REPUBLIQUE ALGERIENNE DEMOCRATIQUE ET POPULAIRE

Ministère de l'Enseignement Supérieur et de la Recherche Scientifique

Université Yahia Farès de Médéa

Faculté de Technologie

Département de Génie des procédés et Environnement

Thèse de Doctorat LMD

En Génie des Procédés

Option : Génie Pharmaceutiques

Présentée par :

GOUDJIL Hadjer

Thème

Utilisation des hydrogels dans les systèmes de délivrance des médicaments : conception de réseau et modélisation basée sur les techniques d'intelligence artificielle.

Devant le jury composé de :

LEFNAOUI Sonia	Prof.	Université de Médéa	Présidente
KERCHICH Yacine	Prof.	ENP. Alger	Examinateur
YAHOUM Madiha Melha	MCA	Université de Médéa	Examinatrice
REBOUH Samia	Prof.	Université de Médéa	Directrice de thèse
BOUHEDDA Mounir	Prof.	Université de Médéa	Co-Directeur de thèse

Année Universitaire

2024 / 2025

PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA

Ministry of Higher Education and Scientific Research

Yahia Fares University of Medea

Faculty of Technology

Department of Process Engineering and Environment



LMD Doctoral Thesis

In Process Engineering

Option : Pharmaceutical Engineering

Presented by:

GOUDJIL Hadjer

Theme

Use of hydrogels in drug delivery systems: network design and modeling based on artificial intelligence techniques.

The jury composed of:

LEFNAOUI Sonia	Prof	University of Medea	President
KERCHICH Yacine	Prof	ENP. Algiers	Reviewer
YAHOUM Madiha Melha	MCA	University of Médéa	Reviewer
REBOUH Samia	Prof	University of Médéa	Supervisor
BOUHEDDA Mounir	Prof	University of Médéa	Co-Supervisor

Academic Year
2024 / 2025

Acknowledgments

The manuscript of this thesis marks the end of my years of doctoral training in Process Engineering at the University of Médéa. More than five years have passed very quickly but they represent a very enriching experience on a scientific level.

I would first like to thank **ALLAH** the Omnipotent for the great energy he has given me all the time… **Alhamdulillah**!

I would like to extend my deepest and most heartfelt gratitude to **my beloved parents**. Your belief in me and your ability to lift me up during difficult moments have been invaluable. I could not have accomplished this without your constant presence, your sacrifices, love, and your sincere prayers. **Mama** and **Abi**, I am profoundly thankful your presence in my life.

I would then like to warmly thank my thesis directors, **Prof. REBOUH Samia** and **Prof. BOUHEDDA Mounir**, Professors at the University of Médéa, for their motivation, their enthusiasm, their immense scientific support and their valuable advice which have helped me enormously throughout the preparation and writing of this thesis. I am profoundly grateful to you and proud to have worked alongside you. I express my gratitude to you for everything you have done for me.

I extend my sincere thanks to **Prof. LEFNAOUI Sonia**, Professor at the University of Médéa, for having done me the honor of presiding my thesis jury, your acceptance to lead the jury has been a privilege for me, and your presence added immense value to this work.

I would also like to thank the honorable members of the jury, **Prof. KERCHICH Yacine**, Professor at the National Polytechnic School of Algiers, and **Dr. YAHOUM Madiha Melha**, MCA at the University of Médéa, for having agreed to devote their precious time to the evaluation of this manuscript and for providing their judicious remarks on the research work presented therein. I deeply appreciate the time you devoted to reviewing my work.

I would like to express my sincere thanks to **Prof. BOUAROURI Djaafar**, the Rector of the University of Medea. Your support demonstrates your kindness and commitment to supporting students at key moments in their academic journey.

I cannot forget to thank **Prof. BOUAZIZ Mohamed Najib**, Director of the Laboratory of Biomaterials and Transport Phenomena (LBMPT) at the University of Médéa, for his invaluable support throughout this research. I also thank the staff of (LBMPT) for their availability and kindness.

A big thank you to **Mr. BACHARI Khaldoun**, Director of the Scientific and Technical Research Center in Physico-Chemical Analysis (CRAPC) in Bou-Ismail wilaya of Tipaza, for having opened the doors of the Laboratories to me. I am very grateful for your help and for all the resources you have made available to me.

I will not forget to thank **Mr. HAMITOUCHE adhya-eddine**, Head of Department at CRAPC and **Mr. LAICHI Yacine**, Analyst at the NMR laboratory at CRAPC, who kindly answered all my questions and helped me to carry out the characterization techniques as part of this study.

I would like to express my deepest gratitude to my sister and close friend **Dr. Sara HAMIOUDA**, MCB teacher and Head of GPE department at the University of Tipaza. You have been by my side in every step of this achievement, offering not only practical help but also emotional strength when I needed it the most. Thank you for being not only a wonderful sister but also a true friend. Your love and support have made all the difference.

I would like to express my heartfelt thanks to my dear son, **Yacine**, for being my source of joy and strength throughout this journey. Your smiles, love, and patience have given me the energy to keep moving forward, even during the most difficult moments. You are my greatest motivation, and everything I do is with you in mind.

I am also deeply grateful to my brothers, **Billel** and **Abdeldjalil**, for their unwavering support, encouragement, and belief in me. You have always been there for me, and I cannot thank you enough for your love and understanding.

I would like to extend my deepest gratitude to my aunt, **Noria**, for being by my side and for her unwavering support. Your presence has been a great source of comfort and strength for me. I am incredibly thankful for all the ways you've supported me. Thank you for always being there for me.

To my **dearest grandparents**, Thank you for your support, encouragement, and your sincere prayers for me. In addition, thank you to my aunt **Leila** and all my family members who truly love me.

I would like to express my sincere gratitude to **Dr. YOUCEF TOUMI Karima**, my life coach, for her guidance, wisdom, and continuous support throughout this journey. Your insights and encouragement have helped me stay focused and motivated, and your belief in my abilities has been a source of great strength. A big Thank you for her daughter **BENZAID Imen** for helping me navigate both the challenges of my academic work and the personal growth that has accompanied this journey. Your mentorship has been invaluable.

I thank from the bottom of my heart my work colleagues, **Mr. BENBRIDA Abdelghani**, **Mr. HENTABLI Mohamed**, **Mr. BASSOUR Ahmed**, **Mr. ZOUAMBIA Fouad**, **Mr. CHERIF Tayeb**, **Mr. ABDELLI Nouar**, **Mr. LAIDI Karim**, **Mr. DJELLALI Abdelkrim**; they have always done everything possible to help me. My thanks also go to my dear friends **BOUKARA Karima**, **MEBARKI Soraya**, **BOUAZIZ Ilhem, BOUKHARI Lynda** and **IRES Nessrine** for their help and sincere prayers for me.

Finally, I would like to express my deep gratitude to all the people who have, directly or indirectly, contributed to the success of this work.

استخدام الهيدروجيل في أنظمة توصيل الدواء: تصميم الشبكات والنمذجة باستخدام تقنيات الذكاء الاصطناعي

ملخص

تركز هذه الدراسة على تطوير تركيبة إنسولين ممتدة الإطلاق عن طريق الاستنشاق وتطوير نموذج عصبي غامض للتنبؤ بحركية إطلاق الأنسولين من الهلاميات المائية المترابطة بالكاربومير والأجار. تم تصنيع الهلاميات المائية عن طريق خلط الكاربومير والأجار، ودمج الجلسرين والبروبيلين جليكول، ثم الربط المتبادل عن طريق التكثيف المتعدد. تم تحديد بنية وشكل الهلام المائي باستخدام مطيافية الرنين النووي المغناطيسي للبروتون (H^1-NMR)، ومطيافية الأشعة تحت الحمراء بتحويل فوري (FTIR) ، والمجهر الإلكتروني الماسح (SEM). أجريت دراسات إطلاق الدواء في المختبر باستخدام كروماتوغرافيا السائل عالية الأداء(HPLC) ، وتم تحليل ملف إطلاق الأنسولين باستخدام نموذج كورسماير-بيباس، لفهم آلية توصيل الدواء بشكل أفضل. تقدم الدراسة نموذجًا لنظام الاستدلال العصبي الضبابي التكيفي (ANFIS) ، والذي تم تدريبه على البيانات التجريبية، للتنبؤ بمعدلات إطلاق الأنسولين من الهلاميات المائية وتحسينها. شمل النموذج متغيرات مثل تركيزات الرابط المتقاطع ووقت التكثيف المتعدد كمدخلات و معدل الإطلاق كمخرجات. أظهر نموذج ANFIS الخاص بنا ارتباطًا قويًا بين النتائج المتوقعة والتجريبية، محققًا مستوى استثنائيًا من الدقة بقيمة R^2تبلغ 0.9999 و $RMSE = 2.159\times10^{-2}$. وهذا يؤكد دقته وموثوقيته في التنبؤ بسلوك إطلاق الأنسولين من الهلاميات المائية. يقدم هذا البحث نهجًا جديدًا لتوصيل الأنسولين عن طريق الاستنشاق المتحكم فيه، مع تأثير تحويلي محتمل على علاج مرض السكري من خلال تحسين الراحة والامتصاص والتزام المريض مقارنة بالطرق التقليدية.

كلمات البحث: النمذجة العصبية الضبابية، الهلاميات المائية؛ توصيل الأنسولين، التكثيف المتعدد، حركية إطلاق الدواء.

Utilisation des hydrogels dans les systèmes de délivrance des médicaments : conception de réseau et modélisation basée sur les techniques d'intelligence artificielle.

RÉSUMÉ

Cette étude porte sur le développement d'une formulation d'insuline à libération prolongée inhalée et sur le développement d'un modèle neuro-floue pour prédire la cinétique de libération d'insuline à partir d'hydrogels réticulés carbomère-agar. Les hydrogels ont été fabriqués en mélangeant du carbomère et de l'agar, en incorporant du glycérol et du propylène glycol, puis en réticulant par polycondensation. La structure et la morphologie de l'hydrogel ont été caractérisées à l'aide de la spectroscopie par résonance magnétique nucléaire du proton (^{1}H-RMN), de la spectroscopie infrarouge à transformée de Fourier (FTIR) et de la microscopie électronique à balayage (SEM). Des études de libération de médicament in vitro ont été réalisées à l'aide de la chromatographie liquide haute performance (HPLC) et le profil de libération d'insuline a été analysé à l'aide du modèle Korsmeier-Pepas, afin de mieux comprendre le mécanisme d'administration du médicament. L'étude présente un modèle de système d'inférence neuro-floue adaptatif (ANFIS), qui a été formé sur des données expérimentales, pour prédire et optimiser les taux de libération d'insuline à partir d'hydrogels. Le modèle comprenait des variables telles que les concentrations des réticulants et le temps de polycondensation comme entrées et le taux de libération comme sortie. Notre modèle ANFIS a démontré une forte corrélation entre les résultats prédits et expérimentaux, atteignant un niveau de précision exceptionnel avec une valeur R^2 de 0,9999 et un RMSE de $2{,}159.10^{-2}$. Cela confirme sa précision et sa fiabilité dans la prédiction du comportement de libération d'insuline à partir d'hydrogels. Cette recherche présente une nouvelle approche pour l'administration contrôlée d'insuline par inhalation, avec un impact transformateur potentiel sur le traitement du diabète en améliorant la commodité, l'absorption et l'adhésion du patient par rapport aux méthodes conventionnelles.

Mots clés : Modélisation neuro-floue ; Hydrogels ; Administration d'insuline ; Polycondensation ; Cinétique de libération de médicament.

Use of hydrogels in drug delivery systems : network design and modeling based on artificial intelligence techniques.

ABSTRACT

This study focuses on the development of an inhaled extended-release insulin formulation and the development of a neurofuzzy model to predict the insulin release kinetics from carbomer-agar cross-linked hydrogels. The hydrogels were fabricated by mixing carbomer and agar, incorporating glycerol and propylene glycol, and then cross-linking by polycondensation. The structure and morphology of the hydrogel were characterized using proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy, Fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM). In vitro drug release studies were performed using high-performance liquid chromatography (HPLC), and the insulin release profile was analyzed using the Korsmeier-Pepas model, to better understand the drug delivery mechanism. The study presents an adaptive neurofuzzy inference system (ANFIS) model, which was trained on experimental data, to predict and optimize insulin release rates from hydrogels. The model included variables such as cross-linker concentrations, polycondensation time as inputs, and release rate as outputs. Our ANFIS model demonstrated a strong correlation between predicted and experimental results, achieving an exceptional level of accuracy with an R^2 value of 0.9999 and an RMSE of $2.159.10^{-2}$. This confirms its accuracy and reliability in predicting insulin release behavior from hydrogels. This research presents a new approach for controlled inhalation insulin delivery, with a potential transformative impact on diabetes treatment by improving convenience, absorption, and patient adherence compared to conventional methods.

Keywords : Neuro-fuzzy modeling ; Hydrogels ; Insulin delivery ; Polycondensation ; Drug release kinetics.

List of publication

The research work included in this thesis has led to the following publications:

International Research Article A category:

H. Goudjil, S. Rebouh, and M. Bouhedda, "*Neuro-fuzzy model for predicting insulin delivery from crosslinked agar-carbomer hydrogels,*" Computer Methods in Biomechanic and Biomedical Engineering, vol. 0, no. 0, pp. 1–16, 2024.

URL : *https://www.tandfonline.com/doi/full/10.1080/10255842.2024.2362863*

Chapter book:

H. Goudjil, S. Rebouh, and M. Bouhedda, "*Adaptative Neuro-Fuzzy Inference System for Predicting the Microbial Fungicide Release from Microcapsules Based on Alginate and Different Gelatin Proportions*, " vol. 174. Springer International Publishing, 2021.

URL : *https://link.springer.com/chapter/10.1007/978-3-030-63846-7_58*

International Conferences

H. Goudjil, S. Rebouh, and M. Bouhedda, "*Adaptative Neuro-Fuzzy Inference System for Predicting the Microbial Fungicide Release from Microcapsules Based on Alginate and Different Gelatin Proportions*" in 4th International Conference on Artificial Intelligence and Renewable Energetic Systems, IC-AIRES 2020, 22-24 November 2020, Tipasa, Algeria.

H. Goudjil, S. Rebouh, and M. Bouhedda, "*Evaluation and optimization of crosslinked hydrogels by polycondensation using design of experiment*" in Polymer and Mediterranean Fiber International Conference, PMFIC 2021, 29-30 September 2021, Bejaia, Algeria.

National Conferences

H. Goudjil, S. Rebouh, and M. Bouhedda, "*Optimisation de la réticulation par polycondensation d'un hydrogel en utilisant les plans d'expérience*" in Scientific Day on Chemistry, Materials and Environment, SDCME 2022, 6 December 2022, Tipaza, Algeria.

H. Goudjil, S. Rebouh, and M. Bouhedda, "Prédiction de la Libération des Cellules Bactériennes à partir de Microcapsules à base de Protéines et de Polysaccharides à l'aide d'un Système d'Inférence Neuro-Floue Adaptatif " in 1er Séminaire National des Sciences du Génie des Procédés : Applications et Innovations, SSGP'22, 14-15 December 2022, USTHB, Algeria.

Table of Contents

Abstract
List of Tables
List of Figures
Abbreviations and Symbols
General Introduction 1

Chapter I : Literature Review

I.1. Introduction 4
I.2. Hydrogels in Drug Delivery Systems 4
I.2.1. Definition and Properties of Hydrogels 4
I.2.2. Types of Hydrogels 5
I.2.3. Applications of Hydrogels in Drug Delivery 6
I.2.4. Crosslinking Techniques 7
I.2.4.1. Chemical Crosslinking Techniques 7
I.2.4.2. Physical Crosslinking Techniques 8
I.2.5. Drug Delivery Systems 9
I.2.5.1. Drug Delivery Systems and Mechanisms 10
I.2.5.2. Advanced Drug Delivery Systems 10
I.2.6. Hydrogel Synthesis 11
I.2.6.1. Natural Hydrogel Synthesis 11
I.2.6.2. Synthetic Hydrogel Synthesis 12
I.2.7. Characterization Techniques 12
I.2.7.1. Mechanical Characterization 13
I.2.7.2. Morphological Characterization 13
I.2.8. Factors Affecting Drug Release from Hydrogels 13
I.2.8.1. Hydrogel Composition 14
I.2.8.2. Crosslinking Density 14
I.2.9. Recent Advances in Hydrogel-Based Drug Delivery 15
I.2.9.1. Smart Hydrogels 15
I.2.9.2. Injectable Hydrogels 16
I.2.9.3. Nasal Hydrogels 16
I.2.9.4. Hydrogel Nanocomposites 18
I.2.10. Advantages and Limitations in Drug Delivery 19
I.3. Traditional Network Design and Modeling of Hydrogels 20
I.3.1. Conventional methods for hydrogel design and modeling 20
I.3.2. Modeling Approaches 21
I.3.3. Limitations of traditional approaches 21
I.4. Artificial Intelligence in Drug Delivery Systems 22

I.4.1. Overview of AI techniques 22
I.4.1.1. Expert Systems 22
I.4.1.2. Neural Networks and Fuzzy Logic 23
I.4.2. Applications of AI in biomedical modeling. 25
I.4.3. Differeces between the AI models and traditional methods 26
I.5. Conclusion 27

Chapter II : Materials and methods
II.1. Introduction 29
II.2. Hydrogel Design and Synthesis 29
II.2.1. Selection of materials 29
II.2.1.1. Chemical reagents and Raw materials 29
II.2.1.2. Equipment 32
II.2.2. Synthesis and characterization methods for hydrogels 32
II.2.2.1. Hydrogel synthesis and drug loading 32
II.2.3. Physico-chemical characterizations 34
II.2.3.1. Fourier-transform infrared spectroscopy (FTIR) 34
II.2.3.2. Scanning Electron Microscopy (SEM) 35
II.2.3.3. Proton nuclear magnetic resonance spectroscopy (NMR) 35
II.3. AI-Based Modeling Techniques 36
II.3.1. Description of AI techniques used 36
II.3.1.1. Neuro-fuzzy modeling for drug release kinetics 36
II.3.1.2. ANFIS architecture 37
II.3.2. Justification for the chosen techniques 39
II.3.3. ANFIS modeling setup 40
II.3.4. Optimizing parameters for ANFIS 42
II.4. Experimental Protocols 44
II.4.1. Experimental design for model validation 44
II.4.1.1. kinetic study 44
II.4.1.2. Microbiological quality control 46
II.4.2. Data Analysis and Korsmeyer-Peppas kinetic Modeling 47
II.5. Conclusion 47

Chapter III: Results and Discussion
III.1. Introduction 49
III.2. Hydrogel Network Design 49
III.2.1. Key design parameters 49
III.2.2. Influence of different parameters on hydrogel properties 50
III.2.2.1. Effect of the propylene glycol concentration 50
III.2.2.2. Effect of the glycerol concentration on the insulin release kinetic 50
III.2.2.3. Effect of the PG/Gly ratio 51
III.2.2.4. Effect of the polycondensation time 52
III.3. Hydrogel characterizations technics 54

III.3.1. FTIR hydrogel characterization 54
III.3.2. SEM analysis 55
III.3.3. NMR analysis 57
III.3.4. Microbiological quality of hydrogel 59
III.4. Development and Validation of AI Models 62
III.4.1. ANFIS prediction of the formulation parameters effect 62
III.4.2. Kinetic release profile and modeling 63
III.4.3. ANFIS modeling results 63
III.4.4. ANFIS predicted insulin release amount as a function of the experimental data 64
III.4.5. Korsmeyer-Peppas modeling results 65
III.5. Comparative Analysis 66
III.5.1. Performance evaluation of the models in another application 66
III.5.2. Comparison with traditional methods 68
III.6. Case Studies on Hydrogel Applications in Insulin Delivery 72
III.6.1. Case 1 : Nanoparticles as an Insulin Delivery System 72
III.6.2. Case 2 : Microparticles (Microspheres and Beads) 73
III.6.3. Case 3 : Hydrogel and Microgel 75
III.6.4. Case 4 : Mucoadhesive Films with PEG-b-PLA/Insulin Nanoparticles 76
III.6.5. Our Case: Agar-Carbomer Hydrogels 76
III.7. Conclusion 77
General Conclusion 80
References 83

List of tables

Chapter II : Materials and methods

Table II.1	List of chemical reagents and solvents.	31
Table II.2	Different equipments used.	33
Table II.3	Composition of insulin-loaded hydrogel formulations.	34
Table II.4	Optimal ANFIS performance achieved during the training phase.	44
Table II.5	Acceptance criteria for the microbiological quality of pharmaceutical forms.	46

Chapter III : Results and Discussion

Table III.1	Acceptable Limit of total aerobic microorganisms count (TAMC), the total mould and yeast count (TYMC), *Staphylococcus Aureus* and *Pseudomonas Aeruginosa*.	60
Table III.2	Korsmeyer-Peppas modeling results.	66
Table III.3	Experimental matrix of Box-Behnken design and response	69
Table III.4	Comparison of the different hydrogel-based insulin delivery systems	77

List of figures

Chapter I : Literature Review

Figure I.1	Various external stimuli, including pH, temperature, electricity, magnetics, light, and biomolecules (including glucose and enzyme), are controlling the drug release from a smart hydrogel.	5
Figure I.2	Schematic representation of classification of hydrogels based on different parameters.	6
Figure I.3	Methods of cross-linking hydrogels.	8
Figure I.4	Schematic illustration of hydrogel fabrication. (a) Physical crosslinking. (b) Chemical Crosslinking.	9
Figure I.5	Nanocomposite hydrogels composed of various polymer matrices filled with different nano-species.	18
Figure I.6	AI into protein product development and customized biologics.	23
Figure I.7	Artificial Neural Network.	24
Figure I.8.	Effect of the formulation composition on the 10%, 50%, and 90% drug release	26

Chapter II : Materials and methods

Figure II.1	Fuzzy inference system.	36
Figure II.2	First-order Sugeno model.	37
Figure II.3	ANFIS architecture.	37
Figure II.4	Studied ANFIS architecture.	40
Figure II.5	Evolution of training and check errors during the learning phase.	42
Figure II.6	*In vitro* study illustration.	45

Chapter III : Results and Discussion

Figure III.1	Effect of the propylene glycol concentration on the insulin release kinetic (experimental and predicted results)	50
Figure III.2	Effect of the glycerol concentration on the insulin release kinetic (experimental and predicted results).	51
Figure III.3	Effect of the proportion of cross-linking agents on the insulin release kinetic (experimental and predicted results).	52
Figure III.4	Effect of the polycondensation time on the insulin release kinetic (experimental and predicted results).	53
Figure III.5	FTIR spectra of Agar (Ag), Carbomer (Ca) and the Ag-Ca-based hydrogel (Hg).	54
Figure III.6	SEM results: (a) 1r/Carbomer hydrogel exposed to polycondensation assisted by microwave; (b) crosslinked Agar/Carbomer hydrogel without microwave exposure; (c) hydrogel with a single crosslinking agent; (d) hydrogel without crosslinking agents; (e) Agar; (f) Carbomer.	56
Figure III.7	NMR spectra: (a) Carbomer; (b) Agar; (c) propylen glycol; (d) glycerol; (e) hydrogel F10.	58
Figure III.8	Chemical structures pattern of the crosslinking reaction.	59
Figure III.9	Total aerobic germ count (TAMC) in the hydrogel H10 on TSA medium.	60
Figure III.10	The enumeration of these microorganisms (TYMC) in the hydrogel H10 on SAB medium.	61
Figure III.11	Macroscopic appearance of the absence of *Staphylococcus Aureus* colonies on mannitol-salt medium (CHAP).	61
Figure III.12	Macroscopic appearance of the absence of Pseudomonas Aeruginosa colonies on cetrimide agar base (CAB) medium.	62
Figure III.13	Insulin release kinetics profiles from the different hydrogel formulas (experimental and predicted results) in PBS 7.4 at 37°C.	64
Figure III.14	Point cloud representing the ANFIS predicted release amount as a function of the experimental data.	65

Figure III.15 Experimental and predicted microcrocapsules biodegradation rate contaning a various concentrations of Gelatin (GE). 67

Figure III.16 Experimental and predicted BaciSub SL-13 viability rate during storage (effect of the Gelatin (GE) concentration on the microcapsules) 67

Figure III.17 Experimental and predicted BaciSub SL-13 cells release from microcapsules containing various concentrations of Gelatin (GE). 68

Figure III.18 Actual by predicted plot of viscosity. 70

Figure III.19 Flow curves of the optimal hydrogel. 71

Abbreviations and Symbols

Abbreviation	Definition
3D	Three-dimensional
A1, A2, A3	Fuzzy sets in the antecedent
AI	Artificial Intelligence
ANFIS	Adaptive Neuro-Fuzzy Inference System
AT7	Automated Testing 7 (Sotax dissolution tester)
BaciSub SL-13	Bacillus subtilis SL-13
BBB	Blood-brain barrier
BBD	Box-Behnken design
BBO	Broadband Observe
BSA	Bovine Serum Albumin
Ca	Carbomer
CD	Cellulose Derivative
CFU	Colony Forming Unit
CGM	Continuous Glucose Monitoring
CMC	Carboxymethyl Cellulose
CRAPC	Research Center for Analytical Chemistry and Physics (Algeria)
D2O	Deuterium Oxide
DDS	Drug Delivery Systems
DMAc	Dimethylacetamide
DOE	Design of Experiments
DSC	Differential Scanning Calorimetry
EDTA	Ethylenediaminetetraacetic Acid
FDA	Food and Drug Administration
F	Formulation
FTIR	Fourier-Transform Infrared Spectroscopy
GI	Gastrointestinal
Gly	Glycerol
H^1-NMR	Proton Nuclear Magnetic Resonance
HEC	Hydroxyethyl Cellulose
HPC	Hydroxypropyl Cellulose
HPMC	Hydroxypropyl Methylcellulose
HPLC	High-Performance Liquid Chromatography

Hg	Hydrogel
IB	Ibuprofen
IU	International Unit
LCST	Lower Critical Solution Temperature
LSE	Least Squares Estimation
MDT	Mean Dissolution Time
MF	Membership Function
ML	Machine Learning
N	Number of tested data
NMR	Nuclear Magnetic Resonance
OPUS	Optical Property Spectroscopy
PBS	Phosphate-Buffered Saline
PEG	Polyethylene Glycol
PEG-b-PLA	Poly(ethylene glycol)-block-Poly(lactic acid)
PG	Propylene Glycol
PLA	Polylactic Acid
PNIPAAm	Poly(N-isopropylacrylamide)
pNIPAM	Poly(N-isopropylacrylamide)
pH	Potential of Hydrogen (acid-base balance)
P. aeruginosa	Pseudomonas aeruginosa
PVA	Polyvinyl Alcohol
PVP	Polyvinylpyrrolidone
R^2	Coefficient of Determination
RMSE	Root Mean Square Error
S. aureus	Staphylococcus aureus
S1, S2	Premise and Consequent parameters
SEM	Scanning Electron Microscopy
TAMC	Total Aerobic Microorganisms Count
TEA	Triethylamine
TEM	Transmission Electron Microscopy
TSK	Takagi-Sugeno-Kang (fuzzy model)
TYMC	Total Yeast and Mold Count
UV	Ultraviolet

General Introduction

General Introduction

Hydrogels have revolutionized the field of technology, particularly in healthcare, material science, the food industry, and pharmaceuticals, over the past few decades. This thesis focuses on the applications of hydrogels in sustained-release drug delivery systems, emphasizing their potential to transform the management of chronic diseases such as diabetes [1]. Hydrogels are three-dimensionally hydrated networks composed of water-soluble macromolecules. The term "hydrogel" is derived from the Greek words "hydro" (water) and "gelos" (gel), indicating water-like gels. These materials have the capacity to hold a high quantity of aqueous solutions, up to thousands of times their own weight in water, and due to their cross-linked network arrangement, they can swell but not dissolve [2]. Hydrogels are categorized based on different characteristics and properties. They can be primarily classified into smart and non-smart hydrogels based on their response to stimuli. Furthermore, based on their release behavior, which significantly influences the drug release mechanism, they can be classified into pH-sensitive, biodegradable, and other types. For instance, hydrogels that are sensitive to heat can be designed to deliver drugs to a specific region of the gastrointestinal (GI) tract. Smart hydrogels can withstand the acidity of the stomach and travel to a desired, potentially more vulnerable area of the GI environment to release the drug. Alternatively, smart hydrogels can be engineered to release the active ingredient at a predetermined rate as it travels through the body, providing a consistent plasma drug level [3]. Hydrogels are one of the best materials preferred for controlled drug delivery purposes. This is because hydrogels can swell while still retaining their three-dimensional network structure. They swell when coming into contact with water or biological fluids, similar to the matrix system, ensuring biocompatible applications. [4]. There are many advantages of using hydrogels for drug delivery systems. Hydrogels, due to their high-water content, are very similar to human tissue; hence, they are biocompatible. The drugs are released in a controlled manner and not all at once, known as "burst release." This helps in improving the efficacy of the therapy while minimizing the incidence of adverse events. Hydrogel drug delivery systems can be designed to provide zero-order release, first-order release, or any desired release profile simply by altering the hydrogel matrix. Hydrogels are used to make oral liquid formulations that can also contain more than 99% of the medicament as an oral gel.

The development of various insulin delivery systems to mimic endogenous insulin secretion is critical to the management of diabetes. Disorders in insulin secretion, insulin action, or both

can lead to diabetes. Despite advancements in insulin and glucose management since the first insulin injection in 1922 and the subsequent launch of the first insulin pump in the 1960s, a significant portion of insulin-treated individuals still do not achieve optimal glucose levels [5]. Current insulin delivery devices offer some degree of customization and are available in a variety of styles; in addition, several system innovations have occurred in recent years. No less than half a billion people are currently living with diabetes. In the United States alone, 1.5 million new cases are diagnosed each year, according to the American Diabetes Association. Although there is a steady rise in diabetes incidence, the advent of technological advancements in the area of insulin delivery systems has generated a surge of desire, curiosity, and investment from the research community and affected patients [6]. Traditional insulin delivery systems, mechanical insulin pumps, pen devices, and inhalation devices are the available insulin delivery systems. Various insulin delivery systems and devices have been introduced in the past few decades. Recent technological advancements have given rise to automated systems, including Continuous Glucose Monitoring (CGM) and real-time sensors. Ongoing research will enable development in more areas, resulting in new innovative products with advanced capabilities. Insulin delivery products are being combined with sensors, actuators, and computation, among others. New products include an insulin micro-pump with an automated controller and an advanced insulin pump that delivers insulin as a result of an "onboard" control component. Despite some challenges, automated systems have been shown to be helpful to individuals in achieving appropriate glycemic control [7].

Artificial intelligence (AI) is being used extensively for biological and medical research and development. These functions can be classified into three types: predictive (i.e., using AI algorithms to make predictions about biological systems), descriptive (i.e., using AI algorithms to identify patterns and relationships within biological systems), and generative (i.e., using AI algorithms to design molecules, drugs, or materials from a set of defined criteria). The broad use of AI in drug discovery and clinical trials is expected to speed up the development process, including a 29% reduction in drug development times, and in clinical trials, reduce sample size [8][9][10][11].

Modeling hydrogel networks presents several challenges due to their complex behavior and multi-scale phenomena. Characterizing the dynamic responses of hydrogels is particularly difficult, as their properties can evolve autonomously due to the relaxation of internal stresses. Additionally, integrating multi-scale phenomena, such as the link between connectivity in the network structure and the non-linear mechanical response, remains a largely unexplored area.

The objective of this thesis is, on the one hand, the innovation of an inhaled sustained-release form of insulin, through the synthesis of chemically crosslinked hydrogels using small molecules to link polymer chains. On the other hand, the development of a neuro-fuzzy model specifically tailored to predict insulin release kinetics from polycondensed agar-carbomer hydrogels. The structure and morphology of the hydrogel were analyzed using Scanning Electron Microscopy, Proton Nuclear Magnetic Resonance, and Fourier Transform Infrared Spectroscopy [9].

The significance of this research lies in its potential to overcome existing challenges in diabetes treatment. By palliating issues associated with traditional subcutaneous injections, such as variability and discomfort, this innovative approach could improve patient adherence and overall quality of life. The development of a neuro-fuzzy model for predicting insulin delivery from crosslinked agar-carbomer hydrogels could provide a more accurate and reliable method for managing diabetes, enhancing the efficacy of insulin therapy and reducing the risk of adverse events [9].

In conclusion, hydrogels offer a promising solution for sustained-release drug delivery systems, particularly in the management of chronic diseases such as diabetes. Their unique properties, including biocompatibility, controlled release, and versatility, make them an ideal material for drug delivery applications. The integration of artificial intelligence in drug discovery and development, along with the development of advanced insulin delivery systems, holds great potential for improving patient outcomes and quality of life. This thesis aims to contribute to this field by developing an inhaled sustained-release form of insulin and a neuro-fuzzy model for predicting.

Chapter I

Literature Review

I.1. Introduction

This section aims to establish a foundational understanding of hydrogels, laying the groundwork for exploring their diverse range of applications, specifically in drug delivery. Given the diverse range of hydrogel types, including natural, synthetic, and hybrid hydrogels, it is crucial to comprehend their distinct characteristics and how they influence their behavior in drug delivery systems. Moreover, understanding the properties of hydrogels, such as swelling behavior, mechanical strength, and biodegradability, is vital for predicting and controlling their drug release kinetics and therapeutic efficacy [1]. In the existing modeling of such hydrogel networks, spring point correlations and both bending and stretching anelastic behavior of the chain's chemical bonds are considered. These early models well describe the elasticity of long polymer chains in good solvents for neutral hydrogels and for transient networks. However, their longer networks are challenging to interpret with this simple analytical approach. As a result, a comprehensive data fit procedure might necessarily be employed to model the mechanics of their volume transitions [2]. Artificial intelligence (AI) is a renaissance tool that has the potential to change the drug delivery landscape, from accelerating the development of new delivery systems to enabling complex drug delivery designs to improve therapeutic outcomes. AI is ushering in a new era of digital drug delivery systems that will harness the power of big data, mathematical modeling, and machine learning methods to uncover insights that were previously unattainable. These advancements will streamline, accelerate, and fundamentally transform the way we discover, design, and deliver drugs to patients [3].

I.2. Hydrogels in Drug Delivery Systems

I.2.1. Definition and Properties of Hydrogels

The definition and properties of hydrogels make them highly attractive for drug delivery purposes. Their high-water content and porous structure enable efficient loading and release of drugs (Figure I.1), while their biocompatibility and tunable mechanical properties make them ideal for various administration routes, including oral, transdermal, and intraocular delivery. Furthermore, the ability of hydrogels to protect sensitive drugs from degradation and provide sustained release over time has made them increasingly important in the development of advanced drug delivery systems. Consequently, a thorough understanding of the definition and properties of hydrogels is essential for harnessing their potential in drug delivery applications [4][5].

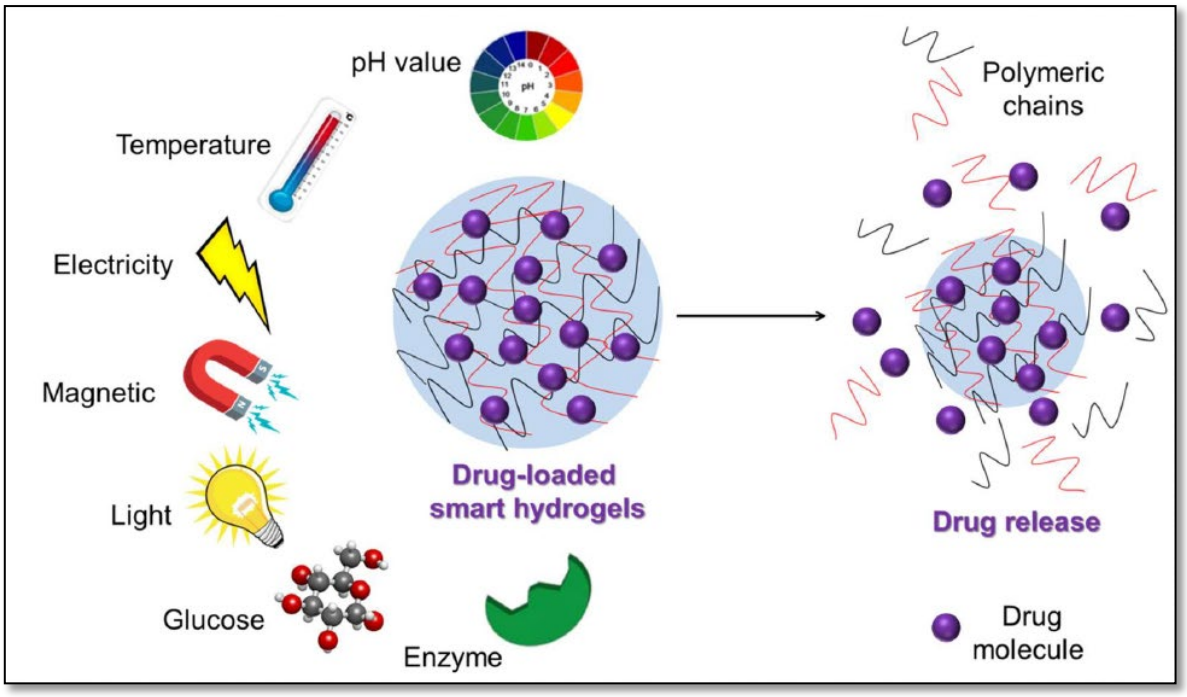


Figure I.1. Various external stimuli, including pH, temperature, electricity, magnetics, light, and biomolecules (including glucose and enzyme), are controlling the drug release from a smart hydrogel [6].

Hydrogels are three-dimensional networks of hydrophilic polymers that have the ability to absorb and retain water. These unique materials are capable of swelling in water without dissolving, making them highly versatile for various applications, particularly in the field of drug delivery. The properties of hydrogels, such as their high-water content and soft, rubbery consistency, mimic natural living tissues, making them suitable for biomedical applications. Additionally, hydrogels can be designed to respond to specific stimuli, such as pH, temperature, or specific ions, allowing for controlled release of drugs in response to various physiological conditions [7].

I.2.2. Types of Hydrogels

Overall, the different types of hydrogels each possess unique properties and characteristics that make them suitable for various drug delivery applications. Understanding these classifications is essential for selecting the most appropriate hydrogel for specific drug delivery needs, considering factors such as biocompatibility, responsiveness, and mechanical properties.

Another classification of hydrogels is based on their responsiveness to external stimuli, leading to the categories of physical and chemical hydrogels (Figure I.2). Physical hydrogels rely on non-covalent

interactions, such as hydrogen bonding, hydrophobic interactions, and physical entanglements, to maintain their network structure. These interactions make physical hydrogels sensitive to changes in environmental conditions, such as temperature, pH, and ionic strength. In contrast, chemical hydrogels are formed through covalent bonds, offering greater stability and mechanical strength. They can also be designed to respond to specific chemical triggers, allowing for controlled drug release [8].

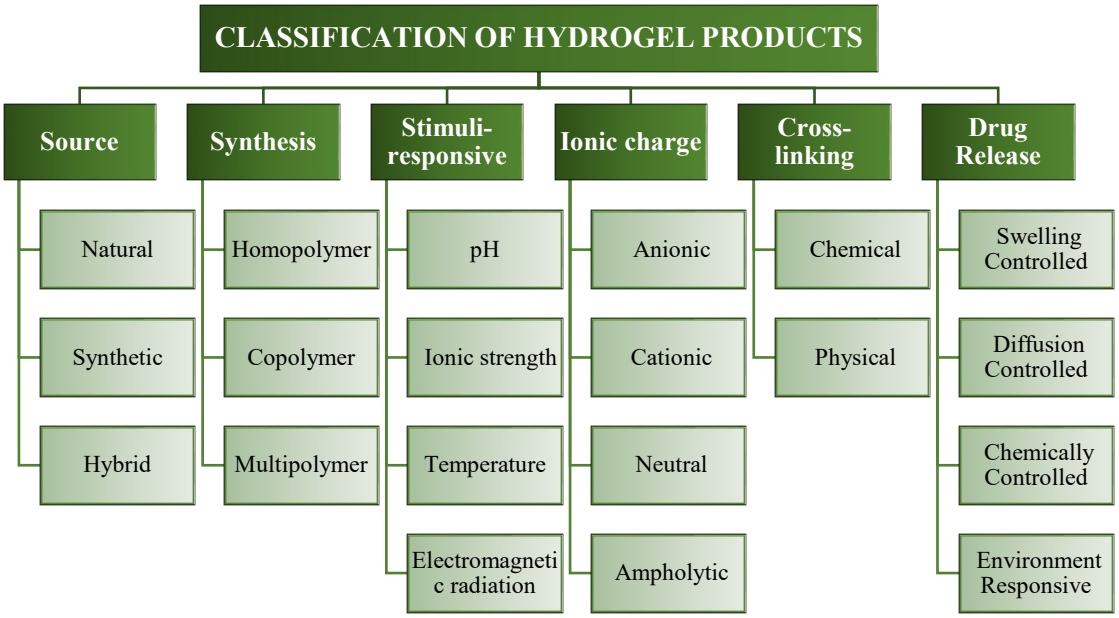


Figure I.2. Schematic representation of classification of hydrogels based on different parameters (based on ref [9]).

Hydrogels can be classified into several types based on their composition and structure. One classification is based on the source of the hydrogel, which includes natural, synthetic, and hybrid hydrogels. Natural hydrogels are derived from biomaterials such as alginate, chitosan, collagen, and hyaluronic acid. These hydrogels offer biocompatibility and biofunctionality, making them suitable for biomedical applications. On the other hand, synthetic hydrogels are created through the polymerization of monomers to form a network structure, allowing for precise control over their properties. Hybrid hydrogels combine natural and synthetic components to harness the advantages of both types [10].

I.2.3. Applications of Hydrogels in Drug Delivery

The applications of hydrogels in drug delivery are diverse and promising, offering solutions for the challenges associated with traditional drug delivery systems. From sustained release to stimuli-responsive delivery and site-specific targeting, hydrogels have the potential to revolutionize the field of pharmaceuticals and improve patient outcomes [6].

Furthermore, the stimuli-responsive nature of hydrogels allows for the development of smart drug delivery systems. By incorporating stimuli-responsive elements into the hydrogel matrix, such as pH, temperature, or enzymes, it is possible to trigger the release of drugs in response to specific physiological conditions.... This level of precision and control in drug release is particularly advantageous for targeted therapies and personalized medicine. Moreover, hydrogels can be designed to adhere to biological tissues, providing localized drug delivery at the site of interest, minimizing systemic side effects [6].

Hydrogels have gained significant attention in the field of drug delivery due to their unique properties that make them suitable for various pharmaceutical applications. One of the key applications of hydrogels in drug delivery is their ability to provide sustained and controlled release of drugs. This is achieved by the incorporation of drugs within the hydrogel matrix, allowing for gradual release over an extended period of time. Additionally, the porous structure of hydrogels enables the encapsulation of a wide range of drugs, including small molecules, proteins, and peptides, making them versatile delivery systems for different therapeutic agents [11].

I.2.4. Crosslinking Techniques

I.2.4.1. Chemical Crosslinking Techniques

There are various chemical methods for executing the above process. The first is the UV irradiation technique, which is one of the most widely used chemical crosslinking techniques (Figure I.3). It utilizes a water-soluble photoinitiator which should be dissolved with polymer chains in the solution. Once this system is irradiated, the photoinitiator absorbs the UV light and accordingly generates radicals. These radicals can generate various free radicals from the polymer chains [12]. The second method is gamma-irradiation. The advantage of this technique is that the irradiated polymer can be sterilized because the ionizing radiation that is generated during the irradiation process is germicidal too. The third category is the electron beam exposure of the macromer. After applying this, the free radicals originate from the polymer chains. The electron beam can penetrate the system without generating heat. The fourth category is the use of physical thermal stimuli in the presence of photostable photoinitiator. Finally, redox initiators can also be utilized [13].

Chemical crosslinking is the most commonly used method of hydrogel crosslinking. In a chemical crosslinking process, multifunctional molecules that can react with the reactive moieties present in the polymer chains are used. A macromer is a compound which has the functional groups that take part in

the crosslinking reactions. By this chemical process, the quantity of network sites is controlled. The gelation and swelling of the hydrogel are controlled by the polymer type itself and the degree of crosslinking. This type of crosslinking is often performed after the desired shape of the hydrogel is fabricated. Therefore, one advantage of the chemical crosslinking method is that it can be used to prepare hydrogels with various shapes [14][15].

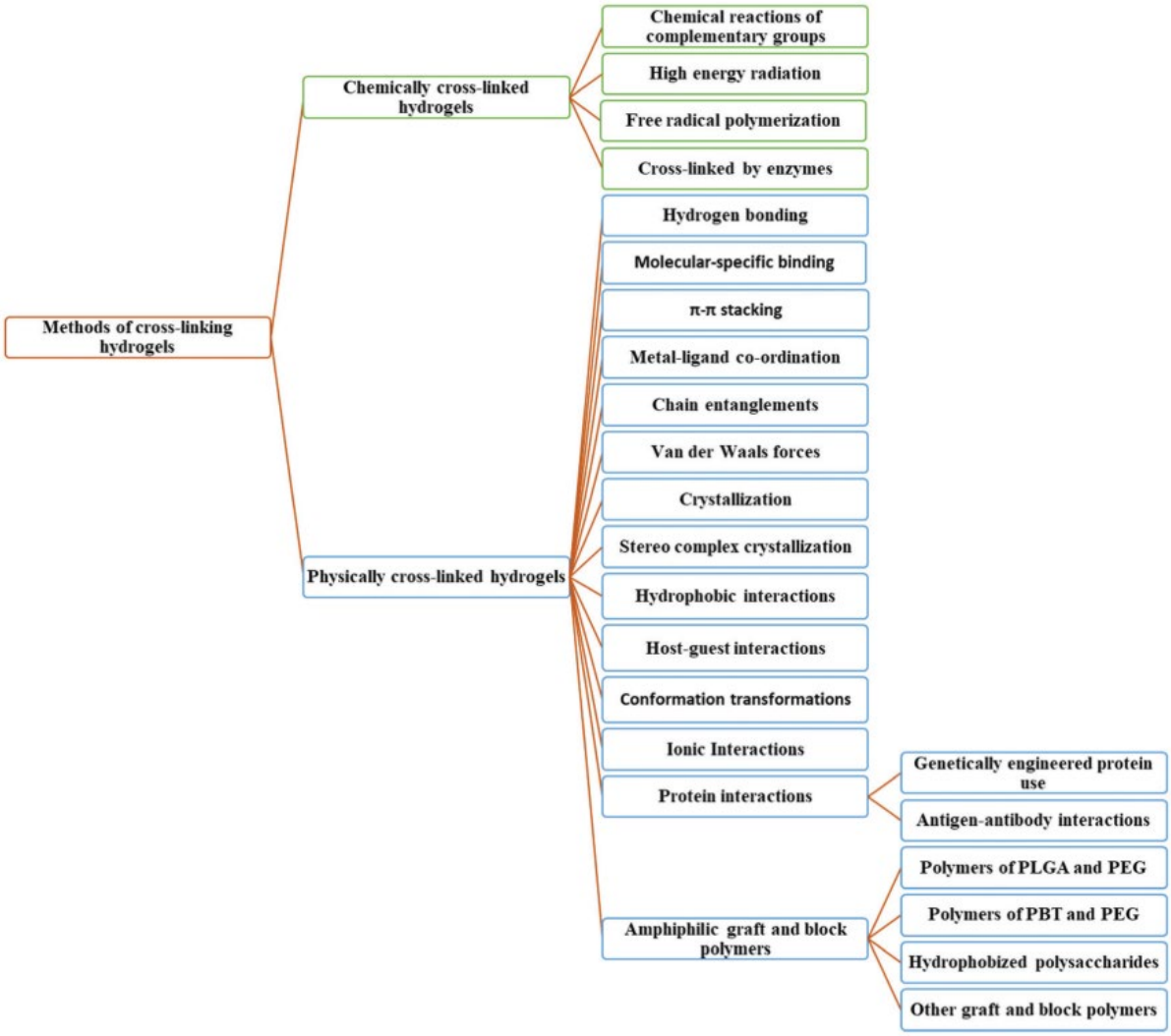


Figure I.3. Methods of cross-linking hydrogels [16]

I.2.4.2. Physical Crosslinking Techniques

Physical techniques of hydrogel crosslinking generally rely on the utilization of specific physical factors and phenomena to fix hydrogel polymer chains in space (Figure I.4). In turn, physical crosslinks are formed through short-range interactions between intra- and inter-chain groups in most types of hydrogels, also called "junction zones." During the early stages of physical hydrogel crosslinking,

junction zones form due to transient interactions between functional groups or segments of insoluble macromolecules (e.g. the crystallization of macromolecular chains in double and triple helices or lamellae), which, in contrast to permanent covalent associations in chemically crosslinked hydrogels, lack effective crosslinking. Besides, the presence of reversible physical crosslinks can lead to temporal deswelling of hydrogels. In turn, hydrogel crosslinked by reversible physical interactions remains in the gel state only under certain conditions and can experience reversible transitions [17][18].

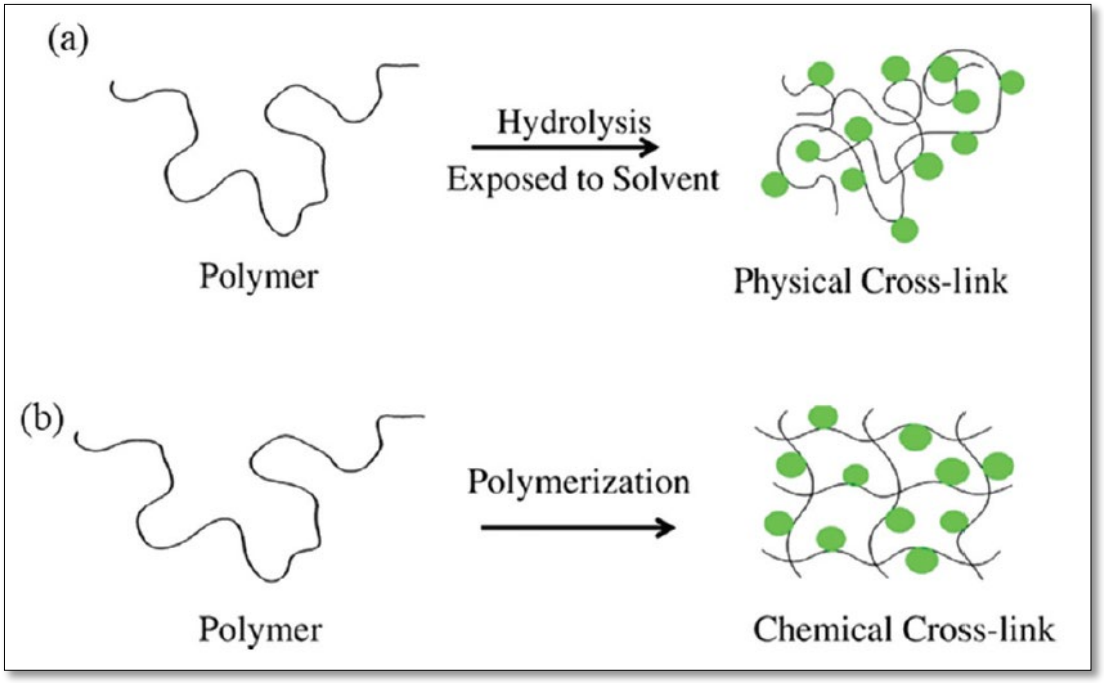


Figure I.4. Schematic illustration of hydrogel fabrication. (a) Physical crosslinking. (b) Chemical Crosslinking [16].

I.2.5. Drug Delivery Systems

Hydrogels made of natural or synthetic polymers are extensively exploited as drug carriers in various applications. They can sustain drug releases in a controlled manner. This article gives a brief review of hydrogels for drug delivery. It includes drug release mechanisms, the effects of the properties and external environments of hydrogels on drug releases, and some examples of hydrogels for drug delivery [19].

Drugs can be administered through different routes such as oral, injectable, transdermal, and inhalable. Each method faces its own limitation. Oral drug deliveries require the movement of the drug through the gastrointestinal tract, and the drugs can be exposed to harsh conditions such as acidic pH and

digestive enzymes [19]. Injectable drug deliveries have challenges in maintaining the drug at constant release rates and spreading the drugs for a wide therapeutic window. For the transdermal route, the molecular size and lipophilicity of the drugs are limited. It is difficult to deliver high molecular weight and hydrophilic drugs. For the inhalable drug delivery, it requires the patients' coordination for effective delivery. To overcome these limitations, drug delivery agents can be designed to improve the performance. One of the most promising and adaptable methods is to develop hydrogels as drug carriers [20].

I.2.5.1. Drug Delivery Systems and Mechanisms

In order to facilitate drug delivery, a variety of drug delivery systems have been developed. The release of drugs from various drug delivery systems is primarily based on different drug diffusion rates, solubility rates, and dissolution rates in different media (water, biological fluid, etc.) or different physical and chemical transformation mechanisms [21]. The release of drugs from most conventional drug delivery systems depends on physical principles such as passive diffusion, filtration, osmosis, and chemical principles such as ion exchange, hydrolysis, or chemical bond cleavage. However, due to the environment-altering characteristics of reactions based on these principles, the drugs can be released in fixed locations, uncontrolled locations, or non-target locations inside the body, which lead to drug waste, degradation, and ineffective drug administration [22].

Also, the drugs released using these principles can be eliminated because they are rapidly removed from the body. Also, it is difficult to intake some drugs due to their hydrophobic/hydrophilic nature or low permeability. Hence, to produce a better drug delivery system with a reduced rate of drug removal, previous research studies of drug release systems concentrated on the development of nanoparticle structures that release a drug into specific target areas with time- or concentration-controlled release [19].

I.2.5.2. Advanced Drug Delivery Systems

The next generation of drug delivery systems (DDS) is based on cell growth and regeneration, attempted by the use of biomimetic or tissue-engineered systems. Such advanced DDS can be giant in order to treat injuries, prevent scar tissue formation, deliver drugs continuously for several weeks, and avoid syringe administration. Polymeric DDS is a powerful foundation for these systems. Some of these advanced delivery systems need to be implanted in order to function, and hydrogels are a desirable implant material due to their soft, water-swollen nature [6]. Small biomolecules, peptides,

and genetic material like DNA or RNA can be used as drugs to control tissue responses. Such high-molecular-weight or charged drugs require physical or biochemical assistance to reach therapeutic concentrations [23].

I.2.6. Hydrogel Synthesis

The synthesis of hydrogels is dependent upon both the particular monomer and the crosslinking agent used. The reaction choice is determined by the crosslinking agent used. Hydrogels have been reported to contain either physical or chemical crosslinks, or a combination of the two. Most smart or stimuli-responsive hydrogels contain chemical crosslinks, which provide better mechanical strength [24].

First, macromer monomers undergo a free radical or anionic polymerisation by initiators as the crosslinker to form the hydrogel network. However, the crosslinking reaction can be limited by the material conversion, especially for the manufacturing of large hydrogel structures. The introduction of extra photosensitive groups by side chain functionalisation is one of the strategies for primary macromers to be polymerised by photopolymerisation, of numerous advantages like low harm, high spatial and temporal resolution. These properties allow the prepolymers to readily encapsulate or suspend the drug cargo and create the final hydrogels directly around the cargo by photopolymerisation with high directionality and control, so as to achieve better therapeutic efficiency and minimised off-target effects, as well as fast swelling-drug release response [25].

I.2.6.1. Natural Hydrogel Synthesis

In recent years, the attention of the scientific community has been attracted by the use of molecules derived from natural sources in the preparation of hydrogels. These molecules often possess extremely interesting properties, such as biocompatibility, gelation under mild conditions, processability, and functionalization with organic or inorganic compounds. The use of natural peptides to prepare gels is not new, and in many cases, hydrogels obtained naturally from these peptides are employed. In this context, it was reported the use of an octapeptide to obtain a hydrogel that could change its mechanical property by binding to bovine serum albumin (BSA) [26].

Many synthesized molecular structures can be collected under the general name of "peptidic", given that they indeed often possess in their structure the presence of amide bonds, amino acid units, and carboxylic acid groups. Besides being common in nature, peptides are extremely versatile chemical entities that can be employed in high added-value products. Due to their diverse composition, and

consequently different three-dimensional structures, peptides possess unique biochemical activity and can carry out a wide variety of biological functions [27].

I.2.6.2. Synthetic Hydrogel Synthesis

Synthetic hydrogels are developed using either free radical polymerization, which involves the use of a chemical initiator to generate radical sites on monomeric units, which reacts functionalized polymeric chains. Free radical polymerization is the most common reaction used in the development of synthetic networks, with UV or thermal generation mechanisms typically employed. Hydrogels synthesized through this method are either copolymerized or end-crosslinked, by utilizing different functional groups on the monomer or by incorporating a bi-functional small molecule, respectively [28]. The advantages of this approach are the addition of different functional groups, tailoring the hydrogel response, such as high strength, using low concentrations of polymeric monomers, and the formation of a homogeneous network. Conversely, the disadvantages include the lack of control over the hydrogel structure, the creation of a toxic environment when crosslinked in situ, the application of harsh processing conditions, and the need for radical inhibitors to prevent premature hydrogel formation [28].

I.2.7. Characterization Techniques

Pure drug, polymer, and drug-loaded hydrogel possess some similarities in their main characteristic peaks in FTIR analysis, with different strengths or some shifted wavenumbers. The presence of the drug can also be confirmed in this test. Particle size, particle distribution, and zeta potential of the nanosuspension of drug-polymer and drug-polymer-hydrogel can be tested using dynamic laser scattering. The enthalpy of the drug, polymer, and drug-loaded hydrogel is measured by DSC. The drug release curve of the drug-loaded hydrogel in vitro and the cumulative release percentage can be analyzed by UV. The UV instrument can be used to determine the drug concentration in blank hydrogel without the drug and drug-loaded hydrogel at different time points. The swelling ratio of different formulas of the drug-loaded hydrogel after water absorption for a certain time is calculated as:

(drug-loaded hydrogel-drug-loaded hydrogel shell / dry hydrogel shell) × 100%.

The stability and particle size of PNIPAAm hydrogel are tested by UV-visible spectrophotometry, laser granulometry, and scanning electron microscopy. The sample of the drug-loaded hydrogel was tense glucose solution for testing glucose-sensitive response in vitro [29][30].

I.2.7.1. Mechanical Characterization

Hydrogels can be covalently or physically connected, allowing for their use as substrates for aging cells and drug delivery. Physical hydrogels with reversible chemical bonds are especially of interest in cell and drug delivery, enabling the encapsulated material to change shapes or to sense signals that cause it to weaken or dissolve rather than be degraded by the encapsulated material. This information is helpful in the development and characterization of PVA/DMAc for tunable hydrogels. For drugs attached to hydrogels, this information is crucial at the point of penetration of the hydrogel into the body [6].

I.2.7.2. Morphological Characterization

Hydrogels are three-dimensional cross-linked hydrophilic networks capable of absorbing a large amount of water relative to their dry weight, while preserving their structural integrity. Over several decades, several hydrogels based on natural origin polymers such as dextran, hyaluronic acid, chitosan, collagen, gelatin, alginate, carrageenan, schizophyllan, and agarose, and synthetic biocompatible polymers such as poly (ethylene glycol), poly(vinyl alcohol), poly(acrylamide), and poly(2-hydroxy ethyl methacrylate) were successfully developed. The whey protein gel is an example of a biopolymeric hydrogel. Whey protein is famously used in the food and pharmaceutical industry, with good mechanical and controlled release properties. It is thermally stable over a wide range of pH values, and either non-toxic or even essential for the human body according to FAO/WHO (1989). Due to their biocompatibility and non-toxic nature, hydrogels were well studied and largely utilized for drug delivery, tissue engineering, and regenerative medicine [31].

I.2.8. Factors Affecting Drug Release from Hydrogels

Hydrogels are extensively used as drug delivery systems to achieve sustained and controlled drug release. In such applications, drug release profiles are determined by diffusion of drug molecules through the hydrogel network and dissolution of drug molecules in water. However, a complex drug release profile can be achieved by adjusting various factors, such as properties of therapeutic agents and hydrogels, and external environmental factors [32].

Factors affecting drug release from hydrogels include various properties of therapeutic agents, hydrogels, and the exterior. Understanding and utilizing these factors are essential for the creation of hydrogels with precisely controlled release profiles for specific therapeutic agents. The choice of the

therapeutic agent is the first determinant of the release profile of hydrogel drug delivery systems. To date, various therapeutic agents have been released from hydrogels. In a controlled release application, the drug release profile is one of the most characteristic properties of the drug delivery system. The same therapeutic agent could exhibit any release profile from any hydrogel [33].

I.2.8.1. Hydrogel Composition

Hydrogels are formed by the covalent or non-covalent association of hydrophilic polymer chains. The various crosslinking methods are reflected in the wide range of compositions from which hydrogels can be synthesized. The challenge remains to control the hydrogels' molecular composition sufficiently to tailor their structure, to meet the constraints imposed by the nature of targeted tissue as well as the physiological state of the process which affects the process of interest. The most common hydrogels are based on poly(2-hydroxyethylmethacrylate) that is modified with hydrophilic comonomers (e.g. N-vinylpyrrolidone) [34].

Other modifiers providing hydrogel compositions for drug delivery applications include 2-hydroxyethylmethacrylic acid. These hydrogels have a density of hydrophilic groups on the polymer chains that can be modified so that they swell in response to chemical or physical changes of their environment (by modifying pH, ionic strength, temperature, etc.). Another group of water-swelling polymers are biodegradable hydrogels utilized for the controlled release of potent biomacromolecules. In general, these polymers originate from the family of polylactic acid (PLA), polyglycolic acid (PGA), and their copolymers.

I.2.8.2. Crosslinking Density

Crosslinking density plays a crucial role in modulating the morphological structure and, therefore, the drug loading and release performances of the hydrogels. It has been demonstrated that the mesh size of a hydrogel network is closely related to the crosslinking density. A hydrogel whose mesh size is much smaller than the drug molecule cannot load and release the drug effectively. Thus, the mesh size of a hydrogel should be suitable for the drug loaded [35].

A series of non-covalently imprinted hydrogels on polyacrylic acid modified with amino groups were investigated for the sustained release of theophylline, and the template molecules were successfully released from the cationic copolymers under physiological conditions of pH 7.4. Recent studies

indicated that the structure of the hydrogel is related to the mole ratio of the monomer and the polymerization time.

I.2.9. Recent Advances in Hydrogel-Based Drug Delivery

Acknowledging the limitations, the past few years have seen the development of bioactive hydrogels that actively respond to the body environment and provide well-regulated drug release or direct healing signals, which can greatly expedite the healing procedure compared to conventional formulations. Many different strategies are employed to develop smart hydrogel-based formulations, and the properties are tailored to various types of applications [36]. In the following, we discuss the recent advances in smart hydrogel systems for drug delivery chronologically: thermosensitive hydrogels, pH/thermosensitive hydrogels, glucose-sensitive hydrogels, photo-sensitive hydrogels, size-sensitive hydrogels, reduction-sensitive hydrogels, and hydrogels with multiple properties.

Conventional drug release formulations cannot release the loaded drugs in a specific and controlled manner, and they are found to have weak regenerative abilities and have limited application. In these years, it has been found that when the materials interact with the surroundings, they can release drugs for a prolonged period, and the materials can not only enhance the therapies but also expedite the healing process. Hydrogels, as drug delivery materials, possess many super properties such as good biocompatibility, a unique three-dimensional structure, large carrying capacity, and most importantly, they can be tailored to release drugs in response to specific stimuli. Upon the stimulation of various endogenous or exogenous factors, smart hydrogels actively release drugs to the sites in need with well-regulated manners [37].

I.2.9.1. Smart Hydrogels

By simply increasing the crosslinking density of the delivery system, the creation of 'smart' hydrogels that can change swelling behavior in response to environmental signals has developed over the last years. Since their porosity and the free amount of water inside them may both be influenced by these modifications, smart hydrogels are especially interesting for the field of on-off drug releasing, where the release is regulated by a specific trigger (e.g. pH, glucose, temperature, chemical and biochemical change) to change their swelling and water holding capacity under on-demand conditions. By tailoring the field sensitive and its responsiveness, these hydrogels may be prepared for a wide range of external stimuli of interest [16].

I.2.9.2. Injectable Hydrogels

Hydrogels have been under extensive investigation for tissue engineering scaffolds, tissue bulking, and drug delivery, etc. They are appealing for biomedical applications likely due to their high-water adsorbing, good biocompatibility, biodegradability, and highly tunable physicochemical and biological properties. Scaffold, tissue bulking, and drug delivery refer to the applications that hydrogels function as a template to guide cell growth and biological tissue regeneration, treating symptoms that originate from structural losses of certain tissues or organs, and as vehicles to deliver drugs to the desired sites to prevent, treat, or diagnose certain diseases, respectively [38].

Injectable hydrogels have attracted growing scientific and clinical interests in the last few years, likely due to their unique effectiveness in terms of simplifying clinical operations by eliminating surgical procedures, reducing treatment time and damage, improving surgical precision, and having less chance of chronic exposure. They are capable of changing solution properties shortly after injection to form an actual hydrogel within the patient's body [39].

I.2.9.3. Nasal Hydrogels

Mucoadhesive hydrogels are an attractive delivery system for precise nasal drug release due to their ability to attach to the mucus governing the nasal passage. They provide solutions for several concerns related to drug formulation, which can be treated using nasal drug delivery. Many hydrogel polymers have been suggested for nasal delivery. These materials are based on their hydrophilic structure, biocompatibility, and ability to be loaded within their gel structure [40]. The delivery of hydrogel to the nasal passage can be enhanced by mixing the gel with the drug, cooling it into a hydrogel-drug mixture, or simply incorporating the drug into the hydrogel structure. Additionally, by using hydrogels, one can explore other advantages, such as absorption enhancers to protect the gel structure, hypo-osmotic agents to preserve the permeability of the mucus-epithelium complex, and metabolic enzymes to speed up drug metabolism and elimination [40][41].

Nasal drug delivery represents an alternative route for drug administration. Historically, nasal delivery has mainly been used for the treatment of local conditions, but it has gained attention as a potential route for more rapid delivery of drugs to the systemic circulation in comparison to oral delivery. It has been found that the nasal route achieves better absorption in comparison to the oral route, thus avoiding drug metabolism in the liver and delivering drugs to the systemic circulation quickly. Additionally, it can be used to bypass the blood-brain barrier and avoid hepatic first-pass metabolism. However, nasal

drug delivery has its own barriers that restrict its clinical routine usage, like limited residence time of the drug due to its high clearance from the nasal cavity surface. To address these drawbacks, researchers over time have worked on several strategies like sophisticated drug delivery systems, such as nanoemulsions, thermoreversible gels, and mucoadhesive nanoparticles [42].

The Advantages of nasal drug delivery systems are :

- Rapid absorption: The nasal mucosa is highly vascularized, which allows for rapid absorption of the drug and a shorter onset of action, particularly useful for emergency treatments.
- Non-invasive administration: More comfortable for the patient than injections, especially for pediatric patients or those with needle phobia.
- Avoids first-pass hepatic administration: The drug is directly absorbed into the systemic circulation without passing through the liver, which improves its bioavailability.
- Local or systemic targeted action: Local: Effective for nasal conditions (e.g. allergic rhinitis, sinusitis). Systemic: Used for drugs with a systemic effect (e.g. nasal insulin, vaccines, analgesics).
- Ease of use: Ideal for self-administration by the patient, thus improving therapeutic compliance.
- Access to the brain via the nasal route (neural route): Molecules can cross the blood-brain barrier via the olfactory nerve, providing a pathway for neurological or neurodegenerative treatments (e.g. Alzheimer's, Parkinson's).

The Barriers of nasal drug delivery systems are :

- Limitation of the administrable volume: The maximum volume per nostril is approximately 200-500 µL, which limits the amount of drug that can be delivered.
- Low absorption for certain molecules: Large molecules (e.g. proteins and peptides) have difficulty crossing the nasal mucosa without the use of absorption promoters.
- Physiological and environmental factors: Variability of absorption due to nasal secretions, the pH of the mucosa or pathological conditions such as nasal congestion.
- Irritation of the nasal mucosa: Some excipients or formulations may cause irritation, sneezing or tissue damage in case of prolonged use.
- Rapid mucociliary clearance: The natural clearance of the cilia reduces the contact time between the drug and the mucosa, thus decreasing the efficacy.

- Formulation stability: Nasal formulations, especially for biological molecules, can pose challenges in terms of stability and preservation.
- Immunological barrier: The nasal mucosa has a local immune system that can degrade certain molecules before their absorption.

The nasal route has considerable potential to deliver drugs with rapid local or systemic action, especially for diseases requiring immediate responses or neurological treatments. However, physiological and technological barriers must be overcome, especially to improve the absorption of large molecules and the stability of formulations [42].

I.2.9.4. Hydrogel Nanocomposites

The word 'composite' refers to the combination of two distinct materials to create a new material that has the best friend's properties included. In hydrogel nanocomposites (Figure I.5), a hydrogel is resulted by the mixing of the nanodimensional filler which can involve the enhancement of the mechanical, swelling, elastic, permeation and thermal properties of the initial hydrogel. Due to the great extents of the advantages of the different hydrogels having the nanocomposite structures for drug delivery systems, these materials are explained and covered in this review. Of the different nanocomposite-based hydrogels, including inorganic materials, nanocarbons, and exohedral fillers, in this section, the improvements of the material properties of the hydrogels are depicted. In the field of nanomaterials, the variety of nanodimensional fillers that are available leads to a widespread designing of the hydrogels for desired applications and final requirements. [43][44].

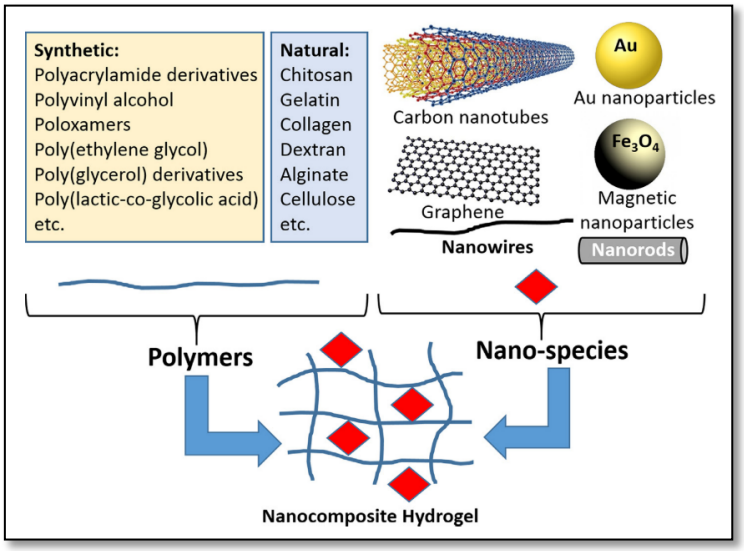


Figure I.5. Nanocomposite hydrogels composed of various polymer matrices filled with different nano-species [44].

The formation of hydrogels with nanocomposite structures generally begins with the administration of monomeric materials. A hydrophilic polymer is usually used during the base polymerization to cover a three-dimensional polymer network inside the nanofiller structure. Heterogeneous properties seen in these composite materials are provided by the addition of these varying materials into the hydrophilic polymer network and the reinforced property which could lead to broader application fields [45]. The different performance of nanostructured fillers has unique individual attributes and is generally due to their nanoscopic dimensions. The unique role of the nanocomposites that are achieved by the prepared nanostructured materials in these hydrogels has to be evaluated. A nanoscaled ball is lost in a bed of hydrophilic matrix molecules and spent the interior of the hydrogel in the morphology. These nanocavities or nanovoids might typically consist of gas molecules or water molecules executed within the hydrogel [46].

I.2.10. Advantages and Limitations in Drug Delivery

The various advantages of hydrogels in drug dosage support include their innocuous, robust, and biocompatible nature, which supports the premise that these hydrogels have fine prospects for clinical drug uses. Both synthetic and natural polymers can be incorporated to create biodegradable hydrogels, and they generally possess very affluent, porous, three-dimensional, and colloidal structures. These diverse types of networks usually encompass hydrophilic repeat units that favorably interact with water, thus exhibiting a high sorption level. Inclusion of hydrophilic polymers will increase the gel swelling capacity as well as the merit for using in a large variation of biomedical applications [47]. Hydrophilic polymer-based hydrogel biomaterials show special promise as ideal candidates for delivery carriers in various studies and research work for drug delivery purposes. The size and shape of the hydrogel can be manipulated to produce submicron, micron, or millimeter-sized particles, which produce a variety of drug release profiles. These hydrogels possess numerous characteristics, including high swelling ratios and accessibility to externally triggered stimuli. External stimuli trigger controlled-release kinetics [48][11].

However, hydrogels have limitations in their use to mimic the body's natural extracellular matrix structure. Generally, neutral and negatively charged synthetic polymers degrade throughout the release mechanism without promoting cell growth and regenerative capabilities. In the case of neutral and negatively charged synthetic polymers, cell growth and the regeneration of tissues cannot be fully promoted. Furthermore, additional concerns still exist, which include significant variations in pore size

distribution and a lack of cellular activities leading to overall limitations in promoting tissue remodeling [11].

I.3. Traditional Network Design and Modeling of Hydrogels

To date, network design and fabrication of hydrogels mostly depend on empirical strategies. Formal theories to model polymer gels have maintained an acute interest for over half a century and are continuously evolving. Two generic approaches based on random media and statistical physics may currently be distinguished: traditional mean-field treatments and Monte Carlo simulations [49] (Monte Carlo method, is an algorithmic method for calculating an approximate numerical value using random processes, i.e. probabilistic techniques).

Hydrogels can be formed not only through equilibrium polymerization mechanisms but also by nonequilibrium reactions, leading to nonhomogeneous and temporally evolving gels. The focused network design aims at unraveling universal design rules on how the mechanical properties of hydrogels can be controlled through the tuning of the history of networks formation under external boundary conditions [35]. This involved using a minimal lattice-gel model of random networks with a set of parameters describing the topological and dynamical aspects of a gel formation under nonequilibrium conditions [50]. The approach is generic and independent of any type of polymer network.

I.3.1. Conventional methods for hydrogel design and modeling

Traditional hydrogels are dual-responsive by temperature and solvent composition. Dual responsiveness results from large changes in the solubility of the polymer at certain conditions. In the case of poly(N-isopropylacrylamide) (pNIPAM), a temperature increase above the lower critical solution temperature (LCST) drives phase separation [51]. For healthy connective tissues like cartilage, concentration gradients in metallic ions influence the architecture and thus mechanical properties of 3D hydrogels from composite ink. Gravitational field was used to induce steady shear in the polymer network and enhance chain orientation, increasing strength. However, the design of patterns is challenging in terms of reproducibility and versatility. A 3D molded drug delivery device with tunable release rates was demonstrated. The tunability arises from differences in LCST and drug solubility, but only three distinct coatings could be achieved. Current conventional methods provide simple pathways but lack versatility and reproducibility. Viscoelasticity, light responsiveness, or stiffness gradients cannot be implemented using basic molds [52][53].

I.3.2. Modeling Approaches

In the quest to decipher the interactions that dictate the properties of gelatin and non-gelatin hydrogels at different thermal states, progress was made in elucidating its architecture by taking advantage of idealizations. These simplifications were employed to explore the underlying physics and to understand the precise connections between the monomer and polymer processes that perfectly cooperatively form cross-links while being responsive to temperature. The aim was to develop a comprehensive argument concerning the network architecture relevant to both systems, establishing an underlying mechanism that explains the property differences at a microscopic level [54][55].

I.3.3. Limitations of traditional approaches

The real synthetic approaches and experimental measurements involve significant complexities that cannot be captured by this traditional modeling and design framework.

a) Mesoscopic scale : Most synthetic and experimental works are performed outside the mesh size scale of the networks where they appear uniform and macroscopically isotropic. The design and characterization approaches of polymeric networks, however, operate on and transform the networks on the molecular/mesoscopic scale. This fundamental disconnect compromises access and controllability at the experimentally relevant scale [56].

b) Heterogeneities : The alkyne/azide click chemistry used for network synthesis and the traditional modeling approaches treat networks as perfect or homogeneous objects, although they are typically very polydisperse in almost all aspects, including chain length, elastic constant of chains, and density of crosslinks. The emergence of defects due to pre-ensnared chains in conjugated reactions or dangling arms in grafted networks adds another level of complexity. Heterogeneities have a significant impact on the swellable and elastic properties of networks that cannot be captured using traditional approaches [56].

c) Memory: Networks made of strongly crosslinked soft polymers possess long timescale dynamics that are currently not trivial to incorporate within traditional modeling.

d) Anisotropies: Recent inspiration from biological systems and soft robotics has led to the design of architected networks with controlled anisotropies that cannot be captured using traditional approaches [57].

e) Off-equilibrium state: Networks under external fields, such as flow or compressed between surfaces, break the assumption of self-equilibrium state that underlies traditional modeling and create entirely nontrivial problems. For example, a swollen polymer gel, on removing a confining pressure, would relax resulting in non-equilibrium and heterogeneous osmotic pressure distribution, making it hard to predict its ensuing dynamics [58].

f) Multiple length and time scales: The structural evolution of one or more networks, their intertwining, and reactions taking place at their overlap involve multiple length scales ranging from chemical, mesoscopic, to macroscopic scales with separated time scales from very fast, fluid phase to slow viscous, solidifying phases [56].

I.4. Artificial Intelligence in Drug Delivery Systems

I.4.1. Overview of AI techniques

I.4.1.1. Expert Systems

Expert systems are artificial intelligence programs introduced in theory and applied through techniques available for synthesizing scientific knowledge by generating an apparatus of systems, rules, and facts. Thus, the expert systems present answers and solutions for the described problems. The purpose of expert systems is to mimic the specialist opinion on a specific field or aim at certain goals effectively. The distinctive characteristics of this type of artificial intelligence are mobility, the capacity for combining rules, fault tolerance, and automated training in partially sensed environments. The available literature shows that artificial intelligence methods are a powerful tool to solve both theoretical and application problems in drug delivery systems, from building and focusing on formulations to drug targeting [59][60].

Regarding development work, in drug delivery systems, expert systems are expert modules that store information on drug-drug and excipient-drug interactions, which data are transparent to other systems in the development process and calculate costs at different production scales, thus optimizing the product development. This being based on a knowledge-based expert system, after the development of design and requirements, the expert system points to the ideal and most flexible formulation for mass production [61].

AI can contribute to protein development and customized biologics by using data analysis (Figure I.6), predictive modeling, and pattern recognition tools for better improvisation in the protein development

process and customized proteins. Knowledge of altered biological pathways and finding disease targets are required for the same. The prediction of protein folding from amino acid sequences and the use of deep learning and molecular dynamic simulation for better understanding can be performed by AI. The prediction of protein/peptide binding affinity and toxicity studies can be performed effectively by AI with the help of SAR and toxicological datasets [61].

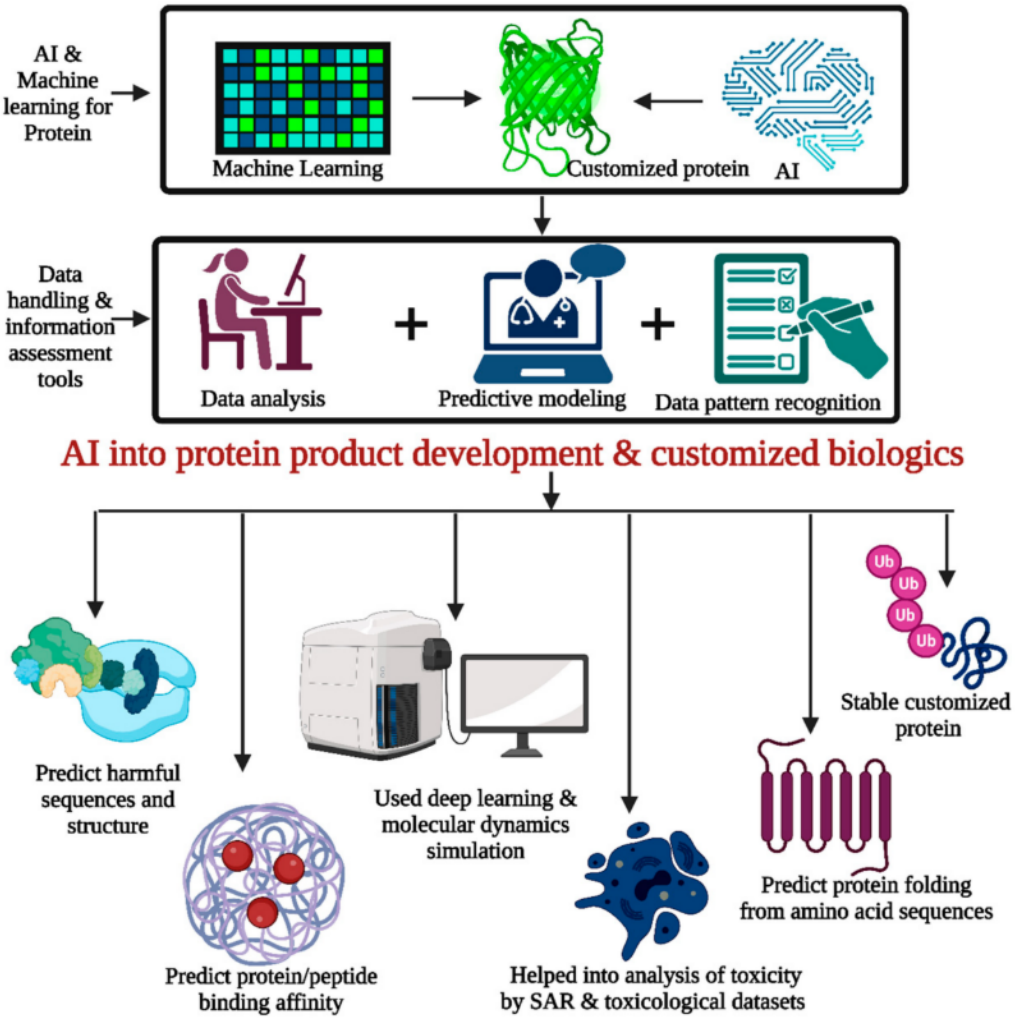


Figure I.6. AI into protein product development and customized biologics [61].

I.4.1.2. Neural Networks and Fuzzy Logic

Neural Networks (NN) is a digital representation platform and computational model in the biological imitation field. NN extremely simulates the way that neurons act, which operate in multicellular organisms' brains. Neurons can be considered the connecting cells of a neurosystem (Figure I.7). In the context of AI, a neural network has been developed to acquire abilities to recognize various multidimensional, iterative structures which are not computationally demanding because of their

memory or storage. Neural networks process information and learn patterns that represent experiences, in other words, they facilitate the development of computational models. In the pharmaceutical field, this type of modeling technology has been applied for drug development optimization, competitive assessment, diagnosis of pathologies, medicine, oral drug delivery, and development of improved materials for pharmaceutical and cosmetic uses [62][63].

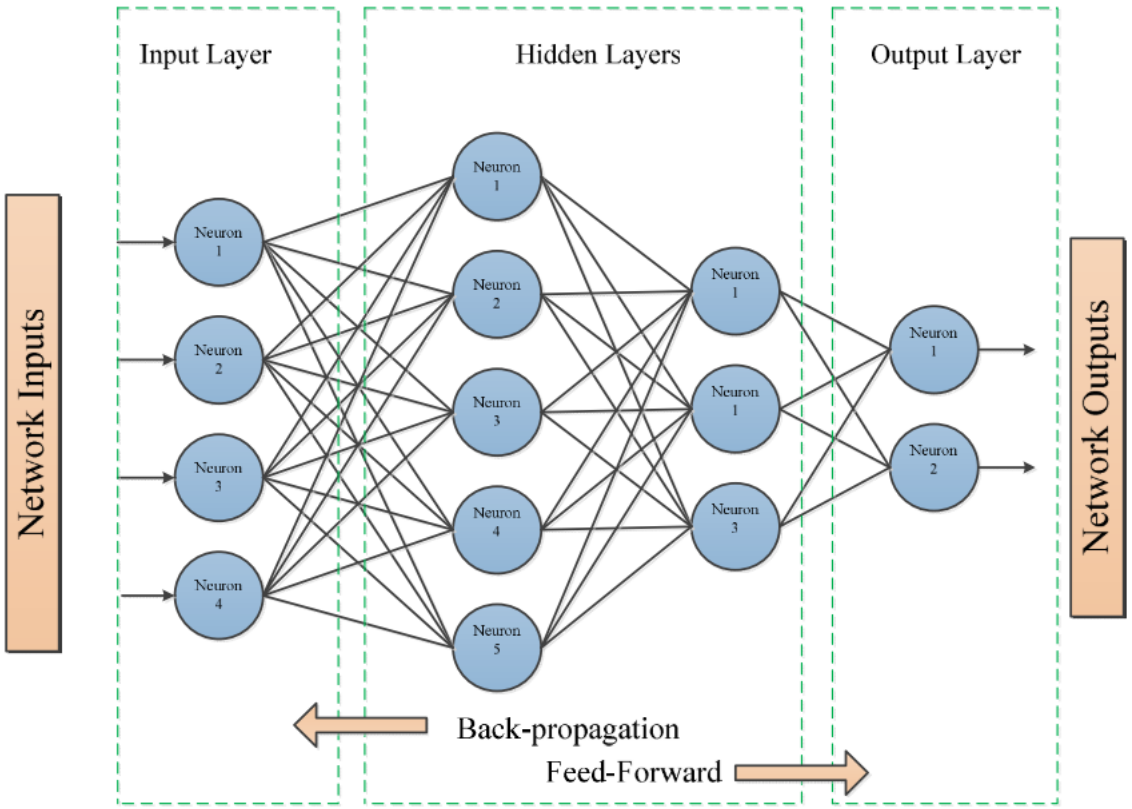


Figure I.7. Artificial Neural Network [64].

In drug delivery research, neural networks identify the nature, structure, and parameters obtained from variables that resolved different problems which evolution contains valuable input/output learning mechanisms [64]. This model's difficulty diminishes analysis of the relationship between these variables which associated methodologies link systems optimization in complex, costly, and time-consuming techniques considering NN's capacity to integrate response surface models. This model for controlled drug release systems was developed. Several input variables like generation one and two, also for the release terpolymer matrices spatial network mesh diameter, and drug loading, were incorporated to predict the release of hydrophilic solvents [61].

Fuzzy logic is an extension of classical two-valued logic that defines the intermediate states of truth between the two logical states, such as on or off, true or false, and processes based on the degree to

which a statement is true or false. The foundation of fuzzy reasoning lies with the construction of fuzzy sets, allowing one to specify a particular value or argument in a word form based on a membership function. This approach, even if not strictly logical, allows the capture and modeling of human reasoning. Fuzzy sets are an advancement in the field of data categorization. Fuzzy rules are the core of fuzzy reasoning, allowing reasoning about data and uncertainty [65][66][67].

I.4.2. Applications of AI in biomedical modeling

The particular challenge of drug delivery systems lies in the different ways in which they release drugs. The timing, dosing, or location of release can all very well affect the effectiveness and safety of the treatment. For that reason, it is a relatively recent and interesting field in which AI is being utilized. The idea of using artificial intelligence in drug delivery systems is no longer new. Although it is still a very new idea, many new products of this technology and platform either reached clinical trials or introduced to the market. On the other hand, a more specialized application, patient monitoring and drug treatment, may also be suitable and could benefit from many others developed main algorithms and techniques [68].

Artificial intelligence (AI) and machine learning (ML) can automate and link together the many different stages in pharmaceutical discovery and drug delivery. Since the number of chemical compounds that researchers need to investigate is so high, any computational approach that reduces the number of experiments that have to be conducted in the lab is of great practical advantage [68]. At a fundamental chemical level, AI can help:

- Generate new molecular components for use in drug delivery;
- Identify new potential therapeutic targets;
- The computational prediction of binding conformations.

Speed is another critical factor: shorter discovery times mean reduced costs and faster times to market for potential new drugs, enabling the development of highly safe, effective, and affordable drug delivery systems. AI is helping researchers achieve that goal, providing a systematic strategy for streamlining discovery and delivery.

The study of Samia Rebouh et al. focuses on the drug release behavior of cellulose derivative (CD) matrices in tablet form using ibuprofen (IB) as the model drug. To optimize sustained drug delivery, the researchers employed an Adaptive Neural-Fuzzy Inference System (ANFIS) to predict the best formulation parameters. Various cellulose derivatives (CMC, HEC, HPC, HPMC, MC) were used,

with the active ingredient ranging between 20-50%. The tablets were evaluated for their flow properties, pharmacotechnical qualities, and drug release characteristics [69].

Results showed that the drug release rate increased with higher drug loads (Figure I.8). The Korsmeyer-Peppas equation revealed that multiple drug release mechanisms were involved, influenced by the strength of the gel layer formed by CDs. HPMC had the highest mean dissolution time (MDT), sustaining drug release for over 14 hours. The ANFIS model accurately predicted the drug release behavior (with high R^2 and low error rates), demonstrating that this system can be an effective tool for designing new dosage systems [69].

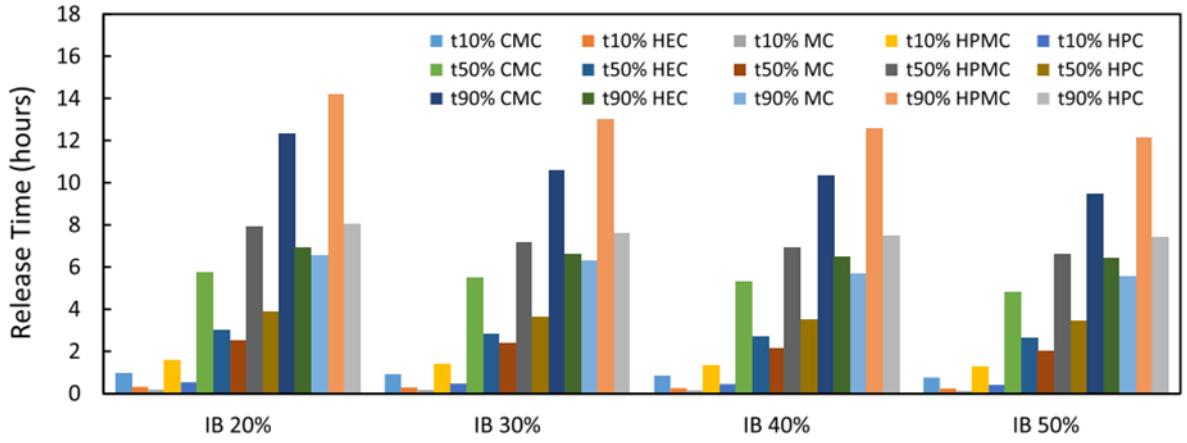


Figure I.8. Effect of the formulation composition on the 10%, 50%, and 90% drug release [69].

I.4.3. Differeces between the AI models and traditional methods

Before the emergence of AI, the modern data analysis community relied on traditional methods for the purpose of problem-solving, planning, prediction, and other activities.

These traditional methods are recognized by both academicians and practitioners, and some of them are used as a benchmark to compare the performance of modern AI systems. Although it is difficult to make a clear definition of traditional methods, they are defined here as the methods developed before the advent of AI as a subject. The major contribution of modern methods is that they have enhanced the deficiencies of traditional methods [70].

Traditional methods are classified as rule-based systems and heuristics-based models. However, a claim can be made that traditional methods were not data-driven; the performance of the earlier methods was based on domain-specific rules and expert knowledge. The results were dependent on

the set of rules, and the model gave different results if the rules were changed. Traditional methods were static. Developing a new rule-based approach or training a new model from scratch for new data points cannot be applied easily in traditional methods. If the domain knowledge grows or new facts are discovered, a large chunk of the existing model might have to be changed; models based on correlations or rules could fail when presented with new data points [71].

AI models are capable of managing and adapting to different types of datasets, unlike traditional methods. Several types of AI already exist, such as weak AI, strong AI, supervised AI, unsupervised AI, semi-supervised AI, and reinforcement AI. Furthermore, AI can be divided into three primary categories: machine learning, neural networks, and deep learning; a mixture of the last two items is also popularly referred to as deep learning. The main difference between these is in the first item's capacity: machine learning, in general, is the least flexible and least adaptive system, while deep learning is the most adaptable [72]. A high-performing AI system's accuracies range from 90% to 99%.

The performance of a model in both advanced analytics and traditional methods relies on three criteria: effectiveness, efficiency, and accuracy. These metrics are mainly used to compare models. Effectiveness is the measurement of a method's success in achieving its purpose. The success of a classification model is assessed by measuring different performance measures such as accuracy, recall, precision, ROC AUC, and F1 score [72]. Efficiency is the measurement of how effectively the method achieves its purpose. Accuracy is the measurement of how close the estimated classification of class probability is.

I.5. Conclusion

The versatility of hydrogels, stemming from their tunable properties and biocompatibility, has made them importants in the field of drug delivery. Innovative strategies must be developed to improve drug delivery. Hydrogels hold promise in this area because they can protect drugs from deactivation and increase shelf life. They can provide a controlled and sustained release of drugs, protect sensitive molecules from the environment, and be engineered to biodegrade and release their cargo over time, increasing patient compliance [73]. Traditionally, several networks of hydrogels have been modeled by a spring chain/rubber lattice model, freely jointed chain model, a model, and later by the other general models that contain the anharmonic contributions of the bonds in the monomer unit. This is due to a need to predict and control dynamic and complex behaviors of the polymeric materials employed in different areas [35]. It is clear that we are now at a point where the existing computational and mathematical delivery tools can be powered by big data and machine learning methods to uncover

not only where and when the drug delivery occurs in the body, but more importantly, predict how these processes happen at cellular and molecular activity, redesign these processes, and predict and even stop diseases in advance of their occurrence [74]. To promote further development in this area, integrating other disciplines such as material science, biomedical engineering, and pharmacology can be important. Collaborative and interdisciplinary work can quickly advance the materials science and development of hydrogels and their properties [75].

As the application of AI in drug delivery systems continues to evolve, it is essential to address the challenges and embrace the opportunities that lie ahead. By fostering collaboration between AI experts, pharmaceutical scientists, and healthcare professionals, we can harness the power of AI to develop innovative, personalized, and effective drug delivery solutions. The future of drug delivery is bright, and AI will undoubtedly play a pivotal role in shaping it.

Chapter II

Materials and methods

II.1. Introduction

This chapter is dedicated to the presentation of all materials and methods used in each experimental step of this study. A detailed description is provided of the operating procedures applied for the synthesis and characterization of hydrogels and drug loading, including various physico-chemical methods such as FTIR, SEM and NMR analyses.

We explore the application of Neuro-Fuzzy Modeling to elucidate the intricate dynamics of drug release kinetics, aiming to enhance our comprehension of the hydrogel's performance in drug delivery applications. Furthermore, the hydrogel's drug release kinetics are systematically investigated using Korsmeyer-Peppas Kinetic Modeling, providing a fundamental understanding of the release mechanism. The combined utilization of these methodologies ensures a thorough analysis of the synthesized hydrogel's structure, composition, and drug release behavior [1].

II.2. Hydrogel Design and Synthesis

II.2.1. Selection of materials

II.2.1.1. Chemical reagents and Raw materials

a) **Insulin glargine solution:** Provided by Sanofi (Algeria), insulin glargine is a synthetic liquid form of insulin designed to provide a slow, steady release to help control blood sugar levels over an extended period. It is used in the management of type I and type II diabetes. Insulin glargine is stable in the presence of an acidic pH, and it is liquid under these acidic conditions. After injection, the acidity is rapidly neutralized in the hypodermis, and glargine then precipitates, forming microcrystals [2]. This insulin causes the body tissues to absorb glucose from the blood and decreases the production of glucose by the liver. Basal insulin requirements in diabetes are met by insulin glargine due to its unique properties. It is insulin derivative due to the addition of two arginine molecules to the amino acid asparagine of native human insulin at position A21. The substitutions have been: Asn (A21) replaced by Gly and two arginine molecules attached to Gly at the B-chain through a lysine spacer [3]. Insulin Glargine is derived from human insulin. It has two polypeptide chains:
A-Chain (21 amino acids) modified A21 (Glycine) replaces native asparagine.
B-Chain (30 amino acids) extended with two arginine residues at the C-terminus (B31 and B32). Insulin Glargine differs from native human insulin through two main changes. The first one is the replacement at the C-terminus of the B-chain: Two arginine residues are added at positions B31 and B32. The arginine residues shift the isoelectric point of insulin glargine,

making it less soluble at neutral pH. The second one is the substitution in the A-chain at position A21, asparagine is replaced with glycine. Insulin glargine precipitates in the tissue, allowing slow, continuous release over time and providing a prolonged duration of action.

Insulin glargine is soluble in an acidic solution (pH 4). Upon injection into the neutral subcutaneous tissue (pH ~7.4), it forms micro-precipitates which are slowly release small amounts of insulin glargine, providing a basal level of insulin for up to 24 hours. This insulin's molecular formula and Molecular Weight are $C_{267}H_{404}N_{72}O_{78}S_6$ and ~6063Da, respectively.

b) **Carbomer 941:** Provided by Orkila (Algeria), carbomers are synthetic high molecular weight non-toxic polymers primarily derived from acrylic acid and cross-linking esters bonded with divinyl glycol/allyl ethers of sucrose or pentaerythritol [4]. The chemical formula of carbomer 941 is $C_{42}H_{80}O_8$. The molecular weight of this type of carbomer is 713.1 g/mol. The pKa of Carbomer 941 is 6.0 ± 0.5, indicating that the carboxyl groups on the polymer are predominantly ionized at pH values near this value. Carbomers are used to stabilize emulsions and thicken with the appearance of an aqueous gel in the pharmaceutical or cosmetic industry. Aminomethyl propanol allows them to be stabilized in the form of a gel.

c) **Agaros**e: Supplied by Orkila (Algeria), agarose is a polysaccharide derived from agar, which is a substance extracted from certain types of red algae, where the repeat unit consists of D-galactose and 3,6-anhydro-L-galactose.. It is a yellowish-white, odorless solid powder, readily soluble in hot water. Agarose has widespread usage in the biological industry, particularly in the areas of separation of biological products, production of prescription drugs, and the pharmacological industry. the typical molecular weight for agarose is approximately 630.5 g/mol, with a formula of $C_{24}H_{38}O_{19}$.

d) **Glycerol:** Provided by Sigma Aldrich (Germany), glycerol's international chemical name is trihydroxypropane glycerol, 1,2,3-propanetriol. Glycerol is a simple polyol compound, a colorless, odorless, viscous liquid that is sweet-tasting and non-toxic, containing three hydroxyl groups, allowing the possibility of creating rigid polymeric networks after crosslinking polycondensation reactions. It is miscible with water, alcohols, and glycols, with insolubility in oils and hydrocarbons [5]. Glycerol is used as an emollient; antimicrobial conservator or solvent in parenteral and non parenteral preparations.

e) **Propylene glycol:** Also known as 1,2-propanediol, 1,2-dihydroxypropane, methyl glycol or methyl ethylene glycol, propylene glycol is a colorless, clear and viscous liquid that is virtually odorless with a mild and slightly bitter taste. It is a hygroscopic liquid that is soluble in water, acetone, and chloroform. The versatility of propylene glycol in polymer chemistry, particularly regarding its utilization as a crosslinking reagent, extends to numerous applications across various industries [6]. It is used in pharmaceutical products, including intravenous, oral and topical medications as conservator, solubilizer or extractant. Propylene glycol was donated by Sigma Aldrich (Germany).

f) **Reagents:** The solvents and chemical reagents used in this study (Table II.1) are of analytical grade and supplied by Sigma Aldrich (Germany).

Table II.1. List of chemical reagents and solvents.

Reagents	Chemical formulas
Sodium chloride	$NaCl$
Sodium hydroxide	$NaOH$
Disodium phosphate	Na_2HPO_4
Potassium phosphate monobasic	KH_2PO_4
Potassium chloride	KCl
Triethylamine (TEA)	$N(CH_2CH_3)_3$
Deuterium oxide	D_2O
Hydrogen peroxide	H_2O_2
Anhydrous sodium sulphate	Na_2SO_4
Phosphoric acid	H_3PO_4
Ethanolamine	$NH_2CH_2CH_2OH$
Acetonitrile	CH_3CN

II.2.1.2. Equipment

All the equipment used in this present study is listed in Table II.2.

II.2.2. Synthesis and characterization methods for hydrogels

II.2.2.1. Hydrogel synthesis and drug loading

Phosphate-buffered saline (PBS) is a buffer solution (pH ~ 7.4) prepared by mixing 8g of Sodium chloride, 0.2g of Potassium chloride, 1.42g of disodium hydrogen phosphate and 0.24g of potassium dihydrogen phosphate in 1 liter of distilled water.

The hydrogel was prepared through a microwave- assisted polycondensation reaction between two polymers: carbomer (Ca) and agarose (Ag), along with cross-linking reagents – propylene glycol (PG) and glycerol (Gly). This was carried out according to the method outlined by Santoro et al.[7], with some minor modifications. To start, 1g of Ca was dissolved in 100ml of Phosphate-Buffered Saline (PBS) solution (pH = 7.4), which is a solution commonly used in biological and chemical research to maintain the pH of a solution and mimic the ion concentration of the human body. PBS solution contained Gly and PG at a determined concentration. After that, Triethylamine (TEA), a commonly used organic base in chemistry, was added gradually until the solution reached neutral pH.

Table II.2. Different equipment used.

Photo			
Name	pH meter	Vacuum oven	HPLC
Brand	Metrohm - 827 ph lab (Switzerland)	Thermo Scientific™ Vacutherm	Waters alliance 2695 (United States)
Photo			
Name	FTIR Spectroscopy	Microwave Solo 23L	Dissolutest
Brand	Bruker Tensor II (United States)	Samsung (South Korea)	Sotax At7 (United States)
Photo			
Name	NMR Spectroscopy	Scanning electron microscopy	Analytical balance
Brand	Bruker 400 MHz (United States)	ESEM-FEI QUANTA 250 (Switzerland)	Radwag AS 220.R2 PLUS (Poland)

Subsequently, 1gr of Ag was added to the above solution, which was kept at 80°C under magnetic stirring, and the mixture was left to rest at room temperature. The mixture was then left to rest at room temperature before undergoing a fixed microwave irradiation of 300W using a Samsung MS23F301TAW SOLO microwave oven. The hydrogels were then formulated and left at room

temperature for 24h. The hydrogel compositions of the formulations that were tested can be found in Table II.3.

The insulin glargine-based solution was then added to the polymeric system that had been prepared earlier using a volumetric ratio of 50/50 v/v.

Table II.3. Composition of insulin-loaded hydrogel formulations.

Hydrogel formulas	[PG] (%)	[Gly] (%)	PT: Polycondensation time (s)
F1	1	0	0
F2	0	1	10
F3	0	1	30
F4	0	2	20
F5	30	0	10
F6	30	0	20
F7	30	0	30
F8	30	1	10
F9	30	1	20
F10	30	1	30
F11	30	2	10
F12	30	2	30
F13	60	0	20
F14	60	1	10
F15	60	1	30
F16	60	2	20

II.2.3. Physico-chemical characterizations

The obtained hydrogels underwent a series of physico-chemical characterizations.

II.2.3.1. Fourier-transform infrared spectroscopy (FTIR)

Infrared spectrometers are universal tools for the characterization and identification of solid, liquid, and gaseous substances. Of all infrared (IR) tools and techniques, the FT-IR is the most important one. Its advantages include speed, low detection limit, sensitivity, and a broad range. One of the most important parameters for FTIR spectrometers is spectral resolution [8]. FTIR is classified as a computational technique for infrared spectroscopy. The principle is derived from the acquisition of signals in the time domain, which can then be mathematically transformed into a frequency domain spectrum. The FTIR analysis was performed on the polymers and hydrogels using an FTIR spectrophotometer. Approximately 10 mg of each sample was placed on the diamond cell of the spectrophotometer. The spectra were recorded over a range of wavenumbers between 4000 - 400 cm^{-1} using the data processing software (OPUS 6.0).

II.2.3.2. Scanning Electron Microscopy (SEM)

The scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning its surface with a focused beam of electrons. The electrons interact with the atoms of the sample, producing various signals that contain information about the surface topography and composition of the sample. The beam position is combined with the intensity of the detected signal to produce a three-dimensional image [9].

The morphological study of each hydrogel was conducted at 25 ± 0.5 °C using the scanning electron microscope with the environmental instrument (ESEM-FEI QUANTA 250) at CRAPC in Algeria. After desiccation in a vacuum oven, the samples were assessed at a voltage of 10 kV and a magnification of x3000 [1]. The micrographs were analyzed using the data processing software (xT microscope control 1.0).

II.2.3.3. Proton nuclear magnetic resonance spectroscopy (NMR)

Proton nuclear magnetic resonance (NMR) spectroscopy is a powerful tool for studying the structure of organic and bio-organic compounds. Proton NMR uses the magnetic properties of the nucleus of the hydrogen (proton) atom to determine the physical and structural properties of chemicals [10]. Nuclear Magnetic Resonance (NMR) is based upon the interaction of monochromatic electromagnetic radiation having a wavelength within the radiofrequency range with atomic nuclei, which behave like spinning charges generating magnetic moments [11].

Polymers, Cross-linking system and hydrogels were analyzed by a Bruker Avance III HD spectrometer operating at a proton frequency of 400 MHz, equipped with a 5 mm diameter liquid BBO probe, set for the observation of nuclei between ^{31}P and ^{15}N for the internal coil and the external coil is adjusted to H^1 for decoupling and observation at the same time [1].

Before NMR analysis, chemical depolymerization is required using hydrogen peroxide in alkaline solution to reduce the viscosity of aqueous solutions of the agar. 7.4 µL of NaOH (1 M) and 25 µL of H_2O_2 are added to 1.5 mL of a 6 g/L agar solution in D_2O. The solution is heated to 80°C for 1 hour with stirring then cooled in an ice bath for 30 minutes before drying.

The analyzed samples were prepared by dissolving an amount of 10 mg of each in a volume of 500 µl of deuterated solvent D_2O, and placing them in a 5 mm diameter borosilicate glass NMR tube. All 1H spectra were acquired with the conditions: Pulse program zg 30°, TD:65536, Acquisition time: 4 sec,

Rga:181, DE: 6 u sec, Spectral width: 20 ppm, Number of scans NS =128 scan, P1: 9 u sec and PLW1: 18.86 W.

II.3. AI-Based Modeling Techniques

II.3.1. Description of AI techniques used

II.3.1.1. Neuro-fuzzy modeling for drug release kinetics

This study proposes an Adaptive Neuro-Fuzzy Inference System (ANFIS) model to predict pharmaceutical properties, renowned for effectively modeling complex data sets [12][13]. Fuzzy logic assesses relationships between uncertain input variables x and output membership functions l ranging from 0 to 1 [14]. Fuzzy "if-then" rules take the format "if x is A, then y is C" where A and C are linguistic values defined by membership functions like triangular, trapezoidal, or Gaussian [14][15]. ANFIS combines the learning capabilities of neural networks with the interpretability of fuzzy logic to model nonlinear pharmaceutical systems like hydrogels for optimized drug delivery, aiming to overcome limitations in current treatment methods.

$$A = \{x, \mu_a(x)/x \in X\} \quad \text{(II.1)}$$

The model elements of a fuzzy inference system are shown in Figure II.1.

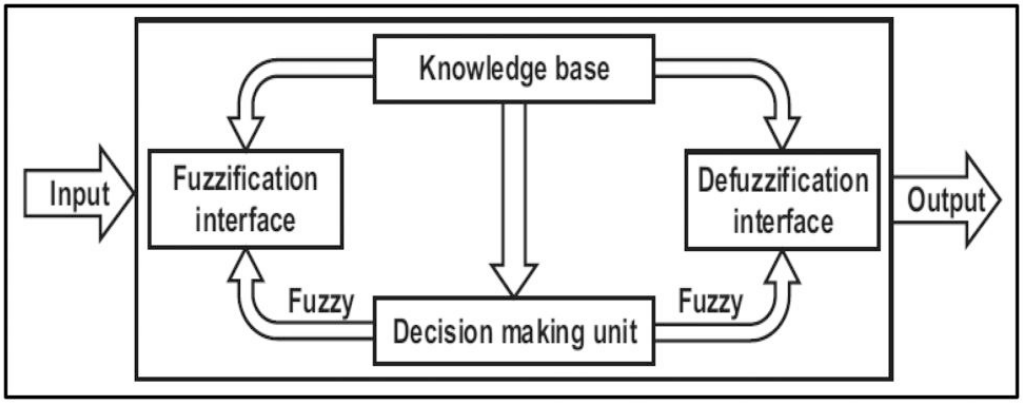


Figure II.1. Fuzzy inference system.

The ANFIS model contains a knowledge base of if-then fuzzy rules defining membership functions [16][17]. It fuzzifies crisp inputs into degrees of linguistic values, executes inference on rules, then defuzzifies results into a crisp output. This study uses a first- order Takagi-Sugeno model, suitable for adaptive modeling combined with neural networks to predict properties of complex systems. If x_1 is A_1 and x_2 is A_2 and x_3 is A_3 then :

$$y = f(x_1,\ x_2,\ x_3) \tag{II.2}$$

A_1, A_2 and A_3 are fuzzy sets in the antecedent, y is a crisp function in the consequent. A fuzzy inference known as first-order Sugeno fuzzy model occurs when $f(x,y)$ is a first-order polynomial (Figure II.2). Particularly, a zero-order Sugeno fuzzy model is obtained when $f(x,y)$ is a constant.

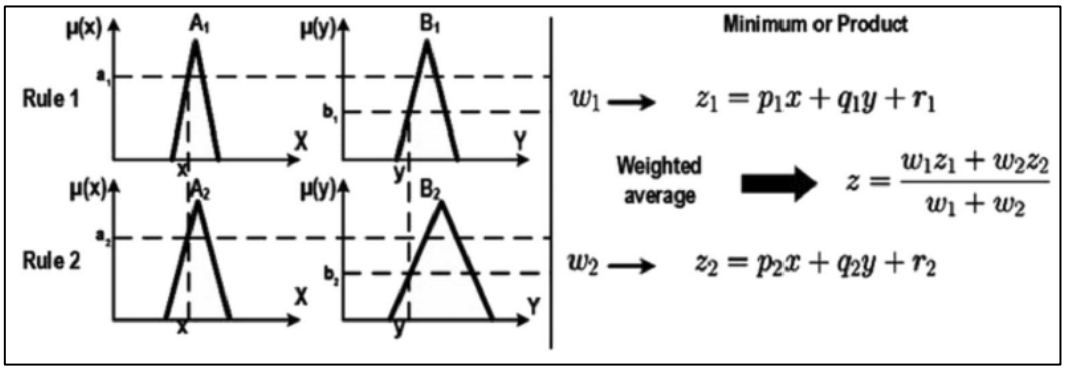


Figure II.2. First-order Sugeno model.

II.3.1.2. ANFIS architecture

The architecture of ANFIS is illustrated in Figure II.3. Circular nodes represent fixed operations, while square nodes portray adaptive functions with learnable parameters. The ANFIS consists of five layers, each performing specific functions [18].

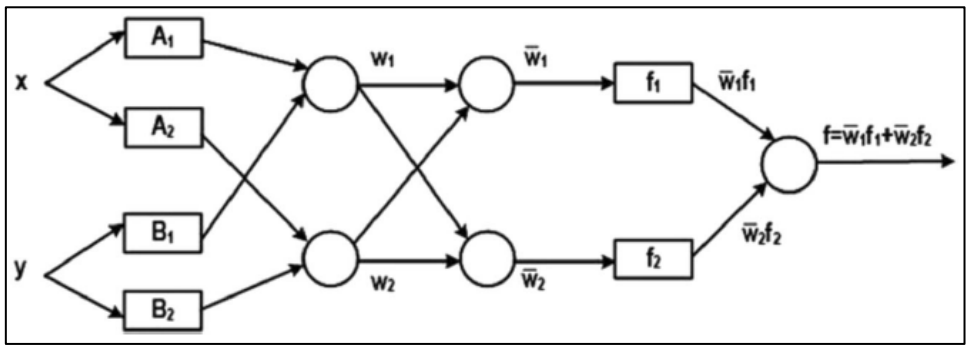


Figure II.3. ANFIS architecture.

Layer 1: The fuzzification process occurs in this layer where all the nodes are adaptive. The outputs of this layer constitute the membership values of the assumption part. Each node's output was given by Equation (II.3):

$$O_{1,i}(x) = \mu_{A_i}(x) \quad \text{For } i = 1, 2$$
$$O_{1,i}(y) = \mu_{B_i}(y) \quad \text{For } i = 3, 4 \tag{II.3}$$

$O_{1,i}(x)$ and $O_{1,i}(y)$ are the membership functions for *x* and *y*, respectively.

The Gaussian membership function, given by Equation (II.4), was used because it provides better results according to Table II.4.

$$\mu_A(x) = e^{\frac{-(x-c)^2}{2\sigma^2}} \quad \text{(II.4)}$$

Where σ is the standard deviation and c represents the mean.

Layer 2: In this layer, each node output represents the firing strength of a rule, and all nodes are fixed. The t-norm operator is used to compute the AND operation of the membership grades, as given by Equation (II.5).

$$O_{2,i}(x) = \omega_i = \mu_{A_i}(x)\mu_{B_i}(y) \quad \text{For} \quad i = 1,2 \quad \text{(II.5)}$$

Layer 3: In this layer the nodes are also fixed. The ratio between the firing weight of the i^{th} and the sum of all rule weights is calculated for the corresponding node. It contains fixed nodes which compute the ratio of the firing strengths of the rules according to Equation (II.6).

$$O_{3,i} = \overline{w_i} = \frac{w_i}{w_1 + w_2} \quad \text{(II.6)}$$

Layer 4: The nodes run as a function block in this layer, with its parameters being adaptive and variables (input values). TSK (Takagi-Sugeno-Kang) outputs are formed from the output of this layer; the nodes are adaptive and execute the consequences of the rules as represented by Equation (II.7).

$$O_{4,i} = \overline{w_i} = \overline{w_i}\,(p_i x + q_i y + r_i) \quad \text{(II.7)}$$

p_i, q_i and r_i are parameters to be learnt. These are the premise parameters. All this layer parameters (p_i, q_i, r_i) should be determined and are identified as the consequent parameters.

Layer 5: It sums all the input signals to generate the output. In this layer, there is only one node that calculates the overall output, as given by Equation (II.8).

$$O_{5,i} = \sum_i \overline{w_i} f_i = \frac{\sum_i w_i f_i}{\sum_i w_i} \quad \text{(II.8)}$$

The hybrid learning algorithm proposed by Jang et al. was used for ANFIS training in this work. This algorithm combines the gradient descent backpropagation and least squares estimation (LSE) to optimize the ANFIS parameters [19].

Two sets of parameters are considered: premise parameters (S1) and consequent parameters (S2). S1 contains the parameters for all the selected input membership functions, while S2 contains the parameters of the output membership function [18].

ANFIS employs two passes for the learning algorithm:

Forward pass: S1 is fixed and S2 is calculated using LSE.

Backward pass: S2 is fixed and S1 is calculated using backpropagation.

The validation of the trained ANFIS model is performed using the remaining data for testing. Root mean square error (RMSE), from Equation (II.9), is used to assess the model's predictive performance [19].

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(y_i - \hat{y})^2} \quad \text{(II.9)}$$

Where N is the count of the tested data, $y_i - \hat{y}_i$ is the difference between the i^{th} desired and predicted outputs of the model, respectively.

II.3.2. Justification for the chosen techniques

The selection of ANFIS as the modeling technique for predicting insulin release from the hydrogel formulations was based on several key factors:

1. ANFIS combines the benefits of fuzzy logic, which can handle uncertainty and imprecision in the input parameters, with the learning capabilities of neural networks [12,13]. This allows it to model the complex nonlinear relationships between the formulation parameters and insulin release rate.

2. ANFIS has demonstrated success in various pharmaceutical applications, such as modeling drug release from sustained release tablets and predicting adsorption of drugs on modified excipients. These previous studies support its suitability for our hydrogel drug release modeling task.

3. The interpretability of ANFIS models, owing to the fuzzy rules, provides insights into the impact of each input parameter on the output [14,18]. This is valuable for understanding the underlying mechanisms and optimizing the hydrogel formulations.

4. ANFIS is computationally efficient compared to other AI techniques like deep learning, requiring fewer training samples and converging faster during training. This is advantageous given the limited experimental data available in our study.

Therefore, ANFIS was chosen as a powerful yet interpretable technique that aligns with the characteristics of our modeling problem and dataset. Its ability to handle uncertainty, model nonlinear relationships, and provide insights make it a suitable choice for predicting and optimizing insulin release from the hydrogel formulations.

II.3.3. ANFIS modeling setup

The ANFIS model, which played a crucial role in predicting the insulin release rate from hydrogel formulations, was developed based on three crucial formulation parameters: glycerol (Gly) concentration (%), propylene glycol (PG) concentration (%), and polycondensation time. These parameters were selected due to their significant influence on the hydrogel's structural properties and consequent drug release behaviour, as evidenced by previous studies [1][7]. The glycerol and propylene glycol concentrations affect the crosslinking density and pore size of the hydrogel network, which in turn impact the diffusion and release of insulin. The polycondensation time determines the extent of the crosslinking reaction and the resulting mechanical strength of the hydrogel, also influencing the drug release kinetics. Therefore, these three parameters collectively capture the key factors governing insulin release from the Ag-Ca hydrogel system. The single output of the ANFIS model was dedicated to predicting the corresponding insulin release rate, providing a valuable tool for understanding the relationship between formulation parameters and drug release behaviour (Figure II.4).

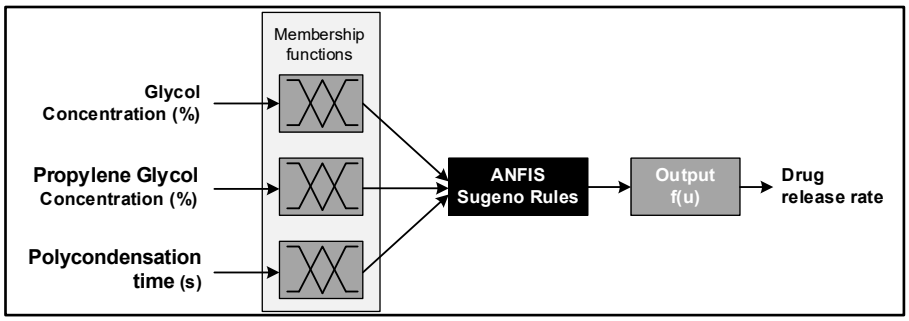


Figure II.4. Studied ANFIS architecture.

Figure II.4. Studied ANFIS architecture for predicting insulin release rate based on glycerol concentration, propylene glycol concentration, and polycondensation time. In the creation of the ANFIS models, a systematic approach was employed. We initiated the process by collecting a comprehensive dataset encompassing the specified formulation parameters and their corresponding insulin release rates. Following the acquisition of this dataset, thorough preprocessing was applied to address missing data, outliers, and the normalization of input features. This preprocessing step is crucial to ensure data quality and improve model performance. Missing data was handled using

appropriate imputation methods, while outliers were identified and treated based on statistical techniques. Feature normalization brings all inputs within a similar range, preventing any feature from dominating the learning process due to scale differences. This meticulous approach ensures equal contribution of all features to the learning process, preventing discrepancies in scales and fostering faster convergence, ultimately leading to enhanced performance in machine learning models.

The selection of input features was a critical step, involving careful consideration and application of feature selection techniques to enhance the models' predictive performance. Feature selection helps in identifying the most informative input variables, reducing model complexity and improving generalization. In this study, the three chosen formulation parameters (Gly concentration, PG concentration, polycondensation time) were confirmed to be the most relevant features based on domain knowledge and their known impact on hydrogel properties and drug release [1][7]. The ANFIS architecture was designed with a hybrid-learning algorithm that seamlessly integrates fuzzy logic and neural networks. This architecture underwent iterative refinement, using Matlab 2018a Software, to optimize its structure for accurate predictions identification [18]. The hybrid-learning algorithm combines the least squares method and backpropagation to optimize the ANFIS parameters. It helps in fine-tuning the membership functions and consequent parameters to minimize the prediction error. Figure II.5 depicts the evolution of training and checking errors using the RMSE formula defined in Equation (II.9). The training error steadily decreases over the epochs, indicating the model is learning the patterns in the training data. The checking error initially decreases but then starts to increase after a certain point, signifying potential overfitting. The optimal model is selected at the epoch with the lowest checking error before it begins to rise. Figure II.5. Evolution of training and checking errors during the ANFIS learning phase, showing decreasing training error and identification of optimal model at lowest checking error to avoid overfitting.

For model training and validation, the experimental dataset was strategically split, allocating 90% for training the ANFIS models and reserving the remaining 10% for validation purposes. This split ratio ensures sufficient data for model training while providing an unbiased evaluation of its predictive performance on unseen data. The training set is used to learn the model parameters, while the validation set helps in assessing the model's generalization ability and preventing overfitting. This approach ensured that the synthesized model was effectively trained while also providing a robust evaluation of their predictive capabilities.

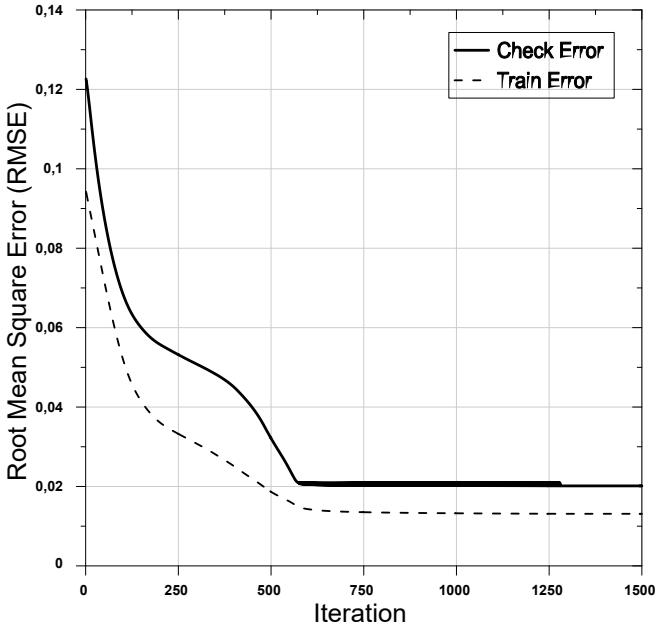


Figure II.5. Evolution of training and check errors during the learning phase.

Performance evaluation metrics based on RMSE (Equation (II.9)) was employed to assess the neuro-fuzzy models' accuracy. RMSE quantifies the average magnitude of prediction errors, providing a measure of how close the predicted values are to the actual observations. A lower RMSE indicates better model performance and accuracy. The correlation coefficient (R^2) is also used later for the final prediction test, to evaluate the model's efficacy in capturing and explaining variance in the predicted outcomes. R^2 represents the proportion of variance in the output variable that is predictable from the input variables. An R^2 value closer to 1 suggests a strong predictive relationship between the inputs and output, indicating the model's goodness of fit. This comprehensive evaluation strategy ensures a thorough assessment of the model's performance across various dimensions, contributing to the robustness and validity of our findings.

II.3.4. Optimizing parameters for ANFIS

The core parameters optimized in developing our ANFIS models include the number of membership functions (MFs), MF type, epoch number, and the hybrid learning algorithm approach. Various membership functions, such as trapezoidal, triangular, Gaussian, and Bell, are subjected to testing. The choice of membership function influences the model's ability to capture the input-output relationships. Gaussian and Bell MFs are commonly used due to their smoothness and differentiability, which facilitate the learning process. To select the optimal number of MFs for each input, we tested from 2 to 10 MFs, evaluating the validation data fit after ANFIS training, a standard methodology as discussed in reference [20]. Increasing the number of MFs enhances the model's flexibility to capture complex

patterns but may lead to overfitting. The optimal number of MFs strikes a balance between model complexity and generalization performance. We selected the Gaussian MF based on its common effectiveness for prediction modeling [21]. The Gaussian MF is defined by two parameters: mean and standard deviation. It provides a smooth and continuous representation of the input space, enabling the model to learn intricate relationships.

The tuning of the number of epochs aimed to prevent overfitting to the training data, as highlighted in reference [22]. An epoch represents a complete pass through the training dataset. Too many epochs can cause the model to memorize noise in the training data, leading to poor generalization. The optimal number of epochs is determined by monitoring the validation error and stopping the training when it starts to increase, indicating overfitting. Additionally, the utilization of the hybrid learning algorithm involved a combination of both backpropagation and least squares methods in an adaptive manner. This approach has demonstrated enhanced model convergence compared to using either method alone, as mentioned in reference [23]. The hybrid learning algorithm leverages the strengths of both techniques - least squares for optimizing consequent parameters and backpropagation for tuning premise parameters. This synergistic combination accelerates convergence and improves prediction accuracy.

Through a process of iterative model training and parameter tuning, we identified that [3 3 4 4] is an optimized combination of the number of membership functions for the ANFIS model. This configuration, with 3 MFs for Gly concentration, 3 MFs for PG concentration, and 4 MFs each for polycondensation time and insulin release rate, provided the best balance between model complexity and predictive performance on the validation set. This configuration exhibited robust predictive capabilities. Validation metrics substantiate its strong accuracy in predicting outcomes on previously unseen test data. Based on the RMSE criteria presented in Table II.4, the conclusive findings reveal that the final variant demonstrates a significant performance superiority over the alternative configurations.

Table II.4 compares the RMSE values of different ANFIS configurations, highlighting the selected optimal model with the lowest RMSE. This model, with the [3 3 4 4] MF configuration, Gaussian MF type, and optimized epoch number, outperforms other variants, confirming its effectiveness in predicting insulin release rates. Table II.4. Optimal ANFIS performance achieved during the training phase, comparing different model configurations based on RMSE. The selected model with the lowest RMSE is highlighted in grey

Table II.4. Optimal ANFIS performance achieved during the training phase.

N°	Input MFs	Output MF	Iterations	Number of Membership functions	RMSE_Train	RMSE_Check
1	Tri	Cst	31	3 3 3 3	0.0432336	0.0672334
2	Tri	Lin	37	3 3 3 3	0.0101527	0.3228540
3	Tra	Cst	225	3 3 3 3	0.0438314	0.0663807
4	Tra	Lin	703	3 3 3 3	0.0161192	0.0574563
5	Bell	Cst	878	3 3 3 3	0.0146106	0.0250171
7	Bell	Lin	1579	3 3 3 3	0.0051974	0.0741582
8	Gauss	Cst	612	3 3 3 3	0.0318528	0.0563337
9	Gauss	Lin	378	3 3 3 3	0.0135806	0.0469595
10	Gauss2	Constant	1359	3 3 3 3	0.0133545	0.0218342
11	Gauss2	Lin	839	3 3 3 3	0.0065507	0.0853260
12	Gauss2	Constant	709	4 3 3 4	0.0121275	0.0217536
13	Gauss2	Constant	451	4 4 4 4	0.0123172	0.0217685
14	Gauss2	Constant	821	3 4 4 3	0.0130822	0.0214744
15	Gauss2	Constant	516	3 3 4 4	0.0121218	0.0216965
16	Gauss2	Constant	1500	4 4 3 3	0.0132978	0.0215965

II.4. Experimental Protocols

II.4.1. Experimental design for model validation

II.4.1.1. kinetic study

The kinetic experiment followed the procedure specified in the European Pharmacopoeia [1]. The hydrogel samples containing the drug were loaded into a polypropylene bag and placed in the basket of a Sotax AT7 dissolution tester. A dissolution medium of 900 ml, PBS 7.4 was added to the rotating cylinder, and the system was maintained at 37°C with a rotation speed of 50 rpm. 5 mL samples were regularly collected from the dissolution medium every hour for chromatographic analysis using the Waters Alliance 2695 HPLC.

Mobile Phase A: 28.4 g of anhydrous sodium sulfate R in the water R for 1000 ml. Add 2.7 ml of phosphoric acid and adjust it, if necessary, at pH 2.3 with the ethanolamine R. filter and degasify. (European Pharmacopoeia, 2016)

Mobile Phase B: Mix 550 ml of the mobile phase A and 450 ml of acetonitrile R, heat the solution at 20°C or more, to avoid precipitation.

Proceed with the elution with a mixture of:

- 42 Volumes of mobile phase A

- 58 Volumes of mobile phase B

At the Operating conditions :

- Wavelength : λ = 214 nm.
- Column Temperature : T = 40°C.
- Injection volume : 20 µl.
- flow rate : 1ml/min.
- A steel column (25cm, 4.6mm) filled with octadecylsilyl silica gel for R chromatography.
- An equal volume of fresh PBS 7.4 solution was added to maintain a constant volume.

The in vitro insulin release study from a hydrogel, conducted using a Sotax AT7 dissolution tester and analyzed through HPLC, involves a controlled laboratory experiment to mimic and measure the release of insulin from the hydrogel under specified conditions. Consequently, an ANFIS model is used in order to predict and optimize the insulin release based on the experimental data obtained from these in vitro studies. The ANFIS model is trained with diverse experimental data encompassing various formulation parameters and their corresponding insulin release profiles. This allows the model to learn the complex relationships between the input variables and the drug release behavior, enabling it to make accurate predictions for new formulations [12,13]. Figure II.6 depicts the *in vitro* insulin release study conducted using a hydrogel [1].

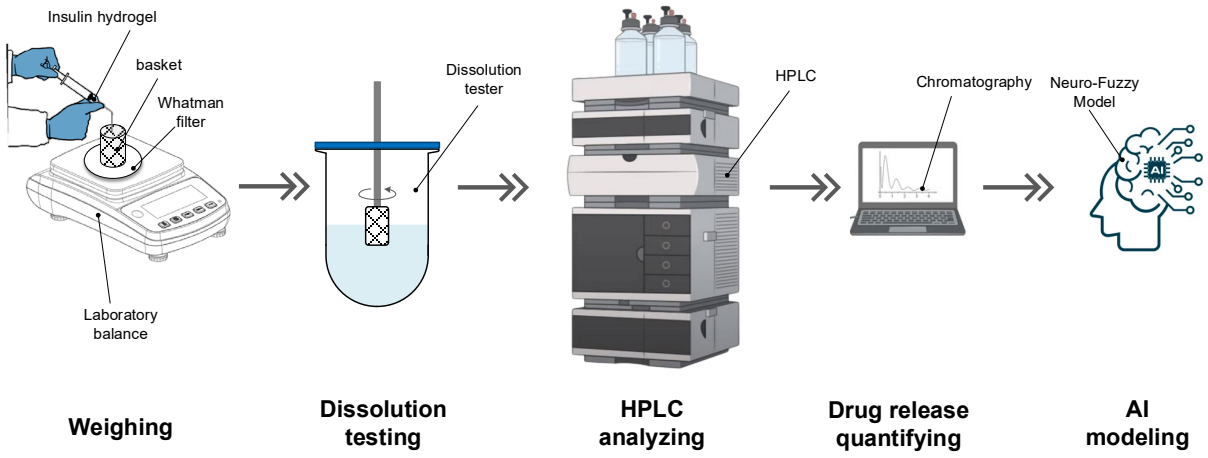


Figure II.6. *In vitro* study illustration.

II.4.1.2. Microbiological quality control

The manufacture of sterile or non-sterile medicinal products imposes specific requirements in order to reduce the risks of microbial and particulate contamination. The microbiological quality of medicinal products is one of the major concerns during formulation, in order to ensure the proper conservation and stability of the prepared pharmaceutical form. To this end, microbiological controls must ensure good hygienic and marketable quality of the manufactured product, and check for possible contamination that may be due to poor manufacturing conditions. The enumeration of bacteria, moulds and yeasts capable of growing aerobically makes it possible to verify the microbiological quality of the products [9].

At the level of the finished product, the pharmacopoeia [24] sets contamination limits based on the pharmaceutical form of the medication to ensure maximum safety. The pharmaceutical form is related to the stability of the active ingredient and the route of administration of the product. These two parameters explain why the acceptance criteria for microbiological quality differ depending on the pharmaceutical form. Microbiological quality focuses on the enumeration of total viable aerobic microorganisms (bacteria, yeasts, and molds) and the detection of specific microorganisms: Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans, Salmonella, and enterobacteria. The acceptance criteria applicable to pharmaceutical forms administered via the nasal mucosa, based on the total aerobic microorganisms count (TAMC), the total mould and yeast count (TYMC), as well as the detection of certain specified microorganisms, are provided in Table II.5. [24]. The microbiological quality of the F10 hydrogel containing insulin was evaluated according to the standards of the European Pharmacopoeia [24], by counting the total viable aerobic microorganisms, moulds and yeasts, Staphylococcus aureus, and Pseudomonas aeruginosa. This assessment ensures that the developed hydrogel formulation meets the required microbiological safety criteria for pharmaceutical products.

Table II.5. Acceptance criteria for the microbiological quality of pharmaceutical forms.

Microbiological control	**Acceptance criteria**
TAMC (UFC.g^{-1} or ml^{-1})	10^2
TYMC (UFC.g^{-1} or ml^{-1})	10^1
Specified microorganisms	Absence of Staphylococcus aureus
(1 g or 1 ml of sample)	Absence of Pseudomonas aeruginosa

II.4.2. Data Analysis and Korsmeyer-Peppas kinetic Modeling

The Korsmeyer-Peppas model equation is often used to describe drug release kinetics from pharmaceutical dosage forms. The equation is commonly cited in the context of drug delivery and release studies. One of the key references for the Korsmeyer-Peppas model is the original paper by Korsmeyer, Gurny, Peppas. It is given by Equation (II.10) [25]:

$$\frac{M_t}{M_\infty} = kt^n \qquad \text{(II.10)}$$

M_t/M_∞ being the released drug rate, k is the kinetic constant, n is an exponent indicating the release mechanism. When n = 0.43, Fickian controlled release happens, when 0.43< n <0.85, anomalous transport that refers to cases where the drug release behavior cannot be accurately described using traditional models of diffusion, such as Fick's law, and when n = 0.85, this indicates transport relaxation/erosion during drug release, which is the process of drug release caused by the deformation and relaxation of the polymeric network, and can ultimately result in a time-dependent release profile. The Korsmeyer-Peppas model was applied to the experimental drug release data obtained from the in vitro studies to elucidate the underlying release mechanisms of insulin from the Ag-Ca hydrogel system. The model parameters, k and n, were determined by fitting the equation to the release profiles using non-linear regression analysis. The value of the release exponent, n, provides insights into the dominant release mechanism. Fickian diffusion (n=0.43) suggests that the drug release is primarily governed by the concentration gradient, while anomalous transport (0.43<n<0.85) indicates a combination of diffusion and polymer relaxation. Case II transport (n=0.85) implies that the drug release is controlled by polymer relaxation and erosion. By analyzing the release kinetics using the Korsmeyer-Peppas model, we can gain a deeper understanding of the factors influencing insulin release from the hydrogel and optimize the formulation accordingly. This information is valuable for designing controlled release systems that meet the desired therapeutic requirements.

II.5. Conclusion

Hydrogels containing insulin were synthesized and subjected to in vitro drug release testing using HPLC. To validate the success of the polycondensation reaction, we employed FTIR spectroscopy, and our study delved into the impact of various polycondensation techniques on hydrogel microstructure through comprehensive SEM and NMR analysis. Our research involved the development of an ANFIS model specifically designed to predict insulin release from Ag-Ca based hydrogels. This model takes into account crucial factors such as cross-linking reagent concentration and polycondensation time, ensuring a high level of accuracy in its predictions. By leveraging the

power of neuro-fuzzy modeling, we can optimize the hydrogel formulation to achieve the desired drug release profile, ultimately improving the efficacy of insulin delivery [12][13]. Furthermore, the microbiological quality of the optimized hydrogel formulation was assessed according to the European Pharmacopoeia standards, confirming its suitability for pharmaceutical applications. The Korsmeyer-Peppas kinetic modeling provided valuable insights into the release mechanisms of insulin from the hydrogel, aiding in the rational design of controlled release systems. In conclusion, this study demonstrates the successful synthesis and characterization of insulin-loaded Ag-Ca hydrogels, along with the development of an ANFIS model for predicting and optimizing drug release. The combination of experimental techniques and computational modeling approaches enables the creation of advanced drug delivery systems with enhanced performance and safety [1].

Chapter III

Results and Discussion

III.1. Introduction

In this section a comprehensive analysis of the obtained, experimental data including FTIR, SEM, and NMR, as well as mathematical modeling using Korsmeyer-Peppas and ANFIS approaches. This section critically examines the influence of key formulation parameters – namely, glycerol and propylene glycol concentrations, PG/Gly ratio, and polycondensation reaction time – on the release kinetics of insulin from the developed matrix. Detailed interpretation of the results is provided to elucidate the underlying mechanisms involved in insulin release and optimize formulations to achieve a better release profile. The insights from this section advance our fundamental understanding of drug release from these matrix systems and provide key knowledge to further the development of optimized formulations for controlled insulin delivery [76].

III.2. Hydrogel Network Design

III.2.1. Key design parameters

Key design parameters include material and process factors that influence hydrogel synthesis, as well as those that affect insulin release kinetics [76].

Crosslinkers Concentrations (inputs)

The concentrations of crosslinkers (propylene glycol and glycerol) used in the polycondensation process are crucial to influence the degree of crosslinking, which affects the swelling behavior, stability, and insulin release rate of the hydrogel.

Polycondensation Time (input)

It is the duration of the chemical reaction that creates crosslinks between the agar and carbomer molecules. The polycondensation time influences the density and mechanical strength of the hydrogel network, which affects how quickly insulin can be released over time.

Release time (output)

The release time is the period during which the release of insulin from the hydrogel is monitored and controlled. It allows understanding the sustained release behavior of the hydrogel. Longer or shorter release times have an impact on the therapeutic profile of the inhaled insulin.

III.2.2. Influence of different parameters on hydrogel properties

III.2.2.1. Effect of the propylene glycol concentration

In this part of study, the polycondensation time and the Gly concentration were kept constant while the PG concentration was varied and the release results are shown in Figure III.1. The hydrogel formulation (F8), comprising 30% PG, exhibited a longer release of insulin over a period of 9.5h compared to the hydrogel with no PG added (F2), which took 8.5h to release insulin. However, the hydrogel formula with an excessive amount of PG (F14) exhibited similar behavior to the hydrogel formula without PG (F2). This suggests that an excess of PG may have an adverse effect on the release properties of Agar-Carbomer-based hydrogels [76].

These findings highlight the importance of optimizing the PG concentration to achieve the desired sustained release profile.

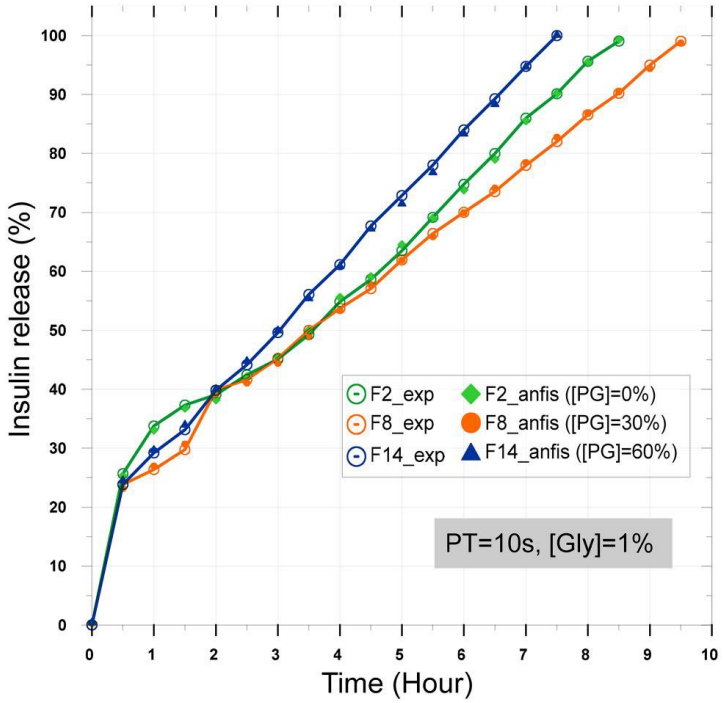


Figure III.1. Effect of the propylene glycol concentration on the insulin release kinetic (experimental and predicted results) [76].

III.2.2.2. Effect of the glycerol concentration on the insulin release kinetic

Figure III.2 demonstrates that, with a consistent polycondensation time and PG concentration, F5 without Gly exhibited the fastest release of insulin (7.5 h), accompanied by a burst release effect within

the first hour. Nevertheless, the hydrogels F8 (1%) and F11 (2%) showed prolonged release by the gradual addition of Gly, with release periods of 9.5 and 11h, respectively [76].

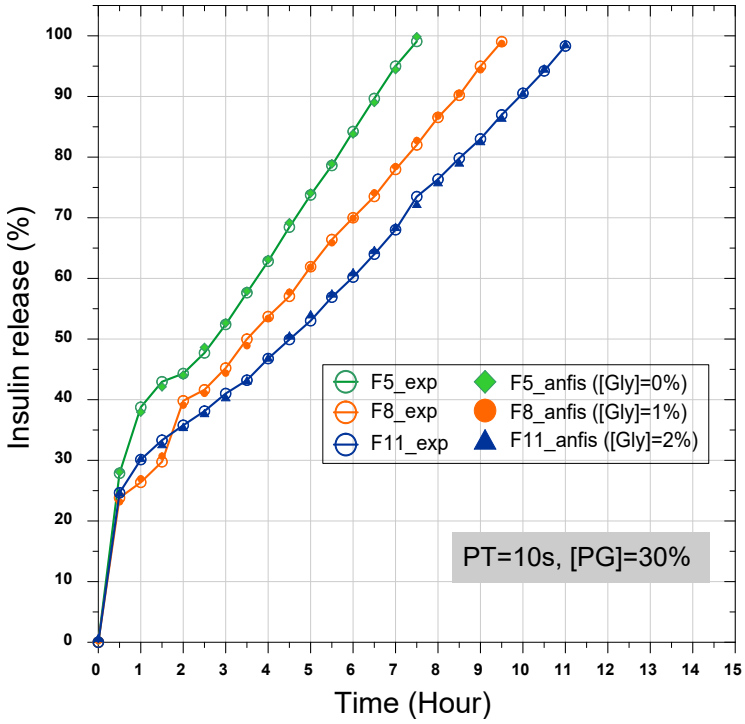


Figure III.2. Effect of the glycerol concentration on the insulin release kinetic (experimental and predicted results).

This indicates that the presence of glycerol plays a crucial role in modulating the release kinetics and mitigating the initial burst release of insulin.

III.2.2.3. Effect of the PG/Gly ratio

The PG/Gly ratio represents the concentration variation of both PG and Gly. By fixing the polycondensation time, we can analyze the impact of different PG/Gly ratios on insulin release. Figure III.3 presents the results obtained. It has been demonstrated that F4, which doesn't contain PG but only Gly (2%), gave the fastest release of insulin in 6.5h. It seems that formulation 15, which had no PG and only 2% Gly, gave the fastest release of insulin, while formulations 13 (with 60% PG and no Gly) and 16 (with the maximum levels of both PG and Gly) showed similar release profiles but reached the total amount of drug released in 8h, which cannot be considered as sustained release [76].

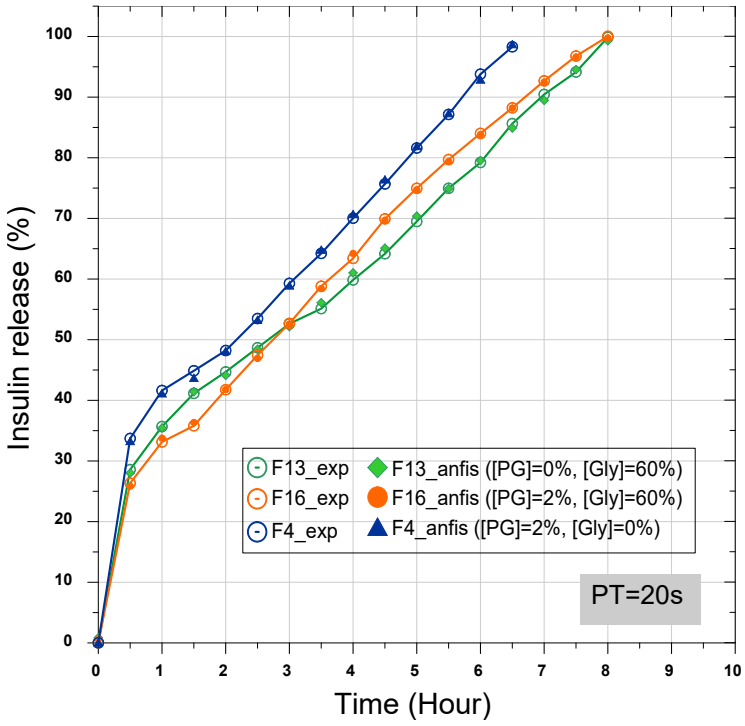


Figure III.3. Effect of the proportion of cross-linking agents on the insulin release kinetic (experimental and predicted results).

These results suggest that an optimal balance between PG and Gly concentrations is necessary to achieve the desired sustained release profile. Excessive amounts of either component can lead to suboptimal release characteristics.

III.2.2.4. Effect of the polycondensation time

The polycondensation time was varied while keeping the concentrations of PG and Gly fixed, and the increased release of insulin was observed. Figure III.4 demonstrates that the polycondensation time greatly affects the release profile. Specifically, F10 (crosslinked with 30% PG, 1% Gly, and subjected to 30s of microwaves irradiation) released insulin over a longer period compared to F8 and F9, which were crosslinked for 10 and 20s, respectively, and had identical crosslinking agent proportions. The insulin release time increased with increasing polycondensation time, with F8, F9 and F10 providing release times of 9.5, 11 and 13h respectively [76].

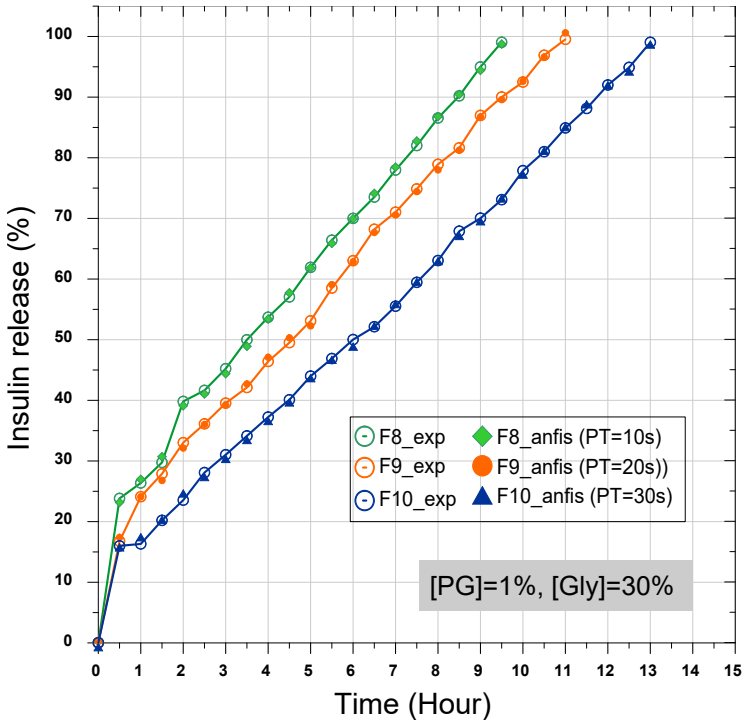


Figure III.4. Effect of the polycondensation time on the insulin release kinetic (experimental and predicted results).

This highlights the significance of the polycondensation time in controlling the crosslinking density and, consequently, the drug release kinetics. Longer polycondensation times lead to more extensive crosslinking, resulting in prolonged release profiles. The findings suggest that formulations with insufficient PG/Gly crosslinkers exhibited burst release and weak hydrogel formation. Gly alone facilitated higher rates of drug release during the burst effect, owing to its inherent ability to allow prompt drug release [99]. Moreover, a brief polycondensation reaction time thwarted the formation of hydrogen bonds with Ca.

An enhancement in drug release profiles was noted by increasing PG and Gly concentrations simultaneously up to 30 and 1%, respectively, showing sustained release over time. Formula F10, which comprised moderate amounts of PG and Gly and was allowed a maximum polycondensation time of 30s, displayed the longest release time of 13h. The strong crosslinking characteristics of the hydrogel allowed for a more pronounced controlled release of insulin from F8. This was facilitated by sufficient condensation time, which provided high irradiation energy and facilitated efficient crosslinking without adding additional Gly and PG [100].

These results emphasize the importance of optimizing the concentrations of crosslinking agents and the polycondensation time to achieve the desired sustained release profile.

When surplus amounts of PG and Gly were integrated into hydrogel formulation (F16), they exhibited similar behavior to those with insufficient amounts of cross-linking agents. This is probably due to the

fact that an excess of cross-linking agents can lead to the formation of denser network structures, which can result in unstable gels [101][102]. This underscores the need to carefully balance the concentrations of crosslinking agents to avoid adverse effects on hydrogel stability and release properties.

III.3. Hydrogel characterizations technics

III.3.1. FTIR hydrogel characterization

FTIR analysis confirmed the cross-linking reaction of the Agar-Carbomer-based hydrogel, with individual species and their cross-linked mixture spectra depicted in Figure III.5. The Ag spectrum displayed characteristic peaks related to -C-O- bending and 3, 6-anhydrogalactose at 1039, 929, and 889cm^{-1}, respectively, consistent with previous research [103].

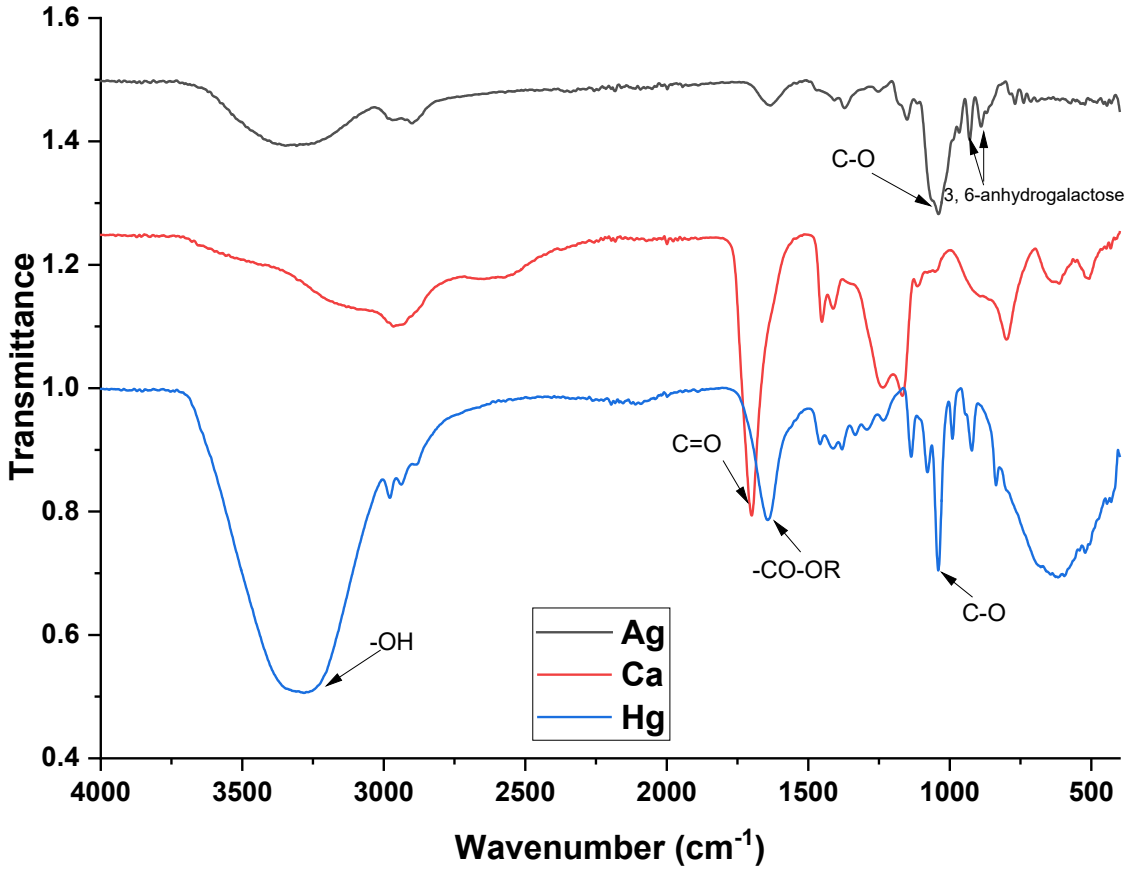


Figure III.5. FTIR spectra of Agar (Ag), Carbomer (Ca) and the Ag-Ca-based hydrogel (Hg).

The Ca spectrum exhibited a prominent peak at 1699cm^{-1}, attributed to C=O stretching of carboxylic groups [104]. In the synthesized hydrogel spectrum, new peaks emerged, including a broad absorption band at 3280cm^{-1} corresponding to -OH bonds and peaks at 2978cm^{-1} linked to C-H methyl groups of PG and Gly molecules. The peak at 1699cm^{-1} was diminished and replaced by a peak at 1642cm^{-1}, indicating the formation of an ester function (-CO-OR) between Ag-Ca. Additionally, an elongation of the peak at 1039cm^{-1} indicated the formation of a -C-O- bond linking PG-Ca [82]. These results provide strong evidence for the successful chemical crosslinking of the Ag-Ca hydrogel in the presence of PG and Gly, confirming the formation of the desired hydrogel network structure.

III.3.2. SEM analysis

The choice of crosslinking method, particularly microwave-assisted polycondensation, has a significant impact on hydrogel morphology and crosslinking stability. These findings are highly relevant and provide valuable insights for optimizing hydrogel synthesis in the pharmaceutical industry, enabling the development of hydrogels with well-controlled microstructures and enhanced stability.

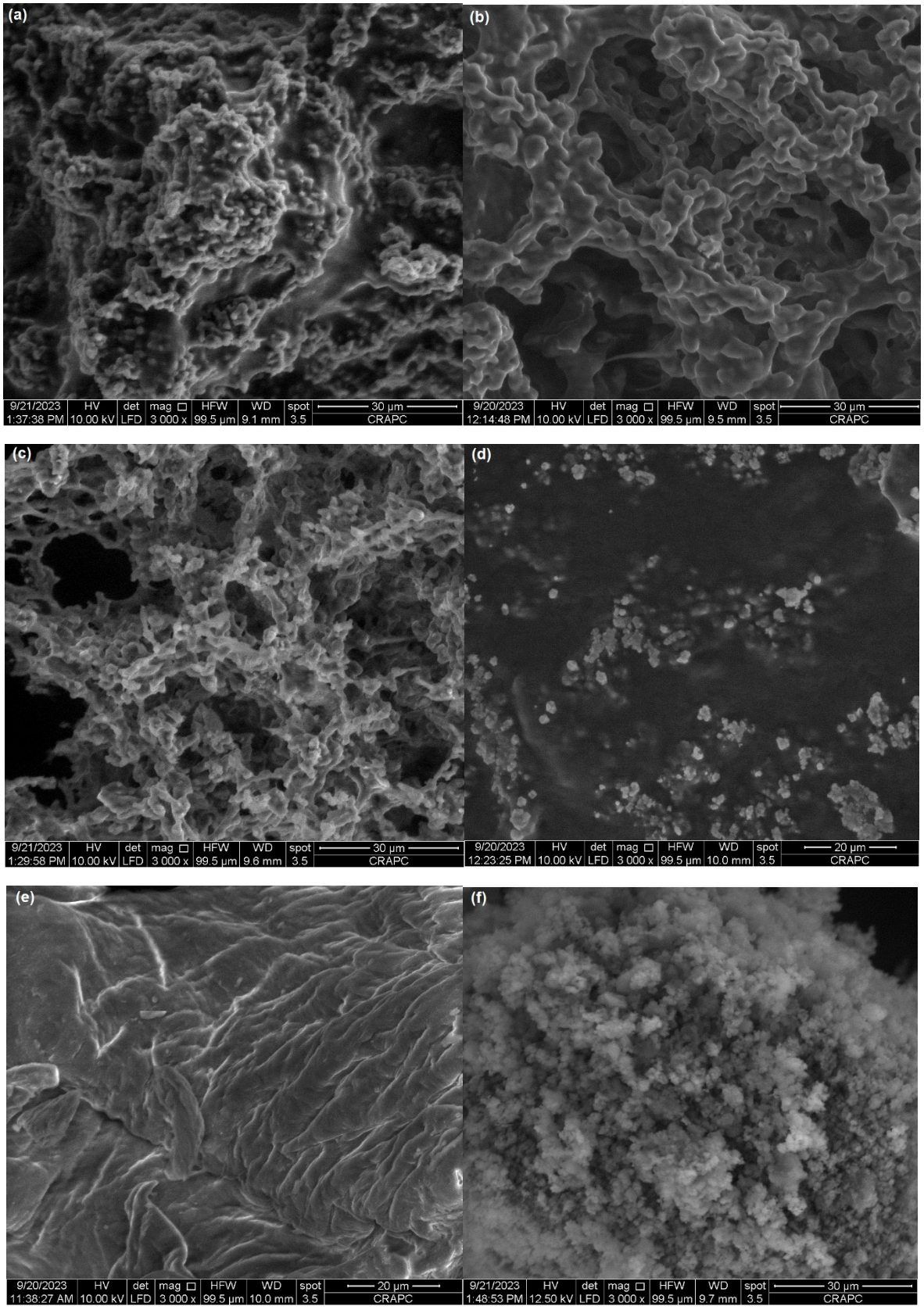


Figure III.6. SEM results: (a) 1r/Carbomer hydrogel exposed to polycondensation assisted by microwave; (b) crosslinked Agar/Carbomer hydrogel without microwave exposure; (c) hydrogel with a single crosslinking agent; (d) hydrogel without crosslinking agents; (e) Agar; (f) Carbomer.

Microstructural properties of hydrogels were analyzed using scanning electron microscopy (SEM). In Figure III.6(a,b), two hydrogels with the same polymer and crosslinking agent amounts were compared. The first (in Figure III.6(a), was produced with microwave-assisted polycondensation, while the second (in Figure III.6(b) followed conventional methods without microwave exposure. Significant differences in microstructure were observed. Figure III.6(a) displayed a compact, tightly linked network, indicating a uniform and interconnected structure. In contrast, Figure III.6(b) revealed a porous network with voids, suggesting a less extensively linked structure. When a single crosslinking agent was used (Figure III.6(c)), an open, porous network was evident, implying a less densely interwoven structure [76]. Furthermore, Figure III.6(d) displayed SEM images of a hydrogel prepared without crosslinking agents, showing dispersed polymers in a fused state without forming a cohesive network [105]. These SEM results provide visual evidence of the impact of crosslinking agents and polycondensation methods on the microstructure of the hydrogels. The use of microwave-assisted polycondensation and the incorporation of both PG and Gly as crosslinking agents result in a more compact and interconnected network structure, which is crucial for achieving the desired drug release properties.

III.3.3. NMR analysis

The H1-NMR analysis was carried out to prove that the hydrogel was successfully crosslinked. The results of the H1 NMR analysis are illustrated by the spectra of Figure III.7.

The spectra in Figure III.7(a,b) represent two polymers, Carbomer and Agarose, respectively. Figure III.7(b,c) display the spectra of the crosslinking system with Propylene glycol and Glycerol, separately. Both spectra show a peak around 4.7 ppm, corresponding to the anomeric proton of deuterium oxide (D_2O) [106].

The H^1NMR spectrum of the hydrogels formed by combining Agarose and Carbomer is characterized by wide signals due to dipole-dipole coupling. The spectrum of the cross-linked hydrogel F10 (Figure III.7(e)) shows characteristic peaks: Peaks at 1,126 and 1,577 ppm correspond to aliphatic protons and methyl protons (CH2), respectively. The chemical shift of the proton related to the acetyl group (R-C=O) is observed at 2,216 ppm. Peaks at 3.425 and 3.507 ppm are typical for protons near electronegative atoms such as oxygen, related to protons in multiple hydroxyl groups (HO-) [76].

New peaks around 3,760 ppm and 4,106 ppm in the spectrum are associated with the ester function (-(C=O)-O-). The appearance of peaks in the 3,760 ppm and 4,106 ppm region indicates the formation of an ester function (-(C=O)-O-). This suggests that the carboxyl group (–C(O)OH) of Carbomer has

been connected with the Hydroxyl group (HO-) of Agarose, forming the ester function. This is shown in Figure 7. The successful formation of the ester function provides strong evidence for the crosslinking of the hydrogel. Comparing with Previous Research, the results align with findings from Santoro et al [82], indicating the consistency of the observed peaks. These NMR results are consistent with the FTIR findings and further confirm the successful development of the crosslinked hydrogel network.

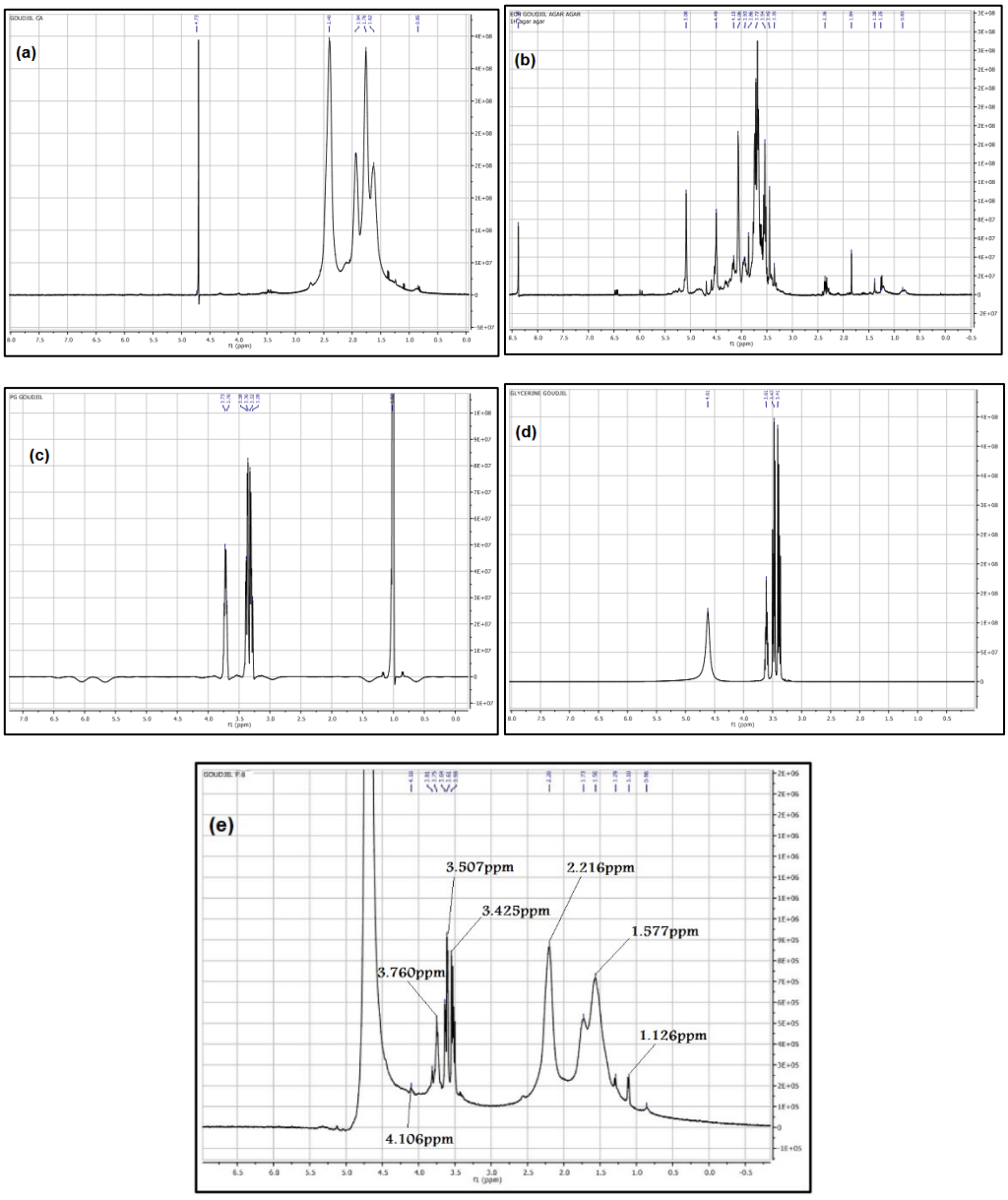


Figure III.7. NMR spectra: (a) Carbomer; (b) Agar; (c) propylen glycol; (d) glycerol; (e) hydrogel F10.

By combining this information, the chemical structure of the elaborated cross-linked hydrogel macromolecule can be provided in Figure III.8. This proposed structure illustrates the key functional

groups and crosslinking bonds formed during the hydrogel synthesis, providing a clear understanding of the molecular architecture of the developed system.

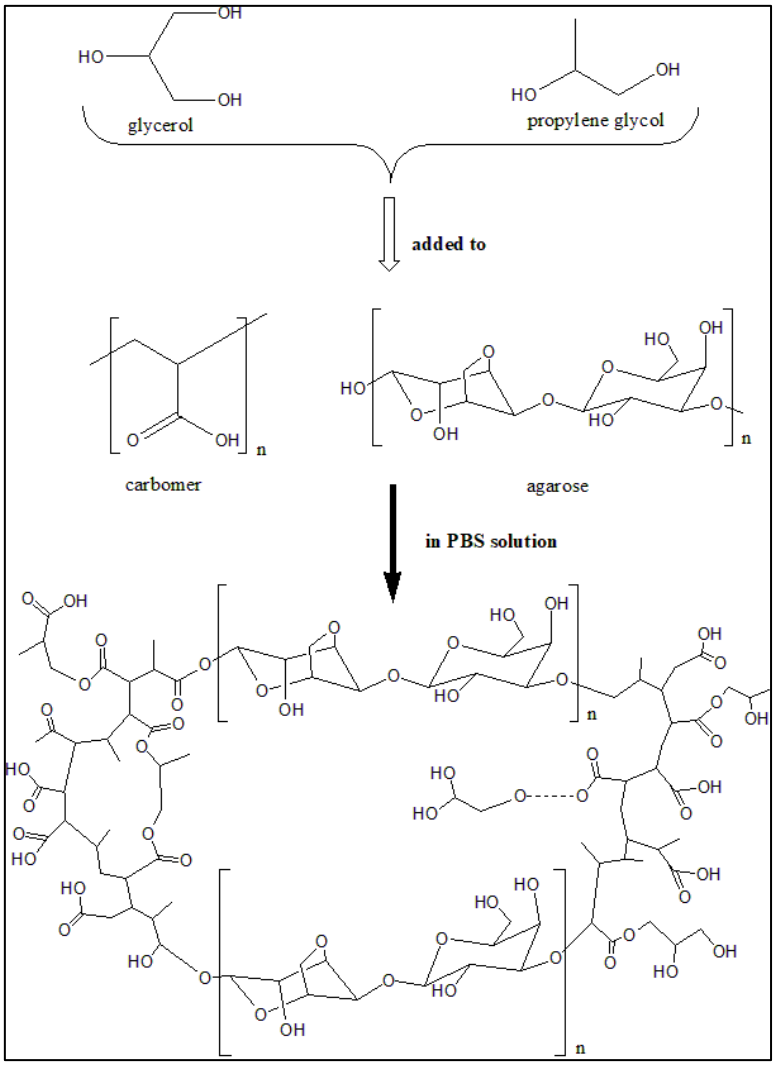


Figure III.8. Chemical structures pattern of the crosslinking reaction.

III.3.4. Microbiological quality of hydrogel

This study allowed to carry out the total aerobic microorganisms count (TAMC), the total mould and yeast count (TYMC), the search for *Staphylococcus Aureus* and *Pseudomonas Aeruginosa* in the F10 hydrogel containing insulin. The results are grouped in Table III.1.

Table III.1. Acceptable Limit of total aerobic microorganisms count (TAMC), the total mould and yeast count (TYMC), *Staphylococcus Aureus* and *Pseudomonas Aeruginosa*.

Presence of microorganisms (CFU.g-1)	Acceptable Quality Limit	F10 Hydrogel
TAMC	**≤ 100**	00
TYMC	**≤ 10**	00
Staphylococcus Aureus	**Absence**	Absence
Pseudomonas Aeruginosa	**Absence**	Absence

Aerobic germs are all microorganisms capable of multiplying in air. This group includes pathogenic microorganisms and spoilage microorganisms [107]. The results of the total aerobic germ count (TAMC) in the hydrogel F10 show that the microbiological load of the sample is 00 CFU.g^{-1} respectively (Figure III.9). These values are lower than the maximum limit of the standard which is 100 CFU.g^{-1} [97].

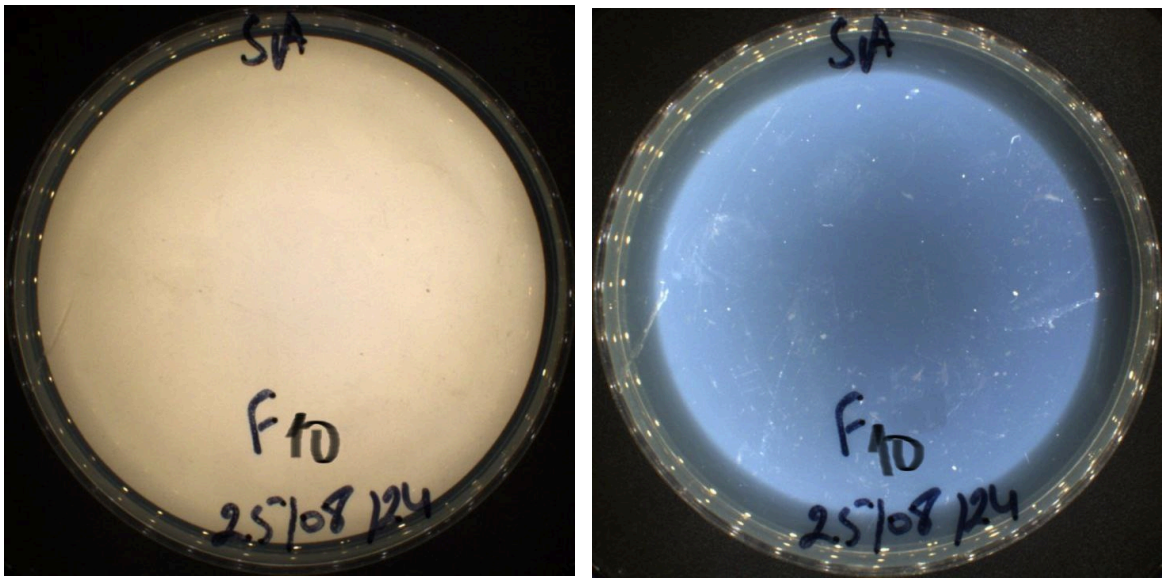


Figure III.9. Total aerobic germ count (TAMC) in the hydrogel H10 on TSA medium.

Molds are aerobic and filamentous microorganisms, oxidative and fermentative, while yeasts are microorganisms that have an exclusively oxidative metabolism [84]. The results of the enumeration of these microorganisms (TYMC) indicate that the hydrogel F10 have loads of 00 CFU.g^{-1} (Figure III.10). These results are acceptable and in accordance with the standards of the European Pharmacopoeia [97], the maximum authorized of which is 10 CFU.g^{-1} [97].

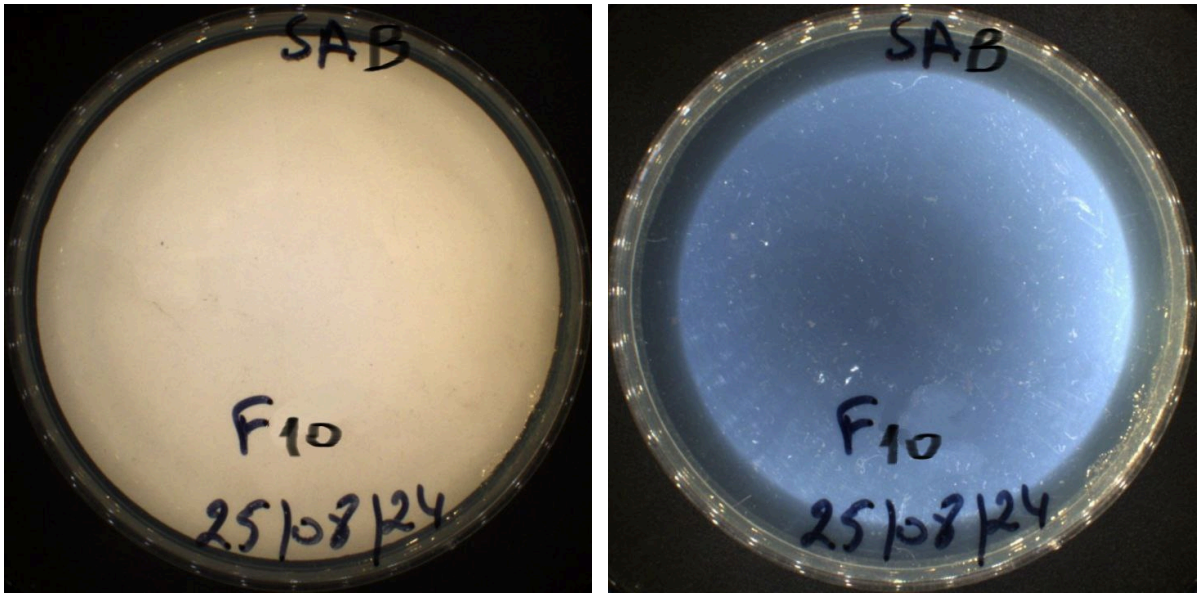


Figure III.10. The enumeration of these microorganisms (TYMC) in the hydrogel H10 on SAB medium.

Figure III.11 shows the results of subculturing on a mannitol-salt medium (CHAP), incubated at 30 – 35 °C for 72 hours for the hydrogel F10. Staphylococcus aureus are Gram-positive, facultative aerobic-anaerobic cocci that tend to group together in clusters. It is the most pathogenic species of the genus Staphylococcus [108]. The results obtained show the absence of Staphylococcus aureus in Hydrogel F10, which complies with the European Pharmacopoeia [97].

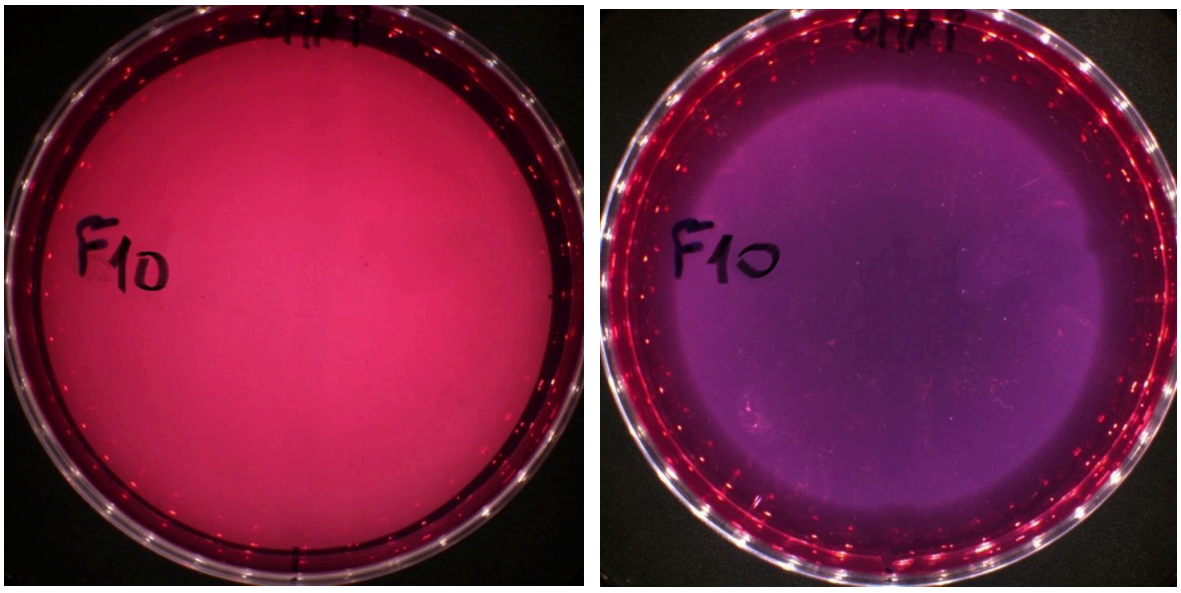


Figure III.11. Macroscopic appearance of the absence of *Staphylococcus Aureus* colonies on mannitol-salt medium (CHAP).

Regarding Pseudomonas aeruginosa, these are Gram-negative bacteria of the Pseudomonas genus [109]. Figure III.12 shows the results of subculturing on a cetrimide agar base (CAB) medium

incubated at 30–35 °C for 72 hours for Hydroger F10. The results obtained show the absence of Pseudomonas Aeruginosa in Hydroger F10, which is in accordance with the European Pharmacopoeia [97].

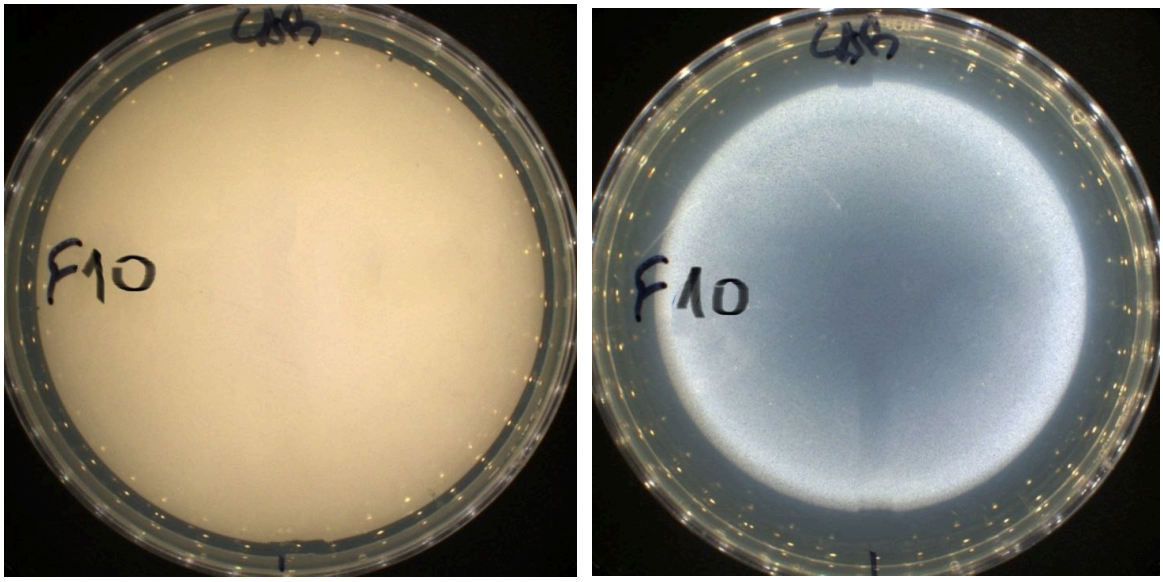


Figure III.12. Macroscopic appearance of the absence of Pseudomonas Aeruginosa colonies on cetrimide agar base (CAB) medium.

According to the results of the microbiological analyses conducted, the F10 hydrogel containing insulin has satisfactory microbiological quality, meeting the standards set by the European Pharmacopoeia [97]. This is a crucial aspect in the development of pharmaceutical formulations, as it ensures the safety and suitability of the hydrogel for potential clinical applications.

III.4. Development and Validation of AI Models

III.4.1. ANFIS prediction of the formulation parameters effect

The ANFIS model, as demonstrated in Figures 1–4, is capable of predicting the impact of formulation parameters on insulin release kinetics. This model allows for the consideration of various factors influencing insulin release, including the composition of the drug delivery system [76].

Researchers can leverage the ANFIS model to simulate different scenarios and forecast the effects of adjusting formulation parameters on insulin release. This is particularly valuable in developing drug delivery systems to identify optimal formulations for controlled and sustained insulin delivery. A high-performing hydrogel derived from the Fuzzy Takagi-Sugeno model is suitable for inhalation delivery.

The reliability of the ANFIS model depends on the quality and quantity of the training data, so selecting high-quality data and appropriate features is crucial to ensure accurate predictions. Future studies should focus on expanding the dataset and incorporating additional relevant parameters to further enhance the predictive capabilities of the ANFIS model.

III.4.2. Kinetic release profile and modeling

An *in vitro* study was conducted to analyze the drug release of insulin-loaded Ag/Ca-based hydrogels prepared by polycondensation in PBS at 37°C. The impact of PG and Gly concentrations, along with the polycondensation time was also investigated. The experimental results presented in Figure III.13 clearly show that the fastest release was observed in hydrogel F1 [76].

In fact, this formulation did not contain Gly and was not subjected to microwave irradiation, and consequently there was no polycondensation reaction (no hydrogen bonding with Ca). Hydrogels created without any added PG – F2 to F4 – displayed comparable trends to F1, with a release time between 6.5 and 7.5 h, depending on the concentration of Gly and the polycondensation time. In contrast, hydrogels F5-F7 that do not contain Gly, but contain 30% PG, have shown extended-release profiles of over 7.5–8.5 h, which increase with increasing polycondensation time, indicating the formation of ester bonds. In addition, it should be noted that all of the above formulations showed a burst release in the first 30 min, which may be due to the insufficient amount of PG/Gly cross-linking agents to form strong hydrogels capable of retaining drugs through their cross-linked networks [99]. Furthermore, a higher rate of drug release was observed in formulations containing only Gly, as its characteristics enable a rapid release of drugs [99].

III.4.3. ANFIS modeling results

Concerning the ANFIS modelling performance, it appears that the ANFIS model adeptly predicted the insulin release rate, as demonstrated by the substantial concordance between the experimental and predicted values depicted in Figure III.13.

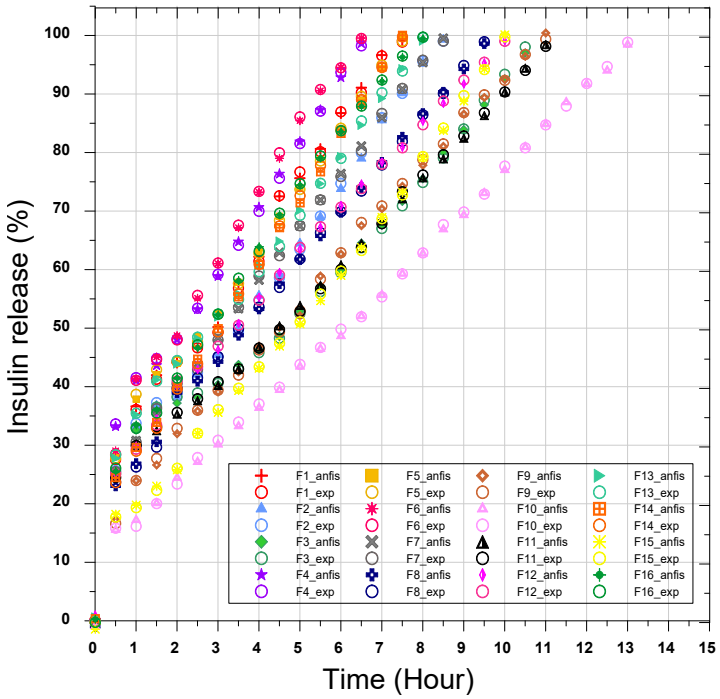


Figure III.13. Insulin release kinetics profiles from the different hydrogel formulas (experimental and predicted results) in PBS 7.4 at 37°C.

III.4.4. ANFIS predicted insulin release amount as a function of the experimental data

Figure III.14 shows the correlation plot of the predicted release rate against the experimental release rate. It is evident that the data points cloud is very close to the first bisector, which indicates the reliability and excellent performance of the ANFIS model, as shown by the narrow gap between the experimental and predicted values. To comprehensively assess the accuracy and reliability of the ANFIS model, a correlation coefficient and root mean squared error were calculated. The achieved results indicate that the model performed very well with a high correlation coefficient close to unity (R^2=0.9999) and an RMSE of 2.159 x 10^{-2}. This signifies the successful development of a high-quality ANFIS model [76].

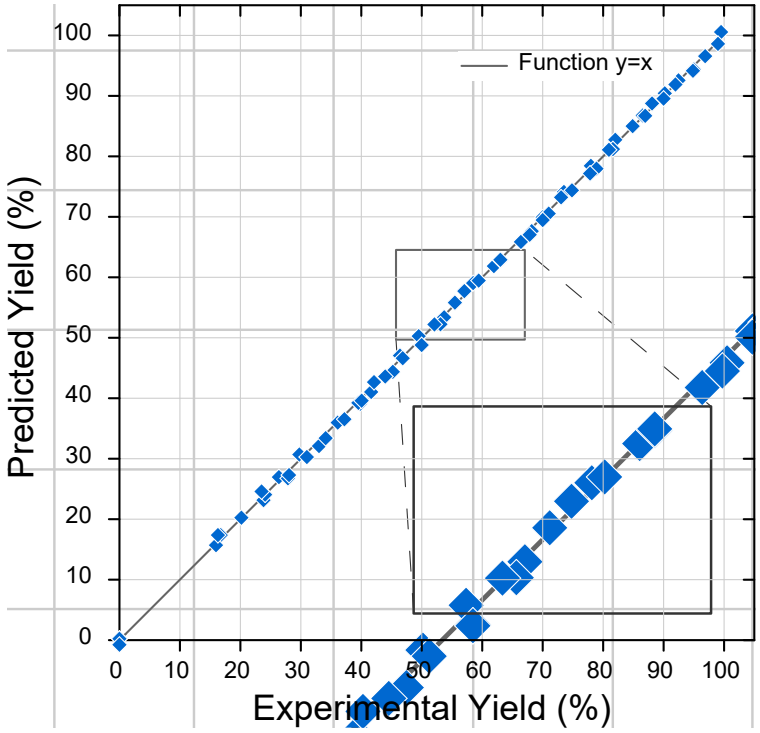


Figure III.14. Points cloud representing the ANFIS predicted release amount as a function of the experimental data.

The neuro-fuzzy model was established based on the parameters used in this particular study. However, other relevant factors, such as insulin concentration, are not taken into account. On the other hand, the model relies on three input characteristics: crosslinking agent's concentrations, polycondensation time, and release. If these characteristics are not measured accurately, the model predictions may be limited or inaccurate. The properties of hydrogels can change over time due to various environmental factors. The model may not account for these dynamic changes, leading to discrepancies between predicted and actual insulin release kinetics in real-world scenarios. The study mentions an excellent correlation with experimental data, but experimental conditions may exhibit inherent variability. In the future, the study needs to address the long-term stability of hydrogel formulations and their impact on insulin release. This is particularly important for extended drug delivery applications [76].

III.4.5. Korsmeyer-Peppas modeling results

The release kinetics of all formulations adhere to the Korsmeyer-Peppas model with a correlation coefficient between 0.95 and 0.99, as stated in Table III.2. This indicates that the primary release mechanism is controlled by diffusion and relaxation.

Previous studies have demonstrated that drug release is controlled by diffusion when the value of *n* equals 0.43, while a value of 0.85 indicates control by swelling [101]. In this study, as long as the

values of *n* for the formulated hydrogels range between 0.43 and 0.85, drug release is conducted by the concurrent phenomena of diffusion and relaxation (anomalous transport).

Table III.2. Korsmeyer-Peppas modeling results.

Formula Number	Korsmeyer Peppas kinetic model			Insuline release mechanism
	k	n	R^2	
F01	33.97	0.49	0.97	Anomalous transport
F02	30.61	0.49	0.97	Anomalous transport
F03	28.85	0.44	0.95	Anomalous transport
F04	39.96	0.43	0.97	Anomalous transport
F05	35.20	0.46	0.98	Anomalous transport
F06	37.99	0.49	0.99	Anomalous transport
F07	30.46	0.51	0.98	Anomalous transport
F08	27.41	0.53	0.98	Anomalous transport
F09	22.21	0.60	0.99	Anomalous transport
F10	17.16	0.62	0.98	Anomalous transport
F11	27.41	0.47	0.97	Anomalous transport
F12	29.68	0.49	0.99	Anomalous transport
F13	34.50	0.46	0.98	Anomalous transport
F14	29.18	0.57	0.98	Anomalous transport
F15	19.74	0.64	0.97	Anomalous transport
F16	32.12	0.52	0.99	Anomalous transport

III.5. Comparative Analysis

In this section, we present some studies to show the practicality of the AI models and the traditional methods in the real world. The AI models and traditional methods are thoroughly discussed and analyzed.

III.5.1. Performance evaluation of the models in another application

The previous chapter book of H. Goudjil et al. [110] describes the application of an Adaptive Neuro-Fuzzy Inference System (ANFIS) to predict the behavior of microcapsules containing Bacillus subtilis SL-13 (BaciSub SL-13) bacteria. ANFIS model was used to predict three key outcomes:

- Microcapsule biodegradation (Figure III.15) (i.e., the rate at which the microcapsules degrade and release the bacteria)
- Viability of the bacteria after being released (i.e., the number of live bacteria)
- Release kinetics profile of the bacteria (i.e., how the bacteria are released from the microcapsules over time)

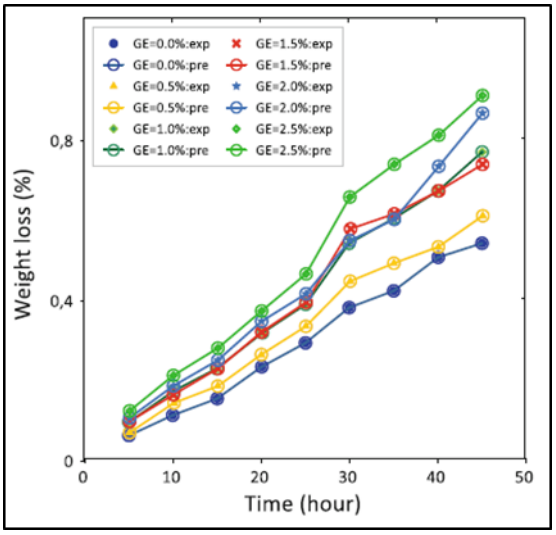


Figure III.15. Experimental and predicted microcrocapsules biodegradation rate contaning a various concentrations of Gelatin (GE).

Time (duration of the experiment), and Gelatin concentration (different levels of gelatin used in the formulation) are the two inputs used for the ANFIS model.

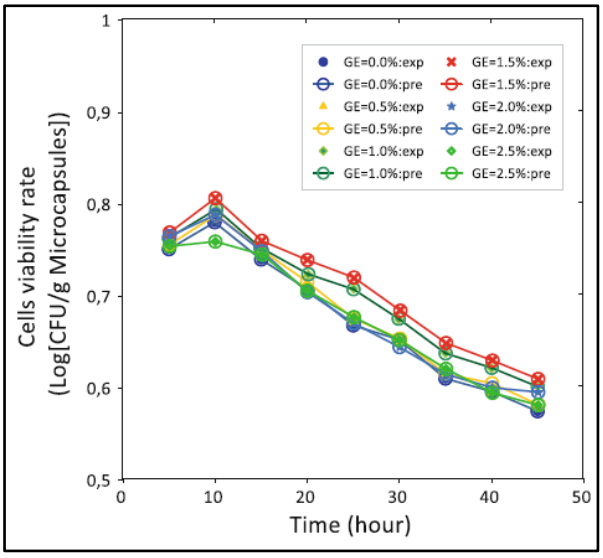


Figure III.16. Experimental and predicted BaciSub SL-13 viability rate during storage (effect of the Gelatin (GE) concentration on the microcapsules)

The ability of ANFIS to handle complex, nonlinear relationships makes it particularly well-suited for this type of prediction task.

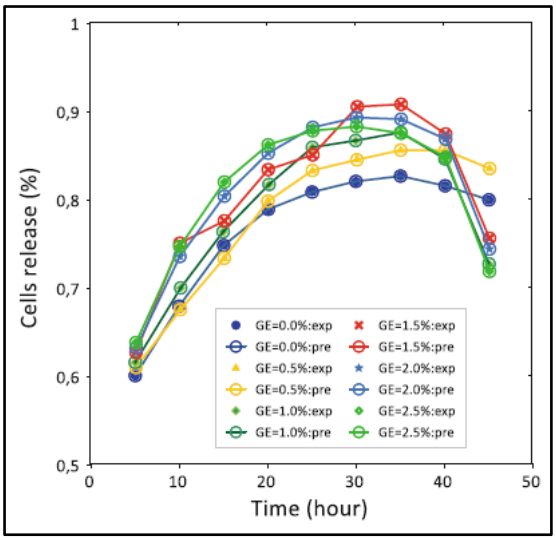


Figure III.17. Experimental and predicted BaciSub SL-13 cells release from microcapsules containing various concentrations of Gelatin (GE).

The root means square error (RMSE), which measures the difference between predicted and actual values, was also very low ($3.26 \times 10^{-4} < RMSE < 1.79 \times 10^{-4}$). A lower RMSE value indicates higher prediction accuracy, and in this case, the error was minimal.

The correlation coefficient (R^2), which measures how close the predicted values are to the experimental values, was extremely high ($R^2 = 0.9999$), indicating an almost perfect agreement between predicted and actual results.

These findings highlight the power of AI models like ANFIS for accurately predicting complex biological processes, such as microcapsule behavior and bacterial release kinetics, based on limited experimental inputs.

III.5.2. Comparison with traditional methods

The Design of Experiments (DOE) is a systematic approach to understanding the relationship between factors affecting a process and the output of that process. It is a structured, organized method for determining the relationship between factors affecting a process and the output of that process. DOE aims to optimize processes, improve product quality, and reduce development time and costs. The main objective of one of our previous studies is to optimize and evaluate the properties of cross-linked

hydrogels, focusing specifically on viscosity, through a systematic approach known as Design of Experiments (DOE). In this case, the Box-Behnken design (BBD) was chosen, which is commonly used to study the effects of three or more independent factors and how they interact to influence the outcome, or response (the hydrogel viscosity in this study). The Box-Behnken design (BBD) is a type of response surface methodology (RSM) that allows efficient estimation of first- and second-order coefficients. It's an alternative to central composite designs and is particularly useful when the number of factors is three or more. The BBD has several advantages:

1. It requires fewer experimental runs than a full factorial design
2. It avoids extreme conditions (all factors at their highest or lowest levels simultaneously)
3. It ensures that all factors are not set at their extreme values at the same time

Table III.3. Experimental matrix of Box-Behnken design and response

	Factors			**Réponses**
Experiments	**Propylene glycol (%)**	**Glycerol (%)**	**polycondensation time (s)**	**Viscosity (Pa.s)**
1	-1	-1	0	0.616
2	-1	0	-1	0.687
3	-1	0	+1	0.694
4	-1	+1	0	0.609
5	0	-1	-1	1.039
6	0	-1	+1	1.103
7	0	0	0	1.452
8	0	0	0	1.436
9	0	0	0	1.42
10	0	0	0	1.45
11	0	+1	-1	1.224
12	0	+1	+1	1.263
13	+1	-1	0	1.148
14	+1	0	-1	1.048
15	+1	0	+1	1.098
16	+1	+1	0	1.097

Sixteen experiments were conducted (Table III.3), and the results were used to create a statistically significant second-order polynomial model (p=0.0003) that describes the viscosity as a function of these factors.

The experimental model uses three independent factors (denoted by X_1, X_2, and X_3), which represent the concentrations of crosslinking agents: propylene glycol and glycerol, and the polycondensation time, respectively. The general form of the second-order polynomial equation in the BBD is:

$$Y = \beta_0 + \Sigma\beta_i x_i + \Sigma\beta_{ii} x_i^2 + \Sigma\Sigma\beta_{ij} x_i x_j + \varepsilon \quad \text{(III.1)}$$

Where Y is the predicted response, β_0 is the model constant, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, β_{ij} is the interaction coefficient, and ε is the error term.

The Box-Behnken model was used to create response surfaces and optimize processes with fewer experimental runs compared to a full factorial model. This efficiency is particularly valuable in situations where experiments are time-consuming or expensive.

The development of a statistically significant second-order polynomial model ($p = 0.0003$) indicates that the relationship between the factors and the hydrogel's viscosity is well-represented (Figure III.18). This demonstrates that the chosen factors are indeed critical in determining the rheological properties of the hydrogel. At specific concentrations of crosslinking agents, the hydrogels showed high viscosity at low shear rates and a more pronounced pseudoplastic behavior, meaning viscosity decreases as shear increases.

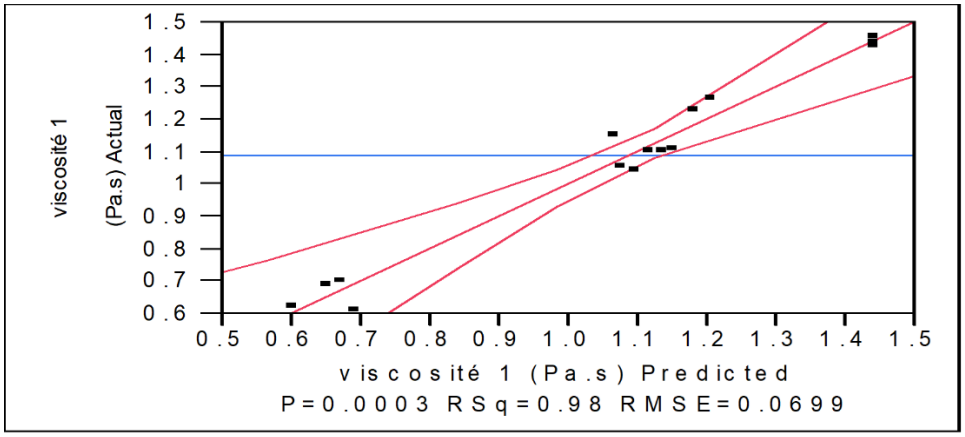


Figure III.18. Actual by predicted plot of viscosity.

The optimal formula was proposed based on the desired responses, and the accuracy of the model was confirmed through analysis of variance (ANOVA). The model demonstrated a correlation coefficient ($R^2 = 0.98$) and a normalized root mean square error (NRMSE) close to zero (Figure 18). These values suggest that the model should predict viscosity with more high precision and minimal error. It's important to note that while a high R^2 value is generally desirable, it should be interpreted cautiously. An R^2 of 0.98 indicates that 98% of the variability in the response variable (viscosity) can be explained by the model. However, this high value could potentially indicate a risk of overfitting, where the model fits the experimental data too closely but may not generalize well to other, untested conditions. This risk could be mitigated by conducting additional validation experiments or by incorporating more data points to test the model's robustness across a broader range of factor values.

Another approach could be to use cross-validation techniques, which involve partitioning the data into subsets, performing the analysis on one subset (the training set), and validating the analysis on the other subset (the testing set).

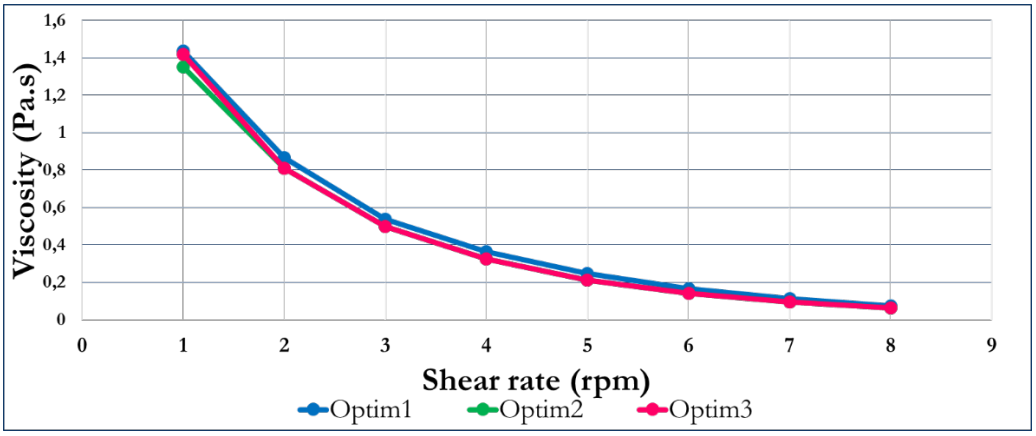


Figure III.19. Flow curves of the optimal hydrogel.

Three validation points of the optimal formula proposed by the design were carried out (Figure III.19). Where the error is in the acceptance limit (≤ 0.05). A high viscosity value at low shear speeds are recorded and the pseudoplastic character of the hydrogel is more pronounced.

In Conclusion, while the Box-Behnken design was effective in predicting optimal hydrogel viscosity and providing valuable formulation insights, further work is needed to validate the model under a wider range of conditions. This could involve testing the model's predictions under different environmental conditions or with slightly different formulations to ensure its robustness. The choice between DOE and AI for optimizing hydrogels or similar systems depends on the system's complexity, data availability, and the need for interpretability. For relatively simple systems with limited factors, BBD is highly effective. It provides a clear understanding of how each factor affects the response, which can be crucial for process optimization and quality control.

However, in more complex, multi-variable environments, AI-based optimization can unlock deeper insights and provide more robust solutions with highest accuracy. AI models can handle non-linear relationships and interactions that might be difficult to capture with traditional DOE approaches. AI models like ANFIS have demonstrated remarkable performance in predicting complex biological processes, making them a promising tool for optimizing and understanding intricate systems in the real world. However, it's worth noting that AI models often require larger datasets for training compared to DOE approaches, and their "black box" nature can make it challenging to understand the underlying

mechanisms driving the predictions. In future studies, a hybrid approach combining DOE for initial experimentation and AI for fine-tuning and prediction could potentially leverage the strengths of both methodologies, leading to more robust and accurate models for complex systems like hydrogels.

III.6. Case Studies on Hydrogel Applications in Insulin Delivery

Real-world applications using hydrogel-based systems to deliver insulin to diabetes patients are an effective way to demonstrate their benefits. The utilization of hydrogels in these case studies resulted in better insulin release profiles when compared to insulin alone.

Within the following case studies, we will explore the use of hydrogel systems to significantly improve insulin release profiles, resulting in long-term normoglycemic control, often without hypoglycemia. This section is dedicated to findings across a variety of hydrogel systems designed to deliver insulin. The section will ascertain what mechanisms have contributed to improvements, providing insight and a basis for a fundamental understanding.

III.6.1. Case 1 : Nanoparticles as an Insulin Delivery System

Nanoparticles have emerged as a promising approach for insulin delivery, offering improved bioavailability, targeted delivery, and protection from degradation. Three key studies highlight the potential of this approach [111] :

1. Polyelectrolyte Complex Nanoparticles:

Al-Kurdi et al. developed nanoparticles using a polyelectrolyte complex of insulin and low molecular weight chitosan [112]. The study found that:

- Lower molecular weight chitosan (13 kDa) enhanced insulin stability in a tris-buffer at pH 6.5.
- This counterintuitive finding suggests that lower molecular weight chitosan provides a more flexible and adaptable matrix for insulin encapsulation.
- The optimal molecular weight likely strikes a balance between sufficient electrostatic interactions for complex formation and maintaining a flexible structure that effectively protects insulin molecules.

2. Carboxymethyl-Cyclodextrin and Chitosan Nanoparticles:

Krauland et al. created nanoparticles from chitosan and carboxymethyl-β-cyclodextrin [113]. Key findings include:

- The nanoparticles demonstrated remarkable stability for at least 4 hours in simulated intestinal fluid (pH 6.8, 37°C).
- Cyclodextrins' unique structure, featuring a hydrophobic interior cavity and hydrophilic exterior, allows them to form inclusion complexes with drug molecules, enhancing stability and solubility.
- The carboxymethyl modification increases cyclodextrins' water solubility, making them more suitable for nanoparticle formation.
- The ionotropic gelation process used for fabrication is particularly advantageous for insulin delivery as it avoids harsh conditions that could potentially denature the protein.

3. Chitosan and Arabic Gum Nanoparticles:

Avadi et al. developed a nanoparticulate system using chitosan and Arabic gum [114]. Their research revealed:

- The ionic gelation technique was employed to combine the mucoadhesive and permeation-enhancing properties of chitosan with the protective and stabilizing effects of Arabic gum.
- Arabic gum serves as a protective coating for insulin against the harsh gastric environment and intestinal enzymes.
- A 23 factorial design was used to systematically test different combinations of chitosan, Arabic gum, and insulin concentrations.
- Increasing the concentration of Arabic gum improved the association efficiency, suggesting its crucial role in stabilizing the nanoparticle structure and enhancing insulin encapsulation.

III.6.2. Case 2: Microparticles (Microspheres and Beads)

Microparticles were developed to achieve sustained insulin release, protect against degradation, and provide controlled release kinetics. Three approaches demonstrated promising results:

1. Chitosan Microspheres with Glutaraldehyde Cross-Linking:

Jose et al. produced microspheres using the emulsion cross-linking method [115]. Their findings include:

- Glutaraldehyde acted as a cross-linker, strengthening the microspheres by chemically bonding the chitosan polymer chains.
- The microspheres exhibited swelling ratios between 1.30 and 2.30, indicating their ability to expand in aqueous environments.
- The insulin release profile showed a burst release during the first 3 hours, followed by controlled release for the next 5–6 hours.
- The release data fit well with the Higuchi model ($R^2 \approx 0.98$), suggesting a primarily diffusion-controlled drug release mechanism.

2. Water-Soluble Chitosan/Insulin/Tripolyphosphate Microparticles:

Wu et al. prepared microparticles using polyelectrolyte complexation [116]. Key observations include:

- At pH 4.0, these microparticles achieved a high association efficiency of 48.28 ± 0.90%.
- The insulin release profile showed three distinct phases: 50% release within the first 2 hours, a significant slowdown for the next 12 hours, and then a gradual, controlled release of the remaining insulin.
- The rapid initial release can be attributed to loosely bound insulin molecules on the surface of the microparticles.
- The subsequent plateau phase suggests a potential barrier to insulin release, possibly due to the formation of a dense polymer layer or depletion of surface-bound insulin.

3. Insulin-Loaded Alginate/Chitosan Blend Gel Beads:

Tahtat et al. prepared gel beads from a blend of alginate and chitosan [117]. Their research showed:

- Dual crosslinking with $CaCl_2$ and glutaraldehyde enhanced the mechanical properties of the beads, making them more resistant to degradation in harsh environments.
- The beads released 90.5% of the insulin within 6 hours, indicating a rapid release profile suitable for intestinal absorption after withstanding gastric conditions.
- In gastric media, the presence of chitosan reduced the cumulative insulin release, likely due to its stability in acidic conditions and its ability to protect insulin from degradation.
- In the intestinal medium, the blend beads demonstrated improved insulin release, attributed to the crosslinking process.

III.6.3. Case 3: Hydrogel and Microgel

Two innovative approaches demonstrated the potential of hydrogel and microgel systems for insulin delivery:

1. Chitosan/Polyvinyl Alcohol Hydrogel for Nasal Insulin Delivery:

Agrawal et al. developed a hydrogel system for nasal insulin delivery [118]. Their findings include:

- The hydrogel was made by mixing chitosan and PVA in different proportions, with pH adjusted to near neutral using sodium bicarbonate.
- Insulin was added at a concentration of 1 IU per 200 µL of solution.
- The hydrogel remains liquid at room temperature but undergoes gelation at 37°C within 12 minutes.
- In vitro tests showed glucose level maintenance for 6 hours.
- Animal testing revealed maximum glucose reduction 4 hours after nasal administration, indicating a slow-release profile.

2. Microgels with Enzyme Nanocapsules and Recombinant Human Insulin:

Gu et al. created a glucose-responsive insulin delivery system using microgels [119]. Key features include:

- Monodisperse microgels were created using a chitosan matrix incorporating enzyme nanocapsules and recombinant human insulin.
- Glucose-specific enzymes were covalently encapsulated into nanocapsules to protect them from denaturation and immunogenicity.
- The system releases more insulin in response to higher glucose concentrations, mimicking the natural response of the pancreas.
- Enzymes convert glucose into gluconic acid, causing a local pH reduction within the microgel, triggering swelling and insulin release.

III.6.4. Case 4: Mucoadhesive Films

Two types of mucoadhesive films showed promise for insulin delivery:

1. PEG-b-PLA/Insulin Nanoparticles in Chitosan Films:

Giovano et al. developed mucoadhesive films loaded with PEG-b-PLA/insulin nanoparticles [120]. Their research revealed:

- Free insulin dispersed in chitosan films was almost completely released within 6 hours (98 ± 14%).
- Nanoparticle-encapsulated insulin showed a more prolonged and controlled release:
- 51 ± 14% released in the first 72 hours
- 70% released after 15 days
- This sustained release is critical for long-term management of blood glucose levels.

2. Bilaminated Hydrogel Films for Buccal Insulin Delivery:

Cui et al. developed a two-layered film for buccal insulin delivery [121]. Key findings include:

- The mucoadhesive layer was made from chitosan-EDTA hydrogel film containing insulin.
- An impermeable protective layer of ethylcellulose prevented insulin leakage.
- The mucoadhesive layer demonstrated a strong adhesion force of over 17,000 N/m², stable for 4 hours in vitro.
- In healthy rats, buccal administration resulted in 17% pharmacological availability compared to subcutaneous injections.

III.6.5. Our Case: Agar-Carbomer Hydrogels

The thesis presented a novel insulin delivery system using agar-carbomer hydrogels:

- Synthesis: Microwave-assisted polycondensation with glycerol and propylene glycol as crosslinking agents.
- Structure: SEM analysis revealed a compact, tightly linked network structure.
- Chemical Confirmation: FTIR and NMR analyses confirmed successful crosslinking with ester bond formation between agar and carbomer.
- Release Profile: Sustained insulin release lasting 6.5 to 13 hours.

- Key Factors: Glycerol and propylene glycol concentrations, and polycondensation time significantly influenced release kinetics.
- Optimization: Precise formulation control is crucial, as both insufficient and excessive crosslinking led to suboptimal release profiles.

Table III.4 provides a concise comparison of the different hydrogel-based insulin delivery systems, highlighting their main components, release durations, key features, delivery routes, and unique advantages.

Table III.4. Comparison of the different hydrogel-based insulin delivery systems

Aspect	**Case 1: Nanoparticles**	**Case 2: Microparticles**	**Case 3: Hydrogel/Microgel**	**Case 4: Mucoadhesive Films**	**Our Case: Agar-Carbomer Hydrogels**
Main Components	Chitosan, cyclodextrins, Arabic gum[112][113][114]	Chitosan, alginate[115][116][117]	Chitosan, PVA, enzymes[118][119]	Chitosan, PEG-b-PLA[120][121]	Agar, carbomer, glycerol, propylene glycol
Release Duration	4+ hours[113]	6-14 hours[115][116][117]	6+ hours[118]	Up to 15 days[120]	6.5-13 hours
Key Features	Improved bioavailability, targeted delivery[111]	Sustained release, protection from degradation[115][116][117]	Glucose-responsive, nasal delivery[118][119]	Prolonged release, mucoadhesion[120][121]	Microwave-assisted synthesis, controlled release
Delivery Route	Oral, nasal[113]	Oral[115][116][117]	Nasal, subcutaneous[118][119]	Buccal[120][121]	Not specified
Unique Advantage	Enhanced stability in GI tract[112][113][114]	Controlled release kinetics[115][116][117]	Self-regulated insulin release[119]	Strong mucoadhesion[120][121]	Optimized crosslinking for sustained release

III.7. Conclusion

In this chapter, we thoroughly analyzed the experimental data concerning the release kinetics of insulin from various hydrogel formulations, emphasizing the significant influence of key formulation parameters such as glycerol and propylene glycol concentrations, their ratio, and the polycondensation reaction time. The results highlighted that optimal concentrations of PG and Gly are crucial for achieving a sustained release profile, with specific formulations demonstrating extended-release periods. For example, formula F10, which contained moderate amounts of PG (30%) and Gly (1%) and was subjected to the maximum polycondensation time of 30s, exhibited the longest insulin release time of 13 hours. This prolonged release is attributed to the strong crosslinking characteristics of the hydrogel, facilitated by sufficient condensation time and optimal concentrations of crosslinking agents. Our study provided an in-depth analysis of the hydrogel's physicochemical and microbiological characterization using FTIR, NMR, SEM, and microbiological testing. The results from each technique validate the successful development of a stable and efficient insulin-containing hydrogel. FTIR analysis confirmed the chemical crosslinking of the Ag-Ca hydrogel in the presence of PG and Gly,

while NMR spectroscopy revealed the formation of ester bonds between the carboxyl group of Carbomer and the hydroxyl group of Agarose. SEM imaging demonstrated the impact of microwave-assisted polycondensation on the hydrogel's morphology, resulting in a compact and tightly linked network compared to conventional methods. Microbiological testing confirmed the satisfactory microbiological quality of the F10 hydrogel containing insulin, meeting the acceptable limits set by the European Pharmacopoeia. This chapter presents the development and validation of two predictive models—ANFIS and Korsmeyer-Peppas—used to model the release kinetics of insulin from hydrogels. Both models demonstrated high predictive performance and provided valuable insights into the mechanisms influencing insulin release. The ANFIS model accurately predicted the insulin release rate, as evidenced by the substantial concordance between experimental and predicted values (R^2 = 0.9999, RMSE = 2.159×10^{-2}). The Korsmeyer-Peppas model revealed that the primary release mechanism is controlled by diffusion and relaxation (anomalous transport), with release exponent values (n) ranging between 0.43 and 0.85. Despite the promising results, further research is needed to optimize this system for clinical use, particularly in terms of long-term stability and efficiency compared to traditional insulin delivery methods.

In conclusion, this study demonstrates the successful development of an insulin-loaded Ag-Ca hydrogel system with tunable release kinetics, influenced by key formulation parameters. The comprehensive characterization and predictive modeling approaches employed provide a solid foundation for further optimization and translation of this promising drug delivery system. With continued research and development, these hydrogel-based systems have the potential to revolutionize insulin delivery, improving the quality of life for millions of individuals living with diabetes worldwide.

Traditional methods such as Design of Experiments (DoE) are powerful and well-established statistical tools for optimization in experimental design, particularly when dealing with a limited number of factors and relatively small datasets. They generate clear and interpretable models while being computationally efficient, making them a sound choice for simple experiments where robustness and simplicity are key. However, these methods show limitations when faced with complex, high-dimensional systems, or when relationships between variables are non-linear or difficult to model using classical approaches. This is where artificial intelligence (AI) models truly shine. AI models offer superior flexibility and are especially well-suited for handling complex problems, often characterized by large datasets, non-linear interactions, and interdependent factors.

Unlike traditional approaches, AI models, such as neural networks or machine learning algorithms, do not require strict assumptions about the form of relationships between variables. This allows for a more dynamic modeling process, capable of capturing subtle interactions and processing much larger data volumes. However, AI models demand significantly higher computational power and can sometimes be more difficult to interpret. Thus, while AI provides undeniable advantages in solving complex problems, it does not fully replace traditional methods but rather complements them depending on the specific requirements of the experiment or study at hand.

The choice between these two approaches depends on the experimental context. For simpler studies, Design of Experiments remains the optimal choice, while for more complex problems, AI becomes an indispensable alternative.

General Conclusion

General Conclusion

This thesis has explored the development and characterization of a novel insulin delivery system based on agar-carbomer hydrogels, with a focus on optimizing formulation parameters and leveraging artificial intelligence for predictive modeling.

We successfully synthesized agar-carbomer hydrogels using microwave-assisted polycondensation, incorporating glycerol and propylene glycol as crosslinking agents. The use of microwave irradiation proved crucial in forming a compact, tightly linked network structure, as evidenced by SEM analysis. This microwave-assisted technique represents a significant advancement over conventional methods, potentially offering more efficient and controlled hydrogel synthesis for pharmaceutical applications.

FTIR and NMR analyses confirmed the successful crosslinking of the hydrogel components, with the formation of ester bonds between agar and carbomer. These findings not only validate our synthesis approach but also provide valuable insights into the molecular structure of the hydrogel, which is essential for understanding and predicting its drug release behavior.

In vitro release studies revealed that the hydrogel formulations exhibited sustained insulin release profiles, with durations ranging from 6.5 to 13 hours. The ability to achieve such prolonged release times is a significant improvement over many existing insulin delivery systems, potentially offering better glycemic control and reduced dosing frequency for diabetes patients.

We identified that the concentrations of glycerol and propylene glycol, as well as the polycondensation time, were critical factors influencing insulin release kinetics. Notably, we found that an optimal balance of these parameters is crucial, as both insufficient and excessive crosslinking led to suboptimal release profiles. This highlights the importance of precise formulation control in developing effective hydrogel-based drug delivery systems.

The development and application of an Adaptive Neuro-Fuzzy Inference System (ANFIS) model for predicting insulin release kinetics represents a significant advancement in the field. The high accuracy of the ANFIS model ($R^2 = 0.9999$, RMSE = 2.159×10^{-2}) demonstrates its potential as a powerful tool for optimizing hydrogel formulations, potentially reducing the need for extensive experimental work and accelerating the development of new drug delivery systems.

Korsmeyer-Peppas modeling revealed that insulin release from the hydrogels follows an anomalous transport mechanism, involving both diffusion and polymer relaxation. This understanding of the release mechanism provides valuable guidance for future formulation strategies, allowing for more targeted approaches to controlling drug release.

The optimized hydrogel formulation (F10) met the microbiological quality standards set by the European Pharmacopoeia, indicating its suitability for pharmaceutical use. This finding is crucial for the potential clinical application of the developed hydrogel system, as it ensures the safety and stability of the formulation.

While this research has made significant strides in developing an advanced insulin delivery system, several areas warrant further investigation:

- Long-term stability studies to assess the shelf-life and storage conditions of the hydrogel formulations.
- In vivo studies to evaluate the bioavailability and pharmacokinetics of insulin delivered via this hydrogel system.
- Exploration of alternative administration routes, such as transdermal or oral delivery, to further improve patient compliance.
- Integration of glucose-responsive elements into the hydrogel matrix to develop a smart insulin delivery system capable of responding to blood glucose levels.
- Expansion of the AI model to incorporate additional parameters and predict long-term performance, enhancing its utility in formulation development.

In conclusion, this research has successfully developed and characterized a promising agar-carbomer hydrogel system for sustained insulin delivery. The combination of advanced synthesis techniques, comprehensive physicochemical characterization, and innovative AI-based modeling represents a significant contribution to the field of controlled drug delivery. These findings not only offer potential improvements in diabetes management but also provide a framework for developing similar systems for other therapeutic proteins. As we move forward, the integration of smart materials and AI-driven design approaches is likely to revolutionize the landscape of drug delivery, offering more personalized and effective treatment options for patients.

Unlike traditional approaches, AI models, such as neural networks or machine learning algorithms, do not require strict assumptions about the form of relationships between variables. This allows for a more dynamic modeling process, capable of capturing subtle interactions and processing much larger data volumes. However, AI models demand significantly higher computational power and can sometimes be more difficult to interpret. Thus, while AI provides undeniable advantages in solving complex problems, it does not fully replace traditional methods but rather complements them depending on the specific requirements of the experiment or study at hand.

The choice between these two approaches depends on the experimental context. For simpler studies, Design of Experiments remains the optimal choice, while for more complex problems, AI becomes an indispensable alternative.

References

References

[1] T. C. Ho *et al.*, "Hydrogels: Properties and Applications in Biomedicine," *Molecules*, vol. 27, no. 9, pp. 1–29, 2022, doi: 10.3390/molecules27092902.

[2] A. D. Drozdov and J. deClaville Christiansen, "Mechanical response and equilibrium swelling of thermoresponsive copolymer hydrogels," *Polym. Int.*, vol. 69, no. 10, pp. 974–984, 2020, doi: 10.1002/pi.6051.

[3] P. C. Tiwari, R. Pal, M. J. Chaudhary, and R. Nath, "Artificial intelligence revolutionizing drug development: Exploring opportunities and challenges," *Drug Dev. Res.*, vol. 84, no. 8, pp. 1652–1663, 2023, doi: 10.1002/ddr.22115.

[4] M. Kamaci, "Polyurethane-based hydrogels for controlled drug delivery applications," *Eur. Polym. J.*, vol. 123, no. December 2019, p. 109444, 2020, doi: 10.1016/j.eurpolymj.2019.109444.

[5] D. Systems, "Cellulose-Based Hydrogels as Sustained," no. i, 2020. doi : org/10.3390/ma13225270.

[6] A. Bordbar-Khiabani and M. Gasik, "Smart Hydrogels for Advanced Drug Delivery Systems," *Int. J. Mol. Sci.*, vol. 23, no. 7, 2022, doi: 10.3390/ijms23073665.

[7] Y. Guo, J. Bae, Z. Fang, P. Li, F. Zhao, and G. Yu, "Hydrogels and Hydrogel-Derived Materials for Energy and Water Sustainability," *Chem. Rev.*, vol. 120, no. 15, pp. 7642–7707, 2020, doi: 10.1021/acs.chemrev.0c00345.

[8] X. Xu, V. V. Jerca, and R. Hoogenboom, "Bioinspired double network hydrogels: From covalent double network hydrogels: Via hybrid double network hydrogels to physical double network hydrogels," *Mater. Horizons*, vol. 8, no. 4, pp. 1173–1188, 2021, doi: 10.1039/d0mh01514h.

[9] A. Raeisi and F. Farjadian, "Commercial hydrogel product for drug delivery based on route of administration," *Front. Chem.*, vol. 12, no. February, pp. 1–14, 2024, doi: 10.3389/fchem.2024.1336717.

[10] F. Abasalizadeh *et al.*, "Correction to: Alginate-based hydrogels as drug delivery vehicles in cancer treatment and their applications in wound dressing and 3D bioprinting," *J. Biol. Eng.*, vol. 14, no. 1, pp. 1–22, 2020, doi: 10.1186/s13036-020-00239-0.

[11] R. Narayanaswamy and V. P. Torchilin, "Hydrogels and their applications in targeted drug delivery," *Molecules*, vol. 24, no. 3, 2019, doi: 10.3390/molecules24030603.

[12] Y. W. Fu, W. F. Sun, and X. Wang, "UV-initiated crosslinking reaction mechanism and electrical breakdown performance of crosslinked polyethylene," *Polymers (Basel).*, vol. 12, no. 2, 2020, doi: 10.3390/polym12020420.

[13] P. Bhattacharjee and M. Ahearne, "Significance of crosslinking approaches in the development of next generation hydrogels for corneal tissue engineering," *Pharmaceutics*, vol. 13, no. 3, pp. 1–24, 2021, doi: 10.3390/pharmaceutics13030319.

[14] A. GhavamiNejad, N. Ashammakhi, X. Y. Wu, and A. Khademhosseini, "Crosslinking Strategies for 3D Bioprinting of Polymeric Hydrogels," *Small*, vol. 16, no. 35, pp. 1–30, 2020, doi: 10.1002/smll.202002931.

[15] H. Nasution *et al.*, "Hydrogel and Effects of Crosslinking Agent on Cellulose-Based Hydrogels: A Review," *Gels*, vol. 8, no. 9, 2022, doi: 10.3390/gels8090568.

[16] P. Sikdar *et al.*, "Recent advances in the synthesis of smart hydrogels," *Mater. Adv.*, vol. 2, no. 14, pp. 4532–4573, 2021, doi: 10.1039/d1ma00193k.

[17] J. Li, X. Jia, and L. Yin, "Hydrogel: Diversity of Structures and Applications in Food Science," *Food Rev. Int.*, vol. 37, no. 3, pp. 313–372, 2021, doi: 10.1080/87559129.2020.1858313.

[18] W. Cui, M. H. Pi, Y. S. Li, L. Y. Shi, and R. Ran, "Multimechanism Physical Cross-Linking Results in Tough and Self-Healing Hydrogels for Various Applications," *ACS Appl. Polym. Mater.*, vol. 2, no. 8, pp.

3378–3389, 2020, doi: 10.1021/acsapm.0c00464.

[19] N. H. Thang, T. B. Chien, and D. X. Cuong, “Polymer-Based Hydrogels Applied in Drug Delivery: An Overview,” *Gels*, vol. 9, no. 7, pp. 1–38, 2023, doi: 10.3390/gels9070523.

[20] B. Tian, S. Hua, Y. Tian, and J. Liu, “Chemical and physical chitosan hydrogels as prospective carriers for drug delivery: A review,” *J. Mater. Chem. B*, vol. 8, no. 44, pp. 10050–10064, 2020, doi: 10.1039/d0tb01869d.

[21] S. Wang, R. Liu, Y. Fu, and W. J. Kao, “Release Mechanisms and Applications of Drug Delivery Systems for Extended-Release,” *Expert Opin. Drug Deliv.*, vol. 0, no. 0, 2020, doi: 10.1080/17425247.2020.1788541.

[22] M. Bermejo, B. Sanchez-Dengra, M. Gonzalez-Alvarez, and I. Gonzalez-Alvarez, “Oral controlled release dosage forms: dissolution versus diffusion,” *Expert Opin. Drug Deliv.*, vol. 17, no. 6, pp. 791–803, 2020, doi: 10.1080/17425247.2020.1750593.

[23] H. Ijaz, F. Azam, U. R. Tul-Ain, and J. Qureshi, “Polymeric Hydrogels for Controlled Drug Delivery,” *Appl. Encapsulation Control. Release*, pp. 37–59, 2019, doi: 10.1201/9780429299520-3.

[24] F. Cadamuro, L. Russo, and F. Nicotra, “Biomedical Hydrogels Fabricated Using Diels–Alder Crosslinking,” *European J. Org. Chem.*, vol. 2021, no. 3, pp. 374–382, 2021, doi: 10.1002/ejoc.202001042.

[25] M. Mueller, C. Bandl, and W. Kern, “Surface-Immobilized Photoinitiators for Light Induced Polymerization and Coupling Reactions,” *Polymers (Basel).*, vol. 14, no. 3, 2022, doi: 10.3390/polym14030608.

[26] Y. Wang *et al.*, “Recent advances in the fabrication, functionalization, and bioapplications of peptide hydrogels,” *Soft Matter*, vol. 16, no. 44, pp. 10029–10045, 2020, doi: 10.1039/d0sm00966k.

[27] F. G. Blanco *et al.*, “From residues to added-value bacterial biopolymers as nanomaterials for biomedical applications,” *Nanomaterials*, vol. 11, no. 6, pp. 1–41, 2021, doi: 10.3390/nano11061492.

[28] Y. Gao, K. Peng, and S. Mitragotri, “Covalently Crosslinked Hydrogels via Step-Growth Reactions: Crosslinking Chemistries, Polymers, and Clinical Impact,” *Adv. Mater.*, vol. 33, no. 25, 2021, doi: 10.1002/adma.202006362.

[29] A. M. Mohammed, S. K. Osman, K. I. Saleh, and A. M. Samy, “In Vitro Release of 5-Fluorouracil and Methotrexate from Different Thermosensitive Chitosan Hydrogel Systems,” *AAPS PharmSciTech*, vol. 21, no. 4, pp. 1–11, 2020, doi: 10.1208/s12249-020-01672-6.

[30] M. Suhail, C. W. Fang, A. Khan, M. U. Minhas, and P. C. Wu, “Fabrication and in vitro evaluation of ph-sensitive polymeric hydrogels as controlled release carriers,” *Gels*, vol. 7, no. 3, pp. 1–18, 2021, doi: 10.3390/gels7030110.

[31] S. Bashir *et al.*, “Fundamental concepts of hydrogels: Synthesis, properties, and their applications,” *Polymers (Basel).*, vol. 12, no. 11, pp. 1–60, 2020, doi: 10.3390/polym12112702.

[32] Z. Zou *et al.*, “A sodium alginate-based sustained-release IPN hydrogel and its applications,” *RSC Adv.*, vol. 10, no. 65, pp. 39722–39730, 2020, doi: 10.1039/d0ra04316h.

[33] M. Vigata, C. Meinert, D. W. Hutmacher, and N. Bock, “Hydrogels as drug delivery systems: A review of current characterization and evaluation techniques,” *Pharmaceutics*, vol. 12, no. 12, pp. 1–45, 2020, doi: 10.3390/pharmaceutics12121188.

[34] M. Bustamante-Torres, D. Romero-Fierro, B. Arcentales-Vera, K. Palomino, H. Magaña, and E. Bucio, “Hydrogels classification according to the physical or chemical interactions and as stimuli-sensitive materials,” *Gels*, vol. 7, no. 4, pp. 1–25, 2021, doi: 10.3390/gels7040182.

[35] J. Lei, Z. Li, S. Xu, and Z. Liu, “Recent advances of hydrogel network models for studies on mechanical behaviors,” *Acta Mech. Sin. Xuebao*, vol. 37, no. 3, pp. 367–386, 2021, doi: 10.1007/s10409-021-01058-2.

[36] W. F. Lai, “Development of Hydrogels with Self-Healing Properties for Delivery of Bioactive Agents,” *Mol. Pharm.*, vol. 18, no. 5, pp. 1833–1841, 2021, doi: 10.1021/acs.molpharmaceut.0c00874.

[37] E. Askari *et al.*, “Stimuli-responsive hydrogels for local post-surgical drug delivery,” *Gels*, vol. 6, no. 2, pp. 1–31, 2020, doi: 10.3390/gels6020014.

[38] S. H. Aswathy, U. Narendrakumar, and I. Manjubala, “Commercial hydrogels for biomedical applications,” *Heliyon*, vol. 6, no. 4, p. e03719, 2020, doi: 10.1016/j.heliyon.2020.e03719.

[39] A. Mandal, J. R. Clegg, A. C. Anselmo, and S. Mitragotri, “Hydrogels in the clinic,” *Bioeng. Transl. Med.*, vol. 5, no. 2, pp. 1–12, 2020, doi: 10.1002/btm2.10158.

[40] V. Garg and S. Jindal, “Nasal Bioadhesive Drug Delivery Systems and Their Applications,” *Bioadhesives Drug Deliv.*, pp. 213–243, 2020, doi: 10.1002/9781119640240.ch8.

[41] T. Adnet *et al.*, “Pharmacotechnical development of a nasal drug delivery composite nanosystem intended for alzheimer’s disease treatment,” *Pharmaceutics*, vol. 12, no. 3, 2020, doi: 10.3390/pharmaceutics12030251.

[42] F. Laffleur and B. Bauer, “Progress in nasal drug delivery systems,” *Int. J. Pharm.*, vol. 607, no. July, p. 120994, 2021, doi: 10.1016/j.ijpharm.2021.120994.

[43] C. A. Barbero, M. V. Martínez, D. F. Acevedo, M. A. Molina, and C. R. Rivarola, “Cross-Linked Polymeric Gels and Nanocomposites: New Materials and Phenomena Enabling Technological Applications,” *Macromol*, vol. 2, no. 3, pp. 440–475, 2022, doi: 10.3390/macromol2030028.

[44] K. Kaniewska, M. Karbarz, and E. Katz, “Nanocomposite hydrogel films and coatings – Features and applications,” *Appl. Mater. Today*, vol. 20, p. 100776, 2020, doi: 10.1016/j.apmt.2020.100776.

[45] W. Xing and Y. Tang, “On mechanical properties of nanocomposite hydrogels: Searching for superior properties,” *Nano Mater. Sci.*, vol. 4, no. 2, pp. 83–96, 2022, doi: 10.1016/j.nanoms.2021.07.004.

[46] A. P. Dhand, J. H. Galarraga, and J. A. Burdick, “Enhancing Biopolymer Hydrogel Functionality through Interpenetrating Networks,” *Trends Biotechnol.*, vol. 39, no. 5, pp. 519–538, 2021, doi: 10.1016/j.tibtech.2020.08.007.

[47] H. A. S. Tohamy *et al.*, “Temperature- and pH-Responsive Super-Absorbent Hydrogel Based on Grafted Cellulose and Capable of Heavy Metal Removal from Aqueous Solutions,” *Gels*, vol. 9, no. 4, 2023, doi: 10.3390/gels9040296.

[48] A. Kasiński, M. Zielińska-Pisklak, E. Oledzka, and M. Sobczak, “Smart hydrogels – synthetic stimuli-responsive antitumor drug release systems,” *Int. J. Nanomedicine*, vol. 15, pp. 4541–4572, 2020, doi: 10.2147/IJN.S248987.

[49] R. Chandrasekaran, M. Hillgärtner, K. Ganesan, B. Milow, M. Itskov, and A. Rege, “Computational design of biopolymer aerogels and predictive modelling of their nanostructure and mechanical behaviour,” *Sci. Rep.*, vol. 11, no. 1, pp. 1–10, 2021, doi: 10.1038/s41598-021-89634-1.

[50] R. Kubota and I. Hamachi, “Cell-Like Synthetic Supramolecular Soft Materials Realized in Multicomponent, Non-/Out-of-Equilibrium Dynamic Systems,” *Adv. Sci.*, vol. 11, no. 8, pp. 1–21, 2024, doi: 10.1002/advs.202306830.

[51] D. N. Hu *et al.*, “Injectable Temperature/Glucose Dual-Responsive Hydrogels for Controlled Release of Insulin,” *Ind. Eng. Chem. Res.*, vol. 60, no. 22, pp. 8147–8158, 2021, doi: 10.1021/acs.iecr.1c01277.

[52] H. N. A. Tuan and V. T. T. Nhu, “Synthesis and properties of pH-thermo dual responsive semi-IPN hydrogels based on N,N’-diethylacrylamide and itaconamic acid,” *Polymers (Basel).*, vol. 12, no. 5, 2020, doi: 10.3390/POLYM12051139.

[53] Z. Deng, R. Yu, and B. Guo, “Stimuli-responsive conductive hydrogels: Design, properties, and applications,” *Mater. Chem. Front.*, vol. 5, no. 5, pp. 2092–2123, 2021, doi: 10.1039/d0qm00868k.

[54] A. M. Padma, *Tissue engineering for novel female infertility treatments Studies on small and large animal models*. 2021.

[55] S. L. Loo, L. Vásquez, A. Athanassiou, and D. Fragouli, “Polymeric Hydrogels—A Promising Platform in Enhancing Water Security for a Sustainable Future,” *Adv. Mater. Interfaces*, vol. 8, no. 24, 2021, doi: 10.1002/admi.202100580.

[56] E. Van Der Giessen *et al.*, “Roadmap on multiscale materials modeling,” *Model. Simul. Mater. Sci. Eng.*, vol. 28, no. 4, 2020, doi: 10.1088/1361-651X/ab7150.

[57] N. N. Vlassis, R. Ma, and W. C. Sun, *Geometric deep learning for computational mechanics Part I: anisotropic hyperelasticity*, vol. 371. 2020. doi : org/10.1016/j.cma.2020.113299

[58] C. Y. Hui, F. Cui, A. Zehnder, and F. J. Vernerey, “Physically motivated models of polymer networks with dynamic cross-links: Comparative study and future outlook,” *Proc. R. Soc. A Math. Phys. Eng. Sci.*, vol. 477, no. 2255, 2021, doi: 10.1098/rspa.2021.0608.

[59] S. Z. Alshawwa, A. A. Kassem, R. M. Farid, S. K. Mostafa, and G. S. Labib, “Nanocarrier Drug Delivery Systems: Characterization, Limitations, Future Perspectives and Implementation of Artificial Intelligence,” *Pharmaceutics*, vol. 14, no. 4, 2022, doi: 10.3390/pharmaceutics14040883.

[60] R. Gupta, D. Srivastava, M. Sahu, S. Tiwari, R. K. Ambasta, and P. Kumar, *Artificial intelligence to deep learning: machine intelligence approach for drug discovery*, vol. 25, no. 3. Springer International Publishing, 2021. doi:org/10.1007/s11030-021-10217-3

[61] L. K. Vora, A. D. Gholap, K. Jetha, R. R. S. Thakur, H. K. Solanki, and V. P. Chavda, *Artificial Intelligence in Pharmaceutical Technology and Drug Delivery Design*, vol. 15, no. 7. 2023. doi: org/10.3390/pharmaceutics15071916

[62] S. Mansouri, “Application of Neural Networks in the Medical Field,” *J. Wirel. Mob. Networks, Ubiquitous Comput. Dependable Appl.*, vol. 14, no. 1, pp. 69–81, 2023, doi: 10.58346/JOWUA.2023.I1.006.

[63] J. Xiong, Z. Xiong, K. Chen, H. Jiang, and M. Zheng, “Graph neural networks for automated de novo drug design,” *Drug Discov. Today*, vol. 26, no. 6, pp. 1382–1393, 2021, doi: 10.1016/j.drudis.2021.02.011.

[64] M. G. M. Abdolrasol *et al.*, “Artificial neural networks based optimization techniques: A review,” *Electron.*, vol. 10, no. 21, 2021, doi: 10.3390/electronics10212689.

[65] P. Murinová and V. Novák, “The theory of intermediate quantifiers in fuzzy natural logic revisited and the model of ‘Many,’” *Fuzzy Sets Syst.*, vol. 388, pp. 56–89, 2020, doi: 10.1016/j.fss.2019.12.010.

[66] H. Goudjil, S. Rebouh, and M. Bouhedda, *Adaptative Neuro-Fuzzy Inference System for Predicting the Microbial Fungicide Release from Microcapsules Based on Alginate and Different Gelatin Proportions*, vol. 174. Springer International Publishing, 2021. doi: org/10.1007/978-3-030-63846-7_58

[67] S. Rebouh, M. Bouhedda, and S. Hanini, “Neuro-fuzzy modeling of Cu(II) and Cr(VI) adsorption from aqueous solution by wheat straw,” *Desalin. Water Treat.*, vol. 57, no. 14, pp. 6515–6530, 2016, doi: 10.1080/19443994.2015.1009171.

[68] D. Paul, G. Sanap, S. Shenoy, D. Kalyane, K. Kalia, and R. K. Tekade, “Artificial intelligence in drug discovery and development,” *Drug Discov. Today*, vol. 26, no. 1, pp. 80–93, 2021, doi: 10.1016/j.drudis.2020.10.010.

[69] S. Rebouh, S. Lefnaoui, M. Bouhedda, M. M. Yahoum, and S. Hanini, “Neuro-fuzzy modeling of ibuprofen-sustained release from tablets based on different cellulose derivatives,” *Drug Deliv. Transl. Res.*, pp. 162–177, 2019, doi: 10.1007/s13346-018-00592-0.

[70] A. Aldoseri, K. N. Al-Khalifa, and A. M. Hamouda, “Re-Thinking Data Strategy and Integration for Artificial Intelligence: Concepts, Opportunities, and Challenges,” *Appl. Sci.*, vol. 13, no. 12, 2023, doi: 10.3390/app13127082.

[71] T. Wang, Y. Wu, J. Lamothe, F. Benaben, R. Wang, and W. Liu, “A Data-Driven and Knowledge-Driven Method towards the IRP of Modern Logistics,” *Wirel. Commun. Mob. Comput.*, vol. 2021, 2021, doi: 10.1155/2021/6625758.

[72] S. Suryadevara, “Energy-Proportional Computing: Innovations in Data Center Efficiency and Performance Optimization,” *... J. Adv. Eng. Technol. ...*, vol. 01, no. 02, pp. 44–64, 2021, [Online]. Available: https://ijaeti.com/index.php/Journal/article/download/399/410.

[73] S. Peers, A. Montembault, and C. Ladavière, “Chitosan hydrogels incorporating colloids for sustained drug delivery,” *Carbohydr. Polym.*, vol. 275, 2022, doi: 10.1016/j.carbpol.2021.118689.

[74] L. Wang and C. A. Alexander, “Big data analytics in medical engineering and healthcare: methods,

advances and challenges," *J. Med. Eng. Technol.*, vol. 44, no. 6, pp. 267–283, 2020, doi: 10.1080/03091902.2020.1769758.

[75] S. Almawash, S. K. Osman, G. Mustafa, and M. A. El Hamd, "Current and Future Prospective of Injectable Hydrogels—Design Challenges and Limitations," *Pharmaceuticals*, vol. 15, no. 3, 2022, doi: 10.3390/ph15030371.

[76] H. Goudjil, S. Rebouh, and M. Bouhedda, "Neuro-fuzzy model for predicting insulin delivery from crosslinked agar-carbomer hydrogels," *Comput. Methods Biomech. Biomed. Engin.*, vol. 0, no. 0, pp. 1–16, 2024, doi: 10.1080/10255842.2024.2362863.

[77] M. Buysschaert and V. Preumont, "Place du Suliqua ® , une association à ratio fixe d ' insuline glargine et de lixisénatide , dans le traitement du diabète de type 2."

[78] G. Vishwakarma, N. Nupur, and A. S. Rathore, "Assessing the Structural and Functional Similarity of Insulin Glargine Biosimilars," *J. Diabetes Sci. Technol.*, vol. 17, no. 2, pp. 417–427, 2023, doi: 10.1177/19322968211058482.

[79] F. Rehman *et al.*, "Optimization, in vitro release and toxicity evaluation of novel pH sensitive itaconic acid-g-poly(acrylamide)/sterculia gum semi-interpenetrating networks," *DARU, J. Pharm. Sci.*, vol. 29, no. 1, pp. 171–184, 2021, doi: 10.1007/s40199-021-00395-8.

[80] M. S. Azerêdo *et al.*, "Environmentally friendly adhesives derived from glycerol-based polymers," *J. Adhes. Sci. Technol.*, vol. 36, no. 1, pp. 98–108, 2022, doi: 10.1080/01694243.2021.1915619.

[81] S. Srinivasan and V. Manoj, "A decade of effective dry eye disease management with systane ultra (Polyethylene glycol/propylene glycol with hydroxypropyl guar) lubricant eye drops," *Clin. Ophthalmol.*, vol. 15, pp. 2421–2435, 2021, doi: 10.2147/OPTH.S294427.

[82] M. Santoro *et al.*, "Smart approach to evaluate drug diffusivity in injectable agar-carbomer hydrogels for drug delivery," *J. Phys. Chem. B*, vol. 115, pp. 2503–2510, 2011. doi : org/10.1021/jp1111394

[83] Y. Song, Y. Cong, B. Wang, and N. Zhang, "Applications of Fourier transform infrared spectroscopy to pharmaceutical preparations," *Expert Opin. Drug Deliv.*, vol. 17, no. 4, pp. 551–571, 2020, doi: 10.1080/17425247.2020.1737671.

[84] "European Pharmacopoeia,", 2016.

[85] S. Schütz and R. Sprangers, "Methyl TROSY spectroscopy: A versatile NMR approach to study challenging biological systems," *Prog. Nucl. Magn. Reson. Spectrosc.*, vol. 116, no. xxxx, pp. 56–84, 2020, doi: 10.1016/j.pnmrs.2019.09.004.

[86] M. Li, W. Xu, and Y. Su, "Solid-state NMR spectroscopy in pharmaceutical sciences," *TrAC - Trends Anal. Chem.*, vol. 135, p. 116152, 2021, doi: 10.1016/j.trac.2020.116152.

[87] M. Bouhedda, S. Lefnaoui, S. Rebouh, and M. M. Yahoum, "Predictive model based on Adaptive Neuro-Fuzzy Inference System for estimation of Cephalexin adsorption on the Octenyl Succinic Anhydride starch," *Chemom. Intell. Lab. Syst.*, vol. 193, no. July, p. 103843, 2019, doi: 10.1016/j.chemolab.2019.103843.

[88] S. I. Abba *et al.*, "Emerging evolutionary algorithm integrated with kernel principal component analysis for modeling the performance of a water treatment plant," *J. Water Process Eng.*, vol. 33, p. 101081, 2020. doi : org/10.1016/j.jwpe.2019.101081

[89] M. S. TOMOHIRO TAKAGI, "Fuzzy Identification of Systems and Its Applications to Modeling and Control," *IFAC Proc. Vol.*, vol. 15, pp. 116–132, 1985, doi: 10.1016/s1474-6670(17)42481-5.

[90] Z. Huang and J. Hahn, "Fuzzy modeling of signal transduction networks," *Chem. Eng. Sci.*, vol. 64, no. 9, pp. 2044–2056, 2009, doi: 10.1016/j.ces.2009.01.041.

[91] S. Rebouh, M. Bouhedda, and S. Hanini, "Neuro-fuzzy modeling of Cu(II) and Cr(VI) adsorption from aqueous solution by wheat straw," *Desalin. Water Treat.*, vol. 57, pp. 6515–6530, 2016. doi : org/10.1080/19443994.2015.1009171

[92] J. Zhou, C. Li, C. A. Arslan, M. Hasanipanah, and H. Bakhshandeh, "Performance evaluation of hybrid FFA - ANFIS and GA - ANFIS models to predict particle size distribution of a muck - pile after blasting," *Eng. Comput.*, vol. 37, pp. 265–274, 2021. doi: org/10.1007/s00366-019-00822-0

[93] D. Karaboga and E. Kaya, "Adaptive network based fuzzy inference system (ANFIS) training approaches: a comprehensive survey," *Artif. Intell. Rev.*, vol. 52, no. 4, pp. 2263–2293, 2019, doi: 10.1007/s10462-017-9610-2.

[94] N. Talpur, M. N. M. Salleh, and K. Hussain, "An investigation of membership functions on performance of ANFIS for solving classification problems," *IOP Conf. Ser. Mater. Sci. Eng.*, vol. 226, no. 1, 2017, doi: 10.1088/1757-899X/226/1/012103.

[95] N. Walia, H. Singh, and A. Sharma, "ANFIS: Adaptive Neuro-Fuzzy Inference System- A Survey," *Int. J. Comput. Appl.*, vol. 123, no. 13, pp. 32–38, 2015, doi: 10.5120/ijca2015905635.

[96] J. S. R. Jang, "ANFIS: Adaptive-Network-Based Fuzzy Inference System," *IEEE Trans. Syst. Man Cybern.*, vol. 23, no. 3, pp. 665–685, 1993, doi: 10.1109/21.256541.

[97] Council of Europe, "European Pharmacopoeia 8th Edition," *Eur. Pharmacopoeia*, p. 2565, 2014.

[98] R. W. Korsmeyer, R. Gurny, E. Doelker, P. Buri, and N. A. Peppas, "Mechanisms of solute release from porous hydrophilic polymers," *Int. J. Pharm.*, vol. 15, no. 1, pp. 25–35, 1983, doi: 10.1016/0378-5173(83)90064-9.

[99] S. Lefnaoui and N. Moulai-Mostefa, "Investigation and optimization of formulation factors of a hydrogel network based on kappa carrageenan-pregelatinized starch blend using an experimental design," *Colloids Surfaces A Physicochem. Eng. Asp.*, vol. 458, no. 1, pp. 117–125, 2014, doi: 10.1016/j.colsurfa.2014.01.007.

[100] J. Maitra and V. K. Shukla, "Cross-linking in Hydrogels - A Review," *Am. J. Polym. Sci.*, vol. 4, no. 2, pp. 25–31, 2014, doi: 10.5923/j.ajps.20140402.01.

[101] J. Siepmann and N. A. Peppas, "Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC)," *Adv. Drug Deliv. Rev.*, vol. 64, no. SUPPL., pp. 163–174, 2012, doi: 10.1016/j.addr.2012.09.028.

[102] G. He *et al.*, "Preparation and properties of quaternary ammonium chitosan-g-poly(acrylic acid-co-acrylamide) superabsorbent hydrogels," *React. Funct. Polym.*, vol. 111, pp. 14–21, 2017, doi: 10.1016/j.reactfunctpolym.2016.12.001.

[103] P. R. Sivashankari and M. Prabaharan, "Three-dimensional porous scaffolds based on agarose/chitosan/graphene oxide composite for tissue engineering," *Int. J. Biol. Macromol.*, vol. 146, pp. 222–231, 2020, doi: 10.1016/j.ijbiomac.2019.12.219.

[104] X. Zhang, X. Gu, X. Wang, H. Wang, and S. Mao, "Tunable and sustained-release characteristics of venlafaxine hydrochloride from chitosan–carbomer matrix tablets based on in situ formed polyelectrolyte complex film coating," *Asian J. Pharm. Sci.*, vol. 13, pp. 566–574, 2018, doi: 10.1016/j.ajps.2018.01.004.

[105] K. Baysal, A. Z. Aroguz, Z. Adiguzel, and B. M. Baysal, "Chitosan/alginate crosslinked hydrogels: Preparation, characterization and application for cell growth purposes," *Int. J. Biol. Macromol.*, vol. 59, pp. 342–348, 2013, doi: 10.1016/j.ijbiomac.2013.04.073.

[106] H. Sara, M. M. Yahoum, S. Lefnaoui, H. Abdelkader, and N. Moulai-Mostefa, "New alkylated xanthan gum as amphiphilic derivatives: Synthesis, physicochemical and rheological studies," *J. Mol. Struct.*, vol. 1207, p. 127768, 2020, doi: 10.1016/j.molstruc.2020.127768.

[107] Y. Morono *et al.*, "Aerobic microbial life persists in oxic marine sediment as old as 101.5 million years," *Nat. Commun.*, vol. 11, no. 1, 2020, doi: 10.1038/s41467-020-17330-1.

[108] A. S. Lee *et al.*, "Methicillin-resistant Staphylococcus aureus," *Nat. Rev. Dis. Prim.*, vol. 4, no. May, pp. 1–23, 2018, doi: 10.1038/nrdp.2018.33.

[109] Z. Pang, R. Raudonis, B. R. Glick, T. J. Lin, and Z. Cheng, "Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies," *Biotechnol. Adv.*, vol. 37, no. 1, pp. 177–192, 2019, doi: 10.1016/j.biotechadv.2018.11.013.

[110] M. Hatti, *Lecture Notes in Networks and Systems 174 Artificial Intelligence and Renewables Towards an Energy Transition*. .

[111] F. C. Barbosa *et al.*, "Progress in the development of Chitosan based insulin delivery systems: A systematic literature review," *Polymers (Basel).*, vol. 12, no. 11, pp. 1–21, 2020, doi: 10.3390/polym12112499.

[112] Z. I. Al-Kurdi, B. Z. Chowdhry, S. A. Leharne, M. M. H. Al Omari, and A. A. Badwan, "Low molecular weight chitosan-insulin polyelectrolyte complex: Characterization and stability studies," *Mar. Drugs*, vol. 13, no. 4, pp. 1765–1784, 2015, doi: 10.3390/md13041765.

[113] A. H. Krauland and M. J. Alonso, "Chitosan/cyclodextrin nanoparticles as macromolecular drug delivery system," *Int. J. Pharm.*, vol. 340, no. 1–2, pp. 134–142, 2007, doi: 10.1016/j.ijpharm.2007.03.005.

[114] M. R. Avadi *et al.*, "Preparation and characterization of insulin nanoparticles using chitosan and Arabic gum with ionic gelation method," *Nanomedicine Nanotechnology, Biol. Med.*, vol. 6, no. 1, pp. 58–63, 2010, doi: 10.1016/j.nano.2009.04.007.

[115] S. Jose *et al.*, "Predictive modeling of insulin release profile from cross-linked chitosan microspheres," *Eur. J. Med. Chem.*, vol. 60, pp. 249–253, 2013, doi: 10.1016/j.ejmech.2012.12.011.

[116] D. Psimadas, P. Georgoulias, V. Valotassiou, and G. Loudos, "Molecular Nanomedicine Towards Cancer :," *J. Pharm. Sci.*, vol. 101, no. 7, pp. 2271–2280, 2012, doi: 10.1002/jps.

[117] D. Tahtat, M. Mahlous, S. Benamer, A. N. Khodja, H. Oussedik-Oumehdi, and F. Laraba-Djebari, "Oral delivery of insulin from alginate/chitosan crosslinked by glutaraldehyde," *Int. J. Biol. Macromol.*, vol. 58, pp. 160–168, 2013, doi: 10.1016/j.ijbiomac.2013.03.064.

[118] A. K. Agrawal *et al.*, "Development and characterization of in situ gel system for nasal insulin delivery," *Pharmazie*, vol. 65, no. 3, pp. 188–193, 2010, doi: 10.1691/ph.2010.9188.

[119] Z. Gu *et al.*, "Glucose-responsive microgels integrated with enzyme nanocapsules for closed-loop insulin delivery," *ACS Nano*, vol. 7, no. 8, pp. 6758–6766, 2013, doi: 10.1021/nn401617u.

[120] C. Giovino, I. Ayensu, J. Tetteh, and J. S. Boateng, "An integrated buccal delivery system combining chitosan films impregnated with peptide loaded PEG-b-PLA nanoparticles," *Colloids Surfaces B Biointerfaces*, vol. 112, pp. 9–15, 2013, doi: 10.1016/j.colsurfb.2013.07.019.

[121] F. Cui *et al.*, "Preparation and evaluation of chitosan-ethylenediaminetetraacetic acid hydrogel films for the mucoadhesive transbuccal delivery of insulin," *J. Biomed. Mater. Res. - Part A*, vol. 89, no. 4, pp. 1063–1071, 2009, doi: 10.1002/jbm.a.32071.