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# FORMULATION, OPTIMIZATION, AND CHARACTERIZATION OF A THERMOSENSITIVE IN SITU NASAL GEL INCORPORATING CHITOSAN NANOPARTICLES OF ZOLMITRIPTAN FOR THE MANAGEMENT OF ACUTE MIGRAINE ATTACKS

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## ABSTRACT

The current study concentrated on creating and refining a zolmitriptan in situ thermosensitive nasal gel with chitosan nanoparticles for improved migraine treatment. Sodium tripolyphosphate (TPP) was used as the cross-linking agent in the ionic gelation process to create chitosan nanoparticles. To make a formulation that changes from sol to gel at nasal cavity temperature, the nanoparticle suspension was mixed with a gel matrix that contained Poloxamer 407 and Poloxamer 188. Particle size, zeta potential, mucoadhesive strength, gelation temperature, viscosity, gel strength, and in vitro drug release were all assessed for a number of formulations (TSGF1–TSGF7). Among the formulations, TSGF3 was identified as the best optimized due to its ideal gelation temperature (35.45 ± 0.37°C), balanced viscosity (493 cP at 40°C and 3523 cP at 34°C), and satisfactory mucoadhesive strength (3614 ± 14.82 dynes/cm²). The drug release study revealed that TSGF3 provided a sustained release of 90.79% over 420 minutes, indicating its potential for prolonged therapeutic effect. The drug and excipients did not significantly interact, according to FTIR analysis. These findings support that the zolmitriptan-loaded thermosensitive chitosan nanoparticle gel is a promising system for intranasal delivery, offering both rapid onset and sustained migraine relief through improved bioadhesion and controlled drug release.

## INTRODUCTION

A complex neurological condition, migraine is typified by frequent, throbbing headaches that are frequently accompanied by phonophobia, photophobia, nausea, and vomiting. With an estimated incidence of over one billion people worldwide, it has a substantial influence on patients' quality of life and productivity.

Because of its neuropeptide-inhibitory and vasoconstrictive properties, zolmitriptan, a selective 5-HT1B/1D receptor agonist, is a popular therapy choice for acute migraine attacks. However, conventional oral administration of zolmitriptan often suffers from delayed onset of action and reduced bioavailability, particularly during migraine episodes, where gastrointestinal motility is impaired. This restriction highlights the necessity of finding alternate delivery methods that can provide quick therapeutic relief without going via the gastrointestinal system (Gu et al., 2020; Khan et al., 2010; Li et al., 2014; Qian et al., 2014; Xia et al., 2020).

A promising method for systemic drug absorption, particularly when targeting the central nervous system (CNS), is intranasal drug delivery. A wide surface area, robust vascularization, avoidance of hepatic first-pass metabolism, and quick absorption into the systemic circulation are just a few benefits of the nasal cavity. However, obstacles including limited residence time and mucociliary clearance may reduce the effectiveness of nasal medication delivery. The creation of thermosensitive and mucoadhesive in situ gel systems has drawn interest as a solution to these problems. These systems can improve medication retention and absorption by staying liquid during delivery and changing from a liquid to a gel when exposed to nasal cavity temperature (32–35°C) (Adnet et al., 2020; Akilo et al., 2019; Gholizadeh, Cheng, et al., 2019; Gholizadeh, Messerotti, et al., 2019; Omar et al., 2019).

The use of nanoparticle technology in this situation enhances the results of drug delivery much more. For drug delivery using nanoparticles, chitosan—a naturally occurring, biodegradable, and biocompatible polymer—has been thoroughly investigated. Drug transport across the nasal epithelium is facilitated by its exceptional mucoadhesive qualities and capacity to temporarily open tight connections. In addition to providing protection against enzymatic degradation, the use of chitosan nanoparticles for zolmitriptan encapsulation enables controlled and prolonged drug release. To enhance the thermosensitive behavior of the formulation, Poloxamer 407 and Poloxamer 188 are incorporated, which form a reversible gel in response to temperature changes. This dual strategy—nanoparticulate encapsulation and thermosensitive gelling—creates a synergistic effect for efficient intranasal delivery (Kumar et al., 2019; Su et al., 2020).

The goal of the current study was to create and improve an in situ thermosensitive nasal gel based on chitosan nanoparticles loaded with zolmitriptan. Various formulations were developed by varying the concentrations of chitosan, sodium tripolyphosphate (as cross-linker), and poloxamers. Particle size, zeta potential, viscosity, mucoadhesive strength, gelation temperature, gel strength, and in vitro drug release were among the physicochemical properties that were assessed. FTIR spectroscopy was also used to evaluate the formulation's compatibility with the excipient (Browne, 1976, 1978; Greenblatt et al., 1987; Morishita, 2009; Nardi et al., 2013; Raggi et al., 2023).

This work suggests a unique delivery strategy that offers quick onset and sustained action, potentially improving patient compliance and treatment success by addressing the drawbacks of traditional migraine medicines. The results not only demonstrate the feasibility of this approach but also highlight the promising role of chitosan-based thermosensitive gels in nasal drug delivery for neurological conditions such as migraine.

**EXPERIMENTAL:**

**Materials, drugs and chemicals:**

Zolmitriptan, the active pharmaceutical ingredient for the study, was kindly supplied as a gift sample by a reputable pharmaceutical company, ensuring its authenticity and pharmaceutical-grade quality. The nanoparticle formulation was based on chitosan, a naturally derived, biodegradable polymer known for its mucoadhesive and biocompatible properties. Specifically, low molecular weight chitosan was selected to optimize particle size and enhance drug encapsulation efficiency. This polymer was sourced from a certified supplier to guarantee consistency and reliability in formulation outcomes. To achieve thermosensitive gelation suitable for intranasal drug delivery, two non-ionic surfactants, Poloxamer 407 and Poloxamer 188, were incorporated. Because of their well-known capacity to experience reversible sol-gel transitions in response to temperature fluctuations, these poloxamers are perfect for in situ gel systems that can gel at body temperature and stay fluid at room temperature. To help chitosan nanoparticles form through ionic gelation, sodium tripolyphosphate (TPP) was employed as an ionic cross-linking agent. The stabilization of the nanoparticulate system was largely dependent on the interaction between the negatively charged phosphate groups of TPP and the positively charged amino groups of chitosan. All reagents, including solvents and excipients, were of analytical grade to ensure the accuracy, reproducibility, and safety of the experimental procedures. To reduce the possibility of interference from contaminants or ions found in untreated water, distilled water was utilized consistently for all preparations, dilutions, and analytical procedures during the investigation. This meticulous material selection demonstrates the dedication to quality and dependability in the creation of an intranasal drug delivery device loaded with Zolmitriptan.

**Drug excipient interaction study:**

***Fourier Transform Infrared Spectra (FTIR) spectroscopy:***

To assess potential interactions between the medicine and formulation excipients, Fourier Transform Infrared (FTIR) spectroscopy was utilized. Based on their distinctive absorption peaks, this method is frequently used to determine which functional groups are present in a molecule. Zolmitriptan, the chosen polymer (chitosan), excipients (such as Poloxamers), their physical mixes, and the finished nanoparticle formulation were all subjected to FTIR analysis in this investigation.The primary objective was to detect any potential chemical incompatibilities or undesirable interactions that could affect drug stability or hinder entrapment efficiency. To determine whether the medicine and excipients were compatible, the spectra were carefully analyzed for the presence or absence of notable peak changes or new peaks. Using a Shimadzu 8400 IR Spectrophotometer, the FTIR spectra were captured between 400 and 4000 $cm^{-1}$. In order to verify the drug's integrity and the lack of new bonds or degradation products, this spectral window includes the essential regions for recognizing functional groups like N-H, O-H, C=O, and C-H. The outcomes supported effective drug encapsulation within the nanoparticulate gel system and helped guarantee that the formulation procedure maintained Zolmitriptan's chemical stability (Lawrie et al., 2007)..

**Preparation of Chitosan Nanoparticles:**

With minor adjustments, the ionic gelation process, as reported by Shard et al. (2014) and Fan et al. (2012), was used to create chitosan nanoparticles encasing zolmitriptan (Fan et al., 2012; Shard et al., 2014). To create a transparent solution, precisely weighed amounts of low molecular weight chitosan (as shown in Table 1) were dissolved in 1% v/v aqueous acetic acid while being magnetically stirred. To guarantee even medication dispersion, zolmitriptan (5 mg) was then added to the chitosan solution and carefully mixed. To create the cross-linking solution, sodium tripolyphosphate (TPP) was separately dissolved in distilled water. At room temperature, the TPP solution was gradually added dropwise while being continuously stirred magnetically in the drug-loaded chitosan solution. Ionic gelation produced spontaneous nanoparticle formation as a result of the ionic interaction between the negatively charged phosphate groups of TPP and the positively charged amino groups of chitosan. To guarantee full cross-linking and stability of the nanoparticles, stirring was maintained for a further thirty to forty-five minutes. A probe sonicator was then used to sonicate the resultant nanoparticle suspension in order to improve homogeneity and decrease particle size. The final formulations (ZOLNF1–ZOLNF6) were stored at 4°C until further evaluation. This method allowed efficient encapsulation of zolmitriptan and formation of stable chitosan nanoparticles with varying polymer and cross-linker concentrations to optimize the formulation characteristics.

**Table 1.** Composition of Chitosan Nanoparticles Loaded with Zolmitriptan (ZOLNF1–ZOLNF6):

| Ingredients | ZOLNF1 | ZOLNF2 | ZOLNF3 | ZOLNF4 | ZOLNF5 | ZOLNF6 |
|---|---|---|---|---|---|---|
| Zolmitriptan (mg) | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Chitosan (mg) | 10 | 15 | 20 | 25 | 30 | 35 |
| Sodium Tripolyphosphate (mg) | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 | 4.5 |
| Acetic Acid (1% v/v, mL) | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Distilled Water (mL) | q.s. to 10 | q.s. to 10 | q.s. to 10 | q.s. to 10 | q.s. to 10 | q.s. to 10 |

**Formulation of In Situ Thermosensitive Gel:**

Poloxamer 407 and Poloxamer 188, thermoresponsive polymers that can undergo sol-to-gel transition at physiological temperatures, were used to manufacture the in situ thermosensitive gel. Both Poloxamers were added gradually, in precisely weighed proportions, to cold distilled water that was kept at around 4°C while being constantly stirred. The resulting solution was refrigerated overnight to facilitate complete dissolution and to maintain polymer integrity. Following complete dissolution, the previously prepared chitosan nanoparticle suspension containing zolmitriptan was gently incorporated into the cold polymeric solution under slow stirring. Care was taken to ensure uniform dispersion while avoiding the formation of air bubbles. The solution stays fluid at room temperature and quickly turns into a gel when it comes into contact with the nasal mucosa thanks to the formulation's optimization to reach a gelation temperature between 32°C and 35°C. This thermosensitive characteristic improves drug absorption through the nasal route by strengthening mucosal adhesion and extending the formulation's residence time (Kamali et al., 2024; Mikhel et al., 2024; Trivedi et al., 2024).

**Table 2.** Formulation table for in situ thermosensitive gel with chitosan nanoparticles:

| Ingredients | TSGF1 | TSGF2 | TSGF3 | TSGF4 | TSGF5 | TSGF6 |
|---|---|---|---|---|---|---|
| Chitosan Nanoparticles (CNF, mL) | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Poloxamer 407 (% w/v) | 18 | 19 | 20 | 20 | 21 | 22 |
| Poloxamer 188 (% w/v) | 1 | 1.5 | 2 | 2.5 | 3 | 3.5 |
| Distilled Water (mL) | q.s. to 10 | q.s. to 10 | q.s. to 10 | q.s. to 10 | q.s. to 10 | q.s. to 10 |

**Evaluation of Chitosan Nanoparticles:**

***Particle Size and Zeta Potential:***

Using dynamic light scattering (DLS) technology, the formed chitosan nanoparticles' average particle size and zeta potential were ascertained. A Malvern Zetasizer (Malvern Instruments Ltd., UK), a dependable tool for assessing nanoparticle properties such hydrodynamic diameter and surface charge, was used for the measurements. To prevent multiple scattering effects, the nanoparticle suspension was suitably diluted with distilled water before examination. While the zeta potential readings revealed the surface charge and stability of the colloidal system, the particle size data shed light on the uniformity and distribution of the nanoparticles. A higher magnitude of zeta potential (either positive or negative) is generally indicative of better stability due to electrostatic repulsion between particles. These parameters were crucial in optimizing the formulation for enhanced mucosal penetration and prolonged retention time (Kamali et al., 2024; Mikhel et al., 2024; Trivedi et al., 2024).

***Drug Loading and Encapsulation Efficiency:***

Using UV-visible spectrophotometry, an indirect technique was used to assess the drug loading (DL) and encapsulation effectiveness (EE) of chitosan nanoparticles loaded with zolmitriptan. To extract the unentrapped medication, the nanoparticle dispersion was centrifuged at 15,000 rpm for 30 minutes at 4°C after formulation. A UV-Vis spectrophotometer was used to measure the amount of free zolmitriptan at its maximum absorbance wavelength (λmax) of 225 nm after the supernatant had been carefully collected. To ascertain the concentration of the unencapsulated medication, a calibration curve of zolmitriptan in the appropriate solvent was created. By deducting the amount of free drug in the supernatant from the total amount of drug originally employed in the formulation, the amount of drug entrapped within the nanoparticles was determined (Kamali et al., 2024; Mikhel et al., 2024; Trivedi et al., 2024).

**Drug Loading (%):**

Drug loading was calculated using the following formula:

$$\text{Drug loading}\ (\%) = \frac{\text{Amount of drug present in nanoparticles}}{\text{Amount of drug loaded nanoparticles}} * 100$$

**Encapsulation Efficiency (%):**

Encapsulation efficiency was determined using the formula:

$$\text{Entrapment efficiency}\ (\%) = \frac{\text{Amount of drug present in nanoparticles}}{\text{Intial amount of drug added}} * 100$$

These parameters were essential to evaluate the formulation's capability to retain the drug within the nanoparticle matrix and to ensure efficient drug delivery upon administration.

***Morphological Analysis Using Scanning Electron Microscopy (SEM):***

To visually verify the shape, surface properties, and approximate particle size of the prepared chitosan nanoparticles, scanning electron microscopy (SEM) was used to examine their surface morphology and size distribution. To improve surface conductivity, a tiny layer of gold was vacuum-coated on a small portion of the freeze-dried nanoparticle sample that had been mounted onto an aluminum stub using double-sided sticky carbon tape. After that, the samples were viewed at the appropriate magnifications using a scanning electron microscope. High-resolution micrographs from SEM images showed the nanoparticles' exterior morphology. The majority of the particles had smooth surfaces, seemed spherical, and aggregated very little, indicating effective formulation and uniform distribution. The results of DLS measurements were corroborated by this study, which also offered comprehensive structural data that was essential for comprehending the behavior of the formulation and its potential for drug administration (Mankar et al., 2024; Mathure et al., 2023; Trivedi et al., 2024).

**Evaluation of Thermosensitive Gel:**

***Gelation Temperature:***

The tube inversion approach was used to find the in situ thermosensitive gel's gelation temperature. In a test tube, a predetermined volume of the gel formulation was heated at a regulated pace over time in a water bath. To monitor the sol-to-gel transition, the tube was tilted horizontally at regular temperature intervals. The gelation temperature was defined as the temperature at which the formulation stopped flowing upon inversion. This temperature is necessary to guarantee that the formulation stays liquid at room temperature and quickly gels at the temperature of the nasal cavity (32–35°C), improving mucosal adherence and extending the duration of residence (Mankar et al., 2024; Mathure et al., 2023; Trivedi et al., 2024).

***Viscosity:***

A Brookfield viscometer with a suitable spindle and a fixed rotation speed was used to evaluate the viscosity of the thermosensitive gel at ambient temperature and physiological temperature. To assess the formulation's flow characteristics and its capacity to change from sol to gel, measurements were made both before and after gelation. Successful gel formation, which is essential for reducing nasal outflow and lengthening drug retention duration, was shown by a notable rise in viscosity at higher temperatures. To guarantee simplicity of administration and the best possible therapeutic outcome, the rheological characteristics of each formulation were assessed (Mankar et al., 2024; Mathure et al., 2023; Trivedi et al., 2024).

***Mucoadhesive Strength:***

To ascertain the thermosensitive in situ gel's capacity to stick to the nasal mucosa and withstand mucociliary clearance, its mucoadhesive strength was assessed. Freshly removed goat nasal mucosa was placed on glass slides and subjected to a modified balancing technique. Two mucosal tissues were sandwiched with a specified amount of the gel formulation, and they were kept together for a predetermined amount of time using gentle pressure. Using weights, the force needed to separate the tissues

was calculated and reported as mucoadhesive strength (g). This parameter is essential for ensuring that the formulation remains in the nasal cavity for an extended period of time, which improves medication absorption and bioavailability (Hard et al., 2024; Mura et al., 2022; Pham et al., 2021).

**In Vitro Drug Release:**

The dialysis bag diffusion method was used in in vitro drug release studies to evaluate the zolmitriptan release profile from the thermosensitive gel loaded with nanoparticles. A pre-soaked dialysis membrane was filled with a known volume of the gel and submerged in phosphate buffer (pH 6.4) that was kept at 37 ± 0.5°C while being constantly stirred. To maintain sink conditions, samples were taken out of the release medium and replaced with new buffer at prearranged intervals. At 225 nm, the amount of zolmitriptan released was measured with a UV-Visible spectrophotometer. The release kinetics and formulation's effectiveness in maintaining drug delivery over time were assessed by analyzing the release data (Hard et al., 2024; Mura et al., 2022; Pham et al., 2021).

**Statistical analysis:**

Based on triplicate calculations (n=3), all experimental data were presented as mean ± standard deviation (SD). In order to find significant differences between groups, one-way analysis of variance (ANOVA) and Tukey's post-hoc test were used to compare various formulations and experimental groups statistically. The p-value was deemed statistically significant if it was less than 0.05 ($p < 0.05$). GraphPad Prism program (version 8) or a comparable statistical analysis tool was used for statistical computations and graphic data visualization. This analysis ensured the reliability, reproducibility, and significance of the results obtained throughout the evaluation of the chitosan nanoparticle-loaded thermosensitive nasal gel.

**RESULTS AND DISCUSSION:**

**Drug excipient compatibility study:**

The TSGF3 formulation's FTIR spectral analysis was performed to evaluate the chemical compatibility of zolmitriptan with the formulation's excipients. Around 3350 $cm^{-1}$ (N–H stretching), 2940 $cm^{-1}$ (C–H stretching), 1675 $cm^{-1}$ (C=O stretching of amide group), and 1450 $cm^{-1}$ (aromatic C=C stretching) were the characteristic peaks of zolmitriptan. The pure drug's FTIR spectrum made these functional group peaks easily recognizable. The primary zolmitriptan absorption bands in the TSGF3 spectrum were unaltered, albeit with minor changes in position or intensity. The peaks corresponding to Poloxamer 407 and 188 appeared near 1100–1150 $cm^{-1}$ (C–O–C stretching), and chitosan showed broad O–H and N–H stretching between 3200–3500 $cm^{-1}$, along with amide II bending around 1580 $cm^{-1}$. The absence of any chemical interaction between the drug and excipients is suggested by the formulation's retention of the main zolmitriptan characteristic peaks, without the emergence of any new peaks or the notable disappearance of any existing ones. These results validate the formulation's integrity and support effective drug entrapment by confirming that zolmitriptan remained chemically stable and compatible within the thermosensitive gel matrix.

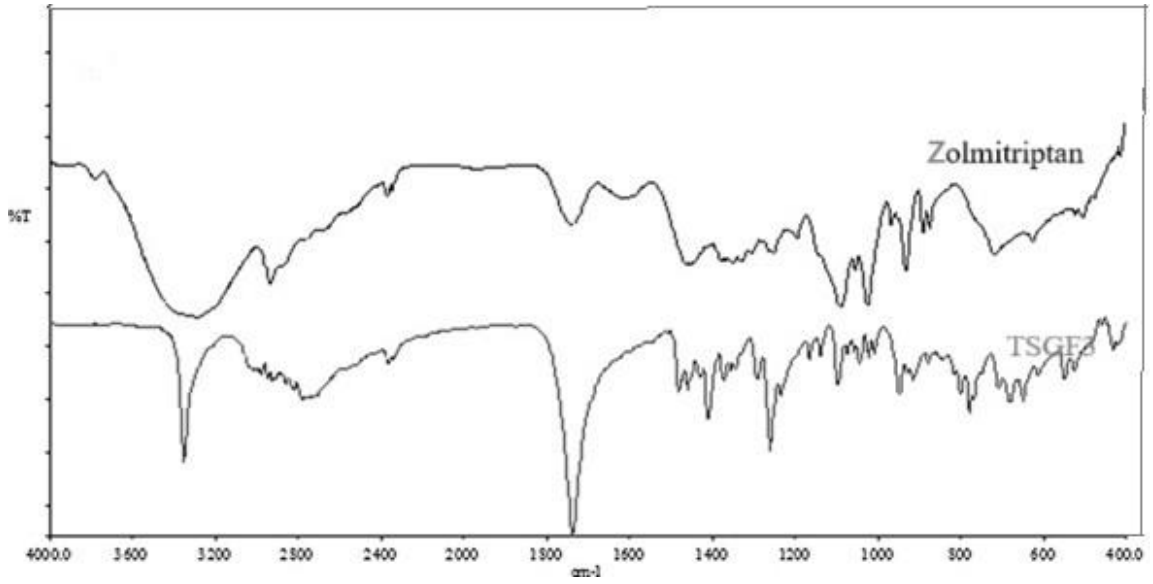


**Figure 1.** FTIR spectra of the formulation (TSGF3) indicating drug excipient compatibility.

**Evaluation of Chitosan Nanoparticles:**

***Particle Size and Zeta Potential:***

For seven formulations with the codes ZOLNF1 through ZOLNF7, the chitosan nanoparticles' zeta potential, polydispersity index (PDI), and particle size were assessed. Particle sizes varied according on formulation composition, ranging from 305.80 nm (ZOLNF3) to 795.33 nm (ZOLNF1). Among these, ZOLNF2 exhibited the smallest size among the sub-400 nm particles (315.51 nm), suggesting better potential for permeation and cellular uptake. The PDI values, which indicate size distribution uniformity, ranged from 0.051 (ZOLNF2) to 0.808 (ZOLNF3 and ZOLNF7), with ZOLNF2 showing the most monodisperse distribution. Higher PDI values in ZOLNF3 and ZOLNF7, on the other hand, indicated a wider size distribution and possible aggregation. All formulations showed negative surface charges with respect to zeta potential, ranging from –14.58 mV (ZOLNF4) to –22.68 mV (ZOLNF2). Higher negative charge magnitudes, such those found in ZOLNF2 and ZOLNF7, indicate better colloidal stability because of electrostatic repulsion. With the best combination of compact size, low PDI, and high zeta potential, ZOLNF2 stood out overall and demonstrated its appropriateness for effective and reliable drug administration via nanoparticles.

**Table 3.** Particle size (nm), PDI, and Zeta potential (mV) of the chitosan nanoparticles

| Formulation | Particle size (nm) | PDI | Zeta potential (mV) |
|---|---|---|---|
| ZOLNF1 | 795.33 | 0.091 | -20.18 |
| ZOLNF2 | 315.51 | 0.051 | -22.68 |
| ZOLNF3 | 305.80 | 0.808 | -16.48 |
| ZOLNF4 | 370.45 | 0.590 | -14.58 |
| ZOLNF5 | 717.17 | 0.205 | -15.68 |
| ZOLNF6 | 594.56 | 0.138 | -21.38 |
| ZOLNF7 | 382.11 | 0.808 | -22.38 |

* n= 3

***Drug Loading (%) and Encapsulation Efficiency (%):***

The evaluation of drug loading and entrapment efficiency for the chitosan nanoparticle formulations (ZOLNF1–ZOLNF7) revealed notable differences among the batches. Drug loading (%) ranged from 0.466 ± 0.084 (ZOLNF3) to 0.830 ± 0.072 (ZOLNF2), indicating that ZOLNF2 achieved the highest drug content per unit of nanoparticles. In terms of entrapment efficiency, values varied from 59.47 ± 2.35% (ZOLNF6) to 78.27 ± 3.08% (ZOLNF2), further establishing ZOLNF2 as the most efficient formulation in terms of encapsulating the drug. Formulations such as ZOLNF1 and ZOLNF7 also showed relatively high drug loading (0.806 ± 0.059 and 0.726 ± 0.104, respectively) and decent entrapment efficiency (>60%), making them potentially viable candidates. However, ZOLNF6, despite a moderate drug loading (0.664 ± 0.073), demonstrated the lowest entrapment efficiency, possibly due to formulation parameters that hindered effective drug encapsulation. Overall, ZOLNF2 emerged as the most optimized formulation, combining maximum drug incorporation and efficient entrapment, crucial factors for therapeutic effectiveness and sustained release.

**Table 4.** % Drug loading and % Entrapment efficiency of the chitosan nanoparticles:

| Formulation code | % Drug loading (Mean + S.D) * | % Entrapment efficiency (Mean + SD) * |
|---|---|---|
| ZOLNF1 | 0.806 ± 0.059 | 72.47 ± 2.22 |
| ZOLNF2 | 0.830 ± 0.072 | 78.27 ± 3.08 |
| ZOLNF3 | 0.466 ± 0.084 | 71.27 ± 2.15 |
| ZOLNF4 | 0.587 ± 0.092 | 74.47 ± 3.25 |
| ZOLNF5 | 0.637 ± 0.067 | 69.57 ± 3.17 |
| ZOLNF6 | 0.664 ± 0.073 | 59.47 ± 2.35 |
| ZOLNF7 | 0.726 ± 0.104 | 63.37 ± 2.77 |

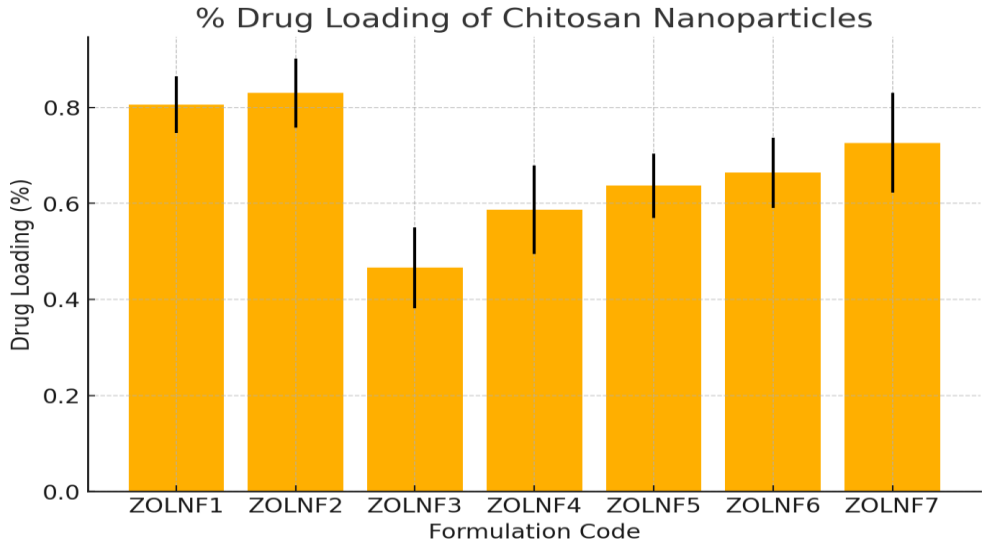


**Figure 2.** % Drug loading of the chitosan nanoparticles

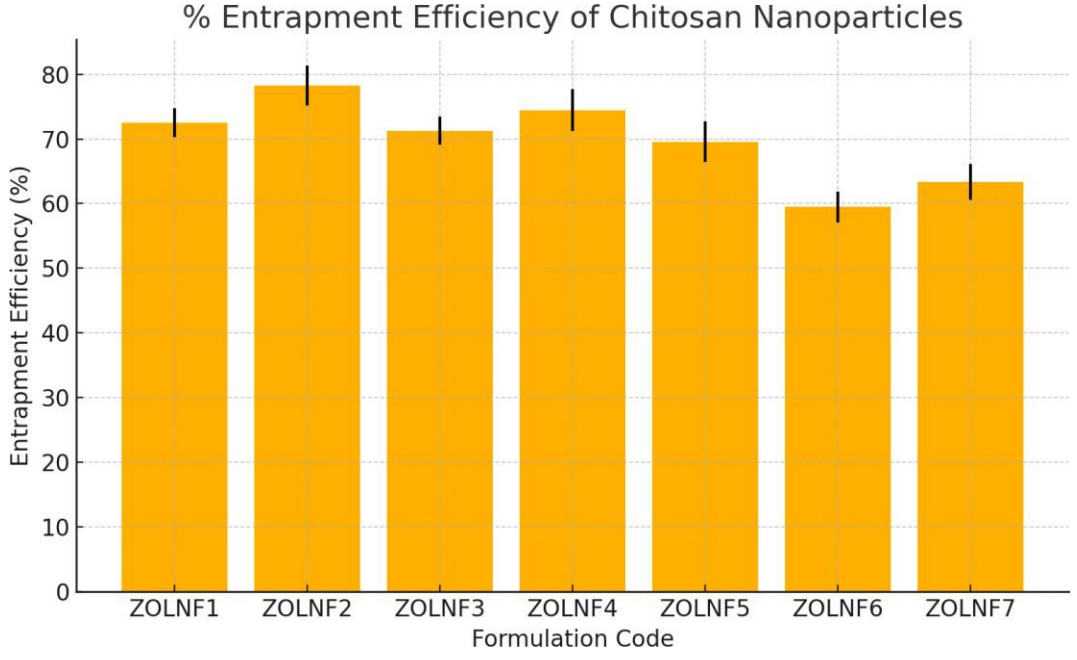


**Figure 3.** % Entrapment efficiency of the chitosan nanoparticles

***Morphological Analysis: SEM analysis:***

The scanning electron microscopy (SEM) photomicrographs of the nasal gel formulation TSGF3 revealed detailed insights into its surface characteristics and particle morphology. As observed in Figure 3, the SEM images exhibited a relatively smooth and uniform surface topography with spherical and well-distributed nanoparticles embedded within the gel matrix. The absence of surface cracks or agglomeration indicated a stable formulation with good structural integrity. Additionally, the uniform and well-defined shape of the particles indicated that the medication had been well encapsulated within the chitosan matrix, which helped to maintain the homogeneity of the gel. When administered by the nose, this morphology is essential for improving mucoadhesive qualities and promoting prolonged drug release. The formulation's potential for effective mucosal penetration and bioavailability increase is supported by the nanoscale characteristics verified by SEM. Overall, the SEM analysis validated the physical uniformity and nanoscale architecture of TSGF3, which are critical for effective nasal drug delivery.

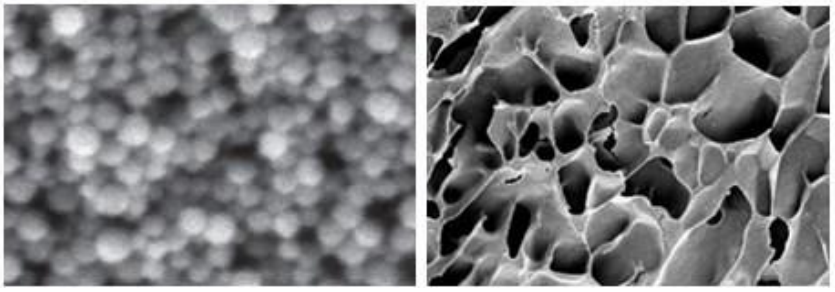

**Figure 4.** Scanning electron microscopy (SEM) photomicrographs of the nasal gel formulation (TSGF3).

**Evaluation of Thermosensitive Gel:**

***Drug Content and pH:***

To verify consistent medication distribution and compatibility with the nasal mucosa, the pH and drug content of thermosensitive gel formulations (TSGF1–TSGF7) were examined. The pH values of all formulations ranged between 6.17 ± 0.012 (TSGF3) and 7.07 ± 0.052 (TSGF5 and TSGF6), falling within the acceptable physiological range for nasal administration (generally 4.5–7.4). This indicates that the formulations are unlikely to cause mucosal irritation and are suitable for nasal application. In terms of drug content, all formulations demonstrated high loading efficiency, with values ranging from 97.97 ± 1.273% (TSGF1) to 101.71 ± 0.223% (TSGF5). The highest drug content was observed in TSGF5, followed closely by TSGF4 and TSGF3, suggesting consistent and effective incorporation of the drug in these formulations. Low standard deviations across the formulations indicate uniform dispersion and minimal batch variability. Overall, the results confirm that all TSGF formulations exhibit both pH compatibility with nasal tissues and satisfactory drug content, reinforcing their potential as stable and effective nasal drug delivery systems.

**Table 5.** Drug Content and pH

| Formulation | pH (mean ± S.D) | Drug Content (mean ± S.D) |
|---|---|---|
| TSGF1 | 6.67 ± 0.012 | 97.97 ± 1.273 |
| TSGF2 | 6.37 ± 0.022 | 99.30 ± 1.023 |
| TSGF3 | 6.17 ± 0.012 | 100.83 ± 0.133 |
| TSGF4 | 6.77 ± 0.042 | 101.26 ± 0.613 |
| TSGF5 | 7.07 ± 0.052 | 101.71 ± 0.223 |
| TSGF6 | 7.07 ± 0.032 | 100.15 ± 0.343 |
| TSGF7 | 6.67 ± 0.012 | 99.94 ± 0.923 |

***Mucoadhesive Strength and Gel Strength:***

The concentration of thermoresponsive polymers (Poloxamer 407 and 188) and the added chitosan nanoparticles were directly correlated with the mucoadhesive strength and gel strength of the in situ thermosensitive gel formulations (TSGF1–TSGF7). Mucoadhesive strength increased progressively from 2925 ± 14.80 dynes/cm$^2$ in TSGF1 to 5207 ± 15.14 dynes/cm$^2$ in TSGF7, indicating enhanced interaction between the formulation and mucosal surface with increasing polymeric content. This enhancement is attributed to the higher viscosity and cohesive gel network formed at elevated concentrations, which promotes stronger bioadhesive interactions with nasal mucosa. Similarly, gel strength followed a consistent upward trend, increasing from 81 ± 1.58 seconds in TSGF1 to 141 ± 1.98 seconds in TSGF7. This suggests that higher concentrations of Poloxamer and chitosan nanoparticles improved the structural integrity and mechanical resistance of the gel, ensuring prolonged residence time at the site of administration. Among all, TSGF6 and TSGF7 demonstrated the highest mucoadhesive and gel strengths, making them the most promising formulations in terms of nasal retention and mechanical stability. However, while TSGF7 had superior values, TSGF6 may be preferable if ease of administration and patient comfort are considered alongside performance.

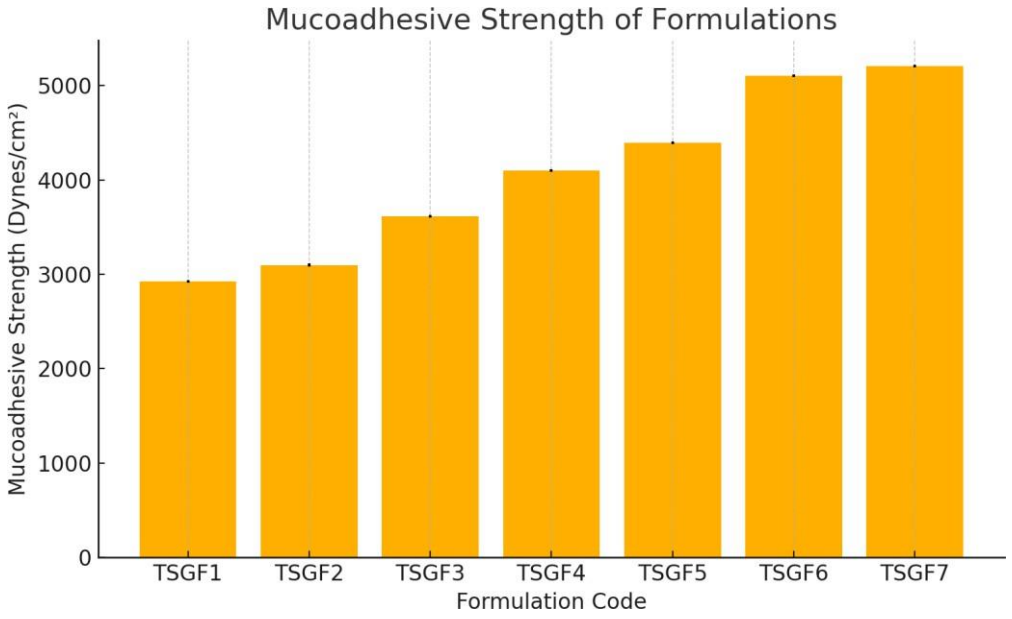


**Figure 5.** Mucoadhesive Strength

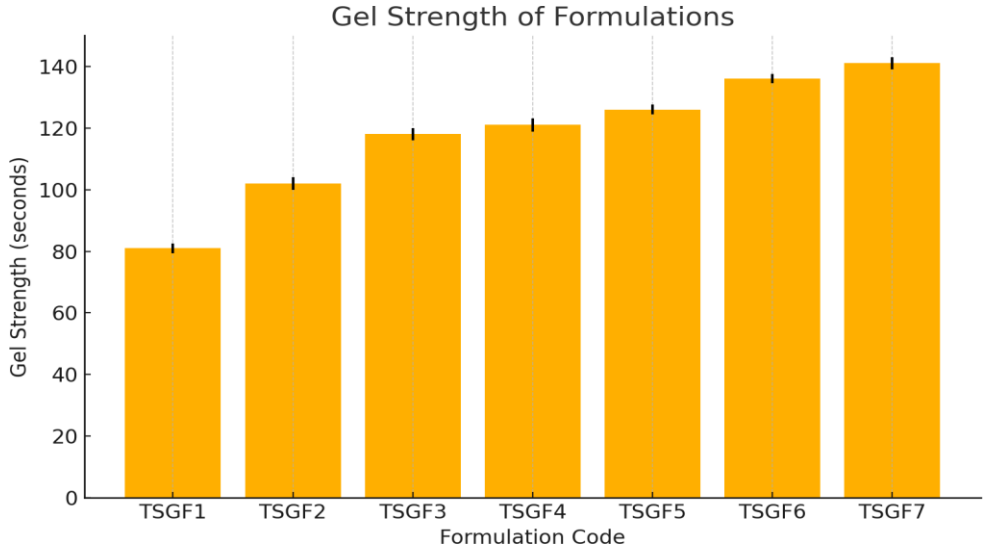


**Figure 6.** Gel Strength

***Gelation Temperature ($T_1$ ºC) and Gel Melting Temperature ($T_2$ ºC):***

The gelation temperature ($T_1$) and gel melting temperature ($T_2$) varied across the formulations, reflecting the impact of differing concentrations of Poloxamer 407, Poloxamer 188, and chitosan nanoparticles. The gelation temperature ($T_1$) is a critical parameter that determines the sol-to-gel transition upon administration. An ideal nasal formulation should gel between 32°C to 35°C, aligning with nasal cavity temperature. Among all, TSGF2 (33.64 ± 1.05°C) and TSGF3 (35.45 ± 0.37°C) fell within this optimal range, suggesting efficient in situ gelation post-administration. Formulations like TSGF4 and TSGF5 exhibited higher gelation temperatures (36.82 ± 0.65°C and 39.46 ± 0.85°C), which may delay gel formation and reduce mucoadhesive retention. On the other hand, TSGF1, TSGF6, and TSGF7 showed lower gelation temperatures, below the physiological threshold, indicating premature gelation prior to application. Gel melting temperature ($T_2$), which reflects the thermal stability of the gel, increased with higher concentrations of poloxamers and chitosan. The highest $T_2$ was observed in TSGF6 (59.45 ± 0.75°C), indicating strong gel integrity and resistance to temperature-induced breakdown, followed closely by TSGF7. Although TSGF6 and TSGF7 provided robust gel strength and thermal stability, their gelation temperature being below optimal may compromise dosing accuracy. Therefore, TSGF3 emerged as the most suitable formulation, combining an ideal gelation temperature (35.45°C) with adequate thermal stability (53.45°C), making it promising for intranasal delivery with precise gelation and sustained retention.

**Table 6.** Gelation Temperature ($T_1$ ºC) and Gel Melting Temperature ($T_2$ ºC)

| Formulation Code | Gelation Temperature ($T_1$ ºC) (mean ± S.D) | Gel Melting Temperature ($T_2$ ºC) (mean ± S.D) |
|---|---|---|
| TSGF1 | 29.49 ± 0.85 | 50.47 ± 0.57 |
| TSGF2 | 33.64 ± 1.05 | 54.75 ± 0.37 |
| TSGF3 | 35.45 ± 0.37 | 53.45± 0.35 |
| TSGF4 | 36.82 ± 0.65 | 55.50 ± 0.75 |
| TSGF5 | 39.46 ± 0.85 | 55.42 ± 0.45 |
| TSGF6 | 31.49 ± 1.35 | 59.45 ± 0.75 |
| TSGF7 | 29.54 ± 0.90 | 57.63 ± 0.70 |

***Viscosity:***

As the temperature dropped from 40°C to 34°C, the viscosity of the in situ thermosensitive nasal gel formulations (TSGF1–TSGF7) increased clearly and consistently, which was consistent with the sol-to-gel transition behavior that thermoresponsive systems are expected to exhibit. At 40°C, all formulations remained in a sol (liquid-like) state, exhibiting relatively low viscosities ranging from 400 cP (TSGF1) to 1065 cP (TSGF7). This low viscosity ensures ease of administration through the nasal route. Upon lowering the temperature to 34°C—close to the average nasal mucosal temperature—all formulations showed a substantial rise in viscosity, indicating successful gelation. The viscosity at 34°C ranged from 2334 cP (TSGF1) to 5899 cP (TSGF7). This transition confirms that the formulations undergo rapid gelation at physiological conditions, which is essential for prolonged nasal residence and controlled drug release. Among all, TSGF6 and TSGF7 demonstrated the highest viscosities at both temperatures, reflecting a denser and more robust gel matrix. However, this may affect sprayability and patient comfort. In contrast, TSGF3 and TSGF4 struck a favorable balance, exhibiting sufficient viscosity rise for in situ gelation while retaining acceptable flow properties at 40°C. These results suggest that TSGF3 or TSGF4 may be ideal for intranasal application, offering effective gelation, mucoadhesion, and patient compliance.

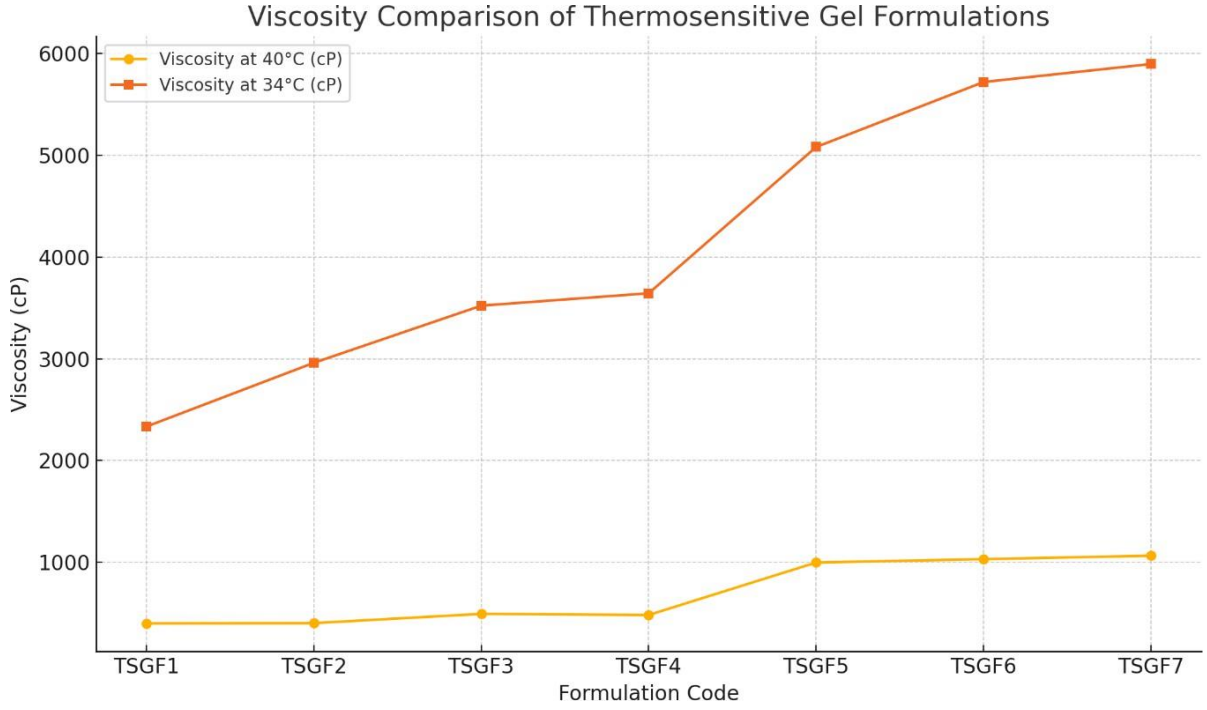


**Figure 7.** Viscosity at 40°C (cP) and at 34°C (cP) data of the in situ thermostatic nasal gel formulation.

***In Vitro Drug Release:***

The in situ thermosensitive nasal gel formulations (TSGF1–TSGF7) showed a regulated and prolonged release pattern over 480 minutes in their in vitro drug release profile. Initially, all formulations exhibited a burst release, with drug release percentages ranging between 19% to 22% at 30 minutes and approximately 28% to 36% at 60 minutes. This early release phase is likely due to the surface-associated drug and rapid hydration of the gel. Among the formulations, TSGF2 and TSGF4 showed a slightly faster initial release, suggesting a relatively looser gel matrix or lower viscosity. In the mid-phase, spanning 120 to 300 minutes, drug release became more sustained and formulation-dependent. TSGF3 released the highest drug amount at 240 minutes (66.81%), indicating an optimal balance between gel structure and diffusion capacity. Conversely, TSGF5 and TSGF6 exhibited comparatively slower release, possibly due to higher gel strength and viscosity, which restrict drug diffusion. In the late phase (360 to 480 minutes), TSGF1 exhibited the highest cumulative release (106.83%), while TSGF2, TSGF3, and TSGF4 also achieved near-complete drug release, ranging from 98% to 104%. These findings suggest faster erosion or weaker gel networks in these formulations. On the other hand, TSGF6 and TSGF7 demonstrated a prolonged and more controlled release, ending with approximately 85% to 88% release, indicating stronger gel matrices and better drug retention over time. Overall, TSGF3 emerged as the most balanced formulation, combining a rapid onset with sustained drug release, making it suitable for effective intranasal drug delivery. While TSGF1 showed the fastest release, it may lack adequate gel stability. TSGF6 and TSGF7, although slower in release, are better suited for prolonged therapeutic action due to their robust gel structure.

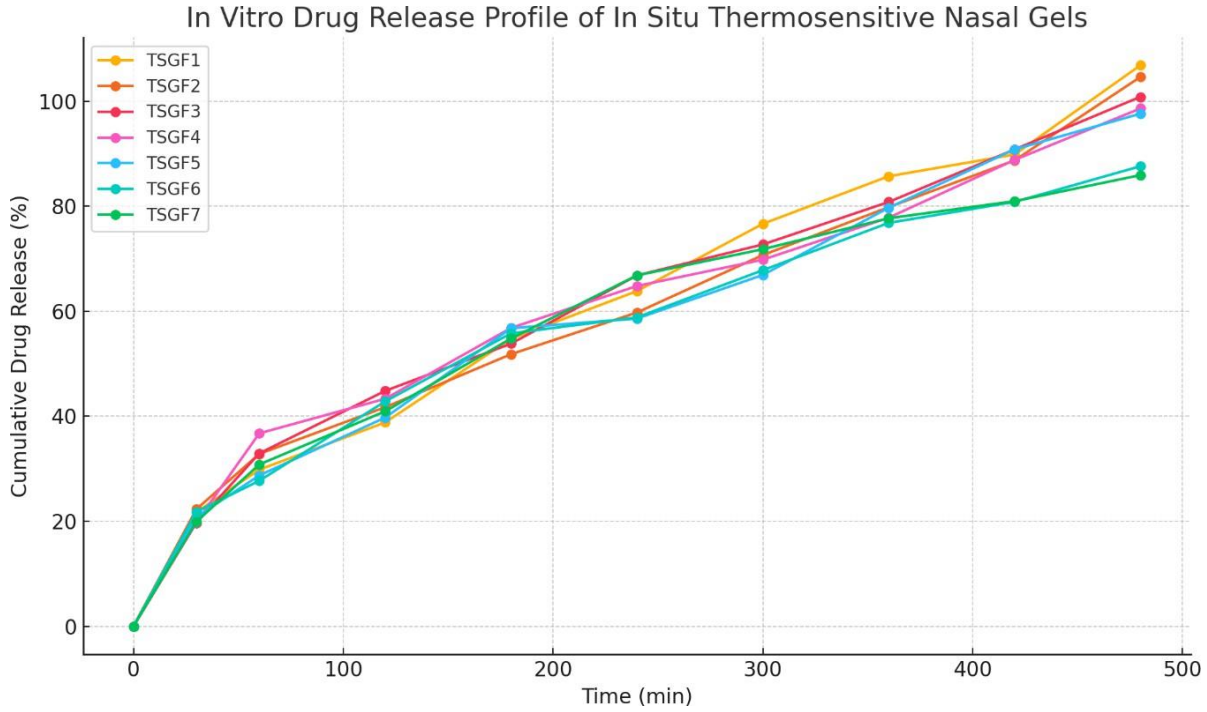


**Figure 8.** Drug release data of the in situ thermostatic nasal gel formulation

**The best optimised formulation:**

The best optimized formulation for in situ thermosensitive nasal delivery of zolmitriptan was found to be TSGF3, taking into account all examined factors, such as particle size, zeta potential, mucoadhesive strength, gelation temperature, viscosity, in vitro drug release profile, and gel stability. With an optimal gelation temperature of 35.45 ± 0.37°C, TSGF3 ensured a successful sol-to-gel transition when administered intranasally. It showed balanced viscosity (493 cP at 40°C and 3523 cP at 34°C), which is crucial for both ease of administration and mucosal retention. Its mucoadhesive strength (3614 ± 14.82 dynes/cm$^2$) and gel strength (118 ± 1.98 sec) indicated firm adhesion to nasal mucosa without being excessively rigid, supporting prolonged residence and sustained drug release. Moreover, TSGF3 presented a controlled and sustained drug release pattern, reaching 90.79% release by 420 minutes, which aligns well with therapeutic requirements for migraine management. It balanced initial burst release with extended delivery, ensuring both rapid onset and prolonged action. Overall, based on comprehensive physicochemical evaluation and release performance, TSGF3 was concluded to be the most suitable and optimized formulation for intranasal delivery of zolmitriptan using a chitosan-based thermosensitive gel system.

## CONCLUSION

In order to improve therapeutic efficacy for migraine therapy, the study successfully developed and assessed a thermosensitive in situ nasal gel containing chitosan nanoparticles loaded with zolmitriptan. Among all tested formulations, TSGF3 demonstrated superior performance across critical evaluation parameters, making it the optimized choice. It demonstrated a gelation temperature that was nearly physiological, guaranteeing a quick sol-to-gel transition when administered intranasally. The formulation showed suitable viscosity and gel strength, which are essential for ease of administration, retention at the mucosal site, and prolonged residence time. Mucoadhesive strength was sufficient to resist mucociliary clearance, further supporting sustained drug presence in the nasal cavity. The most significant benefit of TSGF3 was its controlled and sustained drug release, which reduced the need for frequent dosing by achieving virtually full release within seven hours. The formulation's chemical stability was strengthened by the FTIR confirmation that there was no drug-excipient interaction. The work emphasizes how chitosan nanoparticles and thermosensitive gel systems might be used to get beyond the drawbacks of traditional nasal formulations. To sum up, TSGF3 is a well-suited delivery system for zolmitriptan, providing quick onset, sustained action, and patient compliance. As such, it is a viable substitute for non-invasive intranasal treatment of acute migraine attacks. To confirm its clinical performance, more in vivo research is necessary.

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