Abstract

In this doctoral dissertation ZnO particles of different sizes and shapes were prepared by means of a single- and two-step synthesis by precipitation from zinc nitrate solution after addition of base. Cytotoxity of the prepared samples was compared to commercial ZnO granulate which is classified as safe material (Food and Drug Administration) and with potentially toxic nanoparticles (10 nm). Antibacterial activity was determined for all ZnO particles on the basis of bacteria *(Escherichia coli)* growth suppression. The toxic effects of ZnO particles were evaluated on the culture of mammal epithelial lung cells (Calu-3) and intestine tissue (Caco-2). Approximately 4-times higher level of bacterial activity of ZnO nanoparticles from that of commercial ZnO granulate was observed. However, distinctive toxicity was detected on mammal cell culture (Calu-3 and Caco-2) with nanoparticles in comparison with larger particles where no significant effects were noticed. On the other hand, larger particles (submicron sizes) had only slightly lower antibacterial activity, the effects of which can be additionally increased by increasing the particle concentration. The results obtained suggest that additional *in vivo* tests are needed to assess the toxicity of ZnO nanoparticles prior to their further use.

The synthesised ZnO nanoparticles were additionally functionalised by means of *ex situ* and *in situ* methods. Different amines were used for this purpose (primary, secondary with a short and long chain, amine with dual silane group) and ionic liquid with trimethoxysilane group. Amines are known for their antibacterial activity, in particular quaternary amines, and are very efficient against antibacterial colonisation. Due to the resemblance of ionic liquid structure to quaternary amines, it was presumed that these too can be antibacterially active. We are presenting for the first time covalently bonded ionic liquid to ZnO nanoparticle surfaces with their synergetic antibacterial activity. The successful functionalisation was confirmed with IR spectroscopy, zeta potential measurements and elemental analysis. After testing with bacterial culture, the bacteria growth suppression, ability of colony formation and bacterial cell viability of functionalised ZnO nanoparticles were assessed. Thus their MIC value was obtained. In addition, the multiple sequential antibacterial activity and potential particle ion release into growth media were also checked. By applying ex situ surface modification to the prepared particles, their antibacterial activity on the *Escherichia coli* and *Staphylococcus aureus* bacteria culture was significantly improved.

A short and simplified functionalisation was achieved with an *in situ* preparation of ZnO nanoparticles in the presence of ionic liquid with silane functional group. The

functionalisation level and particle size were verified by ionic liquid concentration; the particle size was up to 4 times smaller in comparison with particles prepared without the presence of the ionic liquid. The size and crystallinity of thus prepared particles were monitored by means of SEM, DLS and XRD methods. The successfully modified surface was verified by IR and NMR spectroscopy, elemental analysis and zeta potential measurements. It has been demonstrated how *in situ* functionalisation can result in an even improved antibacterial activity of ZnO nanoparticles comparable to ZnO particles prepared by *ex situ* method.

Key words: *Zinc oxide, antibacterial activity, functionalisation, ionic* liquid.