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Smart rolled-up capsules for drug release control

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Abstract

The pharmacokinetics of many drugs, namely their absorption, distribution, metabolism and elimination, depend on the time of their administration.Therefore, these drugs are more effective and better tolerated if taken at the right time. The synchronization of drug delivery with the circadian cycle has attracted the attention of many researchers in recent years. Currently, the design of drugs with programmed release of active ingredients is undergoing considerable development within the framework of an emerging practice called personalized medicine[1], [2].

In the frame of this thesis, we are designing a new oral dosage form allowing a time-modulated release of drugs. We combine a theoretical idea of P.Lee [3] suggesting that a non-uniform distribution of a drug in a micro/nanoporous matrix medium can be explored for the programming of drug release, with an experimental system of T.Higuchi [4] which consists of rolling-up a film, soluble in body fluids, and covered by a drug.

The novelty of our system with respect to the one of Higuchi consists in the fact, that the drug release is diffusion controlled, rather than dissolution-controlled. In our work,well-defined radial distributions of the drugs were achieved by winding the strips of thermally crosslinked gelatin, carrying the drug patterns The rolling-up operation transforms the bidimensial distribution of a drug into the spatial distribution according to a well-defined manner, following from the Archimedean spiral shape of the rolled capsules. We concentrate our efforts on the realization of the biphasic drug release systems, which constitute an important subcalss of the systems for chronomodulated drug delivery.

On the other hand**,** the biphasic release systems are used for rapid release of a specific amount of drug for immediate amelioration of a patient's condition, followed by sustained release, in order to avoid repetitive administrations. This type of administration is often necessary to treat many illnesses, such as migraine, hypertension, insomnia, etc.

Compared to traditional biphasic release systems, our system is designed from a single material and which, depending on the initial position of the drug reservoir, can control the time and the rate for its release. Either monodrug or multidrug biphasic release is successfully demonstrated using model fluorescent substances (Fluorescein and Rhodamine B). The incorporation of more than one active ingredient in the formulation is desirable, as this increases patient compliance and reduces the cost of treatment, in particular when separate dosages of active ingredients can be individually adjusted in situ, in order to meet the specific needs of each patient.

The gelatin matrices used for the design of the rolled-up release systems were characterized by a range of materials characterisation methods (FT-IR spectroscopy ; contact angle and absorption study ; Scanning electron microscopy ; atomic force microscopy ; tensile testing ; gas adsorption (BET) ; X-ray diffraction ; differential scanning calorimetry ; thermogravimetric analysis). The crosslinking degree of the gelatin films was determined by the trinitrobenzensulphonic acid (TNBSa) method. The drug release kinetics were studied with the use of the USP2 United States Pharmacopeia dissolution apparatus.The experimental study was completed by a numerical simulation which showed that the drug concentration profiles and the respective release kinetics strongly depend on the radial position of the drug reservoir inside the capsule and that the gain in the release rate through the outer surface prevails the loss of the release rate through the inner surface, so that the net result is the acceleration of the overall release rate.

Résumé

La pharmacocinétique de nombreux médicaments, à savoir leur résorption, leur distribution, leur métabolisme et leur élimination, dépend de l'heure d'administration. Par conséquent, ces médicaments sont plus efficaces et mieux tolérés s'ils sont pris à un moment approprié. La synchronisation de l'administration des médicaments avec le cycle circadien a attiré l'attention de nombreux chercheurs au cours des dernières années. Actuellement, la conception de médicaments à libération programmée de principes actifs connait un développement considérable dans le cadre d'une pratique émergente appelée la médecine personnalisée [1], [2].

Dans le cadre de cette thèse, nous concevons une nouvelle forme galénique orale permettant une libération chronomodulée de médicaments. Nous combinons une idée théorique [3] suggérant qu'une distribution non uniforme d'un médicament dans un milieu matriciel micro/nanoporeux peut être explorée pour la programmation de la libération du médicament, avec un système expérimental [4] qui consiste à enrouler un film, soluble dans les fluides corporels, et couvert par un médicament. Des distributions radiales bien définies des médicaments ont été réalisées par l'enroulement des bandes de gélatine thermiquement réticulés. Cet enroulement transforme la distribution axiale en une distribution spatiale selon une fonction bien définie, suite à la forme spirale archimédienne des rouleaux.

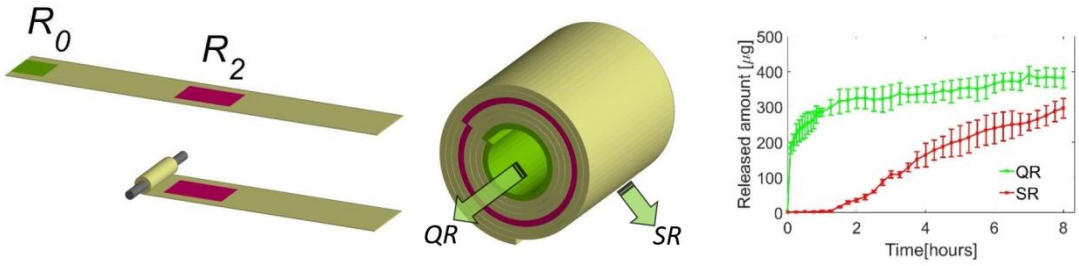
D'un autre coté, les systèmes de relargage biphasique sont utilisés pour une libération rapide d'une quantité spécifique de médicament pour une amélioration immédiate de l'état d'un patient, suivie d'une libération prolongée, afin d'éviter des administrations répétitives. Ce type d'administration est souvent nécessaire pour traiter nombreuses maladies, telles que la migraine, l'hypertension, l'insomnie, etc.

Comparés aux systèmes traditionnels de relargage biphasique, notre système est conçu d'un seul matériau et qui suivant la distribution initiale du médicament peut contrôler le temps nécessaire au déclenchement de la libération du principe actif.

A partir de notre système, une libération biphasique est démontrée avec succès à l'aide de substances fluorescentes modèles (Fluorescéine et Rhodamine B). En effet, la géométrie enroulées des capsules permet un relargage immédiat à partir de la cavité centrale de la capsule, appelé Quick Release et noté QR ainsi que le relargage d'une deuxième dose noté SR qui signifie ''Sustained Release (Figure Résumé).

Par ailleurs, l'incorporation de plus d'un principe actif dans la formulation est souhaitable, car ceci augmente l'observance du patient et réduit le coût du traitement, en particulier lorsque des dosages distincts de principes actifs peuvent être ajustés individuellement in situ, afin de répondre aux besoins spécifiques de chaque patient, les capsules **‘’multidrug’’** ont également été produites par la même approche.

Les matrices polymères qui ont servi à la conception des différents systèmes étudiés ont été caractérisées et les mécanismes de relargage ont été étudiés. L’étude expérimentale à été complétée par une simulation numérique pour le deuxième système conçu.



Capsules for biphasic drug release are designed by the rolling-up approach. Quick release (QR) from the cavity is followed by the sustained release (SQ) through the layers of the roll.

Figure Résumé Conception des capsules enroulées (a) Enroulement de bande à deux reservoirs (b) Section transversal de la capsule

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Table of contents

Abstract 1

Résumé 3

Acknowledgement 5

Table of contents 6

Glossary 8

Preface 10

Chapter 1. State of the art 13

1-1- Pharmacokinetics and chronomodulated release 13

1-2- Biphasic release systems 16

1-3- Thin films for drug release 26

1-4- Biomaterials used as matrices in the controlled drug release formulations 30

1-5- Gelatin films and the methods used to enhance their properties 34

1-6- Methods for drug Release Study 38

Problem statement and thesis challenges 48

Chapter 2. Materials and Methods 49

2-1- DHT effect on gelatin films properties 50

2-2- Fabrication of the Rolled-up capsules with cavity for drug release control 55

2-3- Fabrication of the rolled up capsules without cavity for drug release control 58

Chapter 3. Rolled-up capsules design for drug release control (Matrix characterization) 60

3-1- Preliminary study: Heating time optimisation 60

3-2- Comparative study between untreated, 4h treated and 8h treated films 62

3-3- Selected gelatin films characteristics 72

Chapter 4. Biphasic drug release from rolled-up capsules with a central cavity 78

4-1- Controlled release preliminary study: Proof of concept 78

4-2- Biphasic Monodrug release 85

4-2- Biphasic Multidrug release 86

4-3- Comparison between model drugs release kinetics 87

4-4- pH dependence of drug release 87

4-5- Computer simulation of drug release from the capsule with cavity and a reservoir embedded between the shells of the roll 88

Chapter 5. Programmed drug release from the capsules formed by rolling up self-adhesive flexible gelatin films 93

5-1- Matrix characterization 95
5-2- Drug release study 99
5-3- Kinetic Models 100
5-4- Computer simulation of drug release from the capsule without cavity and a reservoir embedded between the shells of the roll 104
Chapter 6. Conclusion and Outlook 107
Annexes (French summary) 109
Presentations and Publications list 114
References 115

Glossary

ADME	Administration, Distribution, Metabolism and Elimination
API	Active Pharmaceutical Ingredient
BCS	Biopharmaceutics Classification System
CBZ	Carbamazepine
CDD	Controlled Delivery Devices
CMC	Carboxymethyl Cellulose
CR-GRDF	Controlled Release-Gastroretentive Dosage Forms
DEA	Diethanolamine
DEG	Diethylen Glycol
EA	Ethanolamine
EG	Ethylen Glycol
FD	Fluorescein Disodium
FDDS	Floating Drug Delivery System
HPC	Hydroxypropyl Cellulose
HPMC	Hydroxy Propylmethyl Cellulose
HPβCD	Hydroxypropyl-β-Cyclodextrin
IRMT	Immediate Release Mini-Tablets
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
ODF	Orodispersible films
PBS	Phosphate Buffer Solution
PEO	Polyethylen Oxide
PVP	Polyvinylpyrrolidone
PVA	Polyvinyl Alcohol
Rh B	Rhodamine B
SRMT	Sustained Release Mini-Tablets
TEG	Triethylen Glycol

TEA	Triéthanolamine
TAβCD	Triacetyl-β-Cyclodextrin
USP	United States Pharmacopeia
WVP	Water Vapor Permeability

Preface

In order to increase the therapeutic efficacy of a drug by maintaining its concentration at an effective level, controlled release systems which aim to release drugs at a controllable level over an extended period of time have been developed.

In the last years, scheduled drug delivery technology has continued to develop and has shown its effectiveness in ensuring dramatic improvements in the quality of life and well-being of patients as drug administration time is not always comfortable for the patient. The possibility of improving the efficiency of the treatment as well as the toxicological profile of the active substances has prompted many scientists to push their research and favor solutions that are more favorable than the previous ones and which present fewer risks for users.

Indeed, the researchers developed new drug-containing systems with the potential to deliver tailored therapies to different patient populations namely those who need multidose [2] in the context of what is called chronotherapy. Indeed, the synchronization of the drug release with the body biological rhythms, in particular the circadian rhythms [5] can considerably improve the efficiency of the therapy. Non-uniform drug distribution constitutes an advanced approach to programming an optimized diffusion-controlled drug release. In this context, we developed biopolymer capsules with arbitrary complex spatial distributions of drugs. The method is simple; Thin strips of thermally treated gelatin are rolled up. The planar distribution of the drug along the strip changes into different radial positions inside the capsule according to a well-defined relationship, resulting from the Archimedean spiral shape of the capsules. This approach allows more than one drug to be incorporated into the strip to produce multidrug capsules as shown in the Figure-Preface1.

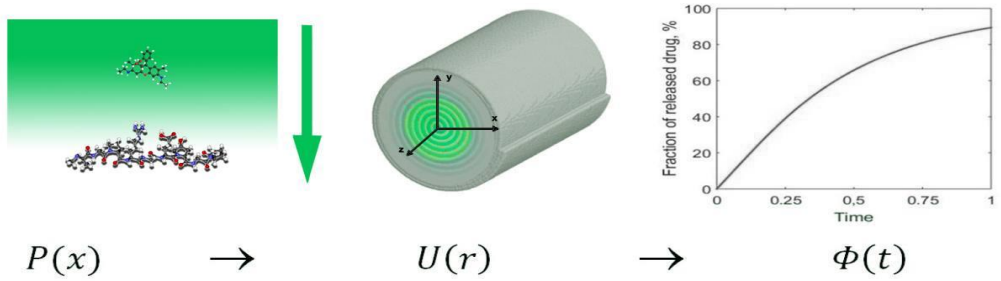


Figure-Preface 1 A drug distribution over a biopolymer strip, P(x), is transformed by rolling into radial distribution in the cylindrical capsule, U(r), which determines the kinetics of the drug release, φ (t).

The method's potential for chronotherapy has been explored by performing in vitro dissolution assays for biphasic and multi-drug systems[6].This approach brings new possibilities for personalized medicine and allows patients to benefit from the advantages of time-modulated therapies.

This PhD thesis therefore aims to develop a prototype of rolled-up systems for drug release control and to understand this novel dosage form release kinetics.The main objective of the PhD thesis was to bring contributions in personalized medicines by creating a new controlled release system, simple and cheap.

Chapter 1 constitutes an overview on scheduled release systems [7], and especially the biphasic release systems. It focuses on challenges facing these cutting-edge technologies. The chapter is ended by thestatement of the thesis objectives.

The second chapter is about the materials and methods used during the study.

Given that the development of these relatively complex delivery systems require the use of materials with specific properties, a deep discussion of the physicochemical properties of the thermocrosslinked gelatin matrix is highlighted in **Chapter 3.** The achievement of this general objective supposes the resolution of several specific objectives, the most important of which is the optimization of the duration of thermal crosslinking carried out on the excipient in order to obtain films which are insoluble during the dissolution study and flexible for the rolling-up, yet mechanically resistant. Gelatin films were characterized structurally, morphologically, mechanically and thermally.

The 4th section represents the basic idea of the thesis which consists of developing the rolled-up capsules for the controlled biphasic drug release. The capsules with a central cavity ensure immediate drug release combined with delayed release. First, the stabilisation of the rolled-up state of the thermocrosslinked gelatin stripes with the use of Transglutaminase is demonstrated. Then, the possibility to incorporate a model fluorescent drug into the capsules via the rolling-up approach, and to release the drug in a physiologically relevant media was investigated. Subsequently, the proof of concept was successfully established through in vitro dissolution tests giving programmed release kinetics. The release kinetics of fluorescent probes from different positions inside the capsules were fitted to different kinetic models.

In the 5th section, a second system is developed by changing the crosslinking method used as well as the geometry of the capsule. This system does not present a central cavity and Transglutaminase is used as a crosslinking agent. Sorbitol is added to the gelatin matrix in order to ensure the flexibility of the films. Consequently, research is directed towards the

effect of this combination on the mechanical properties of the pharmaceutical matrix in order to find a compromise between the stability of the polymer network during dissolution, and the flexibility of the films. The self-adhesiveness of the films of such composition provides the stability of the rolls without the use of the transglutaminase glue. A simple computer model of the drug release from the rolled up capsules is proposed for an idealised case of instant swelling of the capsules

In the **Outlook,** we discuss the future study of the release of vitamin B2 (Riboflafin) in the Fasted-State Simulated Intestinal Fluid (FaSSIF), imitating the upper parts of the gastrointestinal tract.

Chapter 1. State of the art

1-1- Pharmacokinetics and chronomodulated release

The branch dealing with the pharmacological aspects of chronobiology is called **chronopharmacology**. It can be subdivided into chronotherapy, chronopharmacokinetics and chronotoxicity. Chronopharmaco kinetics studies the variation in plasma drug level depending on the time of day and the mechanisms responsible for time-dependent variations [8]. The time of administration of a drug is a key factor that influences the pharmacokinetic process which is divided into absorption, distribution, metabolism and excretion (ADME). In fact, drugs can be more effective if given at a specific time. Their concentration as well as that of the metabolite in tissues and target organs are determined by **circadian pharmacokinetics** which can be translated into chronotoxicity and chronoefficacy[5]. In order to restore disturbed biological rhythms and optimize the effects of drugs, the synthesis of human temporal organization in physiology, pathology, pharmacology and toxicity is required under the name of **chronotherapy.** This notion takes into account homeostasis and the circadian system [9]. Indeed, circadian rhythms significantly influence daily physiological activities including the regulation of sleep patterns, eating behavior, hormone release, hormonal homeostasis, blood pressure and the maintenance of body temperature. The trend of current research is to modulate different circadian rhythms for therapeutic purposes. It is therefore necessary to understand the deep and complex mechanisms of the circadian rhythm as well as the timely coordination of different clocks which is crucial to advance chronotherapy [4]. Circadian rhythms in ADME processes as well as mechanisms related to the regulation of the circadian clock have been revealed and clarified for many drugs, some of which are listed in the Table 1.1 (see also the review of Dong *et al*[5]). Providing the right intervention, including medications, to the right patient at the right time and at the right dose is the foundation of personalized medicine. As part of this practice, Artificial Intelligence systems with acceptable performance, easily interpretable by the clinical community and validated in a large cohort have been developed [10]. Based on indications regarding dosage and timing, optimization of the therapeutic effects of a substance could be achieved. Consequently, the optimal administration schedule is determined by evaluating the chronoefficacy of the active ingredient in relation to its chronotoxicity[9]. Multiple are the examples which prove that the time of drug administration is responsible for a large variability in the efficacy and/or toxicity of the drug.

Table1-1 Drugs and administration times

Drug	Administaration time	Explainations and Benefits	Ref
Theophylline	Morning	Higher Cmax and shorter Tmax	[8]
Propanolol	Morning	More rapid absorption after	[11]
Doxorubicin	9:00 p.m.	A reduced body clearance A longer elimination half-life An increased AUC	[12]
Nifedipine	Evening	Lowerbioavailability	[13]
Amlodipine	It does not matter what time of day you take amlodipine (morning or evening) but it is best to take it at the same time every day,		[14][15]
Valsartan	At bedtime	Improvement of the day/night ratio of blood pressure	[15]
Les anticoagulants	In the evening	Limit the risk of vascular accidents and myocardial infarction	[9]
5-Fu	At 4a.m.	allow a 50% improvement over the non-timed treatment	[16]
Tobramycin	Renal elimination is not affected by the time of day of administration. Urinary KIM-1 raises the possibility of greater nephrotoxicity with evening administration		[17]
Corticosteroids	In the morning	Respecting the physiological rhythm of cortisol, the secretion peak of which is between 7 a.m. and 9 a.m.) ensures better tolerance and better efficacy.	[8]
Prednisolone	At noon	Highest values of the maximum concentration and the area under the curve 18 h: it gives the highest values of half-life and volume of distribution.	[18]
Atorvastatin		Absorption rate and extent are affected by time-of-day	[19]

Rosuvastatin **Simvastatin**	An evening dose	administration Pharmacokinetic properties are unaffected Both drugs, the lipid lowering effects are similar whether administered in the morning or evening. A better lipid lowering effect	
Mattformin	At night	Helps treating high glucose levels overnight by blocking nocturnal hepatic glycogenolysis	[20]
Salbutamol sulphate (nocturnal asthma)	At bed time	Release the contents in the early hours of morning when the asthma symptoms are prevalent.	[21]
Cisplatin	At 6 :00 pm	More efficient	[9]
Acetylsalicylic acid	At bedtime	Superior effects	[8]
Levothyroxin	8 :00 am	Levothyroxine and testosterone show a peak of physiological secretion	[9]
Opiates	In the middle of the night	Maximum analgesicactivity	[9]
Indomethacine	In the evening	Bettertolerance	[8][9]
DA, 8159	10:00 a.m. vs 10:00 p.m. : showed no significant difference		[9]
Aspirine	At dinner or at bedtime	A better digestive tolerance	[9]
Isepamicin	At night : Lower elimination rate constant and longer elimination half-life Morning or evening : Same clinical effects In the evening : Depressed Clearance Morning therapy is desirable because of possible interference from aminoglycoside toxicity.		[22]

Preliminary screening of new drugs for their chronotherapeutic potential may be one way to improve the quality of drug use [21]**.** Knowledge of chronotherapy is growing and current research on chronotherapy shows promise in the design of new drug release systems. Chronotherapy studies should also explore differences related to genetics, gender, and age.

Research for establishing strong synchronization and spatiotemporal dynamics continues to advance in order to improve the efficacy of drugs and reduce their toxicity but synchronization of drug administration with the circadian cycle is not always possible or practical.

1-2- Biphasic release systems

Yeon.H et al. suggested drugs classification in a review called “Controlled Drug Delivery: Historical perspective for the next generation [23]. The first generation of drug release technologies (1950-1980) faced physicochemical problems which are mainly due to the low water solubility of drugs, the high molecular weight of peptide and protein drugs and the difficulty in controlling the kinetics of drug release. The second generation (1980-2010) succeeded in adjusting the physicochemical properties but struggled with biological barriers. In recent years, a growing interest has developed in designing the third generation which must overcome physicochemical and biological barriers. This generation consists in controlled drug release systems (2010-2040).

The biphasic systems that represent the main topic of our study are part of this third generation. So, what is a biphasic system and why is it considered a key in chronotherapy?

The biphasic release systems constitute an important case of the controlled drug release formulations. Up to the date, such systems were designed as the assembly of two excipients with two different characteristic release times. The first excipient, or the « loading dose », provides an immediate-release phase, necessary for reaching a therapeutic level of drug in the blood plasma soon after administration. The second excipient or a sustained-release reservoir called "dose maintenance" which maintains the therapeutic plasma concentration of the drug for a defined period of time. Thus, this configuration is designed to release drug at two different rates or in two different time periods. The first phase responds to a need for maximum and rapid relief and the second is a prolonged-release phase which avoids repeated administration. Nonsteroidal anti-inflammatory drugs (NSAIDs) and antihypertensive agents, antihistamines, antiallergics, antipsychotics and hypnotics are suitable candidates for this type of administration. For example, for migraine and sleep disorders, biphasic treatment rather than a single phase sustained release preparation is highly desirable [24][25]. Below we review the most important formulations for the biphasic drug release.

1-2-1- Tablets

In all of the two-phase release systems, more than one polymer is used and different additives have been incorporated in order to adjust certain properties as well as relatively complex methods have been applied. Careful choice of the components of each layer must be made.

Poly (ethylene oxide) (PEO) and HydroxyPropylMethylCellulose (HPMC) with diclofenac sodium have been compressed into a **three-layer matrix :** a barrier layer, a controlled release layer (~ 84%) and an instant release layer (~ 16%)) [26]. This asymmetric sodium diclofenac release system is one of the oldest biphasic systems which show the importance of the polymers nature in the composition of biphasic systems. Indeed, adjusting certain properties of the polymers control drug release kinetics. Another system confirmed the dependence of the release profile on both the type and amount of polymer in the core tablets; **Compressed Matrix Core Tablets** were formulated to modulate the release of the Ibuprofen. The Ibuprofen contained in the fast releasing component was dissolved within 2 minutes, whereas the drug in the core tablet was released at different times (≈16 or 924 hours), depending on the composition of the matrix tablet [27]. In 2009, an interesting study was performed to design **bilayer regioselective floating tablets** of atenolol and lovastatin to give immediate release of lovastatin and sustained release of atenolol. Bilayer floating tablets having different release profiles for different drugs could be formulated. HPMC and Xanthan gum (alone and in combination) as the release retarding polymers give controlled release of atenolol, and sodium starch glycollate as a super disintegrant gives immediate release of lovastatin [28]. Hydrophilic and hydrophobic cyclodextrins were combined to prepare **bilayer tablets** that can perform as quick/slow biphasic release systems of a poorly soluble drug (Carbamazepine (CBZ)). Hydroxypropyl-β-cyclodextrin (HPβCD) was chosen as complexing agent in the rapid release layer while triacetyl-β-cyclodextrin (TAβCD) was tested as controlling release agent in the sustained release layer. Croscarmellose sodium was utilized as superdisintegrant in the rapid release layer, and sodium stearyl fumarate was applied as anti-adherent lubricant in both layers. The results highlighted the feasibility of the combination of HPβCD/CBZ inclusion complex with croscarmellose sodium in the rapid release layer to achieve fast dissolution for the first 30–45 min, and TAβCD as controlling agent in the sustained release layer of the bilayer tablets to obtain a prolonged release during 720 min[29] . **A 3D printed bilayer oral** soliddosage form combining metformin for prolonged (8 h) and glimepiride for immediate (2–3 h) drug delivery [30].

New double-compartment oral administration devices are designed: One compartment is formulated for rapid drug release, with the aim of achieving high plasma concentration in a

short time and a sustained release compartment designed to maintain an effective plasma level for a prolonged period of time. Generally, compressed tablets are prepared to separate physically or chemically incompatible ingredients or to produce repeated or prolonged action of the drug. They can be classified into compression coated tablets, inlay tablets and layered tablets [31]. .

1-2-1-1- Compression coated tablets

The compression coated tablets consist of a compressed extended release core and an immediate release compressed coating prepared by direct compression (an initial phase of rapid release corresponding to the drug present in the outer layer followed by a slow release corresponding to the drug from the central core of the tablet). Compaction integrity is required. The core and the shell both contain the drug. These tablets have a two-phase release behavior: the rapid-release component dissolves quickly, and depending on the composition of the matrix, different times of drug release from the central tablet may take place. The rate of administration and the ratio of dose fractions can be adjusted according to the therapeutic needs and the desired profile [27].

1-2-1-2- Mini-tablets

Mini tablets can be compressed into a larger tablet or filled into a capsule.

- **Compressed mini-tablets**

The outer layer that fills the empty spaces between the mini-tablets incorporates part of the total drug dose and releases it quickly, and the mini-tablets provide sustained release at different rates [32]. It is possible to adjust the number of mini-tablets in the large tablet to have the desired dosage regimen including sustained release. The polymers used in the composition as well as the number of tablets have been shown to determine the different drug release rates. Particularly, the HPMC is suitable for zero order release over 8 h periods [33].
Of course, the dosage of the drug in the immediate release component can be varied as well. Dimensional uniformity is maintained with smooth surfaces, low porosities and high resistance to forces but if the amounts of powder are insufficient to fill the space between the mini tablets and a fracture may appear on the tablets after compression [34].

- **Encapsulated mini-tablets**

In this type of encapsulated mini-tablets, a hard capsule shell is filled with extended-release mini-tablets. The remaining void volume is filled with powder or granules, for rapid release. In this type of encapsulated mini-tablets, immediate-release mini-tablets (IRMT) instead of rapid-release powder or granules of sustained-release mini-tablets (SRMT) are embedded in a capsule shell [35].

1-2-1-3- Multilayered tablets

Multilayered tablets are one of the important design approaches where the incorporation of incompatible drugs or the same drug with a controlled release rate in a single unit is possible. These tablets have many key advantages [36][37] over conventional immediate-release tablets as they simplify combination therapy regimens and thus improving patient compliance.

In fact, this class of dosage forms overcomes the problems of incompatibility between two or more active pharmaceutical ingredients. Some compounds are physically and chemically incompatible, making multi-layered tablets with split-layered APIs extremely useful. There may be some reactivity to the interface of a bilayered tablet. So if complete physical separation is desired, a trilayered tablet is the ideal choice [31]. Although layered tablets have several advantages, the use of different types of materials in multilayered tablets as well as the geometric limitations of adjacent layers make the mechanical structures of the system quite complex which requires complicated and patient friendly architectures [38].

- **Bilayered/trilayeredtablets**

The bilayer tablet is a new dosage form which overcomes the disadvantages of single layer tablets. It is composed of two layers: One layer may contain a loading dose and the other may provide a maintenance dose of the same drug, or each layer may contain a different sustained release compound [24]. This dosage form is suitable for a sequential release of one or two drugs in combination. There is a variety of approaches to formulating these multilayer tablets [39].

Bilayer tablet technology is demanding and requires precise selection of each component and adjustment of manufacturing parameters in order to avoid common bilayer problems including layer separation, insufficient hardness, improper weight control of individual layers, contamination between coats and reduced performance. The production of quality bilayer

tablets must be carried out on tablet presses specially designed to overcome these problems. Existing but modified tablet presses are used to develop and produce quality tablets [40][41].

- **Floating bilayer tablet**

Controlled release-gastro-retentive dosage forms (CR-GRDF) prolong the retention time of dosage forms in the stomach or upper gastrointestinal tract, in order to improve ; the solubility of drugs that are less soluble in a high pH environment, the bioavailability and the therapeutic efficacy of drugs [38],[42]. Multiple approaches ensure this time extension including floating drug delivery systems (FDDS), also called hydrodynamically balanced systems (HBS), swelling and expansion systems, polymer bioadhesive systems, high density systems and other delayed gastric emptying devices [42],[43]. These systems can be prepared as tablets, capsules by adding appropriate ingredients as well as adding a gas generating agent [43]. Different strategies have been used to develop FDDS by constructing the effervescent and non-effervescent type of floating tablets whose basis is the flotation mechanism [44]. Further studies on the main mechanism of flotation to achieve gastric retention have been conducted [43]. New FDDS methodologies include approaches to design single-unit and multi-unit floating systems, physiological and formulation variability affecting gastric retention as well as the use of recently invented and developed polymers [44]. In particular, systems exhibiting a unique combination of flotation and bioadhesion to prolong residence in the stomach have been developed [42]. Similarly, two-layer floating tablets (BFTs) were made using direct compression technology to release approximately 95% of the captopril in 24 hr in vitro, while the floating lag time was 10 min and the tablet remained floating throughout all the studies [45].

1-2-1-4- Inlay tablets

The inlay tablet represents a variant of a coated tablet with a core which has a partially coated top surface. It is also called, dot, or the bull's eye tablet [31].

1-2-1-5- Single layer tablet

In this dosage form, immediate release and sustained release granulations are blended together and compressed into simple monolithic matrix tablet. Compared to the bilayer structure where the two granular portions interact only at their interface, this dosage form has the advantage of binary mixtures where the two intimately mixed granular portions interact with each other at the particle / particle level. Therefore, the low mechanical strength of bilayer tablets is of

great concern [46][47]. The production of single-layer tablets increases its capacity by at least 60-70% over that of two-layer in terms of time.

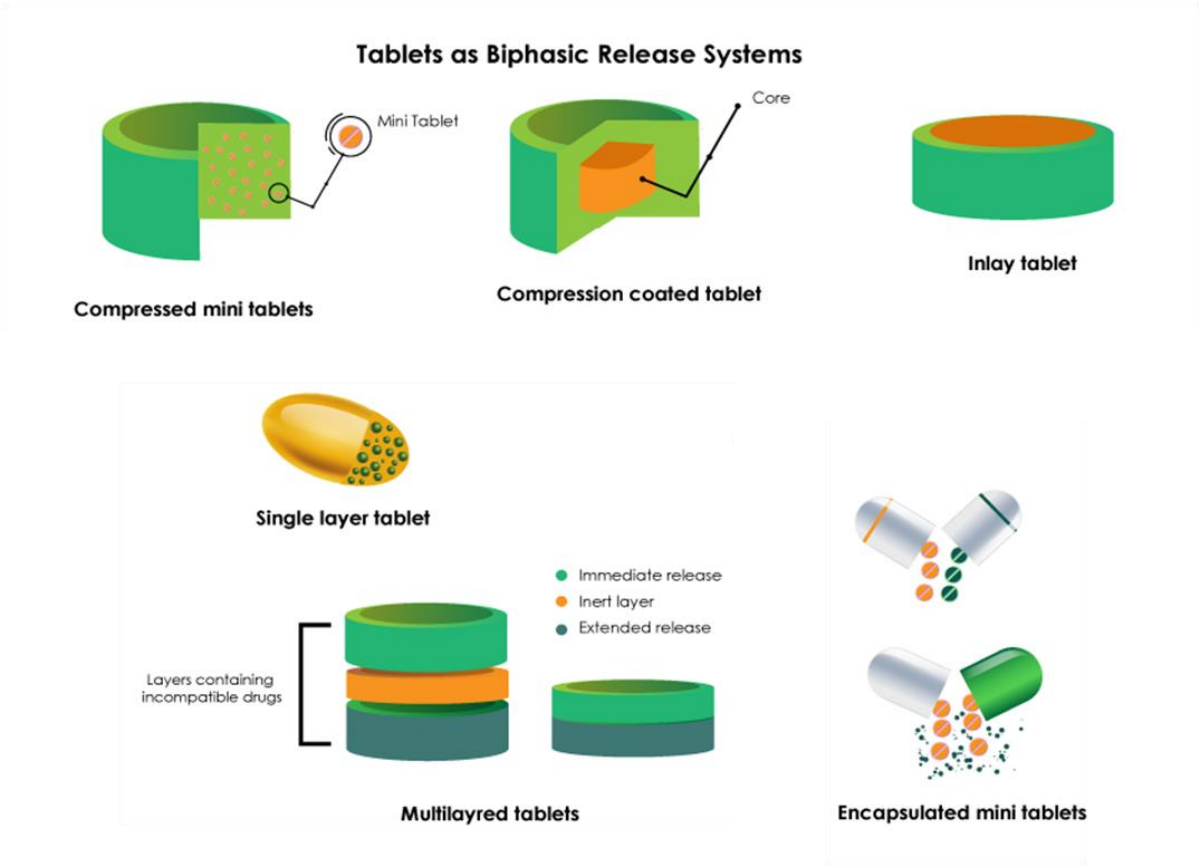


Figure 1- 1 Tablets as biphasic release systems inspired from [48][31][35]

1-2-2- Liquid-filled hard gelatin capsules

Liquid filled capsules are dosage forms useful for administering oily or waxy pharmaceutical preparations. Coating the capsule or adding ingredients to the shell can produce delayed or sustained release properties. The type and amount of substances filled into the capsule should be adjusted to avoid shell erosion and appearance problems due to diffusion and evaporation of the filling material and/or the shell [48].

Figure 1- 2 Liquid-filled hard gelatin capsule

1-2-3- Nanoparticles

Chitosan/fucoidan nanoparticles CS/F NPs are potential carriers for biphasic release of gentamicin GM for pneumonia treatment. The NPs exhibited a zero-order release of GM for the first 10 h, followed by a sustained release of up to 72 h, attaining a value of 99% 77% of encapsulated GM was released in the first phase and the following 22% of encapsulated GM was slowly released allowing the safe use of daily-dose GM-loaded CS/F NPs [49].

1-2-4- Nanofibers

A multilayered zein/PVP-GO/zein electrospun **nanofiber mesh** that achieves time-regulated biphasic drug release behaviour was created. PVP was blended with graphene oxide (GO) to improve ketoprofen release functionality of PVP nanofiber namely by delaying the initial fast release as well as its mechanical properties. The drug release rate and duration can be controlled by adjusting mesh thickness which was achieved by simply regulating the spinning time of the first and third layers [50].

A polyblend electrospun technology was proven to be a new conception for local chemotherapy. By adjusting the weight ratio of the hydrophilic polymer (poly(ethylene oxide), PEO) and the hydrophobic polymer (poly(L-lactide), PLA), (PEO10–PLA90), electrospun polyblend **nanofibers** with typical biphasic release kinetics were successfully prepared. Due to their unique release profile, these fibers can quickly access the tumor site in vivo at a high drug content within 1 h and keep at a high level for longer than two weeks [51].

Emodin-loaded electrospun **nanofibers** with biphasic release profile have been fabricated. Emodin was encapsulated in the core of hydrophilic poly (vinylpyrrolidone), with a hygroscopic cellulose acetate sheath, provided long-term effect against MRSA (Methicillin-resistant Staphylococcus aureus). The excellent film-forming property of CA alllowed the nanofiber membranes to retain integrity during the extended antimicrobial activity experiments [52].

A novel polyvinyl pyrrolidone/poly (ε-caprolactone) (PVP/PCL) **nanofiber mats** using the coaxial electrospinning technology was fabricated. Graphene Ooxide (GO) sheets were blended into the core solution to adjust the drug release behaviour. This core/sheath drug-

loaded nanofibrous carriers based on the GO content in the core matrix showed a biphasic drug release profile[53]**.**

On another side, tri-layered electrospun **nanofiber meshes,** loaded with Ketoprofen (KET) showed a successful biphasic release profile. KET released faster and more from Polyvinylpyrrolidone (PVP) matrix which fitted with the erosion mechanism while KET-Ethyl Cellulose meshes exhibited sustained release. Drug release speed and sustained release duration are controllable by adjusting the fiber diameter and the mesh thickness [54]. A similar study using the same polymers and the same model drug showed that the amount of drug released in the first phase was tailored by adjusting the sheath flow rate, and the remaining drug released in the second phase was controlled by a typical diffusion mechanism. With the selection of suitable sheath and core fluids, the coaxial electrospinning process could be conducted smoothly and continuously [55].

Janus ultrafine fiber consisting of a water-soluble polymer (PVP) and a non-water-soluble polymer PAN exhibited biphasic drug release due to the different properties of both polymers [56] containing two different fluorescent dyes (1,8-naphthalene anhydride and PMI).

1-2-5- Multilayered films

Another study showed that the permeabilities of Pectin/Chitosan/HPMC films to a model drug, paracetamol could be manipulated by changing the HPMC composition. This type of formulation showed a good potential for sigmoidal delivery with an initial slow phase followed by a more rapid phase consistent with the dosage form entering the colon **…**

1-2-6- Other systems

Multi-unit biphasic release systems (BPR MUPS) for diclofenac sodium using different types of neutral starter pellets were designed. The developed systems consisted of two types of drug-layered pellets attaining different release patterns: delayed-release (enteric-coated) and extended-release. The water-insoluble starter pellets (Microcrystalline CelluloseMCC spheres and Dichlorophthalate DCPA-based pellets) were able to control the rate of release much better than soluble sugar spheres or isomalt pellets [57].

Bilayer dissolving **microneedles** containing 5-fluorouracil 5-Fu and triamcinolone TA with biphasic release profile for hypertrophic scar therapy was fabricated. Rapid release of triamcinolone TA from needle tail layer and sustained-release 5-Fu from needle tip layer were successfully realized [58]. Finding a concept easy to implement based on a single polymer is a challenge.

Table 1- 2 Biphasic release systems

Dosage form	Usedpolymers	Technique	Released API, Model drug or applications	Ref
Tablets	PEO/HPMC	Compression	Dicolfenac	[26]
	HPMC/EC		Ibuprofen	[27]
	BAβCD/TAβCD		Carbamazepine (CBZ)	[29]
	Sodium starch glycollate HPMC /Xanthan gum		Atenolol and Lovastatin	[28]
	Pectin/Chitosan/ HPMC	Coating	Paracetamol	[59]
	Eudragit® RL/PVA	Fused Deposition Modelling (Hot-melt extrusion)	Glimepiride and Metformin	[30]
TrilayeredNonofiber meshes	EC/ PVP	Elecrtrospinning (coaxial or sequential…)	Ketoprofen (KET)	[54][55]
Nanofiber mats	PVP + GO/ PCL		VancomycinHydrochloride (VAN)	[53]
Nanofibers	PEO/PLA		Local Chemotherapy	[51]
Nanofiber mats (trilayered)	Zein/PVP-GO		Ketoprofen	[50]
Nanofibers	PVP/ CA		Emodin	[52]
Janus ultrafine fiber membrane	PVP/PAN		Two different fluorescent dyes (1,8-naphthalene anhydride and PMI)	[56]
Multilayered Films	Pectin /Chitosan /HPMC		Paracetamol	[60]

Multiple-Unit Pellet System	Calcium Phosphate-Based Starter Pellets-Microcrystalline cellulose, sucrose and isomalt	Coating	Diclofenac	[57]
Nanoparticles	Chitosan(CS)/fucoidan (F)	Ionotropiccrosslinking	Gentamicin (GM)	[49]
Microneedles	Chitosan(CS)/Dextran (DEX)	Micro-milling/ Casting	5-fluorouracil (5-Fu) and triamcinolone acetonide (TA)	[58]
Layered microneedles	PLGA/PVP	Spray deposition process.	Bovine serum albumin (BSA)	[61]

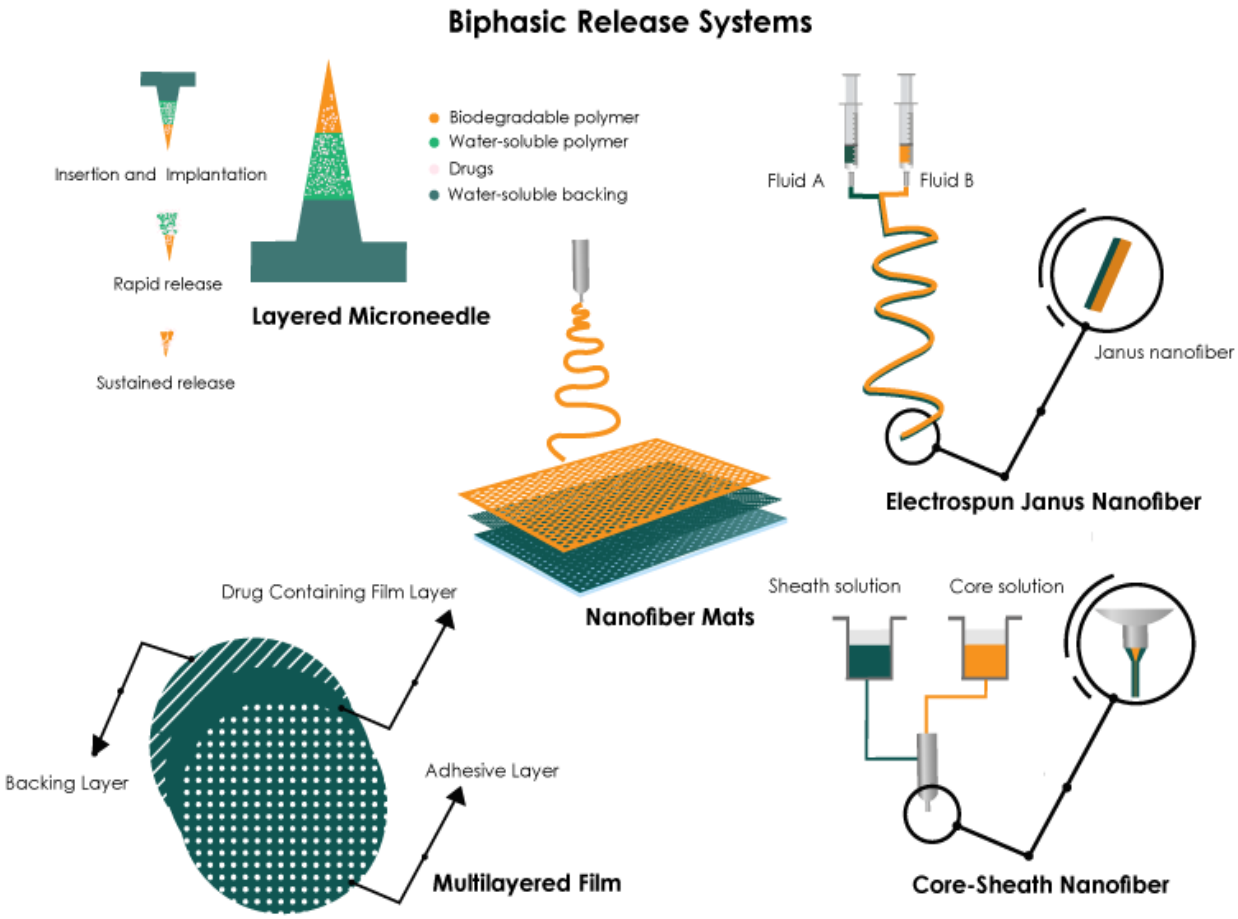


Figure 1-3 Biphasic release systems inspired from [53][55][56][58]

1-3- Thin films for drug release

Given that the films represent the pharmaceutical carrier used to manufacture the rolled-up capsules in our study, this section is dedicated to the specific properties of this dosage form, its pharmaceutical classification in the pharmacopoeia, and in particular its use and potential in chronotherapy.

Thin films represent an innovative drug delivery system and an efficient API delivery platform. They can be defined as a thin and flexible layer based on one polymer or several polymers with or without additives. Thin films were first introduced in the late 1970s to facilitate swallowing of tablets and capsules [62]. Possessing many specific and unique properties, they represent a new versatile option for drug administration. They are characterized by ease of use (self-administration, convenient to swallow), precise dosage, rapid absorption, higher bioavailability of the drug, convenient administration by non-

invasive routes (Buccal, sublingual, ocular and cutaneous), ease of handling and above all a great ability to deliver proteins and peptides. In fact, oral administration of Protein-Peptides (PPs) has become more attractive in drug research and development. Multiple researches have focused on the development of new approaches to overcome the gastrointestinal barriers of PPs including enteric coating, enzyme inhibitors and intestinal microdevices (eg intestinal microneedles) by improving the stability and permeability of PPs [63]. Many researchers have focused on oral polymeric films as oral delivery platforms for PPs that can greatly improve the biological performance of proteins and peptides as well as patient compliance, and disclosed the toxicity issues. possible to overcome by critically analyzing current trends regarding PPs in oral films [64].

In addition, films are adapted to each consumer profile and ensure high compliance of patients, especially children (given a likable flavor and color) and the elderly suffering from disorders of the swallowing [65][66].

The names and classifications of this elegant, stable and efficient administration vehicle are diverse. We mainly find orodispersible films (ODF) and mucoadhesive films [67]. Orodispersible films break down immediately upon contact with saliva while Mucoadhesive ones adhere to various parts of the oral cavity and slowly release the drug into the patient's systemic circulation [68]. Orosidpersible Films (ODF) [69] can be classified into dissolving films which disintegrate and dissolve simultaneously in the mouth (water soluble drugs), and Disintegrating films which disintegrate in the mouth, then dissolve and are absorbed in the GI tract (poorly water-soluble drugs). .Other names have been given to this new dosage form including edible dissolving gelatin strips [70], buccal film, transmucosal film, oral soluble film, wafer oral strip and dissolving films.

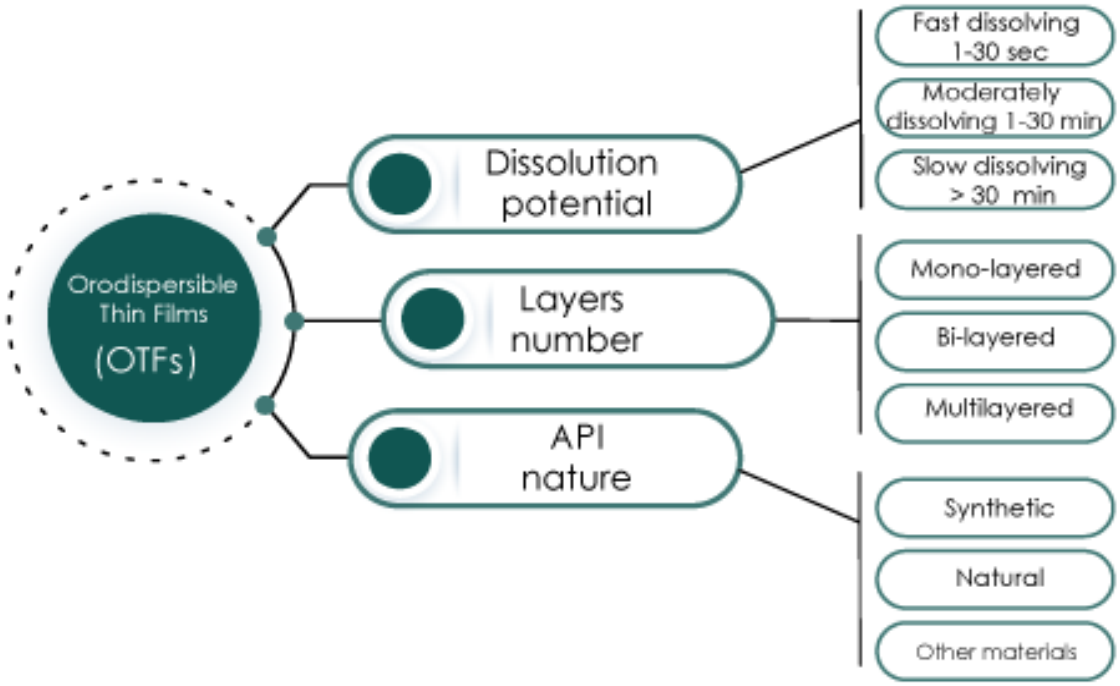


Figure 1- 4 Orodispersible thin films classification

Ideal thin films should have sufficient drug load capacity, rapid dissolution rate or long residence time at the site of administration, acceptable formulation stability and should of course be biocompatible, biodegradable and non-toxic.

Unlike traditional solid dosage forms, thin strips are flexible and are not crumbly, allowing them to resist physical degradation that damages tablets and capsules. Film strips can be individually wrapped in flat, sealed and air-free packages which protect them against atmospheric moisture and oxygen and provide better stability. These films are also able to target sensitive sites otherwise impossible with tablets or liquid formulations [71].

The physicochemical properties of polymers and drugs can affect the formulation of thin films [62].**V**arious polymers allow the development of thin films with specific properties such as weight, texture, solubility, strength and stability. Alone or in combination, these polymers give films with unique properties. Water soluble polymers are used to produce thin films with rapid disintegration, good mechanical strength and good mouth feel effects. The following polymers are commonly used in the manufacture of thin films: (HPMC), (CMC), (HPC), (PVP), (PVA), (PEO), Pullulan, Pectin, Chitosan, Sodium alginate, Carrageenan and Gelatin. For fully promoted absorption and highly improved bioavailability, nanoparticles or complexes inclusion can be incorporated into the films [67]. Indeed, their distribution can

improve bioadhesion to the targeted oral mucosa as well as the solubility and permeability of drugs. High levels of suspended solids can help stabilize the film matrix in the same way that suspended solids can help stabilize a traditional emulsion.

The development of thin film manufacturing approaches continues to advance. This includes solvent casting, semi solid casting, hot melt extrusion, solid dispersion extrusion, electrospinning, 3D Printing (Inkjet printing and FDM 3D print) as well as the electrostatic spray deposition, rolling method. In the case of heat-sensitive APIs, solvent casting is the ideal technique for making thin films. However, residual traces of solvents present regulatory compliance issues. In addition, if the solvent is flammable, special safety measures must be taken. As for the extrusion which is also widely used to manufacture thin films, it subjects the film ingredients to high temperatures, which could cause thermal degradation, the creation of voids in the film affecting its uniformity, strength and appearance [67].

Drug release studies from oral strips loaded with methyl orange with different initial drug amounts were performed using a novel millifluidic continuous flow device. The results showed that the release kinetics are strongly influenced by the initial thickness of the film in its dry state and by the flow rate of the solvent and that it is essentially controlled by the swelling behaviour of the thin film [72]. Gelatin-based thin films have also been used for release control of hydrophobic drugs. Loaded with piperine, thin films were able to deliver hydrophobic drug in a controlled way (fast and slow release profiles) depending on the concentrations of the crosslinker agent and the polymer as well as the pH conditions of the release medium. The polymer concentration contributes to increasing the diffusion path of the film and the crosslinking prevents the polymer matrix from swelling. As the concentration of the polymer and the crosslinking agent increase, the diffusion process slows down considerably which slows down as well the drug release. In addition, the cross-linked gelatin-based thin films are stable under acidic conditions (pH 1.2) of the gastrointestinal tract and are able to release the drug in a controlled manner into the site of absorption (pH 7.4). Control of swelling as well as partial degradation of the films results in sustained release [73]. Another interesting study showed that gelatin films containing ibuprofen-loaded poly-e-caprolactone (PCL) microspheres were developed on organic solvent evaporation from an oil-in-water emulsion followed by crosslinking. This type of microsphere-film system combined good adhesion, typical of gelatin films, with the sustained release performance of PCL microspheres [74]. Montelukast sodium fast dissolving films were made by using gelatin as a film base [75].

This relatively new dosage form of medicine is not yet officially recognized or included in pharmacopoeia of any country. (No detailed monograph nor uniform requirements).

The ODFs first became part of the 7th Ph. Eur. edition in 2012, which included their general monograph. Only the release test is mentioned in the Ph. Eur. to demonstrate the appropriate release of the API. In accordance with pharmacopoeial requirements, ODFs should possess adequate mechanical strength to be handled without being damaged. Although they are not [60][59] adequate for the evaluation of ODFs as they do not relate to the specific characteristics of ODFs, the test methods for solid oral dosage forms (tablets and capsules) are recommended for films. In addition, a large number of individually modified industry methods and guidelines are used in the quality assessment of ODFs. It is therefore very important to unify quality studies, to establish standardized quality control methods in the pharmacopoeia and to develop reference methods for evaluating the properties of films [76].

The large-scale commercialization of thin films is not very well developed. A major limitation of this new dosage form is the low drug load capacity [67]. Although thin films can be manufactured with a relatively high proportion of API without compromising their physical integrity, the relatively low mass of a strip of polymeric film does not allow to provide a sufficient dose for some APIs [71]. Thin films are also hygroscopic by nature which requires special handling for longer storage.

1-4- Biomaterials used as matrices in the controlled drug release formulations

Developing biomaterials with specific physicochemical properties suitable for drug release is a pivotal challenge in the pharmaceutical field. In fact, the chemical nature of the polymer is crucial to the overall design of drug release devices. Commonly used materials include **proteins, carbohydrates and lipids**. These materials have served as pharmaceutical matrices in many drug release systems. Each of these materials has great potential alone, but the combination of certain materials with gelatin makes it possible to enhance the mechanical, thermal, functional and morphological properties of the matrix. In particular, developing films based on compatible biopolymers is a reasonable way to enhance properties of individual biopolymer based films. In this work, the emphasis is on gelatin as it constitutes the polymer chosen to design our pharmaceutical matrix and carry out our release study.

1-4-1- Proteins

Gelatin has many properties which make it an ideal starting material for drug release carriers design. Gelatin can be defined as the product obtained from the acid, alkaline, or enzymatic hydrolysis of collagen [77][78]. It is one of the main components of medical capsules protecting the active ingredients from the harmful effects of light, oxygen and gastric medium. The judicious choice of gelatin's type and its characteristics can involve changes in the surface and structural properties of the films thus influencing the rate of release of the active ingredient during the administration of capsules.Therefore, research efforts for creating gelatin films to be used in the pharmaceutical field have been going on for many years.

Casein is one of the most widely used bipolymers to manufacture matrices with controlled properties. When mixed with gelatin, casein gives films which exhibit significantly higher elongation values compared to films made from gelatin or casein alone [79]. This mixing also causes changes in water vapor permeability [80]. Some functional properties of this composite could be changed using microbial transglutaminase [81].

Zein can also be added to gelatin film forming solution to make multilayer films with rationally designed functionalities. Indeed, by regulating the Zein/Gelatin ratio in the middle layer, we can control the final properties of the matrix [82]**.**

1-4-2- Carbohydrates

Within the class of carbohydrates, **chitosan** has long been recognized as one of the most promising functional biopolymers used for the design of drug release systems. Salomon et al. reported the recent trends in the development of chitosan based drug release systems [83]. So many other studies have focused on the different forms of chitosan-based drug release systems namely nanoparticles [84], films [85][86] and hydrogels [87]. The chitosan was also mixed with the gelatin to give a gel exhibiting properties that make them excellent candidates for drug release control. Many other carbohydrates can impact the properties of gelatin films including **alginate dialdehyde**, **pectin, carrageenan, gellan gum, glucose**, **dextran, cellulose, carboxymethyl cellulose**, **starch**, and **polyols and sugars**.

Alginate dialdehyde (polysaccharides obtained from brown algae**)** has been extensively used for the development of mechanically enhanced mixtures with an improved antioxidative capacity and a better vulnerability on moisture conditions [88][89]. The resulting film had a successful application for localized drug delivery in vivo or in vitro environment [90].

Likewise, the hydrogels of **oxidized pectin** (polysaccharides exclusively of plant origin) and gelatin showed good swelling without any dissolution due to the formation of imine bonds between the aldehyde and amino moieties [91].The mixture of these two natural biopolymers is also suitable for 3D bioprinting by extrusion. The resulting matrix shows adjustable porosity and water absorption [92]. On the other hand, an interesting study has shown that this mixture gives films with good antioxidant properties [93]. The elongation of the films at break and the tensile strength were adjusted by varying the ratio of gelatin and pectin. Furthermore, gelatin and pectin have the ability to trap salts and facilitate their release under simulated gastric conditions [94]**.**

A mixture of **carrageenan** (marine polysaccharide) with gelatin produces physical changes in the resulting gel properties [95]. Being stabilised by electrostatic interactions, the mixture shows an increase in gel strength, gelling and melting temperatures as well as a considerable increase in Young's modulus [96].

Gellan gum which is a linear anionic polysaccharide can be used to enhance gelatin films properties. By modifying the ratio of gellan and gelatine, the mechanical properties of the composite can be improved [97].

Furthermore, the usefulness of **glucose** (the most abundant monosaccharide) addition in gelatine has been successfully demonstrated by the increase of stiffness and the decrease of solubility of the resulting mixture [98].

Dextran (polysaccharide derived from the condensation of glucose) is one of the most used carbohydrates as well. Hydrogels based on an interpenetrating polymeric network of gelatin and dextran have been the subject of several studies [99].

The gelatin-coupled **cellulose** (an organic compound a polysaccharide consisting of a linear chain of several hundred to many thousands of β(1→4) linked D-glucose units) microgel showed excellent dispersibility and stability in water which contributes to hydrophobicity and significantly reduces the moisture absorption of composite films, as well as a decrease in the water vapor permeability of the films [100].

It was also showed that the mixing of Gelatin with **Cellulose-Gum (Carboxy Méthyl Cellulose CMC)** led to the formation of strong film networks of lower solubility and swelling capacity. However, water vapor permeability (WVP) was not significantly influenced by the incorporation of CMC into continuous gelatin films. Furthermore, the study showed that the

physical and mechanical properties of gelatin-CMC composite films are affected by electrostatic interactions and that the polymers were not covalently linked [101]. Similarly, Gelatin and CMC produced films with higher mechanical strength and stiffness compared with plant storage polymers (starch and soy proteins) [102]. Changes in CMC-Gelatin ratios had a significant effect on its mechanical, physical and chemical properties. An interesting study showed that, among the developed formulae, the optimal film was the film formulated with CMC and Gelatin in the ratio 4 to 1[103].

Starch is a polymeric carbohydrate consisting of numerous glucose units joined by glycosidic bonds. An interestin,g study showed that the blends containing up to 50% starch give a compatible microstructure and a continuous phase which enables to produce good films and capsules [104]**.** Orally disintegrating films (ODF) based on starch and gelatin have been made as vitamin C carriers. Films with higher starch concentrations exhibit greater stiffness but disintegrate rapidly. With higher concentrations of starch, the films show greater stability of the active compound as well as a shorter disintegration time [105].

Adding **polyols** (derivatives of oses, obtained by reducing the aldehyde or ketone group of a carbohydrate) **and sugars** (soluble carbohydrates) to gelatine solution increase the melting temperature of gelatin gel which stabilizes the gelatin gel [106]**.** At the molecular level, this is explained by the peptide-peptide hydrogen bond (helix formation) which get enhanced.

1-4-3- Lipids

Lipid materials including waxes, oils as well as fatty acids are incorporated in the gelatine films in order to change their properties.

The incorporation of **Candelilla wax** into gelatin has shown an effect on the physicochemical and morphological properties of the resulting films, in particular an increase in mechanical strength and elongation [102]. The addition of **carnauba wax and beeswax** increased the opacity and yellowing of the gelatin films. The UV/visible light and water vapour barriers were successfully improved with increasing levels of wax. The addition of the wax also improved the thermal stability of the films suggesting an interaction between the wax and the gelatin. From a comparative stand point, beeswax was better than carnauba wax in improving various properties of gelatin films as it gives films with more uniform surfaces and more compact cross sections [107]. By the addition of **fatty acids**, tensile strength of gelatine films decreased. With increasing fatty acids amount, Water Vapor Permeability decreased, light transmission of films

in both UV and visible ranges decreased while elongation at break increased [108].

1-5- Gelatin films and the methods used to enhance their properties

Gelatin is a protein derived from the partial hydrolysis of native collagen, the most abundant structural protein found in animal skin, tendons, cartilage and bone. It has been widely used in the pharmaceutical and medical fields given its interesting properties, in particular its biological origin, its non-immunogenicity, its biodegradability, its biocompatibility and its availability at low cost [109].

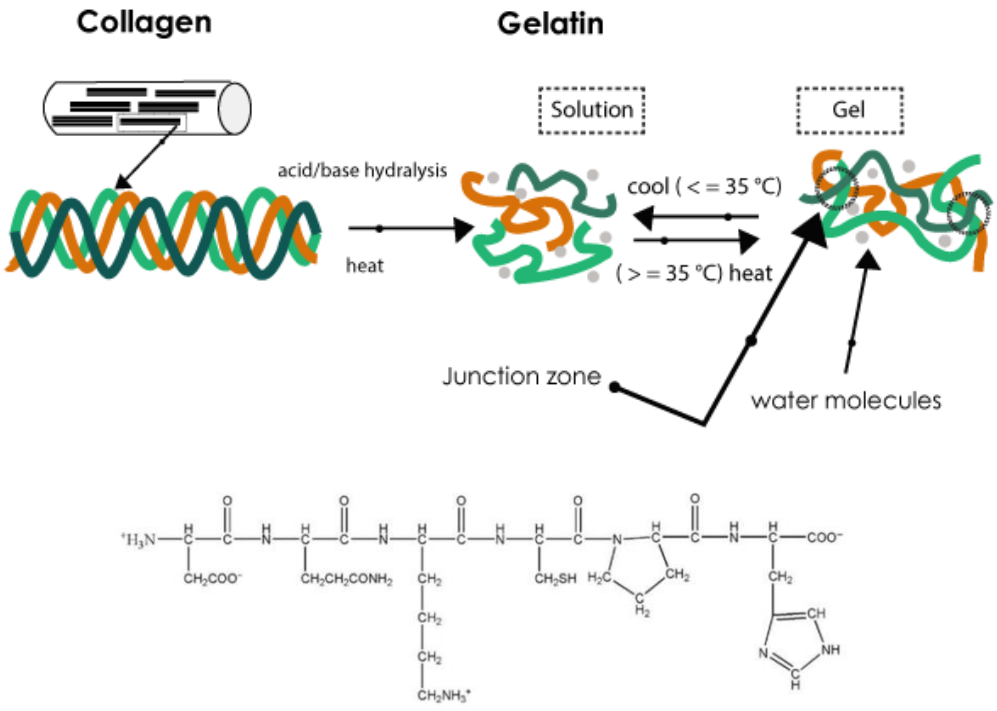


Figure 1- 5 Shematic illustration of the basic structure of gelatin

Depending on the nature of the intended application, specific properties of the gelatine based materials are required. In particular, there is an increasing interest to enhance gelatin film properties using different methods, which enable the preparation of matrices with improved mechanical, thermal and physicochemical properties. Various methods are used to modify gelatin film features, including heating, irradiation, chemical agents, enzymes, phenolic compounds and nanocomposites inclusion, polymers addition and use of plasticizers. The main drawback of gelatin is its water solubility and low mechanical properties. Crosslinking was then employed to prevent its solubility prior to the uses in cell culture and biomedical

applications. Among the crosslinking methods, chemical treatment is the most widely used due to its high efficiency in the stabilization of soluble materials. Generally, crosslinkers are classified into Non-zero-length crosslinkers and zero-length crosslinkers. Non zero are bi-functional or multifunctional molecules that operate by bridging free carboxylic acid groups, amino groups and hydroxyl groups between adjacent polymer molecules (bridging free amine groups of lysine and hydroxylysine or free carboxylic acid residues of glutamic and aspartic acid of the protein molecules). Among these crosslinkers, there are Aldehydes (formaldehyde, glutaraldehyde, and glyceraldehydes), polyepoxides and isocyanates. Zero Length crosslinkers such as Acyl azide, carbodiimide and Transglutaminase present reactive groups such as carboxylic acid and amine groups present in polymer network chains that react with each other leading to the formation of a covalent bond [110]**.**

We have chosen to briefly describe some of the most used methods for modifying the properties of gelatin and we have classified them into ; **Chemical agents, enzymes, phenolic compounds, nanoparticles and dehydrothermal treatment (DHT)** which we used to cross-link our pharmaceutical matrix and whose description will be more developed in the introduction of the section "materials and methods..

1-5-1- Chemical agents

The inclusion of chemical crosslinkers in film forming formulations at different concentrations is one of the most popular approaches for controlling films properties. An interesting study has shown that the employed crosslinking method as well as the crosslinking degree dictate the final properties of the films [111]. Indeed, when the polymer is crosslinked, its molecular mobility is limited as well as the extension of its specific chains to slip which gives it a precise retention capacity depending on its degree of crosslinking [112]. An absolute comparison between the results reported in several papers concerning the effectiveness of crosslinking agents is not possible given that the operating procedures and the film content are different. Indeed, several factors must be taken into account during the preparation of the films, in particular the relative humidity and the temperature, which can considerably affect the final properties of the films. These parameters may change from one preparation to another. Nevertheless, it is possible to determine common points between these different agents essentially those, which belong to the same chemical group.

1-5-2- Enzymes

Different enzymes have been used to crosslink gelatin films, for instance Tyrosinase, Laccasse , Horseradish peroxidase (HRP) and Transglutaminase. These enzymes are capable of creating covalent cross-links in proteinaceous substrates [113]. Transglutamonase was the most used among these enzymes. It is a natural enzyme commonly found in animal tissues and intercellular fluid. It's listed as GRAS (Generally Recognized As Safe) [114]. Due to its cross-linking properties, it has been used as a crosslinking agent to improve gelatin films characteristics**.** An interesting review clearly showed that the main mechanisms involved in the reactions catalysed by microbial transglutaminase are deamidation and polymerisation [115]. These reactions result in significant changes in the physical and chemical properties of proteins and show the considerable potential of Transglutaminase to improve the firmness, viscosity, elasticity and water- binding capacity of gelatin films [116].

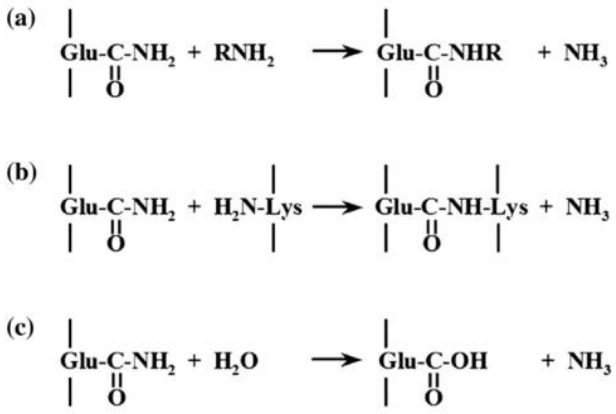


Figure 1- 6 Reactions catalysed by Transglutaminase: a) Acyl transfer; b) cross-linking of lysine and glutamine residues c) deamidation [114]

1-5-3- Phenolic compounds

Phenolic compounds affect gelatine properties. For example, the addition of different oxidised phenolic compounds including caffeic acid, ferrulic acid and tannic acid may result in an enhancement in antioxidative activity, a decrease in surface hydrophobicity with no change in emulsifying properties of the obtained gelatin [117]. Moreover, using phenolic compounds results in a significant decrease in the molecular mobility of the hydrogels, while the modulus of the films remains at high values at high temperatures [118]. Mechanical and barrier properties of gelatin may also be affected using phenolic compounds [119].

1-5-4- Nanoparticles

Efforts to improve the properties of various protein films using nanocomposite technology have been investigated intensely in recent years [120]. For instance, gelatin-based films reinforced with metallic nanoparticles were prepared using Au, Ag, Cu, AuAg, AuCu, and AgCu nanoparticles [121]. The mechanical properties of the resulting films were not significantly influenced. However, all of the composite films exhibited a strong UV light filtering function.

1-5-5- Dehydrothermal treatment (DHT)

As a result of chemical cross-linking, chemical residues that are probably toxic and capable of causing irritation may be present in the formulation. Therefore, physical crosslinking methods such as DHT treatment are the most considered. This method generates a chemical bond between the amino and carboxyl groups of gelatin molecules due to thermal dehydration and can only occur if the amino and carboxyl groups are close to each other and the interchain cross-links formed are a result of condensation reactions either by estrification or by amide formation [122].

In general, by heat-treating proteins, amino acids condense together through their amine and carboxyl groups and form amide bonds between molecules while removing a molecule of water. The hydroxyl groups and the carboxyl groups may be involved in an esterification reaction by dehydration according to studies conducted by Xiao Hua Ma et al [123]. In accordance with the previous findings, an interesting study has reported that Dehydrothermal Treatment removes water from gelatin films which result in the formation of intermolecular crosslinks through condensation reactions. Moreover, chemical bonding between the amino and the carboxyl groups of gelatin molecules which are close to each other can be generated due to thermal dehydration [124]. Wihodo et al, in a review, have reported that when pre-formed films are heated, their functional properties are altered as well. Heat disrupts hydrogen bonds and non polar hydrophobic groups in the proteins , thus, producing a more open structure [120]**.** It was also found that the crosslinking in the gelatin film network between β-chain and α-chain could be induced by heating at 120 °C [125] . In the same study, it was revealed that the main interactions involved in the crosslinked gelatin film formation were changed from ionic bonds and hydrogen bonds to hydrophobic interactions and covalent bonds, leading to improvement water resistance properties of films.

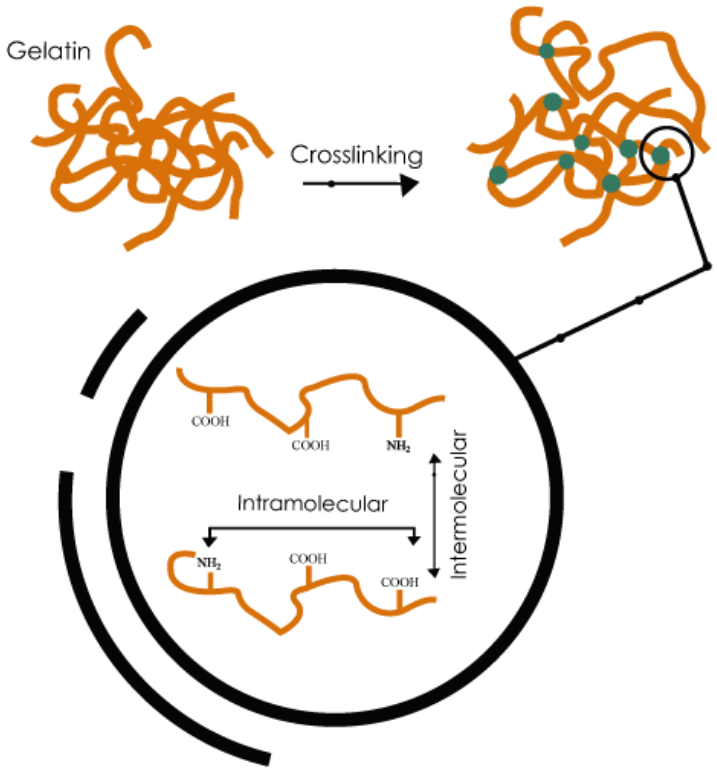


Figure 1- 7 Schematic illustration of gelatin DHT crosslinking

Recently, the effect of DHT on the properties of Tilapia scale gelatin films with 1 µm thick treated at a preferably selected temperature of 120 °C for 0.5 h , 1h, 2h, 4 h and 6h has been investigated [125]. It has been demonstrated that the tensile strength of films was increased gradually with increasing thermal treatment time. Moreover, it has been proved that the film solubility was decreased and thermal stability and water resistance were improved. Some other researchers have used a combined technique to improve gelatin films properties namely DHT with carbodiimide [126] or DHT with plasma treatment [124]. On the other hand, the main disadvantage of the DHT treatment is that it generally provides a low density of cross-links [122].

1-6- Methods for drug Release Study

1-6-1- Dissolution test

Dissolution is the process by which a substance forms a solution. From a pharmaceutical point of view, dissolution is a test used throughout the life cycle of a pharmaceutical product to evaluate the rate of release of a drug substance from the dosage form. Dissolution testing therefore measures the extent and rate of formation of solution from a dosage form, such as a

tablet, capsule, ointment, etc. This test is important for the bioavailability and therapeutic efficacy of a drug [127][128].

Three **main general tests** are used to evaluate a finished pharmaceutical product [127]:

- the assay which determines the overall potency of the batch and guarantees the accuracy of the finished pharmaceutical product,
- the uniformity of dose which determines the consistency between the individual dosage units and guarantees the precision of the manufacturing process,
- the dissolution which ensures that the performance of the finished pharmaceutical product is consistent with API release rates as determined in bioavailability studies during clinical trials.

Nevertheless, the in vitro dissolution is only mandatory for extended-release dosage forms and not for immediate-release forms and it is not necessarily representative of the bioavailability in humans. It depends on whether dissolution or permability through the gastrointestinal epithelium is the limiting factor for drug absorption (i.e. Biopharmaceutics Classification System (BCS) class of the drug). This is only true if dissolution rate is the limiting factor for absorption, (i.e. BCS Class 2 and 4).

The in vitro dissolution test is used primarily to measure the release of an API from its formulation under standardized conditions. In drug development as well as quality control, the performance of an extended-release solid and semi-solid dosage forms is evaluated by the dissolution test only for extended-release forms.

This key parameter also provides control information as part of the process validation approach. The European **Pharmacopeia** (Ph. Eur.) describes dissolution tests in the general part in section 2.9 where the different methods and apparatus for determining compliance with the dissolution requirements for solid dosage forms administered orally are described. The dissolution chapter of the US Pharmacopoeia is harmonized with the corresponding texts of the European Pharmacopoeia and / or the Japanese Pharmacopoeia [128].

1-6-2- Dissolution equipment

Determination of the device's ability to perform dissolution tests depends on the dosage form. Indeed, the devices of the pharmacopoeia have certain drawbacks. For example, the basket apparatus USP 1 presents a problem of adhesion and clogging of the meshes by the films,

while the paddle apparatus USP 2 presents phenomena of flotation of the ODF in the dissolution or adhesion media. local to the bottom of the receiver [129]. The USP 4 (flow cell) apparatus is suitable for the dissolution test of liquid-filled capsules, mainly for drugs with low solubility. For the dissolution of oral strips, the USP 1 and USP 2 devices show comparable trends, qualitatively and quantitatively different from those obtained in a newly proposed millifluidic device [129]. For the dissolution of soft gelatin capsule formulations of a poorly water soluble amino drug, USP 2 and 4 gave similar dissolution profiles. Apparatus 2 tended to give a faster dissolution rate, but Apparatus 4 was better able to distinguish between different formulations [130]. Another study showed that the USP I device was unable to discriminate the dissolution of griseofulvin (GF) particles incorporated into a film strip dosage form, relative to particle size. The results of the study demonstrated the superior discriminating power of USP 4 and suggest that it could be used as a test device in the development of strip films containing drug nanoparticles [131]. For drug release from ODFs, the dissolution study should be performed according to pharmacopoeia requirements for solid oral dosage forms using a basket or paddle device [76]. The choice of dissolution apparatus varies depending on the dosage form and the most important is that the test can be discriminating between various formulations and the differences related to physico-chemical parameters of the films and of the experiment.

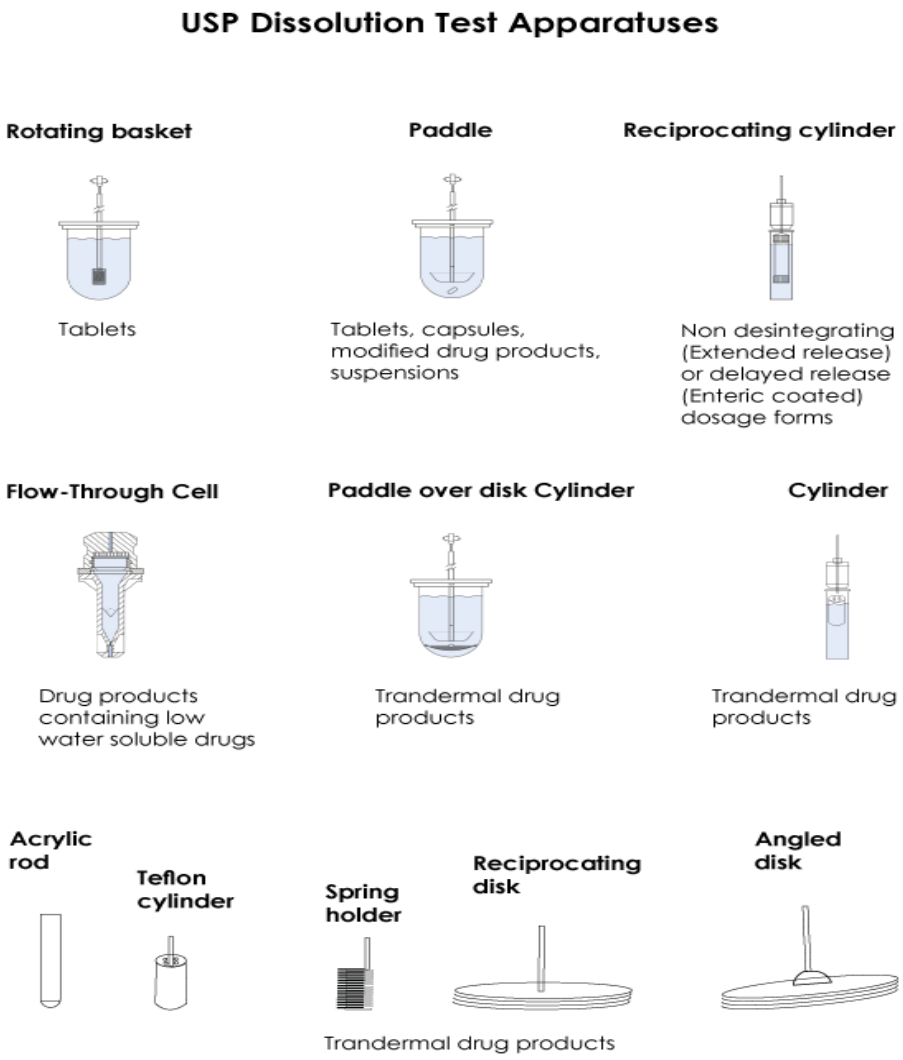


Figure 1- 8 Dissolution apparatuses (USP<711>, Ph. Eur. 2.9.3)

1-6-3- Dissolution Media

The characteristics of the dissolution medium must be carefully adjusted as a function of the galenic form and its characteristics, in particular its composition and its dimensions and of course those of the drug. The selection of the dissolution parameters is therefore necessary to conduct the most suitable dissolution study.

> ***''Critical test parameters that have to be moniored periodically during use include volume and temperatureture of the Dissolution Medium, rotation speed (Apparatus 1 and Apparatus 2), diprate (Apparatus 3), and flow rate of medium (Apparatus 4)''*** [132]**.**

Some cases of dissolution failure have been studied. Dissolution testing of crosslinked gelatin capsules can result in slower release of the drug or no release at all. It's not explicitly

mentioned that the reason for the failure of the dissolution test but the failure is clearly due to the crosslinking of the gelatin which does not rupture and does not release its contents from the capsule into the dissolution medium. Therefore, establishing the type and amounts of enzymes that can be added to the dissolution medium was discussed [133]. When hard or soft gelatin capsules and gelatin coated tablets do not meet dissolution specifications, the Dissolution <711> chapter allows the addition of enzymes to the dissolution medium.

The use of pepsin is recommended when the dissolving medium is water or has a pH below 6.8. This enzyme shows good protease activity up to pH = 4. Possible proteolytic enzymes that could be used for the pH range 4 to 6.8 could be papain or bromelain [133][132]. For media with a pH of 6.8 or higher, pancreatin can be added to produce no more than 1750 USP units of protease activity per 1000 mL [128]. These environments are now increasingly rare and reflect special cases [134].

1-6-4- Dissolution Mechanisms

Drug delivery systems can be divided into diffusion controlled release systems, chemically controlled systems (change their chemical structure when exposed to the biological medium [135], swelling controlled release systems, and environmentally sensitive system.

The mechanisms vary between swelling and dissolution, diffusion, erosion and degradation depending on the nature of the system. Drug release from degradable polymers can be governed by erosion of the surface of the polymer matrix, cleavage of polymer bonds at the surface or bulk of the matrix, or diffusion of the physically entrapped drug. However, drug release is often the result of a combination of the three mechanisms mentioned [136]. Despite its wide range of use, the structure of gelatin and its dissolution and swelling mechanisms have been unsufficiently studied [78]. For example, molecular diffusion and other factors such as film / tablet erosion and drug dissolution are involved in the release of paracetamol film-coated tablets [59]. Two mechanisms can describe drug permeation across polymer membranes: These are the “pore” mechanism and the “partition” mechanism [60]. A typical diffusion mechanism controls the release of drugs from nanofiber surfaces[55]. The drug release mechanism has been found to be a complex mixture of diffusion, swelling and erosion in the case of hard gelatin capsules crosslinked for the release of carmazepine[137].

Table 1- 3 Summary of release mechanisms and kinetic models

Dissolution

The release rate of drug is mainly determined by the slow dissolution of the matrix in a dissolution medium.

Noyes and Whitney equation	**Nernst and Brunner**	**Hixson & Crowell equation**
$\frac{dM}{dt} = K(C_s - C_b)$	$\frac{dM}{dt} = \frac{DA}{h}(C_s - C_b)$	$W_0^{1/3} - W_t^{1/3} = K_s t$

dM/dt = dissolution rate
D=diffusion coefficient
A=surface area
h=thickness stagnant layer
Cs=saturation solubility
C_b=concentration in the dissolution medium
W_0=the initial amount of drug
Wt=the amount of drug at time t
K_s=constant (this constant contains information as density of the matrix and solubility)

Diffusion

The drug release is mainly influenced by the diffusion properties of the drug in the matrix

Steady state conditions **Fick's first law**	**Non steady state conditions** **Fick'ssecond law**
$J = -D\frac{dC}{dx}$ **J** = flux($kgm^{-2}s^{-1}$) **dC/dx**= concentration difference (kgm^{-1})	$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2}$

Homogeneous matrix (dissolution or dispersion)
Higuchi law: Valid for matrices that are inert to the dissolution medium and the planar geometry

$$Q = \frac{M}{A} = \sqrt{((2C_0 - C_s)DC_s t)}\frac{dQ}{dt} = \frac{1}{2}\sqrt{\frac{(2C_0 - C_s)DC_s}{t}}$$

$$\frac{dQ}{dt} = k\sqrt{\frac{1}{t}}$$

C_0=is the initial total drug concentration in the matrix
C_S=the solubility concentration of the drug in the matrix
D=Diffusion constant(m^2s^{-1})

Q=the amount of drug released (M) per surface area (A)
t=time
k=degradation rate constant

The power law :Used If the matrix swells, dissolves or if the diffusivity changes in times

$$\frac{Q}{Q_\infty} = kt^n$$

n=diffusional exponent
Q=the amount of drug released at time t
Q∞=the amount of drug released at infinite Time

Porous matrix $$\frac{dQ}{dt} = \frac{1}{2}\sqrt{\frac{(2C_0 - \epsilon C_s)\epsilon D C_s}{\tau t}}$$

τ=tortuosity
ϵ=porosity of the matrix

Erosion

Surface erosion:
The matrix degrades and drug is released only from the surface,while the internal regions remain unchanged.

Hopfenberg equation $$\frac{Q}{Q_\infty} = 1 - \left[1 - \frac{kt}{C_0}a\right]^n$$

Predicts the drug release from simple surface eroding geometries

Heterogeneous:
The matrix degrades and the drug is released from the surface, but since the polymer matrix is not homogeneous, the surface degradation is not evenly distributed.

Bulk erosion:
The matrix is degraded and drug is released from the entire volume of the system.As the polymer matrix is eroded, drug molecules are free to be released via diffusion as well.

a=system'shalf thickness
n=exponent that varies with geometry n= 1,,2,3 (i.e.slab,cylindrical, spherical geometry)

1-6-5- Modelling of kinetics on drug release from controlled drug release systems

Examining the spectrum of mathematical models developed to describe drug release from pharmaceutical devices is necessary to elucidate mass transport mechanisms, predict the effect of device design parameters on the resulting drug release rate, and accelerate the development of new pharmaceutical products [138]. Different mathematical models are

offered for medical devices such as Zero Order, First Order, Higuchi, Peppas and Hixon**.** These models can be empirical or semi-empirical such as the classical Higuchi equation and the so-called power law, as well as more complex mechanistic theories that support diffusion processes. Monte Carlo simulations were also used for the numerical computation of diffusion-controlled release profiles [139]. Weibull model, Baker-Lonsdale model, and Hopfenberg model are also used as drug release kinetics models.

The common and the most used release Kinetics of pharmaceutical dosage forms are Zero Order [140], First Order, Hixon, Higuchi [141] and Peppas [142][143].

- **Zero-order**

This release profile is distinguished by a drug release rate independent of its concentration, this means that the amount of drug released per unit time is constant during certain time. This method is ideal for obtaining a prolonged pharmacological action and to describe coated dosage forms or membrane controlled dosage forms.

- **First-order**

For this model, the rate of drug release depends on its concentration. Dosage forms which contain a water-soluble drug in a porous matrix follow this profile, which is graphically plotted as the decimal logarithm of drug released as a function of time.

- **Hixon-Crowell**

Hixon-Crowell model is known for the drug release from the dosages having regular surface proportional to the cube root of the dosages volume.The graphical representation of the cubic root of the unreleased fraction of the drug as a function of time will be linear if conditions of equilibrium are not reached and if the geometric shape of the dosage form decreases proportionally with time. This model is used assuming that the rate of release is limited by the rate of dissolution of the excipient and not by diffusion.

- **Higuchi**

Higuchi describes drug release as a diffusion process based on Fick's law, dependent on square root time. To study the release of water-soluble and poorly soluble drugs incorporated into semi-solid and solid matrices, Higuchi developed two models. The first studies the dissolution of a planar system having a homogeneous drug distribution in the matrix as for the

dissolution of drug in suspension from ointment bases. For this geometry, he obtained the release kinetics proportional to the square root of time. He also explored the release from the spherical matrix system. Unfortunately, in this case, the solution is possible only in implicit form and cannot be expressed by a simple dependence on time. As for the cylinder geometry, we are not aware of the application of the Higuchi model to this geometry, but it is unlikely that a simple analytical solution for it exists as well. Dissolution of the drug from certain modified release dosage forms such as some transdermal systems and matrix tablets with water soluble drugs follows this kinetic model which has wide application in polymer matrix systems.

- **Peppas-Korsmeyer**

Korsmeyer et al. developed a simple semi-empirical model, where diffusion is the primary drug release mechanism, exponentially relating drug release to elapsed time (t). Subsequently, Peppas characterized and divided the different release mechanisms into fickian diffusion, mass transfer and abnormal transport. This model is generally used to analyze the release of a polymeric dosage form, when the mechanism of release is not well known or when more than one type of release phenomenon could be involved. When there is the possibility of a burst effect, the Korsmeyer equation is considered inappropriate because the introduction of the latency period is essential to accurately describe the amount of drug released [143][144].

A controlled release study was performed on biphasic polymer hydrogels. Mathematical models of different release mechanisms from biphasic networks have been developed to explain the observed profiles. The results showed the efficacy of biphasic hydrogels as platforms for the zero-order drug release model [145]. In the same context, a kinetic model for controlled drug release has been proposed to describe the sustained release of a solute or drug from a two-phase hydrogel substrate. The solute or drug is believed to be encapsulated in the dispersed microdomains, and diffuses from the interior to the surface of the microdomain [146]. Various models have also been used to study the kinetics of drug release from swellable and non-swellable matrices. Dissolution of the drug from solid dosage forms has been described by some kinetic models including zero kinetics, first order kinetics, a Higuchi and Hixson-Crowell model [142].

Conclusion

The literature review conducted in this chapter highlighted the importance of chronotherapy and the controlled delivery devices (CDD) in the pharmaceutical field. In particular, it provided a state-of-the-art on the dosage forms which ensure biphasic release. Studying these systems has revealed to us certain complexities in the manufacture of the system as well as in the choice of polymers and their combination to ensure different dissolution rates for drug release control.

On another side, the different polymers used in the manufacture of these systems were presented. Gelatin which is widely used in the pharmaceutical and medical fields was chosen as the polymer to manufacture the rolled-up capsules as it is biological, non-immunogenic, biodegradable, biocompatible and available at low cost. We also studied gelatine-based films since they represent the starting matrix to obtain rolled-up capsules.

We have also described the crosslinking methods of gelatin films and we have chosen a physical crosslinking method which is the DHT treatment.

Concerning dissolution study, since our system represents a new form which is based on films and which approaches the form of a classic capsule, we appealed to the Pharmacopeia dissolution chapter to choose the most appropriate and suitable dissolution apparatus for conducting dissolution tests on rolled-up capsules.

We also reviewed the dissolution mechanisms as well as the most studied kinetic models to better understand the drug release mechanism of the new designed system. In fact, to define the mechanism potentially involved in the release of the model drug from the rolled-up capsules, it is important to study the properties of the polymer matrix, mainly its behavior in contact with the dissolution medium. As for the models cited in the bibliography, they will allow us to determine the model that best fits the kinetics of release from the different reservoirs of the rolls-up capsules.

Problem statement and thesis challenges

One of the biggest challenges in the pharmaceutical industry is the release control of therapeutic agents. A relatively constant plasma level of a drug (The so-called zero-order release profile) is often sought in order to maintain the drug concentration within the therapeutic window. However, in conventional dosage forms, soon after administration, the pharmacokinetic profile shows high blood levels causing adverse effects.

On the basis of these considerations, we propose **a new oral delivery device, in the form of a biphasic release rolled-up capsule** which ensure an immediate release to provide rapid onset of action combined with a delayed release by **varying only the position of the reservoir** of drug on the gelatin film which is transformed into radial distribution after rolling-up the strip.

In all of the studied biphasic release systems, relatively complex methods are applied, more than one polymer is used and different additives are incorporated in order to adjust the properties of the matrix. The components of each layer must be carefully chosen. Thus, it is challenging to find a concept that is easy to implement **based on a single polymer.This original, simple and inexpensive** concept does not require complex synthesis or multiple manufacturing processes.

The fundamental concern of our research was also to manufacture **a programmed release system**. We have therefore optimized the formulation of the matrix so that it is insoluble, flexible and self-adhesive by **limiting the ingredients to a polymer, a plasticizer and a crosslinking agent.**

Chapter 2. Materials and Methods

Gelatin has many properties which make it an ideal starting material for drug release systems design. Choosing its type and its characteristics can involve changes in the surface and structural properties of the films thus influencing the rate of release of the active ingredient during the administration of capsules. Various methods may be applied to modify gelatin film features, including heating, irradiation, chemical agents, enzymes, phenolic compounds and nanocomposites inclusion, polymers addition and use of plasticizers. In our study, we chose Dehydrothermal Treatment (DHT) treatment to crosslink gelatin films since it is a physical method that is simple to implement and above all does not cause side effects like chemical crosslinking agents.

Gelatin based systems [147], more particularly, gelatin hydrogels and films may undergo DHT treatment in many applications. About seventy years ago, gelatin hydrogel [148] was subjected to prolonged heating at high temperatures (from 120°C up to 190 °C from one to 16 days). Several changes have taken place in gelatin thus treated namely solubility, weight loss, swelling degree and proteolytic digestion degree. In a similar aspect, a recent study investigated the performance of DHT applied on gelatin hydrogel lyophilized then heated at 160 °C under vacuum for 48 h [149] and reported that thermally treated gelatin scaffold with a crosslinking degree equal to 31 ± 2 % presents a swelling percentage at 72 h equal to 609 ± 6 % , an average pore size equal to 390 ± 14 µm and a maximum of mass loss equal to 19 ± 2 %.

DHT was also applied on gelatin films, which represent the main topic of our study. The impact of DHT treatment (121° C for 48 h) on 500 µm thick gelatin films aimed to be used as sealants for vascular prostheses has been evaluated. It was found that the obtained films possess an optimal crosslinking density of 1,2-1,3 10^{-5} mol/cm^3 , a denaturation temperature of 37.61 ± 2.42 °C and a homogeneous structure devoid of micropores [150].

Recently, the effect of DHT on the properties of Tilapia scale gelatin films with 1 µm thick treated at a preferably selected temperature of 120 °C for 0.5 h , 1h, 2h, 4 h and 6h has been investigated [125]. It has been demonstrated that the tensile strength of films was increased gradually with increasing thermal treatment time. Moreover, it has been proved that the film solubility was decreased and thermal stability and water resistance were improved. Some other researchers have used a combined technique to improve gelatin films properties namely DHT with carbodiimide [126] or DHT with plasma treatment [124].

To our knowledge, a few references appear in the literature concerning DHT crosslinking effect on gelatin films.

2-1- DHT effect on gelatin films properties

2-1-1- Preparation of thermally treated gelatin films

The gelatin (bovine skin Type B 225 g) film forming solution was prepared by dissolving 5 wt.% of bovine gelatin powder (Sigma-Aldrich, Taufkirchen, Germany) in distilled water at 60°C for 30 min (pH = 5.8, surface tension = 39.14 ± 0.23 mN /m). Then, 60-µm thick films were obtained by casting the gelatin film forming solution over a covered Mylar® (DuPont Teijin Films^{TM}, Cotern, Luxembourg) mold. The evaporation of water was carried out at 22± 2°C and at 50 ± 10% relative humidity. Dehydrothermal (DHT) crosslinking of the gelatin films was achieved by drying the films at 100°C for 1 h, then heating them to 150°C for 8 h in a Memmert UFE500 oven (Memmert GmbH, Schwabach, Germany).

2-1-2- Thickness

Film thickness was measured to the nearest 0.1 mm with a digital micrometer (Mitutoyo, Model 406- 305, Japan). Five measurements per film were taken at random positions.

2-1-3- Swelling and mass loss

The swelling tests were conducted according to the Beaker test method. A small piece of gelatin film was weighed and placed inside a beaker. Then, 200 mL of phosphate-buffered saline (PBS) at pH =7.4 was poured into the beaker at 37°C. The swollen capsule was separated using a filter paper. By weighting the film, the swollen degree was determined using the following formula:

$$SD = \frac{W_1 - W_0}{W_0} \times 100 \quad \textbf{(1)}$$

where W_0 is the weight of the sample before immersion in the buffer solution and W_1 is the weight of the sample at time t. The swelling measurements were performed every 30 min for 8 h. The same protocol was applied to the swelling measurements of the capsule without the model drug reservoirs in the buffer solutions at pH=4.5 and at pH=2, prepared according to the French Pharmacopeia [151].

For mass loss experiments, the sample is weighed before immersion in the buffer, then immersed in the buffer for a precise duration, then dried : at 60° C for 2 h then at room temperature for 22 h. This experiment is carried out after different immersion times in order to assess the mass loss by dissolution.

2-1-4- Crosslinking extent determination :Trinitrobenzensulphonic acid (TNBSa) method

The extent of crosslinking was determined by measuring the amount of free or unreacted amino groups in the gelatin films. The following protocol was adapted from a procedure described by Kale and Bajaj. First, 25 µg of thermally crosslinked gelatin film was placed in a 100 mL screw cap test tube. Then, 2 mL of 4% $NaHCO_3$ (Sigma–Aldrich) and 2 mL of 0.5% 2,4,6-Trinitrobenzensulphonic acid **(**TNBS) (Sigma–Aldrich, Taufkirchen, Germany) were added to the tube. The mixture was heated to 40°C for 5 h, then 6 mL of 6 N HCL was added. The mixture was autoclaved at 120°C for 1 h in an oven for total hydrolyzation, diluted to 20 mL with distilled water, then extracted with two 40 mL portions of ether to remove the excess of the unreacted TNBS. To evaporate the residual ether, the mixture was heated for 10 min in a hot water bath. The absorbance was read at 346 nm using a Jasco double-beam spectrophotometer against a reagent blank prepared with same procedure. The number of ε-amino groups, C, expressed in [*moles*]/[*grams of gelatin*] units was calculated as the average of three measurements using the following equation:

$$C = \frac{2 \times Abs \times V}{1.46 \times 10^{-4} \times b \times m_{gel}} \quad \textbf{(2)}$$

where Abs is the absorbance, $1.46 L \cdot mole^{-1} \cdot cm^{-1}$ is the molar absorptivity of TNP-lys, $b = 1\ cm$ is the cell path length, m_{gel} is the sample weight in grams, and $V = 0.02\ L$ is the volume of the water solution containing hydrolyzed protein after the ether evaporation. The ε-amino content of the uncrosslinked gelatin film was estimated to be $29 \cdot 10^{-5}$ moles per gram of gelatin. The average molecular mass of an amino acid is usually taken to be equal to 110 Da. Therefore, the ε-amino content can be estimated to be 32 ε-amino groups per gelatin molecule of 1000 amino acid residues.

The extent of reaction was defined as:

$$f = 1 - \frac{C_{cr}}{C_0} \quad \textbf{(3)}$$

where C_{cr},C_0 are the mean amount of moles of the ε-amino groups per gram of gelatin in the crosslinked and uncrosslinked films, respectively. From the definition, it follows that$f \rightarrow 0$ if $C_{cr} \rightarrow C_0$ and$f \rightarrow 1$ if $C_{cr} \rightarrow 0$. From (1), it also follows that:

$$f = 1 - \frac{Abs_{cr}}{Abs_0} \quad \textbf{(4)}$$

where Abs_{cr}, Abs_0 are the absorptions of the crosslinked and uncrosslinked gelatin. The extent of the reaction was found to be f= 0.36; that is, approximately 36% of the ε-amino groups, or 13 per molecule of 1000 amino residues, was consumed in the crosslinking reaction.

2-1-5- FT-IR Spectroscopy

In order to study structural changes of the films at the molecular level after heating, we used FTIR spectrophotometer (Omni Sampler, Thermo Scientific, iS50) equipped with an ATR accessory with a crystal of Ge monoreflexion /45°. The samples were conditioned at 22 ° C and 50 ± 10% relative humidity (RH) before each run. This measure was done 3 times. The infrared spectrum of each film sample is recorded at room temperature in the range of 690-4000cm-1, using 64 scans and a resolution of 4 cm^{-1}. Spectra are processedusing OMNIC 9.8 software.

2-1-6- Contact angle and absorption rate

In order to assess the ability to wetting film surfaces, a Goniometer DSA100 – Krüss with a camera that can record 200 fps to 2000 fps was used. A drop of 2 µL of PBS 7.4 was deposited on the surface of the film using a microsyringue and the evolution of the contact angle was immediately measured and the rate of absorption of the drop during the 10 first minutes was studied.

2-1-7- Scanning Electronic Microscopy

The morphological examinations of the films were performed using a FEI Quanta 400 electron microscope. The images were made using the high vacuum mode at an accelerating voltage of 30 kV. Strips of dry films were stuck onto a cylindrical aluminum stub by a double-sided tape. The stubs with the film were sputtered with a thin layer of gold (15 nm) in an ion sputter coater (Cressington Sputter Coater 108 auto) and placed into a scanning electron microscope to see the films surfaces before and after crosslinking at different magnifications. Some other images were made using the low vacuum mode (with H_2O

atmosphere, pressure 0.98 torr) at an accelerating voltage of 20kV and without gold layer sputtering.

2-1-8- Atomic Force Microscopy

The morphology of the films was analysed using a FlexAFMNanosurf scanning microscope with Nanosurf C3000 controller and operating in tapping mode. Silicon cantilevers with a stiffness constant of 20-100 N m^{-1} and a silicon tip ACT were used for the measurements.The study was performed in air at room temperature. Images of 2.5 µm x 2.5 µm were made and processed using Gwyddion software.

2-1-9- Tensile testing

In order to study the mechanical properties of films, 8 specimens (50 mm length, 10 mm width and 50 µm thickness) were prepared from each film. The thickness was measured using a micrometer at 10 random points. Young's Modulus (YM), Tensile strength (TS) and elongation at break (E) were measured using a dynamometer (Zwick Roell) with a load cell of 0.2 N. The gap between tensile clamps was 6 cm and tesnsile speed was 10 mm/min

2-1-10-Gas adsorption (BET method)

The measurements are carried out using an ASAP 2020 from MICROMERITICS. The adsorbed gas was nitrogen (N2). The sample was cut into small pieces of approximately 1 cm^2 and placed in the analysis tube until the ball of the tube was compactly filled. A glass insert has been added further to reduce dead volumes. The tube has been degassed beforehand (vacuum of the order of 10 µm Hg} and filled with nitrogen for weighing. The sample was initially degassed for 48 hours at 40 ° C under vacuum in order to get rid of any impurities that could be physisorbed (water or other molecules). At the end of this time, it appeared that the residual pressure in the tube was still high (several hundred µmHg). We then continued its degassing until almost zero pressure was obtained (<5 µmHg). To get there, a time of the order of a week had to be applied . The tube was then filled with nitrogen after degassing and weighed. The degassing temperature was set based on the history and stability of the sample. This one having been treated at 150 ° C to carry out the crosslinking, the temperature of 40 ° C was selected to avoid an equivalent effect (the degassing being carried out here under vacuum) and to bring a minimum of temperature to counterbalance the endothermic effect of withdrawal. The measurements were carried out on a set of points regularly dispersed between the P/P0 values of 0.001 and 1. P/P0 represents the ratio of the measurement pressure

to the saturated vapor pressure of the gas at the analysis temperature. Here, the analyzes were carried out in liquid nitrogen at a temperature of 77.35 K. The saturated vapor pressure associated with nitrogen and used for these analyzes is then P0 = 760 mm Hg. To this set of points is added a set of doses to analyze adsorptions at very low pressures and corresponding to adsorption in micropores. In this case, doses of 0.01 cm3 / g STP were used (minimum possible dose). Doses addition was stopped by the system when the relative pressure P/P0 reached 0.001. The sample was degassed again on the analysis port for 2 hours before recording the isotherm in order to avoid any pollution and start with a perfectly empty porosity. The dead volumes (volume of the tube outside the sample) were measured separately after measuring the isotherm to avoid any helium residue inside the sample when establishing the isotherm.

2-1-11- X-Ray Diffraction

Data were collected with a powder diffractometer D8 ADVANCE A25 from Bruker in Bragg-Brentano reflexion geometry θ-θ (goniometer radius is 280mm). This diffractometer is equipped with the LynxEye XE-T high resolution energy dispersive 1-D detector (Cu Kα1, 2), leading to ultra fast X-ray diffraction measurements. Motorized anti-scatter screen, a device for effective suppression of instrument background, most importantly air-scatter at low angles 2θ, is present. Optical components are limited to two Soller slits (2.5°) for primary and secondary optics, and motorized divergence slits. The instrument is equipped with the Auto-Changer system that consists of a loading station auto-sample, a robotic sample handler with integrated gripper and a rotation sample stage mounted to the goniometer. Conditions for data collection are the following: angular area: 3-70 ° 2 theta (eliminate beyond 50), step size: 0.01°2θ, time per step: 10.7s , variable divergence slits (irradiated sample length: 15mm) , total time for acquisition: 2:05. During the data collection, the sample is rotating at 15 rpm. All the data are converted and presented into fixed divergence slits mode.

2-1-12-Differential Scanning Calorimetry

Thermal properties of gelatin films were determined using differential scanning calorimetry METTLER TOLEDO DSC 822e. The films were powdered and weighed in 40 µl Aluminium pans, sealed then scanned from 30 to 600°C at a basic heating rate of 10°C /min. An empty Aluminium was used as a reference. The Tg was obtained from the inflexion point of the reversible heat flow. Crystallinity percentage and Enthalpy were also determined using this technique.

2-1-13-Thermogravimetric Analysis

TGA METTLER TOLEDO TGA/ DSC 3^+ has been used to study the evolution of mass of gelatin films according to the temperature to assess their thermal stability. The measurements were carried out using a thermal analysis apparatus operating under a stream of nitrogen. The samples were heated from 40 to 600 at a heating rate of 10° C/min.

2-2- Fabrication of the Rolled-up capsules with cavity for drug release control

2-2-1- Fluorescent probes preparation

Fluorescein Disodium (FD) (Alfa Aesar™, Ward Hill, MA, United States) and Rhodamine B (RhB) (Sigma Aldrich, Taufkirchen, Germany) were dissolved in distilled water and used as model drugs**.**The concentration of each solution was 25 µg/µL.

2-2-2- Fluorescent probes incorporation

The model drug reservoirs were formed by spreading solutions of the fluorescent probes over the surface of a gelatin strip at areas corresponding to the prescribed radial positions of the reservoirs inside the capsule after the rolling. 20 µL of a solution per reservoir was spread using a micropipette. The concentration of the solution was 25µg/µL, corresponding to a content of 500 µg within each reservoir. Reservoirs R_0, R_1, $R_2$and R_3 were formed separately or in pairs on the gelatin stripe at precalculated distances so that each reservoir covered a full turn in the capsule. R_0 was located at the end of the stripe and it partially covered the internal surface of the capsule; therefore, it was in direct contact with the central cavity of the capsule after rolling. The R_1, R_2, and R_3 reservoirs were formed in such a way so that after rolling they are located on the 3rd, 6th and 8.5th turn, respectively; the total number of full turns was equal to 10. The coordinates of the boundaries with respect to the end of the gelatin strip, from which the rolling started, are given in Table 2-1.

Table 2- 1 The lateral coordinates of the reservoirs before rolling.

Reservoir	Turn	x_1 [mm]	x_2[mm]
R_0	1	0	17
R_1	3	35	53
R_2	6	91	110
R_3	8.5	140	160

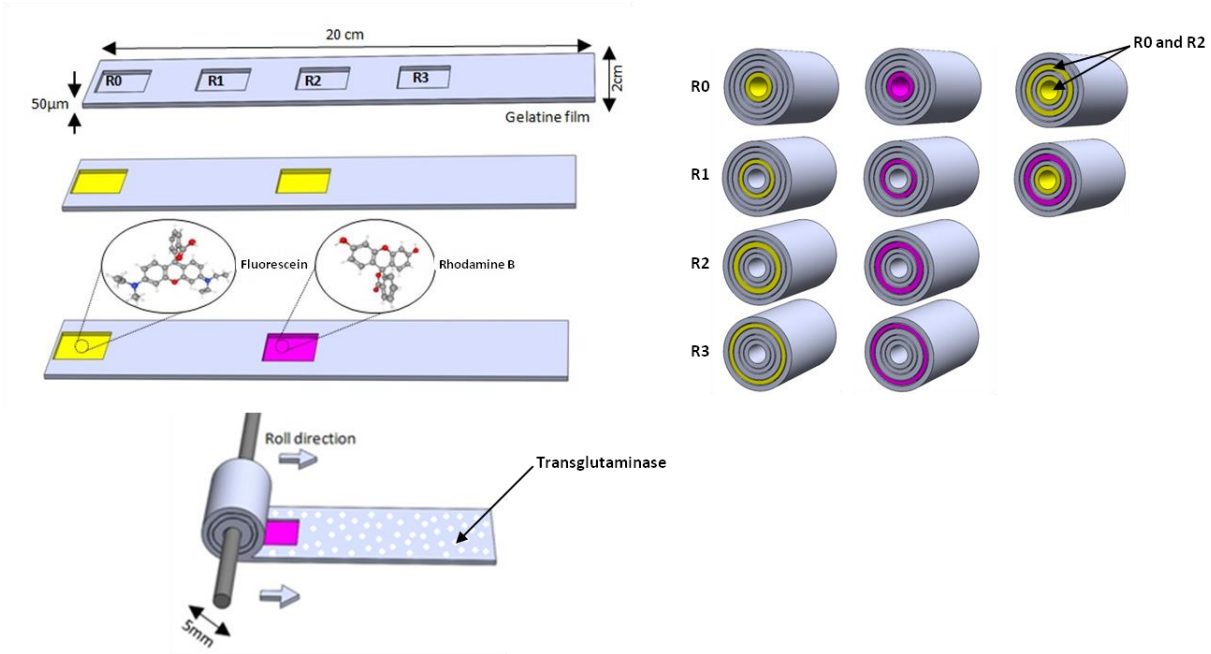


Figure 2-1 Formation of the capsules using the rolling-up approach: Rolling of a gelatin strip with two drug reservoirs around a cylindrical stick.

2-2-3- Calculation of coordinates of the reservoirs

The shape of the roll can be well approximated, in the polar coordinates, by the Archimedean spiral:

$$r = r_0 + \frac{h}{2\pi}\varphi \tag{5}$$

where r is the radius-vector, φ is the angle of rotation of the vector, h is the thickness of the film. Suppose that a reservoir should be placed on the n^{th} turn of the roll. Let x_1, x_2 be the coordinates of the limits of the reservoir along the length of the stripe before rolling. These limits can be found as:

$$x_1 = \int_0^{2\pi(n-1)} \frac{dl}{d\varphi} d\varphi = \int_0^{2\pi(n-1)} \sqrt{\left(r_0 + \frac{h}{2\pi}\varphi\right)^2 + \left(\frac{h}{2\pi}\right)^2}\, d\varphi \quad \textbf{(6)}$$

$$x_2 = \int_0^{2\pi n} \frac{dl}{d\varphi} d\varphi = \int_0^{2\pi n} \sqrt{\left(r_0 + \frac{h}{2\pi}\varphi\right)^2 + \left(\frac{h}{2\pi}\right)^2}\, d\varphi \quad \textbf{(7)}$$

where dl is the element of the length of the spiral corresponding to the rotation of the radius-vector by $d\varphi$. The limits of the reservoirsR_0, R_1, R_2 and R_3, calculated by the formulas above, for the experimental values $h = 0.06mm$, $r_0 = 2.75\ mm$, are given in Table 2-1.

2-2-4- Fabrication protocol

Gelatin films, prepared according to the approach described in the Section 2.2.1, were stored for 1–2 days at room temperature in a vacuum desiccator before being cut into 20 cm long strips using an infrared lasermachine. The gelatin strips were rolled around a 5.5 mm diameter cylindrical stick, which was subsequently removed to form a cylinder-like capsule. To avoid the unrolling of the capsules, point-like drops of 20 wt.% Ajinomoto meat glue (Ajinomoto, Tokyo, Japan) were applied at the extremities of the film (away from the reservoirs) at each turn during the rolling. It is important to note that the Ajinomoto meat glue containedsodium caseinate E469, maltodextrin, transglutaminase and sunflower lecithin E322. Transglutaminase participated in the reaction that catalyzes the formation of the isopeptide bonds between the glutamine residue of γ-carboxamide and the primary ε-amine groups of gelatin [152].

2-2-5- Dissolution media preparation (Classical physiological media defined in the 9th European Pharmacopoeia)

The solution of pH=2 is a phosphoric buffer solution and not a phosphate buffer saline solution (PBS). It was prepared by dissolving 39.2 g of phosphoric acid R, 24 g of acetic acid R and 29.8 g of boric acid R in water R and making up to 1000.0 mL with the same solvent and then diluting 1/10 with distilled water R just before use.

The buffer solution of pH = 4.5 is called phosphate buffer solution according to the French pharmacopoeia. It was prepared by dissolving 13.61 g of monopotassium phosphate R in 750 mL of water R and adjusting the pH if necessary, with 0.1 M sodium hydroxide or 0.1 M hydrochloric acid. then completing to 1000.0 mL with water R.

2-2-6- Dissolution tests

The dissolution testing experiments of the dosage forms were performed using the United States Pharmacopeia (USP) dissolution apparatus 2 (Agilent 708-DS, Agilent Technologies, Santa Clara, CA, USA) with a 50-rpm rotation speed at 37°C. PBS was used as the dissolution medium. The pH of the PBS was adjusted to 7.4. All the experiments were performed in triplicate.

2-2-7- Fluorescence measurements

FD and RhB have an ultraviolet (UV) emission peak at 510 nm and 540 nm, respectively. The amount of the released FD and RhB from the capsules was determined by fluorimetry (Thermo Scientific ™ Varioscan ™ LUX multimode microplate reader) using predetermined calibration curves for FD and RhB. The choice of FD and RhB was based on their emissions at distinct wavelengths, allowing us to avoid overlapping of the emission spectra.

2-3- Fabrication of the rolled up capsules without cavity for drug release control

Contrary to the requirements of the first system designed with a central hole, high flexibility and self-adhesion are two key factors to obtain rolled-up capsules almost without cavity.

As the capsules must be flexible to allow easy rolling, self-adhesive, and stable in the rolled geometry under physiological conditions, we present a system whose properties approach these requirements. We have combined the effect of plasticizers with the effect of crosslinking agents. We also studied the effect of the order of addition of the different components of the film forming solution on the appearance of the films, their topography and their stiffness.

To prepare these films, 10 g of gelatine from porcine skin (Suitable for microbiology, ultrahigh gel strength) supplied by Sigma Aldrich was introduced into 80 mL of DPBS and the mixture was heated at T = 70 ° C. 18 µL of chloroform were introduced into the mixture and then evaporated for 30 minutes. A solution of transglutaminase (1g of Tgase in 10 mL of DPBS) was prepared in parallel.

First, these two solutions were mixed and then spread in a petri dish to form the reference film. Secondly, the previous mixture was mixed with 2 g of Sorbitol (Sigma-S1876-500G) and well mixed and quickly poured into Petri dishes. In the third experiment, the mixing order

was changed: Sorbitol was added to the gelatinous solution before being mixed with the transglutaminase [153]. Then, mechanical properties were studied using AFM and tensile testing.

The films obtained from the second experiment were cut into strips 8 cm long and 2 cm wide. These strips were loaded with FD in 3 positions and then rolled up so that there was no hole in the center. Dissolution tests were performed on these capsules using USP 2 apparatus (PBS 7.4, 37°C, 50 rpm).

Chapter 3. Rolled-up capsules design for drug release control (Matrix characterization)

Studying the properties of the polymer that will be used as a carrier and understanding its behavior are very important for drug release systems design, hence we devoted considerable space in the manuscript to characterize the pharmaceutical matrix. Every factor that may affect surface and structural characteristics of the films was studied. The influence of the crosslinking on the mechanical, thermal and physiochemical properties is investigated for bovine gelatin films, a popular drug delivery platform. It is undertaken to study the changes, which take place as gelatin films are thermally dehydrated (DHT treatment) in order to investigate posteriorly the kinetic release of a model molecule through this crosslinked biomaterial.

3-1- Preliminary study: Heating time optimisation

An important property of untreated gelatin films is that they dissolve in water at a temperature above 30 ° C and release the active ingredient in the digestive tract of humans. Indeed, in wet physiological conditions (37°C, pH=7,4), untreated films exhibit an unstable polymeric network and solubilise. Therefore, films have been crosslinked using dehydro-thermal treatment [154] to avoid concerns about the use of toxic crosslinking agents, such as glutaraldehyde, and their possible release upon degradation in vivo. The stability observed after DHT treatment allowed us to define the optimum crosslinking time for a drug delivery application. The first part of the study is about swelling capacity and mass loss percentage. It was carried out in order to select the optimum heat treatment duration to obtain insoluble films, with a low swelling capacity and a relatively low mass loss. The second part consists of a comparative study of untreated films and those selected in the first part.

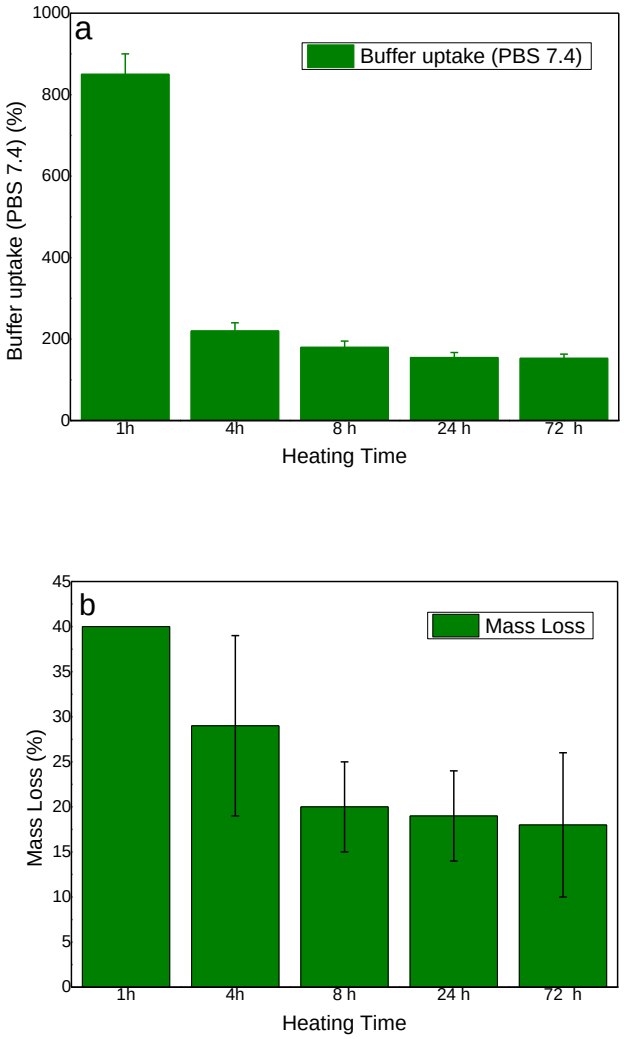


Figure 3- 1 a) Swelling capacity and **b)** mass loss % of gelatin films after immersion in PBS (pH = 7.4)

The results show that the swelling capacity seems to be strongly dependent on the heating time. A preliminary study of swelling was carried out and showed that the swelling phenomenon is linked to the polymer mass loss given that swelling is controlled by crosslinking, and crosslinking limits extraction which generates mass loss. Indeed, films may exhibit not only swelling-deswelling behavior but also polymer dissolution reason for which mass loss percentages were also determined.

The swelling capacity of the untreated films increases during the first 10 min of immersion in physiological conditions then they dissolve. As shown in figure 3-1, the films treated for 1 h swelled up to 850 ± 58% and turned into gel. In contrast, the films treated for 4 h, 8 h, 24 h and 72 h showed swelling percentages equal to 220 ± 20%, 180 ± 15%, 155 ± 12% and 153 ± 10%, respectively, after 24 h of immersion in PBS.

However, mass loss becomes almost constant after 8 h of heat treatment. Therefore, the films treated during 8 h were retained and used as the matrix to design the rolled-up capsules. Obtained films are transparent, non brittle and slightly yellowish.

3-2- Comparative study between untreated, 4h treated and 8h treated films

3-2-1- FTIR Spectroscopy

To highlight the changes mentioned above and further understand the structural modifications induced by DHT treatment within the gelatin film from a molecular point of view, FTIR spectra were collected and studied as shown in figure 3-2.

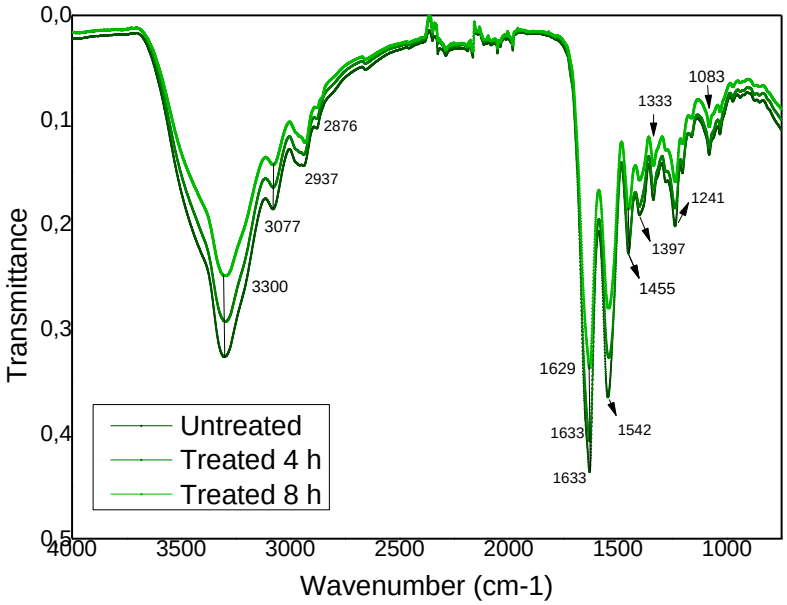


Figure 3- 2 IR spectra of untreated and treated gelatin films

The FTIR spectra of all studied gelatin films clearly show the three typical IR signals that can be considered as marker bands in proteins, corresponding to Amide I at 1629 cm^{-1} due to C=O stretching ,amide II at 1544 cm^{-1} due to NH deformation and Amide III at 1241 cm^{-1} due to CN stretching vibrations. Spectra show many peaks from 3700 cm^{-1} to 3000 cm^{-1} corresponding to OH stretch, free water and NH stretching. The major ones appear at 3296, 3080 and 2939 corresponding respectively to NH stretch of Amide A, to CH

asymmetric/symmetric stretch and Amide B. Table 3-1 contains the most potentially useful information on the structure of 8h treated gelatin films.

The spectra of treated and untreated films (4 h and 8 h) seem to be very similar as also noted by K.Gopal et al who confirmed that there are no distinguishable vibrational changes in the spectral peaks after 48 hours of DHT treatment applied on gelatin scaffolds heated at 160°C under vacuum for 48 hours [149]. However, the peaks between 3700 and 3000 that reflect the amount of water in the films become less intense as the heat treatment time increases as shown in Fig 3-2. In accordance with these findings, an interesting study has reported that DHT removes water from gelatin films, which results in the formation of intermolecular crosslinks through condensation reactions. In fact, during DHT treatment, amino acids condense together through their amine and carboxyl groups and form amide bonds between molecules.

Wihodo et al, have reported that when films are heated, their functional properties are altered as well [120]. In fact, heat disrupts hydrogen bonds and non polar hydrophobic groups in the proteins, thus, producing a more open structure. It was also found that the crosslinking in the gelatin film network between β-chain and α-chain could be induced by heating at 120 °C [125]. In the same study, it was revealed that the main interactions involved in the crosslinked gelatin film formation were changed from ionic bonds and hydrogen bonds to hydrophobic interactions and covalent bonds, leading to improvement water resistance properties of films. Besides, the hydroxyl groups and the carboxyl groups may be involved in an esterification reaction by dehydration [123], [124]. Another effect in the spectra of treated films needs to be explained; that is, the peaks of the gelatin at 1544 cm^{-1} and 1452 cm^{-1} shifted respectively to lower wavenumbers (1525 cm^{-1} and 1444 cm^{-1}).

Table 3- 1 Vibrational peaks of 8 h treated gelatin film spectra

Wavenumber (cm^{-1})	**Functional groupment vibration**	**Wavenumber (cm^{-1})**	**Functional groupment vibration**
3296	Amide A, N-H stretching	**1544**	Amide II, N–H bending Hydrogen Bonds
3080	Overtone amide I and Amide B	**1452**	CH_2 bending
2939	CH_2 Assymetrical	**1242**	C-N stretching, Amide III

	stretching		
1718	C=O Ester	**1082**	C–O stretching
1629	Amide I, C=O stretching		

3-2-2- Scanning Electronic Microscopy

It has been documented that the macroscopic properties of solid materials, such as drug release and permeability, are closely connected to their microporous structure. Small pores in the range of a few nanometers can affect matrix hydration, drug diffusion into the release medium, and polymer degradation. SEM micrographs of the films at 4000X magnification showed smooth surface without pores and without any scratches or striations indicating the formation of uniform and continuous network structure. No significant differences of surface morphology were observed for untreated and treated gelatin films.

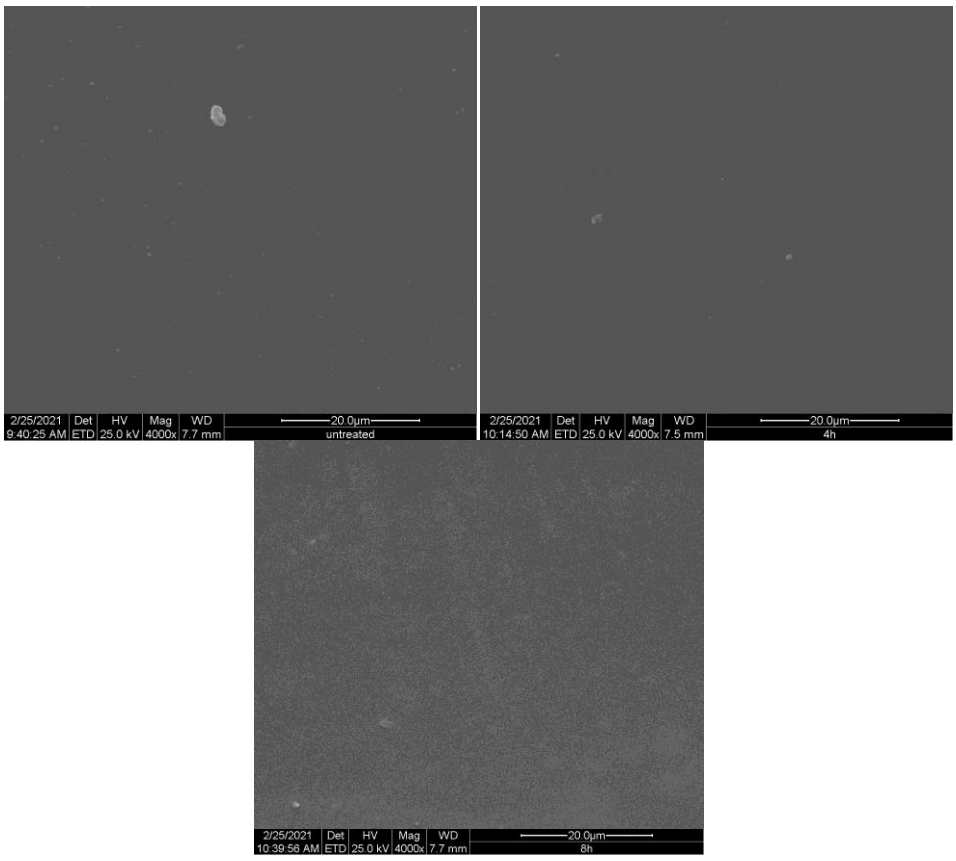


Figure 3-3 SEM micrographs of untreated and treated films

3-2-3- Atomic Force Microscopy

Using atomic force microscopy, we evaluated the topography of the treated and untreated films to study the effect of heat treatment on the average film roughness. The surfaces of all the films were smooth but present some defects probably resulted from water evaporation. The mean surface roughness of all the films was of the order of the nanometer. Further characterization indicates that Young Modulus of the untreated films is about 2, 7 GPa. Films treated 4 h have an elastic modulus of about 5 GPa and the films treated for 8 hours have a modulus equal to 6 GPa. The more the thermal crosslinking time is prolonged, the more the modulus of elasticity increases.

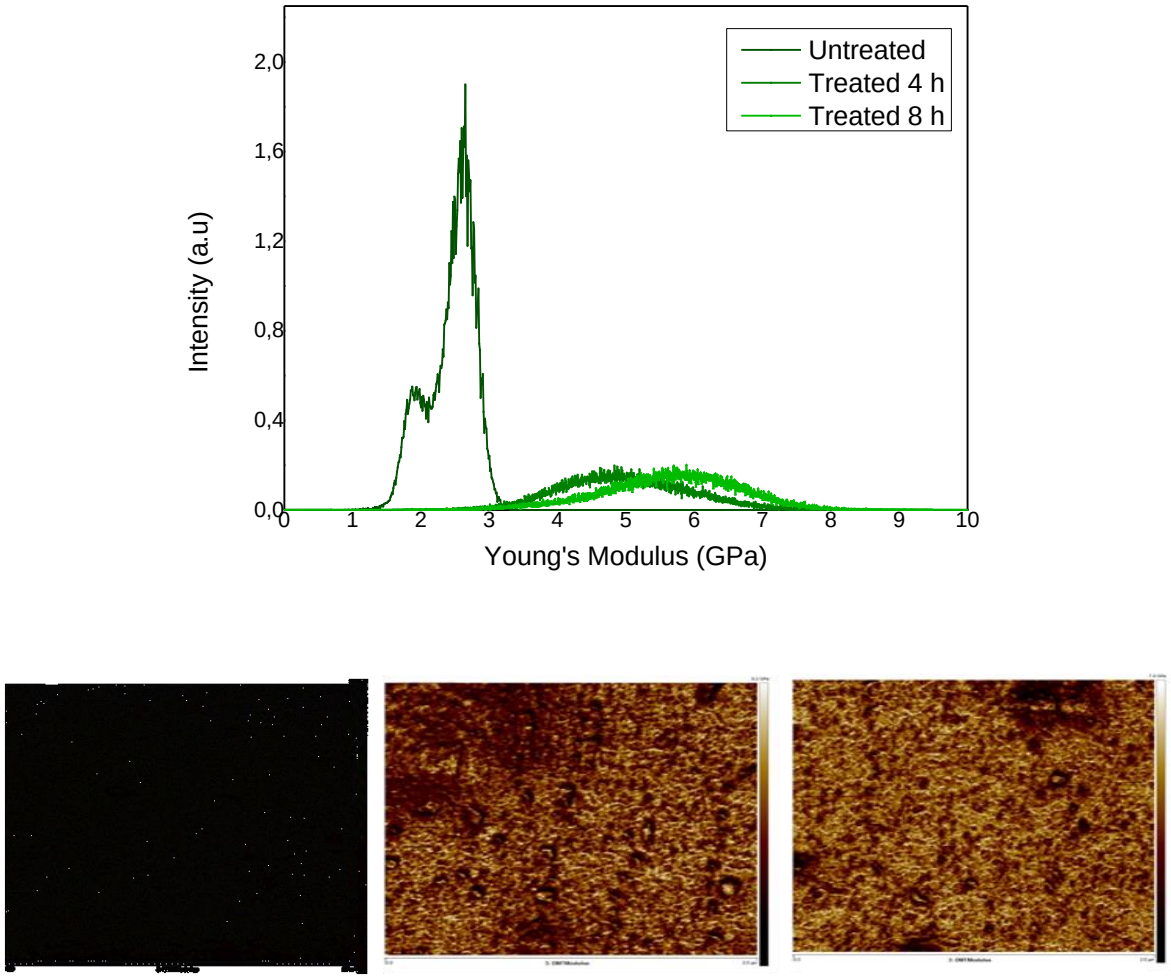


Figure 3- 4 AFM graphs (Roughness and Young's Modulus) of untreated (left), 4 h treated (middle) and 8 h treated films (right)

3-2-4- Tensile testing

Mechanical properties of gelatin films are extremely important since their use, as a drug delivery carrier requires their integrity and flexibility during their passage through the digestive system. In this assay, the film in a dry state is affected by a constant tensile force and resists the mechanical stresses. Unfortunately, we are not equiped for measuring the mechanical properties of the gelatin films in the swollen state. It is supposed in this study, that the mechanical stiffness in the dry state correlates with that in the swollen state. Results show that heat treatment resulted in an increase in stiffness and strength of films. Indeed, treated films had a higher mechanical strength (3000 MPa ± 8 %) in comparison to untreated films (2700 MPa ± 10%) which corresponds to results found previously using AFM. Concerning the elongation, a slight change has been observed (from 3,2 ± 0,8 % to 3,0 ± 0,7 %). In conclusion, treated films displayed a higher mechanical strength in comparison with untreated ones.

3-2-5- Gas adsorption (BET method)

The sorption isotherms for each gelatin film show a sigmoid behaviour associated to the wellknown type II isotherm typical for biopolymers. The result of the specific surface area of the uncrosslinked film shows an area equal to 2.5 m^2/g. DHT treatment reduced the specific surface area as well as the N_2 adsorbed volume by a factor of 10 for both treated samples. In fact, when DHT treatment was undergone, N_2 adsorption showed a reduction in sorption sites availability suggesting a densification of the matrix which in agreement with the crosslinking of the network. Compared to the 4 h treated films, films treated for 8 hours seem to develop a slightly lower but relatively similar volume. On the otherhand, the pore size distribution of the 8-hours-treated sample shows a slight shift of its maximal toward the lowest diameters and a narrower distribution. These results would be consistent with an increase and homogenization in crosslinking with the treatment time and generate a slight decrease of the volume of the pores.

For untreated films, pores distribution shows a fairly narrow peak. It mainly focuses on size W = 2.9-3.1 nm and spans the range [1.5 - 6].Treated films show reduction in pore sizes as well as its range. If the gelatin shows a strong peak, there is nevertheless a slow and long distribution towards the larger diameters. After 4 hours, the size has reduced and so has the volume. On the other hand, the distribution is relatively wide: there is a shift of 1.6 nm

between the average value and the maximum. After 8 h, it would appear that the distribution is narrower and the offset is smaller.

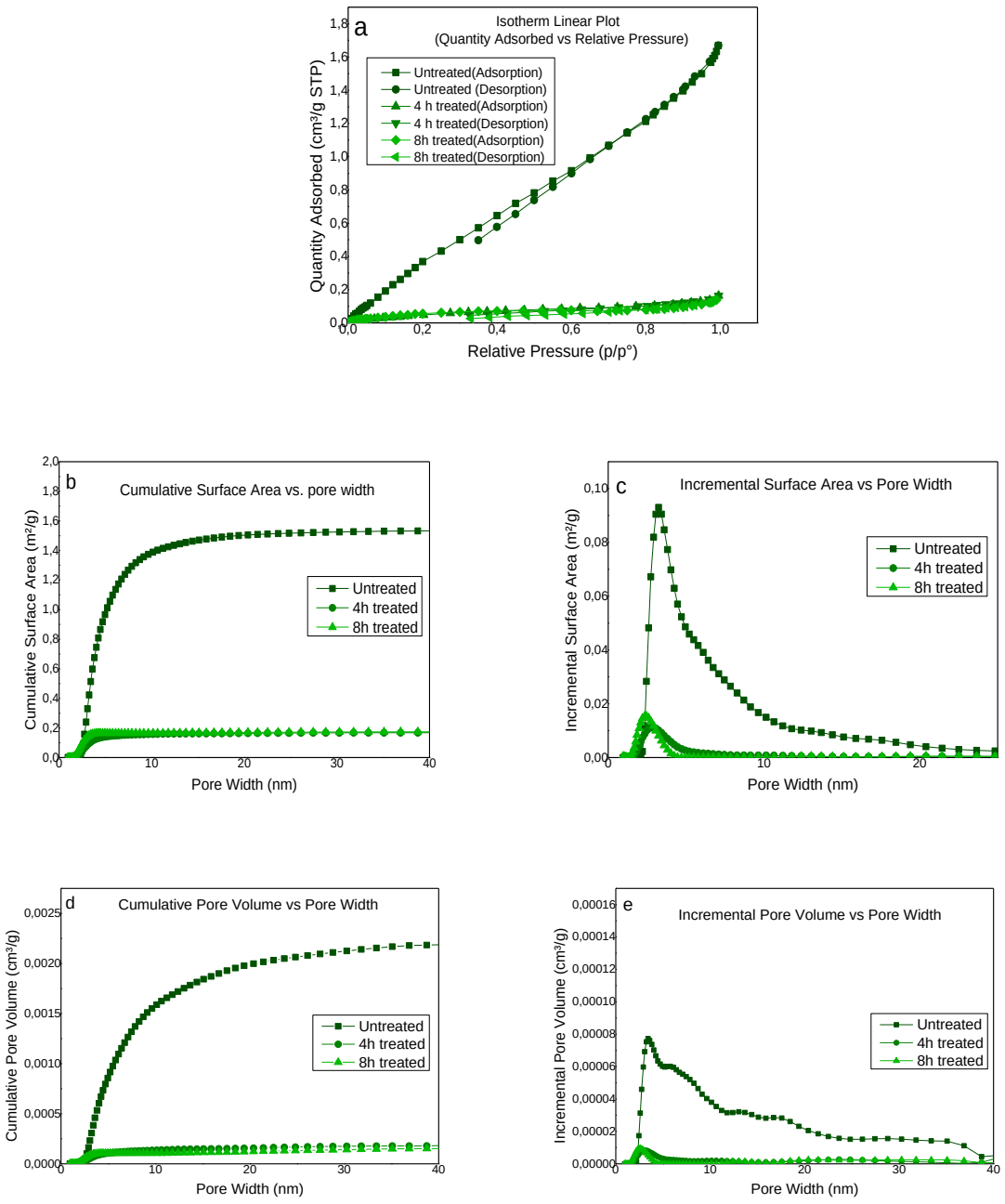


Figure 3-5 a) Isotherm Linear Plot of treated and untreated films b) Cumulative Surface Area vs Pore Width of treated and untreated films c) Incremental Surface area vs Pore Width of treated and untreated films d) Cumulative Pore Volume vs Pore Width e) Incremental Pore Volume vs Pore Width

Table 3- 2 Adsorption data of untreated and treated films

Sample (films)	Specific surface area (m^2/g)	BET constant	Adsorbed volume (cm^3/g)	Total pore volume (mm^3/g)	Pore size maximum (nm)	Average pore size (nm)
Untreated	2,50	3,89	1,6695	2,582	3,2	4,1
4 h Treated	0,24	8,70	0,1637	0,253	2,8	4,2
8 h Treated	0,25	12,05	0,1385	0,214	2,4	3,3

3-2-6- X-Ray Diffraction

XRD pattern has peaks at 2θ ≈7° and 20° corresponding to gelatin type A powder. These peaks demonstrate the reconstitution of the three-dimensional structure of collagen. The peak at 2 θ ≈ 8° is related to the diameter of the triple helix and its intensity will be related to the triple-helix content of the film [155][156]. This is confirmed by another study which shows that the XRD pattern of gelatin has a sharp peak at 2θ = 7.7° and a broad peak at 2θ =19.3° with intensity of 246 and 407 counts respectively, indicating that gelatin possesses both amorphous and crystalline region in its structure [157]. In our study, two diffraction peaks at angles 2θ = 8 ° and 2θ = 20 ° were found on all gelatin samples mainly indicating a partially crystalline structure of gelatin. The peak at 2θ = 8 ° precisely indicates the diameter of the triple helix (refers to the crystal structure of the triple helix from collagen renatured to gelatin) and its intensity is associated with the triple helix content. The second peak is attributed to the amorphous phase with free single helix chains (random coil and, the β sheets). Diffractograms of untreated films and films treated for different time periods show similarities. As can be seen, the position and intensity of these diffraction peaks change slightly after DHT treatment. Contrary to theory, the amount of triple helixes and their heating stability decreased, while the amount of random coil and, β sheets increased. Nevertheless, we can conclude that the films remained essentially amorphous after DHT treatment.

In the literature, XRD analysis performed on gelatin films showed that the diffractograms of chicken skin, porcine and bovine gelatin films show an amorphous character, indicating no tendency to recrystallization, which is probably due to the high stability and to the high moisture content in gelatin films. Diffractograms of chicken skin, porcine and bovine gelatin films showed a diffraction peak at about 2θ = 20° attributed to typical gelatin powder fingerprints. The chicken skin gelatin film diffractograms showed no sign of crystallization,

while the porcine and bovine gelatin films showed a small crystal peak at 8° indicating the diameter of the triple helix; thus, the intensity of gelatin films is associated with triple helix content. In particular, bovine gelatin films possess a larger and more dispersed molecular structure, explaining its crystalline character [158] .

With the help of this structural characterization, the dissolution of the crosslinked films can be predicted. To complete this characterization, the quantification of the percentage of crystallinity would be relevant to better understand the diffusion of the dissolution medium in the gelatinous matrix.

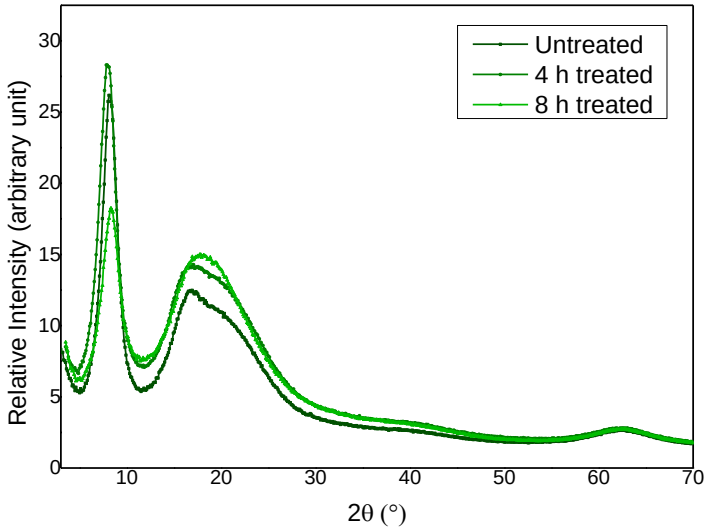


Figure 3- 6 XR diffractograms of untreated and treated films

3-2-7- Differential Scanning Calorimetry

In order to study the thermal properties of treated and untreated gelatin films, DSC analyzes were performed. The graph obtained presents three important transitions in agreement with the DSC curve typical of gelatin which shows a glass transition, melting and deterioration peaks [159].

The first transition that took place around 90°C can be attributed to the glass transition Tg at which the sample undergoes a change in heat capacity and the polymer changes from an elastic material to a brittle material due to the changes in chain mobility.

Increasing the water content of the gelatin film decreases the intensity, goes to a lower temperature and broadens the Tg peak because water has a plasticizing effect on the gelatin

film [160]. This moisture content is difficult to control as the gelatin film is easily dehydrated by water vapor in the air. Therefore, the Tg value can vary considerably from one study to another. In the literature, the glass transition temperature is Tg = 57.51 °C according to [161] and Tg = 59.23 according to [160]. In another reference, bovine bone gelatin film and giant catfish skin gelatin film have glass transition, melting and deterioration peaks around 55–60°C, 83–89°C , 134–140°C, respectively [159].

The first transition is followed by a slight hint of crystallization during which the molecules gain freedom of movement to spontaneously organize themselves into a crystalline form [161]. Gelatin then coexists in its two states, crystalline and amorphous.

The third transition that appears at 223°C corresponds to the melting point at which the polymer chains can move freely. which is in agreement with work also carried out on gelatin films and which showed a Tm = 214.02°C [160].

Our study revealed that the heat treatment has a notable influence on the Tg which results in the reduction of the intensity of the exothermic peak located at 90°C for the reference film. This change may be due to the reduction in water content induced by heating the gelatin. The DSC curves also show a small shift in the melting temperature of the treated samples towards higher temperatures. Previous studies have generally shown that crosslinking does not change the melting point of the gelatin film [160].

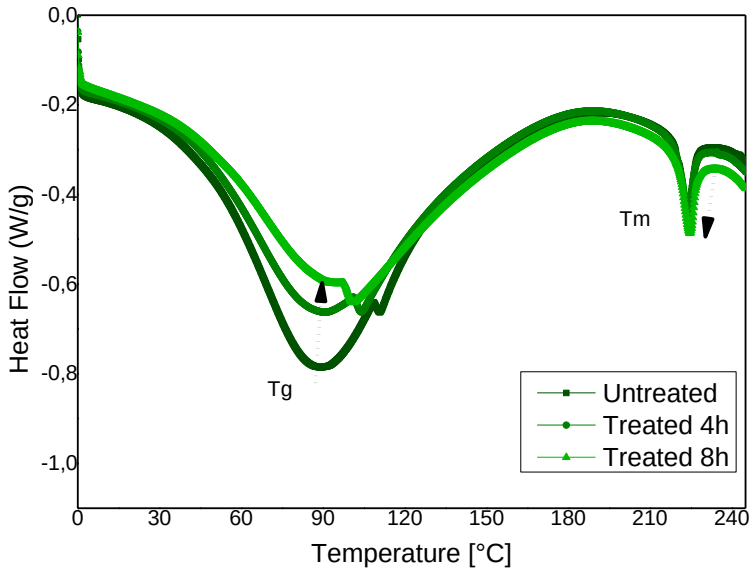


Figure 3-7 Differential Scanning Calorimetry-of untreated and treated gelatin films

3-2-8- Thermogravimetric Analysis

Thermogravimetric analysis was conducted to evaluate the thermal stability of untreated gelatin films and thermally treated ones. Thermograms show the typical sharp denaturation peaks with three steps of mass loss.

The first loss essentially comes from the vaporization of the water trapped in the matrix loss (free and bound water) [155]. This first stage was a 7% –11% weight loss for all the studied films. The peak corresponding to this loss appears at approximately 97 ° C. for the reference film. In literature, gelatin films start to lose weight at 160 ◦C which results in 4% loss of weight [162]. After heat treatment, this peak did not undergo a shift but its intensity decreased in a similar way for the different treatment times.

The second stage showed 5%–7% weight loss (323°C), which is associated with the degradation of low molecular weight protein fraction, as well as structural bound water.

A 78% –89% weight loss was observed at the third stage at 430 °C which depends on the degradation of larger size or higher interacted protein fractions. Indeed, thermal crosslinking offers slightly better thermal stability compared to untreated films. According to bibliographic research, the thermal degradation of the pure gelatin starts at 280°C and 50% weight was lost at around 380° C [163].

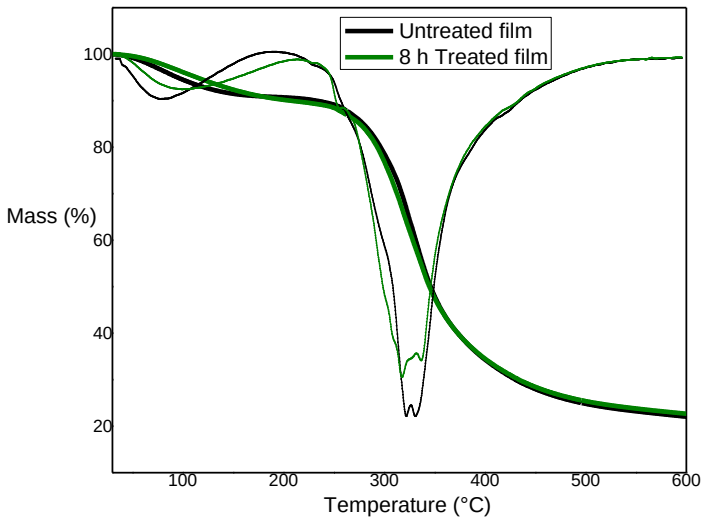


Figure 3-8 Thermogravimetric analysis of untreated and 8h treated films

3-3- Selected gelatin films characteristics

When heated, gelatin undergoes not only structural and mechanical changes but also physico-chemical transformation such as partial or complete loss of solubility in water caused by crosslinking. Such changes can be observed by heating gelatin above 140°C [164]. Therefore, gelatin films were crosslinked for different durations at 150°C. DHT treatment time (8h) was chosen as the compromise between the fabrication convenience and the achieved performance properties of the films (Previous study).

3-3-1- Swelling behaviour

Gelatin forms a thick gel in aqueous solution, which solidifies at room temperature and dries to form regions of crystalline structure within the amorphous film [165]. Its swelling is usually attributed to an osmotic action due to the presence of a soluble form of gelatin or its salt inside the molecular network of the gel [166]. The water molecules diffuse through and within the triple helices and interact particularly with the C = O groups of glycine and proline, the -OH groups of hydroxyproline and the -NH glycine groups giving a swollen film [167]. Aiming to understand the behaviour of the films during the first hours of immersion, swelling degree of films were studied until 8 hours and plotted according to time. Figure 3-9 shows a mass variation curve with ascents and descents suggesting that swelling phenomenon interferes with the polymer mass loss. Indeed, these systems may exhibit not only a swelling-deswelling behavior but also polymer degradation. In this part of the study, it was proposed to evaluate the swelling behaviour of the films by measuring the water retention capacity and to study its mass loss in order to predict films dissolution.

Figure 3-10 compares the swelling behavior of the capsules to that of the thermally crosslinked gelatin films. The crosslinked gelatin film allows water to diffuse easily into it and to reach approximately 250% of its initial mass after 30 min. The water uptake competes with the partial dissolution of the uncrosslinked gelatin. This results in a slight drop in the degree of swelling after 3 h. The capsules show a different swelling behavior, swelling to around 60% of the maximal swelling capacity within 1 h.

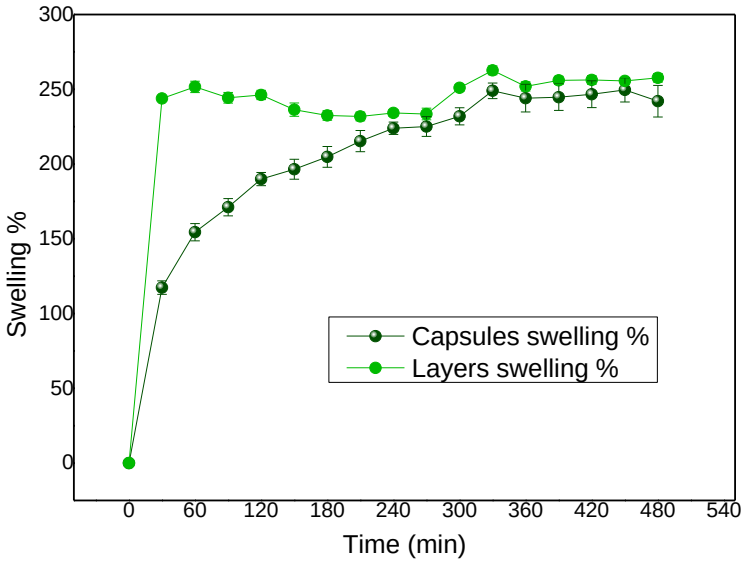


Figure 3-9 Buffer uptake capacity of 8 h treated films and capsules

3-3-2- Crosslinking extent

The extent of crosslinking was determined by measuring the amount of free or unreacted amino groups in each gelatin film [168][169].The degree of crosslinking was estimated as 13 crosslinks per molecule of 1000 amino acid residues.

3-3-3- Contact angle and absorption rate

The wetting behavior was easily measured using the contact angle for PBS (Phosphate Buffer Saline) 7.4 on gelatin films. The evolution of a 2 µL drop deposited on each film was successfully evaluated. It was found that the untreated films exhibit a contact angle equal to 94 ° ± 2 compared to 108 ± 3 ° for the films treated for 4 hours and 112 ° C. for the films treated for 8 hours.

An important question arising from these observations is " Despite the contact angle greater than 90 °, does the film remain hydrophilic and able to absorb the buffer? "

Indeed, gelatin has a hydrophilic character due to the hydrophilic groups exposed in its chains in the absence of crosslinking, but after DHT treatment, the replacement of certain surface amino groups in the gelatin polypeptide by active ester groups results in decrease in hydrophilicity. In addition, the hydrogen bonds formed between the crosslinked gelatin products also contributed to hydrophobicity [170]. Thus, the hydrophilic property of the film has decreased. Heating therefore reduced the ability of the gelatin films to absorb the tampon,

but it still remains hydrophilic. The drop absorption assessment show that the volume absorbed decreases almost linearly with time, indicating that the drop is diffusing through the surface of the gelatin film. When films are dehydrated, they exhibit a lower absorption rate than untreated films suggesting that buffer diffusion becomes more difficult through the film grating due to crosslinking. A comprehensive understanding of the behavior of the gelatin film during wettability allows better control in the choice of the model molecule to be incorporated into the gelatinous matrix.

3-3-4- Drug model formulation and incorporation

The formulation of the ink that serves as the model in the dissolution study is a very important step. This is because the appearance (topography) of the ink incorporated into the gelatinous matrix changes depending on the composition of the model ink. This involves an interaction with the support which strongly depends on the nature of the ink and how it is incorporated into the pharmaceutical matrix. First, carboxymethyl cellulose was added to Rhodamine B and then the mixture was deposited in the form of drops and observed using SEM in order to compare its appearance to that of the ink without CMC. CMC is known to act as a viscosity modifier and water retention agent. Although the amount of CMC added was small in the formulation deposited on the surface of the film, we observed needle agglomerates. This shows crystallization of Rhodamine on the surface of the film after the drop has dried as shown in figure 3-11.

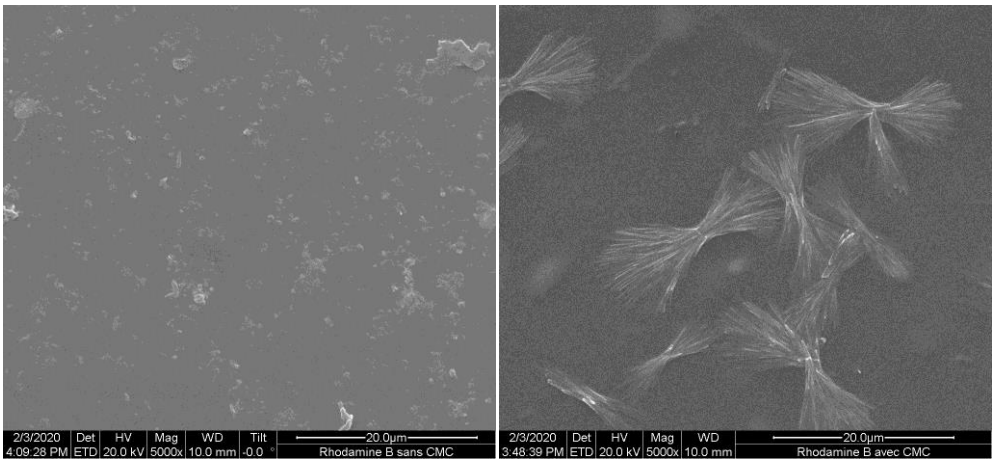


Figure 3-10 SEM images of Rh B Ink with and without CMC

Secondly, we incorporated the model ink in two different ways: by printing (Inkjet printing) and by simple deposition using a micropipette. Magnification of 2500 times shows that the

printed quantity is in the form of similar circles (spread ink) and that the drop deposited is in the form of needles.

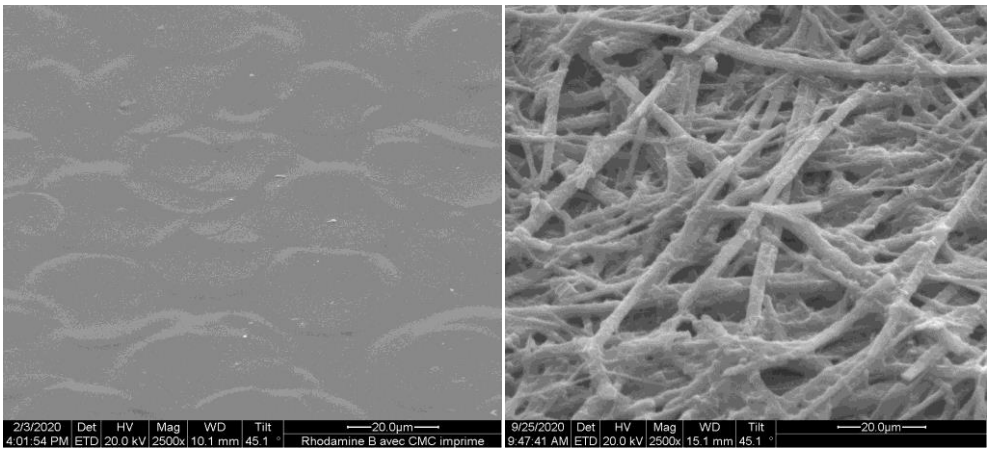


Figure 3-11 SEM images of Rh B ink printed and deposit

The nature of the ink and the technique of its incorporation into the pharmaceutical carrier give different appearance to the surface.This brief study which addresses two formulation-related factors is very important as the dissolution mechanism for such matrices generally involves diffusion and contacting the dissolution medium with the model ink. In other words, the interaction between the ink and the matrix determines its affinity to stay or leave the gelatin film. This interaction depends on the nature of the ink and the method in which it is incorporated. For our dissolution study, we minimized the model ink ingredients so the ink only contains water and fluorescent probe. It remains all the same interesting to compare the dissolution kinetics by varying the components of the formulation as well as their quantities. We also chose to deposit the ink to better control the incorporated amount.

3-3-5- Capsules stabilization with Transglutaminase

The application of the transglutaminase on the surface of the gelatin films formed crosslinks by a condensation reaction between the carboxyl and amino groups in the gelatin. This reaction involves the transfer of acyl to the lysine residue bound in a polypeptide chain resulting in the formation of inter- or intramolecular cross-links of α- (γ-glutamyl) lysine accompanied by the release of ammonia. The isopeptide bonds thus formed contribute to the formation of stable protein networks.

It’s known that Transglutaminase is deactivated or destroyed during the heating of processed foods and cannot survive acidic gastric pH [13]. In agreement with previous reports, the catalytic activity of transglutaminase was significantly reduced at relatively acidic pHs (3.5-

5.5) compared to that shown in buffers close to neutrality [171][115]. Indeed, as shown in Figure 3-13, the swelling study at acidic pHs in particular 4.5 and 2 showed that the capsules remain stable at pH=4.5 and gain about 4 times its initial mass after 8 hours of study while the capsules immersed in a medium of pH=2 deroll because of the deactivation of the Transglutaminase. The swelling of the capsules at neutral pH remained the lowest (approximately 250%) and their stability in the cylindrical shape was maintained.

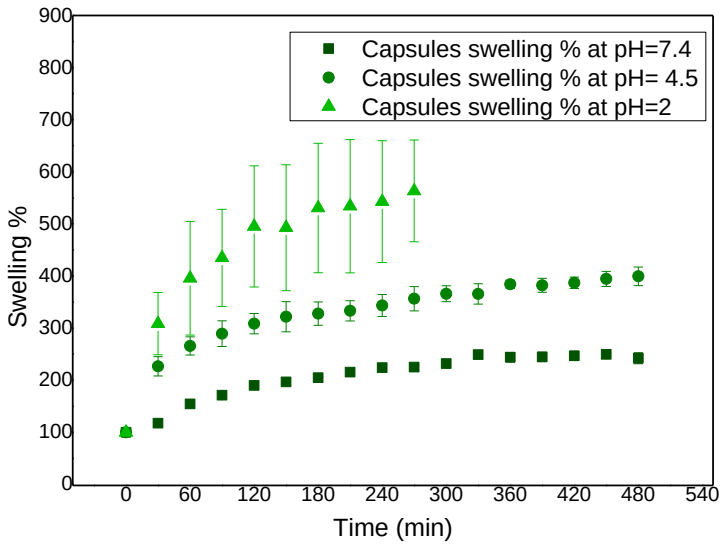


Figure 3-12 Capsules swelling in different pHs

Conclusion

To develop a material with a potential drug delivery application, dehydrothermal treatment (DHT) was applied on crude gelatin films obtained by casting. This physical treatment is an interesting alternative for chemical and enzymatic crosslinking since it diminishes undesirable side effects of chemicals. Typical conditions for DHT are known to involve temperatures between 105 ° C and 140 ° C and a period of about 24 hours or more. Initially, the emphasis is on the influence of the heating time on the swelling kinetics and the loss in mass of the films. It has been observed that films heat treated for 8 hours at 150 ° C. become insoluble in aqueous medium at 37 ° C. and exhibit a buffer uptake of 221% and a maximum mass loss equal to 29%. No significant difference was detected between IR spectra before and after DHT treatment. For these films, which we have recognized as the most promising for drug release application, additional tests have been carried out including mechanical tests. The AFM shows that these films have a smooth surface which facilitates the incorporation of the model ink and are flexible but mechanically resistant which is confirmed by the tensile tests. These observations confirm that 8h of DHT treatment are sufficient to strengthen the gelatin films and make them more robust for our pharmaceutical application.

Given that the development of these relatively complex delivery systems require the use of materials with specific properties, a deep discussion of the physicochemical properties of the thermocrosslinked gelatin matrix is highlighted in this chapter. The achievement of this general objective supposes the resolution of several specific objectives, the most important of which is the optimization of the duration of thermal crosslinking carried out on the excipient in order to obtain films which are insoluble during the dissolution study and flexible for the winding, yet mechanically resistant. Gelatin films were characterized structurally, morphologically, mechanically and thermally using a range of materials characterisation methods (FT-IR spectroscopy ; contact angle and absorption study ; Scanning electron microscopy ; atomic force microscopy ; tensile testing ; gas adsorption (BET) ; X-ray diffraction ; differential scanning calorimetry ; thermogravimetric analysis). The crosslinking degree of the gelatin films was determined by the trinitrobenzensulphonic acid (TNBSa) method.

Chapter 4. Biphasic drug release from rolled-up capsules with a central cavity

In this chapter, rolled-up capsules with a central cavity have been developed for biphasic drug release. This type of capsules provides immediate release and delayed release of the drug.
The proof of concept has been successfully established. Fluorescent inks served as a model for conducting in vitro dissolution studies and gave programmed release kinetics. The release kinetics of fluorescent probes from different positions inside the capsules were fitted to different kinetic models and numerically simulated.

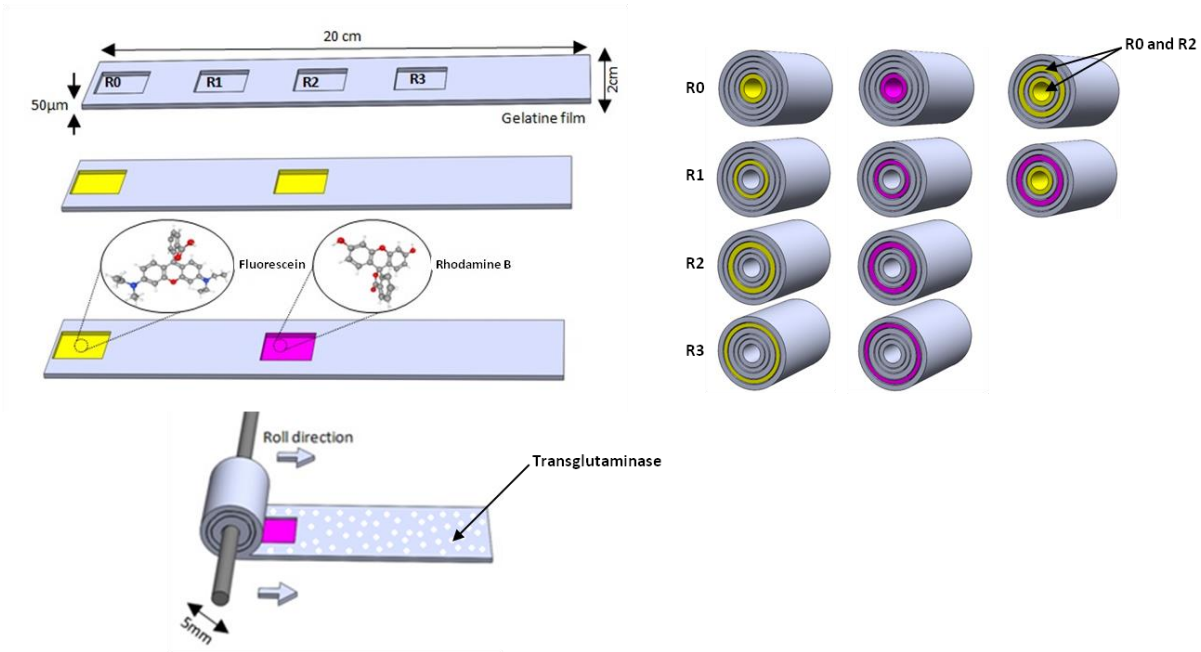


Figure 4-2 Formation of the capsules using the rolling-up approach: Rolling of a gelatin strip with two drug reservoirs around a cylindrical stick.

4-1- Controlled release preliminary study: Proof of concept

The release kinetics of Fluorescein Disodium FD from different radial positions after the immersion of the capsules in the PBS dissolution medium are presented in Figure **4-2**. Diffusion of the drug inside the gelatin matrix and its release from the surface of the capsules are triggered by the penetration of the solvent into the drug reservoir. The experiments were first performed with capsules containing a single reservoir. The release from the reservoir R_0, which is formed on the inner surface of the capsule, starts immediately after the capsule

immersion in the dissolution medium. The release from this reservoir is almost complete within approximately 30 min of dissolution, before the eventual closing of the inner cavity due to the swelling of the capsule. Reservoirs R_1 and R_3 are both separated from the surfaces of the capsule by an average of two layers. Nevertheless, it was systematically observed that the release from R_3, which is closer to the outer surface of the capsule, is faster than the release from R_1, which is closer to the inner surface. After 8 h of dissolution, 95% of the FD is released from reservoir R_0 in comparison to 56% from R_1, 50% from R_2 and 72% from R_3.

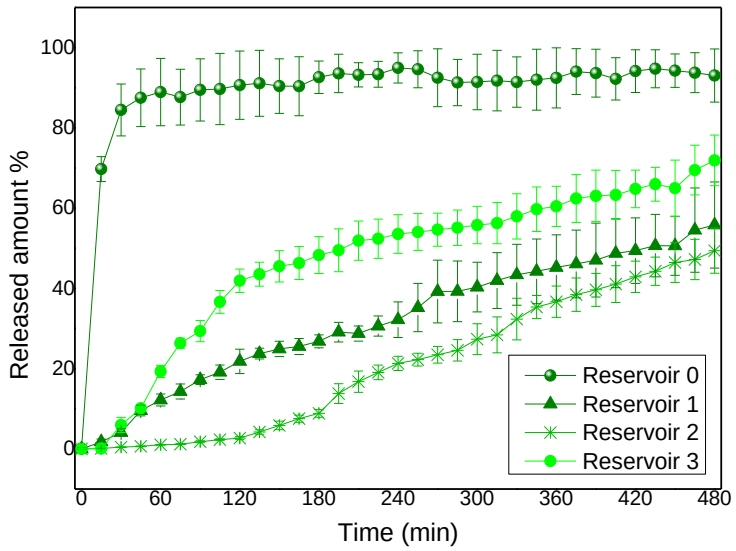


Figure 4-3 The kinetics of FD release from the rolled-up capsules: Monophasic release from a single reservoir formed at different radial positions inside the capsules.

By construction, our system belongs to the class of the reservoir-membrane release systems, and, therefore, the stationary release kinetics is the zero-order one, with the release rate determined by the stationary drug concentration profile inside the capsule (see Section 4-6). But, in the real experiment, the release kinetics are affected by many factors which can hardly be taken into account by a simple theory. Following the common practice, we apply here a formal analysis of the kinetics with the use of the predefined models (zero order kinetics, first order kinetics, the Higuchi model, the Hixon-Crowell model and the Korsmayer-Peppas model), although we are aware that phenomenologically they may not correspond to the system under investigation. Below are given the kinetics for the individual positions of the reservoirs and their fitting by the models.

Position 0 :

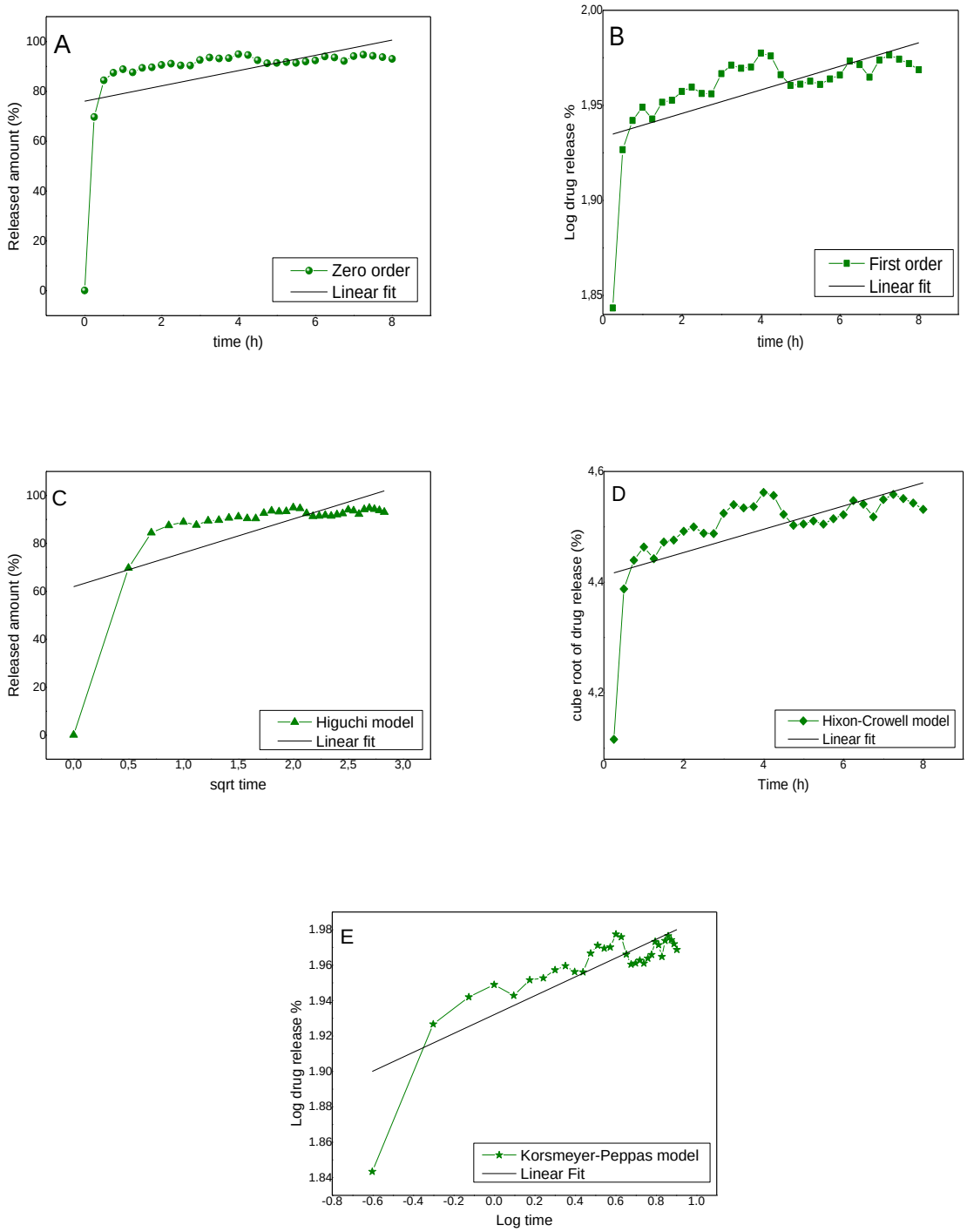


Figure 4-4 Drug release data (position0) fitted to various kinetic models, A) Zero order, B) First order, C) Higuchi model, D)Hixon-Crowell model and E) Korsmeyer-Peppas model

Position 1 :

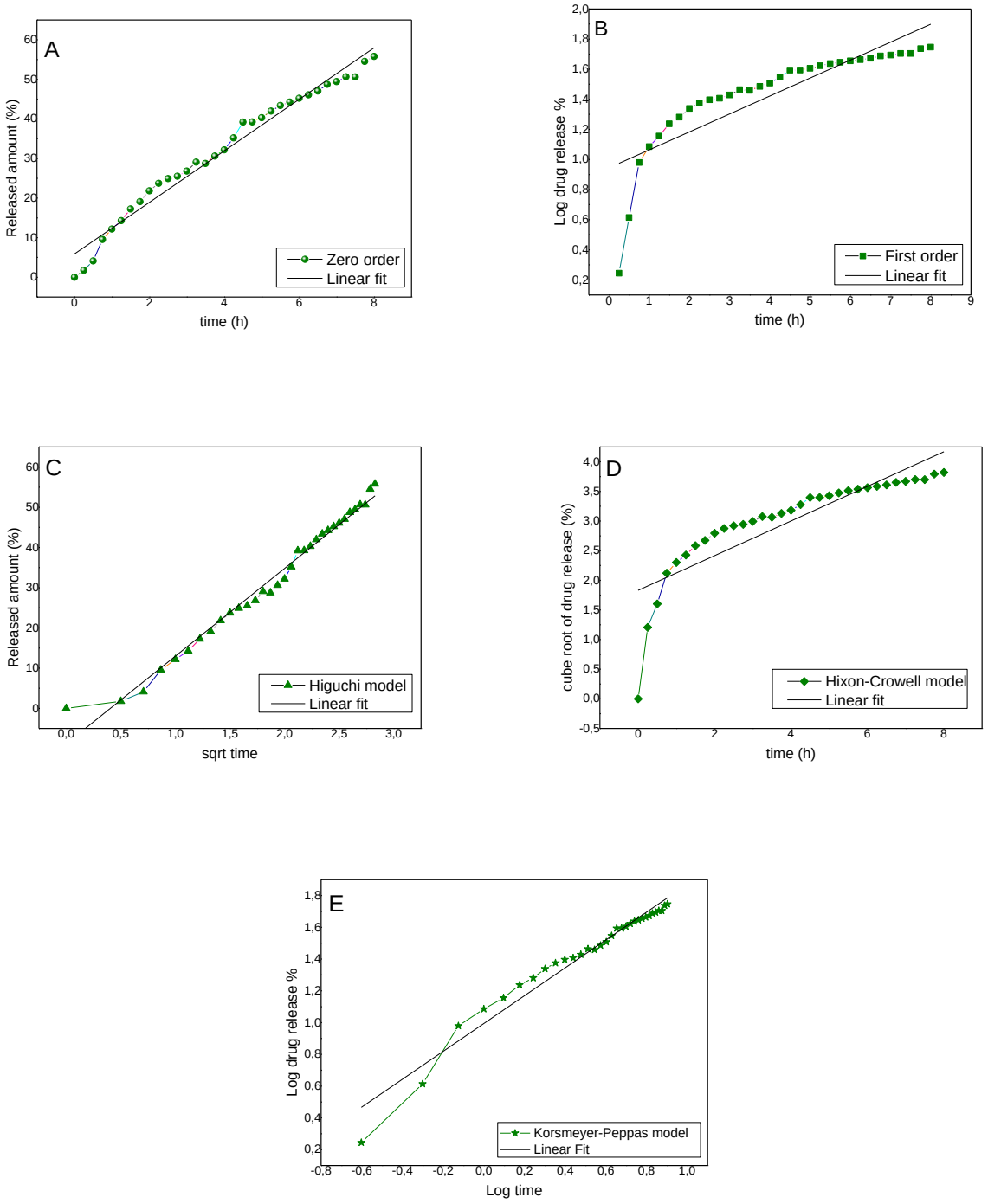


Figure 4-5 Drug release data (position 1) fitted to various kinetic models, A) Zero order, B) First order, C) Higuchi model, D)Hixon-Crowell model and E) Korsmeyer-Peppas model

Position 2 :

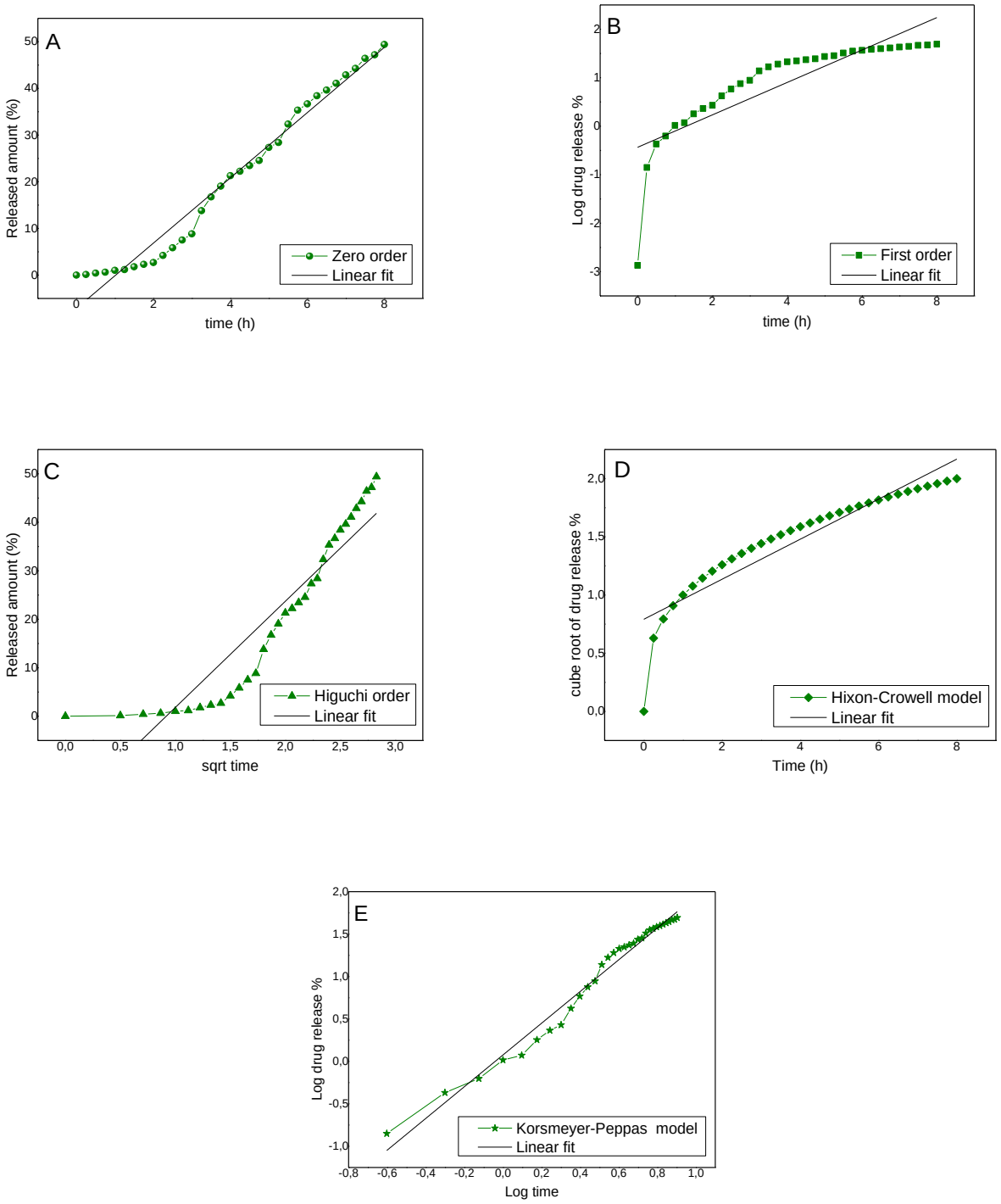


Figure 4-6 Drug release data (position 2) fitted to various kinetic models, A) Zero order, B) First order, C) Higuchi model, D)Hixon-Crowell model and E) Korsmeyer-Peppas model

Position 3 :

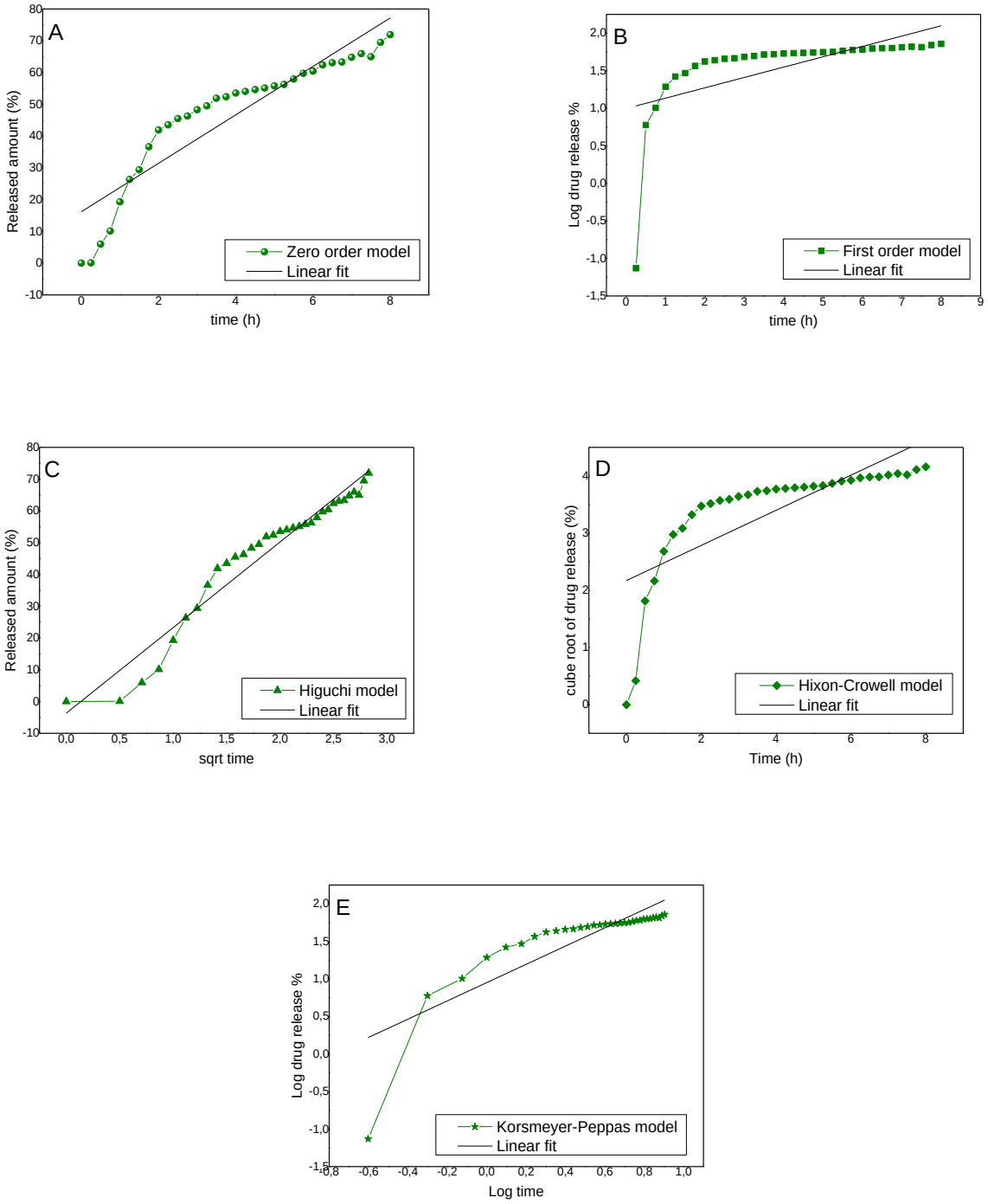


Figure 4-7 Drug release data (position 3) fitted to various kinetic models, A) Zero order, B) First order, C) Higuchi model, D) Hixon-Crowell model and E) Korsmeyer-Peppas model

The criterion for choosing the best model to study the phenomenon of drug dissolution is the use of R-squared (R^2) to assess the fit of a model equation. To characterize the FD dissolution profile from the different positions, the R^2 regression coefficients of each curve were calculated. The standards for a good R^2 reading can be much higher, such as 0.9 or more. An R^2 greater than 0.7 would generally be considered to show a high level of correlation, while a measurement less than 0.4 would show a low correlation. The release curve from position 0 is compatible with the Korsmeyer-Peppas model with an R^2 = 0.67, that of position 1 is compatible with the Higuchi model with an R^2 = 0.98, that of position 2 is compatible with the Peppas model with an R^2 = 0.98 and that of position 3 is compatible with the Higuchi model with an R^2 = 0.95 as shown in table 4-1.

Table 4- 1 DF release data fitted to 5 kinetic models

	Position 0	**Position 1**	**Position 2**	**Position 3**
Zero order	0,17	**0,97**	**0,97**	**0,84**
First order	0,34	0,70	0,69	0,33
Higuchi	0,36	0,98	0,85	0,95
Hixon-Crowell	0,35	0,72	0,84	0,56
Korsmeyer-Peppas	0,67	0,96	0,98	0,68

According to the value of R^2, the model best describing the phenomenon of release from position 0 is that of Peppas. This model describes a prolonged pharmacological action while from position 0, we have a burst release. This model is therefore not the best to take. For the positions 1, 2 and 3, the physically relevant model is the zero-order one, although other models, surprisingly, may fit the release from some positions of the reservoirs. In particular, the Higuchi model (in its version developed for the planar geometry) fits well the release from the position 1, and position 3. Furthermore, the power law may be applied given that the matrix swells and partially dissolves in the long term. For position 2, the Kosmeyer-Peppas model which describes a prolonged pharmacological action responds perfectly to our system.

It should be stressed, that this analysis of the release kinetics has rather formal character. Theoretically, our systems belong to the class of the reservoir-type systems with the membrane-controlled kinetics. Therefore, only the zero-order release, which develops at sufficiently large release times, should be observed. Future simulations, which take into account the swelling

kinetics of the matrix, and possible interaction of the active ingredient with the matrix, will clarify the fact of the better consistency of the Kosmeyer-Peppas and Higuchi models with the experimental data for some positions of the drug reservoirs.

4-2- Biphasic Monodrug release

Biphasic release of FD from reservoirs R_0 and R_2 has been successfully carried out. The dark green curve represents the release kinetics of FD from two reservoirs within the same capsule while the light green curve denotes the superposition of two release kinetics of FD from two reservoirs, each located within a single capsule (Figure 4-6). The curves show a perfect layering and they exhibit a biphasic release profile.

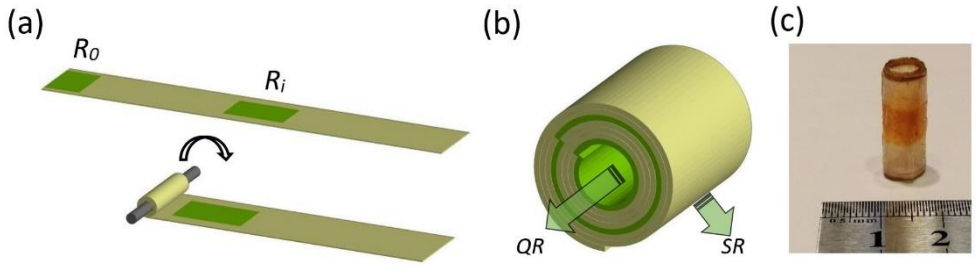


Figure 4-8 Formation of the capsules using the rolling-up approach. (a) Rolling of a gelatin strip with two drug reservoirs around a cylindrical stick. (b) Cross-section of the capsule with two reservoirs. The arrows symbolize the QR from the cavity of the capsule, and SR through the gelatin layers. (c) A photo of a rolled-up cylinder-like capsule.

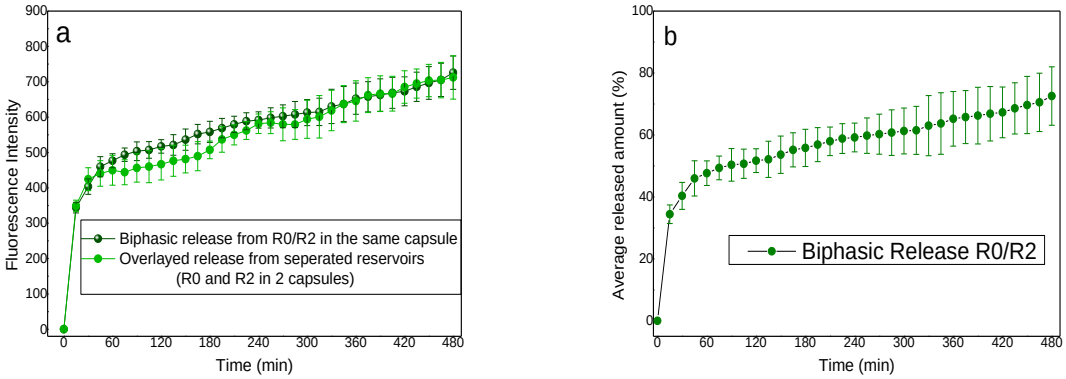


Figure 4-9 Biphasic release of FD from reservoirs R_0 and R_2 ((a) in terms of Fluorescence Intensity, (b) in terms of drug %)

4-2- Biphasic Multidrug release

Different drugs can be loaded in the reservoirs. To demonstrate this feature, we loaded reservoirs R_0 and R_2 with FD and RhB, respectively. Figure 4-9 shows the release profile of FD and RhB from reservoirs R_0 and R_2, respectively. Approximately 70% of FD loaded to R_0 was released during the first hour, while no release of RhB occurred from R_2 during this period. The release of RhB starts with approximately 1.5 h lag time. The release curve of FD was truncated at 2 hours, since the dissolution of RhB, which has a basic nature, might modify the fluorescence intensity of FD due to the change of the portion of energy absorbed by different ionic states and their different quantum yields, which gives the false impression of the continuing release of the dye.

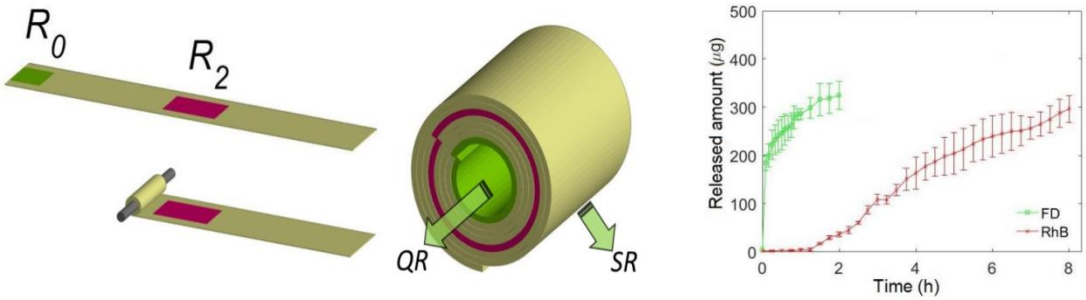


Figure 4-10 Formation of the capsules using the rolling-up approach. (a) Rolling of a gelatin strip with two drug reservoirs around a cylindrical stick. (b) Cross-section of the capsule with two reservoirs. The arrows symbolize the QR from the cavity of the capsule

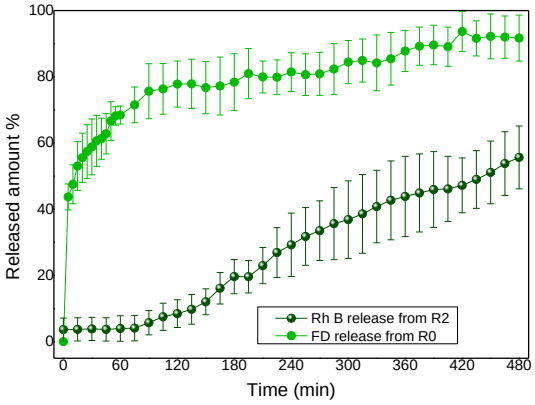


Figure 4-11 Biphasic multidrug release from R_0/R_2

4-3- Comparison between model drugs release kinetics

In this study, we used FD and Rh B not only because they are good tracers but also because they have different characteristics. FD is hydrophilic and RhB is highly lipophilic. Despite their different natures, the ink release kinetics do not show any notable differences, which shows that the interaction between the model ink and the matrix does not vary considerably. In other words, the two model APIs have similar affinities to the pharmaceutical carrier. At the end of the dissolution study, almost all of the amounts introduced are found in the dissolution medium and a very small negligible amount in the matrix reflecting a weak interaction with the matrix. Assigning a specific type of interaction requires further study.

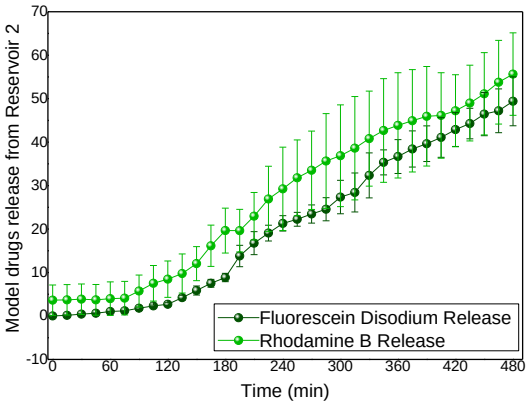


Figure 4-12 Release kinetics from R_2 of two fluorophores

4-4- pH dependence of drug release

In order to cover all the pHs of the digestive medium (intestinal and gastric), dissolution tests have been carried out in 3 pHs from position 2. A sudden increase of the release curve can be observed after 4 hours of dissolution which can be explained by the capsule derolling; Given that Transglutaminase is inactivated at pH = 2, the rolls were derolled, nevertheless, at pH = 4.5, the capsule is maintained in its cylindrical geometric shape and exhibits pseudo first order release kinetics.

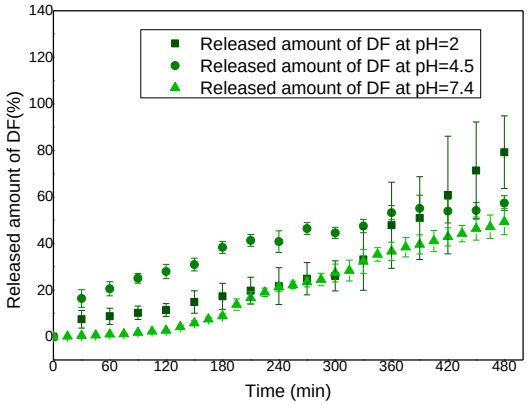


Figure 4-13 Drug release kinetics from R_2 in 3 different pHs

In view of the limited stability of the rolled capsules in the strongly acidic media, they might be more suitable for biphasic drug release in the small intestine, rather than in the stomach. The rolls will be encased in the gastro-resistant capsules, e.g., on the base of Eudragit®L100-55, which is dissolved above pH 5.5 and designed for drug release in the mid to upper small intestine. A good example of such a biphasic release in the small intestine is the release of diclofenac sodium, which belongs to the class of the nonsteroidal anti-inflammatory drugs (NSAIDs) [57]. However, if the stability issues of the rolled up gelatin capsules in the acidic media are resolved in the future, it would be interesting to consider them as a biphasic release gastroretentive dosage form. This application will be favored by the fact that the capsules swell strongly in the highly acidic media. Therefore, the capsules may be designed in such a way that they will be swallowable, but will be retained from passage through the pylorus due to swelling in the gastric juice.

4-5- Computer simulation of drug release from the capsule with cavity and a reservoir embedded between the shells of the roll

An additional insight in the process of the drug release from the rolled-up capsules with the central cavity can be gained by computer simulation (courtesy of V. Luchnikov). The external radius of the capsule is *b*. The internal radius (= the radius of the cavity) is *a*. Cylindrical infinitely thin drug reservoir is embedded inside the capsule at the radial position ρ, $a<\rho< b$

For the sake of simplicity, and in order to enable the analytical soultion of the diffusion equation, we consider the idealized situation, in which it is supposed that

(a) The characteristic time of the capsule's swelling, $t_{sw}$is much less than the characteristic diffusion time, $\tau \sim (b-\rho)^2/D \sim (a-\rho)^2/D$, where $D$is the diffusion coefficient.
(b) The drug is not interacting with the matrix.
(c) The diffusion coefficient is independent of the drug concentration, as well as of the radial, angular and axial position ($D = const$).
(d) The interface of the capsule with the solution can be considered as the perfect sink, because the concentration of the drug in the solution is supposed to be much smaller than the concentration inside the capsule.

It is also assumed that the amount $M$of the model drug is distributed uniformly over the infinitely thin cylindrical layer, at the distance ρ from the capsule's axis.

Under these assumptions, the drug release from the capsule can be simulated using the linear diffusion equation in the cylindrical coordinates:

$$\frac{\partial C}{\partial t} = D\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial C}{\partial r}\right) \quad \textbf{(8)}$$

under the « perfect sink » boundary conditions :

$$C(r,t)|_{r=a} = 0\ ;\ \ C(r,t)|_{r=b} = 0 \quad \textbf{(9)}$$

At the reservoir position $r = \rho$, concentration isequal to the saturation concentration in the gel,C_s, therefore, at this position the boundary condition reads :

$$C(r,t)|_{r=\rho} = C_s \quad \textbf{(10)}$$

The condition (3) holds until the moment τ, at which the drug powder is completely dissolved.

Simulation was done in the arbitrary units, for $D = 1$, $a = 0.1$, $b = 1$, and $M = 5$.
Concentration profile $C(r,t)$was calculated in the Matlab package written on the basis of the ref [172], in which the analytical solution is given in the form of the Fourier series. The drug flux from the surface of the capsule is then calculated as

$$F(t) = F_a(t) + F_b(t) = 2\pi aLD\left.\frac{\partial C(r,t)}{\partial r}\right|_{r=a} - 2\pi bLD\left.\frac{\partial C(r,t)}{\partial r}\right|_{r=b} \quad \textbf{(11)}$$

Where$L = 1$ is the length of the capsule. The drug amount released to the time t is found as

$$\Phi(t) = \int_0^t F(\tilde{t})d\tilde{t} \quad \textbf{(12)}$$

An example of the evolution of the concentration profile is shown on the Figure 4-13. The corresponding flux and the total amount of the released drug are shown on the Figure 4-14 and Figure 4-15, respectively.

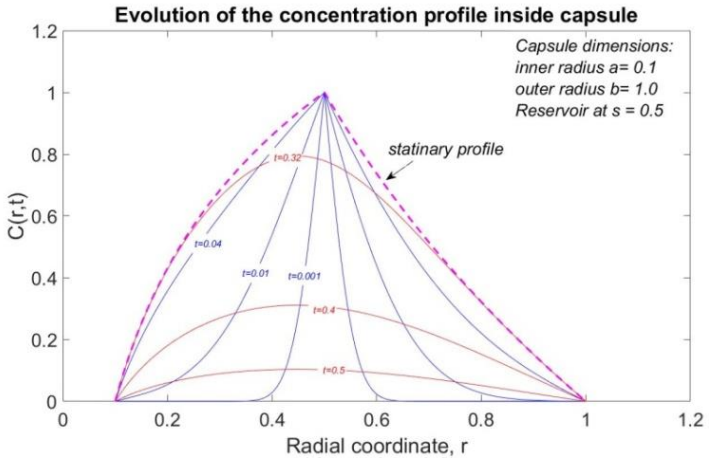


Figure 4-14 Concentration profiles of a drug in the cylindrical capsule with the holeat different times before (blue lines) and after (red lines) the moment of dissolution of the drug powder in the reservoir. Stationary concentration profile is reached before the drug is completely dissolved in the reservoir.

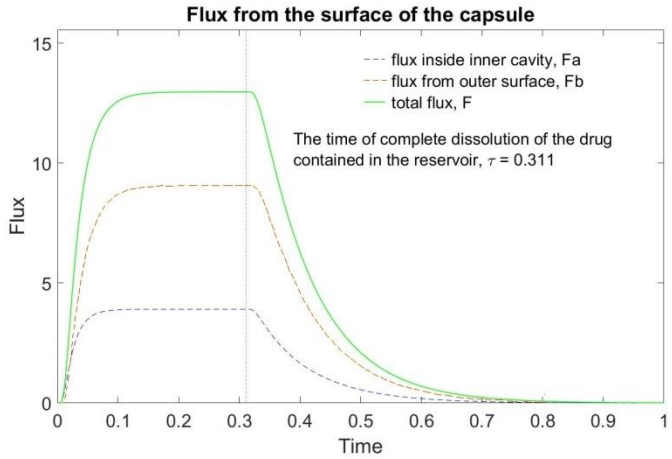


Figure 4- 15 The fluxes from the inner and the outer surfaces of the capsule, and the total flux. The moment of the complete dissolution of the drugpowder in the reservoir is marked by the vertical dashed line.

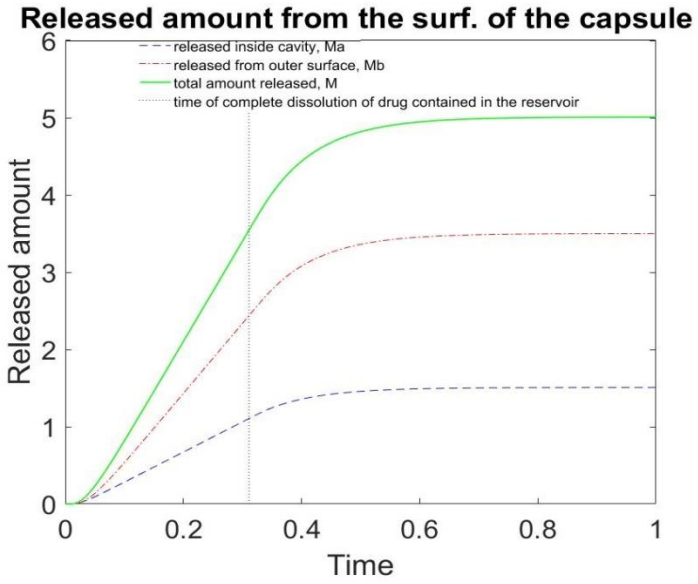


Figure 4-16 Released amount from the inner surface, outer surface, and the total released amount. The dashed line marks the moment, at which the drug powder in the reservoir is completely dissolved.

According to the simulations, the drug concentration profiles and the respective release kinetics strongly depend on the radial position of the drug reservoir inside the capsule. Figure 4-16-a shows the stationary concentration profiles for the capsule with the reservoir at the middle position between the outer and inner surfaces (blue line), and the stationary profile for the case of the reservoir shifted closer to the outer surface of the capsule (pink line). The respective release profiles (with the same color code) are shown on the Figure 4-16-b. The gain in the release rate through the outer surface prevails the loss of the release rate through the inner surface, so that the net resultis the acceleration of the overall release rate. This scenario corresponds, on the qualitative level, to the experimentally observed tendency.

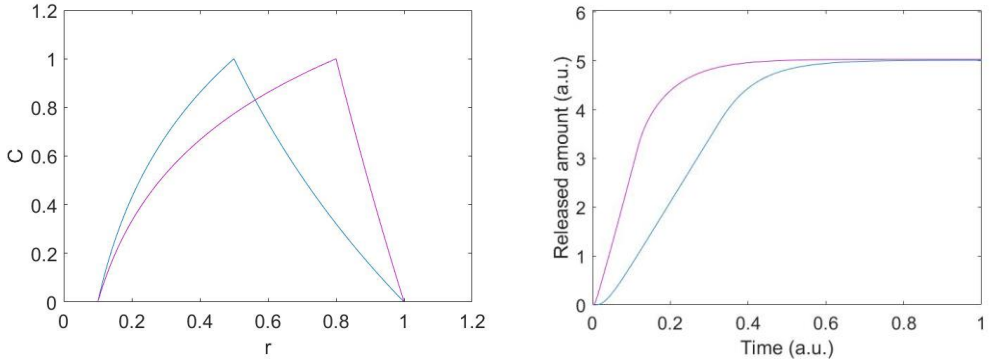


Figure 4- 17 a) Stationary concentration profiles for two positions of the reservoir in the capsules, b) The release kinetics for the tworeservoir positions.

Conclusion

The proof of concept sought during our study has been successfully established: Rolled capsules with a central cavity have been designed. Fluorescent inks served as a model for conducting in vitro dissolution studies and gave programmed release kinetics. In particular, this type of capsules allowed a biphasic release: An immediate release through the central cavity and a delayed release by incorporating the second reservoir in a precise predefined position. In this section, we also showcased a biphasic multidrug release by incorporating 2 different model drugs: one was lipophilic and the other was hydrophilic. On the other hand, it turned out that the pH of the medium impacts the stability of the capsules and consequently the release kinetics of the model drugs which are pH-dependent in nature. The release kinetics of the fluorescent inks from the different radial positions were fitted to different kinetic models and simulated numerically.

Chapter 5. Programmed drug release from the capsules formed by rolling up self-adhesive flexible gelatin films

The system presented in the previous chapter requires stabilization in the rolled-up state by transglutaminase “glue”. This represents significant inconvenience, because distributing the Tgase glue over the stripe after the reservoirs formation should be extremely precise, in order to not smash the reservoirs. This operation can hardly be automatized. Another significant drawback of the system discussed in the previous chapter is the rigidity and fragility of the thermally crosslinked gelatine strip.

An “ideal” material for the rolled-up capsules would be a water-permeable biocompatible polymer stripe, which has 3 important properties:

- ✓ The stripes should be stable above the temperature of human body and in the range of pH corresponding to the human intestine;
- ✓ The stripes should be flexible, to enable easy rolling up with high curvature.
- ✓ The stripes should be self-sticky, and should not deroll at the physiological conditions.

In the present chapter, we present a system whose properties approach these requirements.The treatment undergone by gelatin films consists in combining the effect of plasticizers with the effect of cross-linking agents. To begin with several natural-based and/or biodegradable plasticizers have been used in biopolymer-based films during the last decades [173]. More particularly, for gelatin films, the use of plasticizers was widely reported in the literature. These plasticisers affect the functional properties of gelatin-based film. Their incorporation into gelatin films makes it a continuous matrix and gives it a reinforced structure, flexibility and reduced barrier properties [174], [175]. Among the compounds known by their plasticizing power, mention may be made of **sucrose**, **oleic acid**, **citric acid**, **tartaric acid**, **malic acid**, **sorbitol and mannitol**. The **ethanolamine** compounds **(EA, DEA, TEA)** in turn could improve flexibility of gelatin films. **Polyethylene glycol** with different molecular weights **(EG, DEG, TEG)** also has different effects on gelatin film which implies that the molecular weight of the plasticizer represents a factor to be taken into consideration when choosing the plasticizer. The effect of the plasticizer also varies depending on the nature of the gelatin film in which it is incorporated. For example, following the incorporation of **glycerol**, the stiffness of rabbit gelatin films is slightly lower than that of pork gelatin films, but the flexibility was greater [176]. Other factors may affect the ability to plasticize the gelatin films namely the number and positions of hydroxyl groups, which obviously depend

on the type of a plasticizer, and its concentration. Concerning the molecular mechanisms which control functionality in gelatin films, hydrogen-bonding molecules between water and glycerol call for more exploration [173].

On another side, as we have seen previously, the enzyme transglutaminase (TGase) used as a cross-linking agent can reduce the interaction of gelatin films with water, thus decreasing their solubility while improving their properties. An interesting study showed the increased resistance of gelatin films to biodegradation compared to films without the enzyme. Indeed, the addition of the enzyme promotes the cross-linking of the polymer matrix, maintaining the integrity of the films for longer [177].

Furthermore, a study investigated the combined effect of plasticization and cross-linking on the morphological and mechanical properties of gelatin films. Researchs showed that gelatin films plasticized with different glycerol contents were cross-linked using transglutaminase (TGase). This study showed that the degree of crosslinking of the films decreases linearly with the increase in the glycerol content, which increases the solubility in water. Indeed, glycerol increases the mobility and free volume of the gelatin film matrix [178]. In the next section, we will study the effect of the combination of sorbitol with Transglutaminase on some properties of gelatin films.

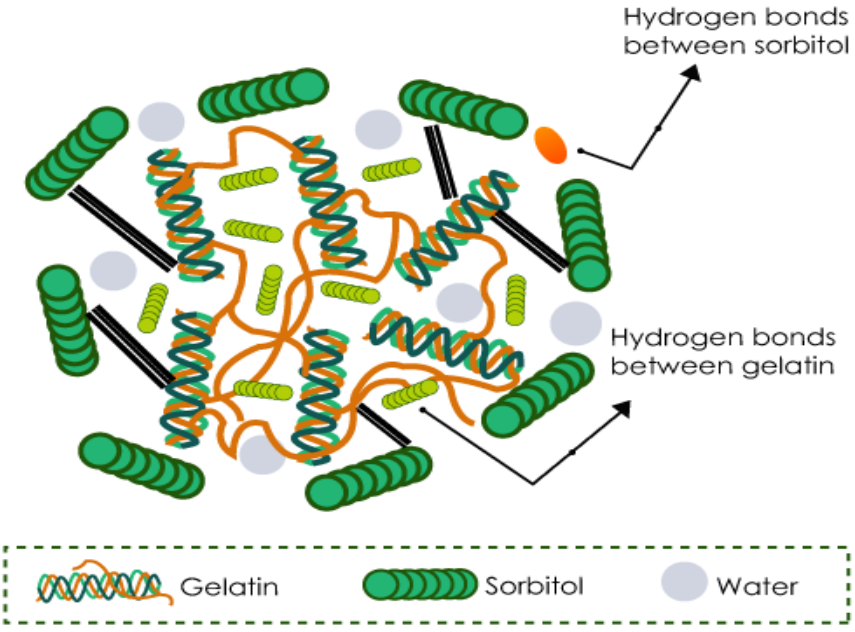


Figure 5- 1 Schematic illustration of the interaction between gelatin and sorbitol

5-1- Matrix characterization

The properties of the matrix treated with Tgase and Sorbitol were studued. The fabrication protocol for these films has been described in detail in Section **2-3.** The influence of the order of introduction of the Tgase and the sorbitol is studied as well.

5.1.1. AFM

Using atomic force microscopy, we evaluated the topography of the different films synthesized in this chapter in order to study the effect of plasticizer addition as well as the order of its addition to the formulation, on the average film roughness. Results show that the surfaces of all the films were smooth except Gel-Tgase/Sor films which was relatively rough.

Indeed, for Gel-Tgase/Sor films, crosslinking occurs after less than 5 minutes after the addition of Tgase to the gelatin solution. As a 3D network forms, the Sorbitol added later cannot be incorporated into the formulation but the agitation makes the formulation cloudy (non-homogeneous formulation) and therefore gives a macroscopically heterogeneous film with a relatively rough surface.

Further characterization indicates that Young Modulus of the Gel-Tgase/Sor films have a modulus equal to 1.1 GPa. The reference Gel/Tgase films have an elastic modulus about 0.6 GPa. Concerning Gel-Sor/Tgase films, they have an elastic modulus of 0.7 GPa which is slightly higher than the reference Young's modulus (Figure 5-2). In conclusion, the plasticizer in the used proportions does not affect the roughness of the films but very slightly increases its elasticity. On the other hand, it is very important to add the sorbitol before the crosslinking reaction takes place.

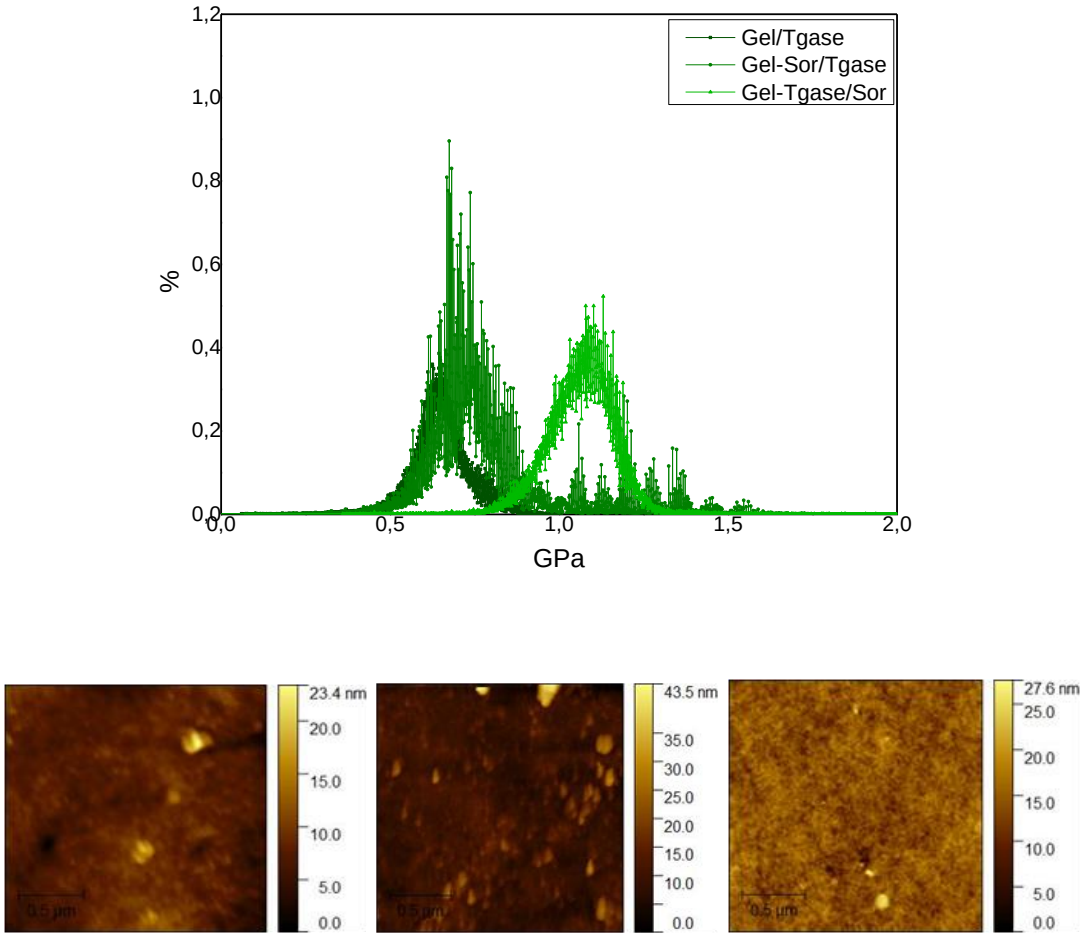


Figure 5-2 AFM images and Young's Modulus

5.1.2. Tensile testing

The macroscopic mechanical properties of the films have been studied. The results show that Gel-Sorbitol-Tgase films exhibit ductile properties as well as Gel-Tgase-Sorbitol films with much lower Young's modulus and similar elongation.The order of addition of this plasticizer therefore affected the modulus of elasticity without significantly varying the elongation of the films. For comparative purpose, an additional study was performed with the Gel-Tgase-Glycerol films. Both films (with Sor and with Gly) show good homogeneity and excellent acceptance of Gly and Sor, with no pores or cracks, which represents a desired property for controlled diffusion. Mechanical tests showed that Gel-Tgase-Glycerol films behave as elastomers with a very important elongation about 160% and especially a much reduced modulus of elasticity. This shows that the nature of the plasticizer in the same proportions has a significant impact on the mechanical properties.

Table 5- 1 Tensile testing results

	Young's Modulus **YM MPa**	Stress max **Fmax/A (MPa)**	Ultimate Strength **(N)**	Elongation **dL %**
Gel-Sor-Tgase	700	47,5	38	44
Gel-Tgase-Sor	326	30	36	57
Gel-Tgase-Gly	5,7	9,59	5,5	164

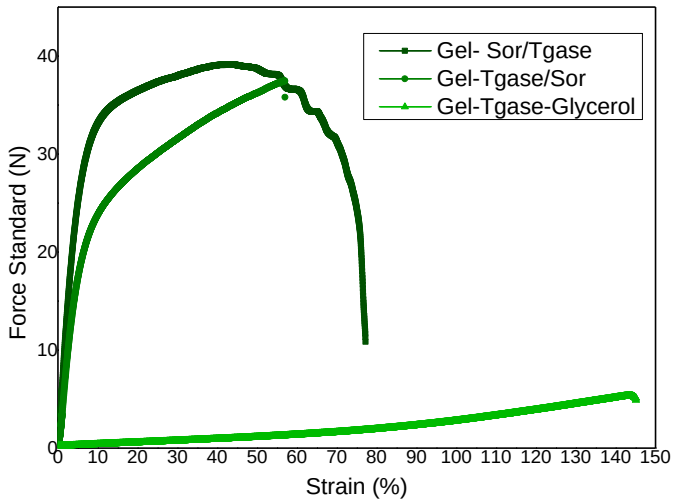


Figure 5- 3 Tensile testing of gelatine films modified with Tgase and sorbitol or glycerol

Although glycerol was not used in our study, the enormous difference in the mechanical behavior of enzymatically crosslinked films by changing the nature of the plasticizer was demonstrated. In literature, a study showed that Gly and Sor have differences when used separately in a rice starch film: Gly has lower tension but a higher elongation. Nevertheless, variations in Young's modulus increase for both plasticizers [174]. So the two plasticizers give the films reduced tensile strength behavior which makes the polymer matrix less dense. As a result, the movement of the polymer chains is easier imparting flexibility or ductility [179]. On another side, adding appropriate amount of sorbitol can facilitate the formation of the triple helix-like structure of gelatin and improve the properties of gelatin gel [180]. It is also possible to mix plasticizers to avoid certain problems related to the use of a single plasticizer. By way of example, films plasticized with mixtures of glycerol and sorbitol showed water vapor permeability, mechanical and viscoelastic properties intermediate to films plasticized only with glycerol or sorbitol [181].

5.1.3. Contact Angle

The water contact angle values were measured 30 seconds after the deposition of the 3 µL drop. Gelatin is hydrophilic in nature; however the contact angle measured on the films crosslinked with transglutaminase (Gel-Tgase) is 118 ± 2°. Indeed, film surface wettability is not only related to the surface chemical properties, but also affected by the film surface microstructure, namely roughness, porosity and pore size. In other words, this measurement does not mean that the crosslinked film is hydrophobic since the dissolution study showed successfully model drug diffusion and release, but that the surface is in a static mode. On the other hand, the plasticizer did not impact the wettability of the films since the contact angle of Gel-Sor/Tgase films is 120 ±2°. On the other hand, Gel-Tgase/Sor has a contact angle of 128 ±2°. Since these films are the roughest, such a result is in agreement with AFM results discussed in the previous section. Unfortunately, there is different data of contact angles on gelatin films. For example, for the untreated ones, the contact angle is equal to ~82° according to [182] but equal to 113.7 ± 4.1 [104]. Therefore, it is impossible to compare our data with those of other authors. These differences of contact angle values is probably caused by possible electrostatic interactions, or more probably by differences in the change of the gelatin hydration state or its molecules conformations upon adsorption [183].

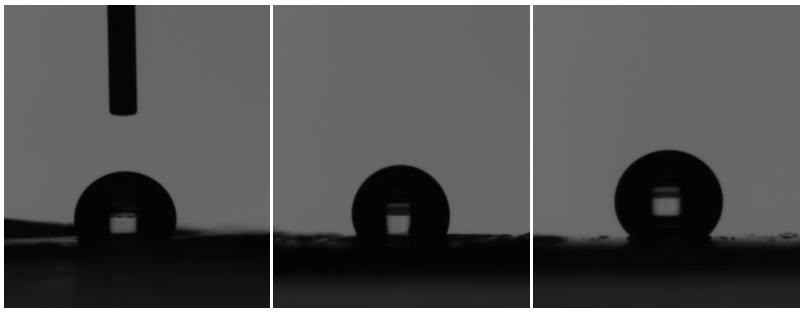


Figure 5-4 Contact angle of matrices a) Gelatin with Tgase (Gel-Tgase), b) Gelatin with Sorbitol and Tgase (Gel-Sor/Tgase), c) Gelatin with Tgase and Sorbitol (Gel-Tgase/Sor)

5-1- Fabrication of the rolled up capsules without cavityby rolling up self-adhesive flexible gelatin films

The films were cut into strips (8 cm long and 2 cm wide, **See section 2-3**). Drug reservoirs were formed by spreading solutions of Fluorescein Disodium over the surface of a gelatin strip at areas corresponding to the prescribed radial positions of the reservoirs inside the capsule after the rolling. Next, 20 µL of a solution per reservoir was spread using a

micropipette. The concentration of the solution was 25 µg/µL, corresponding to a content of 500 µg within each reservoir.The coordinates of the boundaries with respect to the end of the gelatin strip, from which the rolling started, are given in Table 5-2.

Table 5- 2 The lateral coordinates of the reservoirs before rolling.

Reservoir	**Turn**	**x_1 [mm]**	**x_2[mm]**
R_1	2	20	30
R_2	4	35	47
R_3	6	60	74

5-2- Drug release study

The release kinetics of FD from 3 different positions after the immersion of the capsules in the PBS dissolution medium are presented in Figure 5-5. The experiments were performed with capsules containing a single reservoir. After 8 h of dissolution, less than 30 % of the FD is released from reservoir R_1 with a delay of 4 h, in comparison to 70% from R_2 and 80 % from R3. Indeed, the release from the reservoir R_3, which is close to the outer surface of the capsule, starts immediately after the capsule immersion in the dissolution medium. Nevertheless, after 8 h of dissolution, 20% of the model drug remains retained in the matrix. The release from R_2 was slower than the release from R_3 and reached 70% after 8 h. The release kinetic curve has a linear shape which looks like a zero-order release. This assumption will be explored in the next section. In conclusion, controlled release was successfully highlighted with this integrated, insoluble, flexible and self-adhesive system.

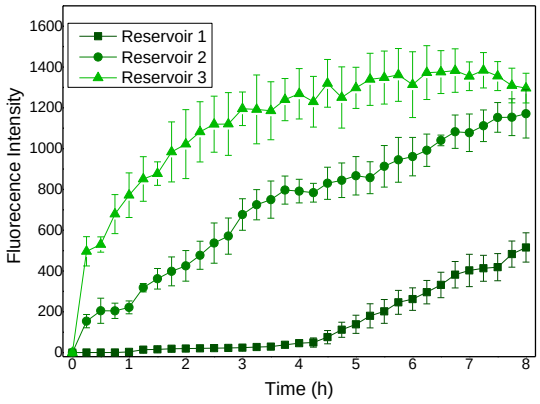
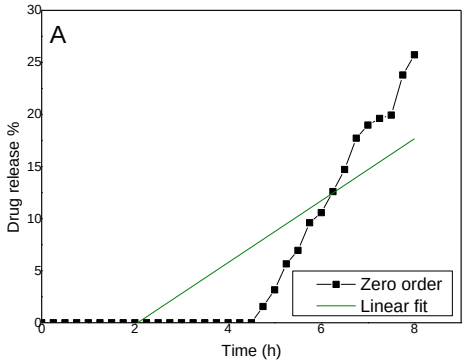


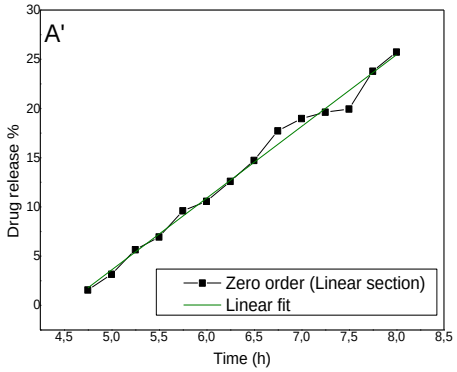
Figure 5- 5 Release kinetics from different reservoir positions

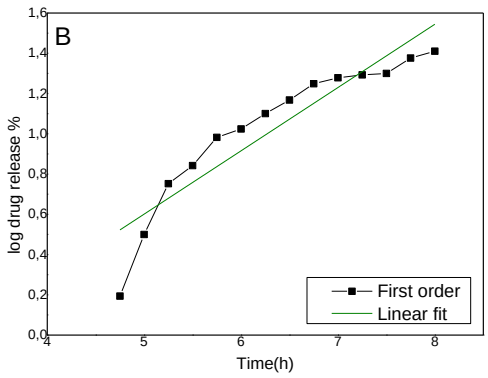
5-3- Kinetic Models

As done previously, we have performed the formal analysis of the experimental release kinetics by fitting them with the use of the best known mathematical models (zero order kinetics, first order kinetics, the Higuchi model, the Hixon-Crowell model and the Korsmayer-Peppas model).

- **Position 1**







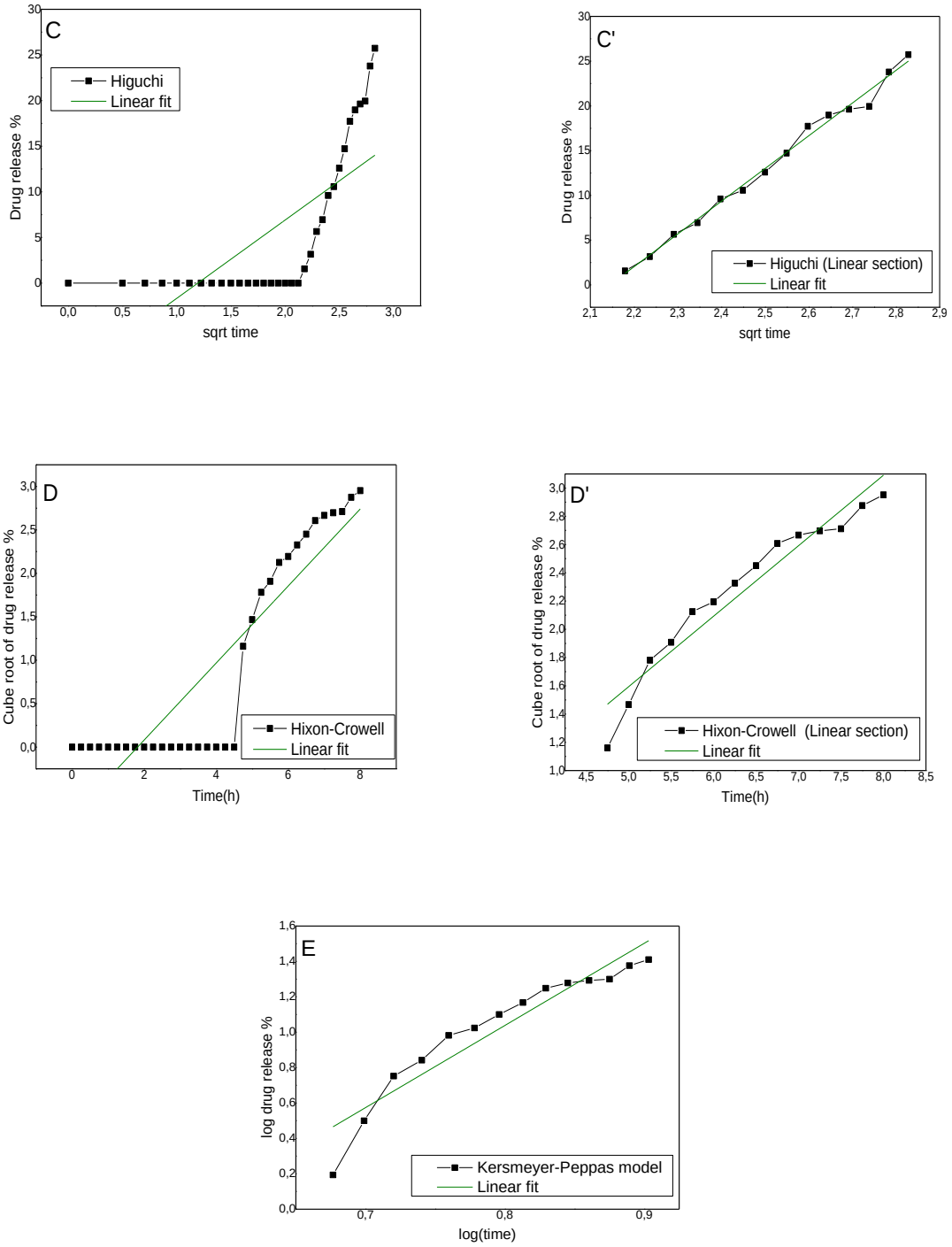


Figure 5-6 Drug release data (position 1) fitted to various kinetic models, A-A') Zero order, B) First order, C-C') Higuchi model, D-D') Hixon-Crowell model and E) Korsmeyer-Peppas model.

- **Position 2**

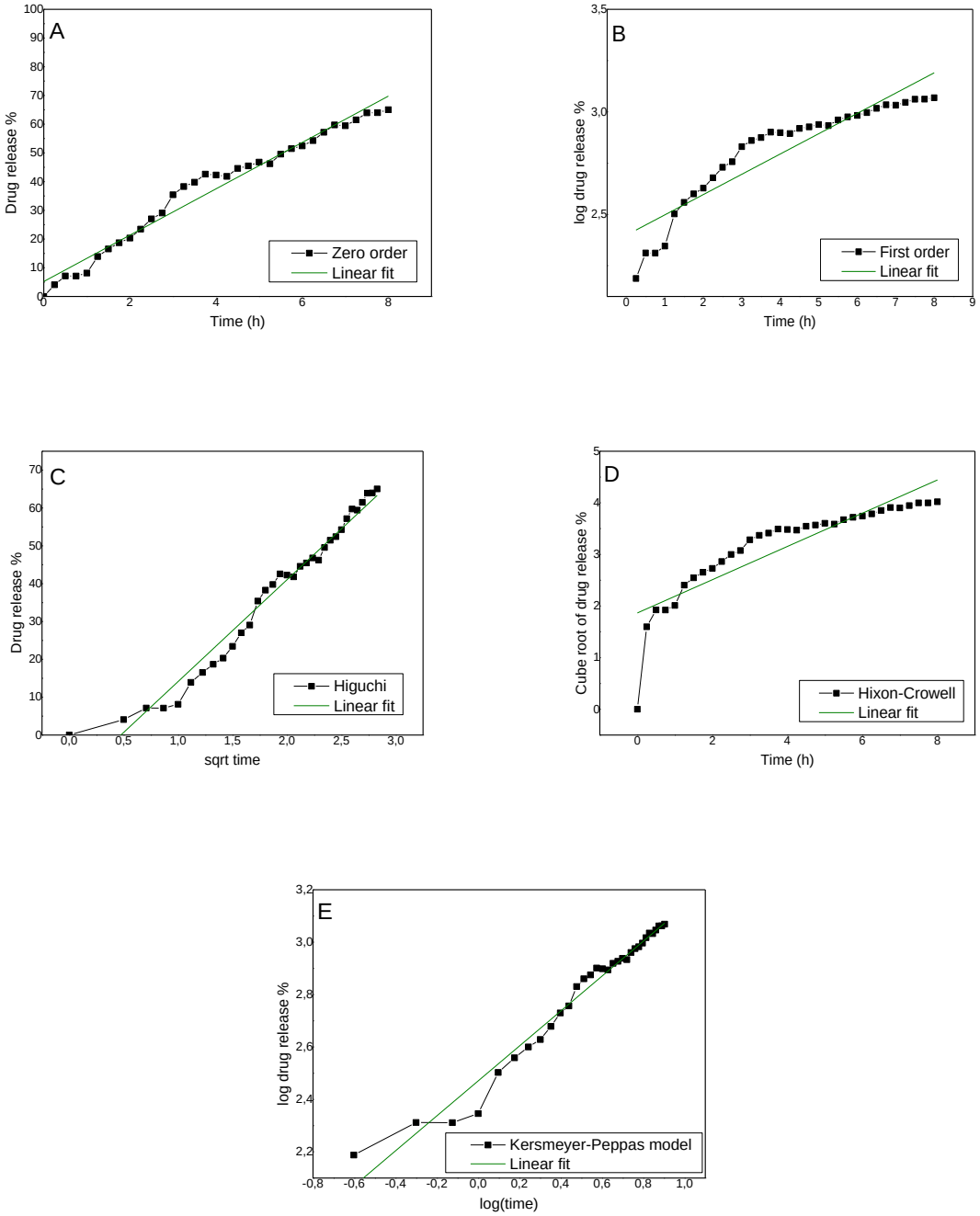


Figure 5-7 Drug release data (position 2) fitted to various kinetic models, A) Zero order, B) First order, C) Higuchi model, D) Hixon-Crowell model and E) Korsmeyer-Peppas model.

- **Position 3**

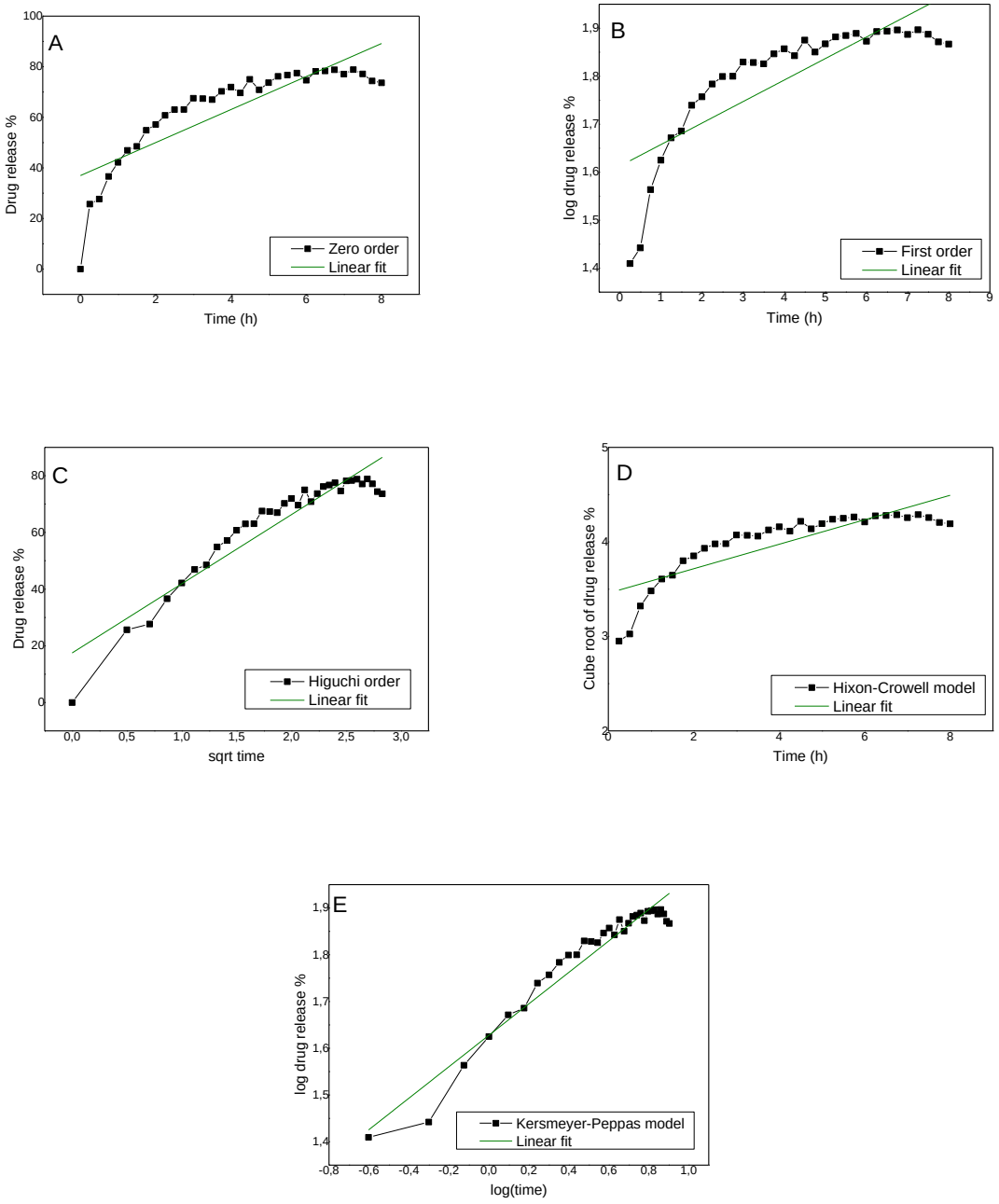


Figure 5-8 Drug release data (position 3) fitted to various kinetic models, A) Zero order, B) First order, C) Higuchi model, D) Hixon-Crowell model and E) Korsmeyer-Peppas model

Table 5-3 DF release data fitted to 5 kinetic models

R^2	Zero order		First order	Higuchi		Hixon		Peppas
Pos 1	0,72	0,99	0,85	0,53	**0,99**	0,79	0,93	0,89
Pos 2	0,97		0,85	0,97		0,76		**0,97**
Pos 3	0,70		0,66	0,89		0,69		**0,94**

Taking into consideration the curve when the release begins, i.e. 4 hours after immersion of the capsule in the dissolution medium, the release curve from position 1 is compatible with the Higuchi model with an $R^2 = 0.99$. Position 2 drug release kinetic may be compatible with Peppas model which describes a prolonged pharmacological action with an $R^2 = 0.97$ as well as that of position 3 with an $R^2 = 0.94$. In order to further develop this theory, a simulation study under these conditions will complete our dissolution study.

As already stressed above, this analysis of the release kinetics has rather formal character, and since theoretically, our systems belong to the class of the reservoir-type systems with the membrane-controlled kinetics. Advanced models, which will take into account the swelling kinetics of the matrix, and the interaction of the active ingredient with the matrix, are necessary for the correct simulations of the release kinetics.

5-4- Computer simulation of drug release from the capsule without cavity and a reservoir embedded between the shells of the roll

In this subsection, we simulate the hypothetical distribution of the drug inside the capsules, under the same assumtions as done in the Subsection 4-6, which enable the analytical solution of the diffusion equation. As above, these simulations, made in arbitrary units, have only illustrative character, and do not pretend to reproduce the experimental kinetics. The results obtained above for the capsules with a cavity are applied here to the capsules without cavity by aspiring the radius of the cavity to zero, $a \to 0$. Cylindrical infinitely thin drug reservoir is embedded inside the capsule at the radial position ρ, $0<\rho< b=1$.

Let us consider the stationary profiles canbefound by resolving the stationary diffusion equation

$$\frac{d}{dr}\left(r\frac{dC}{dr}\right)=0 \qquad \textbf{(13)}$$

whichfollowsfrom the equation (1) by substituting$\frac{\partial C}{\partial t} = 0$. The boundary conditions for the region$\rho \leq r \leq b$read

$$C(r,t)|_{r=\rho} = C_s \; ; \quad C(r,t)|_{r=b} = 0 \tag{14}$$

($C_s$is the saturation concentration of the drug in the capsule's matrix material). (

The solution of (7) reads

$$C(r) = \begin{cases} C_s, 0 \leq r \leq \rho \\ C_s \frac{\ln(r/b)}{\ln\rho/b)}, \rho \leq r \leq b \end{cases} \tag{15}$$

The stationary concentration profiles for two positions of the drug reservoir, $\rho = 0.2$ and $\rho = 0.8$ are shown on the Figure 5-9-a. The respective drug release kinetics (for the stationary and the non-stationary phases) are shown on the Figure 5-9-b.

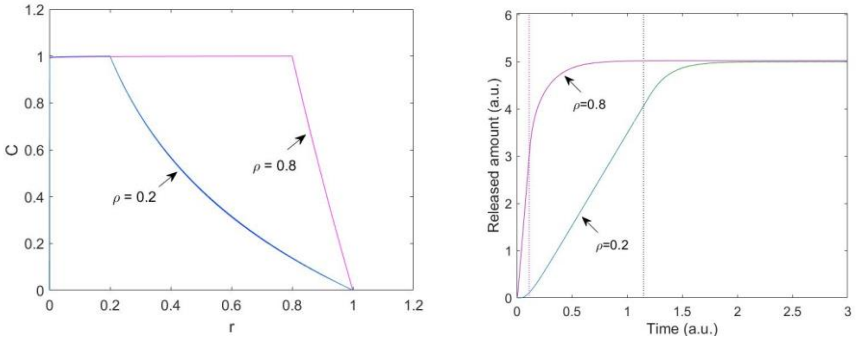


Figure 5-9 a) Stationary concentration profiles for the capsule without the central cavity, for two radial positions of the drug reservoir, b) Release kinetics for the two positions of the drug reservoirs. The vertical dashed lines mark the times of the complete drug dissolution in the reservoirs.

The future simulations of the real capsules and the real release profiles will take into account many factors, such as kinetics of the capsules swelling, the dependence of the diffusion coefficient on the degree of swelling and on the drug concentration, and possible interaction of the drug with the matrix. Since the analytical solution of the diffusion equation under these conditions is impossible, special numerical methods need to be implemented.

Conclusion

First, we studied the properties of the matrix which consists of gelatin films modified by a Transglutaminase and Sorbitol. Combining the effect of plasticizers with the effect of cross-linking agents gives flexible films that are easy to roll up so that capsules can be formed with no central cavity. The capsules were stable and insoluble under physiological conditions, flexible but mechanically resistant and self-adhesive in the dissolution medium. This type of capsules allowed a controlled release from 3 different positions, one of which ensured a delay of 4 hours which can be interesting to exploit for certain APIs. The release kinetics of the fluorescent inks from the different radial positions were fitted to different kinetic models and simulated numerically.

Compared to the first system which represents a simple and effective concept to ensure a biphasic and multidrug release thanks to the central hole which allows an immediate release, the second system allows a delayed and controlled release according to the position of the reservoir with the possibility of incorporating more than one model drug.

As for their mechanical properties, which are those of the films, the thermally crosslinked capsules have a considerably higher modulus of elasticity than that of the enzymatically crosslinked and plasticized system.

The use of transglutaminase in the two systems was not the same. It was used as a glue in the first system and as a crosslinking agent in the second. As for its mode of action, it is rather a surface phenomenon that occurs to maintain the consecutive layers in a rolled-up geometry while in the film forming solution of the second system, it catalyzes a chemical reaction of lysine crosslinking with glutamine residues.

To conclude, the two manufacturing approaches for rolled-up capsules studied in chapters 4 and 5 have shown their effectiveness in drug release control. Without or with cavity, the capsules have made it possible to program different release kinetics, in particular burst, delayed and extended releases.

Chapter 6. Conclusion and Outlook

In the framework of this thesis, rolled up capsules for a programmable controlled drug release have been designed. The release kinetics are determined by the radial position of the reservoirs inside the capsules, while the radial positions of the reservoirs are set by the position of the reservoirs on the gelatin stripes before rolling. This approach allows unprecedented possibilities for the design of the capsules for the mono-or multidrug personnalised chronotherapies.

In order to demonstrate the potential of the approach, we have designed the capsules for the biphasic drug release, which constitutes and important case of the chronomodulated therapies. The biphasic kinetics was achieved due to specific geometry of the capsules, rather than due to the different matrix disintegration rates, which is the method used in traditional bilayer tablet biphasic release systems. The capsules were formed by rolling up thermally crosslinked gelatin strips, on the surface of which different reservoirs were distributed and loaded with FD as the model drug. The capsules were maintained in the rolled state using transglutaminase. Quick release was effectuated from the inner surface of a cylinder-like capsule during the first minutes of immersion in the dissolution media. Sustained release was achieved via embedding a drug reservoir between the layers of the rolls. Moreover, the design of the capsule was suitable for the dual-drug release in a chronomodulated manner, as demonstrated by the experiments with capsules containing FD and RhB in the QR and SR reservoirs, respectively. This concept, which is based on a single polymer, can be implemented on a large scale. Indeed, the incorporation of more than one active principle in the formulation is desirable, as this increases patient compliance and reduces the cost of treatment, in particular when distinct dosages of active principles can be adjusted individually in situ, to meet the specific needs of each patient.

The rolled-up technique was further improved to produce gelatin stripes of improved flexibility, achieved by adding plastisizer to the matrix. Moreover, the transglutaminase-catalysed crosslinking, in synergy with the plasticizer, has provided the films self-adhesiveness, which is a very useful property for the rolls stabilization. With the use of these new films, we have designed a second drug release system, and explored the release of a model drug from 3 different positions. One of the obtained kinetic releases showed a delay of 4 hours which can be interesting to exploit for certain APIs.

In both studied systems, the designed matrices present resistance to dissolution in physiological conditions, appropriate mechanical strength and flexibility to support the rolling up, appropriate smoothness allowing the deposit or the printing of the drug on its surface with an appropriate affinity to the drug and a good wettabilty allowing water diffusion inside the capsule to trigger drug diffusion. DHT treatment effect on gelatin films was investigated in detail. Subsequently, the combination of crosslinking agent with plasticizers effect on gelatin strips was studied.The capsules were stable and insoluble under physiological conditions, flexible but mechanically resistant and self-adhesive in the dissolution medium.

The release kinetics of the fluorescent inks from the different radial positions were fitted to different kinetic models, although this research has very preliminary character.

The hypothetical drug concentration profiles and the drug release kinetics were simulated under the assumption of the instant swelling of the matrices, and the absence of the drug interaction with the matrices. These assumptions allow the analytical solution of the diffusion equation, but they have only illustrative character. In order to reproduce the real kinetics, the simulation should take into account a number of factors, such as the polymer matrix swelling kinetics, and the interaction of the drug with the matrix.

As the next step, the biphasic release from the rolled-up gelatin-based capsules will be explored in vitro with the use of real drugs, such as diclofenac sodium, which belongs to the class of the nonsteroidal anti-inflammatory drugs (NSAIDs).In view of the limited stability of the rolled capsules in the strongly acidic media, they might be more suitable for biphasic drug release in the small intestine, rather than in the stomach. The rolls will be encased in the gastro-resistant capsules, e.g. on the base of Eudragit®L100-55, which is dissolved above pH 5.5 and designed for drug release in mid to upper small intestine. Also, we will study the release of vitamin B2 (Riboflav@in) in the Fasted-State Simulated Intestinal Fluid (FaSSIF), imitating the upper parts of the gastrointestinal tract.The incorporation of vitamin B2 will be carried out in two different ways: once inserted into the matrix in the form of a powder and once in the form of a solution. We also intend to study the release of B2 in its 2 forms in order to know if the diffusion is due to the device itself or to the form of B2. The potential of the method for chronotherapy and chronopharmacology will be explored for drugs, known for their chronokinetic effects, in animal models.

Annexes (French summary)

7. Résultats et discussions

7.1 Etude des propriétés de la matrice réticulée thermiquement

Les propriétés physico-chimiques notamment les propriétés de surface, les propriétés mécaniques ainsi que la mouillabilité des films de gélatine réticulés ont été étudiées. Contrairement aux conditions typiques de la méthode de réticulation thermique (DHT), 8 heures de traitement réalisées à 150°C sont efficaces pour obtenir des films stables à différents pHs notamment 7.4, 4.5 et 2, à 37°C caractérisés par un taux de gonflement limité et une résistance à la dissolution dans des conditions physiologiques.

Comparés aux films non traités, les films réticulés présentent une diminution du taux d'absorption d'eau, une augmentation de l'angle de contact et une capacité de prise en eau réduite avec une perte de masse limitée. L'AFM montre que la rugosité de surface reste presque invariante tandis que les propriétés mécaniques sont améliorées, ce qui est conforme aux résultats des essais de traction réalisés à l'aide d'un dynamomètre. L'analyse BET montre une légère diminution de la taille des pores, de la surface et du volume de N_2 adsorbé. Aucune différence significative n'est détectée entre les spectres FTIR avant et après traitement thermique à l'exception d'un pic potentiellement dû à l'estérification.

7.2 Fabrication des capsules

7.2.1 Incorporation des médicaments modèles dans les films

Les films de gélatine thermiquement réticulés ont été coupés au laser en bandes de 10 cm de long et 2 cm de large. Les réservoirs des médicaments modèles ont été formés en étalant les encres fluorescentes (Fluorescéine (FD) et la Rhodamine B (RhB)) sur la surface de la bande dans des zones correspondant aux positions radiales prescrites des réservoirs à l'intérieur de la capsule après l'enroulement. Chaque réservoir contient 500 µg de substance fluorescente. Les réservoirs R_0, R_1, R_2 et R_3 ont été formés séparément ou par paires sur la bande de gélatine à des distances précalculées afin que chaque réservoir fasse un tour complet dans la capsule. Ensuite, la transglutaminase qui servait de colle biologique a été étalée sur la surface de la bande. Après, la bande était enroulée autour d'un tige qu'on fait retirer une fois que les couches consécutives sont bien collées.

R_0 était situé à l'extrémité de la rayure et recouvrait partiellement la surface interne de la capsule ; par conséquent, il était en contact direct avec la cavité centrale de la capsule après

laminage. Les réservoirs R_1, R_2 et R_3 ont été formés de telle sorte qu'après l'enroulement, ils se trouvent respectivement au 3ème, 6ème et 8,5ème tour ; le nombre total des tours complets était égal à 10. Les coordonnées des limites par rapport à l'extrémité de la bande de gélatine, à partir de laquelle l'enroulement a commencé, sont données dans le tableau Annexe.

Tableau Annexe ; Les coordonnées latérales des reservoirs avant l'enroulement

Reservoir	Turn	x_1 [mm]	x_2 [mm]
R_0	1	0	17
R_1	3	35	53
R_2	6	91	110
R_3	8.5	140	160

La forme du rouleau peut être bien approchée, dans les coordonnées polaires, par la spirale d'Archimède :

$$r = r_0 + \frac{h}{2\pi}\varphi$$

où r est le rayon-vecteur, φ est l'angle de rotation du vecteur, h est l'épaisseur du film. Supposons qu'un réservoir soit placé sur le ième tour du rouleau. Soient x_1 et x_2 les coordonnées des limites du réservoir sur la longueur de la rayure avant l'enroulement. Ces limites peuvent être calculées comme suit:

$$x_1 = \int_0^{2\pi(n-1)} \frac{dl}{d\varphi} d\varphi = \int_0^{2\pi(n-1)} \sqrt{\left(r_0 + \frac{h}{2\pi}\varphi\right)^2 + \left(\frac{h}{2\pi}\right)^2}\, d\varphi$$

$$x_2 = \int_0^{2\pi n} \frac{dl}{d\varphi} d\varphi = \int_0^{2\pi n} \sqrt{\left(r_0 + \frac{h}{2\pi}\varphi\right)^2 + \left(\frac{h}{2\pi}\right)^2}\, d\varphi$$

où dl est l'élément de la longueur de la spirale correspondant à la rotation du rayon-vecteur de dφ. Les limites des réservoirs R_0, R_1, R_2 et R_3 étaient calculées pour les valeurs expérimentales de h=0,06mm et r=0=2,75mm.

7.2.2 Stabilisation des capsules à l'aide de la Transglutaminase

Les capsules obtenues ont été stabilisées dans la géométrie cylindrique à l'aide de la Transglutaminase ; La fonction carboxamide de la glutamine s'échange avec une fonction amine d'un acide aminé (transfert de radicaux acylés), la fonction ε NH_2 d'une lysine ou une amine provenant des réactions de décarboxylation (réaction de réticulation).

7.2.3 Etude de gonflement à différents pHs

Le gonflement des capsules a été exploré à pH = 2, correspondant approximativement au niveau de l'estomac, à pH = 4,5 et à pH = 7,4 (figure 3). Dans le milieu le plus acide (pH = 2), la Transglutaminase est désactivée et n'assure par la réticulation des couches consécutives qui forment la capsule. En conséquence, les capsules se sont déroulées après environ 4 h avec un gain de masse égale à environ 550%. Dans un milieu de pH = 4,5, les capsules ont montré une capacité de gonflement égale à environ 400 %, mais sont restées enroulées dans la plupart des expériences. A pH = 7,4, la prise de poids maximale par les capsules était d'environ 250 % et les capsules ont démontré une excellente résistance au déroulement. Compte tenu des problèmes de stabilité des gélules, nous avons décidé de nous concentrer l'étude sur le gonflement et la libération du médicament à pH = 7,4, ce qui correspond approximativement à la gamme de pH de l'intestin grêle, variant de pH = 6 dans le duodénum à pH = 7,4 dans l'iléon terminal.

7.3 Etude du relargage d'encres modèles (Fluorescéine (FD) et/ou Rhodamine B (Rh B))

Après avoir enroulé le film de gélatine, la position radiale du réservoir chargé en drug modèle détermine le temps de latence et la vitesse de libération. La libération est également contrôlée par les vitesses de dissolution et de diffusion du drug dans la matrice.

7.3.1 Relargage monophasique (Preuve de concept)

Des tests de relargage à l'aide d'un appareil de dissolution **USP 2** [184] ont été conduits.

La libération de la fluorescéine (FD) à partir du réservoir R_0 était rapide. En effet, le positionnement du médicament sur la surface interne (R_0) de la capsule assure une libération immédiate de la FD. La cinétique de relarage à partir du réservoir R_1 est relativement lente en raison de l'obstruction progressive du trou central due au gonflement de la matrice de gélatine. Ce processus réduit l'interface entre la cavité interne et le milieu de dissolution, rendant

difficile le drainage et l'évacuation du drug de la cavité. La libération du réservoir R_2, qui est à la plus grande distance des deux surfaces de la capsule, est la plus lente.

7.3.2 Relargage biphasique

Dans les systèmes de relargage biphasique traditionnels [27], [39], [53], [58], les cinétiques de relargage se basent sur les cinétiques de désintégration de la matrice donc sur les propriétés des polymères qui la constitue. Un choix judicieux des polymères ainsi que des différents additifs s'impose.

Notre système assure un relargage biphasique avec succès sans se soucier de la nature du polymère. C'est plutôt la position du réservoir qui détermine la cinétique de relargage.

Une dose de DF est placée sur la surface de la cavité cylindrique de la capsule permet au milieu de dissolution d'accéder instantanément au réservoir R_0, assurant une dissolution rapide de la DF. Une deuxième dose placée dans un réservoir R_n sur une des couches de la capsule montre un relargage prolongé. Le temps de latence ainsi que le débit peuvent être programmés uniquement par la position radiale du réservoir entre les couches consécutives du rouleau. Cette position radiale est déterminée par la position axiale du réservoir sur la bande avant l'enroulement, le rayon initial de la capsule, et l'épaisseur de la bande. Les positions latérale et radiale sont liées les unes aux autres via la formule de la géométrie spirale d'Archimède.

7.3.3 Relargage multi-drugs

Notre système convient non seulement à la libération biphasique d'un seul médicament, mais également à la libération chronomodulée de deux (ou plus) médicaments différents. Les tests de dissolution effectués ont montré la possibilité d'incorporer plus qu'une substance fluorescente dans la capsule et de détecter le signal fluorescent de chacune pendant 8 h de relargage.

Conclusion générale

Nous avons conçu avec succès un système innovant de relargage contrôlée par la position des réservoirs entre les couches plutôt que par les différentes vitesses de désintégration des polymères qui constituent la matrice des systèmes de libération biphasique traditionnels.

Les capsules sont formées par enroulement de bandes de gélatine réticulées thermiquement, à la surface desquelles différents réservoirs sont répartis et chargés en FD et/ou Rh B comme drug modèle. Les capsules sont maintenues à l'état enroulées à l'aide de la transglutaminase.

La libération biphasique du drug modèle FD a été démontrée à partir d'une capsule contenant un réservoir à relargage immédiat (Quick Release QR) et un réservoir à relargage prolongé (Sustained Release SR). Le QR est effectué à partir de la surface interne d'une capsule en forme de cylindre pendant les premières minutes d'immersion dans le milieu de dissolution. Le SR est obtenu via l'intégration d'un réservoir de médicament entre les couches des rouleaux.

La libération chronomodulée de deux (ou plus) médicaments différents a également été démontrée avec ce système. Ce concept, tout simple, basé sur un seul polymère, peut être mis en œuvre à grande échelle. Un protocole de fabrication plus automatisé utilisant l'impression jet d'encre de matériaux serait préférable, mais il devrait être spécialement adapté pour l'impression rapide de quantités suffisamment importantes de modèles ou de médicaments réels.

Cette nouvelle technologie permet de moduler la libération programmée de médicaments dans le temps ce qui conduira à des améliorations considérables de la qualité de vie et du bien-être des patients auxquels sont destinés des médicaments connus pour leurs propriétés chrono-pharmacocinétiques.

Presentations and Publications list

International colloquium of GFP : Groupe Français des polymères	Kinepolis Mulhouse-	25- 29/11/2019	Poster and flash presentation
Journée IS2M	FST Mulhouse	06-07/06/2019	Poster presentation
Young Scientists Day 2	IS2M Mulhouse	03/12/2019	Oral presentation
13 th edition of International conference on Nanomedicine and Advanced Drug Delivery	London UK	29-30/08/2019	Poster presentation
Formulating Functional Films and Coatings II	Burlington House (RSC) London	11/11/2019	Flash presentation
MIBio 2019 : Stability of biopharmaceuticals	Cambridge	13/11/2019	Poster presentation
Young Scientists Day 3	IS2M Mulhouse	04/02/2021	Oral presentation and poster
15th International conference on materials chemistry Royal Society of Chemistry.	MC 15 Online	12-15/07/2021	Oral presentation
EMCEI-Euro-Mediterranean Conference for Environmental Integration	Online	10-13/06/2021	Oral presentation
European Advanced Materials Congress : EAMC	Stockholm-Sweden	23-26/08/2021	Best Oral presentation award
Controlled Release Society annual meeting	Online	25-29 /07/2021	Oral presentation
Controlled Release Society : Early Career Scientist Meeting	Online	06/04/2021	Pitch presentation
Formulation & Drug Delivery Congress	San Diego-USA	1-3/02/2022	Poster presentation

Jihane Mzoughi, Thierry Vandamme and Valeriy Luchnikov *Pharmaceutics* **2021**, *13* (12), 2040; https://doi.org/10.3390/pharmaceutics13122040.

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