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Preparation and *in Vitro* Blood Cell Compatibility of Chitosan Nanoparticles Incorporated *Salmonella paratyphi* AH Antigen.

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ABSTRACT

Nanoparticle-containing vaccines have attracted tremendous interest in recent years, and a wide variety of nanoparticles have been developed and employed as delivery vehicles or immune potentiators.In the present study,chitosan nanoparticles incorporated typhoid bacilli *Salmonella paratyphi* AH antigen was prepared by modified method of ionic gelation and the prepared nano bacterial cell formulation was characterized by scanning electron microscopy (SEM),fourier transform infrared spectroscopy (FTIR) which showed 200nm nanosphere and formation of absorption bands (Figure 2) at particular wavelength corresponds to functional groups by FTIR respectively. Viability of the nano formulation determined by agglutination of nano formulated cells with specific anti sera revealed agglutination after 30th day of preparation. Hemocompatibility study was done using red blood cells under in vitro condition.Distinct effect on morphology and count was not observed at none of the concentration of tested nano formulation.

Keywords: *Salmonella paratyphi* AH, chitosan nanoparticles, hemocompatibility.

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INTRODUCTION

Vaccine development has a proud history as one of the most successful public health interventions to date. Vaccine development is historically based on Louis Pasteur's "isolate, inactivate, inject" paradigm. As vaccine development moves increasingly to draw on modern concepts of rational design, the number of candidate vaccines is increasing [1,2]. Most candidate vaccines represent "minimalist" compositions [3], which typically exhibit lower immunogenicity. Adjuvants and novel delivery systems that boost immunogenicity are increasingly needed as we move toward the era of modern vaccines. Nanotechnology offers the opportunity to design nanoparticles varying in composition, size, shape, and surface properties, for application in the field of medicine [4,5]. Nanoparticles, because of their size similarity to cellular components, can enter living cells. using the cellular endocytosis mechanism,

The use of nanotechnology in vaccinology, in particular, has been increasing exponentially in the past decade leading to the birth of "nanovaccinology" [6]. In both prophylactic and therapeutic approaches, nanoparticles are used as either a delivery system to enhance antigen processing and/or as an immune stimulant adjuvant to activate or enhance immunity. Polymeric nanoparticles, inorganic nanoparticles, liposomes, self assembled proteins and nanoemulsions have been used in nanovaccinology [7].

A great variety of synthetic polymers are used to prepare nanoparticles, such as poly(d, l-lactide-co-glycolide) (PLG) [8,9],poly(d,l-lactic-coglycolic acid)(PLGA) [10] poly(g-glutamic acid) (g-PGA) [11],poly(ethylene glycol) (PEG) [12] and polystyrene [13] . PLG and PLGA nanoparticles have been the most extensively investigated due to their excellent biocompatibility and biodegradability. These polymeric nanoparticles entrap antigen for delivery to certain cells or sustain antigen release by virtue of their slow biodegradation rate [14].

Chitosan-based nanoparticles have been widely studied due to their biocompatibility, biodegradability, nontoxic nature and their ability to be easily modified into desired shapes and sizes [15].These nanoparticles have been used in the preparation of various vaccines including HBV vaccines, Newcastle disease vaccines and DNA vaccines [16]. Inulin, a well-known activator of complement via the alternative pathway is also a potent adjuvant. Nanoparticle adjuvants derived from inulin, such as Advax TM, have shown enhancement of immune response in vaccines against various viruses including influenza and hepatitis B [17].In the present study, chitosan nanoparticles incorporated Salmonella paratyphi AH antigen was prepared and the prepared nano bacterial preparation was evaluated for hemo compatibility.

MATERIALS AND METHODS

Reagents and Chemicals

Chitosan flakes from crab shells (Practical grade >85% deacetylated; Brookfield viscosity >200 000 cps) were purchased from Sigma– Aldrich Chemical Co. EDTA ($PdCl_2$) and acetic acid were of analytical grade. *Salmonella paratyphi* AH antigen and anti sera were obtained from Tulip. Solutions were prepared with triply distilled water. All compounds were used as received. Chitosan was dissolved in 1% acetic acid solution. Due to the poor solubility of chitosan, the mixture was kept overnight until a clear solution was obtained.

Preparation of chitosan nanoparticles incorporated *Salmonella paratyphi* AH antigen

25ml of chitosan solution prepared from original stock was mixed with 50 ml 0f deionized water and 0.5 ml of *S.paratyphi* AH antigen followed by addition of 0.25ml of glutaraldehyde, kept under magnetic stirring for three hours at 30^{o}C. Slurry thus obtained was lyophilized and stored in screw capped vial. Characterization carried out with fourier transform infrared spectroscopy (FT-IR) and Scanning electron microscope. FT-IR was carried out with KBr palletized dried sample in the range of 4000–500 cm_1 using Bruker OpticGmbH Tensor 27.Particle morphology (Shape and size) and elemental composition was studied by field emission scanning electron microscopy (SUPRA 55-CARL ZEISS ,Germany.

Viability assay

Slide widal agglutination test was carried out to study the viability of nano formulation .0.1 ml of the prepared nano formulation thus obtained was mixed with 0.9 ml of S.paratyphi AH anti sera, mixed well and

observed for agglutination. Positive control, negative control were maintained in each run. Agglutination study was done for 10 days.

In vitro **hemocompatibility assay against Human RBC**

Hemocompatibility of nano formulation under laboratory condition against red blood cells was studied [18]. Using 10ml sterile syringe a peripheral blood was collected in sterile 15 ml centrifuge tube containing 0.1% EDTA and the collected blood was centrifuged at 2500 rpm for 15 min. The supernatant was discarded and the collected RBC was washed with sterile PBS. 0.1 ml of washed cell suspension was incubated with nano formulated cell suspension with different concentrations ranged from 0.25 to 1% in 0.9ml of PBS. The mixture was incubated at 370C for 12-24hrsand RBC count was made using Hemocytometer and microscopic examination with Leishmann stain was done to detect any morphological changes on RB

RESULT AND DISCUSSION

Nanotechnology is currently employed as a tool to explore the darkest avenues of medical sciences in several ways like imaging, sensing, targeted drug delivery, gene delivery systems and artificial implants. Hence, nano sized organic and inorganic particles are catching increasing attention in medical applications due to their amenability to biological functionalization [19].The use of nanoparticles in vaccine formulations allows not only improved antigen stability and immunogenicity, but also targeted delivery and slow release. A number of nanoparticles vaccines varying in composition, size, shape, and surface properties have been approved for human use and the number of candidates is increasing.

Figure 1: Scanning electron microscopic image of chitosan nanoparticles incorporated S.paratyphi AH antigen

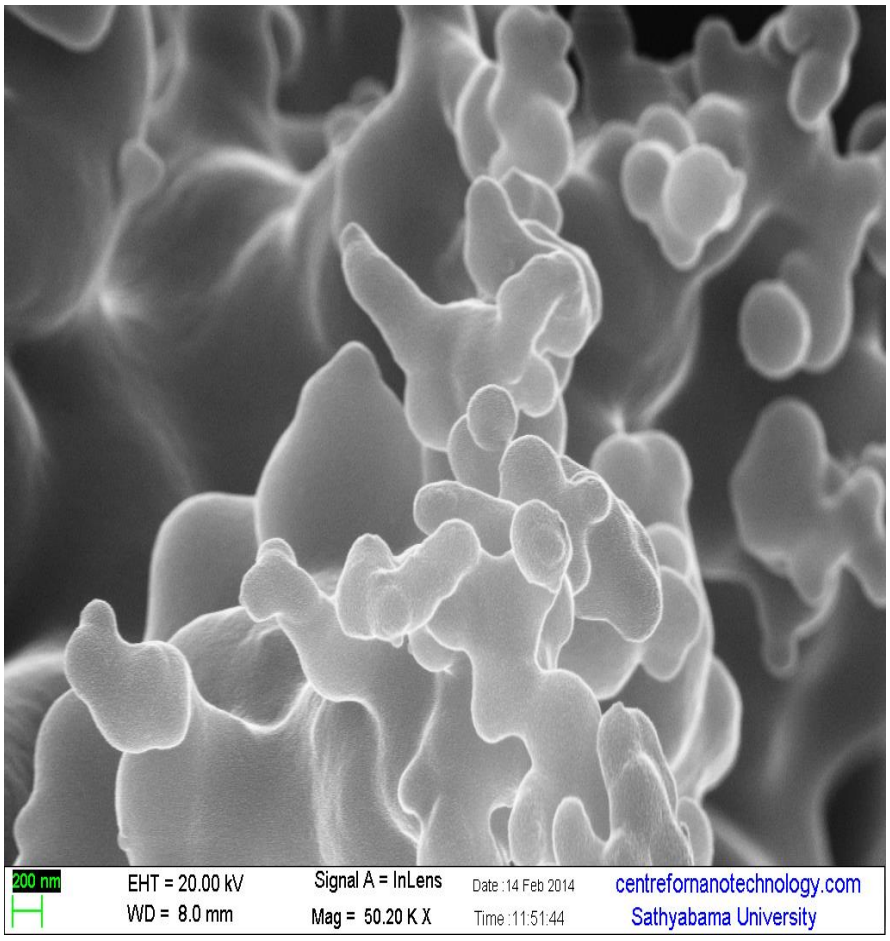

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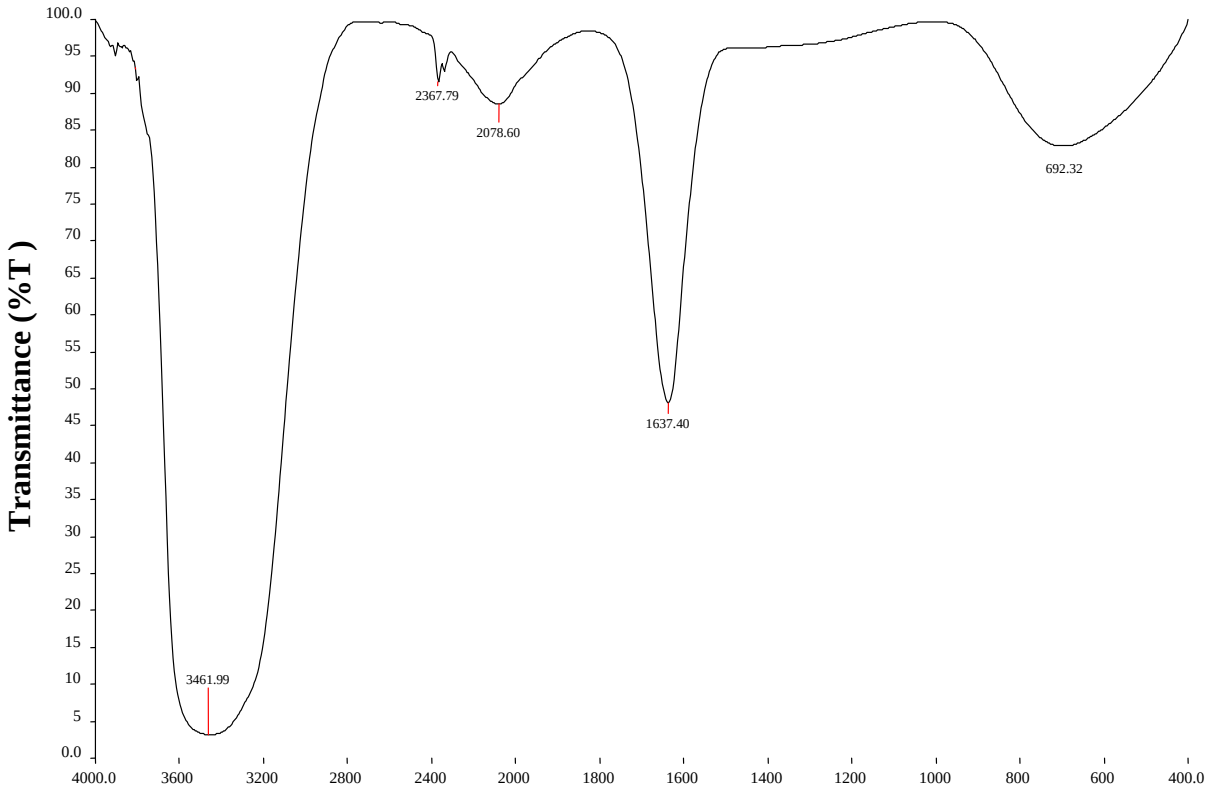
Figure 2: FTIR spectra of chitosan nanoparticles incorporated S.paratyphi AH antigen



In the present study, chitosan nanoparticles incorporated *S.paratyphi* AH antigen was prepared ny the modified method of ionic gelation. Characterization study using SEM showed spheres of 200nm and formation of absorption bands (Figure 2) at particular wavelength corresponds to functional groups of chitosan and antigen was confirmed by FTIR using Bruker Optic GmbH Tensor 27. Chitosan- PEG based formulation of diphtheria toxoid (DT) with the size ranged from 100-400 nm and evaluated mucosal antibody responses at different time post administration [8]. They observed the nano formulated DT induced improved immune response. Effect of formulation on the viability of nano formulation was tested by widal slide test using anti sera which reveals positive agglutination reaction for 30 days. Among the various nanoparticles used in vaccine development, polymeric nanoparticles play a vital role. These polymeric nanoparticles entrap antigen for delivery to certain cells or sustain antigen release by virtue of their slow i_o degradation rate [12].Among the various polymeric nanoparticles, chitosan based nanoparticles are highly used because of its biocompatibility, high rate of entrapment, high release rate [7].

Table 1: RBC count of blood sample treated with nano formulation

Concentration (ug/ml)	RBC count ($X10^6$)
25	1.98
50	1.98
75	1.98
100	1.98
Control	1.98

In vitro hemocompatibility of nano formulated bacterial cells was studied using red blood cells (RBC) reveals no distinct effect in all the tested concentrations. Moreover no lysis, structural changes and reduction was observed. RBC count was found to be stable as in control (Table 1).Chitosan nanoparticles incorporated

S.paratyphi AH cells prepared in the study revealed distinct viability after 30th day of preparation by showing agglutination with S.paratyphi AH anti sera. Further study using suitable animal model will be useful to determine the immune response and helpful to develop biocompatible formulation of typhoid vaccine.

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