

Posters Session II – A
Nanotech in Life Sciences and
Medicine

Assessment of protein aggregates in the presence of nanoscale vaccine adjuvants.

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Abstract: The presence of sub-visible particles in therapeutic protein products has increasingly become a field of concern for the pharmaceutical industry and regulatory agencies [1,2]. Aggregates in the size range of 0.1-10 μm have been implicated in adverse reactions and/or reduction in efficacy of therapeutic products [1–5]. Micro flow imaging techniques have been developed for quantifying and characterizing sub-visible aggregates in the range of 2 to 80 microns [15(6)]. As valuable as these techniques are, the presence of opaque excipients or drug carrier systems (such as liposomes) for therapeutic proteins or vaccine adjuvants (such as lipid emulsions or cationic liposomes) may complicate their use. Presented here are studies to determine the capability of micro flow imaging to characterize and quantify particle standards (polystyrene (PS) microspheres, glass microspheres) or intentionally aggregated proteins when formulated with commercially available nanoscale adjuvants such as squalene based emulsions (approx. 200nm) or cationic liposomes (approx. 100nm). We report that micro flow imaging with a FlowCAM instrument can accurately count and size particle standards up to 50% emulsion or 100mM lipid concentration with 100nm liposomes. However we note that as supplied, squalene based emulsions contained 10-40 μm aggregates that present similar optical properties to PS and glass particle standards making calibration in this size range very difficult (Figure 1A). These aggregates may have implications for the assessment of therapeutic samples. Filtering the emulsion with a 0.22 μm membrane removed the aggregates and easily allows for assessment of standards in this size range. We also report on the ability of the instrument to characterize aggregates of model proteins (Figure 1B) for both squalene and liposome based nanoscale adjuvants.

Keywords: Vaccines, nano adjuvants, liposomes, emulsion

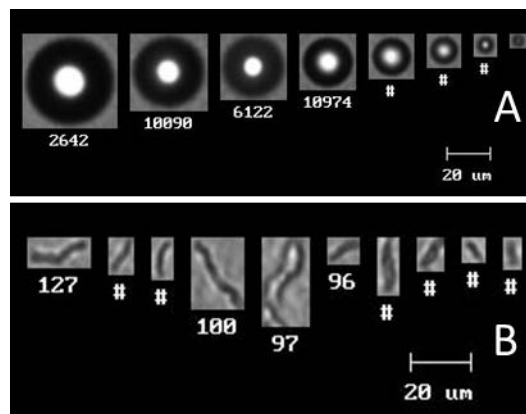


Figure 1. A: Aggregates found in unfiltered squalene based adjuvant showing similar morphology to polystyrene standards. B: Aggregates of BSA identified in the presence of liposome nanoscale adjuvants.

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Kiteplatin Delivery by Hydroxyapatite Nanocrystals for the Treatment of Cancer.

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Abstract: We have recently investigated the use of nanocrystalline hydroxyapatite (HA) as bone-specific platinum-drug delivery device for the treatment of bone tumors by local implantation at the site of an osteosarcoma.[1-3] The inorganic composite materials can act both as bone substitute and as platinum drug releasing agent, with the score of inhibiting locally the tumour re-growth and of reducing the systemic toxicity typical of cisplatin (*cis*-[PtCl₂(NH₃)₂]) and other platinum-based antitumor drugs.

In the search for new Pt(II) derivatives endowed with broader spectrum of activity and inferior toxicity with respect to clinical approved antitumor Platinum drugs, we have recently highlighted that the oxaliplatin analog [PtCl₂(*cis*-1,4-DACH)] (kiteplatin; DACH = diaminocyclohexane) possessed a unique anticancer potential, being effective against several solid cancers. Furthermore, this derivative was able to circumvent both cisplatin and oxaliplatin resistance.[4]

We present in this work our attempts to load injectable HA nanocrystals with kiteplatin and its derivative [Pt(CBDCA)(*cis*-1,4-DACH)] (CBDCA = cyclobutanedicarboxylate) to be delivered at the site of solid cancers or of the skeletal metastases caused by many tumors.

The adsorption and desorption profiles have been determined by measuring, by ICP-MS, the concentration of Pt complexes remaining in the physiological-like buffers as a function of time.

The HAs used in this study form nanostructured aggregates of micrometric dimensions. However, the state of aggregation changes completely after the interaction with the platinum drug. The influence of pH on the release of the Pt-drugs from the composites has also been investigated.

The cytotoxic activity of the apatite-released Pt complexes has been tested against a panel of human carcinoma cell lines and in a human cancer cell line suitably selected for resistance to oxaliplatin or cisplatin. Cytotoxicity profiles of the apatite-released kiteplatin derivatives are discussed and compared with those obtained with the reference Pt chemotherapeutic drugs.

Keywords: platinum-based antitumor drugs, kiteplatin, injectable nanocrystals, hydroxyapatite, in vitro cytotoxicity, colorectal cancer.

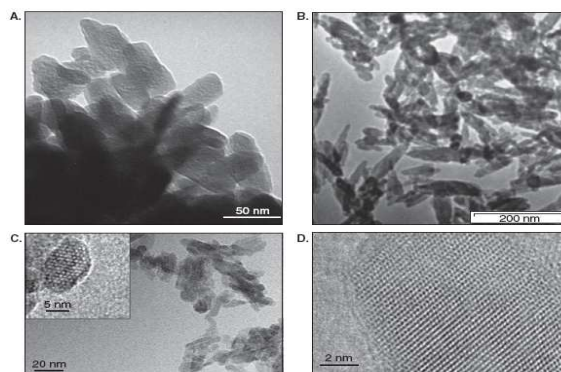


Figure 1: TEM high resolution images of hydroxyapatite nanocrystals loaded with the antitumor Pt-based drug kiteplatin. The controlled release of the platinum drug from the composite material could allow the obtainment of a cytotoxic effect against cancer cells.

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Self Micro-Emulsifying Drug Delivery Systems (SMEDDS) of Clove Oil for Fish Anesthesia

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Abstract: Self micro-emulsifying drug delivery systems (SMEDDS) are specialized form of delivery systems which form fine oil-in-water (O/W) microemulsions when exposed to aqueous media under conditions of gentle agitation or digestive motility that would be encountered in the GI tract (Attama and Nkemnele, 2005). Clove oil has been used as fish anesthetic agent but its lipid nature leads to a problem of aqueous miscibility (Cho and Heath, 2000). In this study, SMEDDS have been developed to enhance the aqueous solubility of clove oil. The formulations of the selected clove oil loaded SMEDDS (C-SMEDDS) were optimized by solubility assay, compatibility tests, and pseudo-ternary phase diagrams analysis. The pseudo-ternary phase diagrams were constructed using water titration method (Chen *et al.*, 2004). Suitable formulations of C-SMEDDS were selected from the pseudo-ternary phase diagrams; Tween20 was used as a surfactant and ethanol or isopropanol was used as a co-surfactant (Figure 1). The formulations were further characterized by electrical conductivity, viscosity, particle size and morphology of the dispersed phase of the microemulsion. Selected C-SMEDDS were O/W microemulsion after mixing with water with the spherical internal droplet size of 11.1-43.6 nm and size distribution of 0.2-0.3 nm. The C-SMEDDS composed of 2:1 ratio of surfactant to co-surfactant showed high electrical conductivity of 17.8-26.6 $\mu\text{S}/\text{cm}$ and low viscosity of 0.02-0.04 Pas. The C-SMEDDS stored at different conditions for 90 days demonstrated high physical stability at 4°C and room temperature. No phase separation was found in all temperature of storage but the color change from pale yellow to intense yellow was found in those kept at 45°C. The *in vivo* anesthetic activity of the selected C-SMEDDS, using clove oil ethanolic solution as a control was investigated in two fish species; *Cyprinus carpio* and *Oreochromis niloticus*. It was found that the C-SMEDDS significantly caused shorter anesthetic induction time than the control solution in both kinds of fish. However, the anesthetic recovery time was depended on the type of the fish. *O. niloticus* received the C-SMEDDS showed shorter time of recovery than those received the control, in contrast with *C. carpio* that showed longer recovery time than the control group.

Keywords: SMEDDS, clove oil, fish anesthesia, Tween20, ethanol, isopropanol

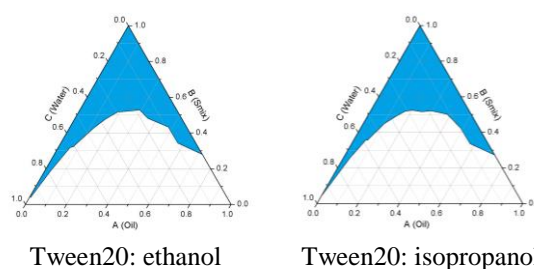


Figure 1: Pseudoternary phase diagrams of clove oil, Tween20, ethanol or isopropanol as co-surfactant and water. The ratio of surfactant and co-surfactant was 2:1.

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Development and characterization of bovine serum albumin nanoparticles of amphotericin B

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Abstract: Amphotericin B (AmB), an antibiotic of polyene class, is naturally produced by *Streptomyces nodosus* (Gold *et al.*; 1956). This antifungal is the main choice for the treatment of systemic fungal diseases, highlighting infections caused by *Cryptococcus neoformans* (Davey *et al.*; 1998). The most important problem for to use AmB is the toxicity. This antibiotic can cause many problems like nephrotoxicity, cardiotoxicity, hepatotoxicity, haematotoxicity and neutropenia (Annaloro *et al.*; 2009). The pharmaceutical nanotechnology is a good choice to reduce the problems with the toxicity of AmB because the nanoparticles can control the delivery of this polyene and can increase the pharmacokinetics properties for many kind of compounds (Mainardes *et al.*; 2010). The aim of this work is to develop and to characterize the AmB nanoparticles to treat meningitis caused by *C. neoformans*. The chosen method to obtain the nanoparticles was the coacervation method using bovine serum albumin. After the nanoparticle size and the polydispersity index were measured and the stability in freezer for seven weeks was tested. The obtained results were a good nanoparticle size with mean of $197,8 \pm 28,7$ nm and the polydispersity index with mean of $0,235 \pm 0,085$. The stability test was performed in freezer and the results was promising because the nanoparticle size maintained nearby 200 nm (Figure 1) and the polydispersity index was around $0,285 \pm 0,021$ during the seven weeks. It is possible to deduce with this study is that the bovine serum albumin nanoparticles with AmB are stable in freezing conditions and this can help the continuation of the study of this kind of nanoparticle to develop a new carrier for AmB to treat meningitis caused by *C. neoformans*.

Keywords: Amphotericin B, Antifungal Activity, Bovine Serum Albumin Nanoparticles, Toxicity.

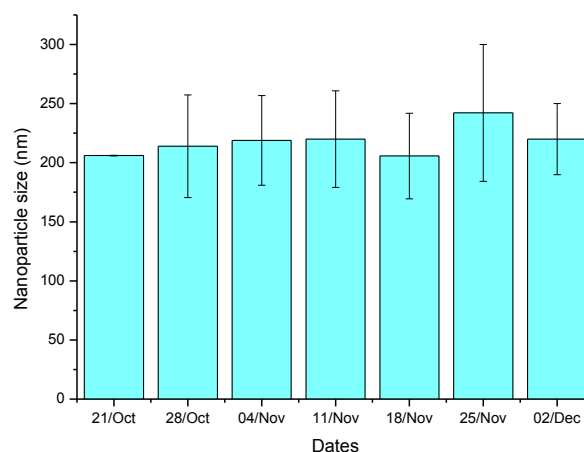


Figure 1: The nanoparticle size of bovine serum albumin nanoparticles of AmB after stability test in freezer during seven weeks.

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Curcumin-loaded Bovine Serum Albumine Nanoparticles: Development and evaluation of stability and antioxidant activity.

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Abstract: The polyphenol curcumin widely used as a spice, present several biological activities such antioxidant, antitumor, antiinflammatory, antifungal (Goel et al.; 2008). Curcumin present low bioavailability due its poor water solubility, instability in physiological to basic pH and the sensitive to the light (Khalil et al.; 2013). The use of nanostructured system as curcumin carriers has been described to improve its bioavailability and water solubility (Kim et al.; 2011), and the Bovine Serum Albumine (BSA) presents technical features to its became an excellent material for nanoparticles obtaining (Kratz; 2008). The curcumin-loaded BSA nanoparticles were obtained by the desolvation method. The mean size obtained was 149.63 ± 7.86 nm, the particle-size distribution presented a bimodal profile, with a polydispersity index of 0.16 ± 0.02 . The curcumin entrapped efficiency was measured by HPLC and it was $45.09 \pm 10.76\%$. The zeta potencial, -31.98 ± 2.74 mV presented, characterizes the nanoparticles solution stability, that was maintained without size and polydispersity index changes over room (21°C) and refrigerate (5°C) temperatures during 45 days, the stability wasn't maintained in freeze (-5°C) temperature. Curcumin scavenger activity was tested on ABTS⁺ at physiological pH for 48h. The free curcumin, at 10 and 20 $\mu\text{g/mL}$, in T0 presented $27.27 \pm 1.45\%$ and $37.81 \pm 2\%$ of ABTS⁺ inhibition respectively, and posteriorly occurred a significant decrease in its activity. After 4h, free curcumin had no presented scavenger activity on ABTS⁺. The curcumin-loaded BSA nanoparticles, at 10 and 20 $\mu\text{g/mL}$, in T0 presented $55.08 \pm 1.696\%$ and $59.05 \pm 1.4163\%$ of ABTS⁺ inhibition respectively, and posteriorly the scavenger activity on ABTS⁺ was maintained, with few oscillation over 48 h. The curcumin fluorescence was also maintained in curcumin nanoparticulated. This study will permit us to evaluate the improve of the curcumin water solubility and its consequently bioavailability.

Keywords: curcumin, bovine serum albumine, nanoparticles, desolvation method, scavenger activity, bioavailability, water solubility, stability assay.

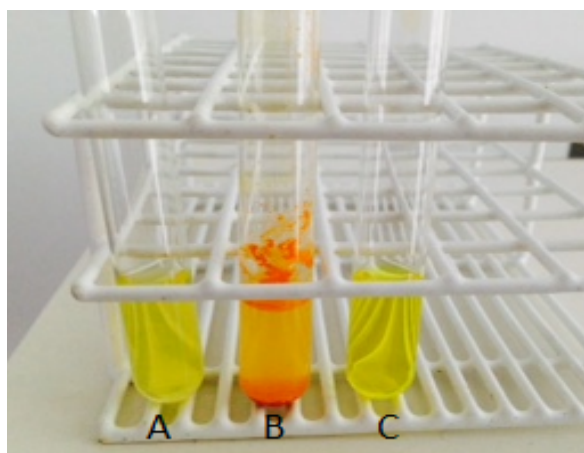


Figure 1: Curcumin-loaded Bovine Serum Albumine nanoparticles solution 50mg/mL in PBS pH 7.4 (A), free Curcumin solution 50mg/mL in PBS pH 7,4 pH solution (B) and Curcumin solution 50mg/mL dissolved inethanol (C).

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Silver Sub-nanometric Quantum Clusters as Potential Therapeutic Agents in Fight against Cancer

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Abstract

One of the most important factor in the design of treatments against cancer is the degree of penetration of the drugs into the tumors to be treated. This parameter affects directly the effectiveness of antitumor drugs, decreasing or completely nullifying their antitumor activity. For this reason, it is necessary to find alternatives for drugs transport or develop new antitumor drugs with greater degree of penetration into the tumors. Atomic quantum clusters (AQC) exhibit very interesting physical and chemical properties that make them particularly attractive for this purpose. This kind of clusters can become very small, even below 10 atoms, and with thicknesses under 0.5nm due to their planar structure. Their small size, comparable to Fermi wavelength of the electron (~0.52 nm for silver), places AQC in the scale range where quantum confinement effects govern the material properties. Because of this, AQC behave like molecules and do not exhibit charges in their structure. Their low molecular weight and the aforementioned absence of charge, increase the diffusivity of these sub-nanometric structures in biological tissues. In addition, AQC below 10 atoms, have planar structures which make them especially interesting to interact with DNA. In order to evaluate the biological activity of AQC against cancer, an easy and versatile method for synthesizing these type of particles has been developed, on the basis of a previous electrochemical method for the synthesis of nanoparticlesⁱ. Additionally, the first preclinical tests to evaluate the effectiveness of small silver AQC have been carried out on isolated tumor cells.

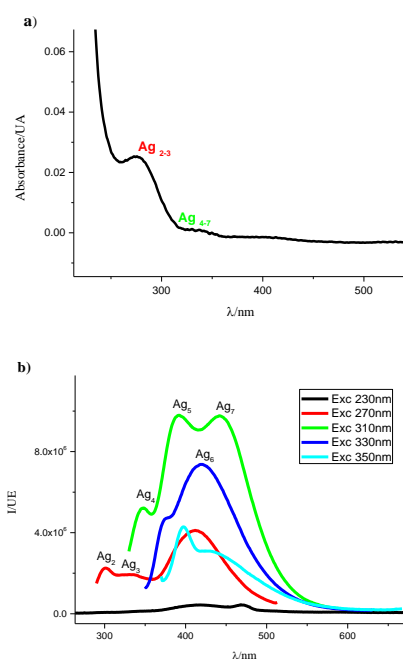


Fig 1. Characterization by Absorption and Fluorescence spectrophotometer of a sample of silver AQC. **a)** UV-Vis absorption spectrum, **b)** Emission spectrum exciting between 230 and 350nm.

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ⁱ *J. Am. Chem. Soc.*, **1994**, *116* (16), pp 7401–7402

Development of the nanoconjugate for liver fluke targeting

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Abstract: Nowadays, there are no biomedical tools for liver fluke direct imaging and targeting. The research aimed at the development, preparation and characterization of nanoconjugate based on superparamagnetic iron oxide (Fe₃O₄) nanoparticles (MNP), modified by silane derivatives and functionalized due to covalent bond formation with a pH-sensitive peptide (pHLIP) designed to detect the local acidosis zone formed around the fluke in vivo in opisthorchiasis infected liver.

The MNP with average diameter less than 20 nm were obtained by co-precipitation from the solutions of Fe³⁺ and Fe²⁺ salts. The surface of MNP were modified using (3-aminopropyl)trimethoxysilane (APTMS) and MNP with amino groups on the surface (MNP-APS-NH₂) were received. The APTMS quantity was calculated according to element analysis data that was reached 0.60±0.06 mmol/g (PE 2400, II, Perkin Elmer). Then, pHLIP was bound on the MNP-APS-NH₂ surface using hetero-functional cross-linker 6-Maleimidohexanoic acid *N*-hydroxysuccinimide ester (EMCS). The obtained nanoconjugate structure was proved by TEM (Philips CM30) and FTIR spectroscopy (Nicolet 6700, Thermo). According to TEM data there were no essential changes in the nanoparticles morphology after surface fictionalization: the average diameter and phase composition have not changed after processing. In FTIR spectra of obtained nanoconjugate the characteristic adsorption bands of Fe₃O₄ nanoparticles at 546 cm⁻¹ (Fe-O) and pHLIP at 1638 (amide I) and at 1531 (amide II) as well as at 1445, 1402 cm⁻¹ (amide III) were indicated. The stable water suspension of MNP modified by APTMS was obtained; the received nanoconjugate in suspension has average hydrodynamic diameter equal 155 nm (PdI 0.09) and z-potential – -22 mV (Zetasizer Nano ZS, Malvern Instruments Ltd.). The MRI contrast properties of nanoconjugate were tested in vitro and in vivo (Bruker, Biospec 117/16 USR), based on received data, the relaxivity coefficients r1 and r2 were calculated. The low cytotoxicity of nanoconjugate was proved using MTT-assay.

Keywords: nanoconjugate, superparamagnetic iron oxide nanoparticles, (3-aminopropyl)trimethoxysilane, MRI, liver fluke, cytotoxicity.

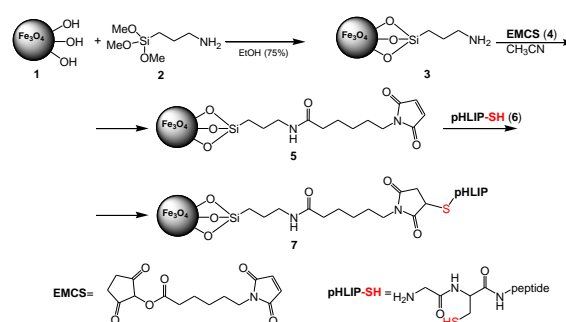


Figure 1: Figure illustrating the general scheme of nanoconjugate synthesis

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A Peptide-Based Drug Design to Overcome Major Challenges on Cancer Treatments

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Abstract: Cancer diagnosis is increasing at exponential rates. Patients and physicians are in need of novel and promising therapies to overcome the fatal disease. A promising option is a peptide-based drug design that would work by tricking the cell and interfering with the body's metabolic pathways. An example of these metabolic pathways that have awakened the researchers' attention is the SP/NK1R pathway. SP (Substance P), is a neuropeptide with multiples contributions in body regulation as: psychological stress, pain, inflammatory response, anti-apoptotic effects, and cell proliferation among others. Substance P receptor (NK1-R) is known to be overexpress in several cancer cell lines, and is involved in anti-apoptotic response (Muñoz and Coveñas, 2014).

This investigation is center in developing promising peptide-based drugs that can selectively attack cancer cells by two different ways: 1) blocking metabolic pathways that are known to promote cell proliferation and anti-apoptosis behavior, and 2) by interfering with the cancer cell's iron dependence mechanism. Cancer cells have a higher need of iron, therefore removing iron from them has been tested to slower tumor growth, and is a promising anti-tumor technique (Buss *et al.*, 2003). The herein purposed lethal weapon is a peptide-based drug that can selectively get to the malignant cells and work as a drug by interfering with the cell's metabolic pathway, plus depleting the iron from the cells; to obtain an optimum result of cell death. A library of peptide-based drugs will be synthesized, tested, and characterized in order to target different types of malignancies by interfering with different metabolic pathways (Figure 1). Our preliminary efforts are center in the synthesis of a complex containing Ti(IV)-HBED-SP_{homologous} peptide. HBED has a good affinity for titanium (IV), but an even greater affinity for iron (III); which can function as the iron depleting agent by releasing Ti (IV) and up taking the Fe (III) (Parks *et al.*, 2014). NK1R are over expressed in some cancer cell lines as for example pancreatic cancer cells. A drug that functions as a NK1 antagonist (to prevent proliferation of malignant cells, and trigger apoptosis), and that also has the ability of

releasing titanium (IV) and uptake iron (III) from the cancer cells would be a powerful and promising treatment.

Keywords: peptide-based drug design, substance p, neurokinin 1 receptor (NK1R), cancer treatments, metabolic pathway, apoptosis, iron cell balance.

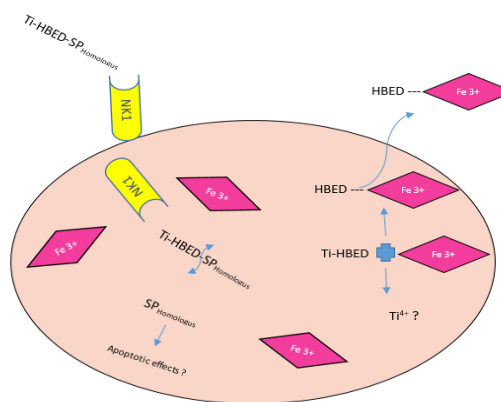


Figure 1. Peptide-based drug design. The complex enters the cell by the overexpressed receptor, and deliver the drug into the cell where the peptide stops cell proliferation and trigger apoptosis; while the HBED or other's high iron affinity molecules take iron out of the cell.

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Superparamagnetic Iron Oxide Nanoparticles for Stem Cell Tracking by Magnetic Resonance Imaging

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Abstract: Stroke is the second leading cause of death worldwide and the major cause of disability in Europe. This disease originates from a reduced blood supply into a region of the brain. The repercussions for the patient vary depending on the extent of the stroke and its location, but most patients suffer from body dysfunctions, such as disabilities in movement, speech, thought processes, and memory. In less severe cases, the healthy areas of the brain are able to assume the functions of the damaged ones. However, the major issue is the inability of the body to replace dead cells and therefore stem cell treatment may boost the repair systems within the brain.

Nowadays, mesenchymal stem cells (MSCs) are one of the most commonly used types of stem cells in clinical trials on stroke. They can be easily obtained and grown from bone marrow of the patient. The beneficial effect of MSCs is not related with producing new brain cells but rather, as suggested in some studies, with the release of substances from the injected MSCs that reduce inflammation and stimulate self-repair. More research is necessary to fully understand all the processes involved before safe therapies can be developed.

One of the main questions to be addressed is how and where the MSCs migrate within the body once they are injected. Cell tracking is used to visualize the cells when they are inside a living organism, and thus there is a need of labeling the cells. Therefore, cell-contrast agents and imaging technique chosen should accomplish several requirements: *e.g.* noninvasiveness, biocompatibility, and no alteration of stem cells properties should be necessary.

Hence, our approach relies on the use of Magnetic resonance imaging (MRI) as a highly convenient method due to its widespread availability in hospitals, as well as its remarkable characteristics like high spatial resolution, rapid acquisition and the absence of exposure to ionizing radiation. However, the sensitivity of MRI is still lower than other imaging techniques, *e.g.* fluorescence or bioluminescence. Therefore, the development of highly efficient contrast

agents is crucial in order to enhance the soft tissue contrast (Fig. 1).

Here, we report on the use of superparamagnetic iron oxide nanoparticles (SPIONs) as MRI probes for stem cell tracking. Data will be shown regarding SPION magnetic properties, MSC uptake, cytotoxicity, and MRI visualization of the labeled MSCs.

Keywords: biomedical applications, cell tracking, contrast agent, MRI, MSC, SPION, stem cells, stroke.

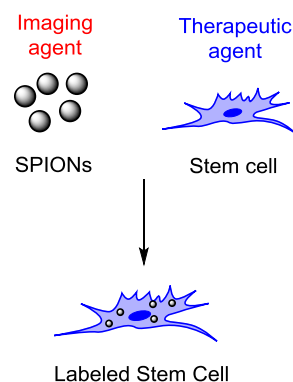


Figure 1: The basic concept of stem cell tracking using SPION labels.

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Phenylethyl Resorcinol in Niosomes for Cosmetic formulation

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Abstract: Niosomes are one of drug delivery system which made from non-ionic surfactant and have ability to entrap both hydrophilic and lipophilic drug. Cosmetic industry used niosomes to prevent anti-oxidant ingredient from oxidation and enhance penetration of poor permeable ingredient (Mahale *et al.*, 2012). Property of non-ionic surfactant is also mild to skin. Phenylethyl Resorcinol was entrapped by niosomes using BrijTM72 as non-ionic surfactant, it exhibited good physical appearance and the particle size was 0.97 to 2.44 μm . At surfactant: cholesterol ratio is 80:1, which had higher entrapment efficiency was $98.65 \pm 0.001\%$. The value of zeta potential of niosomes was -10.61 to -16.87 mV. Cholesterol content, HLB value of surfactant have effect to characterization of niosomes such as particle size, entrapment efficiency, zeta potential (Essa, 2010). Hydrophilic-lipophilic balance is also effect to formation of niosomes vesicle (Kamboj *et al.*, 2014). Therefore, niosomes was used to encapsulate phenylethyl resorcinol for used in cosmetic formulation.

Keywords: Niosomes, non-ionic surfactant, phenylethyl resorcinol, BrijTM72, drug delivery, entrapment efficiency, zeta potential, particle size.

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Nanostructured lipid carriers containing amazon natural lipids for the encapsulation of benzophenone-3

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Abstract: Exposure to ultraviolet (UV) radiation is the main cause of various skin problems, including sunburn, photo-aging and skin cancer. Sunscreens are widely used to protect skin against the deleterious effects of UV radiation. 2-hydroxy-4-methoxybenzophenone, commonly known as benzophenone-3 (BP-3) or oxybenzone, is a strong UV absorber and one of the most important sunscreen agents, and has been available for over 40 years. BP-3 can penetrate skin and its absorption has been associated to endocrine disruption (Kim and Choi, 2014) and other problems. Nanostructured lipid carriers (NLCs) are promising systems which can provide, among other advantages, enhanced drug stability, controlled drug release and targeting and high drug loading (Chen *et al.*, 2014). In this study, we aim to produce NLCs which may avoid penetration of BP-3 in skin, using natural lipids from amazon plants. We produced NLCs from the natural lipids cupuassu (*Theobroma grandiflorum*) butter, buriti (*Mauritia flexuosa*) oil and lanolin, containing the sunscreen BP-3 via hot high pressure homogenization. Dynamic light scattering was used to determine average particle size, polydispersity index and zeta potential of the suspensions. BP-3 entrapment efficiency was estimated using UV absorption. The average size of the particles was around 170 nm, with polydispersity index of less than 0.2, which indicates a narrow size distribution. Zeta potential varied between -30 and -25 mV, indicating good stability. BP-3 entrapment efficiency was higher than 85%. Figure 1 shows the evolution of average particle size and zeta potential of the formulation containing BP-3 during 2 months. No significant changes were observed, which indicates good stability. The results show that the NLCs produced are promising carriers for the sunscreen BP-3. The evaluation of skin penetrability showed very low concentration at the stratum corneum level. Low cytotoxicity of NLCs was found. The sun protection factor (SPF) of formulations containing BP-3 NLCs will be discussed.

Keywords: benzophenone-3, nanostructured lipid carriers, cupuassu butter, buriti oil.

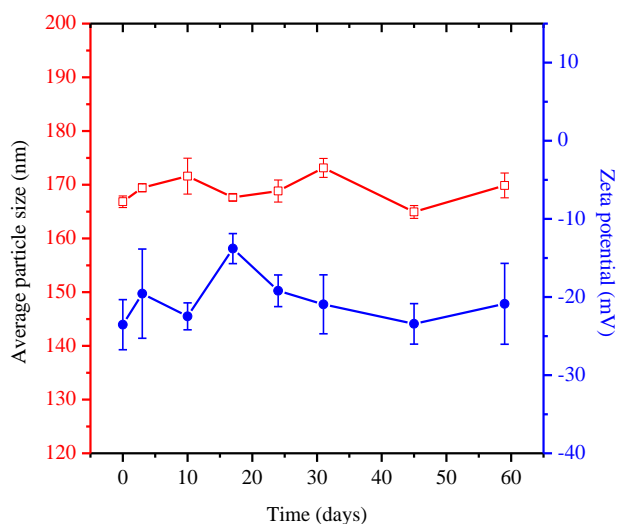


Figure 1: Evolution of average particle size and zeta potential of the formulation containing 10 % of benzophenone-3.

Acknowledgements: Support from FAPESP, CNPq, CAPES, INOMAT (MCTI/CNPq), NanoBioss (MCTI) and Brazilian Network on Nanotoxicology (MCTI/CNPq) are acknowledged.

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Effect of phospholipid and ethanol concentrations on physical property of phenylethyl resorcinol loaded ethosome

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Abstract: Phenylethyl resorcinol (4-(1-phenylethyl) 1, 3-benzenediol) is a synthetically produced from resorcinol which inhibits the conversion of tyrosinase to L-3, 4-dihydroxyphenylalanine (L-DOPA). It is a newly skin-lightening agent. The aim of this research was to develop ethosome containing phenylethyl resorcinol to enhance water solubility and stability, and decrease skin irritation. In addition, the effect of phospholipid and ethanol on the physical appearance was investigated to obtain their suitable concentrations in the ethosome for application as a topical delivery system. The ethosome was prepared by thin-film hydration method and composed of 5% w/v phenylethyl resorcinol, 0.5% w/v cholesterol (CHOL), 3-6% w/v phospholipid (L- α -phosphatidylcholine from soybean; SPC), 10-50% v/v absolute ethanol and water up to 100% v/v. The results show that formulations containing 3-6% w/v SPC and 20-50% v/v ethanol had good physical appearance with yellow colloidal. This phenomenon may be due to a synergistic effect between ethanol and water as a co-solvent system with lipid vesicles of phospholipids, which can increase the solubility of phenylethyl resorcinol in the formulations. However, increasing the ethanol concentration up to 60% v/v resulted in precipitation in ethosome. Besides, after 1 week the formulations containing 50% v/v ethanol had precipitate too which indicate instability in these formulations. These could be explained by the effects of high ethanol concentrations on disruption of lipid vesicle which lead to decreasing drug entrapment efficiency and increasing precipitation of ethosome formulations. Therefore, these results clearly demonstrated that the suitable ethanol concentration in preparation of the phenylethyl resorcinol ethosome was between 20-40% v/v ethanol when combining with 3-6% w/v SPC and 0.5% w/v CHOL.

Keywords: phenylethyl resorcinol, ethosome, ethanol, vesicular drug delivery system

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Clove Oil Loaded Nanoemulsions for Fish Anesthesia

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Abstract: Fish anesthesia is a biological state in fish induced by an external agent to ease handling and reduce fish stress. Clove oil is composed mainly of eugenol which can be used as natural fish anesthetic agent without any chemical hazard to the user and environment (King *et al.*, 2005). Due to the water insoluble property of clove oil, some organic solvents such as ethanol has to be used before mixing in fresh water. However, behavioral anesthesia of fish exposed to clove oil solution has been hyperactivity because of these organic solvents (Songkaew *et al.*, 2007). Nanoemulsions are emulsions with droplet size in the nanometric diameter range of approximately 20–200 nm. Nanoemulsions appear transparent or translucent, low viscosity, low surfactant, high kinetic stability against creaming or sedimentation (Solans *et al.*, 2005). Delivery of clove oil by nanoemulsions, therefore, could reduce the problem of using organic solvent. The purpose of this study was to develop nanoemulsions of clove oil. The effects of clove oil and surfactant on the emulsion characteristics and anesthetic activity in fish were investigated. The obtained emulsion properties were evaluated in terms of droplet size, size distribution, and zeta potential. The suitable formulations of clove oil loaded nanoemulsions (CLN) were selected to investigate for anesthetic induction time and anesthetic recovery time of koi carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*). The clove oil in ethanol solution was used as a positive control. The results showed that CLN formulations composed of 10% clove oil with 5% Tween20 and 20% clove oil with 5-15% Tween20 yielded the stable nanoemulsions with rapidly dispersed into fine droplets having a mean size of 128.9-188.1 nm with the narrow size distribution of 0.2-0.3 nm and zeta-potential in the range of -25 to -30 mV. It was found that the fish anesthetic activity of CLN was dose dependent. Both kinds of fish that received CLN showed shorter anesthetic induction time than those received the control. However, the anesthetic recovery time was varied, depended on dose and type of fish.

Keywords: nanoemulsion, clove oil, fish anesthesia, induction time, recovery time, *Cyprinus carpio*, *Carassius auratus*

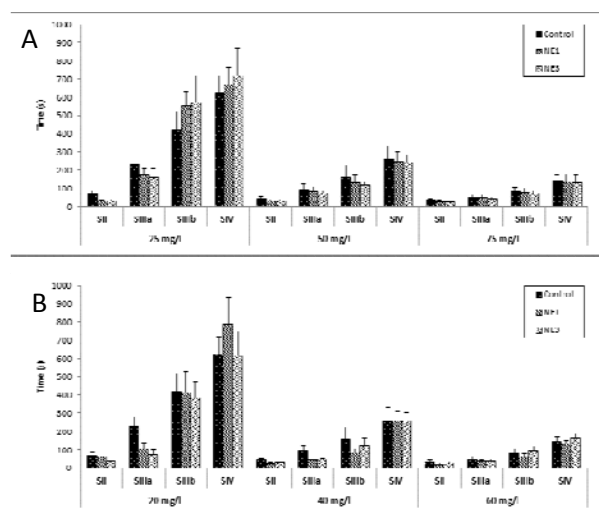


Figure 1: Induction time of anesthetic stage of koi carp (A) and goldfish (B) anaesthetized by clove oil loaded nanoemulsions with different concentrations of clove oil.

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Optimization of *in vitro* conditions to treat cancer with magnetic hyperthermia

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Abstract: According to the latest reports from the World Health Organization, cancer is one of the leading causes of death worldwide having killed nearly 8.2 million people in 2012 (1). Therefore finding new ways to cure cancer or prolong life is a great challenge for scientists. In this sense, magnetic hyperthermia (MHT) has emerged as an experimental anti-cancer strategy that maybe used either alone or as a sensitizing strategy. The aim of this study is to evaluate the ability of magnetic nanoparticles to kill tumour cells with MHT.

Superparamagnetic iron-oxide nanoparticles coated with poly-acrylic acid, having an average core diameter around 18nm and a surface charge of $97\pm 3\text{mV}$, were used as heat generators when submitted to an external magnetic field (EMF). Different combinations of amplitude and frequency of field were tested in order to obtain heating curves reaching deadly temperatures (between 41-46°C), using low, well-tolerable, magnetite concentrations (0.5 g/L). MHT assays were performed with U87MG cells, an *in vitro* model of glioblastoma multiforme, the most aggressive type of primary brain tumours, in the presence of the above-described MNPs. When applying 869kHz+225Oe until the sample reached 46°C, no significant difference was observed in cell

viability, as estimated using the PrestoBlue metabolic rate assay. Nevertheless, decreasing the intensity of the applied EMF (499kHz+275Oe or 688kHz+250Oe until 44 or 46°C were reached), therefore prolonging the exposure time to deadly temperatures, leads to a significant decrease in cell viability (of more than 50%). This fact was confirmed microscopically by double staining the cells with Annexin-V and Propidium Iodide, cell death indicators of apoptosis and necrosis pathways, respectively.

The results suggest that prolonging the time of exposure to temperatures above 41°C for at least 30 minutes may turn MHT more efficient as a strategy to treat cancer.

Keywords: superparamagnetic iron-oxide nanoparticles; magnetic hyperthermia; cancer treatment; cell viability

References: (1) World Cancer Report 2014, Edited by Bernard W. Stewart and Christopher P. Wild

Acknowledgments: V. Vilas-Boas acknowledges Fundação para a Ciência e Tecnologia (FCT) for her PhD Grant SFRH/BD/82556/2011.

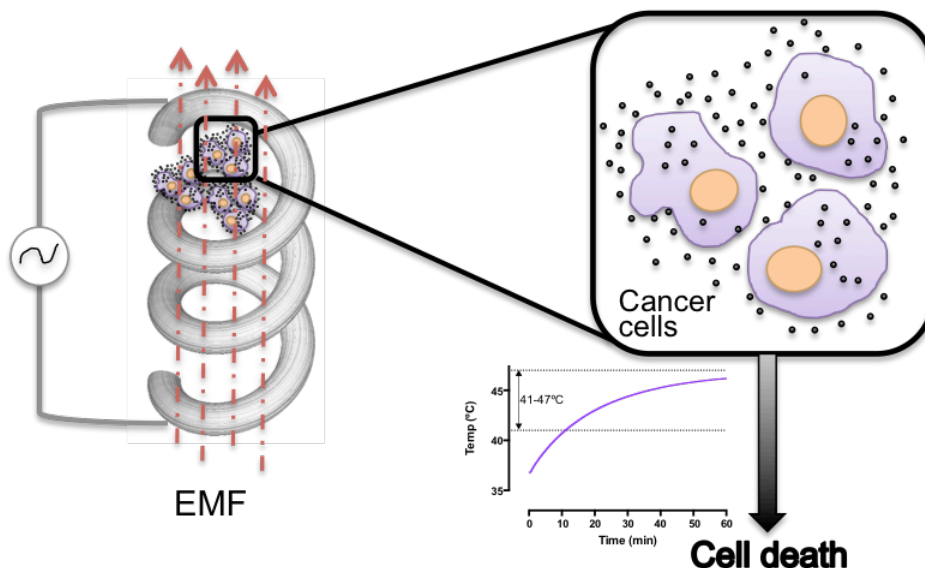


Figure 1: Schematic representation of the experimental design – cancer cells are incubated with the MNPs and then submitted to an external magnetic field that makes them vibrate, generating heat. If the temperatures reached are high enough and sustained for a certain period, significant cell death is observed.

Effective VEGF Binding to Au Nanocrystals with {111} Facets

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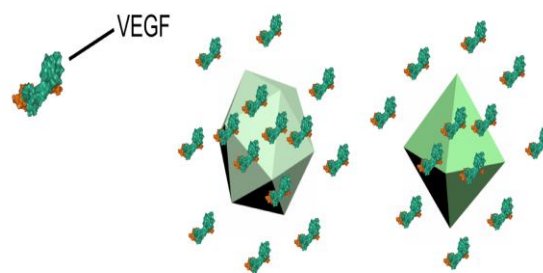
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Abstract: Nanocrystals with well-defined shapes and crystallographic facets can provide novel opportunities to improve efficacy of nanoparticle-based biomedical applications. (Kim *et al.*, 2011; Jo *et al.*, 2012, 2014) This study demonstrates binding of vascular endothelial growth factor (VEGF), a main factor of pathological angiogenesis, to single-crystalline Au nanocrystals with {111} facets. Icosahedral and octahedral Au nanocrystals effectively scavenge VEGF molecules in culture media as their spherical counterparts with similar diameter. Furthermore, they suppress *in vitro* VEGF-induced activation of VEGF receptor and significantly inhibit *in vivo* VEGF-mediated retinal permeability. These results suggest that Au nanocrystals other than nanospheres provide a useful platform for detection and scavenging of VEGF for various human diseases with VEGF-driven pathological angiogenesis, including cancer, age-related macular degeneration, and diabetic complications. (Figure 1)

Keywords: Au nanocrystals, {111} facets, vascular endothelial growth factor, nanoparticle-protein interaction

Figure 1: Gold nanospheres are known to bind to VEGF and suppress VEGF-mediated angiogenesis. In this study, Jo et al. reported gold nanocrystals with well-defined crystallographic {111} facets also exhibited anti-angiogenic effects. These results suggest that gold nanocrystals with (111) facets provide a useful platform for nanoparticle-based treatment of VEGF-driven pathological neovascularization in various human diseases including age-related macular degeneration, cancer, and diabetic retinopathy, beyond their current optical and catalytic applications.



Nanocrystals with {111} facets bind to VEGF and inhibit VEGF-driven pathological neovascularization.

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Kim, J.H., Kim, M.H., Jo, D.H., Yu, Y.S., Lee, T.G., Kim, J.H. (2011) The inhibition of retinal neovascularization by gold nanoparticles via suppression of VEGFR-2 activation. *Biomaterials*, 32, 1865-1871.

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Toxicological Aspects of Graphene Oxide on Gill Cells of Adult Zebrafish (*Danio rerio*)

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Abstract: Graphene exhibits unique physical and chemical properties, which have motivated a wide range of applications. However, the use of graphene could result in its accumulation in aquatic environment where the risks for organisms are still unknown. In this study, zebrafish were exposed to graphene oxide for assessment of toxic effects on gill cells. Adult zebrafish (*Danio rerio*), including males and females (6 - 7 month old; body weight $0.31 \pm 0.09\text{g}$; body size $3.34 \pm 0.31\text{cm}$) were exposed to 0; 2 or 20 mg L^{-1} of graphene oxide during 24 h. Exposure were performed in glass containers under conditions of continuous aeration, 12:12 light:dark photoperiod, $24 \pm 2^\circ\text{C}$ of temperature, 60% of dissolved oxygen, without feeding, including four replicates per treatment, and one fish per replicate (OECD 1992). After exposure, fish were collected, anesthetized, and gill tissues from each group were harvested and treated with 4% of dispase solution for 15 min at room temperature (Ji *et al.*, 2011). As revealed by flow cytometric analysis (BD FACSCalibur Flow Cytometer equipped with 488 nm laser), there was a significant increase in the number of cell in early apoptotic phase (PI negative, FITC annexin-V positive), and by late apoptotic or already dead cells (FITC annexin-V and PI positive), compared to the control (Figure 1). Graphene oxide nanoparticles are small enough to pass through the secondary lamellae of the gills and achieve the gill surface layers. The gill epithelium is similar to the basic features of epithelial design of other vertebrate animals (e.g., lung). Our findings are important, since they may raise concerns of risks to other animals (Handy *et al.*, 2008) and human beings.

Keywords: nanotoxicity, nanoparticles, fish, aquatic environments, risk assessment, graphene oxide, cell viability.

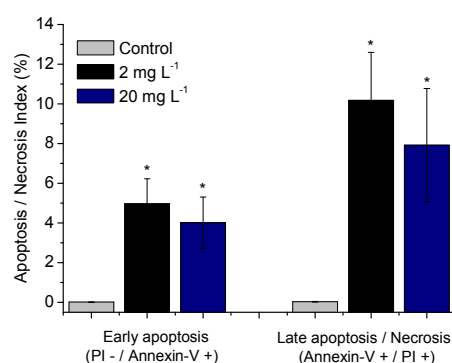


Figure 1: Percentage of number of gill cells of adult zebrafish in early apoptosis phase, late apoptosis or necrosis, relative to the total population after 24 h of exposure to zero, 2 and 20 mg L^{-1} of graphene oxide. * Means significant difference ($p < 0.05$ Tukey test) compared to the control.

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Effect of Particle Size on Oral Absorption, Tissue Distribution, and Excretion of Food Grade Titanium Dioxide and Silica Nanoparticles

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Abstract: Nanoparticles have been widely applied to many industries. Silica has been used as an anticaking agent in powdered mixes, seasonings, and coffee whiteners (Villota *et al.*; 1986) and titanium dioxide has been applied to enhance the white color of certain foods, such as dairy products and candies (Weir *et al.*; 2012). However, the question as to whether nanoparticles have potential toxic effects on human health remains to be answered. Moreover, little information is actually available about biokinetic behaviors of nanoparticles. In this study, biokinetics of titanium dioxide and silica nanoparticles was evaluated after single oral administration to rats, as compared with bulk sized materials. Effects of the presence of food ingredients on oral absorption were also evaluated. Quantitative analysis of titanium and silicon was performed with inductively coupled plasma-atomic emission spectroscopy (ICP-AES) and lithium metaborate fusion, followed by molybdenum blue method, respectively. The results showed that oral absorption of titanium dioxide was not significantly different between nano and bulk-sizes. However, rapid absorption rate and high absorption efficiency of silica nanoparticles was found as compared with bulk-sized silica. No significant effect of particle size on tissue distribution and excretion was found in all the cases. On the other hand, oral absorption was highly affected by the presence of food ingredients. These findings will provide basic information to predict potential toxicity of nanoparticles as well as new insight on toxicity evaluation of nanoparticles applied to foods.

Keywords: titanium dioxide, silica, pharmacokinetics, interaction.

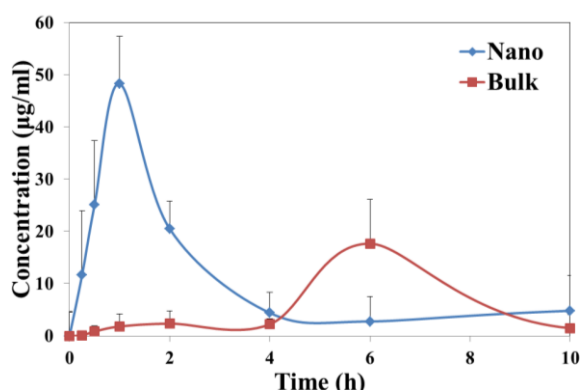


Figure 1: Oral absorption of food grade nanoparticles in rats after single dose administration.

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Anti-angiogenic Effect of Gold and Silica Nanoparticles on Choroidal Neovascularization: Size Matters, Core does not

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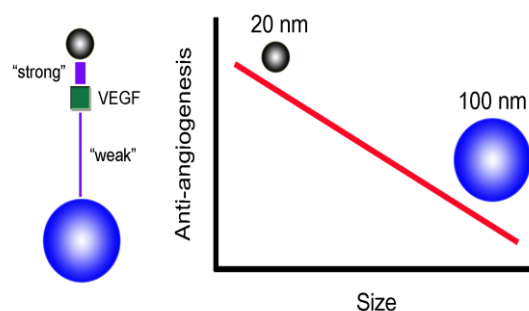
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Abstract: Nanoparticles can be involved in biological activity such as apoptosis, angiogenesis, and oxidative stress by themselves. In particular, inorganic nanoparticles such as gold and silica nanoparticles are known to inhibit vascular endothelial growth factor (VEGF)-mediated pathologic angiogenesis. (Kim *et al.*, 2011; Jo *et al.*, 2012) In this study, we show that anti-angiogenic effect of inorganic nanospheres is determined by their own sizes. We demonstrate that 20 nm size gold and silica nanospheres suppress VEGF-induced activation of VEGF receptor-2, *in vitro* angiogenesis process, and *in vivo* pathologic angiogenesis more efficiently than their 100 nm size counterparts. Our results suggest that size determines inhibitory activity of gold and silica nanospheres to VEGF-mediated angiogenesis. (Figure 1)

Keywords: nanospheres, anti-angiogenesis effects, inorganic nanoparticles, vascular endothelial growth factor, pathologic angiogenesis

Figure 1: Figure 1 illustrates that the size determines inhibitory activity of monodisperse nanospheres to VEGF-mediated angiogenesis regardless of whether they were composed of gold or silica. 20 nm size nanospheres inhibit VEGF-induced angiogenesis more efficiently than their 100 nm size counterparts. We believe that material-independent, but size-dependent anti-angiogenic effect of inorganic nanospheres on pathologic angiogenesis will give insights to researchers who struggle to find out ways to utilize nanoparticles as therapeutic materials.



References:

Kim, J.H., Kim, M.H., Jo, D.H., Yu, Y.S., Lee, T.G., Kim, J.H. (2011) The inhibition of retinal neovascularization by gold nanoparticles via suppression of VEGFR-2 activation. *Biomaterials*, 32, 1865-1871.

Jo, D.H., Kim, J.H., Yu, Y.S., Lee, T.G., Kim, J.H. (2012) Antiangiogenic effect of silicate nanoparticle on retinal neovascularization induced by vascular endothelial growth factor. *Nanomedicine*, 8, 784-791.

Synthesis and surface modification of $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{Au}$ NPs as theranostic agents for Nanomedicine applications.

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Abstract: In the last decades, metallic nanomaterials represent a promising agent in cancer treatment such as hyperthermia therapy. In this study metallic nanoparticles consisting of multiple shells of iron oxide, silica and gold were synthesized ($\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{Au}$). In this way we obtained a complex nanostructure that simultaneously possess magnetic properties of Fe (Lu *et al.* 2007) and optical properties of Au (Gao *et al.* 2009) to develop a novel theranostic system for the treatments of cancer diseases. The resulting nanoparticles have been completely characterized by different techniques such as UV-Vis, DLS and TEM.

Stability of nanoparticles is a crucial requirement for almost any application especially in the nanomedicine field. Native metals nanoparticles, are generally very sensitive to external environment and subjected to agglomeration if not in presence of stabilizing agents. Therefore, it is necessary to develop efficient strategies to improve the chemical stability of this nanosystem. The most straight forward method seems to be protection by an organic layer, which shields the surface of the particles from the environment.

In the case of metallic nanoparticles, organic ligands can be chemically anchored or physically adsorbed on nanoparticles' surface to form a single or double layer, which creates repulsive (mainly as steric repulsion) forces to balance the magnetic and the van der Waals attractive forces acting on the nanoparticles. The obtained $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{Au}$ NPs were coated with an organic ligand by exchange ligand reaction to study their stability and final properties. The organic ligand selected for this purpose presents a thiol as functional group in order to maximize interaction with the gold surface (**Figure 1**). The resulted coated nanoparticles are now suitable for entrapment into biocompatible polymeric matrix to form a targetable water soluble nanocarrier for nanomedicine applications. As polymer the well-known poly(lactic-co-glycolic-co-polyethylene glycol) (PLGA-PEG) was selected due to the Food and Drug Administration (FDA) approval in biomedical formulation and for its ability to create micelles (PMs) with a lipophilic core, which can host our lipophilic particles (Locatelli *et al.* 2015). With oil-in-water technique $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{Au}@PMs$ were obtained. These polymeric micelles were then purified and characterized by different techniques to verify both stability and maintainance of the magnetic and optical properties from native metallic nanoparticles. In conclusion the final nanosystems underwent biological studies aimed at the exploitation of both magnetic and op-

tical properties, thus confirming the possibility to use this multishell system as theranostic agent.

Keywords: nanoparticles, iron, gold, silica, surface chemistry, micelles, theranostic agent.

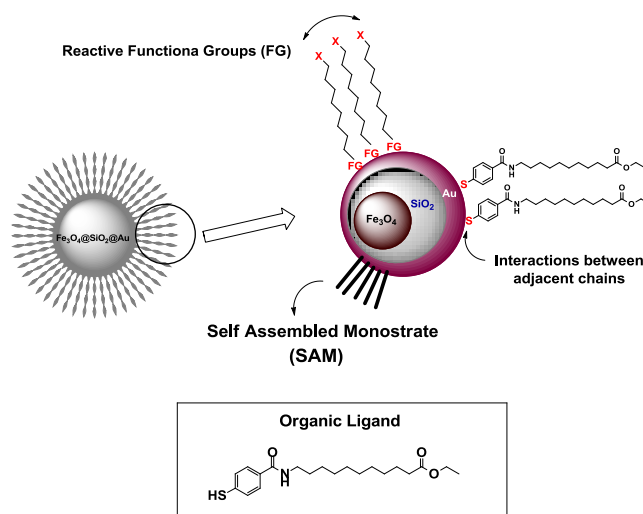


Figure 1: Self Assembled Monolayer (SAM) formation on NPs surface.

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Interactions of Zinc Oxide Nanoparticles with Dispersants: Cytotoxicity, Uptake, and Pharmacokinetics

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Abstract: Zinc oxide (ZnO) nanoparticles have been extensively applied to various commercial products, such as catalyst, electronics, medical diagnosis, cosmetics, sunscreens, and food additives due to their catalytic, semi-conducting, magnetic, antimicrobial, and UV protection properties as well as their thermal conductivity (Fan *et al.*, 2005). Along with growing concern about potential toxicity of ZnO nanoparticles, many researches have been recently focused on their toxicological effects in cultured cell lines as in animal models (Paek *et al.*, 2013). For toxicity evaluation, nanoparticles should be well dispersed in aqueous solution where some dispersants are indispensably added for better stability. However, dispersants used for biological evaluation can also affect cellular response, uptake behaviors, and toxicity of nanoparticles (Haniu *et al.*, 2011). In the present study, the effects of ZnO nanoparticles prepared in different dispersing agents, such as citrate, carboxymethyl cellulose, and cell culture medium or distilled water were investigated in terms of cytotoxicity, cellular uptake, pharmacokinetics, and tissue distribution. We also compared biological behaviors of ZnO nanoparticles with those of zinc ions. The results demonstrated ZnO nanoparticles prepared under different conditions had almost the same particle size, morphology, and solubility, but their zeta potential was highly affected by the presence of dispersants. In terms of cellular response, ZnO dispersed in citrate exhibited the highest toxicity (Figure 1) and enhanced cellular uptake, although dispersants did not influence on energy-dependent uptake mechanism of ZnO. Pharmacokinetic study also showed that oral absorption efficiency differed from dispersants used, but significantly different tissue distribution was not found. These findings suggest that more careful caution is necessary for choice of appropriate dispersants to evaluate biological responses of nanoparticles.

Keywords: ZnO nanoparticles, dispersant, cytotoxicity, cellular uptake, pharmacokinetics, tissue distribution.

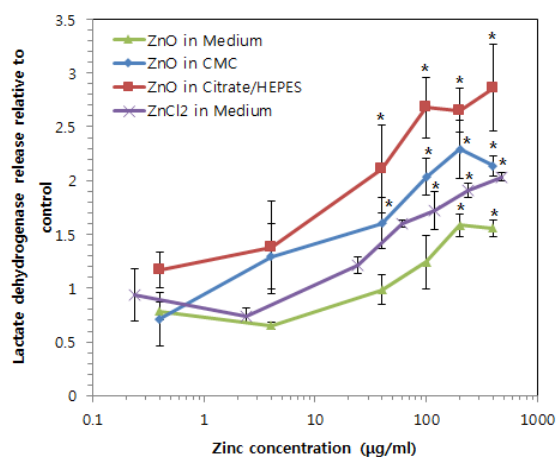


Figure 1: Cytotoxicity of ZnO nanoparticles prepared in different dispersants or cell culture medium in lung epithelial A549 cells after 24 h exposure.

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Transdermal resveratrol nanoethosomes; Preparation, Optimization; *In-vitro*, and *In-vivo* evaluation

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Abstract: Resveratrol is a prominent substance in currently pharmaceutical research. It is a naturally occurring non-flavanoid phenolic compound produced by some spermatophytes, notably grapes. It has been reported to possess antioxidant, neuroprotective, antiphotaging and antiviral activities, and it also seems to play a role in the prevention and reduction of pathological processes such as inflammation. Resveratrol has been demonstrated to show benefits against skin disorders (Zhang *et al.*, 2007). However, trans-resveratrol has poor oral bioavailability, mainly because it is extensively metabolized in the body. The bioavailability of orally administered resveratrol is insufficient to permit high enough drug concentrations for systemic therapy (Hung *et al.*, 2008). The aim of the present study is to prepare, optimize, and evaluate resveratrol as transdermal nanoethosomal formulation, in order to enhance its bioavailability. Several factors as drug to phospholipid molar ratio, phospholipid to ethanol ratio, hydration medium pH, hydration time, and the temperature of hydration were investigated to study their effect on the particle size and entrapment efficiency (EE%) of resveratrol within nanoethosomes. The optimized formula was subjected to *in vitro* release, *Ex-vivo* permeation, and *in vivo* pharmacokinetic studies. The results indicated that optimum nanoethosomal formula was successfully formed with particle size 53 nm and entrapment efficiency 86%, also the bioavailability increased by more than 6-folds after transdermal application of optimized nanoethosomal formula in

comparison with commercially available product. From the above results it can be concluded that nanoethosomal formulation represents an efficacious carrier for transdermal delivery of Resveratrol.

Keywords: Resveratrol, nanoethosomes, *in vitro*, *ex vivo* and *in vivo* characterization, optimization of transdermal permeation.

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Incorporation and release of gemcitabine prodrug in mesoporous silica nanoparticles

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Abstract: In recent decades, mesoporous silica nanoparticles (MSNs) have been subjected of intense research (Mamaeva *et al.*; 2013). Compared to traditional organic nanocarriers (such as liposomes or other colloidal systems) these vehicles exhibit unique properties of inorganic nanomaterials, such as thermal and chemical stability and ease of chemical modification of surface silanol groups. MSNs show very interesting properties for the application in the development of drug delivery devices, such as stable mesoporous structure, high specific surface area (600-1000 m²/g), large pore volume (0.6-1 cm³/g), regular and tunable mesopore diameters (1.6–30 nm) and pore channel systems homogeneously organized in hexagonal mesostructures. Moreover, many types of MSNs have been shown to be nontoxic in many biological systems if they are prepared with certain optimized structural features and are applied in right dosage (Vivero-Escoto *et al.*; 2010).

Based on these considerations, the aim of this work was to explore the ability of different MSNs to be employed as a convenient vehicle for the delivery of antitumoral drugs. In particular, MSNs characterized by different surface functionalizations were studied as vehicles for the delivery of the antitumoral drug gemcitabine (GEM) and of its lipophilic prodrug to improve drug metabolic stability and *in vitro* and *in vivo* cytotoxic activity.

For this purpose, MSNs as such or with grafted aminopropyl and carboxyethyl groups were prepared and characterized. MSNs were produced by a sol-gel procedure in presence of the surfactant cetyltrimethylammonium bromide (CTAB) as structure directing agent and functionalized by post-synthesis grafting as previously reported (Sapino *et al.*; 2015). The obtained materials exhibited quasi-spherical particle morphology with an average particle size of ca. 100 ± 23 nm and regular and ordered cylindrical channels with hexagonal symmetry. Representative high resolution transmission electron microscopy (HRTEM) images of the samples are reported in Figure 1.

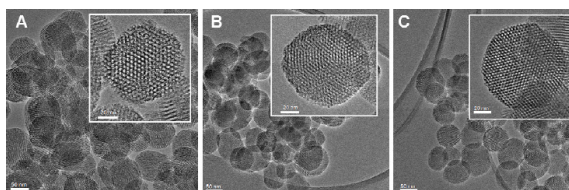


Figure 1: HRTEM images of A) MSNs; B) Carboxy-MSNs and C) Amino-MSNs.

The presence of the functional groups on the surface of Amino-MSNs and Carboxy-MSNs was confirmed by thermogravimetric analysis (TGA) and FTIR measurements.

Then, GEM loading capacity of different MSNs in relation with the nature of the functional group exposed was determined by HPLC. We observed that in our experimental conditions GEM was not loaded in any MSNs whatever tested ratios and incubation time. Thus, we decided to encapsulate a gemcitabine lipophilic prodrug, the 4-(*N*)-lauroylgemcitabine (C12GEM), whose calculated log P value is 4.84, higher than that of the parent drug (-1.4) (Stella *et al.*; 2007). C12GEM was efficiently loaded in the MSNs. Moreover, the results showed that the drug loading efficiency increased in the case of functionalized MSNs, in the order Carboxy- > Amino- > MSNs. This indicates that the insertion of functional groups on the MSN surface lead to an increase in the loading capacity, probably in relation to hydrophobic and hydrogen bonding interactions that stabilize the drug-MSNs complexes.

Similarly, the presence of functional groups on MSN surface influenced the drug release profile. *In vitro* C12GEM release experiments from drug-loaded MSNs were carried out by soaking samples in PBS buffer at 37 °C. As reported in Figure 2, all samples showed a very gradual and slow release, which is influenced by the presence of surface functional groups. In particular, the release rate of the MSNs was the fastest one, followed by Amino-MSNs and by Carboxy-MSNs that was the lowest. This indicates that the diffusion of the prodrug from the three samples to the buffer solution depends on the functionalization with a trend inverse to that found in the drug loading experiments. Carboxy-MSNs showed the

highest drug loading (23 $\mu\text{g}/\text{mg}$) and the lowest release in percentage, suggesting a relatively high interaction between C12GEM and the functional groups.

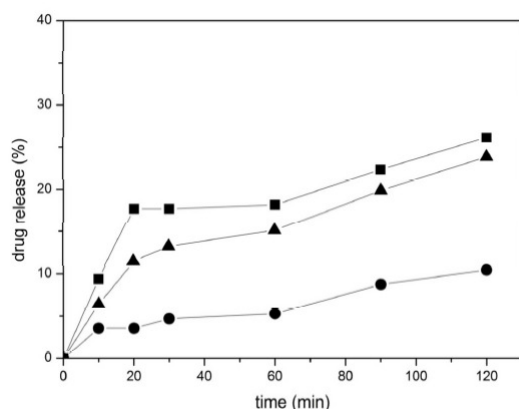


Figure 2: *In vitro* release profiles of MSN-C12GEM (■), Amino-MSN-C12GEM (▲) and Carboxy-MSN-C12GEM (●) in PBS medium at 37 °C. All experiments were done in triplicate. SD: $\pm 10\%$.

Finally, the cytotoxicity of the different preparations was evaluated on MDA-MB-231 (human breast adenocarcinoma) and A2780 (human ovarian carcinoma) cells at different times (24, 48 and 72 h). Data showed that C12GEM-loaded MSNs were less cytotoxic than the free drug with an activity that increased with the incubating time indicating that all these systems are able to release the drug slowly and in a controlled manner.

Altogether the results demonstrate that these MSNs could be an interesting system for the delivery and extended release of anticancer drugs. In particular, our results highlight a dependence of the loading ability of MSNs on molecular properties of the drug, in particular the lipophilic character and steric hindrance, and on the MSN surface characteristics.

Keywords: nanoparticles, drug delivery systems, cancer chemotherapy, prodrugs, silica-based biomaterials, physicochemical properties, surface chemistry.

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Visual Analysis of Water permeability in Aquaporin Z incorporated Giant Unilamellar Vesicles

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Abstract: Water permeability of aquaporin Z was investigated using Giant Unilamellar Vesicles (GUV). Because aquaporin Z has a selective permeability for water molecule in and out of the cell in biological system, It can be applied to design biological separation system. However, like other transmembrane protein, manipulation and application of aquaporin Z has many problems associated with expression, purification and integration of the protein into the membrane system. To solve these problems, we developed variable methods. Aquaporin Z was fused with Green fluorescence Protein (GFP) to visualize aquaporin Z in system. Hydrophobic nature of Aquaporin Z is another hurdle for purifying the protein in native form. Detergents are commonly used additives to facilitate the purification of transmembrane protein (Simons *et al.*; 1975). In this study, we purified GFP-fused aquaporin Z using triton X-100 as surfactant and observed green fluorescence of fused protein to track the location of aquaporin Z in the system. To the study of water permeability, GFP-fused aquaporin Z was needed to be reconstituted into the membrane platform. According to the report (Philippe *et al.*; 2004), membrane protein can be reconstituted into GUV using sequential step. GUV reconstituted GFP-fused aquaporin Z was formed by electroformation method and those vesicles had green fluorescence (Figure 1). When outside solution of aquaporin Z-containing GUV was replaced to hypertonic salt, the volume of vesicle was changed as time-dependent. Reduced volume ratio after 20 minute was less ~50% compare with that of initial time, it agree with the report (Sheereen *et al.*; 2013). In this study, we established manipulation method of aquaporin Z that include visualization, purification, and reconstitution. Also water permeability of aquaporin Z was verified. This study will help to understanding of aquaporin Z character and apply to design of noble water purification system

Keywords: aquaporin Z, transmembrane protein, water permeability, green fluorescence protein, giant unilamellar vesicle, electroformation, water purification system

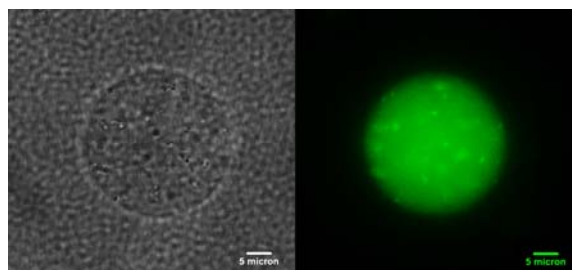


Figure 1: Figure illustrating the microscopy images of GUV reconstituted GFP-fused aquaporin Z. Left image was transmission image. Right image was fluorescence image. All images were same vesicle. Successfully, GFP fused aquaporin Z was incorporated into the GUV by electroformation method.

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Acknowledgments:

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THE PRODUCTION AND THE CHARACTERIZATION OF SPIO NANOPARTICLES FOR MEDIATED TRANSFECTION OF PLASMID DNA

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Abstract: Transfection of eukaryotic cells is one of a key technology in cell biology being used in fundamental and applied research. To achieve a high transfection efficiency, reproducibility and low-toxicity on targeted cells when DNA plasmids is transfected, is highly desired. Several approaches using various nanoparticles (NPs) have been already evaluated but the comparison with standard chemical methods such as polyethylenimine (PEI) is still poor. In our work, we produce small monodispersed superparamagnetic nanoparticles (SPIO) as the suitable candidate for the assisted transfection of DNA expression plasmids. The synthesis of nanoparticles by co-precipitation method is presented and optimized in time, size, temperature and rate of base addition of core formation. The nanoparticles were stabilized with chitosan under physiological pH. DNA plasmids are expected to be integrated into the polymer coat, making the DNA-SPIO complex ready for the transfection via applied magnetic force and subsequently taken up by cells via endocytosis. Here, the DNA-SPIO complex is introduced in HEK cells to analyse the cellular toxicity and the effect on cell proliferation. The uptake of DNA plasmid was also evaluated by fluorescence microscopy to estimate the transfection efficiency by comparing fluorescently positive cells to the total quantity of cells and to see that no evident negative effect on cells is induced. The SPIO-mediated transfection will be compared with commercially available magnetic nanoparticles and PEI transfection agent.

Keywords: magnetic nanoparticles, plasmid DNA, polyethylenimine, transfection

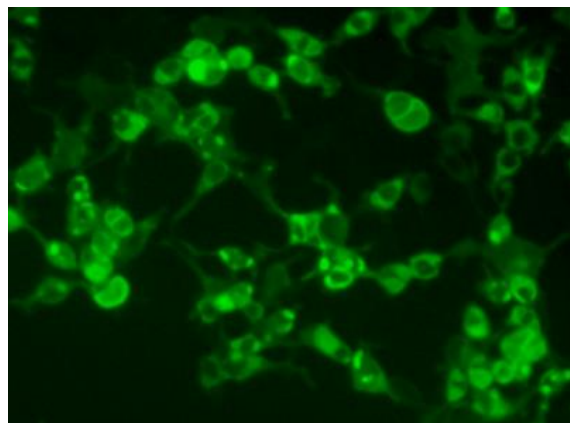


Figure 1: Figure illustrating the transfection of HEK293 cells with ChR-2 plasmid using magnetic nanoparticles.

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Gold nanoparticle-based Immunoprecipitation (IP) sensor for Detection of Shiga toxin (Stx) from pathogenic *Escherichia coli*

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Abstract: Ensuring food safety is critical in food industry. For decades, detection of toxins relevant to foodborne diseases have been widely performed in order to secure food safety. Shiga toxins (Stx) produced by *Escherichia coli* O157:H7 are one of the most virulence factors related to foodborne illness causing life-threatening conditions including kidney failure, neurological complications. Herein, we designed a method for rapid detection of foodborne virulence factors that can be applied to the field diagnosis. We developed gold nanoparticles (GNP) functionalized with specific antibodies (Ab) against Stx. GNP-Ab binds to Stx and creates GNP-Ab-Stx complexes through the specific antigen-antibody interactions. The complexes can bind to other GNP-Ab complexes via antigen-antibody bridging (Min-Cheol et al.; 2011). The antigen mediated complexes and free GNP-Ab are clearly distinguished by Radial chromatography (RC) because mobility of the complexes was affected by the formation of network structure in silica gel plate. When a drop of sample solution was placed onto the surface of silica gel plate, free GNP-Ab were spreading out from the drop site along with the solution. In the presence of target toxin, GNP-Ab forms complexes and their mobility decreases compared to free GNP-Ab. Therefore the sample with target toxin forms a small spot in the droplet site whereas the sample without target toxin forms a larger red circle derived from the unbound free GNP-Ab. The differences in mobility of the GNP-Ab in the absence or presence of target toxin is determined by radial chromatography. From the results, we could detect the existence of target toxins with naked eyes by checking the color distribution. Furthermore, we could apply this system to quantify the target toxin in test sample based on the color gradients of GNP. This method has advantages not only in detection time, cost efficiency but also in portability which is suitable for field diagnosis. This novel method has potential for ensuring food safety in field without conventional lab equipments.

Keywords: Gold nanoparticle, immunoprecipitation sensor, Shiga toxin, Radial chromatography, silica-gel

plate, field diagnosis, color gradient.

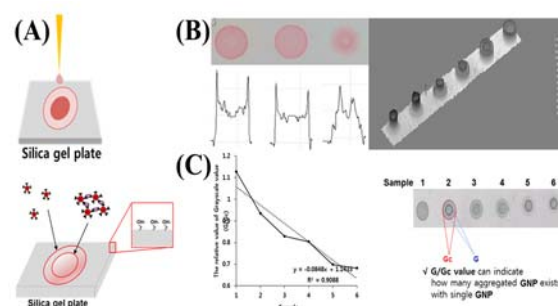


Figure 1: Whole scheme of the detection device based on immunoprecipitation. (A) Separation of GNP-Ab-Stx complexes and free GNP-Ab by radial chromatography (RC) on silica gel plate through capillary action. (B) RC results were analyzed by Image J software with line profiling and 3D image structuring. (C) Grayscale value of inner ring and outer ring (control) was used to calculate G/Gc value. G/Gc value shows the ratio of aggregated BNP-Ab-Stx complexes and unbound free GNP-Ab.

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Development of intelligent Drug Delivery Systems based on thermoresponsive gold nanoparticles

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Abstract: Thermoresponsive polymers have attracted much attention over the last decades because of their numerous potential applications in the biomedical field, for example as Drug Delivery Systems (DDS). These polymers exhibit a drastic influence of T on their conformation in solution: for example, Lower Critical Solution Temperature (LCST) polymers shift from a expanded hydrophilic coil conformation at low temperatures (below their LCST) to a hydrophobic globular conformation at higher temperature (above their LCST).¹

Our aim is to develop a thermocontrolled DDS (see Figure 1) by coupling a thermoresponsive polymer to gold nanoparticles (AuNP). AuNPs present a Localised Surface Plasmon Resonance (LSPR) band in the visible range and, by irradiating the AuNP at this band, it should be possible to induce the phase transition in the polymer.²

PNIPAM is a LCST polymer which displays good properties for the development of such kind of hybrid system.¹ PNIPAM of different sizes were synthesized with a thiol end-group and characterized by GPC, MTDSC and NMR. AuNP were synthesized following the Turkevich method and characterized by UV-Vis, DLS, TEM and SEM.

The different polymers were grafted on the particles, leading to AuNP-PNIPAM hybrids. The organic content of these hybrids was determined by TGA and their thermal behavior was studied in details by UV-Vis spectroscopy and DLS. The results show an important influence of the polymer size and of the ionic strength on the thermal behavior of the AuNP-PNIPAM hybrid systems.

With the aim to improve the control of the thermal behavior of the polymer, different strategies, such as the co-grafting of a hydrophilic polymer, were envisaged.

Keywords: gold nanoparticles, thermoresponsive polymer, PNIPAM, LSPR, Drug Delivery Systems

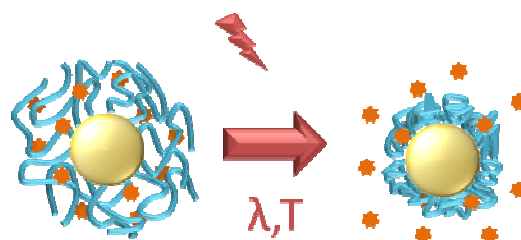


Figure 1: Controlled thermoresponsive drug delivery system.

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Development of a Mdm2 specific colorimetric biosensing platform using gold nanoparticles

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Abstract: Functionalized gold nanoparticles (GNPs) have attracted much attention during the past decade due to their potential applications in the biomedical field. GNPs present interesting optical properties such as their Localized Surface Plasmon Resonance (LSPR) which make them good candidate for the development of sensors (Taylor *et al.*; 2012). Our aim is to exploit the LSPR band of GNPs to develop a platform to detect the oncoprotein Mdm2.

Mdm2 plays a critical role in human cells as it is the main negative regulator of the tumor suppressor protein p53. This latter protein is deeply involved in the cellular response to the stress that can lead to the apparition of cancerous cells (Vousden *et al.*; 2002). In a number of human cancers, overexpression of the Mdm2 protein has been observed (Momand *et al.*; 1998) and its detection could be used in cancer diagnosis. Its regulation could furthermore lead to new therapies.

The platform is made of two sets of GNPs, each functionalized with a different peptidic aptamer, that can bind the targeted protein simultaneously. In the presence of Mdm2 the GNPs aggregate with the protein playing the role of linking agent. The GNPs aggregation is an unambiguous signal with the color of the solution changing from red to purple.

GNPs were synthesized and characterized (by TEM, DLS, UV-Vis) and then functionalized with peptide aptamers containing sequences identified in proteins known to interact naturally with Mdm2. A protocol was developed for the grafting of the aptamers on the GNPs surface and for the quantification of the grafting level. In the presence of Mdm2, but not of a control protein (BSA), GNPs aggregated and a significant change in the LSPR band was observed (Figure 1).

Keywords: protein detection, Gold nanoparticles, UV-vis spectroscopy, Biosensor, Peptidic aptamer, TEM, biomedical applications, Emission spectroscopy, Gold nanoparticles aggregation, LSPR

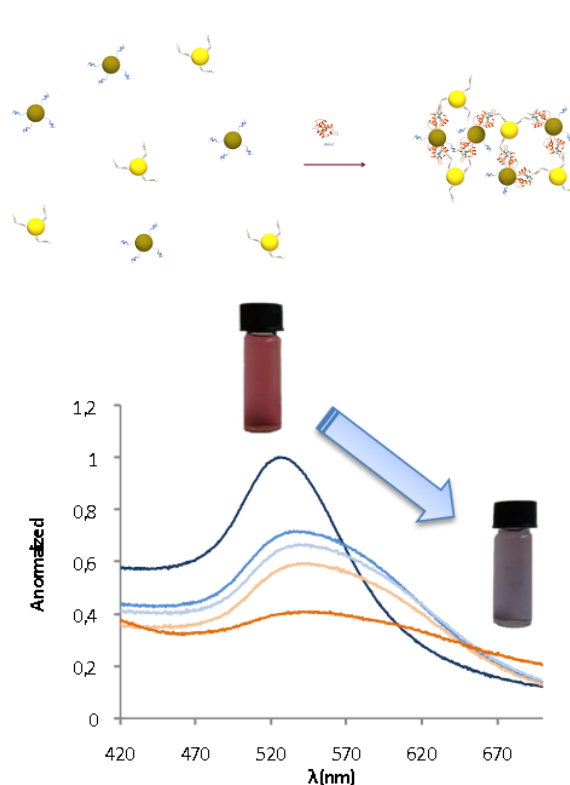


Figure 1: Aggregation of the two sets of gold nanoparticles in presence of the targeted protein (Mdm2)

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Enhanced Antibacterial Activity of Antibiotics in Combination with Silver Nanoparticles against Animal Bacteria

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Abstract: Antibiotics are excessively used to treat and prevent animal infectious diseases which results in development and dissemination of bacterial resistance to antibiotics (Schwarz *et al.*, 2001). Due to the decrease of antibiotics efficiency another ways of infection control has gained importance. Silver nanoparticles (AgNPs) are well-known for their strong antibacterial activity (Panacek *et al.*, 2006) and they can also help to enhance the antibacterial effect of antibiotic therapy in humans (Hwang *et al.*, 2012). Therefore, we studied combined therapy of AgNPs and antibiotics against veterinary bacteria. For this purpose, antibiotics with different modes of action were used. AgNPs 28 nm or 8 nm in size were synthesized through the reduction of complex cation $[\text{Ag}(\text{NH}_3)_2]^+$ by D-maltose or sodium borohydride, respectively. The comparison of minimal inhibitory concentration of AgNPs and antibiotics alone and in combination of each other was realized on the base of standard methods used in practice for evaluation of synergistic interaction of antibiotics with adjuvants (microdilution checkerboard method). The results showed that AgNPs possess synergistic, additive and indifferent activities against bacteria. No antagonistic interaction was observed. Moreover, we observed in some cases that antibiotic resistant bacteria become sensitive when antibiotic is combined with AgNPs. Our results point out to possibility of utilization of AgNPs in combination with antibiotics for treatment diseases caused by antibiotic resistant bacteria.

Keywords: silver nanoparticles, antibiotic resistant bacteria, synergy, microdilution checkerboard method.

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The mechanisms for the radiosensitizing effects in high linear energy transfer radiation on colon cancer cells

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Abstract: The purpose of this study was to investigate the efficacy of Gold Nanoparticle as a radiosensitizer for use in combination therapy for colon cancer cells. Two human colon cancer cell lines (HCT116, HT29) were treated with GNP alone or with radiation followed by GNP. In vitro tests were evaluated by clonogenic survival assay, FACS analysis, western blotting, immunofluorescence, and comet assay. GNP significantly enhanced radiation efficacy under high and low Linear Energy Transfer (LET) radiation conditions *in vitro*. GNP, in combination with radiation, increased G2/M arrest and increased the cell population in the sub-G1 phase and the ROS level, ultimately increasing cellular apoptosis. GNP inhibits the repair of DNA damage caused by radiation and synergistically suppressed cell migration and invasion. The radiosensitizing effects of GNP are much higher in neutron (high LET)-irradiated cell lines than in γ (low LET)-irradiated cell lines. GNP synergistically enhances the radiosensitivity of colon cancer cells, suggesting it may have clinical utility in combination cancer treatment with high LET radiation.

Keywords: GNP, high LET radiation, radiosensitivity, colon cancer cells, DNA damage

Advantages and Obstacles of Using Microfluidic Based Immunoassay for the Detection of Cancer Biomarkers from Biofluids

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Abstract: Microfluidic devices also known as lab-on-chip devices have been a prime candidate for replacing popular biomarker detection techniques such as Enzyme-Linked-Immunoassays (ELISA) for advantages they provide such as reduced reagent and sample consumption, reduced time required, and reduced cost (Ng et al., 2010). In addition, microfluidic devices provide the potential of incorporating more than one process on the same device, one of which is filtration of biofluids such as blood before analyte detection (Sollier et al., 2009). In this work we present the design optimization process of a lab-on-chip device for the detection of Alfafetoprotein (a liver cancer biomarker) from blood (Figure 1). We report the effect of a variety of designs and parameters on the filtration of red blood cells from blood samples before the detection of AFP using chip based immunoassay. Furthermore, we report differences in using different polymer substrates for the fabrication of such devices including polydimethylsiloxane (PDMS) and polymethylmethacrylate (PMMA) and study the material's effect on phenomena such as non-specific binding, blood clotting, and autofluorescence. We also study the device's ability to detect multiple proteins simultaneously. This study also considers the effect of detecting protein from a water sample versus detecting it from a whole blood sample in order to enable better implementing such technologies in the field.

Keywords: microfluidics, biomarker detection, Alfafetoprotein, immunoassay, biofluids, microfabrication, surface treatments, microchannels, fluorescence microscopy, blood filtration, polymethylmethacrylate, polydimethylsiloxane

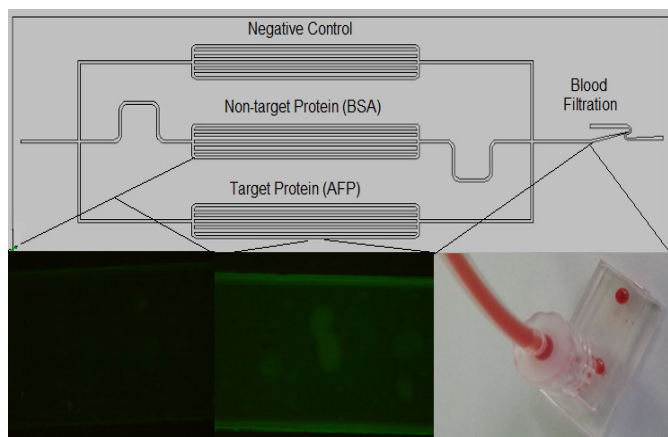


Figure 1: Microfluidic device design, where the device has a blood filtration region on the right and three detection zones to the left. The detection zones can be functionalized with antibodies of biomarkers of interest but in the above case the three regions two of the regions are functionalized with the target analyte antibody (anti-AFP), and the third region is not functionalized with any antibodies as a negative control. Fluorescently tagged bovine serum albumin (BSA) was pumped through the middle section – indicated on the figure, while fluorescently tagged AFP was pumped through the bottom section to ascertain the test's specificity.

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Efficient Encapsulation of Carboplatin Anticancer Molecule into Boron Nitride Nanotube : a Promising Drug Nanovector

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Abstract: Chemotherapy is frequently used to treat cancer patients. However, serious adverse effects are observed when drugs are systemically administered since they exhibit poor specificity in reaching tumor tissues (Yang *et al.*, 2011). More, the efficacy of many potent and promising drug molecules is limited by their low water solubility, by the increased drug resistance and highly cytotoxic side effects (Samori, *et al.*, 2010). To circumvent such important drawbacks, an efficient way of systemic transportation needs to be developed. Many of the pharmacological properties of conventional drugs can be improved through the use of nanocarriers (Allen *et al.*, 2004). Anticancer drug transport is now become a central research since it would allow to localize the drug release near the tumor cell, avoiding secondary medical effect. We report a theoretical study based on molecular dynamics simulations to demonstrate that encapsulation of anticancer carboplatin molecule (CPT) is favored in boron nitride nanotube (BNNT). The ability of BNNT to vectorize CPT is improved since several drug molecules can be adsorbed inside the nanotube. Our simulations demonstrate that CPTs molecules are spontaneously attracted to BNNT nanotube and are stable once encapsulated, forming cluster inside the nanotube. The storage capacity of BNNT is thus very large due to high confinement effects and hydrophobic interactions, favoring its filling with drug molecules until completion. Our calculations show in particular that we can fill the capsule by three drug molecules, opening the way to a very efficient drug transportation. To demonstrate it, the interaction of the filled nanovector with a membrane cell will be depicted in details. In particular, we will show that the CPT release is only effective when the BNNT penetrates the cell (Figure 1).

Keywords: boron nitride nanotube, therapeutic agents, cell membrane, all-atom molecular dynamic.

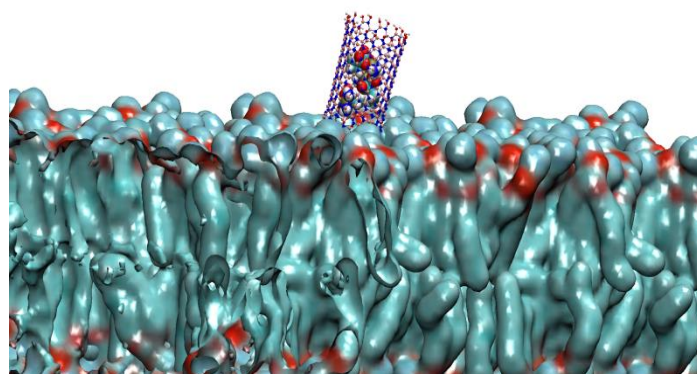


Figure 1: Completion of BNNT nanotube with CPTs drug.

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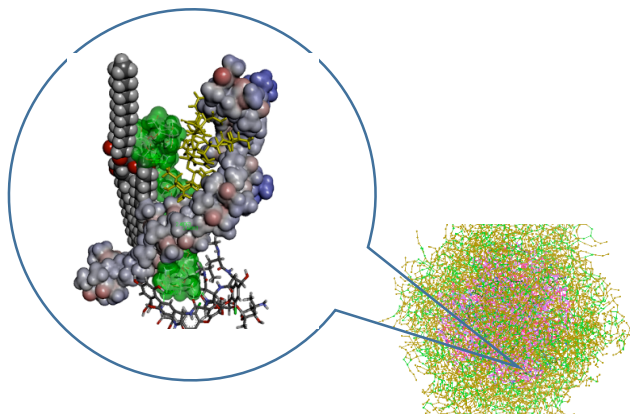
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Atomistic binding energy and Coarse grained simulation studies to understand the structure and drug release activity of Vancomycin loaded Lipid Polymer Nanoparticles (LPNs)

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Abstract: Drug delivery is the most desired application of nanotechnology in medical and health sciences because of their advantages in target delivery, enhancement of bioavailability and potency. Our laboratory has been developing various antibacterial nano systems to combat drug resistance (Kalhapure *et al* 2015). The stability of nanoparticles is strictly governed by the free energy produced during nanoscale organization of molecules whilst the activity/drug release is influenced by the intermolecular forces at atomistic level. We performed a mesoscale simulation study and atomistic binding energy calculations to assess the stability and drug release pattern of the vancomycin (VAM) loaded lipid-polymer nanoparticles (LPNs) that were developed in our laboratory (Seedat *et al.* 2015) The LPNs were composed of Eudragit RS100 (EUD) as the core and tripalmitin (GTP) as encapsulated lipid. Chitosan (CHT), Alginate (ALG) and Oleic acid (OA) were used as auxiliary agents to improve the encapsulation efficiency (EE). The coarse grained methodology of mesoscale simulation was successfully adopted for the above systems and the results suggested that the partially charged bead type polymeric core containing the charged and nonpolar bead type antibiotic is stabilized by the surface packing of nonpolar bead type tripalmitin. Optimized meso structures of all the LPNs were stable at their composition and the difference in free energy of nanoparticle formation among the LPNs clearly demonstrated their stability and ease of degradation. To identify the intermolecular forces governing the drug release mechanism of LPNs, binding free energy studies were performed on various complexes of the drug-polymer-lipid systems. The binding affinity data suggested that the encapsulation efficiency of EUD was increased due to supramolecular linking of the helper polymers by hydrogen bonding. In the presence of OA the drug was encapsulated preferably inside the fatty acid network. The high free binding energy for the OA system ($\Delta G_{\text{bind}} = -3.48$ Kcal/mol) compared to ALG ($\Delta G_{\text{bind}} = -3.23$ Kcal/mol) and native EUD systems ($\Delta G_{\text{bind}} = -3.09$ Kcal/mol) gave more stability for the complex and released the drug at a slower rate. Comparison of binding affinities among the helper polymer complexes revealed that CHT binding mode was relatively tighter than ALG due to higher number of electrostatic bonds in tetra and pentamo-



lecular complexes (Figure 1). Though the ALG facilitated the EE as similar to CHT, the lower free energy of binding for the ALG system at high molecular level allowed the components to dissociate faster than CHT and hence the drug was released much faster than that of CHT and OA system. No major difference in drug release rate was observed among ALG and the native EUD system as the helper polymer was not tightly entangled with the EUD. Together with atomistic and mesoscale simulations this study was successful in assessing the stability of LPNs and explaining the performance of LPNs with respect to their experimental EE and DR.

Keywords: lipid polymer nanoparticle, mesostructure, free energy of binding, intermolecular forces, encapsulation efficiency (EE), drug release (DR), stability, supramolecule.

Figure 1: Figure illustrating the stable mesostructure of LPN produced by coarse grained simulation and the atomistic level intermolecular interactions of the supramolecular complex (EUD-CHT-VAM) of the nanoparticle.

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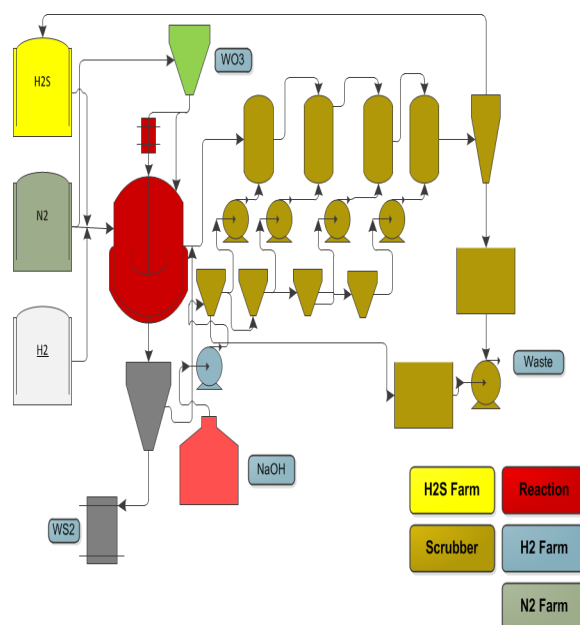
Reliability, Availability, Maintainability and Safety Analysis for the Development of a Nano-material Plant

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Abstract: Emerging technologies could potentially cause some side effects with negative impact on environment, health, safety and sustainability. These impacts can destabilize the business strategies of the companies, which adopt technological innovations. Policies such as Reliability, Availability, Maintainability and Safety Analysis (RAMS) can reduce the previously mentioned risks and improve the competitiveness of those companies, which adopt this methodology in order to create safe and socially acceptable products (A.R. Köhler, C. Som., 2014). Availability and maintainability are important concepts in the early phase of plant construction in order to ensure the achievement of strategically business plan. Therefore, a series of design and operative decisions have to be taken to optimize the plant objectives (E. Godoy et al., 2015). For instance, applying RAMS analysis to a plant producing nano-sized materials for lubricants/oil products proves to be fundamental for design and management evaluation of the system performance. One relevant important issue is to address the detailed study of the failures, which are more probable to occur inside this type of nano plant. Common type of failures, namely system faults, emergency/interruptions and unmanaged accidents which happen due to technical breakdowns. Thus, improving the system reliability is a key chain for developing a productive system in a cost-effective manner. (M.C. Eti et al., 2007). In this paper, the advantages of applying the aforementioned methodology to a nano-material plant are explained. The case study is a real plant producing WS_2 powder from WO_3 in a batch process. The production process is based on the conversion of tungsten oxide into tungsten disulfide within specially designed chemical reactor. The approach reveals the plant design deficiencies, which could compromise the system functionality leading to production loss, human safety issues and environment negative consequences. The results show the most critical subsystems: reactor, hot pre-filter, H_2S and H_2 farms. Nevertheless, optimal logistic solutions for the procurement/supply of the maintenance/spare parts are evaluated in order to improve the system reliability and integrity.



Keywords: RAMS analysis, system reliability, nano-material plant, maintenance/spare part logistics, operative strategies.

Fig 1: Figure demonstrates briefly the main sectors and connections of the used nano-material plant as a case study.

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Development of Liposomal Nanocarriers for Near-Infrared Dye: Preparation and Physico-Chemical Characterization

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Abstract

Indocyanine green (ICG) is the only near-infrared (NIR) fluorescence dye approved by FDA for clinical applications. Fluorescence emission at 820 nm pre-determines ICG to be eligible contrast agent for *in vivo* imaging with minimal interference from blood and tissue auto-fluorescence (450-600 nm). In aqueous environment, rapid degradation of ICG results in significant decrease of absorption and emission. Non-covalent association with human albumin, hydrophobic polymers or lipid vesicles (liposomes) stabilizes ICG, enhances emission intensity and shifts absorption peak to higher wavelengths. The versatility of liposomes to cargo either hydrophobic or hydrophilic entities in combination with tunable size and surface modification have been proven clinically useful. We have previously developed liposomal formulations of anticancer hydrophobic drugs and hydrophilic contrast agents for CT (Koudelka *et al.*, 2010, 2014).

Now we report the preparation and physico-chemical characterization of liposomal ICG. ICG formulated either as cationic or as long-circulating liposomes (lipid/dye molar ratio, 200/1) was prepared by freeze-thaw extrusion followed by purification. Dynamic light scattering showed liposome mean size of 90-100 nm and polydispersity about 0.08. ICG entrapment efficacy of 50 and 41% was found for cationic and long-circulating liposomes, respectively. Association of ICG within the liposome bilayers resulted in shift of absorption peak from 776 to 804 nm (Figure 1). During 3 days at 4 and 37 °C, any decrease of ICG absorption peak was not observed for cationic liposomes while low and significant decrease was found for long-circulating liposome ICG and free ICG, respectively. The decrease rates were faster at 37° C. Lyophilization (lipid/sucrose molar ratio, 1:5) was used to stabilize ICG liposomes during storage. Upon lyophilizate rehydration, neither loss of entrapped ICG nor changes in original size was found for both types of liposomes. We have prepared stable cationic and long-circulating liposome ICG formulations. These developed ICG formulations represent suitable contrast agents for use in NIR fluorescence imaging *in vivo*.

Keywords: drug delivery, indocyanine green, liposomes, nanodelivery systems, nanotechnology, near-infrared (NIR) dyes, NIR fluorescence imaging, stability.

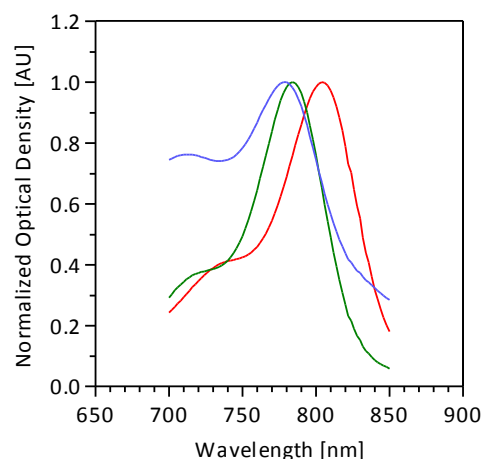


Figure 1: Normalized absorption spectra of different ICG formulations. Blue line represents ICG solubilized in 0.9% sodium chloride solution with peak at 776 nm. The shift of absorption peak to higher wavelength of 784 and 804 nm was observed for ICG solubilized in methanol (green line) and liposomal ICG (red line), respectively.

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Acknowledgement

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Improved Antimicrobial Activity of Electrospun Graphene-Chitosan/Gelatin Nanofibrous-Based Nanocomposite Scaffolds

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Abstract: Chitosan has attracted a lot of attention in the past few years particularly in biomedical and antimicrobial applications. Due to its noticeable antibacterial, biocompatibility, biodegradability, and swelling capacity, a number of wound dressings and antibacterial scaffolds have been developed by chitosan alone or in combination with other polymers, and/or nanofillers. A particular promising example for biomedical engineering and antimicrobial applications is the chitosan/gelatin-based composite. Electrostatic interaction occurring between the positively charged chitosan surface and the negatively charged surface of gelatin molecules blocks the chitosan interaction with various negative moieties on the cellular membranes, and thus, leading to enhancement of cellular migration and proliferation on the material surface. Gelatin was reported to increase the material's hydrophilicity and consequently cell adhesion and spreading (Mao et al.). Electrospinning proved to be one of the most convenient techniques for fabricating nano/microfibrous mats that are highly porous interconnected nanocomposites through manipulating electrospinning parameters (Huang et al.). In this work, the preparation, characterization and antimicrobial evaluation of chitosan/gelatin bulk film, their corresponding electrospun nanofibrous nanocomposites, and chitosan/gelatin nanofibers reinforced with different amounts of graphene nanosheets will be presented. Spectroscopic and morphological characterizations were carried out by using Fourier Transform Infrared (FT-IR) spectroscopy, Raman spectroscopy, transmission electron microscope (TEM), and Scanning electron microscopy (SEM) (the later is shown in Figure 1). Antimicrobial studies of the fabricated composites and nanocomposites against *Staphylococcus aureus* and *Escherichia coli* will also be presented. A reported drawback of gelatin is its bacterial growth enhancement effect. However, our results reveal that presence of chitosan in the the bulk film composite significantly masked the gelatin's bacterial growth enhancement effect, and the overall composites showed bacterial growth inhibition around 60 %. The antimicrobial activity of chitosan/gelatin electrospun nanofibers was enhanced by more 10% when compared to the corresponding

casted film, which was attributed to increasing the aspect ratio of the produced electrospun nanofibers exposed to the bacteria compared to the bulk film. Finally, chitosan/gelatin/graphene nanosheets nanofibers showed enhanced antimicrobial activity of the produced nanofibers that exceeded 80% with both strains. These developed antibacterial scaffolds represent promising candidates that could be tailored and used for different biomedical and environmental applications such as wound dressing, skin regeneration, antibacterial coating, antibacterial food packaging, medical textiles, etc.

Keywords: chitosan, gelatin, electrospinning, nanocomposites, nanofibers, graphene nanosheets, antimicrobial, *Staphylococcus aureus*, *Escherichia coli*, biomedical, environmental applications.

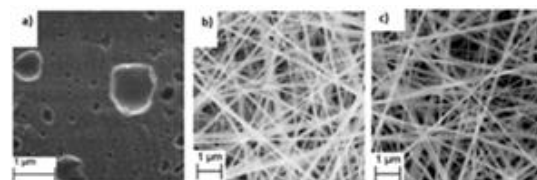


Figure 1: SEM images of : a.Chitosan/Gelatin casted film, b. Chitosan/Gelatin nanofibers, c. Chitosan/Gelatin nanofibers reinforced with graphene nanosheets.

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Honey Chitosan Nanofibers Loaded With Natural Antimicrobials for Wound Dressing Applications

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Electrospun nanofibers have emerged as a promising candidate for wound dressing applications. Electrospun nanofibers show increased and controlled porosity allowing for enhanced exudate management. Moreover, nanofibers mimic the extracellular matrix of the skin thus enhance cell proliferation and migration and show increased surface to volume ratio allowing efficient loading of different hydrophilic and hydrophobic materials (Zahedi, et al., 2010).

Recently, we have managed to electrospin polyvinyl alcohol (PVA), chitosan and high honey concentration (40%) nanofibers using biocompatible solvents (1% acetic acid) (Sarhan & Azazzy., 2015). Such concentration that was previously reported unspinnable (Maleki, et al., 2013). High honey concentration, chitosan nanofibers represent an attractive candidate for advanced wound dressing due to their wound healing and antibacterial abilities. Thus, through the present work the Honey/Chitosan/PVA nanofibers (30%: 3.5%:7%) were loaded with two plant extracts to enhance their wound healing and antibacterial properties.

The first extract was obtained from a traditional plant well known for its enhanced antibacterial property. Whereas, the second extract was obtained from a wild herb that was collected from the mountains of Sinai. The Honey/chitosan/PVA nanofibers loaded with the two extracts were characterized using Scanning electron microscope (SEM), Fourier Transform Infrared (FT-IR) spectroscopy, X-ray (XRD) diffractometer & differential scanning calorimetry (DSC). Moreover, the fibres were tested for their swelling abilities, porosity, antibacterial activity, cytotoxicity and wound healing ability. Remarkably, the inclusion of the plant extracts resulted in complete killing of *Staphylococcus aureus* and *Escherichia coli* and in increasing the swelling ability and porosity of the nanofibers to 500% & 97.7% respectively. Moreover, animal study revealed that the plant extract loaded Honey/Chitosan/PVA nanofibers showed improved wound healing of a full excisional 5mm wound in 15 days compared to 18 days for the control. No cytotoxicity was recorded for the developed

nanofibers on cultured fibroblasts. In conclusion, novel, antimicrobial, biocompatible naturally based nanofibrous wound dressing was developed with enhanced antibacterial and wound healing ability.

Key words: Honey, Chitosan, Electrospinning, Plant extracts, Antimicrobial, Wound dressing.

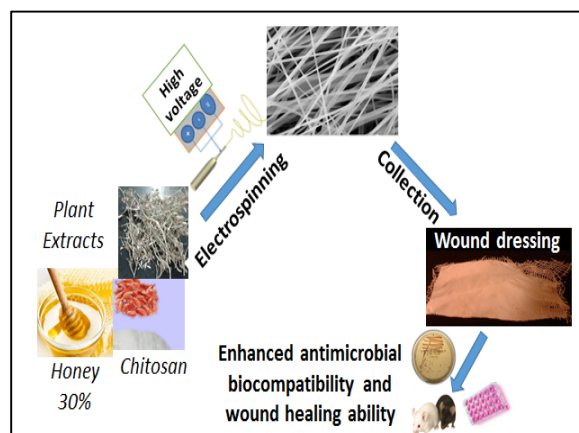


Figure 1: Illustrative diagram of the research and the main results. Honey, chitosan & plant extracts were electrospun with polyvinyl alcohol into nanofibers that were collected as prototype wound dressing. Experimental testing showed enhanced antimicrobial, biocompatibility and wound healing ability.

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Doxorubicin loaded, pH-sensitive biodegradable nanogels and their bio-evaluation

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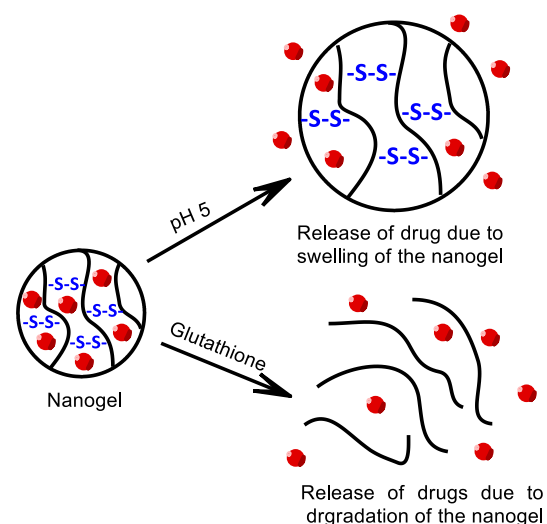
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Abstract: The pH-sensitive delivery system has been widely used in cancer therapy. The pH in tumors is lower than the normal tissues due to high rate of glycolysis in cancer cells. Therefore, pH-sensitive delivery systems can play important role for controlling delivery of drug in cancerous diseases. In biological systems, glutathione (GSH, a tripeptide) having a pendant sulfhydryl (-SH) groups as a cellular reducing agent is found at different concentrations in extracellular (2-20 μ M) and intracellular (2-10 mM) compartments in living cells. This large difference in the redox potential between extracellular and intracellular compartments as well as the further increased concentration of GSH in cancer cells promotes the disulfidethiol degradation platform in the development of glutathione responsive degradable polymeric nanocarriers for drug delivery system (Pan YJ et al., 2012). We herein present the pH and Glutathione responsive biodegradable nanogels for the delivery of doxorubicin (Figure 1). Nanogel synthesis was carried out by AGET ATRP via cyclohexane-water inverse miniemulsion which was insoluble in various solvents including THF and water (Oh et al., 2006). Morphology and Size distribution characterization of the nanogels was carried out by TEM and DLS respectively, which were found to be almost spherical and their mean diameter was in the nanometre range. Doxorubicin was loaded into the nanogels by physical encapsulation method. Loading efficiency and loading level of doxorubicin was determined by UV spectroscopy and was found to be 28.73 % and 8.52 % respectively for 10 weight percent of the drug.

Keywords: biodegradable, nanogels, biomedical application, drug delivery, doxorubicin, glutathione



Fourier Transform Infrared (FTIR) spectra of doxorubicin, blank nanogels, and dox-nanogels were recorded. The Doxorubicin release study was performed at physiological (7.4) pH and acidic (5) pH. At pH 7.4, higher doxorubicin release was achieved as compare to release at pH 5. In-vitro cytotoxicity assay was performed by MTT assay on U87 cell-lines.

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Figure 1: Figure illustrating the release of doxorubicin due to degradation and swelling of nanogels.

Increasing the efficiency of anticancer therapies using mono-dispersed chitosan nanoparticles

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Abstract: This study describes the synthesis of hydrophobically-modified chitosan nanoparticles utilized for the enhanced delivery of radiolabelled [¹⁴C]-doxorubicin. Factors influencing doxorubicin encapsulation were investigated, including drug concentration, TPP reactivity, as well as ionic interactions between various molecules during formation of CNP-[¹⁴C]-doxorubicin involved in encapsulation efficiency. Time-dependent accumulation of released doxorubicin in individual cells treated with CNP-[¹⁴C]-doxorubicin and drug release kinetics from the synthesized nanoparticles were also explored. CNP samples were synthesized through ionic gelation routes with sodium tripolyphosphate. A hydrophobic anchor was then linked to the chitosan polymer, by conjugation with palmitic acid via NHS-amine bridges. Estimation of drug loading and release were done, while time-dependent cell toxicity and accumulation studies were performed using Fluorescent-Activated Cell Sorting (FACS) analysis. Encapsulation efficiency of [¹⁴C]-dox into hydrophobically modified CNP increased by up to 2-fold, potentiated through interactions of the amphiphilic drug with the palmitic chains of hydrophobically-modified CNP. This led to a retention of [¹⁴C]-doxorubicin by a further 6 h (50% release, pH 7) compared to normal CNP, which demonstrated the potential of using hydrophobically-modified chitosan nanoparticles as a controlled release vector for doxorubicin, an advantageous characteristic for long term drug administration. Nanoparticle-mediated delivery of [¹⁴C]-doxorubicin also led to an increase in toxicity at low doses of administration. The efficacy of the drug increased a further 2.4 fold using pCNP-[¹⁴C]-doxorubicin. A hydrophobically-modified CNP system for the encapsulation of doxorubicin was developed, with the ability to prolong administration of the drug and increase its efficacy at lower doses.

Keywords: chitosan nanoparticles, doxorubicin, nanobiotechnology, nanomedicine, nanoparticles, drug delivery, biomedical applications.

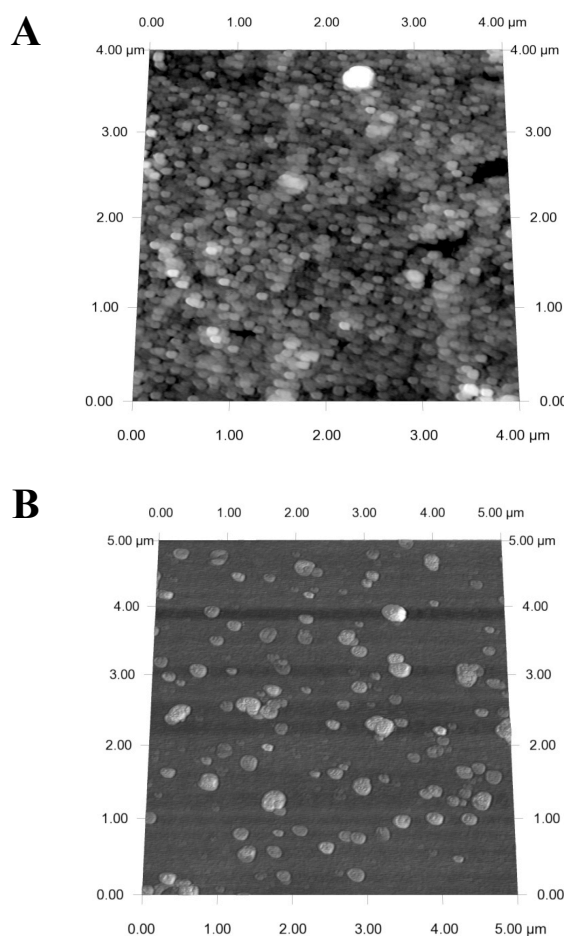


Figure 1: Figure illustrating morphological differences of (A) hydrophobically modified CNP, and (B) hydrophobically modified CNP loaded with [¹⁴C]-doxorubicin, analyzed using Atomic Force Microscopy.

siRNA and miRNA-based SNAs to target canonical and non-canonical Bcl-2 signaling in glioblastomas

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Despite decades of basic and clinical research, survival of patients diagnosed with glioblastoma multiforme (GBM), the most prevalent and aggressive form of malignant gliomas, has not changed significantly. A plethora of ‘undruggable’ genetic events that drive tumor progression together with the lack of drug delivery systems to overcome blood-brain and blood-tumor barriers conspired to make GBM an incurable disease.

We have sought to address these challenges by (a) identifying genetic mechanisms that act as roadblocks preventing therapies to induce tumor regression, and (b) by targeting these roadblocks using Spherical Nucleic Acids (SNAs). These are polyvalent gold nanoparticle loaded with small RNA interference (RNAi) molecules to silence aberrant gene expression.

We identified Bcl2L12 and Bcl-xL as oncogenes enabling tumor progression and micro-RNA-182 as a potent tumor suppressor. Bcl2L12, Bcl-xL and microRNA-182 are important cancer genes and therapeutic targets that regulate therapy susceptibility and tumor progression. Manipulating their expression made tumor cells vulnerable to anti-glioma therapies. Thus, we designed SNAs to silence Bcl2L12 and Bcl-xL expression, and to reconstitute GBM tumors with microRNA-182. We preclinically validated these SNAs *in vitro* in glioma cell lines and glioma-initiating cells (GICs) as well as in GBM mouse models *in vivo*.

RNAi has the potential to silence the expression of cancer genes, but has never entered the clinic due to the fact that conventional RNAi cannot silence gene expression persistently, has significant cytotoxic side effects, and cannot cross the blood brain barrier. On the contrary, SNAs were able to cross the blood-tumor barrier following intravenous administration, and disseminated throughout glioma tissue while reducing GBM burden without any toxicity. They silenced Bcl2L12 and Bcl-xL gene expression, and enabled expression of microRNA-182.

SNA treatment sensitized glioma cells toward several anti-glioma therapies, and when tested in GBM mouse models, SNAs slowed down tumor progression and increased survival of glioma-bearing mice.

Taken together, these results reveal that SNAs represent a promising therapeutic approach to overcome therapy resistance in GBM and, more broadly, SNA administration may represent a universal strategy to target aberrantly expressed cancer genes.

Nanosensors and nanomaterials for biomedicine

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Abstract: Nanotechnology presents a transverse and multidisciplinary approach that envelopes all scientific disciplines. This technology allows the creation of materials and devices with unique properties that find applications in various fields such as: electronics, the development of polymers, materials science, ceramics, biomedicine science, etc. Many problems of biological and medical interest arise from specimens in the range of the nanometers. In these nanoscale level problems, nanotechnology rises as the main path to achieving results. In particular project, we are carrying out multidisciplinary work in which we produce new materials and high-sensitivity sensors intended for use in biomedicine. We are considering the use of biosensors based on defects in solids and nanoporous membranes. I will show the advances obtained in nanoporous alumina films capable of detecting the peptide A β associated to Alzheimer's disease. Also the progress in the implementation of optical tweezers to know the mechanical properties of molecules related to red tide toxicity. Finally, I will show the progress that has been achieved in the creation of biocompatible materials through plasma discharge.

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Keywords: nanosensors, nanomaterials, biomedicine, alumina, peptide A β , nanodiamonds, NVC, red tide plasma discharge.

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