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Formulation Development and Evaluation of Dextran Nanoparticles Enclosed with Recombinant Typhoid (Ty21a) Antigen

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ABSTRACT

Developing efficacious vaccines for Typhoid fever continues to be a paramount concern in global health, particularly in areas with a significant prevalence of the illness. This research focused on the synthesis, refinement, and analysis of recombinant Typhoid (Ty21a) antigen-encapsulated Eudragit-coated dextran nanoparticles. These nanoparticles function as an innovative platform for delivering the Ty21a antigen, which has the potential to enhance vaccination durability and effectiveness. The synthesis process was methodically adjusted to get optimal nanoparticle size, shape, and surface qualities by manipulating several formulation factors. The physicochemical evaluation verified the generation of precisely specified nanoparticles that effectively encapsulate the Ty21a antigen. The in vitro release assays shown that the Eudragit-coated nanoparticles can release antigens in a sustained and regulated manner, suggesting that they have the ability to provide prolonged immune activation. This study demonstrates the potential of using recombinant Typhoid antigen-encapsulated Eudragit-coated dextran nanoparticles as a promising platform for advanced vaccines. This platform offers improved ability to stimulate an immune response and controlled delivery of the antigen. These findings have significant implications for global health efforts focused on preventing Typhoid fever.

Keywords: Antigen, Controlled release, Immunogenicity, Optimization. Nanoparticles, Vaccine

INTRODUCTION

Vaccines have been crucial in the prevention of infectious illnesses, and they are classified into three primary types for human immunization: live-attenuated, inactivated, and subunit vaccines. Advancements in gene therapy technology have brought about a new phase in the field of vaccinology, shown by the recent creation of mRNA vaccines, including those designed for COVID-19. Live-attenuated vaccines consist of attenuated (weaker) versions of either viral or bacterial pathogens. These weakened pathogens are able to replicate inside a host and trigger an immune response. Some notable examples of vaccinations are the MMR (Measles, Mumps, and Rubella), chickenpox, rotavirus, seasonal influenza, and oral polio vaccines.[1] On the other hand, inactivated vaccinations, such as inactivated polio and hepatitis A vaccines, consist of viruses that have been made non-infectious in order to trigger an immune response. Although both types of vaccines have shown clinical safety and have been used for many years, they have certain limitations. These include the requirement for multiple doses, suboptimal immunity, and less-than-desirable ability to produce an immune response, potential inflammation, uncontrolled replication, and the possibility of attenuated vaccines reverting back to a disease-causing state. [2]

Subunit vaccines include inanimate antigens, such as distinct antigenic proteins or epitopes that possess the ability to identify and attach to antibodies or T-cells. These vaccines have several benefits, including being cost-effective, stable, and safe. As a consequence, they have reduced immunogenicity and fewer adverse responses compared to live-attenuated and inactivated vaccines. Subunit vaccinations, such as the hepatitis B and pertussis vaccines, serve as examples. Subunit vaccines may be classified as

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protein-based, polysaccharide-based, conjugate-based, and toxoid-based vaccines.[3]

Within the protein-based classification, a particular protein derived from the antigen is used to elicit an immunological response. Polysaccharide subunit vaccines imitate the polysaccharide capsules of disease-causing bacteria, which in turn stimulate an immune response.[4] Nevertheless, both protein-based and polysaccharide subunit vaccines are vulnerable to denaturation and breakdown caused by changes in pH or the presence of proteolytic enzymes. Conjugate subunit vaccines may elicit immune responses that are comparable to those of polysaccharide subunit vaccines. However, conjugate subunit vaccines often include a carrier protein to prolong the duration of protective immunity. Prominent instances include the diphtheria and tetanus vaccinations. Toxoid vaccinations are composed of deactivated bacterial toxins and are known to be both safe and resistant to infections, as shown by the diphtheria and tetanus vaccines.[5] Subunit vaccinations often demonstrate lower immunogenicity in comparison to live-attenuated vaccines. The current research focused on the synthesis, development, and characterisation of recombinant typhoid (Ty21a) antigen encapsulated eudragit coated dextran nanoparticles.[6]

MATERIALS AND METHODS

From SD Fine Chemicals in Mumbai, we acquired Dextran and Eudragit S100. Merck, Mumbai, supplied the typhoid (Ty21a) antigen. Qualigens Pharma Private Limited of Mumbai was chosen as the supplier of the bovine serum albumin. The membranes used in dialysis were sourced from Himedia in Mumbai, India. The remaining chemicals used were all of analytical quality. The research consistently used double distilled water (DDW) whenever it was necessary.

Identification of Antigen for Protein using Bradford reagent by UV Spectroscopic Method

The current research used Bovine Serum Albumin (BSA) as a representative antigen and Recombinant Typhoid (Ty21a) Antigen as the antigen under consideration. The BSA antigen is valuable due to its affordability, high level of purity, and easy accessibility. It is particularly helpful for studying the immunogenic characteristics of carrier-associated, covalently connected, or entrapped macromolecular antigens inside the carrier. Due to the high cost of the Typhoid (Ty21a) Ag molecule, it was deemed necessary to use it only after conducting optimization and evaluation of the formulation. The absorbance spectra of the Bradford reagent and the Bradford reagent when it is bound to 20µg of BSA standard. The reagent that is provided at no cost has a peak absorbance at a wavelength of 595nm. The unbound dye partly coincides with the bound form of the reagent, resulting in the non-linear response of the Bradford test.[7]

Formulation of Dextran nanoparticles

The dextran-based nanoparticles were synthesized using the following procedure: A solution of 5.0 mg dextran was prepared by dissolving it in 50 ml of deionized water at 25°C. The solution was stirred gently and nitrogen gas was bubbled through it. Then, a certain quantity of cerium ammonium nitrate (CAN) dissolved in 1.25 ml of 0.1 N nitric acid and a specific amount of acrylic acid (AA) were added to the solution in sequence. After twenty minutes, MBA (N, N-Methylene bisacrylamide) was introduced into the reaction, which was then maintained at a temperature of 30°C for a duration of 4 hours. Subsequently, a solution of 1 M NaOH was used to achieve neutralization of the reaction system.[8]

Ultimately, the reaction solution underwent dialysis against deionized water for a duration of 3 days, using a membrane bag with a molecular weight cut-off (MWCO) of 14,000. This process effectively eliminated any remaining unreacted monomers and ungrafted PAA. A volume of 1 milliliter (1 milligram per milliliter) of BSA Solution was added to the dextran nanoparticles for loading and swirled at a speed of 900 revolutions per minute for a duration of 4 hours. The ultimate aqueous solution was subjected to lyophilization in order to get solid nanoparticles. The same process was used to create dextran nanoparticles loaded with typhoid antigen. To examine the impact of the reagent ratio on the resulting nanoparticles, several types of

nanoparticles were synthesized using different reagent ratios. To investigate the significance of the complexation of AA and dextran during synthesis, N-isopropylacrylamide (NIPAM) was selected as a substitute for AA in the one-step synthesis of dextran-based nanoparticles. NIPAM, unlike AA, is a monomer that does not form complexes with dextran.

Eudragit coating on dextran nanoparticles

The coating dispersion included a 2% concentration of coating material (Eudragit S 100) mixed in an organic solvent mixture of acetone to ethanol in a ratio of 2:1. A polymer solution was created by dissolving 0.8 grams of Eudragit in a mixture of 100 milliliters of acetone and ethanol, with a ratio of 2 parts acetone to 1 part ethanol. The polymer solution was used to suspend 0.8 g of dextran nanoparticles. An ultrasonicator operating at a frequency of 40 KHz was used to disperse any clumps in the suspension of polymer and nanoparticles.[9]

The nanoparticles carrying BSA and Ty Ag were coated with an enteric coating utilizing the Supercritical Anti-solvent method. The enteric coating solution was produced according to the method described by The enteric coating solution was formulated by first creating a milky emulsion of Eudragit-S 100 using an organic solvent (acetone: ethanol, ratio 2:1). After a duration of 1 hour, Triethylamine citrate (60%) was introduced and the mixture was agitated for an additional 30 minutes. An antitacking ingredient, talc, was included into the milky latex. During the coating procedure, the coating dispersion was agitated using a magnetic stirrer. Subsequently, the nanoparticles were immersed in a coating solution, followed by filtration and drying of the coated formulation.[10]

Optimization of formulation

Process variable on the basis of particle size and entrapment efficiency

Nanoparticles were created utilizing various concentrations of Acrylic acid (AA), such as 0.1, 0.3, 0.5, 0.7, and 0.9. The shape and size of the formulations were assessed using transmission electron microscopy (TEM). At a concentration of 0.5% weight/volume of AA, the particles exhibited a size of about 192±34 nm. The entrapment effectiveness was determined to be 68±2.7%.[11]

Table 1: Effect of AA concentration on the particle size

Formulation Code	A1	A2	A3	A4	A5
Dextran: AA (w/v)	5:0.1	5:0.3	5:0.5	5:0.7	5:0.9
Particle Size (nm)	486 ±25	266 ±25	192 ±34	280 ±30	370 ±30
% Entrapment	54 ±2.3	61 ±1.6	68 ±2.7	71 ±3.1	62 ±2.4

Various stirring durations were used to improve the size of nanoparticles and enhance their entrapment effectiveness. Various formulations were created by agitating for 30, 60, 90, 120, and 150 minutes. These formulations were then analyzed to determine their size and entrapment efficiency.[12]

Table 2: Effect of stirring time on the particle size

Formulation Code	A3a	A3b	A3c	A3d	A3e
Stirring Time (min)	30	60	90	120	150
Particle Size (nm)	780±30	470±30	380 ±30	192±33	281±30
% Entrapment	28±1.7	48±3.2	61±3.3	68±2.2	69±3.4

Optimization of Coating Materials

The core: coat ratio was optimized in terms of particle size. The core: coat ratio was varied from 1: 5 to 1: 25. The results are given in table 3.

Table 3: Effect of core: coat ratio on particle size and shape on Eudragit coated dextran nanoparticles

S.No.	Formulation Code	Core:coat ratio	Particle size (nm)	% Entrapment
1	A3d41	(1: 5)	284±40	65±2.3
2	A3d42	(1:10)	304±46	67±1.9
3	A3d43	(1:15)	324±52	69±1.5
4	A3d44	(1:20)	344±58	71±1.1
5	A3d44	(1:25)	364±64	73±0.7

Final Optimized Formulas

The final formula obtained after optimizing AA concentrations, core: coat ratio, stirring time and stirring speed (Table 4).

Table 4: Final optimized formula of Eudragit coated dextran nanoparticles

Formulation Code	AA Conc.	Coat:coat ratio	Stirring Time	Stirring Speed	Particle Size	% Entrapment
A3d41	0.5	(1: 5)	120 min.	1600 rpm	203±33 nm.	71±1.7

Characterization of Nanoparticles

Transmission Electron microscopy (TEM)

The Transmission Electron Microscopy (TEM) was conducted using an accelerating voltage of 100 kilovolts (kV). A little amount of the sample was deposited onto a copper grid that had been coated with carbon, resulting in the formation of a thin layer on the grid. The film underwent negative staining with 15 phosphotungstic acid. A little amount of the staining solution was applied onto the film, and any surplus solution was removed using filter paper. The grid was dried completely by exposure to air, and the samples were examined using a transmission electron microscope (TEM).[13]

Zeta (ζ) potential

Doppler electrophoresis (Zetasizer Nano serie.Malvern Instrument Ltd., Worcestershire, UK) in HEPES buffer pH 7.4 after appropriate dilution (1/200 (v/v)) of the various nanoparticle suspensions allowed us to determine the zeta potential of the polymer and nanoparticles based on their electrophoretic mobility.[14]

Size measurement

The hydrodynamic mean diameter and size distribution of the nanoparticles were measured at a temperature of 25°C using quasielastic light scattering using a Zeta Nanosizer device (Malvern, UK). The previously disorganized angle was adjusted to a precise measurement of 900 degrees. The calculated count rate (kcps) was 38128.5250.[14]

Loading Efficiency of BSA Loaded Nanoparticles

The protein content encapsulated inside the nanoparticles was determined by subtracting the quantity of protein left in the supernatant from the total amount initially supplied to the loading solution. The quantities of BSA in the supernatant were determined using the Bradford protein assay technique. A portion of the resultant nanoparticles solution was then isolated using centrifugation for a duration of 20 minutes. Subsequently, the liquid portion was isolated from the nanoparticles. The quantity of untrapped protein present in the supernatant was determined using the Bradford protein assay technique. An unloaded suspension of nanoparticles was used as a blank.[15]

In Vitro Release Studies

10 mg of drug nanoparticles were placed in a 250 ml conical flask. Then, 100 ml of pH 1.2 and pH 7.4 phosphate buffer saline were added to the flask. The flask was then placed in a metabolic shaker and the shaker was set to 80 horizontal shakes per minute at a temperature of 37ºC ± 0.5ºC. A total of 10 milliliters of the medium was extracted at different time intervals of 1, 2, 4, 8, 16, and 24 hours, and then substituted with an equal amount

of phosphate buffer saline. The samples were appropriately diluted using methanolic PBS, and the amount of medication released was measured in each batch using a UV spectrophotometer at a wavelength of 595 nm.[16]

RESULTS AND DISCUSSION

The dextran nanoparticles were effectively synthesized utilizing a one-step approach and an ion-crosslinking technique, with AA serving as the crosslinker. The formula for manufacturing nanoparticles was derived by optimizing the particle size and entrapment efficiency by the manipulation of variables such as AA concentration, stirring duration, and stirring speed. The objective of optimization was to synthesize polymer nanoparticles with a particle size appropriate for internalization by antigen presentation cells and the cells of Gut-associated lymphoid tissue, while also achieving a spherical morphology and maximizing antigen loading.

The process variables (dextran quantity, stirring duration, and % AA) were optimized in terms of average particle size, shape, and % entrapment efficiency. The nanoparticles saw a little drop in size initially as the quantity of dextran increased. However, a subsequent rise in the amount of dextran resulted in an increase in particle size. The nanoparticles' size dropped as the concentration of AA increased. However, the excess AA proved challenging to neutralize using 1 M NaOH. Consequently, a medium concentration of AA was chosen, which resulted in the optimal size of nanoparticles. The optimal formulations were determined to be (A3) for dextran and AA content (5:0.5), formulations (A3d) for stirring duration (120 min.), and formulations (A3d4) for stirring speed (1600 rpm).

The nanoparticles were evaluated for their size, shape, zeta potential, and size distribution using Transmission Electron Spectroscopy (TEM) for size, Scanning Electron Microscopy (SEM) for form, and Zeta Nanosizer for zeta potential and size distribution. The TEM investigation revealed that the size of the palin and coated nanoparticles ranged from 150 to 282 nm, with an average size of 207.67 nm. The percentage of loading efficiency was a crucial factor to ensure the economic viability of the operation. The loading efficiency was determined to be 71±1.7%, which is the most favorable outcome.

The average size of the nanoparticles was found to be 216 nm. The polydispersity index of the nanoparticles was 0.331 (count rate (kcps) was 169.4) (Table 5).

Table 5: Zeta potential of Dextran Nanoparticles

S. No.	Formulation	Zeta Potential	Particle size (nm)	P.D.I
1.	Dextran Nanoparticles	-11.7±0.3	208±34	0.331
2.	Eudragit coated dextran nanoparticles	-14.1±0.2	267±30	0.353

The zeta potential of the final Nanoparticles formulation was determined to be negative. The zeta potential is a crucial parameter of particles, since it has the power to affect both the stability and absorption of particles. Zeta potential values, whether positive or negative, have a tendency to stabilize the suspension of particles. The electrostatic repulsion arising from particles with identical electric charges hinders the aggregation of the spheres. In contrast, absorption may be enhanced by a negative zeta potential value.

The in-vitro release profile was assessed in PBS at pH 1.2 and pH 7.4. The results are shown in table 6 and graphically represented in figure 1. The release of antigens was systematically observed over a period of 24 hours. The coated formulation exhibited sustained release at pH 7.4, but there was little release of the antigen at pH 1.2. This coated formulation effectively shields the antigen from acidic pH and facilitates the targeted delivery of the antigen to the M-cells in the intestinal area. The in-vitro release assays demonstrated that around 20% of the protein that was loaded was promptly released into PBS pH 7.4. This release might perhaps be attributed to the antigen being linked with the surface.

Table 6: In vitro % Release

Time (hrs)	% Release			
	pH 1.2		pH7.4	
	DNPs	ED.DNPs	DNPs	ED.DNPs
1	3±0.87	0.3±0.42	5±0.89	11±0.72
2	8±0.93	0.5±0.79	11±0.63	13±0.75
4	13±1.29	1±1.37	25±1.25	27±1.43
8	22±1.93	3±0.15	47±1.21	52±1.14
16	31±1.32	7±1.12	56±0.98	61±1.73
24	39±0.16	13±1.24	64±0.81	75±0.87

DNPs (dextran nanoparticles); ED.DNPs (Eudragit coated dextran nanoparticles)

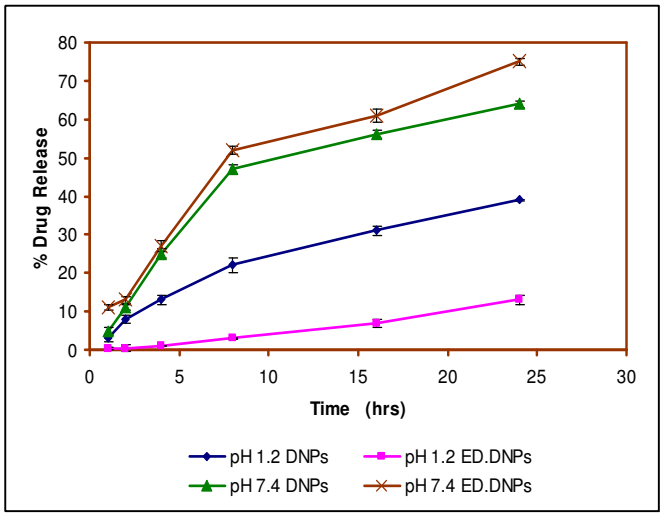

Fig 1: Graph of In vitro % Release

CONCLUSION

For the purpose of drug delivery applications, we create a group of hydrophobically modified dextrans as part of our study to develop nanoparticles.The solubility of modified dextrans in organic solvents is contingent upon their hydrophilic-lipophilic balance (HLB), which is determined by the substitution ratio and the hydrophobicity of grafted alkyl chains. Regarding the enzymatic breakdown of chemically altered dextrans. The oral administration might serve as an appropriate method for priming and enhancing. Antigens for oral administration may assume many forms, such as whole organisms (virus, bacterium), surface proteins, synthetic peptides, and DNA. Oral administration is a favorable option as it allows for the use of lower dosages and targeted delivery of the formulation to the specific place (GALT-Gut associated lymphoid tissue). Furthermore, due to the characteristics of the typical intestinal immune system, the oral pathway may serve as both a stimulator and an active location for the intestines, lungs, and vagina. Therefore, oral vaccinations have a crucial role in preventing Typhoid infections.

Conflict of Interest- No conflict of interest was declared by the authors. The authors alone are responsible for the content and writing of the paper.

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