

Novel contrast agents and increasing tools for radicals generation in low intensity ultrasound based on porous oxides

Original

Novel contrast agents and increasing tools for radicals generation in low intensity ultrasound based on porous oxides / Troia, Adriano; Cauda, Valentina; Canavese, Giancarlo; Ancona, Andrea; Zagallo, Federico; Leonetti, Giuseppe; Galati, Simone. - In: PROCEEDINGS OF MEETINGS ON ACOUSTICS. - ISSN 1939-800X. - 39:(2019), p. 020015. (Intervento presentato al convegno 2019 International Congress of Ultrasonic) [10.1121/2.0001172].

Availability:

This version is available at: 11583/2837703 since: 2020-06-30T15:52:32Z

Publisher:

Acoustical Society of America

Published

DOI:10.1121/2.0001172

Terms of use:

This article is made available under terms and conditions as specified in the corresponding bibliographic description in the repository

Publisher copyright

(Article begins on next page)

**2019 International Congress on Ultrasonics**

Bruges, Belgium

3-6 September 2019

Biomedical Acoustics: MDD (2/2) Presentation 4**Novel contrast agents and increasing tools for radicals generation in low intensity ultrasound based on porous oxides****Adriano Troia***Department of Metrology of Innovative Materials and Life Science, Istituto Nazionale di Ricerca Metrologica, Turin, 10135, ITALY; a.troia@inrim.it***Valentina Cauda, Giancarlo Canavese, Andrea Ancona, Federico Zagallo and Giuseppe Leonetti***Department of Applied Science and Technology, Politecnico di Torino, Corso duca degli Abruzzi 10129 Torino, Turin, ITALY; v.cauda@polito.it, g.canavese@polito.it, a.ancona@polito.it, federico.zagallo@studenti.polito.it, s233125@studenti.polito.it***Simone Galati***Istituto Nazionale di Ricerca Metrologica, Turin, 10135, ITALY; simonegalati94@gmail.com*

The use of porous oxide nanoparticles for diagnostic and therapeutic applications of ultrasound is growing interest owing to their unique surface properties, which can be exploited to get more sensitive and long life stable contrast agents as well as to increase radical generation through their exposure to low-intensity ultrasound, pointing out their possible applications in the oncological field, for both imaging applications and therapeutic functions. It has been largely demonstrated that the presence of porous nanoparticles (NPs) in aqueous solutions decreases the cavitation threshold. NPs are indeed able to stabilize nanobubbles on their surface and inside well-defined cavities, acting as cavitation nuclei. Here we report result the enhancement of radicals generation and lowering effect of cavitation threshold, with respect to pure water, using functionalized mesoporous silica NPs and functionalized ZnO NPs. Cavitation activity was quantified by analyzing the cavitation noise detected by a needle hydrophone and correlated with the analysis of images acquired with an ultrasound scanner probe to monitor the bubbles dynamics. These investigations were finally correlated with electron paramagnetic resonance (EPR) measurements in order to measure the enhancement of radical generation given the presence porous nanoparticles respect to water at same ultrasound intensity

I. Introduction

Porous oxide nanoparticles as a tool for promoting inertial cavitation when exposed to ultrasound have recently attracted much attention since they could be exploited both as ultrasonic triggered drug-delivery system, as contrast agents by means of the oscillating air bubbles which nucleate on their surfaces, revealing their possible use for theranostic applications. Different works conducted on mesoporous silica nanoparticles established the importance of hydrophobicity and surface roughness or porosities, to trap and stabilize the bubbles against dissolution in the fluid, evidencing, for example, how the cavitation threshold depends on the surface functionalization and the presence of impurities [1, 2]. However, most of these nano-contrast agents show different drawbacks, such as the high acoustic pressures [3], or the low biocompatibility, thus limiting their potential employment for in-vivo applications. Despite these limitations, recent studies have evidenced the possibility investigate different porous nanoparticles as ultrasonic responsive contrast agents which are also able to increase OH^\bullet radical formation [4], probably as a consequence of their lowering effect on cavitation threshold [5]. In this paper we report the experimental investigations on the increase of radical formation by using silica mesoporous nanoparticles and more biocompatible porous ZnO nanoparticles. The results were correlated with different techniques (Electron paramagnetic resonance (EPR), diagnostic ultrasound imaging and cavitation noise signal) and analyzed respect of the use of simple pure water..

II. Methodology

II. 1 Synthesis and functionalization of porous oxides nanostructures

Zinc oxide nanoparticles were synthesized through a microwave-assisted synthesis, as previously reported [6] and functionalized with NH_2 group through heating treatment of ZnO in ethanol in presence of aminopropyltrimethoxysilane (APTMS), (1% conc.). Mesoporous Silica Nanoparticles (MSNs) were synthesised through a well-know hydrolysis and condensation reaction of TetraEthylOrthoSilicate (TEOS) catalysed by TriEthanolAmine in presence of liquid-crystal templates, CetylTrimethylAmmonium Chloride (CTAC) in water [7]. MSN sample were also functionalised by using TriEthoxyVinylSilane (TVES) and 3-AminoPropyl-TriEthoxySilane (APTES) as reported in [8]. Functionalization with vinyl group was performed in order to cover or encapsulate MSN particles with hydrophobic species in order to prevent the release of air trapped into the pores; amino group have been chosen to have a better dispersion and increase chemical stability of the nanoparticles. All the samples have been stored as stable colloidal suspensions in ethanol. Characterization of both nanostructures was performed by using Transmission Electron Microscopy (TEM, FEI Tecnai operating at 200 kV) investigations (see Figs. 1A and 1B). From these images, ZnO nanoparticles can be ascribed to single nanocrystalline structures, with an average diameter of $20^{+/-}5$ nm, whereas the MSNs show a diameter of 50-60 nm and a very high porous texture. The particles size was analyzed also by the Dynamic Light Scattering (DLS) technique (Zetasizer Nano ZS90, Malvern), see Fig. 2.

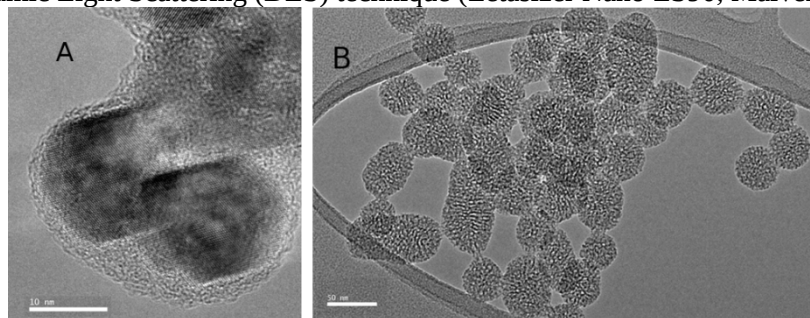


Figure 1: (A) TEM images of ZnO nanocrystals, scale bar 10 nm and (B), TEM image of MSN nanostructure, scale bar is 50 nm.

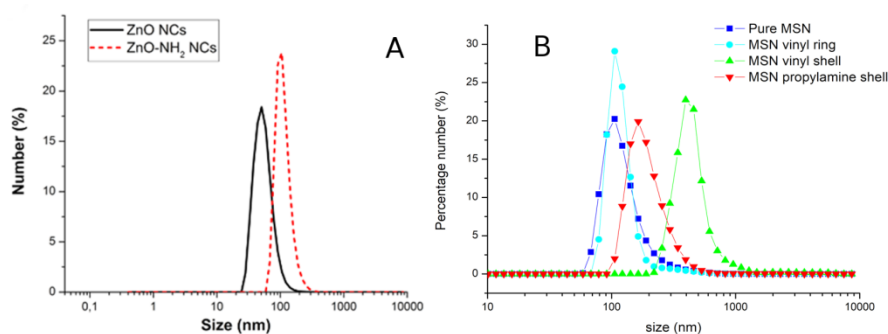


Figure 2: (A) DLS of ZnO and ZnO-NH₂ nanoparticles; (B) DLS of MSN nanostructures with different functionalization by using TEVS and APTES .

II.2 Detection of cavitation activity

Ultrasound excitation was provided by a single-element homemade focused ultrasound transducer placed in a water bath orthogonal to the sample and driven at the fundamental harmonic ($f = 985$ kHz). All reported acoustic pressures are Peak Rarefactional Pressures (PRPs) measured using a needle hydrophone (SN2195, sensor diameter 1.0mm) from Precision Acoustic, coupled to an oscilloscope (Agilent DSO-X2022A) and stored on a PC using Labview software. A scheme of the whole experimental apparatus is reported in Fig. 4. The 1 mL solution composed by the ZnONCs or MSNs in concentration range between 50-200 μ M, was placed inside a single plastic 24-well used for cell cultures. The acoustic cavitation activity in presence of nanoparticles, respect to pure water, was monitored by recording the broad band acoustic emissions generated by collapsing bubbles by using a needle hydrophone Dapco NP 10-3 coupled to a spectrum analyzer (Agilent N9320B). In order to assess quantitatively the cavitation noise, integration of the FFT spectra in a frequency range of 0.8-5.0 MHz performed on three or more spectra for each experiment. On Figure 3 the acoustic spectra and correlation of cavitation noise with acoustic pressure of focused transducer are shown.

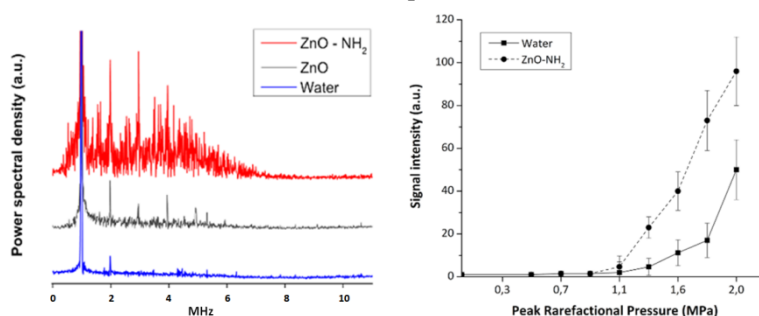


Figure 3: On the left, an example of the FFT spectra obtained with DAPCO hydrophone, using ZnO and functionalized ZnO nanocrystals. Spectrum refer to signal detected in pure water at same acoustic pressure; on the right, cavitation noise measurement as a function of different acoustic pressure for ZnO-NH₂ nanoparticles (dot line) respect to pure water

II.3 Ultrasound imaging

Imaging was performed with a research ultrasonic scanner (Ultrasonix Sonic Touch) equipped with linear probe (L14-5/38) operating at 10MHz in high resolution mode immersed in water bath and positioned along the axis of a single plastic well filled with 1 ml of solution (see Fig 5). The imaging transducer was aligned to the excitation transducer focus. Real-time videos of the system response to ultrasound irradiation were recorded and videos were analyzed using MATLAB which calculated the relative average intensity of the bright spots in the region of interest (ROI) of each frame of the videos. Three videos were recorded for each sample.

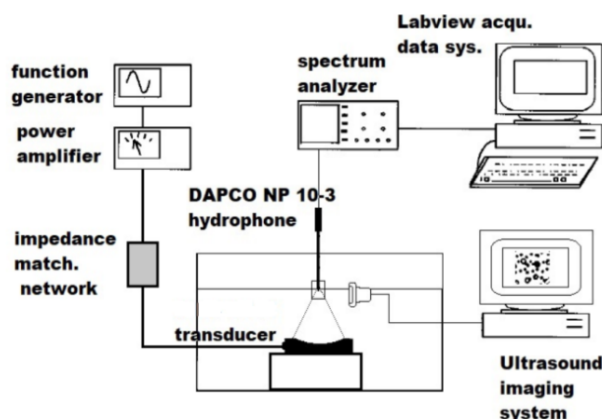


Figure 4: Scheme of the experimental apparatus.

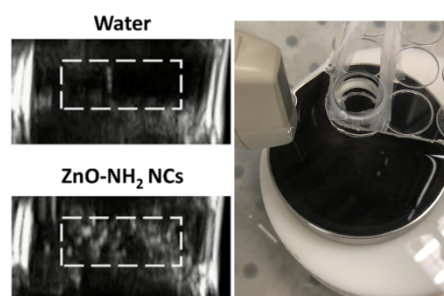


Figure 5: B-mode images taken from movies acquired during ultrasound irradiation of water and a ZnO suspension. The rectangles indicate the region where acoustic scattering was analyzed. On the right a close picture of the experimental set-up

II.4 EPR measurements

Detection of Reactive Oxygen Species (ROS), in particular OH^\cdot , was performed by Electron Paramagnetic Resonance (EPR) Spectrometry (EMXNano from Bruker) using the spin trapping technique with 5,5-DiMethyl-1-Pyrroline-N-Oxide (DMPO), a trap molecule which forms the more stable and detectable DMPO- OH^\cdot spin adducts. The spin adducts were quantified by fitting the EPR spectra using the SpinFit software (Bruker). Fig. 6 shows on the left panel the hydroxyl radicals generation during the insonation of water in the presence of functionalized MSN respect to the water. Unfortunately a meaningful increment radicals production in the presence of MSN with respect to pure water was not observed. The highest intensity is shown by the MSN with the amino-propyl shell. This result can be explained by their higher Z-potential value (around +35 mV in pure water) which allows to remain in suspension for prolonged time. In contrast the ROS generation in the presence of ZnO nanocrystals was clearly different as can be noted in the right panel of Fig. 6. These results confirm that the addition of ZnO particles in the water suspension provides nucleation sites for cavitation of the gas bubbles, lowering the cavitation threshold, which in turns enhances the generation of ROS, in particular of OH^\cdot radicals.

III. Results and discussion

Our experiments with the use of different metal oxides nanostructures, either crystalline (i.e. ZnO NCs) or porous amorphous ones (i.e. MSNs) have demonstrated the possibility of using these nanomaterials as "lowering tools" for the cavitation threshold. Furthermore these nanomaterials are traceable owing to their detection ultrasound imaging. Data relative to ROS production have evidenced some factors which may affect radical production with respect to the case of pure water. Unclear results obtained with MSN samples could be ascribed to low sensitivity of measurement technique or as a consequence of a degassing effect of the air trapped onto the porous structure. Since it has been reported that the acoustic cavitation originates from the nanobubbles

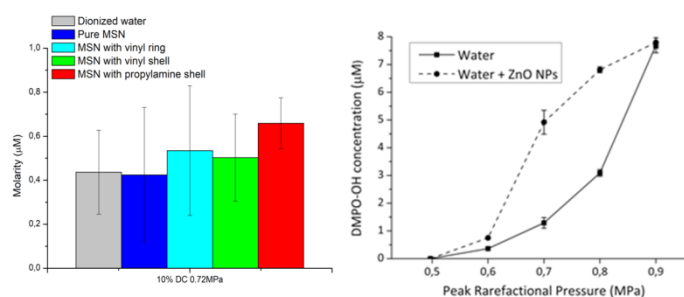


Figure 6: On the left, the radical production of MSN nanostructures exposed to fixed ultrasound pressure (0–72 MPa) as function of different functionalization. On the right, the EPR measurements of ZnONCs water suspensions exposed to different level of ultrasonic pressures. A clear differential from the pure water in the amount of DMPO-OH \cdot spin adducts is observed.

stabilized at the nanoparticles surface rather than into the mesopores [2], it is to suppose that in the case of MSN samples the air trapped into the mesopores do not take a role on increasing the cavitation as it remains sequestered by the structure. Porous ZnO nanocrystals, instead, have revealed as further studies on these oxides could open a new path for develop of ultrasound triggered and monitored nanoparticles. They can last for longer time within the body respect to typical contrast agents and their dimensions could allow to reach tissue regions that are usually precluded.

IV. Conclusion

We have reported the use of nanocrystalline ZnO functionalized nanoparticles and mesoporous functionalized silica nanostructures as novel echographic contrast agents. A correlation between the increase of cavitation activity, the contrast imaging and ROS generation by EPR measurements been found. Further experiments will be carried with ZnO nanocrystals since they appear to be the most promising candidate for these kind of applications, possibly with future tests in vitro.

References

- [1] X. H. Zhang, N. Maeda, V. S. J. Craig, Physical properties of nanobubbles on hydrophobic surfaces in water and aqueous solutions, *Langmuir* **22**, **2006**, 5025–35.
- [2] A. Yildirim, R. Chattaraj, N. T. Blum, A. P. Goodwin, Understanding Acoustic Cavitation Initiation by Porous Nanoparticles: Toward Nanoscale Agents for Ultrasound Imaging and Therapy, *Chem. Mater.* **28**, **2016**, 5962–5972.
- [3] A. Yildirim, D. Shi, S. Roy, N. T. Blum, R. Chattaraj, J. N. Cha, A. P. Goodwin, Nanoparticle-Mediated Acoustic Cavitation Enables High Intensity Focused Ultrasound Ablation Without Tissue Heating, *ACS Appl. Mater. Interf.*, **10**, **2018**, 36786–36795
- [4] G. Canavese, A. Ancona, L. Racca, M. Canta, B. Dumontel, F. Barbaresco, T. Limongi, V. Cauda, “Nanoparticle-assisted ultrasound: A special focus on sonodynamic therapy against cancer”, *Chemical Engineering Journal*, **340**, **2018**, 155–172.
- [5] A. Yildirim, R. Chattaraj, N. T. Blum, G. M. Goldscheitter, A. P. Goodwin, Stable Encapsulation of Air in Mesoporous Silica Nanoparticles: Fluorocarbon-Free Nanoscale Ultrasound Contrast Agents., *Adv. Healthcare Mater.* **5** **2016**, 1290–1298.
- [6] N. Garino, T. Limongi, B. Dumontel, M. Canta, L. Racca, M. Laurenti, M. Castellino, A. Casu, A. Falqui, V. Cauda, A Microwave-Assisted Synthesis of Zinc Oxide Nanocrystals Finely Tuned for Biological Applications, *Nanomaterials*, **9**, **2019** 212–229.
- [7] Beck, J. S.; Vartuli, J. C.; Roth, W. J.; Leonowicz, M. E.; Kresge, C. T.; Schmitt, K. D.; Chu, C. T. W.; Olson, D. H.; Sheppard, E. W. (1992). "A New Family of Mesoporous Molecular Sieves Prepared with Liquid Crystal Templates". *Journal of the American Chemical Society*. **114** (27):
- [8] V. Cauda, A. Schlossbauer, J. Kecht, A. Zürner, T. Bein Multiple core-shell functionalized mesoporous silica nanoparticles, *J. Amer. Chem. Soc.*, **131**, **2009**, 11361–11370.