

NOVEL DRUG-LOADED PAPER TABLETS FOR IMPROVED ORAL DRUG DELIVERY

Thesis

Submitted in the fulfilment of the requirements of degree of

Doctor of Natural Sciences

(Dr. rer. nat.)

equivalent to

Doctor of Philosophy (Ph.D)

to

Faculty of Pharmacy

Philipps University of Marburg

by

Ayat Abdelkader Mohamed Aboelela

from Assiut / Egypt

Marburg (Lahn)

2023

NOVEL DRUG-LOADED PAPER TABLETS FOR IMPROVED ORAL DRUG DELIVERY

Thesis

Submitted in the fulfilment of the requirements of degree of

Doctor of Natural Sciences

(Dr. rer. nat.)

equivalent to

Doctor of Philosophy (Ph.D)

to

Faculty of Pharmacy

Philipps University of Marburg

by

Ayat Abdelkader Mohamed Aboelela

from Assiut / Egypt

Marburg (Lahn)

2023

First Supervisor: Prof. Dr. Cornelia M. Keck

Second Supervisor: Prof. Dr. Martin Koch

Date of Submission: 01.02.2023

Date of Defense: 16.03.2023

Hochschulkennziffer: 1180

NOVEL DRUG-LOADED PAPER TABLETS FOR IMPROVED ORAL DRUG DELIVERY

Dissertation

zur

**Erlangung des Doktorgrades
der Naturwissenschaften
(Dr. rer. nat.)**

eingereicht im

**Fachbereich Pharmazie
der Philipps-Universität Marburg**

vorgelegt von

Ayat Abdelkader Mohamed Aboelela

aus Assiut / Ägypten

Marburg an der Lahn

2023

Erstgutachter: Prof. Dr. Cornelia M. Keck

Zweitgutachter : Prof. Dr. Martin Koch

Eingereicht am: 01.02.2023

Tag der mündlichen Prüfung: 16.03.2023

Hochschulkennziffer: 1180

Author's declaration

I declare that this doctoral thesis titled

“Novel Drug-Loaded Paper Tablets For Improved Oral Drug Delivery”

has been written entirely by myself and is a record of work performed by myself. The research was carried out mainly at the Institute of Pharmaceutics and Biopharmaceutics, Philipps-University of Marburg, Germany under the supervision of Prof. Dr. Cornelia M. Keck. Parts of the research was carried out at the Institute of Mechanics and Materials, Technische Hochschule Mittelhessen, Giessen, Germany and Medelpharm, Science Lab, Beynost, France under the supervision of Prof. Dr. Stefan Kolling.

This thesis has not been submitted to any other university in the current or any similar form and has not served any other purpose.

Marburg, 0.1.02.2023

Ayat Abdelkader Mohamed Aboelela

Eidesstaatliche Erklärung

Ich versichere, dass ich meine Dissertation

“Novel Drug-Loaded Paper Tablets For Improved Oral Drug Delivery“

selbständig ohne unerlaubte Hilfe angefertigt und mich dabei keiner anderen als der von mir ausdrücklich bezeichneten Quellen bedient habe. Alle vollständig oder sinngemäß übernommenen Zitate sind als solche gekennzeichnet. Die Dissertation wurde in der jetzigen oder einer ähnlichen Form noch bei keiner anderen Hochschule eingereicht und hat noch keinem sonstigen Prüfungszwecken gedient.

Marburg, den 01.02.2023

Ayat Abdelkader Mohamed Aboelela

Die vorliegende Arbeit entstand unter der Leitung von

Prof. Dr. Cornelia M. Keck

im Fachbereich Pharmazie am Institut für Pharmazeutische Technologie und Biopharmazie

der Philipps-Universität Marburg.

To my Sun “*Mama*”

To my Moon “*Baba*”

Table of contents

Abstract.....	i
Chapter 1 Introduction.....	1
1.1. Oral drug delivery	2
1.2. Tablets: an overview.....	2
1.2.1. Compressed tablets.....	3
1.2.2. Manufacturing methods of compressed tablets.....	3
1.2.2.1. Direct compression	3
1.2.2.2. Granulation	3
1.3. Role of excipients in tablets.....	4
1.3.1. Excipients as solubilization enhancers	5
1.3.1.1. Chemical solubilization strategies.....	7
1.3.1.2. Physical solubilization strategies	7
1.3.2. Excipients: a source of instability	10
1.4. SmartFilms.....	11
1.4.1. History and properties of smartFilms	11
1.4.2. Applications of smartFilms.....	11
1.4.2.1. Applications of smartFilms in dermal drug delivery	11
1.4.2.2. Applications of smartFilms in oral drug delivery	12
1.4.3. Current state and development of smartFilms as an oral dosage form:	15
1.5. References.....	17
Chapter 2 Aim of work	26
Chapter 3 Results.....	28
3.1. Tablets Made from Paper—An Industrially Feasible Approach	29
3.2. SmartFilm Tablets for Improved Oral Delivery of Poorly Soluble Drugs.....	54
3.3. Improving the Bioactivity of Norfloxacin with Tablets Made from Paper	84
Chapter 4 Summary and Discussion	116
Chapter 5 Appendix	132
5.1. Acknowledgments.....	133
5.2. Publications	135
5.3. Oral and poster presentations.....	135
5.4. Curriculum Vitae	136

Abstract

Abstract

The oral route is the most preferred route of drug administration. Tablets are the most prominent oral dosage form as they can provide greater dose precision, higher stability, simplicity and lower cost of manufacturing and suitability for large-scale production. Compressed tablets, which are the most widely used tablets, consist of a blend of one or more active pharmaceutical ingredients (APIs) with suitable excipients. The excipients in tablets, in particular dissolution enhancing excipients, play a vital role in ensuring an efficient oral drug delivery (i.e., high oral bioavailability). These excipients are usually utilized as a part of a solubilization strategy to enhance the drug solubility, and thus its oral bioavailability. However, various excipients in tablets are associated with instability issues, hence, a comprehensive, costly, and time-consuming investigation of excipients is essential to develop stable and efficient tablets .

SmartFilms technology is an innovative strategy which enhances the drug aqueous solubility via embedding the drug within a matrix of cellulose-based paper in an amorphous state. Despite its proven effectiveness, smartFilms technology remains unrecognized by the pharmaceutical industry due to the difficulty of large-scale production of paper tablets from paper cut outs with limited flowability. The inadequate flowability might obstruct the compression process due to the adherence of the paper to the tablet press, which might result in dose variation of the tablets. In addition, the influence of the smartFilm tablets on the bioactivity of the loaded drug is still ambiguous.

In this thesis, smartFilm tablets were investigated as a potential, industrially feasible approach for an improved solubility and bioactivity of poorly water-soluble APIs.

The first part of the thesis investigated the possibility of transforming unloaded smartFilms (i.e., paper) into a flowable physical form and the influence of sucrose as a binder (i.e., amounts and forms) on the behavior of the material under compression as well as the properties of the obtained tablets. Cellulose-based paper utilized in this work was successfully transformed into granules via a wet granulation process. The obtained unloaded paper granules exhibited a slightly elongated shape, demonstrated good flowability and allowed the production of tablets in a continuous mode. The results also showed that using sucrose as a dry powder during the granulation process was the most suitable for obtaining paper granules that can be compressed in large scale into tablets with good pharmaceutical properties (i.e., in accordance with the European Pharmacopoeia). Investigating the mechanical behavior of paper granules under compression indicated that the compaction behavior of these granules

was comparable to the behavior of classical binders and compression enhancers. These findings indicate that the obtained paper granules have good flowability, a suitable compression behavior and propose paper granules as suitable intermediate products for the production of tablets made from paper on a large, industrial scale.

The second and the third part of the thesis studied the impact of smartFilm tablets on the oral delivery and bioactivity of two poorly water-soluble APIs (i.e., curcumin and norfloxacin) using an ex vivo porcine intestinal model. Curcumin-loaded smartFilms and norfloxacin-loaded smartFilms were successfully transferred into smartFilm granules and smartFilm tablets, respectively. Results also showed that the curcumin-loaded smartFilm granules and smartFilm tablets preserved the amorphous state of the incorporated drug. The obtained tablets also fulfilled the criteria according to the European Pharmacopoeia regarding hardness, friability, content uniformity, mass uniformity, and disintegration time. The incorporation of curcumin or norfloxacin into smartFilm tablets resulted in increasing the dissolution rate (approx. two-fold) especially at the beginning of the release. The ex vivo intestinal permeability of curcumin from the smartFilm tablets was also studied and compared to a physical mixture of curcumin and paper and to a classical and an innovative commercial product, respectively. The innovative product contains curcumin in a micellar form and has previously demonstrated an exceptional oral bioavailability. The findings showed an enhanced intestinal permeability of curcumin from the smartFilm tablets, as compared to the physical mixture tablet and the classical marketed product that contains curcumin as a raw powder (approx. two-fold increase). No difference in the total amount of permeated curcumin was found between the smartFilm tablets and the innovative commercial product (i.e., micellar curcumin). Nevertheless, a trend towards a deeper intestinal permeation of the curcumin from the smartFilm tablets was observed. These outcomes indicate that smartFilm tablets can be equally efficient as innovative and classical curcumin formulation approaches in improving the oral bioavailability of curcumin. The ex vivo bioactivity of norfloxacin from the smartFilm tablets was also investigated and showed a similar trend (i.e., a two-fold higher antibacterial activity of norfloxacin from the smartFilm tablets when compared to the physical mixture tablet).

The findings of this thesis provide evidence that smartFilm tablets are a cost-effective, universal, industrially feasible formulation approach for improved solubility and enhanced bioactivity of poorly water-soluble APIs, i.e., BCS class II and IV drugs.

Abstrakt

Die orale Applikation ist der bevorzugteste Weg der Arzneimittelverabreichung. Tabletten sind die bekannteste orale Dosierungsform, da sie eine größere Dosierungspräzision, höhere Stabilität, Einfachheit und niedrige Herstellungskosten und Eignung für die Produktion in großem Maßstab bieten können. Direktkomprimierte Tabletten, die am häufigsten verwendeten Tabletten, bestehen aus einer Mischung von einem oder mehreren pharmazeutischen Wirkstoffen mit geeigneten Hilfsstoffen. Die Hilfsstoffe in Tabletten, insbesondere Freisetzungsbeschleuniger, spielen eine entscheidende Rolle bei der Sicherstellung einer effizienten oralen Arzneimittelabgabe (d. h. hohe orale Bioverfügbarkeit). Diese Hilfsstoffe werden normalerweise als Teil einer Freisetzungsstrategie verwendet, um die Löslichkeit des Wirkstoffes und damit einhergehend die orale Bioverfügbarkeit zu verbessern. Allerdings sind Hilfsstoffe in Tabletten mit Instabilitätsproblemen verbunden, daher ist eine umfassende Untersuchung der Hilfsstoffe unerlässlich, um stabile, effiziente und sichere Tabletten zu entwickeln.

Die smartFilm Technologie ist eine innovative Strategie, die die Wasserlöslichkeit des Wirkstoffes verbessert, indem der Wirkstoff in einem amorphen Zustand in eine Papiermatrix auf Zellulosebasis eingebettet wird. Trotz ihrer nachgewiesenen Wirksamkeit blieb die smartFilm Technologie von der pharmazeutischen Industrie aufgrund von Umsetzungsschwierigkeiten bislang unerkannt.

In dieser Dissertation wurden smartFilm Tabletten als potenzieller, industriell umsetzbarer Ansatz für eine verbesserte Löslichkeit und Bioaktivität von schwer wasserlöslichen Wirkstoffen untersucht.

Der erste Teil der Dissertation untersuchte die Möglichkeit unbeladene smartFilms (d. h. Papier) in eine fließfähige physikalische Form zu überführen als auch den Einfluss von Saccharose als Bindemittel (d. h. Menge und Form) auf das Verhalten des Materials unter Druck sowie auf die Eigenschaften der erhaltenen Tabletten. Papier auf Zellulosebasis wurde über die Feuchtgranulierung erfolgreich in Granulate umgewandelt.

Die Ergebnisse belegten auch, dass die Verwendung von Saccharose als trockenes Pulver während der Granulierung am Besten geeignet war, um Papiergranulate zu erhalten, die zu Tabletten mit guten pharmazeutischen Eigenschaften (d. h. in Übereinstimmung mit dem Europäischen Arzneibuch) gepresst werden können. Die Untersuchung des mechanischen Verhaltens von Papiergranulaten unter Kompaktion zeigte, dass das

Kompaktionsverhalten dieser Granulate mit dem Verhalten klassischer Bindemittel und Verdichtungsverstärker vergleichbar war. Diese Befunde indizieren, dass die erhaltenen Papiergranulate eine gute Fließfähigkeit und ein geeignetes Kompaktionsverhalten aufweisen und empfehlen Papiergranulate als geeignete Zwischenprodukte für die großtechnische Herstellung von Tabletten aus Papier.

Der zweite und dritte Teil der Dissertation analysierte den Einfluss von smartFilm Tabletten auf die orale Verabreichung und Bioaktivität von zwei schwer wasserlöslichen Wirkstoffen (d. h. Curcumin und Norfloxacin) unter Verwendung eines ex vivo Schweinedarmmodells. Mit Curcumin- beladene smartFilms und mit Norfloxacin-beladene smartFilms wurden erfolgreich in smartFilm Granulate bzw. smartFilm Tabletten überführt. Die Ergebnisse zeigten, dass die mit Curcumin- beladenen smartFilm Granulate und - smartFilm Tabletten den amorphen Zustand des eingearbeiteten Wirkstoffes bewahrten. Die erhaltenen Tabletten erfüllten auch die Kriterien gemäß des Europäischen Arzneibuches hinsichtlich Bruchfestigkeit, Friabilität, Gleichförmigkeit der Masse, Gleichförmigkeit des Gehalts und Zerfallszeit. Die Einarbeitung von Curcumin oder Norfloxacin in smartFilm Tabletten führte zu einer Erhöhung der Auflösungsgeschwindigkeit. Die ex vivo Darmpermeabilität von Curcumin aus den smartFilm Tabletten wurde ebenfalls untersucht und mit einer physikalischen Mischung aus Curcumin und Papier bzw. mit einem klassischen und einem innovativen kommerziellen Produkt verglichen. Das innovative Produkt enthält Curcumin in mizellarer Form und hat zuvor eine außergewöhnliche orale Bioverfügbarkeit gezeigt. Die Ergebnisse zeigten eine verbesserte Darmpermeabilität von Curcumin aus den smartFilm Tabletten im Vergleich zu den Tabletten mit physikalischer Mischung und dem klassischen vermarkteten Produkt, das Curcumin als Rohpulver enthält. Zwischen der smartFilm-Tablette und dem innovativen kommerziellen Produkt wurde kein Unterschied in der Gesamtmenge an eingedrungenem Curcumin festgestellt. Dennoch wurde ein Trend zu einer tieferen Darmpermeation des Curcumins aus den smartFilm Tabletten beobachtet. Diese Ergebnisse zeigen, dass smartFilm-Tabletten bei der Verbesserung der oralen Bioverfügbarkeit von Curcumin ebenso effizient sein können wie innovative und klassische Curcumin-Formulierungsansätze. Die ex vivo Bioaktivität von Norfloxacin aus den smartFilm Tabletten wurde ebenfalls untersucht und zeigte einen ähnlichen Trend .

Die Ergebnisse dieser Dissertation belegen, dass smartFilm Tabletten ein kostengünstiger, universeller, industriell durchführbarer Formulierungsansatz für eine verbesserte Löslichkeit und verbesserte Bioaktivität von schwer wasserlöslichen Wirkstoffen, d. h. Wirkstoffe der BCS-Klassen II und IV, sind.

Chapter 1

Introduction

1.1. Oral drug delivery

The oral route is the most preferred route of drug administration. It can be used for both systemic drug delivery and for treating local gastrointestinal tract (GIT) diseases [1,2]. Oral drug delivery offers several advantages, such as minimal risk of infection and low sterility limitations, which simplifies the production process and reduces costs. Oral delivery systems are also associated with a high patient compliance as they are easy to use, non-invasive, and convenient for self-administration [3,4]. Efficient oral drug delivery (i.e., high oral bioavailability) can be affected by several factors, including aqueous solubility, stability, dissolution rate, permeability, first-pass metabolism, presystemic metabolism, and the susceptibility to efflux mechanisms of the drug molecules [5].

Oral drug delivery can be achieved using solid dosage forms such as tablets, capsules, granules, or liquid dosage forms such as suspensions, emulsions, and a variety of pharmaceutical solutions [1]. Solid dosage forms have been widely used as they are versatile, provide flexible dosing, relatively stable, associated with less complications during formulation and packaging. In addition, solid dosage forms provide the best protection of the drug against light, temperature, humidity, oxygen, microbial contamination (i.e., due to absence of water) and stress, therefore they can be simply stored, handled, and used [1,6].

1.2. Tablets: an overview

Tablets are the most popular oral dosage form as they represent 50% of all oral drug delivery systems and 70% of the produced pharmaceutical formulations [7,8]. Tablets can be defined as a solid unit dosage form that contains one or more active pharmaceutical ingredients (APIs) with suitable excipients [6]. They differ greatly in shape, size, and weight, depending on the amount of the API and the intended route of administration.

Tablets provide several advantages over other oral dosage forms such as i) greater dose precision and lower content variability, ii) simplicity and low cost of manufacturing, iii) compaction and light weight, iv) easier to swallow, v) the possibility of producing sustained release products via enteric coating, vi) more suitable for large-scale production, vii) higher chemical, mechanical and microbial stability, viii) tamper-proofness compared to capsules, ix) the possibility of formulating tablets with two or more drug substances, even if they are physically or chemically incompatible, and thus reducing multiple tablet use [9,10].

Orally administrated tablets can be categorized into i) tablets ingested orally (e.g., compressed tablets (i.e., uncoated tablets), coated tablets (i.e., film-coated, sugar-coated, gelatin-coated and enteric-coated tablets), multiple compressed tablets, layered tablets, extended-release tablet, rapid released tablets, effervescent tablets) and ii) tablets used in

the oral cavity (e.g., buccal tablets, sublingual tablets, troches and lozenges, dental cones) [6,11]. Compressed tablets are the most widely used type of tablets and they were intensively investigated in this work.

1.2.1. Compressed tablets

Compressed tablets represent a significant fraction of tablets that are clinically used, mainly in an uncoated state, to provide a systemic delivery of APIs. Compressed tablets are manufactured by compression of powdered, crystalline, or granular materials into the required shape via applying high pressures and utilizing various shaped steel punches and dies [11].

1.2.2. Manufacturing methods of compressed tablets

1.2.2.1. Direct compression

Direct compression can be defined as the process in which tablets are compressed directly from a blend of powdered APIs and suitable excipients without modifying the physical nature of these materials. This method is applicable for crystalline materials that exhibit good compressible characteristics and flow properties as well as when a drug constitutes a major portion of tablet total weight [7].

Direct compression offers several advantages such as i) fewer manufacturing steps, less processing time, and thus reducing labor cost and validation process, ii) no moisture, heat or high compaction pressure is required, so it is a suitable method for moisture or thermolabile API, iii) optimization of tablet disintegration as all drug particles is released from the tablet mass, and thus available for the dissolution [6,11]. However, there are several challenges associated with this process, including i) high weight and dose variation of the tablets due to differences in particle size and bulk density, ii) production of large sized tablets, which are difficult to swallow and also costly (i.e., tablets with large drug doses), iii) possible formation of a static charge with a powdered material which may result in uneven distribution of the drug, iv) low mechanical strength of the tablets, v) critical selection of the utilized excipients as they must demonstrate good homogeneity and segregation tendency, friction and adhesion properties, flowability, compression properties and compactability [7,12].

1.2.2.2. Granulation

Granulation is a process in which fine or coarse particles are converted into large agglomerates called granules [13]. This process is considered when the properties of a powder blend (i.e., flowability, static charge, dust formation ability) are not suitable for direct

compression tableting [7]. Granulation presents several advantages such as i) reduction of the particle size distribution of a powdered material, hence eliminating the segregation issues and ensuring superior compressibility in the tableting process, ii) allowing higher quantities of the API to be used and enhancement of the uniformity of the API in the final tablet, iii) increasing the density of the blend so that it occupies less volume per unit weight, hence better storage and shipment, iv) facilitating metering or volumetric dispensing, v) reduction of dust formation, and thus reducing toxic exposure and process-related hazards, vi) improvement of the appearance of the tablet [7,9,12]. The granulation process can be mainly categorized into two types: dry granulation and wet granulation [14].

- **Dry granulation:** it can be defined as the process of forming granules by mechanical compression (slugs) or compaction (roller compaction). After weighing and blending the API with excipients, the powder blend is either slugged (i.e., compressed) into large flat tablets with a diameter of one inch or compacted using powder compactors, in which the powder density is increased by pressing it between rollers at 1 to 6 tons of pressure. Following that, the slugs or the compacted material are milled and screened to produce granules, then a lubricant is added, and the blend is compressed into tablets [11,14]. Dry granulation method is especially applicable for materials that degrade in moisture or at the elevated temperatures required for the drying of the granules [11,15].
- **Wet granulation:** it is the process in which a granulation liquid (i.e., binder or solvent) is added to a powdered material (i.e., API and excipients) in a vessel that is equipped with any type of agitation which will produce agglomeration or granules [6]. It is the most widely used granulation method despite the fact that it involves multiple processing steps [9,13]. These steps include weighing and blending the ingredients, preparing a dampened powder or a damp mass, screening of damp pass into granules, drying the granules in drying chambers with a circulating air current and thermostable heat controller (i.e., tray dryer or fluidized bed dryer), sizing the granules by dry screening, addition of a lubricant to promote the flow of granules, and forming tablets by compression [11,14].

1.3. Role of excipients in tablets

Excipients are defined according to the International Pharmaceutical Excipients Council (IPEC America and IPEC Europe) as “These are the substance(s) other than the API in the finished dosage form, which have been appropriately evaluated for safety and are included in a drug delivery system to i) aid the processing of the drug delivery system during its manufacture, ii) protect, support, enhance stability, bioavailability or patient acceptability, iii) assist in product identification, iv) or enhance any other attributes of the overall safety and effectiveness of

the drug delivery system during storage or use” [16,17]. Excipients in tablets can be classified into formulation aids (e.g., diluents, binders, lubricants, disintegrants, pH adjustors) and functional excipients (e.g., taste masking agents, drug release rate modifiers, mucoadhesives, solubility and dissolution enhancers) [1].

Diluents are normally used as fillers to increase the bulk of a formulation to prepare tablets of a desired size (e.g., lactose, starch, and mannitol) [1,18]. Binders promote plasticity as well as the adhesion of the particles of the formulation by cohesive and adhesive forces (i.e., Vander Waals and electrostatic forces, hydrogen bonding, solid bridges, mechanical interlocking). They are either added as a liquid or a dry form (e.g., starch, microcrystalline cellulose, and acacia). The amount and type of the added binder influences the tablet properties [1,19]. Lubricants and glidants enhance the flow of the powder or granules into the tablet dies and aid in the production of glossy tablets. They act by forming a stable layer around particles/surfaces and they also prevent sticking, picking and capping issues (e.g., talc, stearic acid, and magnesium stearate) [1]. Disintegrants promote the disintegration or the breakup of the tablets in GIT (e.g., starch, clays, and cellulose) [20]. Upon tablet disintegration, the pH-adjusting excipients dissolve in the medium of the GIT and adjust the pH of the stagnant boundary layer that surrounds the particles of the API, and thus improving the dissolution of the API (e.g., sodium carbonate and succinic acid) [18]. Taste maskers can hide the bitter taste of the API and improve patient compliance (e.g., sweeteners, flavors) [1]. Drug release rate modifiers can be added to ensure optimal plasma concentrations, prolong the duration of the drug therapeutic action, reduce the frequency of drug administration, and thus enhance patient compliance (e.g., chitosan and polyethylene glycol (PEG)) [21,22]. A mucoadhesive agent/polymer adheres to the mucosal surface and extends the residence time of formulation within the GIT (e.g., chitosan and hydroxypropyl cellulose (HPC)) [1].

1.3.1. Excipients as solubilization enhancers

The aqueous solubility of the drug is a fundamental property that plays a crucial role in insuring high oral bioavailability [5]. Solubility can be defined as the quantity of solute, which dissolves in a quantity of solvent to form a homogeneous solution of the solute in the solvent. The term quantity indicates the concentration of the solute in a saturated solution at a certain temperature [23,24]. “Lipinski's rule of 5” considers the solubility of drug molecules when rejecting inadequate drug candidates at early stages of the drug discovery process [25]. However, it is estimated that 40% of approved drugs and nearly 90% of drugs in development consist of poorly soluble molecules [26]. Such drugs often require high doses to reach therapeutic plasma concentrations after oral administration, leading to inadequate and variable bioavailability and gastrointestinal mucosal toxicity [5].

Biopharmaceutics Classification System (BCS) also recognizes the aqueous solubility and the permeability of drug molecules as fundamental parameters to control the rate and extent of oral drug bioavailability (**Figure 1**) [27]. According to the BCS, the drug is considered to be highly soluble when the highest dose strength can dissolve in a glass of water (i.e., 250 ml) or less of aqueous media over a pH range of 2–7.5 [28]. Permeability is often referred to as the ability of the drug to diffuse across the apical membrane of enterocytes into the cytosol, and it depends on drug characteristics such as polarity, charge, and hydrophobicity [29]. A drug is described as highly permeable when the percentage of absorption is $\geq 90\%$ of the administered dose. BCS Class I drugs (e.g., metoprolol and propranolol) that exhibit high solubility and permeability are good candidates for oral delivery [30]. In contrast, other BCS classes are challenging candidates for oral delivery due to their low solubility (BCS Class II such as ketoprofen and rifampicin) [31], low permeability (BCS Class III such as atenolol and cimetidine) [30], or both (BCS Class IV such as curcumin and norfloxacin) [32,33].

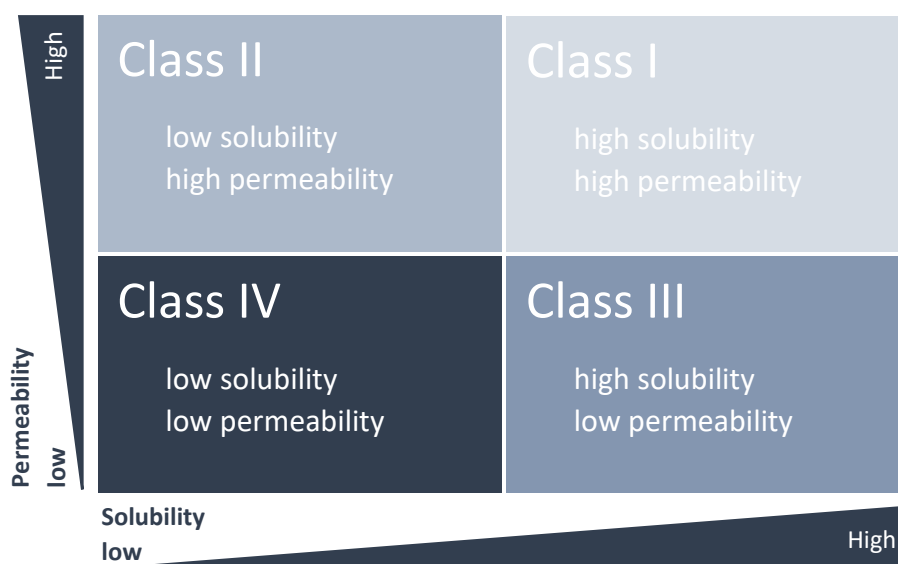


Figure 1. Biopharmaceutics Classification System (BCS) of drugs according to their solubility and permeability (adapted from [24]).

All this indicates that solubilization enhancers play a vital role in improving oral drug delivery of poorly water-soluble drugs. Solubility enhancing excipients can be categorized into three groups, including polymer-based excipients, surfactant-based excipients, and lipid-based excipients. These excipients are usually utilized as a part of a solubilization strategy to enhance the drug solubility, and thus its oral bioavailability [34]. Drug solubilization strategies can be categorized, according to the nature of drug modification involved, into chemical and physical solubilization strategies [35].

1.3.1.1. Chemical solubilization strategies

- pH modification and salt formation

The pH adjustment represents a first line strategy for the formulation of poorly soluble drugs. Ionizable drugs, nearly 70% of drugs are ionizable, demonstrate pH-dependent solubility, where weakly acidic drugs are soluble at $\text{pH} > \text{pK}_a$ (i.e., ionization constant) and weakly basic drugs are soluble at $\text{pH} < \text{pK}_a$ [26,36]. Salt formation results in similar outcomes, where salts are formed via an ionic interaction between weakly acidic or basic drugs and an oppositely charged basic or acidic counterion [37]. Upon dissolution of salts in water, their counter ions provide favorable pH conditions [36,38]. Nevertheless, this solubilization approach is not suitable for neutral drug molecules and usually associated with precipitation tendencies after oral administration [2,39].

- Prodrug formation

A prodrug is an inert, chemical derivative of a parent drug that exhibits improved physicochemical properties and is able to convert into the active parent drug through an enzymatic biotransformation [35]. The prodrug strategy provides improved drug solubility, lipophilicity, transporter-mediated absorption, and the potential to achieve site-specific delivery [40]. Despite being a powerful and versatile solubilization strategy, the use of prodrugs is usually associated with chemical instability and a higher risk for the formation of degradation by-products [41].

1.3.1.2. Physical solubilization strategies

- Cosolvency

In this approach, the drug dissolution is enhanced by using a mixture of water and a water-miscible organic solvent that is less polar than water, which results in lowering the polarity of the bulk solvent (i.e., the dielectric constant) to a level that resembles the polarity of the nonpolar solute (i.e., drug) [36]. Though the cosolvency strategy is one of the oldest and widely used technique for the formulation of poorly soluble drugs, it presents some disadvantages. These disadvantages are linked to the i) taste, stability, and the limited number of cosolvents, ii) concomitant solubilization of other components such as preservatives which might affect the stability and effectiveness of the drug, iii) alteration of the pharmacokinetic profile of the drug, iv) potential drug precipitation upon dilution with aqueous media or physiological fluids [26,35,42].

- Particle size reduction

Reduction of the particle size of poorly water-soluble drugs results in an increase in the drug surface area available for solvation and an increase in the dissolution rate of the drug particles, which results in an improved bioavailability and a reduced toxicity [35]. Micronized and nanosized drug particles can be obtained using various strategies, which can be categorized into: “top-down” techniques, where larger drug particles are fragmented into smaller particles, or “bottom-up” techniques, where small drug particles are obtained after controlled crystallization or precipitation of a drug from a supersaturated solution [36,43]. Nevertheless, several challenges are associated with particle size reduction, including the need for specialized equipments especially for nanonization [44,45] as well as physicochemical-related stability issues such as aggregation or change in the solid state of the drug [2,36].

- Drug carrier systems

A drug carrier is a system that has the capability of incorporating a specific amount of drug molecules to enhance their selectivity, bioavailability, and efficiency. Drug carrier systems are generally designed at nanometric and micrometric levels, to combine the advantages of a carrier and particles with reduced size [46]. There are several types of drug carrier systems, including micelles and polymeric micelles, inclusion complexes, lipid-based formulations (e.g., lipid solutions, lipid suspensions, emulsions, self-emulsifying drug delivery systems, liposomes, and solid lipid nanoparticles), and polymeric nanocarriers [2]. Drug carrier systems present several advantages, including i) taste masking, ii) better tolerability and reduction of drug toxicity, iii) protection of the incorporated drugs from degradation in the GIT, iv) target drug delivery and controlled drug release, v) their nanosized nature which allow active and passive tissue targeting [47–49], vi) longer drug retention as they are less susceptible for reticuloendothelial scavenging (e.g., polymeric micelles) [50], vii) intrinsic biocompatibility, viii) suitability for scaling-up and cost-effectiveness (i.e., lipid-based formulations) [48,51].

Although drug carriers provide promising strategies for the improvement of the oral bioavailability, obtaining the desired physicochemical properties, drug encapsulation, drug-release kinetics, and particle size is difficult, especially with liposomes and solid lipid nanoparticles [52]. They are also associated with various challenges such as i) storage stability issues (e.g., lipid-based formulations) [53], ii) high cost of materials and the need for comprehensive characterization (e.g., inclusion complexes) [35], iii) in vivo instability upon dilution in the GIT (e.g., micelles and inclusion complexes) [54–57], iv) biocompatibility and toxicity issues (e.g., non-biodegradable polymeric nanocarriers) [58,59].

- Crystal engineering (i.e., polymorphism and cocrystal formation)

Polymorphism is defined as the ability of a drug to form one or more crystalline solids that differ only by the molecular arrangement of drug molecules in the crystal lattice, and the different crystalline solids of the same drug are described as polymorphs [60,61]. Metastable polymorphs demonstrate a higher dissolution rate and solubility than the most stable polymorphs, and thus increasing the oral bioavailability of poorly water-soluble drugs [62,63]. However, different polymorphs are usually associated with drug instability [35].

Cocrystals can be described as mixed crystalline solids, which exist as crystals at ambient temperature, consisting of a drug and a cocrystal former (coformer) held together by noncovalent forces [63]. These bonds are able to reduce the strength of the drug-drug interactions, decrease the lattice energy, and increase the solvent affinity, as compared to the pure drug crystal structure, hence enhancing the solubility of a drug [64]. It is a simple strategy, however, most of the utilized coformers are not safe as they could cause membrane irritation and toxicity, particularly at high doses [35,65].

- Amorphization and solid dispersions

Amorphous solids are partially disordered solids where the drug-drug interactions are weaker than in the crystals. They are obtained either by preventing the formation of a crystalline structure or by disrupting an already existing crystal. The amorphous forms of drugs can theoretically provide solubilities up to 1600 times higher than the crystalline forms. The amorphization is usually combined with other small drug particle strategies such as solid dispersions [35].

Solid dispersion formulations consist of dispersing one or more poorly water-soluble drugs in an inert excipient or matrix which is usually a hydrophilic, high molecular weight polymer [2,66]. Solid dispersions are usually prepared using the melting (fusion), solvent evaporation, coprecipitation, melting extrusion, or spray drying method. The melting method is commonly used for developing large quantities of pharmaceutical formulations, but it can't be used with thermolabile substances [67].

Combining amorphization with the solid dispersion technique enhances the dissolution of drugs due to the formation of a high-energy amorphous form and the reduction of the particle size, hence increasing the surface area and wettability of the produced amorphous solid dispersion [2,35]. These amorphous forms are rarely eutectic, and thus remain metastable and thermodynamically active, leading to their supersaturation in the gastrointestinal fluid and obtaining a greater concentration gradient which increases the dynamic force of drug transport across the cellular membrane [2].

Amorphous solid dispersions provide enhanced solubility and dissolution rate compared with traditional crystal modification techniques. It also retards the agglomeration/crystallization of drug molecules due to its molecular level dispersion and steric hindrance interactions within the polymeric matrices [2]. However, their use is associated with many complexities, in particular the intrinsic crystallization tendency of the high-energy amorphous drug which is difficult to predict at early stages of production. In addition, the production process of amorphous solid dispersions is complicated, and several factors should be considered. These factors include i) the choice of excipients that are used to formulate solid dispersions, ii) the generation of homogeneous dispersions with a high degree of miscibility between drug and polymer matrices, iii) identification of systems with high glass transition temperatures relative to storage temperatures [36,68].

1.3.2. Excipients: a source of instability

Excipients are mainly pharmacologically inert, however they can initiate or participate in physical or chemical interactions with the drug molecules, and thus affecting the properties of the product (i.e., such as disintegration, dissolution, and shelf life). They can be affected by chemical, physical, or microbiological instability [17]. Physical instability involves phase transformation of the excipients such as polymorphic variations, hydration and dehydration, precipitation, or variations in the amorphous or crystalline nature. The phase transformation can occur due to aggregation, coagulation, melting, solvent-mediated mechanisms, or thermal stresses during manufacturing processes (e.g., milling, dry granulation, and compaction) [69]. Chemical instability may occur due to thermolytic (i.e., hydrolysis), oxidative (i.e., transition metal-mediated electron transfer or free radical-initiated chain reactions), or photolytic degradation [70,71]. Microbiological instability may occur due to the failure of the preservative in the formulation due to interaction, degradation, or depletion from the system [17]. Several studies reported instability issues of various excipients in tablets, therefore, a comprehensive investigation of excipients during preformulation and formulation studies is essential to develop a stable, efficient, and safe tablets [72–81]. However, such a thorough investigation is usually time-consuming and can increase the costs of tablet production, and thus it is preferred to utilize a system that requires the usage of less excipients to manufacture tablets.

1.4. SmartFilms

1.4.1. History and properties of smartFilms

SmartFilms technology was introduced for the first time in 2016 by Lemke and his colleagues as an innovative approach to improve the solubility and the dissolution rate of the poorly water-soluble drugs [82]. SmartFilms can be defined as sheets of cellulose-based paper in which the API is loaded in an amorphous form within the pores of cellulose matrices. The production of smartFilms involves dissolving a poorly water-soluble drug in an appropriate solvent, adding the resulting solution on a cellulose-based paper, and drying the obtained drug-loaded smartFilms [82,83]. SmartFilms technology offers several advantages as a potential technique for enhancing the solubility of poorly water-soluble drugs, as depicted in **Figure 2**. In addition, smartFilms were able to attain the amorphous state of various drugs for at least 18 months, indicating their long-term stability [82,83].

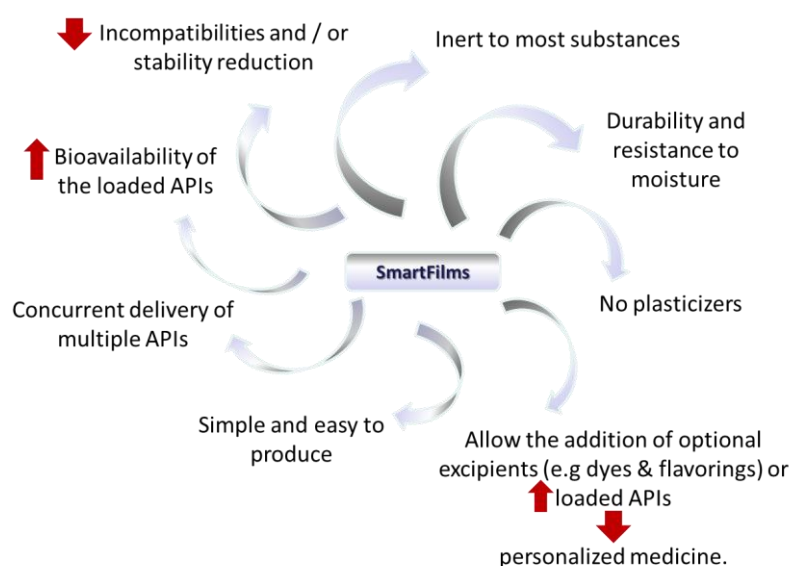


Figure 2. Advantages of smartFilms as a solubilization enhancing technique [82, 83].

1.4.2. Applications of smartFilms

The positive impact of smartFilms on the solubility of drugs encouraged further investigations of smartFilms for improved dermal and oral drug delivery.

1.4.2.1. Applications of smartFilms in dermal drug delivery

Eckert *et al* investigated the ability of smartFilms to improve the passive diffusion of a poorly water-soluble API into and through the skin after dermal application. In that study, curcumin-loaded smartFilms were produced and characterized [84]. The study demonstrated that the

cellulose matrix of smartFilms were able to maintain the amorphous state of curcumin, which resulted in an increased dissolution rate and an increased kinetic solubility when compared to curcumin bulk material [84]. The study also compared the dermal and transdermal penetration of curcumin from smartFilms to curcumin bulk material or curcumin nanocrystals that contain the same amount of curcumin. The authors reported that smartFilms were superior when compared to curcumin bulk material (i.e., seven-fold higher penetration efficacy) or when compared to curcumin nanocrystals (i.e., eight-fold higher penetration efficacy and five-fold deeper curcumin penetration) [84]. These results were confirmed by Keck *et al* who described the ability of smartFilms with lower curcumin content, to provide an improved dermal and transdermal penetration of curcumin when compared to curcumin nanocrystals [85]. Both studies confirmed the ability of smartFilms as a potential drug delivery system for efficient dermal and transdermal delivery of poorly water-soluble drugs.

1.4.2.2. Applications of smartFilms in oral drug delivery

Despite being a promising oral drug delivery system, administration of paper (i.e., smartFilms) is not convenient for the patient. Therefore, incorporation of smartFilms into appropriate oral dosage forms can improve the patient compliance to the novel technology. Preliminary results proved that smartFilms can be compressed into tablets without the addition of any further excipients [82,83]. A scheme of conventional production of drug-loaded smartFilm tablets is illustrated in **Figure 3**. Following that, several studies reported the production of different drug-loaded smartFilm tablets, and the properties of the produced tablets were also studied [86–89].

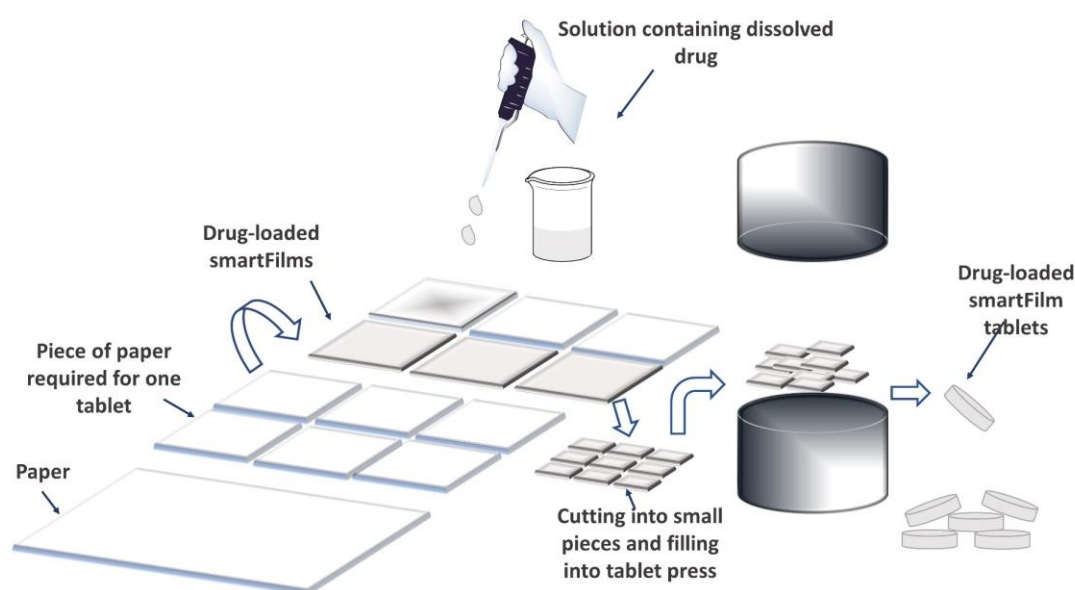


Figure 3. Scheme of production of drug-loaded smartFilm tablets (adapted from [86]).

Stumpf and Keck conducted the first systematic study which investigated the possibility of compressing different types of unloaded paper (e.g., disposable handkerchief, kitchen roll, disposable washing cloth, cosmetic facial tissues, coffee filter) into tablets [86]. The authors used caffeine and rutin as model drugs to produce loaded smartFilm tablets and studied the pharmaceutical properties of the produced tablets (i.e., thickness, mass uniformity, friability, hardness, disintegration time, content uniformity, and dissolution profile). The studies also assessed the crystallinity and morphology of caffeine and rutin loaded within the paper matrix [86,87]. The authors were able to produce smooth tablets using six different types of paper without addition of any excipients. All the unloaded and loaded smartFilm tablets exhibited good pharmaceutical properties which adhered to the European Pharmacopoeia requirements in terms of thickness, mass uniformity, friability, hardness, disintegration time, and content uniformity [86,87]. Scanning electron microscopy (SEM) and X-ray diffraction (XRD) analysis results indicated that caffeine and rutin might be loaded within the paper matrix in an amorphous form. The authors also reported that rutin-loaded smartFilm tablets showed a 10-fold faster drug release, as compared to the bulk material, indicating that the dissolution rate and consequently the bioavailability of rutin can be improved with smartFilm tablets.

Subrahmanyeswari *et al* prepared paper tablets loaded with efavirenz, which is used in treatment of human immunodeficiency virus (anti-HIV drug), and it is considered a class II drug according to the BCS (i.e., low solubility and high permeability) [90]. The authors used the cosolvency technique to prepare the smartFilm tablets and utilized various papers such as kitchen roll, disposable handkerchief, and facial tissues. The results of the study indicated that efavirenz-loaded smartFilm tablets that were prepared from kitchen roll met the requirements of the European Pharmacopoeia and showed an improved release of efavirenz over 180 min, as compared to other formulations. In addition, the stability studies indicated that efavirenz-loaded smartFilm tablets were stable over a period of two months [90].

Recently, Ornik *et al.* investigated the usage of a non-destructive technique to assess the amorphous state of API loaded within the smartFilms and smartFilm tablets during their storage [88,89]. In these studies, terahertz (THz) time-domain spectroscopy, which is a non-destructive, sensitive technique which is able to detect minor changes in the crystalline state of many molecular crystals, was used. In addition, L-tartaric acid and indomethacin were used as model drugs and the obtained THz results were compared to the results obtained from XRD and differential scanning calorimetry (DSC) measurements. In case of L-tartaric acid, the study showed that the DSC measurements failed to detect the crystalline form of L-tartaric acid in

the smartFilms, due to the presence of moisture in the paper basis. XRD and THz-spectroscopy showed similar results and indicated that it was possible to prepare smartFilms loaded with > 23% (w/w) amorphous L-tartaric acid [88]. In case of indomethacin, the study showed that the smartFilms maintained the amorphous form of indomethacin for low loading concentrations. The results also showed that at higher loading concentrations, indomethacin recrystallizes to α - crystalline form (another crystalline polymorph of indomethacin) and the amount of amorphous form of indomethacin in the smartFilms reduces [89]. The authors reported that the usage of smartFilm tablets loaded with the recrystallized form of indomethacin (α - crystalline form) and the amorphous form of indomethacin is expected to increase the oral bioavailability of the drug more than the bulk crystalline indomethacin. The authors explained that this is due to the higher water solubility of these two forms in comparison to the conventionally used γ - crystalline form of indomethacin [89]. The results of both studies indicated the great potential of THz time-domain spectroscopy for non-destructive crystallinity assessment of smartFilms and smartFilms tablets and can be considered as an alternative to the established analytical methods, such as DSC or XRD. The studies also suggest the usage of THz time-domain spectroscopy for long-term monitoring of the API stability within a smartFilm or a smartFilm tablet, as it allows for multiple measurements of one sample at different time points and at different sample positions.

All the above-mentioned studies demonstrated the feasibility of compressing drug-loaded smartFilms into tablets without the addition of any excipients, the ability of smartFilms to maintain the loaded drug in an amorphous form, and the improvement of dissolution rate of the drug loaded within smartFilm matrix. Nevertheless, smartFilms technology remained unrecognized by the pharmaceutical industry due to their unsuitability for high-speed tablet manufacturing and large-scale tablet production. This might be attributed to the inadequate flowability of smartFilms in their original state (i.e., paper cut outs), as they might adhere to the tablet press, and thus obstructing the compression process and resulting in dose variation of the produced tablets. Furthermore, there is a lack of knowledge of the mechanical behavior of smartFilms under compression and the influence of the smartFilm tablets on the behavior of the loaded drug under physiological conditions. Therefore, the transformation of smartFilms into a free-flowing physical form along with providing data regarding the bioactivity of drug-loaded smartFilm tablets are major prerequisites for a wide exploitation of smartFilms as a valid oral dosage form for the delivery of poorly water-soluble drugs.

Granulation is a key processing step in the production of many pharmaceutical tablets and has been widely studied to improve the poor flowability of solids, enhance the

compressibility of materials, or control the release of drugs from solid dosage forms [14,91]. More details about the granulation methods are provided in section 2.1.1.2. Consequently, the transformation of smartFilms into granules was expected to improve their limited flowability. Preliminary data, that produced granules from unloaded paper (i.e., without API) using purified water as a binder and without the addition of further excipients, could already prove this assumption [87]. However, this study reported that the obtained granules bounced out of the die during the production of tablets in a continuous mode, indicating the high elasticity of the granules, which is a non-suitable property for high-speed tablet manufacturing. In addition, the resulting tablets were extremely fragile and could not fulfil the requirements of the European Pharmacopoeia [87].

1.4.3. Current state and development of smartFilms as an oral dosage form:

Up till now, there is a lack of a systemic study which identifies and describes the parameters required for a successful production of paper-based granules and their mechanical behavior under compression (i.e., production of smartFilm tablets). In addition, the influence of the smartFilm tablets on the bioactivity of the loaded drug is still ambiguous, therefore conducting studies that provide information about the drug-loaded smartFilm tablets from a biological point of view is an essential prerequisite.

Sucrose is a disaccharide sugar which composes of glucose and fructose subunits. It can be obtained from sugar cane (*Saccharum officinarum* Linne (Fam. Gramineae)), sugar beet (*Beta vulgaris* Linne (Fam. Chenopodiaceae)), and other sources [78]. Sucrose is widely used in oral pharmaceutical dosage forms as a diluent, binder, taste masking agent, sweetener, and an agent for controlled drug release (e.g., sugar beads) [92]. Sucrose syrups can be used as tablet-coating agents at concentrations between 50% and 67% (w/w) and they are widely used as vehicles in oral liquid dosage forms to enhance palatability or to increase viscosity [78,93]. In pharmaceutical granulation processes, sucrose is used as a dry binder (2–20% (w/w)) where it is granulated with water or hydroalcoholic solutions and it can also be utilized as a liquid binder (i.e., sucrose syrup) containing 50–67% (w/w) sucrose [92,94]. Sucrose can promote the adhesion of the granules of the formulation and maintain the integrity of the final tablet. Therefore, it was hypothesized that using sucrose during the formation of paper-based granules might improve the compressibility of the produced granules (i.e., decrease their elasticity and increase their plastic deformation). However, it was reported that tablets that contain large amounts of sucrose may harden, resulting in poor disintegration and inhibition of the drug release [78]. Therefore, a thorough investigation of the optimum

amounts and forms of sucrose (i.e., dry or liquid) is essential to obtain smartFilm tablets with good pharmaceutical properties in accordance with the European pharmacopoeia.

There are several poorly water-soluble drugs that can serve as ideal candidates for studying the impact of smartFilm tablets, which is produced via granulation with sucrose as a binder, on their dissolution rate and bioactivity. In this work, the selection criteria depend on the innate properties of the drug which allowed the use of simple, efficient analysis methods that can prove the bioactivity of the loaded drug.

Curcumin is a natural compound obtained from the rhizome of the turmeric plant *Curcuma longa* L.. It is a promising molecule for photodynamic therapy, and it has been widely used as a preventive therapeutic agent against numerous diseases [95]. This can be attributed to the intrinsic pharmacological characteristics of curcumin, including antioxidant, anti-inflammatory, antibacterial, antiviral, antihepatotoxic, antidepressant, and anticancer activities [96,97]. However, the therapeutic use of curcumin is limited which is mainly attributed to its poor aqueous solubility and low oral bioavailability [96,98,99]. Curcumin is considered a BCS class IV drug [33,100] and possesses autofluorescence properties, which is beneficial to trace its permeation into the tissues with the established ex vivo model in Marburg, Germany [85,101,102].

Norfloxacin is a broad-spectrum antibiotic that belongs to the class of fluoroquinolone antibiotics. It is effective against actively dividing as well as inactive Gram-positive and Gram-negative bacteria by inhibiting the bacterial DNA gyrase [103]. Norfloxacin is widely used for urinary and genital tract infection, and it is also approved for bacterial diarrhea and gonorrhea [103,104]. According to BSC, norfloxacin is classified a class IV drug with poor aqueous solubility and intestinal permeability [32], and thus only 35-45% of orally administered drug is absorbed [103,105]. In order to enhance the extent of absorption, it is essential to improve the solubility of norfloxacin.

1.5. References

- [1] Arshad, M.S.; Zafar, S.; Yousef, B.; Alyassin, Y.; Ali, R.; AlAsiri, A.; Chang, M.-W.; Ahmad, Z.; Elkordy, A.A.; Faheem, A. A review of emerging technologies enabling improved solid oral dosage form manufacturing and processing. *Adv. Drug Deliv. Rev.* **2021**, *178*, 113840, doi:10.1016/j.addr.2021.113840.
- [2] Alqahtani, M.S.; Kazi, M.; Alsenaidy, M.A.; Ahmad, M.Z. Advances in oral drug delivery. *Front. Pharmacol.* **2021**, *12*, 618411, doi:10.3389/fphar.2021.618411.
- [3] Hodayun, B.; Lin, X.; Choi, H.-J. Challenges and Recent Progress in Oral Drug Delivery Systems for Biopharmaceuticals. *Pharmaceutics* **2019**, *11*, 129, doi:10.3390/pharmaceutics11030129.
- [4] Hua, S. Advances in Oral Drug Delivery for Regional Targeting in the Gastrointestinal Tract - Influence of Physiological, Pathophysiological and Pharmaceutical Factors. *Front. Pharmacol.* **2020**, *11*, 524, doi:10.3389/fphar.2020.00524.
- [5] Savjani, K.T.; Gajjar, A.K.; Savjani, J.K. Drug solubility: importance and enhancement techniques. *Int. Sch. Res. Notices* **2012**, *2012*, 1–10, doi:10.5402/2012/195727.
- [6] Sharma, R.; Garg, R.; Sharma, R.; Kachawa, V.; Dwivedi, J. A brief review on tablet as a dosage form with special reference to fast dissolving tablets (fdts). *World j. pharm. life sci.* **2022**, *8*, 58–62.
- [7] Harbir, K. Processing technologies for pharmaceutical tablets: a review. *Int Res J Pharm* **2012**, *3*, 20–23.
- [8] Maghsoodi, M. How spherical crystallization improves direct tableting properties: a review. *Adv. Pharm. Bull.* **2012**, *2*, 253–257, doi:10.5681/apb.2012.039.
- [9] *The science of dosage form design*; Aulton, M.E., Ed., 2nd ed.; Churchill Livingstone: Edinburgh, 2002, ISBN 9780443055508.
- [10] Gohel, M.C.; Jogani, P.D. A review of co-processed directly compressible excipients. *J. Pharm. Pharm. Sci.* **2005**, *8*, 76–93.
- [11] Allen, L.; Ansel, H.C. *Ansel's pharmaceutical dosage forms and drug delivery systems*, 10th ed.; Lippincott Williams & Wilkins: Philadelphia, 2013, ISBN 1451188765.
- [12] Augsburger, L.L.; Hoag, S.W. *Pharmaceutical dosage forms-tablets*, 3rd ed.; CRC Press: Boca Raton, 2008, ISBN 9780429191794.
- [13] Parikh, D.M. *Handbook of pharmaceutical granulation technology*, 2nd ed.; CRC Press: Boca Raton, 2005, ISBN 9780429132872.
- [14] Shanmugam, S. Granulation techniques and technologies: recent progresses. *BiolImpacts* **2015**, *5*, 55–63, doi:10.15171/bi.2015.04.
- [15] Mudbidri, A. Tablet compression principles. *Pharma Times* **2010**, *42*, 44–47.

- [16] Blecher, L. Excipients-The important components. *Pharm. Process* **1995**, *12*, 6–7.
- [17] Darji, M.A.; Lalge, R.M.; Marathe, S.P.; Mulay, T.D.; Fatima, T.; Alshammari, A.; Lee, H.K.; Repka, M.A.; Narasimha Murthy, S. Excipient stability in oral solid dosage forms: a review. *AAPS PharmSciTech* **2018**, *19*, 12–26, doi:10.1208/s12249-017-0864-4.
- [18] van der Merwe, J.; Steenekamp, J.; Steyn, D.; Hamman, J. The role of functional excipients in solid oral dosage forms to overcome poor drug dissolution and bioavailability. *Pharmaceutics* **2020**, *12*, 393, doi:10.3390/pharmaceutics12050393.
- [19] Adolfsson, Å.; Caramella, C.; Nyström, C. The effect of milling and addition of dry binder on the interparticulate bonding mechanisms in sodium chloride tablets. *Int. J. Pharm.* **1998**, *160*, 187–195, doi:10.1016/s0378-5173(97)00307-4.
- [20] Lowenthal, W. Disintegration of Tablets. *J. Pharm. Sci.* **1972**, *61*, 1695–1711, doi:10.1002/jps.2600611102.
- [21] Wen, H.; Park, K. *Introduction and overview of oral controlled release formulation design*; John Wiley & Sons: Hoboken, 2010, ISBN 9780470253175.
- [22] Vasvári, G.; Kalmár, J.; Veres, P.; Vecsernyés, M.; Bácskay, I.; Fehér, P.; Ujhelyi, Z.; Haimhoffer, Á.; Rusznyák, Á.; Fenyvesi, F.; et al. Matrix systems for oral drug delivery: Formulations and drug release. *Drug Discov. Today Technol.* **2018**, *27*, 71–80, doi:10.1016/j.ddtec.2018.06.009.
- [23] Nidhi, K.; Indrajeet, S.; Khushboo, M.; Gauri, K.; Sen, D.J. Hydrotropy: A promising tool for solubility enhancement: A review. *Int. J. Drug Dev. Res.* **2011**, *3*, 26–33, doi:10.52711/2231-5713.2022.00025.
- [24] Bhalani, D.V.; Nutan, B.; Kumar, A.; Singh Chandel, A.K. Bioavailability Enhancement Techniques for Poorly Aqueous Soluble Drugs and Therapeutics. *Biomedicines* **2022**, *10*, 2055, doi:10.3390/biomedicines10092055.
- [25] Lipinski, C.A.; Lombardo, F.; Dominy, B.W.; Feeney, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* **2012**, *64*, 4–17, doi:10.1016/j.addr.2012.09.019.
- [26] Kalepu, S.; Nekkanti, V. Improved delivery of poorly soluble compounds using nanoparticle technology: a review. *Drug Deliv. Transl. Res.* **2016**, *6*, 319–332, doi:10.1007/s13346-016-0283-1.
- [27] Amidon, G.L.; Lennernäs, H.; Shah, V.P.; Crison, J.R. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharm. Res.* **1995**, *12*, 413–420, doi:10.1208/s12248-014-9620-9.

- [28] Yu, L.X.; Amidon, G.L.; Polli, J.E.; Zhao, H.; Mehta, M.U.; Conner, D.P.; Shah, V.P.; Lesko, L.J.; Chen, M.-L.; Lee, V.H.L. Biopharmaceutics classification system: the scientific basis for biowaiver extensions. *Pharm. Res.* **2002**, *19*, 921–925.
- [29] Lennernäs, H. Intestinal permeability and its relevance for absorption and elimination. *Xenobiotica* **2007**, *37*, 1015–1051, doi:10.1080/00498250701704819.
- [30] Tsume, Y.; Amidon, G.L. The biowaiver extension for BCS class III drugs: the effect of dissolution rate on the bioequivalence of BCS class III immediate-release drugs predicted by computer simulation. *Mol. Pharm.* **2010**, *7*, 1235–1243, doi:10.1021/mp100053q.
- [31] Tsume, Y.; Mudie, D.M.; Langguth, P.; Amidon, G.E.; Amidon, G.L. The Biopharmaceutics Classification System: subclasses for in vivo predictive dissolution (IPD) methodology and IVIVC. *Eur. J. Pharm. Sci.* **2014**, *57*, 152–163, doi:10.1016/j.ejps.2014.01.009.
- [32] Breda, S.A.; Jimenez-Kairuz, A.F.; Manzo, R.H.; Olivera, M.E. Solubility behavior and biopharmaceutical classification of novel high-solubility ciprofloxacin and norfloxacin pharmaceutical derivatives. *Int. J. Pharm.* **2009**, *371*, 106–113, doi:10.1016/j.ijpharm.2008.12.026.
- [33] Visser, M.R.; Baert, L.; van't Klooster, G.; Schueller, L.; Geldof, M.; Vanwelkenhuysen, I.; Kock, H. de; Meyer, S. de; Frijlink, H.W.; Rosier, J. Inulin solid dispersion technology to improve the absorption of the BCS Class IV drug TMC240. *Eur. J. Pharm. Biopharm.* **2010**, *74*, 233–238, doi:10.1016/j.ejpb.2009.10.004.
- [34] Vadlamudi, M.K.; Dhanaraj, S. Significance of excipients to enhance the bioavailability of poorly water-soluble drugs in oral solid dosage forms: A Review. *IOP Conf. Ser. Mater. Sci. Eng.* **2017**, *263*, 22023, doi:10.1088/1757-899x/263/2/022023.
- [35] Rodriguez-Aller, M.; Guillarme, D.; Veuthey, J.-L.; Gurny, R. Strategies for formulating and delivering poorly water-soluble drugs. *J. Drug Deliv. Sci. Technol.* **2015**, *30*, 342–351, doi:10.1016/j.jddst.2015.05.009.
- [36] Williams, H.D.; Trevaskis, N.L.; Charman, S.A.; Shanker, R.M.; Charman, W.N.; Pouton, C.W.; Porter, C.J.H. Strategies to address low drug solubility in discovery and development. *Pharmacol. Rev.* **2013**, *65*, 315–499, doi:10.1124/pr.112.005660.
- [37] Lam, K.W.; Xu, J.; Ng, K.M.; Wibowo, C.; Lin, G.; Luo, K.Q. Pharmaceutical salt formation guided by phase diagrams. *Ind. Eng. Chem. Res.* **2010**, *49*, 12503–12512, doi:10.1021/ie902080k.
- [38] Serajuddin, A.T.M. Salt formation to improve drug solubility. *Adv. Drug Deliv. Rev.* **2007**, *59*, 603–616, doi:10.1016/j.addr.2007.05.010.

- [39] Vioglio, P.C.; Chierotti, M.R.; Gobetto, R. Pharmaceutical aspects of salt and cocrystal forms of APIs and characterization challenges. *Adv. Drug Deliv. Rev.* **2017**, *117*, 86–110, doi:10.1016/j.addr.2017.07.001.
- [40] Shah, K.; Chauhan, D.N.; Chauhan, N.S.; Mishra, P. *Recent Advancement in Prodrugs*, 1st ed.; CRC Press: Boca Raton, 2020, ISBN 9780429328275.
- [41] Sanches, B.M.A.; Ferreira, E.I. Is prodrug design an approach to increase water solubility? *Int. J. Pharm.* **2019**, *568*, 118498, doi:10.1016/j.ijpharm.2019.118498.
- [42] Kawakami, K.; Oda, N.; Miyoshi, K.; Funaki, T.; Ida, Y. Solubilization behavior of a poorly soluble drug under combined use of surfactants and cosolvents. *Eur. J. Pharm. Sci.* **2006**, *28*, 7–14, doi:10.1016/j.ejps.2005.11.012.
- [43] Waard, H. de; Frijlink, H.W.; Hinrichs, W.L.J. Bottom-up preparation techniques for nanocrystals of lipophilic drugs. *Pharm. Res.* **2011**, *28*, 1220–1223, doi:10.1007/s11095-010-0323-3.
- [44] van Eerdenbrugh, B.; van den Mooter, G.; Augustijns, P. Top-down production of drug nanocrystals: nanosuspension stabilization, miniaturization and transformation into solid products. *Int. J. Pharm.* **2008**, *364*, 64–75, doi:10.1016/j.ijpharm.2008.07.023.
- [45] Wu, L.; Zhang, J.; Watanabe, W. Physical and chemical stability of drug nanoparticles. *Adv. Drug Deliv. Rev.* **2011**, *63*, 456–469, doi:10.1016/j.addr.2011.02.001.
- [46] Trucillo, P. Drug carriers: Classification, administration, release profiles, and industrial approach. *Processes* **2021**, *9*, 470, doi:10.3390/pr9030470.
- [47] Loftsson, T.; Brewster, M.E. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. *J. Pharm. Sci.* **1996**, *85*, 1017–1025, doi:10.1021/js950534b.
- [48] Hauss, D.J. Oral lipid-based formulations. *Adv. Drug Deliv. Rev.* **2007**, *59*, 667–676, doi:10.1016/j.addr.2007.05.006.
- [49] Ottenbrite, R.M.; Kim, S.W. *Polymeric drugs and drug delivery systems*, 1st ed.; CRC Press: Boca Raton, 2001, ISBN 9780429136405.
- [50] Francis, M.F.; Cristea, M.; Winnik, F.M. Polymeric micelles for oral drug delivery: Why and how. *Pure Appl. Chem.* **2004**, *76*, 1321–1335, doi:10.1351/pac200476071321.
- [51] Liu, P.; Chen, G.; Zhang, J. A review of liposomes as a drug delivery system: current status of approved products, regulatory environments, and future perspectives. *Molecules* **2022**, *27*, 1372, doi:10.3390/molecules27041372.
- [52] Phan, S.; Salentinig, S.; Prestidge, C.A.; Boyd, B.J. Self-assembled structures formed during lipid digestion: characterization and implications for oral lipid-based drug delivery systems. *Drug Deliv. Transl. Res.* **2014**, *4*, 275–294, doi:10.1007/s13346-013-0168-5.

- [53] Dahan, A.; Hoffman, A. Rationalizing the selection of oral lipid based drug delivery systems by an in vitro dynamic lipolysis model for improved oral bioavailability of poorly water soluble drugs. *J. Control. Release* **2008**, *129*, 1–10, doi:10.1016/j.jconrel.2008.03.021.
- [54] Brewster, M.E.; Loftsson, T. Cyclodextrins as pharmaceutical solubilizers. *Adv. Drug Deliv. Rev.* **2007**, *59*, 645–666, doi:10.1002/jps.21861.
- [55] Yano, K.; Masaoka, Y.; Kataoka, M.; Sakuma, S.; Yamashita, S. Mechanisms of membrane transport of poorly soluble drugs: role of micelles in oral absorption processes. *J. Pharm. Sci.* **2010**, *99*, 1336–1345, doi:10.1002/jps.21919.
- [56] Keskin, D.; Tezcaner, A. Micelles as delivery system for cancer treatment. *Curr. Pharm. Des.* **2017**, *23*, 5230–5241, doi:10.2174/1381612823666170526102757.
- [57] Kim, D.-H.; Lee, S.-E.; Pyo, Y.-C.; Tran, P.; Park, J.-S. Solubility enhancement and application of cyclodextrins in local drug delivery. *J. Pharm. Investig.* **2020**, *50*, 17–27, doi:10.1007/s40005-019-00434-2.
- [58] Hu, J.; Johnston, K.P.; Williams III, R.O. Nanoparticle engineering processes for enhancing the dissolution rates of poorly water soluble drugs. *Drug Dev. Ind. Pharm.* **2004**, *30*, 233–245, doi:10.1081/ddc-120030422.
- [59] Merisko-Liversidge, E.M.; Liversidge, G.G. Drug nanoparticles: formulating poorly water-soluble compounds. *Toxicol. Pathol.* **2008**, *36*, 43–48, doi:10.1177/0192623307310946.
- [60] Grunenberga, A.; Henck, J.-O.; Siesler, H.W. Theoretical derivation and practical application of energy/temperature diagrams as an instrument in preformulation studies of polymorphic drug substances. *Int. J. Pharm.* **1996**, *129*, 147–158, doi:10.1016/0378-5173(95)04283-0.
- [61] Datta, S.; Grant, D.J.W. Crystal structures of drugs: advances in determination, prediction and engineering. *Nat. Rev. Drug Discov.* **2004**, *3*, 42–57, doi:10.1038/nrd1280.
- [62] Pudipeddi, M.; Serajuddin, A.T.M. Trends in solubility of polymorphs. *J. Pharm. Sci.* **2005**, *94*, 929–939, doi:10.1002/jps.20302.
- [63] Blagden, N.; Matas, M. de; Gavan, P.T.; York, P. Crystal engineering of active pharmaceutical ingredients to improve solubility and dissolution rates. *Adv. Drug Deliv. Rev.* **2007**, *59*, 617–630, doi:10.1016/j.addr.2007.05.011.
- [64] Thakuria, R.; Delori, A.; Jones, W.; Lipert, M.P.; Roy, L.; Rodríguez-Hornedo, N. Pharmaceutical cocrystals and poorly soluble drugs. *Int. J. Pharm.* **2013**, *453*, 101–125, doi:10.1016/j.ijpharm.2012.10.043.

- [65] Varshosaz, J.; Ghassami, E.; Ahmadipour, S. Crystal engineering for enhanced solubility and bioavailability of poorly soluble drugs. *Curr. Pharm. Des.* **2018**, *24*, 2473–2496, doi:10.2174/1381612824666180712104447.
- [66] Newman, A. *Pharmaceutical amorphous solid dispersions*, 1st ed.; John Wiley & Sons: New Jersey, 2015, ISBN 9781118901380.
- [67] Vasanthavada, M.; Gupta, S.S.; Tong, W.-Q.T.; Serajuddin, A.T.M. *Water-Insoluble Drug Formulation: Development of solid dispersion for poorly water-soluble drugs*, 3rd ed.; CRC Press: Boca Raton, 2018, ISBN 9781315120492.
- [68] Baghel, S.; Cathcart, H.; O'Reilly, N.J. Polymeric amorphous solid dispersions: a review of amorphization, crystallization, stabilization, solid-state characterization, and aqueous solubilization of biopharmaceutical classification system class II drugs. *J. Pharm. Sci.* **2016**, *105*, 2527–2544, doi:10.1016/j.xphs.2015.10.008.
- [69] Lachman, L.; Lieberman, H.A.; Kanig, J.L. *The theory and practice of industrial pharmacy*; Lea & Febiger: Philadelphia, 1976, ISBN 081210532X.
- [70] Zhou, D. Understanding physicochemical properties for pharmaceutical product development and manufacturing II: physical and chemical stability and excipient compatibility. *J. Valid. Technol.* **2009**, *15*, 36.
- [71] Chaudhari, S.P.; Patil, P.S. Pharmaceutical excipients: a review. *Int J Adv Pharm Biol Chem* **2012**, *1*, 21–34.
- [72] Cotton, M.L.; Wu, D.W.; Vadas, E.B. Drug-excipient interaction study of enalapril maleate using thermal analysis and scanning electron microscopy. *Int. J. Pharm.* **1987**, *40*, 129–142, doi:10.1016/0378-5173(87)90058-5.
- [73] Al-Nimry, S.S.; Assaf, S.M.; Jalal, I.M.; Najib, N.M. Adsorption of ketotifen onto some pharmaceutical excipients. *Int. J. Pharm.* **1997**, *149*, 115–121, doi:10.1016/s0378-5173(96)04857-0.
- [74] Eyjolfsson, R. Lisinopril-lactose incompatibility. *Drug Dev. Ind. Pharm.* **1998**, *24*, 797, doi:10.3109/03639049809082729.
- [75] Jackson, K.; Young, D.; Pant, S. Drug-excipient interactions and their affect on absorption. *Pharm. Sci. Technol. Today* **2000**, *3*, 336–345, doi:10.1016/s1461-5347(00)00301-1.
- [76] Fitzpatrick, S.; McCabe, J.F.; Petts, C.R.; Booth, S.W. Effect of moisture on polyvinylpyrrolidone in accelerated stability testing. *Int. J. Pharm.* **2002**, *246*, 143–151, doi:10.1016/s0378-5173(02)00375-7.

- [77] Laroque, D.; Inisan, C.; Berger, C.; Vouland, É.; Dufossé, L.; Guérard, F. Kinetic study on the Maillard reaction. Consideration of sugar reactivity. *Food Chem.* **2008**, *111*, 1032–1042, doi:10.1016/j.foodchem.2008.05.033.
- [78] Rowe, R.C.; Sheskey, P.; Quinn, M. *Handbook of pharmaceutical excipients*, 6th ed.; Pharmaceutical Press: London, 2009, ISBN 9780853697923.
- [79] Bindra, D.S.; Stein, D.; Pandey, P.; Barbour, N. Incompatibility of croscarmellose sodium with alkaline excipients in a tablet formulation. *Pharm. Dev. Technol.* **2014**, *19*, 285–289, doi:10.3109/10837450.2013.778869.
- [80] Panakanti, R.; Narang, A.S. Impact of excipient interactions on drug bioavailability from solid dosage forms. *Pharm. Res.* **2012**, *29*, 2693–2659, doi:10.1007/s11095-012-0767-8.
- [81] Patel, S.; Kaushal, A.M.; Bansal, A.K. The effect of starch paste and sodium starch glycolate on the compaction behavior of wet granulated acetaminophen formulations. *J. Excip. Food Chem.* **2016**, *2*, 1120.
- [82] Lemke, S.; Strätling, E.J. SmartFilms-oral and peroral films for optimized delivery of nanoparticulate or amorphous drugs. *Controlled Release Society Local Chapter, Saarbrücken/Germany* **2016**, *7*.
- [83] Lemke, S.; Strätling, E.J.; Welzel, H.P. Cellulosefaserbasierte Trägermatrices (smartFilms) zur Applikation von Inhaltsstoffen sowie deren Herstellung. *German Patent Application DE102016000541A1* **2017**.
- [84] Eckert, R.W.; Wiemann, S.; Keck, C.M. Improved dermal and transdermal delivery of curcumin with smartfilms and nanocrystals. *Molecules* **2021**, *26*, 1633, doi:10.3390/molecules26061633.
- [85] Keck, C.M.; Abdelkader, A.; Pelikh, O.; Wiemann, S.; Kaushik, V.; Specht, D.; Eckert, R.W.; Alnemari, R.M.; Dietrich, H.; Brüßler, J. Assessing the Dermal Penetration Efficacy of Chemical Compounds with the Ex-Vivo Porcine Ear Model. *Pharmaceutics* **2022**, *14*, 678, doi:10.3390/pharmaceutics14030678.
- [86] Stumpf, F.; Keck, C.M. Tablets made from paper. *Int. J. Pharm.* **2018**, *548*, 812–819, doi:10.1016/j.ijpharm.2018.05.071.
- [87] Stumpf, F. Tabletten aus Papier – tablets made from paper – zur oralen Applikation schwerlöslicher Wirkstoffe. PhD-thesis; Philipps-Universität Marburg, Marburg, 2019.
- [88] Ornik, J.; Knoth, D.; Koch, M.; Keck, C.M. Terahertz-spectroscopy for non-destructive determination of crystallinity of L-tartaric acid in smartFilms® and tablets made from paper. *Int. J. Pharm.* **2020**, *581*, 119253, doi:10.1016/j.ijpharm.2020.119253.
- [89] Ornik, J.; Heidrich, L.; Schesny, R.; Castro-Camus, E.; Keck, C.M.; Koch, M. Non-destructive crystallinity assessment of indomethacin in tablets made from smartFilms® using

- terahertz time-domain spectroscopy. *Sci. Rep.* **2022**, *12*, 1–7, doi:10.1038/s41598-022-10041-1.
- [90] Subrahmanyeswari, C.D.; Prasanth, Y.; Sameeda, R. Formulation and development of efavirenz tablets by paper technique using co-solvency method. *Int. J. Curr. Pharm. Res.* **2019**, *11*, 87–92, doi:10.22159/ijcpr.2019v11i6.36349.
- [91] Sirisha, V.R.; Sri, K.V.; Suresh, K.; Reddy, G.K.; Devanna, N. A review of pellets and pelletization process-A multiparticulate drug delivery system. *Int. J. Pharm. Sci. Res.* **2013**, *4*, 2145, doi:10.13040/ijpsr.0975-8232.4(6).2145-58.
- [92] Santomaso, A.C.; Baggio, R.; Zorzi, F.; Salviulo, G.; Realdon, N.; Franceschinis, E. Sugars with different thickening power in high shear granulation. *Powder Technol.* **2017**, *317*, 391–399, doi:10.1016/j.powtec.2017.05.017.
- [93] Cooper, J.M. A question of taste-uses of sucrose. *Manuf Chem* **2003**, *74*, 71–72, 74.
- [94] Allen, L.V. Featured excipient: capsule and tablet diluents. *Int. J. Pharm. Compd.* **2000**, *4*, 306–312.
- [95] Tsuda, T. Curcumin as a functional food-derived factor: degradation products, metabolites, bioactivity, and future perspectives. *Food Funct.* **2018**, *9*, 705–714, doi:10.1039/c7fo01242j.
- [96] Hewlings, S.J.; Kalman, D.S. Curcumin: A review of its effects on human health. *Foods* **2017**, *6*, 92, doi:10.3390/foods6100092.
- [97] Kotha, R.R.; Luthria, D.L. Curcumin: Biological, pharmaceutical, nutraceutical, and analytical aspects. *Molecules* **2019**, *24*, 2930, doi:10.3390/molecules24162930.
- [98] Paolino, D.; Vero, A.; Cosco, D.; Pecora, T.M.G.; Cianciolo, S.; Fresta, M.; Pignatello, R. Improvement of oral bioavailability of curcumin upon microencapsulation with methacrylic copolymers. *Front. Pharmacol.* **2016**, *7*, 485, doi:10.3389/fphar.2016.00485.
- [99] Jantarat, C.; Sirathanarun, P.; Boonmee, S.; Meechoosin, W.; Wangpittaya, H. Effect of piperine on skin permeation of curcumin from a bacterially derived cellulose-composite double-layer membrane for transdermal curcumin delivery. *Sci. Pharm.* **2018**, *86*, 39, doi:10.3390/scipharm86030039.
- [100] Wang, Y.; Wang, C.; Zhao, J.; Ding, Y.; Li, L. A cost-effective method to prepare curcumin nanosuspensions with enhanced oral bioavailability. *J. Colloid Interface Sci.* **2017**, *485*, 91–98, doi:10.1016/j.jcis.2016.09.003.
- [101] Pelikh, O.; Pinnapireddy, S.R.; Keck, C.M. Dermal Penetration Analysis of Curcumin in an ex vivo Porcine Ear Model Using Epifluorescence Microscopy and Digital Image Processing. *Skin Pharmacol. Physiol.* **2021**, *34*, 281–299, doi:10.1159/000514498.

- [102] Keck, C.M.; Chaiprateep, E.; Dietrich, H.; Sengupta, S. Influence of Mechanical Skin Treatments on Dermal Penetration Efficacy of Active Ingredients. *Pharmaceutics* **2022**, *14*, 1788, doi:10.3390/pharmaceutics14091788.
- [103] Dua, K.; Ramana, M.V.; Singh Sara, U.V.; Himaja, M.; Agrawal, A.; Garg, V.; Pabreja, K. Investigation of enhancement of solubility of norfloxacin β -cyclodextrin in presence of acidic solubilizing additives. *Curr. Drug Deliv.* **2007**, *4*, 21–25, doi:10.2174/156720107779314776.
- [104] Takács-Novák, K.; Noszál, B.; Hermecz, I.; Keresztúri, G.; Podányi, B.; Szasz, G. Protonation equilibria of quinolone antibacterials. *J. Pharm. Sci.* **1990**, *79*, 1023–1028, doi:10.1002/jps.2600791116.
- [105] Fael, H.; Barbas, R.; Prohens, R.; Ràfols, C.; Fuguet, E. Synthesis and characterization of a new norfloxacin/resorcinol cocrystal with enhanced solubility and dissolution profile. *Pharmaceutics* **2021**, *14*, 49, doi:10.3390/pharmaceutics14010049.

Chapter 2

Aim of work

Aim of work

The overall aim of this work was to introduce smartFilm tablets as a promising, industrially feasible and cost-effective approach for an improved solubility and an enhanced bioactivity of poorly soluble drugs. The aim of this thesis was fulfilled in three subsequent steps:

- The first step was to obtain paper-based granules that can be used for the production of smartFilm tablets on a large, industrial scale. For that, the most suitable sucrose amounts and forms, the granulation parameters, and the granules mechanical behavior under compression (i.e., production of smartFilm tablets) were investigated. In addition, the pharmaceutical properties of the obtained unloaded smartFilm tablets were studied (**cf. Chapter 3, section 3.1**).
- The second step was to assess the ability of smartFilm tablets to improve the oral delivery of a poorly water-soluble drug. For that, curcumin, which is a BSC-class IV drug with poor solubility and low intestinal permeability, was selected for the production of curcumin-loaded smartFilm tablets. The tablets were characterized regarding their physico-chemical and pharmaceutical properties, and the intestinal permeability of curcumin was determined with an ex vivo porcine intestinal model. The ex vivo intestinal permeability of curcumin from the smartFilm tablets was compared to a physical mixture of curcumin and paper and to a classical and to an innovative commercial product, respectively (**cf. Chapter 3, section 3.2**).
- The third step was to determine the ability of smartFilms to improve the bioactivity of smartFilm tablets loaded with a poorly water-soluble drug. For that, norfloxacin, which is a BSC-class IV drug with poor solubility and intestinal permeability was used as model drug for the production of norfloxacin-loaded smartFilm tablets. The crystalline state of norfloxacin loaded within the matrix of smartFilm tablets as well as the pharmaceutical properties of the produced tablets were investigated. The bioactivity of smartFilm tablets was assessed by determining the antimicrobial activity of norfloxacin in vitro and in an infected ex vivo porcine intestinal model. Results were also compared to the antimicrobial activity of a physical mixture of norfloxacin and paper (**cf. Chapter 3, section 3.3**).

Chapter 3

Results

3.1. Tablets Made from Paper—An Industrially Feasible Approach

Ayat Abdelkader ^{1,2}, Christoph Moos ³, Adrien Pelloux ⁴, Marcus Pfeiffer ³, Christian Alter ³,
Stefan Kolling ³ and Cornelia M. Keck ¹

¹ Department of Pharmaceutics and Biopharmaceutics, Philipps-Universität Marburg, Robert-Koch-Str. 4, 35037 Marburg, Germany.

² Assiut International Center of Nanomedicine, Al-Rajhi Liver Hospital, Assiut University, Assiut 71515, Egypt.

³ Institute of Mechanics and Materials, Technische Hochschule Mittelhessen, Wiesenstr. 14, 35390 Giessen, Germany.

⁴ MEDELPHARM, Science Lab, rue du Chat Botté 615, 01700 Beignost, France.

(adapted from *Pharmaceuticals* 2022, 15(10), 1188)

Abstract

Many orally administrated drugs exhibit poor bioavailability due to their limited solubility. The smartFilms technology is an innovative approach to improve the drug aqueous solubility, where the drug is embedded within the matrix of cellulose-based paper in an amorphous state, hence increasing its solubility. Despite its proven effectiveness, smartFilms, i.e., pieces of paper, exhibit limited flowability and are not easy to swallow, and thus oral administration is not convenient. In addition, there is a lack of knowledge of their mechanical behavior under compression. This study aimed to transform unloaded smartFilms, i.e., paper, into a flowable physical form and investigated its mechanical behavior when compressed. Granules made of paper were prepared via wet granulation and were compressed into tablets. The influence of using different amounts and forms of sucrose, as a binder, on the pharmaceutical properties of the produced granules and tablets was studied and the most suitable composition was identified by using instrumented die experiments. For this, the Poisson's ratio and Young's modulus were determined for different compaction force levels and the deformation behavior was estimated with the Heckel mathematical model. All granule batches showed good flowability with angle of repose values between 25–35°. Granule batches with ≤30% dry sucrose content produced tablets that fulfilled the European Pharmacopoeia requirements, and the compaction behavior of the granules was found to be comparable to the behavior of classical binders and compression enhancers. Paper can be transferred into granules. These granules can be used as suitable intermediate products for the production of tablets made of paper in large, industrial scale.

1. Introduction

Oral administration is the most common route of drug delivery, with advantages including high patient compliance, low risk of infection and minimal sterility constraints, which simplifies the production process and reduces costs [1]. Efficient oral delivery of various active pharmaceutical ingredients (API) is extremely influenced by their physicochemical and/or biopharmaceutical properties, e.g., solubility, chemical stability, intestinal permeability, metabolic stability, etc.

Recently, smartFilms were introduced as a novel oral drug delivery system that can incorporate API in an amorphous state within the matrix of cellulose-based paper, leading to the improvement of the dissolution rate and the kinetic solubility of the API [2]. SmartFilms can be obtained by dissolving a poorly soluble API into a suitable solvent and by adding this solution to a cellulose-based matrix, i.e., paper. After drying, the paper, i.e., the resulting “smartFilm”, contains the API in amorphous state, resulting in an enhanced dissolution rate and kinetic solubility of the API [2,3]. Despite being a promising oral drug delivery system, the administration of paper sheets (i.e., smartFilms) is not convenient for the patient. Due to this, the transfer of the smartFilms into appropriate oral dosage forms is important for improving patient compliance. Several studies already reported the successful compression of drug-loaded smartFilms into tablets without the addition of any excipients and demonstrated that the produced tablets fulfilled all requirements of the European Pharmacopoeia.[6–4]

Nevertheless, paper sheets and smartFilms in their original state exhibit inadequate flowability, rendering them unsuitable candidates for high-speed tablet manufacturing and large-scale tablet production [4]. In light of this, the transformation of smartFilms into a free-flowing physical form along with understanding their mechanical behavior under compression is a major prerequisite for a wider exploitation of the smartFilms technology, because it opens the possibility to produce smartFilm tablets on an industrial, large scale.

Granulation is a key processing step in the production of many pharmaceutical tablets [7]. In comparison to raw powders, granules exhibit enhanced flowability and compaction characteristics, and thus can be compressed easily into tablets with a uniform API content [7]. Consequently, the transformation of paper and/or smartFilms into granules was expected to improve their limited flowability.

Preliminary data, that produced granules from paper without API (i.e., unloaded paper granules) without further excipients while using purified water as binder, could already

prove this assumption but showed that the resulting granules possess a high elasticity. The high elasticity of the granules proved to be a non-suitable characteristic for the production of tablets in continuous mode, because during the production, the granules jumped out of the die. In addition, the resulting tablets were too soft and could not fulfil the requirements of the European Pharmacopoeia [8]. Therefore, to decrease the elasticity and to increase the plastic deformation of the paper-based granules, it was hypothesized that the addition of sucrose might improve the compressibility of the paper granules. The aim of this study was to prove or disprove this theory and to identify the most suitable sucrose contents and the most suitable production process for granules made of paper that can be used for the production of smartFilm tablets in large, industrial scale.

Sucrose can be used as a dry binder, i.e., prior to granulation, it is added as dry powder to the other ingredients of the tablet. However, it can also be utilized as a liquid binder. In this case, aqueous sucrose solutions, containing 50–67% (w/w) sucrose, are used as granulation liquid for the granulation of the powdered ingredients of the tablet [9]. In order to identify the most suitable process, granules made of paper were produced with different sucrose contents and by using sucrose as either dry binder (batches B2–B6, Table 1) or liquid binder (batches B7–B11, Table 1), respectively. B1 granules were composed of paper without sucrose and served as benchmark control. B2–B6 granules were produced while using sucrose as dry binder and B7–B11 granules were produced while using sucrose as liquid binder, i.e., aqueous solutions of sucrose. The sucrose content was varied from 10% to 50%, respectively (Table 1).

Table 1. Overview of compositions of the different batches of paper granules produced.

Batch Code	Sucrose Content (Dry Form)	Granulation Liquid
B1	-	distilled water
B2	10%	distilled water
B3	20%	distilled water
B4	30%	distilled water
B5	40%	distilled water
B6	50%	distilled water
B7	-	10% sucrose solution
B8	-	20% sucrose solution
B9	-	30% sucrose solution
B10	-	40% sucrose solution
B11	-	50% sucrose solution

After the production of the granules, size, shape, and the pharmaceutical properties according to the European Pharmacopoeia, i.e., bulk density, tapped density, Hausner's ratio, Carr's index and angle of repose were determined [10]. In the next step, the paper granules were

used for the production of tablets from which the pharmaceutical properties according to the European Pharmacopoeia were determined .

In addition, the mechanical behavior of paper-based granules under compression was investigated and compared to the properties of conventional binders .

Mechanical properties, such as elastic and plastic behaviors, play important roles for the understanding of the granule's mechanical behavior under compression. In addition, mechanical characterization is also pivotal for the simulation modeling of pharmaceutical materials (e.g., granules) under compression. The use of simulation modelling in pharmaceutical industry is becoming not only important to improve the quality of the product but can also be used to understand the influence of materials and processes on the final pharmaceutical product properties. In addition, it can also help to reduce the productions costs [11]. Therefore, in the second step of this study, instrumented die compression tests were performed to characterize the mechanical behavior of the paper granules under compression .[12]

2. Materials and Methods

2.1. Materials

A commercially available, cellulose-based paper (Soft & Sicher, dm-drogerie markt GmbH + Co. KG, Karlsruhe, Germany) was used to produce the granules and tablets. Sucrose was purchased from Carl Roth GmbH + Co. KG (Karlsruhe, Germany). Purified water was freshly obtained from a PURELAB Flex 2 (ELGA LabWater, Veolia Water Technologies GmbH, Celle, Germany).

2.2. Methods

2.2.1 .Production of Paper Granules

Paper granules were prepared via the wet granulation method. Distilled water or sucrose aqueous solutions with various concentrations (10, 20, 30, 40 and 50% w/w) were used as a granulation liquid. To prepare paper granules, a paper sheet that weighted around 2.50 g, was dry milled for 1 min by using a knife mill (Moulinex DP8108, Groupe SEB Deutschland GmbH, Frankfurt, Germany). The milled paper was used to prepare 11 granule batches coded as B1–B11 (Table 1). B1 was the control, i.e., paper granules without sucrose. For the batches B2–B6 sucrose (10%, 20%, 30%, 40% and 50%) was used as dry binder and for the batches B7–B11 sucrose (10%, 20%, 30%, 40% and 50%) was used as liquid binder. B1 was prepared by spraying

small amounts of water onto the milled paper to wet the paper and to increase its density. The wetted mixture was again milled for 1 min, transferred to a plastic sieve, wetted with distilled water to obtain snowball consistency while shaking at 300 rpm in a universal shaker SM-30 control (Edmund Bühler GmbH, Bodelshausen, Germany) for a period of 3–8 min. The wet granules were dried in a drying oven (Heraeus GmbH, Hanau, Germany) at 70 °C overnight. Afterwards, the granules were sieved (mesh size 2.8 mm, Retsch GmbH, Haan, Germany) to obtain a size fraction ≤ 2.8 mm. B2–B6 were produced by adding fine sucrose powder to the dry milled paper to obtain dry paper/sucrose mixtures that contained 10%, 20%, 30%, 40% and 50% (w/w) sucrose. The mixtures were further processed as described for B1. B7–B11 were produced by adding sucrose aqueous solutions to the dry milled paper to obtain mixtures that contain 10%, 20%, 30%, 40% and 50% (w/w) sucrose, respectively. Afterwards, they were processed as described for B1, while using the respective sucrose solutions instead of purified water.

2.2.2 .Characterization of Paper Granules

Particle Size and Shape Analysis

The Feret's diameter of the granules was determined via digital image analysis using ImageJ software (National Institutes of Health, Bethesda, MD, USA) as described previously [34], with slight modifications. For each batch, 10 representative images that contained approximately 200–300 granules were taken by using a Canon IXUS 190 digital camera (Canon Europe Ltd., Uxbridge, UK). The images were color-adjusted, and threshold analysis was performed to label the granules individually. Afterwards, the Feret's diameter of each granule was assessed by the software (Supplementary Material, Table S1). From the results obtained the number based median particle size distribution, i.e., $d(n) 0.10$, $d(n) 0.50$, $d(n) 0.90$, $d(n) 0.95$ and $d(n) 0.99$, was calculated. In addition, the sphericity of the granules was determined via calculating the aspect ratio as follows:

$$\text{Aspect ratio} = \frac{d_{\max}(\text{Feret})}{d_{90^\circ}(\text{dmax})} \quad (1)$$

where $d_{\max}(\text{Feret})$ is the maximum Feret's diameter and $d_{90^\circ}(\text{dmax})$ is the Feret's diameter perpendicular to $d_{\max}(\text{Feret})$.

Determination of Bulk and Tapped Density

A mechanical tapping device, the tap density tester TD200 (Pharma Test Apparatebau AG, Hainburg, Germany), was used to determine the bulk and tapped density of various batches

of the granules according to the test method 2.9.34 of the European Pharmacopoeia [10]. A total of 10 g of the granules were placed into a 250 mL measuring cylinder. The starting volume and the final volume, after carrying out 10, 500 and 1250 taps on the same granules sample, were recorded and used to calculate the bulk and tapped density, respectively. In addition, the flowability of the granules was determined from the tapped and bulk density by calculating Hausner's ratio and the Carr's index using the following equations :[35]

$$\text{Hausner's ratio} = \frac{\rho_{\text{tapped}}}{\rho_{\text{bulk}}} \quad (2)$$

$$\text{Carr's index} = 100 \left(\frac{\rho_{\text{tapped}} - \rho_{\text{bulk}}}{\rho_{\text{tapped}}} \right) \quad (3)$$

where ρ_{tapped} is the tapped density and ρ_{bulk} is the bulk density.

Determination of Angle of Repose

The angle of repose of the granules was determined by using the flowability tester (Karg GmbH, Baden-Württemberg, Germany) according to the test method 2.9.36 of the European Pharmacopoeia [10]. A total of 20 g of the granules from various batches were filled into the funnel of the tester. Then, the granules were stirred carefully to run through the funnel and accumulate on a fixed base to form a heap. The height of the granules heap was measured and the angle of repose (" α ") was determined using the following equation:

$$\tan \alpha = \frac{h}{0.5 d_b} \quad (4)$$

where h is the height of the heap and d_b is the diameter of the base.

2.2.3 .Production of Tablets Made from Paper

Flat-faced bevel-edged tablets were produced by applying compression forces of about 30 kN by using a single punch tablet press (EK0, Korsch GmbH, Berlin, Germany) equipped with a 10 mm flat-faced punch (Ritter Pharma-Technik GmbH, Stapelfeld, Germany). Subsequently, the properties of the produced tablets were assessed by subjecting the tablets to various tests, as described in the European Pharmacopoeia [10]. Details of the methods used are given below.

2.2.4 .Characterization of tablets made from paper

Thickness

A total of 10 tablets were selected randomly from each batch and the thickness was determined using IP67 ABS digimatic caliper (Mitutoyo, Kanagawa, Japan) .

Mass Uniformity

Mass uniformity was evaluated according to the test method 2.9.5. of the European Pharmacopoeia [10]. A total of 20 tablets were randomly selected from each batch and weighed. Then, the average mass was calculated and compared to the mass of each individual tablet to determine the percentage deviation; following this, the results were compared to the European Pharmacopoeia limits.

Friability

Friability of the tablets was determined according to test method 2.9.7 of the European Pharmacopoeia [10] by using a friability tester equipped with an abrasion drum (PTF 10ER, Pharma Test Apparatebau AG, Hainburg, Germany). From each batch, 20 tablets were randomly selected, weighed, then placed into a drum rotating at 25 rpm for 4 min. Following that, the tablets were removed, dedusted, reweighed and the percentage weight loss was calculated using the following equation:

$$\% \text{ weight loss} = 100 \left(\frac{W_1 - W_2}{W_1} \right) \quad (5)$$

where W1 is the weight of the tablets before test and W2 is the weight of the tablets after test.

Resistance to Crushing

The crushing strength or hardness of a tablet is the force required to break down a tablet under compression and it was evaluated according to the test method 2.9.8. of the European Pharmacopoeia [10]. In this study, 10 tablets were randomly selected from each batch and each tablet was placed horizontally between the jaws of a hardness tester PTB 311E (Pharma Test Apparatebau AG, Hainburg, Germany). The results obtained were expressed in Newton (N) as mean, minimum and maximum values of the forces measured .

Disintegration

Disintegration of the tablets was evaluated in water according to the test method 2.9.1 of the European Pharmacopoeia [10]. From each batch, 6 tablets were individually placed into the cavities of a disintegration tester PTZ-S (Pharma Test Apparatebau AG, Hainburg, Germany) that was operated at $37^\circ\text{C} \pm 2^\circ\text{C}$. The time required for complete tablet disintegration was recorded and compared to the limits of the European Pharmacopoeia.[10]

Mechanical Behavior of Paper Granules under Compression

The mechanical characterization was performed by using the single punch tableting press (Styl'One Evo, Medelpharm, Beynost, France) via displacement control. Axial forces of the upper and lower punch were measured using strain gauges and axial displacements were determined by a potentiometric displacement transducer. The global axial elastic deformation of the machine was considered when evaluating the measurement data of the tests. Radial stress components on the die wall were measured using strain gauges attached to the die. Calibration of the instrumented die was conducted by the compression of a nearly incompressible elastomer.

Standard Euro B flat-faced punches with a diameter of 11.28 mm were used for the tests. The maximum possible filling height of the die was 23.5 mm and 250 mg of paper granules were manually filled into the die for each compression test. To determine the strain rate effects, experiments were carried out at low (0.1 mm/s) and high (500 mm/s) displacement velocities. Die wall friction (μ) was calculated during the instrumented die tests using Coulomb's friction law:

$$\mu = \frac{D(\sigma_{up} - \sigma_{lo})}{4h\sigma_{rad}} \quad (6)$$

where D is the diameter of the die, h is the tablet height, σ_{up} and σ_{lo} are the upper and lower punch stress, respectively, and σ_{rad} is the radial die wall stress[30]

Double-compaction tests were carried out to identify Young's modulus and Poisson's ratio at different compaction force levels [23]. The granules were pre-compacted, unloaded and directly followed by a main compaction (i.e., no ejection of the produced tablets during the entire process). The pre-compaction maximum force was set to 90% of the desired main-compaction force. During the loading of the main-compaction, linear elasticity was assumed when the forces remain lower than the maximum pre-compaction force and allows for linear stress—strain relations. A detailed description of the process and the determination of the constants is described in Mazel et al., 2012 [12]. The porosity at the different stress levels, at which the values of the elastic constants (i.e., Young's modulus and Poisson's ratio) were determined, was calculated. This allows for the interpolation of the elastic constant values with porosity during the compression. The true density ρ_0 was determined before the tests by a gas pycnometer (AccuPyc II 1340, Micromeritics, Norcross, GA, USA) and was used to calculate the porosity of the materials (ϕ), where ρ is the actual density.

$$\phi = 1 - \frac{\rho}{\rho_0} \quad (7)$$

Finally, the Heckel Plot was used to determine the yield pressure, as it is widely used for the characterization of the compression and deformation behavior of powders .

$$\ln \left(\frac{1}{\phi} \right) = kP + A \quad (8)$$

where constant k is the slope of the linear plastic deformation section and A is the intercept and is suggested to represent particle rearrangement. The reciprocal of the k is the mean yield pressure P_y , defining the pressure at which the material undergoes plastic deformation. The Heckel plot is used to describe the volume reduction of the paper granules during compression, as the porosity is proportional to hydrostatic pressure P (i.e., compression pressure).

$$P = \frac{\sigma_{ax} + \sigma_{rad} + \sigma_{rad}}{3} \quad (9)$$

With axial stress σ_{ax} and radial stress σ_{rad} measured during the instrumented die test.

2.2.5 .Statistical Analysis

Experiments were performed in triplicates unless otherwise noted and descriptive statistics were calculated using Microsoft Excel® and are reported as mean \pm standard deviation (SD). Normal distribution (Shapiro-Wilk test) and variance homogeneity (Levene's test) were determined for each data set prior to statistical assessment of differences between the granule batches. The comparison of the means was performed by two-sided Student's t-test for pairwise comparison or one-way analysis of variance and post-hoc tests with Tukey correction using JASP software, version 0.16.2 [36]. Differences between means were considered statistically significant if the p value was <0.05 .

3. Results and Discussion

3.1 .Preparation and Characterization of Paper Granules

The paper granules without sucrose possessed a particle size of 3 mm (Table 2). The addition of sucrose resulted in slightly larger sizes. No significant differences ($p > 0.05$) were observed between the batches where sucrose was used as dry or liquid binder (Table 2). Figure 1 shows the numeric particle size distributions of the different paper granules. The results indicate similar $d(n) 0.9$, $d(n) 0.95$ and $d(n) 0.99$ values for all the prepared granules either with or without sucrose, with a slight trend towards larger sizes for the granules that were prepared

Chapter 3. Results

with the liquid sucrose binder. An aspect ratio in the range of 1.38–1.52 was observed for all the prepared granules either with or without sucrose. According to literature, a value of 1.2 is considered to present spherical particles. With this, data indicate that the paper granules possess a slightly elongated shape.[13]

Table 2. Physico-chemical properties of the different paper granules produced in this study.

Batch Code	Feret's Diameter (mm)	Bulk Density (g/cm ³)	Tapped Density (g/cm ³)	Hausner's Ratio	Carr's Index (%)	Angle of Repose
B1	3.0 ± 1.2	0.11 ± 0.003	0.13 ± 0.003	1.12 ± 0.01	11.4 ± 0.4	43° ± 4
B2	3.4 ± 0.67	0.12 ± 0.001	0.14 ± 0.001	1.15 ± 0.00	13.3 ± 0.1	34° ± 4
B3	3.3 ± 0.85	0.14 ± 0.003	0.15 ± 0.002	1.13 ± 0.04	11.7 ± 3.6	31° ± 0
B4	3.2 ± 0.79	0.14 ± 0.01	0.17 ± 0.01	1.16 ± 0.02	13.8 ± 2.0	31° ± 0
B5	3.4 ± 0.83	0.17 ± 0.008	0.20 ± 0.006	1.13 ± 0.00	11.4 ± 0.5	25° ± 5
B6	3.3 ± 0.89	0.21 ± 0.005	0.22 ± 0.007	1.13 ± 0.03	11.9 ± 2.8	25° ± 5
B7	3.3 ± 0.90	0.15 ± 0.015	0.18 ± 0.017	1.14 ± 0.00	13.0 ± 0.0	34° ± 4
B8	3.3 ± 0.88	0.16 ± 0.004	0.18 ± 0.004	1.15 ± 0.01	13.1 ± 0.9	31° ± 0
B9	3.0 ± 0.86	0.18 ± 0.001	0.20 ± 0.005	1.13 ± 0.03	11.8 ± 2.3	31° ± 0
B10	3.55 ± 0.98	0.20 ± 0.017	0.22 ± 0.014	1.13 ± 0.03	11.7 ± 2.3	25° ± 5
B11	3.41 ± 6.75	0.17 ± 0.016	0.20 ± 0.014	1.15 ± 0.02	13.4 ± 1.8	25° ± 5

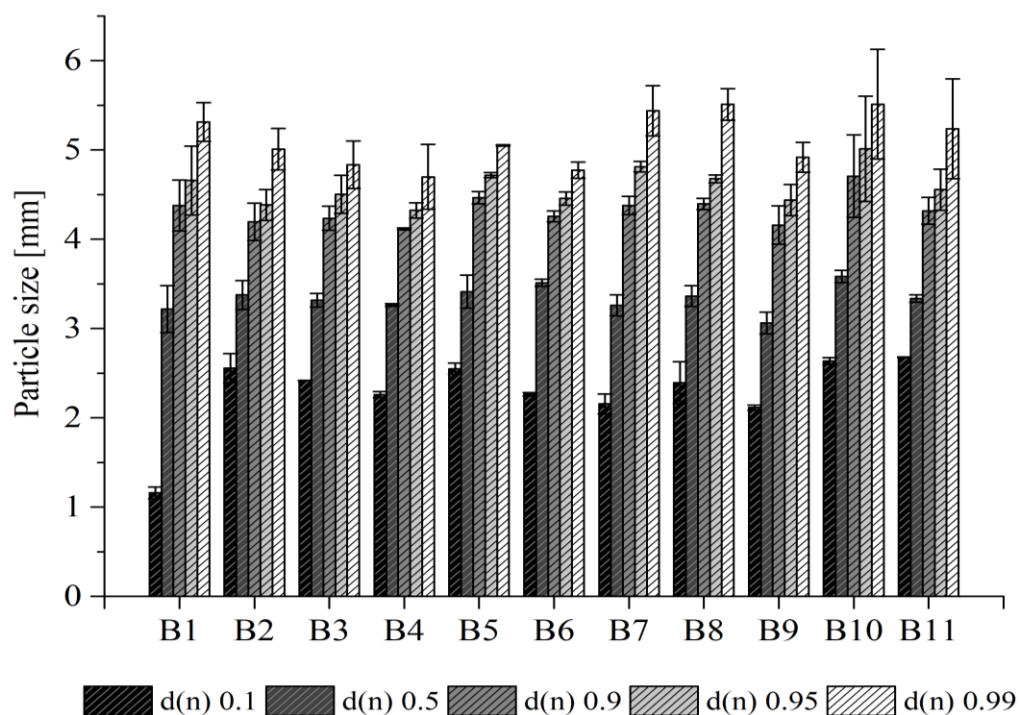


Figure 1. Numeric particle size distribution of paper granules from different batches based on the predetermined Feret's diameter. A $d(n) 0.5$ represents the size where 50% of all granules are smaller or equal to the given number.

Bulk and tapped density of B2–B6 increased significantly ($p < 0.01$) as the amount of sucrose increased. On the other hand, B7–B11 exhibited an inconsistent increase in terms of bulk and tapped density, especially in case of sucrose aqueous solutions of high concentration (Table 2). This can be attributed to the high viscosity of such solutions which might result in an inhomogeneous distribution of the granulation liquid among the formed granules, hence affecting their properties. Hausner's ratio and Carr's index were used as an indication of the flowability of the granules. Table 2 shows that Hausner's ratio and Carr's index values were in the range from 1.12 to 1.18 and from 11 to 15.0, respectively, which indicates good flowability and compressibility of the prepared granules according to the European Pharmacopoeia.[10]

Regarding the angle of repose, B1 (no sucrose) exhibited a value in the range of 41–45°. This indicates only passable flowability and that the produced granules might hang up inside the hopper during tablet manufacturing. Indeed, the adherence of the granules to the hopper was recognized during the production of the tablets and it was attributed to the electrostatic effects of paper [14]. The addition of sucrose decreased the angle of repose. B3, B4, B8 and B9 showed values in the range of 31–35°, which suggests good flowability behavior. B5, B6, B10 and B11 exhibited values in the range of 25–30° (Table 2), which indicate excellent flowability according to the European Pharmacopoeia [10]. Interestingly, the electrostatic adherence of the granules to the hopper disappeared when sucrose was added to the granules. The effects can be a superposition of many different effects [15]. In this study the reduced adherence can be for example due to an increase in size, rigidity and/or density and more research is needed to understand the effects in detail. In spite of this, the obtained results suggest that the die filling process using B2–B11 during high-speed tablet manufacturing will run efficiently with no major complications (e.g., without variations in tablet weight due to inconsistent die filling).[16]

3.2 .Preparation and Characterization of Paper-Based Tablets Using the Produced Granules

In the next step, paper-based tablets were produced from all different paper granule batches. All sucrose containing granules led to smooth, slightly porous, and pale tablets (Figure 2). The manufactured tablets were then evaluated for their thickness, mass uniformity, friability, hardness and disintegration time, and the obtained data are summarized in Table 3 .

Regarding the thickness of the tablets, it was observed that the thickness of the produced tablets from the B1 batch was significantly higher ($p < 0.01$) when compared to the tablets produced from granule batches with the lowest sucrose content (B2 and B7). This indicates that tablets made from paper are less compact in the absence of a binder. The effect

was expected, as the B1 batch tablets contained no sucrose and thus they were expected to possess the highest elasticity. The high elasticity of the material causes a stronger elastic relaxation of the tablet after the compression and, thus results in a higher thickness of the tablets. Moreover, it was found that the tablet thickness increased ($p < 0.01$) with increasing sucrose content (Table 3).

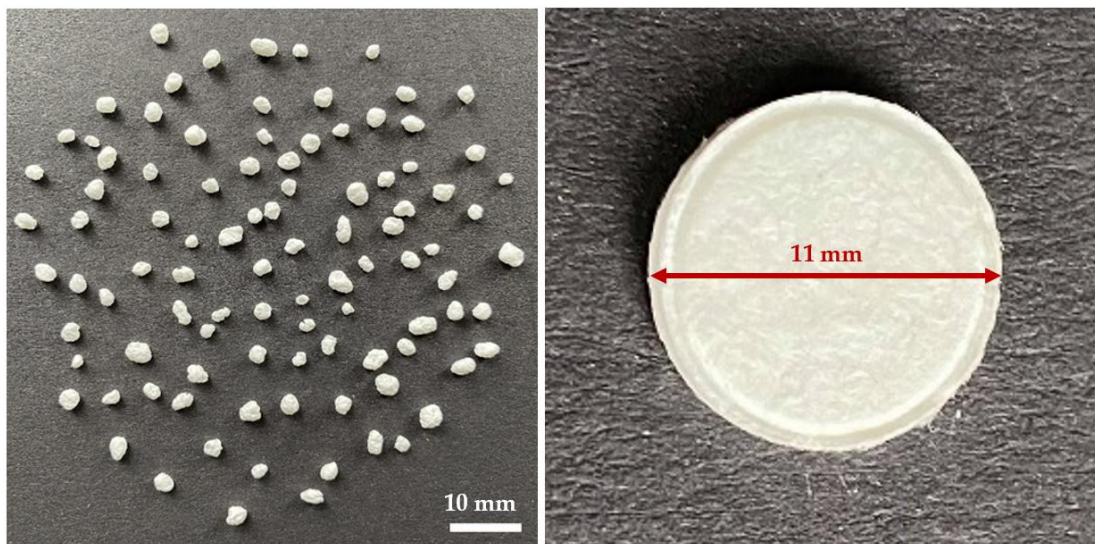


Figure 2. Paper granules with 20% sucrose content (B3) and the produced tablet made from the B3 paper granules.

Table 3. Physico-chemical properties of the different paper granules produced in this study.

Batch Code	Thickness (mm)	Mass Uniformity (%)	Friability (%)	Hardness (N)	Disintegration
B1	1.8 ± 0.01	4.9 ± 2.8	<0.001	20.8 ± 7.6 min.: 10.8 max.: 32.6	all tablets disintegrated within 10 s
B2	1.3 ± 0.02	1.8 ± 1.6	0.14	51.5 ± 12.9 min.: 35.2 max.: 71.5	all tablets disintegrated within 2 min
B3	1.7 ± 0.04	1.8 ± 1.6	0.23	112.8 ± 18.6 min.: 84.2 max.: 129.5	all tablets disintegrated within 5 min
B4	1.8 ± 0.02	3.1 ± 1.8	0.03	123.4 ± 22.2 min.: 97 max.: 142	all tablets disintegrated within 15 min
B5	2.0 ± 0.04	3.2 ± 2.9	0.09	154.2 ± 15.3 min.: 127.1 max.: 173.3	all tablets disintegrated within 35 min
B6	3.3 ± 0.18	4.0 ± 2.9	0.10	250.3 ± 24.6 min.: 210.2 max.: 279	all tablets disintegrated within 50 min
B7	1.4 ± 0.03	3.1 ± 1.9	0.11	71.1 ± 14.6 min.: 52.5 max.: 99.9	all tablets disintegrated within 20 min
B8	1.8 ± 0.04	2.4 ± 2.1	0.04	166.1 ± 29.3 min.: 116.1 max.: 217.2	all tablets disintegrated within 20 min
B9	1.9 ± 0.06	3.8 ± 2.3	0.01	221.7 ± 60.4 min.: 122.9 max.: 288.2	all tablets disintegrated within 45 min
B10	2.0 ± 0.04	2.6 ± 2.1	0.14	258.6 ± 28 min.: 200.9 max.: 290.7	all tablets disintegrated within 55 min
B11	2.0 ± 0.07	4.6 ± 2.3	0.01	271.5 ± 29.2 min.: 221.9 max.: 300	all tablets disintegrated within 60 min

The tablet masses increased with increasing amounts of sucrose and were in the range between 172 and 283 mg. According to the criteria of the European Pharmacopoeia, tablets fulfil the criteria of mass uniformity if not more than 2 of the individual masses (out of 20) deviate from the average mass by (i) more than 7.5% if the tablet mass of the tablets is in the range between 80–250 mg and by (ii) more than 5% if the tablet mass of the tablets is ≥80–250 mg.[10]

The weight of the tablets produced from batches B1-B5 and B7-B10, i.e., tablets containing 0–40% sucrose, was <250 mg and all tablets fulfilled the criteria of the European Pharmacopoeia. In contrast, tablets produced from batches B6 and B11, i.e., tablets that contained 50% sucrose, weighted more than 250 mg and 7 out of 20 tablets exceeded the 5% limit. Accordingly, the tablets produced from batches B6 and B11, i.e., tablets from paper granules with very high sucrose content (50%), could not fulfil the criteria (Table 3).

The friability of the produced tablets from all batches fulfilled the criteria according to the European Pharmacopoeia, as a weight loss of less than 1.0% was observed for all tablets (Table 3). Such results suggest that all the produced tablets attain a sufficient mechanical strength to withstand handling and shipping .

Regarding the resistance to crushing, the produced tablets from B1 were extremely fragile and exhibited very low values for the crushing strength (i.e., 20.81 ± 7.57), indicating low compaction of the tablets in the absence of sucrose (Table 3). On the other hand, the remaining batches, prepared with sucrose as a binder, produced tablets with higher values for the crushing strength. In addition, the produced tablets from batches B2–B6 or B7–B11 exhibited a significant increase in the crushing strength ($p < 0.01$) with increasing the sucrose content due greater deformation and better bonding between granules [17]. Such changes in tablets hardness are extremely important as they might affect disintegration behavior, the dissolution profile and bioavailability of the incorporated drug, hence its therapeutic efficacy .[18]

The disintegration time of tablets produced from B1 was extremely rapid (i.e., 10 s, Table 3), which is consistent with previously reported data that indicated that such disintegration behavior might hinder the administration process of paper-based tablets and decrease patient compliance [4]. In contrast, the remaining batches, prepared with sucrose as a binder, produced tablets with a slower disintegration time. Moreover, the produced tablets from batches B2–B6 or B7–B11 showed a significant increase in the disintegration time ($p < 0.01$) with increasing the sucrose content. These results are consistent with the crushing strength data, indicating that harder tablets attain less porosity, and thus experience a slower disintegration. Furthermore, all the tablets that were prepared from batches B1–B4 fulfilled the criteria according to the European Pharmacopoeia, as all tablets disintegrated within 15 min. On the other hand, all of the tablets that were prepared from batches B5 and B6 failed the criteria according to the European Pharmacopoeia for uncoated tablets, as they did not disintegrate within 15 min. Tablets produced from batches B5 and B6 attain higher crushing

strength values in comparison to batches B1–B4, which might be attributed to the presence of higher amounts of sucrose (i.e., 40% or 50%), which slows down the disintegration. It was also observed that using sucrose aqueous solution as a granulation liquid in the manufacturing of B7-B11 resulted in the production of rigid tablets that failed to disintegrate within 15 min. Solutions of binders are usually used in tablet production [19]. Disintegration of the tablets depends on many factors, including the compression force, nature of the binder, method of tableting and mechanism of tablet disintegration [19]. All of these parameters were kept constant during the production of all tablets, so it can be assumed that the incorporation method of the binder (i.e., the solid state or the aqueous solution) was responsible for the observed increase in disintegration time.

Based on the data, it can be summarized that the addition of sucrose in dry form is most suitable, as it resulted in granules that can be compressed into tablets with good pharmaceutical profile. Paper granules with sucrose contents in the range between 20–30% (w/w) were identified to result in paper-based tablets with optimal pharmaceutical properties.

3.3 Mechanical Behavior of Paper Granules under Compression

The evaluation of the mechanical behavior of paper granules under compression was carried out in the next part of the study by using three selected granule batches. B1 was selected to understand the mechanical behavior of granules in absence of a binder. B3 was chosen as it was able to produce paper-based tablets with an excellent pharmaceutical profile (i.e., optimum disintegration behavior). B6 was selected as a representative of paper-based tablets prepared using granules with a high sucrose content .

The instrumented die compression tests were performed in three steps. In the first step, single compression experiments were performed by applying low and high compression velocities to the different formulations (Figure 3a) .

The results indicated that the compression velocity has only limited effects on the material behavior, i.e., strain rate effects are assumed to be negligible. Based in these findings, all other experiments were continued with one constant compression velocity (0.1 mm/s). The next step investigated the influence of the sucrose content within the granules on the die wall friction coefficient (Figure 3b). For all three formulations, i.e., the granules made of paper without sucrose (B1) and granules with medium (B3) and high sucrose content (B6), the friction coefficient was low for all of the tests. With this, it can be concluded that the

compression of paper granules does not cause a remarkable friction between the granules and the wall of the die. In addition, as no significant differences were found between the friction coefficients from the different granules, it can be concluded that the influence of the sucrose content on the friction coefficient is neglectable.

The next step was the assessment of the material behavior during compression, i.e., the influence of the axial true strain on the axial stress and the radial stress, respectively (Figure 3c,d). Tests were performed within the press machine limits of 5 to 40 kN with compression force steps of 5 kN. Results show that an increase in sucrose content causes an increase in the stiffness of the material along with achieving a similar stress level at lower strains. Hence, it can be assumed that an increase in sucrose causes an increase in plastic hardening since powder compression is mainly due to plastic deformation. The effect is considered to occur due to the higher density that is obtained for the paper granules with higher sucrose content.

The higher density of the granules with higher sucrose content was confirmed by the decrease in the die filling height of the mean curves, which was 18.6 mm for B1, 14.44 mm for B3 and 11.75 mm for B6, respectively. Consequently, an increase in sucrose content caused not only a higher density but also decreased the porosity. Prior to the compression, the measured true density was 1.56 kg/m³ for all three batches, which is comparable to the true density of microcrystalline cellulose [20]. The mean curves resulting porosity of the die filling before compression was 0.914 for the batch without sucrose (B1), 0.889 for the batch with medium sucrose content (B3) and 0.864 for the batch with high sucrose content (B6).

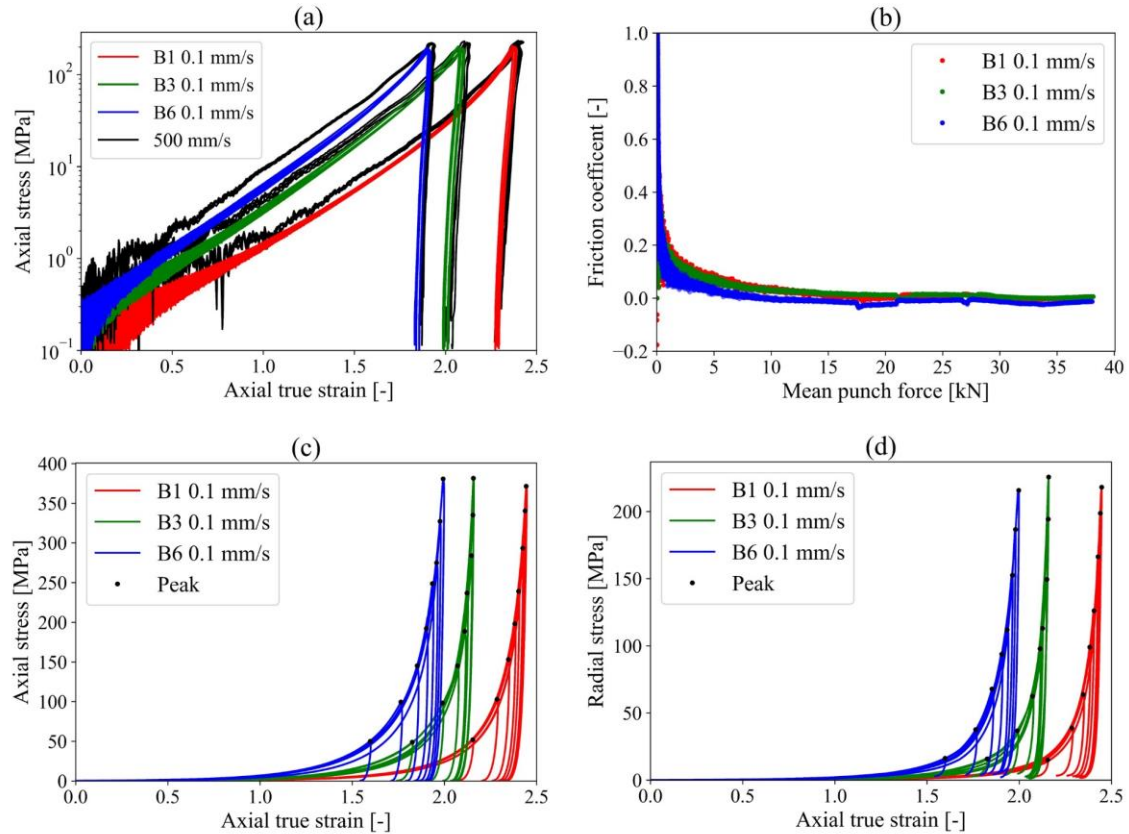


Figure 3. Experimental results of the instrumented die compression test of granules batches B1, B3 and B6. Strain rate dependency in logarithmic scale axial stress—axial true strain (a), friction coefficient—mean punch force (b), typical loading-unloading curves of granules compaction process indicating the influence of the sucrose content on axial stress—axial true strain (c) and radial stress—axial true strain (d) at 0.1 mm/s velocity.

Furthermore, the results also indicated that the compaction behavior of paper granules is comparable to the behavior of industrial powder under compression [20,21]. The compression of powder into tablets is generally divided into different stages [21,22]. The first stage is a rearrangement of the paper granules. In this stage, the rearrangement contributes most to the overall stress and hence the axial measured axial true strain is low. In the second step, an increasing densification of the granules takes place, hence, elastic, and plastic deformation of the granules becomes more dominant, which leads to an exponential increase in the stress—axial true strain curves. During the third stage, the unloading process, a nonlinear response of the stress—axial true strain can be observed (Figure 3c,d).

The next step aimed at identifying the elastic properties of the paper granules. For this, double compaction tests were carried out for 5–30 kN compaction levels via 5 kN steps.

From the results, the respective Poisson's ratio and Young's modulus were determined to investigate the influence of compression forces on the elastic properties and the compressibility of the paper granules with different sucrose content (Figure 4).

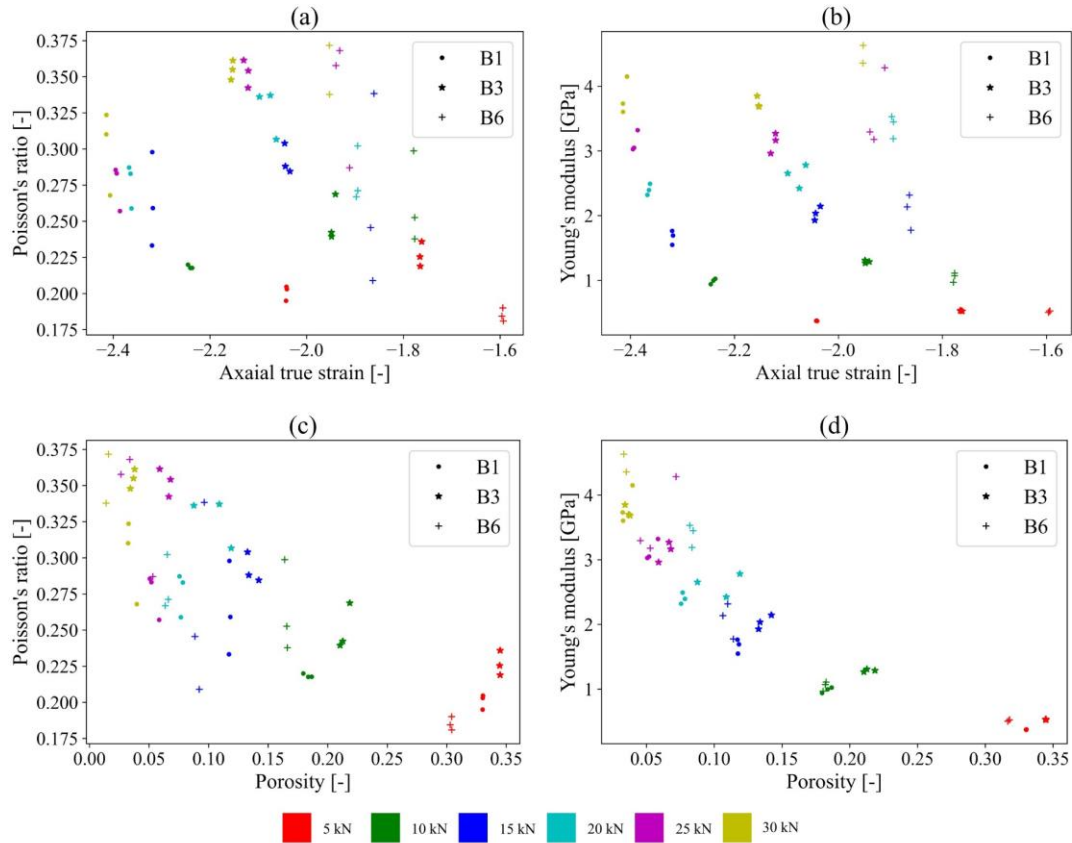


Figure 4. Poisson's ratio and Young's modulus of granule batches B1, B3, B6 at different compaction force levels. Poisson's ratio—axial true strain (a); Young's modulus—axial true strain (b); Poisson's ratio—porosity (c); Young's modulus—porosity (d).

The main-compaction loading curves are not linear when low forces and forces greater than the pre-compaction force level are applied to the granules. This can be attributed to the porosity of the material at the beginning of the compression process [23]. Therefore, to determine the elastic constants, only the linear part of the main-compaction loading curves was used for the calculation of the elastic material properties, i.e., Poisson's ratio and Young's modulus. The linear part was determined to be within 30–80% compared to the pre-compaction force levels. Young's modulus represents the stiffness of a material. Hence, the higher the Young's modulus, the higher is the stiffness of the material. The Poisson's ratio is defined to be the deformation of a material in directions perpendicular to the specific direction of loading. A high Poisson's ratio indicates that the material has a high perpendicular deformation, meaning that—under axial force—the material “escapes” due to low

compressibility. Data obtained from the double compaction tests (Figure 4a,b) show that, for all batches, both the Poisson's ratio and the Young's modulus increase nonlinearly with increasing compaction. Hence, during compaction, the paper granules become more compact and stiffer, which also results in an increased density and reduced porosity (Figure 4c,d). The Poisson's ratio at higher compaction force levels increased with increasing sucrose content and Young's modulus remained almost constant for the different sucrose contents at similar compaction force levels (Figure 4a,b). However, the different sucrose contents had no influence on the changes in porosity (Figure 4a,b). Hence, after compression, independent of the sucrose content, similar porosities were obtained.

In the next step, the data obtained were used to judge if paper granules possess sufficient binding properties rendering them into suitable intermediate products for the production of tablets in large, industrial scale. Binders or compression enhancers with excellent binding properties should be made from soft materials that undergo plastic deformation during compaction [24]. Examples for this are microcrystalline cellulose and various cellulose ethers [20,24]. Today, in many cases, the Heckel mathematical model is used to judge the compressibility of a material [24]. The Heckel equation describes the relationship between the process relating porosity (ϕ) and the hydrostatic pressure (P) (cf. 3.2.4.6.) and allows us to determine the so-called yield pressure (P_y , cf. formula 8), which describes the necessary pressure to plastically deform a material. Low yield pressures represent a soft/plastic behavior, whereas higher values indicate a hard/brittle and behavior [20]. The yield pressures of the paper granules ranged from 79–86 MPa (Figure 5). With this they are in the upper limit (80 MPa) to be classified as soft/plastic material, indicating a very good compressibility of the paper granules [20]. However, they are also close to the lower limit of a hard/brittle behavior. The trend towards a higher hardness and a more brittle behavior can especially be seen for the batches with higher sucrose content .

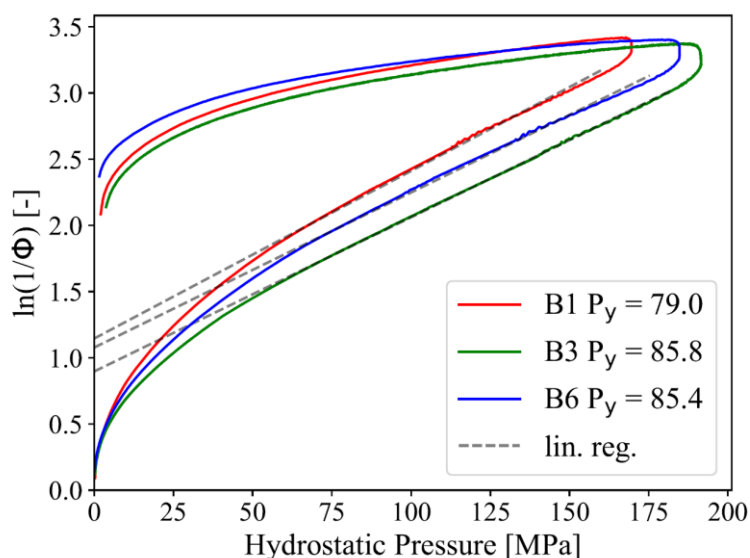


Figure 5. Heckel plots calculated for the three batches and linear regression fit to determine the yield Pressure (P_y). Coefficient of determination for the fits are: $R^2 = 0.999$ for B1, $R^2 = 0.9999$ for B3 and $R^2 = 0.9989$ for B6.

This is reasonable as sucrose is a crystalline and brittle material [25]. Hence, higher contents of sucrose within the paper-based granules results in a higher proportion of crystalline and brittle material within the granules, which should then result in a higher cracking propensity [20]. However, the trend is not significant. This means that it can be summarized that the sucrose content had no significant influence on the yield pressure. Moreover, it can be stated that the deformation behavior of paper granules—independent on the sucrose content—is comparable to other pharmaceutical binders. In fact, paper granules were found to be suitable intermediate products for the production of tablets in large, industrial scale. The material properties of the paper granules are also sufficient for yielding tablets that fulfill the criteria according to the European Pharmacopoeia .

In addition, the data acquired here also provide the base for the establishment of numeric models that are able to simulate the compression process of powder into tablets. Different methods are available for this, i.e., the Discrete Element Method (DEM) [26] and the Finite Element Method (FEM) [27]. FEM is more commonly used to simulate powder compression as it represents the powder as an isotropic continuous media [28–31]. One of the most commonly used models in FEM is the Drucker-Prager Cap plasticity model (DPC) [32,33] and it has been previously utilized for compression simulations for the production of tablets with optimal pharmaceutical properties [28–31]. Such modeling allows us, for example, to predict the shape and the size of the resulting tablets. It also enables us to predict

inhomogeneous properties within the tablets which can result in insufficient pharmaceutical quality of the tablets (for example capping of the tablets) .

The understanding and modulation of such processes thus allows for the development of efficient compression processes that result in tablets with optimal physico-chemical and pharmaceutical properties. Such simulations can also predict the hardness of tablets, which then provides a close link to the disintegration of the tablet and with this also a close link to the dissolution profile of the active ingredient out of the tablet. Future work should now use the data acquired here to establish simulation models that help to predict the above-mentioned parameters. These parameters should then be linked to the biopharmaceutical properties, i.e., bioavailability and pharmacokinetic profiles. The link between simulation and biopharmaceutical performance would then allow for a fast and efficient development of drug-loaded smartFilm tablets with optimal pharmaceutical properties .

4 .Conclusions

The aim of the study was to transform paper into a flowable physical form and to investigate the influence of sucrose on the resulting material properties. Paper was successfully transformed into paper granules via a wet granulation process. The resulting granules had a size of about 3 mm with a slightly elongated shape. The sucrose granules possessed good flowability and allowed for the production of tablets in continuous, i.e., high-speed, tableting mode. The addition of sucrose as a dry powder was found to be the most suitable production process for the paper granules. The tablets made of these paper granules possessed pharmaceutical properties according to the European Pharmacopoeia. The deformation behavior of the paper granules was determined and compared to the properties that are required for excipients that are used as binders and/or compression enhancers. Based on the results, it can be concluded that paper granules possess a suitable compression behavior, thus rendering them suitable intermediate products for the production of tablets made from paper on a larger, industrial scale .

Acknowledgments: Authors would also like to extend thanks to Yousef Jameel Foundation for providing the Scholarship funding for Ayat Abdelkader. Authors thank MEDELPHARM Science Lab for using their facility to conduct the compaction experiments.

5. References

- [1] Hodayun, B.; Lin, X.; Choi, H.-J. Challenges and Recent Progress in Oral Drug Delivery Systems for Biopharmaceuticals. *Pharmaceutics* **2019**, *11*, 129. <https://doi.org/10.3390/pharmaceutics11030129>.
- [2] Lemke, S.; Strätling, E.J. *SmartFilms-Oral and Peroral Films for Optimized Delivery of Nanoparticulate or Amorphous Drugs*; Controlled Release Society Local Chapter: Saarbrücken, Germany, 2016; Volume 7.
- [3] Lemke, S.; Strätling, E.J.; Welzel, H.P. Cellulosefaserbasierte Trägermatrices (smartfilms) Zur Applikation Von Inhaltsstoffen Sowie Deren Herstellung. German Patent Application DE102016000541A1, 20 July 2017.
- [4] Stumpf, F.; Keck, C.M. Tablets made from paper. *Int. J. Pharm.* **2018**, *548*, 812–819.
- [5] Subrahmanyeswari, C.D.; Prasanth, Y.; Sameeda, R. Formulation and development of efavirenz tablets by paper technique using co-solvency method. *Int. J. Curr. Pharm. Res.* **2019**, *11*, 87–92. <https://doi.org/10.22159/ijcpr.2019v11i6.36349>.
- [6] Ornik, J.; Knoth, D.; Koch, M.; Keck, C.M. Terahertz-spectroscopy for non-destructive determination of crystallinity of L-tartaric acid in smartFilms® and tablets made from paper. *Int. J. Pharm.* **2020**, *581*, 119253.
- [7] Shanmugam, S. Granulation techniques and technologies: Recent progresses. *BiolImpacts* **2015**, *5*, 55–63.
- [8] Stumpf, F. Tabletten Aus Papier—Tablets Made from Paper—Zur Oralen Applikation Schwerlöslicher Wirkstoffe. Ph.D. Thesis, Philipps-Universität Marburg, Marburg, Germany, 2019.
- [9] Santomaso, A.C.; Baggio, R.; Zorzi, F.; Salviulo, G.; Realdon, N.; Franceschinis, E. Sugars with different thickening power in high shear granulation. *Powder Technol.* **2017**, *317*, 391–399.
- [10] C.H. Beck. *European Pharmacopoeia*, 8th ed.; 2.09: Pharmaceutical Technical Procedures; C.H. Beck: Nördlingen, Germany, 2016.
- [11] Wang, Z.; Pan, Z.; He, D.; Shi, J.; Sun, S.; Hou, Y. Simulation Modeling of a Pharmaceutical Tablet Manufacturing Process via Wet Granulation. *Complexity* **2019**, *2019*, 3659309. <https://doi.org/10.1155/2019/3659309>.
- [12] Mazel, V.; Busignies, V.; Diarra, H.; Tchoreloff, P. On the links between elastic constants and effective elastic behavior of pharmaceutical compacts: Importance of Poisson's ratio and use of bulk modulus. *J. Pharm. Sci.* **2013**, *102*, 4009–4014.

-
- [13] Chopra, R.; Michael Newton, J.; Alderborn, G.; Podczek, F. Preparation of pellets of different shape and their characterization. *Pharm. Dev. Technol.* **2001**, *6*, 495–503.
- [14] Sappi etc. Printer Tech Tips—Cause & Effects of Static Electricity in Paper. Available online: [Sappi-Printer-Tech-Tips-Cause-And-Effects-Of-Static-Electricity-In-Paper.pdf](#) (accessed on 1 January 2012).
- [15] Boschini, F.; Delaval, V.; Traina, K.; Vandewalle, N.; Lumay, G. Linking flowability and granulometry of lactose powders. *Int. J. Pharm.* **2015**, *494*, 312–320. <https://doi.org/10.1016/j.ijpharm.2015.08.030>.
- [16] Chen, H.; Wang, C.; Liu, S.; Sun, C.C. Development of piroxicam mini-tablets enabled by spherical cocrystallization. *Int. J. Pharm.* **2020**, *590*, 119953.
- [17] Fu, Y.; Jeong, S.H.; Park, K. Fast-melting tablets based on highly plastic granules. *J. Control. Release* **2005**, *109*, 203–210.
- [18] Loscertales, H.R.; Modamio, P.; Lastra, C.F.; Mariño, E.L. Assessing the stability of lamotrigine dispersible/chewable tablets when stored in blister punch cards. *Curr. Med. Res. Opin.* **2017**, *33*, 949–953.
- [19] Alanazi, F.K. Utilization of date syrup as a tablet binder, comparative study. *Saudi Pharm. J.* **2010**, *18*, 81–89. <https://doi.org/10.1016/j.jsps.2010.02.003>.
- [20] Cabiscol, R.; Finke, J.H.; Zetzener, H.; Kwade, A. Characterization of Mechanical Property Distributions on Tablet Surfaces. *Pharmaceutics* **2018**, *10*, 184. <https://doi.org/10.3390/pharmaceutics10040184>.
- [21] Shang, C.; Sinka, I.C.; Pan, J. Constitutive model calibration for powder compaction using instrumented die testing. *Exp. Mech.* **2012**, *52*, 903–916.
- [22] Leuenberger, H.; Rohera, B.D. Fundamentals of powder compression. I. The compactibility and compressibility of pharmaceutical powders. *Pharm. Res.* **1986**, *3*, 12–22.
- [23] Mazel, V.; Busignies, V.; Diarra, H.; Tchoreloff, P. Measurements of elastic moduli of pharmaceutical compacts: A new methodology using double compaction on a compaction simulator. *J. Pharm. Sci.* **2012**, *101*, 2220–2228.
- [24] Ghorri, M.U. Powder Compaction: Compression Properties of Cellulose Ethers. *Br. J. Pharm.* **2016**, *1*, 19–29. <https://doi.org/10.5920/bjpharm.2016.09>.
- [25] Sugimoto, M.; Narisawa, S.; Matsubara, K.; Yoshino, H.; Nakano, M.; Handa, T. Development of manufacturing method for rapidly disintegrating oral tablets using the crystalline transition of amorphous sucrose. *Int. J. Pharm.* **2006**, *320*, 71–78. <https://doi.org/10.1016/j.ijpharm.2006.04.004>.
-

- [26] Cundall, P.A.; (Ed.). A computer model for simulating progressive, large-scale movement in blocky rock system. In Proceedings of the International Symposium on Rock Mechanics, Nancy, France, 4–6 October 1971.
- [27] Moghaddam, M.; Darvizeh, R.; Davey, K.; Darvizeh, A. Scaling of the powder compaction process. *Int. J. Solids Struct.* **2018**, *144*, 192–212.
- [28] Sinka, C. Modelling powder compaction. *Kona* **2007**, *25*, 4–22.
- [29] Han, L.H.; Elliott, J.A.; Bentham, A.C.; Mills, A.; Amidon, G.E.; Hancock, B.C. A modified Drucker-Prager Cap model for die compaction simulation of pharmaceutical powders. *Int. J. Solids Struct.* **2008**, *45*, 3088–3106.
- [30] Diarra, H.; Mazel, V.; Boillon, A.; Rehault, L.; Busignies, V.; Bureau, S.; Tchoreloff, P. Finite Element Method (FEM) modeling of the powder compaction of cosmetic products: Comparison between simulated and experimental results. *Powder Technol.* **2012**, *224*, 233–240.
- [31] Krok, A.; Peciar, M.; Fekete, R. Numerical investigation into the influence of the punch shape on the mechanical behavior of pharmaceutical powders during compaction. *Particuology* **2014**, *16*, 116–131.
- [32] Drucker, D.C.; Prager, W. Soil mechanics and plastic analysis or limit design. *Q. Appl. Math.* **1952**, *10*, 157–165.
- [33] Drucker, D.C.; Gibson, R.E.; Henkel, D.J. Soil mechanics and work-hardening theories of plasticity. *Trans. Am. Soc. Civ. Eng.* **1957**, *122*, 338–346.
- [34] Pelikh, O.; Eckert, R.W.; Pinnapireddy, S.R.; Keck, C.M. Hair follicle targeting with curcumin nanocrystals: Influence of the formulation properties on the penetration efficacy. *J. Control. Release* **2021**, *329*, 598–613.
- [35] Aslani, A.; Jahangiri, H. Formulation, characterization and physicochemical evaluation of ranitidine effervescent tablets. *Adv. Pharm. Bull.* **2013**, *3*, 315–322. <https://doi.org/10.5681/apb.2013.051>.
- [36] JASP Team. JASP, Version 0.13.1; Computer Software; 2020. Available online: <https://jasp-stats.org/> (assessed on 20 September 2022).

6. Supplementary material

Table S1: Macro used for the determination of the Feret's diameter.

```
run("8-bit");
```

```
setAutoThreshold("Default");
```

```
//run("Threshold...");  
  
//setThreshold;(180 ,0)  
  
setOption("BlackBackground", false);  
  
run("Convert to Mask");  
  
run("Analyze Particles..." , "size=0.09-Infinity show=Masks display");
```

3.2. SmartFilm Tablets for Improved Oral Delivery of Poorly Soluble Drugs

Ayat Abdelkader ^{1,2}, Eduard Preis ¹ and Cornelia M. Keck ¹

¹ Department of Pharmaceutics and Biopharmaceutics, Philipps-Universität Marburg, Robert-Koch-Str. 4, 35037 Marburg, Germany.

² Assiut International Center of Nanomedicine, Al-Rajhi Liver Hospital, Assiut University, Assiut 71515, Egypt.

(adapted from Pharmaceutics 2022, 14(9), 1918)

Abstract

Numerous oral drugs exhibit limited bioavailability due to their poor solubility and poor intestinal permeability. The smartFilms technology is an innovative approach that improves the drug aqueous solubility via incorporating the drug in an amorphous state into a cellulose-based matrix, i.e., paper. smartFilms can be transformed into a free-flowing physical form (i.e., paper granules) that can be compressed into tablets with optimum physico-chemical and pharmaceutical properties. The aim of this study was to investigate if smartFilm tablets are suitable for improved oral delivery of poorly water-soluble drugs. Curcumin is a poorly soluble drug with low intestinal permeability and was used for the production of curcumin-loaded smartFilms. The curcumin-loaded smartFilms were transferred into smartFilm granules which were then compressed into curcumin-loaded smartFilm tablets. The tablets were characterized regarding their physico-chemical and pharmaceutical properties, and the intestinal permeability of curcumin was determined with the ex vivo porcine intestinal model. The ex vivo intestinal permeability of curcumin from the smartFilm tablets was compared to a physical mixture of curcumin and paper and to a classical and to an innovative commercial product, respectively. The produced curcumin-loaded smartFilm tablets fulfilled the European Pharmacopoeia requirements, incorporated curcumin in amorphous state within the cellulose matrix and exhibited an enhanced dissolution rate. The ex vivo intestinal permeation data were shown to correlate to the in vitro dissolution data. The ex vivo intestinal permeation of curcumin from the smartFilm tablets was about two-fold higher when compared to the physical mixture and the classical commercial product. No differences in the ex vivo bioavailability were found between the smartFilm tablets and the innovative commercial product. SmartFilm tablets are a cost-effective and industrially feasible formulation approach for the formulation of poorly water-soluble drugs, i.e., BCS class II and IV drugs.

1. Introduction

Oral delivery is the most preferred route for drug administration [1]. The efficient delivery of oral drugs to the systemic circulation is primarily affected by the physico-chemical properties of the drug (e.g., solubility, stability) and/or the physiological properties of the gastrointestinal tract (GIT), e.g., the harsh acidic environment, the abundance of digestive enzymes, and the intestinal mucosal physical absorption barrier [2].

Various approaches have been utilized to increase the poor aqueous solubility of drugs, including micelle formation, lipid-based formulations, nanoemulsions, self-emulsifying drug delivery systems, solid dispersions, inorganic nanocarriers, or nanocrystals [3–5]. Recently, smartFilms have emerged as novel oral delivery systems to improve the poor aqueous solubility of drugs [6]. The smartFilms technology utilizes the pores of cellulose matrices as loading sites in which the therapeutic agent is loaded in an amorphous form, resulting in enhancing the dissolution rate of the loaded drug. The production of smartFilms involves dissolving a poorly soluble drug in an appropriate solvent, applying the resulting solution on a porous cellulose-based paper, and drying the obtained drug-loaded smartFilms (Figure 1). The matrix of the paper retains the drug in an amorphous state, and thus improves its solubility [6,7]. Several studies highlight the smartFilms technology as a potential oral drug delivery system and were able to transform smartFilms into an appropriate oral dosage form (i.e., paper tablets), which can be conveniently administered. These tablets were manually produced, without the addition of any excipients and fulfilled all requirements of the European Pharmacopoeia [8–10].

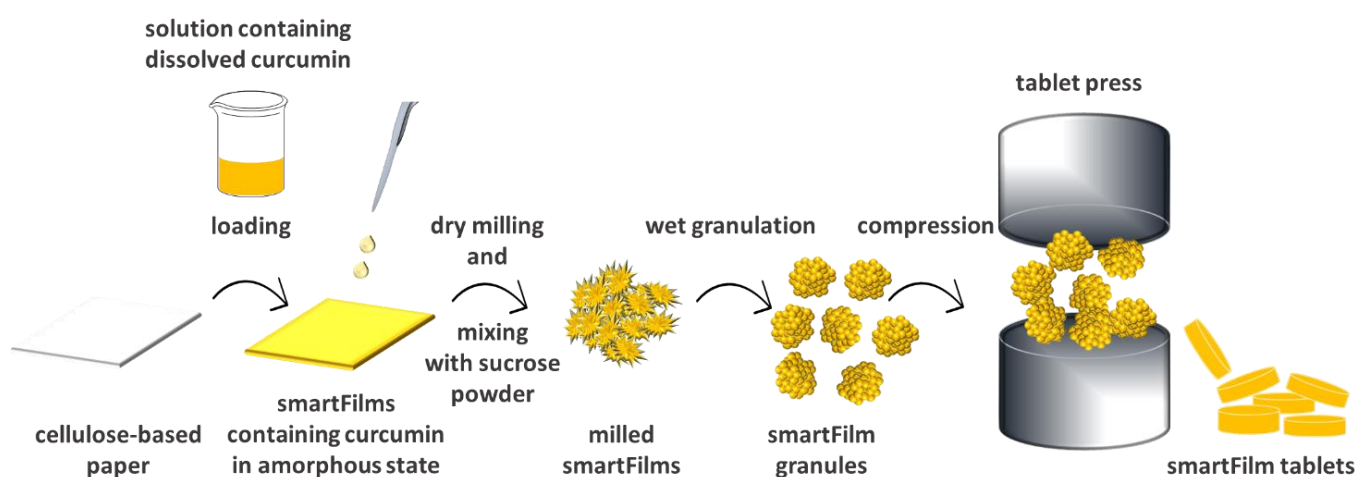


Figure 1. Scheme of production of curcumin-loaded smartFilm granules and smartFilm tablets.

Despite its proven effectiveness, still, the smartFilms technology remained unrecognized by the pharmaceutical industry, because a large-scale production of paper tablets from paper cut outs—due to the poor flowability of the paper cut outs—was not possible. To address this issue, unloaded smartFilms were transformed into a free-flowing physical form (i.e., paper granules), rendering them highly convenient for high-speed, large-scale tablet manufacturing [10,11].

The transfer of paper into paper granules is required to allow for a large-scale production of the tablets. However, this granulation process requires wetting steps [10,11]. These wetting steps are not critical for the production of non-loaded, i.e., drug free, paper tablets, but might be critical if drug-loaded smartFilms—that contain drug in amorphous form—are transferred into tablets. The wetting might cause a (partial) dissolution of the drug during the wetting process and a subsequent re-crystallization upon the drying. The changes in crystalline state can then affect the dissolution velocity of the drug and—consequently—the oral bioavailability of the drug that was loaded into the smartFilms.

The aim of this study was therefore to investigate (i) if drug-loaded smartFilms can be transferred into smartFilm granules and smartFilm tablets and (ii) if these smartFilm tablets can maintain the amorphous state of the incorporated drug, which then should result in an improved dissolution rate and in an enhanced intestinal permeability of the poorly water-soluble drug.

Curcumin is a natural compound that can be obtained from the rhizome of the turmeric plant (*Curcuma longa* L.) and has been widely used as a preventive and therapeutic agent against numerous diseases [12]. This can be attributed to the endogenous pharmacological properties of curcumin, which include antioxidant, anti-inflammatory, antibacterial, antiviral, antihepatotoxic, antidepressant, and anticancer activities [13,14]. The biopharmaceutical classification system (BCS) classifies curcumin as class IV drug, indicating that curcumin is poorly soluble, with limited oral bioavailability and intestinal permeability [15]. These characteristics render curcumin the drug of choice for studying the impact of smartFilm tablets production via wet granulation on the dissolution rate and intestinal permeability of a poorly soluble drug.

The study was performed in two steps. In the first step, curcumin-loaded smartFilms were prepared and transferred into smartFilm granules. The granules were transformed into smartFilm tablets and their physico-chemical and pharmaceutical properties (e.g., thickness, content uniformity, mass uniformity, friability, hardness, disintegration time, and dissolution

profile) were determined. The crystalline state of curcumin was also assessed and compared to that within the smartFilm granules and the smartFilm tablets (Figure 1). In the second step, the oral bioavailability, i.e., the intestinal permeability of curcumin, was determined ex vivo in a porcine intestinal model. The results obtained were compared to a physical mixture tablet, that contained the paper matrix and crystalline curcumin bulk powder material. In addition, the results were compared to two commercially available curcumin products. Commercial product I represented a classical formulation principle, i.e., it contained curcumin as raw powder in a hard capsule. Commercial product II contained “micellar curcumin”, i.e., curcumin and high concentrations of an o/w surfactant filled into a soft capsule. The surfactant solubilizes the lipophilic curcumin in micelles and thus provides excellent solubility [16]. Despite this, micellar curcumin was also found to be superior when compared to other drug delivery systems, i.e., oils, liposomes, phytosomes, cyclodextrines, or sub-micron particles. The major reason for this was attributed to its excellent digestive stability and increased post-digestive solubility, when compared to the other formulation strategies [16]. In fact, at present, the commercial product II, i.e., the “micellar curcumin”, is considered to be the most effective curcumin formulation available and was therefore selected as benchmark control for the curcumin-loaded smartFilm tablets.

2. Materials and Methods

2.1. Materials

Curcumin, which was used as *Curcuma longa* extract powder with a curcumin content of 80%, was obtained from Receptura Apotheke (Cornelius-Apothekenbetriebs-OHG, Frankfurt, Germany). Based on previous studies, commercially available, cellulose-based paper (Soft & Sicher, dm-drogerie markt GmbH + Co. KG, Karlsruhe, Germany) was used as paper matrix [8,10,17]. The paper was approved for food and skin contact and consisted of 100% fresh cellulose pulp that was made from four different tree species (Eucalyptus, Fagus, Picea and Pinus). Sucrose and sodium dodecyl sulfate (SDS) were purchased from Carl Roth GmbH + Co. KG (Karlsruhe, Germany). Anhydrous tribasic sodium phosphate was obtained from Fisher Scientific GmbH (Schwerte, Germany). Purified water was freshly obtained from a PURELAB Flex 2 (ELGA LabWater, Veolia Water Technologies GmbH, Celle, Germany). The commercial products were purchased from dm-Drogeriemarkt (Marburg, Germany). Commercial product I—representing the classical formulation principle—was a hard capsule with 750 mg *Curcuma longa* extract powder that contained 35 mg curcumin. Each capsule also contained 2 mg piperine from black pepper and 25 µg cholecalciferol, along with hydroxypropyl cellulose and

magnesium stearate as excipients. Commercial product II—representing the innovative formulation principle with excellent oral bioavailability—was a soft capsule with 40 mg *Curcuma longa* extract that contained 35 mg curcumin. Each capsule also contained 40 mg ascorbic acid and 5 µg cholecalciferol, along with polysorbate 80 and hydroxypropyl cellulose as excipients.

2.2. Methods

2.2.1. Production of Curcumin-Loaded smartFilms and smartFilm Granules

Curcumin-loaded smartFilms were prepared as described previously [18], with slight modifications. Curcumin was dissolved in ethanol to produce a solution of 2.5 mg/mL. Small paper sheets (approx. 5 cm × 5 cm), with an individual mass of approximately 200 mg, were separately loaded with 0.5 mL of curcumin solution using an automatic micropipette. Following drying, the procedure was repeated several times to prepare 20 mg curcumin-loaded paper sheets (i.e., smartFilms with 10% (w/w) loaded curcumin). Subsequently, the dried curcumin-loaded smartFilms were used to prepare curcumin-loaded smartFilm granules.

The granules were prepared using the wet granulation method and purified water was used as a granulation liquid [11,19]. For this, the curcumin-loaded smartFilms were dry milled using a knife mill (Moulinex DP8108, Groupe SEB Deutschland GmbH, Frankfurt, Germany) for 1 min. Afterwards, sucrose was added to the blend to obtain milled smartFilms that contained 20% (w/w) sucrose [11]. The resulting mixture was slightly wetted by spraying purified water on top of the milled smartFilm/sucrose mixture. This was performed to dissolve the sucrose and to increase the density of the milled paper [11]. The wetted mixture was further grinded with the knife mill (1 min) and then transferred to a plastic sieve, where it was further wetted with purified water under shaking at 300 rpm (universal shaker SM-30 control, Edmund Bühler GmbH, Bodelshausen, Germany) for a period of 3–8 min to obtain the smartFilm granules [11]. The wet curcumin-loaded smartFilm granules were dried in the oven for 30 min at 120 °C (UN 30, Memmert GmbH + Co. KG, Schwabach, Germany). Afterwards they were sieved manually (mesh size 2.8 mm, Retsch GmbH, Haan, Germany) to obtain a size fraction ≤ 2.8 mm.

2.2.2. Characterization of Curcumin-Loaded smartFilm Granules

The smartFilm granules were characterized regarding size and shape. In addition, bulk and tapped density and the angle of repose were determined according to previously described protocols [11]. Details to the methods used are given below.

Determination of Particle Size and Shape

The Feret's diameter of the curcumin-loaded smartFilm granules was measured via digital image analysis using ImageJ software (National Institutes of Health, Bethesda, MD, USA) as described previously [20]. Ten representative images that contained approximately 200–300 granules were obtained with a Canon IXUS 190 digital camera (Canon Europe Ltd., Uxbridge, UK). The images were color-adjusted, and threshold analysis was performed to label the granules individually, then the Feret's diameter of each granule was assessed by the software (Table S1). From the results obtained, the number based median particle size diameters $d(n)$ 0.10, $d(n)$ 0.50, $d(n)$ 0.90, $d(n)$ 0.95, and $d(n)$ 0.99 were calculated. Furthermore, the sphericity of the granules was determined via calculating the aspect ratio as follows:

$$\text{Aspect ratio} = \frac{d_{\max(\text{Feret})}}{d_{90^\circ(d_{\max})}} \quad (1)$$

where $d_{\max(\text{Feret})}$ is the maximum Feret's diameter and $d_{90^\circ(d_{\max})}$ is Feret's diameter perpendicular to $d_{\max(\text{Feret})}$. An aspect ratio of 1.2 is usually accepted to describe spherical particles [21].

Determination of Bulk and Tapped Density

A mechanical tapping device (tap density tester TD200, Pharma Test Apparatebau AG, Hainburg, Germany), was used to determine the bulk and tapped density of curcumin-loaded smartFilm granules according to the test method 2.9.34. of the European Pharmacopoeia [22]. Ten grams of the granules was placed into a 250 mL measuring cylinder. The starting volume and the final volume, after carrying out 10, 500, and 1250 taps on the same granules sample, were recorded and used to calculate the bulk and tapped density, respectively. Moreover, the flowability of the granules was determined from the tapped density (1250 taps) and bulk density via calculating Hausner's ratio and the Carr's index using the following equations [23]:

$$\text{Hausner's ratio} = \frac{\rho_{\text{tapped}}}{\rho_{\text{bulk}}} \quad (2)$$

$$\text{Carr's index} = 100 \left(\frac{\rho_{\text{tapped}} - \rho_{\text{bulk}}}{\rho_{\text{tapped}}} \right) \quad (3)$$

where ρ_{tapped} is the tapped density and ρ_{bulk} is the bulk density.

Angle of Repose

The angle of repose of curcumin-loaded smartFilm granules was determined using the flowability tester (Emmeram Karg Industrietechnik, Krailling, Germany) according to the test method 2.9.36. of the European Pharmacopoeia [22]. Twenty grams of the granules was placed in the funnel of the tester. Then, the granules were stirred carefully to run through the funnel and accumulate on a fixed base to form a heap. The height of the granules heap was measured, and the angle of repose (α) was determined using the following equation:

$$\tan \alpha = \frac{h}{0.5 d_b} \quad (4)$$

where h is the height of the heap and d_b is the diameter of the base.

2.2.3. Production of Curcumin-Loaded smartFilm Tablets

The smartFilm granules obtained were filled into the hopper of a single punch tablet press (EK0, Korsch GmbH, Berlin, Germany) and compressed with a compression force of 30 kN into flat-faced bevel-edged smartFilm tablets with a 10 mm flat-faced punch (Ritter Pharma-Technik GmbH, Stapelfeld, Germany). The properties of the produced tablets were assessed as described earlier [11], i.e., by subjecting the tablets to various tests, as described in the European Pharmacopoeia [22]. Details to the methods used are given below.

2.2.4. Characterization of Curcumin-Loaded smartFilm Tablets

Macroscopic and Microscopic Analysis

The smartFilm tablets obtained were first inspected visually and were then analyzed by scanning electron microscopy (SEM, Hitachi S-510, Hitachi-High Technologies Europe, Krefeld, Germany, equipped with a secondary electron detector) to gain detailed information on the distribution of curcumin within the pores of the paper matrix. For this, a horizontal section of the curcumin-loaded smartFilm tablets and their corresponding references (curcumin raw bulk material, physical mixture of paper and bulk curcumin, curcumin-loaded smartFilm granules, unloaded granules with 20% sucrose content) were sputter-coated with a thin layer of gold (10 mA for 1 min) using Edwards S150 Sputter Coater (Edwards Vacuum, Crawley, UK). An acceleration voltage of 5 kV was used to visualize the samples [24].

Determination of Crystalline State of Curcumin

X-ray diffraction (XRD) patterns were studied to evaluate the crystalline state of curcumin loaded within the smartFilm tablets. X-ray diffraction patterns from smartFilm tablets and

from the according references (curcumin raw bulk material, physical mixture of paper and bulk curcumin, curcumin-loaded smartFilm granules, unloaded granules with 20% sucrose content) were recorded by using an X'Pert Pro MDP X-ray powder diffractometer (PANalytical/Philipps BV, Netherlands). The instrument was equipped with CuK α radiation ($\lambda = 1.7903 \text{ \AA}$) and operated at a voltage of 40 kV and 35 mA current. Samples were scanned at room temperature from $2\theta = 10^\circ$ to $2\theta = 55^\circ$ with a step of $0.03^\circ/\text{sec}$.

Determination of Tablet Thickness and Mass Uniformity

The thickness of ten randomly selected tablets was determined using the IP67 ABS digimatic caliper (Mitutoyo, Kanagawa, Japan). Mass uniformity was evaluated according to the test method 2.9.5. of the European Pharmacopoeia [22]. The test was performed on 20 randomly selected tablets. The tablets were weighed, then the average mass was calculated and compared to the mass of each individual tablet to determine the percentage deviation, followed by comparing the result to the European Pharmacopoeia limits.

Determination of Friability

Twenty randomly selected tablets were used to evaluate the friability of the tablets according to test method 2.9.7. of the European Pharmacopoeia using a friability tester equipped with an abrasion drum (PTF 10ER, Pharma Test Apparatebau AG, Hainburg, Germany) [22]. The tablets were weighed, then placed into a drum rotating at 25 rpm for 4 min. Following that, the tablets were removed, dedusted, reweighed and the percentage weight loss was calculated using the following equation:

$$\% \text{ weight loss} = 100 \left(\frac{W_1 - W_2}{W_1} \right) \quad (5)$$

where W_1 is the weight of the tablets before test and W_2 is the weight of the tablets after test.

Resistance to Crushing

The crushing strength or hardness of a tablet is the force required to break down a tablet under compression and it was assessed according to the test method 2.9.8. of the European Pharmacopoeia [22]. In this study, ten tablets were randomly selected, and each tablet was placed horizontally between the jaws of a hardness tester PTB 311E (Pharma Test Apparatebau AG, Hainburg, Germany), then the result was expressed in Newton (N) as mean value of the forces measured.

Disintegration

Disintegration of the tablets was evaluated in water according to the test method 2.9.1. of the European Pharmacopoeia [22]. Six tablets were individually placed into the cavities of a disintegration tester PTZ-S (Pharma Test Apparatebau AG, Hainburg, Germany) that was operated at $37\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ and the time required for complete tablet disintegration was recorded.

Content Uniformity

Curcumin content uniformity within the smartFilm tablets was investigated according to test method 2.9.6. [22]. Ten randomly selected curcumin-loaded smartFilm tablets were individually immersed in a mixture of 0.1 N hydrochloric acid (HCl) and 0.2% w/w sodium chloride (NaCl) solution containing 1% w/w SDS, under stirring, until complete disintegration of each tablet. 1% w/w SDS was added to ensure complete dissolution of the loaded curcumin. Samples were withdrawn and filtered, followed by discarding the initial ~6 mL of filtrate to ensure saturation of the filter. Following that, the amount of curcumin within each tablet was assessed spectrophotometrically at 425 nm using UV-vis spectroscopy (Multiskan™ GO, Thermo Fischer Scientific, Waltham, MA, USA) and a preconstructed calibration curve (5–10 mg/L).

Dissolution

The dissolution studies were carried out according to the test method 2.9.3. of the European Pharmacopoeia in simulated gastric fluid without pepsin and in simulated intestinal fluid without pancreatin [22]. Six curcumin-loaded smartFilm tablets were individually placed into the vessels of a paddle apparatus PTWS 120D (Pharmatest, Apparatebau AG, Hainburg, Germany), which were filled with 750 mL of a mixture of 0.1 M HCl and 0.2% w/w NaCl solution containing 1% w/w SDS. The paddle speed was adjusted to 100 rpm and the temperature was kept at $37\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$. Samples (10 mL) were taken after 0, 30, 45, 60, 75, 90, and 120 min and replaced with an equal volume (10 mL) of the fresh dissolution medium. After 2 h, 250 mL of 0.2 M tribasic sodium phosphate (TSP) buffer containing 1% w/w SDS, that has been equilibrated to $37\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$, were added to the fluid in each vessel and within 5 min the pH was adjusted to 6.8. Samples (10 mL) were then taken after 1, 2, 4, 6, 10, and 18 h and replaced with an equal volume (10 mL) of a fresh mixture of 0.2 M TSP buffer containing 1% w/w SDS (pH 6.8), 0.1 M HCl and 0.2% w/w NaCl solution containing 1% SDS in a ratio of 1:3. All samples were filtered using a syringe filter with a pore size of 0.22 μm , then samples were analyzed

regarding the amount of dissolved curcumin by using the UV-vis spectroscopy (cf. 2.2.4.7.) The same experiment was conducted by using physical mixture tablets that were produced using a mixture of unloaded paper granules with 20% sucrose content and the same content of curcumin powder.

2.2.5. Determination of Intestinal Permeability

The physiology and the morphology of the porcine GIT bear a strong resemblance to that of the humans [25]. Therefore, the intestinal permeability of curcumin from the different formulations was determined on porcine intestines and the data analysis of permeated curcumin into the intestinal tissue was performed as previously described [26–31], with slight modifications. Fresh intact porcine gastrointestinal tracts were obtained from a local slaughterhouse and were used for the experiments within 2 h after slaughter. From the tissues obtained, small sections (approximately 15 cm in length) of intestinal tissue (i.e., 15–20 cm away from the pylorus) were isolated, dissected longitudinally and spread as a sheet. The intestinal sheets were gently wiped without affecting the villi structure and the pre-existing mucus and, subsequently 50 μ L of the pre-digested formulations were applied onto it without any rubbing or mechanical stress. After 30 min permeation time, punch biopsies (\varnothing 15 mm) were obtained from the differently treated intestinal sections. The intestinal punches were immediately embedded in liquid embedding material (Tissue-Tek[®] O.C.T.TM, Sakura Finetek Europe B.V., Alphen aan den Rijn, The Netherlands), and frozen at –20 °C until further use. Untreated intestinal samples were also biopsied and served as control. Each formulation was tested in triplicate and on different, independent intestinal tissues, i.e., on intestines from different pigs.

Prior to the intestinal application, all formulations (physical mixture tablets, curcumin-loaded smartFilm tablets, commercial products I+II) were subjected to a pre-digestion (i.e., dissolution) procedure. In the first step, adequate amounts of each formulation, i.e., each containing an equivalent curcumin content (~30 mg), were incubated in 100 mL phosphate-buffered saline (PBS, pH 6.8, 37 °C) that contained 1% w/w SDS. The mixtures were stirred for 15 min (300 rpm, universal shaker SM-30 control (Edmund Bühler GmbH, Bodelshausen, Germany) and after this time, 50 μ L aliquots were withdrawn and immediately applied onto the intestinal tissues (cf. 2.2.5.). In addition, similar samples, i.e., 50 μ L from each pre-digested formulation, were withdrawn to analyze the exact amount of dissolved curcumin within these samples (Multiskan[™] GO, Thermo Fischer Scientific, Waltham, MA, USA, 425 nm).

Digital Image Analysis

Curcumin possesses autofluorescence properties, that can be used to trace the permeation of it into tissues [26–28]. For this, the frozen intestinal biopsies were cut into vertical intestinal sections with a thickness of 40 μm by using a Frigocut-2700 cryomicrotome (Reichert-Jung, Wetzlar, Germany). The sections were placed onto microscopic slides and were subsequently examined by inverted epifluorescence microscopy (Olympus CKX53, Olympus Deutschland GmbH, Hamburg, Germany, equipped with Olympus DP22 colour camera, Olympus, Hamburg, Germany). The intensity of the fluorescent light source (130 W U-HGLGPS illumination system, Olympus Deutschland GmbH, Hamburg, Germany) was set to 50% and the exposure time was 50 ms. All samples were analyzed with a 40-fold magnification while using the DAPI HC filter block system (excitation filter: 340–390 nm, dichroic mirror: 410 nm, and emission filter: 420 nm (LP)). From each biopsy, at least 12 intestinal sections were obtained and from each section 3 images were acquired. This resulted in a total of at least 108 images for each formulation tested ($n = 3, 3 \times 12 \times 3 = 108$).

In the next step, the images were subjected to digital image analysis using ImageJ software [32,33]. The first step was an automated threshold that subtracted the autofluorescence of the intestinal tissue (class II pixel) from the autofluorescence of the permeated curcumin (class I pixel, cf. Table S2). After application of the automated threshold, the remaining pixels within each image represent the amount of permeated curcumin as mean grey value/pixel (MGV/px), and thus surrogate the total amount of permeated (TAP) curcumin into the intestinal tissue semi-quantitatively [26–31]. The mean permeation depth of curcumin into the intestine was assessed from the thresholded images with the scale function of the software with the scale set to 0.55 pixel/ μm .

2.2.6. Statistical Analysis

Experiments were performed in triplicates and data were represented as mean \pm standard deviation, unless otherwise noted. Descriptive statistics and statistical assessment of differences between the mean values were performed with JASP software version 16.2 (Universiteit van Amsterdam, Amsterdam, The Netherlands) [34]. After analyzing the data regarding normal distribution (Shapiro–Wilk test) and variance homogeneity (Levene’s test), data were subjected to one-way analysis of variance (ANOVA) or Kruskal-Wallis tests and adequate post hoc tests, such as Tukey’s, Games–Howell, Dunnett and Dunn, were performed to compare the mean values with each other. In some cases, Student’s t-tests for pairwise comparison were performed and the correlation between the in vitro dissolution data with

the data obtained from the ex vivo permeation data was determined by calculating the Spearman's rank correlation coefficient ρ , respectively [34,35]. Differences between means were considered statistically significant if the p -value was < 0.05 .

3. Results and Discussion

3.1. Production and Characterization of Curcumin-Loaded smartFilm Granules

Curcumin-loaded smartFilm granules with 20% sucrose content were successfully prepared (Figure 2A). The particle size ($d(n) 0.5$) was $3 \text{ mm} \pm 0.8 \text{ mm}$ (Figure 2B), and the aspect ratio was 1.4. According to the literature, a value of 1.2 for the aspect ratio is usually appropriate to describe spherical particles [21]. This means the smartFilm granules produced in this study can be considered to possess a slightly elongated shape.

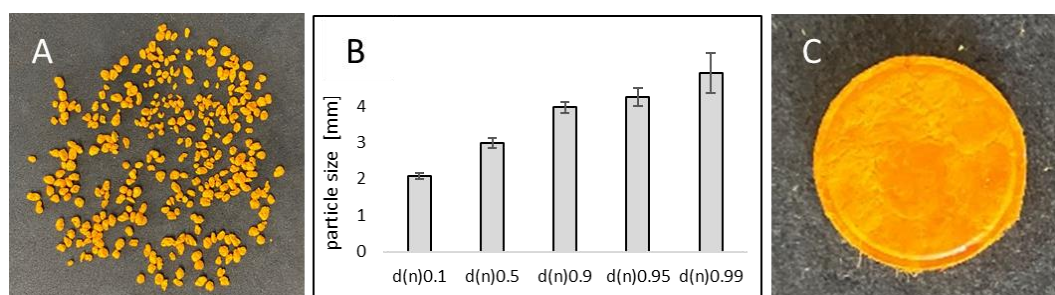


Figure 2. (A) Macroscopic image of smartFilm granules loaded with curcumin. (B) Numeric size distribution of the curcumin-loaded smartFilm granules. (C) Macroscopic image of a smartFilm tablet produced from the smartFilm granules loaded with curcumin.

The bulk and tapped density of the smartFilm granules were 0.18 ± 0.0 and 0.21 ± 0.0 , respectively. These values were significantly higher (Student's t -test, $p < 0.01$) when compared to unloaded paper granules that were previously prepared using the same sucrose content (i.e., 20%) [11]. Hence, loading of curcumin increased the density of the granules. This is reasonable, because curcumin can be considered to be located in the pores of the cellulose matrix. This reduces the volume of the pores and thus increases the density of the formulation. The microscopic images obtained from scanning electron microscopy confirm this assumption. Curcumin bulk powder material is composed of small cubic particles (Figure 3A) and the non-loaded paper granules possess pores but contain no cubic particles (Figure 3B). The physical mixture shows that the cubic particles of the curcumin raw material are mixed with the paper matrix, i.e., both structures can be observed in this image (Figure 3C). For the smartFilm granules, no cubic particles are visible, and the pores of the paper appear

“filled” (Figure 3D). Thus, indicating that the curcumin was loaded into the pores of the paper in non-crystalline state.

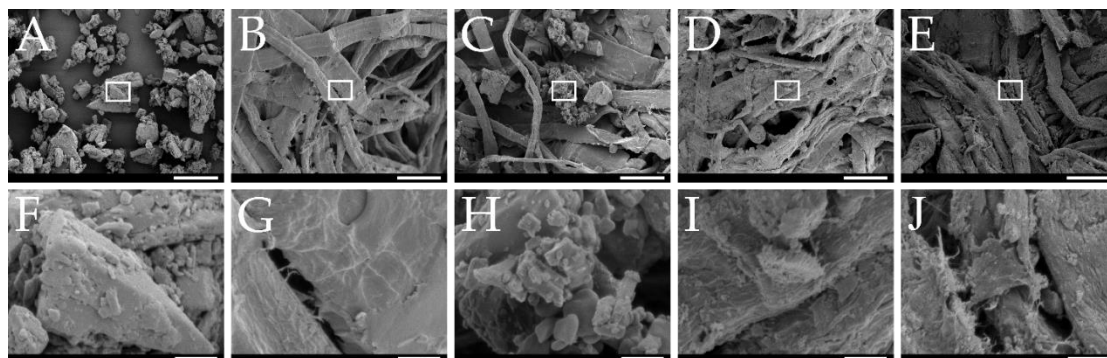


Figure 3. SEM micrographs of (A) pure curcumin, (B) unloaded paper granules, (C) physical mixture, (D) curcumin-loaded smartFilm granules, and (E) curcumin-loaded smartFilm tablets with the respective magnification (F–J) corresponding to the white square. Scale bars represent 60 μm in (A–E) and 7 μm in (F–J).

The calculated Hausner’s ratio, Carr’s index and angle of repose were used as an indication of the flowability of the granules. Hausner’s ratio, Carr’s index and angle of repose values were 1.16 ± 0.0 , $14.4 \pm 0.0\%$, and $31^\circ \pm 0.0$, respectively. According to the European Pharmacopoeia, this indicates good flowability and compressibility of the prepared smartFilm granules [22]. These results were comparable to the results obtained previously from evaluating unloaded paper granules [11]. Thus, suggesting that the filling procedure of curcumin-loaded smartFilm granules, during high-speed tablet manufacturing, will operate efficiently with no major complications [36].

X-ray data (Figure 4) revealed the typical reflexes for the crystalline curcumin bulk material [37]. The reflexes were also visible in the physical mixture that contained identical amounts of curcumin to that of the curcumin loaded smartFilm granules. In contrast, the X-ray pattern of the smartFilm granules showed no reflexes. Thus, suggesting that curcumin was embedded in the paper matrix of the smartFilms granules in amorphous state (Figure 4). This means, wet granulation of the smartFilms that contained curcumin in amorphous state [18] did not alter the crystalline state of the curcumin. Data of this part of the study therefore provided sufficient evidence that curcumin-loaded smartFilm granules can be transferred into smartFilm granules with sufficient pharmaceutical properties and showed that these smartFilm granules are able to maintain the amorphous state of the incorporated curcumin. The smartFilm granules obtained were therefore used as intermediate product for the production of curcumin-loaded smartFilm tablets (cf. 3.2.).

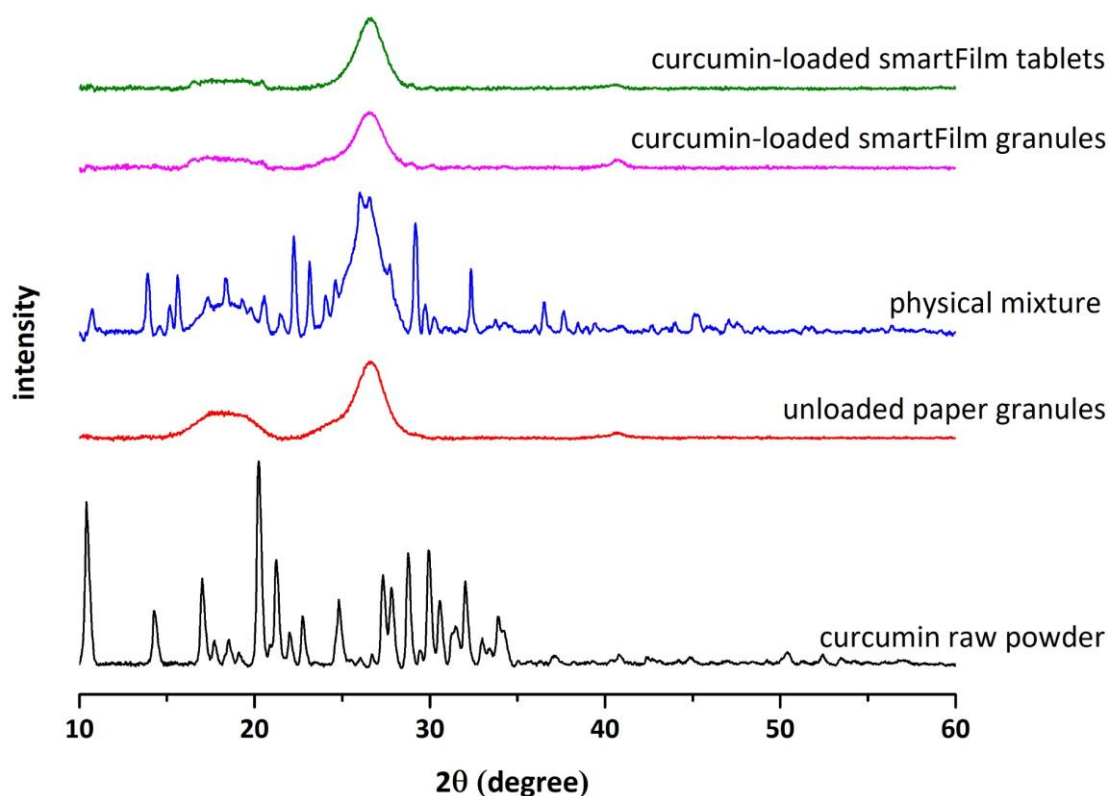


Figure 4. X-ray diffraction patterns of pure curcumin raw powder, unloaded paper granules, physical mixture, curcumin-loaded smartFilm granules, and curcumin-loaded smartFilm tablets.

3.2. Production and Characterization of Curcumin-Loaded smartFilm Tablets

The compression of the curcumin-loaded smartFilm granules resulted in smooth, slightly porous, and yellow-orange tablets with a height of 2.11 ± 0.05 mm (Figure 2C). Microscopic images from scanning electron microscopy showed the absence of crystalline curcumin particles within the tablets (Figure 3E), indicating that curcumin was embedded into the paper matrix in amorphous state. The data obtained from X-ray analysis confirmed this, i.e., no reflexes were found for the curcumin-loaded smartFilm tablets (Figure 4 upper).

In the next step, the produced tablets were evaluated regarding their pharmaceutical properties, i.e., mass uniformity, friability, hardness, disintegration time, content uniformity, and dissolution. The tablets fulfilled all criteria according to the European Pharmacopoeia [22]. The average mass uniformity value was $2.0 \pm 1.2\%$ and the average weight of the produced tablets was 235 ± 0.005 mg. No tablet out of the 20 weighed tablets had an individual mass that differed by 7.5% from the average mass. The friability of the curcumin-loaded smartFilm tablets was found to be 0.086% and the European Pharmacopoeia allows a maximum value of 1.0% [22]. Hence, the smartFilm tablets were within this limit. The crushing

strength value was 115 ± 22 N, indicating a sufficient mechanical strength of the produced tablets. Interestingly, when compared to unloaded paper tablets [11], the data show an insignificant increase (Student's t-test, p value > 0.05) of the force required to crush the curcumin-loaded tablets. It can be assumed that the effect is caused by the incorporation of curcumin into the pores of the paper matrix, which already resulted in an increased density of the smartFilm granules (cf. 3.1.).

Also, regarding the disintegration time, results show that curcumin-loaded smartFilm tablets fulfilled the criteria according to the European Pharmacopoeia, as all tablets disintegrated within 15 min [22]. It was also noticed that the disintegration time of curcumin-loaded tablets exhibited a slight increase when compared to the previously reported data that showed a faster disintegration time for unloaded-paper tablets with the same sucrose content [11]. This might be attributed to the hydrophobic nature of curcumin, which is abundantly loaded within the matrix of the paper. Hence, these data also show a trend towards a lower porosity of the smartFilm tablets, which results not only in an increased hardness of the tablets but also in a slower disintegration time.

The average amount of curcumin loaded within the produced tablets was 15.6 ± 0.5 mg. In addition, curcumin-loaded smartFilm tablets fulfilled the criteria according to the European Pharmacopoeia with an average content uniformity value of $97.6 \pm 2.0\%$, and no tablet out of the ten examined tablets exhibited a content value that differed by 15% from the average content [22].

For the cumulative release profiles of curcumin, significant differences between the physical mixture tablets and curcumin-loaded smartFilm tablets were found for all time points (Figure 5).

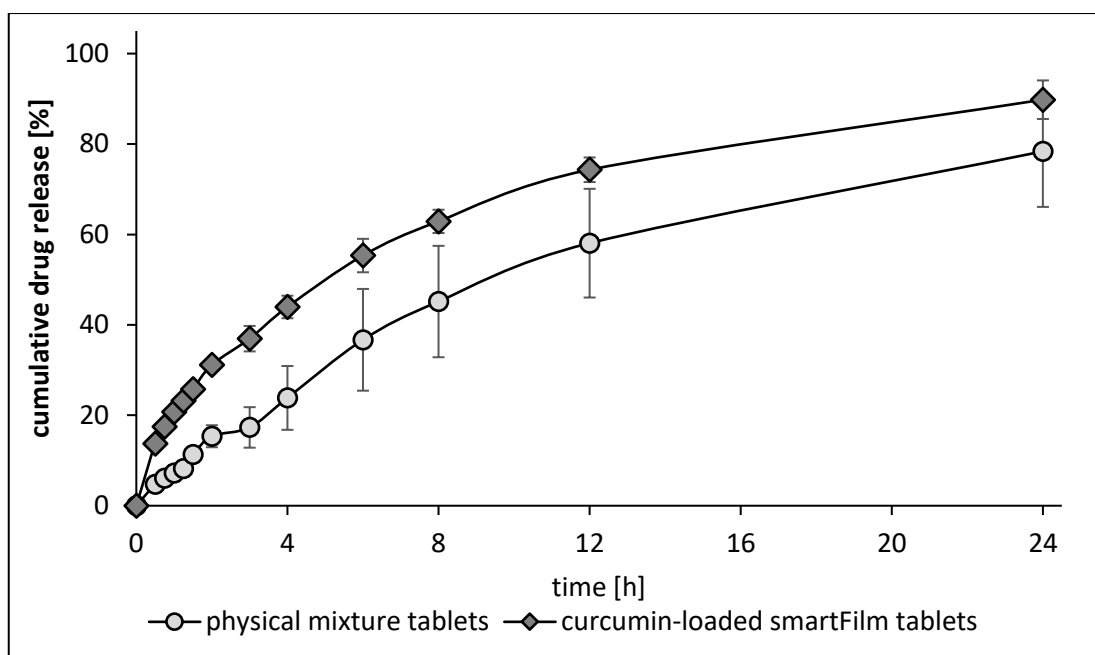


Figure 5. The dissolution profiles of curcumin from physical mixture tablets and curcumin-loaded smartFilm tablets (explanations and details cf. text and Figure S1).

The differences in the dissolution velocity were most pronounced in the beginning. For example, after 1 h, the amount of released curcumin from the smartFilm tablets was about $20.8\% \pm 1.1\%$ and was only $7.2\% \pm 1.8\%$ from the physical mixture tablets (Figure 5). Within 24 h, curcumin-loaded tablets released $89.8\% \pm 4.3\%$, compared to $78.3\% \pm 12.3\%$ of curcumin that was released from the physical mixture tablets (Figure 5).

The results demonstrate that the differences in dissolution rate between smartFilm tablets and physical mixture tablets changed over time, i.e., decreased over time. The increase was about three-fold in the beginning (30 min–1 h) and was about two-fold between 2 h and 4 h dissolution time. After 12 h, the amount of dissolved curcumin was about 30% higher for the smartFilm tablets and about 15% higher after 24 h dissolution time (Figure 5). The findings therefore prove that the dissolution rate of curcumin can be improved with smartFilm tablets and show that the dissolution rate increasing effect of the smartFilms is most pronounced at early time points. The fast dissolution at early time points can be considered to be advantageous, because not only an improved but also a fast dissolution of poorly soluble drugs is considered to be important to improve their oral bioavailability [38–40].

3.3. Determination of Intestinal Permeability

The small intestine is the main absorption site for the majority of orally administered drugs [41]. The intestinal epithelium is arranged into crypts and villi and is covered by a mucus layer,

that acts as a barrier for the diffusion of drugs to the underlying epithelium [42,43]. Due to the complexity of the intestinal tissue, simple in vitro release data cannot cover all aspects that occur during the absorption of drugs into the gut. Therefore, besides in vivo studies that cannot always be performed, ex vivo intestinal models that are able to closely mimic the physiological conditions of the small intestine, are important tools to predict the intestinal permeability of drugs from different formulations [44]. In this study, the porcine intestines were treated with the different formulations and the permeation of curcumin into the intestinal tissue was inspected with epifluorescence microscopy (Figure 6.).

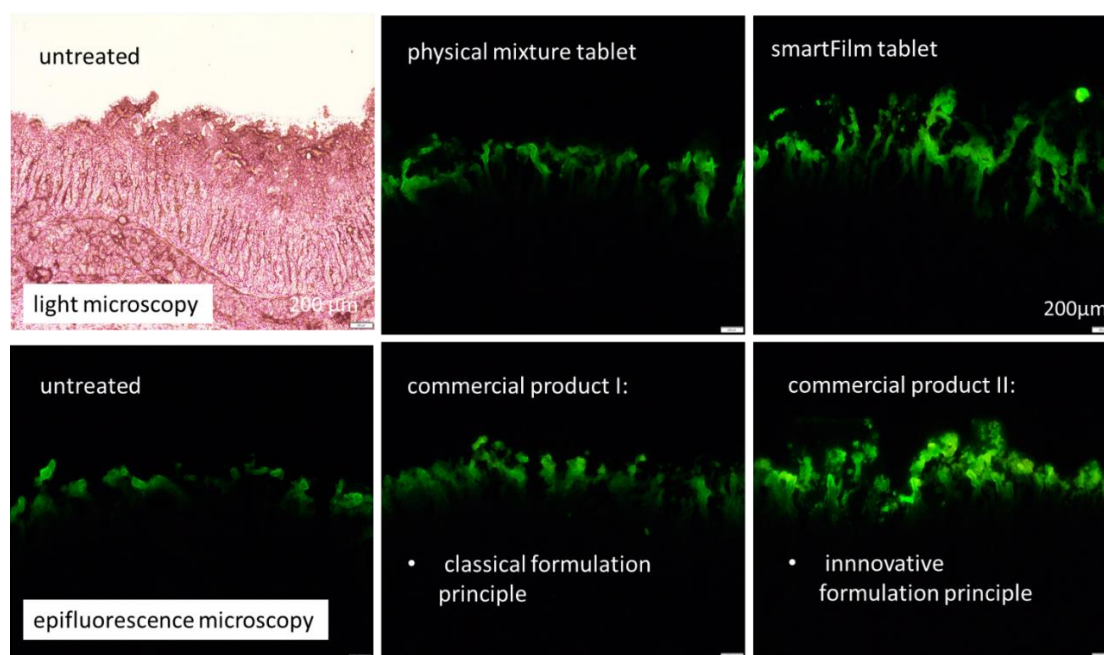


Figure 6. Epifluorescence microscopic images (40-fold magnification) of porcine intestinal sections treated with different curcumin-loaded formulations, as compared to the untreated intestinal tissue. In addition, an image of the intestinal tissue with similar magnification taken with light microscopy is shown to visualize the morphology of the intestinal tissue used for the study (**upper left**). Scale bars correspond to 200 μm.

The images show different permeation profiles of curcumin from the different formulations and show a trend towards an improved permeation of curcumin from the smartFilm tablets and the commercial product II (Figure 6). The trends observed from the images became clearer and proved being significant after digital image analysis of the images that assessed the total amount of permeated curcumin (TAP) and the mean permeation depth (MPD), (Figures 7 and 8).

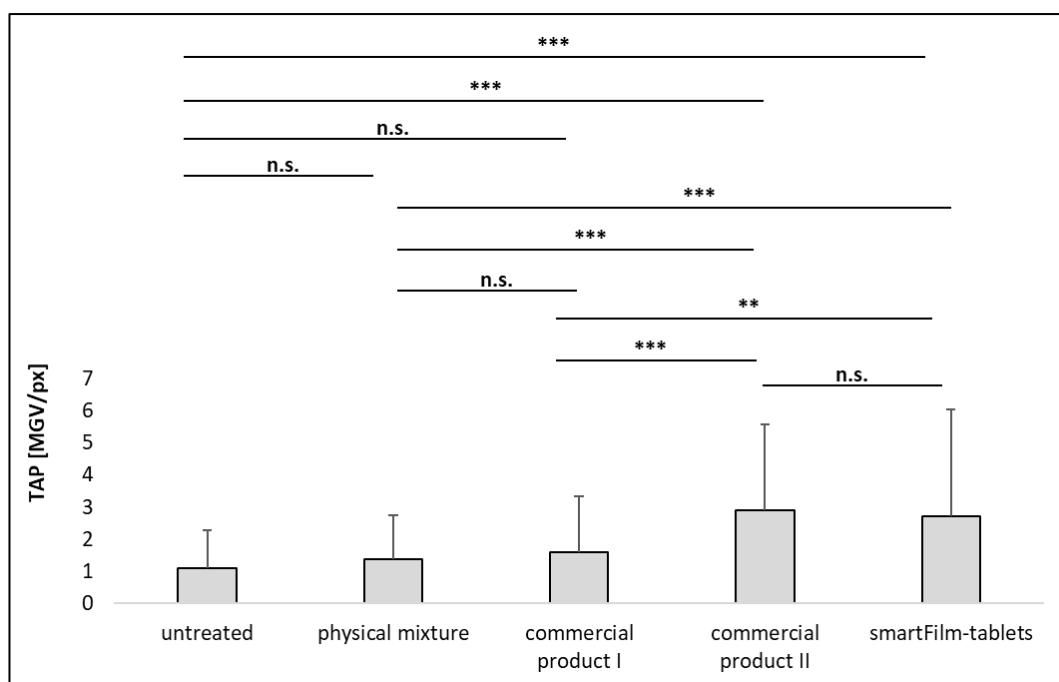


Figure 7. Permeated amount of curcumin from different formulations. The total amount permeated (TAP, MGV/px) is a semi-quantitative parameter that surrogates the intestinal permeability, i.e., oral bioavailability, of curcumin (n.s.—nonsignificant, ** $p < 0.01$, *** $p < 0.001$).

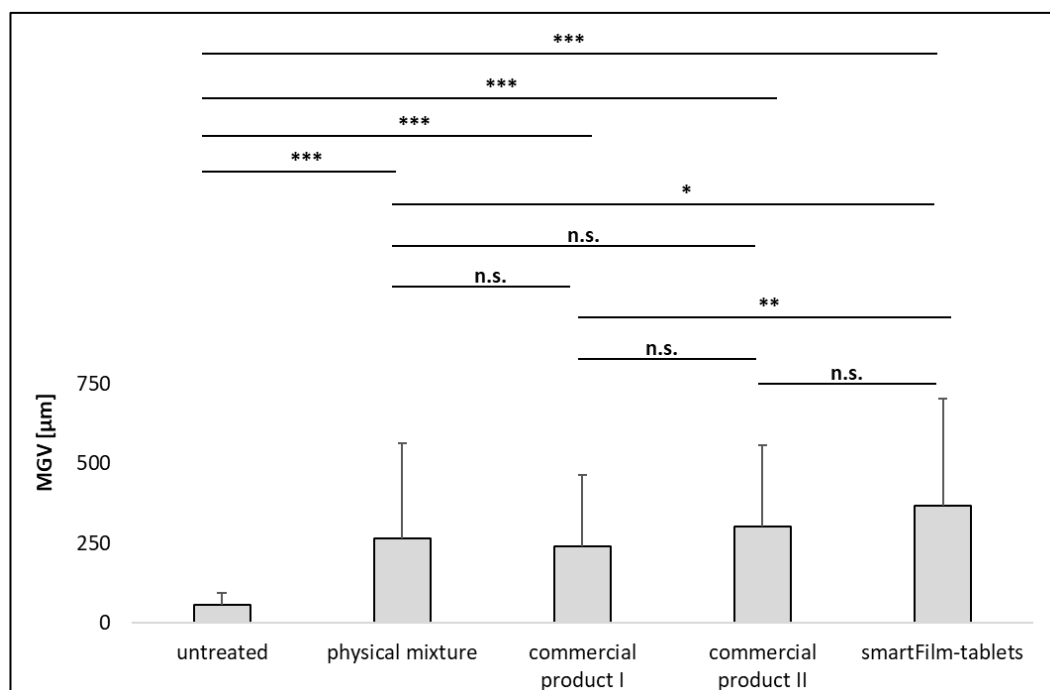


Figure 8. Mean permeation depth of curcumin from different formulations. (n.s.—nonsignificant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

No significant differences in the signal intensity (MGV/px) were found between untreated intestine, the physical mixture tablet, and the commercial product I (Figure 7). However, the permeation depth was significantly higher for the physical mixture tablet and the commercial product I when compared to the untreated tissue (Figure 8). Data indicate that both formulations can be considered to possess a very poor intestinal permeability for curcumin. This was expected, because both formulations contain the curcumin as powder, which is known to possess poor solubility and poor intestinal permeability [15].

Commercial product II, the micellar curcumin, which is considered to result in an optimal oral bioavailability and intestinal absorption of curcumin (cf. 1., [16]), resulted in significantly higher amounts of permeated curcumin than the physical mixture tablet and commercial product I (Figure 7). However, the permeation depth was not altered when compared to the physical mixture tablet and commercial product I (Figure 8).

The smartFilm tablets resulted in similarly high amounts of permeated curcumin as commercial product II (Figure 7). Interestingly, in comparison to the physical mixture and commercial product I, it was found that the permeation depth was significantly enhanced (+38% and +52%, respectively) when curcumin was applied with the smartFilms. The increase in permeation depth was 22% but nonsignificant in comparison to commercial product II (Figure 8).

With this, the smartFilm tablets can be considered to yield comparable intestinal permeation values for curcumin to that of the micellar curcumin, with a slight trend towards a deeper permeation of curcumin. The deeper permeation from the smartFilm tablets is reasonable, because the pre-digestion procedure fully disintegrated the smartFilm tablet, but left behind some pieces of paper. The paper adhered to the gut and can be considered to have caused a locally higher concentration gradient for curcumin between the smartFilm and the intestine, which then promoted a deeper permeation into the intestinal tissue. Improved permeation via a locally high concentration gradient was previously shown for particles and smartFilms that were applied on skin [18,45] and it is very likely that similar effects also occur in the gut. However, more research is needed to investigate and understand this observation in detail.

In the last part of the study the relationship between the in vitro dissolution data (cf. Figure 5) and the ex vivo permeation data (TAP and MPD) was assessed for the physical mixture and the smartFilm tablets (Figure 9). In addition, to gain more detailed information on the in vitro ex vivo correlation, the amount of released curcumin, that was assessed from

all different formulations after the in vitro pre-digestion step and immediately prior to the application onto the intestinal tissues, was correlated to the ex vivo permeation data for all formulations tested (cf. Figure 9, right).

The correlation of the data from physical mixture and smartFilm tablets show significant relations between the in vitro data and the ex vivo data (Figure 9). A very strong correlation (>0.8) was found between the dissolution data after 30 and 45 min of dissolution. With longer dissolution times the correlation coefficient decreased to <0.5 after 24 h, which is still considered to be a relatively strong correlation [46]. However, the decrease in correlation coefficient indicates that the release of curcumin is not following a constant function.

Reasons for this might be, for example, a partial oversaturation of the system and subsequent re-crystallization of the curcumin. Another possibility is the non-linear release of the curcumin from the paper matrix of the smartFilms (cf. Figure 5), which were recently shown to follow a super case II release kinetics, which is caused by the changes that occur after the wetting of the paper matrix [18].

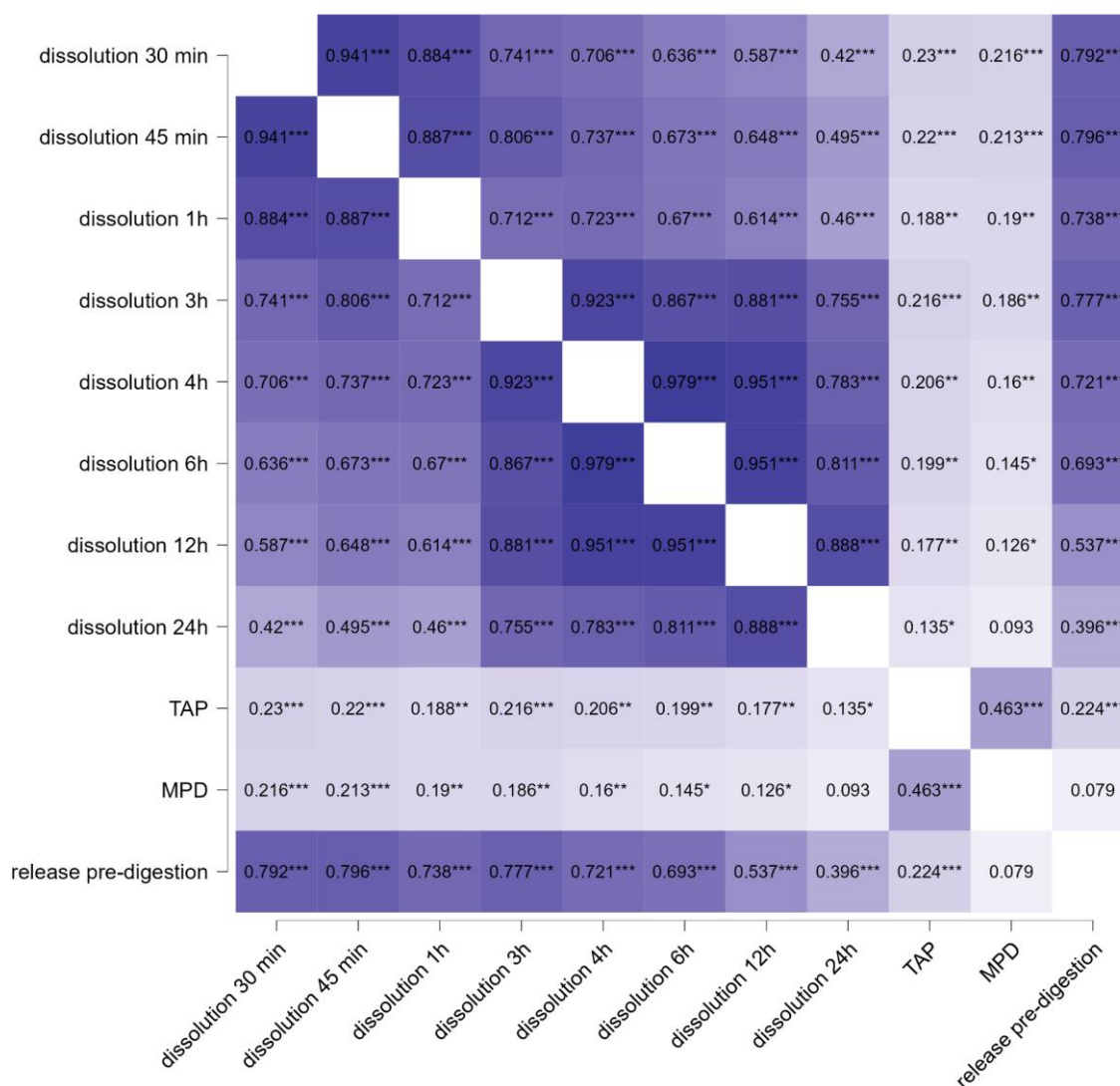


Figure 9. Heatmap of Spearman's rank correlation coefficients ρ that assess the relationship between the in vitro dissolution data and the ex vivo permeation data for the physical mixture and the smartFilm tablets. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

In this study, the formulations were applied onto the intestinal tissue after a 15 min pre-digestion step. This is closest to the 30 min time point for the in vitro dissolution data. The correlation coefficient between the in vitro data 30 min is 0.233 ($p < 0.001$) for the TAP and 0.216 ($p < 0.001$) for the MPD (Figure 9), which represents a moderate correlation according to Rea and Parker [46]. The correlation coefficient decreases, and the p -values increase with increasing dissolution time, indicating that the in vitro ex vivo correlation between dissolution data and ex vivo model declines for longer dissolution times. This trend was expected and points towards a high sensitivity of the intestinal model because the dissolution of curcumin

is nonlinear. Therefore, a good in vitro ex vivo correlation can only be yielded if similar dissolution/pre-digestion times are used for the correlation of the data.

For the comparison between the in vitro and ex vivo data for all formulations tested, the correlation between curcumin released after the pre-digestion and the amount of permeated curcumin into the intestine was calculated. The resulting Spearman's rank correlation coefficient ρ was 0.224 ($p < 0.001$), which also indicates a moderate in vitro ex vivo correlation for the total amount of permeated drug [46]. However, the correlation coefficient was only 0.079 ($p = 0.05$) for the MPD, indicating no in vitro ex vivo correlation between the in vitro dissolution data and the ex vivo permeation depth. This might be explained by the deeper permeation depth of the curcumin from the smartFilm tablets, which was probably caused by the parts of paper that adhered to the intestinal tissue (cf. above). To prove this theory, the Spearman's rank correlation coefficient was re-calculated while excluding the data obtained from the smartFilm tablet formulation. The resulting correlation coefficient was 0.128 ($p = 0.01$), which indicates a weak but significant in vitro ex vivo correlation [46]. The results further substantiate the theory that the smartFilm tablets can cause an improved permeation depth of curcumin via a locally high concentration gradient that resulted from the adherence of pieces of paper to the intestinal tissue.

According to Kinam Park and co-workers BCS class IV drugs are very unlikely to yield strong in vitro ex vivo correlations between dissolution profiles and in vivo permeation, whereas good and very strong in vitro ex vivo correlations can be established for BCS class II drugs [40]. In most cases, curcumin is considered a BCS class IV drug [47,48]. However, sometimes it is also referred to be a BCS-class II drug [49]. Thus, while considering curcumin as an intermediate drug substance with BCS class II and IV properties, the in vitro ex vivo correlations found in this study become very reasonable. Nevertheless, when compared to the in vitro data, the ex vivo data obtained here allowed for a more detailed investigation of the effect of the different formulation principles on the intestinal permeation of curcumin. Therefore, the use of an intestinal ex vivo model can be recommended as a simple and cost-efficient approach to judge the intestinal permeation efficacy of drugs from different formulations in early formulation development. The ex vivo data obtained in this study could clearly discriminate between the formulations possessing poor or good intestinal permeation properties for curcumin. Thus, providing a base for an efficient development of optimized formulations with excellent oral bioavailability for curcumin.

4. Conclusions

The study showed that drug-loaded smartFilms can be transferred into smartFilm granules and smartFilm tablets. Results also showed that the curcumin-loaded smartFilm granules and smartFilm tablets maintained the amorphous state of the incorporated drug. Hence, wet granulation was found to be a feasible approach for the production of smartFilm granules from which smartFilm tablets can be compressed in industrial, large scale. The resulting tablets fulfill the criteria according to the European Pharmacopoeia regarding resistance to crushing, friability, content uniformity, mass uniformity, and disintegration time. The formulation of curcumin in smartFilm tablets resulted in an improved dissolution rate and enhanced intestinal permeability when compared to a physical mixture tablet. The intestinal permeability of curcumin from a marketed product that contained curcumin as raw powder was not significantly higher than the physical mixture but was at least two-fold higher for a commercial product that contained micellar curcumin. A two-fold higher intestinal permeability was also found for the smartFilm tablets. Between the commercialized micellar curcumin (for which previous in vivo studies already demonstrated a superior oral bioavailability in comparison to other innovative formulation principles, e.g., liposomes, phytosomes, submicron crystals, or cyclodextrines) and the smartFilm tablets no significant differences in the total amount of permeated curcumin were found. However, a trend towards a deeper permeation of the curcumin from the smartFilms was found. The effect was significant, thus rendering the smartFilm tablets to be the most effective formulation for the intestinal permeation of curcumin. Based on the results it can therefore be concluded that smartFilm tablets are an industrially feasible formulation approach for improved oral delivery of poorly soluble drugs.

Acknowledgments: Authors would also like to extend thanks to Yousef Jameel Foundation for providing the Scholarship funding for Ayat Abdelkader. Authors would like to extend thanks to Udo Bakowsky for providing the SEM facilities.

5. References

- [1] Homayun, B.; Lin, X.; Choi, H.-J. Challenges and Recent Progress in Oral Drug Delivery Systems for Biopharmaceuticals. *Pharmaceutics* **2019**, *11*, 129. <https://doi.org/10.3390/pharmaceutics11030129>.
- [2] Hua, S. Advances in Oral Drug Delivery for Regional Targeting in the Gastrointestinal Tract-Influence of Physiological, Pathophysiological and Pharmaceutical Factors. *Front. Pharmacol.* **2020**, *11*, 524. <https://doi.org/10.3389/fphar.2020.00524>.
- [3] Da Silva, F.L.O.; Marques, M.B.D.; Kato, K.C.; Carneiro, G. Nanonization techniques to overcome poor water-solubility with drugs. *Expert Opin. Drug Discov.* **2020**, *15*, 853–864. <https://doi.org/10.1080/17460441.2020.1750591>.
- [4] Boyd, B.J.; Bergström, C.A.S.; Vinarov, Z.; Kuentz, M.; Brouwers, J.; Augustijns, P.; Brandl, M.; Bernkop-Schnürch, A.; Shrestha, N.; Préat, V.; et al. Successful oral delivery of poorly water-soluble drugs both depends on the intraluminal behavior of drugs and of appropriate advanced drug delivery systems. *Eur. J. Pharm. Sci.* **2019**, *137*, 104967. <https://doi.org/10.1016/j.ejps.2019.104967>.
- [5] Fathi, H.A.; Allam, A.; Elsabahy, M.; Fetih, G.; El-Badry, M. Nanostructured lipid carriers for improved oral delivery and prolonged antihyperlipidemic effect of simvastatin. *Colloids Surf. B* **2018**, *162*, 236–245.
- [6] Lemke, S.; Strätling, E.J. SmartFilms-oral and peroral films for optimized delivery of nanoparticulate or amorphous drugs. *Controlled Release Society Local Chapter, Saarbrücken/Germany* **2016**, *7*.
- [7] Lemke, S.; Strätling, E.J.; Welzel, H.P. Cellulosefaserbasierte Trägermatrices (smartFilms) zur Applikation von Inhaltsstoffen Sowie Deren Herstellung. German Patent Application DE102016000541A1, 20 July 2017.
- [8] Ornik, J.; Knoth, D.; Koch, M.; Keck, C.M. Terahertz-spectroscopy for non-destructive determination of crystallinity of L-tartaric acid in smartFilms® and tablets made from paper. *Int. J. Pharm.* **2020**, *581*, 119253.
- [9] Subrahmanyeswari, C.D.; Prasanth, Y.; Sameeda, R. Formulation and development of efavirenz tablets by paper technique using co-solvency method. *Int. J. Curr. Pharm. Res.* **2019**, *11*, 87–92. <https://doi.org/10.22159/ijcpr.2019v11i6.36349>.
- [10] Stumpf, F.; Keck, C.M. Tablets made from paper. *Int. J. Pharm.* **2018**, *548*, 812–819. <https://doi.org/10.1016/j.ijpharm.2018.05.071>.

- [11] Abdelkader, A.; Moos, C.; Pelloux, A.; Pfeiffer, M.; Alter, C.; Kolling, S.; Keck, C.M. Granulation as a reliable approach for large scale production of paper tablets. *Pharmaceuticals* 2022, *submitted*.
- [12] Tsuda, T. Curcumin as a functional food-derived factor: Degradation products, metabolites, bioactivity, and future perspectives. *Food Funct.* **2018**, *9*, 705–714.
- [13] Kotha, R.R.; Luthria, D.L. Curcumin: Biological, pharmaceutical, nutraceutical, and analytical aspects. *Molecules* **2019**, *24*, 2930.
- [14] Hewlings, S.J.; Kalman, D.S. Curcumin: A review of its effects on human health. *Foods* **2017**, *6*, 92.
- [15] Carvalho Henriques, M.; Faustino, M.A.F.; Santos Braga, S. Curcumin innovative delivery forms: Paving the ‘Yellow Brick Road’ of antitumoral phytotherapy. *Appl. Sci.* **2020**, *10*, 8990.
- [16] Flory, S.; Sus, N.; Haas, K.; Jehle, S.; Kienhöfer, E.; Waehler, R.; Adler, G.; Venturelli, S.; Frank, J. Increasing Post-Digestive Solubility of Curcumin Is the Most Successful Strategy to Improve its Oral Bioavailability: A Randomized Cross-Over Trial in Healthy Adults and In Vitro Bioaccessibility Experiments. *Mol. Nutr. Food Res.* **2021**, *65*, 2100613.
- [17] Ornik, J.; Heidrich, L.; Schesny, R.; Castro-Camus, E.; Keck, C.M.; Koch, M. Non-destructive crystallinity assessment of indomethacin in tablets made from smartFilms® using terahertz time-domain spectroscopy. *Sci. Rep.* **2022**, 6099.
- [18] Eckert, R.W.; Wiemann, S.; Keck, C.M. Improved dermal and transdermal delivery of curcumin with smartfilms and nanocrystals. *Molecules* **2021**, *26*, 1633.
- [19] Stumpf, F. Tabletten aus Papier—Tablets Made from Paper—Zur Oralen Applikation Schwerlöslicher Wirkstoffe. Ph.D. Thesis, Philipps-Universität Marburg, Marburg, Germany, 2019.
- [20] Pelikh, O.; Eckert, R.W.; Pinnapireddy, S.R.; Keck, C.M. Hair follicle targeting with curcumin nanocrystals: Influence of the formulation properties on the penetration efficacy. *J. Control. Release* **2021**, *329*, 598–613.
- [21] Chopra, R.; Michael Newton, J.; Alderborn, G.; Podczek, F. Preparation of pellets of different shape and their characterization. *Pharm. Dev. Technol.* **2001**, *6*, 495–503.
- [22] C.H. Beck. *European Pharmacopoeia*, 8th ed.; 2.09: Pharmaceutical Technical Procedures; C.H. Beck: Nördlingen, Germany, 2016.
- [23] Aslani, A.; Jahangiri, H. Formulation, characterization and physicochemical evaluation of ranitidine effervescent tablets. *Adv. Pharm. Bull.* **2013**, *3*, 315–322. <https://doi.org/10.5681/apb.2013.051>.

-
- [24] Sitterberg, J.; Ozcetin, A.; Ehrhardt, C.; Bakowsky, U. Utilising atomic force microscopy for the characterisation of nanoscale drug delivery systems. *Eur. J. Pharm. Biopharm.* **2010**, *74*, 2–13. <https://doi.org/10.1016/j.ejpb.2009.09.005>.
- [25] Zhang, Q.; Widmer, G.; Tzipori, S. A pig model of the human gastrointestinal tract. *Gut Microbes* **2013**, *4*, 193–200.
- [26] Keck, C.M.; Abdelkader, A.; Pelikh, O.; Wiemann, S.; Kaushik, V.; Specht, D.; Eckert, R.W.; Alnemari, R.M.; Dietrich, H.; Brüßler, J. Assessing the Dermal Penetration Efficacy of Chemical Compounds with the Ex-Vivo Porcine Ear Model. *Pharmaceutics* **2022**, *14*, 678. <https://doi.org/10.3390/pharmaceutics14030678>.
- [27] Keck, C.M.; Chairateep, E.; Dietrich, H.; Sengupta, S. Influence of Mechanical Skin Treatments on Dermal Penetration Efficacy of Active Ingredients. *Pharmaceutics*. **2022**, *14*:1788. <https://doi.org/10.3390/pharmaceutics14091788>.
- [28] Pelikh, O.; Pinnapireddy, S.R.; Keck, C.M. Dermal penetration analysis of curcumin in an ex-vivo porcine ear model using epifluorescence microscopy and digital image processing. *Skin Pharmacol. Physiol.* **2021**, *34*, 281–299.
- [29] Kaushik, V.; Ganashalingam, Y.; Schesny, R.; Raab, C.; Sengupta, S.; Keck, C.M. Influence of Massage and Skin Hydration on Dermal Penetration Efficacy of Nile Red from Petroleum Jelly-An Unexpected Outcome. *Pharmaceutics* **2021**, *13*, 2190. <https://doi.org/10.3390/pharmaceutics13122190>.
- [30] Kaushik, V.; Keck, C.M. Influence of mechanical skin treatment (massage, ultrasound, microdermabrasion, tape stripping and microneedling) on dermal penetration efficacy of chemical compounds. *Eur. J. Pharm. Biopharm.* **2021**, *169*, 29–36. <https://doi.org/10.1016/j.ejpb.2021.09.003>.
- [31] Keck, C.M.; Specht, D.; Brüßler, J. Influence of lipid matrix composition on biopharmaceutical properties of lipid nanoparticles. *J. Control. Release* **2021**, *338*, 149–163. <https://doi.org/10.1016/j.jconrel.2021.08.016>.
- [32] Rasband, W.S. ImageJ: Image processing and analysis in Java. Astrophysics Source Code Library: Cambridge, MA, USA, 2012; ascl: 1206.013.
- [33] Schneider, C.A.; Rasband, W.S.; Eliceiri, K.W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **2012**, *9*, 671–675.
- [34] JASP Team. *JASP*; Version 0.162; JASP Team: Amsterdam, The Netherlands, 2022.
- [35] Cohen, J. *Statistical Power Analysis for the Behavioral Sciences*; Taylor and Francis: Hoboken, NJ, USA, 2013; ISBN 9781134742707.
-

- [36] Chen, H.; Wang, C.; Liu, S.; Sun, C.C. Development of piroxicam mini-tablets enabled by spherical cocrystallization. *Int. J. Pharm.* **2020**, *590*, 119953.
- [37] Chereddy, K.K.; Coco, R.; Memvanga, P.B.; Ucakar, B.; Des Rieux, A.; Vandermeulen, G.; Pr  at, V. Combined effect of PLGA and curcumin on wound healing activity. *J. Control. Release* **2013**, *171*, 208–215.
- [38] Jinno, J.; Kamada, N.; Miyake, M.; Yamada, K.; Mukai, T.; Odomi, M.; Toguchi, H.; Liversidge, G.G.; Higaki, K.; Kimura, T. In vitro-in vivo correlation for wet-milled tablet of poorly water-soluble cilostazol. *J. Control. Release* **2008**, *130*, 29–37. <https://doi.org/10.1016/j.jconrel.2008.05.013>.
- [39] Jinno, J.; Kamada, N.; Miyake, M.; Yamada, K.; Mukai, T.; Odomi, M.; Toguchi, H.; Liversidge, G.G.; Higaki, K.; Kimura, T. Effect of particle size reduction on dissolution and oral absorption of a poorly water-soluble drug, cilostazol, in beagle dogs. *J. Control. Release* **2006**, *111*, 56–64.
- [40] Lu, Y.; Kim, S.; Park, K. In vitro-in vivo correlation: Perspectives on model development. *Int. J. Pharm.* **2011**, *418*, 142–148. <https://doi.org/10.1016/j.ijpharm.2011.01.010>.
- [41] Mackie, A.R.; Round, A.N.; Rigby, N.M.; Macierzanka, A. The role of the mucus barrier in digestion. *Food Dig.* **2012**, *3*, 8–15.
- [42] Lock, J.Y.; Carlson, T.L.; Carrier, R.L. Mucus models to evaluate the diffusion of drugs and particles. *Adv. Drug Deliv. Rev.* **2018**, *124*, 34–49. <https://doi.org/10.1016/j.addr.2017.11.001>.
- [43] Boegh, M.; Nielsen, H.M. Mucus as a barrier to drug delivery–understanding and mimicking the barrier properties. *Basic Clin. Pharmacol. Toxicol.* **2015**, *116*, 179–186.
- [44] Xu, Y.; Shrestha, N.; Pr  at, V.; Beloqui, A. An overview of in vitro, ex vivo and in vivo models for studying the transport of drugs across intestinal barriers. *Adv. Drug Deliv. Rev.* **2021**, *175*, 113795.
- [45] Wiemann, S.; Keck, C.M. Particle-Assisted Dermal Penetration-A Simple Formulation Strategy to Foster the Dermal Penetration Efficacy. *Pharmaceutics* **2022**, *14*, 1039. <https://doi.org/10.3390/pharmaceutics14051039>.
- [46] Rea, L.M.; Parker, R.A. *Designing and Conducting Survey Research: A Comprehensive Guide*, 4th ed.; Jossey-Bass: San Francisco, CA, USA, 2014; ISBN 9781118767009.
- [47] Visser, M.R.; Baert, L.; Klooster, G.V.; Schueller, L.; Geldof, M.; Vanwelkenhuysen, I.; Kock, H. de; Meyer, S. de; Frijlink, H.W.; Rosier, J.; et al. Inulin solid dispersion technology to improve the absorption of the BCS Class IV drug TMC240. *Eur. J. Pharm. Biopharm.* **2010**, *74*, 233–238. <https://doi.org/10.1016/j.ejpb.2009.10.004>.

-
- [48] Wang, Y.; Wang, C.; Zhao, J.; Ding, Y.; Li, L. A cost-effective method to prepare curcumin nanosuspensions with enhanced oral bioavailability. *J. Colloid Interface Sci.* **2017**, *485*, 91–98. <https://doi.org/10.1016/j.jcis.2016.09.003>.
- [49] Wan, S.; Sun, Y.; Qi, X.; Tan, F. Improved bioavailability of poorly water-soluble drug curcumin in cellulose acetate solid dispersion. *AAPS PharmSciTech* **2012**, *13*, 159–166. <https://doi.org/10.1208/s12249-011-9732-9>.

6. Supplementary material

Table S1: Macro used for the determination of the Feret's diameter, with a scale set to 30.9 $\mu\text{m}/\text{px}$.

```
run("8-bit");

setAutoThreshold("Default");

//run("Threshold...");

//setThreshold;(180 ,0)

setOption("BlackBackground", false);

run("Convert to Mask");

run("Analyze Particles...", "size=0.09-Infinity show=Masks display");
```

Table S2: Macro used for the automated threshold to subtract the autofluorescence of the intestinal tissue from the fluorescence of the permeated curcumin.

```
//Color Thresholder 1.53k

//Autogenerated macro, single images only!

min=newArray;(3)

max=newArray;(3)

filter=newArray;(3)

a=getTitle();

run("RGB Stack");

run("Convert Stack to Images");

selectWindow("Red");
```

```
rename;"0")

selectWindow("Green");

rename;"1")

selectWindow("Blue");

rename;"2")

min[0]=0;

max[0]=0;

filter[0]="stop;"

min[1]=75;

max[1]=255;

filter[1]="pass;"

min[2]=0;

max[2]=0;

filter[2]="stop;"

for (i=0;i<3;i++){

    selectWindow(""+i);

    setThreshold(min[i], max[i]);

    run("Convert to Mask");

    if (filter[i]=="stop") run("Invert");

{

imageCalculator("AND create", "0","1");

imageCalculator("AND create", "Result of 0","2");

for (i=0;i<3;i++){

    selectWindow(""+i);

    close;()
```

```

{
selectWindow("Result of 0");

close;()

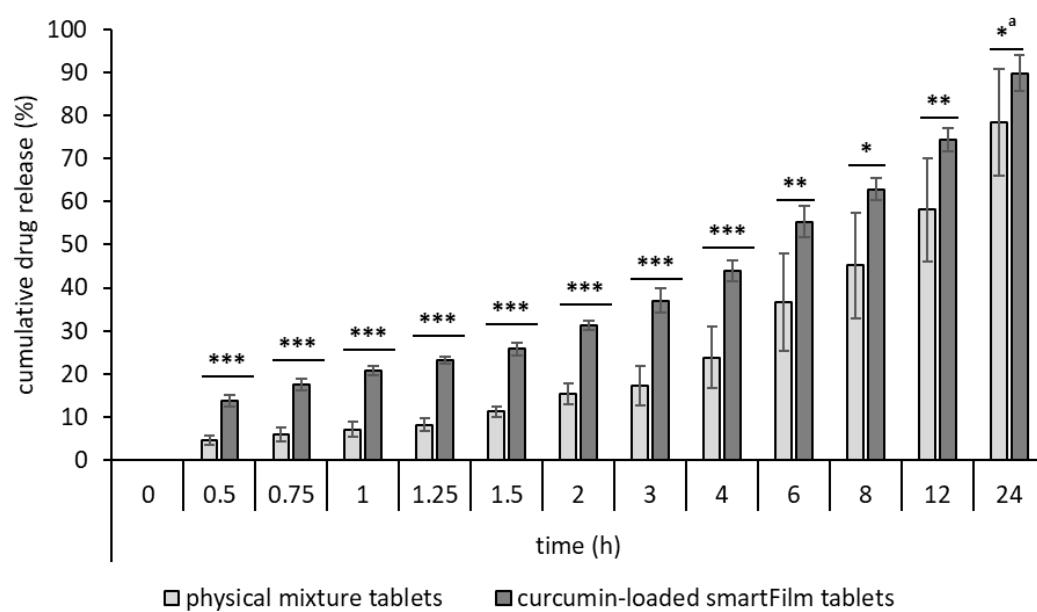
selectWindow("Result of Result of 0");

rename(a);

//Colour Thresholding-----

run("Invert");

```



^a one-tailed t-test

Figure S1. The dissolution profiles of curcumin from physical mixture tablets and curcumin-loaded smartFilm tablets. * $p < .05$, ** $p < .01$, *** $p < .00$

3.3. Improving the Bioactivity of Norfloxacin with Tablets Made from Paper

Ayat Abdelkader ^{1,2}, Laura Nallbati ¹ and Cornelia M. Keck ¹

¹ Department of Pharmaceutics and Biopharmaceutics, Philipps-Universität Marburg, Robert-Koch-Str. 4, 35037 Marburg, Germany.

² Assiut International Center of Nanomedicine, Al-Rajhi Liver Hospital, Assiut University, Assiut 71515, Egypt.

(adapted from Pharmaceutics 2023,)

Abstract

Many drugs possess poor bioavailability, and many strategies are available to overcome this issue. In this study, smartFilm technology, i.e., a porous cellulose matrix (paper), in which the active compound can be loaded onto in an amorphous state was utilized for oral administration to improve the solubility and bioactivity of a poorly soluble BSC class IV antibiotic. Norfloxacin was used as the model drug and loaded into commercially available paper. The resulting norfloxacin-loaded smartFilms were transformed into smartFilm granules via wet granulation and the resulting norfloxacin-loaded smartFilm granules were transformed into norfloxacin-loaded tablets made from paper, i.e., smartFilm tablets. The crystalline state of norfloxacin was investigated, as well as the pharmaceutical properties of the granules and the tablets. The bioactivity of the smartFilm tablets was assessed in vitro and ex vivo to determine the antibacterial activity of norfloxacin. The results were compared to a physical mixture tablet that contained non-loaded paper granules and equal amounts of norfloxacin as a crystalline powder. Norfloxacin-loaded smartFilm granules and norfloxacin-loaded smartFilm tablets contained norfloxacin in an amorphous state, which resulted in an improved and faster release of norfloxacin when compared to the physical mixture tablet. The bioactivity was up to three times higher when compared to the physical mixture tablet. The ex vivo model was demonstrated to be a useful tool that allows for a fast and cost-effective discrimination between “good” and “bad” formulations. It provides realistic physiological conditions and can therefore yield meaningful, additional biopharmaceutical information that cannot be assessed in classical in vitro experiments. SmartFilm tablets are a promising, universal, industrially feasible and cost-effective formulation strategy for improved solubility and enhanced bioactivity of poorly soluble drugs.

1. Introduction

Many drugs and many new chemical entities possess poor solubility which is associated with poor oral bioavailability [1]. Therefore, strategies that overcome this issue are important to allow for efficient oral drug delivery of these compounds. In recent years, various formulation approaches have been developed for this. Examples include incorporation into micelles, cyclodextrins, self-emulsifying drug delivery systems, microemulsions, liposomes, lipid nanoparticles, solid dispersions, nano-milling or the loading of the active ingredient into porous materials [2,3]. A novel formulation approach is smartFilm technology [4,5]. The smartFilm technology utilizes ordinary paper in which active compounds can be loaded onto in an amorphous state. For this, the active ingredient is dissolved in an appropriate solvent. The resulting solution is then applied to the cellulose-based paper matrix. After drying, the drug is located in the pores of the paper in an amorphous state. This technique is simple and requires no costly equipment [6,7].

The superiority of the smartFilms over other classical (bulk powder suspension) and innovative formulation principles (nanocrystals) has already been demonstrated for dermal applications [8]. For oral drug delivery such a proof-of-concept study has not yet been performed. One reason is the inability to transform smartFilms, i.e., pieces of paper, into a convenient-to-swallow dosage form. This has been overcome by manually transferring paper into paper tablets [6,7]. These tablets were shown to possess sufficient pharmaceutical properties, i.e., they fulfilled the criteria required by the European Pharmacopeia. However, these tablets were produced by manually cutting pieces of paper into small pieces that were then placed into the die of a tableting machine to be compressed manually. Hence, at that stage, the paper tablets could not be produced on a larger, industrial scale. A technology that cannot be produced on a large, industrial scale cannot be transferred into real world products. Therefore, a method that allows for the production of paper tablets on a larger scale was developed [9]. This method was a three-step process. In the first step the paper was milled. The milled paper was transformed into paper granules via a wet granulation process. The paper granules then served as an intermediate product for the production of paper tablets.

Paper granules without additional excipients can be produced but will not result in paper tablets with sufficient pharmaceutical properties. Such paper granules possess a high elasticity and can therefore jump out of the die during the compression process. The elastic properties can be decreased, and plastic deformation can be improved by using sucrose as a binder during the wet granulation process. This results in paper granules with optimal

flowability and compressibility. The compression of these paper granules results then in paper tablets with optimal pharmaceutical properties.[9]

The above-mentioned results were obtained from non-loaded paper. Hence, with these drug-free tablets it was not possible to prove that smartFilms can indeed improve the bioactivity of poorly soluble drugs after oral application. Therefore, in a following study, curcumin-loaded smartFilms were produced and transferred into smartFilm granules. The curcumin-loaded smartFilm granules were transferred into curcumin-loaded smartFilm tablets and characterized regarding their pharmaceutical properties. In addition, the oral bioavailability, i.e., intestinal permeability, was assessed in an ex vivo porcine intestinal model. The results demonstrated that wet granulation did not impair the amorphous state of the curcumin and resulted in curcumin-loaded smartFilm granules with good flowability which could be transferred into curcumin-loaded smartFilm tablets with good pharmaceutical properties. The ex vivo bioavailability was superior when compared to physical mixture paper tablets. Therefore, data currently provide evidence that smartFilm tablets are an industrially feasible approach for the formulation of poorly soluble drugs [10]. In the previously mentioned study, the smartFilm tablets were already shown to increase the dissolution rate and the intestinal permeability of curcumin but no data were available for other active ingredients. A study that proves that smartFilms and smartFilm tablets can really improve the pharmacological efficacy in the gastrointestinal tract is also not available. These data are considered to be essential to provide further evidence for the usefulness of the smartFilm technology and to prove that the formulation principle is universal, i.e., can be used to enhance the solubility and bioactivity for different types of poorly soluble active ingredients. Such a proof-of-concept study was therefore performed here.

Norfloxacin is a BSC class IV drug with poor solubility and poor intestinal permeability [11]. It is a broadband antibiotic that belongs to the class of fluoroquinolone antibiotics. It affects Gram-positive and Gram-negative bacteria and thus can be used for the treatment of various diseases [12]. In this study, according to the previously established protocols [9,10], norfloxacin-loaded smartFilms were prepared and transformed into smartFilm granules from which smartFilm tablets were prepared. The crystalline state of norfloxacin within the granules and the tablets was determined and the pharmaceutical properties of the granules and tablets were assessed according to the European Pharmacopeia. The antibacterial activity of the norfloxacin-loaded tablets was assessed in vitro and in an ex vivo model. The results obtained were compared to physical mixture tablets that contained non-loaded paper granules and identical amounts of norfloxacin as a crystalline powder.

2. Materials and Methods

2.1. Materials

Norfloxacin, 95%, was purchased from abcr GmbH (Karlsruhe, Germany). Commercially available, cellulose-based paper (Soft & Sicher, dm-drogerie markt GmbH + Co. KG, Karlsruhe, Germany) was utilized as the paper matrix. Sucrose, peptone, beef extract, sodium chloride and potassium chloride were acquired from Carl Roth GmbH + Co. KG (Karlsruhe, Germany). Agar was purchased from Sigma-Aldrich Pty Ltd. (Darmstadt, Germany). Magnesium sulfate heptahydrate, magnesium chloride hexahydrate, calcium chloride dihydrate, sodium hydrogen carbonate, di-potassium hydrogen phosphate and di-sodium hydrogen phosphate were acquired from VWR International GmbH (Darmstadt, Germany). *Aliivibrio fischeri* (A. fischeri) bacterial strain (ATCC 7744/ NCMB 1281) was obtained from Dr. G. Schuchardt® (Göttingen, Germany). Purified water was freshly obtained from a PURELAB Flex 2 (ELGA LabWater, Veolia Water Technologies GmbH, Celle, Germany).

2.2. Methods

2.2.1. Production and Characterization of Norfloxacin-Loaded smartFilms and smartFilm Granules

Norfloxacin-loaded smartFilms and smartFilm granules were prepared as described previously [9,10], with slight modifications. In the first step, norfloxacin was dissolved in a mixture of acetone and ethanol (ratio of 1:1) to produce a solution that contained 2.5 mg/mL norfloxacin. The obtained norfloxacin solution (0.5 mL) was loaded onto paper sheets (5 × 5 cm²) with an individual mass of approximately 200 mg by using an automatic micropipette. The paper sheets were left to dry, then the process was repeated several times to prepare 20 mg norfloxacin-loaded paper sheets (i.e., smartFilms). The dried norfloxacin- loaded smartFilms were then used to produce norfloxacin-loaded smartFilm granules. For this, the smartFilms were dry milled using a knife mill (Moulinex DP8108, Groupe SEB Deutschland GmbH, Frankfurt, Germany) for 1 min. The milled smartFilms were mixed with sucrose to obtain milled smartFilms that contained 20% (w/w) sucrose. Purified water was sprayed on top of the milled smartFilm/sucrose mixture in order to dissolve the sucrose and to increase the density of the blend. The mixture was further ground for 1 min and then transferred to a plastic sieve. On the sieve, the mixture was further wet with purified water under shaking at 300 rpm (universal shaker SM-30 control, Edmund Bühler GmbH, Bodelshausen, Germany) for a period of 3–8 min. The resulting wet, norfloxacin- loaded smartFilm granules were dried in an oven for 30 min at 120 °C (UN 30, Memmert GmbH + Co. KG, Schwabach, Germany).

Afterwards, the granules were sieved (mesh size 2.8 mm, Retsch GmbH, Haan, Germany) to obtain a size fraction ≤ 2.8 mm. The smartFilms granules were characterized regarding their size and pharmaceutical characteristics (i.e., bulk density, tapped density, Hausner ratio, Carr Index, and angle of repose). The crystalline state of norfloxacin was determined by X-ray diffractometry. Details of the methods used are given below.

Particle Size

The particle size of the smartFilm granules was determined from macroscopic images of the granules. For this, ten representative images that contained approximately 250–300 granules were taken by using a Canon IXUS 190 digital camera (Canon Europe Ltd., Uxbridge, UK). The images obtained were subjected to digital image analysis by using ImageJ software (National Institutes of Health, Bethesda, MD, USA), as described previously [13]. In the first step, the images were color-adjusted, and threshold analysis was performed to mark the granules individually. Then, the Feret diameter of each granule was evaluated by an automated software algorithm (Supplementary Materials, Table S1). The results were then used to calculate the number based median particle size diameters $d(n) 0.10$, $d(n) 0.50$, $d(n) 0.90$, $d(n) 0.95$ and $d(n) 0.99$ with JASP software, version 0.16.2 [14].

Pharmaceutical Characteristics

The pharmaceutical characteristics of the norfloxacin-loaded smartFilm granules that were assessed included bulk density, tapped density, Hausner ratio, Carr index and the angle of repose.

Determination of bulk and tapped density

The bulk and tapped density of norfloxacin-loaded smartFilm granules were determined according to the test method 2.9.34. of the European Pharmacopeia by using a mechanical tapping device (tap density tester TD200, Pharma Test Apparatebau AG, Hainburg, Germany) [15]. An amount of 10 g of the granules was placed into a 250 mL measuring cylinder. The initial volume and the final volume, after performing 10, 500 and 1250 taps on the same granule sample, were noted, and used to calculate the bulk and tapped density, respectively.

Determination of Hausner ratio and Carr index

From the obtained density results, the Hausner ratio and the Carr index, i.e., flowability parameters, were calculated using the following equations:[16]

$$\text{Hausner ratio} = \frac{\rho_{\text{tapped}}}{\rho_{\text{bulk}}} \quad (1)$$

$$\text{Carr index} = 100 \left(\frac{\rho_{\text{tapped}} - \rho_{\text{bulk}}}{\rho_{\text{tapped}}} \right) \quad (2)$$

where ρ_{tapped} is the tapped density and ρ_{bulk} is the bulk density.

Angle of repose

The angle of repose was assessed according to the test method 2.9.36. of the European Pharmacopeia [15]. An amount of 20 g of the granules was placed in the funnel of a flowability tester (Emmeram Karg Industrietechnik, Krailling, Germany). Then, the granules were gently stirred to pass through the funnel and piled up on a fixed base to form a heap. The height of the granule heap was measured, and the angle of repose (α) was calculated using the following equation:

$$\tan \alpha = \frac{h}{0.5 d_b} \quad (3)$$

where h is the height of the heap and d_b is the diameter of the base.

Crystalline State of Norfloxacin

X-ray diffraction (XRD) patterns were studied to assess the crystalline state of norfloxacin loaded within the smartFilm granules and the smartFilm tablets. For this, an X-ray powder diffractometer (X'Pert Pro MDP, PANalytical/Philipps BV, Netherlands) was used to record the X-ray diffraction patterns from smartFilm tablets from the corresponding references (norfloxacin raw bulk material, physical mixture of paper and bulk norfloxacin, norfloxacin-loaded smartFilm granules and unloaded paper granules with 20% sucrose content). The instrument was equipped with CuK α radiation ($\lambda = 1.7903 \text{ \AA}$) and operated at a voltage of 40 kV and a current of 35 mA at room temperature. Samples were scanned from $2\theta = 10^\circ$ to $2\theta = 55^\circ$ with a step of $0.03^\circ/\text{s}$.

2.2.2. Production and Characterization of Norfloxacin-Loaded smartFilm Tablets

The norfloxacin-loaded smartFilm granules were compressed into flat-faced bevel-edged tablets by using a single punch tablet press (EKO, Korsch GmbH, Berlin, Germany) equipped with a 10 mm flat-faced punch (Ritter Pharma-Technik GmbH, Stapelfeld, Germany). The compression force was 30 kN. Physical mixture tablets that contained similar amounts of norfloxacin as raw powder and unloaded paper granules were also obtained via this process. The crystalline state of norfloxacin within the smartFilm tablets was determined with X-ray

diffractometry (cf. Crystalline State of Norfloxacin) and the pharmaceutical characteristics of the produced smartFilm tablets were evaluated according to the tests described in the European Pharmacopeia [15]. Details of the methods used are given below.

Pharmaceutical Characteristics

Tablet thickness and mass uniformity

Ten tablets were randomly selected, and their thickness was determined with a IP67 ABS digital caliper (Mitutoyo, Kanagawa, Japan). The mass uniformity was evaluated according to the test method 2.9.5. of the European Pharmacopeia [15]. The test was conducted using 20 randomly selected tablets. The tablets were weighed, then the average weight was calculated and compared to the weight of each individual tablet to determine the percentage deviation. Afterwards, the results obtained were compared to the European Pharmacopeia limits.

Friability

The friability of 20 randomly selected tablets was evaluated according to test method 2.9.7. of the European Pharmacopeia. The tablets were dedusted and accurately weighed, then placed in the drum of a friability tester PTF 10ER (Pharma Test Apparatebau AG, Hainburg, Germany) [15], and the drum was set to rotate 100 times. Subsequently, the tablets were dedusted, accurately weighed and the percentage weight loss was calculated using the following equation:

$$\% \text{ weight loss} = 100 \left(\frac{W_1 - W_2}{W_1} \right) \quad (4)$$

where W_1 is the weight of the tablets before the test and W_2 is the weight of the tablets after the test.

Resistance to crushing

The force required to break down a tablet under compression, i.e., the crushing strength or hardness, was assessed according to the test method 2.9.8. of the European Pharmacopeia [15]. Tablets ($n = 10$) were individually placed in a horizontal plane between the jaws of a hardness tester PTB 311E (Pharma Test Apparatebau AG, Hainburg, Germany), then the result was expressed in Newtons (N) as a mean value of the forces measured.

Disintegration

Disintegration of tablets ($n = 6$) was evaluated in water according to the test method 2.9.1. of the European Pharmacopeia [15]. Tablets were individually positioned in the cavities of a

disintegration tester PTZ-S (Pharma Test Apparatebau AG, Hainburg, Germany) operating at a temperature of $37\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$, and the time required for full tablet disintegration was noted.

Content uniformity

The content uniformity within the smartFilm tablets was investigated according to test method 2.9.6. of the European Pharmacopeia [15]. Norfloxacin-loaded smartFilm tablets ($n = 10$) were individually immersed in phosphate-buffered saline (PBS, pH 6.8) under stirring until complete disintegration of each tablet. Samples were withdrawn and filtered using a syringe filter with a pore size of $0.22\text{ }\mu\text{m}$ (Otto E. Kobe KG, Marburg, Germany). Afterwards, the amount of norfloxacin within each tablet was determined spectrophotometrically at the predetermined λ_{max} of norfloxacin (320 nm) using UV–Vis spectroscopy (Multiskan™ GO, Thermo Fischer Scientific, Waltham, MA, USA) and a preconstructed calibration curve (10–20 mg/L).

Dissolution

Dissolution studies were conducted according to the test method 2.9.3. of the European Pharmacopeia [15] using PBS solution (pH 6.8) as a dissolution medium and a paddle apparatus PTWS 120D (Pharmatest, Apparatebau AG, Hainburg, Germany). Norfloxacin-loaded smartFilm tablets ($n = 6$) were individually dropped into the apparatus vessels which were filled with 900 mL PBS solution (pH 6.8). The paddle speed was set at 100 rpm and the temperature was maintained at $37 \pm 0.5\text{ }^{\circ}\text{C}$. Samples (10 mL) were withdrawn after 5, 10, 20, 40, 60, 90, 120, 180, 240, 360 and 480 min and replaced with an equal volume (10 mL) of the fresh dissolution medium. All samples were filtered using a syringe filter ($0.22\text{ }\mu\text{m}$) and were then divided in two equal aliquots. One aliquot was analysed regarding the amount of dissolved norfloxacin (UV–Vis spectroscopy, Multiskan™ GO, Thermo Fischer Scientific, Waltham, USA, $\lambda = 320\text{ nm}$, preconstructed calibration curve from 10–20 mg/L) and the second aliquot was used for the determination of the in vitro antibacterial activity (cf. 2.2.4.1.). A similar experiment was carried out by using a physical mixture of tablets that was produced from a blend of unloaded paper granules with 20% sucrose content and the same content of norfloxacin powder.

2.2.4. Evaluation of Antibacterial Activity

The antibacterial activity of the formulations was tested against *A. fischeri*. *A. fischeri* is a Gram-negative, rod-shaped bacterium with bioluminescent properties [17]. The luminescence intensity is directly proportional to the metabolic activity of the bacteria and can therefore be

used as a sensitive indicator to determine the toxicity, i.e., antimicrobial activity, of chemical compounds [18]. In this study, the changes in bioluminescence upon treatment with the different formulations were determined in vitro and ex vivo. For both setups, the bacteria were prepared following a previously established protocol [19]. *A. fischeri* cultures were grown overnight in photobacterium medium (28.13 g NaCl, 0.77 g KCl, 1.2 g CaCl₂·2 H₂O, 3.6 g MgCl₂ 6 H₂O, 0.0825 g NaHCO₃, 2.625 g MgSO₄·7 H₂O, 10 g peptone, 10 g beef extract per 1 L distilled water) under shaking at 24 °C (Incubator Hood TH 15, Edmund Bühler, Bodelshausen, Germany) until an optical density of 0.6 at 600 nm (OD600) was achieved (UVmini-1240 Spectrophotometer, Shimadzu™ Europa GmbH, Duisburg, Germany).

In Vitro Antibacterial Activity

The in vitro antibacterial activity was determined by bioluminescence assay, i.e., by assessing the bioluminescence inhibition (%) of *A. fischeri* over time [18]. For this, 100 µL of sample was added into a well of a 96-well flat bottom white polystyrene microtiter plate. Then, the well was inoculated with 100 µL of *A. fischeri* culture that was freshly diluted (1:1) with photobacterium medium prior to use. The resulting luminescence intensity was measured with an integration time of 1000 ms per well after 12 h by using an Infinite 200Pro microplate reader equipped with an OD2 filter (Tecan Group Ltd., Männedorf, Switzerland). The experiment was performed in triplicate. An amount of 100 µL photobacterium growth medium with 100 µL *A. fischeri* culture was used as a positive control and 200 µL photobacterium growth medium without *A. fischeri* culture was used as a negative control. The bioluminescence inhibition (% BL inhibition) was calculated using the following equation:

$$\% \text{ BL inhibition} = 100 - \left(\frac{100 \cdot (BL_{\text{sample}} - BL_{\text{negative control}})}{BL_{\text{positive control}} - BL_{\text{negative control}}} \right) \quad (5)$$

where BL_{sample} is the measured bioluminescence intensity of the respective sample, $BL_{\text{negative control}}$ is the measured bioluminescence intensity of the negative control and $BL_{\text{positive control}}$ is the measured bioluminescence intensity of the positive control.

Ex Vivo Antibacterial Activity

The ex vivo antibacterial activity was determined from porcine intestines [10]. Fresh intact porcine gastrointestinal tracts were acquired from a local slaughterhouse and were used for the experiments within 2 h after slaughter. From the intestines obtained, small sections (approximately 15 cm in length) of intestinal tissue (i.e., 15–20 cm away from the pylorus) were isolated, dissected longitudinally and spread as a sheet. The intestinal sheets were gently wiped without affecting the villi structure and the pre-existing mucus. Subsequently, 2 mL of

A. fischeri culture was taken and separately centrifugated at 1000 rpm for 10 min at room temperature (MIKRO 120 centrifuge, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany). The resulting pellets were individually resuspended in 30 µL fresh medium and 60 µL of the obtained bacterial suspension was applied to each intestinal test area. The *A. fischeri*-infected intestinal tissues were incubated at room temperature for two hours to allow the bacteria to grow.

During the incubation time, the norfloxacin-loaded smartFilm tablets and the physical mixture tablets were subjected to a pre-digestion procedure to mimic the digestion and changes in the tablets after oral administration [10]. For this, one tablet of each formulation was separately immersed in 500 mL phosphate-buffered saline (pH 6.8, 37 °C) and stirred for 10 min at 300 rpm (universal shaker SM-30 control, Edmund Bühler GmbH, Bodelshausen, Germany). After this time, aliquots (100 µL) were withdrawn from each formulation and applied onto the *A. fischeri*-infected intestinal tissues (i.e., 2 h post infection). One infected area on each intestinal tissue was left untreated and served as control. Swabs from the surface of all intestinal areas were taken using a sterilised cotton swab at 0, 2, 4, 6, 8, 12 and 24 h post-treatment. The bacterial swabs were separately cultured on artificial seawater agar plates using the spread plate technique and incubated at room temperature overnight [20]. The experiments were performed in triplicate and the luminescence intensity of each plate was determined after 18 h.

For this, each plate was photographed in the dark (Figure 1A) using a Nikon D7200 Digital SLR camera equipped with an 18–300 mm lens (Nikon Europe B.V., Amsterdam, The Netherlands). The luminescence intensity from each plate was then determined semi-quantitatively by subjecting the images to digital image analysis described previously [13,21–24], with slight modifications. ImageJ software, version 1.53 k, was used for the analysis [25,26].

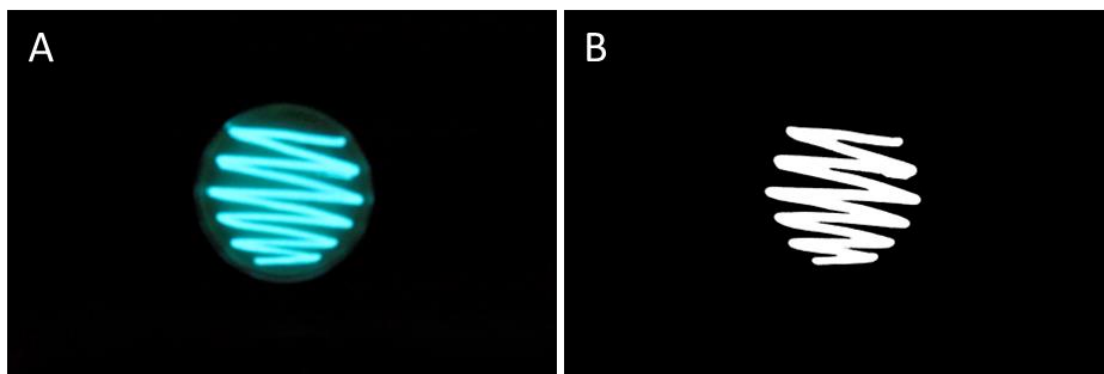


Figure 1. (A): Macroscopic image of an agar plate in the dark cultured with *A. fischeri* swabs after 18 h. (B): Macroscopic image after the automated threshold algorithm (Supplementary Materials, Table S2) that subtracted the background of the image. The remaining white pixels correspond to the bioluminescence intensity of *A. fischeri*.

The first step was an automated color adjustment (Supplementary Materials, Table S2) of the image that allowed for the separation between class I (foreground) and class II (background) pixels. The foreground pixels correspond to the luminescence of the bacteria and the background pixels correspond to the surrounding media. The second step subtracted the class II pixels from the class I pixels (Supplementary Materials, Table S2). The remaining pixels in the resulting image (Figure 1B) represent the bioluminescence intensity of the bacteria after the respective treatment. In this way, the ex vivo luminescence intensity could be assessed as mean grey value ((MGV)/px) from each image. The results are presented as mean values of the bioluminescence intensity \pm standard deviation from the different replicates of each formulation and from the different time points of incubation.

2.2.5. Statistical Analysis

Descriptive statistics and statistical assessment of differences between the mean values were performed using JASP software version 0.16.2 (Universiteit van Amsterdam, Amsterdam, The Netherlands) [14]. Normal distribution was checked with the Shapiro–Wilk test and variance homogeneity was checked with Levene’s test. Subsequently, the data were subjected to one-way analysis of variance (ANOVA, normally distributed data) or the Kruskal–Wallis tests (non-parametric data). Adequate post hoc tests (Tukey’s, Games–Howell and Dunnett and Dunn [27]) were performed to compare the mean values with each other. In some cases, for a more detailed comparison of the data, t-tests for pairwise comparison were also performed. The Pearson correlation coefficient is a measure of linear correlation between two sets of data. It was determined in between the in vitro dissolution data, the in vitro antibacterial activity data and the ex vivo antibacterial activity data [28]. Data are represented as mean \pm standard

deviation, unless otherwise stated. Differences between means were considered statistically significant if the p value was < 0.05 .

3. Results

3.1. Production and Characterization of Norfloxacin-Loaded smartFilm Granules

Norfloxacin smartFilm granules were successfully produced from the norfloxacin-loaded smartFilms (Figure 2 A, B). The granules were further characterised regarding size, their pharmaceutical characteristics and regarding the crystalline state of norfloxacin within the granules.

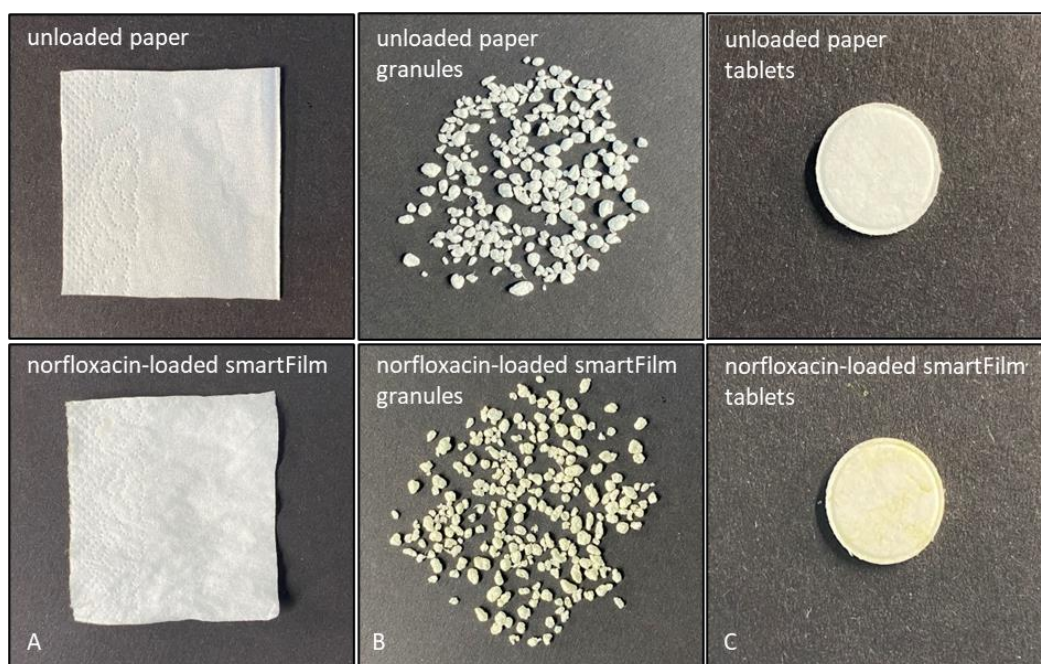


Figure 2. Macroscopic images of (A): unloaded paper (upper) and paper loaded with norfloxacin (smartFilms, lower), (B): unloaded paper granules (upper) and norfloxacin-loaded smartFilm granules (lower) (C): unloaded paper tablets (upper) and norfloxacin-loaded smartFilm tablets (lower).

3.1.1. Particle Size

The Feret diameter of the unladed paper granules was 3.3 ± 0.9 mm and was 2.6 ± 1.0 mm for the norfloxacin-loaded smartFilm granules. The numeric size distribution showed that the $d(n)0.1$, $d(n)0.5$ and $d(n)0.9$ values were significantly lower for the smartFilm granules. The $d(n)0.95$ values were similar but the $d(n)0.99$ value was higher for the smartFilm granules (Figure 3). This means the norfloxacin granules possessed a slightly smaller mean particle size and a slightly broader size distribution than the unloaded paper granules. The smaller size of

the smartFilm granules can be explained by an increased density of the cellulose matrix that is caused by loading the drug into the pores of the paper [10]. The trend towards some larger particles can also be explained by the increased density of the drug-loaded smartFilm granules, i.e., due to the reduced pore size, the uptake of the binding agent into the pores is reduced, which then reduces the granulation efficiency of the binder.

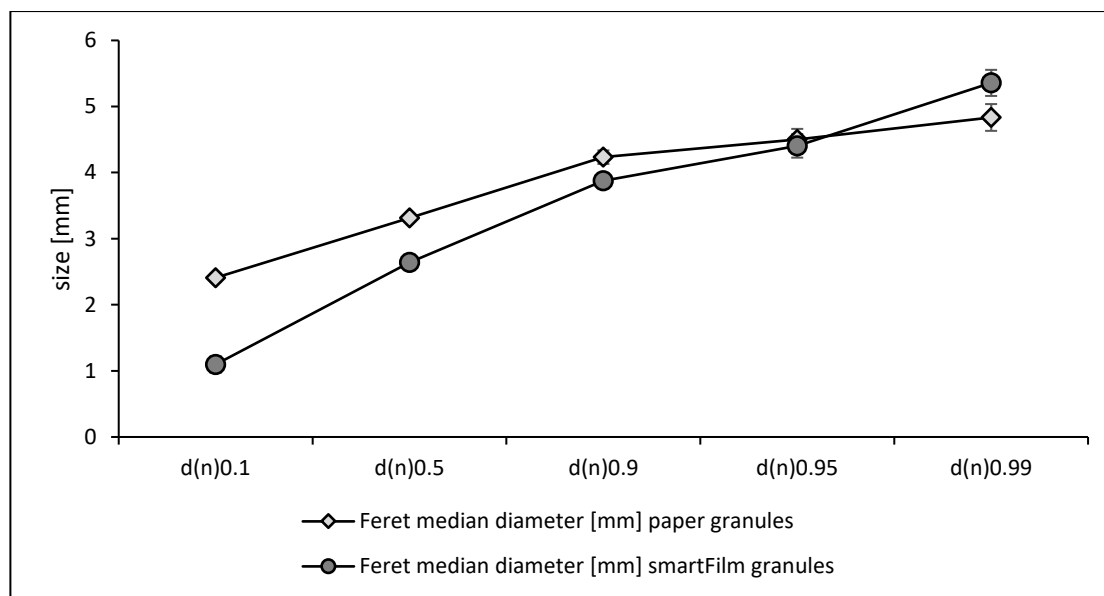


Figure 3. Particle size (numeric median diameters) of norfloxacin-loaded smartFilm granules in comparison to unloaded paper granules.

3.1.2. Pharmaceutical Characteristics

The pharmaceutical characteristics of the norfloxacin-loaded smartFilm granules, i.e., bulk density, tapped density, Hausner ratio, Carr index and angle of repose, were determined in the next step (Table 1). The differences in bulk and tapped density were small, which resulted in a Hausner ratio of < 1.19 , a Carr index of < 15 and an angle of repose of < 40 . The data indicate good to moderate flowability [29]. Hence, industrial compression of the granules into tablets was considered to be possible without complications. The data also support the theory that the smaller particle size of the smartFilm granules is due to their higher density when compared to unloaded paper granules. Both, the bulk and the tapped density increased in the paper granules upon loading the paper with norfloxacin when compared to the unloaded paper granules. As expected, the resulting smaller particle size and the slightly larger size distribution changed the particle interaction forces, which was observed in a slightly reduced flowability of the smartFilm powder (i.e., increased Hausner ratio and Carr index), when compared to the unloaded paper granules (Table 1). Similar effects were also found when curcumin was loaded into paper [10]. Based on these results, it can therefore be concluded

that the loading of active compounds into paper resulted in an increased density of the paper. The transfer of drug-loaded paper into granules resulted in granules with a smaller size, broader size distribution and lower flowability.

Table 1: Pharmaceutical properties of norfloxacin-loaded smartFilm granules in comparison to unloaded paper granules.

	Bulk Density (g/cm ³)	Tapped Density (g/cm ³)	Hausner Ratio	Carr Index	Angle of Repose
Paper granules *	0.14 ± 0.00 *	0.15 ± 0.00 *	1.13 ± 0.04 *	11.7 ± 3.6 *	31° ± 0 *
smartFilm granules	0.15 ± 0.00	0.18 ± 0.00	1.17 ± 0.00	14.9 ± 0.0	31° ± 0

* data from [9].

3.1.3. Crystalline State of Norfloxacin

The smartFilm technology aims to increase the solubility of poorly soluble drugs by transferring and maintaining the drug in its amorphous state. The production of smartFilm granules via wet granulation runs the risk that the drug is (partially) dissolved during the granulation process and might re-crystallize upon drying. The formed crystals might trigger further crystallization of amorphous norfloxacin. X-ray analysis, however, showed that the granulation process did not alter the crystalline state of norfloxacin (Figure 4).

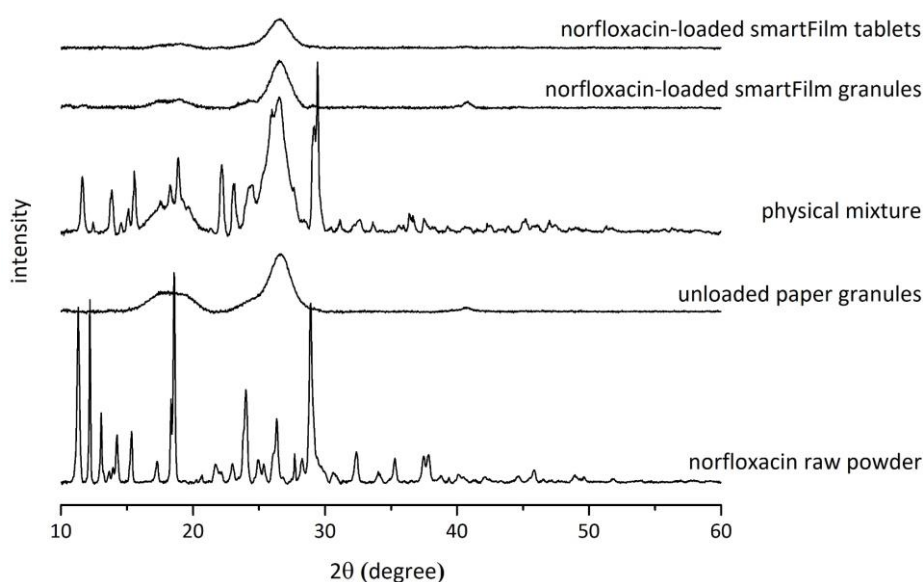


Figure 4. X-ray diffraction patterns of norfloxacin-loaded smartFilm granules and smartFilm tablets in comparison to norfloxacin raw powder, unloaded paper granules and the physical mixture of norfloxacin and paper.

The diffractogram of norfloxacin raw powder revealed the typical reflexes of norfloxacin in a crystalline state. The physical mixture of norfloxacin raw powder and unloaded paper granules also showed these reflexes. In contrast, no reflexes were found for the norfloxacin-loaded smartFilm granules that contained similar amounts of norfloxacin as the physical mixture. This therefore indicates that norfloxacin is loaded into the smartFilm granules in an amorphous state.

Based on these data, it was concluded that the norfloxacin-loaded smartFilm granules are suitable intermediate products for the production of norfloxacin-loaded smartFilm tablets. Therefore, in the next step of the study, the norfloxacin-loaded smartFilm granules were compressed into tablets and their resulting properties were analysed.

3.2. Production and Characterization of Norfloxacin-Loaded smartFilm Tablets

3.2.1. Crystalline State of Norfloxacin

The compression of the norfloxacin-loaded smartFilm granules into smartFilm tablets resulted in smooth tablets with a shiny surface (Figure 2C) and did not alter the crystalline state of the norfloxacin (Figure 4). No reflexes and no changes in the diffractogram in comparison to the norfloxacin-loaded smartFilm granules were observed for the smartFilm tablets, indicating that norfloxacin was loaded into the smartFilm tablets in an amorphous state.

3.2.2. Pharmaceutical Characteristics

The pharmaceutical properties (e.g., thickness of tablet, mass uniformity, content uniformity, friability, etc.) of the norfloxacin-loaded smartFilm tablets are summarised in Table 2 and show that the norfloxacin-loaded smartFilm tablets fulfil the requirements according to the European Pharmacopeia. The average mass uniformity value was $2.9 \pm 1.2\%$ and no tablet out of the 20 weighed tablets had an individual mass that differed by more than 7.5% from the average mass (tablet mass was in the range of 80–250 mg). The average content uniformity value was $97.5 \pm 1.5\%$, and no tablet out of the ten examined tablets exhibited a content value that differed by more than 15%. The disintegration time was < 15 min, the friability was $< 1\%$ and the crushing strength value was about 111 ± 12 N. Thus, the disintegration time of the tablets was sufficiently fast and their mechanical strength was sufficiently high [29].

A comparison of the pharmaceutical properties of the norfloxacin-loaded smartFilm tablets to the properties of the tablets obtained with non-loaded paper granules shows that the properties of the tablets are altered upon loading with norfloxacin (Table 2). Upon loading, the thickness of the tablets, the friability and the disintegration time increased, while the mass

uniformity decreased. The crushing strength was not altered. The changes in mass uniformity can be linked to the reduced flowability of the smartFilm granules, which results in a less constant flow of the granules into the die of the tablet press and thus leads to stronger variation in the tablet mass. The increased thickness reduced friability and increased disintegration time can be linked to the higher density of the smartFilm granules.

Table 2. Pharmaceutical properties of norfloxacin-loaded smartFilm tablets in comparison to unloaded paper tablets.

	Thickness (mm)	Mass Uniformity (%)	Content Uniformity (%)	Friability (%)	Resistance to Crushing (N)	Disintegration
Paper tablets *	1.7 ± 0.04 *	1.8 ± 1.6 *	n.a.	0.23 *	112.8 ± 18.6 * min.: 84.2 max.: 129.5	All tablets disintegrated within 5 min *
smartFilm tablets	2.3 ± 0.05	2.9 ± 1.8	97.5 ± 1.5 max: 99.3	0.07	110.7 ± 12.2 min.: 82.5 max.: 125.9	All tablets disintegrated within 15 min

* data from [9].

The higher density was due to the loading of the drug into to the pores of the paper, which also reduces the porosity of the paper matrix. Hence, the loaded smartFilm granules contain less air within the pores of the paper; therefore, the compression at similar compression forces yielded thicker tablets. The friability, crushing strength and disintegration are influenced by different parameters that can interfere with each other. In the case of the smartFilm granules loaded with norfloxacin, the increased density when compared to the unloaded paper granules seems to be responsible for the increased disintegration time, i.e., the reduced pore size seems to decrease the wettability of the tablet and thus increases its disintegration time. The increased density can be considered to increase the hardness of the material, which then seems to reduce the friability of the tablets. However, such an increase in hardness should also lead to an increase in crushing strength. An increase in crushing strength for smartFilm tables was indeed observed in a recent study when curcumin was loaded into paper [10]. However, such an increase was not seen in this set of data. A possible explanation is a less efficient plastic deformation of the norfloxacin-loaded smartFilm granules during compression due to the lower porosity of the loaded smartFilm granules and the lower predicted compressibility due the increased Carr index. For the production of tablets with optimal and tailor-made pharmaceutical properties, more systematic research is needed to investigate and understand the interplay between granule properties, drug loading and resulting tablet properties in detail. Nonetheless, the data from this study clearly confirm that it is possible to produce norfloxacin-loaded smartFilm tablets that fulfil the requirements

according to the European Pharmacopeia. The resulting biopharmaceutical properties of the smartFilm tablets (release kinetics and antibacterial activity) were therefore determined in the next step of the study.

3.2.3. Dissolution Profile

The dissolution profile of the smartFilm tablets showed a biphasic release profile, i.e., a very slow release at early time points (< 10 min) and a faster release after ≥ 20 min. A biphasic release profile was also obtained for the physical mixture tablets. However, the onset of the fast release was delayed, and the total amount of dissolved active material was lower when compared to the smartFilm tablets (Figure 5). The amount of released norfloxacin was significantly higher for the smartFilm tablets after 5min, 10min, 20min, 40 min, 1h and 1.5h.

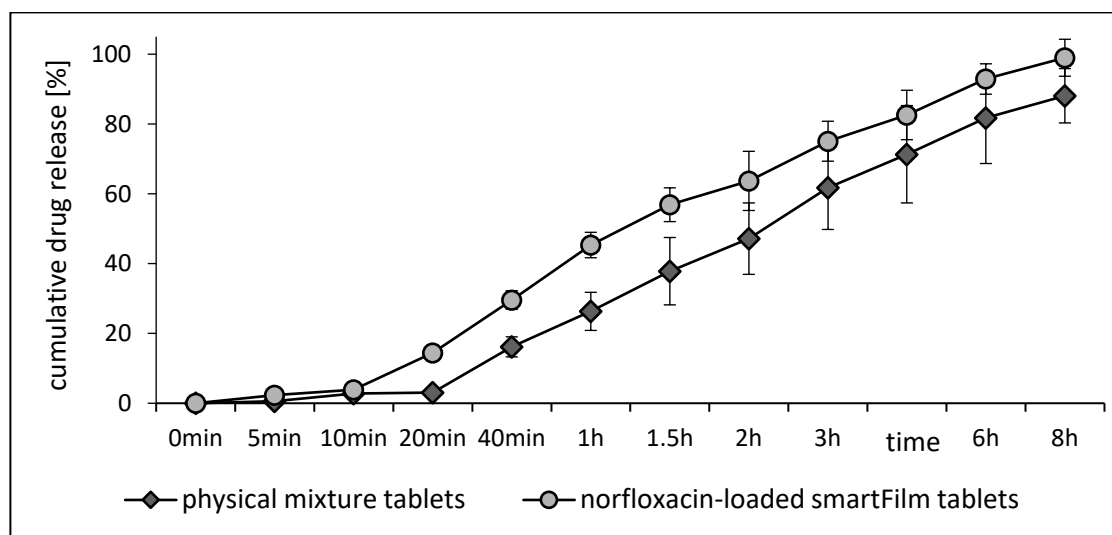


Figure 5. Cumulative drug release of norfloxacin from norfloxacin-loaded smartFilm tablets in comparison to physical mixture tablets.

The difference was most pronounced between 10 and 40 min (about a 2-fold difference) and declined and became non-significant with higher dissolution times (2 h–6 h). After 8 h, the amount of released norfloxacin from the smartFilm tablets was about 12% higher when compared to the released norfloxacin from the physical mixture (Student's *t*-test, one-tailed, $p < 0.05$). From the obtained data, it can be concluded that the smartFilm tablets show an enhanced release of norfloxacin when compared to the physical mixture. Hence, in comparison to the physical mixture, the data suggest a higher bioactivity of the smartFilm tablets. This assumption was therefore tested in the next part of the study by determining the antibacterial activity of the smartFilm tablets *in vitro* and in an *ex vivo* model.

3.2.4. Determination of Antibacterial Activity

In Vitro Antibacterial Activity

The in vitro antibacterial activity of norfloxacin from norfloxacin-loaded smartFilm tablets was determined and compared with the in vitro antibacterial activity of norfloxacin physical mixture tablets (Figure 6). The tests were performed with *A. fischeri* that possesses bioluminescent properties [17]. The bioluminescence extinguishes if the treatment is toxic to the bacterium. Therefore, the bioluminescence inhibition can be used to compare the antibacterial activity of different formulations [18]. A bioluminescence inhibition of 100% means that 100% of the bacteria were deactivated by the treatment.

Treatment with the norfloxacin-loaded smartFilm tablets caused a fast increase in the bioluminescence inhibition. After 5 min, 85% of the bacteria showed an extinguished luminescence and after 10 min, 90% of the bacteria were affected by the treatment, i.e., the decimal reduction time can be estimated to be 10 min. A proportion of 99% of the bacteria luminescence was inactivated after 4h (Figure 6). The physical mixture tablets possessed a less efficient antibacterial activity. After 5 min, about 60% of the bacteria were affected by the treatment and after 10 min, 85% of the bacteria were affected. The decimal reduction time was about 30 min and 99% of the bacteria luminescence was depleted after 8h. Hence, based on the decimal reduction time and the time needed to affect 99% of the bacteria, the antibacterial activity of norfloxacin from smartFilm tablets was about 2- to 3-fold higher when compared to the physical mixture tablets. The differences in antibacterial activity between smartFilm tablets and physical mixture tablets were expected and can be related to the faster release of norfloxacin from the smartFilm tablets (cf. Figure 5).

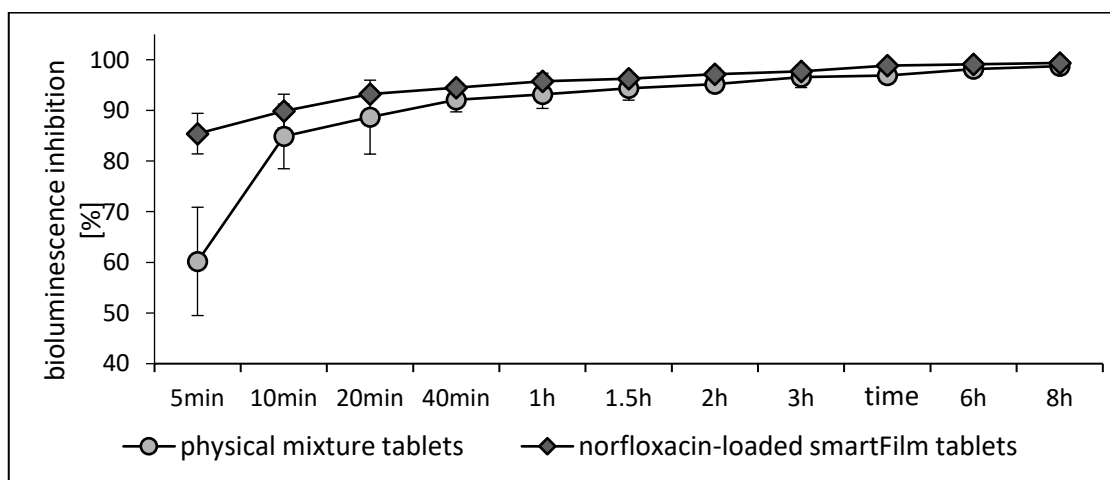


Figure 6. Antibacterial activity (bioluminescence inhibition of *A. fischeri*) of norfloxacin from smartFilm tablets in comparison to physical mixture tablets.

Ex Vivo Antibacterial Activity

The ex vivo antibacterial activity was assessed in porcine intestines that were infected with *A. fischeri*. The formulations were applied onto the infected intestinal tissue and the number of active bacteria was determined by remaining bioluminescence at different time points. The first experiment was performed for a period of 24 h, where samples were taken after 0 h, 12 h and 24 h. The experiment was performed to roughly estimate the time frame for the ex vivo method that can yield reliable and discriminative results between different samples and application setups. Samples applied were the pre-digested smartFilm tablets and the pre-digested physical mixture tablets. The two different formulations were applied on the intestinal tissue either once (application time = 0 h) or twice (application time = 0 h and 8 h), with the untreated infected intestine sections serving as the control (Figure 7).

The data show that the number of active bacteria was similar for all infected intestines tested at the beginning of the experiment. After 12 h, significant differences were found between the untreated and differently treated tissues and after 24 h, the luminescence was extinguished in all samples. The results show that the model yields reproducible results (similar numbers of bacteria at the beginning from different intestinal tissues) and that it remains sensitive within a time frame of at least 12 h (i.e., significant differences and changes can be found in between different formulations and application setups). After this time, all bacteria were inactive, independent of whether the tissues were treated with antibacterial formulations or not (Figure 7).

The data also show that the smartFilm tablets exhibit a significantly higher antibacterial activity than the physical mixture tablets when the formulations were applied once. The decrease in bacterial luminescence after 12 h was about one-third for the physical mixture tablets ($-39 \pm 13\%$) and about two-thirds ($-68 \pm 17\%$) for the smartFilm tablets. Hence, an approximate 2-fold increase in the antibacterial activity of the smartFilm tablets was found when compared to the physical mixture tablets. With this observation, the data are in line with the results obtained in the in vitro bioluminescence assay, which showed a similar trend for the decimal reduction time and the time needed to deactivate 99% of the bacteria (cf. Figure 6). The less efficient and slower deactivation of the bacteria in the ex vivo model when compared to the data from the in vitro model might be caused by the lower amounts of norfloxacin being available after the pre-digestion step and/or a slower diffusion of the released norfloxacin within the tissue. An additional possibility is the metabolism of the norfloxacin via intestinal enzymes or other interactions that might (partially) inactivate it.

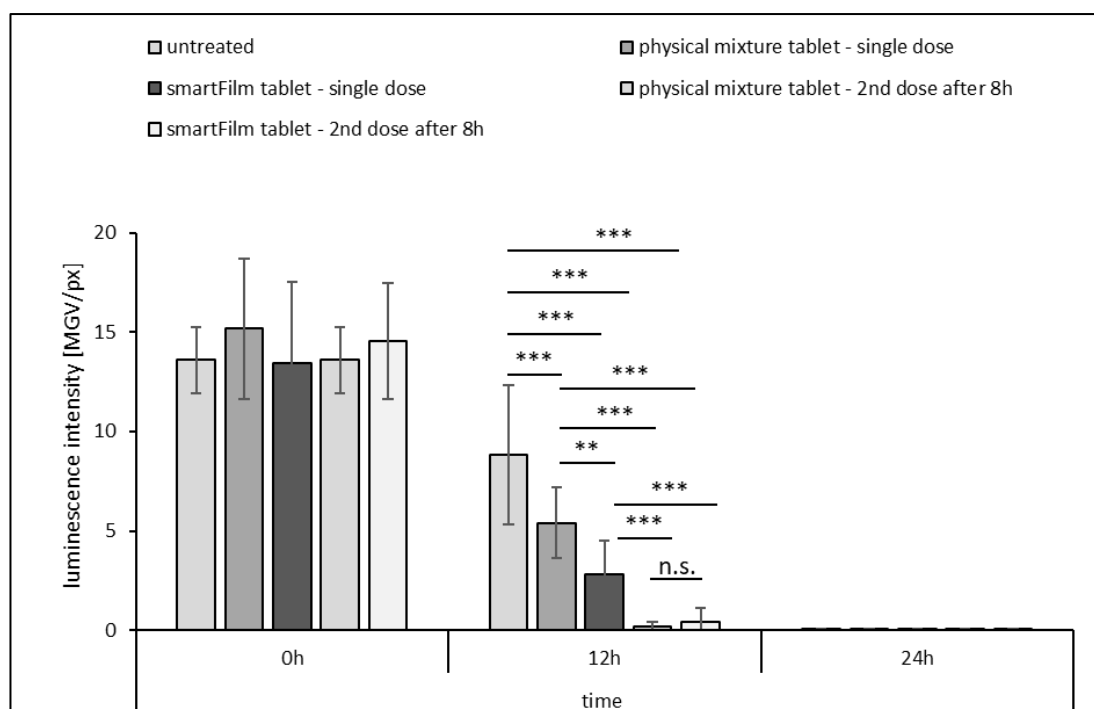


Figure 7. Ex vivo antibacterial activity from infected intestinal tissue (bioluminescence of *A. fischeri*). Non-treated vs. treated with norfloxacin-loaded smartFilm tablets in comparison to physical mixture tablets applied as a single dose or in second (booster) dose setup after 8 h (explanations cf. text). ** $p < 0.01$, *** $p < 0.001$, n.s.: not significant.

The half-life of norfloxacin was about 3 h, but might be prolonged with decreased enzyme activity [30]. As the ex vivo antibacterial activity was assessed after 12 h, which is much later than the half time of norfloxacin, it was reasonable to hypothesize that the norfloxacin might have been partially deactivated during this time. This would mean that the antibacterial effect of the two formulations that were applied only once was not optimal. In addition, it would also mean that the antibacterial effect could be enhanced by applying a second dose. This theory is substantiated by the results obtained when the formulations were applied twice, which resulted in a decrease in luminescence of $\geq 95\%$ for both formulations (Figure 7).

In the next step, we aimed to investigate the differences between the two formulations in more detail, i.e., at different time points within the predetermined time frame of the ex vivo model. For this, the formulations were applied on the intestines, and samples were taken at 2 h, 4 h, 6 h, 8 h and 12 h after application. After 8 h, a second dose, i.e., a booster dose, was applied and final samples were taken after 12 h. Untreated tissues served as controls (Figure 8). At the beginning, slightly fewer active bacteria were located on the non-treated intestinal sections. After 2 h, the differences were cancelled out between untreated controls and the physical mixture tablets, i.e., all sections had a similar luminescence intensity. The tissue

sections treated with the smartFilm tablets resulted in a lower luminescence intensity (Mann–Whitney test, $p < 0.05$). The results therefore indicate that the smartFilm tablets acted faster than the physical mixture tablets. After 4 h, all treated intestine sections showed a lower luminescence intensity than the untreated intestine sections and the effect was significant for both formulations. The smartFilm tablets resulted in significantly less luminescence intensity than the physical mixture tablets. After 6h and 8h, the differences between the formulations became smaller, but remained significant. For both formulations, after 4 h, a plateau in antibacterial activity was reached. Hence, in between 4 h and 8 h no further decrease in the antibacterial activity was observed. After 12 h, and after application of the second dose, the activity of the bacteria declined to almost zero, with no significant differences between the smartFilm tablets and the physical mixture tablets (Figure 8).

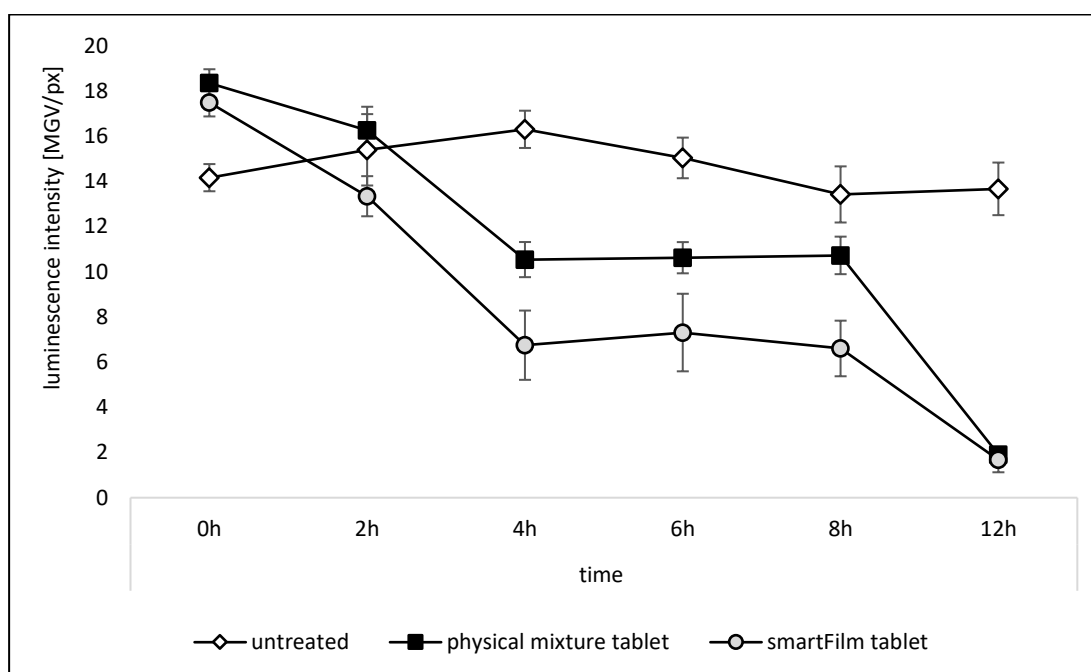


Figure 8. Ex vivo antibacterial activity (luminescence of *A. fischeri*) after treatment with norfloxacin-loaded smartFilm tablets in comparison to physical mixture tablets (explanations cf. text).

The obtained data are in line with the expectations, i.e., both the physical mixture tablets and the smartFilm tablets were expected to possess antibacterial properties. The smartFilm tablets, due to the better solubility of norfloxacin, were expected to possess a more pronounced antibacterial activity. In addition, due to the faster release of norfloxacin from the smartFilm tablets, it was expected that the antibacterial effect would be detectable at earlier time points when compared to the physical mixture with slower release kinetics for norfloxacin. The results proved the faster onset of antibacterial activity for the smartFilm

tablets and their more pronounced antibacterial activity. However, they also showed that the antibacterial effect reaches a plateau after 4 h. The effect is considered to be related to the short half-life of the norfloxacin, which seems to become (partially) inactive after this time. Only the application of a 2nd dose (booster dose) could further reduce the bacterial activity. After 12 h, and after the application of the booster dose, the antibacterial activity was similar for the smartFilm tablets and the physical mixture tablets. This indicates that the smartFilm tablets are especially useful if a fast antibacterial action is required. For longer, multiple dose treatments, the use of a classical formulation approach seems to be sufficient. From an ecological point of view, it might be most efficient to apply the initial dose with a smartFilm tablet to yield a fast onset of the antibacterial activity and to apply the classical formulation as maintenance dose. In this way, the treatment would be highly efficient and cost-effective (intellectual property protected formulations are more costly) at the same time. Future research is now needed to investigate this in more detail.

A correlation between the *in vitro* data and the *ex vivo* data can be typically analysed to judge the predictability of a model [31]. However, norfloxacin is a BSC IV drug [11]; therefore, a good *in vitro in vivo* (*ex vivo*) correlation cannot be expected [31]. The correlation of the data from this study confirmed this (Figure 9, Supplementary Materials, Figure S1). Figure 9 represents some selected time points indicates that the correlation coefficient between the different dissolution (Diss) time points is not constant. This is due to the anomalous release (non-Fickian) diffusion, i.e., biphasic release, of norfloxacin (cf. Figure 5). Hence, only the time points from the slow release in the beginning show a good correlation to each other but result in low correlation coefficients with higher dissolution times that are associated with a faster drug release, and vice versa. A similar effect can be seen for the *in vitro* bioluminescence data (BL), i.e., the BL data from early time points correlate well with early time dissolution data but result in poor correlation with higher dissolution time points. Higher BL time points correlate well with higher dissolution times (Figure 9).

The *ex vivo* data were acquired with pre-digested formulations that were applied to the intestine after 10 min pre-digestion (cf. 2.2.4.2). Hence, in contrast to the correlation between dissolution data and bioluminescence inhibition, where the norfloxacin was released throughout the entire experiment, in this part of the study, only one drug concentration, corresponding to a dissolution time of 10 min, was used. Therefore, the best correlation between dissolution, bioluminescence inhibition and *ex vivo* antibacterial activity should be found when the *ex vivo* antibacterial activity is assessed after 10 min. However, this correlation is not possible, because the *ex vivo* antibacterial activity was assessed after 2 h, 4

h, 6 h, 8 h and 12 h post application of the formulations onto the intestinal tissue. Hence, the early time points that can be expected to correlate well with the early dissolution data were not acquired. Such an early determination of the ex vivo antibacterial activity is also not feasible, because an ex vivo model should estimate the biological effect in a realistic setup and environment [32].

Therefore, based on the chemical nature of norfloxacin and the related considerations, a strong and linear correlation between the in vitro data and the ex vivo data is not possible. However, the model should still provide a clear trend that shows the effect of a formulation in relation to an in vitro property. In this case, it is expected that the higher amount of drug released causes a more pronounced antibacterial activity. This would result in a positive correlation between dissolution data and in vitro bioluminescence inhibition and would result in a negative correlation between the in vitro data and the ex vivo antibacterial activity, which was assessed as the remaining luminescence of the bacteria after the treatment, i.e., a low remaining ex vivo luminescence of the treatment indicates good ex vivo antibacterial activity. This expected negative correlation between in vitro and ex vivo data was found for all time points (Figure 9, Supplementary Materials, Figure S1).

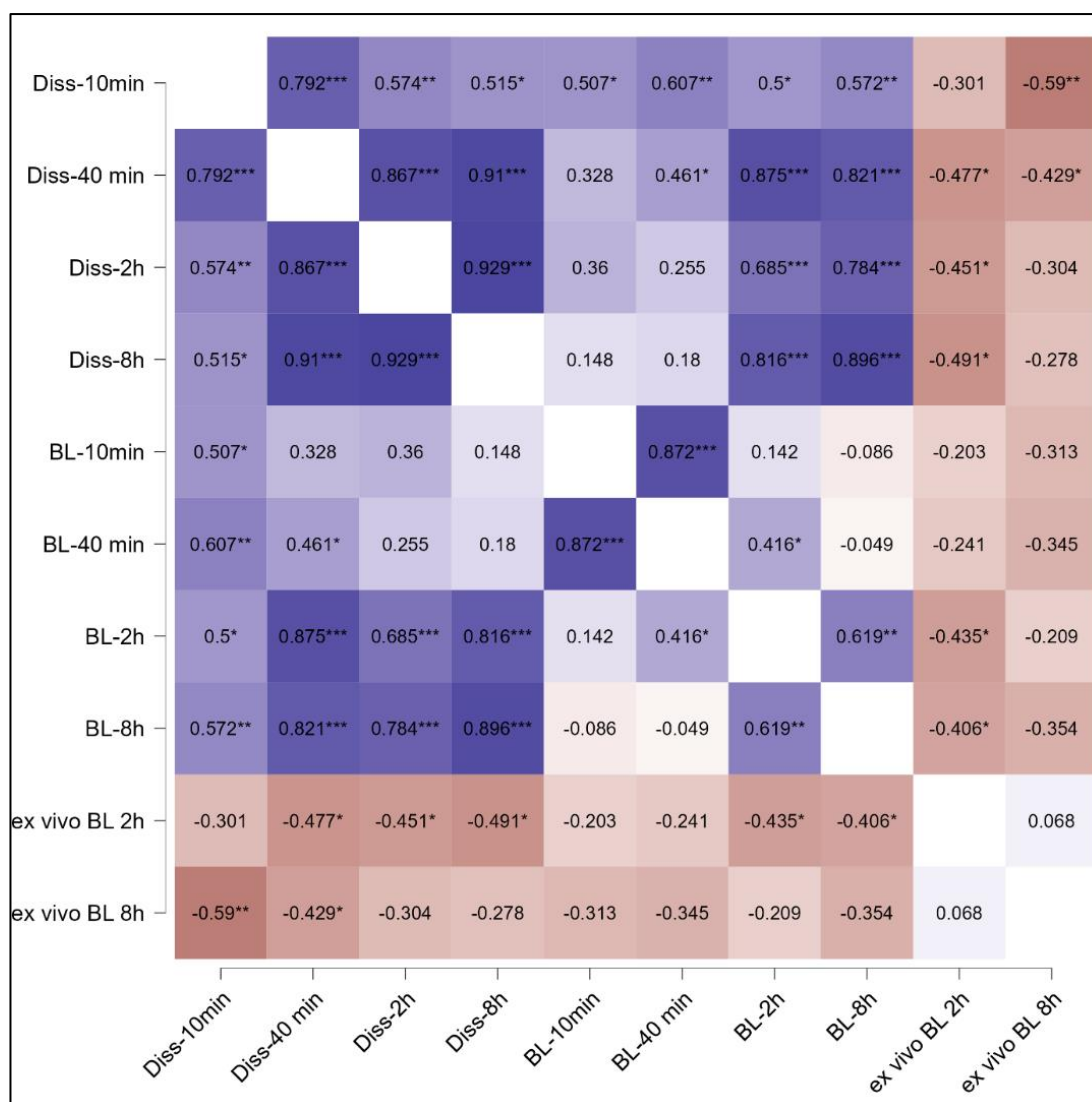


Figure 9. Heatmap of Pearson correlation coefficients (selected time points) that assess the relationship between the in vitro dissolution data (Diss), the in vitro bioluminescence inhibition (BL) and the ex vivo antibacterial activity (ex vivo BL) data for the physical mixture and the norfloxacin-loaded smartFilm tablets at different time points. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Based on the correlation data, it is not possible to estimate the real accuracy of the model because many parameters are not linear. Despite this, the data clearly demonstrate that the ex vivo model is able to judge the antibacterial activity from different formulations. It enables a rough estimation of differences in pharmacokinetic profiles and provides a clear link to the in vitro data, thus rendering the ex vivo model a useful tool that allows for a fast and cost-effective discrimination between “good” and “bad” formulations.

In contrast to the in vitro test methods, the ex vivo model bears manifold parameters that provide a close link to real physiological conditions (i.e., mucus, pH, active enzymes, etc.).

Therefore, its use can be considered to yield meaningful, additional biopharmaceutical information that cannot be assessed from classical in vitro experiments. The model can be considered to be especially useful in early formulation development, because at this stage it is often not necessary to have highly accurate results. In most cases, it is sufficient to obtain a rough estimate of whether a formulation is effective or not and whether changes in the formulation and/or treatment improve the performance or not. In this way, effective formulations can be developed in a cost-efficient and time-saving way.

The data acquired here provide evidence that smartFilm tablets can be utilised as a simple, efficient formulation strategy for the improved solubility and enhanced bioactivity of norfloxacin. Nevertheless, future work should now focus on providing a detailed picture about the underlying mechanisms for this. For this, systematic studies that investigate, e.g., the interactions between the paper matrix and the active pharmaceutical ingredients (API) and the influence of the paper matrix and production parameters on the chemical stability of the API and the pharmaceutical and biopharmaceutical properties, are required. These mechanistic data will then be the base for a sound development of effective smartFilm tablets that allow for an improved bio-efficacy of poorly water-soluble API.

4. Conclusions

Norfloxacin-loaded smartFilm tablets were successfully produced in this study. The tablets maintained the norfloxacin in an amorphous state and possessed sufficient pharmaceutical properties (resistance to crushing, mass uniformity, content uniformity, friability, and disintegration time). The tablets showed a biphasic release profile of the norfloxacin. The dissolution rate was up to three times higher when compared to the physical mixture tablet. This also resulted in an up to three-fold increase in in vitro antibacterial activity. The ex vivo antibacterial activity showed a similar trend, i.e., a higher bioactivity of norfloxacin in the smartFilm tablets when compared to the physical mixture tablet. The more physiological environment of the ex vivo model could provide a more detailed understanding on the effects (formulation aspects and application aspects) that influence the bioactivity of the norfloxacin. The results provide further evidence that smartFilm tablets are a universal, industrially feasible formulation strategy for the improved solubility and enhanced bioactivity of poorly soluble drugs.

Acknowledgments: The authors thank the Yousef Jameel Foundation for providing scholarship funding for Ayat Abdelkader.

5. References

- [1] Stegemann, S.; Leveiller, F.; Franchi, D.; de Jong, H.; Linden, H. When poor solubility becomes an issue: From early stage to proof of concept. *Eur. J. Pharm. Sci.* 2007, *31*, 249–261.
 - [2] Kalepu, S.; Nekkanti, V. Insoluble drug delivery strategies: Review of recent advances and business prospects. *Acta Pharm. Sin. B* 2015, *5*, 442–453. <https://doi.org/10.1016/j.apsb.2015.07.003>.
 - [3] Williams, R.O.; Watts, A.B.; Miller, D.A. *Formulating Poorly Water Soluble Drugs*, 3rd ed.; Springer: New York, NY, USA, 2012; ISBN 9783030887193.
 - [4] Lemke, S.; Strätling, E.-J.; Welzel, H.-P.; Keck, C.M. Cellulose Fibre Based Support Matrices for Layered Products for Oral and Peroral Application and Their Preparation. 2016.
 - [5] Lemke, S. Cellulosebasierte Filme (smartFilms®) als Alternative Orale Oder Perorale Applikationsform; Herstellung und Prüfung. Ph.D. Thesis, Freie Universität Berlin, Berlin, Germany, German Patent Application DE102016000541A1, 20 July 2017.
 - [6] Stumpf, F.; Keck, C.M. Tablets made from paper. *Int. J. Pharm.* 2018, *548*, 812–819. <https://doi.org/10.1016/j.ijpharm.2018.05.071>.
 - [7] Stumpf, F. Tabletten aus Papier—Tablets made from paper—Zur oralen Applikation schwerlöslicher Wirkstoffe. Ph.D. Thesis, Philipps-Universität Marburg, Marburg, Germany, 2019.
 - [8] Eckert, R.W.; Wiemann, S.; Keck, C.M. Improved Dermal and Transdermal Delivery of Curcumin with SmartFilms and Nanocrystals. *Molecules* 2021, *26*, 1633. <https://doi.org/10.3390/molecules26061633>.
 - [9] Abdelkader, A.; Moos, C.; Pelloux, A.; Pfeiffer, M.; Alter, C.; Kolling, S.; Keck, C.M. Tablets Made from Paper—An Industrially Feasible Approach. *Pharmaceutics* 2022, *15*, 1188. <https://doi.org/10.3390/ph15101188>.
 - [10] Abdelkader, A.; Preis, E.; Keck, C.M. smartFilm tablets for improved oral delivery of poorly soluble drugs. *Pharmaceutics* 2022, *14*, 1918. <https://doi.org/10.3390/pharmaceutics14091918>.
 - [11] Breda, S.A.; Jimenez-Kairuz, A.F.; Manzo, R.H.; Olivera, M.E. Solubility behavior and biopharmaceutical classification of novel high-solubility ciprofloxacin and norfloxacin
-

- pharmaceutical derivatives. *Int. J. Pharm.* 2009, 371, 106–113. <https://doi.org/10.1016/j.ijpharm.2008.12.026>.
- [12] Padeřskaia, E.N. Norfloksatsin: Bolee 20 let v klinicheskoj praktike, itogi i mesto v riadu ftorkhinolonov pri sovremennoj khimioterapii infektsij. *Antibiot. Khimioter.* 2003, 48, 28–36.
- [13] Pelikh, O.; Eckert, R.W.; Pinnapireddy, S.R.; Keck, C.M. Hair follicle targeting with curcumin nanocrystals: Influence of the formulation properties on the penetration efficacy. *J. Control. Release* 2020, 10, 598–613. <https://doi.org/10.1016/j.jconrel.2020.09.053>.
- [14] JASP Team. *JASP*, Version 0.13.1; Computer Software; Amsterdam, The Netherlands, 2020.
- [15] C.H. Beck. European Pharmacopoeia, 8th ed.; 2.09: Pharmaceutical Technical Procedures; C.H. Beck: Nördlingen, Germany, 2016.
- [16] Aslani, A.; Jahangiri, H. Formulation, characterization and physicochemical evaluation of ranitidine effervescent tablets. *Adv. Pharm. Bull.* 2013, 3, 315–322. <https://doi.org/10.5681/apb.2013.051>.
- [17] Miyashiro, T.; Ruby, E.G. Shedding light on bioluminescence regulation in *Vibrio fischeri*. *Mol. Microbiol.* 2012, 84, 795–806. <https://doi.org/10.1111/j.1365-2958.2012.08065.x>.
- [18] Molnár, M.; Fenyvesi, É.; Berkl, Z.; Németh, I.; Fekete-Kertész, I.; Márton, R.; Vaszita, E.; Varga, E.; Ujj, D.; Sente, L. Cyclodextrin-mediated quorum quenching in the *Aliivibrio fischeri* bioluminescence model system—Modulation of bacterial communication. *Int. J. Pharm.* 2021, 594, 120150. <https://doi.org/10.1016/j.ijpharm.2020.120150>.
- [19] Nallbati, L. A bioluminescent wound microbiome modification through *Aliivibrio fischeri* and effect of the topical application of norfloxacin nanosuspension. Master's Thesis, Philipps-Universität Marburg, Marburg, Germany, 2021.
- [20] Fathi, H.A.; Abdelkader, A.; AbdelKarim, M.S.; Abdelaziz, A.A.; El-Mokhtar, M.A.; Allam, A.; Fetih, G.; El Badry, M.; Elsabahy, M. Electrospun vancomycin-loaded nanofibers for management of methicillin-resistant *Staphylococcus aureus*-induced skin infections. *Int. J. Pharm.* 2020, 586, 119620. <https://doi.org/10.1016/j.ijpharm.2020.119620>.
- [21] Pelikh, O.; Pinnapireddy, S.R.; Keck, C.M. Dermal penetration analysis of curcumin in an ex-vivo porcine ear model using epifluorescence microscopy and digital image processing. *Ski. Pharmacol. Physiol.* 2021, 34, 281–299.

-
- [22] Keck, C.M.; Abdelkader, A.; Pelikh, O.; Wiemann, S.; Kaushik, V.; Specht, D.; Eckert, R.W.; Alnemari, R.M.; Dietrich, H.; Brüllner, J. Assessing the Dermal Penetration Efficacy of Chemical Compounds with the Ex-Vivo Porcine Ear Model. *Pharmaceutics* 2022, 14, 678. <https://doi.org/10.3390/pharmaceutics14030678>.
- [23] Kaushik, V.; Ganashalingam, Y.; Schesny, R.; Raab, C.; Sengupta, S.; Keck, C.M. Influence of Massage and Skin Hydration on Dermal Penetration Efficacy of Nile Red from Petroleum Jelly-An Unexpected Outcome. *Pharmaceutics* 2021, 13, 2190. <https://doi.org/10.3390/pharmaceutics13122190>.
- [24] Kaushik, V.; Keck, C.M. Influence of mechanical skin treatment (massage, ultrasound, microdermabrasion, tape stripping and microneedling) on dermal penetration efficacy of chemical compounds. *Eur. J. Pharm. Biopharm.* 2021, 169, 29–36. <https://doi.org/10.1016/j.ejpb.2021.09.003>.
- [25] Rasband, W.S. ImageJ: Image processing and analysis in Java. *Astrophysics Source Code Library*: Cambridge, MA, USA, 2012, ascl: 1206.013.
- [26] Schneider, C.A.; Rasband, W.S.; Eliceiri, K.W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 2012, 9, 671–675.
- [27] Dinno, A. Nonparametric pairwise multiple comparisons in independent groups using Dunn’s test. *The Stata Journal* 15, 2015, 292–300.
- [28] Cohen, J. *Statistical Power Analysis for the Behavioral Sciences*; Taylor and Francis: Hoboken, NJ, USA, 2013; ISBN 9781134742707.
- [29] Bauer-Brandl, A.; Ritschel, W.A. *Die Tablette: Handbuch der Entwicklung, Herstellung und Qualitätssicherung*, 3ed.; vollständig überarb. und erw. Aufl.; ECV Editio Cantor Verl.: Aulendorf, Germany, 2012; ISBN 9783871934070.
- [30] Stein, G.E. Review of the bioavailability and pharmacokinetics of oral norfloxacin. *Am. J. Med.* 1987, 82, 18–21. [https://doi.org/10.1016/0002-9343\(87\)90613-9](https://doi.org/10.1016/0002-9343(87)90613-9).
- [31] Lu, Y.; Kim, S.; Park, K. In vitro-in vivo correlation: Perspectives on model development. *Int. J. Pharm.* 2011, 418, 142–148. <https://doi.org/10.1016/j.ijpharm.2011.01.010>.
- [32] Xu, Y.; Shrestha, N.; Pr  at, V.; Belouqui, A. An overview of in vitro, ex vivo and in vivo models for studying the transport of drugs across intestinal barriers. *Adv. Drug Deliv. Rev.* 2021, 175, 113795. <https://doi.org/10.1016/j.addr.2021.05.005>.
-

6. Supplementary material

Table S1: Macro used for the determination of the smartFilm granules particle size.

```
run("8-bit");

setAutoThreshold("Default");

//run("Threshold...");

//setThreshold(0, 180);

setOption("BlackBackground", false);

run("Convert to Mask");

run("Analyze Particles...", "size=0.09-Infinity show=Masks display");
```

Table S2: Macro used for the automated threshold to subtract the background from the luminescence of the *A. fischeri*.

```
// Color Thresholder 1.53k

// Autogenerated macro, single images only!

min=newArray(3);

max=newArray(3);

filter=newArray(3);

a=getTitle();

run("RGB Stack");

run("Convert Stack to Images");

selectWindow("Red");

rename("0");

selectWindow("Green");

rename("1");

selectWindow("Blue");

rename("2");
```

```
min[0]=98;

max[0]=255;

filter[0]="pass";

min[1]=186;

max[1]=255;

filter[1]="pass";

min[2]=199;

max[2]=255;

filter[2]="pass";

for (i=0;i<3;i++){

selectWindow(""+i);

setThreshold(min[i], max[i]);

run("Convert to Mask");

if (filter[i]=="stop") run("Invert");

}

imageCalculator("AND create", "0","1");

imageCalculator("AND create", "Result of 0","2");

for (i=0;i<3;i++){

selectWindow(""+i);

close();

}

selectWindow("Result of 0");

close();

selectWindow("Result of Result of 0");

rename(a);
```

```
// Colour Thresholding-----

run("Invert");min[1]=75;

max[1]=255;

filter[1]="pass";

min[2]=0;

max[2]=0;

filter[2]="stop";

for (i=0;i<3;i++){

selectWindow(""+i);

setThreshold(min[i], max[i]);

run("Convert to Mask");

if (filter[i]=="stop") run("Invert");

}

imageCalculator("AND create", "0","1");

imageCalculator("AND create", "Result of 0","2");

for (i=0;i<3;i++){

selectWindow(""+i);

close();

}

selectWindow("Result of 0");

close();

selectWindow("Result of Result of 0");

rename(a);

// Colour Thresholding-----

run("Invert");
```

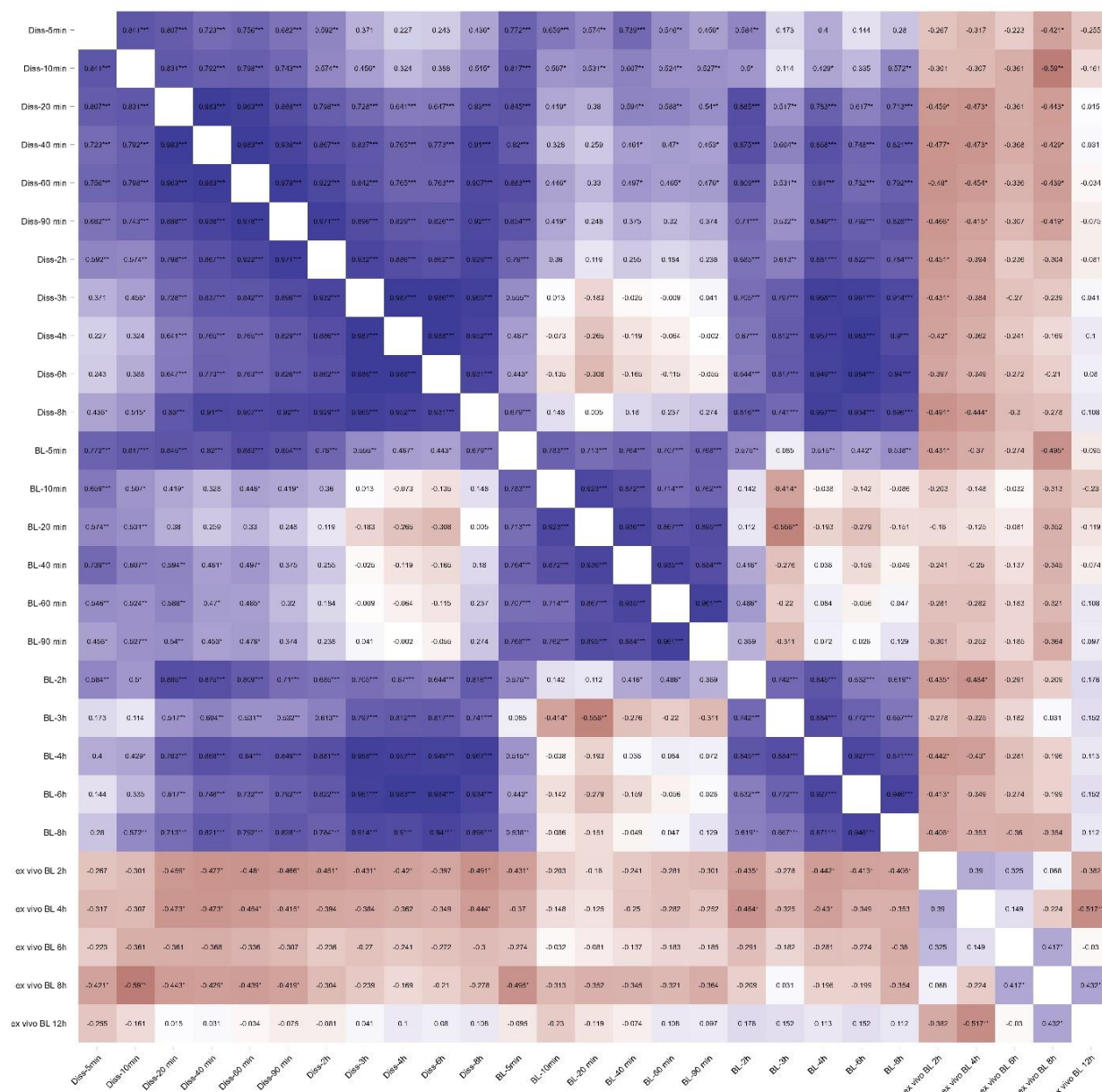


Figure S1: Heatmap of Pearson correlation coefficients that assess the relationship between the in vitro dissolution data (Diss), the invitro bioluminescence inhibition (BL) and the ex vivo antibacterial activity (ex vivo BL) data for the physical mixture and the norfloxacin-loaded smartFilm tablets at different time points. * $p < .05$, ** $p < .01$, *** $p < .001$.

Chapter 4

Summary and Discussion

4.1. Summary and Discussion

The overall aim of this thesis was fulfilled in three consecutive steps:

First step

Preparation of paper-based granules that can be used for large-scale production of smartFilm tablets

To achieve this, sucrose was used as a binding agent during the production of paper-based granules and the influence of sucrose on the pharmaceutical properties and compressibility of the obtained granules was studied. Paper-based granules were prepared via wet granulation technique using different amounts (i.e., 10% - 50%) and forms (i.e., dry or liquid) of sucrose and the produced paper granules were compressed into tablets. The physical properties of the obtained granules, such as particle size and shape, bulk and tapped density, Hausner's ratio and the Carr's index, and angle of repose were assessed. In addition, the pharmaceutical properties of the produced tablets, including thickness, mass uniformity, friability, hardness, and disintegration time, were determined according to the European Pharmacopoeia. Granules and tablets consisting of paper without sucrose were also prepared and served as benchmark control. Moreover, the mechanical behavior of paper-based granules under compression was investigated and compared to the properties of conventional binders. The compressibility of the obtained granules was determined by investigating their mechanical properties, such as elastic and plastic behaviors, using instrumented die compression tests.

The data obtained in this thesis regarding the properties of paper granules showed that the preparation of granules using sucrose resulted in slightly larger sizes as compared to paper granules without sucrose. The data also demonstrated non-significant differences between the granule batches prepared with the dry or the liquid form of sucrose, however, a slight trend towards larger sizes for the granules that were prepared with the liquid form of sucrose was observed. Results also indicate that the paper granules, either with or without sucrose, exhibit aspect ratio values that are slightly higher than the usually obtained aspect ratio values for spherical particles (i.e., 1.2) [1]. This indicates that all granules possess a slightly elongated shape. In addition, bulk and tapped density of the granules that were prepared with the dry form of sucrose increased significantly as the amount of sucrose increased. In contrast, granules that were prepared with the liquid form of sucrose exhibited an inconsistent increase in terms of bulk and tapped density. This behavior was particularly observed in case of highly concentrated sucrose aqueous solutions, as the high viscosity of

such solutions might result in an inhomogeneous distribution of the granulation liquid among the obtained granules, and thus affecting their properties. For all granule batches, the calculated flowability parameters (i.e., Hausner's ratio and Carr's index) indicated good flowability and compressibility of the prepared granules according to the European Pharmacopoeia [2]. Further experimental investigations demonstrated that the granules prepared without sucrose exhibited only passable flowability as the angle of repose values was in the range of 41–45°, which indicates that the produced granules might adhere to the hopper wall during tablet manufacturing. This behavior was indeed observed during the compression of the granules without sucrose into tablets and it was attributed to the electrostatic effects of paper [3]. The data also showed that the angle of repose decreased with increasing the amount of added sucrose either in the dry or the liquid form, which suggests an improved flowability behavior. The enhanced flowability behavior was particularly observed in case of granule batches with high sucrose content as they exhibited values in the range of 25–30°, which indicate excellent flowability according to the European Pharmacopoeia [2]. Interestingly, the improved flowability behavior of the granules containing sucrose was also associated with the disappearance of the electrostatic adherence of the granules to the hopper wall. The reduction of an electrostatic effect is the result of a combination of factors [4], and in this study it might be particularly attributed to the increase in size, rigidity and/or density of the granules, however, more research is needed to understand this outcome. Nevertheless, these findings suggest that the die filling process, using the granule batches containing sucrose, during high-speed tablet manufacturing will run efficiently with no major complications (e.g., without variations in tablet mass due to inconsistent die filling) [5].

The data in this thesis also showed the possibility of compressing all the granule batches into smooth, slightly porous, and pale tablets. Regarding the thickness of the tablets, it was observed that the thickness of the produced tablets from the granules without sucrose was significantly higher when compared to the tablets produced from the granules with the lowest sucrose content. This suggest that paper tablets are less compact and possess high elasticity in the absence of a binder. The high elasticity of the material causes a stronger elastic relaxation of the tablet after the compression, and thus leads to a higher thickness of the tablets. Moreover, it was found that the tablet thickness increased significantly with increasing the sucrose content indicating that tablets with high sucrose content require a higher compaction force. All the produced tablets from granule batches with sucrose content < 50% (w/w) fulfilled the criteria according to the European Pharmacopoeia. In contrast,

tablets produced from granule batches with the highest sucrose content (i.e., 50% (w/w)) weighted more than 250 mg and the individual mass of 7 tablets out of 20 differed by 5% from the average mass, and thus these tablets didn't fulfill the European Pharmacopoeia requirements [2]. The friability of the produced tablets from all granule batches fulfilled the criteria according to the European Pharmacopoeia, which suggests that tablets made from paper demonstrate a sufficient mechanical strength to withstand handling and shipping. The tablets obtained from the granules without sucrose were extremely fragile and exhibited very low hardness values, indicating low compaction of the tablets in the absence of sucrose. In contrast, the granule batches containing sucrose produced tablets that exhibited a significant increase in hardness with increasing the sucrose content due to a superior deformation and enhanced bonding between granules [6]. Such changes in the hardness of tablets are critical as they might affect the disintegration behavior, the dissolution profile, and bioavailability of the incorporated drug, hence its therapeutic efficacy [7]. The disintegration time of tablets produced from granules without sucrose was extremely rapid. This disintegration behavior might hamper the administration process of paper-based tablets and decrease patient compliance [8]. In contrast, the granule batches containing sucrose produced tablets that demonstrated a significant increase in the disintegration time with increasing the sucrose content. Moreover, all tablets that were prepared with high amounts of the dry form of sucrose (i.e., $\geq 40\%$ (w/w)) failed the criteria according to the European Pharmacopoeia for uncoated tablets, as they did not disintegrate within 15 min [2]. The slow disintegration behavior is coherent with the hardness data and suggest that harder tablets are less porous, which delays their disintegration. Data also suggested that using sucrose aqueous solution as a granulation liquid in the manufacturing of the granules resulted in the production of rigid tablets that failed the criteria according to the European Pharmacopoeia for uncoated tablets. All parameters that can affect tablets disintegration (e.g., the compression force, nature of the binder, method of tableting, and mechanism of tablet disintegration) [9] were kept constant during the production of all tablets. Therefore, it can be assumed that the incorporation method of the binder (i.e., as a solid form or an aqueous solution) was responsible for the slow disintegration behavior.

Based on the data obtained in this part of the thesis, it can be concluded that using the dry form of sucrose is the most suitable method to obtain granules which can be compressed into tablets with good pharmaceutical profile. In particular, paper granules with a sucrose content in the range of 20–30% (w/w) were found to produce paper-based tablets with optimal pharmaceutical properties.

Following that, the mechanical behavior of paper granules under compression was investigated via conducting instrumented die compression tests on three selected granule batches. One granule batch was selected to understand the mechanical behavior of granules in absence of a binder, a second batch was chosen as it was able to produce paper-based tablets with an excellent pharmaceutical profile (i.e., optimum disintegration behavior) and the third batch was selected as a representative of granules with a high sucrose content. The obtained data indicated that the compression velocity has only limited effects on the material behavior. In addition, the friction coefficients exhibited low values and demonstrated non-significant differences between different granule batches. This indicates that the compression of paper granules does not cause a remarkable friction between the granules and the wall of the die and that the influence of the sucrose content on the friction coefficient is neglectable. Furthermore, results show that increasing the sucrose content causes an increase in the stiffness of the material along with achieving a similar stress level at lower strains. This can be explained by the higher density and lower porosity values of the granules with high sucrose content, as compared to the values of microcrystalline cellulose [10]. The higher density of the granules with high sucrose content was confirmed by the decrease in the die filling height. The data obtained in this thesis indicated that the compaction behavior of paper granules is comparable to the behavior of industrial powder under compression [10,11]. The compression of powder into tablets is generally divided into different stages [11,12]. The first stage is the rearrangement of the paper granules, this process contributes the most to the overall stress, and thus the axial measured axial true strain is low. In the second stage, an increasing densification of the granules takes place, hence, elastic, and plastic deformation of the granules becomes more dominant, which results in an exponential increase in the stress—axial true strain curves. During the third stage, the unloading process, a nonlinear response of the stress—axial true strain occurs. In this thesis, the influence of compaction forces on the elastic properties (i.e., Poisson's ratio and Young's modulus) and the compressibility of the paper granules with different sucrose content, was also investigated. Young's modulus represents the stiffness of a material. Hence, the higher the Young's modulus, the higher is the stiffness of the material. The Poisson's ratio is the deformation of a material in the direction perpendicular to the specific direction of loading. A high Poisson's ratio indicates that the material has a high perpendicular deformation, meaning that—under axial force—the material “escapes” due to low compressibility [13]. For all granule batches, data showed that both the Poisson's ratio and the Young's modulus increase nonlinearly with increasing compaction, resulting in a more compact and stiffer paper granules with higher density and lower porosity. In contrast, the Poisson's ratio increased with increasing sucrose content while

Young's modulus remained almost constant for the different sucrose contents at similar compaction force levels, indicating that different sucrose contents had no influence on the changes in porosity. The obtained data were also used to compare the binding properties of paper granules to conventional binders using the Heckel mathematical model. The Heckel equation allows us to determine the yield pressure, which describes the necessary pressure to plastically deform a material [14]. Low yield pressure values represent a soft/plastic behavior, whereas higher values indicate a hard/brittle behavior [10]. The findings showed that the yield pressure values of all granule batches were in the upper limit to be classified as soft/plastic material, indicating a very good compressibility of the paper granules [10]. However, the values were also close to the lower limit of a hard/brittle behavior. The trend towards a higher hardness and a more brittle behavior can especially be seen for the batches with higher sucrose content. This can be explained by the crystalline and brittle nature of sucrose [15]. Hence, higher content of sucrose within the paper-based granules results in a higher proportion of crystalline and brittle material within the granules, which should then result in a higher cracking propensity [10]. Nevertheless, this trend was not significant, indicating that sucrose content had no significant influence on the yield pressure. With this, it can be stated that the deformation behavior of paper granules—independent on the sucrose content—is comparable to other pharmaceutical binders.

The outcomes of this step confirmed the potential of paper granules as a suitable intermediate product for the production of paper-based tablets in large, industrial scale. The physical properties of the paper granules are also sufficient for yielding tablets that fulfill the criteria according to the European Pharmacopoeia. In addition, the data acquired here provide the base for establishing numeric models that are able to simulate the compression process of paper granules into tablets. One of the most commonly used models is the Drucker-Prager Cap plasticity model (DPC) [16,17], and it has been previously utilized for compression simulations that aid in the production of tablets with optimal pharmaceutical properties [18–21]. Such modeling allows the prediction of the shape and the size of the resulting tablets as well as their inhomogeneous properties which can lead to tablets with a poor pharmaceutical quality (e.g., capping of the tablets). These simulations can also predict the hardness of tablets, which provides a close link to the disintegration of the tablet and to the dissolution profile of the API loaded within the tablet. These parameters can further be linked to the biopharmaceutical properties of the drug-loaded tablets, i.e., bioavailability and pharmacokinetic profiles. Consequently, these links would allow for a rapid and efficient development of drug-loaded smartFilms tablets with optimal pharmaceutical properties.

Therefore, future work should utilize the data provided here to establish simulation models which can predict all the abovementioned parameters.

Second step

Assessment of smartFilm tablets as a potential strategy for improving the oral delivery of a poorly water-soluble drug

For that, curcumin, a BSC class IV drug with poor solubility and low intestinal permeability, was selected for the production of smartFilm granules which were then compressed into tablets. The physical properties of the produced granules were assessed, and tablets were characterized regarding their physico-chemical and pharmaceutical properties. The morphology and the crystalline state of curcumin loaded within the smartFilm granules and the smartFilm tablets were determined (i.e., SEM and X-ray analysis). An ex vivo porcine intestinal model was used to determine and compare the intestinal permeability of curcumin from the smartFilm tablets to a physical mixture of curcumin and paper as well as to a classical and an innovative commercial curcumin product, respectively.

The findings of this thesis indicated the successful preparation of curcumin-loaded smartFilm granules with 20% (w/w) sucrose content and that the granules possessed a slightly elongated shape. The bulk and tapped density of the smartFilm granules were significantly higher when compared to unloaded paper granules that were previously prepared using the same sucrose content, i.e., 20% (w/w) [22]. This can be attributed to the presence of curcumin within the pores of the cellulose matrix, which reduces the volume of the pores, and thus enhances the density of the granules. The microscopic images obtained from SEM confirm this assumption. For the smartFilm granules, the characteristic shape of curcumin crystals (i.e., cubic shape) was absent and the pores of the paper appear “filled”. Thus, it indicates that the curcumin was loaded within the pores of the paper in non-crystalline state. Curcumin-loaded smartFilm granules exhibited Hausner’s ratio, Carr’s index, and angle of repose values that indicated good flowability and compressibility according to the European Pharmacopoeia [2]. These results suggest that the filling procedure of curcumin-loaded smartFilm granules, during high-speed tablet manufacturing, will operate efficiently with no major complications [5]. X-ray data showed that the typical reflexes of the crystalline curcumin bulk material [23] disappeared in the X-ray pattern of the smartFilm granules. The obtained data suggest that curcumin was embedded in the paper matrix of the smartFilms granules in an amorphous state. The data also indicate that the amorphous state of curcumin loaded within the smartFilms [24] wasn’t altered by the wet granulation process.

Data in this thesis showed that the compression of curcumin-loaded smartFilm granules resulted in smooth, slightly porous, and yellow-orange tablets. Microscopic images from SEM and x-ray data also demonstrated the absence of curcumin crystalline particles and crystalline reflexes within the tablets, indicating that curcumin was embedded into the paper matrix in an amorphous state. In addition, the pharmaceutical properties (i.e., mass uniformity, friability, hardness, disintegration time and content uniformity) of the produced tablets fulfilled all criteria according to the European Pharmacopoeia [2]. The hardness data indicated a sufficient mechanical strength of the curcumin-loaded smartFilm tablets as well as an insignificant increase when compared to the unloaded paper tablets [22]. This effect might be attributed to the incorporation of curcumin into the pores of the paper matrix, which resulted in an increased density of the smartFilm granules. The disintegration time of curcumin-loaded tablets showed similar data (i.e., a slight increase when compared to unloaded-paper tablets with the same sucrose content) [22]. This effect might be due to the hydrophobic nature of curcumin, which is abundantly loaded within the matrix of the paper. The data also demonstrated that the dissolution rate of curcumin can be improved with smartFilm tablets and showed that the enhanced effect of the dissolution rate is most pronounced at early time points. The enhanced dissolution as well as the rapid dissolution behavior at early time points are essential factors for the overall improvement of the oral bioavailability of poorly water-soluble drugs [25–27].

The data obtained in this thesis demonstrated a two-fold enhanced ex vivo intestinal permeability of curcumin from the smartFilm tablets, as compared to the physical mixture of curcumin and paper and to the classical commercial curcumin product (i.e., a hard capsule filled with curcumin as raw powder). This poor intestinal permeability of curcumin was expected because both formulations contain the curcumin as a powder, which is known to possess poor solubility and poor intestinal permeability [28]. Curcumin-loaded smartFilm tablets were also compared to an innovative commercial curcumin product. This innovative commercial curcumin product consists of a soft capsule that contained curcumin and high concentrations of an o/w surfactant, that solubilizes the lipophilic curcumin in micelles [29]. This “micellar curcumin” was found to provide excellent digestive stability and increased post-digestive solubility, when compared to other drug delivery systems, i.e., oils, liposomes, phytosomes, cyclodextrines, or sub-micron particles [29]. The smartFilm tablets were able to yield comparable intestinal permeation values to that of the “micellar curcumin”, with a slight trend towards a deeper permeation of curcumin. The deeper permeation can be explained by the adherence of the curcumin-loaded smartFilm residuals (i.e., pieces of paper), that

remained after the pre-digestion procedure (i.e., disintegration) of the smartFilm tablet, to the intestinal tissues. The adhered paper can be considered to have created a locally higher concentration gradient for curcumin between the smartFilm surface and the intestinal tissue, which then promoted the curcumin deeper permeation. Improved permeation via a locally high concentration gradient was previously shown for smartFilms that were applied on skin [24,30] and it is very likely that similar effects also occur in the intestine. However, more research is needed to investigate this phenomenon in detail. All these outcomes indicate that smartFilm tablets can be equally efficient as innovative and classical curcumin formulation approaches in enhancing the oral delivery of curcumin. The ex vivo permeation data for the physical mixture and the smartFilm tablets was correlated to their in vitro dissolution data and they showed a significant correlation. The correlation was very strong after 30 and 45 min of dissolution, then with longer dissolution times (i.e., after 24 h) the coefficient decreased, however, it was still considered to be a relatively strong correlation. Such a decrease in the correlation coefficient value indicates that the release of curcumin is not following a constant behavior. This might be attributed to the non-linear release of the curcumin from the paper matrix of the smartFilms and it was recently shown to follow a super case II release kinetics, which occur after the wetting of the paper matrix [24]. Another correlation was created between the amount of released curcumin from all different formulations, after the in vitro pre-digestion step, and their ex vivo permeation data and it showed a moderate correlation with the amount of permeated curcumin and no correlation with the permeation depth of curcumin through the intestinal tissue, respectively. This might be explained by the deeper permeation depth of the curcumin from the smartFilm tablets, which was probably caused by the parts of paper that adhered to the intestinal tissue. This was proved by recalculating the correlation while excluding the data obtained from the smartFilm tablet formulation, which then resulted in a weak but significant in vitro ex vivo correlation [31].

Data of this part of the thesis provide sufficient evidence that curcumin-loaded smartFilm can be transferred into smartFilm granules and tablets with sufficient physico-chemical and pharmaceutical properties. It also showed that the smartFilm granules are able to maintain the amorphous state of the incorporated curcumin, and thus it can be used as an intermediate product for the production of curcumin-loaded smartFilm tablets. The obtained ex vivo data could clearly differentiate between the formulations ability to provide poor or good intestinal permeation of curcumin. Therefore, an intestinal ex vivo model can be recommended as a simple and cost-efficient approach to determine the intestinal permeation efficacy of drugs from different formulations in early formulation development.

Third step

Determination of the bioactivity of smartFilm tablets loaded with a poorly water-soluble drug

For that, norfloxacin, which is a BSC class IV antimicrobial drug with poor solubility and low intestinal permeability, was used as model drug for the production of norfloxacin-loaded smartFilm tablets. The crystalline state of norfloxacin loaded within the matrix of smartFilm tablets as well as the pharmaceutical properties of the produced tablets were investigated. The bioactivity of smartFilm tablets was determined by evaluating the antimicrobial activity of norfloxacin in vitro and in an infected ex vivo porcine intestinal model.

Norfloxacin smartFilm granules were successfully produced from the norfloxacin-loaded smartFilms. The obtained data from the digital analysis of the macroscopic images of the granules indicated that the norfloxacin granules possessed a slightly smaller mean particle size and a slightly broader size distribution, as compared to the unloaded paper granules. The broad size distribution can be explained by the increased density of the drug-loaded smartFilm granules and the reduction of the pore size, and thus decreasing the uptake of the binding agent into the pores as well as the granulation efficiency of the binder. The pharmaceutical characteristics of the norfloxacin-loaded smartFilm granules, i.e., bulk density, tapped density, Hausner's ratio, Carr's index, exhibited higher values and similar angle of repose values when compared the values of the unloaded granules. The higher values of the calculated flowability parameters (i.e., Hausner's ratio, Carr's index) indicated reduced flowability, as compared to the unloaded paper granules, however, the data indicated good to moderate flowability [32]. The reduced flowability can be explained by the smaller particle size and the slightly larger size distribution of the norfloxacin-loaded granules, as compared to the unloaded granules, which changed the particle interaction forces. Similar effects were also found when curcumin was loaded into paper [33]. Based on these results, it can be concluded that the loading of APIs into paper results in an increased density of the paper and that the transfer of drug-loaded paper into granules results in granules with smaller size, broader size distribution, and relatively lower flowability. Nevertheless, the good flowability behavior of norfloxacin-loaded smartFilm granules will allow the industrial compression process of tablets to occur without complications. The X-ray analysis showed that the wet granulation process did not alter the amorphous state of norfloxacin, as no typical reflexes of norfloxacin were found in the diffractogram of the norfloxacin-loaded smartFilm granules.

The compression of the norfloxacin-loaded smartFilm granules into smartFilm tablets resulted in smooth tablets with a polished surface. It also did not alter the amorphous state of the loaded norfloxacin as the typical reflexes of norfloxacin were absent in the diffractogram of norfloxacin-loaded smartFilm tablets. The pharmaceutical properties of the norfloxacin-loaded smartFilm tablets fulfilled the requirements according to the European Pharmacopoeia [2] and showed some differences when compared the properties of the unloaded paper tablets. Loading norfloxacin into smartFilms resulted in increasing the thickness, the friability, and the disintegration time of the produced tablets, while the crushing strength was comparable. In contrast, the mass uniformity decreased, and this can be linked to the reduced flowability of the smartFilm granules, which resulted in a less constant flow of the granules into the die of the tablet press, and thus leading to a greater variation in the tablet mass. The increase of tablet thickness and disintegration time can be linked to the higher density of the smartFilm granules. The loading of the drug into the pores of the paper lead to the formation of these dense granules which exhibit a reduced pore size and contain less air within the paper pores. Therefore, the compression of such granules will yield thicker tablets and decrease their wettability (i.e., longer disintegration time). The improved friability behavior can also be explained by the high density of the granules which might increase the hardness of the material and reduce the friability values. Such an increase in hardness of the smartFilm tablets was indeed observed in a recent study when curcumin was loaded into paper [33]. However, such an increase was not observed here, and it can be attributed to the lower porosity of the loaded smartFilm granules and the reduced compressibility (i.e., higher Carr's index values), leading to a less efficient plastic deformation of the norfloxacin-loaded smartFilm granules during the compression process. The dissolution profile of the smartFilm tablets and the physical mixture tablets showed a biphasic release (i.e., a very slow release at early time points followed by a faster release). However, the dissolution of physical mixture tablets showed a delayed onset of the fast release and a lower amount of dissolved norfloxacin. In addition, the data obtained in this thesis demonstrated a significant two-fold increase of the amount of released norfloxacin from the smartFilm tablets, as compared to physical mixture tablets. This increase later declined and became non-significant with higher dissolution times ($\geq 2\text{h} - 6\text{h}$).

The improved release of norfloxacin from the smartFilm tablets suggest a higher antibacterial activity of the norfloxacin-loaded smartFilm tablets against *Aliivibrio fischeri* bacteria (*A. fischeri*), in comparison to physical mixture tablets. *A. fischeri* bacteria possess bioluminescent properties [34], which extinguishes if a compound is toxic to the bacterium.

Therefore, the bioluminescence inhibition can be used as a simple way to compare the antibacterial activity of different formulations [35]. Data indicated that using norfloxacin-loaded smartFilm tablets caused a rapid increase in the bioluminescence inhibition with the decimal reduction time, i.e., 90% of the bacteria are inactive, estimated to be 10 min and 99% of the bacteria luminescence inactivated after 4h. In contrast, the decimal reduction time was about 30 min and 99% of the bacteria luminescence was depleted after 8h in case of physical mixture tablets. Based on these results, the antibacterial activity of norfloxacin from smartFilm tablets was about 2-3-fold higher, when compared to the physical mixture tablets.

The findings in this thesis demonstrated the successful development of an infected ex vivo porcine intestinal model using *A. fischeri*. The results showed that the model yields reproducible results (i.e., similar bacteria count from different intestinal tissues at the beginning of the experiment) and that it remains sensitive within a time frame of at least 12h (i.e., significant differences and changes can be found between different formulations and application set ups). Data also showed that the smartFilm tablets result in a significantly higher antibacterial activity (2-fold increase) than the physical mixture tablets. The acquired data are in line with the results obtained from the in vitro bioluminescence assay, which showed a similar trend for the decimal reduction time and the time needed to eradicate 99% of the bacteria. However, the eradication of the bacteria in the ex vivo model was less efficient and slower, when compared to the eradication of the bacteria in the in vitro model. This might be caused by the lower amounts of released norfloxacin after the pre-digestion step and/or the slower diffusion of the released norfloxacin within the tissue. An additional possibility is the metabolism of the norfloxacin via intestinal enzymes or other interactions that might partially inactivate it. The half-life time of norfloxacin is about 3h [36], therefore a second dose of the formulations was applied to compensate for the norfloxacin that might have been partially deactivated and it resulted in decreasing the bioluminescence $\geq 95\%$ for both formulations. The differences between the antibacterial activity of the two formulations at different time points indicated that the smartFilm tablets resulted in a significantly less luminescence intensity faster than the physical mixture tablets. The faster onset and more pronounced antibacterial activity of the norfloxacin-loaded smartFilm tablets can be explained by the enhanced solubility and the faster release of norfloxacin from the smartFilm tablets. The results also showed that the antibacterial effect of both formulations reaches a plateau after 4h which can be related to the short half time of the norfloxacin. This indicates that the smartFilm tablets are especially useful if a fast antibacterial action is required, however, the use of classical formulation approaches seems to be sufficient for longer, multiple dose

treatments. From an environmental point of view, it might be more efficient to apply the initial dose with smartFilm tablets to yield a fast onset of the antibacterial activity followed by applying the classical formulation as a maintenance dose. Nevertheless, future research is needed to investigate this approach in more detail. It was also found that the correlation between the *in vitro* dissolution data and the *ex vivo* data was not constant, which was expected as a good *in vitro in vivo* (*ex vivo*) correlation cannot be achieved in case of a BSC IV drug [27]. This is mainly due to the anomalous, biphasic release of norfloxacin, as a good correlation was only found at early time points where a slow drug release occurred, and low correlation coefficients were obtained at late time points which were associated with a faster drug release. A similar effect can be seen for the *in vitro* bioluminescence data, i.e., the bioluminescence data from early time points correlate well with early time points of the dissolution data but result in poor correlation with higher dissolution time points.

Based on the obtained data, the production of smartFilm tablets with optimal and tailor-made pharmaceutical properties require a thorough investigation to understand the interplay between granule properties, drug loading, and the resulting tablet properties. Nonetheless, the data also confirm the possibility of producing norfloxacin-loaded smartFilm tablets that fulfil the requirements according to the European Pharmacopoeia and capable of enhancing the dissolution of norfloxacin. The data also demonstrate that the *ex vivo* model is able to judge the antibacterial activity from different formulations. The findings enable a rough estimation of differences in pharmacokinetic profiles and provide a clear link to the *in vitro* data. In addition, the *ex vivo* model can provide a close link to real physiological conditions (i.e., mucus, pH, active enzymes, etc.). Therefore, its use can be considered to yield a valuable, additional bio-pharmaceutical information that cannot be assessed using classical *in vitro* experiments. The model can be considered to be especially useful in early formulation development, because at this stage it is often not necessary to obtain highly accurate results. In most cases, it is sufficient to get a rough estimate if a formulation is effective or not and if changes in the formulation and/or treatment setup improve the performance or not. Thus, this *ex vivo* model can be considered as useful tool which allows for a fast and cost-effective discrimination between “good” and “bad” formulations.

4.2. References

- [1] Chopra, R.; Michael Newton, J.; Alderborn, G.; Podczek, F. Preparation of pellets of different shape and their characterization. *Pharm. Dev. Technol.* **2001**, *6*, 495–503, doi:10.1081/PDT-120000288.
- [2] C.H. Beck. *European Pharmacopoeia, 8th ed.; 2.09: Pharmaceutical Technical Procedures*; C.H. Beck: Nördlingen, Germany, 2016.
- [3] Sappi etc. Printer Tech Tips—Cause & Effects of Static Electricity in Paper. Available online: [Sappi-Printer-Tech-Tips-Cause-And-Effects-Of-Static-Electricity-In-Paper.pdf](#) (accessed on 1 January 2012).
- [4] Boschini, F.; Delaval, V.; Traina, K.; Vandewalle, N.; Lumay, G. Linking flowability and granulometry of lactose powders. *Int. J. Pharm.* **2015**, *494*, 312–320, doi:10.1016/j.ijpharm.2015.08.030.
- [5] Chen, H.; Wang, C.; Liu, S.; Sun, C.C. Development of piroxicam mini-tablets enabled by spherical cocrystallization. *Int. J. Pharm.* **2020**, *590*, 119953, doi:10.1016/j.ijpharm.2020.119953.
- [6] Fu, Y.; Jeong, S.H.; Park, K. Fast-melting tablets based on highly plastic granules. *J. Control. Release* **2005**, *109*, 203–210, doi:10.1016/j.jconrel.2005.09.021.
- [7] Loscertales, H.R.; Modamio, P.; Lastra, C.F.; Mariño, E.L. Assessing the stability of lamotrigine dispersible/chewable tablets when stored in blister punch cards. *Curr. Med. Res. Opin.* **2017**, *33*, 949–953, doi:10.1080/03007995.2017.1297927.
- [8] Stumpf, F.; Keck, C.M. Tablets made from paper. *Int. J. Pharm.* **2018**, *548*, 812–819, doi:10.1016/j.ijpharm.2018.05.071.
- [9] Alanazi, F.K. Utilization of date syrup as a tablet binder, comparative study. *Saudi Pharm. J.* **2010**, *18*, 81–89, doi:10.1016/j.jsps.2010.02.003.
- [10] Cabiscol, R.; Finke, J.H.; Zetzener, H.; Kwade, A. Characterization of mechanical property distributions on tablet surfaces. *Pharmaceutics* **2018**, *10*, 184, doi:10.3390/pharmaceutics10040184.
- [11] Shang, C.; Sinka, I.C.; Pan, J. Constitutive model calibration for powder compaction using instrumented die testing. *Exp. Mech.* **2012**, *52*, 903–916, doi:10.1007/s11340-011-9542-8.
- [12] Leuenberger, H.; Rohera, B.D. Fundamentals of powder compression. I. The compactibility and compressibility of pharmaceutical powders. *Pharm. Res.* **1986**, *3*, 12–22, doi:10.1023/a:1016364613722.

- [13] Mazel, V.; Busignies, V.; Diarra, H.; Tchoreloff, P. On the links between elastic constants and effective elastic behavior of pharmaceutical compacts: importance of Poisson's ratio and use of bulk modulus. *J. Pharm. Sci.* **2013**, *102*, 4009–4014, doi:10.1002/jps.23710.
- [14] Ghori, M.U.; Conway, B.R. Powder compaction: compression properties of cellulose ethers. *Br. J. Pharm.* **2016**, *1*, 19–29, doi:10.5920/bjpharm.2016.09.
- [15] Sugimoto, M.; Narisawa, S.; Matsubara, K.; Yoshino, H.; Nakano, M.; Handa, T. Development of manufacturing method for rapidly disintegrating oral tablets using the crystalline transition of amorphous sucrose. *Int. J. Pharm.* **2006**, *320*, 71–78, doi:10.1016/j.ijpharm.2006.04.004.
- [16] Drucker, D.C.; Prager, W. Soil mechanics and plastic analysis or limit design. *Q. Appl. Math.* **1952**, *10*, 157–165, doi:10.1090/qam/48291.
- [17] Drucker, D.C.; Gibson, R.E.; Henkel, D.J. Soil mechanics and work-hardening theories of plasticity. *Trans. Am. Soc. Civ. Eng.* **1957**, *122*, 338–346, doi:10.1061/taceat.0007430.
- [18] Sinka, C. Modelling powder compaction. *KONA Powder Part. J.* **2007**, *25*, 4–22, doi:10.14356/kona.2007005.
- [19] Han, L.H.; Elliott, J.A.; Bentham, A.C.; Mills, A.; Amidon, G.E.; Hancock, B.C. A modified Drucker-Prager Cap model for die compaction simulation of pharmaceutical powders. *Int. J. Solids Struct.* **2008**, *45*, 3088–3106, doi:10.1016/j.ijsolstr.2008.01.024.
- [20] Diarra, H.; Mazel, V.; Boillon, A.; Rehault, L.; Busignies, V.; Bureau, S.; Tchoreloff, P. Finite Element Method (FEM) modeling of the powder compaction of cosmetic products: Comparison between simulated and experimental results. *Powder Technol.* **2012**, *224*, 233–240, doi:10.1016/j.powtec.2012.02.058.
- [21] Krok, A.; Peciar, M.; Fekete, R. Numerical investigation into the influence of the punch shape on the mechanical behavior of pharmaceutical powders during compaction. *Particuology* **2014**, *16*, 116–131, doi:10.1016/j.partic.2013.12.003.
- [22] Abdelkader, A.; Moos, C.; Pelloux, A.; Pfeiffer, M.; Alter, C.; Kolling, S.; Keck, C.M. Tablets Made from Paper-An Industrially Feasible Approach. *Pharmaceuticals* **2022**, *15*, doi:10.3390/ph15101188.
- [23] Chereddy, K.K.; Coco, R.; Memvanga, P.B.; Ucar, B.; Des Rieux, A.; Vandermeulen, G.; Pr  at, V. Combined effect of PLGA and curcumin on wound healing activity. *J. Control. Release* **2013**, *171*, 208–215, doi:10.1016/j.jconrel.2013.07.015.
- [24] Eckert, R.W.; Wiemann, S.; Keck, C.M. Improved dermal and transdermal delivery of curcumin with smartfilms and nanocrystals. *Molecules* **2021**, *26*, 1633, doi:10.3390/molecules26061633.

-
- [25] Jinno, J.; Kamada, N.; Miyake, M.; Yamada, K.; Mukai, T.; Odomi, M.; Toguchi, H.; Liversidge, G.G.; Higaki, K.; Kimura, T. Effect of particle size reduction on dissolution and oral absorption of a poorly water-soluble drug, cilostazol, in beagle dogs. *J. Control. Release* **2006**, *111*, 56–64, doi:10.1016/j.jconrel.2005.11.013.
- [26] Jinno, J.; Kamada, N.; Miyake, M.; Yamada, K.; Mukai, T.; Odomi, M.; Toguchi, H.; Liversidge, G.G.; Higaki, K.; Kimura, T. In vitro–in vivo correlation for wet-milled tablet of poorly water-soluble cilostazol. *J. Control. Release* **2008**, *130*, 29–37, doi:10.1016/j.jconrel.2008.05.013.
- [27] Lu, Y.; Kim, S.; Park, K. In vitro–in vivo correlation: Perspectives on model development. *Int. J. Pharm.* **2011**, *418*, 142–148, doi:10.1016/j.ijpharm.2011.01.010.
- [28] Carvalho Henriques, M.; Faustino, M.A.F.; Santos Braga, S. Curcumin innovative delivery forms: Paving the ‘Yellow Brick Road’ of antitumoral phytotherapy. *Appl. Sci.* **2020**, *10*, 8990, doi:10.3390/app10248990.
- [29] Flory, S.; Sus, N.; Haas, K.; Jehle, S.; Kienhöfer, E.; Waehler, R.; Adler, G.; Venturelli, S.; Frank, J. Increasing Post-Digestive Solubility of Curcumin Is the Most Successful Strategy to Improve its Oral Bioavailability: A Randomized Cross-Over Trial in Healthy Adults and In Vitro Bioaccessibility Experiments. *Mol. Nutr. Food Res.* **2021**, *65*, 2100613, doi:10.1002/mnfr.202100613.
- [30] Wiemann, S.; Keck, C.M. Are lipid nanoparticles really superior? A holistic proof of concept study. *Drug Deliv. Transl. Res.* **2021**, 1–12, doi:10.1007/s13346-021-01021-5.
- [31] Rea, L.M.; Parker, R.A. *Designing and conducting survey research: A comprehensive guide*; John Wiley & Sons, 2014, ISBN 1118767039.
- [32] Bauer-Brandl, A.; Ritschel, W.A. *Die Tablette: Handbuch der Entwicklung, Herstellung und Qualitätssicherung*; ECV Editio Cantor Verlag, 2012, ISBN 3871934070.
- [33] Abdelkader, A.; Preis, E.; Keck, C.M. SmartFilm Tablets for Improved Oral Delivery of Poorly Soluble Drugs. *Pharmaceutics* **2022**, *14*, 1918, doi:10.3390/pharmaceutics14091918.
- [34] Miyashiro, T.; Ruby, E.G. Shedding light on bioluminescence regulation in *Vibrio fischeri*. *Mol. Microbiol.* **2012**, *84*, 795–806, doi:10.1111/j.1365-2958.2012.08065.x.
- [35] Molnár, M.; Fenyvesi, É.; Berkl, Z.; Németh, I.; Fekete-Kertész, I.; Márton, R.; Vaszita, E.; Varga, E.; Ujj, D.; Szenté, L. Cyclodextrin-mediated quorum quenching in the *Aliivibrio fischeri* bioluminescence model system – Modulation of bacterial communication. *Int. J. Pharm.* **2021**, *594*, 120150, doi:10.1016/j.ijpharm.2020.120150.
- [36] Stein, G.E. Review of the bioavailability and pharmacokinetics of oral norfloxacin. *Am. J. Med.* **1987**, *82*, 18–21, doi:10.1016/0002-9343(87)90613-9.
-

Chapter 5

Appendix

5.1. Acknowledgments

Alhamdulillah, my deepest praise and thanks are due to ALLAH Almighty, for endowing me with health, patience, and knowledge to complete this work.

First of all, I would like to express my sincere gratitude and appreciation to Prof. Dr. Cornelia M. Keck, department of Pharmaceutical technology and Biopharmacy at Philipps University of Marburg, for her supervision, guidance and endless support, personally and academically, during my Ph.D. studies.

To Prof. Dr. Martin Koch, department of Physics, Prof. Dr. Tanja Pommerening, department of History of Pharmacy and Medicine, and Prof. Dr. Raphael Reher, department of Pharmaceutical Biology and Biotechnology at Philipps University of Marburg, thank you for your kindness and professionalism. It is a great honor to have you as my dissertation committee .

Prof. Dr. Stefan Kolling, Dr. Christian Alter, Christoph Moos, and Dr. Marcus Pfeiffer from the Institute of Mechanics and Materials, Technische Hochschule Mittelhessen in Giessen/Germany: thank you for your help, your valuable contribution to this work and for initiating this whole journey.

Many thanks to the Yousef Jameel Foundation and my mentor Prof. Dr. Mahmoud Elsabahy, vice dean of the school of Biotechnology and head of the Science Academy at Badr University in Cairo/ Egypt, for supporting and encouraging me to continue my Ph.D. studies .

I would like to thank my colleagues and the staff at the Institute of Pharmaceutical technology and Biopharmacy at Philipps University of Marburg, the big IPTB family, for their generous help, for accepting my “strong” physique and the impact I have on my surroundings, and most importantly for our fruitful discussions and pleasant conversations. I wish to thank in particular, “my dear” Dr. Alice Abudayyih, Ibrahim Awak, Dr. Muhammad Umair Amin, Abdallah Ayoub, Lena Bender, Martina Bonsu, Dr. Konrad Engelhardt, Matteo Grussu, Ayesha Ishfaq, Susanne Lüttebrandt, the kindest Julia Michaelis, Eva Mohr, Dr. Eduard Preis, Valeri Roschenko, Ramzi Saeed, Dr. Jens Schäfer, Jan Schulze, Tan Shi and Ahmed Somaïda.

To my teammates and “Amigos” who left a positive impact on me and paved the way for this thesis to see the light. Thank you for the laughs, the tears, and all the science in between. Special thanks to “Ostazy” Dr. Abraham Abraham, Muzn Alkhaldi, Noor Almohsen, Dr. Reem Alnemari, Dr. Jana Brüssler, Em-on Chaiprateep, the M.O.M Henriette Dietrich, Dr. Ralph Eckert, Verda Farida, Viktoriya Grikh, Louisa Hermes, Maximilian Jacobi, Vasudha Kaushik, Davide Merella, Laura Nallbati, Dr. Olga Pelikh, Christian Raab, Robert Schesny, Tehseen Sehra, Dr. Soma Sengupta, Orchid Soliman, Dr. David Specht, Dr. Florian Stumpf, Lennart Westermann, and Dr. Sabrina Wiemann.

I would also like to extend my gratitude to my colleagues and friends at Assiut International Center of Nanomedicine, Alrajhy Liver Hospital, and the “Good Companions” in Assiut/Egypt, for believing in me and inspiring me to complete this work.

To my beloved friends, Fayroze Abdelaleem, Dr. Shorok Abdelhameed, Sherouk Elsonbaty, Heba Fathi, Sara Gadallah, Dr. Shaima Hashem, Melek Levent, my “Einstein” Chiraf Souilah, thank for your valuable advice, encouragement, unparalleled support, and for sharing this wonderful voyage with me.

I'm deeply indebted and forever grateful to my family in Marburg, Ahmed Mohamed and Reham Elgendy, for welcoming me to their home, for having my back in every step of the way, and for being there when I had no one.

To Yameera Ganashalingam, my "Sagothari" from another mother, no words could ever express my everlasting gratitude & love towards you, your lovely mom, and the entire family. You are going to live forever in me, *"ya Anisaa"*.

Finally, to my big family, I could not have embarked on this journey without your support, motivation over the years, and for that I am eternally grateful. To my DFANS group and their families, thank you being the friends of yesterday, today, tomorrow, and eternity. To my late grandmother, Bahia, who witnessed the start but not the end, I love you and I miss you. To Arwa, my cousin, and my look alike, thank you for making memories with me and for your "critical eye". To Asma, Youssef, Habiba and Yassin, thank you for loving me through video calls, for making me part of your little gang and for making me feel like the coolest aunt in the world. To Mohamed and Heba, my backbone and my first friend, thank you for holding down the fort and for your family who sprinkled joy, madness and laughter at Abdelkader's house. To Ali, my first child, thank you for being my guy who gets the job done and for sharing all these hilarious "memes" with me. To Omar, my shark slayer, thank you for being at the beginning, the end and everything in between. To Ola, my second mom, and my partner in crime, thank you for not allowing me to miss out on all the joyful moments that happened when I was away. To my mom, my "Basbosa", and my warrior, thank you for your prayers and your blessings which I felt in every step along my path.

In loving memory of my dearest dad, my king, my first mentor, and the reason I started this adventure. He lived his life in the pursuit of knowledge and left behind big shoes to fill, so this is me trying. I hope I made you proud *"ya Baba"*.

5.2. Publications

Abdelkader, A., Nallbati, L., Keck, C.M. Improving the Bioactivity of Norfloxacin with Tablets Made from Paper. *Pharmaceutics* 2023, 15, 375, doi:10.3390/pharmaceutics15020375.

Abdelkader, A.; Moos, C.; Pelloux, A.; Pfeiffer, M.; Alter, C.; Kolling, S.; Keck, C.M. Tablets Made from Paper-An Industrially Feasible Approach. *Pharmaceutics* 2022, 15, 1188, doi:10.3390/ph15101188.

Abdelkader, A.; Preis, E.; Keck, C.M. SmartFilm Tablets for Improved Oral Delivery of Poorly Soluble Drugs. *Pharmaceutics* 2022, 14, 1918, doi:10.3390/pharmaceutics14091918.

Keck, C.M., Abdelkader, A., Pelikh, O., Wiemann, S., Kaushik, V., Specht, D., Eckert, R.W., Alnemari, R.M., Dietrich, H., Brüßler, J., 2022 Assessing the Dermal Penetration Efficacy of Chemical Compounds with the Ex vivo Porcine Ear Model. *Pharmaceutics*, 14, 678, doi:10.3390/pharmaceutics14030678.

Ayoub, A.M., Gutberlet, B., Preis, E., Abdelsalam, A.M., Abu Dayyih, A., Abdelkader, A., Balash, A., Schäfer, J. and Bakowsky, U., 2022. Parietin Cyclodextrin-Inclusion Complex as an Effective Formulation for Bacterial Photoinactivation. *Pharmaceutics*, 14, 357, doi:10.3390/pharmaceutics14020357.

5.3. Oral and poster presentations

Abdelkader, A., Nallbati, L., Keck, C. M. (2022) Evaluation of the in vitro antimicrobial activity of antibiotic-loaded paper tablets. DPHG Jahrestagung 2022/Annual meeting. Marburg/Germany. 13-16 September.

Abdelkader, A., Keck, C. M. (2022) Effect of increasing the azithromycin content on the crystallinity and disintegration behavior of azithromycin-loaded paper tablets. DPHG Jahrestagung 2022/Annual meeting. Marburg/Germany. 13-16 September.

Nallbati, L., Abdelkader, A., Keck, C. M. (2022) A fast, cost-effective antimicrobial testing method for developing efficient pharmaceutical formulations. DPHG Jahrestagung 2022/Annual meeting. Marburg/Germany. 13-16 September.

Abdelkader, A., Keck, C. M. (2022) Determination of curcumin permeation in an ex vivo intestinal model. Controlled Release Society (CRS) 2022 Annual Meeting. Montreal/Canada. 11-15 July.

Abdelkader, A., Keck, C. M. (2022) Smartfilms and tablets made from paper loaded with indomethacin: effect of granulation on the crystallinity behaviour. HIPS Symposium 2022. Saarbrücken/Germany. 12 May.

Abdelkader, A., Keck, C. M. (2021) Smartfilms and paper tablets loaded with caffeine: effect of granulation on the crystallinity behaviour. 13th Central European Symposium on Pharmaceutical Technology. Gdańsk/Poland. 16-18 September.

Abdelkader, A., Keck, C. M. (2021) Smartfilms and paper tablets loaded with rutin: effect of granulation on the crystallinity behaviour. 13th International Conference on Biological Barriers. Saarbrücken/Germany. 7-8 September.

Abdelkader, A., Keck, C. M. (2021) Reproducibility of smartFilms® technology for large scale production of paper tablets. Controlled Release Society - Local Chapter Germany. Aachen/Germany. 3-5 March

5.4. Curriculum Vitae

Personal information

Name	Ayat Abdelkader Mohamed Aboelela
Date of birth	02.09.1989
Place of birth	<i>Assiut, Egypt</i>
Nationality	Egyptian

Working experience

11/2019 – 03/2023	Philipps University of Marburg • PhD student • Institute of Pharmaceutical Technology and Biopharmacy	<i>Marburg, Germany</i>
04/2014 - 10/2019	Assiut University • Researcher • Assiut International Center of Nanomedicine	<i>Assiut, Egypt</i>
07/2012 – 03/2014	Assiut University • Pharmacist • Alrajhy Liver Hospital	<i>Assiut, Egypt</i>
07/2010	Pfizer GmbH • Internship • Pharmaceutical sales	<i>Cairo, Egypt</i>

Education

08/2018	Cairo University • Master of Pharmacy • Pharmaceutical Sciences - Industrial Pharmacy	<i>Cairo, Egypt</i>
01/2014	Assiut University • Diploma of Pharmacy • Clinical Pharmacy	<i>Assiut, Egypt</i>
06/2011	Assiut University • Bachelor of Pharmacy • Pharmaceutical Sciences	<i>Cairo, Egypt</i>

Scholarships & awards

11/2019 - present	Jameel Education Foundation Scholarship • PhD	<i>Marburg, Germany</i>
-------------------	---	-------------------------