



Nadifloxacin-Loaded Thermosensitive *In Situ* Gel Formulations with a Quality by Design Approach

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ABSTRACT

Objectives: In our study, we aimed to prepare topical *in situ* gels containing nadifloxacin (NDF), used in the treatment of acne and superficial skin infections, and to evaluate them using characterization studies.

Materials and Methods: Thermosensitive (temperature-sensitive) *in situ* gels were produced by the cold method using poloxamer 188 (P188) and poloxamer 407 (P407) after methylcellulose (MC) and gellan gum (GG) were dissolved. Finally, the NDF concentration in the gels was 1% (w/w). The prepared gels exhibited desirable properties, as shown by Fourier-transform infrared spectroscopy, and in clarity, pH, gelation temperature, gelling capacity, viscosity, mechanical properties (springiness, resilience, and cohesiveness), spreadability (work of adhesion, work of shear, stickiness, and firmness), drug content uniformity, *in vitro* release, and cytocompatibility (MTT cytotoxicity assay). The impact of critical materials in the formulation on the results was evaluated using quality by design.

Results: The gelation temperatures of all NDF-included *in situ* gels were found to be in the range of 30–35.2 °C. Furthermore, the cytotoxicity of these gels was evaluated using MTT analysis. Based on the observed results, the most suitable gel was identified as N3, which contains 17.5% P407, 7% P188, 0.7% MC, 0.1% GG, and 1% NDF.

Conclusion: After further studies to determine its effectiveness against acne, it could become an alternative to existing creams for acne and superficial skin infections on the market.

Keywords: Nadifloxacin, thermosensitive, *in situ* gel, quality by design, poloxamer

INTRODUCTION

The skin is the largest organ in adults (surface area: ~2 m² and weight: ~3.6 kg), and has three layers: dermis, hypodermis, and epidermis.^{1,2} Skin diseases include scabies, superficial mycoses, eczema (dermatitis), pigment anomalies, and acne.³ Acne involves inflammatory lesions (papules and pustules), noncomedones, and seborrhea.⁴ Acne vulgaris (acne) is a chronic inflammatory disease that affects the pilosebaceous follicles. It ranks eighth among skin diseases and affects

9.4% of the world's population.⁵ Clinical and psychological consequences can occur, including negative psychological effects resulting from potential scarring and pigmentary changes.⁶

Nadifloxacin (NDF) is a newer quinolone-derivative antibiotic commonly used topically to treat inflammatory acne lesions.^{7,8} NDF is antibacterial and anti-inflammatory, making it beneficial for treating inflammatory acne. It is highly effective

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Received: 23.09.2025 Accepted: 23.09.2026 Publication Date: 27.10.2026

Cite this article as: Özcan Bülbül E, İzbudak B, Bal Öztürk A, Üstündağ Okur N. Nadifloxacin-loaded thermosensitive *in situ* gel formulations with a quality by design approach. Turk J Pharm Sci. 2026;23(3):150-164



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against Gram-negative and Gram-positive bacteria, such as *Propionibacterium acnes*. NDF 1% cream is available for the treatment of acne.⁸ It is applied twice a day for 1 or 2 weeks.⁷ However, NDF has very low water solubility ($\log p = 2.47$), and, because of the large droplet size of dermal creams, penetration into the skin layers is limited, which has led to patient non-compliance with treatment. These problems may be overcome by different drug delivery systems.⁸

In situ gel is a drug-delivery system that transitions from “sol to gel” upon application, providing sustained drug release. While their advantages include extended drug residence time, improved patient compliance, and localized drug delivery, they also have some known disadvantages, such as limited mechanical strength and drug degradation. *In situ* gels are divided into three groups: temperature-sensitive (thermosensitive), pH-sensitive, and ion-sensitive.^{9,10} Temperature-sensitive *in situ* gel systems are in the “sol” state at room temperature. Depending on the application site, they transition from sol to gel at different temperatures (32–37 °C). As the temperature increases, polymer-water interactions change. Polymers undergo gelation as temperature increases at concentrations above the critical micelle concentration.¹¹ Poloxamers, well-known polymers, are thermosensitive and are liquid at 4–5 °C, turning into a gel as temperature rises. Poloxamers are suitable for topical, nasal, ophthalmic, and vaginal application routes.¹² NDF was homogeneously dispersed within the prepared *in situ* gels. Compared with conventional cream formulations, this formulation offers the advantage of prolonged residence time at the application site due to its adhesive properties and *in situ* gelation behavior. *In situ* gel systems are a special class of polymeric sustained-release formulations that are prepared in liquid form and transform into a gel after administration via various physicochemical triggers. In these systems, the drug may be present in the polymeric matrix either in dissolved form or as a suspension.¹³ *In situ* gels transform from a solution or suspension into a gel at the target site under the influence of factors such as interactions with body fluids, temperature, pH, ion concentration, UV radiation, and the presence of specific molecules and ions.¹⁰ In the literature, dexamethasone-dispersed *in situ* gels for oral mucosal application,¹⁴ fusidic acid-loaded ophthalmic *in situ* gels⁹, and econazole nitrate-loaded vaginal *in situ* gels¹⁵ prepared using this approach have been reported.

Gellan gum (GG) is a microbial polysaccharide produced by *Pseudomonas elodea*, characterized by its water solubility and mucoadhesive properties.¹⁴ Initially utilized in the food industry as a stabilizing, thickening, and gelling agent, this biopolymer has recently attracted considerable attention in pharmaceutical applications due to its biocompatibility, biodegradability, low toxicity, and structural stability.^{14,16,17} In the present study, GG was preferred not only because of these advantageous properties but also due to its ability to form soft gels even at low concentrations.¹⁴

Quality by design (QbD) is an approach to development that emphasizes understanding of process and product through quality risk management and starts with predefined targets. Pharmaceutical development includes the critical quality

attribute (CQA) and the quality target product profile (QTPP).^{18–20} Critical material attributes (CMAs) may include excipients, such as polymers in the formulation, and these affect the CQAs.²¹

Our study aims to propose a novel approach for the topical application of NDF by preparing temperature-sensitive *in situ* gel formulations to treat acne and superficial skin infections. *In situ* gels were prepared using poloxamers (Poloxamer 407 and 188) and different polymers [methyl cellulose (MC) and GG] for evaluation using the QbD approach. Fourier-transform infrared (FTIR) were recorded, and the gels were characterized for gelation temperature (Tsol-gel, sol-gel temperature), clarity, gelling capacity, pH, viscosity, mechanical properties (springiness, cohesiveness, and resilience), spreadability (work of adhesion, work of shear, firmness, and stickiness), drug content, cytotoxicity, and drug release assays.

MATERIALS AND METHODS

Ethics committee approval and informed consent were not required for this study because the study did not involve human participants or animals.

Materials

Medium-viscosity MC was obtained from BioBasic (Canada). GG was obtained from Thermo Scientific. Poloxamers 188 (P188) and 407 (P407) were provided by BASF. NDF was provided by Berko Pharmaceutical Company (İstanbul, Türkiye) and Santa Farma Pharmaceutical Company (İstanbul, Türkiye). Dialysis membrane (diameter 16 mm, Spectra/Por® 2, Vol/Lg 2 mL/cm, molecular weight of 12–14 kDa) was obtained from Spectrumlabs. Methanol was supplied by Isolab. The phosphate-buffered saline (PBS, pH 7.4) tablet was obtained from Sigma-Aldrich (United States). The L929 mouse fibroblast cell line was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Penicillin-streptomycin solution (10,000 U/mL penicillin, 10,000 µg/mL streptomycin) and fetal bovine serum (FBS) were also acquired from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). Dulbecco's modified Eagle medium (DMEM) was purchased from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). Dimethyl sulfoxide (DMSO) was supplied by Tekkim (Ankara, Türkiye). Trypsin-EDTA solution (0.25%) was purchased from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was supplied by Biomatik (Wilmington, DE, USA).

Implementation of the QbD

The first step in development using the QbD approach is to define the target product profile (TPP), which includes the intended characteristics such as the dosage form, route of administration, and dosage strength.²² To achieve the quality properties, we must summarize the QTPP.^{19,20,23} The QTPP includes dosage form (*in situ* thermosensitive gel), route of administration (topical), drug content (90–110%), dosage strength (1%), pH (~5), gelling capacity (immediate), and gelation temperature (32–35 °C) for dermal application of *in situ* gels. According to the QTPP, the CQAs were determined. Then, CMAs were determined. In our study, different types and

ratios of polymers (GG and MC) were considered inputs (CMAs), and gelation temperature, pH, viscosity, mechanical properties, drug content, spreadability, and *in vitro* drug release (CQAs) were considered outputs.^{21,24} CQAs were tested. The results were imported into Minitab® software, and the relationships between input and output were evaluated.

Preparation of NDF loaded in situ gel formulations

Gels were produced by mixing distilled water and polymers. First, 2/3 of the required distilled water was preheated to 90 °C. MC was added in portions as it dissolved slowly, and the mixture was continuously stirred for 1 hour. Then the remaining distilled water, at room temperature, was added, and the mixture was continuously stirred using a magnetic stirrer until it appeared clear.²⁵ The temperature was reduced to 50 °C, GG was added, and stirring continued for about 20 minutes. The drug was dispersed at 25 °C with continuous stirring.¹⁴ After that, poloxamers were added using the cold technique.^{26,27} The mixture was cooled by covering the outside of the beaker with ice, and the required amounts of P188 and P407 were added while stirring continuously with a magnetic stirrer for about 1 hour. It was stored overnight in the refrigerator until all the polymers were completely dissolved. The gel formulations are presented in Table 1.

Characterization of NDF loaded in situ gel formulations

Gels were characterized using FTIR and by measuring gelation temperature, pH, clarity, viscosity, drug content, mechanical properties, spreadability, cytotoxicity, and *in vitro* drug release.

FTIR spectroscopy analysis

The infrared spectra of the drug (NDF), the polymers (GG, MC, P188, and P407), drug-free *in situ* gels and NDF-loaded *in situ* gels were recorded using an FTIR spectrometer (FT/IR-4600, Jasco, Tokyo, Japan). Samples were placed on the sample plate. The study was performed at room temperature with 32 scans over the spectra range from 4,000 to 400 cm⁻¹ at a resolution of 4.0 cm⁻¹.²⁸⁻³⁰

Clarity

Clarity was determined by visual inspection as follows: very clear (glassy), +++; clear, ++; and turbid, +.³¹

Determination of gelation temperature (Tsol-gel, sol-gel temperature)

After the prepared gels were refrigerated overnight, a magnetic bar was placed inside the container, which was then placed

on a magnetic stirrer at 80 °C. The temperature at which the magnetic bar stopped was recorded (n = 3).³²

Gelling capacity

The gelation capacity of the formulation was evaluated by introducing a drop of the sample into a beaker maintained at 32–34 °C, and the gel formation time was determined visually. The results were categorized according to gel behavior as follows: #, gel developed after a short delay but quickly liquefied; ##, gel formed instantly and lasted for several minutes; ###, rapid gel formation that remained intact for approximately one hour.³¹⁻³⁴

Determination of pH

The pH was determined using a pH meter (Mettler Toledo S220-K, Switzerland). The device probe was directly immersed in the gel, and after 10 seconds, the value read from the device was recorded (n = 4).

Viscosity

The viscosity was measured using a Brookfield DVE digital viscometer. To ensure that the torque value was between 10% and 90% and did not cause errors, the speed value was determined experimentally.³⁵ *In situ* gels were placed in a 100 mL beaker, and the measurement was performed.³¹⁻³³ Formulations were analyzed at 32 °C, at speeds of 60 rpm (for N1, N2, and N3) and 10 rpm (for N4), using spindle number s03 (n = 4).

Mechanical properties

The mechanical properties (springiness, resilience, and cohesiveness) were measured using a TA-XT plus texture analyzer. The test was performed with a Perspex probe (P/25P, θ : 25 mm) and at 32 ± 0.5 °C (n = 4). The pre-test, test, and post-test speeds were each 2.00 mm/s with a trigger force of 0.001 N; the target mode distance and the compression depth in each run were 10.00 mm; and the delay time between two compressions was 10 s.^{12,36}

Spreadability

Spreadability (work of shear, stickiness, work of adhesion, and firmness) was measured using the TA-XT plus texture analyzer. Both female and male cones were used. The gel was placed into the female cone. The distance was 23.00 mm, the test speed was 3 mm/s, and the post-test speed was 10.00 mm/s at 32 ± 0.5 °C (n = 3).³⁶

Drug content uniformity

For the drug content experiment, a 100 µg/mL solution was prepared by dissolving 100 mg of the NDF-loaded gels (N1, N2, N3, and N4) in 10 mL of methanol, was then diluted to 6 µg/mL with methanol: pH 7.4 PBS (30:70, v/v) solution, and was analyzed by UV spectrophotometry using a spectrophotometer (JASCO V-730 BIO, Tokyo, Japan).^{37,38} A stock solution of NDF (100 µg/mL) was prepared by sonication in methanol. The calibration standards were prepared at concentrations of 6–16 µg/mL. A wavelength of 290 nm was used, and methanol: PBS (30:70 v/v) was used as a blind (n = 3).

Table 1. Formulations of the NDF-loaded thermosensitive *in situ* gels (%w/w).

Formulation	P407	P188	MC	GG	NDF
N1	17.5	7	0.3	0.1	1
N2	17.5	7	0.3	0.3	1
N3	17.5	7	0.7	0.1	1
N4	17.5	7	0.7	0.3	1

P407, poloxamer 407; P188, poloxamer 188; MC, methyl cellulose; GG, gellan gum; NDF, nadifloxacin.

In vitro drug release studies

Dialysis membrane method was used for *in vitro* drug release studies for N1, N2, N3, N4 formulations and the commercially available marketed product (MP) (Magnis,[®] Santa Farma Pharmaceutical Company, İstanbul, Türkiye). In this method, physical separation of the dosage forms is achieved by using a dialysis membrane, which facilitates sampling at periodic intervals. *In vitro* release experiment of formulations was performed in 100 mL methanol and pH 7.4 PBS (30:70 v/v ratio) at 320 rpm. Methanol and pH 7.4 PBS (30:70 v/v ratio) to ensure sink conditions were used as a medium in the study. The beakers were sealed with parafilm to prevent evaporation. The temperature was adjusted at 34 ± 1 °C to imitate skin temperature.³¹ Two g of formulations were separated from the medium by means of a dialysis membrane (Spectra/por, MW of 12–14 kDa). 0.1 mL of sample was withdrawn at predetermined time intervals of 0.5, 1, 2, 3, 4, 6, 8, 24, 30, and 46 h, and the same volume of fresh medium was replaced.^{31,36,39} Gels were measured using the UV method as described above.

Cytotoxicity studies

Cell culture

The murine fibroblast cell line (L929) was used. The cells were cultured in high-glucose DMEM supplemented with 1% (v/v) penicillin-streptomycin and 10% (v/v) FBS. Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO₂. When the cells reached 70–80% confluence, they were detached with 0.25% trypsin-EDTA and used for subsequent studies.

MTT cytotoxicity assay

L929 cells were seeded in 96-well plates at a density of 1×10^4 cells/well and incubated for 24 hours at 37 °C, 5% CO₂. Following this period, four hydrogel formulations containing NDF (N1, N2, N3, and N4) were extracted by incubation in cell culture medium for 24 hours at 37 °C. After release, the hydrogel extracts were sterilized using a membrane filter (pore size of 0.22 µm). The resulting sterile hydrogel extracts were applied to the cells at concentrations of 50, 125, 250, 500, 750, and 1000 ppm, and the cells were incubated for 24 hours. Cells treated only with cell culture medium were used as negative controls. At the end of the incubation period, 10 µL of MTT solution (5 mg/mL prepared in PBS) was added to each well, and the plates were incubated for an additional 4 hours at 37 °C. The medium was then carefully removed, and 100 µL of DMSO was added to each well to dissolve the formed formazan crystals. Absorbance values were determined with a BMG SPECTROstar microplate reader at a wavelength of 570 nm. Cell viability (%) was calculated using the following formula:⁴⁰

Statistical analysis was performed using two-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test in GraphPad Prism version 8.4.3. A *p*-value of less than 0.05 was considered statistically significant.

Statistical analysis

All data are expressed as mean $\pm$ standard deviation (SD); the number of replicates (*n* = 3 or 4) is given in each table legend.

Physicochemical, spreadability, and mechanical parameters were analyzed using ordinary one-way ANOVA followed by Tukey's multiple comparisons test. *In vitro* release data were analyzed by two-way ANOVA followed by Dunnett's multiple comparisons test. Multiplicity-adjusted *p*-values are reported (**p* < 0.05, ***p* < 0.01, ****p* < 0.001). Analyses were performed with GraphPad Prism 10.2.1 (GraphPad Software, Boston, MA, USA); *p* < 0.05 was considered significant.

RESULTS

Preparation of NDF loaded in situ gel formulations

The United States Food and Drug Administration has approved two poloxamers (P407 and P188), which are widely used in the preparation of thermosensitive *in situ* gels. The sol-gel temperature of the formulation varies with the polymer concentration. Normally, they are liquid at room temperature and undergo gelation at physiological temperatures.⁴¹ During the preparation of *in situ* gels, MC was dispersed at 90 °C according to the method in the literature.²⁵ The temperature was reduced to 50 °C, and GG was added and mixed.¹⁴ Poloxamers were added to this mixture using a cold technique.^{26,27}

In this study, we formulated thermosensitive *in situ* gels that can be effortlessly applied to the skin and are appropriate for use in treating acne vulgaris and superficial bacterial skin infections. *In situ* gels contain 1% NDF, 17.5% P407, and 7% P188. The effect of CMAs was investigated within the framework of QbD, using variable ratios of MC and GG. *In situ* gels are carrier systems that can be prepared using poloxamers and that play a vital role in delivering drugs to a specific area of the skin.^{31,42} In our study, opaque, white, and homogeneous *in situ* gels were produced (Figure 1).

FTIR spectroscopy analysis

FTIR studies were conducted on the pure drug (NDF), the polymers (GG, MC, P188, and P407), and the prepared drug-free

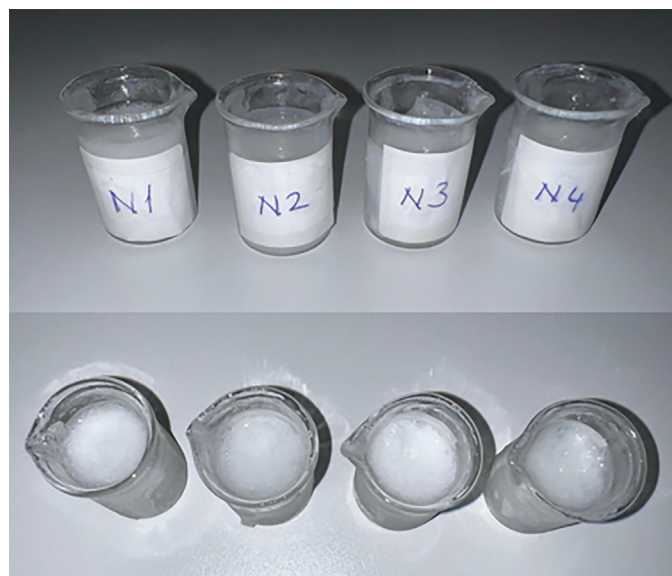


Figure 1. Photo of prepared nadifloxacin-loaded thermosensitive *in situ* gel formulations.

and NDF-loaded gels (Figure 2). No peak shift or peak loss was observed in the spectra of the gels and substances, indicating that the excipients and the drug were compatible.

Determination of gelation temperature, gelling capacity, clarity, viscosity, and pH

The gelation temperatures, pH, and viscosity of the gels prepared with NDF are presented in Table 2. Results of the clarity evaluation, showing that all the gels were turbid, are presented in Table 2. The photograph of the four formulations, which provides an idea of their clarity, is presented in Figure 1.

Mechanical properties

The textural parameters of the four prepared dermal gel formulations, including springiness, cohesiveness, and resilience, were evaluated using a texture analyzer. Table 3 shows the TPA data of the formulations.

Spreadability

Table 4 presents the results of our study on spreadability. The spreadability study characterizes the formulation's work of adhesion and work of shear, as well as its stickiness and firmness by measuring force fluctuations over time.³⁶

Drug content uniformity

The maximum wavelength observed with the method was 290 nm. The average NDF values were 97.1% and 100.3% (Table 2).

In vitro drug release studies

Release from NDF gels of N1, N2, N3, N4, and the MP was carried out and is shown in Figure 3. During the first hour of

the study, the NDF release rates were observed for N4 (6.1%), N3 (9.5%), N2 (10.4%), N1 (11.1%), and MP (11.9%), in ascending order (Table 5). Within 8 h, *in vitro* NDF release from N1, N2, N3, N4, and MP was 96.1%, 95.1%, 92.4%, 77.5%, and 42.8%, respectively. Within 24 h, approximately 100% of the NDF was released from all *in situ* gels, and 78.9% was released from MP.

Cytotoxicity studies

The cytotoxic effects of the developed NDF-loaded hydrogel formulations on L929 fibroblast cells were evaluated using MTT analysis. The results are presented in Figure 4. Cells were exposed to increasing concentrations of hydrogel extracts (50–1000 ppm), and cell viability remained above 90% in all groups, indicating that the formulations are generally well tolerated and highly biocompatible.

Evaluation of quality by design approach

Table 6 shows that the R^2 values and p -values.

Contour plots show the effects of MC and GG concentrations on various parameters (Figure 5).

DISCUSSION

In the literature, the peaks of the polymers used and of NDF have already been reported. GG FTIR spectra described O–H stretching vibration (hydroxyl groups, hydrogen bonding) at 3200 cm^{-1} – 3400 cm^{-1} . The band at about 2927 cm^{-1} is due to CH_2 group, and 1153 and 1024 cm^{-1} are due to C–O stretchings. Bending vibration of the carboxylate group appeared at about 1627 and 1409 cm^{-1} .⁴³ MC had an absorption band at about

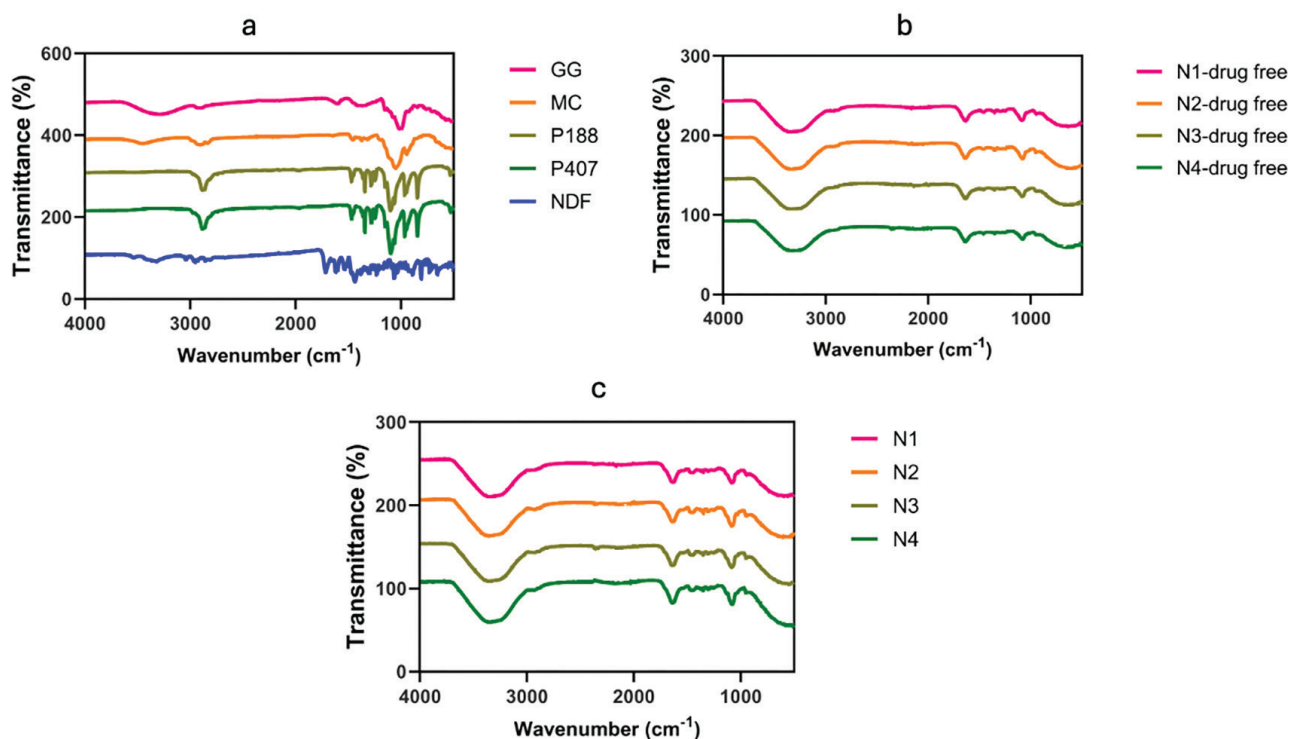


Figure 2. FTIR spectra of a) the different starting components (GG, MC, P188, P407, and NDF); b) drug-free *in situ* gels and c) NDF-loaded *in situ* gels. MC, methyl cellulose; GG, gellan gum; NDF, nadifloxacin.

Table 2. Values of gelation temperature (n = 3), gelling capacity (n = 3), pH (n = 4), viscosity (n = 4), drug content (n = 3), and score of clarity (n = 3).

Formulation	Gelling capacity	Gelling temperature (°C)	Clarity	pH	Viscosity (cP)	Drug content (%)
N1	###	35.167 ± 0.289 ^A	+	7.095 ± 0.013 ^B	422.425 ± 0.846 ^{B*}	98.686 ± 0.315 ^B
N2	###	32.500 ± 0.500 ^B	+	7.085 ± 0.013 ^B	883.750 ± 3.676 ^{C*}	97.697 ± 0.116 ^C
N3	###	32.000 ± 0.000 ^B	+	7.093 ± 0.036 ^B	1196.250 ± 4.272 ^{B*}	97.072 ± 0.075 ^D
N4	###	30.000 ± 0.000 ^C	+	7.198 ± 0.005 ^A	3622.500 ± 15.000 ^{A**}	100.348 ± 0.046 ^A

Values are expressed as mean ± standard deviation.

^{A-D}Within each column, values not sharing a common superscript letter differ significantly (one-way ANOVA followed by Tukey's multiple comparisons test, $p < 0.05$). Letters are assigned in descending order of the mean and are independent for each column. Gelling capacity and clarity were scored qualitatively and were identical for all formulations; therefore no statistical comparison was performed for these parameters.

#, gel developed after a short delay but quickly liquefied; ##, gel formed instantly and lasted for several minutes; ###, rapid gel formation that remained intact for approximately one hour.

+++, very clear (glassy); ++, clear; +, turbid.

*measured at 60 rpm; **measured at 10 rpm. These symbols denote the rotational speed of the measurement only and carry no statistical meaning.

ANOVA, analysis of variance.

Table 3. The mechanical properties of the NDF-loaded thermosensitive *in situ* gels (n = 4).

Formulation	Springiness (%)	Cohesiveness	Resilience (%)
N1	3.985 ± 0.001 ^A	2.227 ± 0.104 ^A	0.005 ± 0.000 ^C
N2	2.656 ± 0.161 ^B	0.957 ± 0.039 ^B	0.005 ± 0.001 ^C
N3	0.997 ± 0.000 ^C	0.805 ± 0.031 ^C	0.038 ± 0.002 ^B
N4	0.977 ± 0.003 ^C	0.770 ± 0.029 ^C	0.080 ± 0.004 ^A

Values are expressed as mean ± standard deviation (n = 4).

^{A-C}Within each column, values not sharing a common superscript letter differ significantly (one-way ANOVA followed by Tukey's multiple comparisons test, $p < 0.05$). Letters are assigned in descending order of the mean and are independent for each column.

ANOVA, analysis of variance; NDF, nadifloxacin.

Table 4. The spreadability results of the NDF-loaded thermosensitive *in situ* gels (n = 3).

Formulation	Work of shear (g.sec)	Stickiness (g)	Firmness (g)	Work of adhesion (g.sec)
N1	13.312 ± 0.294 ^C	-60.250 ± 1.578 ^C	35.289 ± 0.545 ^C	-8.155 ± 0.218 ^C
N2	27.500 ± 0.357 ^C	-79.102 ± 0.938 ^B	50.959 ± 0.192 ^C	-12.327 ± 0.078 ^C
N3	71.756 ± 11.401 ^B	-110.277 ± 2.354 ^A	135.704 ± 12.501 ^B	-33.512 ± 4.539 ^B
N4	104.857 ± 3.550 ^A	-110.579 ± 5.881 ^A	162.308 ± 10.204 ^A	-43.721 ± 3.699 ^A

Values are expressed as mean ± standard deviation (n=3).

^{A-C}Within each column, values not sharing a common superscript letter differ significantly (one-way ANOVA followed by Tukey's multiple comparisons test, $p < 0.05$). Letters are assigned independently for each column, with "A" denoting the highest magnitude of the corresponding property.

Stickiness and work of adhesion are reported as negative values because they are derived from the withdrawal phase of the probe; more negative values therefore indicate stronger adhesive interaction. Superscript letters for these two parameters were assigned according to the absolute magnitude of the response.

ANOVA, analysis of variance.

3400 cm⁻¹ associated to hydrogen bonding. The bands at about 2900 cm⁻¹ 2800 cm⁻¹ are attributed to vibration of C-H. The pics at 1063 cm⁻¹ are assigned to C-O-C stretching mode from glycosidic units. The absorption band at 944 cm⁻¹ is ascribed to OCH₃ groups.^{44,45} P188 and P407 peaks at around 2800-2900 cm⁻¹ (C-H stretch). Other peaks are seen at 1341 cm⁻¹ (in-plane O-H bend) and around 1100 cm⁻¹ (C-O stretch). Our spectra of P188 and P407 were comparable to previously recorded spectra.^{46,47} The characteristic peak of P188 and P407 shifted from 2856 cm⁻¹ (Figure 2a) to 2871 cm⁻¹ (Figure 2c) as previously reported, which was shown to the physical interaction between drug and water-soluble polymers.⁴⁷ The spectrum of NDF

demonstrated an intense vibration observed at 2976 cm⁻¹ can be associated with the carboxyl O-H stretching. At the same time was observed the presence of large bands at 1748 cm⁻¹ was observed, attributed with ketone C = O stretching. Other large vibrations observed at 1624 cm⁻¹ can be related to the aromatic C = C stretching.⁴⁸ All characteristic bands were shown in the FTIR spectra of polymers, NDF, and gels with or without the drug and all excipients (Figure 2). The FTIR spectra of drug-free gels and NDF-containing gels did not show significant differences across formulations, indicating that the polymer blends do not support the formation of additional interactions among their components.³⁰

Table 5. *In vitro* cumulative release (%) of NDF from the thermosensitive *in situ* gel formulations and the marketed product (n=3).

Time (h)	N1	N2	N3	N4	Product
0.5	4.2 ± 0.4	4.3 ± 1.2	4.5 ± 1.5	2.3 ± 0.5	7.7 ± 2.0
1	11.1 ± 0.6	10.4 ± 3.6	9.5 ± 4.5	6.1 ± 1.5	11.9 ± 2.9
2	27.6 ± 1.4	27.6 ± 8.6	24.6 ± 6.2	18.2 ± 2.3	20.4 ± 4.0
3	42.9 ± 2.9*	43.9 ± 12.3	43.8 ± 4.4	27.0 ± 5.5	27.2 ± 3.9
4	51.4 ± 5.4*	57.7 ± 3.1***	58.2 ± 4.7	35.8 ± 3.2	28.5 ± 1.4
6	76.9 ± 4.4	74.5 ± 3.7	70.8 ± 11.1	65.1 ± 15.8	37.0 ± 1.4
8	96.1 ± 11.8*	95.1 ± 7.4*	92.4 ± 2.3**	77.5 ± 8.7	42.8 ± 5.1
24	100.5 ± 1.3**	100.2 ± 1.9***	98.8 ± 2.6	98.5 ± 0.6	78.9 ± 2.4
30	100.5 ± 1.3**	100.2 ± 5.0	103.6 ± 1.7	98.5 ± 0.5	90.5 ± 0.5
46	100.5 ± 1.3	100.2 ± 7.0	102.9 ± 0.1	98.5 ± 0.5	102.5 ± 2.3

Values are expressed as mean ± standard deviation (n = 3). Statistical comparisons were performed by two-way ANOVA followed by Dunnett's multiple comparisons test, with each formulation compared against the marketed product at every sampling time.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus the marketed product. Absence of a symbol indicates no statistically significant difference. ANOVA, analysis of variance.

Table 6. Regression equation, model summary, and p -values of NDF-loaded *in situ* gels.

CQA	Regression equation	Model summary			p -value	
		R ² (%)	R ² adj (%)	R ² pred (%)	p -value of MC	p -value of GG
Gelation temperature (°C)	38.338 - 7.125 MC% (w/w) - 11.75 GG% (w/w)	99.11	97.33	85.76	0.078	0.094
pH	7.008 + 0.125 MC% (w/w) + 0.250 GG% (w/w)	58.14	0.00	0.00	0.558	0.558
Viscosity 32°C (cps)	-2108 + 4391 MC% (w/w) + 7219 GG% (w/w)	84.26	52.79	0.00	0.325	0.380
Springiness (%)	5.75 - 5.83 MC% (w/w) - 3.37 GG% (w/w)	93.23	79.69	0.00	0.174	0.490
Cohesiveness	2.85 - 2.01 MC% (w/w) - 3.26 GG% (w/w)	73.78	21.34	0.00	0.417	0.482
Resilience (%)	-0.0565 + 0.1350 MC% (w/w) + 0.105 GG% (w/w)	88.39	65.17	0.00	0.236	0.500
Firmness (g)	-57.43 + 264.7 MC% (w/w) + 105.7 GG% (w/w)	99.74	99.23	95.90	0.033	0.161
Work of shear (g.sec)	-54.2 + 169.8 MC% (w/w) + 118.2 GG% (w/w)	98.30	94.90	72.80	0.088	0.242
Stickiness (g)	-29.5 - 101.9 MC% (w/w) - 47.9 GG% (w/w)	95.32	85.96	25.14	0.142	0.490
Work of adhesion (g.sec)	18.24 - 70.94 MC% (w/w) - 36.0 GG% (w/w)	98.95	96.85	83.21	0.067	0.253
Drug content (%)	96.73 + 1.25 MC% (w/w) + 5.5 GG% (w/w)	24.87	0.00	0.00	0.851	0.693
Release 6 th hour (%)	85.56 - 19.38 MC% (w/w) - 20.25 GG% (w/w)	96.56	89.69	44.99	0.134	0.246

MC, methyl cellulose; GG, gellan gum; NDF, nadifloxacin; CQA, critical quality attribute.

All four formulations (N1–N4) exhibited rapid sol-to-gel conversion upon exposure to the simulated physiological conditions, and the resulting gels remained structurally intact for approximately one hour (###). None of the formulations liquefied prematurely, indicating that the concentrations employed in this study were sufficient to establish a coherent gel network in every composition tested. With respect to visual appearance, all four formulations were scored as turbid (+); no formulation reached the clear (++) or glassy (+++) levels on the clarity scale. The *in situ* gels produced were turbid. The

literature reports that the clarity of drug-loaded *in situ* gels reaches this level.³²

Rapid gel formation, which maintained structural integrity for approximately one hour, was observed in all formulations. If the gelation temperature is below 25 °C, the gel may form at room temperature before application. When the gelation temperature is above 38 °C, it remains liquid at body temperature and therefore does not gel. Therefore, an average gelation temperature of 32 °C–35 °C is preferred.⁴⁹ The gelation temperature decreased progressively from N1 to N4, and the differences among the

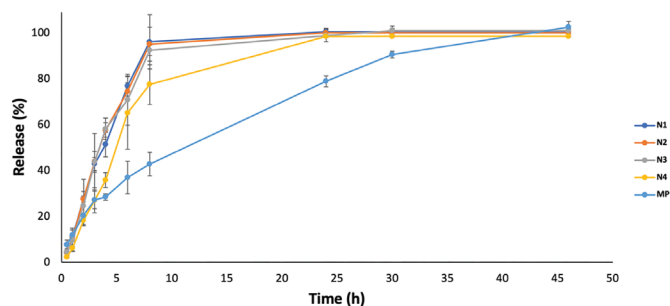


Figure 3. *In vitro* drug release profiles of NDF-loaded *in situ* gels (N1, N2, N3, and N4) and marketed product (MP) ($n = 3$, $\pm$ SD).

NDF, nadifloxacin; SD, standard deviation.

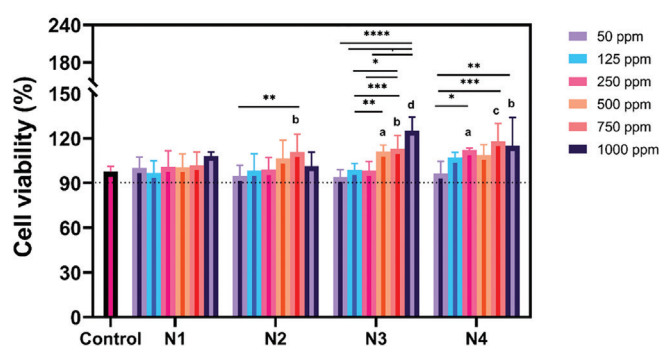


Figure 4. The cell viability (%) of L929 fibroblast cells treated with NDF-loaded hydrogel extracts (N1–N4) at various concentrations (50–1000 ppm) for 24 hours. Data are shown as average $\pm$ standard deviation ($n = 7$). Statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ according to the hydrogel content at different concentrations and ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$; ^d $p < 0.0001$, compared with the control group.

NDF, nadifloxacin.

formulations were statistically significant (one-way ANOVA, $F(3,8) = 163.0$, $p < 0.0001$). Tukey's multiple comparisons test showed that N1 (35.167 ± 0.289 °C) gelled at a significantly higher temperature than all other formulations ($p < 0.0001$), whereas N4 (30.000 ± 0.000 °C) gelled at the lowest temperature and differed significantly from N1, N2 ($p < 0.0001$) and N3 ($p = 0.0001$). The only pairwise comparison that did not reach statistical significance was that between N2 (32.500 ± 0.500 °C) and N3 (32.000 ± 0.000 °C) (adjusted $p = 0.2252$), indicating that these two compositions behave equivalently with respect to the sol-gel transition.

The pH value is a significant factor in dermal applications. The normal pH range for non-neonatal skin is acidic, typically between 4 and 6.⁵⁰ Accordingly, the formulations' skin-nonirritating pH and the pH of the gels are suitable for topical application. The formulations had pH values of approximately 7.1–7.2. These values were compatible with the skin pH, which ranges from 5.0 to 7.0.^{51,52} Although the average pH of healthy skin is around 5.5, the pH of wounded, burned, or acne-affected skin may shift toward neutral or slightly alkaline values. Previous studies have reported that the wound surface pH is around 7 during the healing process. Therefore, the pH

values of the developed formulations (7.1–7.2) were considered acceptable for dermal *in situ* gel application and were not expected to cause irritation.⁵³ In other dermally applied *in situ* gel studies, the pH values were reported to range from 5.777 ± 0.07 to 6.027 ± 0.06 .³¹ If gels contain GG, their pH is primarily determined by the pH of the solution used and by the presence of acidic or basic additives. GG is a weak acid, and when used at concentrations of 0.1–2% w/w, as in our study, increasing GG concentration in a formulation has only a minor effect on the pH of the final gel.⁵⁴ Another study reported that altering the proportion of methylcellulose in gels did not significantly change the pH of the formulation within the typical operating range. pH is primarily determined by the solvent and any acidic or basic components, but not by the methylcellulose concentration itself.⁵⁵ The pH values of all formulations were within the near-neutral range (7.085–7.198). Although one-way ANOVA indicated a statistically significant difference among the groups ($p < 0.0001$), Tukey's test revealed that this effect was driven entirely by N4 (7.198 ± 0.005), which differed significantly from N1, N2 and N3 ($p < 0.0001$ for all three comparisons). No significant differences were detected among N1, N2, and N3 (adjusted $p = 0.8965$, 0.9980 , and 0.9521 for N1 vs. N2, N1 vs. N3, and N2 vs. N3, respectively). The absolute magnitude of the difference attributable to N4 was only 0.10–0.11 pH units.

Similar studies in the literature on *in situ* gel formulations report viscosity measurements performed at 32 °C. For example, viscosity analyses were also carried out at 32 °C in pycnogenol-loaded⁴² and vanillic acid-loaded⁵⁶ *in situ* gel formulations. In the vanillic acid-loaded *in situ* gel study, the gelation temperature was below 32 °C, but the viscosity was evaluated at 32 °C, as in our study. As observed in other gel studies, viscosity increased with the increase in polymer content.^{57,58} Viscosity increased markedly across the formulation series, and all six pairwise comparisons were statistically significant ($p < 0.0001$ for each). The measured values followed the order N1 (422.425 ± 0.846 cP) < N2 (883.750 ± 3.676 cP) < N3 (1196.250 ± 4.272 cP) < N4 (3622.500 ± 15.000 cP). It should be noted that N1–N3 were measured at 60 rpm, whereas N4 was measured at 10 rpm because its consistency exceeded the working range of the spindle at the higher rotational speed (inference). Because *in situ*-gelling systems of this type typically display pseudoplastic (shear-thinning) flow, the apparent viscosity recorded for N4 at the lower shear rate is expected to be higher than the value obtained at 60 rpm; the difference between N4 and the remaining formulations should therefore be interpreted as a combined effect of composition and measurement conditions, rather than a composition effect alone.

Suitable mechanical properties are crucial for dermal formulations. TPA is a standard technique for assessing mechanical properties.⁵⁹ Springiness is a textural parameter attributed to the flexibility of the product.⁶⁰ The gel sample deforms during initial compression and restructures to regain elasticity.⁵⁹ In our study, the springiness decreased as GG increased in the N3 and N4 formulations, which were in gel form at 32 °C. Similarly, one study reported that the addition of GG increased hardness but significantly decreased springiness

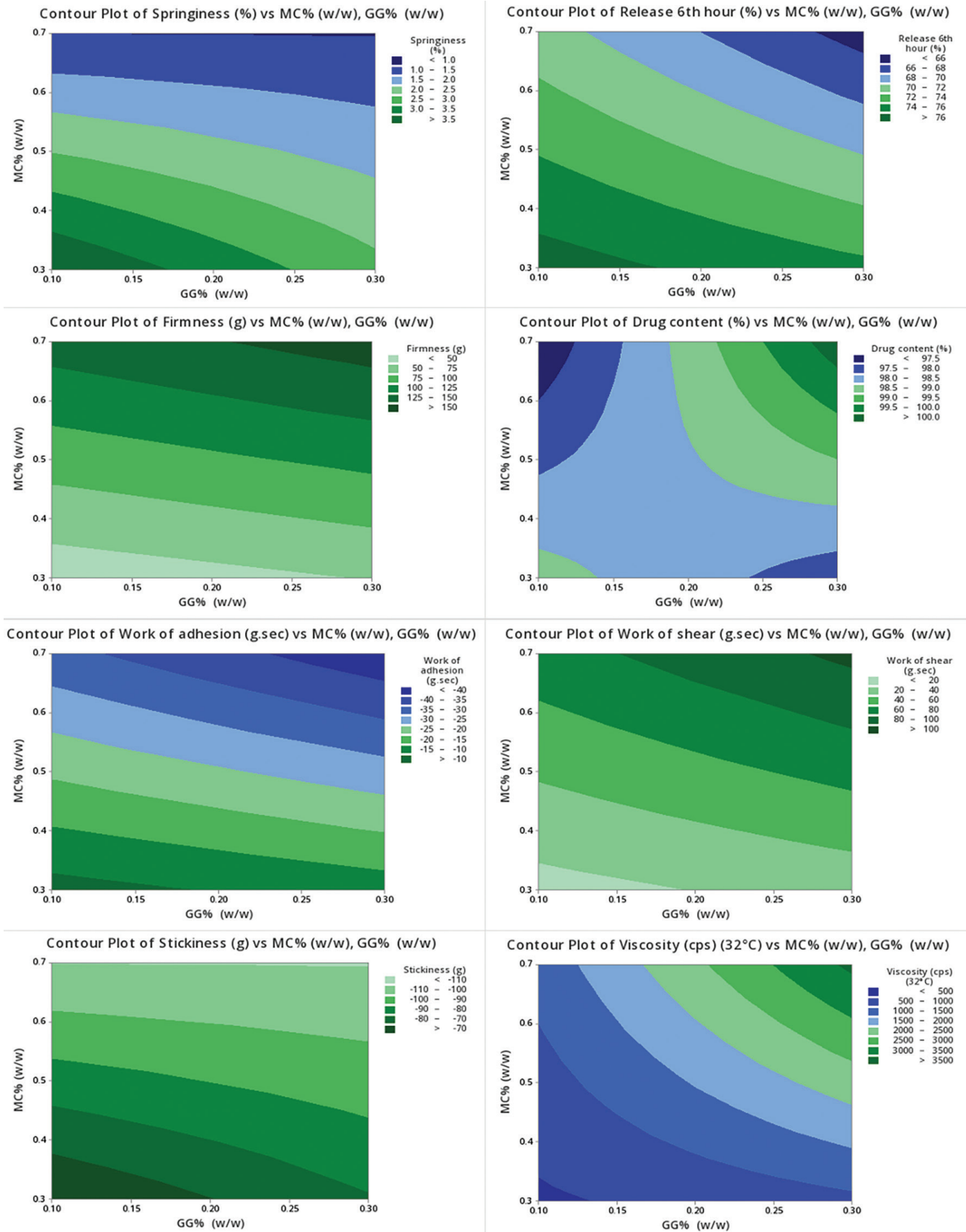


Figure 5. Contour plot graphics on various CQAs of NDF-loaded *in situ* gels.

CQA, critical quality attribute; MC, methyl cellulose; GG, gellan gum; NDF, nadifloxacin.

compared to the control group.⁶¹ Similarly, as MC increased, springiness decreased. Cohesion describes how well a sample resists a second deformation relative to its resistance to the first deformation.⁵⁹ Elasticity indicates the rate at which a sample returns to its original form after deformation, while cohesiveness provides insight into the sample's response to repeated stresses. Higher values indicate lower sample elasticity.⁹ A lower cohesion value indicates a weaker gel structure that breaks more readily.⁶² As can be seen from Table 3, cohesiveness values range from 0.770 to 2.227. In our study, cohesiveness decreased as the MC ratio increased (N2: 0.3% and N4: 0.7%). This decrease was also observed in another *in situ* gel study prepared with 0.1% and 0.5% Na CMC.⁹ In our study, cohesiveness decreased as the GG ratio increased (N3, 0.1% and N4, 0.3%). A similar decrease was observed in GG/Chitosan formulations and in GG formulations. While GG alone exhibited lower cohesiveness, the addition of chitosan increased cohesiveness.⁶² Resilience reflects the capacity of gels to recover their shape after penetration.⁶³ Resilience is an indicator of how quickly and forcefully a product recovers from deformation. It's the product's elastic recovery.⁶⁰ The elastic recovery was highest in N4 gels, which had the highest polymer concentration in the sample. In our study, we observed that resilience increased with increasing MC (N2, 0.3% and N4, 0.7%). Similarly, other studies reported that the MC in hydrogels showed improved resilience as weight percentage increased.^{64,65} In our study, resilience increased as the GG ratio increased (N3, 0.1%; N4, 0.3%). One study reported that increasing the GG content improved resilience and reduced swelling and water uptake.⁶⁶ The mechanical properties of the NDF-loaded thermosensitive *in situ* gels were evaluated by texture profile analysis, and the results are summarized in Table 3. All three parameters—springiness, cohesiveness, and resilience—differed significantly among the formulations (one-way ANOVA, $p < 0.0001$).

Springiness decreased markedly across the formulation series. N1 exhibited the highest value (3.985 ± 0.001), followed by N2 (2.656 ± 0.161); both differed significantly from each other and from the remaining formulations ($p < 0.0001$ for all comparisons involving N1 or N2). N3 (0.997 ± 0.000) and N4 (0.977 ± 0.003) displayed the lowest springiness values and did not differ significantly from each other (adjusted $p = 0.9838$), indicating that the elastic recovery of the gel network reached a plateau in these two compositions. A similar pattern was observed for cohesiveness. N1 showed a distinctly higher value (2.227 ± 0.104) than all other formulations ($p < 0.0001$), while N2 (0.957 ± 0.039) differed significantly from both N3 (0.805 ± 0.031 ; adjusted $p = 0.0157$) and N4 (0.770 ± 0.029 ; adjusted $p = 0.0038$). As with springiness, no significant difference was detected between N3 and N4 (adjusted $p = 0.8424$). Resilience followed the opposite trend, increasing from N1 and N2 to N4. N1 (0.005 ± 0.000) and N2 (0.005 ± 0.001) exhibited the lowest values and were statistically indistinguishable from one another (adjusted $p = 0.9978$), whereas N3 (0.038 ± 0.002) and N4 (0.080 ± 0.004) showed significantly higher resilience than both of these formulations and than each other

($p < 0.0001$ for all remaining comparisons). N4, therefore, displayed approximately sixteenfold greater resilience than N1. Taken together, the texture profile data indicate an inverse relationship between the elastic parameters (springiness and cohesiveness) and resilience across the formulation series.

Spreadability is a fundamental property of topically applied dermal formulations. If the formulation is used in this manner, it should exhibit sufficient spreadability to enable the patient to apply it easily to the skin.^{36,67} When the male cone is removed from the sample, a negative region is formed. The maximum negative value represents the gel's adhesion force; that is, the force needed to separate the gel from the tube.⁶⁸ In one study, stickiness and the work of adhesion of drug-loaded hydrogels prepared with Carbopol as the gelling polymer increased with increasing polymer ratio.³⁶ Similarly, in our study, the work of adhesion and stickiness of the formulations increased with increasing polymer ratios. Firmness is the maximum force required to deform the formulation, indicating its strength. Higher firmness values are more favorable for the formulation's power.⁶⁸ In our study, as the polymer content in the gels increased, the firmness and viscosity increased. As a result, the spreadability of the gels decreased. Another study emphasized this similar relationship between the polymer content, viscosity, firmness, and spreadability. The same study reported that higher firmness values corresponded to greater gel consistency, which negatively affected its spreadability.⁶⁹ The field up to the positive force represents the work of shear, indicating the spreadability of the formulation.⁶⁸ Spreadability is inversely related to both work of shear and firmness.³⁶ The suitability of a gel for the skin is related to its spreadability, which is influenced by its resistance to external forces. Even if two products have similar firmness, their skin applications may differ. Accordingly, shear work and firmness are usually evaluated together.⁶⁷ Shear work and firmness values are N4, N3, N2, and N1, in order from highest to lowest (Table 4). The spreadability follows an opposite order, i.e., from highest to lowest: N1, N2, N3, and N4. The data showed that the work of shear and firmness were higher in formulations N3 and N4 than in formulations N1 and N2, resulting in lower spreadability. The spreadability and adhesion-related mechanical characteristics of the NDF-loaded thermosensitive *in situ* gels are presented in Table 4. All four parameters differed significantly among the formulations (one-way ANOVA, $p < 0.0001$ in each case).

The work of shear, which reflects the energy required to spread the gel, increased markedly across the formulation series. N4 required the greatest energy (104.857 ± 3.550 g·sec) and differed significantly from all other formulations ($p < 0.0001$ versus N1 and N2; $p = 0.0006$ versus N3), while N3 (71.756 ± 11.401 g·sec) in turn differed significantly from both N1 and N2 ($p < 0.0001$). The two formulations at the lower end of the series, N1 (13.312 ± 0.294 g·sec) and N2 (27.500 ± 0.357 g·sec), were not statistically distinguishable from one another (adjusted $p = 0.0758$), although their means differed approximately twofold.

Firmness followed an essentially identical pattern. The highest value was recorded for N4 (162.308 ± 10.204 g), followed

by N3 (135.704 ± 12.501 g); these two formulations differed significantly from each other (adjusted $p = 0.0159$) and from both N1 and N2 ($p < 0.0001$ for all four comparisons). As with work of shear, no significant difference was detected between N1 (35.289 ± 0.545 g) and N2 (50.959 ± 0.192 g) (adjusted $p = 0.1593$).

Stickiness became progressively more negative from N1 to N4, indicating a stepwise increase in adhesive strength. N1 exhibited the weakest response (-60.250 ± 1.578 g) and differed significantly from N2 (-79.102 ± 0.938 g; adjusted $p = 0.0005$) as well as from N3 and N4 ($p < 0.0001$). N3 (-110.277 ± 2.354 g) and N4 (-110.579 ± 5.881 g) produced almost identical values and were statistically indistinguishable (adjusted $p = 0.9994$), indicating that the stickiness response reached a plateau in these two compositions.

The work of adhesion showed the same directional trend but a different grouping. N4 exhibited the highest adhesive work (-43.721 ± 3.699 g·sec) and differed significantly from all other formulations, including N3 (-33.512 ± 4.539 g·sec; adjusted $p = 0.0117$). N1 (-8.155 ± 0.218 g·sec) and N2 (-12.327 ± 0.078 g·sec) displayed the lowest adhesive work and did not differ significantly from each other (adjusted $p = 0.3633$). Stickiness reached a plateau between N3 and N4, whereas the work of adhesion continued to increase.

The within-group variability observed for N3 was consistently higher than that observed for the other formulations for work of shear, firmness, and work of adhesion (relative SDs of 15.9%, 9.2%, and 13.5%, respectively, compared with values below 5% for N1 and N2).

When considered together with the texture profile data (Table 3), the results indicate that N3 and N4 form denser and more strongly adhesive gels at the expense of markedly reduced spreadability, whereas N1 and N2 spread readily but adhere weakly.

Two hundred ninety nm is consistent with the 291 nm,⁷⁰ 294 nm,³⁷ 295 nm,⁷¹ 296 nm,³⁹ and 296.5 nm³⁸ values found in the literature. The maximum SD for NDF content was found to be 0.3. NDF is homogeneously distributed in the gels. Drug content ranged from $97.072 \pm 0.075\%$ to $100.348 \pm 0.046\%$ across the four formulations. All six pairwise comparisons were statistically significant, with N4 showing the highest content and N3 the lowest (N1 vs. N2, $p=0.0006$; N2 vs. N3, $p=0.0112$; all remaining comparisons, $p<0.0001$). Despite these statistically significant differences, every formulation complied with the (90.0–110.0%) acceptance range specified in (Ph. Eur./USP monograph), confirming that the drug was distributed homogeneously and the preparation procedure was reproducible.

Taken together, these results indicate that all four formulations satisfy the basic requirements for an *in situ* gelling system in terms of gelling capacity, pH, and drug content, but differ substantially in gelation temperatures and viscosity profiles.

Release studies are commonly carried out in media such as water, PBS, mixtures of organic solvents with PBS, or combinations of inorganic components with PBS.⁷² The choice of release medium is primarily determined by the solubility

characteristics of the drug being investigated.⁷³ NDF is insoluble in ethanol⁷⁴ and soluble in methanol. In NDF studies, methanol was used as a solvent.^{39,75} Therefore, a 30:70 (v/v) mixture of methanol and PBS (pH 7.4) was chosen as the release medium. Based on the release results, the gels sustained drug release for 8 hours. N1 and N2 formulations showed higher release than N3 and N4 formulations; this difference can be attributed to the polymer ratio. The *in vitro* cumulative release profiles of the four thermosensitive *in situ* gel formulations and the MP are presented in Table 5. Two-way ANOVA revealed significant effects of formulation, sampling time, and their interaction ($p < 0.0001$ in each case), and Dunnett's multiple comparisons test was subsequently applied at each sampling time to compare each formulation against the MP.

During the first two hours, all four formulations and the MP released comparable proportions of the incorporated drug (2.3–7.7% at 0.5 h and 18.2–27.6% at 2 h). The MP showed the highest initial release at 0.5 h ($7.7 \pm 2.0\%$), whereas N4 exhibited the slowest onset ($2.3 \pm 0.5\%$). The profiles began to diverge from the third hour onwards. Between 3 and 8 h, N1, N2 and N3 released the drug substantially faster than the MP, reaching 92.4–96.1% at 8 h compared with $42.8 \pm 5.1\%$ for the product. The differences were statistically significant for N1 at 3, 4 and 8 h ($p < 0.05$), for N2 at 4 h ($p < 0.001$) and 8 h ($p < 0.05$), and for N3 at 8 h ($p < 0.01$). Between 3 and 8 h, N4 released the drug more gradually than the other three formulations and did not differ significantly from the MP at any point.

At 6 h, none of the four comparisons reached statistical significance, despite mean differences of 28–34 percentage points in favour of the formulations. By 24 h all four formulations had released essentially their entire drug load (98.5–100.5%), whereas the MP had reached $78.9 \pm 2.4\%$. The difference remained statistically significant for N1 ($p < 0.01$) and N2 ($p < 0.001$) at this time, and for N1 alone at 30 h ($p < 0.01$). At 46 h the MP had also achieved complete release ($102.5 \pm 2.3\%$) and no significant differences remained between any formulation and the product. Overall, the release data indicate that three of the four formulations (N1, N2, and N3) accelerated drug release relative to the MP over the 3–24 h interval, while N4 produced a profile that remained statistically indistinguishable from the MP throughout.

Viability increased with dose, particularly in the N3 group, suggesting that the methylcellulose and GG create a microenvironment supportive of cell proliferation. The literature reports that polymers such as MC and GG provide controlled drug release while supporting fibroblast activity and wound healing.⁷⁶ Although cells and medium were used as controls, the high viability values indicate that the carrier polymer system is not cytotoxic and provides a cell-friendly environment. It has been previously reported that MC and GG facilitate cell migration and proliferation by maintaining moisture balance at the wound surface.⁷⁷ The controlled release of NDF allows for local treatment while reducing the risk of systemic toxicity. Such topical systems offer benefits for both effective drug delivery and cell viability, particularly in infected or chronic wounds.⁷⁸

The definitions of QbD and its elements are given in ICH Q8 (R2).¹⁹ In our study, the effect of material properties on CQA was investigated.¹⁹⁻²¹ Two different concentrations of MC (0.3% and 0.7%) and GG (0.1% and 0.3%) were used to prepare gels within the QbD framework. The adjusted R^2 (R^2 adj), regression coefficient (R^2), and predicted R^2 (R^2 pred) values were calculated from the program. The interaction between output and input variables can be assessed using p -values.²⁰ Based on the analysis results, R^2 is an estimate of the ratio of the input factors to the variance in the output response. The R^2 adj varies with the number of terms in the analysis. The R^2 pred indicates how well the analysis predicts the output responses. The R^2 pred and the R^2 adj are always less than R^2 .⁷⁹ R^2 values for gelation temperature, viscosity, springiness, cohesiveness, resilience, firmness, stickiness, work of adhesion, work of shear, and release at 6 hours are greater than 70%, indicating that the model captures more variation in the results and is reliable.⁸⁰ A regression model is considered a good fit when $p < 0.05$.⁷⁹ The p -value for the firmness parameter for MC is <0.05 . Therefore, MC is identified as an influential factor for this parameter. Consequently, the effects of the CMAs can be assessed using the QbD concept.

Study limitations

The present study focused on the development and characterization of NDF containing topical *in situ* gels. However, microbiological studies were not performed to evaluate the efficacy of the developed formulation against acne and superficial skin infections. Further investigations are therefore needed to assess its antimicrobial activity and determine its potential as an alternative to conventional creams used to treat these conditions.

CONCLUSION

Our study evaluated the potential of *in situ* gels containing NDF as topical drug carriers. It was concluded that NDF *in situ* gels could be prepared successfully by hot dissolution of MC and GG followed by cold dissolution of poloxamers. The prepared formulations were investigated and evaluated using QbD. FTIR studies revealed compatibility between the excipients and the drug. The drug content, viscosity, and pH of all formulations were demonstrated to be satisfactory. Formulations with appropriate gelation temperatures (32 °C–35 °C) are N1, N2, and N3. The two formulations with the highest spreading parameters and redeformation capacity are N3 and N4, which both have high MC ratios (0.7%). Their release profiles were similar. Cytotoxic effects were assessed using MTT assays, and a dose-dependent increase in viability was observed, particularly in the N3 formulation. This study may open new avenues for the topical application of NDF-loaded *in situ* gels. It may serve as a superior alternative to traditional creams for acne and dermal infections. Furthermore, the impact of critical materials in the formulation on the results was evaluated using QbD, which has been shown to avoid unnecessary studies that waste time and money in industry.

Ethics

Ethics Committee Approval: Ethics committee approval was not required for this study because the study did not involve human participants or animals.

Informed Consent: Informed consent was not required for this study because the study did not involve human participants or animals.

Acknowledgements

The authors would like to thank Berko Pharmaceutical Company (İstanbul, Türkiye) and Santa Farma Pharmaceutical Company (İstanbul, Türkiye) for kindly providing nadifloxacin and BASF (Türkiye) for kindly getting poloxomers.

Footnotes

Authorship Contributions

Surgical and Medical Practices: E.Ö.B., B.İ., N.Ü.O., Concept: E.Ö.B., N.Ü.O., Design: E.Ö.B., N.Ü.O.,

Data Collection or Processing: E.Ö.B., B.İ., N.Ü.O., Analysis or Interpretation: E.Ö.B., B.İ., A.B.Ö., N.Ü.O., Literature Search: E.Ö.B., B.İ., A.B.Ö., N.Ü.O., Writing: E.Ö.B., B.İ., A.B.Ö., N.Ü.O.

Conflict of Interest: The authors declare no conflicts of interest.

Financial Disclosure: The authors declared that this study received no financial support.

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