

Preparation of probiotic-loaded solid lipid nanoparticles and in vitro survival in gastrointestinal conditions

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Abstract. In the present study, Probiotics (*Lactobacillus plantarum*) loaded-solid lipid nanoparticles were prepared and characterized. Probiotics have proven to possess significant potential in addressing a range of conditions, encompassing neurodegenerative disorders, cancers, cardiovascular diseases, and inflammatory conditions. Additionally, they show efficacy in sustaining a harmonious gut microbiota ecosystem. SLNs were prepared by using solvent emulsification-diffusion technique. The size and morphology of prepared probiotic-loaded SLNs were examined by transmission electron microscopy. The activity or viability of probiotics might be compromised in the challenging gastric conditions of the stomach or in the presence of bile salts. Furthermore, they may face vulnerability to thermal or oxidative stress during preparation and storage. Hence, there is a need for stable probiotic formulations to surmount diverse physicochemical, biopharmaceutical, and biological barriers, ensuring maximal therapeutic effectiveness and clinical applicability. In vitro experiments were carried out with the aim of examining the viability of bacterial cells under gastrointestinal conditions. Encapsulation of the cells protect the cell numbers (colony forming unit) when compared to free bacteria. Nevertheless, the number of probiotic cells decreased in gastrointestinal acidic condition in contrast to free cells. Overall encapsulation of *Lactobacillus plantarum* in SLN's plays an important role in enhancing viability and stability which consequently enhance the survival of bacteria against gastrointestinal environmental conditions.

Keywords: Probiotic, nanoparticles, GIT, cell viability, encapsulation

1 Introduction

Nanotechnology is indispensable for creating new techniques for transporting symptomatic and therapeutic molecules to specific areas of CNS [1, 2]. These formulations improve therapeutic efficacy by increasing drug concentration at the site of action and minimising long-term side effects linked to the abnormal distribution of drugs. Various nanoparticles (NPs) have been suggested to improve drug penetration across BBB, including lipid nanoparticles, gold nanoparticles, liposomes, polymeric nanoparticles, dendrimers, and carbon nanotubes. [3]. The suitability of nanodelivery systems for brain delivery is determined by factors such as nanometric size, surface charge, shape, molecular recognition, and the interaction between a particular ligand attached to the surface of nanoparticles and the substance overexpressed at the brain target area [20, 21]. Particle size, zeta potential, and percent drug concentration are examples of intrinsic characterisation characteristics of nanoparticles (NPs) that are critical in defining their physiological effects and, ultimately their safety and efficacy. [4].

Probiotics are live bacteria that extend to the distal digestive tract and, when given in appropriate amounts, improve the host's health. The two most popular probiotic bacteria are *Lactobacilli* and *Bifidobacterium*. The flexible lactic acid bacteria *L. plantarum* is present in a variety of environmental settings, where it ferments dairy, meat, and a wide range of crops. Furthermore, it is frequently found in the digestive system of humans. [5]. However, because these bacteria prevent GIT pathogens from adhering to the intestinal mucosal membrane

through competitive colonisation, there may be an issue with the probiotics' capacity to remain stable in storage and effectively distribute to the distal part of the gastrointestinal tract. These probiotics stop the growth of bacteria by lowering the pH of the intestine through the release of acids like lactic and acetic acid. Additionally, these organic acids encourage the digestive tract's peristalsis [6]. Encapsulation is the optimum way to maintain the vitality of probiotics during storage and early passage through the gastrointestinal tract [7]. The biological effects of probiotics are diverse. Nevertheless, they also hold promise as therapeutic adjuvants and drug delivery systems for a range of illnesses, such as cancers, inflammatory diseases, neurological disorders, and cardiovascular disorders [8]. In this research, chitosan loaded nanoparticles were prepared. Finally, the viability of probiotic cells in simulated intestinal conditions was studied. Essential advantages of nanoparticles include good acceptability, high biocompatibility and protection of integrated drugs from enzymatic or chemical degradation [9].

2 Material and methods

2.1 Drug and other chemicals

Lactobacillus plantarum was purchased from Proen Biopharma (Maharashtra, India) in the form of gift sample. Chitosan derived from shrimp shells was acquired from Sigma (St. Louis, USA). In addition, all other chemicals used in this research work were of analytical grade.

2.2 Preparation of probiotic loaded nanoparticles

Chitosan nanoparticles were prepared with ionic gelation of chitosan with tripolyphosphate (TPP) anions [22, 27]. Briefly, different concentrations of chitosan (0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL) were dissolved in acetic acid solution. Subsequently, a solution of TPP at a concentration of 0.1 mg/mL was prepared. Following this, 10 mL of the TPP solution was slowly added drop by drop under continuous stirring to a 20 mL chitosan solution. The result was the spontaneous formation of a milky-colored suspension under these conditions, signifying the successful preparation of chitosan nanoparticles. The nanoparticles loaded with probiotic cells were also created by introducing the TPP solution into a chitosan solution that included approximately 1 mL of bacteria, which had been diluted in the preceding step with normal saline [12]. In short, the bacterial solution and a fixed volume of normal saline (1 mL) were combined with the chitosan solution under sonication to minimize the size of bacteria, facilitating their encapsulation in nanoparticles. Following approximately 2 hours of stirring, the TPP solution was introduced into the mixture. The impact of various parameters, such as chitosan concentration, on encapsulation and loading efficiency was assessed. Simultaneously, the viability of probiotic bacteria in a simulated gastrointestinal system was examined.

2.3 Particle size and zeta potential

Dynamic light scattering measured the particle size of produced nanoparticles with a Zeta sizer Nano ZS (Malvern, UK). Using photon correlation spectroscopy, 1 mL of nanoparticle suspension was diluted tenfold with 10 mL of distilled water, and the average particle size was then determined in triplicate ($n = 3$) [13]. The surface charge of probiotic loaded nanoparticles, also known as the zeta potential, was measured using the Zetasizer to measure the electrophoretic mobility of nanoparticle in a U-shaped tube at 25 °C.

2.4 Shape and surface morphology

TEM (Talos L120c G2, United States) was utilized to investigate the morphology and shape of probiotic loaded nanoparticles [14].

2.5 Stability study

Using a stability chamber (Stericox, India), the stability of the probiotic-loaded nanoparticle formulation was evaluated. In addition, formulation stability experiments were conducted at 25°C/60% RH and 40°C/75% RH. During storage, samples were taken at 0, 15, 30, and 60 days intervals. During sampling, the developed formulation's physical properties, including particle size was monitored at room temperature [15]. All measurements were performed in triplicate ($n=3$).

2.6 Simulation of the intestinal conditions

The artificial intestinal syrup was simulated according to Pinto et al. [16]. The solution was comprised of 6.4 g/L NaHCO₃, 0.239 g/L KCl, 1.28 g/L NaCl, 0.5% bile salts (Oxgall, Merck, Darmstadt, Germany), and 0.1% pancreatin (Fluka Biochemika, Steinheim, Germany) [17]. The pH of the solution was adjusted to 7.2. Subsequently, the encapsulated bacteria were introduced into the solution. After disrupting the cell wall through agitation, the samples were spread-plated onto MRS agar to determine the colony-forming units per milliliter (CFU/mL).

2.7 Statistical analysis

Statistical analysis was performed and expressed as mean \pm SD. All examined samples underwent statistical analysis through one-way analysis of variance (ANOVA), followed by a Post-Hoc Tukey test. Statistical significance was established for P values less than 0.05.

3 Results and Discussion

3.1 Surface morphology

The TEM analysis indicated that the developed nanoparticles have a uniform surface, and all particles are spherical in shape [18]. Figure 1 illustrates the TEM imaging of probiotic loaded nanoparticles.

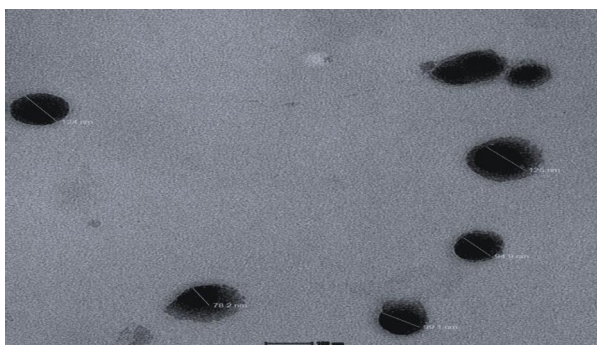


Fig. 1 TEM imaging of probiotic loaded nanoparticles

3.2 Particle size and Zeta potential

Average size of Probiotic loaded nanoparticles were found to be ranging from 100 to 250 nm. Different concentrations of chitosan, specifically 0.05, 0.1, 0.2, and 0.3 mg/mL, were examined in conjunction with 0.1% TPP. The smallest particle size was attained at a chitosan concentration of 0.05 mg/mL. Moreover, an increase in the chitosan concentration led to a larger particle size. Thus, it was evident that the chitosan concentration played a crucial role in influencing the formation of nanoparticles.

Measuring the zeta potential enables predictions regarding the storage stability of colloidal dispersions. Typically, particle aggregation is less likely to occur for charged particles (those with a high zeta potential) due to the presence of electric repulsion forces [19]. The mean zeta potential was -23.54 ± 2.72 mV ($n=3$). Hence, this method has achieved relatively high stability and good dispersion quality.

3.3 Viability of Free and Encapsulated *Lactobacillus plantarum* in Intestinal Conditions

The viability of the free and encapsulated probiotic bacteria in intestinal syrup was evaluated and illustrated in Table 1. The experiments were performed under intestinal conditions over a time span of 0 to 120 minutes, with intervals of 30 minutes. The count of free cells exhibited a significant decrease from 1.74 to 1.18 Log CFU/mL over the 120-minute period. In contrast, the count of encapsulated cells showed no significant reduction during the entire testing duration. Initially, the count of encapsulated cells was 1.66 Log CFU/mL, and after exposure to biliary salt conditions for 120 minutes, it decreased to 1.43 Log CFU/mL. The encapsulation of probiotic bacteria with chitosan demonstrated an enhancement in the resistance of cells within the intestinal environment, consequently extending the lifespan of the cells.

Table. 1 Viability of free and encapsulated cells versus time in the intestinal conditions
 Stability study

Test	Time (min)				
	0	30	60	90	120
Free probiotic (Log CFU/mL)	1.74±0.13 ^a	1.63±0.12 ^b	1.54±0.12 ^c	1.21±0.14 ^d	1.18±0.11 ^e
Encapsulated probiotic (Log CFU/mL)	1.66±0.1 ^a	1.61±0.2 ^b	1.53±0.2 ^c	1.49±0.3 ^c	1.43±0.1 ^d

Table 2 shows the results of particle size of probiotic loaded nanoparticles after 15, 30 and 60 days of storage at room temperature. Chitosan-based probiotic-loaded nanoparticles demonstrated adequate long-term stability, exhibiting only slight particle growth ($P>0.05$) after storage at room temperature for 60 days. Moreover, no observable aggregation was noted in the system during this period.

Table. 2 Particle sizes of SLN after 1, 2 and 4 weeks of storage at room temperature ($n=3$)

Sample	Average diameter (nm)		
	15 days	30 days	60 days
Encapsulated probiotic	120±5.6	128±7.3	136±3.3

4 Conclusion

The current study has shown that *Lactobacillus plantarum* probiotic bacteria are more stable and viable when encapsulated in chitosan nanoparticles. Additionally, in a simulated gastric and intestinal environment, encapsulation significantly increases bacterial survival. The results also indicated that an enhancement in the concentrations of chitosan resulted to the larger size of nanoparticles. Moreover, the survival of encapsulated probiotic bacteria was also higher rather than free probiotics. The results obtained from in-vitro experiments indicated that chitosan is a good substance for encapsulation of probiotics in stomach and gastric conditions. Furthermore, nanoencapsulation can be considered as a good tool for establishing viable probiotics in foods and maintaining their survival during simulated gastric and intestinal conditions.

5 References

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