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RESEARCH ARTICLE

Synthesis and characterization of insulin-loaded nanoparticles fortified milk

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ABSTRACT

Type 1 diabetes caused by the destruction of the pancreatic cells, leads to reduced or no production of insulin, the hormone responsible for lowering blood glucose levels. Insulin is a protein administered subcutaneously to humans for treating, type I diabetes mellitus to control glucose homeostasis when pancreatic β-cells production is not sufficient to ensure daily needs of this hormone. The desire for a more convenient and socially compatible route of insulin administration other than subcutaneous injection has originated several approaches to attempt its oral delivery. The aim of this project is to research and obtain a therapeutic food product containing an oral insulin delivery system. The method used to synthesize the insulin loaded nanoparticles is a two step Ionic pre-gelation method for the preparation of alginate/chitosan nanoparticles. The sizes of the alginate-chitosan nanoparticles were estimated by Scanning Electron Microcopy to range from 326-850nm. The characterization of the synthesized nanoparticles was done using Scanning electron microsopy (SEM), Fourier Transform Infrared spectroscopy (FTIR) and X-Ray Diffraction (XRD) studies. The interaction between the Alginate and Chitosan was confirmed by the FTIR studies. From the results of the XRD studies, it was observed that there was a decline in the crystal structure of the Chitosan after the formation of the nanoparticles. These Nanoparticles were homogenized with Ultra High Temperature (UHT) sterilized milk containing 4.5% fat. The standard milk (control) and the milk containing nanoparticles (product) was subjected to sensory analysis for the color, aroma and consistency. From the sensory analysis of color, aroma and consistency, it was found that there was no significant difference between the control and the product.

Keywords: *Alginate, Chitosan, Insulin, Therapeutic Milk, Oral Delivery System*

INTRODUCTION

Diabetes is referred to a collection of disorders defined by elevated blood glucose levels. The condition arises from an insufficiency in insulin synthesis or activity, or both, due to various factors, leading to metabolic abnormalities of proteins and lipids. The prolonged consequences of hypoglycemia include harm to tissues and organs. Individuals with type 1 diabetes are incapable of producing sufficient insulin. This category accounts for around 5%–10% of all diabetes cases. This type involves the cellular death of beta cells in the pancreas. In type 1 diabetes, the pancreas fails to secrete insulin. Insulin is delivered via subcutaneous injection to manage individuals with type 1 diabetes (Mobasseri *et al*., 2020).

In addition to the psychological barriers for the use of insulin in injectable form, its use is accompanied

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by different complications such as hypoglycemia, lipoatrophy at the injection site and all other risks associated with injections. These complications make the search into alternative routes for insulin delivery is necessity. The other most studied routes are oral, nasal, buccal and pulmonary. Buccal route is based on the micellar solublisation. Nasal route was fully investigated but no commercial exploitation is taking place. Pulmonary route was commercially exploited but unfortunately was withdrawn from the market. Nevertheless, oral route is the most desired and has been investigated thoroughly (Elsayed *et al.,* 2011).

Oral delivery system is the preferred route for administration because it is non-invasive, avoids injections and decreases the risk of infections. It is also physiologically desirable since the exogenous protein imitates the physiological pathway undergoing first hepatic bypass. The intestinal absorption of proteins has been reported in a combination of mechanisms described to explain how the protein cross the intestinal mucosa (Sarmento *et al*., 2007). Peptide and protein drugs are intrinsically poorly absorbable through the intestinal membrane owing to high molecular weight and hydrophilicity. Moreover, they are highly susceptible for enzymatic degradation in the gastrointestinal (GI) tract after oral administration. Entrapment of peptides drugs in micro- and nanoparticulate carriers protects them against the harsh environment of the GI tract until they are absorbed in released or intact particular form. In addition, formulations of the carrier system using mucoadhesive polymers prolongs the direct contact of the particles to the mucosal surface, achieves higher local drug concentration in the mucus layer, and minimizes drug dilution and degradation by the luminal content (Li *et al*., 2008).

Nanoparticles consisting of synthetic biodegradable polymers, natural biopolymers, lipids and polysaccharides have been developed and tested over the past decades. Recently, the idea of using nanoparticles made from natural biodegradable polymers to deliver drugs has provoked great interests. Among them, alginate and chitosan are very promising and have been widely exploited in pharmaceutical industry for controlling drug release (Makhlof *et al*., 2011). Sodium Alginate has a unique property of cross linking in the presence of multivalent cations such as calcium ions in aqueous media. Alginate forms a reticulated structure in contact with calcium ions and this network can entrap proteins. Chitosan is a linear copolymer polysaccharide and it is produced commercially by deacetylation of chitin, which is the structural element in the exoskeleton of the crustaceans. The strong electrostatic interaction of the amino groups of the chitosan with the carboxylic groups of the alginate leads to the formation of the complex chitosan/alginate that becomes the polyelectrolyte complex between chitosan and alginate has been widely used in order to obtain microcapsules for cell encapsulation and devices for the controlled release of drugs or other substances (Finotelli *et al*., 2010).

There is a growing awareness nowadays of the health benefits of a category of bioactive food constituents known as nutraceuticals. This has created society demand for products (functional foods) and preparations rich in these constituents to improve public health. It is well documented that their consumption produces physiological benefits or reduce the long-term risk of developing degenerative diseases. On the other hand, the added bioactive constituents may have some undesirable effects on the taste and odour of the food matrix used as many of these constituents have undesirable taste and odour. Therefore, the objectives of food manufacturers and nutritionists have been to maximize the availability of administered nutraceuticals without compromising consumer acceptability (Salam *et al*., 2012). This study aims to develop functional dairy food (milk) with oral insulin delivery system to overcome the limitations of the painful subcutaneous injections of insulin administration and produce a more patient friendly method of insulin administration. It involves the synthesis, characterization and mixing of the insulin loaded nanoparticles into milk and observation of the settling and dispersion in it.

MATERIAL AND METHODS

Materials

Sodium alginate, chitosan and calcium chloride were purchased from Himedia Laboratories. Recombinant human isophane insulin was purchased under the brand name of Humulin N®from Biocon Laboratory, India. Milk used in this study was purchased from the local market. UHT sterilized standard milk (4.5% fat) which was marketed by Amul Dairy under the Brand name of Amul Gold was used in this study. Deioinzed water (Milli-Q®) was used throughout the process as a medium.

Preparation of Nanoparticles

Insulin loaded Alginate-Chitosan Nanoparticles were produced by dilute alginate solutions containing insulin by inducing an ionotropic pre-gel with calcium counter ions followed by polyelectrolyte complex coating with Chitosan as described by Sarmento *et al,* 2007*)*. with isophane insulin as source of insulin.

Nanoparticle Characterization

The prepared nanoparticles were characterized using scanning electron microscopy, Fourier transform infrared spectroscopy and x-ray diffraction studies. The morphology such as the shape, size and the occurrence of aggregation of particles were studied by scanning electron microscopy. The interaction between the various components of the nanoparticulate systems were studied using Fouriertransform infrared spectroscopy. The crystalline nature of the components of the nanoparticles namely, the alginate and the chitosan were studied using x-ray diffraction studies before and after the formation of the nanoparticles.

Scanning Electron Microscopy

Scanning electron microscopy was done using a low vacuum FEI QuantaFEGSEM (5kV) at 80 Torr. Alginate chitosan nanoparticles were synthesized with three different concentrations of chitosan. This synthesis was carried out and the influence of the chitosan concentration on the size of the nanoparticles formed was studied using scanning electron microscopy. The lyophilized nanoparticles powders were mounted on a stub using a carbon tape and then examined for the shape, size and the aggregation nature of the nanoparticles.

Fourier Transform Infrared Spectroscopy

FTIR spectra were measured using a Bruker-α spectrometer. The samples were gently mixed with micronized KBr powder and compressed into discs at a force of 10 kN for 1 minute using a manual tablet presser. The interferogram was collected in absorption from 400 cm^{-1} and 4000cm^{-1} region at room temperature.

X-Ray Diffraction

X-Ray diffraction of the lyophilized powder samples was done using PANalyticalXpert Pro XRD in powder mode. The scanning was done at the rate of 0.05°/ step and 2 sec/step with CuKα as the source of x-ray with a wavelength of 0.154 nm. The powdered samples of chitosan, alginate, alginate/chitosan nanoparticles and insulin loaded alginate/chitosan nanoparticles were analysed for the crystalline nature.

Association Efficiency (AE)

The association efficiency of the insulin to the nanoparticles was analysed to find out the amount of insulin bound to the nanoparticles. The insulin determinations were done in triplicates. It was calculated using the following formula:

$$AE = \frac{(total amount of insulin) - (amount of insulin in supernatant)}{total amount of insulin} X\ 100$$

In-Vitro Insulin Release Studies

The insulin release profile from nanoparticles in simulated gastric and intestinal pH was carried out to study the amount of insulin released from the nanoparticles in to the medium simulating the stomach and intestinal conditions. The nanoparticles were placed into test tubes containing 20 mL of HCl buffer pH 1.5 or PBS buffer pH 7.4. At appropriate intervals of 30 minutes, aliquots of 500 µL were taken and replaced by fresh buffer. Aliquots were centrifuged at 5000g for 15 minutes. The amount of insulin released from the nanoparticles was evaluated by HPLC.

Incorporation of nanoparticles into milk

The lyophilized nanoparticles in the form of powders were added to UHT pasteurized milk. The total yield of powders from a single batch of prepared nanoparticles was added to 100 mLmLof UHT pasteurized milk using a blender. The milk containing the insulin loaded nanoparticles was kept at 4°C. The addition of the nanoparticles was done under sterile conditions to prevent the spoilage of the product.

Sensory analysis

The standard milk and the milk containing insulin loaded nanoparticles were evaluated for acceptability by a panel of judges for aroma, color and appearance, consistency according to 9-point hedonic scale wherein a score of 1 represented dislike extremely and score of 9 represented like extremely.

RESULTS AND DISCUSSION

Synthesis and characterization of Insulin loaded nanoparticles by Spontaneous Emulsification Solvent Diffusion Method

Chitosan solutions at concentrations of 0.05%, 0.07%, and 0.09% were prepared for analysis, with the dimensions and characteristics of the resulting nanoparticles detailed in Table 1. Each experiment was conducted in triplicate.

Scanning electron micrographs revealed that the synthesis process using the lowest chitosan concentration (0.05%) produced cylindrical nanofibers, as shown in Figure 1. Table 2 presents the variance analysis for nanoparticle sizes at chitosan concentrations of 0.07% and 0.09%, indicating that the size reduction between these concentrations was statistically insignificant. The scanning electron micrographs of the nanoparticles produced from the three chitosan concentrations—0.05%, 0.07%, and 0.09%—are shown in Figures 1, 2, and 3, respectively.

The size of the nanoparticles was measured to be 68.4±2.800 nm and 66.7±1.201 nm when synthesizing nanoparticles with starting chitosan concentrations of 0.07% and 0.09%, respectively. Particles of a size smaller than 100 nm shown a 2.5-fold increase in uptake compared to microparticles measuring 1µm (Panyam and Labhasetwar, 2003).

Preliminary attention was given to polyelectrolyte interactions and insulin trapping in order to investigate the linkages between components of the nanoparticulate systems. The interaction between the carboxyl (-COO-) group of the anionic polymer and the amino group (-NH3+) of chitosan is a known phenomenon. This interaction leads to the formation of an ionic complex between the two molecules, as described by Sarmento *et al,* 2006).

Fourier Transform Infrared (FTIR) spectroscopy

The synthesis of insulin-loaded nanoparticles was carried out, and the interactions between the nanoparticle constituents, specifically chitosan and alginate, were analyzed using Fourier Transform Infrared (FTIR) spectroscopy. Figure 4 shows the FTIR spectrum of pure chitosan, which was examined to identify the highest values within the ranges of 3000–3120 cm^{-1}, 1480–1530 cm^{-1}, and 1590–1620 cm^{-1}. These peaks correspond to the presence of amino groups ($-NH_3^+$) in the spectrum. The observed peaks in Figure 4 confirm the presence of amino groups ($-NH_3^+$) in pure chitosan at wavenumbers of 1501.72 cm^{-1}, 1654.73 cm^{-1}, 3045.94 cm^{-1}, 3079.94 cm^{-1}, and 3147.95 cm^{-1}.

The highest point within the range of 1710 cm^{-1} – 1740 cm^{-1} indicates the existence of saturated carboxylic acids. Figure 5 displays a prominent signal at 1738.31 cm^{-1}, indicating the existence of the carboxylic group in the alginate solution employed for nanoparticle production.

Table 1.The initial concentration of chitosan, size and nature of the nanoparticles

S.No.	Chitosan Concentration% (w/v)	Size (in nm)	Nature
1	0.05	--	Nanofibres like structures
2	0.07	68.4±2.800	Agglomerated
3	0.09	66.7±1.201	Agglomerated

Table 2 Peak Values of FTIR spectrum of Alginate, Chitosan, and Nanoparticles formed with initial chitosan concentrations of 0.05%, 0.07%, and 0.09%.

Samples	Alginate (COO^-) & Chitosan (NH_3^+)	Initial Concentration of Chitosan		
Functional groups		0.05%	0.07%	0.09%
COO^-	1738.31	1738.49	1732.83	1742.75
NH_3^+	1501.72	1517.46	1525.96	1514.23
	1654.73	1640.73	1606.73	1646.40
	3045.94	3284.30	3355.14	2920.16
	3079.94	3315.47	3386.31	2952.75
	3147.95	3348.05	3416.06	3455.74

Fig 1.Scanning Electron Micrographs of nanofibre-like structures formed with initial Chitosan Concentration of 0.05%

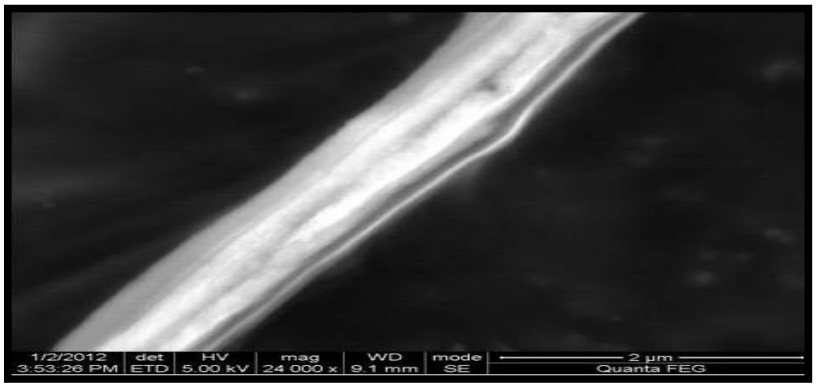


Fig 2. Scanning Electron Micrographs of nanoparticles with initial chitosan concentration of 0.07%

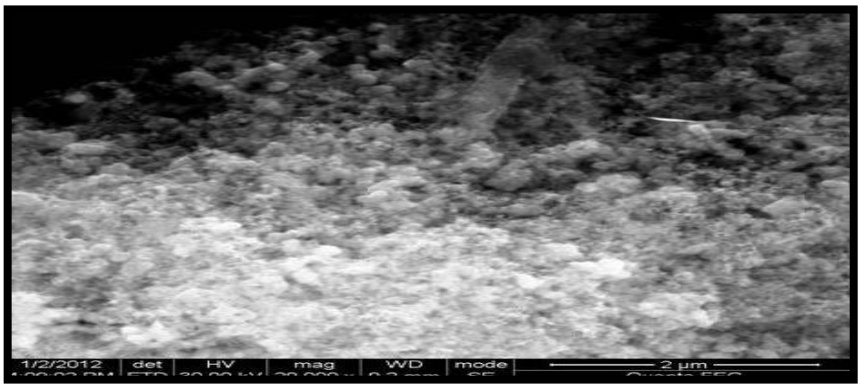
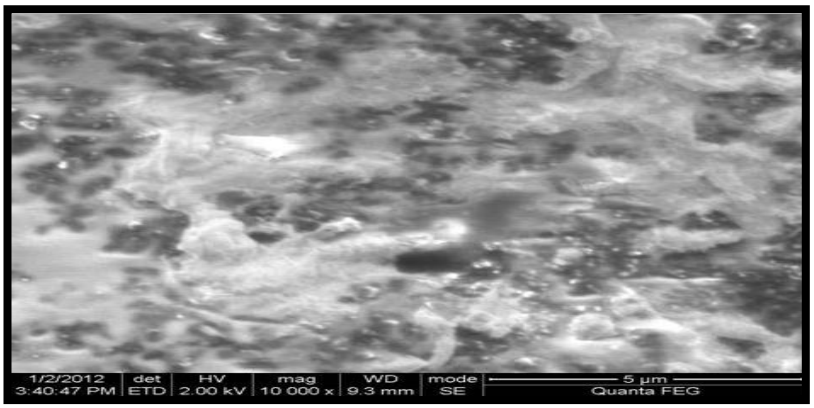


Fig 3. Scanning Electron Micrographs of Nanoparticles synthesized with initial chitosan concentration of 0.09%



An analysis was conducted on nanoparticles synthesized using chitosan concentrations of 0.05%, 0.07%, and 0.09% to examine the presence of amino and carboxyl groups in their respective FTIR spectra. Figure 6 displays the FTIR spectra of alginate, chitosan, and nanoparticles prepared with chitosan concentrations of 0.05%, 0.07%, and 0.09%, respectively. The peak values for chitosan, alginate, and the nanoparticles synthesized with initial chitosan concentrations of 0.05%, 0.07%, and 0.09% are presented in Table 2. Each spectrum was individually analyzed to identify any shifts in the peak values of the amino and carboxyl groups, which would suggest interactions between chitosan and alginate in the nanoparticulate systems.

Fig 4. FTIR spectrum of Chitosan

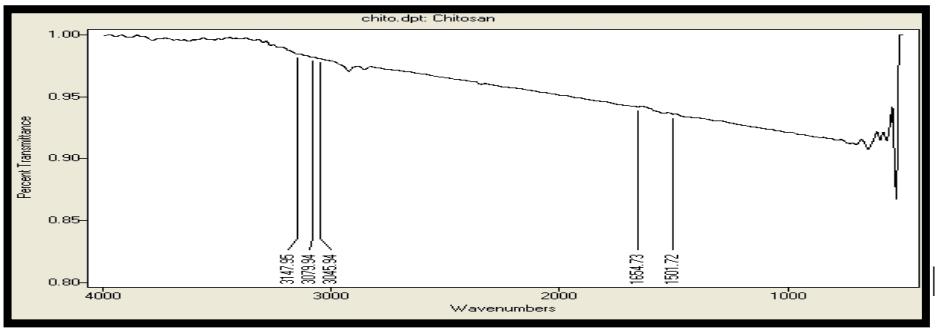
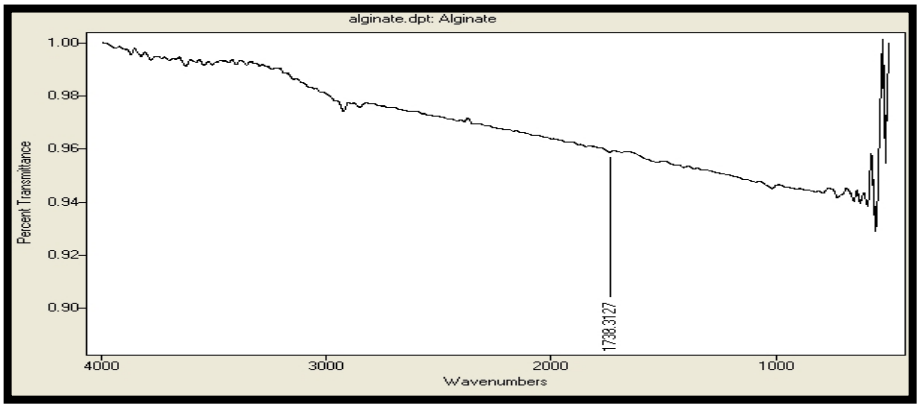


Fig. 5 FTIR spectrum of alginate



The analysis revealed a slight shift in the peak values of the carboxyl group. Specifically, the peak shifted from 1738.31 cm^{-1} in alginate to 1738.49 cm^{-1} in nanoparticles with an initial chitosan concentration of 0.05%. Additionally, there was a shift in the peak values of the amino groups, moving from 1501.72 cm^{-1}.

The wavenumber range for chitosan was observed between 1654.73 cm^{-1} and 3147.95 cm^{-1}, while for nanoparticles synthesized with a chitosan concentration of 0.05%, the range extended from 1517.46 cm^{-1} to 3348.05 cm^{-1}.

The spectra indicate a shift in the peak values of the carboxyl group, moving from 1738.31 cm^{-1} in alginate to 1732.38 cm^{-1} in nanoparticles with an initial chitosan concentration of 0.05%. Similarly, shifts are observed in the peak values of the amino groups, which change from 1501.72 cm^{-1}, 1654.73 cm^{-1}, 3045.94 cm^{-1}, 3079.94 cm^{-1}, and 3147.95 cm^{-1} in chitosan to 1525.96 cm^{-1}, 1606.73 cm^{-1}, 3355.14 cm^{-1}, 3386.47 cm^{-1}, and 3416.06 cm^{-1} in nanoparticles formed with a chitosan concentration of 0.07%.

For nanoparticles prepared with an initial chitosan concentration of 0.09%, the carboxyl group peak shifted from 1738.31 cm^{-1} in alginate to 1742.75 cm^{-1} in the nanoparticles. The amino group peaks also displayed changes, moving from 1501.72 cm^{-1}, 1654.73 cm^{-1}, 3045.94 cm^{-1}, 3079.94 cm^{-1}, and 3147.95 cm^{-1} in chitosan to 1514.23 cm^{-1}, 1646.40 cm^{-1}, 2920.16 cm^{-1}, 2952.75 cm^{-1}, and 3455.74 cm^{-1} in the nanoparticles, respectively.

The results of the study indicate that the shifts in the peaks representing the amino group and the carboxyl groups in the FTIR spectrum of the nanoparticles provide evidence of the interaction between the carboxyl (-COO-) group of the anionic polymer and the amino group (-NH3+) of Chitosan. This interaction leads to the formation of an ionic complex between the two compounds in the nanoparticulate system.

X-ray diffraction (XRD) studies

The nanoparticles containing Humulin N, along with the nanoparticulate system components, chitosan and alginate, were analyzed using X-ray diffraction (XRD) to assess any

Table 3 Peak Values of FTIR spectra of Alginate, Chitosan and Nanoparticles with Humulin N

Samples Functional groups	Alginate (COO^-) & Chitosan (NH_3^+)	Nanoparticles with Humulin N
COO^-	1738.31	1738.49
NH_3^+	1501.72	1510.38
	1654.73	1646.40
	3045.94	2931.50
	3079.94	2964.08
	3147.95	3420.32

changes in the crystalline structure of chitosan and alginate before and after nanoparticle formation. XRD analysis of the individual components showed that chitosan exhibited a crystalline structure, while alginate appeared to be amorphous. The crystalline nature of chitosan was evidenced by a prominent peak at 20.38° in its X-ray diffractogram. In contrast, alginate's diffractogram displayed no distinct peaks, indicating its amorphous nature. Figure 7 shows the X-ray diffractograms of chitosan and alginate before nanoparticle synthesis.

The X-ray diffraction pattern of nanoparticles created with an initial chitosan content of 0.05%., 0.07% and 0.09%. represents the values of Figure 8. The diffractogram shows that a solitary significant peak of chitosan vanished following the creation of the nanoparticles. All three nanoparticles have an amorphous structure.

Thus, the decline in crystallinity of chitosan may be caused due to interaction with alginate and other components of the nanoparticulate system. The result

Fig 6. FTIR spectrum of Chitosan (blue), Alginate (red), and Nanoparticles with initial chitosan concentration of 0.05% (green), 0.07% (orange), and 0.09% (violet)

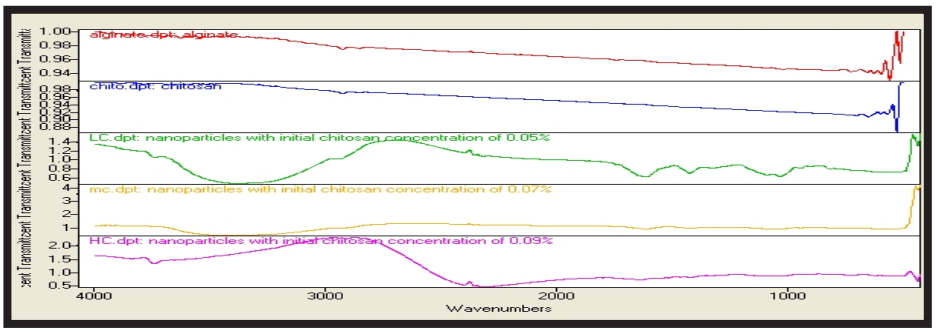


Fig 7. X-Ray Diffractogram of Alginate (red) and Chitosan (black) before the formation of nanoparticles

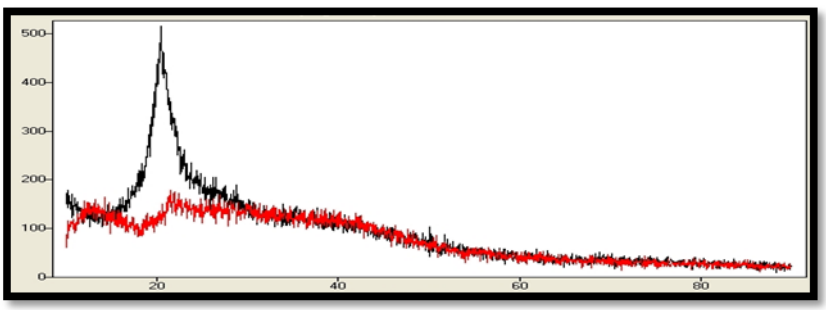
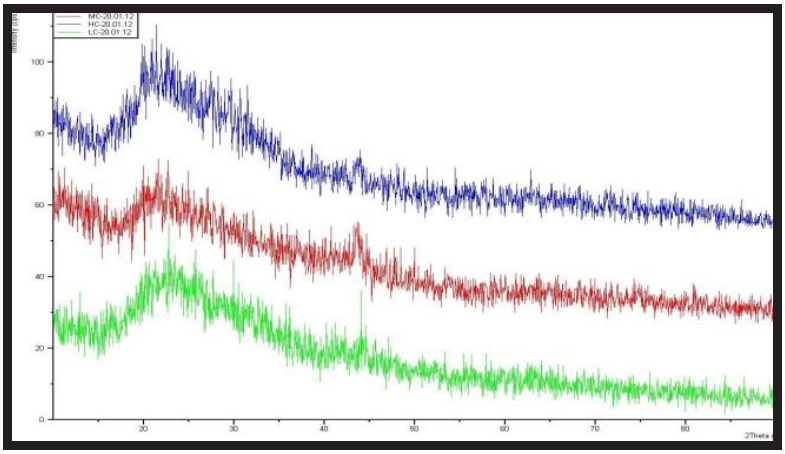


Figure 8 X-Ray Diffractograms of Nanoparticles with initial chitosan concentration of 0.05% (Green), 0.07% (Blue), and 0.09% (Red)



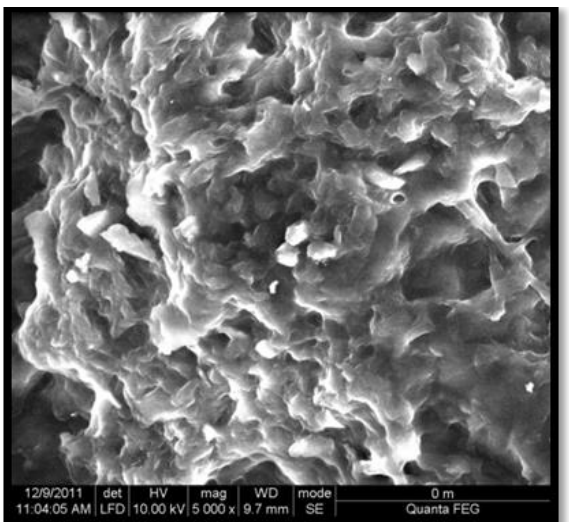
obtained in the current study is in accordance with the observations by Lin *et al*, 2007 where the disruption of the crystal structure of the chitosan was observed after chitosan combined with γ-PGA via electrostatic interactions to form Chitosan-γ-PGA nanoparticles.

Synthesis and characterization of Insulin loaded nanoparticles by Ionic Pregelation with Polyelectrolyte complexation method

Scanning Electron Microscopy studies

Alginate chitosan nanoparticles were synthesized. On slow addition of the chitosan over the pregel mixture (formed by addition of $CaCl_2$ over the alginate insulin solution), the solution changed from clear to opalescent and finally turning turbid indicating the formation of the insulin loaded nanoparticles. This synthesis was carried out and the size of the nanoparticles formed was studied using scanning electron microscopy. Isophane insulin (Humulin N®) suspension was taken as source of insulin for synthesis. The sizes of the nanoparticles formed ranged between 326 nm – 850 nm and the nanoparticles were not regular spheres as observed from the scanning electron micrographs. The scanning electron micrographs of the nanoparticles synthesized is represented in Figure 9. Particles below 1000 nm are desirable as they are better absorbed in the intestinal tract.

Fig 9. Scanning Electron Micrographs of Insulin Loaded Nanoparticles



Fourier Transform Infrared Spectroscopy (FTIR) studies

The nanoparticles synthesized with Humulin N as insulin sources were subjected to FTIR studies. Figure 10 shows a combined representation of the FTIR spectra of alginate, Chitosan and nanoparticles synthesized with Humulin N. The peak values were noted and investigated for the shift in the peak values of amino group and the carboxyl group.

The peak values are displayed in the table 1. The peak values of alginate and chitosan were subjected to comparison with the peak values of nanoparticles with Humulin N individually in order to ascertain the interaction between the components of the nanoparticulate systems.

The following observations were made from the FTIR spectra of Humulin N as insulin source. There is a presence of shifts in the peak values of amino groups from 1501.72 cm^{-1}, 1654.73 cm^{-1}, 3045.94 cm^{-1} , 3079.94 cm^{-1} and 3147.95 cm^{-1} in chitosan to 1510.38

Fig 10. FTIR spectra of Alginate (red), Chitosan (blue), Nanoparticles with Humulin N (green)

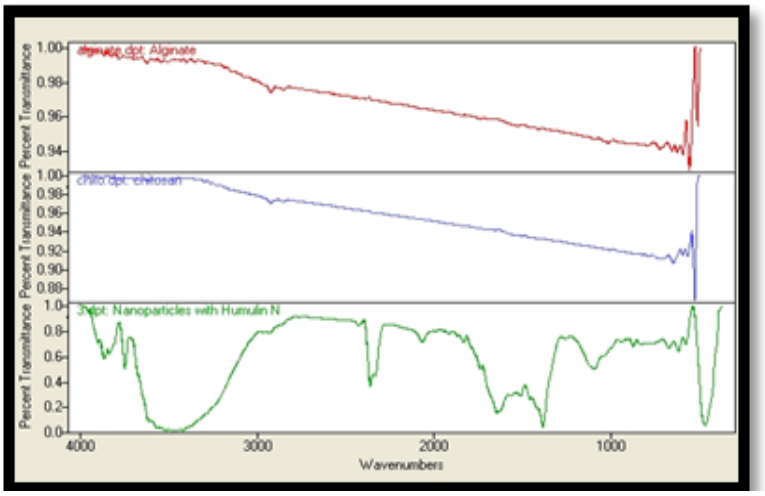
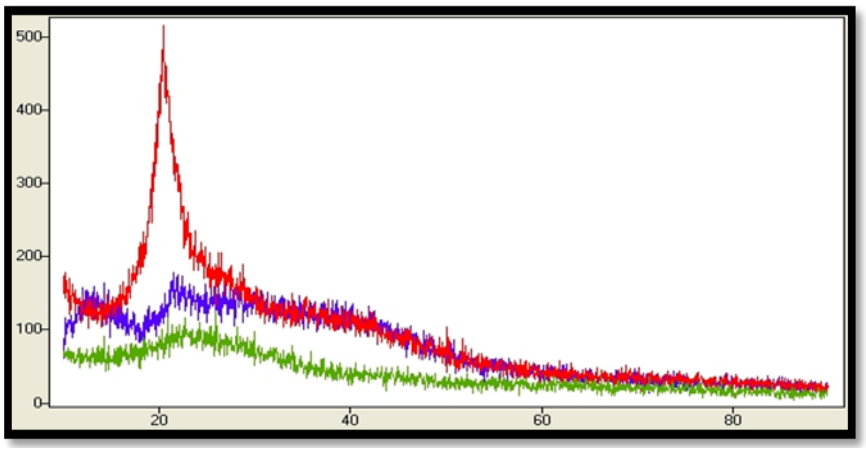


Fig 11. X-Ray Diffractogram of Chitosan (red) , alginate (blue)and Nanoparticles with Humulin N (green)



cm^{-1}, 1646.40 cm^{-1}, 2931.30.66 cm^{-1}, 2964.08 cm^{-1}, 3420.32 cm^{-1} in nanoparticles with Humulin N as insulin source. The shift was also observed in the carboxyl group peaks from 1738.31 cm^{-1} in alginate to 1738.49 cm^{-1} in the nanoparticles with Humulin N.

From the results of the present study, it is evident that the shifts in the peaks representing the amino group and the carboxyl groups in the FTIR spectrum of the nanoparticles confirms the interaction between the the carboxyl ($-COO^-$) group of the anionic polymer and the amino group ($-NH_3^+$) of chitosan to form an ionic complex between the two compounds in the nanoparticulate system in the present study.

X-Ray Diffraction studies

The observations from the X-Ray Diffractogram of nanoparticles with H Humulin N shows the decrease in the crystallinity of the Chitosan which is evident from the absence of a single clear peak at 20.38° in the X-Ray diffractogram with the intensity equal to that of the Chitosan before the formation of the nanoparticles. The X-Ray diffractogram of chitosan, alginate and the nanoparticles with Humulin N is shown in the figure 11.

Association Efficiency

The nanoparticles were analyzed for the amount of insulin associated with it after the synthesis. This study was carried out by investigating the supernatant obtained after the centrifugation of the reaction mixture to HPLC for the presence of insulin. Standard calibration curves for both types of insulin used in the study were constructed by running known amounts of insulin (Humulin N) in the HPLC. The Standard Chromatogram of the Humulin N are as shown in the figure 12. The peak at approximately 11 minutes indicates presence and amount of insulin in the sample.

In the above chromatograms, a peak at approximately 6 minutes is observed. This presence of this peak may not be considered as there is a similar peak observed when deionised water was run in the same column with the same linear gradient program as followed for analysis of standards and the sample .Figure 13 displays the standard calibration curve of Humulin n. The efficiency of the insulin's association with the nanoparticles was determined to be 55.3±3.066 (%)

Invitro Insulin Release Studies

The amount of insulin released in the simulated gastric fluid and simulated intestinal fluid is as shown in the Figure 14. It is observed that 34.7% of the associated insulin was released in the simulated Gastric fluid and that 77.27% of the associated insulin was released in the simulated intestinal fluid.

In the first two hours of the study, a burst release pattern was observed, where a significant amount of insulin was rapidly released from the nanoparticles into both simulated gastric and intestinal fluids. This initial release likely stems from weak interactions between insulin and the nanoparticle surfaces, as the alginate core may lack sufficient strength to fully retain the insulin. Approximately 30% of the insulin maintained a stable interaction with the nanoparticles even after 2 hours in the release media. Comparatively, the type of insulin used for encapsulation did not significantly affect the release pattern, aligning with findings by Sarmento *et al*., 2006b. The standard chromatogram of the in vitro insulin release study is shown in Figure 15

A distinct peak at approximately 11 minutes suggests the presence of insulin without detectable

Fig 12. Chromatogram of standard Humulin N

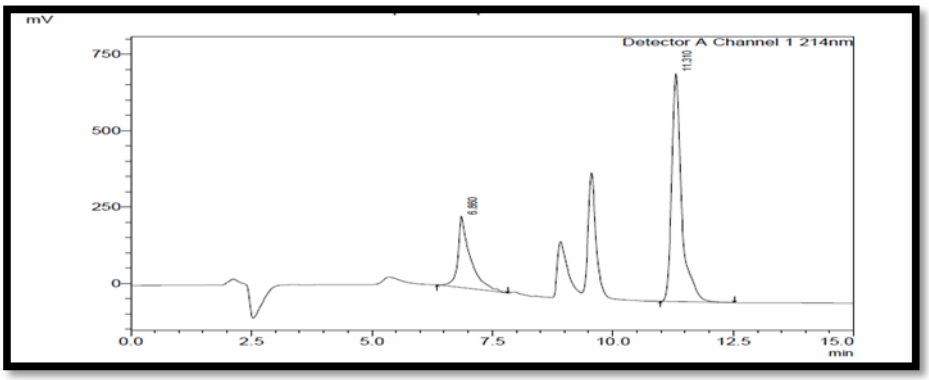


Fig. 13 Standard Calibration curve of Humulin N in HPLC.

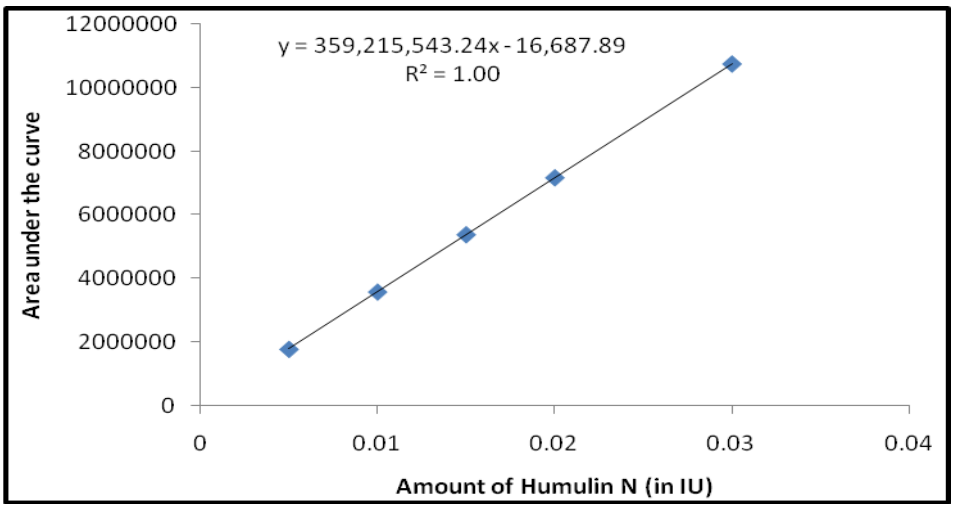
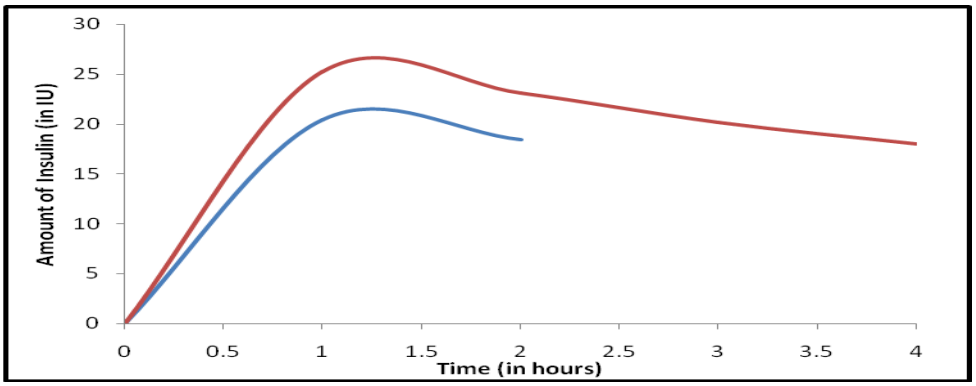


Fig 14. Insulin Release vs Time Profile in Simulated Gastric fluid (Blue) and Simulated Intestinal fluid (Red)



hydrolysis degradation products. This aligns with findings by Sarmento *et al.* (2006b), indicating that nanoparticulate carriers can preserve the primary structure of insulin during encapsulation. Therefore, changing the type of insulin as the core material has minimal impact on the coating material's ability to retain insulin's primary structure.

Nanoparticle incorporated into milk

The insulin loaded nanoparticles which were investigated for their release characteristics were synthesized again and were lyophilized. The lyophilized nanoparticles were then added to 4.5% fat standard milk and mixed thoroughly for few seconds using a blender. There were no settling or sedimentation of the nanoparticles in the milk. The milk containing Insulin loaded nanoparticles are as shown in the figure 16.

In order to study if there are any differences in the physical properties of the control (milk without nanoparticles) and the product (milk containing the insulin loaded nanoparticles), a sensory analysis of appearance (whiteness), aroma and the consistency was conducted on a 9 point hedonic scale from 20 panel members . The results of the sensory analyses of appearance, aroma and consistency are as represented in figure 17.

The nutritional profiles of the milk without and with the nanoparticles are as shown in Figure18 and Figure 19 respectively. From the results of the nutritional analysis studies, it was observed that there is no significant difference in the total fat, ash and moisture content between the control and the product. A difference of 0.2g in protein per 100 mL of milk was observed. The product showed 0.2 g increase in protein compared to the control.

This may be due to the release of insulin which are held on the surfaces of the coating material by weak interactions. Further investigations are needed to be made in the development of a method for estimating the presence and the amount of insulin present in the milk samples.

Figure 15 Chromatogram of Humulin N in simulated intestinal fluid

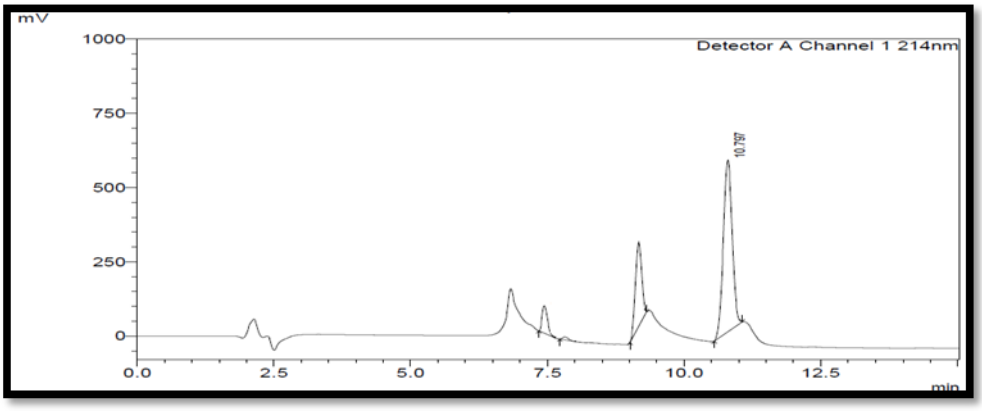


Figure 16 Standard milk (left) and milk containing insulin loaded nanoparticles (right)

Figure 17. Sensory Analysis of Standard milk and Product (Milk Containing Insulin loaded nanoparticle

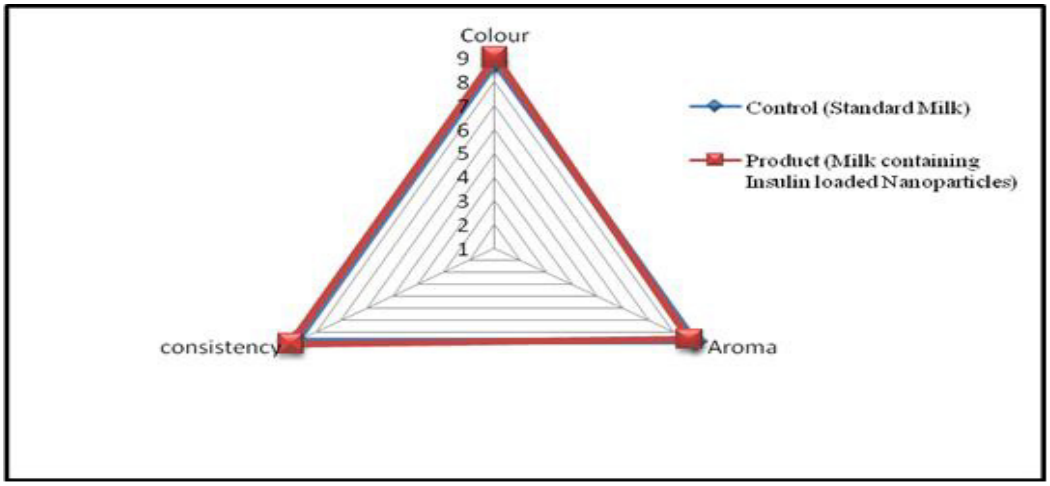
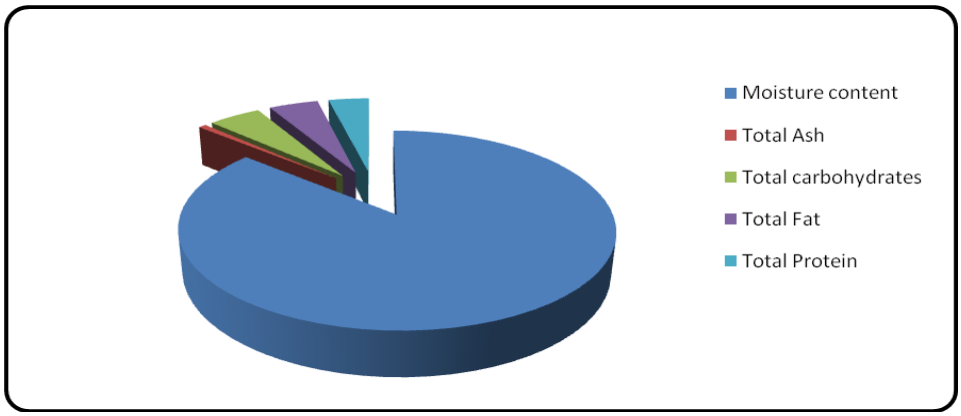


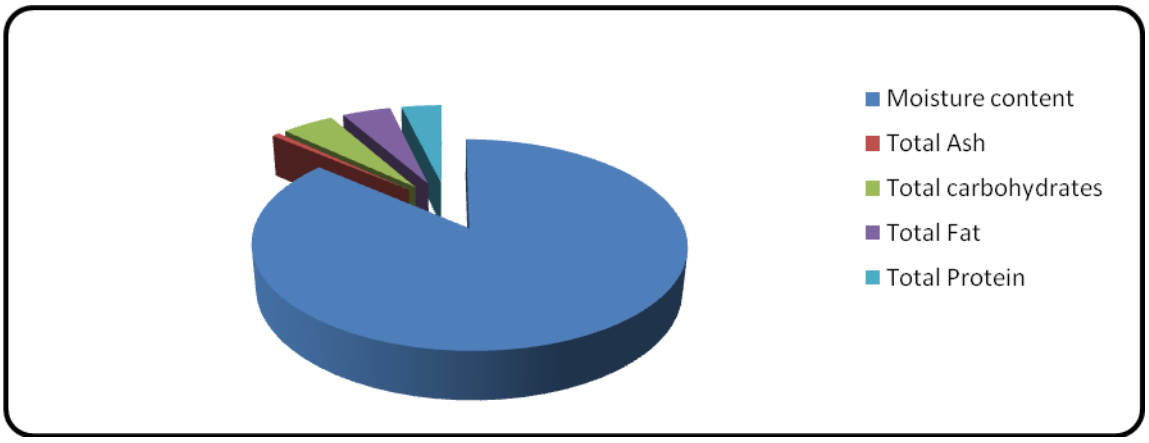
Figure 18 Nutritional Profile of Control (standard milk)



CONCLUSION

The study concludes that nanoparticles synthesized with an initial concentration of 0.05% formed nanofiber-like structures, while those with 0.07% and 0.09% concentrations showed agglomeration in scanning electron micrographs. FTIR analysis revealed well-established interactions between the components across all three nanoparticle variants. Additionally, the crystalline nature of the components diminished or was completely disrupted upon nanoparticle formation. Due to the relatively low insulin association efficiency within the nanoparticles synthesized through this method, further studies, such as in vitro release

Figure 19 Nutritional Profile of the Product (Milk containing Insulin loaded nanoparticles)



testing, were not pursued. The Isophane insulin-loaded nanoparticles were subsequently mixed with milk to produce the final product, which underwent nutritional analysis. This analysis indicated an increase in protein content compared to the control sample. Sensory evaluations of color, aroma, and consistency were conducted on both the product and the control, with results showing no significant differences, suggesting that the inclusion of insulin-loaded nanoparticles did not impact these attributes. Based on this study, it is concluded that the product containing insulin-loaded nanoparticles demonstrates effective insulin release characteristics and exhibits no notable differences in nutritional or sensory attributes, such as aroma, appearance, and consistency, when compared to standard milk.

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