RESEARCH PAPER

Formulation And Evaluation Of Mucoadhesive Microspheres Of Nitroglycerine For Nasal Delivery

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ABSTRACT
Formulate and evaluate mucoadhesive polymeric microspheres of Nitroglycerine for intranasal delivery to bypass hepatic first-pass metabolism, extend mucosal residence time, and provide controlled antianginal therapy.
Microspheres were prepared by emulsification cross-linking using Chitosan with glutaraldehyde. Formulations were evaluated for yield, particle size, entrapment efficiency, swelling index, *in vitro* mucoadhesion, and *ex vivo* permeation across sheep nasal mucosa.
Optimized microspheres were spherical (15-42 µm) with a drug entrapment efficiency of 70.90%–81.76%. They demonstrated high mucoadhesive strength adhesion at 1 hour i.e. 61.17-72.45% and a biphasic release profile: a rapid initial burst within 15 minutes followed by controlled, diffusion-controlled drug release over 8 hours.
Mucoadhesive microspheres are a viable, non-invasive system for intranasal Nitroglycerine delivery, offering a promising alternative for both acute and prophylactic management of angina pectoris.
KEYWORDS: Nitroglycerine, Mucoadhesive Microsphere, Nasal Delivery, Anti-anginal Agent, Chitosan, Emulsification Cross-Linking Technique.
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1. INTRODUCTION

The non-invasive administration of therapeutic agents has remained a pivotal focal point in modern drug delivery design, with an increasing emphasis on bypassing the limitations inherent to conventional oral and parenteral pathways. While the oral route remains highly accepted by patients, it frequently presents challenges such as extensive hepatic first-pass metabolism, enzymatic degradation within the gastrointestinal tract, and delayed onset of action (Chaturvedi et al., 2011; Donnelly et al., 2011). Parenteral administration, although offering rapid systemic availability, suffers from low patient compliance due to its invasive nature, risk of infection, and the need for professional supervision (Dondeti, n.d.). Consequently, the exploration of alternative transmucosal routes has intensified, among which the intranasal route has emerged as a exceptionally viable and patient-friendly strategy for systemic drug delivery (Clementino et al., 2025; Porfiryeva et al., 2026).

The unique anatomical and physiological architecture of the nasal cavity provides a compelling platform for drug absorption. Characterized by a large surface area (approximately 150 to 160 cm^2, a highly permeable epithelial monolayer, and a dense, subepithelial capillary network, the respiratory region of the nasal mucosa enables rapid drug transport directly into the systemic circulation (Krishnarajan, n.d.; Porfiryeva et al., 2026). This immediate absorption bypasses the destructive environment of the gastrointestinal tract and avoids first-pass hepatic clearance entirely, mimicking the rapid pharmacokinetics of an intravenous injection (Chaturvedi et al., 2011; Clementino et al., 2025). Furthermore, the proximity of the olfactory region to the central nervous system offers a unique opportunity for direct nose-to-brain targeting along olfactory and trigeminal nerve pathways, bypassing the restrictive blood-brain barrier (Porfiryeva et al., 2026).

Despite these distinct physiological advantages, the therapeutic efficacy of conventional nasal formulations (such as liquid sprays and drops) is significantly curtailed by natural physiological defense mechanisms. Chief among these is **mucociliary clearance**, a process where the continuous beating of ciliated epithelial cells clears foreign particles and entrapped substances from the nasal cavity every 15 to 20 minutes (Krishnarajan, n.d.; Porfiryeva et al., 2026). This rapid clearance shortens the residence time of the formulation on the absorptive mucosa, leading to low and inconsistent bioavailability. To overcome this limitation, the design of **mucoadhesive drug delivery systems** has been heavily exploited (Donnelly et al., 2011). By utilizing hydrophilic macromolecules capable of establishing physical entanglement, hydrogen bonding, or electrostatic interactions with the native mucin layer, these systems actively prolong the residence time of the

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dosage form at the absorption site, thereby facilitating controlled drug release and enhanced bioabsorption (Chaturvedi et al., 2011; Porfiryeva et al., 2026).

Among the various mucoadhesive strategies, **particulate microspheres** exhibit substantial technical merit. Compared to monolithic gels or simple solution sprays, microspheres spread uniformly across the nasal mucosa, swell upon hydration to temporarily maximize contact intimacy, and protect encapsulated therapeutic candidates from local enzymatic degradation (Chaturvedi et al., 2011; Clementino et al., 2025). This formulation topology is particularly suited for **Nitroglycerine** (glyceryl trinitrate), a potent antianginal agent widely used for the acute management and prophylaxis of angina pectoris. When administered orally, nitroglycerine undergoes near-complete hepatic first-pass degradation, resulting in an abysmal oral bioavailability (less than 10%). While sublingual tablets circumvent this issue, they are limited by a short duration of action, potential mucosal irritation, and the mandatory requirement of patient consciousness during an attack.

Developing mucoadhesive microspheres of nitroglycerine for intranasal delivery presents a robust methodology to achieve both a rapid therapeutic onset—essential during acute cardiac episodes—and a controlled plasma profile for prophylactic management. Accordingly, the objective of the present study is to formulate and systematically evaluate mucoadhesive microspheres of nitroglycerine designed for nasal administration. This investigation focuses on optimizing the polymeric composition using biocompatible polymers, and comprehensively evaluating the resulting formulations with respect to production yield, particle size distribution, surface morphology, drug entrapment efficiency, *in vitro* mucoadhesive strength, and *ex vivo* transnasal permeation characteristics.

2. MATERIALS AND METHODS

2.1 Materials: Nitroglycerine was obtained as a gift sample from Orex Pharma Pvt. Ltd., Mumbai. Chitosan was procured from Sisco Research Laboratory Pvt. Ltd., Delhi. Ethanol, Glutaraldehyde, DOSS, Sodium hydroxide, Sodium chloride, Light Liquid Paraffin, Heavy liquid paraffin and acetic acid were purchased from SD Fine chemicals, Mumbai.

2.2 Compatibility Study: [2,43,60&69] The I.R. Spectroscopy was used to verify the compatibility study. I.R. Spectroscopy was used to get the FTIR spectra of the formulation and chitosan. The resulting FT-IR spectra were used to determine the compatibility between the pure medication and polymer. The sample was scanned over the wave number, and the 4000-400 cm^{-1} wave number was used to record the spectra.

2.3 Method Of Preparation By W/O Emulsion Cross Linking Method [8,44,45,46,62&63]

- Step-1: Taken a 10 ml of 2% aqueous acetic acid solution (2 ml acetic acid dissolved in 100 ml distilled water).
- Step-2: Now taken a given quantity of (0.1/0.2/0.3 gm) of chitosan was dissolved in a 10 ml of 2% aqueous acetic acid solution by continuously stirring until a homogenous solution was obtained.
- Step-3: Then added the drug (0.1 gm) slowly with stirring in prepared chitosan solution. Dispersed phase was prepared.
- Step-4: Now we prepared stabilizing agent with DOSS. Given quantity about 50 mg of DOSS was dissolved in 25 ml glycerine continuously stirring by glass road.
- Step-5: Then 50 ml heavy and 50 ml light liquid paraffin was taken in 500ml beaker, place under electronic stirring machine for 15 mins at 1640 rpm.
- Step-6: Added DOSS (stabilizing solution) as per the given quantity (2 ml or 3 ml) constant stirring at 1640 rpm for 15 minutes. External Phase was prepared.
- Step-7: The dispersed phase (drug + chitosan + acetic acid) was added slowly to the above prepared external phase under constant stirring at 1640-1680 rpm for 15 minutes.
- Step-8: Added Glutaraldehyde was added to above solution using continuously stirring for next 2 or more hours at 1640 rpm.
- Step-9: Microspheres was prepared and filtered using vacuum filtration.
- Step-10: Firstly, washed with the n-hexane and then washed with the water. Kept for air drying about 24 hours and then stored in desiccator until next use.

Table 1: Different Variables of Microspheres

	Formulation and process variables			Constant parameters		
Fo. Code	**Drug: Polymer**	**Vol. of stabilizing agent (DOSS)**	**Vol. of cross-linking agent (Glutaraldehyde)**	**Constant parameter aq. to oil phase**	**Stirring rate**	**Cross linking**
NGN1	1:1	2 ml	2 ml	10 :100	1640-1680 rpm	2 hrs
NGN2	1:2	2 ml	2 ml			
NGN3	1:3	2 ml	2 ml			
NGN4	1:1	2 ml	4 ml			
NGN5	1:2	2 ml	4 ml			
NGN6	1:3	2 ml	4 ml			

2.4 Characterization & Evaluation (1,7, 10,11,22,45,46,47,56,60,61,62,63&70)

2.4.1 Determination of Percentage Yield of Microspheres: [10,11,33&50] By comparing the weight of the finished product after drying to the initial total weight of the medication and polymer used to make the microspheres, the percentage yield of prepared microspheres was calculated. After that, the dried microspheres were gathered and precisely weighed. Next, the formula below was used to compute the % yield.

$$\% \text{ yield} = \frac{\text{Mass of microspheres obtained}}{\text{Total weight of drug and polymer}} \times 100$$

2.4.2 Determination of % Drug Content and % Entrapment Efficiency: [4] 100 mg of precisely weighed microspheres were crushed in a glass mortar and pestle, and with the aid of an ultrasonic stirrer, the powdered microspheres were dissolved in 100 ml of ethanol. The solution was filtered through Whatmann filter paper no. 41 after 12 hours, and the filtrate's drug content was measured at 210 nm using a UV-visible spectrophotometer.

2.4.3 Particle Size Analysis: [18,19&39] Each microsphere was assessed in terms of its dimensions and form. The microsphere-prepared slide was inspected using an optical microscope, and the microsphere's size was measured using the Olympus Master camera and modified Magnus Pro 3.0 software on the microscope (OLYMPUS). Average particle size of dried microspheres suspended in glycerine was calculated.

2.4.4 Shape and Surface Characterisation: Microspheres' form and surface characteristics were examined using a scanning electron microscope (SEM). The Tokyo Scanning Electron Microscope, Joel model JSM 6400, was the tool utilised in this investigation. Using double sided sticky tape, the microspheres were adhered directly to the SEM sample stub. Gold film (200 nm in thickness) was then applied under low pressure (0.001 torr) and captured on camera.

2.4.5 Degree of Swelling: [20&29] Precisely balance After being weighed, 50 mg microspheres (W) were incubated for 24 hours at pH 6. 8 in phosphate buffer saline. Whatman filter paper was used to separate the enlarged microspheres after a 24-hour period. After gathering the microspheres and blotting them to remove extra water, their weight (Wt) was recorded. It was also discovered that the swelling index depended on the particle's surface area. It was discovered that the swelling index rose along with the particle surface area.

2.4.6 Mucoadhesive Property by Wash-Off Test: Microspheres' mucoadhesive properties were assessed using the wash-off method, an in vitro adhesion testing technique. "A freshly cut (2 x 2 cm) slice of goat nasal mucosa was mounted using cyanoacrylate glue on glass slides (3 x 1 inch); about twenty-five microspheres were placed on each wet-rinsed tissue specimen after two glass slides were coupled with an appropriate support and the support was then fastened to the arm of a USP tablet dissolving test machine". "The tissue specimen was placed in the test fluid (phosphate buffer pH 6.8) at 37 ± 0.5°C for a slow, regular up-and-down instant before the disintegration test machine was turned on and the machine was stopped after 30 minutes, 60 minutes at hourly intervals, and up to 6 hours, and the number of microspheres that were still attached to the tissue was counted". The following formula was used to display the adherent percentage:

$$\text{Mucoadhesion} = \text{No. of microspheres adhered} / \text{No. of microspheres applied} \times 100$$

2.4.7 In-Vitro Drug Release or Dissolution Studies: [28&37] All of the formulations were subjected to dissolution experiments using the USP XXIV apparatus (Basket technique) with 900 ml of phosphate buffer (pH 6.8) as the dissolution medium, rotating at a constant speed of 50 rpm and at 37 ± 0.5°C. "For each test, a sample of microspheres equivalent to 10 mg of Nitroglycerine was employed; to keep the sink condition, an aliquot of the sample was periodically taken at an appropriate time interval, and the volumes were replaced with new dissolving medium". At 215 nm, the percentage of the medication that dissolved during various time periods was computed.

2.4.8 Kinetics of Drug Release: [40&49] Regression analysis of the aforementioned plots was used to calculate the coefficient of correlation (r^2) values for the linear curves in the drug release data from the in-vitro dissolution study using a variety of kinetic models, including zero order, first order, Higuchi's, Peppa's, and others. This allowed for a better understanding of the mechanism and kinetics of drug release. In summary, four kinetics models of data treatment were used to plot the findings from in-vitro release investigations.

2.4.9 Stability Study: [53,67&68] For stability investigations, the formulation (NGN3) was created from the produced microspheres. Three sample sets of the formulation were separated and stored at 4±1°C, 25±2°C & 60±5%RH and 37±2°C & 65±5%RH. After 30 days, the samples were tested for drug release. Entrapment effectiveness for the same composition was also examined.

3. RESULTS AND DISCUSSION

3.1 FTIR Spectra: The pure form of Nitro-glycerine's FTIR spectrum was captured. Figure 1 displays the sample drug's FTIR spectrum. FTIR spectroscopy was used to analyse the infrared spectra of pure drugs utilising the KBR.

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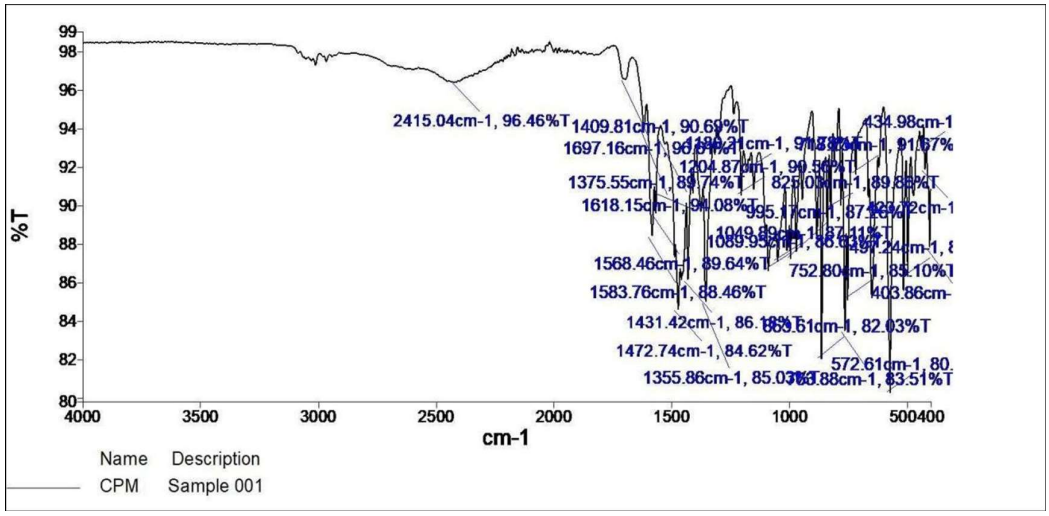

Figure 1 FTIR Spectra of Nitroglycerine

3.2 Compatibility Study: By employing FTIR spectroscopy, the medication and polymer were found to be compatible. For the medication, chitosan, and formulation NGN3, infrared spectroscopy examination was done. Figures 2 and 3 show the FTIR spectra of Formulation NGN3 and chitosan.

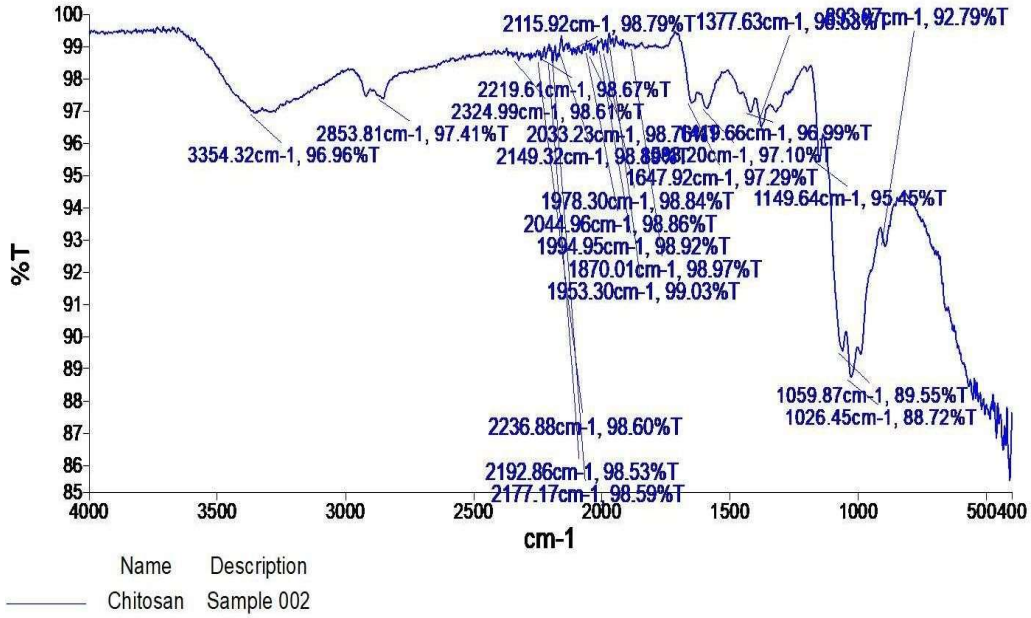

Figure 2: FTIR Spectra of Chitosan

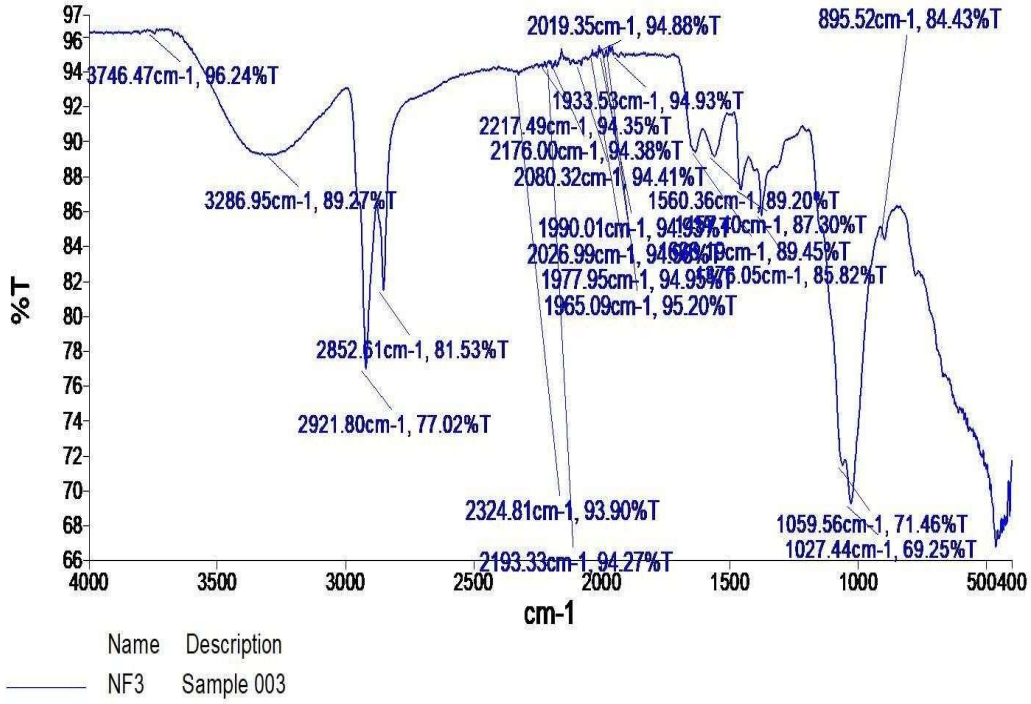

Figure 3: FTIR Spectra of Formulation NGN-3

The FTIR spectra of chitosan and formulation NGNF3 revealed that the distinctive peaks of the medication and polymer did not move or vanish. This implies that the medication and polymer do not interact. Thus, it can be said that the medication keeps its original form without interacting chemically with chitosan.

3.3 Optimization of Process and Formulation Variables

i) Emulsification Cross Linking Method: In the current work, the emulsification crosslinking approach was used to create microspheres. As the aqueous phase, polar organic solvent was used to prepare the w/o kind of emulsion.

ii) Selection of Internal Phase

Selection of Dispersing Agent: The results of this study demonstrated that liquid paraffin was the exterior phase, and DOSS-which is soluble in both liquid paraffin and cone—was employed. It was discovered that 0.2% w/v was adequate for the creation of microspheres. DOSS appears to have shielded organic polymer droplets from one another and kept them from clumping together.

Selection of Washing Solvent: In order to get rid of any last residues of liquid paraffin, microspheres were cleaned. Hexane was tested, in which liquid paraffin is soluble but polymers are not, in an attempt to find a washing solvent that will only dissolve liquid paraffin and not polymers. The resulting microspheres were distinct in character.

3.4 Characterization and Evaluation

3.4.1 Production Yield: Following the microspheres' preparation, the practical yield and percentage yield were determined. Figure 4 displays the % yield of several formulations. It was discovered that NGN3 had the highest percentage yield, followed by NGN1, NGN2, NGN3, NGN4, and NGN5. It was discovered that the percentage yield ranged from **86.20% to 96.97 %**. NGN3 formula demonstrated the highest yield of 96.97 %. Microspheres do not develop at concentrations below or beyond the optimal threshold for the polymer and crosslinking agent, according to observations. Process parameters were the cause of the material loss that occurred during the microsphere preparation. Another region for that may be agglomeration and sticking of polymer to blades of stirrer and to the wall of the beaker during microsphere formulation.

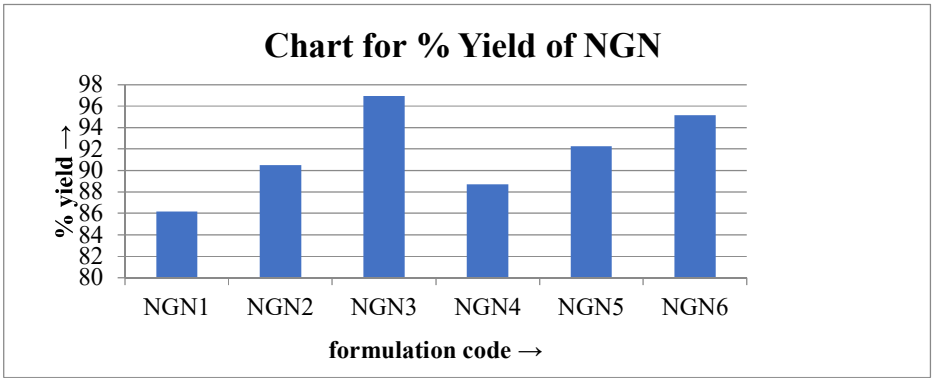

Figure 4. Data For Percentage Yield of Mucoadhesive Microsphere Nitroglycerine

3.4.2 Drug Content and Entrapment Efficiency: The analysis of the drug content revealed that the technique was very effective in producing microspheres with the maximum possible drug content, even when the polymer composition was altered. The range of the drug content percentage (w/w) was found to be **69.17% to 79.97% w/w.** It was discovered that NGN3 has the highest percentage of drug content, followed by NGN1, NGN2, NGN3, NGN4, and NGNS. The best drug content percentage, 79.97% w/w, was displayed by formulation NGN3. Figure 5 displays the microspheres' entrapment efficiency results. For every microsphere, the computed percentage entrapment efficiency varied between **70.9% and 81.76%**. For formulation NGN3, the maximum entrapment efficiency is observed. Roughly speaking, the polymer concentration influences the entrapment efficiency. The formulations with 3%w/v of chitosan (NGN3 and NGN6) had an entrapment efficiency that was higher than the formulations with 1%w/v of chitosan (NGN1 and NGN2). It was shown that the entrapment efficiency increased as the polymer concentration did.

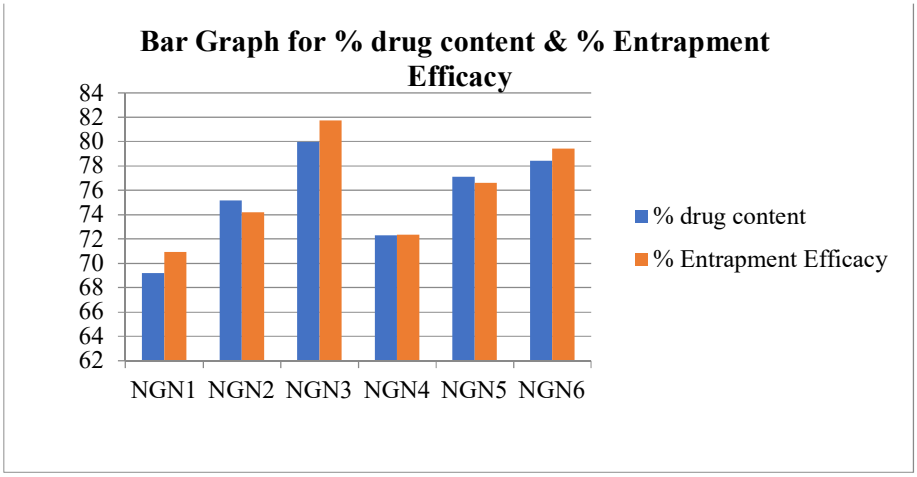

Figure 5: Percentage Drug Content of Prepared Microspheres

3.4.3 Particle Size Analysis of Microspheres: Using OLYMPUS INEA, the particle sizes of all produced microspheres were analysed. Table 2 displays the average particle size of the prepared microspheres. The microspheres measured between 15-42 µm in size. It was discovered that the crosslinking agent concentration had a greater influence on the particle size than the polymer concentration. Up to a certain point, higher chitosan cone causes the development of tiny particles, which may be caused by a high anionic concentration. Out of all the formulations, formulation NGN3 had the best suitable particle size of **21 ± 2.11 µm**, making it suitable for nasal administration.

Table 2: Mean Particle Size Analysis of Microspheres

SR. No.	Formulation code	Particle size
1	NGN1	37 ± 4.98
2	NGN2	31 ± 3.66
3	NGN3	21 ± 2.11
4	NGN4	34 ± 3.92
5	NGN5	32 ± 3.51
6	NGN6	27± 4.96

3.4.4 Surface Morphology by Scanning Electron Microscopy (SEM): The produced microspheres' surface morphology was examined using scanning electron microscopy. Dry microspheres were coated with gold using an ion sputter after being deposited in a brass stub for a scanning electron microscope. Figure 6 displayed the formulation NGN3 SEM figure. According to the batch NGN3 formulation created for SEM investigation, the surface morphology of the microspheres was spherical and smooth.

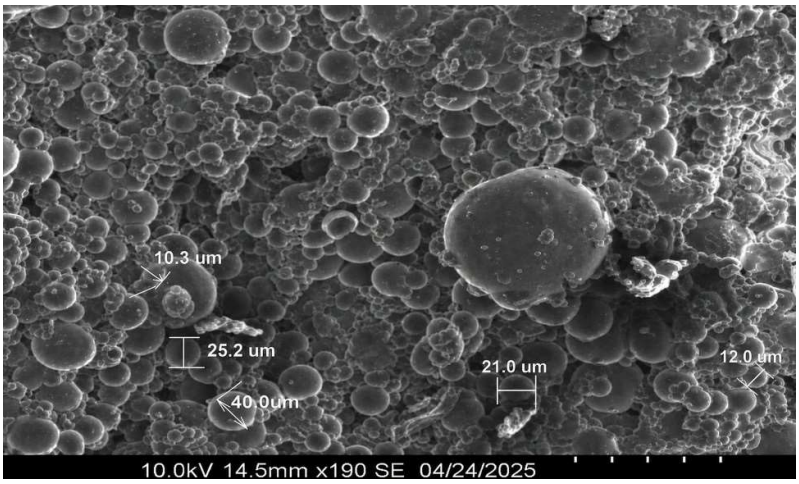

Figure 6: SEM Image of Formulation NGN3

3.4.5 Swelling Property: Figure 7 displays the formulas' Swelling Index. In comparison to formulations NGN l & NGN4 with 1% w/v and NGN2 & NGN5 with 2% w/v polymer concentration, which lost their integrity after 3 hours, formulations NGN3 and NGN6 with higher polymer concentration (3 o/ow/v) demonstrated greater swelling and retained their integrity until 4 hours. This could be as a result of the former's higher density, which allowed for a slower rate of solvent penetration over a longer period of time than the latter. It was also discovered that the swelling index depended on the particle's surface area. It was discovered that the swelling index rose along with the particle surface area.

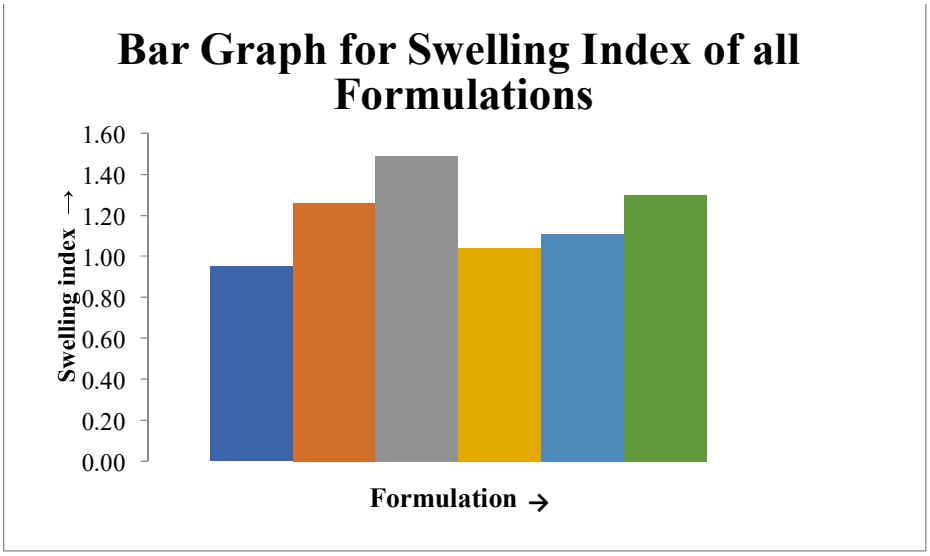

Figure 7: Swelling Index of Microspheres

3.4.6 In-vitro Mucoadhesion Test for Microspheres: Table 3 displays the mucoadhesion test result. The results show that when the concentration of polymer increases, so does the mucoadhesive strength. The formulations with a 3% w/v polymer concentration (NGN3 and NGN6) exhibited greater mucoadhesive strength than the 1% w/v formulations (NGNl and NGN2). It was also discovered that the surface area of the particle affected the mucoadhesion. It was discovered that mucoadhesion increased along with particle surface area.

Table 3: Data for In-vitro Wash-off Test for Mucoadhesion in Phosphate Buffer pH 6.8

SR. No.	Formulation code	Mucoadhesion (%)
1	NGN1	61.17 ± 0.386
2	NGN2	67.17 ± 0.421
3	NGN3	72.45 ± 0.901
4	NGN4	63.72 ± 0.315
5	NGN5	68.98 ± 0.504
6	NGN6	69.87 ± 0.133

In-vitro Release Studies: Figure 8 shows a tabulation of all the formulations' in-vitro release data. After six hours, the total percentage of medication release was supposed to reach 91%. For the formulations NGNl through NGN6, respectively. Figure 8 depicted the release studies of Nitroglycerine microspheres graphically. It was evident that the drug release was significantly impacted by both the polymer concentration and stirring rate. The medication release was greater than the mucoadhesive polymer concentration as the polymer concentration rose. When the stirring rate was increased from a lower to a higher level, the release of drugs rose sharply. This is most likely caused by the microspheres' lower particle size at greater stirring rates, which results in a significantly bigger surface area that is available for release and a shorter pathlength for the medication to diffuse through. the increased release of the drug from the chitosan, which creates a hydrophilic channel inside the microspheres to aid in drug diffusion. Increased hydrophilic holes created by chitosan made it easier for water to enter microspheres, sped up the erosion of the expanding matrix, and combined the erosion and diffusion mechanisms to release drugs from microspheres.

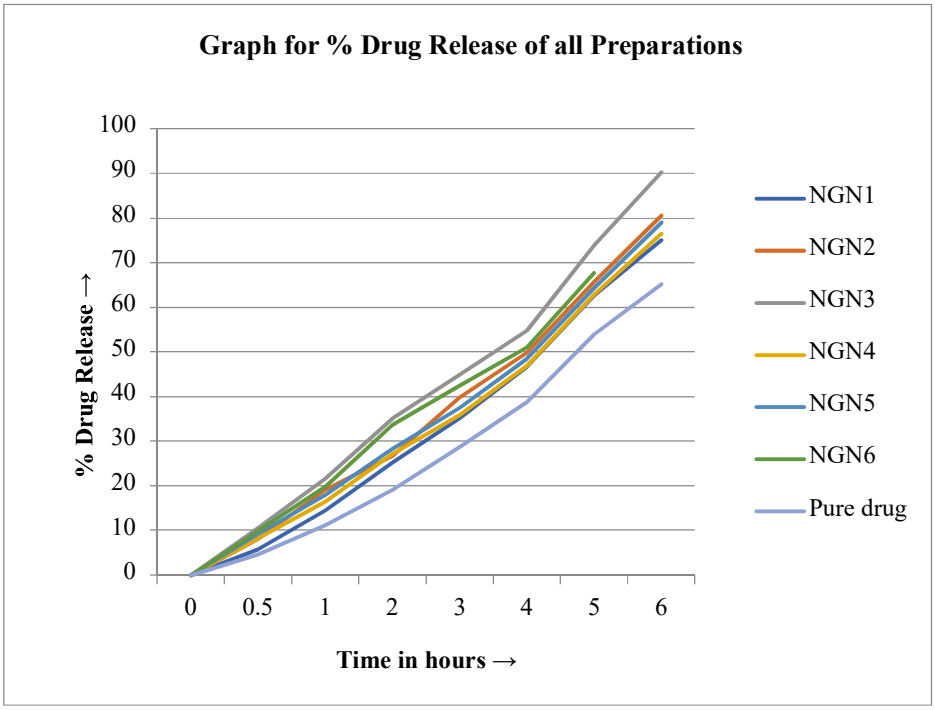

Figure 8: In-vitro Drug Release of Prepared Microspheres Formulations

In-vitro Drug Release Kinetics: Regression analysis revealed that the drug release sequence was zero order. The Korsemeyer Peppas equation's "n" value leads to the conclusion that a non-fickinian diffusion is followed by the drug release. Through the process of diffusion, drugs can be released from microspheres. It was found that drug diffusion predominates as the mechanism controlling the release of Nitroglycerine-loaded chiton microspheres drug delivery system. The outcomes were displayed in Table 4.

Table 4: In-Vitro Release Kinetic Data for Nitroglycerine Mucoadhesive Microspheres

Formula code	Zero order	First order	Higuchi's	Korsmeyer-Peppas	
	R	R	R	N	R
NGN1	0.9666	0.9493	0.9663	0.6264	0.9754
NGN2	0.9688	0.9812	0.9683	0.6585	0.9846
NGN3	0.9897	0.9848	0.9854	0.6654	0.9894
NGN4	0.9742	0.9748	0.9794	0.6388	0.9799
NGN5	0.9767	0.9776	0.9812	0.6466	0.9807
NGN6	0.9799	0.9826	0.9835	0.6633	0.9888

K_0= Zero order constant K_1= First order rate constant r= Coefficient correlation n= diffusion exponent

Stability Study: Six-month stability testing of **NGN3** at **4±1°C, 25±2°C/60 ±5 RH, and 37±2°C/65±5% RH** showed negligible changes in drug content or entrapment efficiency. This confirms formulation stability, likely aided by minimal polymer matrix erosion during storage.

Table 5: Stability Studies of Formulation NGN3

Sr. No.	Time in Months	4±1°C		25±2°C with 60±5% RH		37±2°C with 65±5% RH	
		Z	Y	Z	Y	Z	Y
1	**1**	84.90	83.00	84.90	83.05	82.93	81.03
2	**2**	84.80	82.60	84.80	83.03	82.62	81.01
3	**3**	84.70	82.60	84.70	83.00	82.10	80.00
4	**4**	84.00	82.50	84.50	82.90	81.70	79.80
5	**5**	83.70	82.40	84.30	82.80	81.30	79.50
6	**6**	83.77	82.45	84.37	82.85	81.36	80.12

4. CONCLUSION

In order to prevent first pass metabolism, increase patient compliance, employ an alternative therapy to traditional dosage forms, achieve controlled blood level profiles of the drug, and enhance the therapeutic efficacy. Mucoadhesive microspheres of Nitroglycerine for nasal delivery were developed using the W/O emulsion cross linking method. The mucoadhesive polymer utilised was chitosan. Several metrics were used to assess the manufactured microspheres. Of the formulations created, formulation NGN3 produced the best outcomes. After a thorough analysis of all the experimental findings, it was determined that microspheres made using W/O Emulsion Cross Linking procedures would be a highly promising option for the controlled release of different medications. Use also lessens drug loss and dosage frequency.

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