# Preface

The incredibly serious problem of Hepatitis B is virus-related chronic liver disease. Chronic Hepatitis B Virus (HBV) infections are severe with weak antiviral immune responses. It slowly damages the liver. Virus infection causes acute as well as chronic inflammatory liver disease and, in some cases, develops serious problems such as hepatocellular carcinoma or liver cirrhosis or chronic liver disorders.

The conventional preventive treatment for Hepatitis B requires booster dose of vaccine, which need repeated administration of the vaccine to the subject and also require the appropriate small model for the study of immunotolerance and immunopathogenesis. Thus there is a need to develop formulation which can eliminate the need of booster dose, to enhance patient's compliance and safety.

With this view, a single dose sustained-release poly(d,l-lactide-co-glycolide) (PLGA) nanovaccine/nanoparticles of Hepatitis B virus for Hepatitis B were prepared by double emulsion solvent evaporation technique using central composite design model by optimizing formulation and process parameters. The effect of independent variables such as amount of polymer, stabilizer concentration, aqueous/organic phase ratio and homogenizer speed on two responses like particle size and entrapment efficiency were studied.

Nanoparticles were in vitro characterized for their size, zeta potential, entrapment efficiency, morphology, integrity, haemocompatibility, *in-vitro* release and uptake internalization studies. *In-vivo* immunological evaluation was performed in normal immunogenic BALB/c mice. Nanoparticles at single and multiple doses were compared with booster dose of alum adsorbed HBsAg vaccine and measure the immunological marker and cytokine (interleukin-2 and interferon-Y) levels by using ELISA. The effect

of nanovaccine through different route of administration was also performed to check whether any other viable route of administration could be possible. Further, for enhancing the antibody production efficiency the prepared polymeric HBsAg loaded nanovaccine were tested in immune deficit humanized xenograft mice suffering from chronic Hepatitis B virus. The humanized xenograft mice were engrafted with peripheral blood mononuclear cells (PBMCs) of human and transplanted by the bone marrow of NOD/scid mice. After administration of nanovaccine in developed humanized xenograft mice model, results in enhanced intracellular delivery of antigens to immunocompetent cells and generated long-lasting and an efficient immune response. Rationally, single dose of nanoparticles was sufficient for production of immunoglobulin plus cytokine levels and maintain immunogenicity for longer period of time. Nanoparticles eliminate the booster dose and showed adjuvant properties. Therefore, specific memory responses were elicited by vaccination with Hepatitis B virus surface antigen of humanized mice transplanted with PBMCs derived from HBV donors.

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