

# PREFORMULATION STUDIES OF THERMOSENSITIVE DERMATOLOGICAL HYDROGELS CONTAINING INSULIN

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## Abstract

*This work continues previous research into the development of an insulin (INS) hydrogel carrier for dermatological applications in chronic wound healing. Insulin has been found to play an important role in promoting wound healing, acting at multiple levels. This study aimed to develop an insulin hydrogel carrier based on poloxamer 407 (P407) and chitosan (CS). Rheological and texture parameters of the formulation were analysed. The pharmaceutical availability of insulin was assessed in vitro using the innovative Strat-M<sup>®</sup> membrane, which mimics the skin barrier. The hydrogels showed a favourable balance between rheological and textural properties and ease of application, especially to irregularly shaped wounds and hard-to-reach areas. Insulin was released in a prolonged manner, reducing the need for multiple daily applications. The obtained results will be used further in planning and performing preclinical and clinical studies.*

**Keywords:** hydrogels, insulin, poloxamer 407, chitosan, rheology, texture, Strat-M<sup>®</sup> membrane

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## 1. Introduction

Insulin (INS) plays an important role in wound healing, acting at multiple levels. It enhances the activity of endogenous growth factors such as IGF-1, which accelerates tissue regeneration [1]. INS also stimulates skin fibroblasts for more intensive collagen production and has anti-inflammatory effects, as it reduces pro-inflammatory cytokines and influences immune cell function, which can reduce chronic inflammation that impedes regeneration. In addition, INS improves glucose homeostasis and blood vessel function, creating a favourable environment to promote healing [2]. Studies in animal models have shown that topical application of insulin promotes angiogenesis, accelerates re-epithelialisation and wound closure, and improves the granulation process. One of the key advantages of topical insulin application is its safety; there is no risk of hypoglycaemia. In addition, insulin is readily available, relatively inexpensive, and well tolerated, making it a practical solution for the treatment of hard-to-heal wounds such as diabetic ulcers, bedsores, and burn wounds [3].

Chitosan-based hydrogels can be a potential carrier for insulin application to the skin. According to the Pharmacopoeia, hydrogels are substrates in which the aqueous phase is gelled with a suitable substance, usually a polymer. Hydrogel matrices are characterised by high physical stability, biocompatibility, hydrophilicity, and low sensitisation potential. They can control the release rate of active pharmaceutical ingredients (APIs) and the duration of API action. Furthermore, the technology to prepare this drug formulation is relatively simple.

Chitosan-based dressing materials have been found to promote wound healing at every stage of the process. In the haemostatic phase, chitosan accelerates platelet aggregation and fibrin clot formation. In the inflammatory phase, it stimulates the proliferation of neutrophils and macrophages that cleanse the wound, releasing cytokines that promote regeneration. In the final stage of healing, chitosan mimics the natural extracellular matrix, creating optimal conditions for tissue reconstruction [4].

Ploaxamer 407 (P407), an ethylene oxide/propylene oxide (EO-PO) copolymer, is a non-toxic, non-ionic polymer used as a carrier for various routes of drug administration. Its aqueous solutions ( $\geq 15\%$ ) exhibit thermosensitivity. Solutions stay liquid at low temperatures, and when the temperature limit ( $T_{\text{sol-gel}}$ ) is exceeded, they transform into a semi-solid form by reverse thermal gelation [5]. Chitosan (CS) in combination with other materials can also form the basis for the formulation of thermosensitive hydrogel formulations, e.g., chitosan/sodium glycerophosphate, hydroxybutyl chitosan, chitosan/polyol-polymer, chitosan/polymer amphiphile, and chitosan/inorganic alkali salt [6].

Other studies [7, 8] confirmed the efficacy of the chitosan/ploaxamer and chitosan/hyaluronic acid/ploaxamer hydrogel carrier. They were found to be effective in dihydromyricetin's delivery and therapeutic effect at the wound site. Studies conducted *in vitro* and *in vivo* indicated good adhesion of the formulations at the wound site, prolonged release of API from the polymer matrix, and material biocompatibility in contact with human tissues.

This research aimed to develop a hybrid insulin carrier based on chitosan and Ploaxamer 407, with a view to the preparation of prescription medicines. To the best of our knowledge, no research has been conducted to date on insulin hydrogels in the proposed formulation.

## **2. Materials and Methods**

### **2.1. Materials**

Insulatard<sup>®</sup> Penfill<sup>®</sup> (human insulin (INS), isophane, long-acting) was purchased from Novo Nordisk (Bagsværd, Denmark) at a concentration of 100 IU/mL. Excipients included zinc chloride, metacresol, glycerol, phenol, sodium hydroxide, disodium phosphate dihydrate, protamine sulphate, hydrochloric acid, and water for injection. Chitosan (CS, average molecular weight, degree of deacetylation 75 - 85%, viscosity approximately 200 – 800 cP) was supplied by Sigma-Aldrich (St. Louis, MO, USA). Poloxamer 407 (P407) and PBS buffer (phosphate-buffered saline, pH 7.4) were also provided by Sigma-Aldrich (St. Louis, MO, USA). Acetic acid was purchased from Avantor Performance Materials Poland SA (Gliwice, Poland). All reagents used were of analytical grade. The Strat-M<sup>®</sup> membrane was purchased from Merck Millipore (Burlington, MA, USA).

### **2.2. Methods**

#### *2.2.1. Preparation of Hydrogel Based on Poloxamer 407*

15.0 g of Poloxamer 407 was added to a beaker containing 50.0 ml of cold water (4°C). The formulation was stirred on a magnetic stirrer (Fisherbrand<sup>™</sup> Isotemp<sup>™</sup>, Thermo Fisher Scientific) until the polymer was completely dissolved and a clear solution of 30% (m/v) was obtained. After 5 days of refrigerated storage, the sol was brought to room temperature. When removed from the fridge, the system was in the sol state, with gelation occurring once it reached room temperature. Next, an INS was added, reaching a concentration of 1 mg/g (28.57 IU/g). The formulation was mixed mechanically until clear formulations were obtained (P407-INS). The preparations showed stability both during storage and during the study.

#### *2.2.2. Preparation of Hydrogel Based on Poloxamer 407 and Chitosan*

1.0 g of CS was introduced into 50.0 ml of 0.1 M acetic acid heated to 50°C (which accelerated the dissolution process). The formulation was stirred on a magnetic stirrer (Fisherbrand<sup>™</sup> Isotemp<sup>™</sup>, Thermo Fisher Scientific) until the chitosan was completely dissolved. The formulation was then cooled to 4°C, and 15.0 g of P407 was added under continuous stirring. The finished medium, with a concentration of 30% (m/v) P407 and 2% (m/v) CS, was brought to room temperature after 5 days of storage in the refrigerator, and INS was added, reaching a concentration of 1 mg/g (28.57 IU/g). The formulation was mixed mechanically until clear formulations were obtained (CS/P407-INS). The preparations showed stability both during storage and during the study.

#### *2.2.3. Pharmaceutical Availability Studies*

Pharmaceutical availability testing of insulin from hydrogels was carried out in an Erweka DT600 (Husenstamm, Germany) with a Dissolution Enhancer Cell<sup>™</sup> (exposure area of 3.80 cm<sup>2</sup>). 1 g of INS-containing formulation was introduced into the chambers, covered with a Strat-M<sup>®</sup> membrane (mimicking the skin barrier), and placed in a 200 ml vessel. 50 ml of PBS buffer was used as an acceptor fluid, while the temperature was 32 ± 1°C (temperature at the surface of human skin). The speed of the mini-paddles was set at 100 rpm, ensuring sink conditions. Released insulin was analysed spectrophotometrically ( $\lambda = 271$  nm) using a CECIL UV-VIS spectrophotometer (CE 3021, Cambridge, UK), based on a calibration curve.

#### 2.2.4. Analysis of Release Profiles

INS release profiles were assessed using statistical methods recommended by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). The acceptance criteria were a difference coefficient  $f_1 < 15$  and a similarity coefficient  $f_2 > 50$  [9]:

$$f_1 = \left[ \frac{\sum |R_t - T_t|}{\sum R_t} \right] \cdot 100 \quad (1)$$

$$f_2 = 50 \cdot \log \left\{ \left[ 1 + \frac{1}{n} \sum (R_t - T_t)^2 \right]^{-0.5} \cdot 100 \right\} \quad (2)$$

where:

$R_t$  – mean reference dissolution value;

$T_t$  – mean test dissolution value;

$n$  – number of time points.

#### 2.2.5. Analysis of Release Kinetics

INS release kinetics were analysed using DDSolver 1.0 software (Microsoft Excel 2019 add-on) [9], using zero- and first-order, Higuchi, Korsmeyer-Peppas, Peppas-Sahlin, Hixson-Crowell, Hopfenberg, and Baker-Lonsdale mathematical models. Model fit was assessed using the coefficient of determination ( $R^2$ ), Akaike's criterion (AIC), and the model selection criterion (MSC). Theoretical details on this analysis were described by us elsewhere [10].

#### 2.2.6. Rheological Tests

Rheological parameters of INS-containing hydrogels were analysed using an RM 200 rotational rheometer (Lamy Rheology Instruments, France) with an MK-CP 2445 system (plate/plate, 24 mm, 0.45° angle). Measurements were performed at  $32 \pm 0.1^\circ\text{C}$  using a CP-1 PLUS heating system. The sample (approximately 1 g) was stabilised for 30 min, and then the dynamic viscosity dependence on shear rate ( $1.0 - 100.0 \text{ s}^{-1}$ ) was measured for 15 min. Analysis was performed with Rheomatic-P software using Casson, Bingham, and Herschel-Bulkley models. The fit was assessed by coefficient of determination ( $R^2$ ) analysis. The viscosity of the developed formulations was tested at three shear rates: 30, 50, and  $100 \text{ s}^{-1}$ . Lower shear rates reflect the behaviour of the hydrogel under resting conditions, while higher shear rates represent its behaviour during application to the skin.

#### 2.2.7. Texture Analysis

Texture characteristics of INS-containing hydrogels were assessed using a Texture Analyzer TX-700 (Lamy Rheology Instruments, France) with a hemispherical probe (8 mm). Two tests were performed: CRT (Compression Directe/Relaxation/Tension; measurement parameters: 5.0 mm distance, 0.05 N force to start, 20 s relaxation time, 0.5 mm/s down speed) and TPA (Tension/Penetrometry; measurement parameters: 5.0 mm distance, 0.05 N force to start, 0.5 mm/s down speed). The parameters analysed were: hardness 1 and 2, adhesiveness, cohesiveness, and elasticity. The tests were conducted at  $32 \pm 0.1^\circ\text{C}$ , and the results' analysis was performed in RheoTex (TX-700, version TX-UK01/2019). Theoretical details were described by us elsewhere [10].

#### 2.2.8. pH Measurement

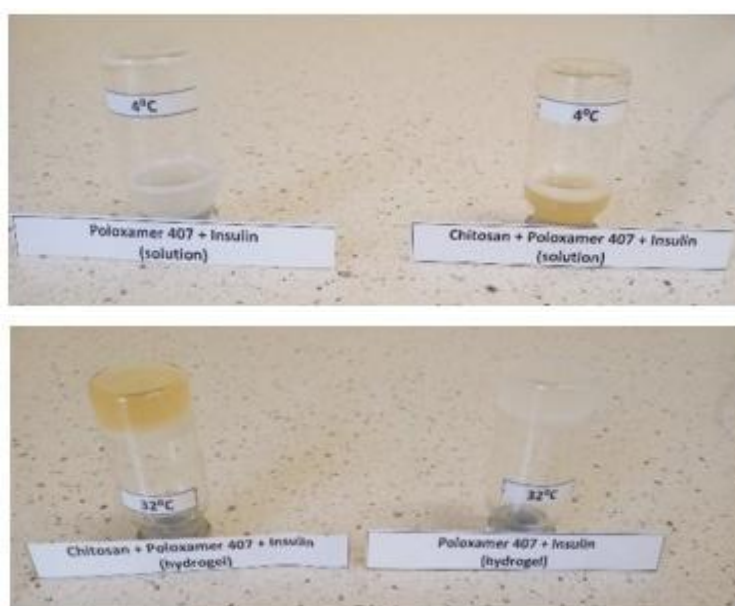
The pH of the hydrogels was determined potentiometrically using a SevenCompact™ S210 laboratory pH-meter equipped with an InLaB® Expert Pro-ISM electrode.

### 2.2.9. Statistical Analysis

The experimental data represent the mean value with standard deviation (SD). Differences were analysed using the two-sample Student's t-test ( $p < 0.05$ ). Calculations were performed using Statistica 13.1 software (StatSoft, Kraków, Poland).

## 3. Results and Discussion

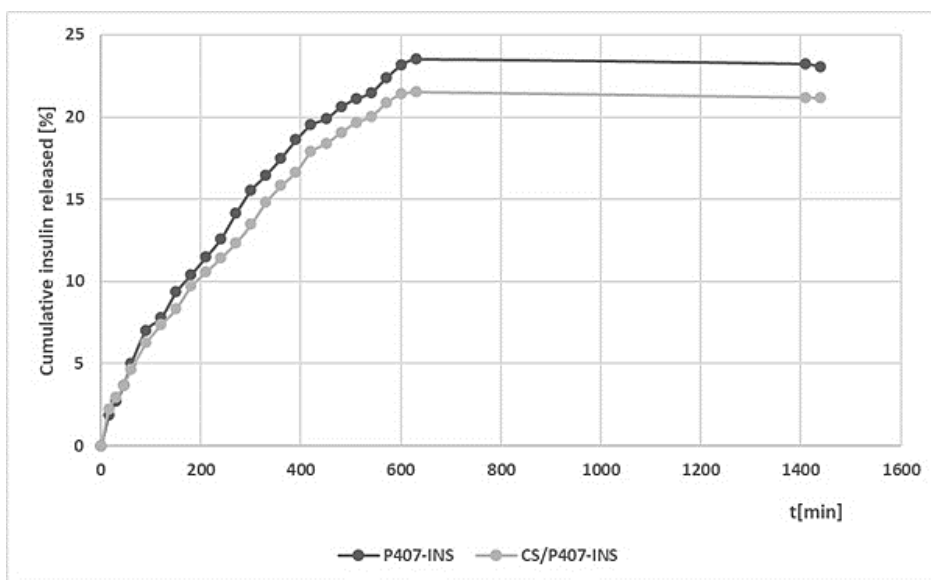
The obtained INS-containing formulation based on P407 (P407-INS) was colourless, while the formulation based on poloxamer 407 and chitosan (CS/P407-INS) was yellow. Both samples were characterised by slight turbidity and were homogeneous in the visual assessment. There was no stratification of the samples or precipitate formation at 4°C and 32°C (Figure 1). The P407-INS hydrogel showed a pH of 7.45, while the pH of the CS/P407-INS-based hydrogel was 5.35.



**Figure 1.** P407-INS and CS/P407-INS formulations at 4°C and 32°C.

Analysis of the insulin release profiles of the developed hydrogel carriers (Figure 2) showed that after 10.5 h, 23% of the initial insulin dose was released from the P407-based hydrogel, while 21% of the initial insulin dose was released from the CS/P407 carrier. A gradual, prolonged release of the hormone was observed. The release profiles of P407-INS and CS/P407-INS were similar ( $f_1$  factor  $< 15$ ;  $f_2$  factor  $> 50$ ; Table 1). According to the guideline, two dissolution profiles are considered similar if the  $f_1$  value falls within the range of 0 to 15 and the  $f_2$  value is between 50 and 100 [10].

The study used the Strat-M<sup>®</sup> membrane, an alternative to human skin in drug permeation studies. The results obtained with the usage of this membrane give similar data to studies on human skin [11, 12]. The advantage of Strat-M<sup>®</sup> is non-animal conditions testing.



**Figure 2.** Release profiles of insulin from P407-INS and CS/P407-INS hydrogels through Strat-M<sup>®</sup> membrane (mean  $\pm$  SD, n = 6).

**Table 1.** Analysis of the difference coefficient ( $f_1$ ) and the similarity coefficient ( $f_2$ ) between the release profiles of developed insulin hydrogels.

Compared hydrogels	$f_1$	$f_2$	Dissolution profile
P407-INS CS/P407-INS	8.57	88.94	Similar

The various mathematical models were applied to describe the INS release from P407-INS and CS/P407-INS hydrogels (Table 2). It was found that insulin is released from the P407-INS hydrogel according to the Peppas-Sahlin model ( $R^2 = 0.9967$ ,  $AIC = 38.3316$ ,  $MSC = 5.4075$ ), suggesting the involvement of two mechanisms. Initially, the release of INS occurs by diffusion and erosion, then is determined by swelling and relaxation of the polymer chain [13, 14]. In turn, the release of INS from the CS/P407-INS hydrogel matrix occurs according to the Korsmeyer-Peppas model ( $R^2 = 0.9972$ ,  $AIC = 29.5771$ ,  $MSC = 5.5838$ ). The  $n = 0.662$  value of the Korsmeyer-Peppas coefficient, within the range  $0.45 < n < 0.89$ , suggests that the polymer matrix erosion is a predominant release mechanism, with an associated Fick diffusion effect. Similar results have been reported by others [13–15].

Rheological analysis of pharmaceutical preparations allows their technology to be optimised, taking into account changes during storage, transport, and use [16]. The rheological properties of hydrogels play a key role in their therapeutic effect. Based on the flow curve (Figure 3) and the data in Table 3, it is shown that the hydrogels studied are non-Newtonian fluids, diluted by shear with a flow limit. An increase in shear rate leads to disintegration of the matrix, lowering the viscosity, facilitating application to the skin, improving API bioavailability, and spreading the formulation [17].

**Table 2.** Mathematical models describing the kinetics of insulin release from P407-INS and CS/P407-INS.

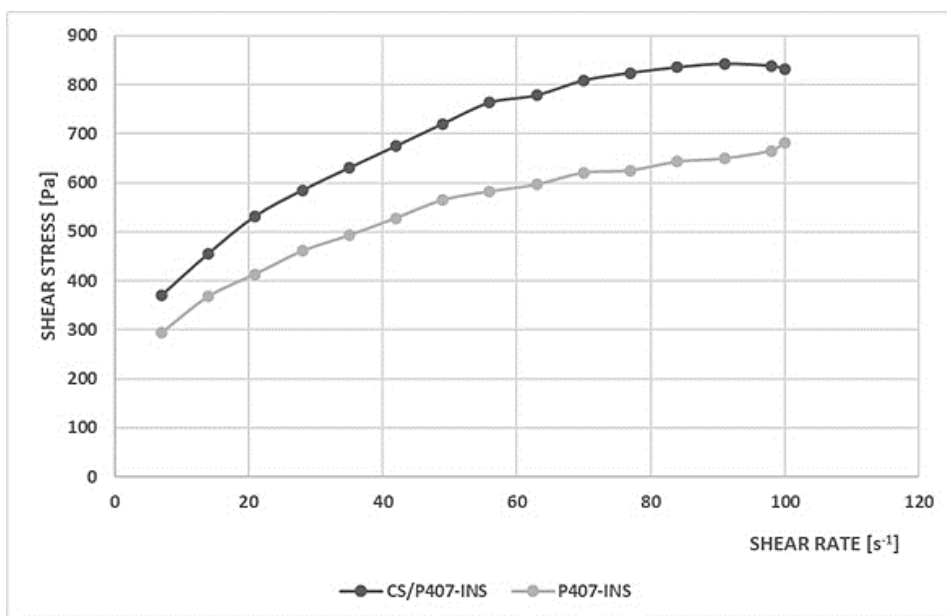
Kinetic model	Hydrogel	Model parameters	R <sup>2</sup> adjusted	AIC	MSC
zero order	P407-INS	k <sub>0</sub> = 0.043	0.9121	115.4781	2.1930
	CS/P407-INS	k <sub>0</sub> = 0.040	0.9187	109.1437	2.2685
first order	P407-INS	k <sub>1</sub> = 0.00045	0.9441	104.60	2.6462
	CS/P407-INS	k <sub>1</sub> = 0.00044	0.9462	99.2484	2.6808
Higuchi	P407-INS	k <sub>H</sub> = 0.901	0.9683	91.0273	3.2118
	CS/P407-INS	k <sub>H</sub> = 0.826	0.9683	86.5427	3.2102
Korsmeyer-Peppas	P407-INS	k <sub>KP</sub> = 0.356 n = 0.656	0.9955	44.8654	5.1352
	CS/P407-INS	k <sub>KP</sub> = 0.314 n = 0.662	0.9972	29.5771	5.5838
Peppas-Sahlin	P407-INS	k <sub>PS1</sub> = -1.838 k <sub>PS2</sub> = 1.270 m = 0.254	0.9967	38.3316	5.4075
	CS/P407-INS	k <sub>PS1</sub> = -0.145 k <sub>PS2</sub> = 0.382 m = 0.320	0.9970	31.4030	5.5077
Hixson-Crowell	P407-INS	k <sub>HC</sub> = 0.000	0.9345	108.41	2.4874
	CS/P407-INS	k <sub>HC</sub> = 0.000	0.9379	102.6843	2.5376
Hopfenberg	P407-INS	k <sub>HB</sub> = 0.000 n = 786.320	0.9415	106.6176	2.5622
	CS/P407-INS	k <sub>HB</sub> = 0.000 n = 498.129	0.9437	101.2696	2.5966
Baker-Lonsdale model	P407-INS	k <sub>BL</sub> = 0.0	0.9615	95.6351	3.0198
	CS/P407-INS	k <sub>BL</sub> = 0.0	0.9618	90.9872	3.0250

Note. R<sup>2</sup> adjusted, adjusted determination coefficient, AIC, Akaike Information Criterion; MSC, Model Selection Criteria.

**Table 3.** Apparent viscosity values at different shear rates.

Hydrogel	η (30 s <sup>-1</sup> ) [Pa·s]	η (50 s <sup>-1</sup> ) [Pa·s]	η (100 s <sup>-1</sup> ) [Pa·s]
P407-INS	10.9 ± 0.129	7.09 ± 0.126	3.96 ± 0.159
CS/P407-INS	17.4 ± 0.452	13.2 ± 0.442	7.89 ± 0.217

Note. Mean ± SD, n = 3, T = 32 ± 0.1°C



**Figure 3.** Flow curves of P407-INS and CS/P407-INS hydrogels at  $32 \pm 0.1^\circ\text{C}$  (mean  $\pm$  SD,  $n = 3$ )

Selected rheological models were applied to describe the rheograms, showing that the studied formulations best fit the Herschel-Bulkley model (highest values of the  $R^2$  determination coefficient). The  $n = 0.314 - 0.323$  flow behaviour index indicates that hydrogels belong to non-Newtonian pseudoplastic fluids ( $n < 1$ ). A lower  $n$  value of P407-INS hydrogel indicates a stronger shear-thinning effect and a more pronounced pseudoplasticity of this hydrogel compared to CS/P407-INS. The yield stress  $\tau_0$  determined from the Herschel-Bulkley model (Table 4) defines the minimum stress necessary to maintain hydrogel flow. A higher  $\tau_0$  value indicates a stronger formulation structure, while a lower value promotes better flowability [18].

**Table 4.** The results of the mathematical modelling of the rheograms.

Hydrogel	Herschel-Bulkley				Bingham		Casson	
	$\tau_0$	$n$	K	$R^2$	$\tau_0$	$R^2$	$\tau_0$	$R^2$
P407-INS	116.2	0.314	161.1	0.996	342.0	0.923	228.7	0.969
CS/P407-INS	121.1	0.323	199.4	0.988	437.7	0.891	287.6	0.950

*Note.*  $\tau_0$ , the yield stress [Pa]; K, the consistency index [ $\text{Pa}\cdot\text{s}^n$ ];  $n$ , the flow behavior index;  $R^2$ , determination coefficient.

The hardness of the hydrogel determines the ease of application. The higher the hardness value, the harder the sample. The low hardness values of both hydrogels, being within a range of 0.294 - 0.188 N (Table 5), indicate easy spreading of the formulation. Cohesiveness, indicating the ability to rebuild the structure, was higher for CS/P407-INS, suggesting a higher drug yield. Adhesiveness, which correlates with retention time at the wound site, was higher for P407-INS, providing its longer adhesion. Greater deformation resistance was demonstrated by CS/P407-INS hydrogel (94.8%). The obtained results are consistent with the literature [14].

**Table 5.** Mechanical parameters of P407-INS and CS/P407/INS hydrogels.

Hydrogel	Relaxation [%]	Hardness 1 [N]	Hardness 2 [N]
P407-INS	92.0	0.294	0.279
CS/P407-INS	94.8	0.188	0.181
<i>p</i>	< 0.05	< 0.05	< 0.05
Hydrogel	Cohesiveness	Adhesiveness [mJ]	Elasticity
P407-INS	0.955	1.0	0.949
CS/P407-INS	1.064	0.8	0.891
<i>p</i>	< 0.05	< 0.05	< 0.05

Note. Mean  $\pm$  SD, n = 3, T = 25  $\pm$  0.1°C

The developed P407-INS and CS/P407-INS hydrogels are characterised by appropriate rheological and textural properties, enabling convenient application, especially to wounds with irregular shapes and hard-to-reach areas [19]. An additional advantage of these formulations is the use of a ready-to-use human insulin preparation that contains metacresol and phenol with antimicrobial activity, which minimises the risk of microbial contamination. An aqueous environment is conducive to the growth of microorganisms that can enter the hydrogel matrix from the skin surface. In addition, the zinc chloride present in the formulation, which exhibits protease inhibitory activity [20], may contribute to insulin stability in the hydrogel matrix and at the wound site.

The study confirmed that 23% of the INS was released from the P407-based hydrogel, while 21% of the initial insulin dose was released from the CS/P407 carrier. The gelation mechanism of poloxamer is based on micelle packing and entanglement [21]. Gratieri *et al.* [22] found that the incorporation of chitosan into the polymer matrix of poloxamer probably increases its entanglement, affecting drug diffusion. Chitosan promotes the absorption of free water derived from the dehydration of the micelle core, enhancing the elastic behaviour of the formulation at 35°C. It also increases the viscosity and cohesiveness of the hybrid hydrogel. The more compact cross-linking of the matrix correlates with impaired API diffusion, leading to reduced hormone release.

The above-discussed results suggest the need for further research into optimising the hydrogel carrier of insulin to increase its bioavailability. The inclusion of sorption promoters in the formulation could be considered. The risk of enzymatic breakdown of insulin in the skin (peptidases are present in epithelial cells) is also an important issue to be aware of. This problem can be potentially eliminated by adding enzyme inhibitors (e.g., aprotinin, bestatin, soybean trypsin inhibitor) to the hydrogel matrix. Importantly, cholic acid salts also show the ability to inhibit aminopeptidase activity [23].

#### 4. Conclusions

The developed P407-INS and CS/P407-INS hydrogels provide a balance between rheological performance, texture, and ease of application. Prolonged release of the hormone eliminates the need for frequent hydrogel replacement.

Further research should include optimisation of the formulation composition, including the use of additional enzyme inhibitors and/or sorption promoters, which may increase the stability and bioavailability of the active substance. We also suggest conducting cytotoxicity and biocompatibility studies on the developed hydrogel

formulations to assess their comprehensive safety for use on the skin. Finally, preclinical and clinical studies are needed to confirm the efficacy of insulin-containing hydrogels in chronic wound management.

## 5. Acknowledgements

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## 6. References

- [1] Carreira LM, Silva R, Alves J, Inácio F, Pires G, Azevedo P; (2024) The Use of Fast-Acting Insulin Topical Solution on Skin to Promote Surgical Wound Healing in Cats. *Animals* 14(9), 1358. **DOI:** 10.3390/ani14091358
- [2] Fadi SIM, Abdelaziz SH, El-Sayed ZM, Khairy HM; (2025) Topical Insulin Versus Insulin Irrigation on Wound Healing among Patients with Venous Leg Ulcer. *Malays J Nurs* 16(3), 170–180. **DOI:** 10.31674/mjn.2025.v16i03.017
- [3] Przybyła M, Dolińska B, Ostróżka-Cieślík A; (2024) Progress of knowledge in the development of chitosan formulations with insulin to promote skin wound healing. *Prog Chem Appl Chitin Deriv* 29, 44–59. **DOI:** 10.15259/PCACD.29.004
- [4] Kędzierska M, Miłowska K; (2019) The use of chitosan-based biomaterials for the treatment of hard-healing wounds. *Postepy Hig Med Dosw* 73, 768–781. **DOI:** 10.5604/01.3001.0013.6823
- [5] Osmalek T, Froelich A, Jadach B, Ancukiewicz K, Gadziński P, Wagner D, Białas W; (2018) Badania reologiczne i analiza tekstury termowrażliwych hydrożeli dopochwowych z chlorowodorkiem benzydamininy. *Farm Współczesna* 11, 72–82.
- [6] Wang J, Huang L, Wu E, Li X, Rao Y, Zhu C; (2025) Recent Advances on Chitosan-Based Thermosensitive Hydrogels for Skin Wound Treatment. *Biology* 14(6), 619. **DOI:** 10.3390/biology14060619
- [7] Zhao Y, Liu X, Peng X, Zheng Y, Cheng Z, Sun S, Ding Q, Liu W, Ding C; (2022) A poloxamer/hyaluronic acid/chitosan-based thermosensitive hydrogel that releases dihydromyricetin to promote wound healing. *Int J Biol Macromol* 216, 475–486. **DOI:** 10.1016/j.ijbiomac.2022.06.210
- [8] Liu X, Ding Q, Liu W, Zhang S, Wang N, Chai G, Wang Y, Sun S, Zheng R, Zhao Y, Ding C; (2024) A Poloxamer 407/chitosan-based thermosensitive hydrogel dressing for diabetic wound healing via oxygen production and dihydromyricetin release. *Int J Biol Macromol* 263(Pt 1), 130256. **DOI:** 10.1016/j.ijbiomac.2024.130256
- [9] Zhang Y, Huo M, Zhou J, Zou A, Li W, Yao C, Xie S; (2010) DDSolver: An add-in program for modeling and comparison of drug dissolution profiles. *AAPS J* 12(3), 263–271. **DOI:** 10.1208/s12248-010-9185-1
- [10] Ostróżka-Cieślík A, Strasser C, Dolińska B; (2024) Insulin-loaded chitosan–cellulose-derivative hydrogels: *in vitro* permeation of hormone through Strat-M<sup>®</sup> membrane and rheological and textural analysis. *Polymers* 16(18), 2619. **DOI:** 10.3390/polym16182619
- [11] Sohn JS, Choi JS; (2024) Development of a tadalafil transdermal formulation and evaluation of its ability to *in vitro* transdermal permeate using Strat-M<sup>®</sup> membrane. *Eur J Pharm Sci* 192, 106615. **DOI:** 10.1016/j.ejps.2023.106615

- [12] Ostróżka-Cieślak A, Wilczyński S, Dolińska B; (2023) Hydrogel formulations for topical insulin application: preparation, characterization and *in vitro* permeation across the Strat-M<sup>®</sup> membrane. *Polymers* 15(17), 3639. **DOI:** 10.3390/polym15173639
- [13] Medeiros TS, Bezerra de Lima LE, Alves-Pereira EL, Alves-Silva MF, Dourado D, Fernandes-Pedrosa MF, Figueiredo RCBQ, da Silva-Junior AA; (2025) Cationic and anionic PLGA-cholesterol hybrid nanoparticles as promising platforms to enhance the trypanocidal efficacy of benznidazole and drug delivery in *Trypanosoma cruzi*-infected cells. *Biomed Pharmacother* 183, 117782. **DOI:** 10.1016/j.biopha.2024.117782
- [14] Inal O, Yapar EA; (2013) Effect of mechanical properties on the release of meloxicam from poloxamer gel bases. *Indian J Pharm Sci* 75(6), 700–706. **DOI:** 10.4103/0250-474X.124770
- [15] Rawat PS, Ravi PR, Mir SI, Khan MS, Kathuria H, Katnapally P, Bhatnagar U; (2023) Design, characterization and pharmacokinetic–pharmacodynamic evaluation of poloxamer and kappa-carrageenan-based dual-responsive *in situ* gel of nebigolol for treatment of open-angle glaucoma. *Pharmaceutics* 15(2), 405. **DOI:** 10.3390/pharmaceutics15020405
- [16] Malkin AY, Isayev A; (2022) *Rheology: Concepts, Methods, and Applications*. 4<sup>th</sup> ed. ChemTec Publishing, Toronto. ISBN 9781927885932.
- [17] Parfenyuk EV, Dolinina ES, Kraev AS; (2024) Synthesis and study of organo-modified silica based hydrogels: rheological properties and drug release kinetics. *J Biomed Mater Res B Appl Biomater* 112(6), e35418. **DOI:** 10.1002/jbm.b.35418
- [18] Ortan A, Dinu-Parvu C, Ghica MV, Popescu LM, Ionita L; (2011) Rheological study of a liposomal hydrogel based on Carbopol. *Rom Biotechnol Lett* 16(Suppl.), 47–54.
- [19] Markovic MD, Spasojevic PM, Pantic OJ, Savic SI, Savkovic MMS, Panic VV; (2024) Status and future scope of hydrogels in wound healing. *J Drug Deliv Sci Technol* 98, 105903. **DOI:** 10.1016/j.jddst.2024.105903
- [20] Maret W; (2013) Inhibitory zinc sites in enzymes. *Biometals* 26(2), 197–204. **DOI:** 10.1007/s10534-013-9613-7
- [21] Cabana A, Ait-Kadi A, Juhasz J; (1997) Study of the gelation process of polyethylene oxide–polypropylene oxide–polyethylene oxide copolymer (Poloxamer 407) aqueous solutions. *J Colloid Interface Sci* 190(2), 307–312. **DOI:** 10.1006/jcis.1997.4880
- [22] Gratieri T, Gelfuso GM, Rocha EM, Sarmiento VH, de Freitas O, Lopez RF; (2010) A poloxamer/chitosan *in situ* forming gel with prolonged retention time for ocular delivery. *Eur J Pharm Biopharm* 75(2), 186–193. **DOI:** 10.1016/j.ejpb.2010.02.011
- [23] Janicki S, Sznitowska M; (1994) Technologiczne i biofarmaceutyczne aspekty przezskórnych systemów terapeutycznych. *Farm Pol* 50(8), 317–334.