

## Full Length Research Paper

# Lung uptake of gold nanoparticles after intraperitoneal administration *in vivo*: Fluorescence study

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**Gold nanoparticles (GNPs) have potential applications in cancer diagnosis and therapy, drug delivery and food industry. Some studies indicate that certain nanoparticles (NPs) may cause adverse effects because of their small size and unique properties. However, little is known about the particle-size and exposure duration of intraperitoneal administration of GNPs in the rat lung tissue *in vivo*. The aim of the present study was to elucidate the particle size and exposure duration effects of intraperitoneal administration of GNPs on the rat lung tissue *in vivo* using fluorescence spectroscopy.**

**Key words:** Gold nanoparticles, sizes, exposure duration, lung tissue, fluorescence spectroscopy.

## INTRODUCTION

Fluorescence has many practical applications, including mineralogy, gemnology, chemical sensors, fluorescent labelling, dyes and biological detectors. Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiation of a different wavelength. In most cases, emitted light has a longer wavelength, and therefore lower energy, than the absorbed radiation. However, when the absorbed electromagnetic radiation is intense, it is possible for one electron to absorb two photons; this two-photon absorption can lead to emission of radiation having a shorter wavelength than the absorbed radiation. Size, surface area and number of particles appear to play important roles in facilitating nano-sized particle-related toxicity. It has been proposed that the size of the particles plays a key role in their adhesion to and interaction with biological cells (Braydich-Stolle et al., 2005; Foster et al., 1997).

The origin of the unique optical properties of GNPs is a phenomenon known as surface plasmon resonance (SPR). When an electromagnetic radiation, of a wavelength much smaller than the diameter of the GNPs, hits the particles, it induces coherent, resonant oscillations of the metal electrons across the nanoparticles. These oscillations are known as the SPR, which lie within visible frequencies and result in strong optical absorbance and scattering properties of the GNPs (Schmid, 1992; Jain et al., 2007). This property allows the

use of GNPs for many applications, for example, they can be used as Raman sensors (Tian et al., 2002), photocatalysts (Kamat, 2002), and photo electrochemical materials (Shipway et al., 2000; Kuwahara et al., 2001). In the bioscience and medical fields, GNPs are used as immunostaining marker particles for electron microscopy, and as chromophores for immunoreactions and nucleic acid hybridization (Mirkin et al., 1996; Huber et al., 2004).

Numerous studies have shown that exposures to nanoscale particles produce grater inflammatory and cytotoxic effects when compared to exposures to larger sized particles at equivalent mass concentration (Wang et al., 2007). It is considered that nanoparticles can be more reactive with biological components and have adverse effects due to large surface area and much particle number (Nel et al., 2006).

Toxicity has been thought to originate from nanomaterial size and surface area, composition, and shape. Size plays a role in how the body responds to, distributes, and eliminates materials (Kamat, 2002; Huber et al., 2004). Particle size can also affect the mode of endocytosis, cellular uptake, and the efficiency of particle processing in the endocytic pathway (Liu, 2006; Baptista et al., 2008).

GNPs can be used in various biomedical applications, however, very little is known about their size-dependence *in vivo* kinetics. Here, we focus our attention on aspects related to fluorescence spectroscopy for different GNP

sizes dissolved in aqueous solution. Moreover, the particles size and shape were monitored by the transmission electron microscopy. Thus, the aim of the present study was to elucidate the period effects of intravenous administration of different GNP sizes on the rat lung tissue *in vivo* using fluorescence spectroscopy.

## MATERIALS AND METHODS

The experimental rats were divided into control and six groups (G1A, G1B, G2A, G2B, G3A and G3B; G1: 20 nm; G2: 10 nm; G3: 50 nm; A: infusion of GNPs for 3 days; B: infusion of GNPs for 7 days). To investigate the period effects of GNPs 10, 20 and 50 nm on the lung tissue of rats, 50  $\mu$ l dose of GNPs (of concentration 0.1% Au) were intravenously injected into rats for periods of 3 and 7 days to identify the toxicity and tissue distribution of GNPs *in vivo* using fluorescence measurements.

### Gold nanoparticles (GNPs)

Different GNP sizes of 10, 20 and 50 nm were purchased (Product MKN-Au-010, MKN-Au-020 and MKN-Au-050 in aqueous solution of concentration 0.01% of gold, Canada) and used in this study. The mean size and morphology of these GNPs in addition to assessment the high electron densities of GNPs as well as the homogeneity of the particles shape and size were addressed from the images taken by the transmission electron microscope (TEM) (Abdelhalim et al., 2011).

### Animals

Healthy, male Wistar-Kyoto rats obtained from the Laboratory Animal Center (College of Pharmacy, King Saud University). 8 to 10 weeks old (approximately 250 g body weight) were housed in pairs in humidity and temperature-controlled ventilated cages on a 12 h day/night cycle. A rodent diet and water were provided. To study the infusion period effects of GNP size, fifty rats were individually caged, and divided into control group (NG: n = 8), group 1 (A: infusion of GNPs of size 20 nm for 3 days; n = 6 and B: infusion of GNPs of size 20 nm for 7 days; n = 6), group 2 (A: infusion of GNPs of size 10 nm for 3 days; n = 6 and B: infusion of GNPs of size 10 nm for 7 days; n = 6) and group 3 (A: infusion of GNPs of size 50 nm for 3 days; n = 6 and B: infusion of GNPs of size 50 nm for 7 days; n = 6). 10, 20 and 50 nm GNPs in aqueous solutions was administered to the animals via intraperitoneal injection. The rats were anesthetized by inhalation of 5% isoflurane until muscular tonus relaxed. Lung tissue was collected from each rat.

### Digestion of rat tissue samples

Lung rat tissue samples were wet digested with nitric acid and converted into acidic digest solutions for analysis by fluorescence spectroscopy. The tissue was freeze dried in order to minimize loss of analytes and to facilitate subsequent sample preparation steps, and then homogenized to a fine powder by ball-milling in plastic containers. Approximately 0.20 to 0.25 g of powdered tissue was weighed into a Teflon reaction vessel and 3 ml of  $HNO_3$  were added. The closed reaction vessel was heated in a 130°C oven until digestion was completed. Samples were then diluted to a final volume of 20 ml with quartz distilled water and stored in 1 oz. polyethylene bottles for later fluorescence spectroscopy analysis.

### Fluorescence spectroscopy

Fluorescence characterization of different GNP sizes 10 and 20 nm) was performed using FluoroMax-2 (JOBAN YVON-SPEX, Instruments S.A., Inc., France). Fluorescence measurements were made over the wavelength range of 250 to 700 nm. The fluorescence measurements were made using 1 cm path length quartz cuvettes which were cleaned before each use by sonicating them for 5 min in deionized water and then rinsing with deionized water.

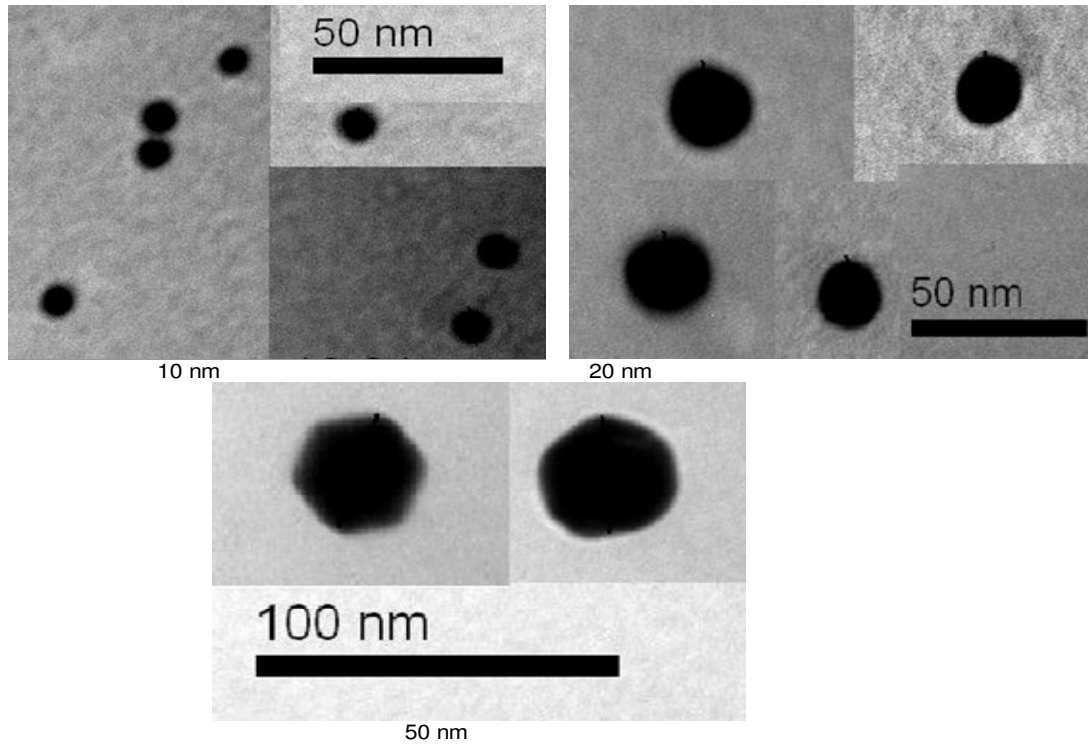
## RESULTS AND DISCUSSION

The fluorescence peak intensity increased for G1A, G2A and G1B compared with the control while it decreased for G3A and G2B, and sharply decreased for G3B compared with the control. Fluorescence peak intensity for 10 and 20 nm GNPs was higher than that of 50 nm GNPs at the infusion period of 3 days; while fluorescence peak intensity for 20 nm GNPs was higher than those for 10 and 50 nm GNPs at the infusion period of 7 days.

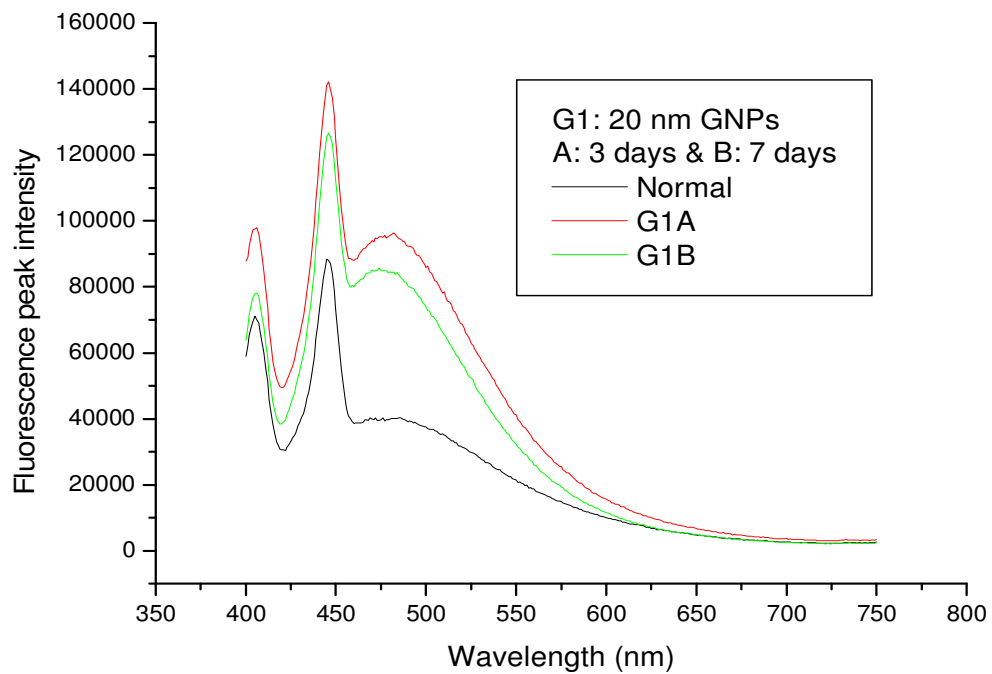
Fluorescence peak intensity increased for smaller GNPs (10 and 20 nm) while it decreased with larger GNPs (50 nm) compared with the control at the infusion period of 3 days. Fluorescence peak intensity increased for G1B, decreased for G2B and sharply decreased for G3B compared with the control at the infusion period of 7 days. Fluorescence peak intensity for 20 nm GNPs was higher than those for 10 and 50 nm GNPs at the infusion period of 7 days. The decrease in fluorescence peak intensity may be attributed to clearance of GNPs via urine and bile, size, shape, nanoparticles number, surface area, and strong quenching of GNPs. Moreover, decreasing the size may lead to an exponential increase in surface area relative to volume, thus making GNPs surface more reactive on itself (aggregation) and to its surrounding environment (biological components). This study demonstrates that fluorescence peak intensity is particle size and exposure duration dependence. The alterations of accumulation of GNPs in the lung tissue may be mediated by dynamic protein binding and exchange. A better understanding of these mechanisms will improve drug delivery and estimate the risk assessment.

### Size and morphology of different GNPs size

The GNPs sizes 10 and 20 nm show spherical morphology with good particle size distribution dispersed in the solution. The mean sizes for these GNPs were calculated from the images taken by the TEM. Mean size was  $9.45 \pm 1.33$  nm for GNPs of size 10 nm and  $20.18 \pm 1.80$  nm for GNPs of size 20 nm while GNPs of 50 nm have no spherical shape, but they have hexagonal shape as shown in Figure 1. The high electron densities of GNPs as well as, the homogeneity of the particles shape and size make them highly conspicuous under the TEM



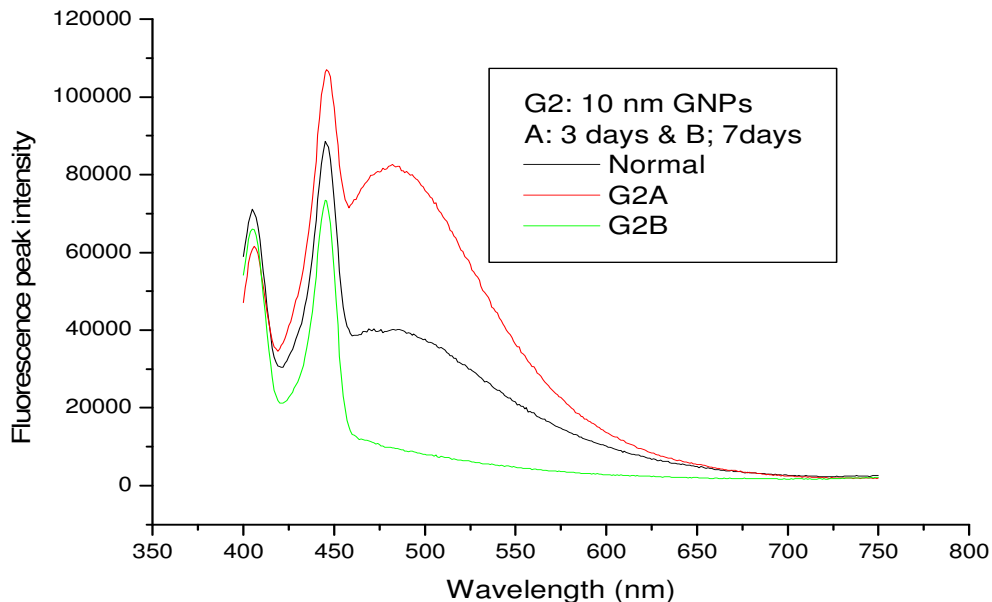
**Figure 1.** TEM images for different GNP samples.



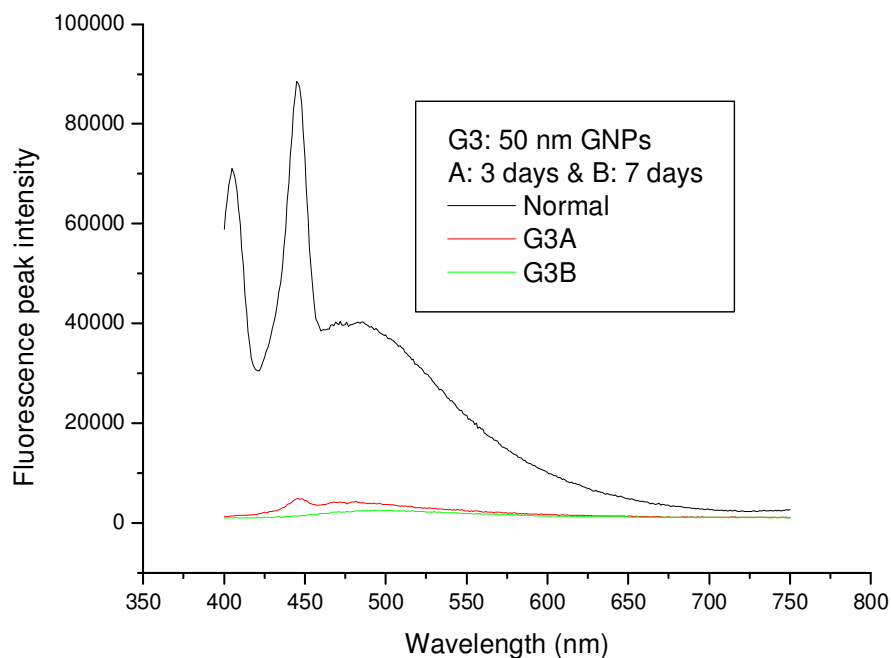
**Figure 2.** The emission fluorescence peak intensity for 20 nm GNPs at infusion periods of 3 and 7 days.

(Abdelhalim, 2011; Abdelhalim et al., 2011). Figure 2 shows that fluorescence peaks intensity at wavelength

445 nm increased for G1A and G1B compared with the control. Fluorescence peak intensity for 20 nm GNPs at



**Figure 3.** The emission fluorescence peak intensity for 10 nm GNPs at infusion periods of 3 and 7 days.

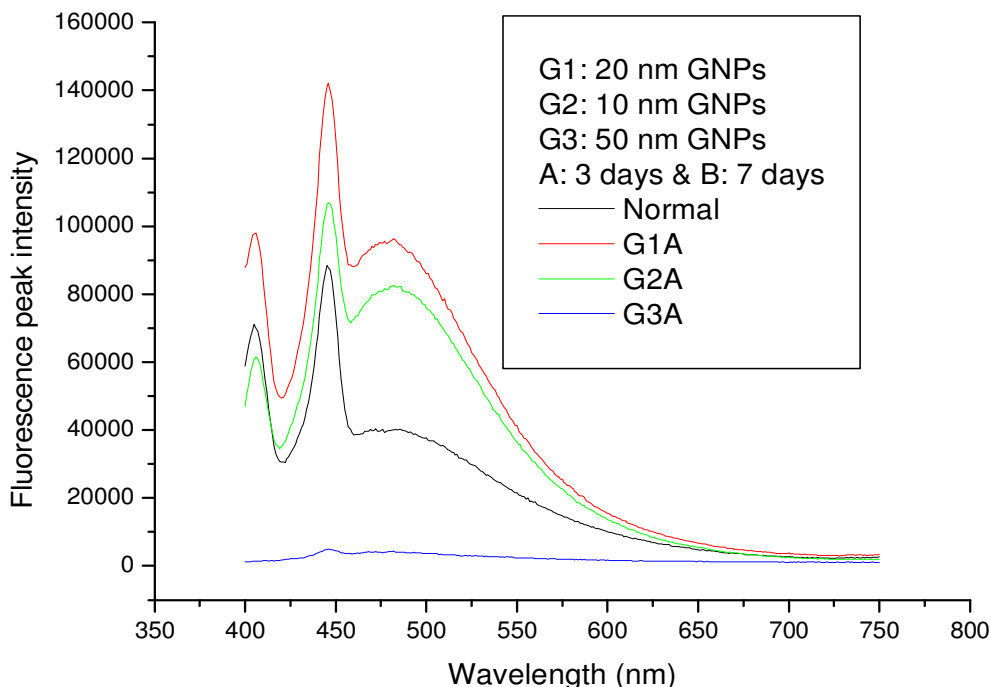


**Figure 4.** The emission fluorescence peak intensity for 50 nm GNPs at infusion periods of 3 and 7 days.

infusion period of 3 days was higher than that at infusion period of 7 days. The decrease in fluorescence peak intensity for G1B may be attributed to clearance of GNPs through the urine and bile.

Figure 3 shows that fluorescence peaks intensity at wavelength 445 nm increased for G2A and decreased for

G2B compared with the control. Fluorescence peak intensity for 10 nm GNPs at infusion period of 3 days was higher than that at infusion period of 7 days. The decrease in fluorescence peak intensity for G2B may be attributed to clearance of GNPs through the urine and bile. Figure 4 shows that fluorescence peaks intensity at



**Figure 5.** The emission fluorescence peak intensity for 10, 20 and 50 nm GNPs at infusion period of 3 days.

wavelength 445 nm decreased for G3A and G3B compared with the control. Fluorescence peak intensity for 50 nm GNPs at infusion period of 3 days was higher than that at infusion period of 7 days which may be attributed to clearance of the GNPs through the urine and bile; while the decrease in fluorescence peak intensity for G3A and G3B may be attributed to clearance of GNPs, size, shape, nanoparticles number, surface area and strong quenching of GNPs.

Figure 5 shows fluorescence peak intensity for 10, 20 and 50 nm GNPs at wavelength 445 and infusion period of 3 days. The fluorescence peak intensity increased for G1A and G2A compared with the control while it decreased for G3A compared with the control. Fluorescence peak intensity for 10 and 20 nm GNPs was higher than that of 50 nm GNPs at the infusion period of 3 days. The decrease in fluorescence peak intensity for G3A compared with G1A and G2A may be attributed to clearance of GNPs, size, shape, nanoparticles number, surface area, and strong quenching of GNPs. Figure 6 shows fluorescence peak intensity for 10, 20 and 50 nm GNPs at wavelength 445 and infusion period of 7 days. The fluorescence peak intensity increased for G1B, decreased for G2B and sharply decreased for G3B compared with the control. Fluorescence peak intensity for 20 nm GNPs was higher than those for 10 and 50 nm GNPs at the infusion period of 7 days. The decrease in fluorescence peak intensity for G3B compared with G1B may be attributed to clearance of GNPs, size, shape, nanoparticles number, surface area, and strong

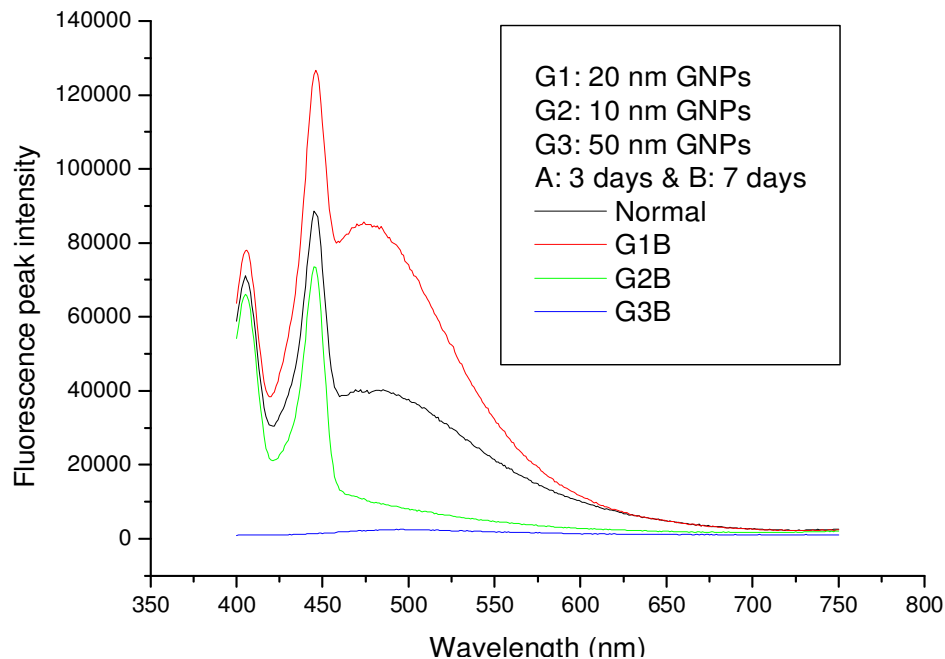
quenching of GNPs. Figure 7 shows fluorescence peak intensity for 10, 20 and 50 nm GNPs at wavelength 445 and infusion periods of 3 and 7 days.

The fluorescence peak intensity increased for G1A, G2A and G1B compared with the control while it decreased for G3A and G2B, and sharply decreased for G3B compared with the control. Fluorescence peak intensity for 10 and 20 nm GNPs was higher than that of 50 nm GNPs at the infusion period of 3 days; while fluorescence peak intensity for 20 nm GNPs was higher than those for 10 and 50 nm GNPs at the infusion period of 7 days.

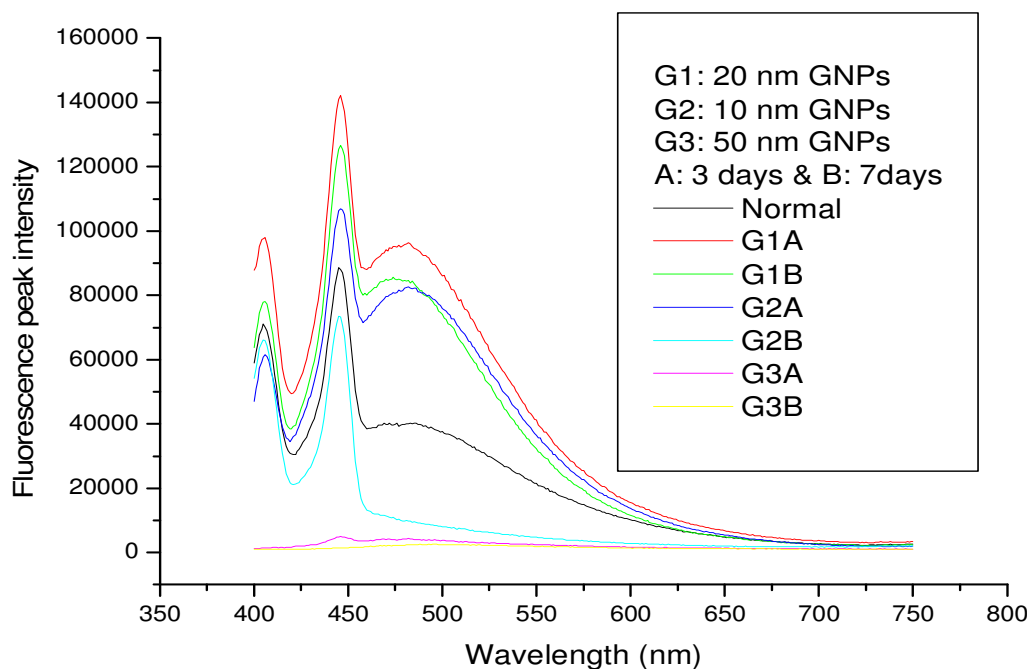
This decrease in fluorescence peak intensity for G3A, G2B and sharply decrease for G3B compared with the control may be attributed to the following:

- 1) The occurrence of a strong quenching of the fluorescence from serum albumins due to the formation of a ground state complex with GNPs (static quenching);
- 2) Electron microscopy revealed that most GNPs of size 50 nm were of hexagonal morphology than spherical morphology (GNPs of sizes 10 and 20 nm).
- 3) The physical and chemical properties as well as the applications of nanoparticles are controlled and limited by their dimensions and shape.
- 4) The 50 nm GNPs may be taken up faster and more intensively than the other sizes by macrophages of the lung and disappeared thereafter.

This study indicates that decreasing size may lead to an



**Figure 6.** The emission fluorescence peak intensity for 10, 20 and 50 nm GNPs at infusion period of 7 days.



**Figure 7.** The emission fluorescence peak intensity for 10, 20 and 50 nm GNPs at infusion periods of 3 and 7 days.

exponential increase in surface area relative to volume, thus making the GNPs surface more reactive on itself (aggregation) and to its surrounding environment (biological components). This study also suggests that

the increase in fluorescence intensity may be attributed to increase in the surface area which is related to number of GNPs and slow clearance via urine and bile from the kidney. Increased uptake into certain tissues may lead to

accumulation, where they may interfere with critical biological functions. The rate of exocytosis of GNPs was size dependent with more accumulation of larger gold nanoparticles in the cell (Liu, 2006; Shrestha et al., 2006).

It has been published that GNPs of very small sizes impart physical and chemical properties that are very different from those of the same material in the bulk form. These properties include a large surface to volume ratio, enhanced or hindered particle aggregation depending on the type of surface modification, enhanced photoemission, high electrical and heat conductivity, and improved surface catalytic activity (Liu, 2006; Shrestha et al., 2006). Since this surface area can interact with biological components of cells, small nanoparticles can be more reactive than larger particles. Incidence and severity of inflammatory response was transiently increased with injection of 200 and 100 nm GNPs within 12 h. GNPs were trapped by macrophages in the spleen and lung and remained there until 4 weeks after the single injection (Cho et al., 2010).

To evaluate the impact of a particle size on tissue distribution, we injected 50  $\mu$ l different sizes of GNPs. The 50 nm GNPs were taken up faster and more intensively than the other sizes by macrophages of the lung and disappeared thereafter. This result is related with the inflammatory response of the lung. Nanoparticles for therapy need to have a long retention time for targeting and therapy. However, a long retention time can evoke the toxic effects *in vivo*. Thus, the clearance rate and route of nanomaterials is an important issue (Cho et al., 2010; Choi et al., 2007).

Absorbed nanoparticles within the systemic circulation can be excreted through various routes. A possible elimination route for nanoparticles could be renal and biliary clearance. Renal clearance of solid nano-sized materials was affected by particle size and surface charge (Choi et al., 2007; Gupta, 2005). In our study, we compared fluorescence intensity and distribution of three different GNPs of varying particle size after intravenous injection into rats for periods of 3 and 7 days. It has been demonstrated that fluorescence intensity and lung tissue distribution was different depending on particle size.

The fluorescence peak intensity increased for G1A, G2A and G1B compared with the control while it decreased for G3A and G2B, and sharply decreased for G3B compared with the control. Fluorescence peak intensity for 10 and 20 nm GNPs was higher than that of 50 nm GNPs at the infusion period of 3 days; while fluorescence peak intensity for 20 nm GNPs was higher than those for 10 and 50 nm GNPs at the infusion period of 7 days. As particle sizes increased, fluorescence intensity decreased because more particles were trapped by macrophages.

These results could serve as a guideline in the rational design of drug nanocarriers with maximized therapeutic efficacy and predictable *in vivo* properties. All injected

GNPs showed a propensity to accumulate in the lung tissue. The lung tissue distribution of GNPs is size-dependence, with the smallest particles showing the most widespread organ distribution. Smaller GNPs showed greater cellular accumulation due to the dose-metric treatment. Therefore, GNPs target organs with many phagocytic cells such as lung, spleen, and mesenteric lymph node should be taken into consideration.

## Conclusions

The aim of the present study was to elucidate the size and exposure duration effects of intraperitoneal administration of GNPs on the lung tissue of rats using fluorescence measurements *in vivo*. The high electron densities of GNPs as well as the homogeneity of the particles shape and size make them highly conspicuous under the TEM. GNPs of sizes 10 and 20 nm have spherical shape, but GNPs of size 50 nm have hexagonal shape.

The fluorescence peak intensity increased for G1A, G2A and G1B compared with the control while it decreased for G3A and G2B, and sharply decreased for G3B compared with the control.

Fluorescence peak intensity for 10 and 20 nm GNPs was higher than that of 50 nm GNPs at the infusion period of 3 days; while fluorescence peak intensity for 20 nm GNPs was higher than those for 10 and 50 nm GNPs at the infusion period of 7 days. Fluorescence intensity of GNPs was size, shape, number, surface area, infusion period dependent in addition to the clearance via urine and bile. The decrease in fluorescence intensity induced in large 50 nm GNPs may be attributed to the occurrence of strong quenching, decrease number and surface area of GNPs in addition to high clearance of GNPs via urine and bile. Moreover, decreasing size may lead to an exponential increase in surface area relative to volume, thus making GNPs surface more reactive on itself (aggregation) and to its surrounding environment (biological components). This may be related to their useful characters, also plays a key role in toxicity.

This study demonstrates that fluorescence peak intensity is particle size and exposure duration dependence. The alterations of accumulation of GNPs in the lung tissue may be mediated by dynamic protein binding and exchange. A better understanding of these mechanisms will improve drug delivery and estimate the risk assessment. This study suggests that extensive further studies in several organs of rats *in vivo* are needed.

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## REFERENCES

- Abdelhalim MAK (2011). The period effects of intraperitoneal administration of different gold nanoparticle sizes on kidney tissue of rat *in vivo* using fluorescence measurements. African Journal of Microbiology Research in publication.
- Abdelhalim MAK, Mady MM, Ghannam MM (2011). Dielectric constant, electrical conductivity and relaxation time measurements of different gold nanoparticle sizes. International Journal of the Physical Sciences in Publication.
- Baptista P, Pereira E, Eaton P, Doria G, Miranda A, Gomes I (2008). Gold nanoparticles for the development of clinical diagnosis methods. Anal. Bioanal. Chem., 391: 943-50.
- Braydich-Stolle L, Hussain S, Schlager JJ, Hofmann MC (2005). In Vitro Cytotoxicity of Nanoparticles in Mammalian Germline Stem Cells. Oxford J. Life Sci. Med. Toxicol., Sci., 88(2): 412-419.
- Cho WS, Cho M, Jeong J, Choi M, Han BS, Shin H-S, Hong J, Chung BH, Jeong J, Cho MH (2010). Size-dependence tissue kinetics of PEG-coated gold nanoparticles. Toxicol. Appl. Pharmacol., 245: 116-123
- Choi CJ, Anantharam V, Saetveit NJ, Houk RS, Kanthasamy A, Kanthasamy AG (2007). Control Cellular Prion Protein Protects against Manganese-induced Oxidative Stress and Apoptotic Cell Death. Section: Neurotoxicology, 52(3): 280-283.
- Foster W, Ruka M, Gareau P (1997). Morphologic characteristics of endometriosis in the mouse model: application to toxicology. Can. J. Physiol. Pharmacol., 75(10-11): 1188-1196.
- Gupta R (2005). System behaviour of wood truss assemblies. Progress Struct. Eng. Mater., 7(4): 183-193.
- Huber M, Wei TF, Muller UR, Lefebvre PA, Marla SS, Bao YP (2004). Gold nanoparticle probe-based gene expression analysis with unamplified total human RNA. Nucleic Acids Res., 32: e137-e145.
- Jain P, El-Sayed I, El-Sayed M (2007). Au nanoparticles target cancer. Nano Today, 2: 18-29.
- Kamat PV (2002). Photochemical and photocatalytic aspects of metal nanoparticles. J. Phys. Chem. B., 106: 7729-7744.
- Kuwahara Y, Akiyama T, Yamada S (2001). Photochemical and analytical applications of gold nanoparticles and nanorods utilizing surface plasmon resonance. Langmuir, 17: 5714-5716.
- Liu WT (2006). Nanoparticles and their biological and environmental applications. J. Biosci. Bioeng., 102: 1-7.
- Mirkin CA, Letsinger RL, Mucic RC, Storhoff JJ (1996). A DNA-based method for rationally assembling nanoparticles into macroscopic materials. Nature, 382: 607-609.
- Nel A, Xia T, Mädler L, Li N (2006). Toxic Potential of Materials at the Nanolevel. Science 3 February 311(5761): 622-627. Schmid G (1992). aaaaa, Chem. Rev., 92: 1709.
- Shipway AN, Eugenii K, Itamar W (2000). Nanoparticle arrays on surfaces for electronic, optical, and sensor applications. Chem. Phys. Chem., 1: 18-52.
- Shrestha S, Yeung C, Nunnerley C, Tsang S (2006). Comparison of morphology and electrical conductivity of various thin films containing nano-crystalline praseodymium oxide particles. Sens. Actuators A Phys., 36: 191-198.
- Tian ZQ, Bin R, Wu DY (2002). Surface-Enhanced Raman Scattering: From Noble to Transition Metals and from Rough Surfaces to Ordered Nanostructures, J. Phys. Chem. B., 106: 9463-9483.
- Wang X, Duan S, Geng B, Cui J, Yang Y (2007). Schmeissneria: A missing link to angiosperms? BMC Evol. Biol., 7: 1-13.