi, M; Endou, H. Hydrogen Peroxide Induces Necrosis, Apoptosis, Oncosis and Apoptotic Oncosis of Mouse Terminal Proximal Straight Tubule Cells. Nephron 1999, 81, 234 - 238

8. Renz, A. Rapid Extracellular Release of Cytochrome C Is Specific for Apoptosis and Marks Cell Death in Vivo. Blood 2001, 98, 1542-1548.

9. C. E. Richeson; P. Mulder; V. W. Bowry; Ingold, K. U. The Complex Chemistry of Peroxynitrite Decomposition: New Insights. J. Am. Chem. Soc. 1998, 120, 7211 - 7219.

10. Chen, Y.-J.; Yuan, R.-M.; Liu, Y.-J.; Yang, H.-J.; Liang, G.-Y.; Tian, L.-X., Dietary vitamin C requirement and its effects on tissue antioxidant capacity of juvenile largemouth bass, Micropterus salmoides. Aquaculture 2015, 435, 431-436.

11. Hong, R.; Han, G.; M, J.; Ferna´ndez; Kim, B.-j.; Forbes, N. S.; Rotello, V. M., Glutathione-Mediated Delivery and Release Using Monolayer Protected Nanoparticle Carriers. J. Am. Chem. Soc. 2006, 128, 1078-9.

12. Soomets, U.; Zilmer, M.; Langel, l., Manual Solid Phase Synthesis of Glutathione Analogs. In Peptide Synthesis and Applications, Howl, J., Ed. Humana Press: 2005; Vol. 298, pp 241-257.

13. Tobwala, S.; Karacal, H.; Ercal, N., Antioxidant Defense Network in the Lens and Benefits of Glutathione Prodrugs in Cataracts. In Studies on the Cornea and Lens, Babizhayev,

M. A.; Li, D. W.-C.; Kasus-Jacobi, A.; Žorić, L.; Alió, J. L., Eds. Springer New York: 2015; pp 327-359.

14. Kim, J. S.; Kuk, E.; Yu, K. N.; Kim, J.-H.; Park, S. J.; Lee, H. J.; Kim, S. H.; Park, Y. K.; Park, Y. H.; Hwang, C.-Y.; Kim, Y.-K.; Lee, Y.-S.; Jeong, D. H.; Cho, M.-H., Antimicrobial effects of silver nanoparticles. Nanomedicine: Nanotechnology, Biology and Medicine 2007, 3, 95-101.

15. Lok, C.-N.; Ho, C.-M.; Chen, R.; He, Q.-Y.; Yu, W.-Y.; Sun, H.; Tam, P. K.-H.; Chiu, J.-F.; Che, C.-M., Silver nanoparticles: partial oxidation and antibacterial activities. JBIC Journal of Biological Inorganic Chemistry 2007, 12, 527-534.

16. Kaegi, R.; Voegelin, A.; Sinnet, B.; Zuleeg, S.; Hagendorfer, H.; Burkhardt, M.; Siegrist, H., Behavior of metallic silver nanoparticles in a pilot wastewater treatment plant. Environ Sci Technol 2011, 45, 3902-8.

17. Lima, E.; Guerra, R.; Lara, V.; Guzmán, A., Gold nanoparticles as efficient antimicrobial agents forEscherichia coliandSalmonella typhi. Chemistry Central Journal 2013, 7.

18. Giljohann, D. A.; Seferos, D. S.; Daniel, W. L.; Massich, M. D.; Patel, P. C.; Mirkin, C. A., Gold nanoparticles for biology and medicine. Angewandte Chemie 2010, 49, 3280-94.

19. Pingarrón, J. M.; Yáñez-Sedeño, P.; González-Cortés, A., Gold nanoparticle-based electrochemical biosensors. Electrochimica Acta 2008, 53, 5848-5866.

20. Dauthal, P.; Mukhopadhyay, M., In-vitro free radical scavenging activity of biosynthesized gold and silver nanoparticles using Prunus armeniaca (apricot) fruit extract. Journal of Nanoparticle Research 2012, 15.

21. Banerjee, U. C.; Mittal, A. K.; Kaler, A., Free Radical Scavenging and Antioxidant Activity of Silver Nanoparticles Synthesized from Flower Extract of Rhododendron dauricum. Nano Biomedicine and Engineering 2012, 4.

22. Mani, A.; S, S. L.; V, G., Bio-Mimetic Synthesis of Silver Nanoparticles and Evaluation of Its Free Radical Scavenging Activity. International Journal of Biological & Pharmaceutical Research 2012, 3, 631-633.

23. Singh, N.; Amateis, E.; Mahaney, J. E.; Meehan, K.; Rzigalinski, B. A., The Antioxidant Activity of Cerium Oxide Nanoparticles is Size Dependant and Blocks Aβ1-42-Induced Free Radical Production and Neurotoxicity. The FASEB Journal 2008, 22.

24. Naveena, B. E.; Prakash, S., Biological Synthesis Of Gold Nanoparticles Using Marine Algae Gracilaria Corticata And Its Application As A Potent Antimicrobial And Antioxidant Agent. Asian Journal of Pharmaceutical and Clinical Research 2013, 6, 179-182.

25. Thanh, N. T. K.; Green, L. A. W., Functionalisation of nanoparticles for biomedical applications. Nano Today 2010, 5, 213-230.

26. Zhang, Z.; Chen, H.; Xing, C.; Guo, M.; Xu, F.; Wang, X.; Gruber, H.; Zhang, B.; Tang, J., Sodium Citrate: A Universal Reducing Agent for Reduction/Decoration of Graphene Oxide with Au Nanoparticles. Nano Research 2011, 4, 599-611

27. Liu, J.; Hurt, R. H. Ion Release Kinetics and Particle Persistence in Aqueous Nano-Silver Colloids. Environmental Science & Technology 2010, 44, 2169 - 2175.

28. Bondarenko, O.; Ivask, A.; Kakinen, A.; Kurvet, I.; Kahru, A., Particle-cell contact enhances antibacterial activity of silver nanoparticles. PLoS One 2013, 8, e64060.

29. Mohammad, A. W.; Hilal, N.; Al-Zoubib, H.; Darwish, N. A.; Ali, N., Modelling the effects of nanofiltration membrane properties on system cost assessment for desalination applications. Desalination 2007, 206, 215-225.

30. Levard, C.; Mitra, S.; Yang, T.; Jew, A. D.; Badireddy, A. R.; Lowry, G. V.; Brown, G. E., Jr., Effect of chloride on the dissolution rate of silver nanoparticles and toxicity to E. coli. Environ Sci Technol 2013, 47, 5738-45.

31. Matsukawa, T.; S. Ikeda, H. I.; Yamada, M., Alleviation of the two-cell block of ICR mouse embryos by polyaminocarboxylate metal chelators. Reproduction 2002, 124, 65–71.

32. Byegatrd, J.; Skarnemark, G.; Skalberg, M., The stability of some metal EDTA, DTPA and DOTA complexes: Application as tracers in groundwater studies. Journal of Radioanalytical and Nuclear Chemistry 1999, 241, 281-290.

33. Sillanpaa, M.; Oikari, A., Assessing the Impact of Complexation by EDTA and DTPA on Heavy Metal Toxicity Using Microtox Bioassay. Chemospher 1996, 32, 1485-1497.

34. Magalhães, L. M.; Segundo, M. A.; Reis, S.; Lima, J. L. F. C., Methodological aspects about in vitro evaluation of antioxidant properties. Analytica Chimica Acta 2008, 613, 1-19.

35. Venkatasubramanian, L.; Maruthamuthu, P. Kinetics and Mechanism of Formation and Decay of 2,2'-Azinobis-(3-Ethylbenzothiazole-6-Sulphonate) Radical Cation in Aqueous Solution by Inorganic Peroxides. International Journal of Chemical Kinetics 1989, 21, 399-421.

36. Rea, R.; Pellegrinia, N.; Proteggentea, A.; Pannalaa, A.; Yanga, M.; Rice-Evansa, C., Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. Free Radical Biology and Medicine 1999, 26, 1231-1237.

37. Ivanov, M. R., Covalently functionalized gold nanoparticles: synthesis, characterization, and integration into capillary electrophoresis. University of Iowa Iowa Research Online 2011.

38. Kakinen, A.; Ding, F.; Chen, P.; Mortimer, M.; Kahru, A.; Ke, P. C., Interaction of firefly luciferase and silver nanoparticles and its impact on enzyme activity. Nanotechnology 2013, 24, 345101.

39. Gupta, R.; Sharma, M.; Lakshmy, R.; Prabhakaran, D.; Reddy, K. S., Improved method of total antioxidant assay. Indian Journal of Biochemistry & Biophysics 2009, 46, 126-129.

40. Lee, Y. J.; Kim, J.; Oh, J.; Bae, S.; Lee, S.; Hong, I. S.; Kim, S. H., Ion-release kinetics and ecotoxicity effects of silver nanoparticles. Environmental toxicology and chemistry / SETAC 2012, 31, 155-9.

41. Damm, C.; Münstedt, H., Kinetic aspects of the silver ion release from antimicrobial polyamide/silver nanocomposites. Applied Physics A 2008, 91, 479-486.

42. Liu, J.; Hurt, R. H., Ion release kinetics and particle persistence in aqueous nano-silver colloids. Environmental science & technology 2010, 44, 2169-2175.

43. Cortada, M. G., L; Costa, S; Gil, FJ; Rodriguez, D; Planell, JA, Metallic ion release in artificial saliva of titanium oral implants coupled with different metal superstructures. Bio-Medical Materials and Engineering 1997, 7, 213-220.

44. Djokić, S., Synthesis and Antimicrobial Activity of Silver Citrate Complexes. Bioinorganic Chemistry and Applications 2008, 2008, 1-7.

45. El-Kheshen, A. A.; El-Rab, S. F. G., Effect of reducing and protecting agents on size of silver nanoparticles and their anti-bacterial activity. Der Pharma Chemica 2012, 4, 53-65.

46. Burnett, L. C, Final Report on the Safety Assessment of Pentasodium Pentetate and Pentetic Acid as Used in Cosmetics. International Journal of Toxicology 2008, 27, 71-92.

47. Caravan, Peter; Ellison, Jeffrey J.; McMurry, Thomas J. ; Lauffer, Randall B., Gadolinium(III) Chelates as MRI Contrast Agents: Structure, Dynamics, and Applications. Chem. Revs. 1999, 99, 2293-2342.

48. Furia, T., EDTA in Foods - A technical review. Food Technology 1964 18, 1874-1882.

49. Shirley, D.G.; Walter, S.J.; Noormohamed, F.H., Natriuretic effect of caffeine: Assessment of Segmental Sodium Reabsorption in Humans. Clinical Science 2002 103, 461–466.

50. Balcerczyk, A.; Sowa, K.; Bartosz, G., Metal Chelators React Also with Reactive Oxygen and Nitrogen Species. Biochem Bioph Res Co 2007, 352, 522-525.

51. D. Feng; Deventer, J. S. J. v., Thiosulphate Leaching of Gold in the Presence of Ethylenediaminetetraacetic acid (EDTA). Minerals Engineering 2010, 23, 143-150.

52. J. Roger Hart., Ethylenediaminetetraacetic Acid and Related Chelating Agents. In Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VCH, Weinheim, 2005.

53. D.M. Puente-Siller; J.C. Fuentes-Aceituno; Nava-Alonso, F., A Kinetic-Thermodynamic Study of Silver Leaching in Thiosulfate-Copper-Ammonia-EDTA Solutions. Hydrometallurgy 2013, 134-135, 124-131.

54. Chappell, M. A.; Miller, L. F.; George, A. J.; Pettway, B. A.; Price, C. L.; Porter, B. E.; Bednar, A. J.; Seiter, J. M.; Kennedy, A. J.; Steevens, J. A., Simultaneous dispersion-dissolution behavior of

concentrated silver nanoparticle suspensions in the presence of model organic solutes. Chemosphere 2011, 84, 1108-16.

55. http://www.dojindo.com/Images/Product%20Photo/Chelate_Table_of_Stability_Constants.pdf.

56. Chappell, M. A.; Miller, L. F.; George, A. J.; Pettway, B. A.; Price, C. L.; Porter, B. E.; Bednar, A. J.; Seiter, J. M.; Kennedy, A. J.; Steevens, J. A., Simultaneous dispersion-dissolution behavior of concentrated silver nanoparticle suspensions in the presence of model organic solutes. Chemosphere 2011, 84, 1108-16.

57. Pearson, R.G. Hard and Soft Acids and Bases, HSAB, Part I. Journal of the American Chemical Education 1968, 45, 581-587

58. Pearson, R.G. Hard and Soft Acids and Bases, HSAB, Part II. Journal of the American Chemical Education 1968, 45, 643-648

59. Pearson, R.G. Hard and Soft Acids and Bases. Journal of the American Chemical Society 1963, 85, 3533-3539

CHAPTER 3

The Antioxidant Activity of Lanthanide Nanoparticles Relates to Their Elemental Periodicity

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Abstract

Lanthanide metal oxide nanoparticles (LnOxNPs) are being exploited for a broad range of catalytic reaction. The catalytic activity between LnOxNPs is different and relates to multiple physicochemical factors. Despite the numerous promising applications of LnOxNPs, design rules for developing materials with optimal antioxidant electrochemistry are not yet defined. There is an immediate need to rapidly assess the physicochemical behavior of these materials in order to identify physicochemical characteristics predictive of anti-oxidative reactivity. In this study, the Trolox Equivalent Antioxidant Capacity (TEAC) assay was used to determine the redox behavior of eight LnOxNPs. We determined the antioxidant activity and normalized it to unit surface area. We assessed the relationships between antioxidant capacity (AOC) and the valance state, the number of unpaired electrons of 4f orbital, ionic radius, band gap, ionization energy, lanthanum-oxygen bond length and electronegativity of the atoms that make up the NP. We hypothesized that the AOC of bare NP was related to their basic elemental periodicity. Two methods were employed to assess the AOC of LnOxNPs: 1) NPs were added to a pre-formed radical solution or 2) NPs were added before the radical formation was initiated by hydrogen peroxide. Our results showed that only CeO_2NP had differed significantly between the two methods; no significance was observed from the others. The AOC of CeO_2NP, Nd_2O_3NP, TbO_2/Tb_2O_3NP, Ho_2O_3NP was reduced in the preformed solution after 8 minutes. Over the entire reaction time, AOC was negatively related to the amount of unpaired electrons in 4f orbital and

lanthanum crystal ionic radius, implying that there is correlation between redox property of LnOxNP and periodicity of their constituent atoms. Ultimately, the ability to predict simple NP chemical activity from their constituent atom properties will improve the a priori of nanomaterials for specific needs.

3.1 Introduction

The Lanthanide metal oxides nanoparticle (LnOxNP) are widely used as catalysts, it can be functionalized as oxidants for forming oxide species which have high electron affinities and is benefit to ionize molecule or activate the preformed ions to release from the surface as free ions. Catalytic properties of LnOxNP have been investigated and applied in a variety of industrial reactions. Such as ortho or para hydrogen conversion,[1] deuterium exchange reactions of hydrocarbons,[2] H_2-D_2 equilibration,[3] alcohol dehydration,[4] olefin isomerization,[5] decomposition of N_2O and NO,[6] and oxidation reactions of hydrogen,[7] carbon monoxide[8] and hydrocarbons.[1]

The catalytic activity of LnOxNP shows difference between each other in previous studies. Minachev suggested the binding energy of oxygen with lanthanide ions could affect the activity in the oxidation of hydrogen and propylene.[9] Also for the oxidation of hydrogen, the activity of neodymium and erbium oxides were found lower than cerium, praseodymium and termium oxides.[10] There are also some studies indicating that the catalytic activity of LnOxNP depends on the periodic electronic configuration of the inner 4f orbital.[9,11] Still, the catalytic activities of LnOxNP was still fragmentary compared to other alkali metal oxides.[12]

Given the importance of this new material class and the unclear relationship between the catalytic activities of LnOxNPs, it is important to have a rapid, reproducible, robust, systematic assay to analyze NP reducing capacity. A number of scientific studies have been devoted to establishing assays based on redox properties of NPs that may prove useful in predicting their catalytic activity. Among them, antioxidant assay is well developed to evaluate the redox properties of both organic compounds and NP. Assays such as the Oxygen Radical Absorbance Capacity (ORAC),[13] 1',1'-Diphenylpicryl-hydrazyle (DPPH),[14] Terephthalic acid (TPA)[15,16] and 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) have been employed to investigate the redox activity NPs.[17] Yet, each method has specific limitations, which preclude its use as a rapid, systematic assay for studying NP redox activity (see **Table 1**).

Table 1. The antioxidant assays of NP used in previous studies their limitations

Assay	Limitation
ORAC	Temperature sensitive,[18,19] time consuming,[20] not easy to calculate AOC
TPA	Poor solubility in water
DPPH	Hydrophobic,[21] time consuming,[22] steric hindrance to large antioxidant[23]
	the spectrophotometric measurements can be affected by compounds[16]
DMPO	Side reactions,[24] Expensive[25]

In this article, a Trolox Equivalent Antioxidant Capacity assay (TEAC), based on $ABTS^{2-}$ /H_2O_2/HRP (Horseradish Peroxidase) decoloration method, was used to assess the AOC of eight LnOxNPs. In this assay, hydroxyl radical (·OH) are generated from the reaction between H_2O_2 and HRP then oxidized colorless $ABTS^{2-}$ to a long-lived green-blue radical anion ($ABTS^{\bullet-}$),[26] The AOC was determined by measuring the ability of LnOxNPs to quench $ABTS^{\bullet-}$ alone. The benefit of this assay over the previously applied assay is that it is very simple and sensitive, and ABTS radical ($ABTS^{\bullet-}$) scavenger used are stable at a broad range of pH, hence it can be used to study pH effect on activity.[27] Moreover, the TEAC assay can be applied in estimation of both hydrophilic and lipophilic samples compares to DPPH assay and the reaction time is short (1-30 min),[16] at room temperature. Because this assay has been widely used and conducted to assess complex food matrixes[28-30], we believe it is amenable to assess complex dispersions of nanoparticles.

This assay can be operated in two methods. The first method (normal method) focuses on the scavenging activity of the NP on preformed $ABTS^{\bullet-}$, rather than the inhibition of $ABTS^{\bullet-}$ formation. The objective of this method is to provide insight into the action of experimental analytes on persistent radicals without competitive formation effects by measuring the ability of LnOxNPs to quench ABTS radicals alone rather than ABTS radicals and free radicals competitively. However, the problem may be that the antioxidant activity of NPs especially for metal oxides are only enough to reduce ·OH rather than ABTS radical, the AOC will be underestimated by the method above. In order to overcome that, another method (preincubation method) is applied with the addition of NPs prior to HRP which allows for the NPs to react with ·OH directly and approach NPs real AOC. Based on the strong periodicity of lanthanide atoms, the specific hypothesis we test is that there is a periodicity to the AOC of LnOxNPs that can be predicted by atomic or ionic parameters displayed in **Table 2**. Due to the chemical structure similarities between LnOxNPs and other metal oxides, if our hypothesis holds, it may be possible to estimate redox properties of other metal oxide NP on the basis of their atomic or ionic periodicity.

Table 2 The atomic parameters that are potential related to the AOC of LnOxNPs.

Ln^{3+}	Ln-O[32]	IR[33]	BG[34]	IE[35]	E-4fn	UnE-4fn	EG[36]
	Angstrom	Angstrom	eV	eV			
Ce	2.27	1.150	4.0	36.76	1	1	2.794
Nd	2.35	1.123	4.7	40.41	3	3	2.808
Sm	2.31	1.098	5.0	41.40	5	5	2.819
Eu	2.30	1.087	4.4	42.70	6	6	2.828
Gd	2.29	1.078	5.4	44.00	7	7	2.809
Tb	2.26	1.063	3.8	39.37	8	6	2.819
Ho	2.25	1.041	5.3	42.50	10	4	2.828
Er	2.24	1.030	5.3	42.70	11	3	2.830

***** Ln-O: bond length between lanthanide and oxygen, IR: crystal radius of trivalent lanthanide ion, BG: band gap, IE: ionic energy, E-4fn: electrons in 4f orbital, UnE-4fn: unpaired electrons in 4f orbital, EG: electronegativity.

3.2 Material and method

3.2.1 Chemicals

The following reagents were purchased from Sigma Aldrich (St. Louis, MO) : $ABTS^{2-}$ (2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonate acid), 98%) obtained as diammonium salt, HRP (Horseradish peroxidase) obtained as lyophilized powder, Ho_2O_3NP (holmium(III) oxide, nanopowder, <100nm, 99.9%), Er_2O_3NP (erbium(III) oxide, nanopowder, <100nm, 99.9%), Sm_2O_3NP (samarium(III) oxide, nanopowder , <100nm, 99.9%), Gd_2O_3NP (gadolinium(III) oxide, nanopowder , <100nm, 99.8%), CeO_2NP (cerium(IV) oxide, nanopowder, <25nm, 99.9%), Eu_2O_3NP (europium(III) oxide, nanopowder, <150nm, 99.5%), Nd_2O_3NP (neodymium(III) oxide, nanopowder, <100nm, 99.9%), Tb_2O_3/TbO_2NP (terbium(III, IV) oxide, nanopowder, <100nm, 99.5%). UW (ultrafine Water) was purchased from Fisher Scientific (St. Clara, CA) and NaH_2PO_4 (sodium phosphate, monobasic anhydrous, 99%) was purchased from Fisher Scientific (Fair Lawn, NJ). H_2O_2 (hydrogen peroxide, 30%, v/v) was obtained from Mallinckrodt Chemicals (Phillipsburg, NJ). Copper chelate resin (50% slurry in 20% ethanol) was purchased from G-Biosciences (Maryland Heights, MO). Na_2HPO_4 (sodium phosphate, dibasic anhydrous, granular, 99.1%) was purchased from Macron Chemicals (Center Valley, PA). All chemicals and materials were ACS grade or better and were used without further purification.

3.2.2 Prepare Metal Oxide Nanoparticle Suspension

Copper chelate resin slurry was poured into a 2.5 ml plastic column and washed with 100 ml UW. Phosphate buffer solution (PBS) was passed through the column with a syringe pump (NE1010, New Era

Pump System, Farmingdale, NY) to achieve pure PBS without Cu^{2+}. PBS solution was made by mixing 50 mM Na_2HPO_4 and NaH_2PO_4 stock solutions at a ratio of 21:4 by volume. The eight uncoated metal oxide NPs investigated in the current study were CeO_2, Nd_2O_3, Sm_2O_3, Eu_2O_3, Gd_2O_3, Tb_2O_3/TbO_2, Ho_2O_3, and Er_2O_3. Each stock was prepared by dispersing nanopowder into UW and sonicating at 750 watts (VCX 750, Vibra-Cell-Sonics&Materials, Newtown, CT) for 15 minutes. NP dispersions with final concentrations of 200 ppm were kept at the room temperature and sonicated again for 3 minutes, just prior to starting the AOC experiment. For the TEAC assay, NP stock dispersions were diluted to concentrations of 40 ppm in PBS.

3.2.3 Characterization of LnOxNP

A Zetasizer (ZEN3600, Malvern Instruments, Westborough, MA) was used to determine the hydrodynamic diameter (HDD) and polydispersity index (PDI) of the NPs in PBS at 0 and 8 minutes based on the reaction time of TEAC assay. The standard deviation of NP size distribution (σ) was determined via equation (5) on the basis of PDI and average hydrodynamic diameter ($\bar{d}$) of NP. Considering the impact of agglomeration on the surface reactivity of LnOxNPs, we report AOC per unit surface area (AOC/Area) to normalize antioxidant activity of NPs based on agglomerate size. The total surface area of NP in PBS was determined using equation (6).

$$PDI = \left(\frac{\sigma}{\bar{d}}\right)^2 \rightarrow \sigma = \bar{d} * \sqrt{PDI} \quad (5)$$

$$\text{Total Suface Area} = 6 * \left(\frac{\frac{1}{\sigma\sqrt{2\pi}} \int_0^\infty x^2 e^{-\frac{(x-\bar{d})^2}{2\sigma^2}} dx}{\frac{1}{\sigma\sqrt{2\pi}} \int_0^\infty x^3 e^{-\frac{(x-\bar{d})^2}{2\sigma^2}} dx} * \frac{m}{\rho} \right) \quad (6)$$

In the equation (6), m, ρ and x represented total NP mass, density of metal oxide and HDD of NP measured by dynamic light scattering (DLS).

3.2.4 Measurement of Total AOC

For the TEAC assay, a SpectraMax M2 spectrophotometer (Molecular Devices, Sunnyvale, CA) was used to test the absorption change of $ABTS^{\bullet-}$ and the inherent absorbance of LnOxNPs at 734 nm. A calibration curve was made for 1 mM $ABTS^{2-}$ with 4 U/ml HRP (one enzyme unit (U) is defined as the amount of the enzyme that catalyzes the conversion of 1 microMole of substrate per minute) and a series of H_2O_2 concentrations (0~51 μM). A 1 ml ABTS radical ($ABTS^{\bullet-}$) working solution was comprised of 1 mM $ABTS^{2-}$, 51 μM H_2O_2, 4 U/ml HRP, 50 mM PBS (pH = 7.45). Temperature was held at 26±1℃. For the AOC assay, $ABTS^{\bullet-}$ solution without NP served as a control. The inherent absorbance of LnOxNPs at

734 nm was tested as a baseline to determine differences from control. The AOCs of eight LnOxNPs were measured by the normal and preincubation methods. The total reaction time was 8 minutes from $ABTS^{\bullet-}$ generation to the end of assay. In both methods, the decrease in absorption compared to control in 0 and 8 minutes as used to measure the AOC of NPs. 1 mM Trolox stock was prepared by dissolving Trolox powder into UW. A calibration curve of a series concentration of Trolox (0 ~ 25 μM) vs. $ABTS^{\bullet-}$ working solution was made to determine the relationship between aborption decrease and an equivalent concentration of Trolox. The antioxidant activity of LnOxNPs is presented as AOC per unit surface area AOC/Area and the unit of AOC/Area was μM Trolox/cm^2. All experiments were performed at least in triplicate.

3.2.5 Statistical Analysis

Statistical analysis was done using SigmaPlot 12.5 (San Jose, CA). Data are expressed as mean ± standard error of mean (SEM). Statistical significance was determined by one-way ANOVA. The correlation between AOC and different explanatory variables was determined by multivariate linear regression using R-studio (Boston, MA) ($p < 0.05$).

3.3 Results

3.3.1 Characterization of Nanoparticle

The size of all the LnOxNPs at 40 ppm is presented in **Fig.1**, The results show the size of LnOxNPs in PBS in 0 and 8 minutes, CeO_2NP was the smallest size at both time points. In addition, the size of Sm_2O_3NP and Tb_2O_3/TbO_2NP decreased significantly whereas the difference in size was not observed or the other material between 0 and 8 minutes. The total surface area of LnOxNPs in PBS was calculated on the basis of HDD using equation (5) and (6). Data are displayed in **Table 3.**

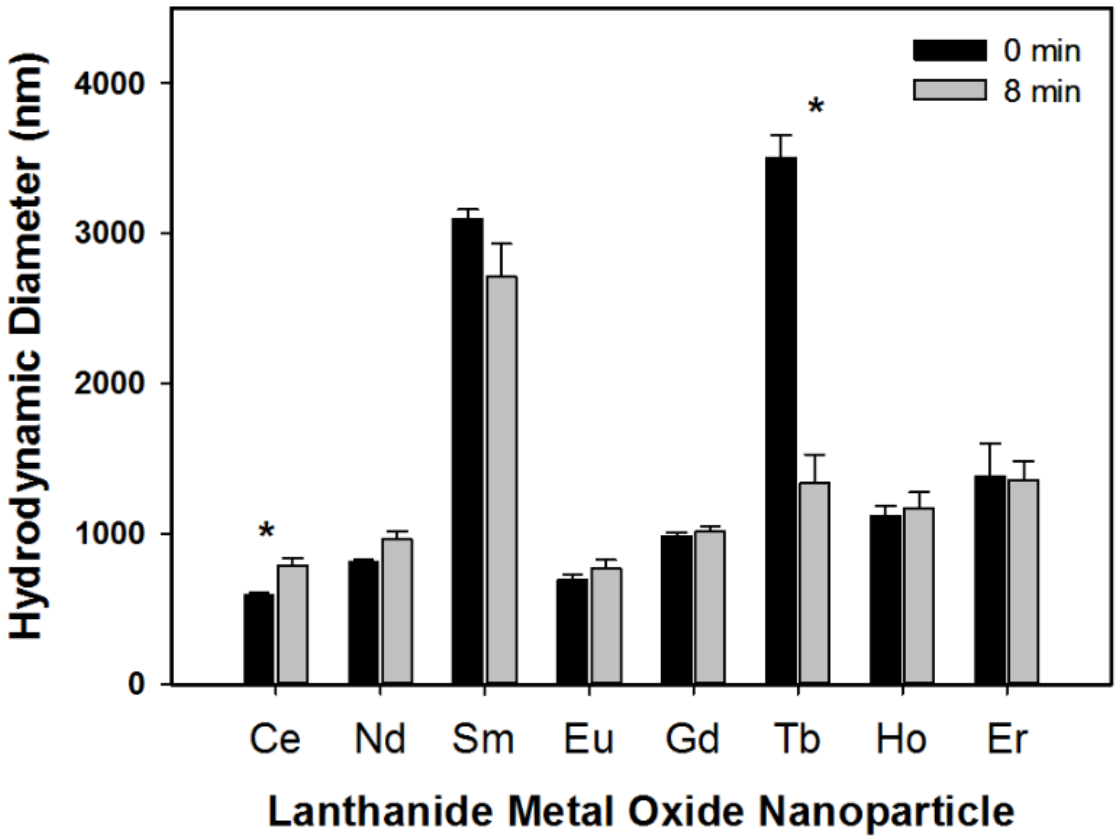

Figure 1. Size of LnOxNPs described above by constituent atoms at 40 ppm in PBS in 0 and 8 minutes. Data are presented as the mean±SEM. * represents a significant difference in size of NPs between 0 and 8 min.

Table 3. The average HDD, PDI and surface area of eight LnOxNPs in PBS at 0 and 8 minutes.

LnOxNP	Density	Average HDD		Average PDI		Average Surface Area	
		0 min	8 min	0 min	8 min	0 min	8 min
	($g \cdot cm^{-3}$)	(nm)	(nm)			(cm^2)	(cm^2)
Ce	6.77	544.60	788.20	0.38	0.37	0.42	0.29
Nd	7.01	735.10	963.90	0.25	0.25	0.33	0.25
Sm	7.52	2411.67	2714.33	0.68	0.59	0.07	0.07
Eu	5.24	808.63	774.17	0.57	0.55	0.32	0.34
Gd	7.90	1340.33	1017.40	0.69	0.38	0.12	0.19
Tb	8.23	4236.67	1339.67	0.96	1.00	0.03	0.10
Ho	8.79	1513.00	1173.67	0.66	0.63	0.10	0.13
Er	9.07	1332.67	1193.53	0.58	0.73	0.11	0.15

3.3.2 TEAC Assay

A calibration curve of H_2O_2 concentration vs. ABTS radical ($ABTS^{\bullet-}$) absorbance had a high linear relationship (R-sqr = 0.9996) that demonstrated the kinetics of scavenging ·OH of ABTS radical, After starting the TEAC assay, the absorbance reached the highest point around 150 s. As $ABTS^{\bullet-}$ began to be reduced to $ABTS^{2-}$, the absorbance decreased by about 1% per minute during the first 2 minutes, and continued to decrease for the last 6 minutes.

3.3.3 Time Effect on AOC

The $ABTS^{\bullet-}$ scavenging activity exhibited over time for the LnOxNPs is shown in **Fig. 2**. The AOC/Area of CeO_2NP, Nd_2O_3NP and Ho_2O_3NP decreased significantly after 8 minutes in normal method, and a negative AOC of TbO_2/Tb_2O_3NP was also observed with its absolute AOC/Area value also decreased during this time. However, no significant differences were observed in preincubation method (data not show).

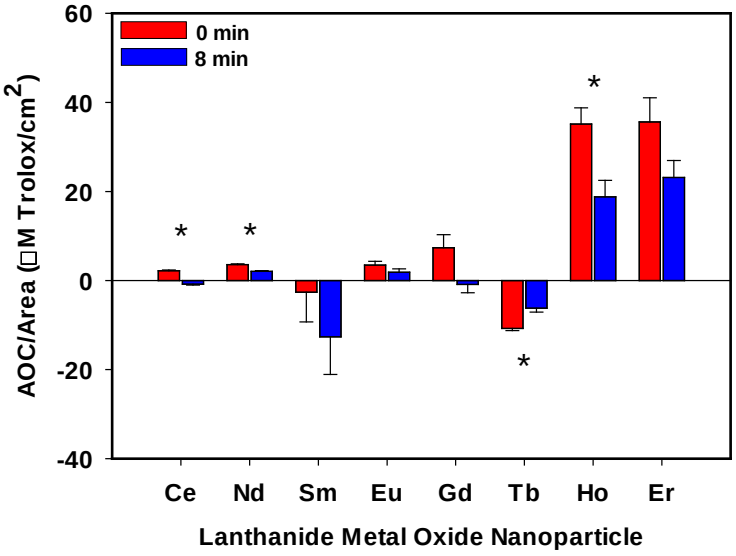

Figure 2. AOC/Area of LnOxNPs described above by constituent atoms at 40 ppm in normal method at 0 and 8 minutes, data are presented as the mean ± SEM. * represents a significant difference in AOC/Area of NP in between start and end points.

3.3.4 Incubation Method Effect on AOC

Fig. 3 shows the effect of preincubation on the AOC/Area of only CeO_2NP, no significant effects were observed from the other NPs (data not show). The result showed the AOC/Area of CeO_2NP in

normal method was significant lower than that in preincubation method. The model in equation (7) used to predict the AOC/Area of LnOxNPs was established via multiple linear regression. From the model, the AOC/Area in both 0 and 8 min could be predicted from a linear combination of the crystal ionic radius (IR) and unpaired electrons in 4f orbital (UnE-4fn) ($P < 0.01$). Moreover, the data points of AOC/Area between 0 and 8 min were also investigated and all the points fit the model well (data not show).

$$AOC/Area = \beta_0 + \beta_1 * IR + \beta_2 * UnE\_4fn \quad (7)$$

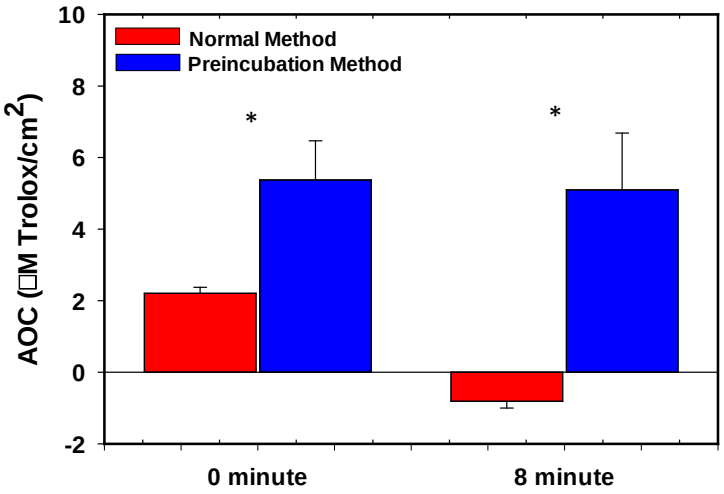

Figure 3. AOC/Area of CeO_2NP at 40 ppm in 0 minute and 8 minutes with normal and preincubation method separately. Data are presented as the mean ± SEM. * represents a significant difference in AOC/Area of NP between the different methods.

3.3.5 Inherent Physicochemical Properties Effect on AOC

Fig. 4 illustrates the relationship between AOC/Area and two explanatory variables (IR and UnE-4fn). AOC/Area of NPs corresponded negatively to crystal ionic radius except for TbO_2/Tb_2O_3NP which had lower AOC/Area compared to that we predicted from model (7), see **Fig. 4A**. In addition, results also showed that the AOC/Area increased first then decreased as unpaired electrons in 4f orbital increased and the maximum value appeared when there were 4 electrons in 4f orbital, see **Fig. 4B**.

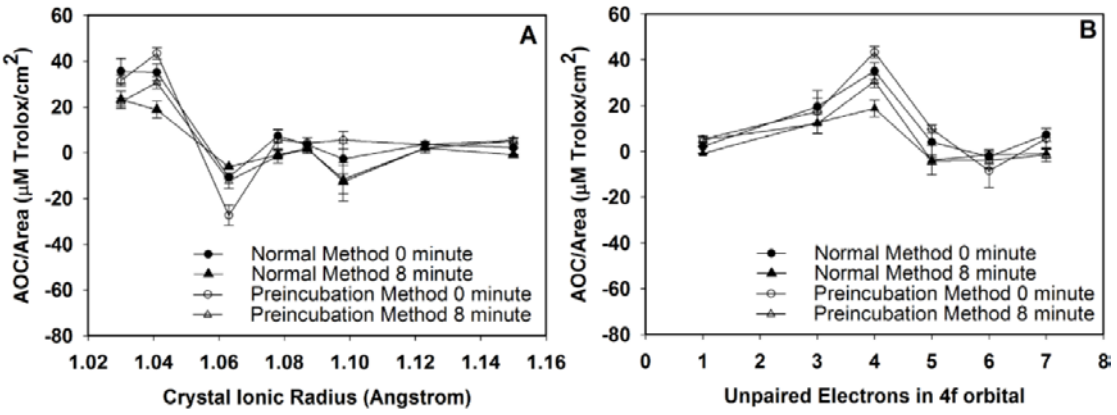

Figure 4. The influence of (A) crystal ionic radius and (B) unpaired electrons in 4f orbital on the AOCs of LnOxNPs at 40 ppm. Data are presented as the mean ± SEM.

3.4 Discussion

Trolox Equivalent Antioxidant Capacity assay (TEAC) based on $ABTS^{2-}$/H_2O_2/HRP (Horseradish peroxidase) decoloration method has been widely applied in food science[26,31] to estimate the total amount of radicals which can be scavenged by antioxidants in food homogenates, i.e. the AOC. Here we employed this assay to investigate the antioxidant activity of LnOxNPs due to their strong elemental periodicity. This research method can also be used to investigate other simple NPs which are in periodic series. In control, $ABTS^{2-}$ was first oxidized to its radical form $ABTS^{\bullet-}$ then began to be reduced back as it was a reversible reaction and $ABTS^{\bullet-}$was less stable than $ABTS^{2-}$.[37] Since the size of NP is negatively correlated with its surface area; when the total AOC of NP was normalized by AOC to surface area, the impact of size on AOC/Area of NP was minimized.

A decrease AOC/Area was observed as the time passed for some NP but only in normal method (**Fig. 2**). The reason may be that some LnOxNP such as Nd_2O_3NP have the ability to stabilized free radicals to form a metastable state complexation, which was reported in a previous study.[38] Thus, it is probable that when $ABTS^{\bullet-}$ was produced via oxidation of $ABTS^{\bullet-}$ by ·OH, it was stabilized by the addition of LnOxNPs to form a complexation (LnOxNP-$ABTS^{\bullet-}$) which decreases the effective concentration of $ABTS^{\bullet-}$ and inhibits the reduction process of $ABTS^{\bullet-}$ to $ABTS^{\bullet-}$. Due to the metastable state LnOxNP-$ABTS^{\bullet-}$complexation, as more $ABTS^{\bullet-}$ is reduced to $ABTS^{\bullet-}$, the concentration of $ABTS^{\bullet-}$ decreases and result in more $ABTS^{\bullet-}$ released from complexation to be further reduced, see **Fig. 5**. However, under preincubated condition, LnOxNPs react with both ·OH and $ABTS^{\bullet-}$, and ·OH will be stabilized by NPs prior to $ABTS^{\bullet-}$ due to its stronger oxidizing ability and smaller steric hinderance which

results in passivation of NP surface and inhibition of further stabilization of $ABTS^{\bullet-}$. Therefore, reaction time effect was not significant for AOC/Area change in preincubation method.

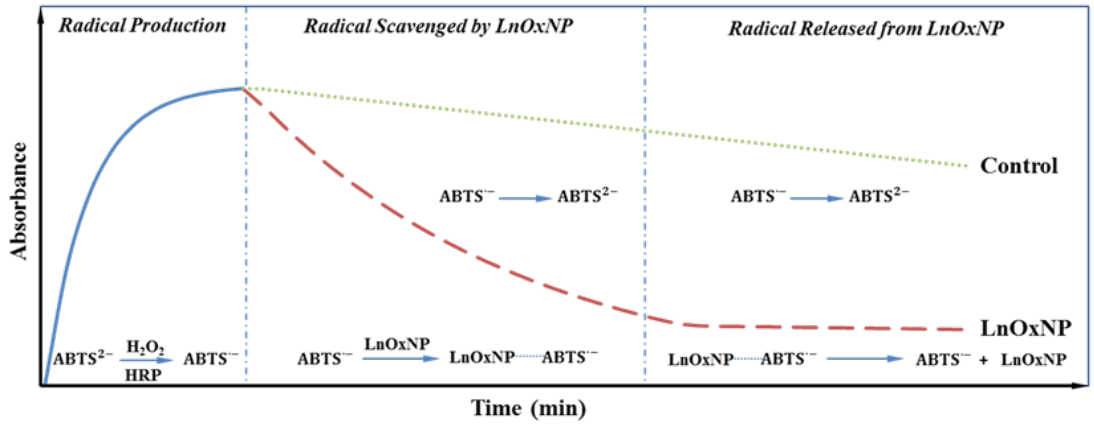

Figure 5. A possible mechanism of stabilization and releasing of $ABTS^{\bullet-}$ by LnOxNP in normal method.

The AOC/Area of CeO_2NP in normal method was significantly lower than in preincubation method, which can be explained that the AOC/Area comes from the impurity of low valence state cerium metal oxide (Ce_2O_3) which is less stable than CeO_2NP and easily oxidized by free radicals.[39] However, at such low concentration, it can not be oxidized by $ABTS^{\bullet-}$ as effectively as ·OH. Since there is only $ABTS^{\bullet-}$ in the working solution of normal method while both $ABTS^{\bullet-}$ and ·OH of preincubation method, thus, CeO_2NP has a higher AOC/Area in the preincubation method (**Fig. 3**). However, no significant results are observed for TbO_2/ Tb_2O_3NP which includes unstable valence metal oxide as CeO_2NP. The reason might be that the precipitation of TbO_2/ Tb_2O_3NP is far greater than CeO_2NP. Much precipitation of TbO_2/ Tb_2O_3NP on the bottom of cuvette could result in exposure surface area reduction to inhibit its functional AOC/Area.

Among the inherent physicochemical properties of lanthanide elements, only crystal ionic radius and unpaired electrons in 4f orbital had a significant effect on the AOC/Area of LnOxNPs. Crystal ionic radius is used since it corresponds more closely to the physical size of ions in a crystal lattice of LnOxNPs, which is helpful to study the AOC/Area of LnOxNPs.[33] We found in that AOC/Area of LnOxNPs decreased as their crystal ionic radius increased (**Fig. 4A**). The reason is that trivalent lanthanides are hard Lewis acids[40] while hydroxyl groups in ·OH and amine groups in $ABTS^{\bullet-}$ are hard Lewis bases,[41] the acidity of trivalent lanthanides with large crystal ionic radius will be softer compared to small one,[42] thus the NPs with larger crystal ionic radius have a more difficult time scavenging free radical (·OH and $ABTS^{\bullet-}$) due to the fact that hard acids prefer binding to the hard bases to give ionic complexes, whereas the soft acids prefer binding to soft bases to give covalent complexes according to

hard and soft acids and bases theory (HSAB).[43] This theory can explain the relationship between AOC/Area of LnOxNPs and crystal ionic radius. Similarly, due to the large TbO_2/Tb_2O_3NP precipitation, its ability to scavenging radical is also reduced thus the AOC/Area of TbO_2/Tb_2O_3NP is lower than the other LnOxNPs.

Unpaired electrons in 4f-orbital of lanthanum atoms are an important factor to estimate redox potential of LnOxNPs because the redox potential of atom is established on the basis of its electron transfer. As there are seven 4f orbitals, the number of unpaired electrons can be as high as 7. As long as unpaired electrons reach 7, the 4f orbitals will be in half-filled state, and the electron transfer from 4f orbital is difficult that makes the lanthanum atoms stable and reduces the redox potential. Therefore, as **Fig. 4B** shows, except for CeO_2NP, the AOC/Area of NP decreased as the number of UnE-4fn increased. The AOC/Area of CeO_2NP with only one UnE-4fn also has much lower AOC/Area compared to Nd_2O_3NP. The reason may be that tetravalent cerium without UnE-4fn is the principal component of CeO_2NP, only tiny amount of trivalent cerium with one UnE-4fn exist in the CeO_2NP. As tetravalent cerium is fairly stable and should not contribute to the redox activity of NP, the AOC/Area of CeO_2NP only comes from trivalent cerium with small amount which is not enough to show as much AOC/Area as Nd_2O_3NP.[38]

3.5 Conclusion

In this study, the AOC of eight LnOxNPs was investigated using a TEAC assay. Two methods were employed to test the interaction between NPs and free radicals. Considering the impact of agglomeration on the AOC of NPs, we used AOC per unit surface area of NPs instead of its total AOC as response and considered the physicochemical properties of component atom of NPs. The importance of this study is to change the traditional way of exploring chemical reactivity of the NPs, which only focus on the external factor such as interaction between solution properties and NPs. Here we discussed why various NPs present different redox properties in the same environmental condition via their inherent physicochemical properties and established a preliminary model to predict this correlation. It could be a useful guidance for studying the influence of inherent properties of NPs on their behavior in the environment.

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References

1. Selwood, P. W., The effect of a weak magnetic field on the rare earth catalyzed parahydrogen conversion rate. Journal of Catalysis 1971, 22, 123-129.

2. Minachev, K.M.; Vakk, E.G.; Dmitrier, R.V.; Nasedkin, E.A., Izv.Akad.nank SSSR, ser khlm, 1964, 3, 421

3. Ashmead, D. R.; Eley, D. D.; Rudham, R., The catalytic activity of the rare earth oxides for parahydrogen conversion and hydrogen-deuterium equilibration. Journal of Catalysis 1964, 3, 280-288.

4. Minachev, K.M., "Catalysis" J.W.High Tower, North Holland, 1973, 219

5. Khodakov,Y.S.; Nosteror, V.K.; Minachev, K.M., Izv Akad Nauk. SSS Rser Khlm 1975, 9, 2012

6. Read, J. F., The decomposition of nitrous oxide on neodymium oxide, dysprosium oxide and erbium oxide. Journal of Catalysis 1973, 28, 428-441.

7. Winter, E. R. S., The catalytic decomposition of nitric oxide by metallic oxides. Journal of Catalysis 1971, 22, 158-170.

8. Read, J. F.; Conrad, R. E., Hydrogen-oxygen reaction on lanthanide oxides. II. Stoichiometric hydrogen-oxygen reaction on neodymium oxide, dysprosium oxide, and erbium oxide. The Journal of Physical Chemistry 1972, 76, 2199-2206.

9. Sazonov, L.A.; Artamonov, E.V.; Mitrofanova, G.N., Kinet.Katal., 1971, 12, 378

10. Minachev, K.M.; Proc.Int.Congr.Catal. 1973, 5th, 219

11. Bakumenko, T.T.; Chachenokova, I.T., Kinet.Katal., 1969, 12, 378

12. Tanabe, K., “Solid Acids and Bases”, Academic Press, 1970, New York

13. Kung, M.; Hsieh, S.; Wu, C.; Chu, T.; Lin, Y.; Yeh, B.; Hsieh, S., Enhanced reactive oxygen species overexpression by CuO nanoparticles in poorly differentiated hepatocellular carcinoma cells. Nanoscale 2014.

14. Li, Z.; Pan, L.-L.; Zhang, F.-L.; Wang, Z.; Shen, Y.-Y.; Zhang, Z.-Z., Preparation and Characterization of Fullerene (C60) Amino Acid Nanoparticles for Liver Cancer Cell Treatment. Journal of Nanoscience and Nanotechnology 2014, 14, 4513-4518.

15. Liu, J.; Lagger, G.; Tacchini, P.; Girault, H. H., Generation of OH radicals at palladium oxide nanoparticle modified electrodes, and scavenging by fluorescent probes and antioxidants. Journal of Electroanalytical Chemistry 2008, 619-620, 131-136.

16. Ishibashi, K.-i.; Fujishima, A.; Watanabe, T.; Hashimoto, K., Quantum yields of active oxidative species formed on TiO_2 photocatalyst. Journal of Photochemistry and Photobiology A: Chemistry 2000, 134, 139-142.

17. Esumi, K.; Takei, N.; Yoshimura, T., Antioxidant-potentiality of gold–chitosan nanocomposites. Colloids and Surfaces B: Biointerfaces 2003, 32, 117-123.

18. Prior, R.; Hahoang; Gu, L.; XianLi Wu; Marabacchiocca; Lukehoward; Maureenhampsch-Woodill; Dejian, Huang; Boxin, Ou; Androbert, Jacob, Assays for Hydrophilic and Lipophilic Antioxidant Capacity(oxygen radical absorbance capacity (ORACFL)) of Plasma and Other Biological and Food Samples. Journal of agricultural and food chemistry 2003, 51, 3273-3279

19. Lussignoli, S.; Fraccaroli, M.; Andrioli, G.; Brocco, G.; Bellavite, P., A Microplate-Based Colorimetric Assay of the Total Peroxyl Radical Trapping Capability of Human Plasma. Anal Biochem 1999, 269, 38-44.

20. Huang, D. O., Boxin; Hampsch-Woodill, Maureen; Flanagan, Judith A; Prior, Ronald L, High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. Journal of agricultural and food chemistry 07/2002, 50, 4437 - 4444

21. Lumata, L.; Merritt, M.; Khemtong, C.; Ratnakar, S. J.; van Tol, J.; Yu, L.; Song, L.; Kovacs, Z., The efficiency of DPPH as a polarising agent for DNP-NMR spectroscopy. RSC advances 2012, 2, 12812-12817.

22. Brand-Williams, W.; Cuvelier, M. E.; Berset, C., Use of a free radical method to evaluate antioxidant activity. Food Science and Technology-Lebensmittel-Wissenschaft & Technologie 1995, 28, 25-30.

23. Prior, R. L. W., Xianli; Schaich, Karen, Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. J. Agric. Food Chem 2005, 53, 4290-4302.

24. Nohl, H.; Jordan, W.; Dietmer, H., Identification of free hydroxyl radicals in respiring rat heart mitochondria by spin trapping with the nitrone DMPO. FEBS Letters 1981, 123, 241-244.

25. M.Weckhuysen, B.; Heidler, R.; Schoonheydt, R. A., Electron Spin Resonance Spectroscopy. Mol. Sieves 2004, 4, 295-335.

26. Scott, S. L.; Chen, W. J.; Bakac, A.; Espenson, J. H., Spectroscopic parameters, electrode potentials, acid ionization constants, and electron exchange rates of the 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) radicals and ions. Journal of Physical Chemistry 1993, 25, 6710 - 6714.

27. Lemańska, K.; Szymusiak, H.; Tyrakowska, B.; Zieliński, R.; Soffers, A. E. M. F.; Rietjens, I. M. C. M., The influence of pH on antioxidant properties and the mechanism of antioxidant action of hydroxyflavones. Free Radical Biology and Medicine 2001, 31, 869-881.

28. Marino B. Arnao; Antonio Cano; Antonio Cano; Acosta, M., The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chemistry 2001, 73, 239-244.

29. Labrinea, E. P.; Georgiou, C. A., Stopped-flow method for assessment of pH and timing effect on the ABTS total antioxidant capacity assay. Analytica Chimica Acta 2004, 526, 63-68.

30. Chen, I.-C.; Chang, H.-C.; Yang, H.-W.; Chen, G.-L., Evaluation of Total Antioxidant Activity of Several Popular Vegetables and Chinese Herbs A Fast Approach with ABTS/H_2O_2/HRP System in Microplates. Journal of Food and Drug Analysis 2004, 12, 29-33.

31. Dauthal, P.; Mukhopadhyay, M., In-vitro free radical scavenging activity of biosynthesized gold and silver nanoparticles using Prunus armeniaca (apricot) fruit extract. Journal of Nanoparticle Research 2012, 15, 1-11.

32. Xue, D.; Zuo, S.; Ratajczak, H., Electronegativity and structural characteristics of lanthanides. Physica B: Condensed Matter 2004, 352, 99-104.

33. Shannon, R., Revised effective ionic radii and systematic studies of interatomic distances in halides and chalcogenides. Acta Crystallographica Section A 1976, 32, 751-767.

34. Gillen, R.; Clark, S.; Robertson, J., Nature of the electronic band gap in lanthanide oxides. Physical Review B 2013, 87.

35. Lang, P. F.; Smith, B. C., Ionization Energies of Lanthanides. Journal of Chemical Education 2010, 87, 875-881.

36. Zhao, X.; Wang, X.; Lin, H.; Wang, Z., Average electronegativity, electronic polarizability and optical basicity of lanthanide oxides for different coordination numbers. Physica B: Condensed Matter 2008, 403, 1787-1792.

37. Majcherczyk, A.; Johannes, C.; Hüttermann, A., Oxidation of aromatic alcohols by laccase from Trametes versicolor mediated by the 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) cation radical and dication. Appl Microbiol Biotechnol 1999, 51, 267-276.

38. Zhu, L.; Tang, G.; Shi, Q.; Cai, C.; Yin, J., Neodymium oxide-assisted melt free-radical grafting of maleic anhydride on isotactic-polypropylene by reactive extrusion. Journal of Polymer Science Part B: Polymer Physics 2006, 44, 134-142.

39. P.O. Maksimchuk, A. A. M., Yu.V. Malyukin, Spectroscopically Detected Formation of Oxygen Vacancies in Nano-Crystalline CeO2-x. J. NANO- ELECTRON. PHYS. 2013, 5, 01004(4pp).

40. T. Imamoto, Lanthanides in Organic Synthesis, 1994.

41. Pearson, R.G., Hard and Soft Acids and Bases, HSAB, Part II. Journal of the American Chemical Education 45 1968, 643-648

42. Pearson, R.G., Hard and Soft Acids and Bases, HSAB, Part I. Journal of the American Chemical Education 45 1968, 581-587

43. Pearson, R.G. Hard and Soft Acids and Bases. Journal of the American Chemical Society 1963, 85, 3533-3539

CHAPTER 4 CONCLUSION

Reactive Oxygen Species (ROS) are important intermediates produced during normal metabolic processes in our human body. However, overproduction of ROS would cause oxidative stress and can induce various disease states. Novel NP antioxidants are extensively studied at present, due to their potential to overcome many of the limitations of traditional antioxidants, such as time, pH sensitivity and bioavailability. It is important to have a rapid, reproducible, robust, systematic assay for the qualitative and quantitative analysis of NP chemical behaviors. This thesis work focused on the antioxidant study of 1) Ag/AuNPs and 2) eight lanthanide metal oxide NPs and used a modified TEAC assay to quantify their total AOC relative to Trolox.

For the eight LnOxNPs, two methods were used to study the interaction between NPs and free radicals. Because most chemical reactivity sites are distributed on the surface of NP, the antioxidant activity of NP is directly driven by the reactive surface area. However, NP is unstable in the buffer solution and form agglomerates that reduce reactive surface area. To reduce the influence of this confounding factor, the data were normalized by using antioxidant capacity per unit surface area of NPs based on their polydispersity index and size in phosphate buffer solution, rather than the total AOC as response. The result showed that CeO_2NP, Nd_2O_3NP, TbO_2/Tb_2O_3NP and Ho_2O_3NP likely form a metastable state LnOxNP-$ABTS^{\bullet-}$ complexation and inhibit the reducing process of $ABTS^{\bullet-}$ to $ABTS^{2-}$. CeO_2NP showed different AOCs between two methods likely due to the small amount of trivalent cerium impurities that can be only oxidized by ·OH rather than $ABTS^{\bullet-}$.

This work revealed that the AOC of LnOxNPs was negatively correlated to the unpaired electrons in 4f-orbtial and the crystal ionic radius of their component ions. The mechanism could be well explained via hard and soft (Lewis) acids and bases (HASB) theory. Crystal ionic radius was an important parameter due to the fact that trivalent lanthanides are hard Lewis acids while hydroxyl groups in ·OH and amine groups in $ABTS^{\bullet-}$ are hard Lewis bases. The acidity of trivalent lanthanides with large crystal ionic radius will be softer compared to small one, thus the NP with larger crystal ionic radius had a difficult time to scavenge free radicals (·OH and $ABTS^{\bullet-}$) due to the fact that hard acids prefer binding to the hard bases to give ionic complexes. Whereas, soft acids prefer binding to soft bases to give covalent ? (what?).

Unpaired electrons in 4f-orbital of lanthanum atoms are more related to the redox potential of LnOxNPs. As there are seven 4f orbitals, the number of unpaired electrons can be as high as 7. As long as unpaired electrons reach 7, the 4f orbitals will be in half filled state that makes the lanthanum atoms fairly

stable and reduces their redox potential. The AOC/Area of CeO_2NP with only one UnE-4fn had a much lower AOC/Area compared to Nd_2O_3NP, This may be because tetravalent cerium without UnE-4fn is the principal component of CeO_2NP, only tiny amount of trivalent cerium with one UnE-4fn exist in the CeO_2NP. As tetravalent cerium is fairly stable and should not contribute to the redox activity of NP, the AOC/Area of CeO2NP comes predominantly from trivalent cerium with small amount which is not enough to show as much AOC/Area as Nd_2O_3NP.

The importance of this study is to change the traditional way of exploring chemical reactivity of NPs, which only focus on the external factor such as interaction between solution properties and NPs. Here we discussed why various NPs present different redox properties in the same condition via their inherent physicochemical properties and established a preliminary model to predict this correlation. Such models can serve as a useful guide for studying the influence of inherent properties of NPs on their behavior in the same condition.

For gold and silver NPs, the AOC of AuNP stabilized with citrate and silver stabilized with citrate and PBS were also assessed via the TEAC assay. In this study, chelators (DTPA and EDTA) were also used to test their impact of on AOC of these NPs. The result showed apparently that both AuNP and AgNP showed their antioxidant capacities which was also reported in previous studies, the AOC of AuNP was stronger because the AuNP with the Au^{3+} ionic state being prevalent in solution had a higher AOC than the AgNP that release predominantly Ag^{1+} ionic species. For silver NP alone with different stabilizers, AgNP-citrate was observed to have smaller AOC than AgNP-PBS. Since PBS and citrate did not significantly alter AOC compared to control (data not show). The reason of AOC difference in AOC between two kinds of AgNP is likely due to the larger size of citrate which covers the NP surface. Silver ions complexed by citrate can be absorbed on the surface of AgNP and inhibited further ion release more efficiently than AgNP-PBS. Chelators such as EDTA and DTPA showed their inherent AOCs due to the reduced capability of tertiary amine group. Compared to EDTA, DTPA even showed higher AOC because it has more tertiary amine group than EDTA. Moreover, they could both significantly increase gold and silver NPs AOC, and the impact on AgNP was more obvious, this is possibly because the dissolution rate of AgNP is much higher than AuNP.

Two methods (normal and preincubation) were used to investigate the effect of interaction time between NP and chelators on the AOC. Results showed that if a chelating environment was built first in 10 seconds prior to NP by addition of chelators into the solution (normal method), a significant increase of when compared to the preincubation method. A potential explanation could be that for both Au and

AgNPs, the surface area available for oxidation declined through the course of the assay. NP release ions during surface oxidation, and as this process progresses the surface dissolves, reducing the effective contact area for ABTS radicals. Therefore, the apparent decrease in antioxidant activity of solutions incubated with a chelator may be attributed to differences in accessible surface area between samples in normal and preincubation method. A proposed mechanism for the Ag/AuNPs studies is presented in **Figure 1**.

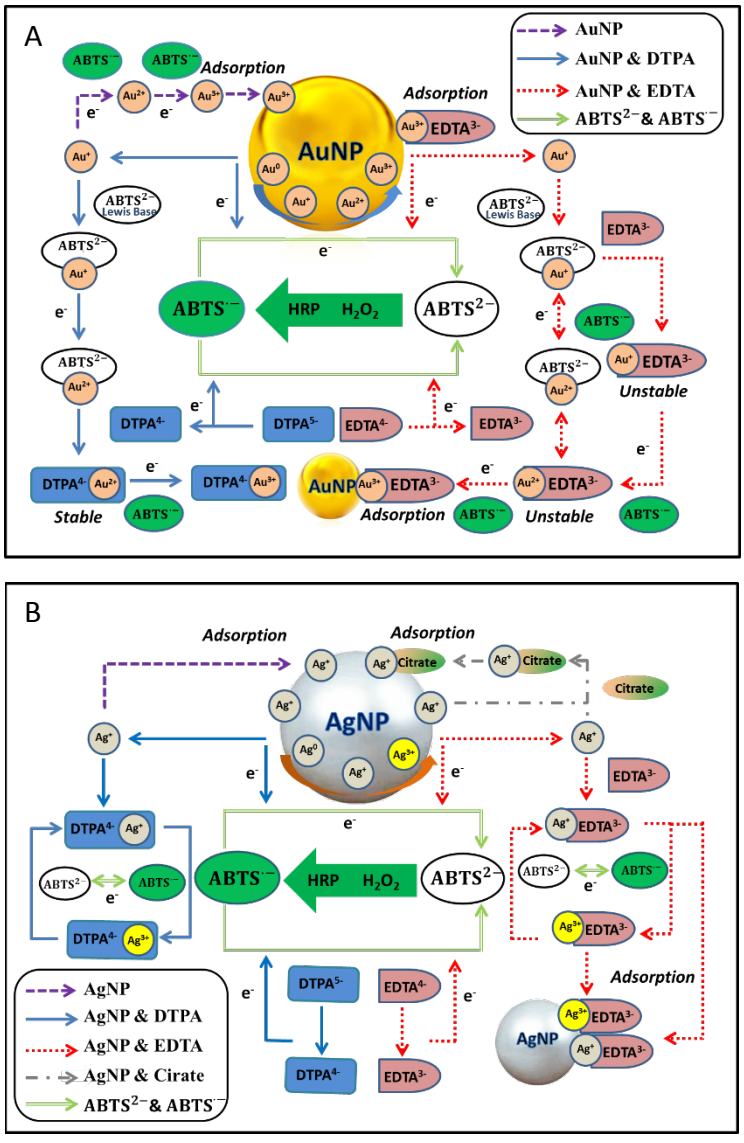

Figure 1. The reaction process and mechanisms of antioxidant activity of (A) gold nanoparticle and (B) silver nanoparticle and the role of chelators on them: including the electron transfer of EDTA/DTPA and Ag/AuNPs; the enhancement of AOC by addition of EDTA/DTPA, and the inhibition of citrate in the process of ion releasing for Ag/AuNPs.

This study demonstrates the use of antioxidant assays to assess the chemical redox potential of NPs and predict their behaviors. This study contributes to our understanding of the antioxidant activity of Ag/AuNPs which could be beneficial for additional promising biomedical applications. In addition, the interaction of Ag/AuNPs and chelators could also be further applied to investigate the interaction of NPs and chemicals with chelating structures including amino group in biological fields such as phytochelatin, hemoglobin and chlorophyll. This information would be beneficial to the nanotoxicology studies on organisms. Therefore, future work could focus on the interaction between NPs with biological systems, for instance, toxicology studies could be involved to test different toxicities of NPs and compare to their antioxidant capacities. Also, some biological molecules like protein and DNA could be used to test their effects on the NP AOCs.

References

1. Lobo, V.; Patil, A.; Phatak, A.; Chandra, N., Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy Reviews 2010, 4, 118-126.

2. Magalhães, L. M.; Segundo, M. A.; Reis, S.; Lima, J. L. F. C., Methodological aspects about in vitro evaluation of antioxidant properties. Analytica Chimica Acta 2008, 613, 1-19.

3. Bagchi K, Puri S. Free radicals and antioxidants in health and disease. East Mediterranean Health Jr. 1998, 4, 350-60

4. Liu T., Stern A., Roberts J., The isoprostanes: Novel prostanglandin - like products of the free radical catalyzed peroxidation of arachidonic acid. J. Biomed Sci; 1999, 6:226-35.

5. Review, R. O. S. a. N. A. A., Reactive Oxygen Species and Natural Antioxidants: A Review. Advances in Bioresearch 2012, 3, 164-175.

6. Ebadi M., Antioxidants and free radicals in health and disease: An introduction to reactive oxygen species, oxidative injury, neuronal cell death and therapy in neurodegenerative diseases. Arizona: Prominent Press; 2001, p. 13-5.

7. Trachootham, D.; Lu, W.; Ogasawara, M. A.; Valle, N. R.-D.; Huang, P., Redox Regulation of Cell Survival. Antioxidants & Redox Signaling 2008, 10, 1343-1374.

8. Droge W. Free radicals in the physiological control of cell function. Physiol Rev. 2002, 82, 47-95.

9. Halliwell, B. Free Radicals and Antioxidants: A Personal View. Nutrition reviews 1994, 52, 253 - 265.

10. Myrtill Simkó, A. G.; Ulrich Fiedeler, M. N. Nanoparticles, Free Radicals and Oxidative Stress. 2011.

11. Mark K. Shigenaga; Hagen, T. M.; Ames, B. N. Oxidative Damage and Mitochondrial Decay in Aging. P Natl Acad Sci USA 1994, 91, 10771 - 10778.

12. Salvatore Cuzzocrea; Michelle C McDonald; Helder Mota Filipe; Giuseppina Costantino; Emanuela Mazzon; Sabrina Santagati; Achille P Caputi; Thiemermann, C. Effects of Tempol, A Membrane-Permeable Radical Scavenger, in a Rodent Model of Carrageenan-Induced Pleurisy. European journal of pharmacology 2000, 390, 209 - 222.

13. Leiro, J. Effects of Cis-Resveratrol on Inflammatory Murine Macrophages: Antioxidant Activity and Down-Regulation of Inflammatory Genes. Journal of Leukocyte Biology 2004, 75, 1156-1165.

14. Takeda, M. S., I; Kobayashi, M; Endou, H. Hydrogen Peroxide Induces Necrosis, Apoptosis, Oncosis and Apoptotic Oncosis of Mouse Terminal Proximal Straight Tubule Cells. Nephron 1999, 81, 234 - 238

15. Renz, A. Rapid Extracellular Release of Cytochrome C Is Specific for Apoptosis and Marks Cell Death in Vivo. Blood 2001, 98, 1542-1548.

16. C. E. Richeson; P. Mulder; V. W. Bowry; Ingold, K. U. The Complex Chemistry of Peroxynitrite Decomposition: New Insights. J. Am. Chem. Soc. 1998, 120, 7211 - 7219.

17. Valko, M.; Leibfritz, D.; Moncol, J.; Cronin, M. T.; Mazur, M.; Telser, J., Free radicals and antioxidants in normal physiological functions and human disease. The international journal of biochemistry & cell biology 2007, 39, 44-84.

18. Frankel, E. N.; German, J. B., Antioxidants in foods and health: problems and fallacies in the field. Journal of the Science of Food and Agriculture 2006, 86, 1999-2001.

19. Frankel, E. N., Antioxidants in lipid foods and their impact on food quality. Food Chemistry 1996, 57, 51-55.

20. Li, F.; Liang, Z.; Zheng, X.; Zhao, W.; Wu, M.; Wang, Z., Toxicity of nano-TiO2 on algae and the site of reactive oxygen species production. Aquatic toxicology 2015, 158, 1-13.

21. Kang, T.; Guan, R.; Song, Y.; Lyu, F.; Ye, X.; Jiang, H., Cytotoxicity of zinc oxide nanoparticles and silver nanoparticles in human epithelial colorectal adenocarcinoma cells. LWT - Food Science and Technology 2015, 60, 1143-1148.

22. Kung, M.; Hsieh, S.; Wu, C.; Chu, T.; Lin, Y.; Yeh, B.; Hsieh, S., Enhanced reactive oxygen species overexpression by CuO nanoparticles in poorly differentiated hepatocellular carcinoma cells. Nanoscale 2014.

23. Li, Z.; Pan, L.-L.; Zhang, F.-L.; Wang, Z.; Shen, Y.-Y.; Zhang, Z.-Z., Preparation and Characterization of Fullerene (C60) Amino Acid Nanoparticles for Liver Cancer Cell Treatment. Journal of Nanoscience and Nanotechnology 2014, 14, 4513-4518.

24. Kauffer, F. A.; Merlin, C.; Balan, L.; Schneider, R., Incidence of the core composition on the stability, the ROS production and the toxicity of CdSe quantum dots. J Hazard Mater 2014, 268, 246-55.

25. Matill HA. Antioxidants. Annu Rev Biochem. 1947, 16, 177-92.

26. German J. Food processing and lipid oxidation. Adv Exp Med Biol. 1999, 459, 23-50.

27. Gilgun-Sherki, Y.; Melamed, E.; Offen, D., The role of oxidative stress in the pathogenesis of multiple sclerosis: the need for effective antioxidant therapy. Journal of neurology 2004, 251, 261-8.

28. Halliwell B. How to characterize an antioxidant- An update. Biochem Soc Symp. 1995, 61, 73-101.

29. Pala, F. S.; Gürkan, H., The role of free radicals in ethiopathogenesis of diseases. Advances in Molecular Biology 2008, 1, 1-9.

30. Shi HL, Noguchi N, Niki N. Comparative study on dynamics of antioxidative action of α-tocopheryl hydroquinone, ubiquinol and α- tocopherol, against lipid peroxidation. Free Radic Biol Med. 1999, 27, 334-46.

31. Levine M, Ramsey SC, Daruwara R. Criteria and recommendation for Vitamin C intake. JAMA. 1991, 281, 1415-23.

32. Sies H. Oxidative stress: Oxidants and antioxidants. Exp Physiol. 1997, 82, 291-5.

33. Magnenat JL, Garganoam M, Cao J. The nature of antioxidant defense mechanisms: A lesson from transgenic studies. Environ Health Perspect. 1998; 106: 1219-28.

34. Meister A. Glutathione-ascorbic acid antioxidant system in animals. J Biol Chem. 1994, 269, 9397-400.

35. Padayatty S, Katz A, Wang Y, Eck P, Kwon O, Lee J, et al. Vitamin C as an antioxidant: Evaluation of its role in disease prevention. J Am Coll Nutr. 2003, 22, 18-35.

36. Smirnoff N. L-ascorbicacid biosynthesis. Vitam Horm. 2001, 61, 241-66.

37. Shigeoka S, Ishikawa T, Tamoi M, Miyagawa Y, Takeda T, Yabuta Y, et al. Regulation and function of ascorbate peroxidase isoenzymes. J Exp Bot. 2002, 53, 1305-19.

38. Herrera E, Barbas C., Vitamin E: Action, metabolism and perspectives. J Physiol Biochem. 2001, 57, 43-56.

39. Traber MG, Atkinson J., Vitamin E, antioxidant and nothing more. Free Radic Biol Med. 2007, 43, 4-15.

40. Wang X, Quinn P., Vitamin E and its function in membranes. Prog Lipid Res. 1999, 38, 309-36.

41. Meister A, Anderson A., Glutathione., Annu Rev Biochem. 1983, 52, 711-60

42. Matill HA. Antioxidants., Annu Rev Biochem. 1947, 16, 177-92.

43. Wolf G., The discovery of the antioxidant function of vitamin E: The contribution of Henry A. Mattill. J Nutr. 2005, 135, 363-6

44. Caniato R, Filippini R, Piovan A, Puricelli L, Borsarini A, Cappelletti E., Melatonin in plants. Adv Exp Med Biol. 2003, 527, 593-7

45. Reiter RJ, Carneiro RC, Oh CS., Melatonin in relation to cellular antioxidative defense mechanisms. Horm Metab Res. 1997, 29, 363-72

Uric acid

46. Tan DX, Manchester LC, Reiter RJ, Qi WB, Karbownik M, Calvo JR., Significance of melatonin in antioxidative defense system: Reactions and products. Biol Signals Recept. 2000, 9, 137-59

47. Jaeschke H, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D, Lemasters JJ., Mechanisms of hepatotoxicity. Toxicol Sci. 2002, 65, 166-76

48. Halliwell B, Gutteridge J. Free Radicals in Biology and Medicine. Oxford, UK: Oxford Univ. Press, 2007

49. Zelko I, Mariani T, Folz R., Superoxide dismutase multigene family: A comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. Free Radic Biol Med. 2002, 33, 337-49

50. Banniste J, Bannister W, Rotilio G. Aspects of the structure, function, and applications of superoxide dismutase. CRC Crit Rev Biochem. 1987, 22, 111-80

51. Johnson F, Giulivi C., Superoxide dismutases and their impact upon human health. Mol Aspects Med. 2005, 26, 340-52

52. Wuerges J, Lee JW, Yim YI, Yim HS, Kang SO, Djinovic Carugo K., Crystal structure of nickel-containing superoxide dismutase reveals another type of active site. Proc Natl Acad Sci. 2004, 101, 8569-74

53. Chelikani P, Fita I, Loewen PC., Diversity of structures and properties among catalases., Cell Mol Life Sci. 2004, 61, 192-208

54. Gaetani G, Ferraris A, Rolfo M, Mangerini R, Arena S, Kirkman H. Predominant role of catalase in the disposal of hydrogen peroxide within human erythrocytes. Blood. 1996, 87, 1595-9

Glutathione systems

55. Eisner T, Aneshansley DJ. Spray aiming in the bombardier beetle: Photographic evidence. Proc Natl Acad Sci USA. 1999, 96, 9705-9

56. Meister A, Anderson M., Glutathione. Annu Rev Biochem. 1983, 52, 711-60

57. Brigelius-Flohe R., Tissue-specific functions of individual glutathione peroxidases. Free Radic Biol Med. 1999, 27, 951-65

58. Hayes J, Flanagan J, Jowsey I., Glutathione transferases. Annu Rev Pharmacol Toxicol. 2005, 45, 51-88

59. Ge, W.; Li, D.; Chen, M.; Wang, X.; Liu, S.; Sun, R., Characterization and antioxidant activity of beta-carotene loaded chitosan-graft-poly (lactide) nanomicelles. Carbohydrate polymers 2015, 117, 169-76.

60. Chen, Y.-J.; Yuan, R.-M.; Liu, Y.-J.; Yang, H.-J.; Liang, G.-Y.; Tian, L.-X., Dietary vitamin C requirement and its effects on tissue antioxidant capacity of juvenile largemouth bass, Micropterus salmoides. Aquaculture 2015, 435, 431-436.

61. Hong, R.; Han, G.; M, J.; Ferna´ndez; Kim, B.-j.; Forbes, N. S.; Rotello, V. M., Glutathione-Mediated Delivery and Release Using Monolayer Protected Nanoparticle Carriers. J. Am. Chem. Soc. 2006, 128, 1078-9.

62. Soomets, U.; Zilmer, M.; Langel, l., Manual Solid Phase Synthesis of Glutathione Analogs. In Peptide Synthesis and Applications, Howl, J., Ed. Humana Press: 2005; Vol. 298, pp 241-257.

63. Tobwala, S.; Karacal, H.; Ercal, N., Antioxidant Defense Network in the Lens and Benefits of Glutathione Prodrugs in Cataracts. In Studies on the Cornea and Lens, Babizhayev, M. A.; Li, D. W.-C.; Kasus-Jacobi, A.; Žorić, L.; Alió, J. L., Eds. Springer New York: 2015; pp 327-359.

64. Saritha, K.; Saraswathi, U., Antioxidant activity of gold nanoparticles synthesized using Lemna minor. World Journal of Pharmaceutical Sciences 2014, 2, 1545-1551.

65. S, V.; P, A.; K, M., Biological Reduction of Silver Nanoparticles Using Cassia Auriculata Flower Extract and Evaluation of Their in vitro Antioxidant Activities. Nanoscience and Nanotechnology: An International Journal 2012, 2, 30-35.

66. Chen, J.; Patil, S.; Seal, S.; McGinnis, J. F., Rare earth nanoparticles prevent retinal degeneration induced by intracellular peroxides. Nat Nano 2006, 1, 142-150.

67. Korsvik, C.; Patil, S.; Seal, S.; Self, W. T., Superoxide dismutase mimetic properties exhibited by vacancy engineered ceria nanoparticles. Chemical communications 2007, 10, 1056-8.

68. Heckert, E. G.; Karakoti, A. S.; Seal, S.; Self, W. T., The role of cerium redox state in the SOD mimetic activity of nanoceria. Biomaterials 2008, 29, 2705-9.

69. Martin, R.; Menchon, C.; Apostolova, N.; Victor, V. M.; Alvaro, M.; Herance, J. R.; Garcia, H., Nano-Jewels in Biology. Gold and Platinum on Diamond Nanoparticles as Antioxidant Systems Against Cellular Oxidative Stress. ACS Nano 2010, 4, 6957–6965.

70. Zu, Y.; Wu, W.; Zhao, X.; Li, Y.; Wang, W.; Zhong, C.; Zhang, Y.; Zhao, X., Enhancement of solubility, antioxidant ability and bioavailability of taxifolin nanoparticles by liquid antisolvent precipitation technique. International journal of pharmaceutics 2014, 471, 366-76.

71. Shea, T. B.; Ortiz, D.; Nicolosi, R. J.; Kumar, R.; Watterson, A. C., Nanosphere-mediated delivery of vitamin E increases its efficacy against oxidative stress resulting from exposure to amyloid beta. Journal of Alzheimer's Disease 2005, 7, 297-301.

72. Duclairoir, C.; Orecchioni, A. M.; Depraetere, P.; Nakache, E., α-Tocopherol encapsulation and in vitro release from wheat gliadin nanoparticles. Journal of Microencapsulation 2002, 19, 53-60.

73. Zigoneanu, I. G.; Astete C. E.; Monroe, W. T. and Sabliov, C. M., Sustained release and antioxidant activity of alpha-tocopherol entrapped in Poly (D, L-lactide-co-glycolide) nanoparticles. IFT Annual Meeting 2006, Orlando, FL.

74. Erica, S.; Daniel, A.; Silvana, A., Artificial Nanoparticle Antioxidants. In Oxidative Stress: Diagnostics, Prevention, and Therapy, American Chemical Society: 2011; Vol. 1083, pp 235-253.

75. Mani, A.; S, S. L.; V, G., Bio-Mimetic Synthesis of Silver Nanoparticles and Evaluation of Its Free Radical Scavenging Activity. International Journal of Biological & Pharmaceutical Research 2012, 3, 631-633.

76. Ranjbar, A.; Ataie, Z.; Khajavi, F.; Ghasemi, H., Effects of silver nanoparticle (Ag NP) on oxidative stress biomarkers in rat. Nanomedicine Journal 2014, 1, 205-211.

77. Shayesteh, T. H.; Khajavi, F.; Ghasemi, H.; Zijoud, S.-M. H.; Ranjbar, A., Effects of silver nanoparticle (Ag NP)on oxidative stress, liver function in rat: hepatotoxic or hepatoprotective? Issues in Biological Sciences and Pharmaceutical Research 2014, 2, 040-044.

78. Barathmanikanth, S.; Kalishwaralal, K.; Sriram, M.; Pandian, S. R.; Youn, H. S.; Eom, S.; Gurunathan, S., Anti-oxidant effect of gold nanoparticles restrains hyperglycemic conditions in diabetic mice. J Nanobiotechnology 2010, 8, 16.

79. Du, L.; Miao, X.; Jiang, Y.; Jia, H.; Tian, Q.; Shen, J.; Liu, Y., An effective strategy for the synthesis of biocompatible gold nanoparticles using danshensu antioxidant: prevention of cytotoxicity via attenuation of free radical formation. Nanotoxicology 2013, 7, 294-300.

80. Onizawa, S.; Aoshiba, K.; Kajita, M.; Miyamoto, Y.; Nagai, A., Platinum nanoparticle antioxidants inhibit pulmonary inflammation in mice exposed to cigarette smoke. Pulmonary pharmacology & therapeutics 2009, 22, 340-9.

81. Kim, J.; Takahashi, M.; Shimizu, T.; Shirasawa, T.; Kajita, M.; Kanayama, A.; Miyamoto, Y., Effects of a potent antioxidant, platinum nanoparticle, on the lifespan of Caenorhabditis elegans. Mechanisms of ageing and development 2008, 129, 322-31.

82. Tarnuzzer, R. W.; Colon, J.; Patil, S.; Seal, S., Vacancy Engineered Ceria Nanostructures for Protection from Radiation-Induced Cellular Damage. Nano Letters 2005, 5, 2573-2577.

83. Dutta, P.; Pal, S.; Seehra, M. S.; Shi, Y.; Eyring, E. M.; Ernst, R. D., Concentration of Ce^{3+} and Oxygen Vacancies in Cerium Oxide Nanoparticles. Chemistry of Materials 2006, 18, 5144-5146.

84. Campbell, C. T.; Peden, C. H. F., Oxygen Vacancies and Catalysis on Ceria Surfaces. Science 2005, 309, 713-714.

85. Pirmohamed, T.; Dowding, J. M.; Singh, S.; Wasserman, B.; Heckert, E.; Karakoti, A. S.; King, J. E.; Seal, S.; Self, W. T., Nanoceria exhibit redox state-dependent catalase mimetic activity. Chemical communications 2010, 46, 2736-8.

86. Xue, Y.; Luan, Q.; Yang, D.; Yao, X.; Zhou, K., Direct Evidence for Hydroxyl Radical Scavenging Activity of Cerium Oxide Nanoparticles. The Journal of Physical Chemistry C 2011, 115, 4433-4438.

87. Dowding, J. M.; Dosani, T.; Kumar, A.; Seal, S.; Self, W. T., Cerium oxide nanoparticles scavenge nitric oxide radical (NO). Chemical communications 2012, 48, 4896-8.

88. Tsai, Y.-Y.; Oca-Cossio, J.; Lin, S.-M.; Woan, K.; Yu, P.-C.; Sigmund, W., Reactive oxygen species scavenging properties of ZrO2-CeO2 solid solution nanoparticles. Nanomedicine: nanotechnology, biology, and medicine 2008, 3, 637-645.

89. Schalow, T.; Laurin, M.; Brandt, B.; Schauermann, S.; Guimond, S.; Kuhlenbeck, H.; Starr, D. E.; Shaikhutdinov, S. K.; Libuda, J.; Freund, H.-J., Oxygen Storage at the Metal/Oxide Interface of Catalyst Nanoparticles. Angew. Chem. Int. Ed. 2005, 44, 7601–7605.

90. Sanjay, S. S.; Avinash C. Pandey; Kumar, S.; Pandey, A. K., Cell membrane protective efficacy of ZnO nanoparticles. SOP TRANSACTIONS ON NANO-TECHNOLOGY 2014, 1, 21-29.

91. Das, D.; Nath, B. C.; Phukon, P.; Dolui, S. K., Synthesis and evaluation of antioxidant and antibacterial behavior of CuO nanoparticles. Colloids and surfaces. B, Biointerfaces 2013, 101, 430-3.

92. Saikia, J. P.; Paul, S.; Konwar, B. K.; Samdarshi, S. K., Nickel oxide nanoparticles: A novel antioxidant. Colloids and Surfaces B: Biointerfaces 2010, 78, 146-148.

93. Schubert, D.; Dargusch, R.; Raitano, J.; Chan, S.-W., Cerium and yttrium oxide nanoparticles are neuroprotective. Biochem Bioph Res Co 2006, 342, 86-91.

94. Paul, S.; Saikia, J. P.; Samdarshi, S. K.; Konwar, B. K., Investigation of antioxidant property of iron oxide particlesby 1′-1′diphenylpicryl-hydrazyle (DPPH) method. Journal of Magnetism and Magnetic Materials 2009, 321, 3621-3623.

95. Medhe, S.; Bansal, P.; Srivastava, M. M., Enhanced antioxidant activity of gold nanoparticle embedded 3, 6-dihydroxyflavone: a combinational study. Applied Nanoscience 2012, 4, 153-161.

96. Nie, Z.; Liu, K. J.; Zhong, C.-J.; Wang, L.-F.; Yang, Y.; Tian, Q.; Liu, Y., Enhanced radical scavenging activity by antioxidant-functionalized gold nanoparticles: A novel inspiration for development of new artificial antioxidants. Free Radical Biology and Medicine 2007, 43, 1243-1254.

97. Cao, G.; Alessio, H. M.; & Cutler, R. G., Oxygen-radical absorbance capacity assay for antioxidants. Free Radical and Biology Medicine 1993, 14, 303–311.

98. Bai, Y.; Qin, B.; Zhou, Y.; Wang, Y.; Wang, Z.; Zheng, W., Preparation and Antioxidant Capacity of Element Selenium Nanoparticles Sol–Gel Compounds. Journal of Nanoscience and Nanotechnology 2011, 11, 5012-5017.

99. Prior, R. L.; Hoang, H.; Gu, L.; Wu, X.; Bacchiocca, M.; Howard, L.; Hampsch-Woodill, M.; Huang, D.; Ou, B.; Jacob, R. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORACFL)) of plasma and other biological and food samples. J. Agric. Food Chem. 2003, 51, 3273-3279.

100. Lussignoli, S.; Fraccaroli, M.; Andrioli, G.; Brocco, G.; Bellavite, P. A microplate-based colorimetric assay of the total peroxyl radical trapping capability of human plasma. Anal. Biochem. 1999, 269, 38-44.

101. Huang, D.; Ou, B.; Hampsch-Woodill, M.; Flanagan, J.; Prior, R. L. High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. J. Agric. Food Chem. 2002, 50, 4437-4444.

102. Fang, X.; Mark, G.; von Sonntag, C., OH radical formation by ultrasound in aqueous solutions Part I: the chemistry underlying the terephthalate dosimeter. Ultrasonics Sonochemistry 1996, 3, 57-63.

103. Liu, J.; Lagger, G.; Tacchini, P.; Girault, H. H., Generation of OH radicals at palladium oxide nanoparticle modified electrodes, and scavenging by fluorescent probes and antioxidants. Journal of Electroanalytical Chemistry 2008, 619-620, 131-136.

104. Ishibashi, K.; Fujishima, A.; Watanabe, T.; Hashimoto, K., Quantum yields of active oxidative species formed on TiO2 photocatalyst. Journal of Photochemistry and Photobiology A: Chemistry 2000, 134, 139-142.

105. Behnajady, M. A.; Modirshahla, N.; Shokri, M., Photodestruction of Acid Orange 7 (AO7) in aqueous solutions by UV/H2O2: influence of operational parameters. Chemosphere 2004, 55, 129-34.

106. C.Galindo; Kalt, A., UV-H_2O_2 oxidation of monoazo dyes in aqueous media a kinetic study. DYES AND PIGMENTS 1998, 40, 27-35.

107. Muruganandham, M.; Swaminathan, M., Photochemical oxidation of reactive azo dye with UV-H_2O_2 process. DYES AND PIGMENTS 2004, 62, 269 - 275.

108. Sharma, O. P.; Bhat, T. K., DPPH antioxidant assay revisited. Food Chemistry 2009, 113, 1202-1205.

109. Blois, M. S., Antioxidant determinations by the use of a stable free radical. Nature 1958, 181, 1199-1200.

110. Brand-Williams, W.; Cuvelier, M. E.; Berset, C., Use of a free radical method to evaluate antioxidant activity. Food Science and Technology-Lebensmittel-Wissenschaft & Technologie 1995, 28, 25-30.

111. Prior, R. L. W., Xianli; Schaich, Karen, Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. J. Agric. Food Chem 2005, 53, 4290-4302.

112. Esumi, K.; Takei, N.; Yoshimura, T., Antioxidant-potentiality of gold–chitosan nanocomposites. Colloids and Surfaces B: Biointerfaces 2003, 32, 117-123.

113. Arroyo, C. M.; Kramer, J. H.; Dickens, B. F.; Weglicki, W. B., Identification of free radicals in myocardial ischemia/reperfusion by spin trapping with nitrone DMPO. FEBS Letters 1987, 221, 101-104.

114. Šnyrychová, I.; Hideg, É., The first application of terephthalate fluorescence for highly selective detection of hydroxyl radicals in thylakoid membranes. Functional Plant Biology 2007, 34, 1105-1111.

115. Miller, N. J.; Rice-Evans, C. A.; Davies, M. J.; Gopinathan, V., & Milner, A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clinical Science 1993 84, 407-412.

116. Scott, S. L.; Chen, W. J.; Bakac, A.; Espenson, J. H., Spectroscopic parameters, electrode potentials, acid ionization constants, and electron exchange rates of the 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) radicals and ions. Journal of Physical Chemistry 1993, 25, 6710 - 6714.

117. Lemanska, K.; Szymusiak, H.; Tyrakowska, B.; Zielinski, R.; Soffer, A. E. M. F.; Rietjens, I. M. C. M. The influence of pH on the antioxidant properties and the mechanisms of antioxidant action of hydroxyflavones.Free Radical Biol. Med. 2001, 31, 869-881.

118. Arnao, M. B.; Cano, A.; Acosta, M., The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chemistry 2001, 73, 239-244.

119. Labrinea, E. P.; Georgiou, C. A., Stopped-flow method for assessment of pH and timing effect on the ABTS total antioxidant capacity assay. Analytica Chimica Acta 2004, 526, 63-68.

120. CHEN, I.-C.; CHANG, H.-C.; YANG, H.-W.; CHEN, A. G.-L.; Haenen, G. R. M. M., Evaluation of Total Antioxidant Activity of Several Popular Vegetables and Chinese Herbs A Fast Approach with ABTS/H_2O_2/HRP System in Microplates. Journal of Food and Drug Analysis 2004, 12, 29-33.

121. Liu, J.; Hurt, R. H. Ion Release Kinetics and Particle Persistence in Aqueous Nano-Silver Colloids. Environmental Science & Technology **2010**, 44, 2169 - 2175.

122. Mohammad, A. W.; Hilal, N.; Al-Zoubib, H.; Darwish, N. A.; Ali, N., Modelling the effects of nanofiltration membrane properties on system cost assessment for desalination applications. Desalination **2007**, 206, 215-225.

123. Levard, C.; Mitra, S.; Yang, T.; Jew, A. D.; Badireddy, A. R.; Lowry, G. V.; Brown, G. E., Jr., Effect of chloride on the dissolution rate of silver nanoparticles and toxicity to E. coli. Environ Sci Technol **2013**, 47, 5738-45.

124. Matsukawa, T.; S. Ikeda, H. I.; Yamada, M., Alleviation of the two-cell block of ICR mouse embryos by polyaminocarboxylate metal chelators. Reproduction **2002**, 124, 65–71.

125. Byegatrd, J.; Skarnemark, G.; Skalberg, M., The stability of some metal EDTA, DTPA and DOTA complexes: Application as tracers in groundwater studies. Journal of Radioanalytical and Nuclear Chemistry **1999**, 241, 281-290.

126. Sillanpaa, M.; Oikari, A., Assessing the Impact of Complexation by EDTA and DTPA on Heavy Metal Toxicity Using Microtox Bioassay. Chemospher **1996**, 32, 1485-1497.