Korean J. Chem. Eng., **30**(6), 1321-1325 (2013)
DOI: 10.1007/s11814-013-0073-0

Physicochemical properties between pristine and aged AgNPs for the evaluation of nanotoxicity

Joongso Choi, Ha Nee Umh, Jaehoon Sim, Hyeon Ho Shin, and Younghun Kim†

Department of Chemical Engineering, Kwangwoon University, Wolgye-dong, Nowon-gu, Seoul 139-701, Korea
(*Received 4 December 2012 • accepted 4 May 2013*)

Abstract−The use of nanomaterials in industrial and commercial applications is growing, and official reports concerning the possible environmental and health effects of nanoparticles are steadily increasing. An understanding of the potential toxicity of nanomaterials is important for creating sustainable and safe nanotechnologies. To test the cytotoxicity of nanomaterials, quantitative and qualitative analyses of raw nanomaterials should be priorities. However, the fundamental properties of raw materials will change compared to those of aged materials in biological media due to the interaction between nanomaterials and media composition. Therefore, the correlation and interdependence between pristine physicochemical properties (PChem) of raw nanomaterials before the toxicity test and aged PChem in biological media were evaluated using modified test guidelines originally suggested by the OECD WPMN (Organization for Economic Cooperation and Development, Working Party on Manufactured Nanomaterials) for peer-reviewed papers concerning silver nanoparticles, during the period of 2005 to 2010. In addition, we investigated whether the suggested analysis tools are applicable to define the PChem of AgNPs with regard to cytotoxicity.

Key words: Nanomaterials, Physicochemical Properties, Silver Nanoparticles, Toxicity

INTRODUCTION

The use of nanomaterials and nano-consumer products is rapidly increasing [1], and a thorough understanding of their potential toxicity for the environment, health, and safety (EHS) is important for sustainable and safe nanotechnologies [2]. Registered papers for nano-EHS in ICON (International Council on Nanotechnology) reached 6,200 in June, 2012, and 800-900 papers regarding hazard, exposure, and fate have been published annually in the last three years. Studies on nano-hazards mainly involve research on the exposure and environmental fate of nanomaterials (Fig. 1). There have been several recent reviews on the current state and oversight of nanotoxicity [3-6]. Many studies have been conducted on nano-EHS, and thus this subject has become a key topic in risk assessment, and has gathered the attention of the users of nano-consumer products as well as researchers who deal with nanomaterials.

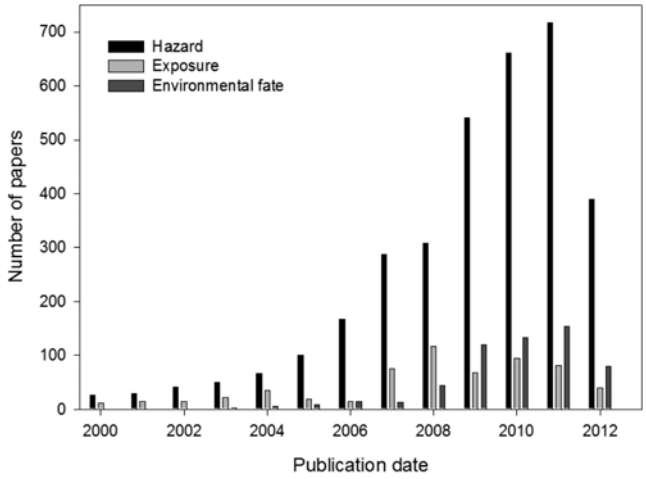

Fig. 1. Time progressive distribution analysis for paper publications from 2000 to 2012 (raw data obtained from ICON).

To carry out the cytotoxicity test of nanomaterials, quantitative and qualitative analyses of raw nanomaterials should be the priority. Until now, studies on *in-vivo* and *in-vitro* cytotoxicity of nanomaterials have progressed with a ratio of 25 to 75, which was analyzed by the ICON data base. In early studies on cytotoxicity, the physicochemical properties (PChem) of nanomaterials were mainly obtained from suppliers, and the possibility of PChem change in biological media was not considered. However, one study has proven that it is possible to have neighboring nanomaterials readily aggregate, which decreases their dispersion in biological media, resulting in the loss of the intrinsic properties of nanomaterials [7]. Therefore, even though toxicity tests have been performed for the same nanomaterials in the same media, researchers have reported different positive or negative data for toxicity. Recently, researchers have recognized that pristine and aged PChem analysis is important in toxicity tests, and PChem before and after *in-vivo* and *in-vitro* tests has been reported.

International organizations such as OECD (Organization for Economic Co-operation and Development) and ISO (International Organization for Standardization) have tried to suggest test guidelines (TG) for standard analysis protocols of nanomaterials' PChem. OECD WPMN (Working Party on Manufactured Nanomaterials) recommended a candidate analyzing method in 2010 through the project, "Manufactured Nanomaterials and Test Guideline," which addressed whether existing TG can be successfully applied to nanomaterials [8]. However, no clear analysis protocols for nanomaterials have

†To whom correspondence should be addressed.
E-mail: korea1@kw.ac.kr

been reported yet. Therefore, the analysis process itself can be a variable in the toxicity test of nanomaterials.

This study was conducted to determine the essential PChem in cytotoxicity tests, and in collected papers on silver nanoparticles (AgNPs) published between the years 2005 and 2010. Twelve kinds of representative PChem were extracted from the OECD's report and collected papers, and the validity of each PChem as an important factor in the toxicity test was evaluated. The correlation between the pristine PChem of raw nanomaterials before the toxicity test and aged PChem in biological media was estimated. The results may suggest that the PChem that defines the properties of nanomaterials according to manufacturers or suppliers are not identical to those determined by toxicologists. These perspectives will provide information for what PChem should be analyzed before and after cytotoxicity tests.

METHODS

We searched the existing TG reported by OECD, ISO, ASTM (American Society for Testing and Materials), and the EPA (Environmental Protection Agency) for the twelve kinds of representative PChem. The existing TG from international organizations was written in the 1980s and 1990s, and mainly focuses on micro or bulk particles and chemicals. We conducted a literature survey to define whether the analysis tools recommended by OECD were successfully applied to analyze target PChem. In addition, the main toxic mechanism for AgNPs in cells and biological organisms was investigated to correlate the representative PChem by a literature survey. Finally, the correlation and interdependence between the pristine PChem of raw nanomaterials before the toxicity test and aged PChem in biological media were evaluated. This study is limited to the cytotoxicity of AgNPs using 12 PChem, but was analyzed qualitatively and quantitatively for the importance of PChem before and after the toxicological study.

RESULTS AND DISCUSSIONS

1. Existing TG and Candidate Tools for PChem

OECD WPMN suggested four categories for 17 kinds of PChem, which are the basic properties required for the toxicity test of nanomaterials. The four categories are as follows: i) information about size distribution and agglomeration (size, shape, and agglomeration), ii) particle information (solubility, crystallinity, surface area, porosity, density, octanol/water partition coefficient, and dustiness, iii) surface chemistry (surface composition, surface charge, surface energy, and surface reactivity), and iv) reactivity (photocatalytic reactivity, redox potential, and radical formation potential). Some properties among the 17 kinds of PChem are not applicable to analyze AgNPs in the aqueous phase. For example, analysis for porosity is not required, because spherical AgNPs have no pores. Therefore, these properties were compressed into 12 representative PChem, as summarized in Table 1. The concentration of AgNPs is a control variable rather than an independent variable, and it was thus excluded from the 12 PChem.

A standard analysis protocol for the 12 PChem was partially suggested by OECD, ISO, ASTM, and the EPA (Table 1). Several studies on whether existing TG can be successfully applied to nanomaterials are underway by OECD WPMN. OECD's sponsorship program recommended possible analysis tools for the 12 PChem. A literature survey of papers on candidate tools was used to determine whether the suggested analyzing tools are applicable to define the 12 PChem of AgNPs in cytotoxicity tests. The key characteristics of the 12 PChem of AgNPs are summarized in Table 2.

Some examples are as follows. DLS was successfully applied to define particle aggregation as a function of ionic strength and the nature of the electrolyte [9]. A crystallographic plane of AgNPs prepared by the polyol method was easily confirmed as metallic AgNP with a face-centered cubic structure by XRD [10]. In addition, the possibility of a twinned crystalline structure of AgNPs could be ana-

Table 1. Standard methods for physicochemical characterizations of silver nanoparticles

Physicochemical properties		Existing protocol*	Candidate method**
PChem 1	Agglomeration/aggregation state		DLS, TEM
PChem 2	Crystalline phase/crystallite size	M 6300	XRD, HR-TEM
PChem 3	Representative TEM	M TEM	TEM
PChem 4	Size & size distribution	TG 100, TC 206	DLS, TEM, SEM, AFM
PChem 5	Zeta potential/surface charge		ELS-zeta
PChem 6	Surface chemistry	TC 201	FT-IR, fluorescence
PChem 7	Photocatalytic activity	TG 316, TC 206, M 21	UV-vis
PChem 8	Dispersion stability in water	TG 112, TC 34/35	MLS
PChem 9	Abiotic degradability/hydrolysis	TG 111, E 895-89	ICP, ISE
PChem 10	Octanol water partition coefficient	TG 107/123, E 1147-92	ICP
PChem 11	Redox potential	TC 190	ORP meter, CV
PChem 12	Radical formation potential		Fluorescence

*M (EPA protocol), TG (OECD protocol), TC (ISO protocol), E (ASTM protocol)

**Guidance manual for the testing of manufactured nanomaterials: OECD's sponsorship programme (ENV/JM/MONO(2009)20/REV)
DLS (dynamic light scattering), TEM (transmission electron microscopy), XRD (x-ray diffraction), HR-TEM (high resolution TEM), SEM (scanning electron microscopy), AFM (atomic force microscopy), ELS (electrophoretic light scattering), FT-IR (Fourier transformation infrared), UV-vis (UV visible spectroscopy), MLS (multiple light scattering), ICP (inductively coupled plasma spectroscopy), ISE (ion selective electrode), ORP (oxidative-reductive potential), CV (cyclic voltammetry)

Table 2. General features of cytotoxicity and PChem of AgNPs

Physicochemical properties		Toxicity trend	Key characteristics
1	Agglomeration/aggregation state	Decreasing antibacterial toxicity with aggregation of AgNPs	Fast flocculation with salts in media
2	Crystalline phase/crystallite size	Relatively toxic for (111) plane	High electron density of (111) plane
3	Representative TEM	Toxicity dependence on the size, shape, and concentration	Limitation to require the sample drying on TEM-grid
4	Size & size distribution	Increasing antibacterial toxicity with decreasing of particle size	The small size, the large surface area
5	Zeta potential/surface charge	Easily adsorption of positive charged AgNPs on the cell-membrane	Easy aggregation of AgNPs when surface charge approaches to zero value
6	Surface chemistry	Cytotoxicity dependence on the toxicity of stabilizer	Influence on the surface charge and dispersion stability
7	Photocatalytic activity	Cytotoxicity induced by release of Ag^+ ion via UV irradiation	Possible to degrade stabilizer via UV irradiation
8	Dispersion stability in water	High dispersion stability, high toxicity due to reduced aggregation	Decrease of dispersed AgNPs for low stability
9	Abiotic degradability/hydrolysis	Possible to release Ag^+ ion via hydrolysis	Release of Ag^+ ion dependence on the pH, temperature, and media
10	Octanol water partition coefficient	Hydrophobic AgNPs easy to adsorb cell-membrane	Hydrophilicity control by stabilizer
11	Redox potential	Increasing Ag^+ ion release with oxidation reaction	Control of ion release via adjustment of redox potential
12	Radical formation potential	High toxic with active radical in media	Generation of reactive oxygen species by Ag^+ ion

lyzed by electron diffraction and XRD [11]. The primary size and morphology of AgNPs were assessed using TEM, and compared with the hydrodynamic diameter obtained by DLS [12]. TEM was used complementarily with XRD and DLS [13]. For PChem 4 (size/size distribution), in an antibacterial test for AgNPs, the particle size distribution was measured by TEM and image analysis software [14]. The surface charge of AgNPs was easily measured by ELS, and the isoelectric point of the particles in test media was determined [15]. Although the verified examples for all PChem are not listed here (see Supplementary information), candidate tools proposed by OECD were reasonably applied in the analysis of AgNP properties. However, because the hydrodynamic diameter measured by DLS does not always match the particle size obtained from TEM, the particle size of the DLS data should be confirmed by TEM or XRD analysis.

2. Toxicity Mechanism of AgNPs with PChem

Although the cytotoxicity mechanisms behind the activity of AgNPs on cells and bacteria are still not well understood, the three most common mechanisms have been proposed [5]: (1) the uptake of free silver ions to disrupt ATP production, (2) the generation of reactive oxygen species by Ag^+ and AgNPs, and (3) the direct damage to the cell membrane by AgNP attack. To understand these cytotoxicity mechanisms, *in-vivo* and *in-vitro* tests using micro-organisms and mammalian cells should be carried out, and many toxicologists have performed relevant investigations.

The toxicity trend of AgNPs is summarized in Table 2 along with pristine PChem. In previous work, the cytotoxicity of AgNPs was investigated using Escherichia coli as a model organism, from the standpoint of the most relevant physicochemical properties used as three key metrics (ionic ratio, size, and agglomeration) [16]. The findings indicated that cytotoxicity is depressed by the agglomeration of AgNPs. The metrics listed in order of toxic sensitivity are as follows: total Ag concentration>ionic ratio>size. This order was inversely related to the extent of agglomeration. In a comparison test of the acute responses of mice livers to short-term exposure to nano-sized or micro-sized silver particles [17], smaller AgNPs were found to be more toxic with short-term exposure in mice. The surface charge is also an important factor for adsorption between AgNPs and the cell-membrane [18]. Qiu et al. reported the importance of surface chemistry for the cellular uptake of nanoparticles [19]. The intrinsic properties of stabilizer on the surface were influenced by cell vitality. Gu et al. reported that reactive oxygen species could be induced by AgNPs and cause oxidative damage and toxicity via proteins or membranes [20].

Three mechanisms could be correlated with the 12 PChem, as summarized in Table 3. The first and second mechanisms are mainly induced by the release of Ag^+ ions from AgNPs, and the PChem related to the dissolution of ions should be defined in cytotoxicity tests. PChem 7, 9, 11, and 12 are adaptable to these mechanisms. Because the third mechanism is direct cell damage by AgNPs, the intrinsic properties of nanoparticles such as PChem 1-6, 8, and 10

Table 3. Toxicity mechanisms of AgNPs exposed to cells and related PChem properties

Toxicity mechanisms of AgNPs to cell-uptake		Relating PChem
M1	Uptake of free Ag^+ followed by disruption of ATP production and DNA replication	7, 9, 11
M2	AgNP and Ag^+ generation of ROS	7, 9, 11, 12
M3	AgNP direct damage to cell membranes	1-6, 8, 10

must be measured before and after the cytotoxicity test.

3. Correlation between Pristine and Aged PChem

Various biological objects from cells to animals, including lung cells, *Escherichia coli*, *Daphnia magna*, zebrafish, and mice, were used in nanotoxicity tests. In toxicokinetic studies involving the exposure route of intravenous injection for mice, AgNPs must be dispersed in a blood isotonic solution. In addition, in cell vitality tests, AgNPs should be stabilized in culturing media such as PBS (phosphate buffered saline) and FBS (fetal bovine serum). Because various salts and proteins in biological media could change the pristine PChem of AgNPs through aggregation between salts or proteins and AgNPs, it is possible that nano-sized particles no longer exist in the media. Therefore, PChem between the initial and final states in media should be clearly verified.

The correlation and interdependence between pristine PChem of raw nanomaterials before the toxicity test and aged PChem in biological media were evaluated based on peer-reviewed papers published from 2005 to 2010. Raw data searched regarding keywords (AgNPs +toxicity) can be found in the supplementary information. Fig. 2 shows the number of articles related to the PChem properties of AgNPs obtained from material suppliers before *in-vivo* or *in-vitro* toxicological tests. The size and size distribution of AgNPs is frequently dealt with in papers, because these factors can be used to confirm that nanoparticles are the target particles. PChem information regarding hydrolysis, zeta potential, and crystalline phase was consistent with the raw properties of AgNPs. However, PChem 10-12 were not provided by suppliers.

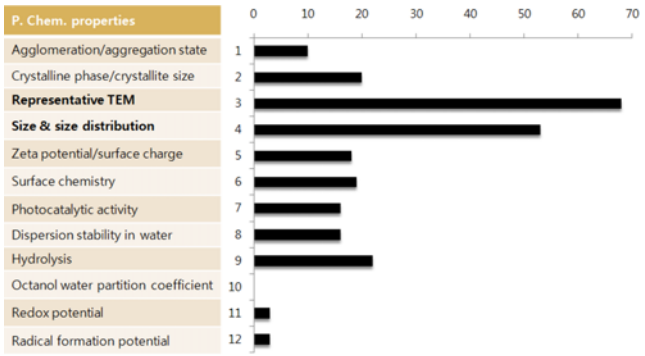

Fig. 2. Number of articles related to PChem properties of AgNPs obtained from materials' suppliers before *in-vivo* or *in-vitro* nanotoxicological tests.

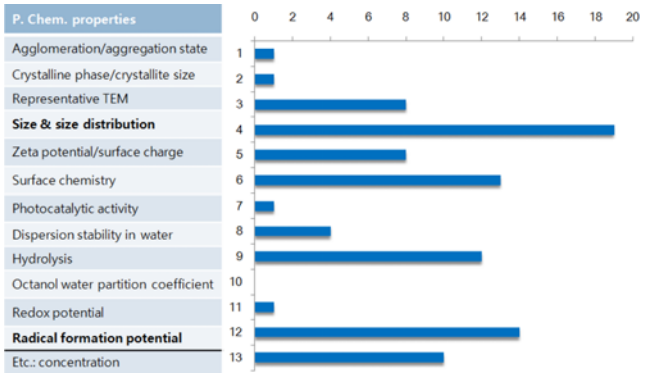

Fig. 3. Number of articles related to key PChem properties of AgNPs, which induced or effected on cytotoxicity of target organisms (cell, bacteria, rat, etc.) during *in-vivo* or *in-vitro* nanotoxicological tests.

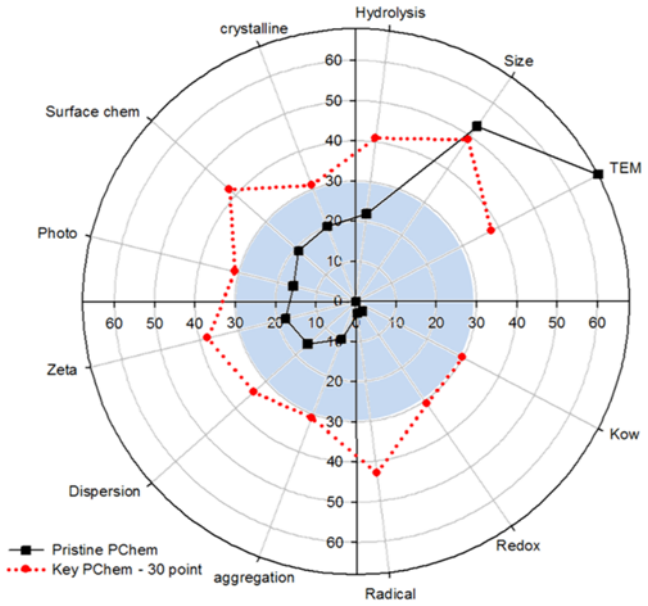

Fig. 4. Rose plot showing the relation between pristine PChem in beaker and key PChem in the toxicity test (deviation of point of key PChem is -30 from pristine PChem).

The aged properties of AgNPs in biological media are required, and thus, the most dominant factors among the 12 PChem affecting on cytotoxicity will be presented. To investigate the key PChem in toxicity tests, as shown in Fig. 3, articles relating to the key PChem that induces or affects the cytotoxicity of target organisms (cells, bacteria, and mice) are analyzed. As with the pristine PChem, information on the size and size distribution is important. The surface chemistry (stabilizer) and the possibility of radical formation have also emerged as key factors for cytotoxicity.

A rose chart plot for the pristine and aged PChem used in other studies (Fig. 4) shows pristine PChem listed in the order of TEM, size, hydrolysis, crystalline, and surface chemistry. Although the size, surface chemistry, and radical formation are the key PChem in media, some PChem in pristine and aged properties are considered equally. Information on radical formation, redox potential, and partition coefficient was not examined in detail. PChem 10-12 are directly correlated with toxicity mechanisms in Table 3. Therefore, characterization for these PChem must be carefully considered in future studies.

The interdependence among the 12 PChem was investigated by evaluating the extent of donors or acceptors for information. TEM data (PChem 3) could provide information about size (PChem 4), aggregation state (PChem 1), and crystalline phase (PChem 2). Based on this concept, the PChem information received from other PChem can be judge as important data. As shown in Fig. 5, the important PChem source is presented as a large circle. The PChem indicated by blue circles act as donors, and those indicated by red circles were considered as key acceptors. Therefore, in cytotoxicity studies, PChem 1, 4, 5, 6, and 8 have the greatest weight, and PChem 3, 7, 9, and 10 provide fundamental information. In cell toxicity, PChem 12

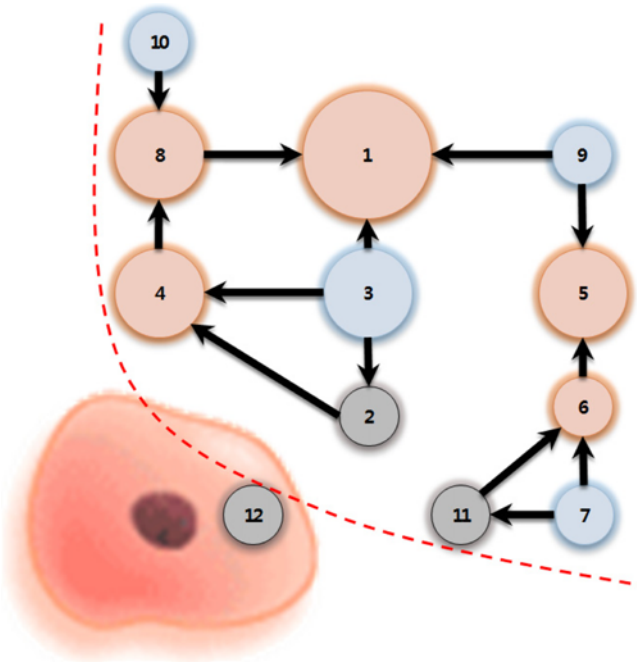

Fig. 5. Interdependence between PChem properties of AgNPs.

must be considered clearly.

CONCLUSIONS

Fundamental information about the PChem of nanomaterials in toxicity tests is required, but the TG suggested by international organizations is too old to apply to nanomaterials. Recently, OECD WPMN suggested candidate tools for the PChem analysis of nanomaterials, so we investigated whether the suggested analysis tools are applicable to define 12 PChem for AgNPs in cytotoxicity tests. Literature studies showed that candidate tools are reasonable for application to the analysis of AgNP properties. It is possible that the properties of AgNPs can change before and after toxicity tests, and thus, the correlation and interdependence between pristine PChem of raw nanomaterials before toxicity tests and aged PChem in biological media were evaluated based on peer-reviewed papers published from 2005 to 2010. While pristine PChem are listed in the order of TEM, size, hydrolysis, crystalline, and surface chemistry, key PChem in media were ordered as size, surface chemistry, and radical formation. Interestingly, the order of pristine PChem and aged PChem was not consistent. Although PChem 10-12 were measured in media, the same PChem were not dealt with in pristine AgNPs. The PChem of AgNPs should be considered before and after toxicity tests, because of the correlation between toxicity mechanisms and the PChem. Finally, a single PChem is not an independent variable but an interdependent one, and thus, complementary analysis is required to clearly analyze the properties of pristine or aged PChem.

ACKNOWLEDGEMENTS

This work was supported by the research fund of the National Institute of Environmental Research (2010) and a Research Grant from Kwangwoon University in 2013.

SUPPORTING INFORMATION

Raw data searched by keywords (AgNPs+toxicity). Additional information as noted in the text. This information is available via the Internet at http://www.springer.com/chemistry/journal/11814.

REFERENCES

1. H. N. Umh, J. Orh, J. Park, B. Kawk, B.-C. Lee, K. Choi, J. Yi and Y. Kim, *Korean Chem. Eng. Res*., **50**, 112 (2012).
2. E. Bae, H.-J. Park, J. Yoon, Y. Kim, K. Choi and J. Yi, *Korean J. Chem. Eng*., **28**, 267 (2011).
3. M. Auffan, J. Rose, J. Y. Bottero, G. V. Lowry, J. P. Jolivet and M. R. Wiesner, *Nat. Nanotech*., **4**, 634 (2009).
4. S. Bhattacharya, Q. Zhang, P. L. Carmichael, K. Boekelheide and M. E. Andersen, *PLoS One*, **6**, e20887 (2011).
5. C. Marambio-Jones and E. M. V. Hoek, *J. Nanopart. Res*., **12**, 1531 (2010).
6. A. D. Ostrowski, T. Martin, J. Conti, I. Hurt and B. H. Harthorn, *J. Nanopart. Res*., **11**, 251 (2009).
7. E. Bae, H. J. Park, J. Park, J. Yoon, Y. Kim, K. Choi and J. Yi, *Bull. Korean Chem. Soc*., **32**, 613 (2011).
8. OECD, *Guidance manual for the testing of manufactured nanomaterials: OECD's sponsorship programme*, ENV/JM/MONO(2009)20/REV (2009).
9. R. A. French, A. R. Jacobson, B. Kim, S. L. Isley, R. L. Penn and P. C. Baveye, *Environ. Sci. Technol*., **43**, 1354 (2009).
10. T. Zhao, R. Sun, S. Yu, Z. Zhang, L. Zhou, H. Huang and R. Du, *Colloids Surf. A*, **366**, 197 (2010).
11. J. Q. Hu, Q. Chen, Z. X. Xie, G. B. Han, R. H. Wang, B. Ren, Y. Zhang, Z. L. Yang and Z. Q. Tian, *Adv. Funct. Mater*., **14**, 183 (2004).
12. R. Flodbjerg, P. Olesen, M. Hougaard, D. A. Dang, H. J. Hoffmann and H. Autrup, *Toxicol. Kett*., **190**, 156 (2009).
13. Y. Sun and Y. Xia, *Science*, **13**, 2176 (2002).
14. D. Roe, B. Karandikar, N. Bonn-Savage, B. Gibbins and J. B. Rollet, *J. Antimicrob. Chemother*., **61**, 869 (2008).
15. T. M. Scown, E. M. Santos, B. D. Johnston, B. Gaiser, M. Baalousha, S. Mitov, J. R. Lead, V. Stone, T. F. Fernandes, M. Jepson, R. van Aerle and C. R. Tyler, *Toxicol. Sci.*, **115**, 521 (2010).
16. E. Bae, H. J. Park, J. Lee, J. Yoon, Y. Kim, J. Choi, K. Park, K. Choi and J. Yi, *Environ. Toxicol. Chem*., **29**, 2154 (2010).
17. K. Cha, H. W. Hong, Y. G. Choi, M. J. Lee, J. H. Park, H. K. Chae, G. Ryu and H. Myung, *Biotechnol. Lett*., **30**, 1893 (2008).
18. W. Jinag, H. Mashayekhi and B. Xing, *Environ. Pollut*., **157**, 1619 (2009).
19. Y. Qiu, Y. Liu, L. Wang, L. Xu, R. Bai, Y. Ji, X. Wu, Y. Zhao, Y. Li and C. Chen, *Biomaterials*, **31**, 7606 (2010).
20. E. T. Hwang, J. H. Lee, Y. J. Chae, Y. S. Kim, B. C. Kim, B. I. Sang and M. B. Gu, *Small*, **4**, 746 (2008).

Supporting Information

Change of the physicochemical properties between pristine and aged AgNPs for the evaluation of nanotoxicity

Joongso Choi, Ha Nee Umh, Jaehoon Sim, Hyeon Ho Shin, and Younghun Kim†

Department of Chemical Engineering, Kwangwoon University, Wolgye-dong, Nowon-gu, Seoul 139-701, Korea
(*Received 4 December 2012 • accepted 4 May 2013*)

Journal	Vol	Page	Year	Materials	1	2	3	4	5	6	7	8	9	10	11	12	Key PChem
Toxicol Sci	115	521	2010	AgNPs		1	1	1	1	1		1					Concentration
J Antimicrob Chemother	65	258	2010	AgNPs		1	1	1		1							Concentration
Environ Toxicol Chem	29	2154	2010	AgNPs	1	1	1	1									Size
J Nanobiotech	8	15	2010	PVP/AgNPs			1	1		1							Size
J Appl Toxicol	30	74	2010	AgNPs			1	1		1							Shape, surface chemistry
Adv Funct Mater	20	1233	2010	PVA/AgNPs			1	1	1						1		Agglomeration
Environ Toxicol	27	42	2012	AgNPs				1		1							Concentration
Toxicol Sci	108	452	2009	AgNPs	1	1	1	1	1								Capping agent, size
Small	5	1553	2009	AgNPs			1	1	1								Shape, concentration
Small	5	1897	2009	AgNPs			1	1		1							Shape
Chem Med Chem	4	1129	2009	PVP/AgNPs			1	1							1		Shape
Environ Health	8	S2	2009	AgNPs		1	1	1	1	1							Shape, size
Bull Korea Chem Soc	29	1179	2008	AgNPs		1	1	1									Shape, size
Small	4	746	2008	AgNPs			1	1					1				Shape, size
J Antimicrob Chemother	61	869	2008	AgNPs/plastic catheter		1	1	1			1						Shape, size
Environ Toxicol Chem	27	1972	2008	AgNPs			1	1	1			1			1		Size, lattice
Nanotech	19	255102	2008	AgNPs			1	1		1							Size
Inhal Toxicol	19	857	2007	AgNPs	1		1	1									Capping agent, size
Appl Environ Microbiol	73	1712	2007	AgNPs		1	1	1									Concentration
J Biomed Nanotechnol	3	203	2007	AgNPs		1	1	1									Media
Nanobiotech	3	55	2007	AgNPs				1									Size, concentration
Nanotech	16	2346	2005	AgNPs		1	1	1									Media
Toxicol Sci	88	412	2005	AgNPs				1									Capping agent, size
J Nanobiotech	3	6	2005	AgNPs		1	1	1									Media, size
ACS Nano	1	133	2007	Citrate AgNP			1	1			1	?					-
Langmuir	24	7457	2008	AgNP			1										-
J Phys Chem C	112	5825	2008	SDS, Tween 80, PEG, PVP, PDDA AgNP	1		1				1	1					Stability
Enviro Sci Technol	42	4583	2008	PVA AgNP				1			1					1	Size, ionic ratio
Enviro Sci Technol	42	8959	2008	Carbonate coating AgNP	1		1		1			1	1				-

J Phys Chem B	112	13608	2008	Hydrocarbon-coated AgNP									1	Size
ACS Nano	3	279	2008	Starch-coated AgNP			1	1		1			1	-
Enviro Sci Technol	43	3933	2009	AgNP			1	1						-
Enviro Sci Technol	43	6046	2009	Polyethylenimine AgNP										-
J Phys Chem C	113	4296	2009	Tween 80, PEG 35000, PVP 360 AgNP			1	1	1	1	1			Surface modification
Enviro Sci Technol	43	7285	2009	Citrate AgNP			1	1				1		pH, Humic acid
Enviro Sci Technol	43	9004	2009	Citrate AgNP			1							pH, Fulvic acid
Enviro Sci Technol	44	2163	2010	Bare, Carbonate coating AgNP			1		1	1				Surface modification
Enviro Sci Technol	44	5649	2010	Ag/SiO_2 Nanoparticle		1	1	1		1		1		Ionic ratio
Enviro Sci Technol	44	7321	2010	AgNP			1	1	1		1	1		Stability
Ind Eng Chem Res	49	852	2010	Biosynthesized AgNP			1			1				-
J Phys Chem C	114	5798	2010	AgNP in ethyleneglycol										-
Enviro Sci Technol	44	5210	2010	Biosynthesized AgNP, Colloidal Ag, Olated capped AgNP			1	1	1	1		1		Synthesis procedure
J Nanop Res	7	145	2005	AgNP		1	1							-
Nanobiotechnol	3	55	2007	Citrate AgNP										-
Biotechnol Lett	30	1893	2008	AgNP			1			1				Size
Biotechnol Biopro Eng	14	490	2009	AgNP			1	1						Different microorganism
Cellulose	16	1147	2009	AgNP: two different stabilizer				1		1				-
Biotechnol Biopro Eng	14	842	2009	Citrate AgNP		1		1		1				Different microorganism
Nanobiotechnol	5	2	2009	AgNP										Size
Langenbecks Arch Surg	394	495	2009	PVP AgNP				1						-
World J Microbiol Biotechnol	26	615	2010	AgNP										-
Colloid J	72	66	2010	AgNP			1			1				Reductant
Arch Toxicol	84	63	2010	AgNP										-
Ecotoxicology	19	185	2010	AgNP			1	1						-
Toxicology	3	179	2009	AgNP	1		1	1	1					Action potential
Adv Drug Del Rev	60	1289	2008	AgNP		1				1			1	Ion release
Biochem Biophys Res Commun	390	733	2009	AgNP	1		1						1	Oxidative stress
Chem Phys Lett	487	92	2010	PVP-AgNP			1			1	1			Surface chemistry
Toxicol Lett	191	305	2009	AgNP			1					1		Radical formation
Aqua Toxicol	96	44	2010	AgNP			1						1	ROS formation
Colloids Surf B	78	334	2010	PVA-AgNP							1		1	UV irradiation
Aqua Toxicol	94	320	2009	AgNP		1	1		1				1	Ion release
Sci Total Environ	407	4184	2009	Citrate-AgNP			1			1				Protein adsorption
Sci Total Environ t	407	5243	2009	AgNP								1		Dispersion, concentration

Toxicol in Vitro	19	975	2005	AgNP							1					ROS generation
Aqua Toxicol	100	151	2010	AgNP			1				1	1				Ion release
Toxicol Appl Pharm	236	310	2009	AgNP	1		1	1								ROS generation
Toxicol inVitro	23	1076	2009	AgNP			1					1				Oxidative stress
Toxicol Lett	190	156	2009	PVP-AgNP			1	1	1							ROS generation
Environ Toxicol Pharm	30	162	2010	AgNP				1								Size
Water Res	43	1879	2009	PVA-AgNP						1	1	1				Surface chemistry
Neurotoxicol Teratol	32	391	2010	AgNP								1				Ion release
Adv Colloid Interf Sci	145	83	2009	Polysaccharide-AgNP			1			1		1				Ion release
Toxicol in Vitro	24	872	2010	AgNP	1		1	1			1					ROS generation
Toxicol Appl Pharm	242	263	2010	Polysaccharide-AgNP			1	1	1		1					Dispersion stability
Biomaterials.	31	680	2010	AgNP								1				Ion release
Nanomed	6	681	2010	AgNP			1	1	1		1					Size
Environ Poll	157	3034	2009	PVP-AgNP			1					1				Ion release
Nanomed	4	237	2008	PVP-AgNP			1	1								Concentration
ToxicologyLetters	179	130	2008	AgNP			1									ROS generation
Biomaterials	30	5979	2009	AgNP		1				1		1				ROS generation
Marin Environ Res	69	S49	2010	Citrate-AgNP							1					Concentration
Appl Clay Sci	48	547	2010	AgNP		1		1								ROS generation
Toxicol Appl Pharm	233	404	2008	AgNP, polysaccharide-AgNP						1						Surface chemistry
LWT-Food Sci Technol	41	1100	2008	PVP-AgNP			1			1						Surface chemistry
Toxicol Lett	179	93	2008	AgNP			1									Oxidative stress
Water Res	42	3066	2008	PVA-AgNP			1					1				Hydrolysis
Internat J Antimicro Agents	36	280	2010	βcyclodextrin-AgNP			1	1		1						Surface chemistry
Colloids Surf B	73	51	2009	AgNP				1								
Mater Sci Eng C	29	2104	2009	SDS-AgNP		1	1			1						Surface chemistry
Toxicol Lett	197	82	2010	Citrate-AgNP			1					1				Ion release
Biomaterials	30	6333	2009	PVP-AgNP			1	1		1		1	1			Ion release
Aqua Toxicol	100	160	2010	PVP-AgNP		1	1	1								Concentration
Neuro Toxicol	30	926	2009	AgNP			1	1	1							Oxidative stress
Toxicol Lett	187	15	2009	AgNP	1		1	1	1							Oxidative stress
		Total			10	20	68	53	18	19	16	16	22	0	3	3	95