

DNA detection based on surface enhanced Raman scattering of gold nanoparticle aggregates coupled with photo-ligation technique

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Current genetic diagnostics suffer from high-cost and time-consuming operations, because they usually need precise temperature control and/or expensive reagents. Recently, various biosensors using metallic nanoparticles (NPs), such as gold and silver, have been proposed and are known as localized surface plasmon resonance (LSPR) sensors. Therefore, easy-to-use and rapid analyses of DNA or protein become to be realized.

In the present study, Au NPs conjugated with oligo DNAs (ODNs) were prepared. After the photoligation between ODNs (primers 1 and 2) in the presence of target DNA (T-DNA), surface enhanced Raman scattering (SERS) spectra obtained with high sensitivity (Fig. 1 and 2).

We are currently developing novel biosensors based on the above-mentioned technique to apply them to ultra-sensitive biodetection systems for DNA sequence detection *in situ*. It is a new solution-type biosensor based on SERS effect in Au NP aggregates coupled with DNA photoligation technique.

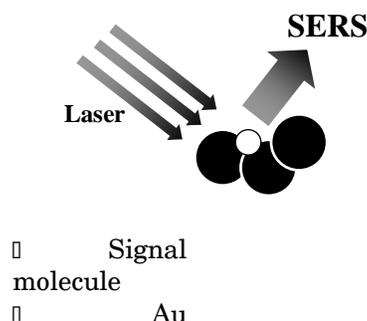


Figure 1. Active SERS mechanism

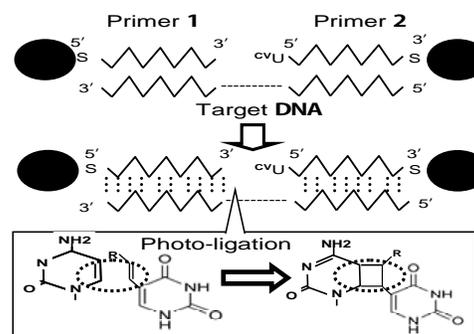


Figure 2. Reaction model for photoligation and hybridization specificity [1]

Reference:

[1] S. Ogasawara, K. Fujimoto *et al.*, ChemBioChem 8, 1520-1525 (2007).

Synthesis of highly dispersible Al-doped ZnO nanoparticles and their electronic properties

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The development of electronics industry today strongly depends on the invention of new materials. ZnO is a very attractive oxide semiconductor which possesses wide and direct band gap (3.3 eV), large excitonic binding energy (60 MeV) and high transparency in the visible range. Doping ZnO with high-valence elements such as Al, Ga and In dramatically increases its conductivity, especially when these nanostructures can be realized. The resulting materials are interesting alternatives for the commercially available transparent conductive semiconductor, indium tin oxide (ITO).

In this study, we synthesized non-doped ZnO and aluminium-doped ZnO (AZO) nanoparticles (NPs) by the solvothermal decomposition of acetylacetonate precursors. This wet chemical route takes the advantages of mild reaction conditions, short time and commercially available chemicals. The as-synthesized NPs has the average size smaller than 15 nm with high crystalline, uniformity and good dispersibility in non-polar solvents. The influence of various factors on the properties of AZO NPs was thoroughly investigated. Al is successfully doped into ZnO crystal lattices without causing significant changes in the phase composition and lattice parameters. After spin-coating and annealing processes, these NPs form the respective thin films on the substrate. Some important properties of these films including thickness, crystal structure, surface morphology, optical transparency and conductivity are characterized. Experimental parameters are optimized to get the NPs and their films with good quality. Base on these results, the practical capability of the above NPs and their films is proposed.

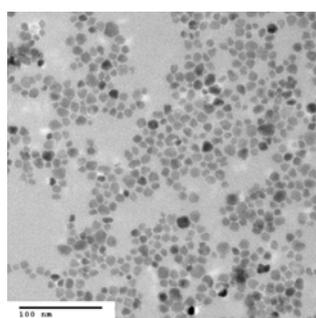


Figure 1. TEM image of AZO NPs (input molar ratio of Al was 5.0 mol%)

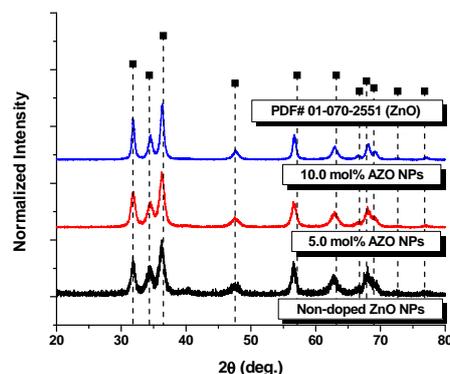


Figure 2. XRD patterns of non-doped ZnO NPs and AZO NPs with respective input molar ratios.

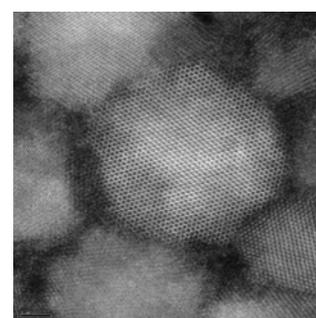


Figure 3. HAADF image of AZO NPs (input molar ratio of Al was 10.0 mol%)

Reference:

[1] Norris, B. J.; Anderson, J.; Wager, J.F.; Keszler, D.A., Spin-coated zinc oxide transparent transistors. *J. Phys. D: Appl. Phys.* 2003, 36, L105-L107.

Characterization and performance of nucleic acid nanoparticles combined with gold and protamine

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The binding, stabilization, and delivery of nucleic acid nanoparticles (nPs) was investigated using cationic polypeptide protamine to attach DNA and RNA to Au nanoparticles. Visualization of the nanoparticles was achieved by Atomic Force Microscopy (AFM) and Transmission Electron Microscopy (TEM). Characterization of the nanoparticles and the binding of nucleic acids was performed by Dynamic Light Scatter (DLS) and UV/Vis Spectroscopy (UVS). Size increase shown by DLS and shifts in UVS confirmed interaction of DNA and RNA with Au nanoparticles mediated by protamine. Stabilization of DNA was demonstrated by gel electrophoresis and immunotiter data where at 25 °C and 60 °C protamine stabilized DNA up to 3 months compared to DNA kept at 4 °C. Gel data confirmed protamine more effectively stabilizes nucleic acids while providing protection from nuclease degradation when compared to spermidine.¹ Delivery by gene gun of Au-protamine particles showed protamine enhanced the delivery of DNA vaccine in conjunction with Au.² Relative light unit (RLU) measurements confirmed that a 1:1 N:P mol:mol ratio of a protamine-RNA splice-site switching oligo (SSO) was most effectively delivered to cells compared to other protamine SSO N:P ratios and standard delivery agent lipofectamine.³ Zeta potential analysis showed that nanoparticle surface potential can be modulated based on N:P ratios, while surface charge may show a correlation with SSO delivery activity.³

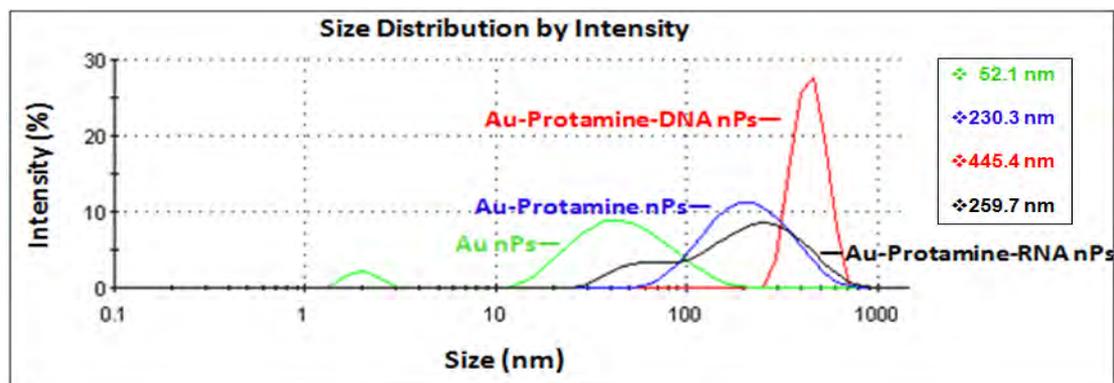


Figure 2. Dynamic Light Scatter (DLS) measurements confirmed binding of DNA and RNA to Au-protamine nanoparticles

Reference:

- [1] "Protamine-mediated DNA coating remarkably improves bombardment transformation efficiency in plant cells", E. Sivamani, R.K. DeLong, and R. Qu. *Plant Cell Reports*. 28(2), 2009, p213-21.
- [2] "Characterization and performance of nucleic acid nanoparticles combined with protamine and gold." *Biomaterials*. (accepted for publication)
- [3] "High throughput experimental design investigation of protamine splice-site switching oligomer nanoparticle delivery." *Journal of Experimental Medicine*. (submitted for publication)

Synthesis of binary metal nanoparticles in micro segmented flow and interaction of nanoparticles with radiation

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Due to their well-defined size- and composition-dependent optoelectronic properties^[1] bimetallic nanoparticles have reached much interest for applications in fields of catalysis, nonlinear optical devices^[2] and DNA sequencing^[3]. Therefore a fast and effortless controllable synthesis procedure is required from which highly monodisperse nanoparticles can be obtained. The micro segmented flow^[4] is predestined for the synthesis of nanoparticles with a narrow size distribution because of a high mixing efficiency and the independence of residence time distribution. Au/Ag core shell nanoparticles were synthesised in a two step mixing process under conditions of the micro segmented flow. Monodisperse gold seeds are injected in a carrier stream of tetradecane, silver nitrate ($c = 0.001 \text{ mol/l}$) and ascorbic acid ($c = 0.1 \text{ mol/l}$) were added by a second and third injector unit, respectively. The typical size of the resulting core shell particles can be adjusted by the reaction parameters, e.g. flow rate. It is shown that the typical particle diameter can be reduced by setting up a higher flow rate. Thus, a different silver shell thickness is reached, reflected in a red shift of the plasmon resonance frequency and a broadened spectral bandwidth (Figure 1). The high homogeneity of binary metal nanoparticles was confirmed by SEM, AFM, and DCS (differential centrifugal sedimentation). The different characteristics of product particles result in different optoelectronic properties reflected by the optical spectra. Optical absorption spectra were taken by UV/VIS spectroscopy and the scattering spectra were measured in a single core-shell particle by dark field microscopy, a spectroscopy setup and a CCD camera.

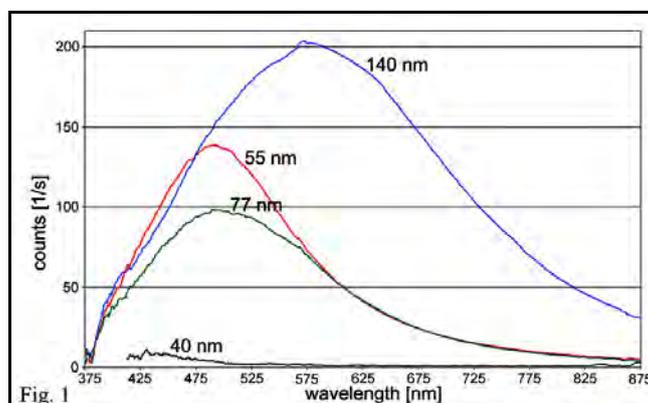


Figure 1. Optical scattering spectra of binary metal nanoparticles with a different sized Ag-shell at a 25 nm diameter Au-core.

Reference:

- [1] K. Lance Kelly, et al. *J. Phys. Chem.* 107, 2003, 668-677
- [2] M. Ringler, et al. *Phys. Rev. Lett.* 100, 2008, 203002(4)
- [3] Csaki, A., et al. *Nano Lett.* 7(2), 2007, 247-253
- [4] G.A. Groß, et al. *Chem. Eng. Technol.* 30, 3, 2007, 341-346

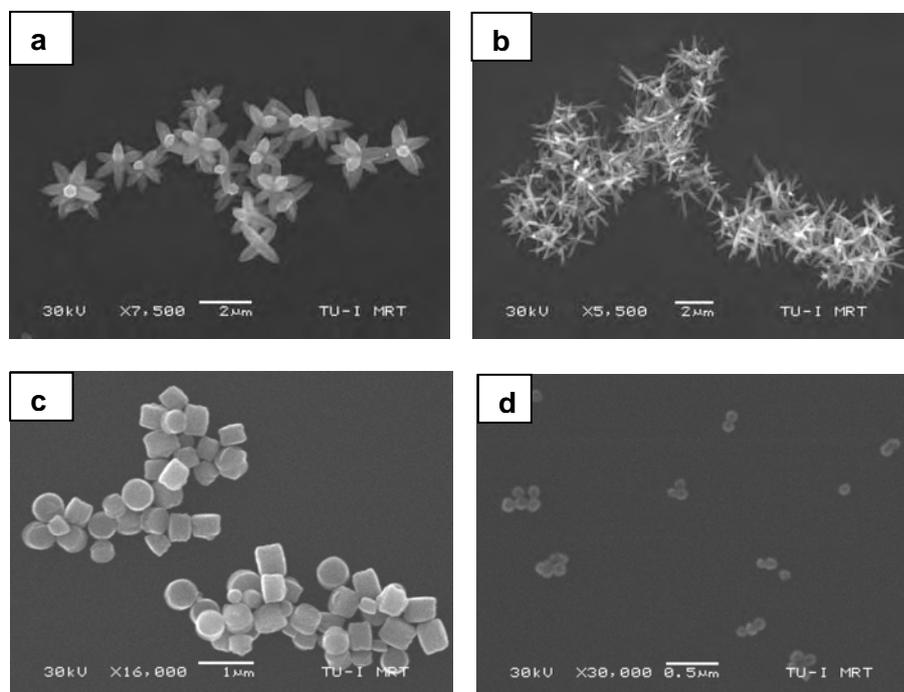
Monodisperse ZnO micro and nanoparticles with different shape and size obtained by continuous flow synthesis

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Synthesis of ZnO micro and nanoparticles has generated a great deal of interest in recent years due to their potential applications such as luminescent light emitters, solar cells and photocatalysts^[1,2]. However, the study on ZnO micro and nanoparticles synthesized by micro flow-through synthesis is still lacking a thorough exploration^[3]. Therefore, we have studied the possibility of generating ZnO particles by aqueous micro fluid segments embedded in an inert organic carrier liquid.

The experimental setup consists of syringe pumps, static mixer, PTFE tubings and heater. ZnO particles were prepared at a high flow rate (5000 $\mu\text{L}/\text{min}$), with increased temperatures (100-150 $^{\circ}\text{C}$). Homogeneous flower-like and needle-like ZnO microparticles (Figure 1a, b) were prepared by use of $\text{Zn}(\text{Ac})_2$ and NaOH as precursors. Cylinder-like ZnO particles (500 nm, Figure 1c) was synthesized in the presence of triethanolamine (TEA) at 120 $^{\circ}\text{C}$. With the assistance of polyacrylamide (PAM), the size of ZnO particles was reduced to 100 nm (Figure 1d). The structure and morphology of the obtained products were characterized by SEM. The results reveal that the reaction temperature, capping agent, different alkalis and molar ratio of alkali to Zn^{2+} play a crucial role in determining the final size and shape of the samples.



Figures (a), (b), (c), (d). SEM images of the obtained ZnO particles

Reference:

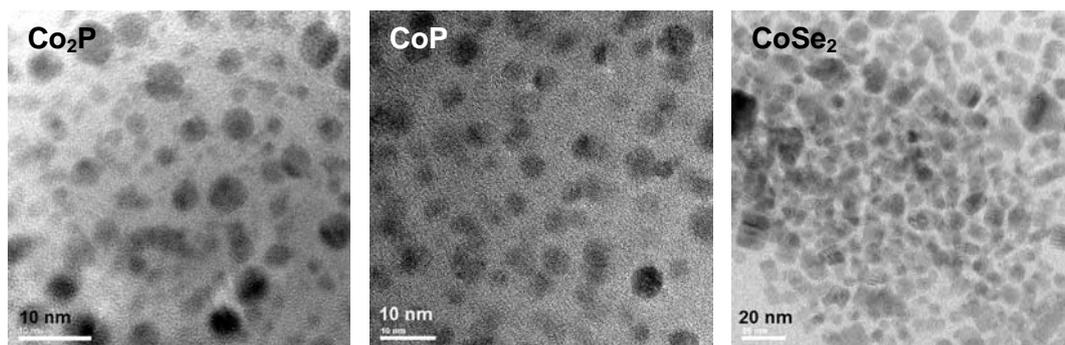
- [1] Pan, Z. W.; Dai, Z. R.; Wang, Z. L. *Science* 291, 2001, 1947-1949.
- [2] Kashyout, A. B.; Soliman, M.; El Gamal, M.; Fathy, M. *Mater. Chem. Phys.* 90, 2005, 230 - 233.
- [3] Li, S.; Günther P. M.; Köhler J. M. *J. Chem. Eng. Jap.* 42, 2009, 338-345

The preparation of cobalt phosphide and cobalt selenide nanoparticles from single source precursor

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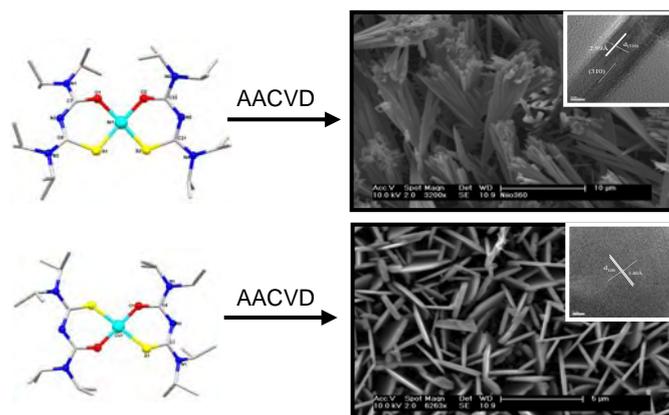
Dialkyldiselenophosphinatocobalt(II), $[\text{Co}(\text{Se}_2\text{PR}_2)_2]$ ($\text{R} = \text{}^i\text{Pr}$, Ph and $\text{}^t\text{Bu}$) complexes were used to grow cobalt selenide or cobalt phosphide nanoparticles by thermolysis in trioctylphosphine oxide (TOPO) or hexadecylamine (HDA) at 300 °C. The orthorhombic CoSe_2 nanoparticles can only be formed in HDA, whereas, in TOP/TOPO or TOP/HDA, only orthorhombic CoP or Co_2P nanoparticles were formed. Characterization of these nanoparticles by transmission electron microscopy, X-ray diffraction, and electron diffraction show highly crystalline spherical monodisperse CoP and Co_2P nanoparticles with a 5 nm diameter, while, CoSe_2 nanoparticles exhibit the cuboid shape of 10 nm length. The optical absorption experiment show that Co_2P is the direct transition phosphide semiconductor with a large band gap (E_g) = 3.15 eV, and CoP nanoparticles give an indirect band gap of (E_g) = 1.61 eV, while CoSe_2 nanoparticles exhibit direct band gap of (E_g) = 1.45 eV.



The deposition of nickel sulfide or copper sulfide thin film nanostructures by AACVD

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The nickel(II) and copper(II) complexes of 1,1,5,5-tetra-*iso*-propyl-2-thiobiuret [$M(C_{14}H_{28}N_3OS)_2$], $M = Ni$ (1), Cu (2) have been synthesized and their single crystal X-ray structures determined. Both complexes were used as single source precursors for the deposition of NiS^1 and CuS^2 thin films by aerosol assisted chemical vapour deposition (AACVD). The X-ray diffraction (XRD) showed deposition of orthorhombic Ni_9S_8 from (1) and a mixture of cubic CuS_2 and hexagonal Cu_2S thin films from (2). Scanning Electron Microscopy (SEM) of nickel sulfide thin films showed the deposition of flower like crystallites at 320 °C and 360 °C and multi-branched crystallites at 400 °C whereas for copper sulfide thin films, spherical crystallites were grown at 280 °C, cuboids at 320 °C and plates at 360 and 400 °C. Transmission Electron Microscopy (TEM) of the material scratched from thin films showed nanobelts of nickel sulfides with 20-25 nm size and nanoplates of copper sulfides with 25-30 nm size. Selected Area Electron Diffraction (SAED) showed single crystal nature of both nickel and copper sulfide thin films.

Reference:

- [1] P. O'Brien and J. Waters, *Chemical Vapor Deposition* 2006, 12, 620.
- [2] K. Kemmler, M. Lazell, P. O'Brien, D. J. Otway, J.H. Park, J. R. Walsh, *Journal of Materials Science: Materials in Electronics* 2002, 13, 531

Synthesis and assembly of inorganic nanostructures at the oil-water interface

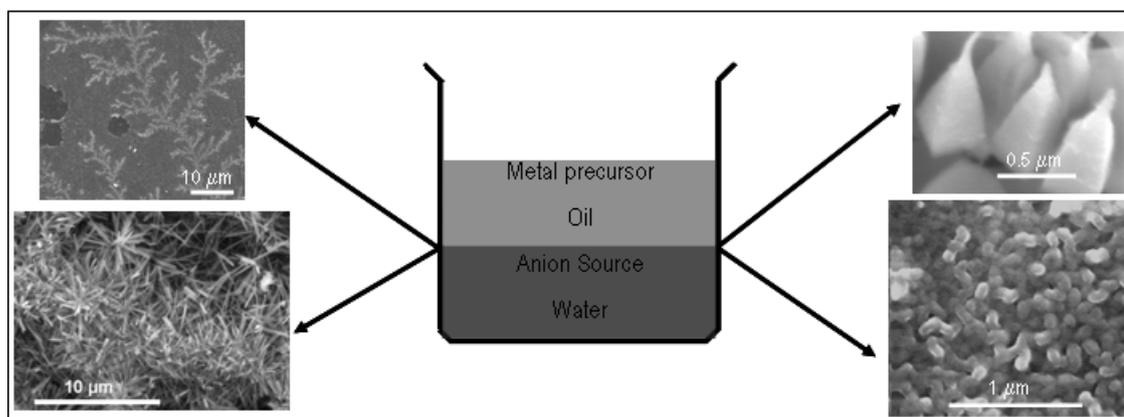
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The areas surrounding the interface between two immiscible liquids is of current interest due to their ability to assemble colloidal as well as nanocrystalline particulates. This has seen renewed interest and has been used to generate assemblies of colloidal particles.

This talk will showcase recent results and make the case that the water-oil interface, for long confined to intellectual backwaters, possesses a number of properties particularly suited for the synthesis of nanostructured materials.

In Manchester, we have been successful in using the water-oil interface to synthesize nanorod arrays of $\text{Cd}(\text{OH})_2$ ¹, assemblies of Au nanocrystals², monolayered films of pyramidal PbS crystallites with high energy {113} facets³, nanocrystalline films of CdS, ZnS⁴ and Bi_2S_3 .⁵ The above structures have been obtained by holding a metal precursor dissolved in toluene in contact with an aqueous layer containing a reducing, oxidizing or precipitating reagent. The reaction is mediated by interfacial processes and results in deposits *suspended* in the interfacial region. The interface has a dual role of controlling charge transport and directing the structure of the deposit.



The talk will show that deposition provides a simple, convenient and inexpensive route to high quality films of nanostructures.

Reference:

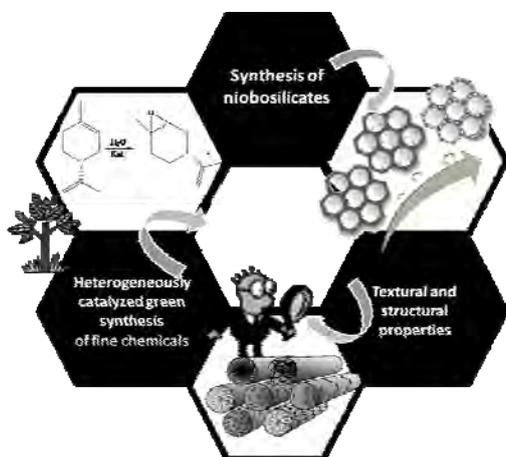
- [1] S. N. Mlondo, P. J. Thomas, P. O'Brien, *Chem. Commun.*, 2008, 2768-2770.
- [2] S.N.Mlondo, P.J. Thomas, P.O'Brien, manuscript under consideration;
- [3] D. Fan, P. J. Thomas, P. O'Brien, *J. Am. Chem. Soc.* 2008, 130, 10892-10894.
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- [5] D. Fan, P. J. Thomas, P. O'Brien, *Chem. Phys. Lett.* 2008, 465, 110-114.

Nanoporous niobosilicates serving as catalysts and drug carriers

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The materials used as catalysts for production of fine chemicals, adsorbents and drug carriers should possess a well defined structure of large pores with dimensions adjusted to the substrate/product sizes, properly attached metal species being active sites and high stability during the above mentioned processes.

The aim of this work was to synthesize the potentially active materials for the liquid-phase oxidation and for drug carrying processes (Scheme 1). For the first purpose the development of new heterogeneous catalysts for the „green chemistry” oxidation processes of terpenes and FAME, with the surface and structural properties assuring fast diffusion, minimal leaching of active phase into solution and easy regeneration, was the key problem. Several important issues related to nanostructured catalysts, e.g., the effect of the precursors used and synthesis conditions on the structural and surface properties of niobium-containing catalysts, will be discussed. As an example, it was found that the epoxidation of limonene gives high selectivity to limonene oxide (99%) and takes place exclusively at the more nucleophilic double bond. Also epoxyderivative of methyl oleate – cis-9,10-epoxyoctadecanoic acid methyl ester – was obtained as the only product, with no traces of 9,10-dihydroxyoctadecanoic acid methyl esters or other products.



Scheme 1. Work strategies.

A protocol for the incorporation of poorly soluble drug – ibuprofen into silicates containing niobium, was also established. All the obtained niobosilicates showed a high amount of incorporated ibuprofen, resulting in both loading methods, i.e., adsorption from drug solution under continuous stirring and “incipient wetness” (soaking of pressed powder in a ibuprofen solution), up to 50 wt % of drug loading. The second procedure, although more time consuming, has reached slightly better results. The dependence of drug amount present in mesopores on the niobium source and on the surfactant used for nanoporous structure formation (i.e., obtained pore size and volume) was also clearly visible.

Limiting ammonia emissions from sheep manure using water suspended nanosilver with different mineral sorbents

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Ammonia emitted from animal bedding is one of the main environmental toxins dangerous form livestock and human.

The aim of the study was to determine ammonia emission patterns from sheep manure using preparations being a mixture of nanosilver water suspension with mineral sorbents.

Nanosilver water suspension (W) [$1000 \text{ ppm Ag} \cdot \text{dm}^{-3}$] (AMEPOX, Poland) was sprayed onto 3 different mineral sorbents: vermiculite (V) – $100 \text{ cm}^3 \text{ W} \cdot \text{dm}^{-3}$, halloysite (H) – $200 \text{ cm}^3 \text{ W} \cdot \text{dm}^{-3}$ and bentonite (B) – $200 \text{ cm}^3 \text{ W} \cdot \text{dm}^{-3}$ of sorbent. Sheep manure with top application of preparation (5 or 10% of manure volume) was fermented in closed containers (vol. 15 dcm^3) at room temperature. Groups analyzed were: C – control; WV5%, WV10%, WH5%, WH10%, WB5% and WB10%. The air samples were collected from headspace of containers and ammonia concentration [ppm] was determined using colorimetric method according to Polish standard. Analysis of ammonia were conducted on the 2-nd and 7-th day after preparation application.

All used preparations and doses limited ammonia emissions – Fig. 1. Significantly lower concentration of ammonia was stated on the 2-nd and 7-th day after preparation application in the group WV5% (0.32 ± 0.02 and 0.86 ± 0.01 ppm) and WV10% (0.19 ± 0.01 and 0.81 ± 0.01 ppm) when compared to group C (2.23 ± 0.03 and 2.53 ± 0.05 ppm). Nanosilver with V sorbent, despite the lowest dose of Ag, limited ammonia the most effectively, while nanosilver with B sorbent was the least efficient, respectively: - 66 – 68% and - 30 – 31% on the 7-th day, when compared to C group. In WV and WH groups efficiency in limiting ammonia (2-nd vs. 7-th day) lowered in time (2-nd vs. 7-th day) while in WB groups increased. Reported data suggest superior action of preparation containing nanosilver with vermiculite. Higher dose (10% vs. 5%) was not positively related in limiting ammonia, what question the use of higher doses of preparations.

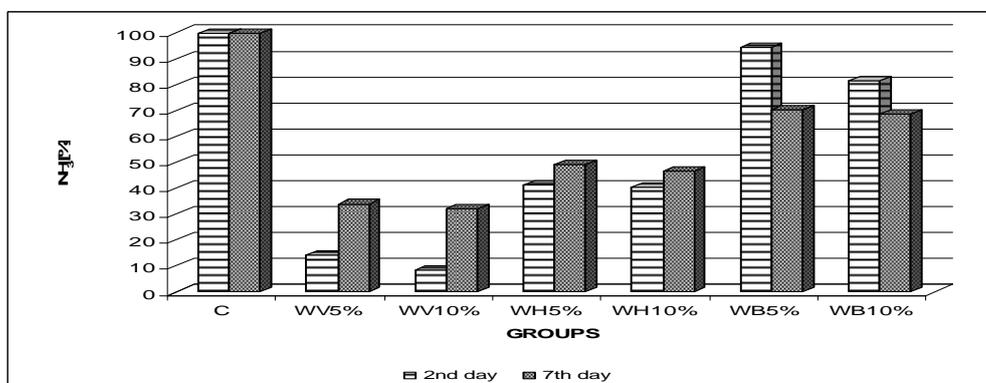


Fig. 1. Percentage share of ammonia emission from sheep manure when compared to control group (C).

The study conducted within the framework of research project number N N205 018634 financed by Polish Ministry of Science and Higher Education.

Methodic aspects of nanoparticles determination in aquatic environment

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Products containing nanosilver create 20% of all available nanotechnology production [5]. Nanosilver has become very important nanoscale antibacterial agent commonly and widely used in wound care products, athletic clothing, food storage containers, washing machines and appliances [3], [4]. The remnants of these products flow with sewage into rivers and water reservoirs [1], [5]. With such a dynamic growth in the number of products containing nanosilver it seems justified to define the notion which does not explicitly determine the solution components. Nanosilver defines any solution containing nanoparticles independent of the exact concentration [2]. The aim of the following research was the determination of the nanoparticles and ions ratio in colloidal nanosilver solution PVP-N-NanoSilver-1000. The research was performed using available methods [2], [3]. 0,01; 0,05; 0,5; 1; 2 concentrations were examined. After samples centrifugation in Beckmann ultra-centrifuge an analysis of nanosilver concentration was performed using Varian SpectrAA 220 atomic spectrophotometer. The results of the research show that the solution of PVP-NanoSilver-1000 consisted of 39,5% of nanoparticles.

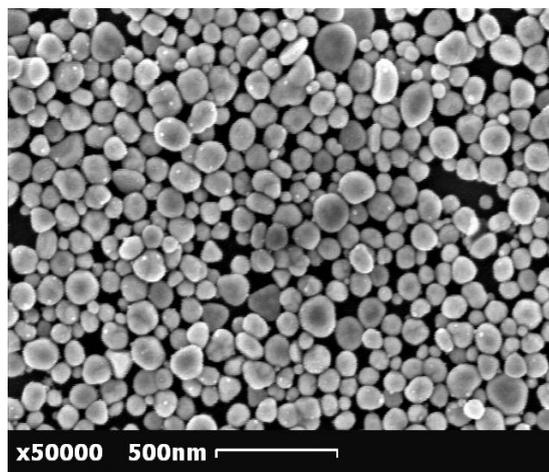


Fig.1. Silver nanoparticles in TEM (Transmission electron microscopy).

Reference:

- [1] Handy R. D., Owen R., Valsami-Jones E. 2008. The Ecotoxicology of Nanoparticles and Nanomaterials: current status, knowledge gaps, challenges, and future needs. *Ecotoxicology* 17, 315- 325.
- [2] Key F. S. , Mass G. 2001. Ions, atoms and charge particles, *SilverColloids*.
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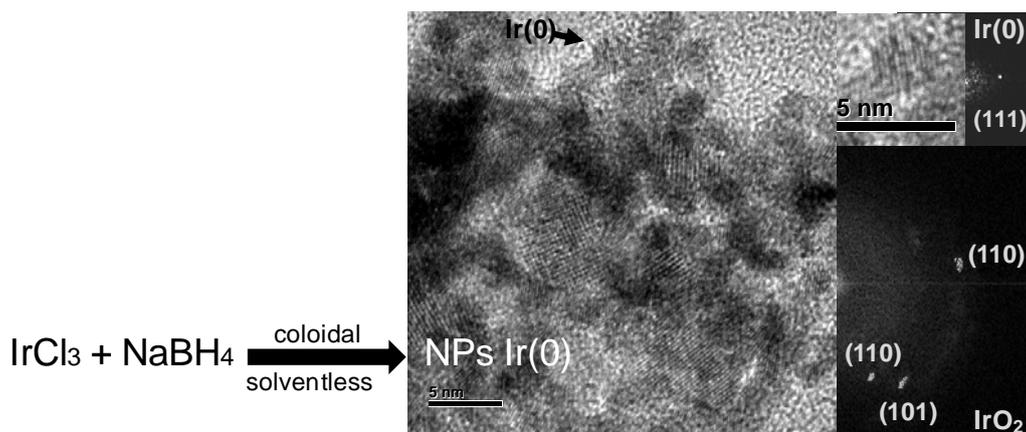
Solventless synthesis of iridium(0) nanoparticles

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Nanoscale materials have been widely studied because of their particular properties and potential applications. In particular, noble metal nanoparticles have been used as catalysts [1, 2] and photocatalysts in solution for many years. The activity of these materials is controlled by their size, crystal structure and the nature of the stabilizer. Nevertheless, metal nanoparticles in solution are hard to maintain on zero oxidation state, thus there had been some attempts to obtain nanoparticles without the use of solvents. In this paper we present the results on a free solvent synthesis of iridium(0) nanoparticles; with and without water washes. In both cases we study the effect of the change in the concentration of reducing agent used and the temperature used after the reaction. If the final product is not washed, in some cases, impurities of NaBH_4 and IrO_2 appeared in the final powder. On the other hand, if the final product is washed at the end of the synthesis, the obtained powder is free of impurities. In all cases the calculated particle size is less than 10 nm.



Reference:

- [1] "Synthesis, Characterization, and Surface Immobilization of Platinum and Palladium Nanoparticles Encapsulated within Amine-Terminated Poly(amidoamine) Dendrimers" H. Yee, R. W. J. Scott, R. M. Crooks, *Langmuir*, 20[7], 2004, 2915-2920.
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Investigation of nanomaterials for detoxification technologies

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Transition metal based nanomaterials have been used in concurrence with UV light and Atmospheric Pressure Non Equilibrium Plasma (APNEP) generated using microwaves to detoxify volatile organic compound (VOC) polluted gas streams. Sol-gel synthesized titania nanostructured surfaces using reverse micelles (figure 1) alone or with further surface modification on alumina and cordierite substrate geometries, have been developed.

By the construction of a pilot reactor which contains the heterogeneous catalyst after the plasma generation chamber, it was shown that the nanostructured titania greatly enhanced the destruction of the model VOC compounds (Toluene and 1,2 dichlorobenzene) as opposed to the plasma stream alone.

Experiments presented show the effect of microwave power, gas stream composition (N_2 , N_2/O_2 and N_2/H_2O) and temperature on the effectiveness of the catalyst. These experimental variables cause a change in the Fermi electron (e^-) and electron hole density (h^+) of the nanostructured material, therefore, causing enhanced redox VOC destruction to occur on the surface of the nanoparticles. It was observed that the catalyst is greatly enhanced at low microwave plasma power by doping the surface of the nanoparticles with noble metals at low concentrations by chemical vapour deposition (CVD). These results demonstrate that APNEP microwave technology performance is greatly enhanced with the use of nanostructured heterogeneous catalysis for detoxification of VOC polluted gas streams.

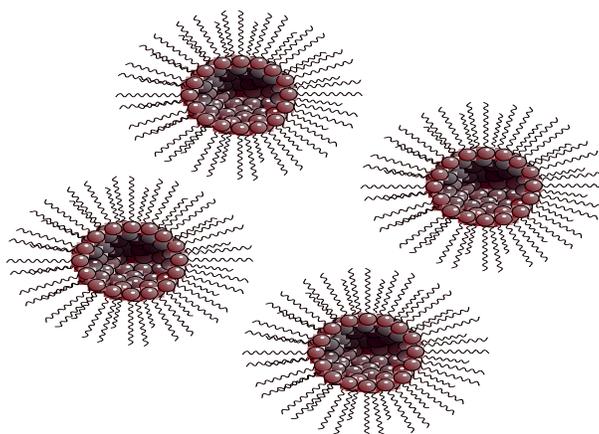


Figure 1. Reverse micelles

The polymer route to monofunctional gold nanoparticles

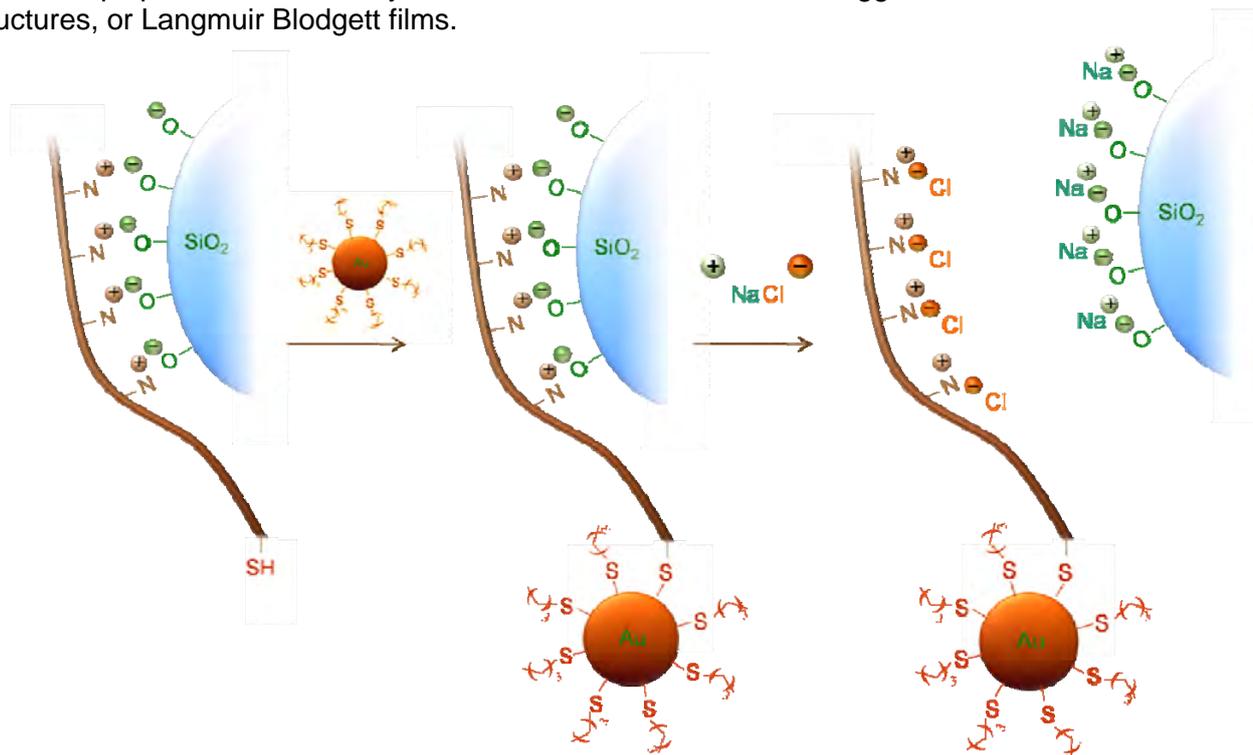
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The ability to control assemblies of building blocks at the nanometer scale into bigger structures with specific properties, and functionalities is critical for the development of new materials and devices. Those functionalities are not only dependent on the size or shape of individual particles, but also on the interparticles distance interactions and conformation.

However, the organization of such nano-assemblies is mainly governed by the chemical functionalities of the building blocks, and therefore, it is important to first achieve a precise control over the chemical functionality of nanoparticle building blocks.

Thereby, we report a method for the solid-phase preparation of monofunctionalized gold nanoparticles, i.e. nanoparticles bearing a single thiol terminated poly(2-(dimethylamino)ethyl methacrylate) polymer (pDMAEMA) attached to each particle. Those gold particles/polymer systems are expected to behave as a giant amphiphile, and ultimately, will be used in the formation of bigger structures, such as micelle like structures, or Langmuir Blodgett films.



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