

# Intranasal Delivery of Nicotine Via Thermoresponsive Hydrogel Systems: Design, Characterization, and *In Vitro* Evaluation

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*Intranasal Delivery of Nicotine Via Thermoresponsive Hydrogel Systems: Design, Characterization, and In Vitro Evaluation*

*Termoresponsif Hidrojel Sistemler ile Nikotin'in İntranasal Uygulaması: Tasarım, Karakterizasyon ve İn Vitro Değerlendirme*

## SUMMARY

Nicotine replacement therapy (NRT) remains a cornerstone strategy in smoking cessation programs, yet conventional nasal sprays are often limited by mucosal irritation and low patient adherence. This study aimed to develop and characterize a novel thermoresponsive hydrogel nasal formulation that combines Kolliphor P 407 and chitosan to enhance mucosal retention, control nicotine release, and improve patient compliance. Nicotine-containing and nicotine-free hydrogels were prepared with varying concentrations of Kolliphor P 407 and a constant chitosan level. Analytical method validation was performed in accordance with ICH Q2 (R1) guidelines. The physicochemical properties of the formulations, including sol-gel transition temperature, pH, FTIR spectrum, viscosity, and homogeneity, as well as *in vitro* drug release, were assessed. FTIR analysis confirmed the molecular interactions among the formulation components. *In vitro* drug release studies demonstrated a controlled, biphasic nicotine release from optimized hydrogel formulations (F2 and F8), characterized by favorable pH and viscosity profiles suitable for nasal administration. The findings suggest that the developed thermoresponsive hydrogel system is a promising and patient-friendly alternative for intranasal nicotine delivery in NRT.

**Keywords:** Nicotine Replacement Therapy (NRT), Thermoresponsive hydrogel, Kolliphor® P 407, Chitosan, Intranasal drug delivery.

## ÖZ

Nikotin replasman tedavisi (NRT), sigara bırakma programlarında temel stratejilerden biri olmaya devam etmektedir. Ancak, konvansiyonel nazal spreylerde sıklıkla mukozal irritasyon ve düşük hasta uyumu gibi sınırlamalarla karşılaşmaktadır. Bu çalışmanın amacı, mukozal tutunmayı artırmak, nikotin salımını kontrol altına almak ve hasta uyumunu iyileştirmek amacıyla Kolliphor® P 407 ve kitosan kombinasyonu ile yeni bir termoresponsif hidrojel nazal formülasyonu geliştirmek ve karakterize etmektir. Farklı konsantrasyonlarda Kolliphor® P 407 ve sabit miktarda kitosan içeren nikotinli ve nikotinsiz hidrojel formülasyonları hazırlanmıştır. Analitik yöntem doğrulaması, ICH Q2 (R1) kılavuzlarına uygun şekilde gerçekleştirilmiştir. Formülasyonların sol-jel geçiş sıcaklığı, pH, FTIR, viskozite ve homojenlik gibi fizikokimyasal özellikleri ve *in vitro* ilaç salımı değerlendirilmiştir. FTIR analizleri ile formülasyon bileşenleri arasındaki moleküler etkileşimleri doğrulamıştır. *In vitro* ilaç salım çalışmaları, nazal uygulama için uygun pH ve viskozite profiline sahip olan optimize edilen hidrojel formülasyonları (F2 ve F8) için kontrollü ve bifazik bir nikotin salımını ortaya koymuştur. Elde edilen bulgular, geliştirilen termoresponsif hidrojel sisteminin, NRT kapsamında intranasal nikotin uygulaması için umut vadeden ve hasta dostu bir alternatif olabileceğini göstermektedir.

**Anahtar Kelimeler:** Nikotin Replasman Tedavisi (NRT), Termoresponsif hidrojel, Kolliphor® P 407, Kitosan, İntranasal ilaç uygulaması.

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## INTRODUCTION

Tobacco smoke is a highly complex mixture, comprising approximately 5,000 chemically active and reactive constituents. Reports from the World Health Organization (WHO) indicate that tobacco consumption is responsible for about 5.4 million premature deaths globally each year. Furthermore, scientific investigations suggest that the total number of distinct compounds present in cigarette smoke may surpass 100,000 (Al-Dahhan et al., 2022). Among these, nicotine is the most prominent psychoactive alkaloid, found almost exclusively in tobacco plants such as *Nicotiana tabacum* and *Nicotiana rustica*. Structurally, nicotine is a tertiary amine composed of pyrrolidine and pyridine rings (Brandon et al., 2009). Nicotine can enter the body through smoking, chewing, or intranasal inhalation of fine tobacco powders. It readily crosses the blood–brain barrier and distributes rapidly to the central nervous system. When inhaled via smoking, nicotine is absorbed through the pulmonary epithelium into the systemic circulation and quickly reaches the brain (Brandon et al., 2009; Wadgave & Nagesh, 2016). Acting as a full agonist, nicotine stimulates neuronal nicotinic acetylcholine receptors in the ventral tegmental area, thereby triggering dopamine release in the nucleus accumbens and reinforcing addiction (Devi et al., 2020; Prochaska & Benowitz, 2019).

The most widely investigated pharmacological approach for nicotine dependence is nicotine replacement therapy (NRT) (Wadgave & Nagesh, 2016). Purified nicotine formulations have long served as the first-line treatment to alleviate withdrawal symptoms and reduce physical dependence (Prochaska & Benowitz, 2019). NRT has been demonstrated to significantly decrease craving and improve cessation outcomes (Stead et al., 2012). A variety of NRT dosage forms are available, including gums, lozenges, transdermal patches, nasal sprays, and oral inhalers. While patches deliver nicotine continuously at a controlled rate, nasal sprays, gums, and tablets provide acute

dosing (Wadgave & Nagesh, 2016). These therapies deliver lower and slower nicotine levels than cigarettes, reducing withdrawal with a diminished risk of dependence and relapse (Stead et al., 2012).

Commercial nasal sprays, such as Nicorette®, deliver 0.5 mg of nicotine per 50 µL spray and are typically administered once per nostril, corresponding to a total of 1 mg per dose. They are recommended for use 1–2 times per hour, with a maximum of five administrations per day (Brandon et al., 2009; Devi et al., 2020). Owing to their rapid absorption, nicotine nasal sprays produce a pharmacokinetic profile similar to that of cigarette smoking (Uzaslan, 2003). However, their clinical utility has been limited due to poor patient compliance, largely attributed to local side effects such as dryness and irritation, which can lead to reduced efficacy (Devi et al., 2020).

Several alternative nicotine delivery systems have been investigated. For instance, cellulose-based hydrogels with ultrasound-triggered release have been developed. However, they were primarily designed for neurological disorders such as Alzheimer's and Parkinson's disease. Cellulose hydrogel drug carrier was created with cellulose concentrations ranging from 0.45 wt% to 1.8 wt%, and 1 wt% nicotine solution was added to the system at a weight ratio of 9:1 (cellulose:nicotine). So the formulation contains a different dose of nicotine from those used in NRT (Iresha & Kobayashi, 2021). In another study, nicotine was incorporated into chitosan hydrogels, with or without glutaraldehyde crosslinking, for evaluation in transdermal delivery systems. While these systems offered prolonged release till 48 h, they did not provide the dosage per hour required for smoking cessation (Lino et al., 2017). Intranasal administration of nicotine at a dose of 1 mg/kg, along with proliposomes containing either nicotine base or nicotine hydrogen tartrate salt, or a mixture of powdered nicotine hydrogen tartrate salt and sorbitol, was applied for the prolonged delivery of nicotine to the systemic circulation. Nasal nicotine absorption from these formulations was ex-

tremely quick (less than 10 minutes to achieve plasma peaks) and resulted in significantly sustained plasma nicotine levels when compared to NB and NS saline solutions, as well as previous nasal nicotine sprays (Jung et al., 2000). The combination of pulsatile and sustained plasma nicotine profiles for smoking cessation was achieved by developing a nasal formulation of a powder containing a nicotine-Amberlite resin complex, which is a cationic exchange material (Cheng et al., 2002). All these formulations were developed for NRT; however, they do not provide mucoadhesion, which is essential to overcome mucociliary clearance for prolonged delivery of nicotine via the nasal route.

Hydrogels, which are three-dimensional hydrophilic polymeric networks, can retain large amounts of water and biological fluids. Their insolubility is ensured by chemical crosslinks (junctions, tie-points) and physical associations (entanglements, crystallites) (Peppas et al., 2000). Some hydrogels exhibit responsiveness to external stimuli such as pH or temperature. For example, Kolliphor® P 407-based formulations are well known for their thermoresponsive properties, undergoing sol-gel transitions in response to small temperature changes (Darge et al., 2019; Dumortier et al., 2006; Kojarunchitt et al., 2011). Hydrogels are also used as carriers that can interact with the mucosa lining in various parts of the body, including the gastrointestinal (GI) tract, colon, vagina, nose, and others, due to their ability to prolong residence time at the delivery location (Peppas et al., 2000). Additionally, thermoresponsive gels undergo a sol-gel transition at nasal temperature, allowing for in situ gelation and prolonged retention at the application site. The polymeric matrix further modulates drug diffusion by forming a three-dimensional network that slows the release of the incorporated drug (Arisoy & Dortunc, 2020).

Chitosan represents a multifunctional polymer, featuring both mucoadhesive and permeation-enhancing properties, and therefore is a widely stud-

ied excipient for mucosal drug delivery. Regarding nasal administration, chitosans have been used for the preparation of gels, solid inserts, powders, and nanoparticles, in which a three-dimensional network can be recognized (Luppi et al., 2010). Its mucoadhesive properties enable it to interact with the negatively charged mucin layer, thereby prolonging residence time and resisting clearance mechanisms (Arisoy et al., 2024; Arisoy & Dortunc, 2020). Consequently, combining chitosan with thermoresponsive polymers offers a rational strategy for sustained nasal delivery.

In the present study, thermoresponsive hydrogel formulations composed of Kolliphor® P 407 and chitosan were developed for nicotine replacement therapy. This system was designed to address the limitations of existing nasal sprays by providing controlled release, reduced dosing frequency, and enhanced patient compliance. Furthermore, the water-retention capacity of the hydrogels supports regional moisturization, potentially improving tolerability. By combining thermoresponsive gelation with mucoadhesive properties, these formulations are expected to maintain longer residence in the nasal cavity, overcome mucociliary clearance, and achieve a sustained release profile, consistent with findings from previous studies on nasal mucoadhesive systems (Arisoy, Sayiner, & Comoglu, 2020).

## MATERIALS AND METHODS

### Materials

Kolliphor® P 407 (102.13 g/mol) was a gift from BASF. Nicotine (d: 1.010 g/mL, liquid form), Sodium dihydrogen phosphate, Sodium Hydroxide, and Medium molecular weight chitosan (Acetylation degree: 75-85%) were from Sigma. Mucin from porcine stomach, Type II was from Sigma.

### Methods

#### Analytical method validation

A high-concentration nicotine stock solution (100 µg/ml) was prepared using a buffer solution (pH 6.5). Standard solutions were obtained at concentrations of

1, 2.5, 5, 7.5, 10, and 12.5 µg/ml with several dilutions (Østergaard et al., 2010). The absorbance of the dilutions, measured at 254 nm using a UV-visible spectrophotometer (Cary 60 UV-vis), was plotted versus the concentration (Al-Dahhan et al., 2022; Østergaard et al., 2010). The study was repeated in three parallel groups, and the average values were graphed with their standard errors. The method was validated according to the ICH guideline Q2 (R1) with the parameters of accuracy, precision (intermediate precision), Limit of Detection (LOD), Limit of Quantification (LOQ), and linearity (Arisoy et al., 2021).

#### Preparation of Kolliphor® P 407-chitosan thermoresponsive hydrogel

0.5 mL of acetic acid was added to 9.5 mL of distilled water, and 0.05 g of chitosan was weighed and added. The mixture was then stirred with a magnetic stirrer until homogeneous. It was then stored in the refrigerator overnight. Different amounts of Kolliphor® P 407 were weighed and mixed with 1 mL of cold buffer 6.5. Then, 0.8 mL of buffer was added 1 hour later (Hulambukie et al., 2022). Half an hour later, 0.2 mL of the 0.5% chitosan solution was added. To achieve a homogenous system, it was agitated in an ice bath at 250 rpm using a magnetic stirrer (Multi-Channel Stirrer, MS-53M) for 24 hours (Sayiner et al., 2020). To obtain 50 mg of nicotine in each formulation, 50 µL of nicotine ( $d = 1.01 \text{ g/mL}$ ) and 50 µL of water were added during the preparation step. Thus, nicotine and nicotine-free formulations were created using Kolliphor® P 407 ratios ranging from 16% to 18% and chitosan at a constant ratio (Table 1.).

**Table 1.** Formulation Parameters

Formulation	Kolliphor® P 407	Nicotine	Chitosan (5 %)
F1	18 %	-	+
F2	18 %	+	+
F3	17 %	-	+
F4	17 %	+	+
F5	16 %	-	+
F6	16 %	+	+
F7	18 %	-	-
F8	18 %	+	-

#### Homogeneity

Nicotine and nicotine-free Kolliphor® P 407 hydrogels were visually examined for homogeneity. The resulting gels were heated to 40°C, which is above the transformation temperature, and gel images were recorded (Sayiner et al., 2020).

#### Determination of sol-gel transition temperature

The hydrogel's sol-gel transition was determined using the inverse test tube method. A test tube containing 1 mL of the hydrogel formulation was put in a water bath (Water Bath, Wisconsin). The temperature of the water bath was raised from 10°C to 40°C at a rate of 1°C per minute. The tubes were inverted every 30 seconds to check for flow, and the temperature showed that no flow was recorded as the transition temperature (Gu et al., 2020; Sayiner et al., 2020; Watts & Smith, 2009). The experiment was conducted in three parallel groups.

#### pH

A drop of the sample was dissolved in 10 mL of water, and the pH was measured using a pH meter (Inolab). The experiment was carried out in 3 parallel (Watts & Smith, 2009).

#### Viscosity

Viscosity was calculated at a speed of 5 rpm at a temperature of 33°C, which is above the sol-gel transition temperature of the gels (Sayiner et al., 2020). The experiment was conducted in three parallel runs using a rotational viscometer (Brookfield DV2-T) with a CPA-51Z spindle.

#### Swelling index

The swelling ratio was evaluated by recording the sample weights before and after swelling at various time intervals. Pre-weighed specimens were immersed in phosphate-buffered saline (pH 6.5). After carefully removing excess surface buffer with filter paper, the swollen samples were weighed at predetermined intervals of 0, 10, and 15 minutes. The swelling ratio at a given time (Wt) was calculated using equation 1:

$$Wt = \frac{Ws - Wd}{Wd} \quad (\text{Eq 1.})$$

where  $W_s$  represents the swollen weight of the sample at a specific time and  $W_d$  is the corresponding dry weight. The point at which no further change in weight was observed was defined as the equilibrium swelling ratio ( $Wt$ ) (Arisoy et al., 2023).

#### Fourier transform infrared spectroscopy (FT-IR) analysis

To investigate the molecular integration of hydrogel and nicotine, it was analyzed using FTIR spectrometry. Briefly, the spectra were recorded with 40 scans in the wave number range from  $4000 \text{ cm}^{-1}$  to  $400 \text{ cm}^{-1}$  at a resolution of  $1 \text{ cm}^{-1}$ . All measurements were performed at room temperature (Akkuş-Dağdeviren et al., 2023).

#### Mucoadhesion test

The mucoadhesive property was evaluated using a modified balance method (Thangarajoo et al., 2023). A 5% mucin solution was prepared in a pH 6.5 buffer (Thirawong et al., 2008). A filter paper impregnated with 0.4 mL of mucin solution was affixed to a clean glass slide, while on the opposite side, the lyophilized formulation was secured to the lower arm of the balance using double-sided adhesive tape in the same manner. Following 10 seconds of contact between the mucin and the formulation, the weight on the balance was gradually increased by adding water dropwise to the empty pan of the balance until detachment occurred. The mucoadhesive strength of each formulation was defined as the minimum weight necessary to separate the mucin-impregnated filter paper from the formulation. This method demonstrates the formulation's ability to remain attached at the site of application, which is crucial for localized therapeutic efficacy. The mucoadhesive property was quantified by calculating the mucoadhesive strength and the corresponding force of adhesion. The force of adhesion for the formulations and chitosan was calculated using the following equation 2:

$$\text{Force of adhesion (N)} = \frac{\text{Mucoadhesive strength (g)} \times 9.81}{1000} \quad (\text{Eq 2.})$$

where mucoadhesive strength (g) represents the minimum weight required for detachment, and 9.81 is the gravitational acceleration constant.

#### In vitro drug release

A phosphate buffer solution with a pH of 6.5 was used as a buffer to simulate the nasal area for in vitro release experiments (Arisoy et al., 2020). Hydrogel, nicotine solution in pH 6.5 buffer, and Nicorette® nasal spray, each containing 2.5 mg nicotine were filled into separate dialysis bags (MWCO of 10 kDa) (Arisoy & Comoglu, 2020). Then, the bags were placed in beakers containing 500 mL of pH 6.5 buffer, the temperature was set at  $37^\circ\text{C}$ , and mixed at 50 rpm using the USP 2 apparatus (dissolution device, PHARMA TEST). At predetermined time intervals, 1 mL samples were taken from the beakers, and the release medium was balanced with an equal amount of pre-heated refreshing medium. Nicotine content of the samples was calculated with UV at 254 nm.

#### RESULTS AND DISCUSSION

Nicotine has a tertiary amine structure and can exist in either ionized or non-ionized forms, depending on the pH of its surroundings. After smoking, nicotine travels rapidly to the arterial circulation and brain in 15 to 20 seconds, where it displays its addictive effects. The speed of delivery to the brain is regarded as playing a key role in the addictive potential of inhaled nicotine when compared to other routes. Nicotine dependence from drugs that give nicotine slowly, such as nicotine patches, gum, and lozenges, seems to be minimal (Prochaska & Benowitz, 2019).

Drugs can be transported directly to the brain via the olfactory and trigeminal nerve pathways, making the intranasal route an exciting alternative to traditional parenteral and oral routes. Additionally, it bypasses hepatic first-pass effects, ensuring a rapid onset of action and providing a patient-friendly method of administration (Koo et al., 2024). Smokers typically absorb about 1 to 1.5 milligrams of nicotine with

each inhalation. While nicotine’s half-life is about two hours, environmental and genetic variables may influence its concentration in blood vs time. Blood nicotine levels climb over four to six hours due to regular smoking, plateau throughout the day, and then fall overnight (Prochaska & Benowitz, 2019).

In this formulation study, a nicotine-loaded nasal spray was prepared to obtain a direct and rapid action compared to the oral route. It contains 0.5 mg of nicotine per 50 µL to sustain steady nicotine levels in the brain, equivalent to smoking one cigarette. Consequently, in a daily smoker, the brain is exposed to nicotine for 24 hours every day, resulting in symptoms and tolerance development (Prochaska & Benowitz, 2019). Formulation characterization studies were conducted to ensure the quality of the developed thermoresponsive nicotine-containing hydrogel, and the results are discussed in this paper.

### Analytical method

All examinations were conducted in 3 parallel groups. The average values were graphed with the standard errors. The deviation of the data obtained as a result of accuracy and precision studies was found to be less than 2%. The accuracy and precision of the method were evaluated at three concentration levels, representing low, medium, and high concentrations

(5 µg/mL, 7.5 µg/mL, and 10 µg/mL, respectively). The accuracy of the analytical method was evaluated based on recovery studies. The mean percentage recoveries were found to be 100.38%, 99.51%, and 103.42% at the tested concentration levels. The corresponding standard deviations were 0.143, 0.094, and 0.047, respectively, with relative standard deviations (RSD%) of 0.143, 0.094, and 0.045. These values are within the acceptable limits, indicating the accuracy and reliability of the method. The precision of the method was assessed at three concentration levels (5 µg/mL, 7.5 µg/mL, and 10 µg/mL). The mean concentrations obtained were 5.0188, 7.4630, and 10.3423 µg/mL, respectively. The standard deviations were 0.01, 0.01, and 0.00, with relative standard deviations (RSD%) of 0.14, 0.09, and 0.04, respectively. These results demonstrate the high precision and reproducibility of the developed analytical method (Arisoy, Sayiner, & Comoglu, 2020). The LOD value was found to be 0.04 µg/mL, and the LOQ value was found to be 0.126 µg/mL. The calibration equation was prepared at 5 points as specified in the ICH guide, and R<sup>2</sup> was evaluated as the linearity parameter. The R<sup>2</sup> value was found to be as suitable as 0.998 (Figure 1.). All results indicate that the method was validated (Arisoy et al., 2021).

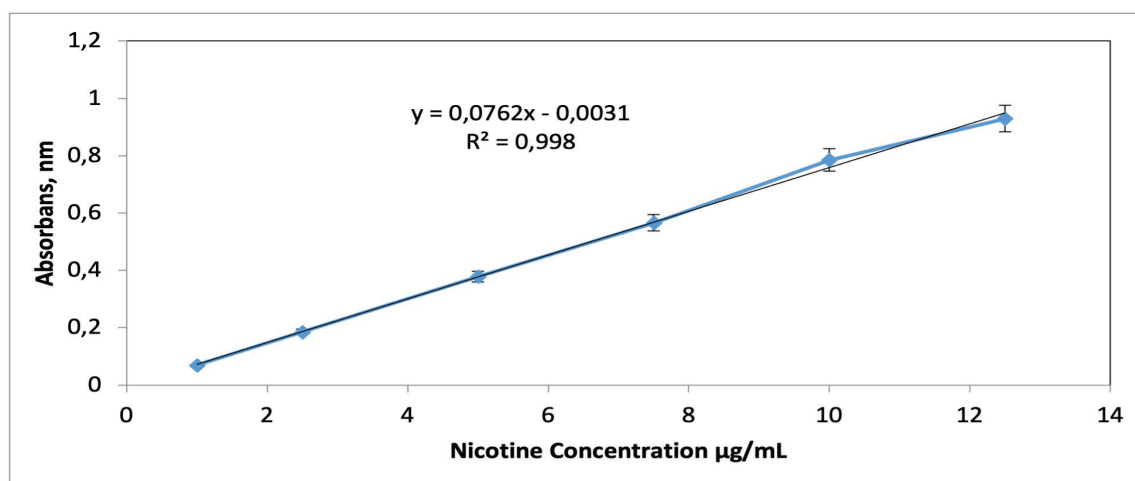


Figure 1. Calibration graph of nicotine concentration (µg/mL) vs. absorbance (nm)

### Characterization studies

The primary limitation of intranasal application is the removal of drugs before they are sufficiently absorbed through nasal drainage (Arisoy et al., 2020). The use of mucoadhesive polymers, such as chitosan, has been proposed in drug delivery to increase the residence time at the mucosal surface and facilitate the permeation of various drugs into the brain (Ojeda-Hernández et al., 2024). Kolliphor® P 407 was used for its thermo-reversible behavior to formulate an intranasal sol-gel that accumulates in the nasal area after easy application in a solution state, while chitosan was used for its mucoadhesive properties (Riaz et al., 2023).









All hydrogels have a homogeneous appearance (Table 2.). All experiments were carried out in three parallel tests to determine the sol-gel transition of the hydrogel using the inverse test-tube method. The turn was observed in one minute for all formulations, as

the experiment showed a 1°C increase in heat over this period.

F5 and F6, with the lowest Kolliphor® P 407 ratio (16%), did not form a gel state during the experiment, which was heated to 40°C. This result is supported by the literature, which shows that higher sol-gel transition temperatures occur with the decreased Kolliphor® P 407 concentration. In this study, nicotine addition, which was + for F6 and - for F5, did not alter this phenomenon, while the increase in pH might be attributed to nicotine's tertiary amine structure (Table 1-2.) (Prochaska & Benowitz, 2019; Riaz et al., 2023).

F1, F2, F3, and F4 showed a sol to gel transition at about 25°C, while F7 and F8 showed it at about 30°C, indicating that nicotine addition did not affect the transition temperature, while chitosan (5%) addition lowered the transition temperature due to it is gel state at 25°C.

**Table 2.** Characterization of formulations

Formulation	Transition temperature (°C)	pH	Appearance
F1	24.3 ± 0.1	6.04 ± 0.006	
F2	23.53 ± 0.16	7.84 ± 0.05	
F3	25.46 ± 0.46	6.02 ± 0.01	
F4	24.93 ± 0.16	8.14 ± 0.02	
F5	-	5.15 ± 0.06	
F6	-	8.02 ± 0.07	
F7	31.43 ± 0.29	7.77 ± 0.03	
F8	29.13 ± 0.15	6.77 ± 0.01	
Nicotine solution in the buffer pH 6.5	-	7.5 ± 0.03	-
Nicorette® nasal spray	-	7.00 ± 0.05	-
Nicotine solution in the water	-	9.72 ± 0.03	-

The pH of the healthy nasal mucosa is between 5.5 and 6.5. Formulations with a pH close to that of the nasal mucosa are associated with reduced discomfort following nasal administration (Watts & Smith, 2009). All formulations have a suitable pH to apply to biological membranes (Table 2.). It was shown that the addition of chitosan lowered the pH of the formulation, whereas the addition of nicotine led to a higher pH in the formulation (Table 2.). Chitosan is a cationic biopolymer that relies on pH for its solubility; it does not dissolve in neutral or alkaline water but can become soluble in acidic aqueous solutions through the protonation of its amine groups (Darge et al., 2019). In the formulation studies, chitosan was initially dis-

solved in an acidic solution and added, resulting in a rise in the pH of the formulation produced with the addition of the chitosan solution. Since nicotine possesses a basic chemical structure, its addition led to an increase in the pH of the chitosan solution.

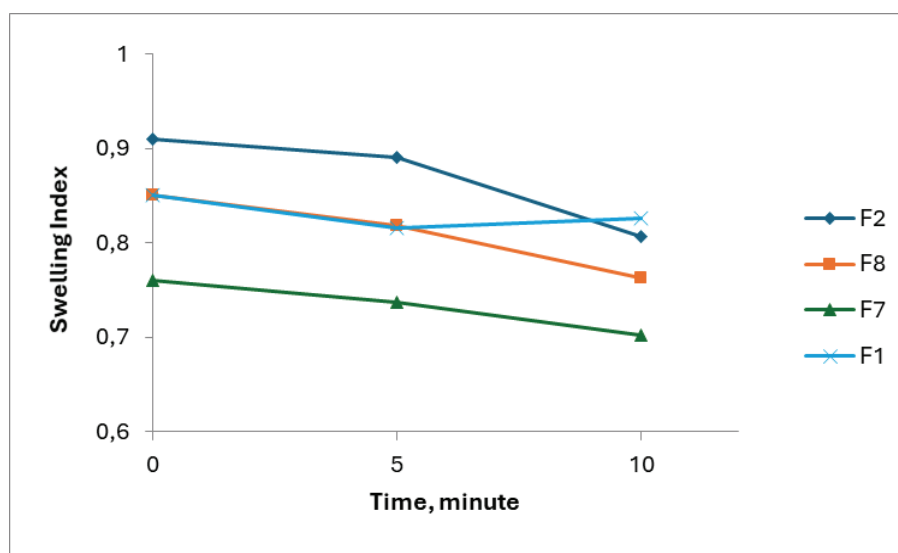
Nicotine is a weak base with a pKa of 8.0, which means that at a physiological pH of 7.4, 69% of it is ionized while 31% remains unionized. The unionized form, referred to as freebase nicotine, easily crosses membranes such as the nasal mucosa (Prochaska & Benowitz, 2019). F1, F2, F7, and F8 have a pH range between 6.04 and 7.84, which favors the non-ionized form of nicotine, facilitating the mucosal transition.

**Table 3.** Viscosity of formulations

Formulation	Viscosity (cP)	Relative standard deviation (RSD) (%)
F1	34126 ± 975	2.86
F2	49373 ± 817.39	1.66
F7	4459.67 ± 207.32	4.65
F8	6255.00 ± 31	0.50
Chitosan (5%)	5.52 ± 2.60	47.11

It was found that as the Kolliphor® 407 concentration increased, the viscosity of the formulation also increased; however, due to the fluidic nature of chitosan solution, chitosan-added formulations showed lower viscosity (Riaz et al., 2023). F1, F2, F7, and F8

have suitable viscosity for a stable state in the nasal area (Gu et al., 2020). F2 and F8, with and without chitosan, were chosen as optimum formulations for further studies due to their acceptable viscosity, pH, transition temperature, and appearance.



**Figure 2.** Swelling index vs. time of formulations

All formulations exhibited a high swelling index close to 1 (Figure 2.). By 15 minutes, however, all formulations degraded and lost their structural integrity. Among them, F7, which lacked both chitosan and nicotine, showed the lowest swelling, whereas F2, containing both chitosan and nicotine, demonstrated the highest swelling. F8 and F1 displayed similar swelling indices.

### FTIR analysis

In the spectrum of Kolliphor® P 407 (Figure 3.), characteristic signals are observed mainly at wavelengths of  $\sim 2878\text{ cm}^{-1}$  (C-H aliphatic stretching),  $\sim 1372\text{ cm}^{-1}$  (O-H bending), and  $\sim 1279\text{ cm}^{-1}$  (C-O stretching). In the F8 (containing nicotine and Kolliphor® P 407), peaks belonging to nicotine were also observed in addition to the peaks belonging to Kolliphor® P 407. The peak observed at  $716.76\text{ cm}^{-1}$  characterizes the different chemical functions of nicotine and corresponds to the out-of-plane bending of the C-H bond of the mono-substituted pyridinic cycle (Al-Dahhan et al., 2022). The vibration band for chitosan at  $3266\text{ cm}^{-1}$  is related to N-H stretching, as well as -OH linked to both intermolecular and intramo-

lecular hydrogen bonding. An absorption band near  $2872\text{ cm}^{-1}$ , associated with asymmetric C-, was detected. The H stretching of the  $-\text{CH}_2$  group appeared as a vibration band at  $1634\text{ cm}^{-1}$ , attributed to C=O stretching of amide I. The N-H deformation of amide II was seen at  $1542\text{ cm}^{-1}$ , with bands corresponding to the C- $\text{CH}_3$  deformation observed at  $1256\text{ cm}^{-1}$ , and the C-O-C stretching of the saccharide structure at  $1152, 1067, \text{ and } 1023\text{ cm}^{-1}$  were recorded (Becerra et al., 2022).

In the FTIR analysis of the formula containing chitosan and Kolliphor® P407 without nicotine, peaks belonging to Kolliphor® P407 were observed next to the peaks belonging to chitosan. The presence of characteristic signals belonging to Kolliphor® P407 was observed at wavelengths of  $\sim 2879\text{ cm}^{-1}$  (C-H aliphatic stretching vibration),  $\sim 1372\text{ cm}^{-1}$  (O-H bending vibration), and  $\sim 1279\text{ cm}^{-1}$  (C-O stretching vibration) (Becerra et al., 2022). Characteristic peaks of nicotine (e.g., C-H stretching at  $\sim 3020\text{--}3100\text{ cm}^{-1}$ , N-H stretching at  $\sim 3300\text{ cm}^{-1}$ , and C-N stretching at  $\sim 1200\text{--}1350\text{ cm}^{-1}$ ) were observed in the nicotine-loaded hydrogel (F2) (Al-Dahhan et al., 2022).

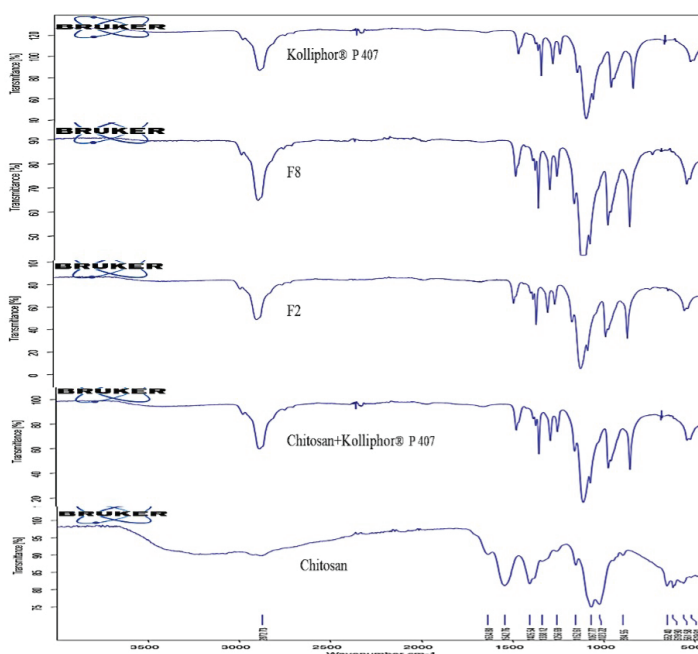


Figure 3. FTIR spectrum

### In vitro mucoadhesive strength

The mucoadhesive property of the formulation helps establish the ability of a topical formulation to retain at the site of action when applied to a specific

area, such as the nasal area (Thangarajoo et al., 2023). In the development of an in situ gel system, the mucoadhesive strength is a crucial factor to be modulated in order to promote the retention of the formulation at the application site.

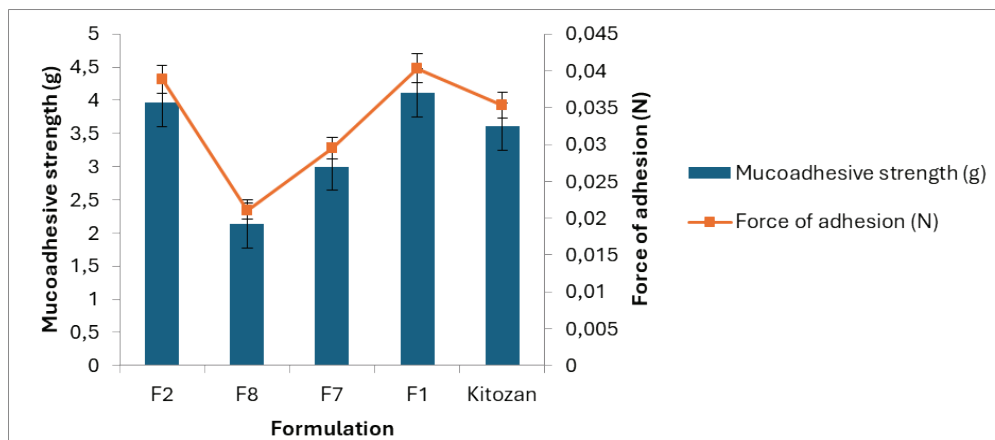


Figure 4. Mucoadhesive strength and force of adhesion of the formulations

Since formulations F1, F2, F7, and F8 exhibited suitable viscosity for maintaining stability in the nasal cavity and contained the same amount of Kolliphor® P 407, their mucoadhesive properties were investigated. Furthermore, these formulations provide a suitable model for investigating the effects of chitosan and poloxamer content, as well as nicotine incorporation, on mucoadhesion. Although Kolliphor® P 407 thermo-responsive hydrogels are not inherently mucoadhesive, they have been widely investigated as mucosal drug delivery systems owing to their thermosensitive

gelation at the application site, which prolongs residence time and supports effective drug delivery. Incorporation of chitosan into Kolliphor®-based gels enhanced both gel strength and mucoadhesive properties, as the protonated amino groups of chitosan can readily interact with the negatively charged mucin components of the mucosal layer (Giuliano et al., 2018). As shown in Figure 4, the addition of chitosan enhanced mucoadhesiveness, whereas nicotine incorporation led to a reduction.

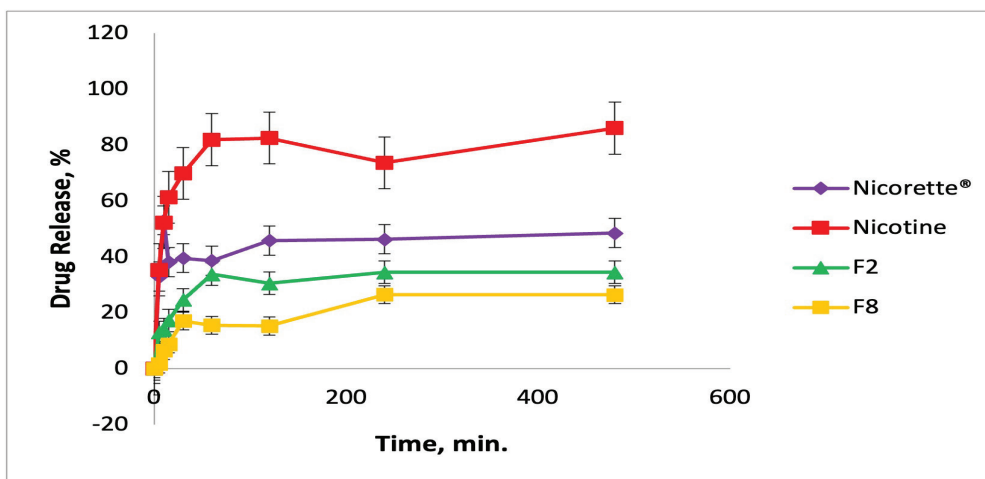


Figure 5. In vitro release profile of nicotine from the drug-loaded formulations in PBS (pH 6.5) at 37°C

### ***In vitro* drug release**

Numerous substances with a range of uses are found in Nicorette® nasal spray; NNS aroma DZ-03226 (B-ionone) as a flavoring agent, sodium chloride as a tonicity adjuster, sodium phosphate dodecahydrate, anhydrous citric acid as buffered, polysorbate 80 as a surfactant, methyl parahydroxybenzoate, propyl parahydroxybenzoate as antimicrobials, disodium edetate as a chelating agent, and purified water as a solvent. It was observed that Nicorette® nasal spray released 53.14% of the loaded nicotine within the first ten minutes. The percentage release rate increased over the first two hours and remained constant until the eighth hour, when the total release rate reached 48.57%.

The nicotine solution was prepared using only a phosphate buffer (pH 6.5) as a solvent. The nicotine solution showed a higher release %, 86.11%, than the formulations and Nicorette® nasal spray, which might be due to the absence of other substances that could potentially affect the release rate of nicotine through molecular interaction (Arisoy et al., 2024).

When the releases of nicotine from formulations, Nicorette® nasal spray and nicotine solution, were evaluated together, it was observed that the release was affected by pH as well as molecular interaction. Since the nicotine solution had a basic pH, nicotine was present in a non-ionized form in this solution and could therefore move more easily from the membrane to the release medium (Table 2, Figure 5.) (Al-Dahhan et al., 2022; Arisoy & Şalva, 2023).

F8 formula released 15.57% of the loaded nicotine into the buffer medium during the first hour. The percentage release increased during the first hour but stabilized during the second hour. It climbed again after the second hour, eventually releasing 26.53% of the loaded nicotine in the fourth hour. It was observed that F8 released the nicotine in its content in a controlled manner, exhibiting biphasic release (Arisoy & Comoglu, 2020).

The F2 formulation released 13% of the loaded nicotine in the first ten minutes and released 33.84% in

the following hour. The release after the first hour continued stably until the eighth hour, and 34.55% of the loaded drug was released in total. It was observed that the F2 formulation released the nicotine content in a controlled manner after the burst release that occurred in the first five minutes (Arisoy & Comoglu, 2022).

These findings are consistent with the literature, as formulations F8 and F2 demonstrated the advantages of in situ gelling systems for intranasal drug delivery: their gel structure prolongs residence time in the nasal cavity, enables sustained drug release, and ultimately enhances bioavailability (Giuliano et al., 2018). The difference in burst release between F2 and F8 can be attributed to the absence of chitosan in F8, which causes a portion of nicotine to remain unincorporated within the gel matrix and localize on the surface, leading to its rapid release (Arisoy & Şalva, 2023).

The difference in the release profile between the F2 and F8 formulas might be due to a pH difference (Table 2.). F2 might have shown a higher release than F8 at the 8th hour due to its higher pH. Additionally, the differences between F2, F8, and Nicorette® may be attributed to the molecular interactions of Kolliphor® and chitosan with nicotine. The discrepancy between the two formulations' release patterns and percentage release rates at the conclusion of the eighth hour may be associated with the hydrophilic nature of chitosan, which is present in F2 but not in F8 (Arisoy et al., 2024).

### **CONCLUSION**

This study successfully formulated and evaluated thermoresponsive hydrogel systems incorporating Kolliphor® P 407 and chitosan for intranasal nicotine replacement therapy. The developed formulations exhibited desirable gelation behavior at physiological temperatures, appropriate viscosity for nasal retention, and a pH compatible with the nasal mucosa. Controlled *in vitro* nicotine release profiles were observed, particularly in F2 and F8 formulations, indicating effective modulation of drug delivery. FTIR spectroscopy confirmed the presence of key function-

al interactions between nicotine, chitosan, and Koliphor® P 407. Overall, these results demonstrate that the hydrogel system holds substantial potential as a patient-compliant and effective alternative to conventional nicotine nasal sprays, overcoming limitations such as rapid drug clearance.

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#### AUTHOR CONTRIBUTION STATEMENT

The conceptualization, methodology, investigation, data curation, formal analysis, visualization, writing – original draft, writing – review & editing, project administration (SA), the investigation, literature review, writing – original draft, data curation, formal analysis, project administration (ÖY), the investigation, literature review, writing – original draft, data curation, formal analysis, project administration (BK), the conceptualization, methodology, formal analysis, data curation, project administration (RB), the formal analysis, data curation, writing – review & editing (DÖ).

#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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