https://doi.org/10.31925/farmacia.2024.5.3 **ORIGINAL ARTICLE**

DEVELOPMENT AND CHARACTERIZATION OF MEFENAMIC ACID EUTECTOGELS FOR CONTROLLED DRUG DELIVERY

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Abstract

The objective of this paper was to design and evaluate of some novel eutectogels based on xanthan gum (XAN), hyaluronic acid (HA) and Natural Deep Eutectic Solvents (NADES) for the topical delivery of mefenamic acid (MA). Mefenamic acid, a poorly water-soluble non-steroidal drug, presents limitations in bioavailability. Integration of NADES into the gel matrix has overcome this drawback by improving the solubility and stability of the drug. A face-centered central composite design was used to investigate the impact of three independent variables, namely the percentages of water, HA and XAN respectively, on some physical-chemical and biopharmaceutical parameters of the designed eutectogels. Thus, rheological and textural analyses revealed that the inclusion of NADES significantly enhanced the viscosity and mechanical stability of the eutectogels, ensuring both ease of application and robust structural integrity under shear stress. *In vitro* drug release studies demonstrated a controlled, sustained release profile essential for maintaining therapeutic concentrations over extended periods. Overall, this study underscores the potential of NADES-based eutectogels as a promising platform for drug delivery, offering enhanced solubility, stability and an adequate mefenamic acid release profile.

Rezumat

Obiectivul acestui studiu a fost de a concepe și evalua unele eutectogeluri noi bazate pe gumă xantan (XAN), acid hialuronic (HA) și solvenți eutectici naturali (NADES) pentru administrarea topică a acidului mefenamic (MA). Acidul mefenamic, o substanță antiinflamatoare nesteroidiană cu solubilitate scăzută în apă, prezintă limitări în ceea ce privește biodisponibilitatea. Integrarea NADES în matricea gelului a permis depășirea acestui dezavantaj prin îmbunătățirea solubilității și stabilității medicamentului. Pentru a investiga impactul a trei variabile independente, și anume procentul de apă, HA și respectiv XAN, asupra unor parametri fizico-chimici și biofarmaceutici ai eutectogelurilor concepute, s-a utilizat un design experimental de tip central compozit. Astfel, analizele reologice și texturale au arătat că includerea NADES a îmbunătățit semnificativ vâscozitatea și stabilitatea mecanică a eutectogelurilor, asigurând atât ușurința aplicării, cât și o integritate structurală robustă în condiții de stres de forfecare. Studiile de eliberare *in vitro* au demonstrat un profil de cedare controlată și susținută a MA. Acest studiu subliniază potențialul eutectogelurilor pe bază de NADES ca formulări promițătoare pentru administrarea medicamentelor, oferind solubilitate și stabilitate îmbunătățite, precum și un profil adecvat de eliberare a acidului mefenamic.

Keywords: eutectogels, Natural Deep Eutectic Solvents (NADES), controlled drug delivery, xanthan gum, hyaluronic acid, mefenamic acid

Introduction

Currently, Natural Deep Eutectic Solvents (NADES) are intensively explored as a promising and sustainable green alternative to classical organic solvents [1]. NADES represent a new class of Deep Eutectic Solvents (DES) consisting of natural, bio-renewable compounds like amino acids, sugars, organic acids and sugar alcohols in different ratios, which interact through hydrogen bonding, and forming stable eutectic mixtures at normal temperatures with a lower melting point that of each individual component [2-4]. Among the remarkable properties of NADES that make them attractive in the pharmaceutical industry are mentioned: non-toxicity, biocompatibility, versatility, stability at high temperature, easy preparation, environmentally friendly nature and especially the ability to dissolve both polar and non-polar substances [5-7] and to improve the solubility of poorly soluble drugs [8-10], improving bioavailability and consequently increasing therapeutic efficacy.

On the other hand, the hydrogels represent a field in continuous development, and the immobilization of NADES in the polymer network leads to innovative pharmaceutical formulations, so-called eutectogels. These hybrid materials can serve as controlled drug

delivery platforms, offering enhanced solubility of bioactive compounds, sustained release, biocompatibility and adhesiveness [11]. These features makes eutectogels as versatile drug delivery systems for different administration routes, especially mucosal ones when a controlled and sustained deliverability are essential for achieving therapeutic efficacy [12].

The use of eutectogel-based delivery systems holds promise for improving the efficacy of the various pharmaceutical compounds, including mefenamic acid (MA) [13]. Mefenamic acid [14], a member of the fenamate class [15, 16] is a non-selective nonsteroidal anti-inflammatory drug (NSAID) derived from anthranilic acid. It is indicated for the management of diverse painful conditions such as dental pain [17, 18], premenstrual syndrome, postpartum and postoperative pain [19-21] and osteoarthritis [22, 23], as well as for conditions such as asthma, Alzheimer's disease and urticaria [20, 24]. However, the poor water solubility of mefenamic acid [25] impedes its absorption and bioavailability, and its short plasma half-life constrains its therapeutic efficacy [15, 26]. Mefenamic acid is insoluble in water and only slightly soluble in alcohol and methyl alcohol, sparingly soluble in chloroform and soluble in alkaline hydroxide solutions. Owing to its limited solubility, it must be stored in light-protected airtight containers to preserve its stability [27].

The correlation between a drug's solubility and its bioavailability and therapeutic efficacy is well-established. Mefenamic acid exhibits distinct solubility profiles that can significantly influence its absorption and clinical effectiveness. Accordingly, the enhancement of mefenamic acid solubility through advanced formulation strategies, such as eutectogels, holds the potential to markedly improve its bioavailability and therapeutic outcomes. Eutectogels, by augmenting drug solubility and providing stable delivery platforms [28], present a promising approach to surmounting these challenges, ultimately enhancing the clinical performance of poorly soluble drugs like mefenamic acid.

One of the crucial factors in formulating eutectogels is the selection of appropriate polymeric components. Xanthan gum, XAN [29] and hyaluronic acid, HA, are two commonly used biopolymers in pharmaceutical formulations due to their complementary and synergistic effects. When combined, XAN and HA create a strong gel network that supports the eutectic solvent system and offers improved therapeutic potential through sustained release and strong adhesion to mucosal surfaces. This study aims to explore the potential of eutectogels [30] based on NADES for mucosal drug delivery by developing novel formulations such as hybrid eutectogels with XAN and HA, and ternary NADES [31, 32], choline chloride: sorbitol: water, 1:1:1, with the incorporation of MA as the active pharmaceutical ingredient. The goal is to create an eutectogel suitable for mucosal administration, providing sustained drug release, enhanced bioavailability and improved patient compliance. The optimization process will lead to the final formulation with favourable rheological and textural properties, ensuring an effective drug delivery system suitable for topical administration.

Materials and Methods

Materials

The NADES systems were prepared using choline chloride (≥ 98%, Sigma-Aldrich, Saint Louis, MO, USA) and D-(-)-sorbitol (≥ 96%, high purity, VWR Chemicals, Leuven, Belgium). Mefenamic acid (98% purity) was sourced from MP Biomedicals (Eschwege, Germany). Xanthan gum (extra pure, Carl Roth GmbH & Co, Karlsruhe, Germany) and sodium hyaluronate (Fagron, Rotterdam, Netherlands) were selected as gelling agents. Ultrapure water was produced using a Milli-Q EQ 7008 water purification system (Merck Millipore, Burlington, MA, USA). For HPLC analysis, acetonitrile and methanol (HPLC grade) were provided by Honeywell Research Chemicals (Seelze, Germany) and formic acid (LC-MS grade) was obtained from Fisher Chemical (Leicestershire, UK).

Obtaining NADES-Based Xanthan-HA Eutectogels

The formulation of the eutectogels was guided by a comprehensive investigation into the solubility of mefenamic acid across diverse eutectic systems, as documented in preliminary research. The ChCl:sorbitol: water 1:1:1 molar ratio NADES was identified as the optimal eutectic system, providing an ideal combination for drug solubility, ensuring biocompatibility and taste masking.

Prior to use, the choline chloride was vacuum-dried at 50°C for at least 24 hours and stored in an airtight container, while the sorbitol was finely ground using a mortar and pestle to ensure a uniform consistency. In order to maintain an anhydrous environment, the weighed components were mixed in a clean, dry, screw-cap glass container within a glovebox. The mixture was maintained at 75°C and 1000 rpm for 2 hours using a ThermoMixer C system euipped with a 50 mL thermoblock (Eppendorf, Hamburg, Germany), until a homogeneous, clear liquid was obtained.

Mefenamic acid was slowly incorporated into the NADES under continuous stirring using an overhead stirrer (Velp Scientifica, Usmate Velate, Italy), until the drug was fully dissolved. Next, varying amounts of xanthan gum and hyaluronic acid (as detailed in Table II) were dispersed into the NADES solution while continuously stirring. Additional ultrapure water was gradually added and the mixture was maintained at 75°C for two hours with continuous agitation. This process ensured complete hydration and uniform distribution of the polymers. Finally, the mixture was allowed to cool to room temperature, initiating gelation. The resulting eutectogels, with a standardized drug concentration of 0.5%, were stored at ambient temperature for further analysis. The concentration

of the solubilized drug in the eutectic mixtures was determined using a validated HPLC method, with UV detection at 280 nm.

Rheological characterization

To evaluate the viscosity and flow behaviour of the eutectogels at 37°C, a RM100 CP2000 Plus cone-plate rheometer (Lamy Rheology Instruments, Champagne au Mont d'Or, France) with a CP6020 measuring cone (Ø 60 mm, angle 2°) was used. Flow curves were built by applying a controlled shear rate increase from 1 to 100 s^{-1}. Rheological parameters such as shear stress (Pa), shear rate (s^{-1}) and viscosity (Pa·s) were measured, and experimental data was fitted with the Power law model (Equation 1):

$$\eta = K \cdot \dot{\gamma}^{n-1}, \qquad (1)$$

where, η – is apparent viscosity (Pa·s), $\dot{\gamma}$ – shear rate (s^{-1}), K – consistency index, corresponding to the viscosity at a shear rate of 1 s^{-1} ($Pa \cdot s^n$) and n – flow behaviour index (dimensionless).

When n = 1, the fluid exhibits Newtonian behaviour, characterized by a constant viscosity across varying shear rates. For values of n < 1, the fluid demonstrates shear-thinning or pseudoplastic behaviour, wherein the viscosity decreases as the shear rate increases. The greater the deviation of n from 1, the more pronounced the shear-thinning effect observed in the fluid.

Texture profile analysis

To assess the textural properties of the samples, Texture Profile Analysis (TPA) was performed under controlled conditions at 37°C. A TX-700 texture analyser from Lamy Rheology Instruments was employed, wherein each gel sample (approximately 30 g) was placed in cylindrical containers and equilibrated to room temperature prior to testing. The analysis consisted of two sequential cycles of compression and decompression. Throughout the compression phase, the probe was driven at a constant velocity of 0.5 mm/s to a depth of 10 mm, with the process beginning once a force threshold of 0.01 N was reached. During the decompression phase, the probe was retracted to a position 20 mm above the sample. The resulting force-time curves were analysed to extract textural parameters, including hardness and adhesiveness. To guarantee precision and dependability, five replicates of each gel formulation were performed, the average results were presented as average ± SD. Data analysis was conducted utilizing RheoTex software version 1.52.0.0.

In vitro release experiments

The *in vitro* release of the eutectogel was evaluated using Franz diffusion cells (Hanson Research Inc., USA) with a capacity of 7 mL, and effective surface area of 1.77 cm^2, employing 0.45 μm pore size Supor® polyethersulfone membrane filters (PES) membrane disc filters (Pall Life Sciences, Portsmouth, UK). The PES membranes were positioned between the donor and receptor compartments of the diffusion cells. The receptor chambers were filled with phosphate-buffered saline containing 20% ethanol. The receptor medium was continuously stirred at 400 rpm and maintained at 37 ± 0.5°C. The eutectogel formulations were carefully applied to the donor compartment. At predetermined time intervals (0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 hours) samples of 1 mL were collected from the receptor medium and analysed by HPLC.

Experimental design

To systematically investigate the influence of the formulation variables on the rheological and drug release properties of the eutectogels, a face-centred central composite design (FCCCD) was employed. This design approach allowed for the assessment of linear, quadratic and interaction effects of three independent variables on the characteristics of the eutectogels, *i.e.* percentage of water (X_1), hyaluronic acid (X_2) and xanthan gum (X_3). These variables were evaluated at three different levels: low (-1), medium (0) and high (+1), as shown in Table I. The response variables, which were critical for assessing the performance of the eutectogels, included: consistency index (Y_1, measured in $Pa \cdot s^n$), hardness (Y_2, measured in N), adhesiveness (Y_3, measured in N·s) and drug release rate (Y_4, expressed as $\mu g/cm^2/min^{1/2}$).

Table I

Experimental variables and responses analysed in the Central Composite design

Variables	Code	Level		
		Low (-1)	Medium (0)	High(+1)
Water (%)	X_1	20	30	40
HA (%)	X_2	0.25	0.5	0.75
XAN (%)	X_3	1	1.5	2

Responses	Code	Measuring unit
Consistency index	Y_1	$Pa \cdot s^n$
Hardness	Y_2	N
Adhesiveness	Y_3	N·s
Release rate	Y_4	$\mu g/cm^2/min^{1/2}$

A total of 17 formulations were prepared, with each formulation reflecting a unique combination of the three variables based on the FCCCD matrix (Table II). Each experiment was performed in triplicate to

ensure data accuracy and reliability, and the results were analysed using Design Expert software (version 13, Stat-Ease Inc., USA). The experimental design also included three replicates of the centre point, allowing for the evaluation of experimental errors and model precision. The results were fitted to a second-order polynomial equation using multiple regression analysis, with non-significant terms removed through backward elimination.

Table II

Experimental design matrix for the study of mefenamic acid eutectogels

Formulation code	X_1 Water (%)	X_2 HA (%)	X_3 XAN (%)
EG_1	20	0.75	1
EG_2	30	0.5	2
EG_3	40	0.75	1
EG_4	20	0.75	2
EG_5	30	0.5	1.5
EG_6	40	0.75	2
EG_7	20	0.25	2
EG_8	30	0.25	1.5
EG_9	20	0.5	1.5
EG_10	30	0.5	1
EG_11	40	0.25	2
EG_12	30	0.5	1.5
EG_13	20	0.25	1
EG_14	40	0.25	1
EG_15	30	0.5	1.5
EG_16	40	0.5	1.5
EG_17	30	0.75	1.5

Statistical analysis

Data obtained in triplicate/duplicate were expressed as means ± SD. Statistical analysis was performed using GraphPad Prism v10.

Results and Discussion

Experimental design

The experimental results obtained for the dependent variables for all 17 experimental formulations are presented in Table III. These results were fitted using a second-order polynomial equation, with non-significant terms removed through backward elimination based on the Akaike information criterion (AIC).

The multiple regression analysis revealed highly significant models ($p < 0.0001$) for all dependent variables, indicating that the polynomial models effectively captured the relationships between the independent variables and the observed outcomes. No significant lack of fit was detected in any of the models, confirming strong alignment with experimental data. The adjusted R-squared values show that the models accounted for a considerable portion of the variability in the response variables. The magnitudes and signs of the regression coefficients reveal key insights into the influence of the independent variables on the response variables. A positive coefficient shows that increasing the respective independent variable leads to a rise in the response variable, while a negative coefficient suggests that an increase in the independent variable results in a decrease in the dependent variable.

Table III

Rheological, textural and kinetic properties of various XAN-HA eutectogel formulations (EG_1- EG_17) obtained by applying a face-centred central composite design, with each column representing a specific parameter: Y_1 (consistency index, Pa·s^n), Y_2 (hardness, N), Y_3 (adhesiveness, N·s), Y_4 (drug release rate, μg/cm²/min$^{1/2}$)

Code	Y_1 (Pa·s^n)	Y_2 (N)	Y_3 (N·s)	Y_4 (μg/cm²/min$^{1/2}$)
EG_1	178.313 ± 3.7	1.201 ± 0.026	3.179 ± 0.113	21.13 ± 1.2
EG_2	226.241 ± 7.5	1.258 ± 0.232	2.822 ± 0.536	22.95 ± 2.2
EG_3	113.501 ± 0.4	0.743 ± 0.011	2.174 ± 0.153	23.38 ± 3.2
EG_4	267.243 ± 8.0	2.192 ± 0.071	5.265 ± 0.129	20.94 ± 1.1
EG_5	150.789 ± 4.5	1.015 ± 0.020	2.434 ± 0.149	21.91 ± 3.7
EG_6	241.927 ± 6.4	1.305 ± 0.156	3.092 ± 0.487	23.96 ± 6.1
EG_7	192.034 ± 4.3	1.518 ± 0.083	2.910 ± 0.286	22.63 ± 5.5
EG_8	119.124 ± 4.0	0.764 ± 0.100	1.861 ± 0.141	24.63 ± 3.8
EG_9	130.906 ± 3.9	1.464 ± 0.068	2.985 ± 0.101	20.15 ± 3.0
EG_10	97.576 ± 0.1	0.667 ± 0.016	1.817 ± 0.051	23.95 ± 2.2
EG_11	137.550 ± 2.0	0.782 ± 0.099	2.046 ± 0.175	22.96 ± 1.6
EG_12	149.727 ± 1.6	1.140 ± 0.061	2.282 ± 0.235	23.62 ± 1.3
EG_13	98.008 ± 1.5	0.875 ± 0.087	1.796 ± 0.062	22.50 ± 1.8
EG_14	64.687 ± 1.2	0.473 ± 0.009	0.877 ± 0.106	22.68 ± 6.5
EG_15	150.341 ± 4.2	1.285 ± 0.103	2.816 ± 0.150	24.37 ± 3.8
EG_16	138.159 ± 6.1	0.921 ± 0.040	1.751 ± 0.226	22.39 ± 2.5
EG_17	208.024 ± 6.1	1.280 ± 0.168	3.047 ± 0.377	24.59 ± 1.9

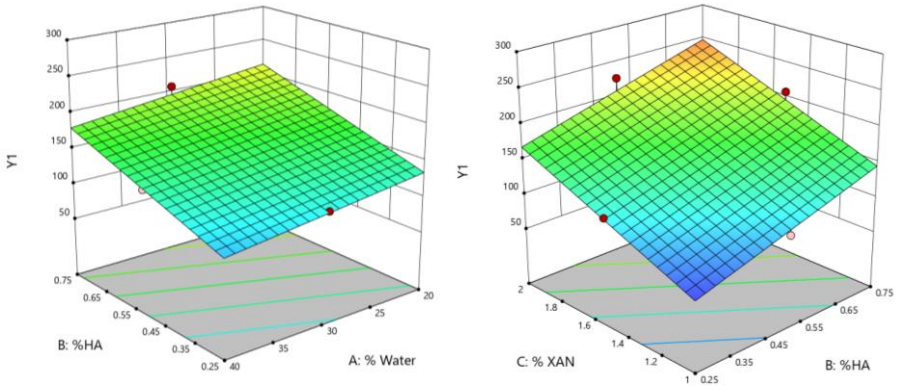
The relationship between consistency index (Y_1, Pa·s^n) and the independent variables was best described by the following second-order polynomial equation:

$$Y_1 = 156.71 - 17.07X_1 + 39.76X_2 + 51.29X_3.$$

Analysis of variance revealed a statistically significant effect of the formulation variables on consistency index ($F = 51.03$, $p < 0.0001$). Increasing XAN concentration (X_3) had the most significant positive impact on

viscosity, as XAN forms a highly structured network in the gel. HA (X_2) also showed a positive effect, with higher concentrations contributing to the gel's viscoelasticity. Conversely, increasing water content (X_1) had a negative effect on viscosity, diluting the gel matrix and reducing its overall thickness. The regression model for consistency index yielded an R^2 value of 0.9037, indicating a strong fit with the experimental data.

The response surface plots with the most significant parameters that influence the viscosity of eutectogels are presented in Figure 1.



(a) (b)

Figure 1.

Response surface plots for consistency index (Y_1) as a function of (a) Water (%) and HA (%) and (b) HA (%) and XAN (%)

The textural features of the XAN-HA eutectogels are crucial for their effectiveness in pharmaceutical and cosmetic applications. Characteristics like hardness and adhesiveness provide valuable information about the mechanical properties and overall user experience of the gels [33].

Gel hardness (Y_2), which serves as a key indicator of structural integrity and resistance to deformation, ranged from 0.473 ± 0.020 N (EG_14) to 2.192 ± 0.004 N (EG_4) and was significantly influenced by the eutectogel composition. Both xanthan gum (X_3) and hyaluronic acid (X_2) positively influenced hardness (Y_2), with higher concentrations leading to firmer gels. The interaction between these two biopolymers demonstrated a synergistic effect, enhancing the structural integrity of the gel. Water content (X_1) had a negative impact on Y_2, further confirming its role as a diluent in the gel matrix. The statistical analysis yielded a highly significant model for the hardness, with an adjusted R^2 value of 0.9289, indicating a strong correlation between the experimental data and the predicted model. The regression equation for Y_2 is as follows:

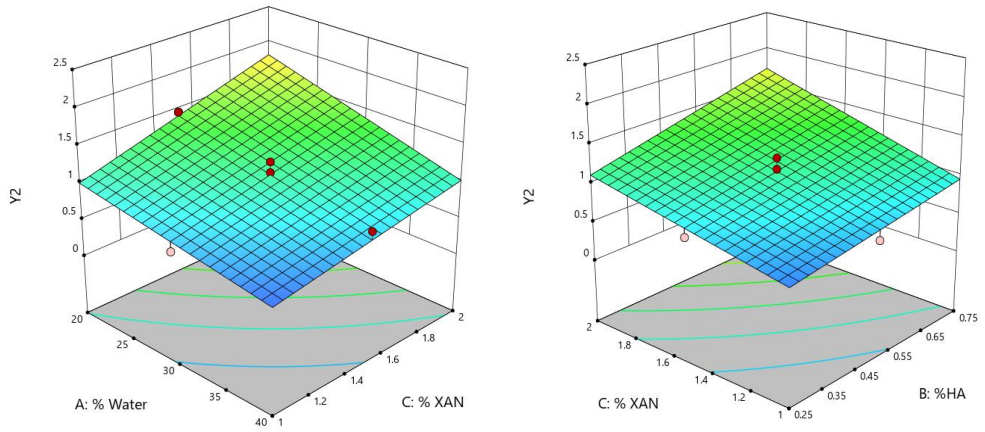
$$Y_2 = 1.11 - 0.3027X_1 + 0.2309X_2 + 0.3095X_3 - 0.0954X_1X_3 + 0.0751X_2X_3.$$

In this model, the negative coefficient for X_1 underscores the detrimental effect of increased water content on the gel's hardness, while the positive coefficients for X_2 and X_3 confirm their role in strengthening the gel matrix. Additionally, the negative interaction between X_1 and X_3 suggests that excessive water may counteract xanthan gum's thickening effect. In order to further investigate the textural properties of the XAN-HA eutectogels, response surface plots were created, focusing specifically on the most statistically significant effects ($p < 0.0001$), are illustrated in Figure 2.

Adhesiveness (Y_3), which quantifies the gel's ability to interact with surfaces, ranged from 0.877 ± 0.013 N·s (EG_14) to 3.480 ± 0.003 N·s (EG_4). The results demonstrated that water content (X_1) exerted a significant negative effect, while both hyaluronic acid (X_2) and xanthan gum (X_3) had substantial positive effects on the gel's adhesive properties. Moreover, the interaction between X_2 and X_3 showed a negative coefficient, suggesting that while both biopolymers individually enhance adhesiveness, excessive concentrations in combination may reduce the synergistic benefit. This implies that there is an optimal balance between xanthan gum and hyaluronic acid for achieving maximum adhesiveness. The regression equation for Y_3 is as follows:

$$Y_3 = 2.43 - 0.441X_1 + 0.548X_2 + 0.451X_3 - 0.133X_2X_3$$

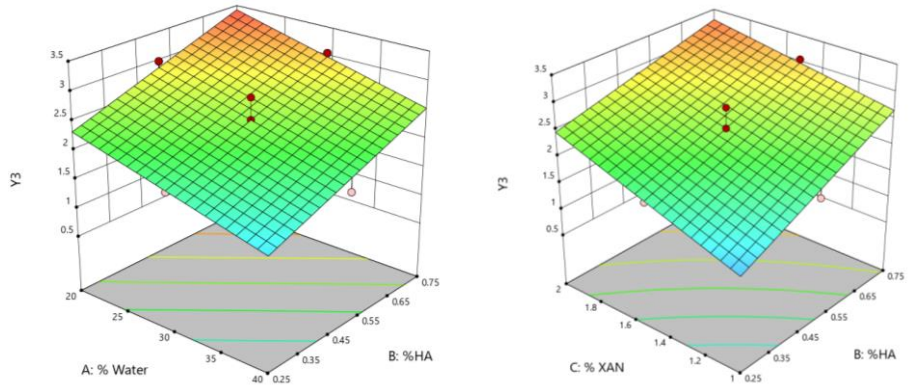
The model demonstrated strong predictive capability with an adjusted R^2 value of 0.9275, indicating a robust correlation between the independent variables and the experimental outcomes. The corresponding response surface plots for Y_3, including the most significant effects ($p < 0.0001$) are illustrated in Figure 3.



(a) (b)

Figure 2.

Response surface plots for hardness (Y_2) as a function of (a) Water (%) and XAN (%) and (b) HA (%) and XAN (%)



(a) (b)

Figure 3.

Response surface plots for adhesiveness (Y_3) as a function of (a) Water (%) and HA (%), (b) HA (%) and XAN (%)

The analysis of both Y_2 and Y_3 highlights the complex interactions between formulation parameters and the textural properties of XAN-HA eutectogels. By carefully adjusting the concentrations of water, HA and XAN, the textural characteristics of the gel can be significantly improved.

The rate and extent of drug release from a delivery system are critical factors influencing therapeutic efficacy. Maintaining a sustained, steady-state release rate is crucial for ensuring therapeutic drug levels over an extended duration, particularly in the management of chronic conditions [34]. In this study, the release rate (Y_4), representing the steady-state diffusion of the drug through the gel matrix, was calculated from the slope of the linear section of the cumulative release *versus* the square root of time plot, following the Higuchi model. This approach is aligned with the SUPAC-SS [35] guidelines for semisolid systems and provides insights into the steady-state drug diffusion behaviour.

The release rate varied between 20.15 ± 3.0 μg/cm²/min$^{1/2}$ (for formulation EG_9) and 24.63 ± 3.8 μg/cm²/min$^{1/2}$ (for formulation EG_8), highlighting the significant influence of formulation variables on drug release kinetics. The following regression equation was derived to describe the effects of these variables on the release rate:

$$Y_4 = 23.42 + 0.8024X_1 - 0.1401X_2 + 0.5970X_1X_2 - 2.12X_1^2 + 1.22X_2^2.$$

An increase in water content (X_1) had a notable positive effect on the release rate (Y4), leading to a faster drug release from the gel matrix. This can be attributed to the increased fluidity and enhanced diffusion properties within the matrix as water content rises. However, the quadratic term for water content (X_1^2) exhibited a negative effect, suggesting that beyond a certain threshold, excessive water dilutes the gel network and compromises its structural integrity, thereby reducing the overall drug release rate. HA (hyaluronic acid) also had an overall positive impact on the release rate, primarily through its second-order interactions with water (X_1X_2) and its own quadratic effect (X_2^2). The thickening properties of HA contribute to the formation of a denser gel matrix, which can slow down the drug diffusion. Nevertheless, the interaction

between X_1 and X_2 indicates that increasing water content can mitigate some of the diffusion-limiting effects of HA, maintaining an optimal release rate. The quadratic effect of HA (X_2^2) further underscores the complexity of its role in the gel matrix, where excessive concentrations could hinder drug diffusion by forming a highly entangled polymer network. The positive interaction between water (X_1) and hyaluronic acid (X_2) is noteworthy, as it suggests that an optimal balance between these two components is crucial for maximizing drug release. Water, while increasing diffusion, helps counteract the thickening effects of HA, thereby facilitating a more efficient drug release. However, exceeding optimal levels of either component can lead to diminished release rates, as shown by the quadratic terms in the model.

Figure 4 (the response surface plot) provides a visual representation of the most significant interactions affecting the release rate. It clearly illustrates that the release rate is maximized when both water and HA are balanced within specific ranges, underscoring the necessity of fine-tuning these formulation variables for optimal drug delivery performance.

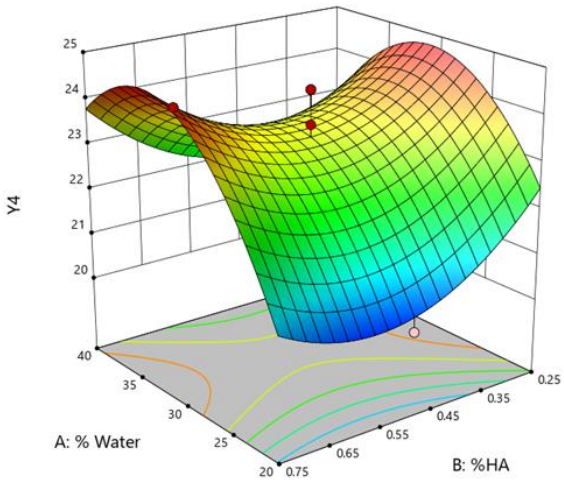


Figure 4.
Response surface plot for release rate as a function of Water (%) and HA (%)

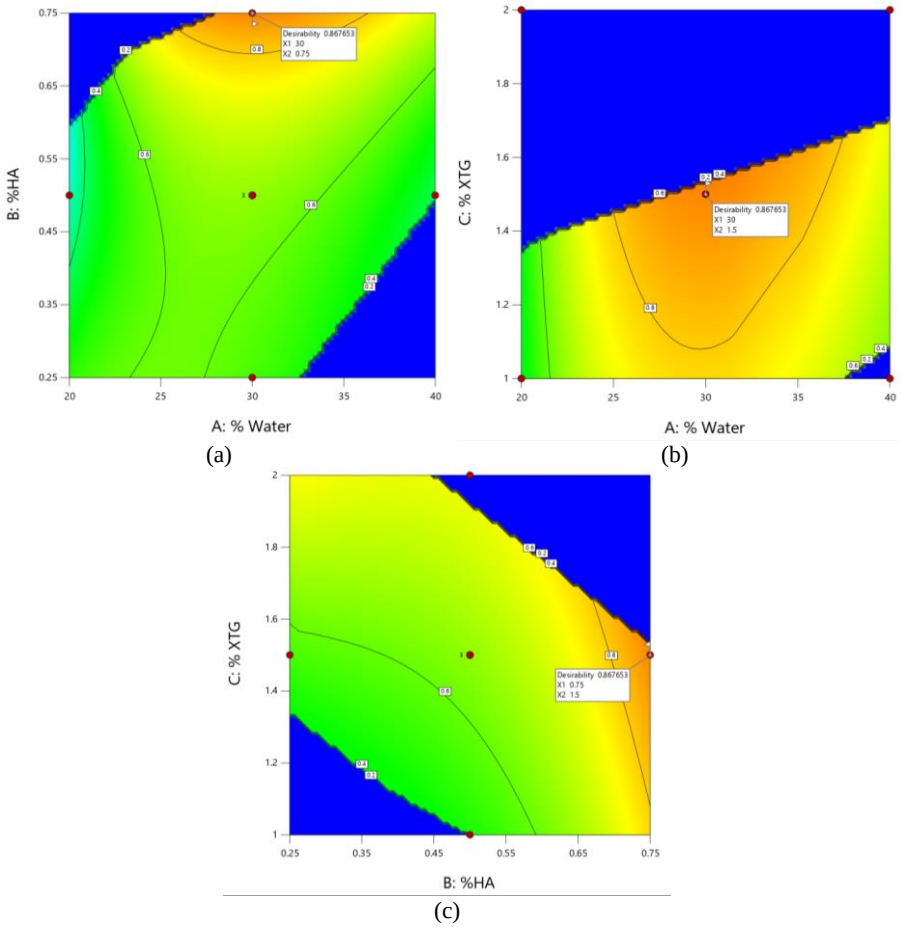


Figure 5.
Contour plots for desirability as a function of: (a) Water (%) and HA (%), (b) Water (%) and XAN (%), (c) HA (%) and XAN (%)

Optimization of XAN-HA-NADES eutectogels

To analyse the effects of formulation factors and responses on the characteristics of the formulation under investigation, a desirability function approach was implemented. This method allowed for the simultaneous optimization of multiple formulation

characteristics by transforming each response variable into a dimensionless desirability score ranging from 0 (undesirable) to 1 (highly desirable). The overall desirability score was calculated as the geometric mean of the individual scores, providing a comprehensive evaluation of the eutectogel performance.

The optimization process aimed to achieve a balance between rheological, textural and drug release properties. The key objectives were to maximize adhesiveness and release rate while ensuring viscosity and hardness remained within a target range. Specifically, consistency index was optimized to be between 100 and 200 Pa·s^n, ensuring the gel remained structurally stable yet easily spreadable during application, while hardness was maintained between 0.8 and 1.5 N, providing sufficient mechanical strength without compromising comfort during topical application.

Figure 5 highlights the optimization contour plot for the desirability score as a function of water, HA and XTG concentrations, demonstrating the optimal ranges for these variables.

The optimization results, as shown in Figure 5, indicate that the optimal concentrations for the formulation variables are 30.8% water, 0.75% HA and 1.55% XAN. These concentrations achieve a balanced outcome for the rheological and drug release properties of the eutectogel.

This specific composition was determined to provide the best compromise among the critical formulation parameters, ensuring both optimal physical characteristics and effective drug delivery performance. The predicted response variables for this optimized formulation were as follows: consistency index of 200 Pa·s^n, hardness of 1.35 N, adhesiveness of 2.98 N·s and a drug release rate of 24.57 µg/cm^2/min$^{1/2}$ (as illustrated in Figure 6). These values reflect the ideal combination of mechanical stability and sustained drug release, making the formulation well-suited for therapeutic applications.

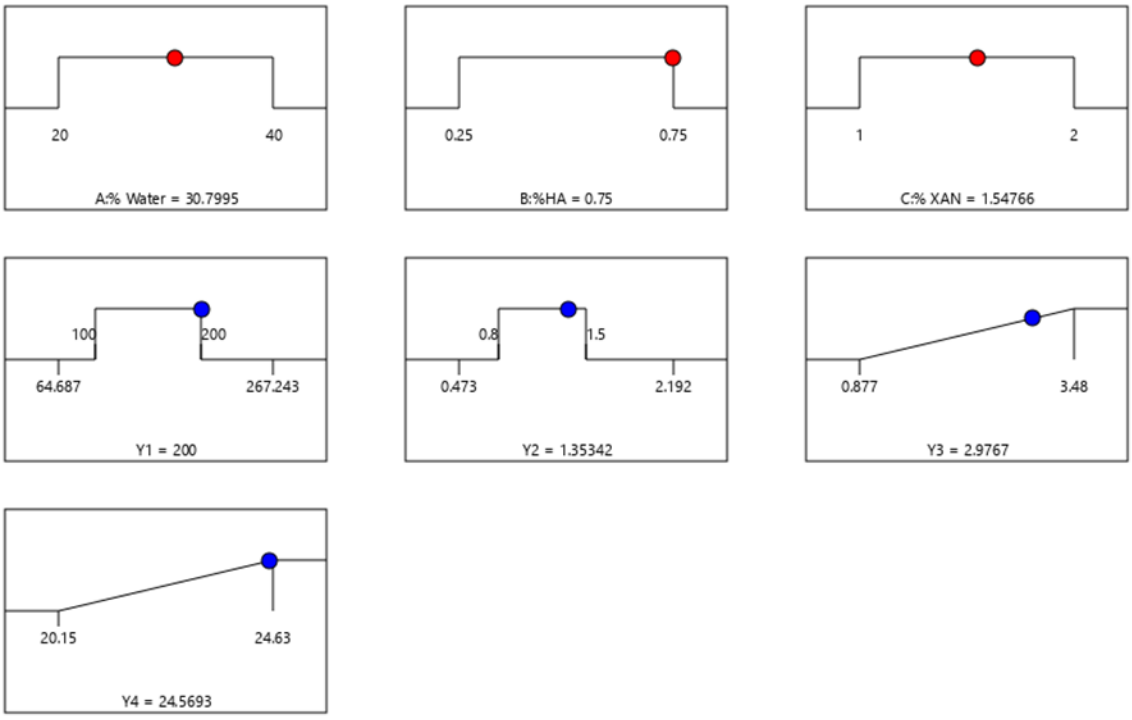


Figure 6.

Optimization plot for the MA eutectogels, illustrating the maximum desirability score. The selected optimal levels of the independent variables are marked in red. The predicted optimal responses for consistency index, hardness, adhesiveness and drug release rate are shown in blue.

The optimized eutectogel formulation, (coded as XAN-HA-NADES-MA), was experimentally prepared in triplicate and evaluated for its rheological, textural and drug release properties. To validate the accuracy the optimization model and its ability to predict the performance of the optimized formulation, the experimentally obtained data were compared with the predicted values for the key response variables, including consistency index, hardness, adhesiveness and drug release rate. Additionally, to establish a baseline for comparison, a gel base formulation containing only the polymers (XAN-HA) and a blank eutectogel without mefenamic acid (XAN-HA-NADES) were prepared and characterized. These formulations provided further insights into the contribution of the NADES system and MA to the overall properties of the optimized eutectogel (as shown in Table IV).

Table IV

Results obtained for characterization of the optimized formulation (XAN-HA-NADES-MF) along with the blank eutectogel formulation (XAN-HA-NADES) and the gel base (XAN-HA)

Sample	Consistency index (Pa·s^n)	Hardness (N)	Adhesiveness (N·s)	Release rate (μg/cm^2/min$^{1/2}$)
XAN-HA-NADES-MF	196.643 ± 9.14	1.359 ± 0.084	2.945 ± 0.156	23.97 ± 1.89
XAN-HA-NADES	193.213 ± 9.70	1.348 ± 0.066	2.988 ± 0.242	-
XAN-HA	30.600 ± 6.79	0.221 ± 0.010	0.404 ± 0.058	-

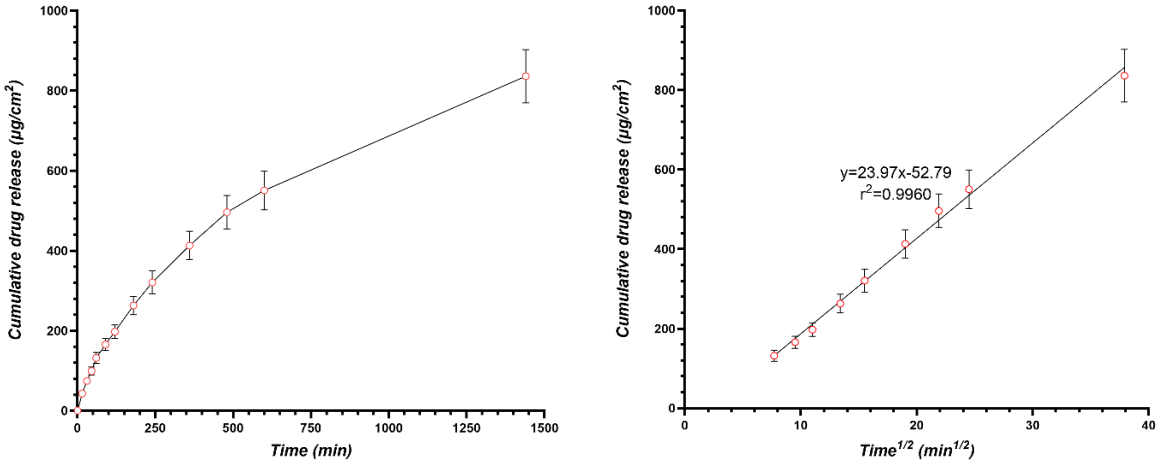
The optimized formulation, XAN-HA-NADES-MA, exhibited a consistency index of 196.643 ± 9.14 Pa·s^n, closely matching the predicted value of 200 Pa·s^n. This result confirms the predictive accuracy of the optimization model and highlights the significant role of NADES in enhancing the gel's consistency index compared to the base formulation (30.600 ± 6.79 Pa·s^n). The substantial increase in viscosity observed in the NADES-containing formulations indicates that the deep eutectic solvent system contributes to a more structured gel matrix.

In terms of hardness, the optimized formulation reached a value of 1.359 ± 0.084 N, very close to the predicted value of 1.35 N. This significantly higher hardness, when compared to the base formulation (0.221 ± 0.010 N), demonstrates significant effect of both NADES and MA on the gel network. The blank eutectogel (XAN-HA-NADES) similarly exhibited enhanced hardness (1.348 ± 0.066 N), indicating that the NADES system contributes greatly to the mechanical strength of the gel even in the absence of the active pharmaceutical ingredient.

The adhesiveness of the optimized formulation (2.945 ± 0.156 N·s) is also in excellent agreement with the predicted value (2.98 N·s). This level of adhesiveness is critical for mucosal applications, where prolonged contact with the mucosal surface is necessary for effective drug delivery. In comparison, the base formulation (0.404 ± 0.058 N·s) displayed significantly lower adhesiveness, further supporting the conclusion that both NADES and mefenamic acid improve the mucoadhesive properties of the eutectogel. The blank formulation (2.988 ± 0.242 N·s) showed comparable adhesiveness to the optimized formulation, suggesting that the NADES system plays a pivotal role in enhancing the gel's ability to adhere to mucosal surfaces, independent of the drug content.

The drug release rate of the optimized formulation (23.97 ± 1.89 μg/cm^2/min$^{1/2}$), is also very close to the predicted value of 24.57 μg/cm^2/min$^{1/2}$. This result further verifies the optimization model's ability to accurately predict the release kinetics of MA from the eutectogel matrix. The optimized release rate is designed to provide sustained and controlled delivery of the active pharmaceutical ingredient, making the formulation ideal for extended therapeutic applications.

The graphical representation of the cumulative drug release as a function of time (Figure 7a) demonstrates a controlled and gradual release of MA from the eutectogel, with the release extending over 24 hours. The curve shows a steady increase in drug release over time, further confirming the sustained release nature of the formulation. The linear plot in Figure 7b indicates a diffusion-controlled release mechanism, following the Higuchi model, consistent with the goal of achieving a sustained release profile for MA, as described in the optimization phase.



(a) (b)

Figure 7.

Cumulative release profiles of MA for XAN-HA-NADES-MA expressed as a function of (a) time and (b) square root of time

Conclusions

Different eutectogels designed for controlled drug delivery, containing mefenamic acid as principal active ingredient, and xanthan gum and hyaluronic acid as gel forming biopolymers, were physical-chemical and biopharmaceutical assessed. The formulation's rheological properties were notably improved by the addition of NADES, leading to a more cohesive gel structure with enhanced mechanical strength and stability. The presence of NADES also contributed to the formation of a robust gel matrix, improving the gel's ability to resist mechanical stress, which is crucial for maintaining its integrity during application. *In vitro* release studies confirmed a controlled and prolonged release of MA, essential for sustaining therapeutic levels over an extended period

The resulting optimized eutectogel exhibited moderate viscosity and hardness, high adhesiveness and a sustained drug release profile, making it highly suitable for pharmaceutical applications requiring a controlled drug delivery.

In summary, the XAN-HA-NADES-MA optimum eutectogel offers a promising platform for drug delivery, by providing enhanced mechanical and rheological properties as well as controlled drug release profile. This innovative formulation has the potential to improve drug deliverability, thereby enhancing treatment outcomes and patient compliance.

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Conflict of interest

The authors declare no conflict of interest.

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