CHARLES UNIVERSITY

Faculty of Pharmacy in Hradec Králové

Department of Pharmaceutical Technology

Development of liquisolid systems for colon targeting

Doctoral Thesis

Doctoral Degree program: Pharmaceutical Technology

Hradec Králové 2024 Mgr. Chiazor Ugo Ogadah

Statement of originality

I hereby declare that this thesis is my own original work, composed independently. All references to literature and other resources are included in the bibliography list and have been properly cited. This work has not been submitted previously to attain another or the same degree.

Date:

Signature:

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1 Abstract

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Colon-targeted drug delivery holds significant promise for the local treatment of colonic ailments or the systemic delivery of drugs. Nevertheless, the success is challenged by the physiological barriers within the gastrointestinal tract. Improving the solubility of the drug prior to targeting will circumvent the rate-limiting step of dissolution and enhance absorption and oral bioavailability. Additionally, retaining the formulation at the target site will optimize the dose and improve the therapeutic efficacy. Therefore, the aim of this thesis was to develop a drug delivery system that will potentially allow the targeting a poorly water-soluble drug, cyclosporine A (CyA), to the colon.

In the first part of the experiments, the preformulation studies related to improving the solubility and dissolution rate of the CyA by the formulation of liquisolid systems (LSS) or interactive mixtures by co-milling was performed. This included the selection of suitable carrier and evaluation of its milling properties as well as the selection of non-volatile solvent for the solubilization of CyA. The carrier, Neusilin® US2 (NEU) emerged as the most suitable one due to its large specific surface area, high flowable liquid retention potential and acceptable milling behaviour. Solubility studies were performed and further, LSS were prepared and evaluated for drug release in different biorelevant media of pH 1.6, 6.5 and 7.8, respectively, which confirmed Transcutol® HP (TRC-HP) as the most suitable solvent. By comparison of NEU-based LSS and co-milled formulations with functionalized calcium carbonate based ones, NEU exhibited better carrier properties regarding its efficiency in

loading higher concentration solutions of CyA as well as by co-milling, due to its high surface area and pore volume. Ultimately, the LSS demonstrated superiority to the co-milled formulations, as the release of CyA was significantly improved from LSS.

To develop a mucoadhesive matrix core for the adequate prolonged release of drug and increased residence time of the dosage form at the target site in the colon, several mucoadhesive polymers were characterized by measuring their rheological properties in different biorelevant media. Subsequently, the swelling and drug release of matrix tablets prepared from selected polymers were studied, using a model freely soluble drug, theophylline. The polymers showing promising controlled release properties, hydroxypropyl methyl cellulose (HPMC) and guar gum (GG) were selected and their mucoadhesion properties was determined by estimation of adhesion force needed to detach the tablet from the mucin layer. Finally, the influence of the LSS carrier, NEU on the swelling and mucoadhesive properties of the matrix systems incorporating each of the selected polymers above was investigated. However, the details of the NEU influence on mucoadhesion were not completely clarified in this thesis and a more detailed study is necessary in future.

In summary, the acceptable excipients for development of a suitable colon-targeted dosage form for improving CyA solubility were achieved. The formulation of LSS proves to be a better approach for improving CyA solubility and drug release over co-milling. The polymers with suitable swelling, drug release and mucoadhesion properties necessary for the matrix core with prolonged release were confirmed. However, the influence of the LSS carrier on the polymer matrix behaviour and drug release requires future studies.

2 Abstrakt

Univerzita Karlova, Farmaceutická Fakulta V Hradci Králové

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Název dizertační práce:	Vývoj liquisolid systémů s cíleným přívodem léčiva do kolonu

V lokální nebo systémové terapii chorob tlustého střeva mají systémy cílící do kolonu slibnou perspektivu. Úspěch však představuje výzvu díky fyziologickým bariérám trávicího traktu. Pokud zvýšení rozpustnosti léčiva předchází cílení, lze rozpouštění, jako krok limitující rychlost, obejít, urychlit vstřebání léčiva a zvýšit jeho perorální biodostupnost. Pro optimalizaci dávky a terapeutického efektu je kromě toho potřebné zadržet přípravek v místě cílení. Cílem této práce je proto vývoj systému pro doručení a cílení omezeně rozpustného léčiva cyklosporinu A (CyA) do kolonu.

V první části experimentu byly využity preformulační studie k dosažení zvýšené rozpustnosti a zrychleného uvolnění CyA pomocí tzv. liquisolid sytému (LSS) nebo přípravou interaktivních směsí společným mletím (komletím). To zahrnovalo výběr vhodného nosiče a hodnocení jeho vlastností při mletí, stejně jako výběr netěkavého rozpouštědla pro solubilizaci CyA. Nejvhodnější vlastnosti nosiče z pohledu velkého specifického povrchu, vysoké schopnosti zadržení kapaliny při zachování sypnosti a akceptovatelných vlastností při mletí vykázal Neusilin® US2 (NEU). Hodnocení rozpustnosti a uvolňování léčiva z připravených LSS do biorelevantních médií s hodnotou pH 1.6, 6.5 a 7.8 potvrdilo Transcutol® HP (TRC-HP) jako nejvhodnější rozpouštědlo. Z pohledu vyššího účinného zabudování koncentrovaného roztoku CyA a komletí byly díky velké ploše povrchu a objemu pórů prokázány lepší nosičové vlastnosti NEU i při porovnání LSS systémů a komletých směsí oproti

funkcionalizovanému uhličitanu vápenatému. Navíc bylo proti komletým směsím prokázáno významně zvýšené uvolňování CyA z LSS.

Při vývoji mukoadhezivní matrice pro prodloužené uvolňování léčiva a zvýšení kontaktního času lékové formy v místě cílení (kolonu) bylo charakterizováno několik mukoadhezivních polymerů pomocí měření reologických vlastností v biorelevantních mediích. Následně byly z vybraných polymerů připraveny matricové tablety a hodnoceno jejich bobtnání a uvolňování modelového dobře rozpustného léčiva theofylinu. Protože hypromelosa (HPMC) a guarová klovatina (GG) vykázaly slibný profil uvolňování, byly dále hodnoceny jejich mukoadhezivní vlastnosti pomocí síly potřebné k odtržení tablety do mucinové vrstvy. Na závěr byl studován vliv přídavku NEU jako nosiče pro LSS systém na bobtnání a mukoadhezivní vlastnosti matricového systému z obou zmíněných polymerů. Vliv NEU na mukoadhezi nebyl v této práci kompletně objasněn a bude třeba detailnější studie v budoucnu.

Lze shrnout, že byly nalezeny vhodné pomocné látky pro vývoj systému se zvýšenou rozpustností CyA cílícího do kolonu. Bylo potvrzeno, že příprava liquisolid systémů představuje lepší možnost pro zvýšení rozpustnosti a uvolňování CyA oproti komletí. Pro přípravu matrice s prodlouženým uvolňováním byly nalezeny polymery s vhodnými vlastnostmi ve vztahu k bobtnání, uvolňování léčiva a mukoadheze. Pochopitelně další studie jsou nezbytné pro objasnění vlivu nosiče pro LSS systémy na chování matric a uvolňování léčiva.

3 Aim of the study

The combination of liquisolid systems and colon-targeted dosage form with prolonged residence time in the colon holds a great potential for success. The aim of this work is to formulate and evaluate liquisolid systems that will potentially allow for colon-specific drug delivery after oral administration. The liquisolid system (LSS) will be developed to incorporate cyclosporine A (CyA), an anti-inflammatory model poorly soluble drug, in order to improve its solubility while reducing the dose and adverse effect after systemic absorption in future therapeutic use. The resulting formulation can be embedded in the mucoadhesive polymer matrix for controlled release and increased residence time of the dosage form at the target site in the colon. Such strategy for colon-targeted drug delivery is undertaken to gain an insight into the potential benefits to be expected from a combination of both approaches.

With the aim in mind, the specific objectives were divided into two parallel studies, first, to develop the efficient dosage system to improve solubility of CyA which would potentially be incorporated into a mucoadhesive matrix core, secondly, to develop a swellable and mucoadhesive matrix core for the colon targeting of CyA. In order to achieve these two aims, the preformulation/formulation studies will include the partial steps as follows:

1. The development of the efficient dosage system to improve solubility of CyA
   - The selection of the suitable excipients for the formulation of the liquisolid systems (LSS)
     - Selection of a drug carrier with a high loading capacity for improving CyA dissolution rate by preparation of LSS
     - Selection of a non-volatile solvent with the highest solubilization capacity for preparation of LSS
   - The selection of a suitable carrier for co-milling
   - The preparation of CyA formulations with improved solubility and dissolution rate of CyA (either by LSS preparation or co-milling) and their comparison in biorelevant media

2. The development of swellable and mucoadhesive matrix core for the colon targeting of CyA

- Selection of a retarding agent with acceptable viscosity
- The evaluation of and swelling, mucoadhesive behaviour and drug release behaviour of the selected polymers or their combination with a well soluble model drug
- The investigation of effect of the addition of the selected LSS carrier on the properties of matrix systems, particularly swelling and mucoadhesive properties

4 List of abbreviations

abbreviation/variable	unit	explanation
5-ASA		5-aminosalicylic acid
AER		Aeroperl® 300 Pharma
ANOVA		Analysis of variance
AOR	°	Angle of repose
API	°	Active pharmaceutical ingredient
BCS		Biopharmaceutics classification system
CCD		Central composite design
CI	%	Compressibility index
CM	-	Co-milled
CMC Na	-	Carboxymethyl cellulose sodium
CyA		Cyclosporine A
F	mN/mm^2	Mucoadhesion force
FaSSGF		Fasted state simulated gastric fluid
FaSSIF		Fasted state simulated intestinal fluid
FaSSCoF		Fasted state simulated colonic fluid
GG		Guar gum
GIT		Gastrointestinal tract
HEC	-	Hydroxyethyl cellulose
HR	-	Hausner ratio
HPC	-	Hydroxypropyl cellulose
HPMC	-	Hydroxypropyl methylcellulose
I-CAR	-	Iota carrageenan
IBD		Inflammatory bowel diseases
K	$Pa.s^n$	Consistency coefficient
L-CAR	-	Lambda carrageenan
LSS		Liquisolid systems
n		Power law index

NEU		Neusilin® US2
PEG		Polyethylene glycol
Phi (ϕ)	-	Flowable liquid retention potential
Ph. Eur.		European Pharmacopeia
PG		Propylene glycol
PM	-	Physical mixtures
SD		Standard deviation
SI	%	Swelling index
SSA	m^2/g m^2/kg	Specific surface area
SYL		Syloid® 244 FP
TH		Theophylline
TRC-HP		Transcutol® HP
V_0	mL	Bulk volume
XRPD		X-ray powder diffraction
η	Pa.s	Dynamic viscosity
x_{10}	µm	Particle size corresponding to 10 % cumulative frequency
x_{50}	µm	Mean particle size (median)
x_{90}	µm	Particle size corresponding to 90 % cumulative frequency
y	s^{-1}	Shear rate

5 Introduction

The therapeutic benefits of colon drug delivery have been acknowledged in recent decades, most notably as a means to enhance therapeutic outcomes while minimizing adverse effects in the treatment of inflammatory bowel diseases such as Crohn's disease and ulcerative colitis. This approach seeks to precisely deliver drugs to the colon, optimize drug efficacy, while reducing exposure to other parts of the gastrointestinal tract (GIT). However, development of colon-targeted drug delivery systems remains a big challenge in drug delivery research. This is attributable to the fact that the colon is situated in the distal part of the gastrointestinal tract. Therefore, the drug delivery system must overcome the pH changes and absorption from the walls of the small intestine and be delivered in sufficient quantities to the colon. Different formulation approaches have been investigated in literature, which leverage the GIT physiology, including pH, gastric transit time and other colon-specific triggers, such as pressure and microbiomes. In summary, one singular approach may not be highly reliable especially in diseased state.

One of the obstacles to colon drug delivery forming the hypothesis of this research is the low fluid volume in the colon, posing a challenge for the dissolution of poorly soluble drugs targeted to this region. Based on this premise, it was necessary to investigate an additional strategy within the context of colon-targeted drug delivery, which tackles the issue of low drug solubility and to integrate it with one of the well-established formulation approaches in the field of colon-targeted delivery.

Among the methods for improving the solubility and dissolution rate of poorly water soluble drugs, the preparation of liquisolid systems, where the drug is incorporated in its dissolved form [1], have gained considerable attention. The combination of LSS and colon drug delivery systems has great potential to improve the success of colon drug targeting. However, more research is required. Therefore, to cover the scope of this project, a brief review of colon-targeted drug delivery and the most common approaches therein is presented. Thereafter, the drug formulation approaches for poorly water-soluble drugs are also reviewed with special focus on the novel

preparation of liquisolid systems. Overall, the research work aimed to highlight the strategies for colon-targeted drug delivery, with a special consideration on the challenges for formulation and targeting of poorly water-soluble drugs and to gain an insight into the potential added value to be expected from a combination of methods.

6 Theoretical section

6.1 Colon-targeted drug delivery

Colon-targeted drug delivery has become an important area of research in the last few years as a promising approach for the local treatment of various colonic ailments, including inflammatory bowel diseases (IBD), colorectal cancer and irritable bowel syndrome [2,3]. By directly targeting the colon, the drug exposure to the upper GIT is reduced, thereby preventing metabolism by the gut walls or degradation by gastric enzymes and ensuring that a higher concentration of the administered drug reaches the desired site of action. Furthermore, if the drug will be directly available at the target site, lower doses might be sufficient and thus a reduction in the systemic side effects as well as an enhanced therapeutic outcome can be observed. [3–5]. In addition, with novel colon drug delivery systems it may be possible to deliver macromolecules (*e.g.*, peptides and proteins) which would otherwise be adversely affected by strong changes between the stomach and the small intestinal environment pH [6].

Colon targeting can be achieved via the oral route or by rectal administration. However, considering the patient compliance and acceptability, the rectal administration is not widely employed and is thus reserved as an alternative in supportive therapy and emergency situations [4]. On the other hand, rectal administration offers the advantage of avoiding harm to the GIT. However, rectally administered formulations, such as enemas or suppositories have difficulty of targeting specific sites in the colonic region, and mostly reach only the lower part of the colon [3,7].

Therefore, the oral route appears to be a more suitable alternative to achieve colon targeting as the dosage form can reach all parts of the ileocecal region and the colon and patient acceptance rate is higher [4,7] Nevertheless, limitations for their successful application stem from the fact that the colon is in the distal part of the gastrointestinal tract and the orally administered formulations must traverse the entire alimentary canal and withstand highly variable conditions in the GIT

environment, such as wide range of pH values, fluid volumes and gastric transit times, which are especially less predictable in diseased state [8] Another limitation of targeting the colonic region is the low amount of free water present in this part of the GIT [7,9] which could hamper the dissolution of poorly water-soluble drugs. With these challenges in mind, several approaches have been investigated to navigate the physiological variations and achieve drug delivery to the colon. These include prodrugs, time-dependent release systems, pH-dependent systems, microflora activated systems and matrix systems, as well as combinations of these approaches. The following chapter 5.2 attempts to summarise the fundamental principles of some of the widely used strategies and highlight their advantages and limitations.

6.2 Drug delivery systems and approaches for colon-targeted drug delivery

The design of colon-targeted dosage forms should consider variabilities in physiology in order to prevent suboptimal delivery. For example, knowledge of GIT pH is important for drug delivery, as it can affect the solubility of drugs. Hence a brief overview of the colon physiological pH is summarised (Table 1) to aid an understanding of how some of the drug delivery systems achieve specific target.

Table 1: Overview of GIT physiological pH ranges in healthy individuals [3,7,9–11].

GIT location		pH ranges
Stomach	Fasted state	0.4 – 4.0
	Fed state	2.0 – 4.5
Small intestine	Duodenum	5.0 – 7.0
	Jejunum	5.0 – 6.5
	Ileum	6.0 – 7.5
Colon	Cecum	5.5 – 7.0
	Ascending colon	5.7 – 6.9
	Transverse colon	5.8 – 7.4
	Descending colon	6.3 – 7.7

6.2.1 Prodrug approach

The prodrug approach entails administration of a pharmacologically inactive derivative of a parent drug molecule which undergoes *in vivo* biotransformation under colon-specific conditions to form the active drug [12]. The prodrug is coupled with the drug molecule by a moiety (*e.g.*, azo conjugates) which minimizes the absorption rate in the small intestine and facilitates its delivery to the colon, where it is converted spontaneously, or through enzymatic cleavage (by azoreductases) into lipophilic drug molecule available for absorption [13]. A specific example is the azo prodrug sulfasalazine which is used in the therapy of IBD [2]. Sulfasalazine consists of the anti-inflammatory drug 5-aminosalicylic acid (5-ASA), linked via an azobond

to sulfapyridine. Following oral administration, a negligible absorption occurs in the small intestine (approximately 12 %), but a substantial amount reaches the colon intact, where cleavage of the bond by azoreductases and release of 5-ASA and sulfapyridine occurs. Although sulfasalazine has been generally effective in IBD treatment, the high incidence of allergic reactions or other adverse events resulting from the sulfapyridine moiety limits its use in several patients [9].

6.2.2 pH-dependent release

In the pH-dependent systems, the GIT pH gradient serves as a triggering mechanism. The pH in the stomach is low and gradually increases going from the small to large intestine. Therefore, in targeting the colon, it is reasonable to coat the drug formulation with pH-sensitive polymers which are insoluble in acidic pH, but dissolve or swell at neutral or alkaline pH in the distal part of the small intestine or in the colon to release the drug [14,15].

Although it was previously accepted that the pH gradient of the GIT increases progressively from the stomach to the colon and would allow to achieve controlled drug release, it has however been shown that the pH increases up until the distal ileum, but then significantly decreases again in the colon to a range between 5.5 and 7.0 [3]. Moreover, in addition to the inter- and intra-individual variability, differences in observed pH values are even more pronounced in IBDs, where pH as low as 2.3 and 5.3 have been reported for ulcerative colitis [16] and Crohn's disease [17], respectively. Therefore, for a successful colon-targeting, the effect of specific diseases on the target site must be considered.

The commonly used pH-dependent polymers considered to coat the drug formulation are polymethacrylates, commercially available as Eudragits®. The different types employed in colon targeting dissolve at a relatively narrow pH range [7]. For example, Eudragit® L and Eudragit® S are soluble at pH 6 - 7 and pH 7 - 8, respectively. The study by Ibekwe et al. reported that Eudragit S lacked specificity in terms of the disintegration site [18], and therefore, it was proposed that its suitability will only be for enteric coating as opposed to colon-targeting. Nevertheless, both Eudragits L and S have been employed in the therapy of IBD for the delivery of the

anti-inflammatory drug mesalazine (5-ASA), to protect it from the stomach or small intestine, as it would otherwise be unstable or be absorbed [19]. Presently, the commercially available colon-targeted oral preparations of mesalazine coated with Eudragit® L or Eudragit® S are sold under the trade names Claversal®, Mesazal®, and Colitofalk®, or Asacol®, respectively. Nevertheless, it has been reported in some studies that Asacol® passed through the GIT without disintegrating [20]. This was attributed to rapid small intestinal transit time, implying that the dosage form was not subjected to a pH of 7 or higher for a long period. Hence the reason why different grades of Eudragits are combined [8,19].

6.2.3 Time-dependent release

Time-dependent release systems for colon-targeted drug delivery are formulated to resist the acidic stomach and release the active ingredient after a predetermined lag time corresponding to the transit time from the mouth to the terminal ileum [21]. One of such systems is the time-dependent formulation Pulsincap® [22]. This consists of a capsule, with an enteric coated half and a non-disintegrating half. The enteric coat dissolves upon entry into the small intestine to reveal a hydrogel plug, which consists of a crosslinked copolymer of polyethylene oxide and polyurethane. This plug, which functions as a stopper for the non-disintegrating part, undergoes a pH-independent swelling, and after a predetermined lag time, which is governed by the length of the hydrogel plug, it is swollen to the extent that it is ejected from the bottom half of the capsule thereby releasing the drug. In the study by Stevens et al. [23], formulations of Pulsincap® were prepared to deliver a dose of the drug, dofetilide following a 5 h delay. The results showed that the drug absorption from the Pulsincap® occured from all GIT sites, however, absorption was generally prolonged from distal sites. On the contrary, *in vivo* data showed a reduction in bioavailability of dofetilide from the Pulsincap®, in the colon, which indicates less efficient absorption than anticipated [23].

6.2.4 Microflora activated systems

The vast quantity of anaerobic bacteria inhabiting the colon has been extensively exploited as a trigger for colon-specific targeting. The approaches range from coating the drug with a biodegradable material (*e.g.*, azo polymer), to the preparation of matrix systems with bacterial degradable polysaccharides. The latter approach relies on the matrix swelling or degradation by the colonic microflora. Several polysaccharides and their derivatives have been studied as candidates for the development of colon-targeted drug delivery system including amylose, chitosan, chondroitin sulphate, dextran, guar gum and pectin [5,24]. However, no marketed products are yet available employing this approach.

In addition to the approaches highlighted above, other strategies including the combination of pH and microbial-triggered systems [18,25], pH and time-dependent systems [26,27], as well as some novel methods such as the use of multiparticulate drug delivery systems [28,29] have also been extensively investigated in literature with respect to for colon-targeted drug delivery. However, their detailed review is beyond the scope of this introduction. Interested readers are referred to [3,4,9,11].

6.3 Formulation of poorly water soluble drugs into colon-targeted drug delivery systems

Generally, a drug must be dissolved prior to absorption from the lumen of the GIT. In the more distal portions of the GIT, such as the colon, the limited water content represents a serious challenge. The dissolution of a drug is subject to highly viscous luminal contents, which can significantly impede its dissolution, particularly wherever drugs are poorly water-soluble [30]. Hence this chapter aims to review the existing technological strategies for improving the solubility and dissolution rate and highlight the most promising ones for colon-targeted delivery of poorly soluble drugs.

6.3.1 Improving dissolution rate of poorly water-soluble drugs

Due to the emerging trends of combinatorial chemistry and API design, many of the potentially new APIs are highly lipophilic and poorly water soluble [31]. Low aqueous solubility in relation to the dose that needs to be administered is one of the most challenging aspects for the development of a pharmaceutical dosage form. The low equilibrium solubility and slow dissolution rate lead to poor intestinal absorption and insufficient bioavailability. Based on this premise, the Biopharmaceutical Classification System (BCS), which classifies active pharmaceutical ingredients (APIs) according to their aqueous solubility and gastrointestinal permeability was proposed by Amidon et al. [32]. The BCS categorizes drug into 4 classes as: Class 1 (high solubility and high permeability), Class II (low solubility and high permeability), Class III (high solubility and low permeability) and class IV (low solubility and low permeability) (Figure 1). A vast majority of drugs on the market (more than 30 %), as well as the drug candidates in the product pipeline (up to 70 %) belong to the BCS class II or IV. This classification system has been relatively recently improved in order to include the influence of pH [33].

For the BCS class II drugs, the solubility and dissolution rate are the limiting factors for their oral bioavailability [34]. Therefore, they can benefit from formulation approaches for solubility and dissolution rate enhancement. Several of such methods

have been studied and are well documented in scientific literature. These include salt formation [35], amorphous solid dispersion [36,37], solubilization in surfactant micelles [38], lipid-based formulations [39,40], supersaturating systems [41], micronization [42] and co-milling [43,44]. The enhanced release is achieved by the increased the drug surface area, improved drug solubility, or due to having the API in its dissolved state.

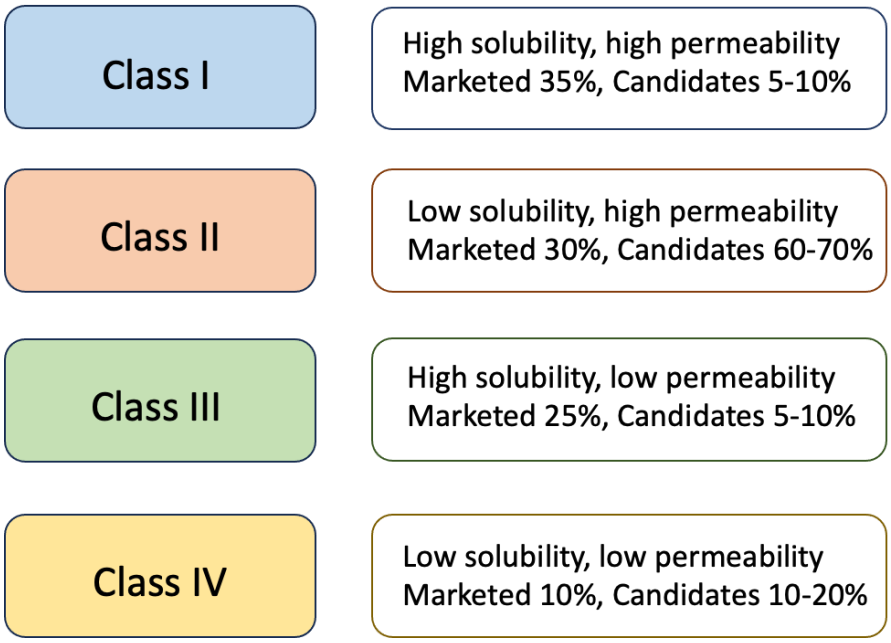

Figure 1: Schematic of Biopharmaceutical classification system. Modified from [45].

Although showing great promise in the laboratory settings, most of the above-mentioned methods are highly challenging to scale up to industry level or translate to clinical settings [46]. For instance, the amorphous solid dispersions face the underlying issues of physical instability, such as conversion of the API from amorphous to a crystalline form. This can be related to the thermal or mechanical stress introduced during the manufacturing process as well as to the storage conditions.

In summary, although more techniques have been introduced for the preparation, the potential for the industry is still limited by high energy consumption, high processing temperatures, and poor reproducibility of the process. [47].

Micronization represents a rather simple method for increasing the surface area. However, during the milling process, there is a tendency for the drug to aggregate as a result of their hydrophobicity and electrostatic forces, thus, reducing their available surface area [42]. On the other hand, co-milling which involves milling the poorly soluble drug with different pharmaceutical excipients, represents a more suitable approach. The excipient may aid in the amorphization of drug or improve the stability of amorphous form, minimize agglomeration, improve wetting, and ultimately improve the solubility of the milled drug particles [43,44,48,49].

Among the variety of technological processes, a novel promising approach to enhance the aqueous solubility and dissolution rate of poorly soluble drugs is by the preparation of liquisolid systems [50–52]. Consequently, liquisolid systems will now be reviewed in more details.

6.3.2 Liquisolid systems as a novel drug delivery technology

Liquisolid systems were first introduced by Spireas and Bolton [53]. The principle of liquisolid system entails dissolving or suspending of the poorly soluble API in a non-volatile solvent to form a liquid medication followed by its subsequent conversion into a free-flowing non-adherent and readily compressible powder by adsorbing on a carrier with high surface area. This admixture is suitable for filling into capsules or compression into tablets after mixing with other excipients [53–55].

The potential of LSS to improve the bioavailability of poorly soluble drugs has been investigated over the past decade. This relatively new technology holds key benefits over other approaches in the drug dissolution rate and bioavailability enhancement of poorly water soluble drugs. In several in vitro studies, incorporating a wide range of model drugs, dissolution profiles were significantly improved with LSS compared to the physical mixtures or directly compressed tablets of similar composition or some marketed products [51,56].

The key advantages stem from the cost-effective and simple preparation method which favours scale up to industrial production capacities. The manufacturing process is similar to conventional tablets and the excipients employed are readily accessible and available on the market [55].

Key steps in the preparation of liquisolid systems

The key steps in the preparation of LSS include dissolving or dispersing the drug in the required amount of a non-volatile solvent and incorporating the resulting blend into calculated amounts of carrier and coating materials. Therefore, the preparation consists of i) a selection of a suitable carrier with a high loading capacity, ii) a selection of an acceptable non-volatile solvent allowing the highest achievable concentration of an intended drug, iii) mixing/loading method; iv) a selection of a coating material to prepare a final solid dosage form with the suitable properties.

The carrier should be a porous powder material with high specific surface area (SSA). Its selection depends on liquid absorption capacity, flowability and compressibility [55,57]. The conventional carriers for the preparation of LSS has been microcrystalline cellulose, due to its availability, well-known acceptability, and stability in pharmaceutical products. Their application in LSS has been demonstrated in several studies[58,59]. However, in more recent times, newer carriers with significantly higher absorption capacity *e.g.,* anhydrous dibasic calcium phosphate (Fujicalin®) [60], amorphous magnesium aluminometasilicate (Neusilin® US2) [61] have become more popular. Moreover, due to their high surface area, much lower amounts compared to the conventional carriers will be required for the conversion of a drug in liquid state into dry, non-adherent and free-flowing powdered form.

The liquid portion in the LSS can be a liquid drug or a drug solution or suspension obtained in an appropriate solvent. The solvent is usually a water miscible, non-volatile solvent with high boiling point since the liquid portion remains loaded on the carrier, with no drying step involved and preferably not highly viscous, in order to facilitate loading into the pores [57,62]. Commonly used pharmaceutical solvents such as liquid polyethylene glycols, propylene glycols, glycerol, polysorbates are suitable in the preparation of LSS and have been employed as vehicles for the solubilization of several poorly soluble drugs [50,63–65]. The impact of the solvent on the drug release has been highlighted in several studies. Notably, the liquid

vehicle in which the drug is most soluble is usually selected for enhanced release [55,66,67].

The preparation of LSS is easy and can generally be done by simple physical mixing of these excipients using a mortar and pestle. The blending process is carried out in three steps. In the first step, the liquid (a solution/dispersion of a drug) is mixed with the powder at an approximate rate to homogenously distribute the liquid throughout the powder carrier. Then the liquisolid admixture is spread evenly as a uniform layer and left to stand and allow the liquid to be absorbed in the interior/pores of the powder particles. Finally, the liquisolid (LS) powder is mixed with other excipients needed to prepare the final dosage form. Schematically represented in Figure 2.

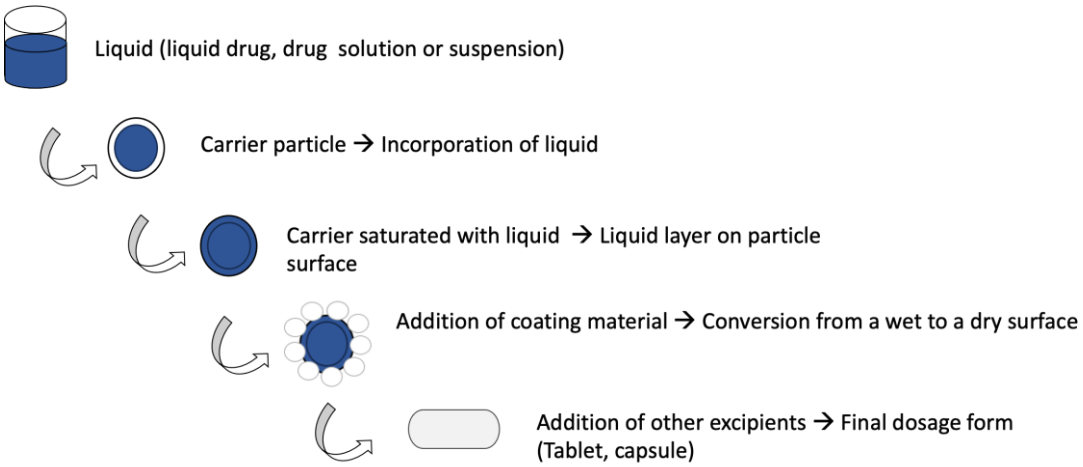

Figure 2: Schematic illustration of the preparation of liquisolid systems.

Some studies have also shown that the liquid drug could be sprayed into the carrier using fluid bed processor to obtain a homogenous distribution of the drug [54,59]. Following the incorporation of the liquid portion into the carrier material, the inner pores of the carrier become saturated with liquid and the excess will adsorb on the carrier surface and form a liquid layer. Such excess could decrease the flowability leading to poorly flowable powder. Therefore, the coating materials are added to cover the wet carrier particles and adsorb the excess liquid to ensure free flowability of the liquisolid powder mixture [53,55]. In LSS, the frequently used coating materials are fine particles (0.01 to 5 µm in diameter) with high SSA and very high

adsorption capacity but lack suitable flow or compression properties to be employed as carriers. Some notable examples include colloidal silicon dioxide (Aerosil® 200), Amorphous silica gel (Syloid® Sylisia ®), granulated silicon dioxide (Aeroperl®) [60,61]. Finally, the liquisolid powder is mixed with other excipients needed to prepare the final dosage form, tablets or capsules.

Based on the above-mentioned, the preformulation research for the preparation of LSS involve studies related to the selection of the suitable powder materials with the requisite flow properties and adequate sorption capacity for liquid loading as well as an appropriate non-volatile solvent for obtaining the drug in the liquid state.
Solubility studies are performed by the shake flask method. The excess of the drug is added to non-volatile solvents to prepare a saturated solution of the drug, which is shaken or stirred until equilibrium is reached. Subsequently, the amount of dissolved drug in a specific solvent is determined by analytical evaluation and the solvent demonstrating the highest drug solubilization capacity are then chosen for formulating LSS with enhanced drug release characteristics [59,66].

To optimize LSS preparation, a mathematical approach was proposed by Spireas and Bolton [53] to calculate required proportions of carrier and coating materials during formulation of LSS, which is based on the determination of the flowable and compressible liquid retention potentials, ϕ-value and Ψ-value, respectively.

The flow properties of powders are evaluated using the **angle of slide** as the main parameter. However, the estimation of this value for the carrier itself is the first step. The angle of slide is evaluated by placing a weighed amount of carrier on one end of a metal polished plate. Then this end is raised gradually until the plate makes an angle with the horizontal surface at which powder is about to slide [68]. According to Spireas et al. [69], this is regarded as the preferred method to determine the flow properties of powders with particles sizes below 150 μm and the value of 33° was referred to as the optimum flow behaviour. A known mass of the powder is then

mixed with varying amounts of the liquid vehicle and the angle of slide of the liquisolid (LS) mixture is evaluated repeatedly. The liquisolid mass ratio of the mixtures with an angle of slide of 33° is taken as the ϕ-value of the powder excipient.

The **flowable liquid retention potential** describes the ability of a powder material to retain a certain amount of liquid while maintaining acceptable flowability. The ϕ-value defines the maximum mass of liquid (m_{max}) that can be retained per unit mass of the powder material to obtain a free flowing liquisolid mixture. The parameters are described in the Equation 1 [50]:

$$\phi_{CA} = \frac{m_{max}}{Q} \; or \; \phi_{CO} = \frac{m_{max}}{q} \quad (1)$$

Where ϕ_{CA} or ϕ_{CO} represent the flowable liquid retention potential of the carrier and coating material, respectively; Q or q is the mass of carrier or mass of coating material.

In addition to the flowable and the compressible liquid retention potential characterizing the flow and compaction behaviour of powder excipients, other important parameters such as the liquid load factor i.e., the ratio of the mass of the drug in the liquid state to the mass of the carrier material is also determined prior to the formulation of LSS in order to accurately calculate the amount of carrier and coating material required in each formulation. Finally, the common powder flow properties including the angle of repose, compressibility index, Hausner ratio, flow through an orifice, are equally evaluated according to the relevant pharmacopoeia to ensure uniform dose and reproducible filling during the production of final dosage forms tablets or capsules.

A major limitation is often encountered in the formulation of high dose poorly soluble drugs which require a large amount of solvent to dissolve. This leads to the need for high amount of the carrier and coating material to absorb the drug solution and maintain a free-flowable material. Consequently, this results in increased tablet weight and the production of bigger tablets sizes which are difficult to swallow. To

overcome this, it is necessary to ensure the selection of non-volatile solvent with high solubilization capacity for the drug [50,70]. In addition, the use of carriers and coating materials with a large specific surface area and high absorption capacity can be employed. For instance, mesoporous magnesium aluminosilicate Neusilin® US2 (NEU), which has demonstrated a high liquid absorption capacity in several studies [68,71,72], may be a very effective carrier. Moreover, NEU has also been reported to act as a coating material in the LSS [60,66], and this will minimize the number of excipients required, thereby further simplifying the preparation of LSS.

6.3.2.1 Enhanced drug release from liquisolid systems

Several poorly soluble drugs have been formulated as LSS to improve their dissolution properties by employing different carriers, solvents and coating materials [50,51,56,59,71]. For example, Cirri et al. [71] compared the dissolution rate of glyburide (glibenclamide), an antidiabetic drug, from LS tablets with that from a commercial tablet (Gliboral®) and directly compressed tablets. The prepared compacts contained either Neusilin® US2 or Aeroperl® 300 as carrier with no coating material, while 2-pyrrolidone or N,N-dimethylacetamide were used as the solvents. The results of dissolution testing showed that the LSS formulations greatly improved the dissolution rate of glyburide, with 90 % of the drug being dissolved from the LSS in 60 min, while 40 % of the API was released from the reference formulations in the same time frame [71].

Several mechanisms have been postulated in literature to explain the enhanced drug release from the LSS as follows:

1. Increased drug surface area available for release

The drug dissolved in the liquid vehicle is situated in the powder carrier in a solubilized, molecularly dispersed state. Hence the surface area for release is much higher than in directly compressed tablets and this contributes to faster drug release [50,66].

2. Enhanced aqueous solubility of the drug

The solvent in the LSS diffuses out of a single liquisolid (LS) particle together with the drug molecules and might act as a co-solvent in the microenvironment at the interface between the LS powder particle and the dissolution medium, thereby increasing the aqueous solubility of the drug [50,66,73].

3. Improved wetting of the drug

The hydrophilic solvent acts either as a surface active agent or has a low surface tension and thus improves the wetting of the LS primary particles [52,57].

In addition to the above-mentioned mechanisms, the dissolved state of the drug in LSS allows to circumvent the most rate-limiting step during drug absorption in the GIT, and in turn may improve bioavailability. Recently, several poorly soluble drugs formulated as LSS have demonstrated improved bioavailability in *in vivo* studies [59,65,74,75].

6.3.2.2 Colon-targeting of poorly water soluble drugs by formulation of liquisolid systems

As previously mentioned, low free fluid volume in the colon is one of the limitations of dissolution and absorption of poorly soluble drugs. Thus, targeting the colon with LSS provides the ability to deliver active substance in already dissolved form, overcoming the rate-limiting step of dissolution which may result in a higher drug absorption and improved oral bioavailability.

There are several ways to prepare LSS with prolonged release. It has been even suggested that can zero-order release kinetics can be achieved from LSS simply by the addition of a matrix forming substance such as HPMC to the formulation of LSS [70]. The use of microbially degradable polysaccharides such as guar gum, pectin, chitosan or their combinations as carriers, may bring even greater benefits for the use in the colon environment. Moreover, the mentioned polymers possess mucoadhesive properties, which may help to prolong the residence time of the dosage form due to better contact with the colonic mucosa.

The approach of combining LSS with matrix tablets for colon targeting has been recently investigated in a few studies [1,76–78]. In one of the studies, the authors prepared liquisolid tablets for the delivery of indomethacin to the colon using a combination of guar gum, pectin and chitosan polysaccharides in varying amounts. The tablets were coated with Eudragit RL 100 to control how much of the drug is absorbed in the upper GIT. Based on the in vitro dissolution studies that the drug release in rat caecal contents was prolonged for up to 16 h by pectin, which is the duration of time that the solid dosage form spends in the colon [1].

Another study that has investigated the colonic drug delivery of LSS utilized a combination of three approaches to achieve the targeting of sulfasalazine to the colon. The prepared liquisolid drug delivery system comprised of pH-dependent polymer, Eudragit® S-100, to prevent the release of sulfasalazine in the gastric region and microbially triggered polymers, pectin and guar gum as core coating to retard the release until reaching the colon. A burst release of the drug was observed in colon upon the digestion of polysaccharide [76].

In summary it has been demonstrated that the combination of LSS with matrix tablet core may be advantageous in the preparation of colon-targeted drug delivery systems.

7 Experimental section

7.1 Materials

Mesoporous carriers

Neusilin® US2 (Fuji Chemical industries Co., Ltd, Japan)

Aeroperl® 300 Pharma (Evonik Industries AG, Germany)

Syloid® 244 FP (Grace GmbH & Co. KG, Germany)

Functionalized calcium carbonate (Omyapharm® 500-OG with average particle size 6.6 µm) (Omya International AG, Switzerland)

Non-volatile solvents

Polyethylene glycol 200 (Sigma-Aldrich Chemie GmbH, Germany)

Polyethylene glycol 400 (Sigma-Aldrich Chemie GmbH, Germany)

Propylene glycol (Dr. Kulich Pharma, Czech Republic)

Polysorbate 80 (Dr. Kulich Pharma, Czech Republic)

Transcutol® HP (Gattefossé, France)

Mucoadhesive release-modifying polymers

Hydroxypropylmethylcellulose - Methocel® K15M (Colorcon ltd. Dartford, UK)

Guar gum (Sigma-Aldrich Chemie GmbH, Germany)

Lambda carrageenan (Sigma-Aldrich Chemie GmbH, Germany)

Iota carrageenan (Sigma-Aldrich Chemie GmbH, Germany)

Carboxymethylcellulose sodium - Blanose™ CMC (Ashland, Netherlands)

Hydroxyethylcellulose - Natrosol™ HEC (Ashland, Netherlands)

Hydroxypropylcellulose - Klucel™ HPC (Ashland, Netherlands)

Mucoadhesion substrate

Mucin from porcine stomach - type III (Sigma-Aldrich Chemie GmbH, Germany)

Substances for buffer and biorelevant media

Hydrochloric acid (Penta chemicals s.r.o, Czech Republic),

Sodium hydroxide pellets (Penta chemicals s.r.o, Czech Republic),

Sodium chloride (Dr. Kulich Pharma, Czech Republic)

Sodium phosphate monobasic dihydrate (Dr. Kulich Pharma, Czech Republic)

Trizma® base (Sigma-Aldrich Chemie GmbH, Germany)

Maleic acid (Sigma-Aldrich Chemie GmbH, Germany)

FaSSIF/FeSSIF/FaSSGF powder for preparation of Fasted State Simulated gastric fluid (FaSSGF), Fasted State Simulated intestinal fluid (FaSSIF) (biorelevant.com Ltd, United Kingdom)

Fasted State Simulated Colon fluid (FaSSCoF) powder for biorelevant media (biorelevant.com Ltd, United Kingdom)

Other solvents

Acetonitrile - HPLC grade (Sigma-Aldrich, Czech Republic)

Methanol - HPLC grade (Sigma-Aldrich, Czech Republic)

Ethanol absolute (Penta chemicals s.r.o, Czech Republic)

Purified water (Faculty of Pharmacy, Hradec Kralove, Czech Republic)

7.2 Methods

7.2.1 Evaluation of flow properties of carriers and liquisolid mixtures

The raw carriers Neusilin® US2 (NEU), Aeroperl® 300 Pharma (AER) or Syloid® 244 FP (SYL) were first characterized alone by evaluation of angle of slide and flow properties (described below), then the liquisolid mixtures for determination of flowable liquid retention potential were prepared by blending of each carrier with solvent Polyethylene glycol 200 (PEG 200). 50 g of NEU or 25 g of AER or SYL was used. The powder was added to a mortar, followed by a dropwise addition of the required starting amount of PEG 200 (20 g for NEU and 0.5 g for AER and SYL, respectively). The samples were blended using a pestle and sieved through 1mm

mesh size (NEU and AER) or 800µm mesh size (SYL) and subsequently homogenized in a cube mixer KB 20 S attached to a motor drive (Type AR 401, Erweka GmbH, Germany) for 10 min at 17 revolutions per minute, to ensure a homogenous distribution of solvent throughout the whole volume of carrier.

After the evaluation, an additional amount in liquid was introduced to the mixture, followed again by mixing, sieving and homogenization as above. The amount of the liquid added was based on the value of angle of slide obtained. When the value of angle of slide of 33^{o} (considered as an optimum [79]), was reached, at least 2 further additions of solvent were made. The experiment was terminated when there was a consecutive increase in the angle of slide in addition to simultaneous worsening of other flow parameters.

7.2.1.1 Angle of slide

The angle of slide (°) was evaluated using the equipment in Figure 3 and according to the method by Vraníková et al. [68]. 10 g of each powder excipient (NEU, AER or SYL) or their mixture with increasing amount of non-volatile solvent was placed at the end of a polished metal plate and then raised gradually until the plate formed with the horizontal surface an angle at which the powder was about to slide. The measurements were repeated five times, and the results are presented as mean values and standard deviation (SD).

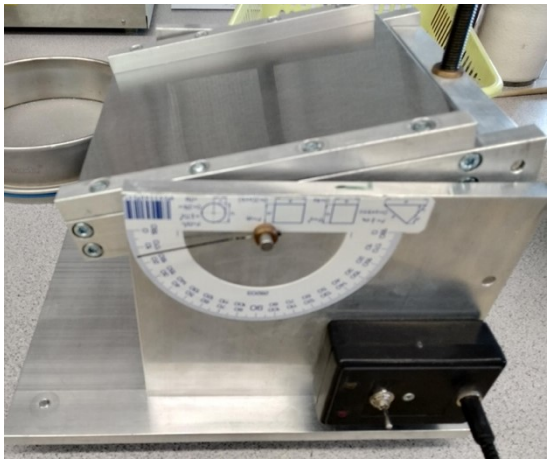

Figure 3: Equipment for angle of slide measurement.

7.2.1.2 Flow through orifice

Flow through orifice was determined on an automated powder and granulate flow tester (GTB, ERWEKA GmBH, Germany) using a 200 mL stainless-steel conical hopper coupled with a suitable orifice (10, 15 or 25 mm diameter). The smallest size of orifice diameter through which the powder was flowable was used for the measurement. The sample (25 g) was carefully filled into the hopper and the time in seconds taken for the entire material to flow out of the hopper though the orifice was recorded with a stopwatch. Each measurement was repeated five times and the mean flow rate and SD were calculated.

7.2.1.3 Angle of repose

The angle (relative to the horizontal base) formed by a cone-like pile of the powder material was measured on the automated powder and granulate flow tester (GTB, ERWEKA GmBH, Germany). 25 g of the sample was measured using a 200 mL stainless-steel conical hopper coupled with an orifice (10 or 15 mm diameter). The measurement was repeated five times and the mean angle of repose (AOR, °) and SD were calculated.

7.2.1.4 Bulk and tapped density

A known mass of powder (20 g) was gradually filled into a 100 mL graduated cylinder avoiding compaction of the powder. The unsettled apparent volume V_0 (i.e., the bulk volume) was recorded. The graduated measuring cylinder was fixed to the base of the tapped density tester (SVM 102, ERWEKA GmBH, Germany). After 10, 500 and 1250 taps were carried out, the corresponding volumes V_{10}, V_{500} and V_{1250} were recorded. The compressibility index (CI) and Hausner ratio (HR) were calculated from the bulk and tapped densities using in agreement with the European Pharmacopeia (Ph. Eur.) 11.0, 2.9.36 Powder flow.

7.2.1.5 Determination of flowable liquid retention potential (ϕ-value)

The flowable liquid retention potential (ϕ-value) of the liquisolid mixtures prepared with varying amounts of PEG 200, and evaluated according to the above methods was determined by Equation 1 (above). The liquid to carrier ratio for the mixtures with an angle of slide closest to 33° was taken as the ϕ-value of each carrier.

7.2.2 Granulometric properties and milling behaviour of carriers

7.2.2.1 Particle size measurement

The particle size distribution of powder excipients was determined based on Mie theory of static light scattering using Mastersizer 3000 (Malvern Instruments Ltd., United Kingdom). An adequate amount of dry sample (sieved through a 500 µm sieve) was filled into the Aero S unit and measured at an air pressure of 2.0 bar and a feed rate of 50 % to control the sample throughput and dispersion. A particle refractive index of 1.50 was used for all substances. The values representing the particle sizes for 10 %, 50 % and 90 % of the cumulative distribution curve (x_{10} µm, x_{50} µm and x_{90} µm) and the specific surface area SSA (m^2/kg) were recorded. *Span*, a parameter characterising the width of the volume particle size distribution was determined according to the Equation 2:

$$\frac{x_{90}-x_{10}}{x_{50}} \qquad (2)$$

7.2.2.2 Preliminary milling of carriers

The powder carriers NEU, AER or SYL were milled for 15 min using a planetary ball mill (PM 100, Retsch, Germany) at 300 rpm. 25 mL stainless steel milling vessels were filled with 100 pieces of 5 mm stainless steel milling balls. Then each powder sample was added to fill approximately two thirds of the vessel volume. 2 g of powder was used for NEU and AER, while for SYL which was voluminous, only 0.5 g of powder was filled to the vessel.

7.2.3 Preparation and evaluation of Cyclosporine A formulations

7.2.3.1 Solubility studies of Cyclosporine A in non-volatile solvents

The solubility of Cyclosporine A (CyA) was determined in five different non-volatile solvents: PEG 200, polyethylene glycol 400 (PEG 400), propylene glycol (PG), polysorbate 80 (PS80), and Transcutol® HP (TRC-HP). To achieve saturation, excess CyA was added to a vial containing 3g of the specified solvent. The vials were sealed and then left stirring (Cimarec™ Multipoint 15, Thermo Scientific™, USA) at laboratory temperature (22.7 ± 0.3° C) for 24 h. The excess undissolved solid was separated by centrifugation at 15000 rpm for 60 min. Aliquots of the supernatant were withdrawn (in duplicates) and diluted with mobile phase to an appropriate concentration for analysis (determined in preliminary studies). The diluted samples were filtered through polytetrafluorethylene syringe filters (pore size: 0.2 µm) and analysed by a validated HPLC method (see chapter 7.2.3.9).

7.2.3.2 Preparation of Liquisolid systems

A prescribed quantity of CyA was dissolved or suspended in non-volatile solvent by stirring for 24 hours at ambient conditions. The resulting dispersion was added dropwise to the weighed amount of powder in a mortar and thoroughly mixed with a pestle until the mortar content started to resemble a dry homogeneous powder to obtain liquisolid systems (LSS). The final mixtures were subsequently sieved (mesh size 630 µm) and left to stand in closed vials for at least 24 hours to ensure the distribution of the drug solution throughout the whole volume of carriers. LSS of CyA were prepared twice in this study as follows:

a. <u>Preparation of LSS with different non-volatile solvents:</u>
   The drug dispersions (20 % w/w) were obtained in five non-volatile solvents PEG 200, PEG 400, PG, PS 80 and TRC-HP and NEU was used as a carrier. Similarly, physical mixtures (PM) of CyA were prepared for comparison, by blending drug (without solvent) and carrier briefly in a mortar, and sieving

(630 µm). The prepared LSS and PM were subsequently filled into capsules prior to dissolution analysis. The code LSS C was assigned to distinguish LSS samples filled into capsules from the subsequent ones (Table 2).

b. Preparation of LSS with two different carriers:

The drug solution (50 % w/w) was obtained using TRC-HP as the non-volatile solvent, and either NEU or functionalized calcium carbonate (FCC) as carriers. The drug loading in the LSS was varied only according to quantity of the carrier, such that at various drug loading, the amount of drug in the carrier was always 25 mg. The preparation of LSS 50 with 50 % loading was not successful for FCC as a dry powder could not be obtained. The final samples were coded based on the processing method, the weight percentage of the drug content and the respective carrier, *e.g.*, LSS15 NEU/FCC (Table 2). Note that for the LSS, the amount of TRC-HP was not included for the expression of the drug loading.

7.2.3.3 Preparation of co-milled formulations

To prepare the powder mixtures for co-milling, 500 mg of abovementioned sample containing CyA at varying concentrations (15, 25 and 50 % w/w) with either NEU or FCC, respectively, was placed in a 25 mL milling jar with two stainless steel balls (Ø 12 mm) and milled for 2 min at a frequency of 30 Hz in an oscillatory ball mill (Mixer Mill MM400, Retsch GmbH & Co., Germany), kept in a cold room (5°C). Similarly, 500 mg of CyA, NEU, or FCC were also milled. The samples were stored in screw cap glass vials at ambient conditions until further analysis.

The final samples were coded based on the processing method, the weight percentage of the drug content and the respective carrier, *e.g.*, CM15 NEU/FCC.

Table 2: Composition of Physical mixtures (PM) of cyclosporine A (CyA) and the liquisolid systems (LSS) and co-milled mixtures (CM) based on Neusilin® US2 (NEU) or functionalized calcium carbonate (FCC).

Code	CyA		Carrier	Solvent (mg)
	(%)	(mg)	(mg)	
CyA	100	25	0	0
PM[a]	20	25	100	0
LSS C[a]	12.5	25	75	100
LSS 50[b]	50	25	25	25
LSS 25[b]	25	25	75	25
LSS 15[b]	15	25	142	25
CM 50[b]	50	50	50	0
CM 25[b]	25	25	75	0
CM 15[b]	15	15	85	0

Note: [a]NEU was used as carrier. [b]either NEU or FCC was used as a carrier. LSS C and PM were filled into capsules.

7.2.3.4 Storage conditions for stability studies

The milled drug CyA, and the LSS and CM mixtures were stored in open vials in desiccators at 25 ± 2 °C for 20 weeks. Storage humidity conditions of 75% RH were maintained using a saturated solution of NaCl. Samples were analysed by XRDP at 0 days and 20 weeks to monitor possible changes in the drug state.

7.2.3.5 Specific surface area and pore properties

Specific surface area (SSA) was determined by the standard BET (Brunauer, Emmett, Teller) method for the raw and milled NEU and FCC using a Gemini VII. 2390 (Micromeritics Instrument Corporation, Norcross, USA) at a relative pressure p/p_0 in the range of 0.05–0.33. The samples (approximately 100 mg) were heated to 150 °C before measurements to remove any residual moisture and kept for 24 h under nitrogen atmosphere using FlowPrep 060 (Micromeritics Instrument

Corporation, Norcross, USA). BJH (Barrett, Joyner, and Halenda) adsorption and BJH desorption methods were employed for the determination of the pore volume and pore size. The measurement of all samples was performed three times; mean values and SD were calculated.

7.2.3.6 Scanning electron microscopy (SEM)

Samples were placed onto a carbon conductive tape, and a layer of gold approximately 10-nm-thick was sputter-coated as a conductive layer. The morphology and surface characteristics of the samples were determined using scanning electron microscope (Phenom Pro, Phenom-World B.V., The Netherlands) with the Back-scattered Electron Detector (BSE) at the accelerating voltage of 5 kV or using a Hitachi TM3030 Tabletop scanning electron microscope (SEM, Hitachi High-Technologies Corporation, Tokyo, Japan) at an accelerating voltage of 15 kV (only for the milled NEU and FCC samples).

7.2.3.7 X-ray powder diffraction (XRPD)

This an analytical method identifies crystallinity of a material by the diffraction patterns. This study was carried out at the University of Copenhagen, Demark. The spectra of samples were investigated using X'Pert PANalytical PRO X-ray diffractometer (PANalytical B.V., The Netherlands). Cu Kα radiation ($\lambda = 1.54187$ Å) was generated using a 45 kV acceleration voltage and current of 40 mA. Samples were scanned in reflectance mode in 2θ range from 5 - 35° with a scan rate of 0.067° /sec and a step size of 0.026°. The data were collected and analysed using the software X'Pert Data Collector (version 2.2.4) (PANalytical B.V., The Netherlands).

7.2.3.8 Study of drug release behaviour of CyA formulations

In vitro dissolution studies were conducted in biorelevant media for CyA physical mixtures and the different formulations (LSS and CM). The experiments were performed using a USP2 (paddle method) in dissolution apparatus (Sotax AT 7 Smart, Sotax AG, Switzerland) coupled with an automatic sampling system. The

paddles speed and temperature were set at 100 rpm and temperature 37 ± 0.5°C, respectively. (Details are summarised in Table 4).

a. For the evaluation of drug release from the physical mixtures and CyA LSS prepared using different non-volatile solvents (method 7.2.3.2a), the amount of powder samples corresponding to 25 mg of drug was filled into two hard gelatin capsules (size 00). Sinkers were used to avoid floating of the capsules and their dissolution was monitored in 900 mL biorelevant media FaSSGF, FaSSIF and FaSSCoF. Samples (5 mL) were withdrawn by the automatic sampling system (followed by media replacement) for pre-determined time intervals (0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12, 16, 20 and 24 h). The concentration of CyA was analysed using the validated HPLC method (see chapter 7.2.3.9). Results are presented as mean values and SD of six determinations.

b. For the comparison of drug release from CyA LSS (method 7.2.3.2b) and co-milled formulations (method 7.2.3.3), prepared using different carriers, powder dissolution study was performed using biorelevant media FaSSIF (pH 6.5). First, the powder samples corresponding to 25 mg of the model drug CyA was introduced into the dissolution vessels, followed by the addition of 500 mL of media pre-warmed to 37 ± 0.5°C in a water bath. Samples collection (5 mL) and media replacement was done by an automatic sampling system at pre-determined time intervals (5, 10, 15, 20, 30, 40, 50, 60, 90, 120, 180 and 240 min). The concentration of CyA was analysed using the validated HPLC method (see section 7.2.3.9). Results are presented as mean values and SD of four determinations.

7.2.3.9 HPLC analysis

The chromatographic analysis was carried out using a Nexera X2 UHPLC system (Shimadzu Corporation, Kyoto, Japan). The system comprised of two pumps (LC-30AD), a degasser (DGU-20A5R), an autosampler (SIL-30AC), a column oven (CTO-20AC), a UV/VIS detector (SPD-M30A), a communication bus module (CBM-20A), and computer software (LabSolution). Chromatographic separation was

achieved at a temperature of 60°C with a Kinetex™ C18 2.1 × 50 mm, 1.7 µm column coupled with a pre-column (Security guard ULTRA Cartridges, UHPLC C18, 2.1 mm) (Phenomenex, CA, USA). The mobile phases were: A - ultrapure water adjusted to pH 2.4 with 85% phosphoric acid, filtered before measurement using Millipore system glass fiber filter (pore size 0.22 µm) and B - a mixture of acetonitrile and methanol in a ratio of 10:90 (v/v). A linear gradient elution was performed at 0.5 mL/min starting at 70% B, then increasing to 100% B over 3.5 min and subsequently returning to 70% B at 3.51 min for column re-equilibration, which was completed at 6 min. CyA was detected at a wavelength of 210 nm using a UV/VIS detector.

7.2.4 Rheological characterization of polymers

Dispersions of all tested polymers carboxymethylcellulose sodium (CMC Na), hydroxypropyl methylcellulose (HPMC) K15M, hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC), guar gum (GG), iota carrageenan (I-CAR) and lambda carrageenan (L-CAR), were prepared by adding 1.0 g of polymer into varying volumes (10 – 50 mL) of biorelevant dissolution media FaSSGF, FaSSIF and FaSSCoF, respectively, to simulate gel layer formed around matrix tablet upon hydration in the gastrointestinal tract. Subsequently, the dispersions comprising of a mixture of two polymers (HPMC and GG) was also tested in varying media volumes (12.5 – 50 mL). The ratio of mixtures corresponded to five polymer concentrations in a range of 0 – 100 % based on the proposed experimental design (see Chapter 8.2.3). The viscosities of the resulting dispersions were determined using a Kinexus Pro+ rotational rheometer (Malvern Instruments Ltd., United Kingdom) equipped with a cone-plate upper geometry (CP 2/20 - 2^{o} angle, 20mm diameter). Flow curves were obtained at the defined temperature of 37 ± 0.5 °C using a shear rate sweep, with shear rates increasing logarithmically from 0.01 to 100 s^{-1}. Approximately 0.5 mL of sample was placed on the lower geometry. After the upper geometry made contact with the sample, the sample excess was carefully trimmed off using a spatula. A sample cover with a solvent trap was used to minimize sample drying.

The rheological profiles were analysed in Kinexus software (version 1.75). Apparent viscosities at a shear rate of $1s^{-1}$ were estimated from the flow curves using the (Ostwald de Waele) power law equation:

$$\eta = Ky^{n-1} \quad (3)$$

Where η is the shear viscosity (Pa.s), K is the consistency coefficient ($Pa.s^n$), numerically equals to viscosity measured at the shear rate of 1 s^{-1}, y is the shear rate (s^{-1}) and n is the power law or flow behaviour index (dimensionless), which takes the values < 1, 1 or >1 for pseudoplastic/ shear thinning, Newtonian materials or dilatant systems, respectively. All samples were analysed at least three times; the mean values and SD were calculated.

7.2.5 Preparation and evaluation of mucoadhesive matrix tablets

Three groups of matrix tablets were prepared in this work. In case the tablets contained the model drug theophylline (TH), the drug made up 50 % of the entire powder mixture.

- **Group 1** was composed of a mixture of theophylline and a raw polymer; either carboxymethyl cellulose sodium (CMC Na), hydroxyethyl cellulose (HEC), hydroxypropylmethyl cellulose (HPMC) K15M, guar gum (GG) or iota-carrageenan (I-CAR).
- **Group 2** contained theophylline in equal ratio with either a polymer GG or HPMC, respectively, or their mixture.
- **Group 3** comprised of either a polymer GG or HPMC, or a carrier NEU, or a polymer in combination with NEU.

The 3 groups are schematically represented in Figure 4 below and the actual composition of the matrix tablets is shown in Table 3.

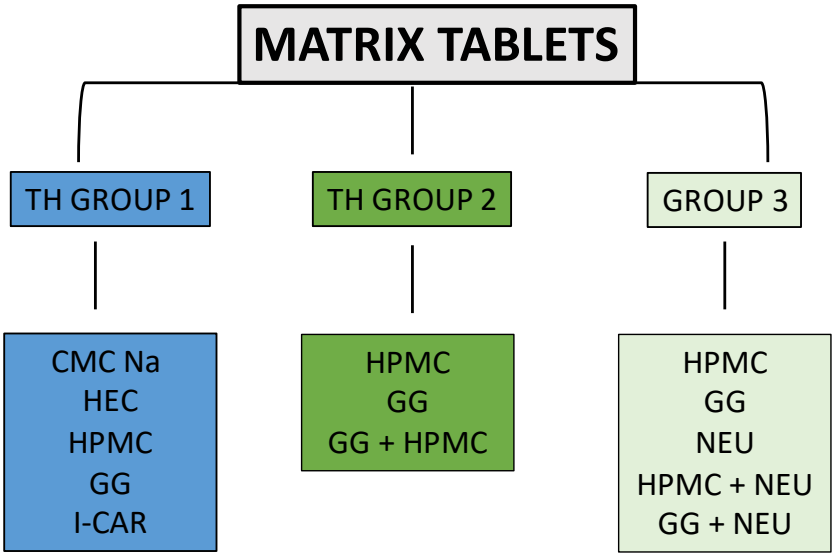

Figure 4: Schematic illustration of the different groups of matrix tablets.

The powders were weighed, sieved (mesh size 400 µm) and subsequently mixed in a three-axial homogenizer (T2F, TURBULA System Schatz, Switzerland) for 10 min at 34 rpm. Matrix tablets weighing 200.0 ± 1.0 mg were compressed using a 7mm diameter stainless steel die and punch assembly (Adamus HT, Adamus HT, Machine Factor Group, Poland) on a material testing machine (Zwick/Roell T1-FRO 50, Zwick GmbH, Germany) at a compression rate of 0.5 $mm \cdot s^{-1}$. The compression pressure was adjusted in a range from 1 to 10 kN for each mixture to obtain tablets with hardness in the range of 60 to 80 N (hardness tester 8M, Dr. Schleuniger Pharmatron AG, Switzerland). The prepared tablets were kept in a polyethylene bag for 24 h before testing. The composition of the prepared tablets is shown in (Table 3).

Table 3: Composition of the different groups of matrix tablets prepared with polymers and or Neusilin® US2 (NEU).

Group	Theophylline (mg)	Polymer 1 (mg)	Polymer 2 (mg)	NEU (mg)
1	100	100	0	0
2	100	0	100	0
	100	14.6	85.4	0
	100	50	50	0
	100	85.4	14.6	0
	100	100	0	0
3	0	0	0	100
	0	14.6	0	85.4
	0	50	0	50
	0	85.4	0	14.6
	0	100	0	0

7.2.5.1 Evaluation of swelling behaviour of matrix tablets

The swelling index (SI) of theophylline matrix tablets containing different polymers was evaluated according to a previously described method with slight modifications [80]. Each tablet was accurately weighed (HR-120, A&D Company, Japan) and placed in a pre-weighed stainless steel mesh dissolution basket. The entire set (tablet and basket) was immersed in a beaker containing 75 g of biorelevant media FaSSGF, FaSSIF and FaSSCoF pre-warmed at 37 ± 0.5 °C in a water bath. The basket containing the swollen tablet was removed from the medium at the specified time intervals (15, 30, 60, 120, 240, and 480 min), dried carefully with an absorbent tissue paper (to remove excess liquid) and weighed. The percent increase in tablet weight due to uptake of liquid was calculated using equation:

$$SI = 100\,\frac{Wt - W0}{W0} \qquad (4)$$

Where SI is the swelling index (%), w_o and w_t represent the initial weight of the tablet and the weight of the tablet at the determined time interval, respectively. Measurements were done six times for each tablet and the mean and SD were calculated.

7.2.5.2 Characterization of mucoadhesive properties

To evaluate a potential interaction between the matrix tablets and mucin, mucoadhesive studies were performed by two *in vitro* methods.

The force required to break the adhesive bonds between the matrix tablets and the mucin layer (mucoadhesive force) was evaluated using a using a modified two-arm physical balance (Figure 5), similar to that described by Gajdziok et al. [80]. In this method, the right pan was replaced with an accessory that serves as a tablet holder and a lower height adjustable platform to hold the mucoadhesive substrate (mucin). The tablet was attached to the lower side of the tablet holder using a double-sided adhesive and the balance was adjusted. 50 µL of 2 % (w/w) mucin dispersion in biorelevant medium, FaSSGF (pH 1.6), FaSSIF (pH 6.5) or FaSSCoF (pH 7.8) was placed on the platform and then the tablet was brought into contact with the mucin-covered layer. After a contact time of 5 min, the weights were gradually added to the left pan until the tablet was separated from the mucin layer. The mucoadhesive force per unit area was calculated according to the equation:

$$F = \frac{m.g}{A} \qquad (5)$$

where F is the mucoadhesive force (mN/mm^2), m is weight added in grams to the left pan, g is the acceleration due to gravity (m/s^2), A is the surface area (38.48 mm^2) of the tablet. Measurements were repeated five times for each tablet and mean and SD were calculated.

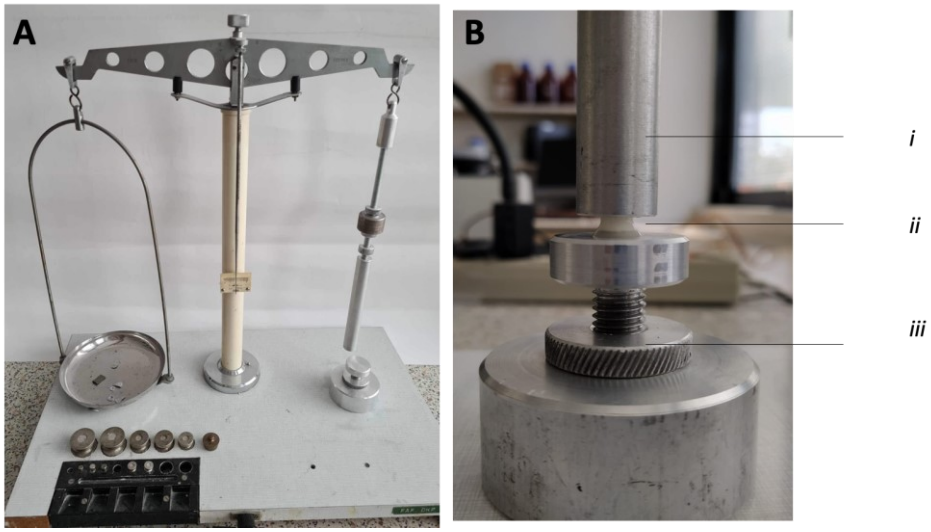

Figure 5: Apparatus for evaluation of mucoadhesion strength. A - modified physical balance. B - Enlarged image of the modified right arm showing an active measurement: *i* – tablet holder, *ii* – mucoadhesive tablet adhered on a mucin layer, and *iii* – height-adjustable platform.

The evaluation of the **mucoadhesion residence time** of the tablets was performed in a modified USP disintegration tester (ZT 301, Erweka GmbH, Germany), following a previously described method, with slight adjustments [81]. The test medium was composed of 900 g of biorelevant medium (FaSSGF, FaSSIF or FaSSCoF), maintained at 37.0 ± 0.5 °C. A plastic slab was fixed to the arm of the disintegration tester in a horizontal position. The 50 µL of 2 % (w/w) dispersion of mucin in biorelevant media was applied on the slab. Subsequently, the tablet was brought into contact with the mucin-coated layer for 30 s. After establishing contact, the arm was fixed vertically to the apparatus and allowed to move up and down in the media. The time for detachment or erosion of the tablet from the mucin layer was monitored four times for each tablet and the mean and SD were calculated.

7.2.5.3 Study of drug release behaviour of matrix tablets

The experiments for the evaluation of theophylline in vitro drug release from the different groups of matrix tablets were conducted in biorelevant media using a USP2 (paddle method) in dissolution apparatus (Sotax AT 7 Smart, Sotax AG, Switzerland)

(Table 4). The tablets were placed in sinkers to avoid sticking to the bottom of the vessel and the drug release was monitored in 900 mL biorelevant media, FaSSGF, FaSSIF and FaSSCoF. The samples were withdrawn by the automatic sampling system (followed by media replacement) for pre-determined time intervals (0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12, 16, 20 and 24 h) and the drug concentration was analysed using a UV-spectrophotometer, Specord 205 (Analytic Jena AG, Germany) at 272 nm. Results are presented as mean values and SD of six determinations.

Table 4: Review of the conditions for in vitro dissolution testing performed in 7.2.3.8 and 7.2.5.3. The apparatus USP2 with autosampler (Sotax AT 7 Smart, Sotax AG, Switzerland), paddle speed of 100 rpm and temperature 37 ± 0.5°C, respectively, were used.

	[a]LSS C [b]PM	[a]LSS [c]CM	Matrix tablets
Media	FaSSGF FaSSIF FaSSCoF	FaSSIF	FaSSGF FaSSIF FaSSCoF
Media volume (mL)	900	500	900
Dosage form	Capsules	Powder	Tablets
Sampling (h)	24	4	24
Analysis	HPLC	HPLC	Spectrophotometry
Replicates	6	4	6

Note: [a]liquisolid systems. [b]Physical mixtures. [c]co-milled mixtures.

7.2.6 Mathematical and statistical evaluation

The dissolution data were subjected to a single factor analysis of variance (ANOVA) using the Data Analysis add-in in Microsoft® Excel for Mac Version 16.77.1 (Microsoft Corporation, WA, United States). $P < 0.05$ was considered as statistically significant.

8 Results and discussion

Drug targeting to the colon can be beneficial in the therapy of inflammatory bowel diseases (IBD) such as ulcerative colitis and Crohn's disease, as well as for drugs which would otherwise be adversely affected in the stomach and the small intestinal environment [4,6]. However, the drug dissolution in this region is often limited by the low free fluid volume in the colon [15]. Therefore, it is necessary to employ strategies which improve the drug solubility and dissolution rate of poorly water soluble drugs in order to maximize the extent of their absorption and overall bioavailability. The formulation of LSS and the preparation of interactive powder mixtures represent some of the potentially suitable strategies out of the possibilities presented in the theoretical section (Chapter 6).

Another challenge associated with colon targeting is the variable residence time of a drug delivery system in GIT, which is especially less predictable in diseased states. For example, in IBD patients, there is higher gastric motility and shorter colonic transit time, due to the diarrhoea associated with the disease [3]. Therefore, it is necessary to modify a drug delivery system for prolonged contact residence time in the colon to avoid premature elimination of the dosage form prior to drug release.

In view of the main target to achieve the colon-specific drug delivery system releasing an efficient amount of cyclosporine A (CyA) in the colon tissue during a specific time, and based on the above-mentioned premises, some of the partial aims were to improve solubility and dissolution rate of CyA, a model poorly soluble drug, and to prolong the system's residence time in the colon by using mucoadhesive polymers. For this reason, the structure of the thesis distinguishes two main steps of the experimental work, which are described in more detail in separate subchapters. Firstly, the choice of a suitable excipients for development of cyclosporine A dosage form with improved solubility (Chapter 8.1); second, development of a mucoadhesive matrix core. (Chapter 8.2).

8.1 Development of the dosage system to improve solubility of CyA

One of the important steps in achieving a well-designed colon drug delivery system is the selection of appropriate excipients to be potentially employed to improve the solubility and dissolution rate of the poorly water-soluble model drug CyA and those that will facilitate its localised and prolonged release in the colon. This includes first, a finding a carrier with an acceptable capacity for loading an efficient amount of drug regardless of its physical state, either in liquid state in LSS or in solid state for co-milling., In the LSS, the selection is mainly based on evaluation of the optimum liquid retention potential of carrier while the suitable milling behaviour is observed to prevent agglomeration in case of co-milling. Second, to find the best solvent for the improvement of CyA solubility for loading in LSS. Hence, the emphasis of this subchapter was placed on several pre-formulation studies performed in relation to this.

8.1.1 Selection of suitable drug carriers

As stated above, different strategies are used for improving solubility such as preparation of LSS and/or interactive mixtures. Both methods employ the carrier materials that play a vital role in incorporating and stabilizing the drug in the formulation. For example, in the formulation of LSS, the carrier is important for obtaining the dry form of the powder from drug in the liquid state, i.e., the drug solution or dispersion in a suitable non-volatile solvent [55]. Appropriate materials for LSS are usually porous carriers with high specific surface area and liquid absorption capacity. On the other hand, in the preparation of interactive mixtures by co-milling, carriers help to minimize agglomeration of a micronized drug and due to the spreading onto the surface of carrier particles improve its wettability and dissolution rate by a more efficient contact with the dissolution medium [48,82].

Therefore, to guide in the selection of suitable carriers for further investigation in relation to improving the drug solubility for colon-targeted delivery, several silica-

based powder excipients were characterized by their flow properties, particle size, specific surface area and milling behaviour.

8.1.1.1 Evaluation of flowable liquid retention potential

The carriers for preparation of LSS are selected based mainly on the specific surface area (SSA), porosity, liquid adsorption capacity, flowability and compressibility [83]. Considering these parameters, there are several substances on the market that can potentially be employed, including magnesium aluminometasilicates, anhydrous dibasic calcium phosphate, mesoporous silica, etc. [84,85]. In this study, three commercially available, high surface area materials considered as potential adsorbents for the preparation of LSS namely Neusilin® US2 (NEU), Aeroperl® 300 (AER), and Syloid® 244 FP (SYL), were investigated as potential carrier materials for the formulations containing cyclosporine A (CyA) as a model drug. NEU is an amorphous highly porous magnesium aluminometasilicate prepared by spray drying. It possesses extremely large specific surface area (>300 m^2/g), high porosity and high absorption/adsorption capacity [55,84]. Neusilin is available in two forms varying in the particle size. NEU selected for this study is a granulated form with the mean particle size of 106 μm [86]. AER is a high purity colloidal silicon dioxide prepared also by the granulation process. According to manufacturer's specifications, it possesses mean particle size of 20 – 60 μm and high SSA of up to 320 m^2/g [87]. Finally, SYL represents a micronized synthetic amorphous colloidal silica material with mean particle size of 3.5 μm [88] and SSA of 358.73 ± 3.26 m^2/g [89].

The preformulation studies for the selection of the carriers for LSS involve the evaluation of the flowable liquid retention potential (ϕ-value), which is the maximum mass of liquid that can be retained per unit mass of the powder while maintaining acceptable flow properties, determined by the angle of slide, flow rate and angle of repose (AOR). Among them, the angle of slide is designated in the scientific literature [56,69] as the main parameter, the value 33° is considered as optimal and is chosen for the calculation of ϕ- value. Other parameters including flow rate, AOR, compressibility index (CI) and Hausner ratio HR are taken into consideration in case

of various mixtures containing different amount of solvent showing a similar value of angle of slide [68].

To select the most suitable carrier for the preparation of LSS, the flowable liquid retention potential of three above-mentioned carrier materials NEU, AER and SYL was investigated. These materials have been already employed in various LSS as carrier and/or coating materials [61,71,90]. The ϕ- value for the carrier NEU was evaluated in a previous study by Vraníková et al. [69]. The results indicated that NEU could retain 1.0 g of the added solvent (propylene glycol) while maintaining acceptable flow properties. While the other tested carriers, Neusilin® UFL2, Neusilin® NS2N, Aerosil® 200, Fujicalin® and Avicel® PH 101 retained 0.97, 0.54, 0.04, 0.25 and 0.12 g, respectively. In another study published by the same authors, NEU demonstrated a high absorption capacity for three different solvents, with 1 g of the powder absorbing up to 1.48 g of polyethylene glycol 200 (PEG 200). However, the flowable liquid retention potential has not yet been evaluated under the same conditions for the carriers deliberated in this doctoral thesis. Considering the previously established highest retention capacity of NEU for the solvent PEG 200 compared to the other solvents tested [68], PEG 200 was chosen for the current evaluation to facilitate a direct comparison between carriers.

As can be seen in Table 5, the results for raw NEU, AER and SYL showed a higher value of angle of slide than the recommended value of 33° for all the carriers. The highest value (53.60 ± 0.37°) was recorded for AER, while NEU and SYL have a value of angle of slide approximately 42°. On the other hand, AER implied faster flow rate and lower AOR than NEU; whereas it was not possible to determine these parameters for SYL, as it did not pass through the orifice, indicating its poor flow character. Although a direct comparison of the carrier flow rates cannot be made because of the different diameter of a hopper orifice used for each measurement, the poor flow character of SYL was confirmed by CI and HR evaluation. The determined AOR of NEU 38.54 ± 0.13° and that of AER 35.74 ±1.42° indicated "fair" flow properties, in contrast to the manufacturers' specification of 30° and 24°,

respectively [86,91]. This considerable difference between these values can be due to several factors, such as the different instrumentation that was used for the determination. The CI and HR of NEU and AER corresponded to “good” and “fair” flow properties (Ph. Eur. 11.0, 2.9.36 Powder flow)

Table 5: Flow properties of raw carriers.

Sample	Angle of slide (°)	Flow rate (s/25g)*	AOR (°)*	CI (%)	HR
NEU	42.70 ± 1.60	1.79 ± 0.27	38.54 ± 0.13	12.60	1.14
AER	53.60 ± 0.37	1.69 ± 0.25	35.74 ± 1.42	16.00	1.19
SYL	42.80 ± 0.68	–	–	18.28	1.22

*For NEU, 25 mm orifice was used for flow rate and 15 mm for AOR measurements, respectively. 10 mm orifice was used for AER and 15 mm orifice was used for SYL for both flow rate and AOR measurements.

The flowable liquid retention potential was estimated by mixing a powder carrier with increasing amounts of PEG 200 and measuring the flow properties, using angle of slide. When the value of angle of slide of 33° [79] was reached, at least 2 further additions of solvent were made, and the flow properties were evaluated. Finally, **ϕ-**value was calculated as mass of solvent divided by mass of carrier (Equation above). The results are summarized in Tables 6 – 8; the optimum value is highlighted in bold script.

ϕ-value of Neusilin® US2

Based on knowledge from the literature [68], that 1 g of NEU could absorb more than 1 g of PEG 200, a higher starting amount of solvent (20 g) was used. The amount of liquid added depended on the angle of slide, when the angle did not decrease after making several 1g increments, the subsequent added amount of

solvent increased by 2 g. When the value was approaching 33°, the amount of PEG added was again adjusted. After the first addition of 20g of PEG 200 to 50 g of NEU (Figure 6), the angle of slide decreased to 38.0 ± 1.6°. This signifies improved flow behaviour and may be explained by the lubricating effect of PEG [92]. After adding a total of 53 g of PEG 200, a value of angle of slide of 33.4 ± 0.6° close to the recommended value of 33° was obtained.

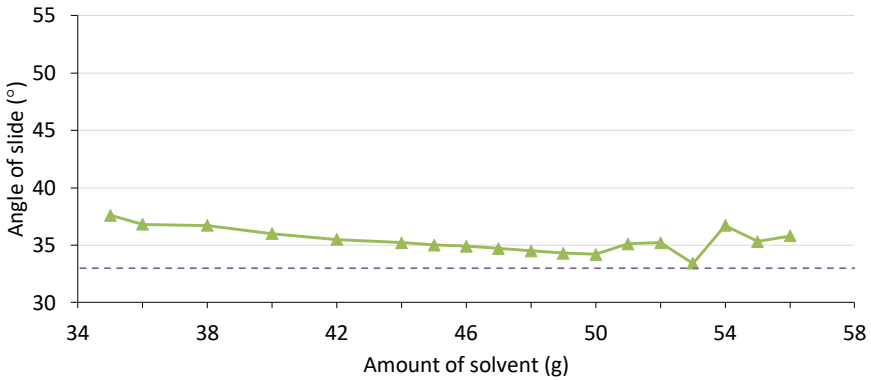

Figure 6: The average values of angle of slide of 50.0 g of Neusilin® US2 (NEU). 33 degree line indicates required optimal value of angle of slide. Note that the starting amount of solvent displayed on the x-axis was adjusted for better readability.

The flow rate and the AOR value both increased after 35 g of solvent was added to 2.83 ± 0.30 s/25 g and 42.18 ± 0.83°, respectively (Table 6) showing poorer flow properties of the liquisolid (LS) powder compared to NEU itself. A similar trend was also detected for values of CI and HR. This can be explained by the saturation of the powder particles with the solvent and the increased moisture content in the sample which results in higher cohesion of the particles to the walls of the hopper. This negative effect of moisture on flow properties is well known and has already been described, for example, in the study of Hou et al. [93]. In general, the flow properties did not show a direct relationship with the amount of solvent added.

Table 6: Flow properties of Neusilin® US2 mixtures loaded with PEG 200.

Mass of solvent (g)	Flow rate (s/25g)*	AOR (°)*	CI (%)	HR
Rows omitted for brevity				
35	2.83 ± 0.30	42.18 ± 0.83	20.04	1.25
36	2.66 ± 0.18	41.60 ± 1.00	20.91	1.26
38	2.66 ± 0.08	42.22 ± 1.01	24.43	1.32
40	2.15 ± 0.15	41.38 ± 1.44	21.05	1.27
42	2.43 ± 0.27	41.54 ± 1.14	22.56	1.29
44	2.09 ± 0.20	40.92 ± 1.14	22.14	1.28
45	2.31 ± 0.18	41.28 ± 1.47	22.08	1.28
46	1.98 ± 0.17	42.12 ± 0.88	20.59	1.26
47	1.98 ± 0.21	42.44 ± 0.86	21.58	1.28
48	1.97 ± 0.22	41.40 ± 1.61	20.30	1.25
49	1.90 ± 0.17	41.02 ± 2.19	20.78	1.26
50	1.81 ± 0.22	41.14 ± 1.32	18.07	1.22
51	1.83 ± 0.11	40.28 ± 1.79	21.19	1.27
52	1.92 ± 0.21	41.78 ± 1.77	21.73	1.28
53	**1.95 ± 0.29**	**42.26 ± 1.39**	**19.97**	**1.25**
54	1.96 ± 0.20	42.26 ± 0.86	16.90	1.20
55	2.25 ± 0.40	42.64 ± 1.05	14.79	1.17
56	1.90 ± 0.20	42.96 ± 0.39	13.64	1.16

*Orifice of diameter 25 mm was used for flow rate and 15 mm for AOR, respectively. Note that the starting mass of solvent displayed on the table was adjusted for better readability.

ϕ-value of Aeroperl® 300

The evaluation of angle of slide revealed that the addition of solvent to 25.0 g of AER decreased the values of angle of slide from the initial value of 53.60 ± 0.37° to 40.9 ± 0.92° after reaching 9 g increment in the amount of PEG 200. This is probably

due to the lubricating effect of PEG described above. However, further addition (above 9 g) led to a continued increase in the angle of slide up to 50.70 ± 1.18° observed for mixtures containing 11.5 g of PEG 200 (Figure 7). This observation may be attributed to oversaturation of the LS powder with the solvent as discussed above.

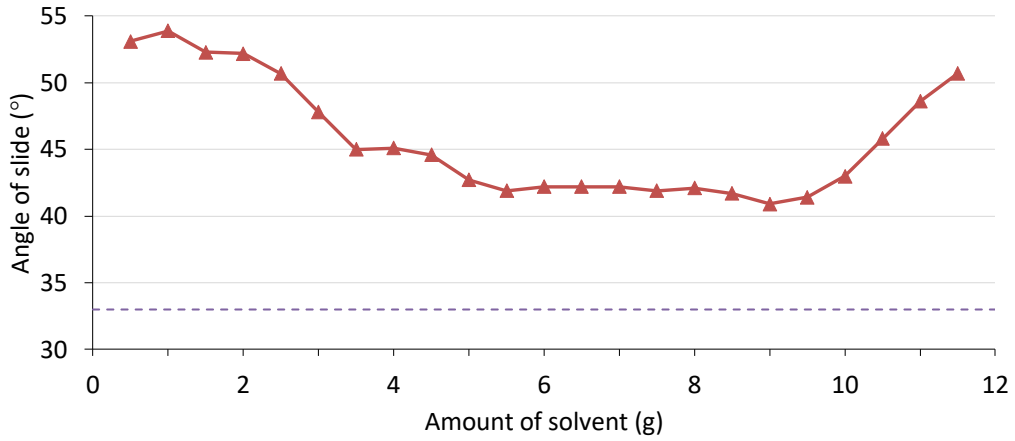

Figure 7: The average values of angle of slide of 25.0 g of Aeroperl® 300 (AER). The dashed line indicates required optimal value of angle of slide 33°.

The flow rate was initially improved after the addition of a small amount of solvent (Table 7) probably due to the increasing mass of the individual particles of AER and the filling of its pores with PEG 200. A similar phenomenon was described by Vraníková et al. for NEU loaded with PEG 400 [68]. However, above 7.5 g of solvent, the value did not improve further, rather a substantial deterioration of the measured parameter was observed for the last two mixtures containing 10.0 and 10.5 g of PEG 200 (18.80 ± 1.41 and 22.98 ± 1.17 s/25 g, respectively. This may also be due to the greater interparticle forces resulting from the higher liquid content in the carrier and an increase in the attractive forces between the particles, similar to the previous observation in NEU LS mixtures.

Table 7: Flow properties of Aeroperl® 300 mixtures loaded with PEG 200

Mass of solvent (g)	Flow rate (s/25g)*	AOR (°)*	CI (%)	HR
0.5	1.54 ± 0.22	28.84 ± 1.88	23.3	1.30
1.0	1.47 ± 0.18	27.30 ± 1.41	24.71	1.33
1.5	1.48 ± 0.09	29.98 ± 1.39	24.42	1.32
2.0	1.33 ± 0.15	33.86 ± 1.18	24.40	1.32
2.5	1.26 ± 0.12	34.08 ± 0.70	24.24	1.32
3.0	1.21 ± 0.09	35.58 ± 2.56	24.54	1.33
3.5	1.24 ± 0.06	35.06 ± 1.87	23.42	1.31
4.0	1.32 ± 0.11	35.22 ± 2.24	23.87	1.31
4.5	1.43 ± 0.15	36.34 ± 1.03	24.84	1.33
5.0	1.48 ± 0.22	33.70 ± 0.37	24.00	1.32
5.5	1.33 ± 0.03	36.36 ± 0.15	22.07	1.28
6.0	1.22 ± 0.20	35.64 ± 0.45	20.86	1.26
6.5	1.27 ± 0.11	34.52 ± 0.83	21.01	1.27
7.0	1.39 ± 0.12	35.10 ± 0.73	22.22	1.29
7.5	1.15 ± 0.13	34.94 ± 0.30	22.90	1.30
8.0	1.20 ± 0.06	34.84 ± 0.45	22.48	1.29
8.5	1.24 ± 0.06	35.72 ± 0.88	22.40	1.29
9.0	**1.34 ± 0.19**	**33.94 ± 0.39**	**24.39**	**1.32**
9.5	1.34 ± 0.22	34.38 ± 1.28	23.77	1.31
10.0	18.80 ± 1.41	34.20 ± 0.57	23.14	1.30
10.5	22.98 ± 1.17	35.12 ± 0.54	23.33	1.30
11.0	–	–	24.17	1.32
11.5	–	–	24.58	1.33

*Orifice of diameter 15 mm was used for flow rate and 10 mm for AOR, respectively

Even the addition of only 1 g of PEG 200 led to a decrease in the measured AOR value from 35.74 ± 1.42° to 27.30 ± 1.41°, which was the lowest value recorded for AER (Table 7) and signifies excellent flow properties. Although the value of AOR

decreased in general due to addition of solvent, no direct correlation was established between the amount of solvent added and the measured parameters. The CI and HR of all AER LS mixtures with PEG 200 were higher than for the raw substance, implying slightly more cohesion in the particles after liquid absorption. The lowest values of both parameters, 20.86% and 1.26, respectively were observed for the sample containing 6 g of PEG 200 signifying "passable" flow properties (Ph. Eur. 11.0, 2.9.36 Powder flow).

ϕ-value of Syloid® 244 FP

Following the gradual addition of PEG 200 to 25.0 g of SYL, the angle of slide did not reach the desired value of 33°. During the measurement, no dependence between the amount of added solvent and the value of the angle of slide was established. The first solvent additions decreased the values to 36.60 ± 0.37° while subsequent solvent addition led to a fluctuation in the values of the determined angle, until after adding up to 16 g the solvent, the parameter again increased to 42.10 ± 0.37° (Figure 8).

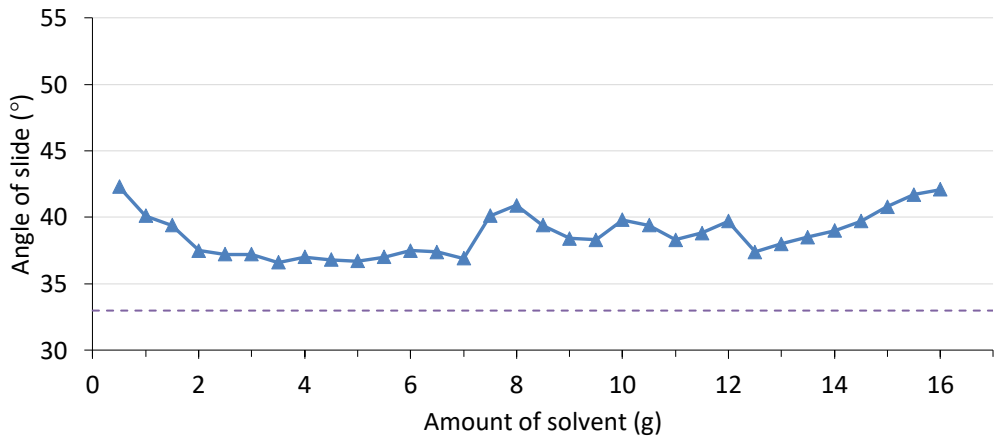

Figure 8: The average values of angle of slide of 25.0 g Syloid® 244 FP (SYL). The dashed line indicates required optimal value of angle of slide 33°.

The flow rate of SYL was improved upon addition of PEG 200, as can be seen from the measured values of 22.34 ± 0.50 s/25 g (Table 8). In general, a continuous

improvement in the flowability was observed and the mixture containing the highest amount of PEG 200 (16 g) showed the best flowability of 4.73 ±0.24 s/25 g. This improvement can be attributed to the filling of carrier irregularities by the adsorbed liquid [68] as well as the increase in the weight of the individual particles resulting in a faster flow through the orifice which was also recorded for NEU and AER mixtures discussed above (Table 7 and 8).

The results in Table 8 indicate that the AOR was improved after the addition of solvent to SYL as it was possible to measure this parameter for the LS powder mixtures. However, following initial addition of 0.5 g solvent, the AOR 46.58 ± 1.02° obtained still indicate poor floor character. The lowest AOR value of 37.54 ± 0.96° (fair flow properties) was detected in samples containing 16 g of solvent. Similar to the case of AER above, it is also possible to attribute the improvement of the flow properties to the filling of the spaces on the surface of SYL by the adsorbed liquid [68]. The measured values of CI and HR (Table 8) showed no relationship with the amount of PEG 200. The values ranged from 13.51 % to 22.22 % and from 1.16 to 1.29, respectively for CI and HR, indicating “good” to “passable” flow properties of the mixture according to the pharmacopeial scale of flowability (Ph. Eur. 11.0, 2.9.36 Powder flow).

Table 8: Flow properties of Syloid® 244 FP mixtures loaded with PEG 200.

Mass of solvent (g)	Flow rate (s/25g)*	AOR (°)*	CI (%)	HR
0.5	22.34 ± 0.50	46.58 ± 1.02	17.20	1.21
1.0	13.42 ± 0.63	44.86 ± 0.37	15.12	1.18
1.5	14.25 ± 0.80	42.32 ± 0.98	15.58	1.18
2.0	13.14 ± 1.00	41.96 ± 0.79	17.61	1.21
2.5	13.70 ± 0.37	42.22 ± 0.42	17.39	1.21
3.0	11.75 ± 0.44	42.70 ± 1.14	15.87	1.19
3.5	**11.66 ± 0.15**	**41.82 ± 0.58**	**13.56**	**1.16**
4.0	11.41 ± 0.30	42.48 ± 0.80	13.51	1.16
4.5	11.43 ± 0.31	41.14 ± 0.67	16.67	1.20
5.0	11.47 ± 0.16	41.72 ± 1.04	16.00	1.19
5.5	10.69 ± 0.21	42.70 ± 0.60	15.22	1.18
6.0	10.87 ± 0.36	42.82 ± 0.13	19.78	1.25
6.5	10.63 ± 0.57	43.08 ± 0.67	18.39	1.23
7.0	10.54 ± 0.44	43.22 ± 0.66	19.77	1.25
7.5	10.05 ± 0.36	43.30 ± 0.37	18.29	1.22
	Rows omitted for brevity			
14.0	5.84 ± 0.23	40.84 ± 0.44	18.18	1.22
14.5	5.33 ± 0.19	39.48 ± 0.27	20.37	1.26
15.0	5.54 ± 0.29	38.96 ± 0.88	18.87	1.23
15.5	5.04 ± 0.07	38.66 ± 0.94	19.61	1.24
16.0	4.73 ± 0.24	37.54 ± 0.96	18.00	1.22

*15 mm orifice was used for both flow rate and AOR measurements. Note that some rows were omitted for better readability.

The average values of angle of slide reflecting the added amount of PEG 200 are illustrated in Figure 6 – Figure 8. In the relevant Tables 6 – 8, the optimum amount of the solvent added is highlighted. It is visible that NEU reached a value of angle of slide 33.4°, close enough to optimum value of angle of slide of 33°, for 53 g of PEG

200; this value was selected for calculation of the ϕ-value. For the other mixtures, the ϕ-value was calculated using the lowest angle of slide obtained for each one, while also considering the other flow parameters as discussed above. To calculate ϕ-value of AER, the mixture containing 9 g PEG 200 was chosen. At this point, the flow rate, AOR, CI and HR values were acceptable signifying "good" and "passable" flow character, respectively. The LS mixture containing 3.5 g of solvent was selected for the calculation of ϕ-value for SYL; the additional parameters of flow rate and AOR were within acceptable flow characters as well as CI and HR values.

The obtained results of ϕ-value are shown in Figure 9. The ϕ-values of NEU, AER and SYL for PEG 200 were 1.06, 0.36 and 0.14, respectively. It implies that 1 g of NEU, AER, or SYL could retain 1.06, 0.36, or 0.14 g of PEG 200 while maintaining acceptable flow properties.

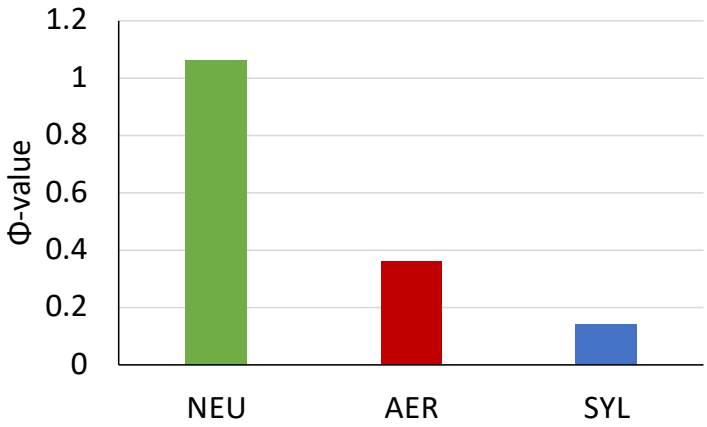

Figure 9: The flowable liquid retention potential (ϕ-value) of Neusilin® US2 (NEU) Aeroperl® 300 (AER) and Syloid® 244 FP (SYL) for PEG 200.

Based on the results, AER and SYL showed a lower flowable retention potential, which is indicative of their lower absorption capacity compared to that of NEU. The highest capacity (retention potential) is expected in view of future loading of a drug solution and to achieve its suitable therapeutically active concentration Consequently, NEU was selected as the best carrier for the further preparation of LSS.

8.1.1.2 Granulometric characterization and milling behaviour

The preparation of interactive powder mixtures using a co-milling of the drug with carrier material represents a suitable method of improving the dissolution rate of active substances. However, many of the pharmaceutical excipients show unacceptable properties during milling, such as the stickiness, which reduces the milling efficiency, or particle agglomeration, which may reduce the surface area available for adsorption of a drug [94,95]. Both finally reduce the contact between the drug and the dissolution medium and decrease the dissolution rate. Therefore, such excipients cannot be considered for the utilization as carrier material in interactive mixtures prepared by co-milling.

Therefore, the previously mentioned silica carriers NEU, AER, and SYL were also assessed to observe their suitability for co-milling by evaluation of their particle size, particle size distribution and specific surface area before and after milling. The results shown in Table 9 highlight their granulometric properties.

Table 9: Granulometric characteristics of raw powder excipients used in preliminary milling study. The code M15 shows the characteristics of milled material [82].

	x_{10} (µm)	x_{50} (µm)	x_{90} (µm)	span	*SSA (m^2/kg)
NEU	7.6	76.8	180.0	2.25	222.6
NEU M15	1.6	12.0	74.7	6.08	860.7
AER	4.2	25.0	55.7	2.06	374.5
AER M15	1.6	7.9	29.8	3.60	978.9
SYL	1.3	2.8	5.6	1.53	1675.0
SYL M15	1.1	3.9	31.8	7.89	1583.0

*SSA values were recorded from Mastersizer.

The median particle sizes of raw NEU, AER and SYL were 76.8 µm, 25.0 µm, and 2.8 µm, respectively, indicating the largest particles in NEU and the smallest in SYL.

This observation is consistent with the information provided by the manufacturers [84,91,96]. Smaller particles of SYL may result in poorer flow properties [89]. This was also confirmed from the results presented above, where SYL showed worse properties than NEU and AER. Apart from the particle size, the parameter conveying the width of the particle size distribution (span) is important; a lower value usually signifies the narrower distribution [97]. The calculated values were similar for NEU (2.25) and AER (2.06) but lower for SYL (1.53). The relatively lower value for SYL indicates a more uniform particle size than in other carriers.

SSA is related to the carrier's capacity to adsorb/absorb the drug on its surface and in open pores [98]. Based on the manufacturers' specifications and scientific literature, the 3 evaluated carriers are within the same range of SSA (more than 300 m^2/g by BET method) [78,82,92]. In this study, the SSA values obtained for NEU, AER and SYL were 222.6 m^2/kg, 374.5 m^2/kg, and 1675.0 m^2/kg, respectively (Table 9). It is important to note here that the reported values of SSA were obtained from the equipment assuming that the materials were non-porous, spherical powders. Nevertheless, these values were used to compare the carriers for their milling behaviour.

Generally, a decrease in x_{50} and an increase in SSA is expected after milling due to the energy input. When the substances were milled with 100 pieces of 5-mm milling balls, at 300 rpm for 15 min in the preliminary study, the particle size x_{50} was evaluated again. The behaviour of the substances is highlighted in different colours in Figure 10. Based on the results, a particle size reduction without any aggregation or agglomeration, illustrated in green script, was detected for AER. On the other hand, some level of agglomeration/aggregation after milling was detected for NEU coloured in yellow. Finally, the red script used for SYL shows even an increase in x_{50}.

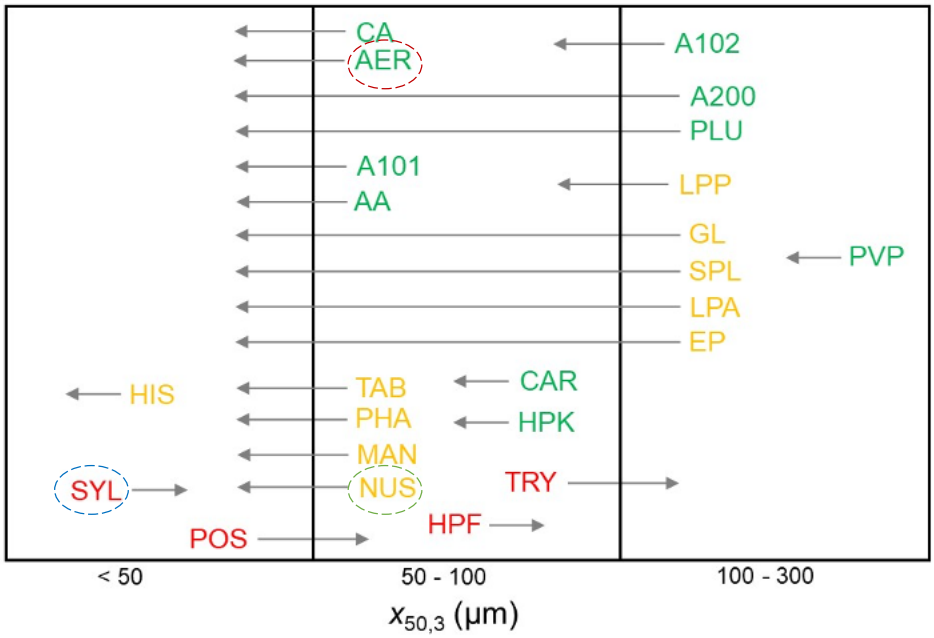

Figure 10: Schematic chart of particle size x_{50} change after preliminary milling under constant conditions [82]. The carriers of interest are encircled for easier identification. Note: NEU = NUS and $x_{50} = x_{50.3}$ in Figure 10.

As summarized in Table 9, the SSA was greatly enlarged by 2.6 and 3.9 folds for both AER and NEU, showing values of 978.9 m^2/kg and 860.7 m^2/kg, respectively after milling. In contrast, the measured value of SSA 1583.0 m^2/kg for SYL indicates decrease in SSA probably due to the particle agglomeration. Milling also resulted in increased particle size distribution, indicated by span of 3.60, 6.08 and 7.89, for AER, NEU and SYL, respectively due to a higher difference between x_{10}, and x_{90}

Both a decrease in particle size and an increase in SSA is expected after milling as these parameters are beneficial for a faster drug dissolution because the larger surface of the carrier is available for the attraction of drug particles [49]. In conclusion, AER showed the best milling properties between these three silica substances. Also, NEU was relatively easy to mill, with some level of particle size reduction, which is promising for further use as a carrier in co-milled mixtures. Finally, SYL showed not acceptable milling properties as well as low absorption capacity and therefore it is not further considered.

In conclusion, NEU remains the better choice considering the absorption capacity. In order to compare the LSS formulations with interactive powder mixtures prepared by co-milling as well as considering other properties, such as the absorption capacity for a liquid solvent (loading), flowability and compressibility, which are equally important for the LSS powder processing and preparation of final formulations, NEU displayed better properties than AER or SYL. Therefore, it was finally selected as the carrier for possible LSS as well as the co-milling (see Chapter 8.1.3.2).

The above-mentioned results were partially included in the published co-author article [82]:

MARUSHKA, J., BROKEŠOVÁ, J., OGADAH C. U., KAZEMI A., TEBBENS, J. D., ŠKLUBALOVÁ, Z.: Milling of pharmaceutical powder carrier excipients: Application of central composite design. *Adv. Pow. Tech.,* 2022, 33, 103881, IF_{2021} 5.2 Q2, ISSN: 0921-8831. https://doi.org/10.1016/j.apt.2022.103881

In this work, twenty-four pharmaceutical excipients, including the above-mentioned silica carriers, were subjected to preliminary mild milling conditions (see Chapter 7.2.2.2). By the evaluation of particle size reduction without any aggregation while maintaining a narrow span, five substances were further milled using circumscribed central composite design to optimize the effect of the factors: milling speed, milling time, and ball size on the response parameters of particle size, span and particle size distribution of the final powder. In 30 experimental combinations of the studied factors, the optimum milling conditions leading to the expected level of particle size x_{90} corresponding to the 90 % of cumulative distribution were detected and a good agreement between the measured x_{90} value and that of predicted by the generated quadratic equation was confirmed.

Author's contribution: Experimental work and data processing, manuscript review and editing.

8.1.2 Selection of non-volatile solvents for the preparation of Cyclosporine A-based LSS

The choice of solvent can significantly impact drug release from a final LSS dosage form [70]. For enhanced drug release from LSS, a solvent in which the active ingredient is most soluble is usually selected [55]. This is also crucial to reduce the amount of powder excipient required to adsorb the drug in liquid state, and to avoid high weight of the final dosage form. Hence, as part of the preformulation studies, the solubility of a model drug CyA in different non-volatile solvents was evaluated as described in method 7.2.2.2, to identify the most appropriate solvent for the preparation of enhanced release LSS of CyA.

The results shown in (Figure 11) indicate the higher solubility of the drug (454.62 mg/ml) in Transcutol® HP (TRC-HP) compared to the other solvents. However, solvent capacity is only one aspect, since additional factors such as dielectric constant, hydrophilic-lipophilic balance (HLB) and micelle formation have been reported to also influence the dissolution rate of the drug [99]. Thus, to confirm TRC-HP as the solvent of choice, the influence of the solvents on the dissolution behaviour of CyA LSS in different biorelevant media was further evaluated (see Chapter 8.1.3.1).

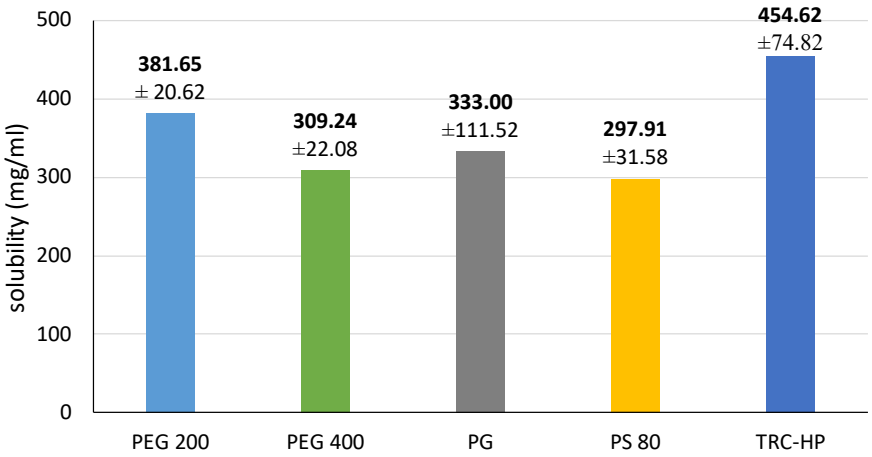

Figure 11: Solubility of cyclosporine A in non-volatile solvents polyethylene glycol 200 (PEG 200), polyethylene glycol 400 (PEG 400), propylene glycol (PG), polysorbate 80 (PS 80) and Transcutol® HP (TRC-HP) after 24 h at laboratory temperature.

Consecutively, the carrier NEU selected based on its absorption capacity and milling behaviour was evaluated for its flowable liquid retention potential (ϕ-value) with TRC-HP, the solvent with the best solubilizing ability for the model drug CyA.
The addition of 20.0 g of TRC-HP to 30.0 g of NEU resulted in the improvement of the angle of slide (Figure 12) compared to that recorded for NEU itself (42.70 ± 1.60). The measured value of angle of slide 34.00 ± 0.35° shows a closeness to the required optimum value of 33° for a mixture containing 24 g of solvent (Table 10). This mixture was chosen to calculate the ϕ-value of NEU for TRC-HP.

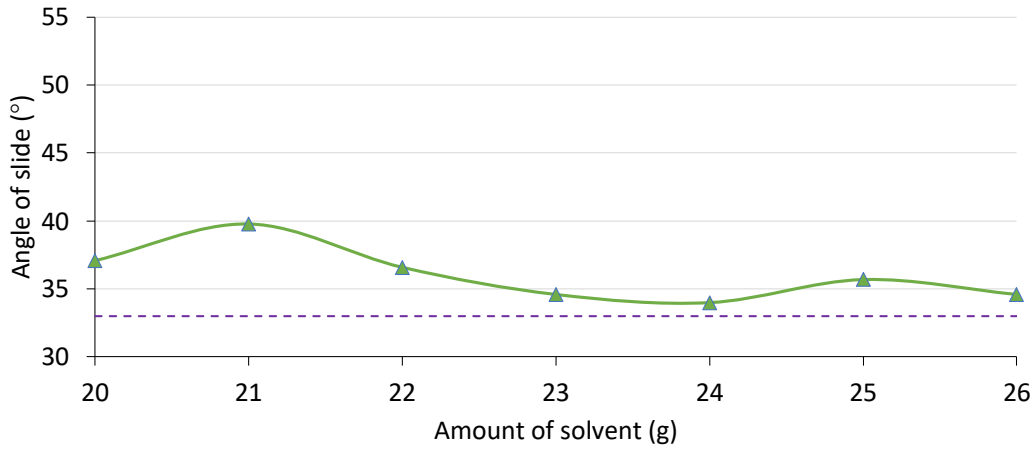

Figure 12: Average value of angle of slide of 30.0 g of NEU mixtures in Transcutol® HP. The dashed line indicates the desired optimum angle of slide 33°.

The calculated ϕ-value of 0.8 implies that 1 g of NEU could retain 0.8 g of TRC-HP while maintaining acceptable flow properties, which is lower than for other tested solvents like PEG 200 (see chapter 8.1.1.1) or PEG 400 and PG [68].

Table 10: Flowable liquid retention potential of Neusilin® US2 for Transcutol® HP.

Mass of solvent (g)	Flow rate (s/50g)*	AOR (°)*	CI (%)	HR
20.0	12.20 ± 0.45	45.50 ± 1.25	18.42	1.23
21.0	10.00 ± 0.71	45.24 ± 0.80	17.33	1.21
22.0	11.60 ± 0.55	46.76 ± 0.74	19.93	1.25
23.0	9.80 ± 0.45	47.48 ± 0.24	18.34	1.22
24.0	**10.20 ± 0.84**	**49.00 ± 0.83**	**21.53**	**1.27**
25.0	10.20 ± 0.84	49.96 ± 1.17	22.05	1.28
26.0	12.00 ± 2.55	49.58 ± 1.26	22.39	1.29

*Orifice diameter 25 mm and 15 mm were used for flow rate and AOR, respectively.

8.1.3 Development of drug delivery systems for cyclosporine A with enhanced dissolution behaviour

This chapter is dealing with improving solubility and dissolution rate of a model poorly soluble drug cyclosporine A (CyA) by preparation of a liquisolid system (LSS). In order to confirm the efficiency of the previously selected solvent Transcutol® HP, even from a possible final dosage form, the influence of non-volatile solvents on the CyA release from LSS filled into capsules was studied in the first part. Subsequently, the efficiency of LSS and co-milling was compared.

8.1.3.1 Development of liquisolid systems of cyclosporine A

The solubility of the drug in a suitable non-volatile solvent plays a role in determining the drug release rate from LSS. As shown in the solubility studies (subchapter 8.1.2), CyA showed a higher propensity to dissolve in Transcutol® HP (TRC-HP), thus it was considered as the appropriate solvent for the preparation of CyA LSS. However, as previously mentioned, in addition to the drug solubility, additional solvent factors such as dielectric constant, hydrophilic-lipophilic balance (HLB) and possible micelle formation may also influence the dissolution rate of the drug [99]. Moreover, some authors have observed that the findings from the solubility studies may not always align with the drug dissolution rate [50]. Since capsules or tablets represent the final dosage forms for LSS powder, a trial dissolution test was performed to observe which of the non-volatile solvents achieves the highest in vitro drug release of CyA when filled in a capsule. Based on the pharmacopeial requirements, capsules should disintegrate within 30 min releasing their content (Ph. Eur. 11.0).

The NEU-based LSS formulations of CyA were prepared using 20 % w/w drug solution or suspension in either PEG 200, PEG 400, PG, PS 80 or TRC-HP as solvent, filled into gelatine capsules (i.e., denoted here as LSS C), evaluated for in vitro drug release in three biorelevant media, and compared to the physical mixtures

of CyA (PM). The results are shown in Figure 13 – Figure 15 in relation to the medium used.

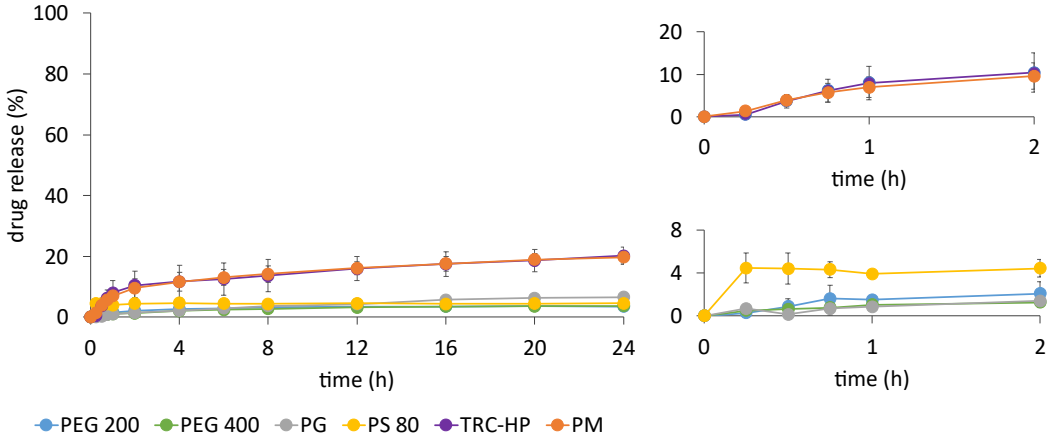

Figure 13: Influence of non-volatile solvents on the dissolution profiles of cyclosporine A LSS filled in capsules compared with physical mixtures (PM) in FaSSGF medium. The inserts on the right side represent a zoom of the 2 h time interval.

The dissolution profiles in FaSSGF (pH 1.6), indicate a low drug release from most of the formulations (Figure 13), where less than 20 % drug concentration was attained after 24 h. This behaviour was expected due to the poor aqueous solubility of CyA. The lowest concentration was observed for LSS C based on PEG 200, PEG 400 and PG with the released amount of 1.50 ± 0.37 %, 1.05 ±0.38 % and 0.94 ± 0.24 %, respectively, after 60 min. The LSS based on PS80 and that of TRC- HP showed a little bit higher drug release of 3.93 ± 0.27 % and 8.04 ± 3.96 within the same time period.

The relatively lower release of CyA from LSS C based on PEG 200, PEG 400 and PG can be mainly attributed to the lower solubilities of CyA in these solvents compared to those in TRC-HP. Even though the drug showed the lowest solubility in PS 80, surprisingly, an initial higher drug release was detected. The explanation may be due to the surfactant properties of PS 80, leading to decrease in surface tension or solubilization via micelles [58]. Interestingly the percentage of drug release after

60min (6.97 ± 2.38 %) from PM was comparable to TRC-HP-based LSS C and after 24h, both samples reached similar high concentration of CyA 19.68 ± 2.23 % and 20.86 ± 2.82 %, respectively (Figure 13). However, the maximum released amount of CyA was only approximately 20 % in this acidic medium.

The enhanced drug release from LSS formulations is often attributed to several factors including the fact that the drug is dissolved or dispersed in the non-volatile solvent, which increases the surface area of the interface between the liquid drug and the dissolution medium; at the same time, the drug is carried in the powder particles with high surface area. Therefore, the dissolution rate is accelerated due to the increased wettability and higher surface area available to the dissolution medium [56,58,73]. Thus, the dissolution rate enhancement seen in the PM may also be attributed to the effect of increased contact area of the drug with the dissolution medium, due to loading on large surface area of NEU carrier particles [100].

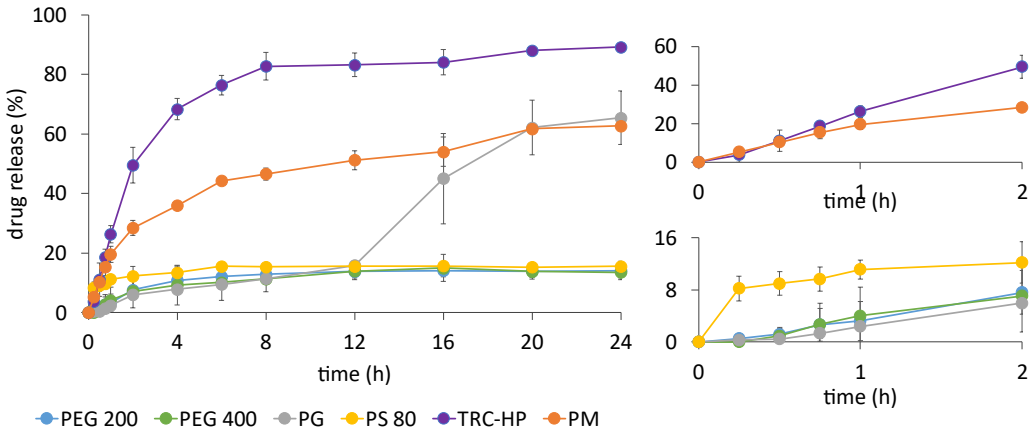

Figure 14: Influence of non-volatile solvents on the dissolution profiles of cyclosporine A LSS filled in capsules compared with physical mixtures (PM) in FaSSIF medium. The inserts on the right side represent a zoom of the 2 h time interval.

The CyA released amount of in FaSSIF (pH 6.5) was generally improved as shown in Figure 14. Although the initial concentration was low for LSS C based on PEG 200 and PEG 400 (3.23 ± 3.00 %, 4.05 ± 4.42 %) during 60 min, the concentrations

achieved after 24 h were higher than those previously observed in FaSSGF (Figure 13). The LSS C of PG showed an initial slow release and after a lag time of approximately 12 h the drug release was increased continuously. Similar to FaSSGF, the release from PS 80 was also higher than from PEG 200 and PEG 400 in which the drug showed better solubility; 11.16 ± 4.17 % was released after 60 min, the drug concentration was increasing slightly then finally reaching finally reaching the concentration of 15.49 ± 1.75 % after 24 h.

The fast release achieving 26.27 ± 2.96 % of released CyA within the first 60 min, 49% in 2 h and above 80 % after 8 h in FaSSIF was observed for the TRC-HP-based LSS. Comparing PM to this sample after the first 60 min, the amount released was similar (19.53 ± 2.66 %). Eventually, LSS surpassed the PM, and the final concentration reached at the end of 24 h was much higher for LSS (89.22 ± 0.22 %) compared to PM (62.67 ± 0.42 %). The initial fast release which was observed for the PM can be explained similarly as in FaSSGF. The overall improved dissolution in FaSSIF for all the LSS and PM may be attributed to the higher concentration of surfactants (*e.g.,* lecithin and sodium taurocholate) present in this medium [101].

Given that all LSS C contained the same concentration of CyA in liquid state (20 % w/w), the drug was not entirely dissolved in all the formulations. The results shown here clearly demonstrate that the drug release profiles were dependent on the drug state in each solvent. Whereby the LSS in which CyA was not dissolved but only dispersed (PEG 200, 400 and PS 80) attained a lower drug concentration in the dissolution medium compared to those in which the drug was completely dissolved (PG and TRC-HP). This observation is in line with the result described in literature for LSS based on different non-volatile solvents [58].

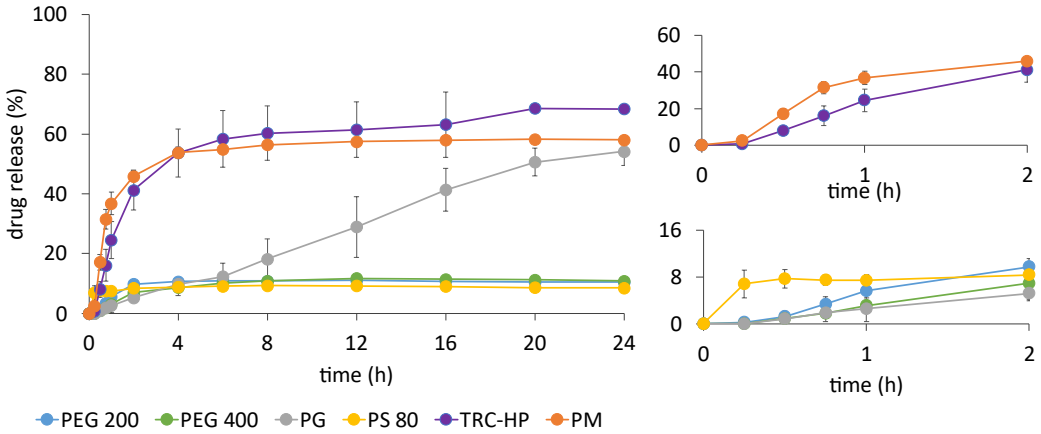

Figure 15: Influence of non-volatile solvents on the dissolution profiles of cyclosporine A LSS filled in capsules compared with physical mixtures (PM) in FaSSCoF medium. The inserts on the right side represent a zoom of the 2 h time interval.

Finally, an improved drug release was also noted in FaSSCoF (pH 7.8) (Figure 15), likely attributed to the bile salts, such as cholate, in the medium [102]. The LSS C formulated with PEG 200, PEG 400 exhibited similar low CyA release as in the other media, reaching only 10.42 ± 0.81, 10.79 ± 1.16 % respectively, after 24 h. At the same time, a poorer drug release was detected even for PS 80 (8.53 ± 1.07 %). An interesting behaviour was observed for the PG-based LSS C sample which showed a gradual, almost linear CyA release up to 20 h, achieving finally 54.19 ± 4.75 % after 24 h. TRC-HP-based LSS released a high drug amount (68.42 ± 0.91%) in FaSSCoF after 24 h, however it should be noted that also PM reached a high released concentration of 58.04 ± 1.56% at the same time point showing good carrier properties of NEU. In both cases, however, the maximum amount was lower than the amounts reached in FaSSIF.

Overall, it is important to state that problems with dissolution of the capsule shell and subsequent release of the drug was encountered, which led to a delayed release rather than an enhanced or immediate release as is expected from LSS. Some of the capsules did not fully disintegrate, and the release of the capsules content occurred mainly by rupture of the weak points of the shell. After the 24 h test interval,

portions of the capsule or its undissolved contents remained in the sinkers or at the bottom of the dissolution vessels. This behaviour was confirmed visually, and the findings are in line with the study by Glube et al. [103], albeit for HPMC capsules. The authors reported a slow disintegration of the HPMC capsule in acidic environment, a slight improvement at higher pH and overall, a delayed release from the capsules, which was attributed to the interaction between the active substance and the wall of the capsule material [103].

To ascertain if the behaviour of the LSS C in the dissolution media could be attributed to the non-volatile solvents, a study conducted in the diploma thesis of Helerová [104] and Bartůňková [105] was dedicated to investigating the influence of different non-volatile solvents on the disintegration of hard gelatine capsules. Helerová reported that the capsules containing PG or PS 80 with NEU as a carrier prolonged the disintegration time compared to those containing only NEU. Moreover, the addition of CyA to the LS mixture further prolonged the disintegration time, especially for PG-based LSS. Similar observations were confirmed also for PEG 200, PEG 400 or TRC-HP when compared with NEU alone [105]. This delay may explain the slow drug release, especially the lag time which was visible in LSS C based on PG (Figure 14 and Figure 15) and also the faster initial release or sometimes even better performance observed for the PM samples in comparison to the LSS in this work. Nevertheless, the capsules in both above-mentioned studies disintegrated within 30 min complying with the Ph. Eur. criteria for disintegration, compared to the lag time of several hours or failure of capsule disintegration in the current study. This may be due to the different hydrodynamics in the apparatus. Further experiments may be necessary in the future for optimizing the LSS formulations of CyA for capsule filling to ensure that the final dosage form does not compromise the benefits of LSS preparations significantly.

In conclusion, the obtained results from the dissolution studies confirmed that the TRC-HP is the most appropriate solvent for the preparation of CyA LSS due to the efficient release profiles in all media compared to LSS C based on other solvents.

The results were presented at the following conference:

OGADAH, C., VRANÍKOVÁ, B., MRŠTNÁ K., ŠKLUBALOVÁ, Z.:
Improving the solubility and dissolution rate of cyclosporine A by the liquisolid technique. 12th Postgraduate and Postdoc Conference, Faculty of Pharmacy in Hradec Králové, Czech Republic, 1 - 2. February 2022. (ORAL PRESENTATION).

8.1.3.2 Comparison of efficiency of LSS and co-milling in improving dissolution of cyclosporine A

The low aqueous solubility and dissolution rate hinder the sufficient bioavailability of orally administered active substances [32]. As mentioned in the Introduction section, the formulation of liquisolid systems (LSS) or the preparation of interactive mixtures by co-milling with a suitable carrier may represent suitable strategies for improving the solubility and dissolution rate of a poorly soluble drug. However, the selected method may influence the amount of loaded drug in the carrier, drug stability and the drug release behaviour in general. Thus, the aim presented in this part is to compare the efficiency of the two methods namely the preparation of LSS and co-milling for drug loading onto a mesoporous carrier for improving the solubility of CyA, as well as to compare Neusilin® US2 (NEU) used here as the optimum carrier for CyA with another carrier, functionalized calcium carbonate (FCC). This experimental work was partially carried out at University of Copenhagen University during the internship stay.

The LSS and co-milled samples were prepared as described in Methodology subchapters 7.2.3. The carriers were loaded with CyA to achieve the drug concentration of 15, 25, and 50 % (w/w) in the LSS as well as the co-milled samples. To prepare LSS, TRC-HP was used as a solvent; it should be noted that 50 % LSS sample was not possible to prepare for FCC due to a limited liquid loading capacity (see below).

While after drug loading, the NEU-based LSS maintained their spherical shape similar to the raw NEU particles, the particles tended to agglomerate already at 15 % drug load in the FCC-based LSS. NEU demonstrated a higher liquid sorption capacity, due to its considerably higher specific surface area and pore volume compared to FCC.

Table 11: Specific surface area (SSA), pore volume and pore diameter of raw and milled Neusilin® US2 (NEU) and functionalized calcium carbonate (FCC) [106].

Sample	SSA (m^2/g)	Pore volume (cm^3/g)	Pore size (nm)
Raw NEU	363.2 ± 10.2	1.8 ± 0.6	18.8 ± 1.9
Milled NEU	27.7 ± 0.3	0.18 ± 0.05	26.3 ± 5.3
Raw FCC	45.8 ± 3.9	0.82 ± 0.05	37.2 ± 6.6
Milled FCC	13.7 ± 0.2	0.07 ± 0.02	36.6 ± 9.6

The size reduction of a material, *e.g.,* by milling, can result to changes in the particle structure and shape as well as other properties such as specific surface area (SSA) and pore size and volume. As shown in Table 11, the SSA of raw larger particles of NEU (Table 8) 363.2 ± 10.2 m^2/g was reduced by 13 times after 2 min of milling to 27.7 ± 0.3 m^2/g. Similarly, FCC showed a 3 times reduction in SSA from 45.8 ± 3.9m^2/g to 13.7 ± 0.2 m^2/g. The decrease in SSA may be attributed to the formation of agglomerates due to the higher surface energy of very small particles [107]. Alternatively, it may be due to the fragmentation of the particles and their subsequent compression by the milling balls during milling, resulting in a decrease in the pore volume (Table 11). The decrease in SSA contrasts with the findings of Lou et al. [108] as well as the author's own previous measurement (Table 9), where an increase in SSA of NEU was recorded. However, the disparate results observed may be explained by the different milling conditions employed in each study. For example, the current study used a higher vibration frequency of (30 Hz) and 2 stainless steel

balls (Ø 12 mm) compared to the study by Lou et al. [108] which used a lower frequency (4 Hz) and a single smaller ball (Ø 5 mm).

The formation of agglomerates leading to some fractions of the milled particles being larger than the starting material was observed for raw FCC after milling. Therefore, the reduction in its SSA may be attributed to the first of the described potential mechanisms.

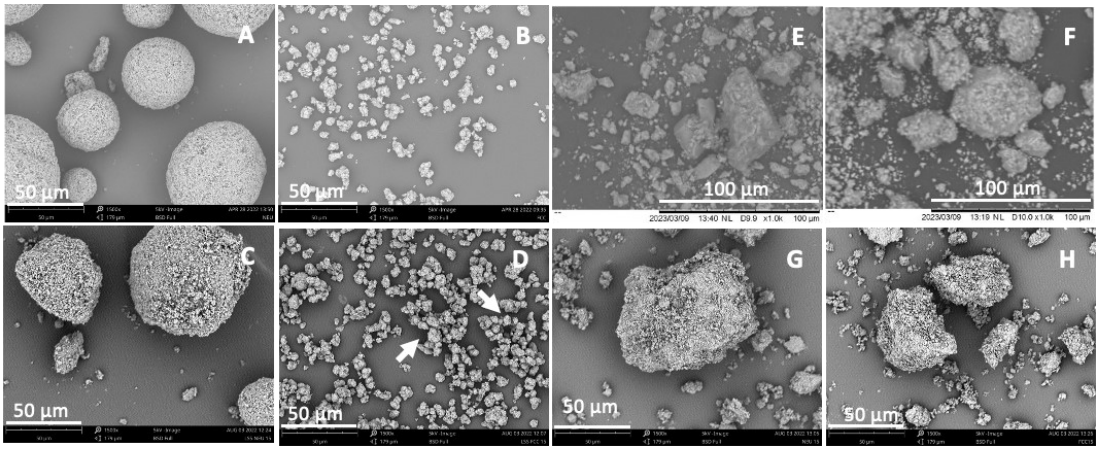

Figure 16: SEM images of the raw and milled carriers, liquisolid systems (LSS) and co-milled mixtures (CM) of cyclosporine A with Neusilin® US2 (NEU) or Functionalized calcium carbonate (FCC) and the indicated amounts of drug load. A - raw (NEU), B - raw FCC, (C) LSS15 NEU (D) LSS15 FCC, E - milled NEU, F - milled FCC, G - CM 15NEU and H - CM15 FCC. (Magnification at 1500× except E and F at magnification 1000×). The inserted arrows indicate the agglomerated FCC particles. Modified from [106].

Figure 16 illustrates the changes in the carrier structure after milling, the CM formulations showed smaller particles distributed among larger ones (Figure 16). The particle size heterogeneity may be attributed to the coexistence of unbroken and partially broken particles. Moreover, the rough surface of CM formulations imply that some portion of the drug was loaded onto the carrier surface.

The formulations of CyA were subjected to in vitro drug release in biorelevant media FaSSIF and compared with the milled amorphous drug (Figure 17). CyA dissolved concentration over the 240 min experiment was 7.9 ± 2.5 µg/mL. This slow

dissolution behaviour was anticipated in advance due to the poor aqueous solubility of the drug and this observation is in line with a previous study [37] where the dissolved concentration after 60 min was found to be approximately 60ng/mL in distilled water (approximately 0.2% of the total).

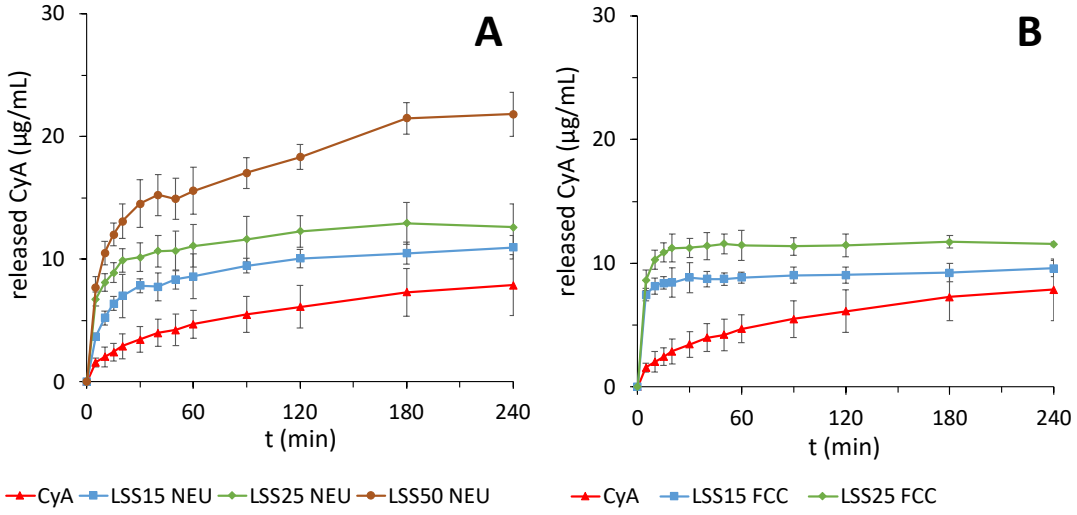

Figure 17: Dissolution profiles of cyclosporine A (CyA) from liquisolid systems (LSS). Comparison of milled CyA and different drug loadings in the carriers. A - Neusilin® US2 (NEU) and B - functionalized calcium carbonate (FCC). Modified from [106].

The dissolution profiles of LSS imply significantly faster ($p < 0.05$) drug release than the milled CyA. For example, 7.8 ± 0.5 µg/mL and 8.9 ± 0.3 µg/mL drug was dissolved from LSS 15 NEU and LSS 15 FCC respectively, within 30 min, compared to only 3.4 ± 1.0 µg/mL achieved for the milled drug. The faster dissolution from the LSS may be attributed to the increased wetting properties and surface area of drug available for dissolution, as previously discussed (See chapter 8.1.3.1). Other authors have also reported similar results for the LSS containing poorly soluble drugs, e.g., prednisolone [50], naproxen [56] and rosuvastatin [54]. The drug release from LSS displayed a biphasic drug release profile, showing, a rapid dissolution during the first 30 min, which was followed by a slower release (Figure 17). This dissolution behaviour is similar to that observed for the profiles of TRC-HP-based LSS C in

FaSSIF above (Figure 14) although it is more visible here, due to higher concentration of drug in the solvent, 50 % w/w compared to 20 % w/w, as well as the different dosage forms tested (powder or capsule). Similar profiles have also been reported in previous findings related to mesoporous silica-based systems [109,110]. It is suggested that the drug can be situated either in the pores or on the surface of the carrier. Therefore, the first phase of the dissolution relates to the drug release from the surface, while the slower release phase is due to drug located in the deeper pores [110]. This behaviour could be potentially beneficial in the preparation of modified release formulations, offering a fast onset of action combined with a more gradual dissolution for longer-term action.

Finally, the amount of CyA released from the LSS formulation was found to increase as the drug loading increased. This can be attributed to the proportion of the liquid phase in relation to the carrier material, which contributes to the better wetting of the formulation by the dissolution medium. Additionally, it has been discussed in other studies, that the solvents diffuse alongside the drug molecules into the dissolution medium, acting as co-solvents in that microenvironment, further improving drug release. [54,56]. After 240 min, the highest dissolved CyA concentration of 21.8 ± 2.8 μg/mLwas recorded for LSS 50 NEU, signifying 2.8 times improvement in the drug release compared to the milled CyA alone.

The dissolution of CyA from the co-milled formulations also showed a higher drug release than the milled CyA (Figure 18) albeit lower in comparison to the LSS. For example, at 15% drug load concentration, the drug release from NEU-based co-milled formulations (CM NEU) was 17.0 ± 0.1 μg/mL, after 240 min, signifying 2.2 fold higher concentration than milled CyA, whereas for FCC-based formulations, the dissolved CyA was 8.8 ± 1.1 μg/mL at the same time point.

The enhanced dissolution behaviour in comparison to the milled amorphous drug alone imply that the carrier plays a role in the improved drug release behaviour. Similar results have been discussed for other poorly water-soluble APIs co-milled with various carriers [40,41]. One of the proposed mechanisms for enhanced dissolution rate is through improved wetting due to the hydrophilicity of the carrier

[40]. Moreover, the presence of the carrier prevents the cohesive interactions and the possible formation of agglomerates, leading to an improved dissolution [49].

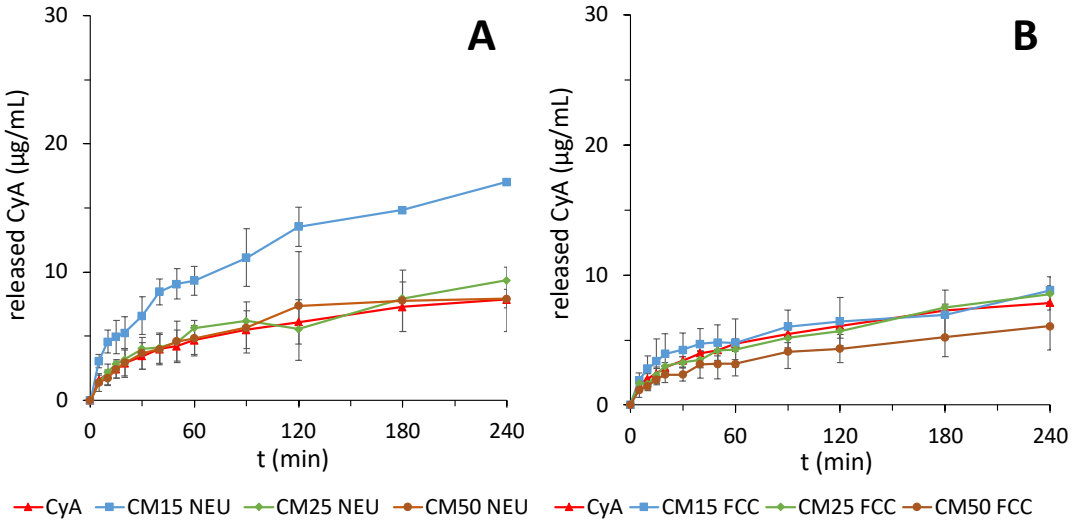

Figure 18: Dissolution profiles of cyclosporine A (CyA) from co-milled (CM) mixtures. Comparison of milled CyA and different drug loadings in the carriers. A - Neusilin® US2 (NEU) and B - functionalized calcium carbonate (FCC). Modified from [106].

Overall, the results indicate that the dissolution rate of CyA was improved by either preparation of LSS or co-milling. However, only LSS increased the dissolution significantly ($p < 0.05$). The enhanced dissolution and release characteristics observed in LSS compared to co-milled systems is attributed to how the drug is dispersed within the formulation. In the LSS, the drug is dissolved in the non-volatile solvent, loaded in a dissolved state within the carrier and remains as such. Hence, upon contact of the formulation with the dissolution medium, the drug is released easily. On the other hand, with co-milling the API is not pre-dissolved (despite being amorphous), and thus the solid drug particles are required to dissolve before being released into the surrounding medium. As previously discussed, the surface area available for contact with the medium is another factor for improving dissolution. Therefore, the faster dissolution from LSS was also attributed to the ability of the carriers to maintain a high surface area after drug loading compared to the co-milled

formulations where the SSA was decreased due to particles fracture, and consequently a slower drug dissolution.

In conclusion, loading CyA on mesoporous carriers is a feasible way to improve its dissolution profile. However, a selection of the loading method and carrier is crucial. The preparation of LSS of CyA represents a more suitable method for improving the solubility and dissolution rate compared to the co-milling. The efficiency of NEU as a carrier was again demonstrated. Moreover, solid state under humid storage conditions remained unchanged for 20 weeks as confirmed by X-ray powder diffraction, which is promising for the future preparation of the final CyA-loaded dosage form.

The results were included in the following first-author publication [106]:

OGADAH C. U., MRŠTNÁ, K., MATYSOVÁ, L., MÜLLERTZ, A., RADES, T., NIEDERQUELL, A., ŠKLUBALOVÁ, Z., VRANÍKOVÁ, B.: Comparison of the liquisolid technique and co-milling for loading of a poorly soluble drug in inorganic porous excipients. *Int. J. Pharm*. 2024, 650, 123702, IF_{2021} 5.8, Q2, ISSN: 0378-5173. https://doi.org/10.1016/j.ijpharm.2023.123702

The efficiency of two preparation methods, formulation of LSS and co-milling using two mesoporous carriers was evaluated. The drug loaded LSS formulations showed a significant improvement in CyA release in comparison to the milled amorphous drug itself. It was confirmed that the loading method, as well as the carrier, influenced the dissolution behaviour. While the efficiency of LSS was attributed mainly to the co-solvency effect of TRC-HP and the fast release of dissolved drug from the carrier surfaces and pores, in the case of co-milling, improved dissolution was achieved by the increased contact area of the formulation and better wetting by the dissolution medium. Although both carriers increased the drug release, NEU-based formulations showed better loading properties due to the larger surface area and greater pore volume. Moreover, the amorphous CyA remained unchanged for 20 weeks as confirmed by X-ray powder diffraction.

Author's contribution: Experimental work, data processing, writing, revision and editing of original manuscript.

The results were presented at the following conferences:

OGADAH, C., ŠKLUBALOVÁ, Z. MÜLLERTZ, A., RADES, T., VRANÍKOVÁ, B.: Comparison of the liquisolid technique and co-milling for loading of a poorly soluble drug in inorganic porous excipients. 13th Postgraduate and Postdoc Conference, Faculty of Pharmacy in Hradec Králové, Czech Republic, 1 - 2. February 2023. (ORAL PRESENTATION)

OGADAH, C., ŠKLUBALOVÁ, Z. MÜLLERTZ, A., RADES, T., VRANÍKOVÁ, B.: Comparison of methods of drug loading in inorganic porous excipients. Improving Drug Solubility: Recent Advances in Pharmaceutical Technology. Faculty of Pharmacy in Hradec Králové, Czech Republic, 6 - 7 February 2023. (POSTER PRESENTATION)

8.2 Development of mucoadhesive matrix core

The oral bioavailability of CyA is generally insufficient as it is a cyclic polypeptide with low aqueous solubility and low permeability (BCS IV group) [111]. To improve its solubility and dissolution rate, the strategies of preparation of liquisolid systems and interactive mixtures have been suggested above. However, to facilitate its absorption and to achieve a sufficient level of the drug at the target site in the colon for a longer duration, additional strategy to sustain the release of the formulation is necessary. The most common approaches for prolonged release in GIT generally include the preparation of matrix tablets using various polymers. However, to address the highly variable colon transit time and to prolong the residence in the colonic mucosa, it is recommended to incorporate certain mucoadhesive hydrophilic polymers. Such mucoadhesive polymers include derivatives of poly (acrylic acid) [112], cellulose derivatives, [81,113] as well as various polysaccharides [114,115]. Most of these polymers possess intensive hydration, gel forming ability as well as mucoadhesive properties [80]. With respect to colon-targeting, the polysaccharides additionally represent remarkable candidates due to their ability to be degraded by the bacteria in the colon [24,116,117].

Based on the literature review, some of the widely used hydrogel agents from the cellulose derivatives, namely, carboxymethylcellulose sodium (CMC Na), hydroxypropyl methylcellulose (HPMC) K15M, hydroxyethyl cellulose (HEC) and hydroxypropyl cellulose (HPC), as well as polysaccharides, guar gum (GG), iota carrageenan (I-CAR) and lambda carrageenan (L-CAR) were therefore considered in this study. [107,108]. However, due to the high hydrophilicity of these polymers, there is a potential risk of premature drug release from matrix tablets. For these reasons, characterization of their properties was performed in biorelevant media.

To select the most suitable polymers to be employed in the swellable and mucoadhesive core matrix for the prolonged release colon-targeted LSS system, the experimental runs were organized as follows.

1. Based on the viscosity evaluation of the pure polymers, the reduced list of substances was used to prepare the matrix tablets with a model, freely soluble drug

theophylline (TH). These tablets were tested for their swelling, drug release and mucoadhesive properties. Then, the substances deemed unacceptable were excluded.

2. To evaluate the viscosity behaviour of the binary mixture of two remaining substances, a Central Composite Design statistical plan was employed. Subsequently, the matrix tablets made of the binary mixture with TH were compressed and evaluated as described above.

3. Finally, the effect of different amount of NEU, simulating the added LSS system, on the properties of the matrix tablet regarding swelling and mucoadhesion ability was tested.

8.2.1 Characterization of viscosity of pure polymers

The formation of the gel layer on the surface of matrix tablets is related to the polymer chosen and the specifics of the movement and flow of surrounding fluids [118]. Hence, to select the appropriate polymers for preparation of sustained release matrix core, the rheological properties, specifically shear (dynamic) viscosity of the polymer dispersions (CMC Na, HPMC, HPC, HEC, GG, I -CAR and L-CAR) were tested in three distinct biorelevant media, FaSSGF, FaSSIF and FaSSCoF of pH value 1.6, 6.5 and 7.8, respectively, representing the different conditions along the GIT. For each polymer, 1 g was dispersed in varying media volumes ranging from 10 to 50 mL to simulate a swollen matrix at various degrees of hydration ranging from the partially hydrated tablet core to the continuously eroding gel layer [119]. The shear viscosity measurements were performed on a rotational rheometer.

The flow curves obtained for the measured polymer dispersions indicated a pseudoplastic behaviour, i.e., decreasing viscosity with increasing shear rate, as illustrated in Figure 19. This is related to the orientation of the polymer chains along the direction of flow and to the disentanglement of polymer molecules with the increasing shear force [120].

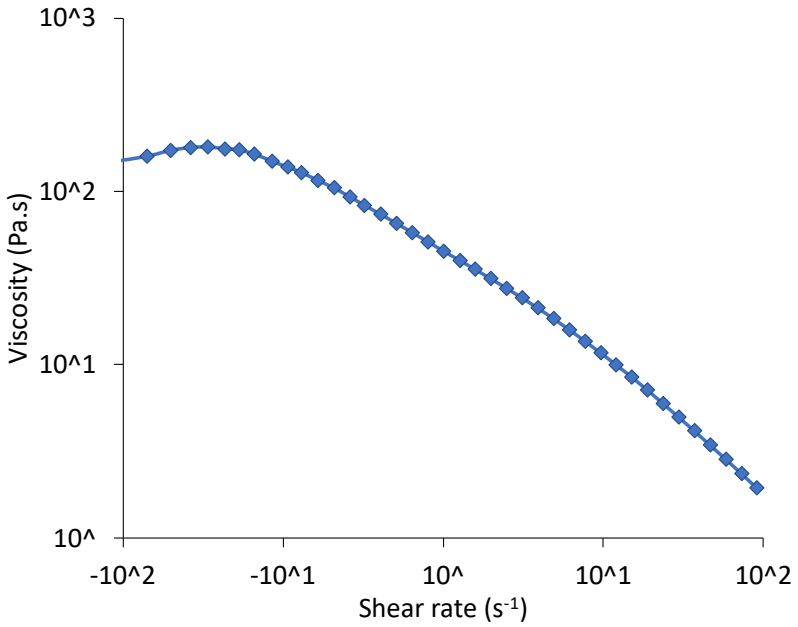

Figure 19: Schematic illustration of viscosity dependence on shear rate for tested polymers showing pseudoplastic (shear thinning) behaviour in biorelevant media. (log-log plot)

To obtain the viscosity data from rheological flow measurements, the linear portion of each flow curve was fitted to the Ostwald de Waele (power law) model [121] described in Equation 3 above. The numerical value of the consistency coefficient or power law parameter *K* in the fitted model is indicative of the shear rate at 1 s^{-1}. This shear rate was chosen to characterise the viscosities of the different samples as it is considered representative of the relevant in vivo conditions [118,122]. The obtained values of apparent viscosity at 1 s^{-1} were plotted for the polymers against increasing the volume of media from 10 to 50 mL and the results have been graphically represented in Figure 20.

It can be clearly observed that the apparent viscosity decreased with increasing volume of media. This reduction in the apparent viscosity is attributed to the decrease in the level of intermolecular interactions between the polymer molecules [123].

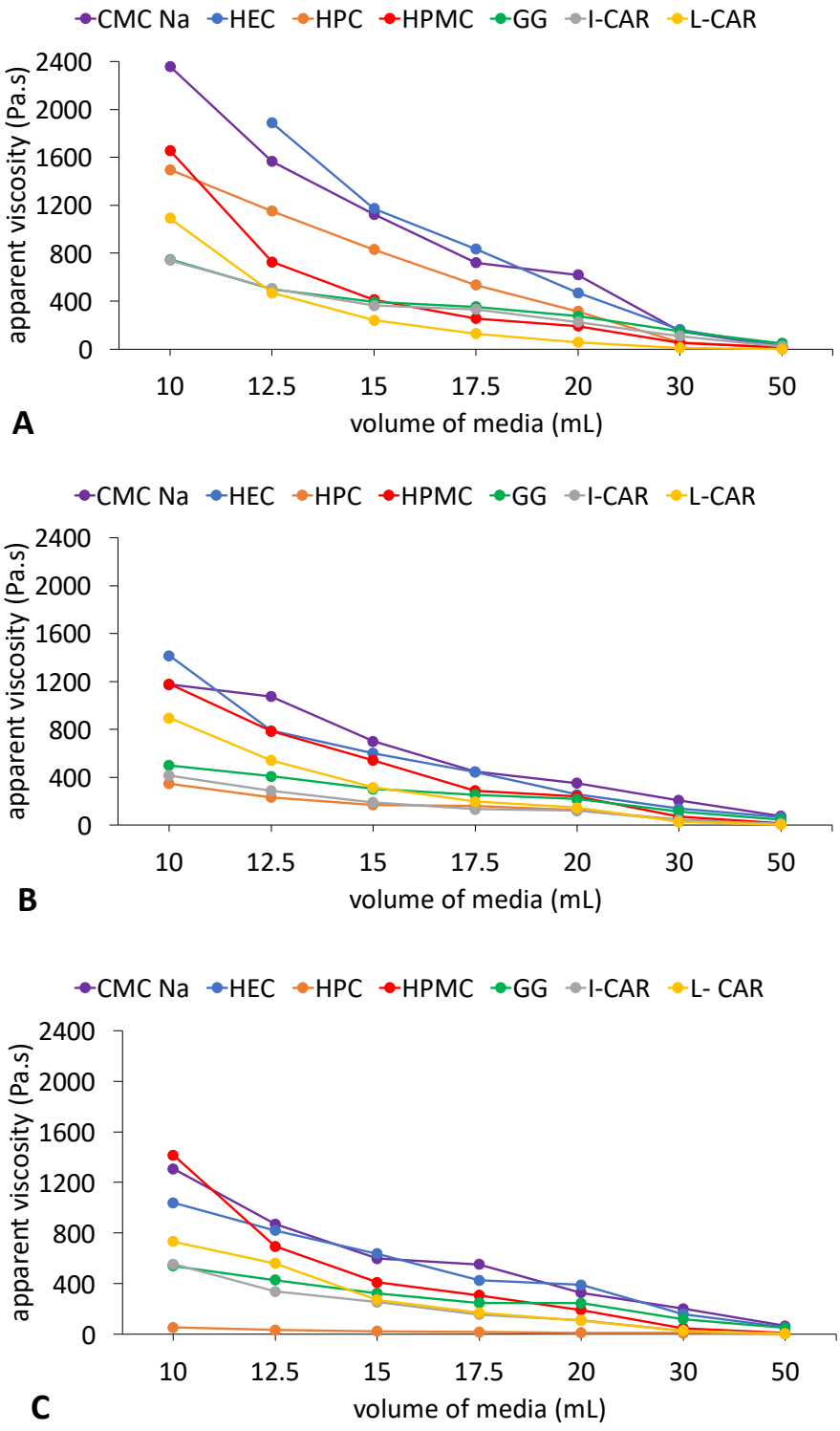

Figure 20: The average values of apparent viscosity of mucoadhesive polymers in biorelevant media. A - FaSSGF, B - FaSSIF, and C - FaSSCoF.

A comparison of Fig. A, B, and C illustrates the effect of pH and dilution by media volume on the viscosity behaviour of polymer samples. With respect to the effect of media type on the apparent viscosity, the highest values were observed in an acidic environment of FaSSGF (Figure 20A). From the measured values, CMC Na dispersions in 10 mL of media showed the highest viscosity (> 2000 Pa.s). Generally, the rank order of the viscosity after the addition of 10 mL of media was Na CMC > HPMC > HPC > L-CAR > GG > I-CAR; it should be noted that to measure in 10 mL of media was problematic for HEC due to insufficient hydration and thus is not mentioned in the ranking. The lowest measured value in 50 mL of FaSSGF (0.72 ± 0.15 Pa.s) was obtained for L-CAR, despite showing a high initial value (1092.33 ± 42.67 Pa.s) in 10 mL dilution. This faster decrease in viscosity upon dilution for L-CAR could imply accelerated loss of the gel layer formed around the tablet and hence less prolonged drug release might be expected from this polymer. On the other hand, the highest value in 50 mL media (47.96 ± 13 Pa.s) was obtained for GG.
All the polymers showed a lower viscosity in FaSSIF in 10 mL of media (Figure 20B). The highest to lowest viscosity values was ranked as follows: HEC > HPMC > Na CMC > L-CAR > GG > I-CAR > HPC. Similar fast decrease in viscosity was again shown by L-CAR in 50 mL of media. Finally, the values of viscosities from highest to lowest in 10 mL of FaSSCoF were HPMC > CMC Na > HEC > L-CAR > I-CAR > GG > HPC (Figure 20C). The viscosity values (53.49 ± 3.32 Pa.s) recorded for HPC in this media was 28 times and 6.5 times lower than observed in FaSSGF and FaSSIF, respectively (Figure 20A and B).

Using the viscosity data as the first criteria for selecting the suitable polymers to prepare the matrix core, the polymers L-CAR and HPC were excluded from further testing. The fast reduction in viscosity of L-CAR with increasing volume of media in all tested media, may signify that the gel layer which will form around the tablet will be very susceptible to erosion and drug release would be difficult to control. Furthermore, HPC was removed from subsequent testing due to the low viscosity of this polymer in the FaSSCoF media based on the assumption that the prolonged

release in the colon will not be achieved due to the low viscous gel. The remaining polymers (CMC Na, HPMC, HEC, GG and I -CAR) which showed suitable behaviour in different biorelevant media were further utilized in the preparation of matrix tablets by direct compression (Chapter 7.2.5). Theophylline was used as a model drug.

The results were presented at the following conferences:

OGADAH, C., VRANIKOVA, B., SKLUBALOVA, Z.: Characterization of rheological properties of polymers for formulation of liquisolid systems targeted to the colon. 10th Postgraduate and Postdoc Conference, Faculty of Pharmacy in Hradec Králové, Czech Republic. 22 - 23 January 2020. (ORAL PRESENTATION)

OGADAH, C., VRANIKOVA, B., SKLUBALOVA, Z.: Characterization of rheological properties of polymers with respect to colon-targeted delivery of liquisolid tablets. 13th Central European Symposium on Pharmaceutical Technology. Gdansk, Poland, 16 -18 September 2021. (VIRTUAL POSTER PRESENTATION)

8.2.2 Characterization of pure polymer matrix tablets with theophylline

The reduced number of substances, CMC Na, HPMC, HEC, GG and I-CAR, was used to prepare the matrix tablets of approximately the same hardness by direct compression using. Theophylline (TH), a freely water-soluble API with pH-independent aqueous solubility was used as the model drug. Apart from the need to have a freely soluble drug which allows to better visualize the hydrophilic polymers' influence on the swelling ability, mucoadhesion and subsequent drug release, the decision to use theophylline was made also based on the need to save cost and to minimize exposure to the highly active substance, CyA. These tablets were tested for their swelling, drug release and mucoadhesive properties.

Swelling behaviour of theophylline pure polymer matrix tablets

Hydrophilic polymers swell in contact with the aqueous medium, to form a continuous gel layer. With more uptake of dissolution medium, the gel layer may undergo continued swelling or erosion or a combination of the two [124]. The extent of swelling and growth of the gel layer, the relative penetration of dissolution medium, drug dissolution rate, and matrix erosion play a crucial role in the mechanism of the drug release [125].

The swelling indices (SI) of the matrix tablets in biorelevant media are displayed in Figure 21. In FaSSGF medium (Figure 21 A), the swelling of the tablets comprising of CMC Na, I-CAR and HPMC increased gradually with time. After 8 h, the highest SI values in this medium was observed for CMC Na, which reached 1301.45 ± 85.55 %, while I-CAR and HPMC SI values were 888.53 ± 46.51 % and 358.61 ± 12.38 %, respectively. This high swelling of CMC Na agrees with other authors [80] and indicates that the polymer has a high water uptake capacity with time. For the HEC tablets, the maximum swelling and in fact the highest among all the tested tablets (1765.08 ± 298.97), was recorded in the first 15 min interval, then erosion occurred slowly until the end of the 8 h test. Similarly, GG also showed a moderately high swelling at 15 min (620.47 ± 138.76 %). However, in contrast to the swelling behaviour of HEC, the SI value decreased only until 1 h, then increased again gradually until 8 h. The initial decrease in the SI of GG may signify that the polymer at the surface of the swollen tablet eroded at a faster rate than the hydration of the core and as more liquid ingress into the matrix, the deeper cores become fully hydrated leading to more swelling later.

Very similar trends for the swelling of the polymer tablets were observed in FaSSIF (Figure 21 B) and FaSSCoF (Figure 21 C). The only exception was I-CAR whose SI values remained almost constant from the initial time point, showing only a slight decrease after 8 h. This behaviour may signify that swelling and erosion were occurring relatively at the same rate. Even though slightly higher swelling was noted for HPMC in FaSSIF, generally its behaviour was pH independent; the same can be concluded for GG.

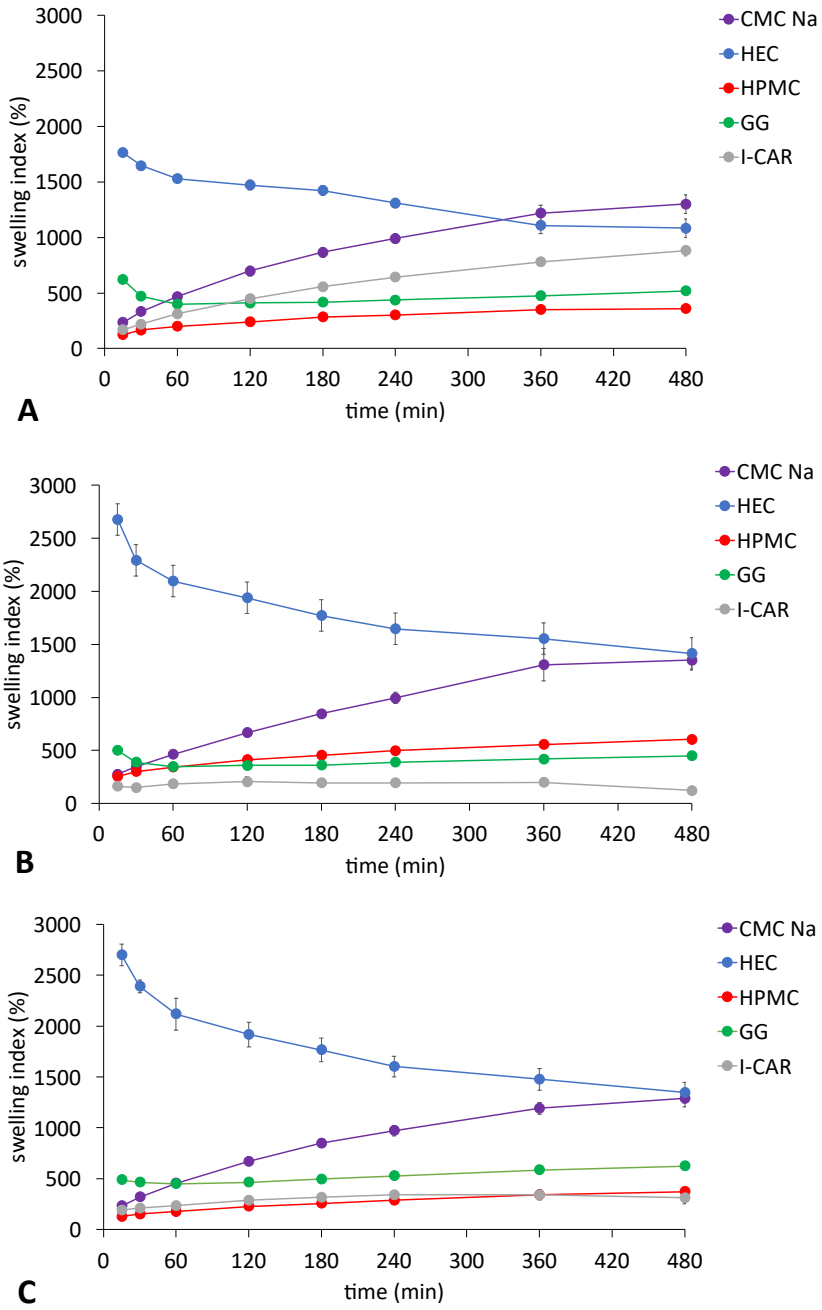

Figure 21: Swelling index of theophylline matrix tablets in biorelevant media. A - FaSSGF, B - FaSSIF and C - FaSSCoF.

The swelling data showed that the polymer HEC was swelling excessively at the initial time intervals. This behaviour is anticipated to result in a high burst effect,

potentially leading to the loss of the sustained release ability of the polymer. Accordingly, HEC was omitted from subsequent testing.

Drug release of theophylline from pure polymer matrix tablets

The mechanism of drug release from hydrophilic matrix tablets generally involves different processes: the ingress of the aqueous medium into the matrix, swelling of the matrix, dissolution of the drug in the medium, diffusion of the drug through the gel layer, and subsequently, the erosion of the swelled matrix [126]. The matrix tablets comprising of the remaining pure polymers CMC Na, HPMC, GG and I-CAR were subjected to dissolution testing in biorelevant media to highlight TH release.

In FaSSGF (Figure 22), the release amount of the drug within 15 min ranged from 2.94 – 19.78 % in relationship to the polymer behaviour.

The matrices comprised of GG showed the highest initial drug release followed by sustained release up to 24 h. Such faster initial drug release (an initial burst release) may be due to surface erosion and shorter diffusion path and confirms the previous observations for swelling (Figure 21). The release rate then decreases as the external layers of the tablet are depleted and the diffusion pathlength for the drug increases [127]. Similar behaviour was observed in HPMC matrix apart from the early time point in which homogeneous water uptake resulted in a regular drug release. After 24 h, the TH released amount achieved approximately 80 % in all media while still increasing. Similar behaviour was registered in FaSSIF (Figure 22 B) and FaSSCoF (Figure 22 C).

The pH-dependent drug release was noted for CMC Na, probably for its ionic nature. Contrary, very fast release for I-CAR, particularly in FaSSIF (Figure 22 B) and FaSSCoF (Figure 22 C), is visible resulting in about 80 and 100 % of the drug already released within 4 - 8 h.

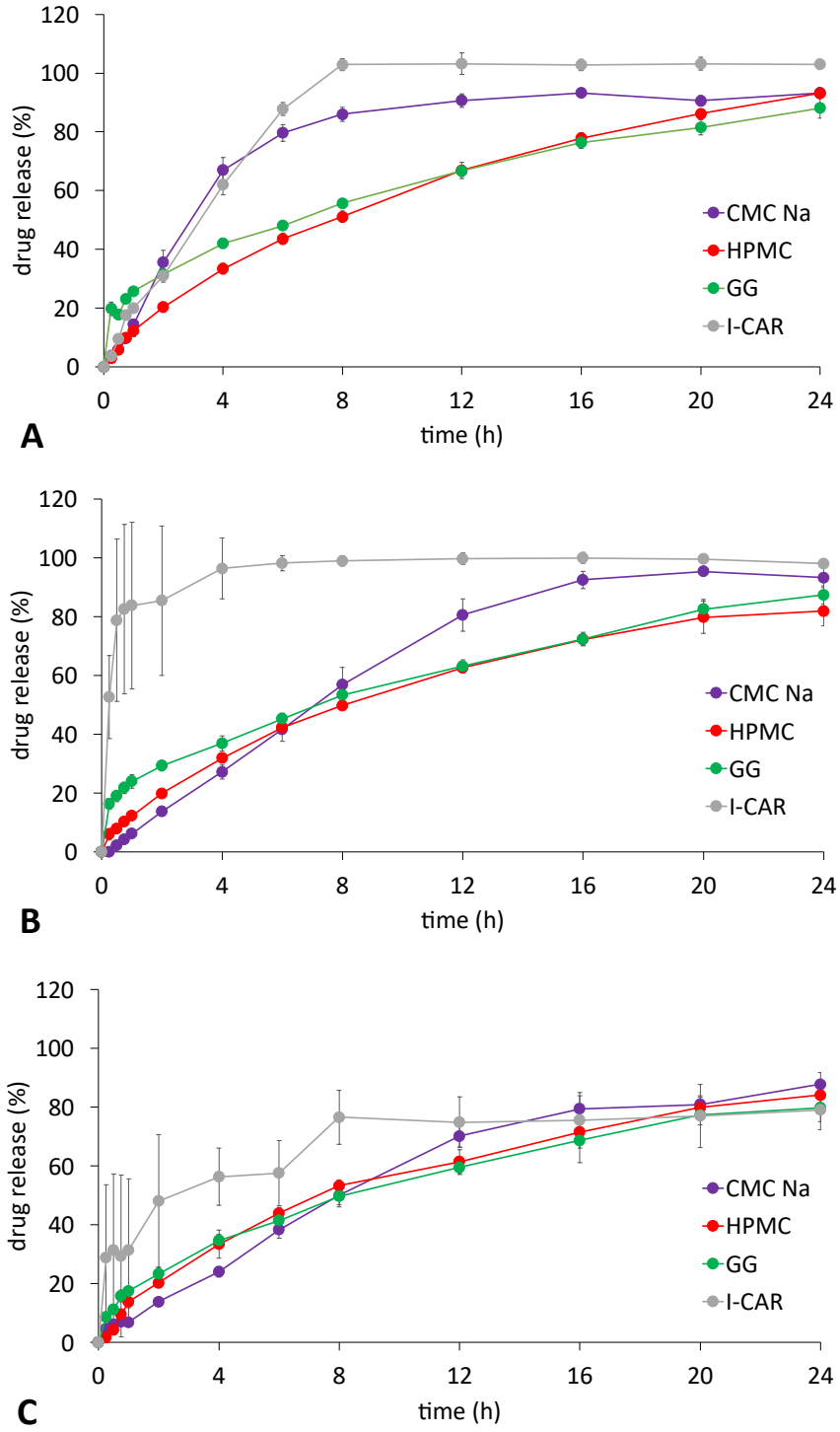

Figure 22: Dissolution profiles of theophylline matrix tablets in biorelevant media A - FaSSGF and B - FaSSIF and C - FaSSCoF.

The drug release was overall poorer in FaSSCoF media having a basic pH value of 7.8 (Figure 22 C). The model drug TH is considered a weak base (pK_a 8.6) [128], hence the aqueous solubility decreases with increasing pH of the solvent.

In summary, I-CAR showed very fast dissolution and high variability within the data set in the release profiles, particularly measured in FaSSIF and FaSSCoF. Similarly, CMC Na also showed fast dissolution in FaSSGF, thus there is a likelihood of rapid drug release in the proximal colon, where acidic pH values are often encountered, especially in the active phases of ulcerative colitis [16]. Based on the dissolution profiles, the polymers I-CAR and CMC Na were excluded from further studies. In opposite, HPMC and GG matrices showed good, promising properties.

Mucoadhesion behaviour of theophylline pure polymer matrix tablets

The formation of adhesive bonds between a polymer and the mucus layer requires first hydration and swelling of polymer surface [129]. It is influenced by the polymer structure, its hydration and hydrogen-bonding capacity leading finally the to different speed of adhesion [80,130]. Mucoadhesion may also be influenced by viscosity due to the better spreading and interpenetration of the gel with the mucin chains [130]. However, there is no direct relationship.

To evaluate the potential interaction between the matrix tablets based on GG and HPMC, their mucoadhesive properties were evaluated based on the measurement of mucoadhesive force required to detach the tablet from a mucin layer and by the time to wash-away tablet from this layer. The tablet was first hydrated in biorelevant media allowing a sufficient time to contact with an adhesive mucin layer as described in chapter 7.2.5.2. The results are summarized in Figure 23.

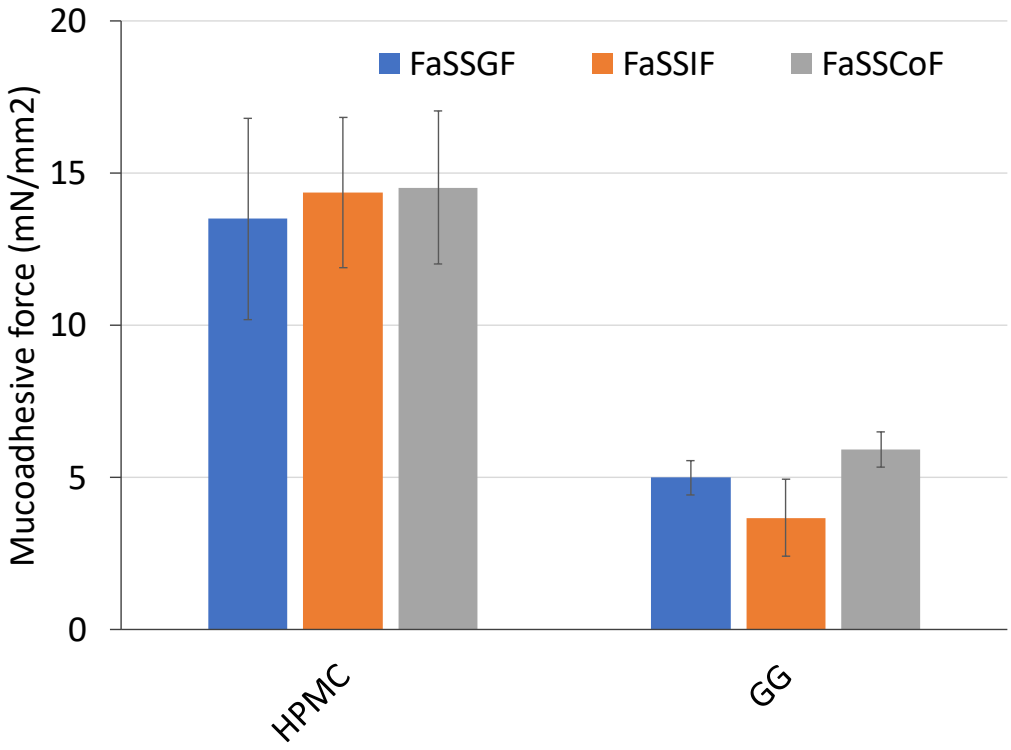

Figure 23: Comparison of mucoadhesion force for hydroxypropyl methylcellulose (HPMC) and guar gum (GG) matrix tablets in biorelevant media FaSSGF, FaSSIF and FaSSCoF.

GG and HPMC are both a long-chained, polymers; the mechanism of adhesive bond formation has been attributed to chain flexibility, which allows effective interpenetration with mucin components as well as the hydrophilic functional groups, primarily hydroxyl groups which facilitate the formation of hydrogen bonds with mucin [116,127]. The results revealed overall a lower mucoadhesion strength of GG in all media compared to HPMC (Figure 23). In this case, the lower mucoadhesion strength (let us recall that the force necessary to detach surfaces is estimated by the careful addition of the weights to the balance) may be explained by the initial fast swelling of GG as commented above, leading to overhydration and surface erosion. Although hydration is necessary for the relaxation and interpenetration of polymer chains with mucin, excess hydration could lead to decreased mucoadhesion and easy

detachment from the mucin surface due to the formation of a slippery mucilage [131].

Regarding the influence of the media pH on mucoadhesion, the mucoadhesive strength of the tablets increased based on the increase in pH in the rank order FaSSGF < FaSSIF < FaSSCoF for HPMC while the lowest force was observed in FaSSIF for GG. However, it should be noted that the measurement of the force to detach the adhered tablet form the mucin surface is strongly dependent on the careful manipulation by operator and shows some level of data variability.

Apart from the force to detach the tablet, the **mucoadhesion time** was also measured by modified disintegration apparatus simulating the transit time in GIT. The tablets were attached to a mucin surface spread onto a plastic slab. The slab was submerged in the water medium, then moved up and down and tablets were monitored for their detachment or washing off. Unfortunately, even after 24 h, all tablets were found still attached to the mucin layer. This implies that the *in vitro* residence time did not directly correlate with the mucoadhesion strength; however, the residence time *in vivo* may also be influenced by other factors. Based on this observation, both GG and HPMC were considered for further studies.

8.2.3 Characterization of binary polymer mixtures

Although the tested polymers individually showed desirable properties in terms of swelling, prolonged drug release and sufficient mucoadhesion strength, it has been frequently reported that a combination of polymers, may exhibit even higher functionality due to the interactions [80,132,133]. The specific properties of the polymers required for the present work was not only gel formation to prolong the drug release, but also the possibility to strengthen the mucoadhesion bond, especially in the colonic region. Therefore, a binary mixture of HPMC and GG in different ratios were evaluated to observe how their combination is affecting all parameters

including the viscosity, and consequently, the swelling, drug release and mucoadhesion of prepared tablets.

An experimental design based on the principle of Central Composite design (CCD) was implemented to determine the influence of polymer ratio, media volume and media pH on the viscosity. This step was done in,collaboration with Mgr. Julia Marushka. For three different media either FaSSGF, FaSSIF and FaSSCoF of pH 1.6, 6.5 and 7.8, respectively, three separate CCDs were used with five levels of polymer mixtures (GG and HPMC) ranging from 0 to 100 % in combination with five levels of media volume in the range from 12.5 to 50 mL. The experimental runs were performed at all 30 possible combinations of the factors. The two independent factors, polymer mixture X_1 and media volume X_2 were varied as required via experimental design (Table 12). The highest and lowest levels of X_1 and X_2 were determined based on results of the preliminary measurements and the rheological data of the individual polymer solutions. The response variable (viscosity) was calculated according to second-order response surface models (Equation 6) and the data were also subjected to 3D response surface plots to visualise the influence of the factors (Figure 24).

$$\gamma = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 + e \quad (6)$$

In the given equation, y is the expected value for the response, X_1 and X_2 as mentioned above and β denotes regression coefficients representing the intercept (β_0), the main (β_1, β_2), quadratic (β_{11}, β_{22}) and the interaction (β_{12}) effects, while the term, e, represents the error of the model [134].

Table 12: The structure of experimental runs according to the CCD with coded and natural values for variables X_1 (polymer mixture), X_2 (media volume) and the measured results of viscosity (Pa·s) in each of the 10 runs performed for FaSSGF, FaSSIF and FaSSCoF media. Note that the order in the mixture is GG/HPMC.

Levels X_1	**Polymer mixture (%)**	**Levels** X_2	**Media volume (mL)**	*FASSGF*	*FASSIF*	*FASSCoF*
(+1)	85.4/14.6	(+1)	15	513.41	242.74	311.49
(+1)	85.4/14.6	(-1)	30	115.22	68.14	101.39
(-1)	14.6/85.4	(+1)	15	395.18	350.47	345.42
(-1)	14.6/85.4	(-1)	30	50.90	63.13	73.16
(0)	50/50	(0)	20	226.09	121.37	255.59
(0)	50/50	(0)	20	226.09	121.37	255.59
(1.414)	100/0	(0)	20	275.91	216.41	245.87
(-1.414)	0/100	(0)	20	191.74	241.16	191.99
(0)	50/50	(1.414)	12.5	748.40	380.38	538.14
(0)	50/50	(-1.414)	50	11.88	12.29	15.08

Upon careful examination of the graphical representations of the results (Figure 24), it becomes evident that neither the types of polymers (GG or HPMC) nor their respective ratios exert a discernible influence on viscosity. Instead, the primary factor influencing viscosity appears to be the nature of the media. In conclusion, the analysis reveals that the pH value emerges as the predominant factor influencing the observed outcomes in the binary mixtures. This observation corresponds to the data in Figure 20 for pure polymers.

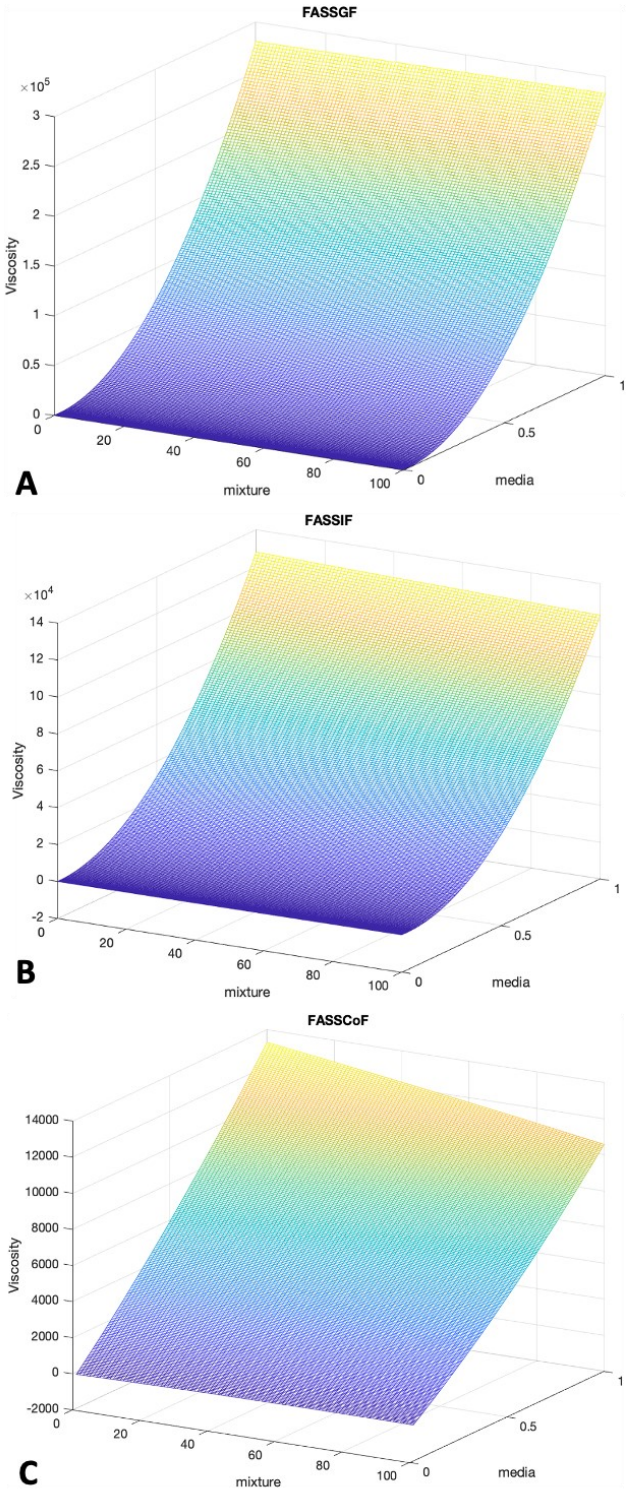

Figure 24: Response surface plots representing the influence of ratio of polymer mixture and volume of media on viscosity measured in A - FaSSGF, B - FaSSIF and C - FaSSCoF.

8.2.4 Characterization of theophylline matrix tablets made of binary polymer mixtures

The use of mixtures of polymers may represent a potential way of achieving required swelling, drug release and mucoadhesive properties. The tablets were compressed using a well soluble theophylline as a model drug again.

Swelling behaviour

In the previous chapters, swelling behaviour of the tablets made of pure individual polymers was already discussed. It was shown that GG swelled rapidly, underwent a partial surface erosion and then experienced another phase of swelling due to hydration of the deeper layers of the matrix core. HPMC on the other hand, shows a gradual consistent swelling. The influence of GG on the swelling of HPMC is shown in Figure 25. If the portion of HPMC is high (mixture 14.6/85.4 and even 50/50), the addition of GG did not influence the swelling and the properties of HPMC dominated showing similar behaviour as HPMC itself in all tested media. A higher content of GG in the matrix (85.4/14.6) led to a swelling profile resembling GG, with a high swelling in 15 min followed by a surface erosion in all tested media again.

From the profile detected, no burst effect is expected in advance for the 14.6/85.4 and the 50.50 mixtures regarding the drug release, while the 85.4/14.6 mixtures should follow the same pattern as previously observed for GG.

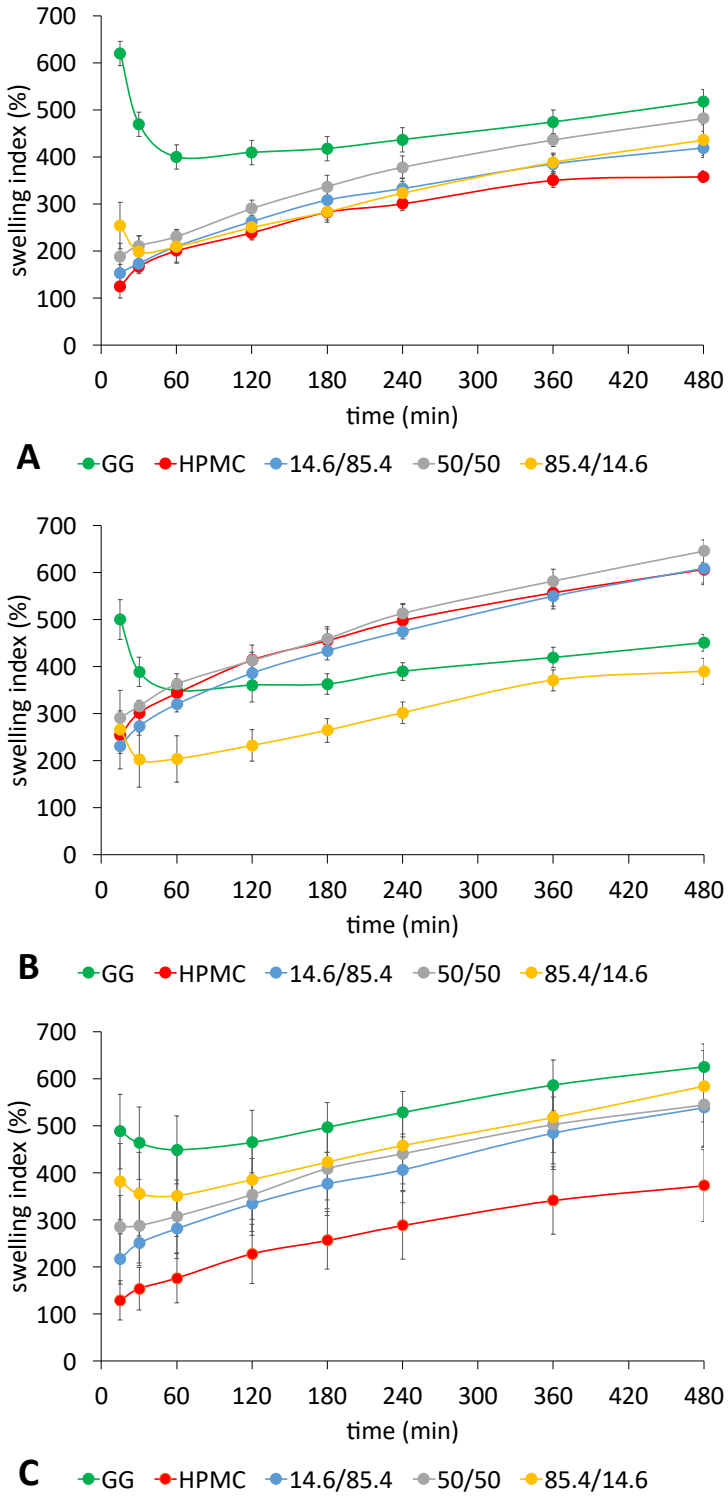

Figure 25: Swelling index of matrix tablets based on polymer mixtures in A - FaSSGF, B - FaSSIF and C - FaSSCoF.

Theophylline release

The in vitro drug release of the polymers in different concentrations is shown in Figure 26.

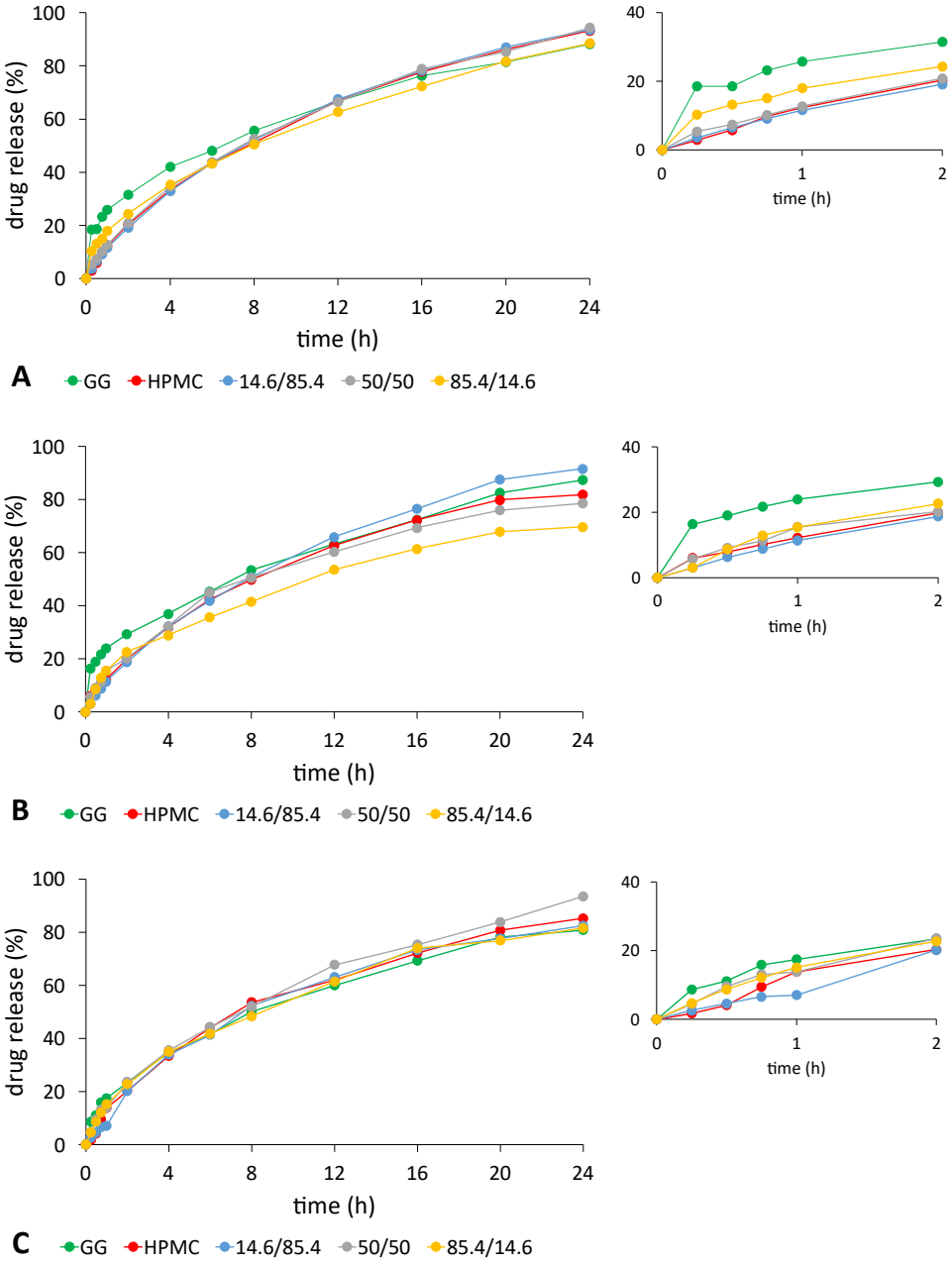

Figure 26: Dissolution profiles of matrix tablets based on polymer mixtures in A - FaSSGF, B - FaSSIF and C - FaSSCoF. The inserts on the right side represent a zoom of the 2 h time interval.

It can be seen from the TH release profiles in three biorelevant media, that the polymer combinations mirror the release behaviour noted when the individual pure polymers were used. With the addition of GG to HPMC in smaller (14.6/85.4) and equal amount (50/50), the influence of HPMC on the drug release remained dominant. The shape of the cumulative concentration curve was pH independent, however, the highest amount of TH release was detected in FaSSGF in agreement with the results for the pure polymer tablets (Figure 22). However, upon the highest addition of GG (85.4/14.6), the initial stage of the release profiles resembles a slight burst effect especially in FaSSGF where the drug exhibits a higher solubility. This is clearly shown by the yellow line in detail zoom views.

Therefore, it can be concluded that appropriate balancing between the concentration of polymers in the matrix may contribute to better results for the optimization of prolonged release of a drug while achieving the wanted burst effect with an initial drug dose. However, it should be noted, that the expected drug CyA has different properties than TH.

Mucoadhesion strength

The mucoadhesion strength for the binary mixtures of the polymers are shown in Figure 27; data for pure polymers are included for clarity. The lower mucoadhesion strength of GG compared to HPMC was ascribed to its high and fast initial swelling and possible surface erosion, likely resulting to overhydration and easy detachment from the mucin layer. Therefore, Figure 21 confirms the expectable behaviour of tablets; the mucoadhesion force directly relates to the amount of GG in the mixture regardless of the medium used. The increasing amount of GG leads to a decreased mucoadhesion in general.

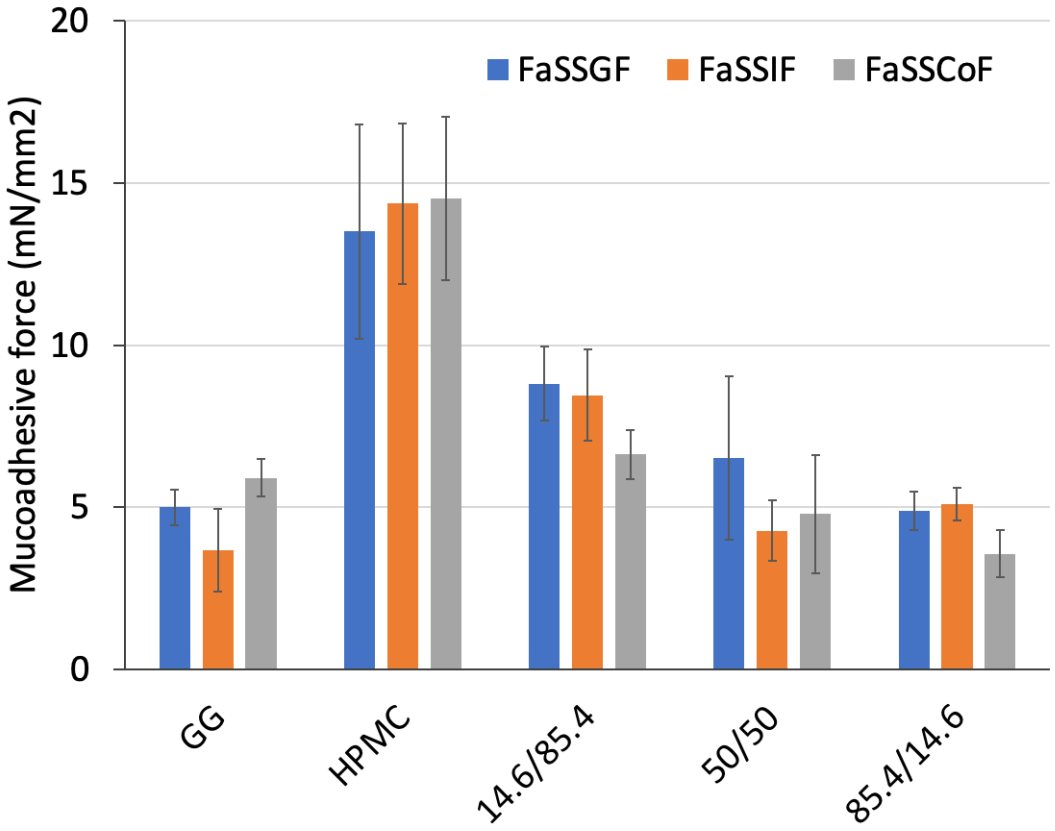

Figure 27: Mucoadhesion force of polymer mixtures guar gum (GG) and hydroxypropyl methylcellulose (HPMC) in biorelevant media. Note that the order in the mixture is GG/HPMC.

The results were presented in the following conference:

OGADAH, C., SKLUBALOVA, Z., VRANIKOVA, B.: Biorelevant dissolution testing of matrix tablets based on combination of mucoadhesive polymers. 3rd Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Science. Szeged, Hungary, 20 – 22 January 2021. (ONLINE ORAL PRESENTATION)

OGADAH, C., VRANIKOVA, B., MARUSHKA J., SKLUBALOVA, Z.: Effect of polymer combinations on the dissolution profiles of a model drug in biorelevant media. 11th Postgraduate and Postdoc Conference, Faculty of Pharmacy in Hradec Králové, Czech Republic, 1 – 2. February 2021 (ORAL PRESENTATION).

8.2.4.1 Influence of mesoporous carrier for liquisolid systems on the properties of the mucoadhesive matrix tablets

It was stated that the strategy of incorporating the system with a sufficient concentration of a drug in a liquid state, such as the LSS, into a mucoadhesive polymer core matrix to achieve the prolonged release drug delivery system is required for retaining the dosage form at the colon mucosa to maintain adequate drug level, minimize its high systemic absorption and limit possible side effects. Some authors *e.g.,* Elkhodairy et al. [1] have previously attempted to formulate a poorly soluble drug into LSS by using polysaccharides as the carriers. However, the major problem encountered with the preparations was that the LSS had poor flow characteristics which limited their formulation into tablet dosage form. The final chapter of this thesis, therefore, aims to the combination of both suitable formulation approaches developed and described above.

To understand the influence of the highly sorptive carrier NEU having the good flow properties on the polymer matrix behaviour, the matrix tablets were prepared by incorporating NEU in different concentrations (simulating the prepared LSS system for CyA) in a preliminary experiment. The pure polymers commented in previous chapters, HPMC or GG, were used and the swelling and mucoadhesive properties of the tablets were evaluated.

Influence of NEU on swelling behaviour

As previously discussed, (see Chapter 8.2.2), the different swelling properties were detected for hydrophilic GG and HPMC. While GG partially erodes at surface of the matrix tablet, HPMC readily hydrates upon contact with aqueous medium showing a slow uniform swelling. The resulting viscous gel layer controls the drug release. On the other hand, NEU is an insoluble mesoporous material excipient possessing a high water absorption capacity [68,135], but with no swelling ability. Since the swelling of the polymer depends on the rate of penetration of fluid into the matrix, the evaluation of the swelling index upon the incorporation of different concentrations of NEU into the matrices was performed to better understand the influence of this

material on the water uptake into the matrix and possibly predict the drug release behaviour from the prepared formulations.

The tablet matrix prepared from the pure polymers, either HPMC or GG; the results are illustrated in one figure to clearly show the differences in polymer matrix behaviour. (Figure 28)An initial increase in SI and later disintegration and erosion were observed for NEU itself (NEU 100), regardless of the media. This observation may possibly be related to the capillary suction of the medium into the pores of NEU [136].

Comparing the swelling of HPMC matrix (Figure 28, left side) with the GG matrix (Figure 28, right side), a negligible influence of NEU addition on the swelling behaviour of HPMC was noted. On the other hand, the incorporation of NEU into GG matrices had a major disruptive effect and generally led to a dramatic increase in the initial swelling followed by erosion or disintegration. Moreover, the matrix behaviour was more affected by the pH value (medium used) in NEU/GG mixtures.

Due to the fast swelling of the matrices consisting of GG and NEU, the formation of a coherent gel layer was interrupted. Additionally, NEU probably increased the porosity of the matrix gel layer such that the physical separation of the polymer occurred, resulting in disintegration. This observation agrees with the scientific literature, stating that that a poorly formed gel, in which the gel layer is disrupted or incompletely formed, will lead to rapid penetration of the medium, which in more extreme cases would lead to the premature disintegration of the matrix [137].

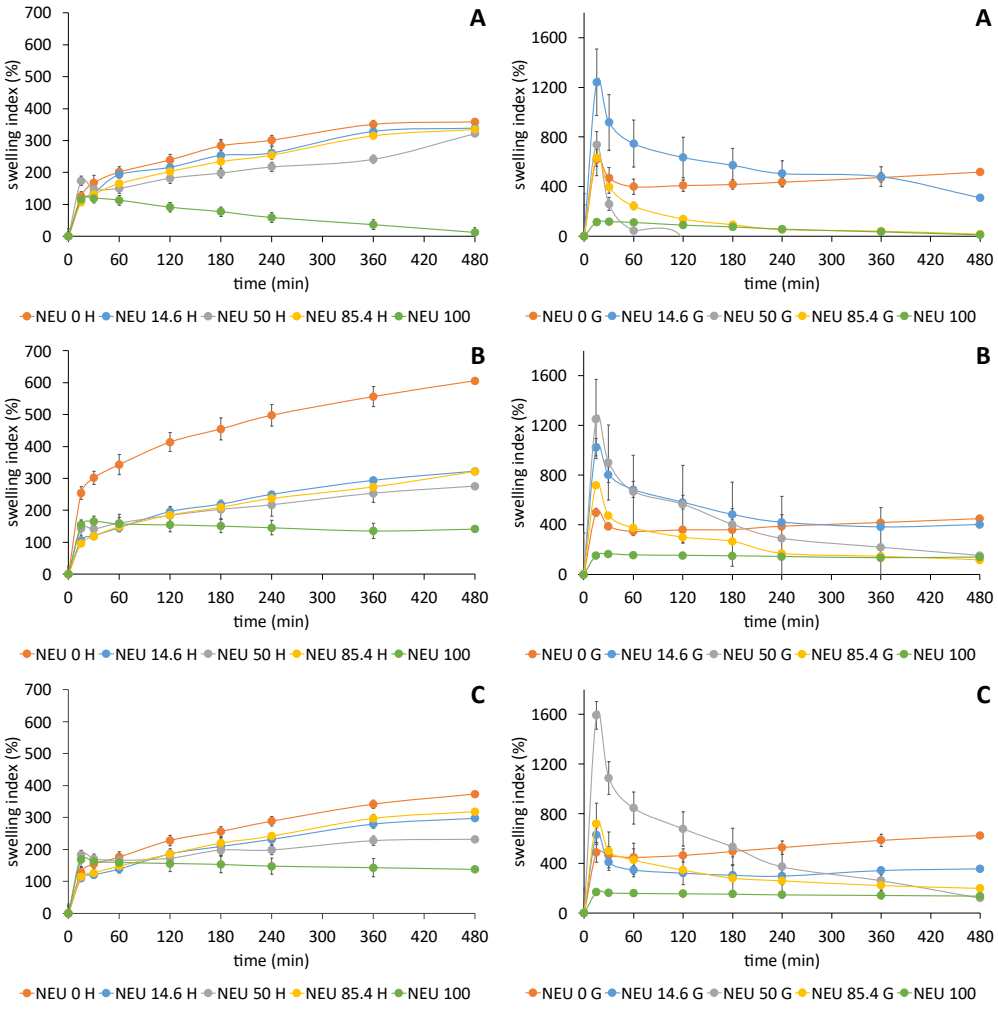

Figure 28: Influence of Neusilin® US2 (NEU) on hydroxypropylmethyl cellulose (HPMC) and guar gum (GG) swelling in biorelevant media A - FaSSGF, B - FaSSIF and C - FaSSCoF. The number represents NEU concentration in the tablet. HPMC - H (left side) or GG - G (right side).

Influence of NEU on mucoadhesion behaviour

Neusilin is generally not considered a mucoadhesive substance on its own. In this preliminary observation it was confirmed (NEU 100) in all used biorelevant media (Figure 29). The result is attributed to NEU's polar components (*e.g.,* silanol groups) likely leading to some degree of interaction with mucin via hydrogen bonding. In opposite, HPMC shows a high mucoadhesion effect, whereas GG a moderate one. The influence of different concentrations of NEU on the mucoadhesion properties of the polymers is shown in Figure 29 A and B. For HPMC matrix, the low NEU

addition did not impact polymer mucoadhesion largely showing that HPMC effect still dominates. The same was noted in the case of GG in this NEU concentration (Figure 29 B). In a high concentration (NEU 85.4 H), mucoadhesion force was decreased for HPMC matrix apart from FaSSGF while the comparable behaviour to GG itself was observed for GG matrix with slight variations.

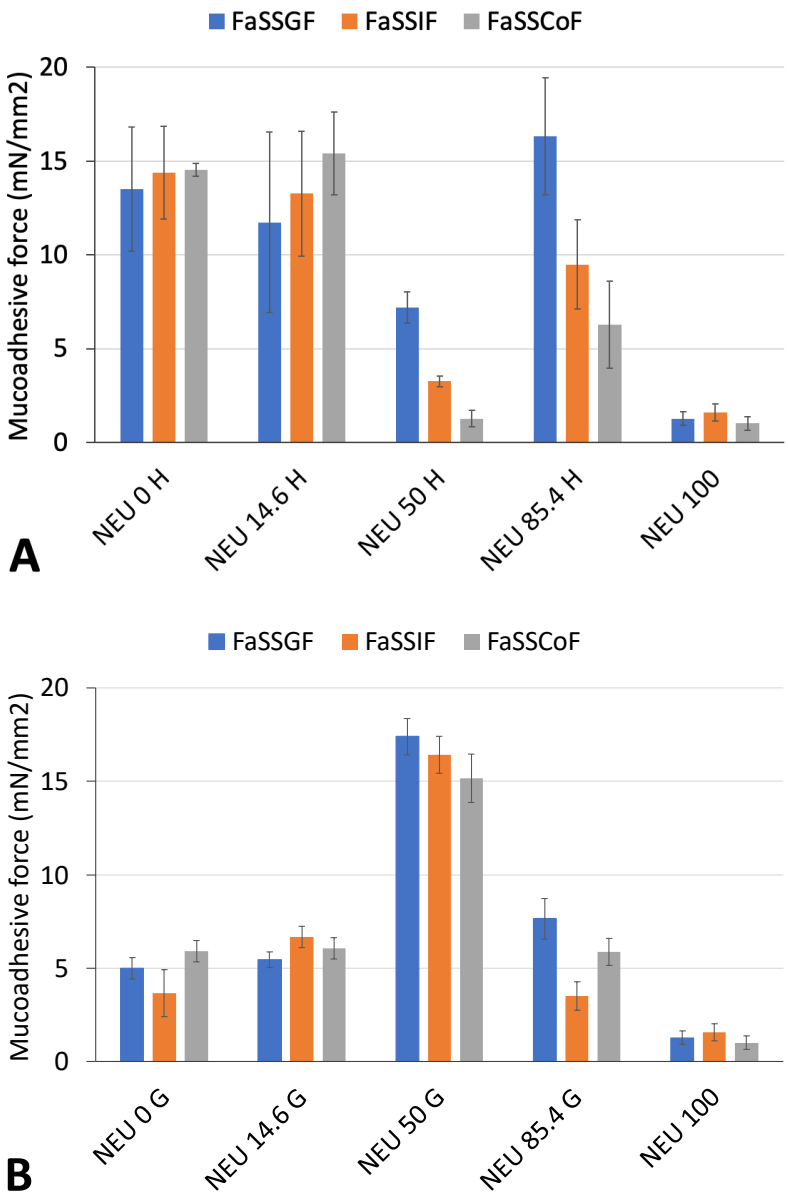

Figure 29: Influence of Neusilin®US2 (NEU) on the mucoadhesion of A - Hydroxypropyl methylcellulose (HPMC or H) and B - Guar gum (GG or G) in biorelevant media FaSSGF, FaSSIF and FaSSCoF.

Surprisingly, both formulations with the equal amount of NEU and polymer (NEU 50 H of NEU 50G), showed an atypical behaviour when compared to other samples, with lower mucoadhesion for HPMC and much higher for GG. This effect is hard to explain based on preliminary observations. However, the measurement of the detachment force is strongly dependent on the careful manipulation with the instrument by the operator as previously mentioned. The unexpected findings for NEU 50 H and NEU 50 G might be related to some experimental errors and should be confirmed in further experiment.

In conclusion, a more detailed study is necessary to achieve the detail view on how the LSS drug delivery system influences the properties of polymer matrix and well as the drug release which was not studied in this experimental step. Based on the results, it seems that the HPMC matrices containing NEU swell still slowly and will show a similar drug release profiles as the matrices containing the polymer alone, being more beneficial for prolonged release. Contrary, a higher burst effect would be achieved in the case of GG. However, if an initial fast release from the matrices is required, then a small portion of GG could be incorporated in the final matrices.

9 Conclusions

This experimental thesis expands the knowledge in the research area of solubility improvement of poorly soluble drug cyclosporine A (CyA) by the preparation of liquisolid system (LSS), specifically aimed to the colon-targeted drug delivery in combination with a swellable, mucoadhesive matrix core. The efficiency of LSS was compared to the binary mixtures of a drug and a carrier prepared by the co-milling technique.

The results of the preformulation/formulation studies regarding first, the development of the efficient dosage system to improve solubility of CyA, and second, the development of a suitable matrix core, can be summarized in the following outlines:

1. The development of the efficient dosage system to improve solubility of CyA

1.1 The angle of slide and the flowable liquid retention potential (ϕ-value) are suitable parameters for evaluation of a carrier capacity to load a sufficient amount of liquid in the preparation of LSS. When the other flowability parameters such as the flow rate through a hopper orifice, AOR, HR, and CI are considered, these parameters allow to clearly specify the optimum carrier with a high capacity for the selected solvent. Out of three high surface area carriers, Neusilin® US2 (NEU), Aeroperl® 300 (AER), and Syloid® 244 FP (SYL), the highest ϕ-value 1.06 was detected for NEU when PEG 200 was used as a model solvent, implying that 1 g of NEU could retain the same mass of PEG 200. In conclusion, NEU was selected as the most suitable carrier as it showed the best properties in terms of its ability to absorb a high amount of solvent while remaining acceptable flowability.

1.2 Based on the solubility studies conducted to identify the most efficient non-volatile solvent for cyclosporine A, polyethylene glycol 200 (PEG 200), polyethylene glycol 400 (PEG 400), propylene glycol (PG), polysorbate 80 (PS 80), and Transcutol® HP (TRC-HP), the highest solubility of CyA in the latter was detected. The calculated ϕ-value showed that 1 g of NEU could retain 0.8 g of TRC-HP. The detail dissolution experiment evaluating the CyA release from the NEU

liquisolid systems prepared based on the above-mentioned solvents and filled into capsules allowed to confirm the most efficient CyA release profiles for TRC-HP based LSS systems even in the three biorelevant media of different pH value, FaSSGF (pH 1.6), FaSSIF (pH 6.5), and FaSSCoF (pH 7.8).

1.3. Using circumscribed central composite design, the effect of the milling factors: speed, time, and ball size on the particle size corresponding to the 90 % of cumulative distribution was optimized for five substances out of twenty-four milled to reach the expected level of particle size x_{90}. Even though NEU did not belong to this group, adequate milling properties were confirmed by comparing the granulometric properties before and after milling in a ball mill showing its promising use also for the preparation of co-milled binary mixtures with a drug.

1.4. Finally, the efficiency of two preparation methods, formulation of LSS and co-milling, using two mesoporous carriers, either NEU or functionalized calcium carbonate (FCC), was evaluated. Although both carriers increased the CyA release, NEU-based LSS demonstrated a higher liquid sorption capacity, due to its considerably higher specific surface area and pore volume compared to functionalized calcium carbonate; even 50 % w/w loading of TRC-HP based CyA solution was possible. The beneficial CyA release was observed regarding the future preparation of formulation for colon targeting with the fast first phase of the drug dissolution from the surface following with the slower release due to drug located in the deeper pores. Also, the co-milled mixtures showed a high drug release by preventing the cohesive particle interactions and the possible formation of agglomerates of the drug, allowing the improved wetting due to NEU hydrophilicity. In conclusion, the results indicate that the increase in the dissolution rate of CyA was achieved by both preparation methods, either LSS or co-milling, however, by the pre-dissolving of CyA in the non-volatile solvent, LSS showed faster release. Moreover, the amorphous CyA remained unchanged for 20 weeks as confirmed by X-ray powder diffraction.

2. The development of a matrix core for LSS colon targeting of CyA

2.1. The selection of the swellable and mucoadhesive polymer excipient for the matrix core was based on the measurement of viscosity in biorelevant media FaSSGF, FaSSIF and FaSSCoF, the volume of which ranged from 10 to 50 mL to simulate various degree of hydration to erosion. Out of four cellulose derivatives and three polysaccharides, five excipients carboxymethylcellulose sodium (CMC Na), hydroxypropyl methylcellulose (HPMC) K15M, hydroxyethyl cellulose (HEC), guar gum (GG), and iota carrageenan (I-CAR) were used to prepare the matrix tablet of similar hardness by direct compression for further studies.

2.2. Out of five polymers, the HPMC and GG based matrix tablets showed the best properties regarding the swelling and mucoadhesion estimated by the adhesion force needed to detach a tablet from the mucus layer. Using a freely water-soluble model drug theophylline (TH), the acceptable drug release profile was also achieved. These substances could be used in further studies.

2.3. Viscosity of the binary mixtures of GG and HPMC of different ratios, was predominantly influenced by pH, as proved by central composite design for the biorelevant media used. The swelling and mucoadhesive properties of tablets prepared using these mixtures showed the predominant effect of HPMC to achieve the prolonged drug release of TH while GG showed mainly surface degradation potentially important in achieving burst release..

2.4. Influence of the mesoporous carrier NEU for liquisolid systems on the properties of the mucoadhesive matrix tablets was not completely clarified in this thesis and a more detailed study is necessary in future, however, some general conclusion can be generated. Based on the results, it seems that the HPMC matrices containing NEU swell still slowly and will show a similar drug release profiles as the matrices containing the polymer alone, being more beneficial for prolonged release. Contrary, a higher burst effect would be achieved in the case of GG if an initial fast release from the matrix is required.

10 Research outputs

10.1 Original articles in impact Journals

OGADAH C., MRŠTNÁ K., MATYSOVÁ L., MÜLLERTZ A., RADES T., NIEDERQUELL A., ŠKLUBALOVÁ Z., VRANÍKOVÁ B. Comparison of the liquisolid technique and co-milling for loading of a poorly soluble drug in inorganic porous excipients. *International Journal of Pharmaceutics*, 2024, vol. 650. 123702
Author's contribution: Experimental work, data processing, writing, revision and editing of original manuscript.

MARUSHKA J., BROKEŠOVÁ J., OGADAH C., KAZEMI A., DUINTJER TEBBENS J., ŠKLUBALOVÁ Z. Milling of pharmaceutical powder carrier excipients: Application of central composite design. *Advanced Powder Technology*, 2022, vol. 33. ISSN 0921-8831
Author's contribution: Experimental work and data processing, manuscript review and editing.

10.2 Poster / oral presentations

OGADAH, C., ŠKLUBALOVÁ, Z., MÜLLERTZ, A., RADES, T., VRANÍKOVÁ, B.: Comparison of methods of drug loading in inorganic porous excipients. Improving Drug solubility: Recent Advances in Pharmaceutical Technology. Faculty of Pharmacy in Hradec Králové, Czech Republic, 6 – 7 February 2023. (POSTER PRESENTATION)
OGADAH, C., ŠKLUBALOVÁ, Z., MÜLLERTZ, A., RADES, T., VRANÍKOVÁ, B.: Comparison of the liquisolid technique and co-milling for loading of a poorly soluble drug in inorganic porous excipients. 13th Postgraduate and Postdoc Conference, Faculty of Pharmacy in Hradec Králové, Czech Republic, 1 – 2. February 2023. (ORAL PRESENTATION)
OGADAH, C., VRANÍKOVÁ, B., MRŠTNÁ K., ŠKLUBALOVÁ, Z.: Improving the solubility and dissolution rate of cyclosporine A by the liquisolid technique. 12th

Postgraduate and Postdoc Conference, Faculty of Pharmacy in Hradec Králové, Czech Republic, 1 – 2. February 2022. (ORAL PRESENTATION)

OGADAH, C., VRANIKOVA, B., SKLUBALOVA, Z.: Characterization of rheological properties of polymers with respect to colon-targeted delivery of liquisolid tablets. 13th Central European Symposium on Pharmaceutical Technology. Gdansk, Poland, 16 – 18 September 2021. (VIRTUAL POSTER PRESENTATION)

OGADAH, C., VRANIKOVA, B., MARUSHKA J., SKLUBALOVA, Z.: Effect of polymer combinations on the dissolution profiles of a model drug in biorelevant media. 11th Postgraduate and Postdoc Conference, Faculty of Pharmacy in Hradec Králové, Czech Republic, 27 – 28 January 2021. (ORAL PRESENTATION)

OGADAH, C., SKLUBALOVA, Z., VRANIKOVA, B.: Biorelevant dissolution testing of matrix tablets based on combination of mucoadhesive polymers. 3rd Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Science. Szeged, Hungary, 20 – 22 January 2021. (ONLINE ORAL PRESENTATION)

OGADAH, C., VRANIKOVA, B., SKLUBALOVA, Z.: Characterization of rheological properties of polymers for formulation of liquisolid systems targeted to the colon. 10th Postgraduate and Postdoc Conference, Faculty of Pharmacy in Hradec Králové, Czech Republic, 22 – 23 January 2020. (ORAL PRESENTATION)

10.3 Grants and internship

Grant agency of the Charles University (GAUK); Grant number: 70119/2019; Title of project: Development of colon-targeted liquisolid systems for the therapy of inflammatory bowel diseases, 2019 – 2021

6-month research internship; Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark. Supervised by Prof. Anette Müllertz and Prof. Thomas Rades. Funded by Rector's Mobility Fund; Grant number: FM/c/2021-1-028, co-funded by GAUK; Grant number: 70119/2019 and Internationalization fund; October 2021 – April 2022

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