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ARTICLE

Comparison of the Loading of Lactoferrin Extracted from Camel Milk on Pectin and Chitosan Nanoparticles

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ABSTRACT

Lactoferrin is a bioactive ingredient that is sensitive to environmental stresses and needs the use of microencapsulation, notwithstanding all the biological activities and effects it exerts. Pectin is a polysaccharide that acts as an anion for ion–ion interactions. Chitosan is a biodegradable and nontoxic material. In this study, lactoferrin was extracted and purified, pectin and chitosan nanoparticles were prepared, and finally, the lactoferrin was microencapsulated in the nanoparticles. After determining the final loading efficiencies of the lactoferrin, SEM and subsequent zeta potential and particle size measurements confirmed the lactoferrin loading onto the pectin and chitosan nanoparticles. The findings confirmed the presence of lactoferrin in the 0.5, 0.6, 0.7, and 0.8 M ($mol \cdot L^{-1}$) NaCl fractions through an SDS-PAGE and tetramethylbenzidine test. The loadings indicated that lactoferrin was indeed loaded, with the final loading efficiencies for pectin and chitosan being 84% and 81.5%, respectively. SEM confirmed the loading and showed that the emulsion structures present at the time of production and attachment were very uniform, with a consistent distribution of spherical particles. The average size of lactoferrin-loaded pectin nanoparticles was 276.3 nm, with a surface charge of –35 mV.

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Loading of lactoferrin onto the polysaccharides pectin and chitosan resulted in a shift toward more negative zeta potential values.

Keywords: Microencapsulation; Camel Milk Lactoferrin; Pectin; Chitosan

1. Introduction

Milk is often thought of as a complete food because it provides a significant and varied spectrum of nutrients and bioactive compounds that significantly improve human health in many ways beyond sustenance. Bioactives may be categorized on the basis of their physiological roles: for instance, regulating digestive system function and microbes; supporting the growth and development of infants; stimulating a person's innate and adaptive immune response; and inducing direct antimicrobial activity [1]. Lactoferrin is among these valuable components, and its many possible biological activities make it a prime target for research regarding nutraceuticals, pharmaceuticals, and functional foods.

Lactoferrin, an iron-binding glycoprotein belonging to the transferrin family, weighs about 80 kDa. It is noted for its diverse bioactivity, which includes immunomodulation, antimicrobial, antiviral, antioxidant, and anti-inflammatory activities [2]. Lactoferrin plays a role in many physiological functions in the body, such as antimicrobial activity, regulation and absorption of iron, and immune responses. It also has antioxidant, anticancer, and anti-inflammatory properties. The antibacterial activity of lactoferrin operates through two mechanisms: it can sequester iron, thereby depriving iron-dependent pathogenic bacteria of a needed nutrient, and it can have direct interactions with microbial and host cell surfaces that affect various aspects of cellular processes [3]. New studies continue to shed light on lactoferrin's promise, and a variety of evidence supports its use for treating gastrointestinal infection, systemic inflammation, and as an anti-cancer agent through its ability to induce apoptosis in some cancerous cell lines [4]. The unique lactoferrin found in camel's milk has been studied extensively with the intent of determining its potential differences in structure and function compared to bovine and human lactoferrin. Results indicate that camel lactoferrin has a much higher degree of iron-binding ability and exhibits significantly greater antimicrobial activity against drug-resistant bacteria and fungi. In addition to this, camel lactoferrin appears to possess a uniquely different amino acid composition and three-dimensional structure, both of which provide camel lactoferrin with its unique properties [5].

While it has many beneficial effects, lactoferrin is a naturally fragile molecule that can be damaged by many environmental stressors experienced during processing, storage, and the gastrointestinal journey. Lactoferrin is easily denatured by extreme pH, various proteolytic enzymes, elevated temperature, and mechanical shear stress, losing a large percentage of bioactivity in the process [6]. The stability of lactoferrin is a significant barrier to effectively incorporating it in oral delivery systems, where it must survive the extreme acidic environment of the stomach and then remain bioactive in the intestines. Thus, it is necessary to develop stable and effective systems for delivering lactoferrin; it is not an enhancement but a prerequisite for successful nutraceutical and pharmaceutical applications.

In order to address the stability limitations of lactoferrin, it is necessary to microencapsulate it within a protective carrier system. Microencapsulation is a well-established technology that has been used successfully to encapsulate many ingredients; however, nanoencapsulation offers specific advantages for the oral administration of lactoferrin, including improved bioavailability, enhanced cellular uptake, and better mucosal adhesion due to the high surface area-to-volume ratio of nanoparticles [7,8].

Developing biopolymer nanoparticles using polyelectrolyte complexation is one of the most promising nanoscale formulation technologies. In general, ionic gelation and/or complex coacervation occur at the nanoscale through the action of electrostatic attractions between two negatively and positively charged biopolymers. Additionally, electric field-induced coacervation can work effectively without solvents (which could compromise protein stability), making this process ideal for highly susceptible proteins like lactoferrin. Compared to other various methods used to produce particles, coacervation generally pro-

vides superior encapsulation efficiencies and controlled release characteristics [9]. Importantly, the properties of the nanoparticles formed, including their size, surface charge, and the profile of their release, can be adjusted precisely by controlling the pH, ionic strength, and ratios of the biopolymers employed [10].

The choice of wall materials for any encapsulation system is critical to its overall success. Generally recognized as safe (GRAS) biopolymers, especially polysaccharides, represent an excellent choice as they are biocompatible, biodegradable, and versatile. Biopolymer-based nanoparticles have a large surface area to volume ratio, which can enhance the bioavailability of the encapsulated agent [10]. Biopolymer nanoparticles can be produced from a single biopolymer or, more effectively, via complex coacervation of proteins and polysaccharides. Complex coacervation is advantageous because it can provide fine-tuning of particle properties (size, surface charge, and release profile) by manipulating conditions (e.g., pH, ionic strength, or biopolymer concentrations) [11].

Pectin, an anionic polysaccharide composed primarily of α-(1→4)-linked D-galacturonic acid residues, is commonly used in these applications. Pectin is derived from plant cell walls and is a common gelling and stabilizing agent. In microencapsulation, at a low pH below the isoelectric point of most proteins, positively charged proteins interact electrostatically with the negatively charged carboxyl groups of the pectin to form insoluble complexes or coacervates [6]. This interaction is highly dependent on specific environmental conditions such as pH, ionic strength, and the amount of the biopolymers. Pectin-based delivery systems have shown potential for protecting bioactives in the gastrointestinal tract [12].

On the other hand, chitosan, a cationic polysaccharide derived from the deacetylation of chitin, presents a different set of properties. Chitosan is non-toxic, biodegradable, and bioadhesive. As a cationic polysaccharide, chitosan has a positive charge that allows it to bind strongly to negatively charged mucosal surfaces and cell membranes, imparting significant mucoadhesiveness and making it extensively studied for improving the absorption of drugs and bioactives through the intestinal epithelium [13]. Additionally, chitosan has its own immunostimulatory and antimicrobial properties, which synergize with the actions of lactoferrin, making it a very appealing option for oral vaccines and therapeutic delivery systems. Due to these properties, recent studies have also examined chitosan-based nanoparticles for delivering a wide range of proteins, demonstrating protection of the proteins and sustained release [7,14].

There is also a large variation in biopolymer types. For example, pectin is an anionic biopolymer that, when mixed with positively charged proteins below the protein's isoelectric point, will form complexes with those proteins that may provide protection against gastric conditions [6,12]. Chitosan, as a cationic biopolymer, is also used for its mucoadhesiveness and permeation-enhancing effects, both of which may allow for enhanced absorption in the intestine [13]. Both have also been used successfully for the creation of protein-loaded nanoparticles [7,14].

Due to the distinct and complementary properties of pectin (anionic, plant-based, gel-forming in the GI tract) and chitosan (cationic, mucoadhesive, permeation-enhancing), a timely and justified comparison of their ability as nanocarriers for a sensitive protein, camel milk lactoferrin, is warranted. However, such a comparison should assess more than loading efficiency, but the physicochemical properties of the nanoparticles resulting from each chemical, including size, zeta potential, and morphology, and their subsequent function in simulated biological environments. Therefore, this study will systematically compare loading efficiency, as well as the key physicochemical properties of camel milk lactoferrin when microencapsulated in either pectin-based nanoparticles or chitosan-based nanoparticles, and provide meaningful information for the selection of optimal delivery systems for such an important bioactive protein.

2. Materials and Methods

2.1. Materials

Camel milk was sourced from the vicinity of Mashhad Province. Chitosan of medium molecular weight, high methoxyl pectin, sodium chloride, trypsin, Sephadex, ammonium sulfate, 3% hydrogen peroxide, tetramethylbenzidine, dimethyl sulfoxide-tetrazolium, hydrochloric acid, and 100X liquid antibiotic were obtained from Merck (Germany). Bovine Serum Albumin (BSA), CM Sephadex

C-50, acrylamide, and bis-acrylamide were obtained from Sigma, and RPMI 1640 and sodium thiosulfate were purchased from Roche, Germany.

2.2. Methods

2.2.1. Extraction and Purification of Lactoferrin

The process of purifying lactoferrin was conducted as reported by Raei et al. (2015) [15]. After defatting by centrifugation (4000× *g* for 25 min) of the milk, 10% acetic acid and 1 M sodium acetate were gradually added to adjust the pH to 4.6. The mixture was then centrifuged (30,000× *g* for 30 min) before it was filtered through Whatman No. 1 filter paper and precipitated with 90% ammonium sulphate.

Cation exchange chromatography utilized CM Sephadex C-50 resin and a column measuring 10 cm and a 3 cm internal diameter. The protein sample from the previous steps was equilibrated in 10 mM sodium phosphate buffer at pH 6.8 and was applied to the CM Sephadex C-50 column. The column was equilibrated with the same phosphate buffer by applying the protein sample. Following the sample application, it was washed with phosphate buffer and then eluted with phosphate buffer containing different salt concentrations (0.1 to 1 M), which were collected. Fractions were collected, and protein content was measured by spectrophotometry at 260 nm.

The purified protein concentration was measured by the Bradford method and NanoDrop. SDS-PAGE electrophoresis was used to check for lactoferrin in the fractions. Fractions that had an SDS-PAGE band of 80 kDa could contain either lactoferrin or lactoperoxidase. To confirm the presence of lactoferrin in the fractions, a Tetramethylbenzidine (TMB) test was performed. Lactoperoxidase catalyzes a reaction with TMB in the presence of hydrogen peroxide, oxidizing TMB and producing a blue colour. The reaction mixture consisted of 30% H_2O_2 with 88 mM TMB and 10 mM sodium phosphate buffer (pH 6). For the assay, 10 µL of each sample was placed in 200 µL of reaction mixture and incubated for 15 min at 37 °C (20), with horseradish peroxidase (HRP) as a positive control [15,16].

2.2.2. Preparation of Pectin Nanoparticles

Nanoparticles were prepared using a method that utilized controlled self-assembly and homogenization adapted from previous studies [10]. In brief, a 0.5% (w/v) pectin solution was prepared by dissolving high methoxyl pectin powder in 100 mL of sterile deionized water. The solution was stirred on a magnetic stirrer for 24 h at room temperature (25 ± 2 ℃) to allow for complete hydration and relaxation of the polymer chains, which is critical to achieving a uniform molecular dispersion. After hydration, the pectin solution was homogenized at high-shear using an Ultra-Turrax homogenizer (IKA, Germany) at 12,000 rpm for 10 min to reduce bulk aggregates and initiate nanosizing. The sample container was placed in an ice bath during homogenization to remove heat and avoid thermal degradation of the pectin, which could alter gelling properties and molecular weight. Following homogenization, the pectin dispersion pH was carefully adjusted to 4.0 with 0.1 N HCl and NaOH. This pH was chosen because it was below the isoelectric point (pI) of lactoferrin (pI ~ 8–9), which allows the protein to have a net positive charge, while pectin will be negatively charged, allowing for the use of electrostatic complexation [17].

Pectin nanoparticles were produced by controlled self-assembly utilizing electrostatic interactions and hydrogen bonding at pH 4.0 (below the isoelectric point of lactoferrin) without requiring chemical cross-linking agents. Homogenization techniques enabled effective particle size reduction and improved dispersion stability.

2.2.3. Lactoferrin Loading into Pectin and Chitosan Nanoparticles

Lactoferrin was loaded onto the pre-formed pectin nanoparticles through electrostatic deposition. First, a stock solution of purified lactoferrin was prepared at 0.2% (w/v) in 10 mM sodium phosphate buffer, pH 6.8. The solution was then placed in a dry heat block and incubated at 60 ℃ for 10 min. This mild heat treatment was used to partially unfold the lactoferrin, thereby increasing the exposure of some hidden cationic amino acid residues, which enhanced the interaction of the lactoferrin with the anionic pectin chains, a method to enhance protein-polyelectrolyte binding. As the lactoferrin solution was being heated, 600 µL aliquots of the pH-adjusted pectin nanoparticle dispersion were gently warmed to 37 ℃ for 10 min, to match the temperature of both solutions and

reduce thermal shock upon mixing, which could potentially cause aggregation. Once both solutions were at the same temperature and under gentle magnetic stirring, the lactoferrin solution was then added dropwise to the pectin dispersion from a height of 10 cm. This controlled, gradual mixing promoted the formation of a lactoferrin-pectin complex coacervate at the interface and progressive build-up of lactoferrin layers on the pectin nanoparticles, rather than instantaneous macroscopic precipitation. The mixture was subsequently stirred at a constant ambient temperature for 24 h to allow complexation to occur and stabilize the loaded nanoparticles [18].

For chitosan nanoparticles, ionic gelation using tripolyphosphate (TPP) as a cross-linker agent was employed. For protonation of the amino/amine ($-NH_3^+$) groups involved in ionic cross-linking, pH was adjusted with NaOH to 5.0. For TPP, a 0.25 mg/mL solution was prepared in deionized water and pH adjusted to 5.0. First, isolated lactoferrin (120 µL from a solution containing 852 µg/mL lactoferrin) was mixed into the TPP solution; this provides the best encapsulation to get the lactoferrin distributed evenly throughout the chitosan-TPP nanoparticles. This mixture of lactoferrin and TPP was then added drop-wise under magnetic stirring to the chitosan solution containing 2.5 mg/mL chitosan (10 mL). This gives the final size ratio of chitosan to TPP (approximately 5:2). The formation of an opalescent suspension as a result confirmed that chitosan-TPP nanoparticles based on lactoferrin had formed. Finally, the resulting mixture was incubated at 57 ℃ for 10 min to allow for solidification of the nanoparticles, thus allowing for increased rigidity and load efficiency of the particles [19].

2.2.4. Determination of Loading Efficiency and Verification of Lactoferrin Loading

To determine the encapsulation efficiency (EE %) of lactoferrin loaded in pectin and chitosan nanoparticles, the amount of protein remaining unbound was quantified [20]. Following the loading of lactoferrin into each nanoparticle suspension, the mixtures were subjected to centrifugal forces of 1000× *g* for 10 min. This relatively low centrifugal force was deliberately chosen to have sufficient force to pellet the nanoparticles while allowing unencapsulated (free) lactoferrin to remain in the supernatant [21]. Loading efficiency of either formulation (LE) was derived from the following calculations:

$$\text{Efficiency (\%)} = (\text{Output Power} \div \text{Input Power}) \times 100$$

2.2.5. Scanning Electron Microscopy (SEM)

The Scanning Electron Microscope (LEO VP 1450, Germany) was used to study the morphology, topography, and approximate dimensions of the nanoparticle suspension. The NP suspension was deposited as droplets onto aluminium stubs and allowed to air dry before being coated with gold/palladium alloy via sputtering to increase conductivity. High vacuum imaging was accomplished using a 15 kV accelerating voltage [22].

2.2.6. Measurement of Zeta Potential, Polydispersity Index (PDI), and Particle Size

The zeta potential of the nanoparticles, which is an indicator of colloidal stability and surface charge, was determined using a HORIBA Nano Particle Analyzer (Model SZ-100, Japan) based on the principle of electrophoretic light scattering. The samples were then appropriately diluted with deionized water to ensure there were no multiple scattering effects. The zeta potential shift after lactoferrin was primary evidence of successful surface attachment or encapsulation [23]. The average hydrodynamic diameter and particle size distribution (polydispersity index, PDI) were determined using Dynamic Light Scattering (DLS) on the same instrument. For verification purposes, particle size analysis was conducted using a VASCO particle size analyzer (Model 1, France). All measurements were performed in triplicate at 25 °C [24].

A Polydispersity Index (PDI) value is generated by the DLS instrument, such as the Malvern Zetasizer, as a means of evaluating the range of a given particle size distribution within a colloid.

Through its analysis of the correlation of fluctuation of scattered light, the DLS software will be able to calculate a particle's hydrodynamic diameter in terms of its Z average (d.nm) and the PDI by calculating the width of an intensity-weighted particle size distribution.

The PDI is calculated as follows: conceptually, PDI = $(\sigma/Z)^2$, where Z is the Z-average mean size, and σ is the standard deviation of the particle size distribution about the Z-average mean size. Both the PDI and hydrodynamic diameter are calculated through the use of the DLS software.

3. Results

3.1. Extraction Confirmation of Lactoferrin

The protein concentration of the eluted fractions was measured at 595 nm, revealing a distinctive elution profile. Protein concentrations in successive NaCl fractions were 110.73 µg/mL (0.4 M), 162.73 µg/mL (0.5 M), 531.1 µg/mL (0.6 M), and 569.9 µg/mL (0.7 M), suggesting the elution of a major protein component. As expected, protein concentrations decreased in the higher salt fractions: 293.98 µg/mL (0.8 M), 255.75 µg/mL (0.9 M), and 135.49 µg/mL (1.0 M).

3.2. Confirmation of Lactoferrin Purification

SDS-PAGE performed on the 0.5, 0.6, 0.7, and 0.8 M NaCl fractions showed a distinct band at 80 kDa corresponding to lactoferrin (**Figure 1**). A positive reaction of TMB (3,3′,5,5′-tetramethylbenzidine) was specifically noted in 0.6 and 0.7 M NaCl fractions, confirming the presence of the active protein. These results confirm that lactoferrin was successfully purified.

The 0.6 and 0.7 M NaCl fractions were tested for the presence of both a TMB-positive reaction and a band of molecular weight 80 kDa. It was determined that both fractions contained pure lactoferrin according to this evidence. However, the highest amounts of active protein were found in the 0.7 M NaCl fraction.

3.3. Loading and Loading Efficiency of Lactoferrin on Pectin and Chitosan

Purified lactoferrin was loaded onto the pectin and chitosan biopolymer carriers. Loading efficiency is an important indicator of the amount of lactoferrin associated with the carriers relative to the initial dose used. The microencapsulation process achieved a high recovery of the initial lactoferrin dose. The loading efficiency for lactoferrin fractions onto pectin particles was 84.0%, and for chitosan particles it was 81.5%. These high percentages indicate that the loading process was effectively optimized for both biopolymers, with most lactoferrin associated with the particulates rather than free in solution.

3.4. Confirmation of Lactoferrin Loading, Using Particle Size, Distribution, and Morphology with Scanning Electron Microscopy

The morphology of both unloaded biopolymers and lactoferrin-loaded particles was characterized via Scanning Electron Microscopy (SEM). The images in **Figure 2** provide a direct visual comparison. The unloaded pectin particles appeared rough and irregular with porous aggregates (**Figure 2b**), while the unloaded chitosan particles appeared spherical and smooth (**Figure 2c**).

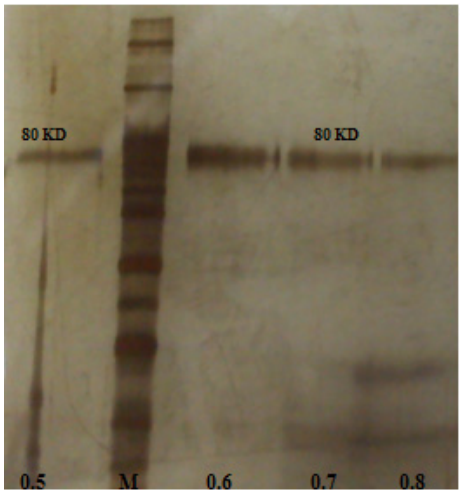

Figure 1. SDS-PAGE electrophoresis results.

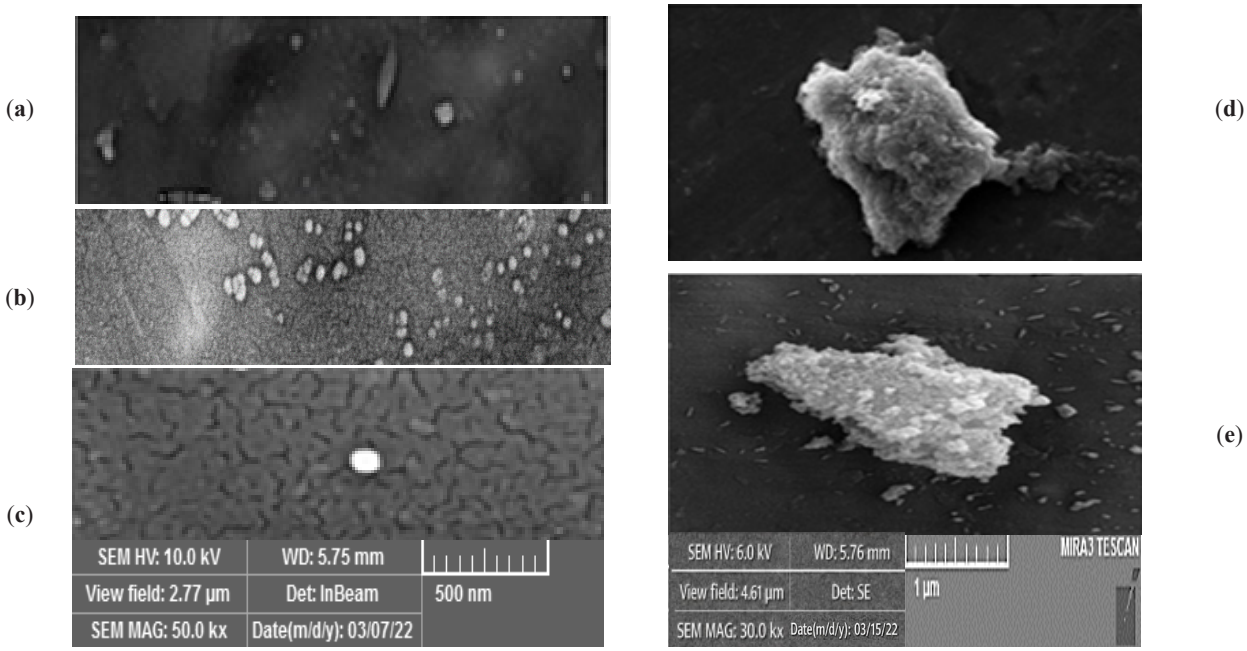

Figure 2. Comparison of SEM images: (**a**) Lactoferrin; (**b**) Pectin; (**c**) Chitosan; (**d**) Lactoferrin loaded onto Pectin; (**e**) Lactoferrin loaded onto Chitosan.

After the loading procedure was completed, clear changes in the physical characteristics of the particles were visible, which acted as direct evidence of successful incorporation of lactoferrin. The lactoferrin-pectin particles (**Figure 2d**) appeared visibly less porous on the surface and more densified into a film-like structure, indicative that lactoferrin molecules had coated the pectin matrix and filled some of the porous voids. Similarly, the lactoferrin-chitosan particles (**Figure 2e**) showed a distinctly altered morphology, lost their clearly defined spherical smoothness, and exhibited a more clumped and roughened surface structure. These morphological differences suggest that the amphiphilic lactoferrin protein was able to integrate and adsorb onto the surface of the chitosan particles. Particle size and zeta-potential analysis support that loaded particles were within a micron-scale size range with relatively consistent distribution, which can promote consistent performance in subsequent applications.

The image indicates that the emulsion structures during production and attachment were very homogeneous with uniform distribution, and the morphology is spherical. One reason for particle uniformity is their surface charge, which allows the particles to remain homogeneous without aggregate formation.

3.5. Results of Comparing Particle Size, Polydispersity Index (PDI), and Zeta Potential of Lactoferrin Loaded onto Pectin and Chitosan

Particle size and zeta potential were analysed for both pectin and chitosan-loaded samples. The results are presented in **Table 1**. The data show that unloaded chitosan produced the largest particles (mean size of 956 nm ± 2). Conversely, lactoferrin-loaded pectin nanoparticles were the smallest, with it producing a mean size of 276.3 nm ± 1.56. Lactoferrin-loaded chitosan nanoparticles have an intermediate average size of 496.09 nm ± 2.01. Regarding zeta potential, a general rule for therapeutic formulations is that a zeta potential equal to or greater than +30 mV is associated with a stable monodispersed formulation. A zeta potential around ± 20 mV indicates only short-term stability, while a value below +5 suggests a rapid tendency to aggregate. Based on the results, lactoferrin-loaded pectin with a zeta potential of −35 mV (± 0.63) is more stable than lactoferrin-loaded chitosan with a zeta potential of −12.9 mV (± 0.11).

Table 1. Results of comparing particle size, Polydispersity Index (PDI), and zeta potential of lactoferrin, pectin, chitosan, and lactoferrin loaded onto pectin and chitosan.

Samples	Average Particle Size (nm)	Polydispersity Index	Zeta Potential (mV)
Lactoferrin	542.18 (± 1.24)	0.489 (± 0.037)	−10.9 (± 0.11)
Chitosan	965 (± 2)	0.544 (± 0.098)	−31.37 (± 0.12)
Pectin	293.06 (± 1.05)	0.397 (± 0.088)	−26.8 (± 0.14)
Lactoferrin loaded onto Pectin	276.3 (± 1.56)	0.305 (± 0.074)	−35 (± 0.63)
Lactoferrin loaded onto Chitosan	496.09 (± 2.1)	0.479 (± 0.081)	−12.9 (± 0.11)

4. Discussion

The purpose of this research project was to create and analyze both cationically charged chitosan nanoparticles and anionically charged pectin nanoparticles containing camel milk lactoferrin (LF). To ensure the loading of LF, the two main objectives were to protect this sensitive protein while at the same time characterizing the physicochemical properties of the resultant nanocarriers, which in turn impact stability and functional efficacy. High loading efficiency was attained for both pectin (84%) and chitosan (81.5%) through the use of ionic gelation and electrostatic complexation. Extensive characterization through scanning electron microscopy (SEM), dynamic light scattering (DLS), and zeta potential gave evidence that both types of nanoparticles formed spherical, stable entities with different size/range and surface charge profiles. The final section discusses how the findings from this research could help future attempts at designing and developing the nanocarrier delivery systems and their uses in biological applications.

Microencapsulation of lactoferrin (LF) is an essential technological step to realize its full therapeutic utility, especially for oral delivery, since LF is extremely sensitive to denaturation and proteolytic digestion in the hostile gastrointestinal tract. The study has shown it is possible to load camel milk LF onto pectin and chitosan nanoparticles primarily by ionic gelation, thereby demonstrating that a polyelectrolyte complexation approach was worthwhile and effective for encapsulation. The SEM image shows uniformly sized spherical nanoparticles, which is a direct result of, and indication of the efficacy of, the electrostatic interactions between the cationic patches on the LF molecule and the anionic/cationic functional groups of the polysaccharides. The creation of nanoencapsulated camel milk lactoferrin (LF) in pectin and chitosan nanoparticles highlights the advantages of utilizing polyelectrolyte complexation in the fabrication of nanoscale protein delivery systems.

High loading efficiencies (84% for pectin and 81.5% for chitosan) indicated an efficient complex was formed, which is important for the economic and therapeutic efficacy of any delivery system [25,26].

While chitosan nanoparticles are stabilized by ionic cross-linking with tripolyphosphate (TPP), pectin nanoparticles are stabilized through electrostatic and hydrophobic interactions. These differences may account for discrepancies in particle stability and release kinetics within simulated biological settings.

The decrease in the zeta potential to more negative values upon encapsulating LF with pectin (from −26.8 mV to −35 mV) indicates polyelectrolyte adsorption. This suggests that the lactoferrin molecules were either adsorbed onto or coated within the pectin matrix, resulting in a more negatively charged colloidal surface. The increased absolute value of the surface charge is particularly valuable, as a greater negative charge increases electrostatic repulsion, minimizing the likelihood of aggregation and ensuring longer-term colloidal stability in suspension [18]. For the chitosan system, the zeta potential changed from a highly positive charge of +31.37 mV to a less positive charge of +12.9 mV (isoelectric point of the Camel LF is at pH 8.14). This reduction in positive charge affects colloidal stability (tendency to aggregate) compared to highly negatively charged pectin particles.

The interaction with LF and chitosan partially neutralizes the positive charge of the nanoparticle at the experimental pH, due to LF's lower positive charge density. However, the resulting zeta potential is still sufficiently positive for therapeutic application. The net positive charge of both lactoferrin and chitosan may facilitate delivery via electrostatic attraction to the negatively charged intestinal

lining, potentially longer residence time, and greater absorption [27].

The data describing the particle sizes from this study are extremely beneficial. Lactoferrin-pectin nanoparticles have been consistently measured at an average size of 276.3 nm, which lies within the optimal range for use in biological applications. Nanoparticles between 200–500 nm in size are generally the most likely candidates to adhere to mucosal tissues and be taken up through clathrin-mediated endocytosis as compared to microparticles that are larger in size or nanoparticles that are smaller in size, which could be eliminated from the body more rapidly [28]. The larger particle size of the chitosan nanoparticles (496.09 nm) could be a result of the molecular weight of chitosan and the cross-linking density of TPP. The larger size of the chitosan nanoparticles is still appropriate for the administration of these nanoparticles by mouth because this size enables them to be taken up by M-cells located in the Peyer's patches of the GALT [29]. Another very important aspect of particle uniformity demonstrated in these SEM photos is the ability of a monodisperse population to release its payloads uniformly in vivo, which is essential in order to achieve reproducible therapeutic outcomes [30].

The major reason to encapsulate lactoferrin (LF) is to protect LF from damage caused by the stomach's acid. The acidity of the stomach (pH 1.5–3) and the action of pepsin will quickly destroy any encapsulated LF that has not yet reached the intestines, thus reducing LF's effectiveness. According to this study, the microencapsulation systems created in this investigation (especially those made from pectin) provide an effective means of protecting LF from stomach acid and ensuring its availability in the intestinal area. Pectin, under acidic environments or when exposed to calcium ions, gels, thereby preventing gastric fluids from easily penetrating the microencapsulated material and consequently yielding a protected core [31]. Moreover, the LF-pectin coacervate shows good structural integrity, providing protection from dissociation during the harsh acidic conditions of the stomach, thereby providing a physical barrier for transport. Even though chitosan is soluble under acidic conditions, when used in conjunction with tripolyphosphate (TPP), ionic interactions provide a stronger matrix. Research has shown that protein encapsulated in the chitosan/TPP nanoparticle formulation remains protected under simulated gastric fluid conditions, with most of the protein released at the higher pH of the small intestine, a point at which chitosan becomes less soluble [32]. The reported synergism between LF and chitosan suggests that release in the stomach may not be a drawback but may provide joint antimicrobial action.

The finding of antimicrobial synergy between lactoferrin and chitosan, as discussed in the work of Duarte et al. (2023) and Zibaee et al. (2024), is a critical finding that promotes their use as active therapeutics rather than merely as delivery systems of nanoparticles [33,34]. This finding could be attributed to a multi-mechanistic assault on bacterial cells [35]. Chitosan, as a positively charged biopolymer, can interact with negatively charged bacterial cell membranes [36]. Co-microencapsulation of the two agents should facilitate their synergistic interaction. This type of combination approach may be useful for combating antibiotic-resistant bacterial strains, as well as in applications for food preservation and topical wound treatment.

The choice between pectin and chitosan as the primary carrier depends on the specific application. Pectin is a negatively charged biopolymer derived from plants, which is a good candidate for a stable delivery system that responds to fluctuations in pH. Pectin offers several advantages over other delivery systems in that it protects lactoferrin (LF) during its journey through the intestinal tract, but also provides a means by which LF can be released into the bloodstream, thereby assisting in immune function or supporting healthy intestinal microbiota population levels [37]. The classification of Pectin as GRAS (Generally Regarded As Safe) is based on its proven safety and usefulness in food applications. Chitosan also has mucoadhesive and permeation-enhancing capabilities that improve LF bioavailability and provide both local and systemic benefits [38]. The immunostimulatory properties of chitosan can augment the effects of LF, providing a synergistic enhancement of the host's immune response [39]. Lastly, the inherent antimicrobial activity of chitosan, as demonstrated, supports using LF-chitosan nanoparticles as effective targeted antimicrobial therapy.

While the findings are promising, potential limitations and future directions must be considered. Although in vitro models provided solid proof of concept, these formulations need to be tested in more complex in vivo

models to determine true bioavailability, pharmacokinetics, and therapeutic effectiveness [40]. Furthermore, the long-term stability of the colloidal systems produced will require further assessment to determine storage techniques under various storage conditions (temperature/humidity), and requires assessment to determine commercial viability [41]. Future exploration of the microencapsulation of LF in a more complex triple complex system, such as a chitosan-pectin-LF composite nanoparticle, may produce a delivery system leveraging the protective and mucoadhesive properties of both polymers. This could capitalize on the coacervation ability of chitosan and pectin at specific pH values to create a multi-layer wall structure for better control over drug release profiles [42].

The combination of ionic gelation and complex coacervation used in this study was successful and advantageous for production. It generally eliminates harsh organic solvents, is water-based, and is a relatively simple and scalable process, making it attractive for pharmaceutical and food applications.

The results presented here are encouraging, but the limitations and future research directions need to be considered. While in vitro models validated the concept, the true bioavailability, pharmacokinetics, and therapeutic activity of the formulations must be substantiated using more sophisticated in vivo models [40]. Also, the stability of these pH-sensitive formulations under long-term storage at different temperatures and humidity levels needs to be evaluated to assess commercial potential [41]. From a technological standpoint, the techniques used here, ionic gelation and complex coacervation in aqueous solution, avoid hazardous organic solvents and constitute an easy-to-produce method. These characteristics, along with the high microencapsulation efficiencies, suggest successful scaling up. However, a detailed economic analysis of materials, process optimization, and estimated industrial yields is necessary to fully evaluate commercial viability [42].

Additionally, future formulation studies should explore hybrid nanoparticle formulations, such as composite nanoparticles made from chitosan/pectin/LF, which would take advantage of the inherent properties of both polymers while providing opportunities for a multilayered formulation with controlled release profiles and functional properties.

In summary, this work provides strong evidence that both pectin and chitosan can be used as polymer matrices for producing lactoferrin-loaded nanoparticles via electrostatic complexation. These nanocomplexes demonstrated a variety of desirable physicochemical properties, including high loading efficiency, desirable nanoscale size, uniform morphology, and suitable surface charge characteristics for colloidal stability. The LF-pectin system yields a stable, negatively charged particle suitable for maximizing protection, while the LF-chitosan system provides a mucoadhesive vehicle with enhanced and synergistic antimicrobial activity. This work provides a comprehensive comparison and supporting evidence for future development of active oral delivery systems for camel lactoferrin. It has implications for next-generation functional foods focused on gut health management as well as therapeutic formulations for preventing infections and nutraceuticals aimed at improving systemic immunity.

5. Conclusions

This study successfully illustrated the efficient extraction of lactoferrin from camel milk, and then its subsequent loading onto both pectin and chitosan nanoparticles with high efficiency. The microencapsulation, driven primarily by electrostatic interactions and ionic gelation, proved to be a highly feasible approach for developing lactoferrin delivery systems. The main results confirmed that lactoferrin was isolated, purified, and then loaded onto nanoparticles with high efficiencies of 84% for pectin and 81.5% for chitosan.

The characterization of nanoparticles as stable spheres with uniform distribution was confirmed by Scanning Electron Microscopy (SEM). The noticeable changes in zeta potential values before and after lactoferrin loading provided strong evidence of successful loading and effective surface attachment to the biopolymers.

When both biopolymers were compared, pectin nanoparticles showed marginally greater loading efficiency and smaller, more negatively charged nanoparticles, characteristics that make them well-suited for the development of a stable protective barrier for lactoferrin as it traverses the gastrointestinal tract.

Author Contributions

Conceptualization, methodology, validation, writing—review and editing, supervision, project administration, formal analysis, investigation, S.Z.; investigation, data curation, writing—original draft, visualization, S.D.A., S.S. and N.S. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

All data are available in the article. All interested people can benefit from the findings of this article.

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Conflicts of Interest

The authors have no conflict of interest.

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