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# Development and characterization of LigA antigen-functionalized silver nanoparticles for the detection of anti-leptospiral IgG antibodies in human sera

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Silver nanoparticles have become a good application in plasmonic technologies owing to its unique properties. Surface plasmon resonance of silver nanoparticles in the Ultraviolet-Visible (UV-Vis) region can be applied to identify nanoparticle-protein interactions. This property is used to detect antibodies against leptospirosis, which is an emerging, infectious, zoonotic disease in many developing countries, including in Sri Lanka. The objective of the study was to design a rapid and efficient identification system for the detection of anti-leptospiral antibodies in human blood using LigA (leptospiral immunoglobulin-like protein A) antigen functionalized silver nanoparticles. Silver nanoparticles, with a size of 80 nm, were synthesized according to the Leopold and Lendl method by reduction of silver nitrate using hydroxylamine hydrochloride at pH levels of 7.0, 8.0, 9.0 and 10.0. The nanoparticles were incubated separately at 25°C and 37°C with LigA recombinant antigen (rLigA) for 1hr for the functionalization. rLigA-functionalized nanoparticles were characterized by UV-Vis spectroscopy, Fourier Transform Infra-red (FT-IR) spectroscopy and Scanning Electron Microscopy (SEM). After that, three samples of human sera containing IgG (Immunoglobulin-G) anti-leptospiral antibodies (groupA) and another three samples without IgG anti-leptospiral antibodies (groupB) were tested separately with functionalized nanoparticles. Aggregation of antigen-coated nanoparticles in the presence of anti-leptospiral antibodies was verified by UV-Vis spectroscopy and SEM. The optimum concentration of 1.5 µg/ml of the antigen was selected for effective functionalization of monodispersed, orange-grey colour spherical silver nanoparticles at 25°C and pH of 7.0. FT-IR spectra verified that nanoparticle-LigA interaction had taken place through carboxylate groups of the protein, as the corresponding peaks showed enhanced intensities. SEM confirmed the aggregation of nanoparticles in groupA upon the addition of antibodies, compared with groupB, which showed no aggregation. No significant difference in UV-Vis absorption was observed between groupB and LigA functionalized nanoparticles, whereas groupA showed a significant reduction in absorption compared with groupB. According to the experimental results, rLigA-functionalized silver nanoparticles can be used to detect anti-leptospiral IgG antibodies in human sera using UV-Vis spectroscopy.

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