# BIOMEDICAL APPLICATIONS OF MICROFLUIDICS AND NANOTECHNOLOGY - REVIEW

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ABSTRACT: The current paper represents a short review of the recent research performed in the Micro- and Nano-Fluidics laboratory from National Institute for Research and Development in Microtechnologies, IMT-Bucharest. We have selected a part of our published results in the domains of activity related with biomedical applications: i) melanoma cells apoptosis obtained using magnetic hyperthermia or localized surface plasmon resonance, ii) CTCs – detection, quantification and capture, iii) graphene: synthesis, transfer, post-processing and iv) advanced nanomaterials with biomedical application.
KEYWORDS: melanoma, localized surface plasmon resonance, apoptosis, CTCs, single layer graphene, advanced nanomaterials.

## 1. INTRODUCTION

The Micro- and Nano-Fluidics laboratory is the result of the multidisciplinary project POSCCE - O.2.1.2, Microfluidic Factory for "Assisted Self-Assembly of Nanosystems" (MICRONANOFAB), which gathered experts from micro- nanotechnology and chemistry, and had the fundamental objective the realization of a prototype of an integrated microfluidic system able to dose, encapsulate and deliver different chemicals for medical diagnosis. Our mission is the research, development and education in the micro and nano-fluidics domain. The primary focus of our research is the design of microfluidic devices for applications in clinical diagnostics and regenerative medicine.

As an interdisciplinary group of researchers our domains of activity are reflecting our expertise. Therefore amongst our interests one can find: Computational Fluid Dynamics (CFD) modelling of Newtonian and non-Newtonian flow; Design of microfluidic devices for applications in clinical diagnostics and regenerative medicine; Investigation of fluid flow and rheology at the microscale; Development of micron-resolution particle image velocimetry (μ-PIV); Bioengineering: Cellular uptake of gold-coated maghemite superparamagnetic nanoparticles; studies of cells apoptosis induced by magnetic hyperthermia; tumour cells investigation using UV fluorescence, microscopy (SEM, SNOM) and spectroscopy (FTIR, Raman, Impedance); Molecular transport in microfluidic devices: Magnetophoretic system for detection of magnetic marked biomolecules; active magnetophoretic systems for cell separation through magnetic fields; filters for separation of microparticles with different morphological, electrical and magnetic properties; nanoparticles separation microfluidic devices; Circulating tumour cells (CTCs) detection, quantification and capture; Graphene: synthesis, transfer, post-processing; Advanced nanomaterials. For this paper, we have selected a few of these interests and we have presented a part of our published results.

## 2. MELANOMA CELLS APOPTOSIS

### 2.1 Magnetic hyperthermia

Apoptosis is the process of programmed cell death strictly controlled by enzymes [1]. When the critical temperature for triggering apoptosis is reached (around 42°C), the sustained thermal stress degenerates the regulation process. A multitude of heat induced deviations from the normal metabolism of a cancer cell can lead to apoptosis (programmed cell death). Although many cancer types are slightly more susceptible to hyperthermia than healthy cells, the normal cells essentially share the same fate when heated [2]. When the critical temperature for triggering apoptosis is reached (around 43°C), the sustained thermal stress degenerates the regulation process. When heated to 46°C, vital proteins of cancer cells are damaged and/or the cell membrane is partially dissolved in the surrounding aqueous medium [2;3].

Our studies examined the apoptosis of B16 mouse melanoma subjected to magnetic hyperthermia. Maghemite superparamagnetic nanoparticles were synthesized and characterized by SEM, X-ray diffraction and FTIR spectrometry. Therapeutic protocol consisted of two sessions of intravenous nanoparticle administration, followed by a local exposure to a magnetic field concentrator and to an external alternating magnetic field (120 kHz and 18

mT) for 30 minutes (Fig. 1). The apoptosis of B16 melanoma cells was studied by FTIR and Raman spectrometry. The results clearly suggest the apoptosis of B16.

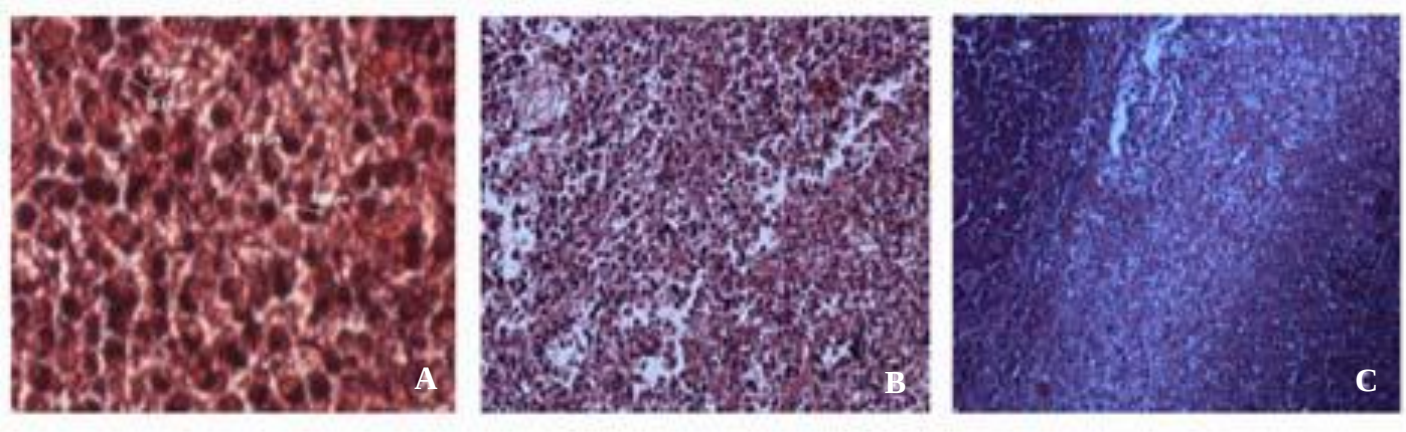


**Figure 1**. a) Untreated melanoma; b) Magnetic hyperthermia treated melanoma (30', 120kHz, 8 mT); c) Magnetic hyperthermia treated melanoma (60', 120kHz, 8 mT).

Magnetic hyperthermia can be a reliable therapeutic alternative to the actual treatment of melanoma, which in the late stages of the disease presents poor results. Due to the properties of magnetic NPs like superparamagnetic behaviour, biocompatibility and the absence of cytotoxicity as well as alternative magnetic field which isn't harmful to the body, important side effects of classical treatment (immunotherapy and chemotherapy) can be avoided. Also due to increased sensitivity of melanoma cells to hyperthermia, resistant tumours to current treatment can benefit from this method. The synthesized maghemite superparamagnetic nanoparticles were successfully tested in the treatment of B16 melanoma induced artificially to lab mice. Our investigations have shown that an aqueous suspension of polyethylene glycol coated maghemite exhibits excellent magnetic properties under the influence of AC fields. Their specific loss power may be well understood on the base of supra-position of Néel and Brown relaxation processes taking into account the actual particle size distribution. FTIR characterization after magnetic hyperthermia treatment to expose mice B16 melanoma to 43°C revealed cellular apoptosis through the occurrence of the oxidative process with fatty acids in the tumour tissues and the externalization of phosphatidylserine. [4]

## 2.2 Localized surface plasmon resonance

Because of their photo-optical distinctiveness and biocompatibility, gold nanoparticles have proven to be powerful tools in various nanomedicine applications. In our research, we revealed the advantage of gold nanoparticles in image diagnostic application of melanoma. We demonstrated the potential role of gold nanoparticles in the study of tumour tissue architecture and the utility of gold nanoparticles in the histopathological exam of B16 melanoma with the benefit of fluorescence emission of gold nanoparticles in UV spectrum. The optical properties of colloidal gold nanoparticles allow spectroscopic detection and identification of melanoma cells.

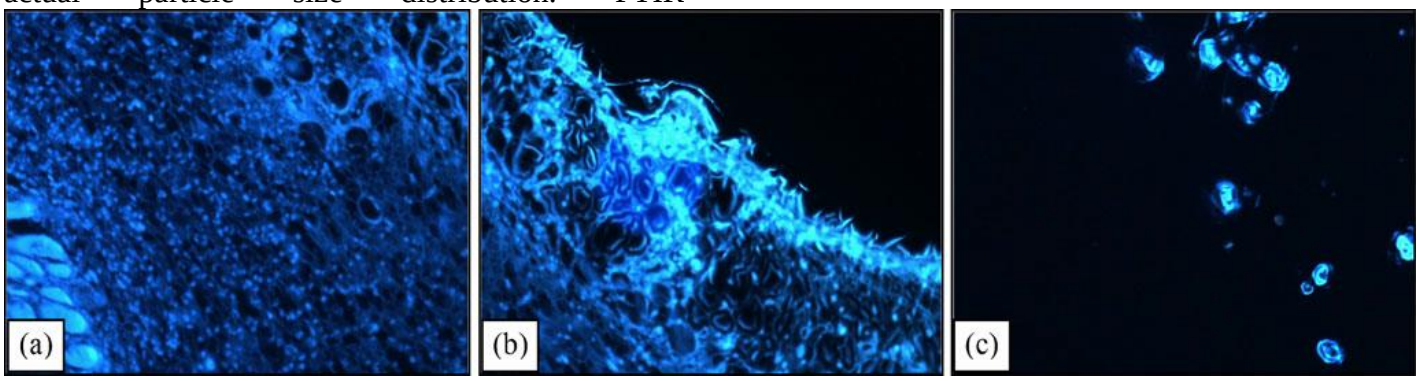


**Figure 2**. UV fluorescence microscopic image of the cryosections of B16 melanoma injected with gold nanoparticles in UV spectrum of: a) The images are shifted from green (see previous image) to blue due to the uptake of gold nanoparticles. b) It can be observed a well-defined border and the high fluorescence from epidermis and intracellular in plasma lemma, but not in cell nucleus. c) The reveal of cellular gold nanoparticles uptake (×60).

The method proposed is easy, inexpensive and reliable for histopathological analysis of melanoma. The fluorescence images of tissues cryosections depicted a strong luminescence property of gold nanoparticles uptaken in melanoma cells, results that confirm the role of the gold nanoparticles in biological labelling and imaging applications (see Fig. 2). To emphasize the AuNPs influence on biological tissues, a study of the chemical bonds configuration was performed using Raman spectrometry.

The special optical property of gold nanoparticles to be fluorescent in UV light was demonstrated in vivo by the intra-tumour injecting of AuNPs-citrate and examining the B16 melanoma cryosections. In addition, in vitro fluorescence study confirmed that the AuNPs are very useful for melanoma cells imaging using their relatively stable fluorescence emission under biological conditions. We have synthesized AuNPs and its in vitro cytotoxicity analysis and fluorescence behaviour was systematically studied in this research. From various cell viability assays and morphological studies, it has been confirmed that the AuNPs have least cytotoxicity even at higher concentrations. In addition, in vivo fluorescence study confirmed that these AuNPs are very useful for melanoma cells imaging using their relatively stable fluorescence emission under biological conditions.

This technique is simple, requiring just a mercury-vapour lamp attached to the light microscope and has a significant potential to be used as histopathological diagnosis method. We plan to improve the results to create specifically functionalized gold nanoparticles in order to detect early cell neoplasia or micro-metastases with peritumoral, subcutaneous, nodular or visceral localization, and also to detect tumour borders and depth with high accuracy. The results certify the applicability of gold nanoparticles in tissue architecture.

The Raman spectrometry investigation was used for a better understanding of the chemical bond modification induced by the presence of tumour cells and of AuNPs. It was also observed how in the case of normal tissue the vibration from acquired spectra can be assigned in general to biomolecules like proteins, lipids and nucleic acids and how in the case of the pathological tissues quantitative variations and structural changes had appeared. Raman spectrometry highlights the difference between normal and maligned tissue and showed the influence of AuNPs on melanoma tissue, leading to the idea of using this technique in cancer diagnosis [5].

## 3. CTCS – DETECTION, QUANTIFICATION AND CAPTURE

Circulating tumour cells (CTCs), are cells found in the bloodstream during the metastasis process, which originate from primary or secondary tumours in patients with cancer. The number of CTCs in the blood can be used for early diagnosis, prognosis evaluation and even treatment efficiency evaluation. CTCs have different surface markers than the normal blood cells and are defined as EpCAM+ /CK+ /DAPI+ /CD45- cells. In our research, we have developed a microfluidic device which can count, and capture CTCs present in a blood sample from cancer patients. The device has a lysis section, a lysis stopping and cell conservation region, two counting microsensors and an antibody functionalized micro-structured capture chamber. The lysis section was used to destroy erythrocytes, whose number is of millions per microliter of blood and can interfere with the count. The electrodes, situated at the entrance and exit of the capture chamber, rely on Coulter Principle to count CTCs, which are larger than other blood cells. The capture chamber was functionalized with anti-EpCAM antibodies, which are going to specifically capture CTCs in the blood sample. By using two types of CTCs detection methods, our device has increased specificity and sensibility and by changing the antibodies we can study and capture different tumour cell subpopulations.

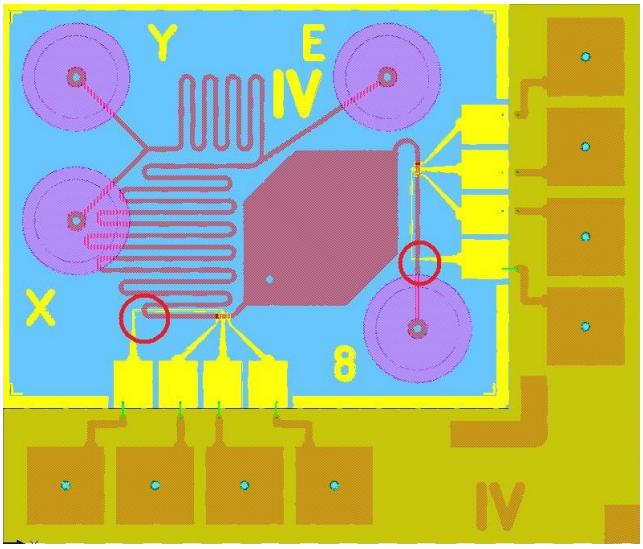


**Figure 3**. The design of the microfluidic device

We fabricated a microfluidic device (Fig. 3) that can be used for the detection, quantification and capture of circulating tumour cells. It has a CTCs enrichment section, two counting sections and a capture section. It uses two methods for CTCs detection which enhances its sensibility and specificity. It can also be used to study tumour cell heterogeneity and tumour subpopulations. The enrichment section lysis erythrocytes in order to enhance cell counting and

capture and to decrease the background noise created by erythrocytes during cell counting. For CTCs quantification we use two electrochemical sensors and the Coulter counting principle. The microstructured capture chamber contains 34446 anti-EpCAM functionalized microposts that are meant to increase the sensibility and specificity of the device by enhancing the interaction between CTCs and the antibodies [6].
Circulating tumour cells can also be captured based on specific antigens found on their surface that differ from those of normal blood cells, they can be captured using specific electrical signatures using dielectrophoresis and they can also be captured using induced magnetic properties and magnetophoresis. We developed a method for synthesizing and functionalizing superparamagnetic nanoparticles. The nanoparticles were covered with polyethylenglicol (PEG) molecules to reduce agglomeration and non-specific cell adhesion or blood proteins fouling. The PEG covered magnetic nanoparticles were functionalized with anti-EpCAM antibodies that are going to make the nanoparticles specifically bind to CTCs present in the blood sample. The samples were further processed in a microfluidic device that separated the targeted cells through magnetophoresis.

The γ-Fe2O3 superparamagnetic nanoparticles coated with PEG-diamine have been synthesized and characterized by FTIR spectrometry and magnetically characterized by using the VSM unit from 7T Mini Cryogen Free Measurement System. Phase analysis was performed by FTIR spectrometry, showing spectral bands at 584 $cm^{-1}$ and 444 $cm^{-1}$ which can be attributed to the Fe-O bond. After PEG-diamine bonding, FTIR spectra demonstrated the formation of Fe-O-C bonds. Magnetic characterization showed that the diameter of the nanoparticles was $d_{magn}$=9.88 nm. The diamagnetic contribution of water and PEG molecules can lower the field produced by the functionalized MNPs when large magnetic fields are used for magnetophoresis and detection experiments. The "magnetic diameter" obtained with the Langevin function of the PEG-diamine coated superparamagnetic nanoparticles was $d_{magn}$=11.48 nm. Future work is focused on optimizing the antibody functionalization process, which will improve CTCs detection.[7]

## 4. GRAPHENE: SYNTHESIS, TRANSFER, POST-PROCESSING

Graphene is a two-dimensional wonder material, used for electronic and medical applications. To date, various synthesis method of graphene layers is proposed, such as: SiC epitaxial growth, Chemical Vapour Deposition (CVD) and mechanical exfoliation. Graphene single layer can be rapidly produced by CVD. Although the transfer process on different substrates has been researched, a post-processing step after graphene growth for understanding influence of radical of carbon unreacted in graphene properties has not been studied. Deposition of graphene on metal transition substrate involves many reactions, and is not clear what happens with the unreacted methane. Scanning electron microscopy is the best method to visualize this unreacted species as a thin film which covers the graphene layer. Graphene post-processing after CVD is a crucial step for the performance of graphene-based devices. The graphene film is characterized morphologically and structurally before and after the post-processing step, with the scope of removing the unreacted film and investigating the influence of this step on the graphene properties. To identify the specific vibration of graphene layer before and after post-processing step, Raman spectroscopy has been used. In our studies, we investigated the quality of CVD graphene before and after removing unreacted hydrocarbon, to better understand the importance of post-processing process for device applications, before the graphene transfer.
We investigated the importance of post-processing for CVD graphene layers with Raman and SEM methods. It has been found that the post-processing step adds to the graphene structure defects. The defects are very useful for chemical applications. The step of introducing defects in the graphene structure is very important when for different applications the structure is needed as such.
The post-processing step has been optimized as a result of the characterizations accomplished during the experiments of CVD graphene synthesis. Our results are beneficial for using graphene in different applications, by controlling defects. [8]

Graphene also exhibits remarkable optical and electric properties that make it versatile for different applications, with special attention in industry. Graphene single layer has been obtained with different methods and on arbitrary substrates. In device application, the essential step is the transfer of graphene on a specific substrate, without a metal catalyst. In our research, single layer graphene was grown on a copper catalyst using chemical vapour deposition (CVD) method and transferred to a dielectric substrate (quartz), by the chemical etching method. The substrate material influences graphene properties. Quartz is a dielectric material with excellent optical transmission. PMMA is used as a support film coated on graphene layer before

dissolving the metal catalyst. The transfer method proposed in this article offers little contamination, without altering the properties of graphene. The quality and structural properties of the graphene transferred on quartz substrate was evaluated by Raman spectroscopy and for morphological evaluation we used scanning electron microscopy. We used UV-VIS spectroscopy for optical transmittance characterization of graphene layer on quartz substrate.

Single layer graphene on copper foil was successfully transferred without polymer residue, and with control over the number of layers, on quartz substrate. SEM micrographs evidence graphene deposition on copper catalyst, and transfer on quartz substrate. Raman spectroscopy was used to investigate the quality of single layer graphene before and after transfer. For application based on graphene understanding its properties and influence of support layer is very important aspect.

Graphene on quartz substrate is an ideal approach for optical and electronical device. Graphene single layer on dielectric substrate demonstrated more adaptable function with remarkable optical properties. The results evidence the capacity of number layer control both in the process of synthesis and in the transfer process. This experimental paper shows the optical properties of graphene single layer on quartz substrate, which can be used in different applications: sensors, optical device, transparent electrodes, medical imaging. [9]

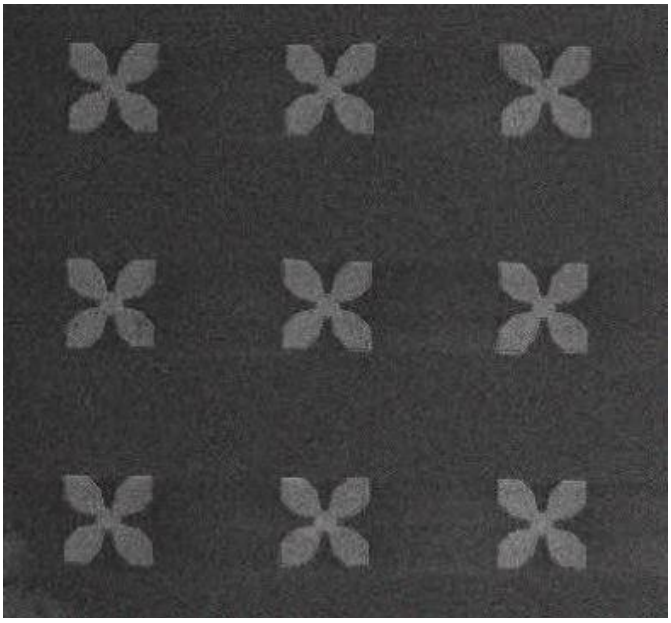

**Figure 4**. SEM micrograph of transferred CVD graphene on gold electrodes

Single layer graphene was synthesized by chemical vapour deposition method (CVD), on copper foil, and then transferred on gold structured electrodes (Fig. 4). The transferred single layer graphene has high electrical conductivity and high specific surface area, these properties are very applicable for enhanced sensor performance. A detailed analysis of the transferred graphene on the gold structured electrodes was performed by Raman spectroscopy, scanning electron microscopy (SEM), cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Raman spectroscopy indicates that the single layer graphene film was transferred successfully on the gold/glass substrate.

The gold structure coverage and the transferred layer uniformity was performed by SEM, in order to determine the graphene morphology after transfer. The influence of single layer graphene on electrochemical performance for working electrodes was studied by CV and EIS. The results established performance through: 1) the capacitance of double layer for gold electrodes reached up to 0.5 mF; 2) the graphene on gold electrodes exhibited significant better capacitance of double layer: 0.13 mF and the superior electrochemical properties. [10]

## 5. ADVANCED NANOMATERIALS

ZnO is a versatile material due to its unique and fascinating properties having numerous applications such as gas sensors, solar cells, light emitting devices, photocatalyst, antibacterial activity, transparent conductive films, transparent UV protection and cancer treatment and so on.[11-12]

In our work, we obtained Cu-doped ZnO particles (Fig. 5) by a wet chemical method in aqueous medium using $Zn(NO_3)_2$, $Cu(CH_3COO)_2$ as basic precursors, NaOH as a precipitator material, in the absence and presence of two polymer surfactants (CTAB, PEO).

This synthesis technique was selected as a potential method for obtaining Cu-doped ZnO nanoparticles with various stoichiometric ratios.

The synthesized nanoparticles were investigated using Fourier Transform Infrared Spectroscopy (FT-IR), X-ray diffraction (XRD), Field Emission Scanning Electron Microscopy (FESEM), UV–Vis absorption and Photoluminescence spectroscopy (PL).

The results suggest that process variables together with the type of surfactant are crucial parameters for the physical, chemical and optical properties of Cu-doped ZnO nanoparticles. Moreover, the use of surfactants significantly improves the quality of synthesized particles by stabilizing particle growth, reducing the surface energy, preventing the

interactions among the particles and decreasing the tendency of agglomeration.

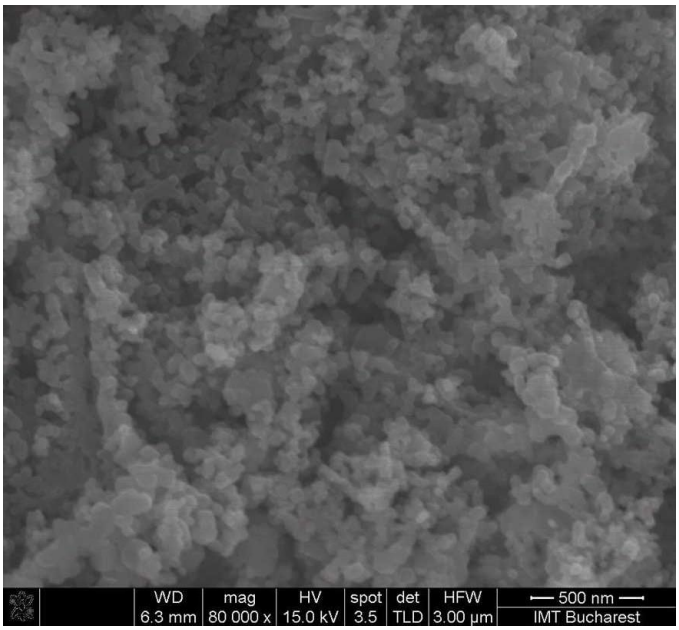


**Figure 5**. SEM micrograph of ZnO:1 % Cu

The results demonstrate that the surfactants depending upon their molecular structure (i.e. nature of head group, length of hydrophobic/hydrophilic tail) exert a strong influence on controlling the ZnO size and morphology.

Moreover, we proposed the co-precipitation method to obtain undoped and doped ZnO, because it is a relatively simple and easily accessible experimental one, which makes it very suitable for obtaining different types of powder oxides having a limited distribution of nanoparticle size [13].

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