DOCTORAL (Ph.D.) DISSERTATION

Preformulation studies and optimization of floating drug delivery systems based on pharmaceutical technological and biopharmaceutical parameters

Design of modified drug delivery systems

Dr. Péter Diós

University of Pécs, Faculty of Medicine

Institute of Pharmaceutical Technology and Biopharmacy

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Dr. Péter Diós

Head of the Doctoral School:

Dr. Erika Pintér

Head of Program:

Dr. Lajos Botz

Supervisior:

Dr. Attila Dévay

Founder and first director of

the Institute of Pharmaceutical Technology and Biopharmacy

University of Pécs, Faculty of Medicine

Pécs

2015

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Preformulation studies and optimization of sodium alginate based floating drug delivery system for eradication of *Helicobacter pylori*
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IV. **P. Diós**, F. Budán, S. Nagy, I. Horváth, K. Szigeti, D. Máthé, A. Dévay:
Nátrium-alginát alapú effervescens úszótabletták *in vitro* és *in vivo* gyógyszertechnológiai és biofarmáciai vizsgálata és optimalizálása (oral presentation, poster presentation, I. prize)
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Új távlatok – technológiai áttöréseken keresztül: úszó effervescens tabletták *in vivo* hatóanyag kioldódás vizsgálata Röntgen-CT-vel (poster presentation)
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VII. **P. Diós,** S. Nagy, V. Bognár, Sz. Pál, A. Dévay:
Hidrofil mátrixképző polimerek alkalmazhatósága effervescens úszó készítményekben (oral presentation)
I. Cholnoky László Szakkollégiumi Szimpózium, Pécs, 2015

VIII. **P. Diós**, S. Nagy, T. Pernecker, Sz. Pál, A. Dévay:
Influence of sodium alginate and low-substituted hydroxylpropyl cellulose quantity in floating behavior and drug release in floating drug delivery systems (poster presentation)
6th BBBB Conference on Pharmaceutical Sciences, Helsinki, 2015

IX. **P. Diós**, F. Budán, S. Nagy, I. Horváth, K. Szigeti, D. Máthé, Sz. Pál, A. Dévay:
Achievement of very high floating force and rapid dissolution of sodium alginate based floating drug delivery systems: *in vitro*, *in vivo* study (poster presentation)
6th BBBB Conference on Pharmaceutical Sciences, Helsinki, 2015

X. **P. Diós**, F. Budán, K. Szigeti, I. Horváth, S. Nagy, T. Pernecker, A. Dévay, G. Gerencsér, Z. Gyöngyi, I. Kiss, Sz. Pál, D. Máthé:
Parameters of floating drug delivery systems - tracked in animal model utilizing *in vivo* X-ray CT imaging (poster presentation)
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XI. D. Máthé, F. Budán, Sz. Pál, I. Kiss, **P. Diós**, K. Szigeti:
X-Ray CT Imaging of Stomach Passage of Contrast-Enhanced Floating Tablets in a New Rat Model (poster presentation)
European Association of Nuclear Medicine Congress, Hamburg, 2015

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List of Abbreviations

ANOVA	Analysis of Variance
API	Active Pharmaceutical Ingredient
$BaSO_4$	Barium sulfate
CAS	Chemical Abstracts Service
CO_2	Carbon dioxide
CV	Coefficient of Variation
DDS	Drug Delivery System
DLVO theory	Derjaguin, Landau, Verwey and Overbeek theory
DOE	Design of Experiments
DTA	Differential Thermal Analysis
EFDDS	Effervescent Floating Drug Delivery System
EUFIC	European Food Information Council
FDA	Food and Drug Administration
FDDS	Floating Drug Delivery System
FOV	Field of View
GIT	Gastrointestinal Tract
GRDDS	Gastroretentive Drug Delivery System
GRT	Gastric Residence Rime
HBS	Hydrodynamically Balanced Systems
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
IST	Isothermal Stress Testing
L-HPC	Low Substituted Hydroxypropyl Cellulose
LUT	Lookup Table
MDDS	Modified Drug Delivery System
MF_OPT	Optimized composition
MMC	Migrating Myoelectric Complex
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate

NEFDDS	Non-Effervescent Floating Drug Delivery System
PTFE	Polytetrafluorethylene
SD	Standard Deviation
SI	International System of Units
VOI	Volume of Interests

List of Symbols

α	Angle of repose
A_f	Filled area
β	Shape parameter of the dissolution curve
C	Concentration
C_i	Carr index
C_s	Solubility of drug in the matrix media
D	Diffusion coefficient
dv_x/dy	Velocity gradient
η_{mucus}	Viscosity of 3 % mucus solution.
η_{total}	Viscosity of mucus/tablet mixture
η_{tabl}	Viscosity of MF_OPT equilibrated to 3 % L-HPC and sodium alginate
f_1	Difference factor
f_2	Similarity factor
F_{detach}	Detachment force
F_{float}	Force expressed vertically upward by a floating tablet
F_{max}	Maximal floating force
$F_{max/100mg}$	Maximal floating force calculated for 100 mg tablet mass
H	Height
H_r	Hausner ratio
HU	Hounsfield Units
K	Proportionality coefficient (rate constant)
n_{diss}	y-intercept
P_{cr}	Crofton perimeter
Ψ	Sphericity
R	Radius
R^2	Regression coefficient
RH	Relative humidity

ρ_{bulk}	Bulk density
ρ_{tapped}	Tapped density
s	Slope of curve
S_i	Swelling index
t	Time
t_0	Lag time of dissolution
t_{lag}	Floating lag time
$t_{F1/2}$	Time required for 50 % of maximal floating force,
t_{Flag}	Lag Time for achieving maximal floating forces
$t_{floating}$	Total floating time
t_{Fmax}	Time needed for maximal floating force,
τ_{yx}	Shear stress
W_0	Initial amount of dissolved drug
W_1, W_2	Tablet weight
W_t	Dissolved drug amount in time
X	Individual factor
γ	Shear rate
Y	Response variable
z	Flow behavior index

1. Introduction

The most frequent application of medicines is the peroral way of administration, which provides easy to take option, relatively low therapeutic cost, various formulations and applicable technologies [1]. Its spread is shown by the fact that more than 50% of commercially available medicines are orally applied preparations [2]. Higher patient compliance may be experienced due to their easy application. Although among the *per os* administered preparations, few are designed with biopharmaceutical aspect meeting with the physiological environment of the dosage forms. While until the 90's not much, however nowadays more frequently modified drug delivery systems are designed containing special excipients and/or manufactured with special technological methods [1]. With novel preparations having controlled release, patient compliance can be increased more, namely multiple daily administrations can be reduced to once a day administration. Another advantage can be a local drug delivery, with which not only the administration of the medicine can be improved, but also the site-specific efficiency of a particular applied active pharmaceutical ingredient (API) may be optimized.

Based on the Dévay's proposal biopharmaceutical classification system of pharmaceutical preparations, the following classes of drug delivery systems can be distinguished [1]:

1. Time controlled systems based on the effect time after their administration and the time interval of effect can be the following:
   1.1. rapid (e.g. solutions, effervescent preparations, fast dissolving or disintegrating tablets),
   1.2. sustained (e.g. extended tablets or tablet implants),
   1.3. delayed (e.g. enteric coated tablets) and,
   1.4. pulsatile drug delivery (e.g. repeated bursts of API dissolution) preparations.
2. Site-controlled systems, which can be:
   2.1. approaches with direct administration of medicine (e.g. directly into muscle or joint) to the target organ or,
   2.2. passive and active targeted drug delivery systems:
      2.2.1. passive targeting: nanotechnological drug delivery systems, which are based on accumulation of the drug in the areas around the tumor due to EPR (enhanced permeability and retention) effect [3],

2.2.2. active targeting: nanotechnological drug delivery systems, which are able to identify target cells (e.g. tumor cells) and to bound and penetrate into them in order to achieve specific effect [3],

3. New types of preparations, i.e. site- and time-controlled systems, the application of the combination of the previously listed two main classes.

The modification of drug release is always performed to achieve a particular therapeutic aim, with which optimized bioavailability of API(s) can be reached by taking the physiological environment into consideration. In the cases of APIs with short elimination half-life, long acting preparations can be designed with the prolongation of API release and absorption. On the other hand, some acute or emergency cases require the possibility of the most rapid effect of the API, which can be developed by the fast API release from preparations.

Modified drug delivery systems (MDDSs) can be classified based on the time and location of drug release. With *per os* administered medicines, the location of drug release in the gastrointestinal tract (GIT) may be in: the mouth (e.g. orodisperse, sublingual, buccal DDSs), the stomach (e.g. floating, expandable DDSs), the small intestine and/or the colon (e.g. intestinosolvent, enterosolvent, colon targeted DDSs). Thus the location of drug release can be controlled with an appropriate modification of the preparation, with which site-controlled systems can be achieved. During drug release in the oral cavity or in the stomach, not only systemic but also local effects may be taken into consideration, while drug release in the small intestine may be expected to be predominantly systemic. In colon-specific therapy, mostly local effects may develop, since absorption is limited/ minimal.

Those modified drug delivery systems, in which the modification is aimed at prolonging the residence time (GRT), are termed gastroretentive drug delivery systems (GRDDS). Via the modification of the time spent in stomach, site- and time-controlled systems may be achieved. Based on the applied technology, gastroretentive systems can be classified into four separate groups:

- expandable -,
- high density -,
- floating -,
- mucoadhesive preparations.

1) Expandable drug delivery systems hinder their transfer through the pylorus with their expansion, swelling via their size without causing gastric obstruction [4, 5].
2) High density drug delivery systems involve formulations of dosage forms having higher average density, than physiological stomach content. Application of high density ingredients are required to use such as barium sulfate (4.50 g/cm³), zinc oxide (5.61 g/cm³), titanium dioxide (4.23 g/cm³). For significant prolongation of GRT, 2.5 g/cm³ average density is necessary [6].
3) Floating drug delivery systems (FDDS) are those preparations, which are capable for buoyancy on the surface of gastric medium after a particular time. The mechanism of flotation depends on the applied technology. During flotation, preparations have bulk density lower than the gastric fluid ($\rho<1.00$ g/cm^3) and can remain buoyant without influencing gastric emptying rate. This results in the prolongation of gastric residence time and better control on drug release.
4) Mucoadhesive drug delivery systems are capable for bioadhesion onto gastric mucosa resulting in sustaining of GRT, which may cause enhancement of drug absorption in a site-specific manner. Special polymers having mucoadhesive ability are indispensable to apply in these systems, which can adhere to the epithelial surface of the stomach [7]. Mucoadhesion may be an approach, which can be combined with former mentioned technologies in order to achieve not only physically but also chemically resulted gastric retention.

2. Literature survey

In the case of *per os* administered controlled release dosage forms, overall gastrointestinal transit time is generally between 8-12 hours, which makes creating once daily drug formulations more difficult [8]. Gastrointestinal transit time may vary based on two main significant physiological parameters: short GRT and unpredictable gastric emptying. Additionally, the latter is influenced by many idiosyncratic factors (e.g. age, race, gender etc.) and several parameters of applied dosage forms as well as the type of dosage forms. Therefore, suitable drug delivery systems should be designed and developed to overcome the former mentioned patient related variables with the application of a suitable and desirably low cost dosage form. Thus in this section, not only principals of floating drug delivery system, and type of flotation based on mechanisms are detailed, but also the physiological and biopharmaceutical factors are highlighted that can have influence on the success of a floating drug delivery systems.

2.1. Principals of floating drug delivery systems

The aim in applying floating preparation is to achieve prolonged GRT. Primary requirements of FDDSs are the following:

- API(s) should be released slowly, thus floating systems behave as reservoir,
- system should maintain lower density, than the physiological density of gastric medium (1.004-1.1010 g/cm^3) [9],
- preparation should form a cohesive barrier [10].

The buoyancy can be formed instantly at particular system with porous structure, while in the most of the cases preparations require certain time to start flotation. After immersion, the preparation is wetted by the gastric medium. Following the wetting, the API could be dissolved and could leave from the preparation via wetted pore system. Depending on the type of the API and the drug release mechanism, specific effect can be achieved, which may be stomach-specific local effect, or prolonged drug release aiming at systemic absorption.

Biopharmaceutical and therapeutic advantages of FDDSs:

1) with dissolution in gastric medium, API gets into absorbable state,

2) fluctuation in plasma concentration of API can be minimized, which has highlighted role in the cases of narrow therapeutic index drugs (e.g. theophylline, warfarin, digoxin, phenytoin),
3) in the case of short intestinal residence, the absorption of APIs may not decrease due to longer gastric retention time,
4) with sustained GRT, low bioavailability of particular APIs could be increased, whose properties can be the following:
    a) acting better locally,
    b) absorbing better in gastric area,
    c) having low solubility in alkaline pH media such as in small intestine,
    d) having rapid absorption in small intestine area,
    e) being absorbed through a short section of the intestinal tract,
    f) inactivating in intestinal tract,
5) reduced dosing frequency.

In addition to the several advantages of floating drug delivery systems, there are some limitations in their applicability.

Biopharmaceutical and therapeutic disadvantages of FDDSs:

1) those APIs could be less applicable:
    a) which cause unwanted effects due to prolonged residence in stomach (e.g. gastric irritants, ulcer promoting materials),
    b) which inactivate or decompose in acidic media,
2) bioavailability of those APIs having high first pass effect decomposition could not be increased (e.g. budesonide),
3) suitable amount of liquid as gastric medium is required for flotation.

At the design of floating preparations, favorable drug delivery system in biopharmaceutical approach can be developed with relatively low manufacturing costs. This is evidenced by a number of floating drug delivery products placed on the market in recent years (e.g. Valrelease®, Madopar® HBS).

2.2. Biopharmaceutical and physiological bases of gastroretentive drug delivery systems

The design of floating preparations is determined by several physiological parameters, from which gastric emptying plays one of the most crucial roles. The emptying of pharmaceutical preparation is a complex process, thus in order to increase the GRT exact and updated knowledge of physiological background is indispensable.

2.2.1. Anatomic structure and physiologic role of the stomach

The stomach is a J-shaped organ localized in the upper left side of the abdomen, whose main role is to store the nutrition temporarily grind it and stir it. After achieving of appropriate particle size, the stomach releases and transfers the gastric content slowly into the small intestines [11]. Predigestion of proteins and peptides also takes place in the stomach.

Anatomically, the stomach can be divided into three main parts: the fundus (fundus), the body of stomach (corpus), and the gastric antrum (antrum). The role of fundus and stomach body is to store the undigested nutrition, while the smooth muscles assist in mixing the gastric content in the antrum region by performing stirring and mixing motions. The end of the gastric antrum is the pylorus.

The structure of stomach is shown at Fig. 1.

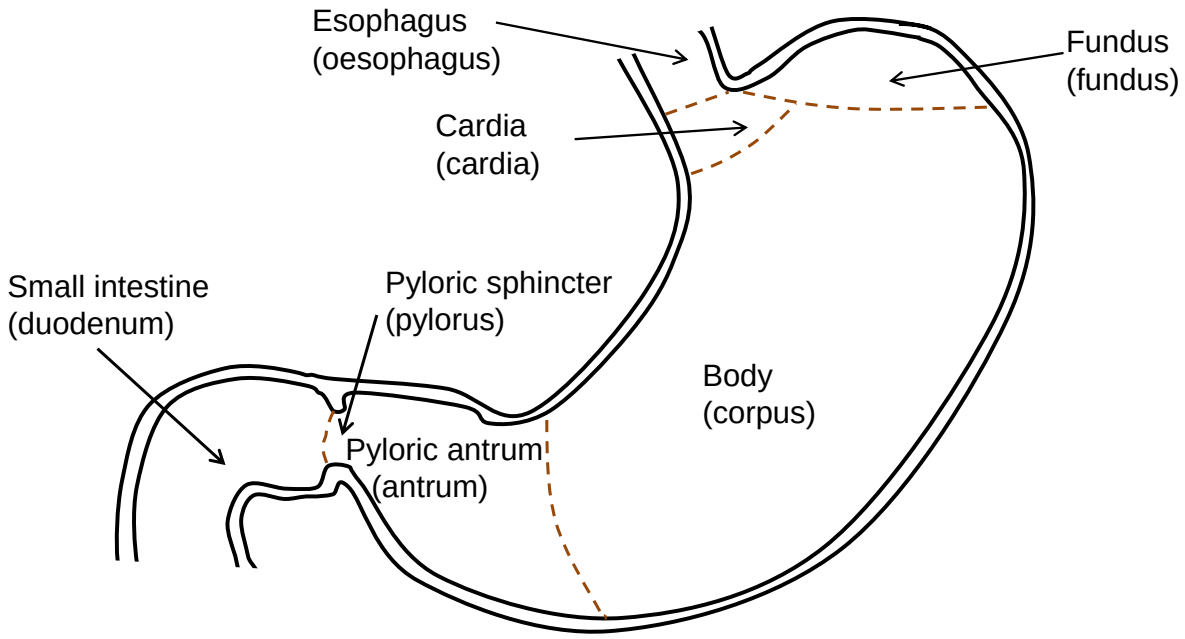

Fig. 1. The structure of the stomach (in bracket the Latin names)

On the surface of the stomach four main types of cells can be distinguished: mucous neck cells (secreting basic mucus gel layer and protecting from gastric acid and shear force), parietal (oxyntic) cells (secreting gastric acid and intrinsic factor), chief (zymogenic) cells (secreting pepsinogen and gastric lipase) and G cells (secreting hormones such as gastrin, histamine, serotonin etc.) [12].

Besides the smooth muscles performing stirring motion, gastric mucosa plays a significant role in protecting of the stomach from gastric acid. Additionally, the mucosae layer could be a possible place for drug delivery by the adhesion of drug delivery system onto it. Epithelia of the gastric mucosae above the connective tissue is single layered, which secrete the mucus directly onto the epithelial surface. The mucus consisting of mucin, glycoproteins, lipids, inorganic salts and water, is a highly hydrated system [13]. Generally mucin creates a gel like, coherent, adhesive structure. The thickness of the mucosal layer is different depending on the location. In the case of gastric mucosae, the layer thickness varies from 50 to 450 μm [14-16].

2.2.2. Parameters influencing gastric motility and emptying

Gastric emptying of pharmaceutical preparations is the process, which depends on applied dosage forms and fasting/fed state. Based on the physiological process, gastric residence time of a preparation may vary from 5 minutes to 2 hours [17].

The gastric motility and emptying are influenced by complex (enteral, sympathetic, parasympathetic) neural and humoral parameters. Among humoral parameters, gastrin and cholecystokinin have prominent role, which cause relaxation of the proximal part of stomach, while cause contraction of distal part concluding gastric emptying. The relaxation or contraction of gastric smooth muscles are determined by the resultant of stimulating and inhibitory signals. Transfer of liquids is done by instant spurting from the stomach, in contrary to solid materials which can be transferred through the pyloric sphincter only after having reached 1-2 mm diameter particle size [18]. Functionally two states of the stomach can be distinguished: the fasting and the fed state. The pH values of states are fundamentally different. Fasting pH is generally between 1.2 and 2.0, while in fed condition it can rise to pH 6 [19]. The increase of pH at fed state is primarily depends on consumed nutrition. At consumption of high amount of liquid, pH of gastric medium can increase to 6-9.

In both states, stomach has motility motions needed to gastric emptying, though the motility pattern differs significantly. In fasting condition, interdigestive electrical events occur

cyclically in every 2-3 hours between two fed states. This phenomenon is called “Interdigestive myoelectric cycle” or “Migrating myoelectric complex” (MMC).
The MMC cycles can be divided into four phases [20]:

- PHASE I: This is a quiescent phase with infrequent contractions lasting form 30 to 60 minutes.
- PHASE II: This section has varying action potentials and repeated contractions, in which the intensity and frequency of the contractions gradually rise. This phase lasts form 20-40 minutes.
- PHASE III: In this section, regular short-term, intense contractions occur lasting for 4-6 minutes. In this phase, the contractions transfer the yet undigested food to the small intestine through the pylorus (‘housekeeper’ wave).
- PHASE IV: The fourth phase is a transient section of MMC between PHASE III and the newly emerging PHASE I.

At fed state, gastric emptying is slowed, hence the beginning of MMC shifts.
In physiological cases, GRT depends on many factors, but the main important influencing factors are the following:

- viscosity -,
- volume -,
- and energy content of the gastric medium.

Several biological factors may have significant role related to gastric emptying and motility.

These idiosyncratic biological factors can be the following:

- gender – gastric emptying in females is slower than in males without the consideration of weight, height and body surface [21],
- posture – in upright position, floating dosage form is protected from postprandial emptying, because it floats above gastric content independently from its size [22], while in supine position there is no protection from early and erratic emptying due to the fact that the floating dosage form can be located anywhere in the longitudinal section of the stomach so that the peristaltic movement may forward it easily [21, 23],
- age – in elderly people (especially over 70 years) low gastric emptying was observed compared to youngers, [21],

- body mass index (BMI) [24],
- physical activity,
- diseases (e.g. Crohn disease, diabetes) and/or medical conditions such as depression which decreases or stressed condition which increases the rate of gastric emptying [8].

Food and liquid intake has one of the most important roles, namely its present or absence can directly affect gastric emptying. Generally presence of nutrition increases the GRT. Stomach retains nutrition independently from its chemical structure (carbohydrates, proteins, fats) by detecting its energy content. Higher the caloric content is, longer the GRT becomes. In more acidic or in gastric medium having higher osmolarity, the food containing energy is retained longer [24]. Oth et al. reported that frequency of food intake may increase GRT by 400 minutes, when successive meals are taken compared to single meal because of low frequency of MMC related contractions. They studied bilayer floating capsules containing misoprostol [25]. In another study, Iannuccelli *et al.* published 5 hours GRT after a single meal compared to control with 3 hours of gastric retention [26]. The food or beverage intake may also vary the pH, which is the medium for dissolution. Drug release of APIs having pH dependent solubility may vary after consumption of different nutrition.
Temperature of nutrition is an affecting factor as well, since nutrition with higher or lower temperature, than the body temperature is retained and tempered until reaching body temperature [27].

Factors of floating dosage form affecting its gastric emptying:

- density – for buoyancy lower than 1.004 g/cm^3 average density is needed. During hydration, generally the average density of floating dosage form shows to decrease, as a result of development of hydrodynamic equilibrium [28],
- size – materials with size between 1-2 mm are suitable to pass through the pyloric valve, thus generally larger the dosage form longer is expected the GRT [29]. In general, small tablets may leave the stomach within digestive phase, while larger ones are transferred during housekeeping waves.
- shape – dosage forms with tetrahedron or ring shape have better GRT compared to other types [30],
- caloric content,

- single-unit or multiple-unit dosage forms – in the case of single-unit dosage forms, unintentional (‘all-or-nothing’) emptying may occur [31], while multiple-unit ones may pass fed state due to their suitable size,
- viscosity grade of applied polymer – decrease of dissolution rate was observed with the increase of viscosity grade of polymers [32].

Another consideration about multiple-unit dosage forms is that may be able to reduce intersubject variability in absorption and lower probability of dose dumping [33]. Furthermore, the manufacturing costs of multiple unit preparations are generally higher than, in the case of single unit dosage forms, due to the need of increased number of process steps. The effect of floating drug delivery systems are also influenced by the concomitant applied other medications as well. APIs (e.g. atropine, propantheline) causing decrease of gastrointestinal motility result in risen GRT. In contrast with other API groups, which may decrease GRT such as prokinetics (e.g. metoclopramide, cisapride), opiates (e.g. codeine) and types of API causing reduced gastric motility as side effects (e.g. erythromycin, bethanechol) [34].

2.3. Classification of floating drug delivery systems based on buoyancy mechanisms

Floating drug delivery systems as concept were first described by David in 1968 in order to overcome patient’s choking sensation caused by swallowing pills. Davis suggested a novel type of tablets having less than 1.0 g/cm^3 average density, consequently floated after immersion into gastric medium [35]. Since then, plenty of novel dosage forms are created and studied in order to achieve a suitable one with optimal floating behavior and drug release.

The flotation of preparations in gastric medium may be achieved by different ways and mechanisms. Subsequent to wetting of preparation and release of API(s), the residual part of the preparation is emptied after a certain time. Depending on the ingredients, preparation structure may be decomposed (e.g. in the case of biodegradable polymers) or excreted in unchanged form (e.g. at application of non-erodible ingredients). Using low density excipients (ρ<1.00 g/cm^3), buoyancy of the preparation may be developed in short time.

When developing of floating drug delivery systems, the preparation should meet the following three basic criteria:

- suitable ingredient composition for creating coherent gel structure,
- less than 1.004 g/cm^3 average density of the dosage form,
- application of polymer having appropriate physicochemical property to ensure the desired drug release profile.

Based on the mechanism of flotation, two different technological approach could be distinguished and utilized: non-effervescent - (NEFDDS) and effervescent floating drug delivery systems (EFDDS). Depending on the ingredients and technology, the preparation can be single-unit or multiple-unit dosage form.

Floating dosage forms involve tablets, granules, capsules, pellets, beads, hollow microspheres (‘microballoons’) and laminated films [8].

Solid floating drug delivery dosage forms may be categorized into the following groups:

1) single-unit dosage forms:
    a) tablets (pills),
    b) coated tablets,
    c) capsules,
    d) laminated films,
2) multiple-unit dosage forms:
    a) minitablets,
    b) granules, pellets,
    c) hollow microspheres (‘microballoons’),
    d) beads,
    e) powders.

Additionally, minimal gastric content is required for achieving of flotation thus gastric residence. Suitable floating force (resultant weight) expressed by the preparation is indispensable in order to be positioned reliably above the gastric content. Floating lag time (t_{lag}) is the time required from immersion of dosage form into medium until reaching the upper surface of the medium. Another determining factor is the total floating time ($t_{floating}$).

Affecting factors of flotation of FDDSs are the following:

- properties of applied excipients,
- quantity of applied polymer(s),
- viscosity grade of applied polymer(s) – grade is generally characterized by the viscosity of 1 or 2 % solution of polymers in 20 °C,
- mass, thickness, porosity of manufactured preparation,
- quantity of effervescent excipients (at EFDDSs),
- pH of dissolution media.

Kinetics of API dissolved from matrix structured systems can be controlled by varying the proportion of excipients (particularly applied polymers), which subsequently result in modification of floating behaviors as well [36].
The categorization of FDDS types are based on single-unit dosage forms especially focused on tablets, since the experimental part of the dissertation deals with the tablet formulations, studies and optimization.

2.3.1. Non-effervescent floating drug delivery systems (NEFDDS)

The most frequently applied excipients in NEFDDS include the gel-forming and swellable cellulose derivative hydrocolloids (e.g. hydroxypropyl methylcellulose, hydroxyethyl cellulose, and high substituted hydroxypropyl cellulose), polysaccharides, matrix forming polymers (e.g. polycarbonate, polyacrylate, polymetacrylate, polystyrene) and chitosan or carbopol derivatives, which have bioadhesive properties. These polymers are used in high concentration. At manufacture, powder blends are made by the mixture of excipients and APIs, then a suitable dosage form is created [37, 38]. At application, firstly the outer surface of the preparation gets into contact with the gastric fluid, which is hydrated, swollen and the aqueous medium diffuses from the medium into the inside of the drug delivery system. The gel forming (gelatinous layer) on the surface entraps the air being in capillaries of preparation inside, consequently the average density of the preparation decreases. The structure can maintain a relative integrity of shape and bulk density less, than the medium. Nevertheless by the increase of the swollen layer thickness, the diffusion of the medium decreases into the preparation [39]. During the process from layer into the next layer, the API(s) can be dissolved and released driven also by diffusion. The creating coherent gel structure plays reservoir function coinciding

the sustained release of the API. The rate of API diffusion is influenced by the relatively dry internal layer of the preparation. In the case of rapid hydrating excipient, gastric medium can diffuse into deeper layer of the dosage form faster [40]. This effect may be assisted by excipient with high hydrophilic properties and rapid water uptake. At hydration, the first hydrated layer is swollen, then is dissolved, after which its detachment may assist the further layers to be hydrated. The properties of former described technology are the principals of Hydrodynamically Balanced Systems (HBS™), which should be highlighted, since there are several commercially available products. HBS™ is generally categorized into the solid single-unit dosage form group (Fig. 2).

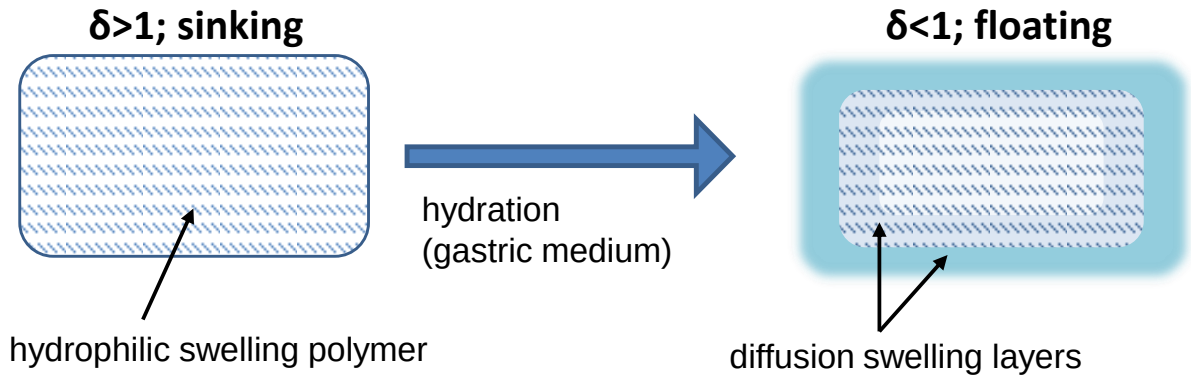

Fig. 2. Mechanism of density drop of Hydrodynamically Balanced Systems (HBS™)

Based on the technology and the dosage forms, the following types are involved in NEFDDS: microporous systems, alginate beads and hollow microspheres.

Microporous systems are also a possible option due to their stable uniform porous structure having high surface. The porous structure may allow the improvement of solubility of poorly water soluble drugs. The base excipients of these systems may be materials such as silica, ethylene vinyl acetate, polypropylene foam powders and titanium dioxide. Their initial average density is lower than 1.0 g/cm^3 , hence may float at touching and remain buoyant in gastric medium [41].

Among multiple-unit dosage forms, alginate beads and hollow microspheres have significant role in development of FDDSs. Alginate beads are created with the aqueous solution of sodium alginate, which added dropwise into aqueous solution containing calcium ions and/or other divalent or polyvalent cations [42]. The other multiple-unit drug delivery systems are the hollow microspheres, which are empty particles having spherical shape [43]. API is dispersed or dissolved in the particle matrix. Microspheres also known as ‘microballoons’, are prepared by

dissolution of applied polymer(s) in organic solution, in which API is dispersed/dissolved, then this organic solution/dispersion is emulsified in aqueous solution containing surfactant(s). An O/W type emulsion is prepared from which organic solvent is removed, the polymer precipitate onto the surface of remaining drops hence forming a cavity resulting in hollow spheres.

2.3.2. Effervescent floating drug delivery systems (EFDDS)

EFDDSs generally consist of swelling/ gelling polymers and effervescent components. The most frequently applied effervescent agent is sodium bicarbonate, but there are studies using calcium bicarbonate as well [44] being able to generate carbon dioxide (CO_2). In non-floating effervescent preparations, acidic ingredients are also applied such as tartaric acid, citric acid or maleic acid. In these cases, touch of water is enough to initiate gas generation, due to the *in situ* developed acidic environment of the preparation. Acidic component is generally omitted from formulations.

The swellable excipient creates a hydrated gel layer when immersed into the gastric medium (similarly to NEFDDS). During this process, HCl reacts with the carbonate creating CO_2 gas bubbles, which are entrapped in swollen gel structure. Due to this process, average density of the preparation is dropped becoming lower than gastric medium and then the preparation starts to float. Therefore floating lag time of effervescent FDDSs may be shorter due to the fast CO_2 generation inside the preparation resulting in accelerated density decrease. The mechanism of flotation in the case of effervescent floating tablets is shown in Fig. 3.

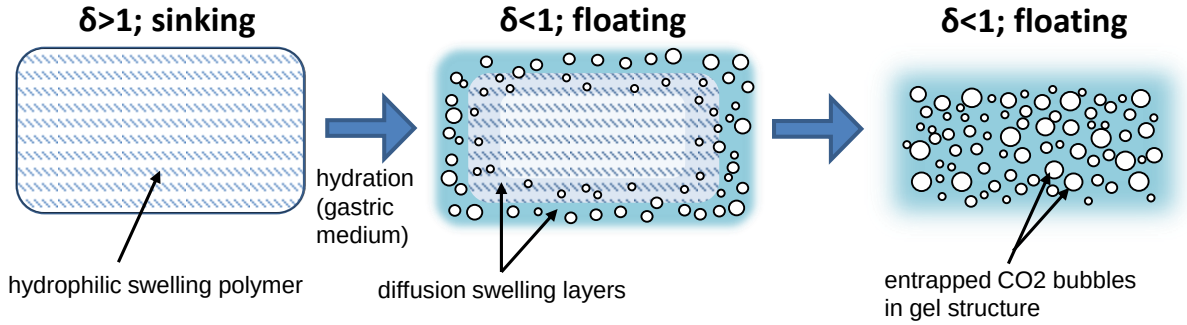

Fig. 3. Mechanism of density drop and structural change in effervescent floating tablets

Additionally, besides assisting of flotation, generation of CO_2 creates alkaline microenvironment suitable for the gelling process of polymers [45] and contributes and accelerates the hydration of the preparation [46]. CO_2 bubbles being in the structure may vary

the release kinetics of API(s) compared to non-effervescent preparations with similar composition. Generally drug release kinetics of non-effervescent floating preparations can be described with Higuchi model [38, 47-49], while the effervescent floating systems have considerably different release kinetics [47, 50, 51].

2.4. Theoretical base of mucoadhesion

Mucoadhesion is the phenomenon, when two surfaces (one of them is mucous membrane) adhere to each other. Mucoadhesive drug delivery systems are developed with special excipients, which are suitable to adhere onto mucous membranes in order to achieve prolonged localized drug release or to deliver sensitive molecules (peptide -, or nucleoid based molecules) into the blood stream [15].

Over the last two decades, several theories of bioadhesion were described as well as the possible types of chemical bonds (e.g. ionic -, covalent -, hydrogen -, Van-der-Waals -, hydrophilic bonds) [52]. Therefore it is important to evaluate the force of the connection between the materials and mucosal surface. For mucoadhesion, six general theories are used: electronic -, wetting -, adsorption -, diffusion -, mechanical -, and fracture theory [53-55]. Although the real, physiological mechanism may possibly be a mixture of previously listed theories. The contact between the mucous surface and dosage form can be divided into two modes based on the hydration level of the preparation: fully hydrated or dry (or partially hydrated) dosage forms are in contact with mucosae.

From the interaction until the creation of mucoadhesive bonds, two steps (Fig. 4) are adapted to describe the behavior of mucoadhesive materials and mucous membrane [56, 57]:

I. contact stage: wetting between the two surfaces,
II. consolidation stage: creation of various physicochemical interactions to consolidate the adhesive connection.

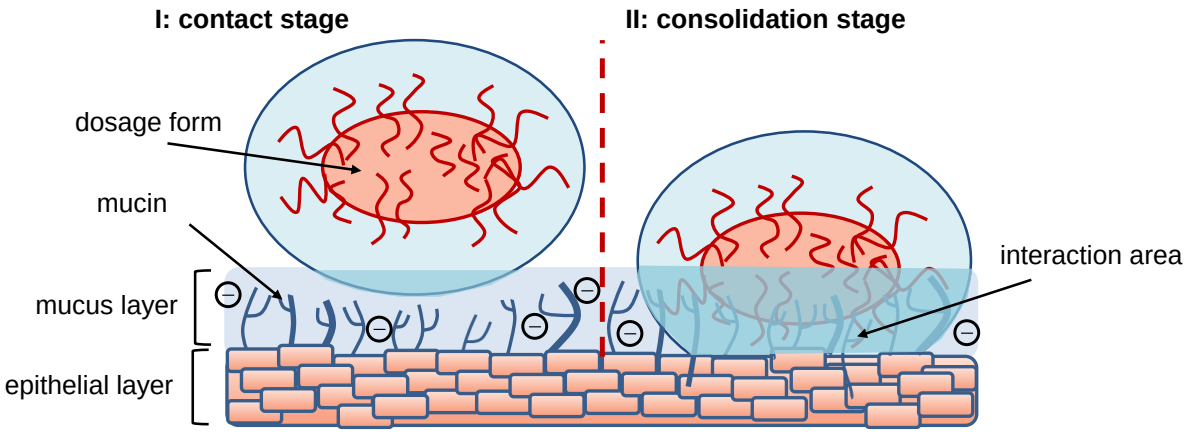

Fig. 4. Mechanism of mucoadhesion

At the contact stage, dosage form touches the mucosal surface. Gastrointestinal mucosae are special from other mucosal surfaces (e.g. nasal, oral, ocular, or vaginal), since it is inaccessible for placing anything directly onto the target mucosa. For larger particles such as tablets, pellets, gastrointestinal peristaltic movements may assist in creating contact onto mucosal surface [15]. One of the most significant parameters is the charge on the surface of the particular dosage form. If the dosage form has the same charge than mucosal surface, then repulsive forces will increase. If the charges are opposite then attractive forces will prevail depending on the nature of dosage form, the medium, and the distance between dosage form and surface (DLVO theory). Approximately 10 nm is the distance when attractive and repulsive forces are balanced up to that point when dosage forms can easily be detached.

The consolidation stage is when the bonds are being created between the touching surfaces. Mucoadhesive materials adhere mostly strongly onto solid dry surfaces in the presence of a wetting medium [58]. Mucoadhesive molecules are become dissolved and conform to the shape of mucosal surface. The bond is mostly van der Waals and hydrogen bonds, however cationic mucoadhesive materials can bond to anionic mucosa (via carboxyl and/or sulfate groups on mucin) with electrostatic interaction. The resultant mucoadhesion is probably a mixed interaction resulting from the combination of different forces discussed above. Two theories describes the consolidation phase: the interpenetration - and dehydration theory. The principals of the interpenetration theory is described by Peppas and Sahlin [54]. The mucoadhesive molecules interpenetrate and bond the glycoproteins of mucin layer. Indirect evidence for interpenetration theory can be measured by rheological methods. These methods measure the additional viscosity over the sum of the viscosities of polymer and mucin, which could be caused by the mucoadhesive interaction between them. The base of consolidation according to

the dehydration theory [57] is that the dry dosage form after coming into contact with mucus surface dehydrates the mucus due to its high swelling ability and osmotic pressure. The consolidation lasts until equilibrium between dosage form and mucosal surface is achieved [59, 60].

There are also limitations of mucoadhesion. In the case of interpenetration, the process is likely to be created at the two touching surface (polymer and mucosal surface), which inhibits any further interpenetration. At theory of dehydration, the dosage form has to be dry or partially hydrated. On the other hand, mucus membranes have highly varied macroscopic and microscopic topography and there are interpersonal physiological difference varying the site and the thickness of mucosae.

2.5. The most frequently used active substances and excipients

2.5.1. Possible active substance candidates

At design and development of FDDSs, physicochemical and pharmacokinetic properties of API(s) have to be known precisely. Several API(s) are not suitable for incorporation into FDDS due to their properties. For instance, proton pump inhibitors (e.g. omeprazole, lansoprazole, pantoprazole, rabeprazole) are inactivated in acidic medium during dissolution due to their protonation [61, 62]. Peptide-type molecules and phospholipids are particularly sensitive to acidic medium. Some API(s) (e.g. acetylsalicylic acid) may cause local irritation, thus their application is debatable. When choosing of API(s), pK value is also a determining parameter in the cases of substances having pH dependent solubility. Substance having pH dependent solubility (e.g. ibuprofen, flufenamic acid, mefenamic acid, niflumic acid, diclofenac sodium and meclofenamic sodium) may poorly dissolved in acidic medium [63].

For example solubility of ibuprofen sharply increases in medium higher than pH 4-5. On the other hand, in acidic medium low solubility of ibuprofen can be observed [64].

Those active substances are possible candidates for floating drug delivery, which [24]:

- absorb locally from stomach and/or proximal section of small intestine (e.g. furosemide, riboflavin-5-phosphate, chlordiazepoxide, cinnarazine [2]),
- have local site-specific effect in stomach (e.g. antacids, antibiotics for eradication of *Helicobacter pylori*, misoprostol),

- have a sleek absorption window in the upper tract of the small intestines,
- are unstable in distal section of GIT (e.g. captopril),
- have low solubility in intestinal fluid (e.g. quinidine, diazepam),
- have short elimination half-life,
- have variable bioavailability (e.g. sotalol hydrochloride).

By carefully choice of active substance, its bioavailability can be increased. Ritschel *et al*. developed a floating delivery system, which was able to increase the absolute bioavailability of furosemide to 42.9%. This novel FDDS was compared to two commercially available furosemide products showing 33.4% (Lasix®) and 29.5% (Lasix long®) absolute bioavailability [65].

2.5.2. Applicable excipients

When designing of FDDSs, the applied swelling polymers have to be carefully chosen as described in the section of possible floating mechanisms. Not only the quality but also the quantity may be determining. Kumar *et al.* studied the effects of different ingredients and polymers and found that ingredients can remarkably modify floatability and drug release kinetics [66].
Polymers have a significant role in the compositions, but other excipients may be necessary to use: fillers, binders, glidants, lubricants, plasticizers etc. Tableting or granulating ingredients should not significantly alter the floating properties and release mechanism of the preparations.

Various polymers (natural, semisynthetic, synthetic) are used for FDDS, but the most frequently applied polymers in floating drug delivery systems are the following [67]:

I. Natural polymers:
    a. alginic acid,
    b. guar gum,
    c. gellan gum,
    d. xyloglucan,
    e. pectin,
    f. chitosan,

II. (Semi)Synthetic polymers:

a. sodium alginate, calcium alginate,
b. hydroxypropyl methyl cellulose,
c. polymethacrylate,
d. poly-caprolactone,
e. hydroxyethyl cellulose.

Inert lipophilic materials may also be used which have low density and/or play role in decreasing hydrophilicity of preparations. Fatty, inert ingredients involve:

- beeswax,
- long-chain fatty alcohols,
- ethylcellulose,
- fatty acids.

At particular systems, low density foam creating excipients are used such as polypropylene foam powders (Accurel MP1000) assisting the flotation [24].

Mucoadhesive excipients are generally hydrophilic macromolecules having groups forming hydrogen bonds [68-70]. Hydroxyl, carboxyl and amine groups after hydration show adhesion. Generally poly(acrylic acid), chitosan, sodium alginate and cellulose derivatives (e.g. sodium carboxy methylcellulose, hydroxypropyl cellulose) are used as 'first generation' mucoadhesive agents due to their easy availability (regulatory approved). Novel mucoadhesive materials (e.g. chitosan–iminothiolane, poly (acrylic acid)–cysteine, chitosan–thioglycolic acid, alginate–cysteine, sodium carboxymethylcellulose–cysteine) are the thiolated polymers (thiomers) being able to create intra- or interchain disulphide bonds [71].

In effervescent preparations, carbonates or bicarbonates assist in flotation. Speed of carbon dioxide generation can be enhanced with acidic materials, but their manufacture requires special technologies and/or environment due to their intense reaction.

The drug release may also be modified by several excipients. Mannitol, lactose as well as sodium chloride can increase the drug release by assisting in hydration process. Materials such as dicalcium phosphate, magnesium stearate and talc may since being practically insoluble in water decelerate the rate of hydration.

2.6. Commercially available floating drug delivery systems

Based on the research results of the past 30-40 years, several floating preparations have received regulatory approval, thus pharmaceutical industry considers them potentially applicable products. For pharmaceutical industries, floating systems can be favorable due to their relatively simple development and cost-effective manufacture. In most of these cases, no special equipment is required for their manufacture. Floating products on the pharmaceutical market are shown by [2, 72, 73]. Until now, none of the floating preparations have become available in Hungary.

Table 1. Commercially available floating drug delivery systems

Brand name	Active substance	Pharmacological effect	Dosage form	Manufacturer
Zanocin® OD	ofloxacin	antibiotic	tablet	Ranbaxy, India
Riomet® OD	metformine hydrochloride	antidiabetic	tablet	Ranbaxy, India
Prazopress® XL	prazosin hydrochloride	sympatholytic	tablet	Sun Pharma, Japan
Metformin® Hcl LP	metformin hydrochloride	antidiabetic	tablet	Galenix, France
Cafeclor® LP	cefaclor	antibiotic	tablet	Galenix, France
Tramadol® LP	tramadol	opioid analgesic	tablet	Galenix, France
Inon Ace®	simethicone	antigas	tablet	Sato Pharma, Japan
Madopar® HBS	benserazide, levodopa	for Parkinson's disease	capsule	Roche Products, USA
Valrelease®	diazepam	sedative, hypnotic	capsule	Hoffmann-LaRoche, USA
Cytotec®	misoprostol	synthetic prostaglandin E1 analog	capsule	Pharmacia, USA
Baclofen® GRS	baclofen	muscle relaxer	capsule	Sun Pharma, India
Cifran® OD	ciprofloxacin	antibiotic	capsule	Ranbaxy, India
Gaviscon®	aluminum hydroxide, magnesium-carbonate	antacid	liquid suspension	Glaxo Smith Kline, India
Topalkan®	aluminum hydroxide, magnesium-carbonate	antacid	liquid suspension	Pierre Fabre Drug, France
Conviron®	ferrous sulfate, folic acid	iron supplementation	colloidal solution	Ranbaxy, India

Table 1 shows floating products being available, with which better bioavailability and patient compliance can be achieved. Some of the brand names are followed by two, three letter abbreviations related to the modification in drug release:

- OD – once daily,
- XL – extended release,
- LP – liberation prolongeé (in English: prolonged liberation),
- HBS – Hydrodynamically Balanced Systems
- GRS – gastroretentive system.

Metformin Hcl® LP, Cafeclor® LP and Tramadol® LP are designed and developed with MINEXTAB® Floating technology meaning **min**imum **ex**cipient **tab**let with gastroretentive effect by the Galenix inc., France. Cifran® OD once daily floating preparation is a bilayer floating capsule, Inon Ace® tablets are foam based floating tablets. Baclofen® GRS system is designed to be a multi-layer floating and swelling capsule.

Gaviscon® and Topalcan® are liquid suspensions containing sodium alginate, which *in situ* turns into a gelatinous, rubbery alginic acid gel after protonation and being able to float and prolong the API release.

The controlled release of Madopar® HBS (in several countries Prolopa® HBS) is a gelatin capsule developed for flotation whose dissolution creates a gelatinous-mucous gel structure. This gel structure constrains the APIs release from the hydrated layers through a diffusion process [74]. In Valrelease®, diazepam is the API, which was a reasonable choice due to its pH dependent solubility. pK_a value of diazepam is 3.4, thus the absorption is better in acidic pH medium compared to the intestinal medium, in which diazepam is practically insoluble. Pharmacokinetic studies revealed that once daily administration of Valrelease® results in similar effect to three times daily administration of Valium® 5 mg tablet [40].

In summary, FDDS technologies may be able to prolong drug release or to sustain local effect in the stomach or proximal section of the small intestines. Additionally, products with regulatory approval may also indicate that patient compliance may increase due to once daily administration as another approach for more convenient medical therapy.

3. Aims

The objective of the dissertation is to summarize the applicability, manufacturing possibilities, excipients and the types of floating drug delivery systems and to optimize a floating, mucoadhesive system aiming at the eradication of *Helicobacter pylori* having desired floating and drug release properties based on preliminary excipient examination. Direct compressed (DC) tablet was chosen as dosage form being a cost-effective technology for pharmaceutical industry requiring less procedures.

Before the implementation of the pharmaceutical technological aims, analysis of critical factors influencing the manufacture was carried out. Reproducible manufacturing processes are required to achieve suitability and tablets uniformity to achieve the uniform properties of tablets, which could influence experimental parameters. Ishikawa diagram [75] evaluation was created, which is a commonly used graphical method to identify factors resulting in an overall effect on product design and quality imperfection. The aim was to reveal affecting factors on uniformity of DC tablets in order to standardize all possible conditions and adjustments. Critical factors are indicated separately in particular method sections.

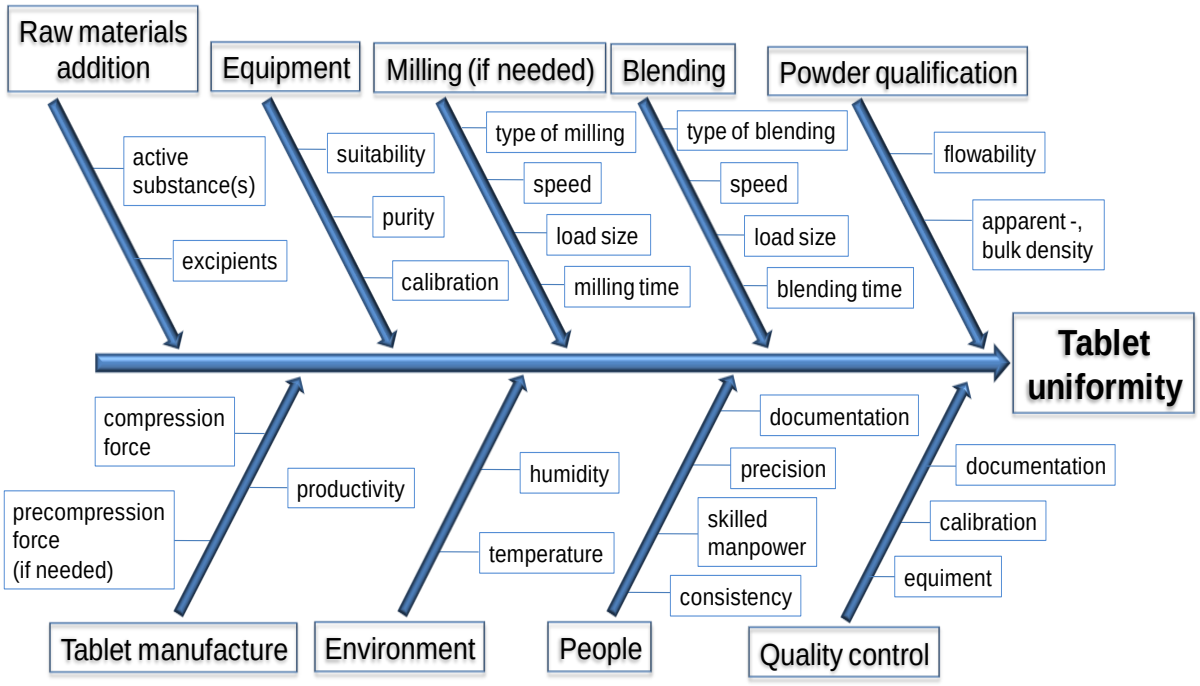

Fig. 5. Summary of most significant influencing factors on uniformity of directly compressed tablets

In order to achieve my goals, the following experimental aims were stated:

- comparison of two types of low substituted hydroxypropyl cellulose (L-HPC 11, L-HPC B1) and their 1:1 mixture based on microscopic, wettability and flowability in order to characterize their role and influence in floating tablets,
- determination of viscosity grade and rheological properties of sodium alginate,
- characterization of parameters related to floating behavior of floating tablets, as well as determination of floating force study parameters,
- evaluation of drug release and floating parameters with variance analysis,
- optimization of floating drug delivery tablets containing metronidazole based on release and floating parameters for better antibacterial effect,
- comparison of dissolution of optimized floating tablet containing metronidazole with commercially available metronidazole products,
- application of a microbiological detected dissolution on blood agar plates, and comparing the results with classic UV spectrophotometric detected dissolution methods,
- determination of possible interactions between API and excipients in optimized tablets with differential thermal analysis and isothermal stress tests,
- application of two *ex vivo* mucoadhesive studies in order to determine the mucoadhesive properties of optimized tablets,
- application of the *ex vivo* detachment force mucoadhesion studies to evaluate the possible effect of L-HPC B1 on *ex vivo* mucoadhesion,
- application of X-ray CT imaging technique for *in vivo* tracking of the optimized floating tablets,
- application of high resolution X-Ray CT imaging technique for better view of floating tablets, with which structure of tablets could be assessed,
- application of X-Ray CT imaging of the optimized floating tablet for quantification by Hounsfield unit attenuation and tablet volume,
- recommendation of the optimized composition in order to achieve a more successful gastroretentive therapy.

4. Materials and Methods

4.1. Materials

In this section, the materials which were applied in the designed formulations are detailed. Their appearances, physicochemical properties, roles and applied concentrations are described. All excipients are registered with E-numbers by European Food Information Council (EUFIC), which can highlight their wide range use in Food industry.
Materials used only for examinations are listed in the end of this section.

4.1.1. Metronidazole

Metronidazole (Fig. 6) is a nitroimidazole type antimicrobial active substance having potent anti-anaerobic, amebicidal, and antiprotozoal activity, which was first synthetized in France in the mid-1950s. At the time of its discovery, it was used as antiprotozoal agent, then later its bactericide effect against anaerobic bacteria was recognized. In gastroretentive dosage forms, prolonged contact with the stomach mucosae can be achieved resulting in better local effect against *Helicobacter pylori*.

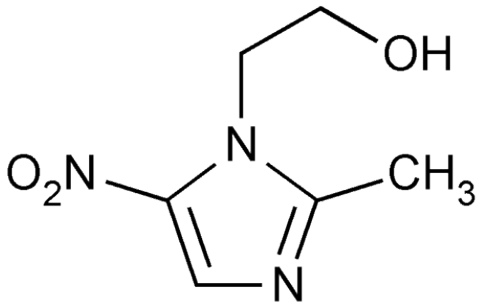

Fig. 6. Chemical structure of metronidazole (1-Hydroxyethyl-2-methyl-5-nitroimidazole; CAS: 443-48-1)

4.1.1.1. Physicochemical properties

Metronidazole is a white, yellowish odorless crystalline powder. Its melting point is between 159 and 163 °C [76]. Solubility of metronidazole depends on pH. Being a weak base, it has high solubility (64.80 mg/ml) in acidic medium (pH=1.2) and lower in higher pH media (~10

mg/ml). It is slightly soluble in purified water, in acetone, in ethanol or in methylene chloride [77].

4.1.1.2. Pharmacodynamics

Metronidazole has a wide bactericide spectrum. The material penetrates via the bacterial cell membrane into the intracellular medium. Nitro functional group of the molecule is reduced intracellularly, which probably turns into hydroxylamine group. This cytotoxic metabolite damages the bacterial DNA, thus DNA synthesis of the cell ceases, which also means the death of the bacterial cell. Metronidazole is effective against certain infections caused by protozoa or obligate anaerobic bacteria [78]. Its indication include: *Stomatitis ulcerosa*, *Amebiasis*; vaginitis; *Trichomonas* infections; *Giardiasis*; anaerobic bacteria; and *Treponemal* infections [78, 79].

4.1.1.3. Pharmacokinetical properties

Absorption of metronidazole is fast from of *per os* administered formulations. Its bioavailability is more than 90 %. Orally applied 250 mg metronidazole results in approximately 5 μg/ml plasma peak concentration. Volume of distribution is high and 20 % bonds to plasma proteins. Metronidazole can penetrate into cerebrospinal liquid as well, in which therapeutic concentration can be achieved. It penetrates into the biliary tract, too.
The average elimination half-life time of metronidazole is 8 hours. 60-80% of metronidazole and its metabolites are excreted through kidneys, 6-15% eliminated via feces. In patients having hepatic impairment, metronidazole plasma clearance is reduced [78].

4.1.1.4. Commercially available metronidazole products

Regulatory approved metronidazole products are summarized in Table 2 based on the Food and Drug Administration (FDA) drug database [80]. The originator brand name of the metronidazole product was Flagyl, which is globally marketed by Sanofi Aventis and in United States by Pfizer.

Table 2. List of commercially available orally applied metronidazole preparations, their dosage forms and strengths based on the FDA database (date of search: 05/10/2015)

Dosage forms	Strengths	FDA proprietary names	Applicants
tablet	250 mg	Flagyl	Gd Searle Llc
tablet		Metronidazole	Alembic Pharms Ltd
tablet		Metronidazole	Appco Pharma Llc
tablet		Metronidazole	Aurobindo pharma Ltd
tablet		Metronidazole	Mutual Pharm
tablet		Metronidazole	Teva Pharms Usa
tablet		Metronidazole	Unichem Labs Ltd
tablet		Metronidazole	Watson Labs
tablet	500 mg	Flagyl	Gd Searle Llc
tablet		Metronidazole	Alembic Pharms Ltd
tablet		Metronidazole	Appco Pharma Llc
tablet		Metronidazole	Aurobindo Pharma Ltd
tablet		Metronidazole	Mutual Pharm
tablet		Metronidazole	Pliva
tablet		Metronidazole	Unichem Labs Ltd
tablet		Metronidazole	Watson Labs Inc
extended release tablet	750 mg	Flagyl ER	Gd Searle Llc
extended release tablet		Metronidazole	Alembic Ltd
capsule	375 mg	Flagyl	Gd Searle Llc
capsule		Metronidazole	Alembic Ltd
capsule		Metronidazole	Par Pharm

Nowadays there are two generics on the pharmaceutical market of Hungary: Klion (Gedeon Richter Plc.), Supplin (Sandoz GmbH). In Hungary, there are other dosage forms as well such as: gel (ROZEX 7,5 mg/g), cream (ROZEX 7,5 mg/g), pessary (KLION, 10x), infusion (KLION 5 mg/ml), vaginal tablet (KLION-D 100, 10x), emulsion (ROZEX 7,5 mg/g) [81].

4.1.2. Sodium alginate

Sodium alginate (CAS number: 9005-38-3) is an odorless and tasteless, white to yellow-brownish colored powder. Slowly dissolves in water and forms viscous colloidal solution [82].

It is a non-toxic, biodegradable copolymer composed of L-guluronic and D-mannuronic acid blocks and extracted from brown seaweed species (*Phaeophyceae* family) by ion-exchange techniques. It is used in various pharmaceutical formulations. In tablet, sodium alginate can play binder and disintegrant role as well as applied in extended release oral preparations. Sodium alginate swells in purified water, since in acidic medium alginic acid is created by protonation, which is a rubbery less water soluble material. Various viscosity grades are available.
Sodium alginate is also applied by food and cosmetic industries.

4.1.3. Low substituted hydroxypropyl cellulose

Low substituted hydroxypropyl cellulose (L-HPC, CAS: 9004-64-2) is an odorless and tasteless, white to yellowish white powder or granules [82].
L-HPCs are widely used water-insoluble cellulose derivatives. In contact with water, they swell and act as disintegrant in solid dosage forms. Additionally they can have binder function as well. Applied concentration is generally between 2.5-5.0 % [83]. The disintegrative effect is due to the rapid water uptake and high swelling force. Particle size and shape of the particles play also significant role in the disintegration process [84]. In our work, L-HPC 11 and B1 were used. L-HPC 11 has the longest and fibrous particles among the different grades and is generally used as anticapping and disintegrant agent. L-HPC B1 is non-fibrous. Hydroxypropyl substitution ratio of both materials are the same, 11%.

4.1.4. Sodium bicarbonate

Sodium bicarbonate ($NaHCO_3$, CAS: 144-55-8) is an odorless, white crystalline powder having slight alkaline taste. Its crystal structure is monoclinic prisms. It is soluble in water, practically insoluble in ethanol [82].
Sodium bicarbonate is generally applied as effervescent agent, but as API it is also used as alkalizing agent for acute relieve of hyperacidity. Its usual effervescent concentration is 25-50%. Carbon dioxide is generated in contact with acidic medium. It is also applied to produce or maintain alkaline pH in preparations within 10-40% concentration.

4.1.5. Talc

Talc (CAS number: 14807-96-6) is a light, very fine, odorless, white to grayish-white crystalline powder. It is a powdered, purified, selected hydrated magnesium silicate. It can contain aluminum silicates and iron. Practically insoluble in dilute acids and alkalis and water [82].

Talc is a widely applied excipient in solid dosage forms as lubricant, diluent and glidant. Glidant and/or lubricant effect is generally achieved by 1.0-10.0 % concentration.

4.1.6. Magnesium stearate

Magnesium stearate (CAS number: 557-04-0) is light, very fine powder having mild odor and characteristic taste. It is greasy to touch and adheres to skin [82] and is practically insoluble in water.

Magnesium stearate is a widely used lubricant in solid state dosage forms. It is applied between 0.25 and 5.0 % concentration.

4.1.7. Colloidal silicon dioxide, hydrophilic

Hydrophilic colloidal silicon dioxide (Aerosil 200, CAS number: 7631-86-9) is a light, amorphous, odorless and tasteless powder having approximately 15 nm particle size. It is practically insoluble in water.

Aerosil is generally used as glidant in 0.1-10 % in solid pharmaceutical preparations. It has small particle size and large specific surface resulting in desirable flow characteristics. Aerosil has anti-caking effect as well due to its hydroscopic property.

4.1.8. Materials used for examinations:

- paracetamol as API in preliminary studies,
- 0.1 M HCl as dissolution medium,
- barium sulfate as X-Ray contrast material,
- methanol for metronidazole assay by high performance liquid chromatography (HPLC),
- potassium dihydrogen phosphate for metronidazole assay by HPLC,

- Krebs-Henseleit buffer containing D-glucose, magnesium sulfate, potassium phosphate monobasic, potassium chloride and sodium chloride for *ex vivo* mucoadhesion studies.

4.2. Methods

Preliminary – and optimization experiments have been carried out. Principal aim of the research work was the optimization of floating drug delivery system, but the preliminary project and its conclusions were needed to begin designing of optimization project. The Method section contains all method descriptions, which were used either during preliminary or optimization studies.

4.2.1. Preformulation methods

4.2.1.1. Comparative physical examination of L-HPC 11 and L-HPC B1

4.2.1.1.1. Microscopic examination

Particle size and shape parameter measurements were done with microscopic examination with the use of 160x and 640x magnification (Zeiss, Axio Imager A1 Microscope, Germany) based on 50 largest separated particles.
Sphericity (Ψ) was calculated with the following formula:

$$\Psi = 4\pi \frac{A_f}{P_{Cr}^2}. \quad (1)$$

Sphericity of particles describes the form of region on the bases of their circularity. Numerical range is from 0 to 1. The value of the sphericity for a perfect round shape particle is 1. Filled area (A_f) is the region including any holes on it. Crofton perimeter (P_{cr}) determines circular region with correction optimized for circular objects. Zeiss Axio Vision Rel. 4.7 software (Carl Zeiss, Germany) was used for digital photo analysis.

4.2.1.1.2. Flowability and density

Flow properties of L-HPC 11 and B1 were examined to highlight further physical differences. Determination of angle of repose was performed according to 2.9.16. test of Ph. Eur. Ed. 5.0. Measurements were carried out in triplicate. Angle of repose was calculated by the following formula [85]:

$$tg(\alpha) = \frac{H}{R}. \quad (2)$$

where, α is the angle of repose, *H* is the height, and the *R* is the radius of the conical pile. Result was considered to be valid, when symmetric cone shape was formed.

Apparent density examination was carried out by a volumetric device (Erweka SVM 121, Germany) according to 2.9.15. Ph. Eur. 5.0. Bulk densities (ρ_{bulk}) were recorded after filling 100.0 g material into graduated cylinder; tapped densities (ρ_{tapped}) were recorded after 1250 taps. Using these measurements Carr indices (C_i) [86] were calculated according to the following formula:

$$C_i = \frac{\rho_{tapped} - \rho_{bulk}}{\rho_{tapped}} * 100. \quad (3)$$

Hausner ratios (H_r) were also determined by using the ratios between tapped and bulk density of powders applying the following formula:

$$H_r = \frac{\rho_{tapped}}{\rho_{bulk}}. \quad (4)$$

4.2.1.1.3. Wettability

Force tensiometer (KSV, Sigma 701) was applied to measure water uptake of the L-HPC types. Glass sample holder vessel was used with 1.15 mm width and with 1.00 mm diameter glass filter at the bottom holding 50.0 mg of samples. The materials were immersed into distilled water and 0.1 M hydrochloric acid for 60 minutes respectively. Sampling was done in every 5 seconds. Accuracy of force tensiometer instrument was 0.01 mg. Wettability tests were performed in triplicate.

4.2.1.2. Rheological behavior of sodium alginate

Viscosity grade of sodium alginate was examined with the use of rotational viscometer (Anton Paar RheolabQC, Austria) with standard measuring system (CC27). Temperature dependence of viscosity and flow curves of 1.0 % sodium alginate solution were also determined at 20, 25, 30, 35, 37 ºC. Viscosity measurements were performed with 100 1/s constant shear rate. Rheological (flow curve) behavior studies were carried out with linear increase of shear rate from 10 to 500 1/s. Three parallel samples were examined.
Flow behavior indices (*z*) of 1 % sodium alginate solutions at different temperature were also calculated based on the Ostwald-de Waele power law model [87]:

$$\tau_{yx} = K(\gamma)^z = K\left(\frac{dv_x}{dy}\right)^z \quad (5)$$

Where, τ_{yx} (F/A) is the shear stress being the proportion of shear force (F) and affected surface (A). Value of γ is the shear rate or the velocity gradient (dv_x/dy) of shearing force in the direction of x.

4.2.1.3. Drug-excipients interaction studies

4.2.1.3.1. Differential Thermal Analysis (DTA)

Differential thermal analyzer (Shimadzu DTA-50, Japan) was applied for thermal analysis of metronidazole and metronidazole-excipient blends. Metronidazole and excipients were analyzed separately as well as blends with drug-excipient ratios according to the optimized composition (MF_OPT). 10.0 mg of individual samples and blends were scanned in the temperature range of 25-400 ºC under air atmosphere. Temperature rate was 5 ºC/ min. Peak shifting and melting point were evaluated on thermograms in order to detect interaction between metronidazole and excipients. Three parallel examinations were carried out.

4.2.1.3.2. Isothermal stress tests

Isothermal stress testing (IST) was implemented with metronidazole and metronidazole-excipients blends according to the excipient composition of optimized formulation. Samples

were weighed separately, directly into 15 ml glass vials and stirred on vortex mixer for 2 minutes [88]. 10 % purified water was added into each vials and mixed on vortex mixer for 2 minutes. The vials were then sealed with rubber cups and stored at 50 °C (Binder BF115, Germany) for 3 weeks. Samples were examined to determine abnormal color change of samples. As reference, samples without added water were stored in refrigerator. Measurements were done in triplicate.

Determination of metronidazole has been performed by HPLC method according to the metronidazole monograph of Ph. Eur. 5th Ed. (2013). Applied parameters were the following: mobile phase: mixture of 300 ml methanol and 0.01 M potassium dihydrogen phosphate (pH=4,3), flow rate: 1 ml/min, detection: at 315 nm, injection: 10 µl. Mobil phase degassing was performed with 15 minutes ultrasound treatment. All samples were previously filtered with 0.2 µm GHP membrane (Acrodisc®, Pall, USA). The liquid chromatographic system consisted of HPLC device (Class-LC10A, Shimadzu, Japan), C18 column (LiChrospher 100 RP-18, Teknokroma, Spain) with 25 cm size and 4.6 mm diameter, pump (LC-10AD, Shimadzu, Japan), auto injector (SIL-10A XL, Shimadzu, Japan) and UV-VIS detector equipped with 8-µl flow cell (SPD-10A, Shimadzu, Japan).

4.2.2. Experimental design, statistical analysis and optimization

Several statistical approaches of experimental design originate from the work of R. A. Fischer. In the early 20th century, he showed how important it is to appropriately consider the design and the execution of experiments before they are actually performed to prevent frequently encountered problems.

Design of experiments (DOE) belongs to the field of applied statistics, which deals with planning, analyzing and interpreting controlled studies [36]. Afterwards the data have been gained from experiments they are objectively evaluated in order to understand the mathematical relation between factors (independent variables) and responses (dependent variables). With the application of DOE, fewer experiments are enough to explore their correlation.

The experimental design contains its settings, sequence and is created with the choice of layout type before beginning the studies. Choice of used layout depends on the experimental aim(s).

One of the most important aim of DOE is the optimization and assessment of the influence of factors on responses. After objective evaluation of result data, optimization criteria have to be determined based on the desirable responses.

In our work, Design Expert 7.0.0 software was used in order to create the experimental design and response surface plots. Data obtained from the floating tablet properties were analyzed with this software, too. Polynomial models were generated for all responses including linear, quadratic as well as interaction terms. The best model was chosen based on the particular statistical parameters involving coefficient of variation (CV), regression coefficient (R_2) and p-value. The following mathematical equation form was used to evaluate numerical effect of independent variables on responses:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2, \quad (6)$$

where Y is the response variable, b_0 is the intercept, b_i is the estimated coefficient of factors. X_1, X_2 and X_3 are the main effects representing how responses change, when an individual factor changes. Interaction term (X_1X_2) shows the effect of simultaneous change of factors on responses. X_i^2 is the quadratic effect for evaluation of non-linear correlations.

In our work, two experimental designs were applied.

I. The preliminary study focused on the influence of L-HPC 11, B1 and their 1:1 mixture on certain properties of sodium alginate based floating drug delivery systems. In this project, face centered central composite design (α=1) was applied with two numerical factors (X_1, X_2) and with three-levels (+1, 0, -1). One categorical factor was used involving the types of two L-HPCs and 1:1 mixture of L-HPCs. The two numerical independent variables were the sodium alginate (X_1) and particular L-HPC type (X_2). Factors mean the concentrations (%) of the materials in the floating tablets. All tablets contained 150 mg paracetamol and fixed amount of excipients contributing effervescent effect and tablet compressibility. Experimental layout is shown in Table 3. Dependent variables were the following: floating time, floating lag time, floating force, swelling capability and drug dissolution.

Table 3. Experimental layout of preliminary project

Exp. No.	Sodium alginate, X_1 (%)	L-HPC 11, X_2 (%)	Exp. No.	Sodium alginate, X_1 (%)	L-HPC B1, X_2 (%)	Exp. No.	Sodium alginate, X_1 (%)	L-HPC 11:B1, X_2 (%)
PFS01	0.50	0.50	PFS10	0.50	0.50	PFS19	0.50	0.50
PFS02	35.15	0.50	PFS11	35.15	0.50	PFS20	35.15	0.50
PFS03	0.50	25.00	PFS12	0.50	25.00	PFS21	0.50	25.00
PFS04	35.15	25.00	PFS13	35.15	25.00	PFS22	35.15	25.00
PFS05	0.50	12.75	PFS14	0.50	12.75	PFS23	0.50	12.75
PFS06	35.15	12.75	PFS15	35.15	12.75	PFS24	35.15	12.75
PFS07	17.82	0.50	PFS16	17.82	0.50	PFS25	17.82	0.50
PFS08	17.82	25.00	PFS17	17.82	25.00	PFS26	17.82	25.00
PFS09	17.82	12.75	PFS18	17.82	12.75	PFS27	17.82	12.75

II. For optimization of sodium alginate based floating tablets, face-centered central composite design (α=1) was used with three factors: sodium alginate (X_1), L-HPC B1 (X_2) and sodium bicarbonate (X_3). Each factors were examined in three levels (+1, 0, -1). Each tablets contained 250 mg metronidazole and constant quantities of excipients contributing effervescent effect and tablet compressibility. Factors mean the concentrations of the materials in the floating tablets. Experimental layout is shown in Table 4. Dependent variables were the following: floating lag time, maximal floating force, maximal floating force calculated to 100 mg tablet mass, time needed for maximal floating force and drug dissolution.

Table 4. Experimental layout of optimization project

Exp. No.	Sodium alginate, X_1 (%)	L-HPC B1, X_2 (%)	$NaHCO_3$, X_3 (%)	Total tablet weight (mg)
MF01	5.00	30.00	8.00	463.82
MF02	15.00	30.00	8.00	569.48
MF03	5.00	45.00	8.00	642.67
MF04	15.00	45.00	8.00	865.05
MF05	5.00	30.00	13.00	511.25
MF06	15.00	30.00	13.00	642.67
MF07	5.00	45.00	13.00	737.46
MF08	15.00	45.00	13.00	1046.03
MF09	5.00	37.50	10.50	569.48
MF10	15.00	37.50	10.50	737.46
MF11	10.00	30.00	10.50	538.79
MF12	10.00	45.00	10.50	796.18
MF13	10.00	37.50	8.00	603.86
MF14	10.00	37.50	13.00	686.81
MF15	10.00	37.50	10.50	642.67

4.2.3. Formulation studies

4.2.3.1. Preparation of effervescent floating tablets

Ingredients of tablets were accurately measured with analytical balance (Kern, ABJ 220-4M, Germany). Powder blends were mixed every time after adding next substance for 3 minutes with the use of mortar and pestle, and finally all the blends were mixed for 10 minutes. The flow properties of blends were qualified to be suitable for direct compression. Eccentric single-punch tablet press (Erweka, EP-1, Germany) was used using 8, 10, 12 mm round concave punches.

Compression forces at all batches were adjusted to achieve 50±5 N tablet hardness. Tableting was performed and stored at 50±10 % relative humidity (*RH*) and at 25±5 °C.

4.2.3.2. Studies of floating behavior

4.2.3.2.1. Determination of floating lag time

Floating lag time (t_{lag}) is the period from the immersion of the tablet until its buoyancy. Experiments were carried out in 450 ml 0.1 M hydrochloric acid at 37±0.5 °C. Durations of floating lag time were visually recorded by camcorder (DCR-SX85E, Sony, Japan). Each test was carried out for 4 hours in triplicate. The process of floating lag time measurements is shown on Fig. 7.

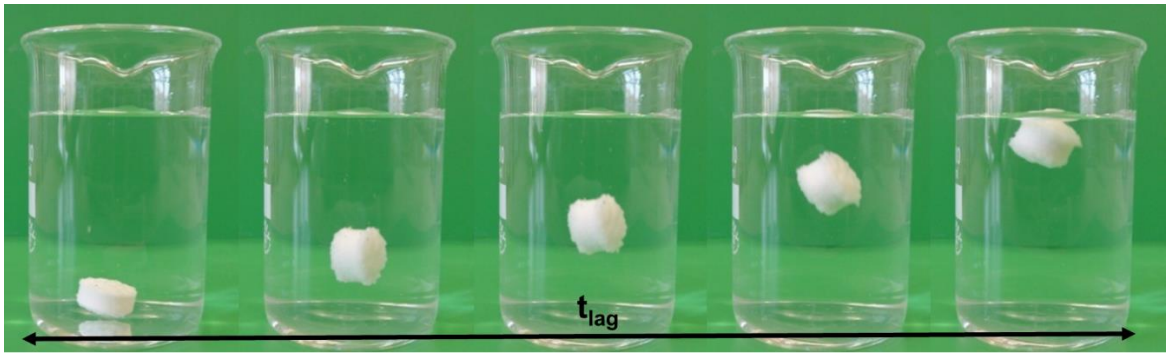

Fig. 7. The process of floating lag time measurements

4.2.3.2.2. Floating force study

Floating force measurements were carried out based on the theoretical base described by Timmermans and Moes [22, 89, 90]. KSV Sigma force tensiometer (KSV Instruments Ltd, Helsinki, Finland) was used with 0.1 mg accuracy. Tablets tested in a standard vessel containing 450 ml 0.1 M HCl, in which a special filtering plate with 2 mm aperture size was applied (Fig. 8). Majority of developing carbon dioxide bubbles passed through the filter plate resulting less noise. Media were exposed with ultrasound to avoid gas formation on filtering plate.

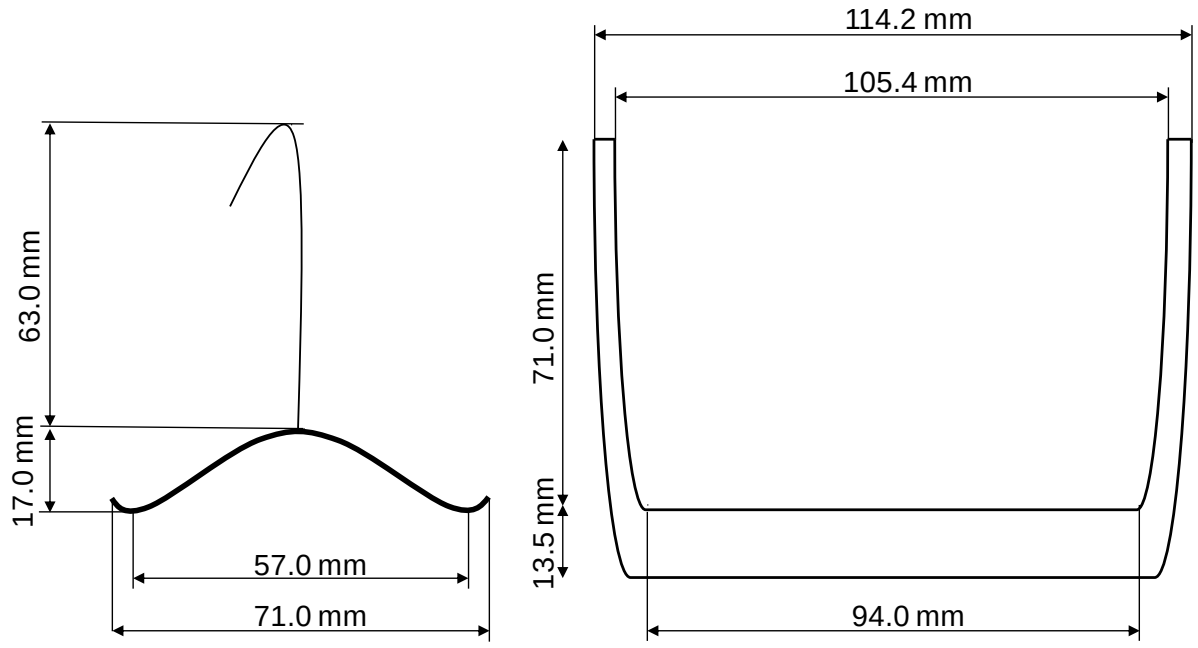

Fig. 8. Structure of standard vessel and filtering plate for floating force studies.

During the test, the weight of the filtering plate was continuously measured. Floating tablets pushed the filtering plate vertically upward, hence change of the weight could be registered as a function of time. Evaluated parameters were the following:

- maximal floating force (F_{max}),
- time (t_{Fmax}) needed for maximal floating force,
- time ($t_{F1/2}$) required for 50 % of maximal floating force,
- maximal floating force calculated for 100 mg tablet mass ($F_{max/100mg}$).

Floating forces were calculated based on the formula described by Cromer [91]:

$$F_{float} = F_0 - F_t = F_0 - m_t * g = F_0 - (F_0 + m_{it} * g) \quad (7)$$

In the equation, F_{float} is the floating force expressed vertically upward by a floating tablet. F_0 equals with the multiplication of filtering plate mass in medium with gravitational acceleration (g), which was constantly 39.84 mN. m_t is the weight measured by the devices, when a tablet pushed the plate upward. m_{it} is the negative weight gradient caused by the tablet directly, which was calculated by subtraction of m_t from m_0. In absolute value, m_t is the weight expressed by the buoyancy of floating tablets. All experiments were performed in triplicate.

4.2.3.3. Determination of swelling capability

Swelling capacities of floating tablets were measured based on the method described by Dorozynski *et al.* [37]. Tablets were weighted (W_1), then immersed into glass beaker filled with 200 ml of 0.1 M hydrochloric acid at 37±0.5 °C. At time 30, 60, 120, 180 and 240 minutes, tablets were removed from the beaker. After wiping the excess liquid from surface, tablets were reweighted (W_2). Swelling index (S_i) was calculated with following formula:

$$S_i = \frac{(W_2 - W_1)}{W_1} \tag{8}$$

Calculated index was corrected with the actual tablet weight in order to standardize the results. Swelling study was performed only on floating tablets having no rapid disintegration in preliminary project. Samples were tested in triplicate.

4.2.3.4. Drug release studies

In vitro dissolution studies of all floating tablets were performed according to Ph. Eur. 2.9.3 dissolution test with paddle apparatus. Stirring speed was 50 RPM (Erweka DT-700, Germany), the medium was 900 ml 0.1 M HCl tempered to 37± 0.5 °C. Dissolution tests for preliminary project were done for 4 hours, for optimization project were performed for 6 hours. During the studies, 2.5 ml samples were taken at 10, 15, 20, 30, 45, 60, 90, 120, 180, 240, 300 and 360 minutes. Each sample was filtered through PTFE membrane.

All measurements were done in triplicate.

Active substance contents of samples were determined with spectrophotometric method (Jasco V-670, Japan) at their absorption maximum (paracetamol, λ^{p}_{max}=243 nm; metronidazole, λ^{m}_{max}=277 nm). Linear calibration curve was previously created, all samples were measured within this concentration interval.

In optimization project, dissolution studies of two commercially available metronidazole tablets (Klion® 250 mg and, Supplin® 250 mg) were also performed for comparison with optimized floating tablets.

4.2.3.5. Kinetics of drug release

Model dependent evaluations of dissolutions were carried out with four mathematical models. Zero order -, first order -, Higuchi - and Weibull kinetic models were used to describe the drug release from floating tablets [1, 92].

Zero order kinetic is used at the dosage forms with slow release, at which release rate is independent from concentration:

$$W_0 - W_t = Kt \tag{9}$$

where W_0 is the initial amount of drug, W_t is the drug amount in time t, K is proportionality coefficient. Fundamentals of first order kinetics were first described by Noyes-Whitney. Their equation is:

$$\frac{dC}{dt} = K(C_s - C) \tag{10}$$

where C is the concentration of the substance in time t, C_s is the concentration of the equilibrium, K is first order proportionality coefficient, t means time. The equation above was later modified several times by Brunner *et al.* [93], then Hixson and Crowell. The model can be applied in dosage forms such as porous matrices containing water-soluble drugs [94], which is described by the following formula:

$$logW_t = logW_0 + \frac{K_1 t}{2.303} \tag{11}$$

where W_0 is the initial drug amount in the dissolution medium, W_t is drug released in time t, K_1 is first order release coefficient. As the first model Higuchi's kinetics for planar homogeneous matrix system was used according to the following equation [95]:

$$Q = \sqrt{D(2C - C_s)C_s t} \tag{12}$$

where Q is the drug released in time t, D is diffusion coefficient, C is the initial drug concentration, C_s is the drug solubility in the matrix media. Weibull's model is a commonly used model, which fits various kinds of dissolution profiles [96]. During the evaluation of dissolution data, the following equation was applied:

$$W_t = W_\infty \left(1 - e^{-\left[\frac{t-t_0}{\tau}\right]^\beta}\right) \quad (13)$$

where W_t is dissolution in time t, W_∞ is dissolution in infinite time, t_0 is lag time of dissolution, τ is mean dissolution time (time when 63.2% of the substance is dissolved), β is shape parameter of the dissolution curve. During evaluation linear transformation was carried out on each dissolution profile and the equation of the fitted linear line was determined according to the following formula:

$$Q_t = st + n_{diss} \quad (14)$$

where Q_t is the drug dissolved in time t, s is the slope of the line, n_{diss} is y-intercept.

4.2.3.6. Microbiologically detected dissolution studies

Microbiologically detected dissolution was only performed for optimized tablets. Antibacterial effect of metronidazole dissolution samples were determined in order to visualize the correlation between dissolution detected with spectrophotometric and microbiological disk diffusion method [97]. A calibration curve was made with standard dilution of pure metronidazole from 0 to 4.0 µg/5 µl. The test bacterium strain *Bacteroides fragilis* (ATCC 25285) was spread on the surface of *Brucella* blood agar plates supplemented with hemin, vitamin K_1 (Becton Dickinson GmbH, Germany) and 5 % defibrinated sheep blood. 30 µl of 10^5 test bacteria/ml suspension was used for inoculation of blood agar plates. Following, Whatman 3MM filter paper disks (diameter 6 mm) (Cole-Parmer Instruments Co., USA) were placed onto the inoculated plates. These disks were impregnated with 5-5 µl of calibration standards and dissolution samples at different times (5, 10, 15, 20, 30, 45, 90, 120, 180, 240, 300 and 360 min). Inoculated plates were placed into an anaerobic jar containing a GENbox anaer (bioMérieux, France) opened just before the jar was closed. The cultures were incubated

at 37 °C for 48 hours. After incubation time, jars were opened, and the diameters of inhibitory zones around the filter paper disk were determined with vernier caliper. Detection limit of the method was 0.5 µl metronidazole in 5 µl solution per disk on blood agar plates. Experiments were carried out in triplicate.

4.2.3.7. *Ex vivo* mucoadhesion studies

Two most frequently performed mucoadhesion studies were done: the detachment force and rheological mucoadhesion measurements. The former was performed with rat gastric mucosa, the latter one with extracted rat gastric mucus.

Wistar rats (250-350 g) were bred in a temperature-controlled room having a 12 h light/dark cycle, provided with standard rodent chow and water *ad libitum*. For harvesting the gastric mucosa, rats were deeply anaesthetized with sodium thiopental (100 mg/kg *i.p.*) and killed by cervical dislocation and exsanguination. The abdomen was opened; the stomach was excised and cut open along the lesser curvature. Stomachs were stored in Krebs-Henseleit solution until their further use. Gastric content was gently emptied and the mucosa was rinsed with 0.1 M HCl solution containing 0.9 % sodium chloride (NaCl).

4.2.3.7.1. Detachment force studies

Detachment force studies were carried out according to the modified surface tensiometer method [98-101]. Inner surface of stomach mucosa was outspread on 10 % agar-agar gel immobilized with pins. Tablets were fixed with ethyl 2-cyanoacrylate on the bottom of a special specimen hanged on a tensiometer arm (KSV Instruments Ltd, Finland). Before measurements, mucosae were wetted with 20.0 µl 0.1 M HCl containing 0.9 % NaCl in order to achieve better mucoadhesive performance. Tablets were left on mucosae surface for 3 minutes.

Maximal detachment forces were recorded and calculated in mN with the following equation:

$$F_{detach} = F_{total} - F_{tablet} \quad (15)$$

Where F_{detach} is the detachment force, F_{total} is the measured total weight and F_{tablet} is the weight of tablet. Structure of measuring method is depicted in Fig. 9. Samples were tested in triplicate.

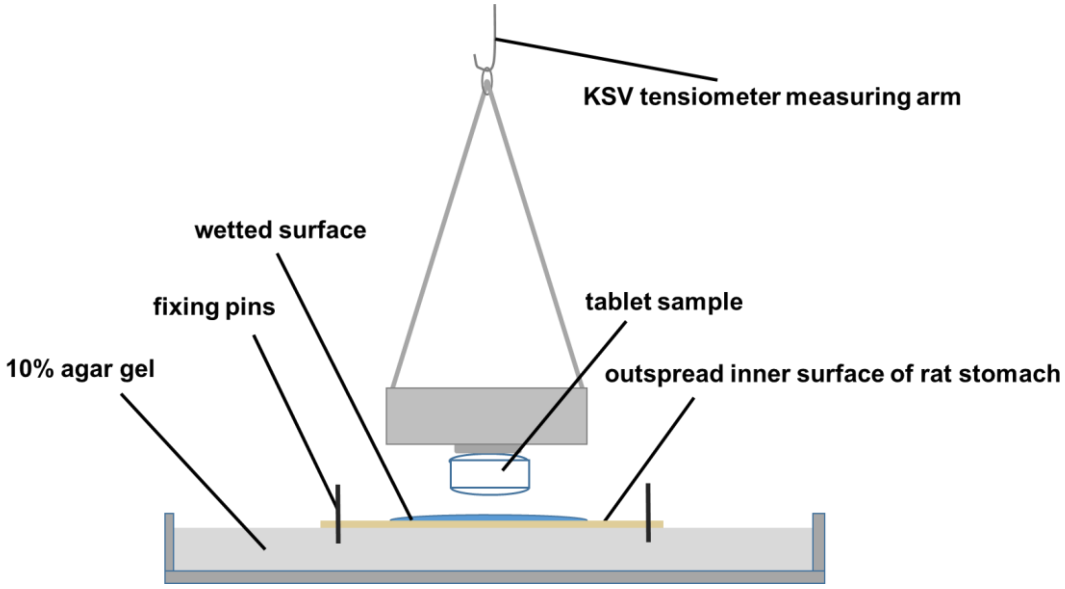

Fig. 9. Structure of tablet detachment force testing apparatus

4.2.3.7.2. Rheological mucoadhesion studies

Rheological *ex vivo* mucoadhesion measurements were carried out based on literature [102, 103].

Gastric mucus was carefully removed under operating microscope, after which it was put into 0.1 M HCl containing 0.9 % NaCl. Dispersion with high speed homogenizer (Ultra-Turrax® T 25, IKA®, Germany) with 9500 RPM for 2 minutes was performed to achieve homogeneous sample. The mucus then was centrifuged with 5500 RPM for 1 hour. Pellets were dialyzed with Membra-Cel® dialysis tubing (Serva MWCO 3500, Germany) on 4 ˚C for 24 hours. Mucus was again centrifuged (Labogene 1524, Denmark) with 15000 RPM for 1 hour. Pellets were stored at -15 ˚C until further use [104, 105].

3 % mucus solution and optimized formulation equilibrated to 3 % L-HPC and sodium alginate were dispersed separately in 20 ml 0.1 M HCl. Then mixture of 3 % mucus and MF_OPT equilibrated to 3 % L-HPC and sodium alginate were also prepared.

Flow curves of samples were examined in a rotational viscometer (Anton Paar Rheolab QC, Austria) at 37 °C. Data were recorded in a 0-25 s^{-1} shear rate interval.

Increase of viscosity due to mucoadhesion (η_m) were calculated with the following formula:

$$\eta_m = \eta_{total} - \eta_{tabl} - \eta_{mucus} \quad (16)$$

Where η_{total} is the viscosity of mucus/tablet mixture, η_{tabl} is the viscosity of MF_OPT equilibrated to 3 % L-HPC and sodium alginate and η_{mucus} is the viscosity of 3 % mucus solution. All experiments were performed in triplicate.

4.2.3.8. *In vivo* X-ray CT evaluation of floating tablets in rat

Metronidazole optimized tablets (optimization project) were studied in Wistar rats (*n*=5). For visualization and detection floating tablets, barium sulfate ($BaSO_4$) X-ray contrast material was used. 10 % of the optimized blend was replaced with $BaSO_4$. The homogenized blend was pressed with 3 mm round concave punches by eccentric single-punch tablet press (TSV-1, OMTKI, Hungary).

The experiment was carried out with the permission from the institutional Animal Ethics Committee of Semmelweis University and in compliance with the relevant European Union and Hungarian regulations (EC Directive 86/609/EEC). Images were acquired with a NanoSPECT/CT^{PLUS} (Mediso Ltd., Hungary). Animals were anesthetized with isoflurane (2 %) and positioned in the center of field of view (FOV). Before the experiment, rats were kept at room temperature in 12 hour light and dark cycle. The animals were not fasted, food and water were supplied *ad libitum*. Imaging was performed at the following sampling times: 5 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h and 48 h. Image at 48 hours after administration was examined that floating tablets did not caused gastrointestinal obstruction.

Experimental parameters of CT were: scan range: 59.8 mm; exposure: 500 ms, 65 kV, projection/ rotation: 360; number of rotations: 2; number of frames: 720; pitch: 1; corrections: offset, pixel, quadratic gain; acquisition time: 6 min 1 sec; reconstruction: butterworth filter, voxel size: 0.22*0.22*0.22 mm. Reconstructed, reoriented and co-registered images were further analyzed with Fusion (Mediso Ltd., Hungary) and VivoQuant (inviCRO LLC, USA) dedicated image analysis software products by placing appropriate volume of interests (VOI) on the tablets. Linear attenuation data were reconstructed into Hounsfield units (*HU*). Then a second, more detailed, lookup table (LUT) (indicated with different colors) was used by image processing to visualize spectacularly the differences of attenuation values of voxels ordered to the tablets VOIs [106].

5. Results and discussion

5.1. Preformulation methods

5.1.1. Comparative physical examination of L-HPC 11 and L-HPC B1

The photo analysis of L-HPC particles showed differences in shape and in size (Fig. 10). L-HPC 11 had longitudinal shape, while L-HPC B1 formed similar to spheroidal particles. Their shapes were characterized numerically with sphericity index. Sphericity index in the case of L-HPC 11 was 0.19±0.08, while in the case of L-HPC B1 was 0.48±0.18. The result showed that L-HPC B1 is more similar to ideal spherical particles (Ψ=1.0) than L-HPC 11, but its shape is far from sphere shape.

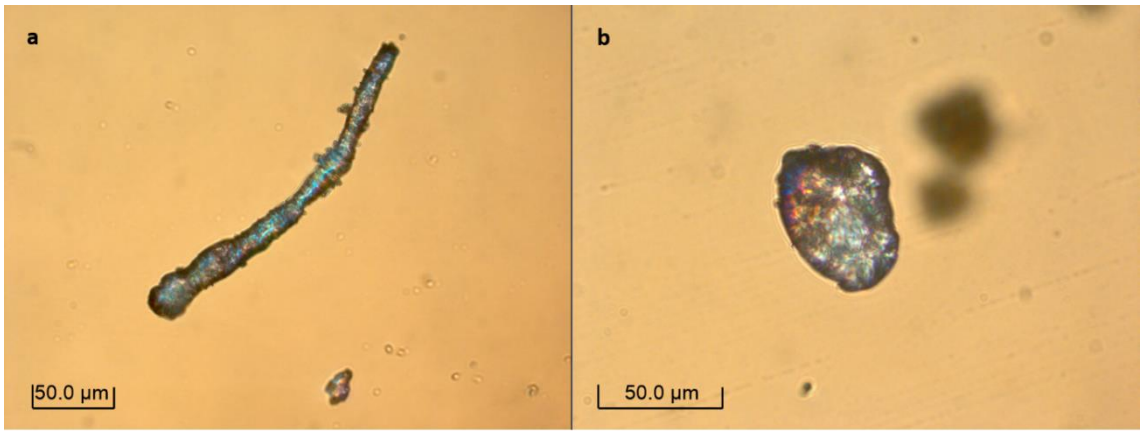

Fig. 10. Microscopic appearance of a) L-HPC 11 and b) L-HPC B1 particles

Particle sizes of L-HPC 11 and B1 were also differed. From both materials, 50 particles were evaluated. In the case of L-HPC B1, average size was 44.01±10.59 µm compared to L-HPC 11 with 246.35±82.03 µm average size.

Wettability of 50.0 mg pure L-HPCs were examined in order to reveal further difference between their physicochemical properties. All tests were performed in purified water and in 0.1 M hydrochloric acid for 1 hour, but the powders absorbed most of the fluid in the first 1 minute. Their liquid uptake and wettability rate as a function of time are shown in Fig. 11.

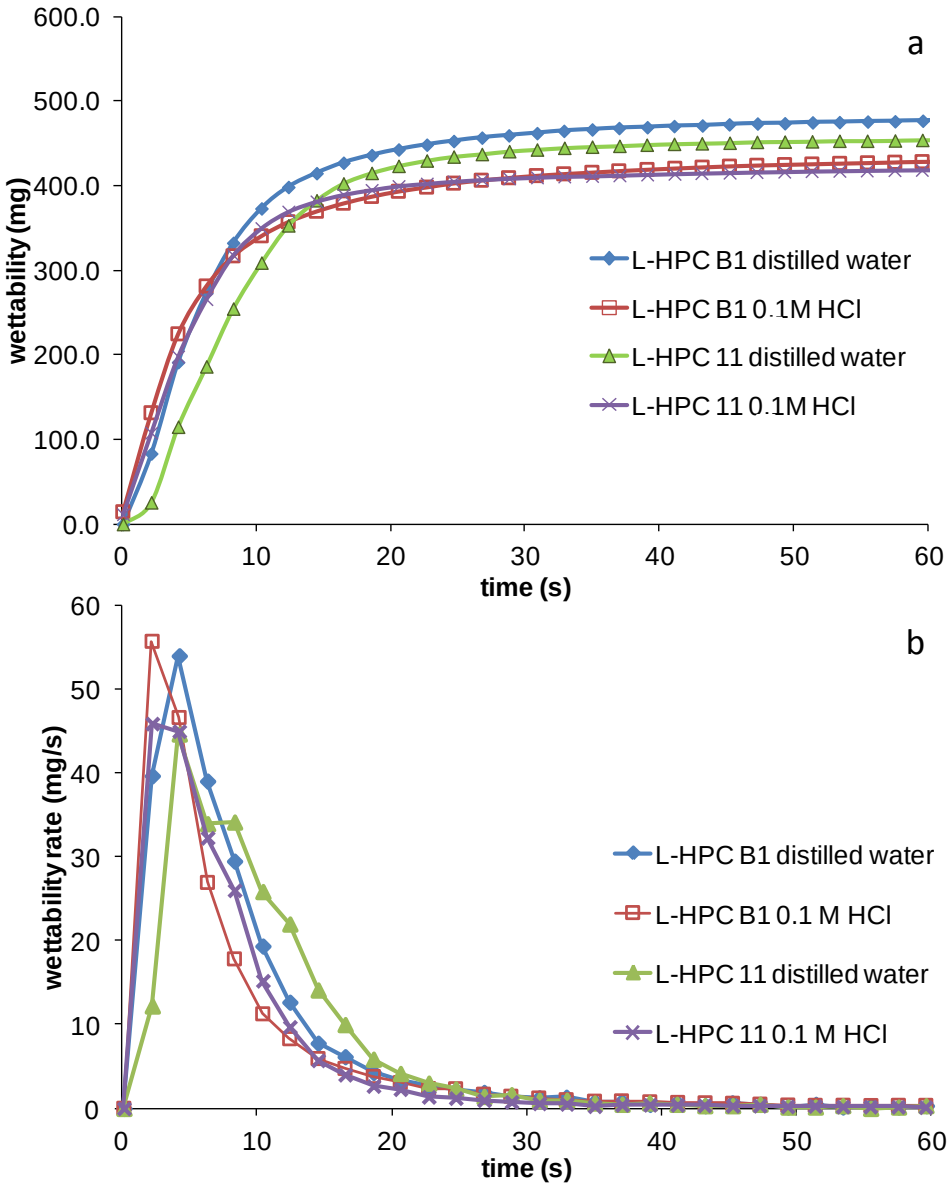

Fig. 11. Wettability and wettability rate of L-HPC 11 and B1 as a function of time

Final wettability values of examined L-HPCs are shown in Table 5. The result showed that wettability of both materials was lower in 0.1 M HCl than in distilled water, however, the rate of fluid absorption was higher in 0.1 M HCl. L-HPC B1 showed faster and more intense liquid absorption compared to L-HPC 11.

Table 5. Total wettability of L-HPC 11 and L-HPC B1 after 1 hour

Total wettability of 50 mg L-HPCs	distilled water (mg)	0.1 M HCl (mg)
L-HPC 11	483.7±22.8	451.0±17.4
L-HPC B1	522.7±36.6	480.0±39.6

Both values (Carr index and Hausner ratio) characterizing powder flowability showed better flowability of L-HPC B1. The differences manifested also in tapped (ρ_t) and bulk (ρ_b) densities.

Table 6. Flow characteristics and densities of L-HPC 11 and L-HPC B1

Samples	ρ_t (g/cm^3)	ρ_b (g/cm^3)	Carr index	Hausner ratio	Angle of repose(°)
L-HPC 11	0.443±0.001	0.356±0.008	22.91±0.32	1.24±0.04	48.77±1.67
L-HPC B1	0.591±0.003	0.496± 1.012	19.05±2.31	1.19±0.02	39.24±0.86

Demonstrated data (Table 6) revealed that flowability values differed between L-HPC types, but the differences are not remarkable. The largest deviation was in the case of angle of repose, which can have significant influence during filling the blend into dies.

5.1.2. Rheological behavior of sodium alginate

At the preformulation stage of experiments, rheological properties of sodium alginate were determined. High viscosity grade sodium alginate was applied during formulation, which was characterized specifically. The result showed significant dependence of sodium alginate viscosities on temperature. Measured viscosities are presented by Table 7.

Flow curve measurements showed non-Newtonian, pseudoplastic behavior showing decrease in viscosity caused by increase of shear rate. The influence of temperature in rheological properties of sodium alginate solutions is shown in Fig. 12.

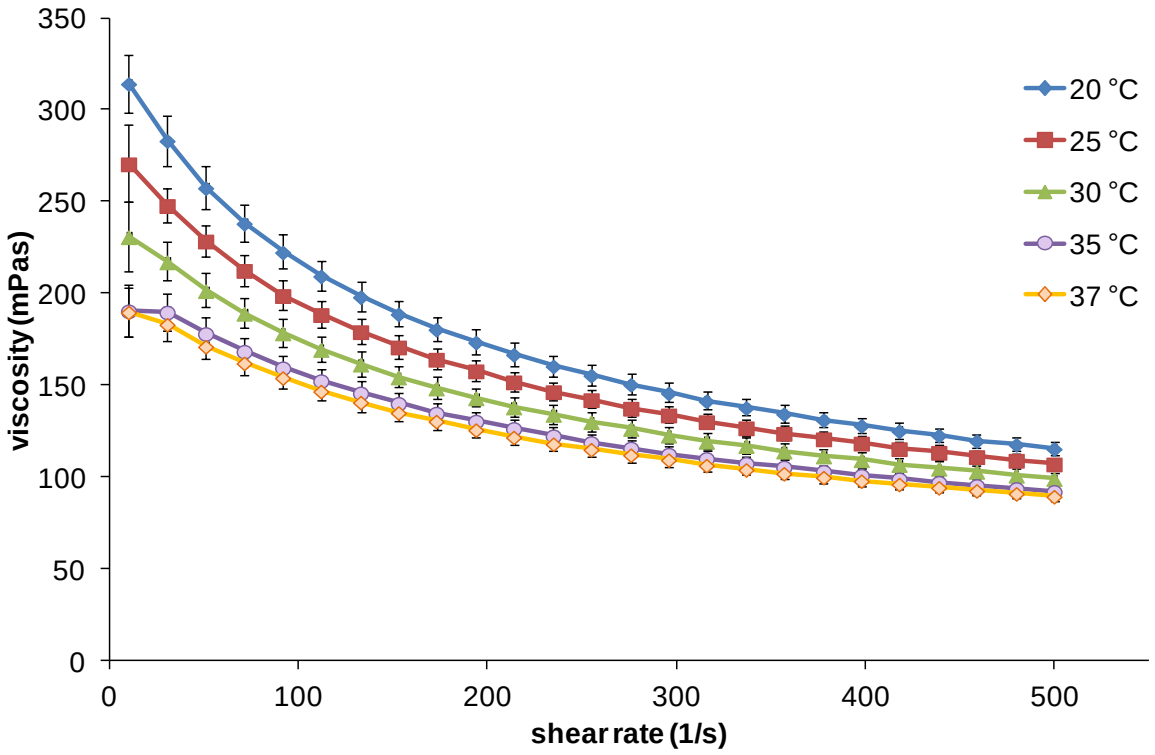

Fig. 12. Temperature dependence of flow curves of 1 % sodium alginate aqueous solutions

Calculated flow behavior indices are shown in Table 7, which demonstrates that rheological behavior changes with temperature. Increasing *z* value results in less change in viscosities caused by increase of shear rate.

Table 7. Temperature dependence of viscosity and flow behavior index of 1 % sodium alginate solutions

t (°C)	η (mPas)	*z*
20	214.73±7.73	-0,6943
25	196.88±7.02	-0,7139
30	175.65±7.82	-0,7352
35	157.12±6.60	-0,7601
37	151.63±6.42	-0,7604

5.2. Formulation results

5.2.1. Preliminary project

5.2.1.1. Studies of floating behavior

In the section of floating behavior examinations, floating lag time, total floating time and vertically expressed floating force studies were performed and evaluated.

The best fitting model on floating lag time (t_{lag}) data was the linear model (p<0.01) and sodium alginate was the only significant factor (p<0.01) in the examined concentration ranges. In the lower level (-1) of sodium alginate (0.5 %) short t_{lag} values were observed, which was 25.77±6.53 s. Increasing amount of sodium alginate resulted in longer time to achieve buoyancy. At higher sodium alginate levels (0, +1), maximal t_{lag} value was 520.30±20.79 s which can be considered to be long time to float, but only 8 % of sodium bicarbonate accelerated the flotation.

On the total floating time ($t_{floating}$) data, quadratic model could be fitted and both numerical factors and their interactions showed significance (p<0.0001). At 0 and +1 levels (17.82, 35.05 %) sodium alginate caused more than 4 hour flotation, since in the cases of tablets having 0.5 % sodium alginate more rapid disintegration could be observed, at which disintegrant effect of L-HPCs could prevail. L-HPC 11 resulted in faster disintegration.

Floating lag time and total floating time data are shown in Table 8.

Table 8. Data of floating lag time and total floating time

Exp. No.	t_{lag} (sec)	$t_{floating}$ (min)	F_{max} (mN)	t_{Fmax} (s)
PFS01	6.44 ± 5.19	21.7 ± 4.6	2.295	415.1
PFS02	343.00 ±28.99	240.0 ± 0.0	1.549	14395
PFS03	0.00 ± 0.00	7.1 ± 0.5	2.037	30.4
PFS04	507.16 ± 100.35	240.0 ± 0.0	3.884	14295
PFS05	0.00 ± 0.00	3.9 ± 0.3	2.363	15.3
PFS06	10.16 ± 75.77	240.0 ± 0.0	1.904	14396
PFS07	109.20 ± 89.32	240.0 ± 0.0	1.626	930
PFS08	16.69 ± 1.58	240.0 ± 0.0	2.557	14398
PFS09	520.30 ± 20.79	240.0 ± 0.0	1.780	13715
PFS10	16.02 ± 6.58	28.6 ± 1.1	1.375	379.2
PFS11	356.55 ± 47.07	240.0 ± 0.0	2.859	14398
PFS12	0.00 ± 0.00	15.5 ± 3.0	1.292	45.6
PFS13	230.16 ± 11.87	240.0 ± 0.0	4.606	3004.8
PFS14	0.00 ± 0.00	15.3 ± 1.9	1.199	141.7
PFS15	507.67 ± 161.30	240.0 ± 0.0	2.164	14386
PFS16	273.45 ± 156.64	240.0 ± 0.0	0.925	14399
PFS17	180.00 ± 51.64	240.0 ± 0.0	2.677	14119
PFS18	106.14 ± 24.18	240.0 ± 0.0	2.06	14389
PFS19	25.77 ± 6.53	35.7 ± 3.1	1.412	475.3
PFS20	238.16 ± 13.25	240.0 ± 0.0	1.324	5450.9
PFS21	13.87 ± 3.25	17.2 ± 1.3	1.273	45.6
PFS22	417.22 ± 18.12	240.0 ± 0.0	3.270	14386
PFS23	8.10 ± 2.62	13.3 ± 0.3	1.172	65.8
PFS24	330.33 ± 27.79	240.0 ± 0.0	2.315	14398
PFS25	307.71 ±174.70	240.0 ± 0.0	1.194	14398
PFS26	344.00 ± 29.70	240.0 ± 0.0	2.217	14313
PFS27	184.00 ± 24.64	240.0 ± 0.0	1.899	14393

The floating force measurements have been performed for 4 hours. Among samples two patterns could be observed the rapid disintegrating and exponentially increasing patterns. On maximal floating force values (F_{max}) data, two-factor interaction model was fitted (p<0.0001). Both numerical factors (p<0.0001) and their interactions (p=0.0004) showed significance. Categorical factor had no significance.

On t_{Fmax} data, quadratic model was used due to its significant fitting. Time values for achieving maximal floating forces were only influenced significantly by sodium alginate (p<0.0001). Response curve of t_{Flag} values as a function of L-HPC 11/B1 mixture, and sodium alginate is depicted by Fig. 13.

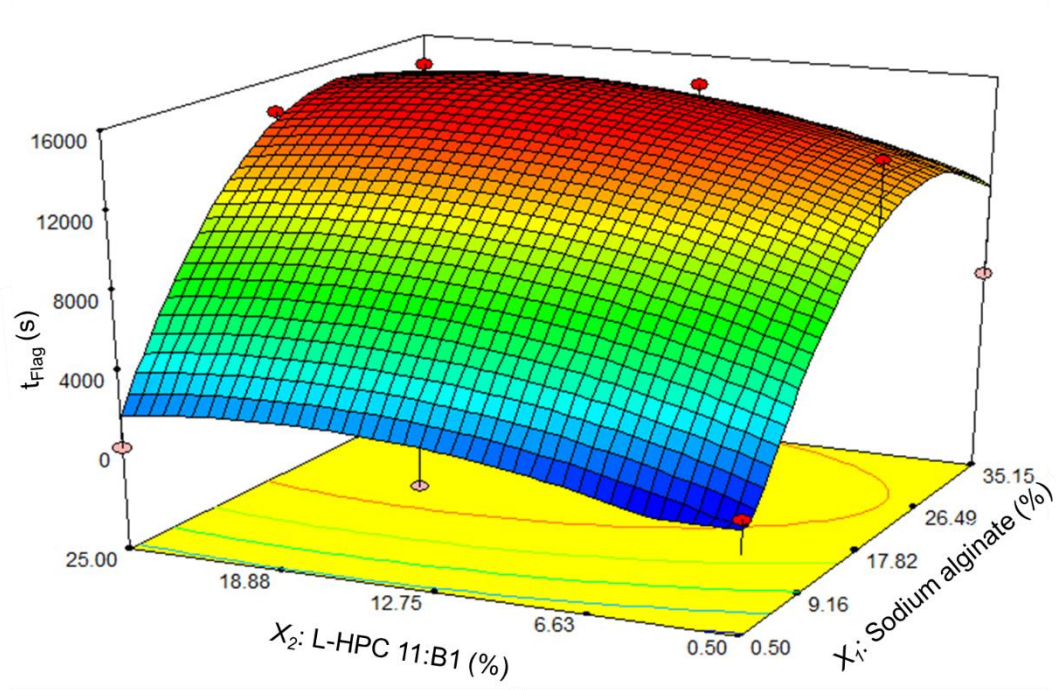

Fig. 13. The effect of L-HPC 11:B1 mixture and sodium alginate on maximal floating force development time

5.2.1.2. Determination of swelling capability

Swelling capabilities of polymers have one of the most important influences on the behavior in aqueous medium of floating tablets, which are basically matrix tablets. In matrix tablets, during the swelling process, aqueous medium reaches deeper layers of tablets with time, which process is generally driven by diffusion. Drug release of tablets are also affected by swelling, since diffusion creases hydration layers in the tablets resulting in outward diffusion of API [39]. Inside the tablets, relatively dry core with low water content is followed by more hydrated layers, which are surrounded by the surface layer in contact with the medium. The hydration layers of PFS04 floating tablet (L-HPC 11: 25.0 %, Sodium alginate: 35.15 %) can be identified at 4 hour depicted by Fig. 14.

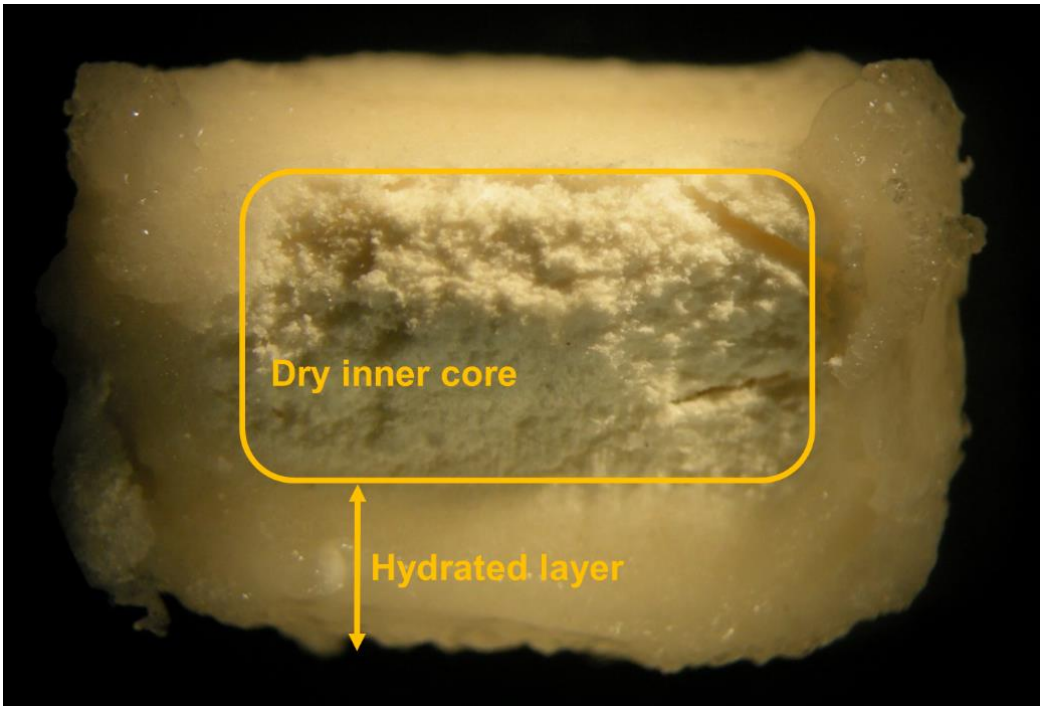

Fig. 14. Macroscopic view of PFS04 floating tablets (L-HPC 11: 25.0 %, sodium alginate: 35.15 %) after 4 hours of hydration

Values of S_i indices show the water uptake of tablets, which additionally were standardized for tablet weights. On the swelling capability data, linear model could be fitted with high significance ($p<0.01$). At S_i values after 30 minutes and 1 hour, sodium alginate was the only significant factor ($p<0.01$), but at 2 hours L-HPC (X_2) was also significant ($p<0.05$). The categorical factor was not significant. Maximal swelling index (S_i=2.357±0.067) was observed at PFS22 (L-HPC 11/B1: 25.0 %, sodium alginate: 35.15 %), which is significantly higher, than published S_i values of sodium alginate based floating tablets [107]. The summary of swelling indices as a function of time is shown in Fig. 15.

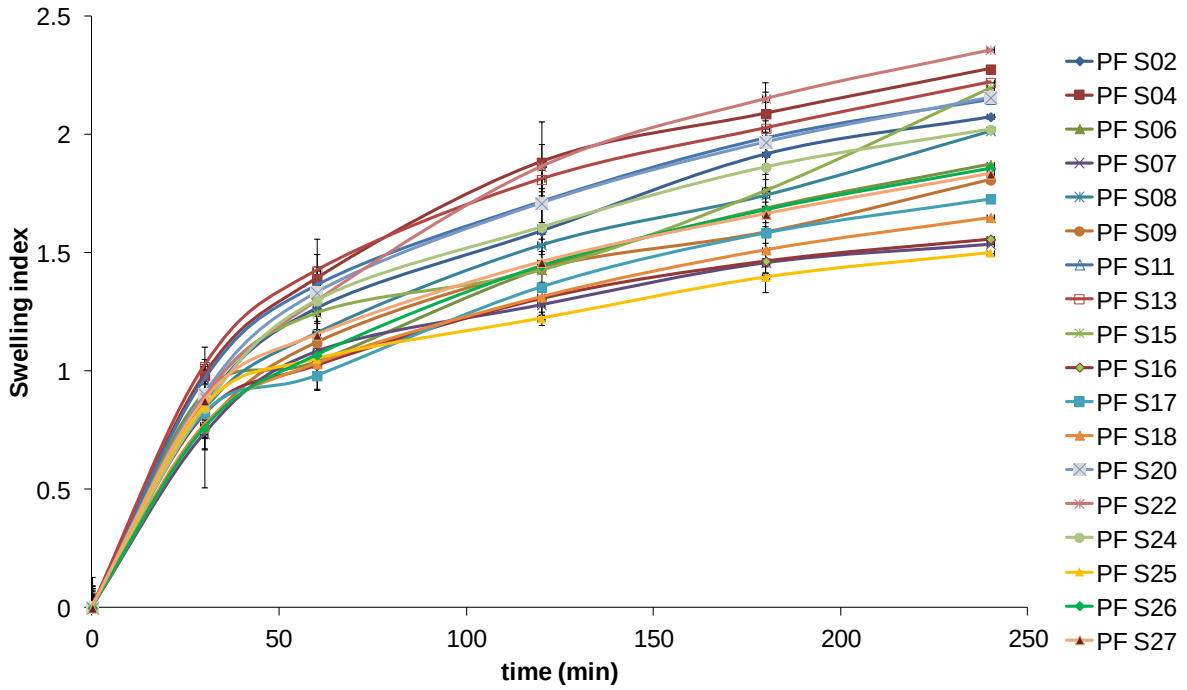

Fig. 15. Result of swelling capability determination studies as a function of time

5.2.1.3. Paracetamol release studies

Dissolution studies of paracetamol were carried out for 4 hours. In this time interval, various release profiles could be observed as shown in Fig. 16, but two main types could be distinguished and identified: rapid disintegrating – and prolonged drug release.

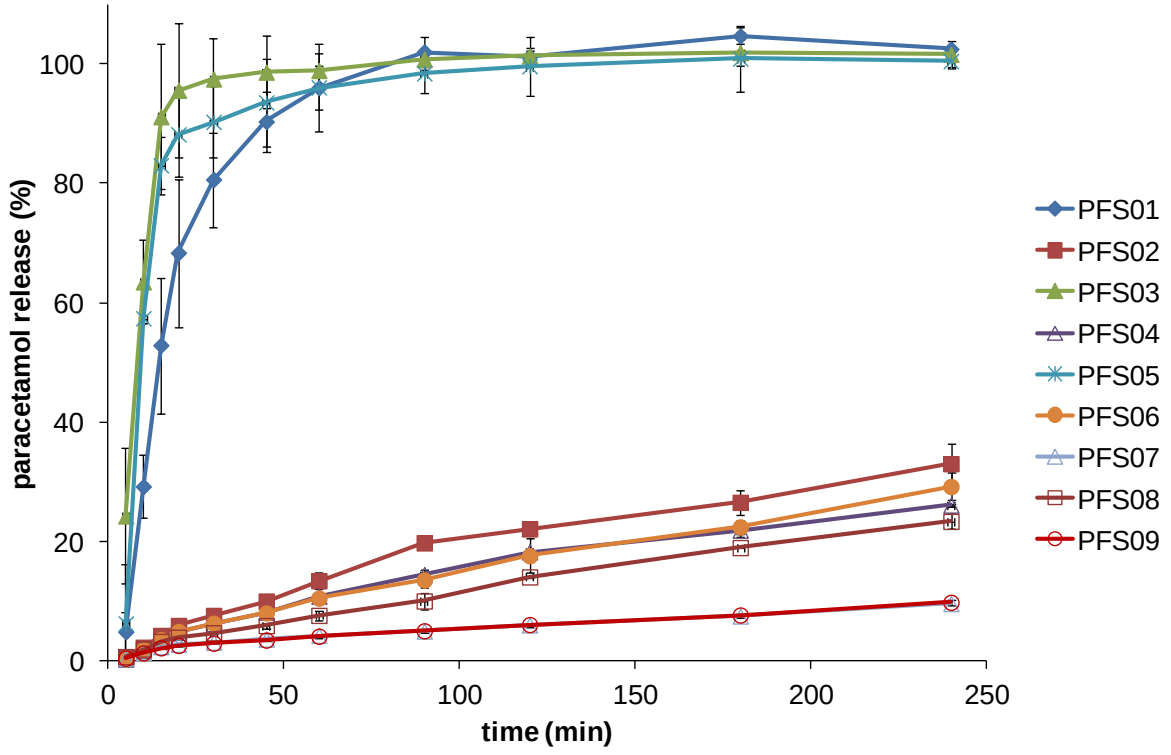

Fig. 16. Dissolution of paracetamol from floating tablets containing L-HPC 11

Those tablets in which sodium alginate concentration was in lower level (-1: 0.5 %) released the API fast. Floating tablets with more than 17.82 % sodium alginate showed sustained release. This phenomenon may be due to that 0.5 % sodium alginate concentration was not enough to create coherent structure, thus the effect of L-HPCs could manifest. On the other hand, floating tablets with sustained release could produce maximally 32.99±3.40 % dissolution after 4 hours. In the analysis of variance (ANOVA), two sections could be identified based on significance of factors. All dissolution data were fitted with quadratic model (p<0.0001). In the first time period (from 5 to 45 min), sodium alginate (X_1), L-HPC (X_2) and their interaction (X_1X_2) were significant (p<0.05). At 45 min, categorical factor (X_3) became significant, too. In the second period (from 1 to 4 hours), sodium alginate and the categorical factor were only significant (p<0.05), which indicated that the influence of L-HPC was not permanently significant on dissolution only until 45 minutes. Difference between L-HPC types could only be observed in this period.

5.2.1.4. Conclusion of the preliminary project

The following conclusion could be drawn from the preliminary project studies:

- the floating-, swelling behavior and dissolution of the preliminary floating tablets were highly affected by the amount of sodium alginate (X_1) in the compositions,
- L-HPC resulted in rapid disintegration, but this effect could only be manifested, when lower quantity of the matrix former (sodium alginate in 0.5 %) was present,
- when sodium alginate was applied in 17.82 and 35.05 %, then high swelling capability and sustained dissolution could be observed as well as longer floating lag time and floating time,
- L-HPC as numerical factor (X_2) was significant in several cases, but its effect was less than the effect of sodium alginate concentration (in this concentration range),
- categorical factor (X_3) was only significant at dissolution after 45 min, hence the significance could be identified but the difference was not remarkable, therefore for further studies L-HPC B1 was used considering its better flowability properties,
- floating lag time data indicated that 8 % sodium bicarbonate may have to be increased in order to achieve faster start of buoyancy,
- the range of L-HPC and sodium alginate concentrations was too broad, thus further adjustment in this interval was required in order to create a floating drug delivery systems with desirable floating-, swelling-, dissolution properties.

5.2.2. Optimization project

In order to utilize the result of the preliminary project, the optimization project was designed with 5.0-15.0 % sodium alginate (X_1) - and 30.0-45.0 % L-HPC B1 (X_2) concentration. 8.0-13.0 % of sodium bicarbonate was applied as another numeric factor (X_3) in this experimental matrix. L-HPC B1 was used in these experiments, since it had better physicochemical properties. Other differences between L-HPC types (L-HPC 11 and B1) were not remarkable.
The experimental setup is shown in Table 4.

5.2.2.1. Studies of floating behavior

Results of floating lag time studies indicated that rapid buoyancy could be achieved, since t_{lag} of 9 formulations was less than 1 minute. The best model fitted on data was the quadratic model (p<0.01). Sodium alginate, L-HPC B1 and the possible interaction of L-HPC B1 and sodium bicarbonate were significant (p<0.01), among which the latter may be explained by the hydration mechanism of the tablets. Low substituted hydroxypropyl cellulose absorbs the water/hydrochloric acid rapidly (Fig. 11) resulting in more intense carbon dioxide creation. Shorter t_{lag} could be observed at floating tablets having 5.0 % of sodium alginate and 37.5 % of L-HPC B1 or more. MF07 showed the shortest floating lag time, which contained the 5.0 % sodium alginate (X_1: -1), 45.0 % L-HPC B1 (X_2: +1) and 13.0 % sodium bicarbonate (X_3: +1). The increase of sodium alginate amount raised floating lag time values, which may be explained by higher coherency of the matrix structure. Floating lag time and floating force data are shown in Table 9.

Table 9. Results of floating behavior studies

Samples	t_{lag} (s)	F_{max} (mN)	t_{Fmax} (s)	$F_{max/100mg}$ (mN)
MF01	29.00±1.00	5.18±0.49	1122.05±400.30	1.12
MF02	217.67±43.39	2.2±0.05	6213.50±338.35	0.39
MF03	16.67±1.15	12.29±0.03	5934.15±1148.90	1.91
MF04	220.00±44.93	6.88±0.60	5179.90±781.42	0.79
MF05	266.00±50.91	6.17±1.02	537.50±334.44	1.21
MF06	321.00±216.37	5.89±0.29	23114.53±1650.89	0.92
MF07	12.00±2.37	26.64±1.18	877.27±21.18	3.61
MF08	82.57±26.39	14.13±0.94	13327.90±2995.73	1.35
MF09	17.33±3.39	16.77±1.86	1352.37±69.55	2.95
MF10	165.33±15.01	3.96±0.41	7058.25±1738.28	0.54
MF11	30.67±0.58	2.68±0.56	2463.40±303.91	0.50
MF12	18.33±1.53	8.05±1.47	7421.10±405.17	1.01
MF13	54.00±2.00	3.62±0.25	4321.07±831.62	0.60
MF14	45.33±3.21	8.49±0.60	21546.00±567.94	1.24
MF15	48.67±4.62	7.04±0.04	20915.05±2881.39	1.09
MF_OPT	13.25±0.50	12.75±1.87	847.68±373.90	2.29

Results of floating force studies indicate remarkably high maximal floating forces and maximal floating forces per 100 mg. The maximum was observed at MF07 expressing 26.64±1.18 mN vertical force. In the literature, floating force is termed as resultant weight or floating strength and is wrongly expressed in the unit of grams or milligrams, since the International System of Units (SI) uses Newtons as derived unit (N=1 kg*m/s^2) to express force/strength. In order to compare the result of our floating force studies, floating force value of MF07 was converted to resultant weight: 26.64±1.18 mN = 2716.52±120.32 mg „resultant weight". The more than 2.50 g floating force is remarkable high compared to the values published in several articles [108-113].

Linear model was applied on floating force data (F_{max}, $F_{max/100mg}$) with appropriate fitting (p<0.01). In the analysis of F_{max}, all numerical factors were significant (p<0.03) pointing out their influence on maximal floating force. Final equation of F_{max} showed that increase of sodium alginate amount decreases -, while increase of L-HPC or sodium bicarbonate increases the vertically expressed floating force values.

$$F_{max} = 8.68 - 3.38X_1 + 4.57X_2 + 3.14X_3 \quad (17)$$

In the case of $F_{max/100mg}$ evaluation, only sodium alginate and L-HPC B1 were significant, but p value of sodium bicarbonate was 0.079 possibly showing a tendency to affect $F_{max/100mg}$. Final equation of $F_{max/100mg}$ data showed the same relation of influences of factors as in Eq. 18.

$$F_{max/100mg} = 1.28 - 0.68X_1 + 0.46X_2 + 0.35X_3 \quad (18)$$

The time needed for maximal floating force (t_{Fmax}) did not show any significant fitting neither on linear, nor quadratic, nor interaction model. The shortest t_{Fmax} values could be observed at tablets having lowest sodium alginate content.

5.2.2.2. Metronidazole release studies

In vitro dissolution studies of all floating tablets were performed for 6 hours and its result are depicted in Fig. 17.

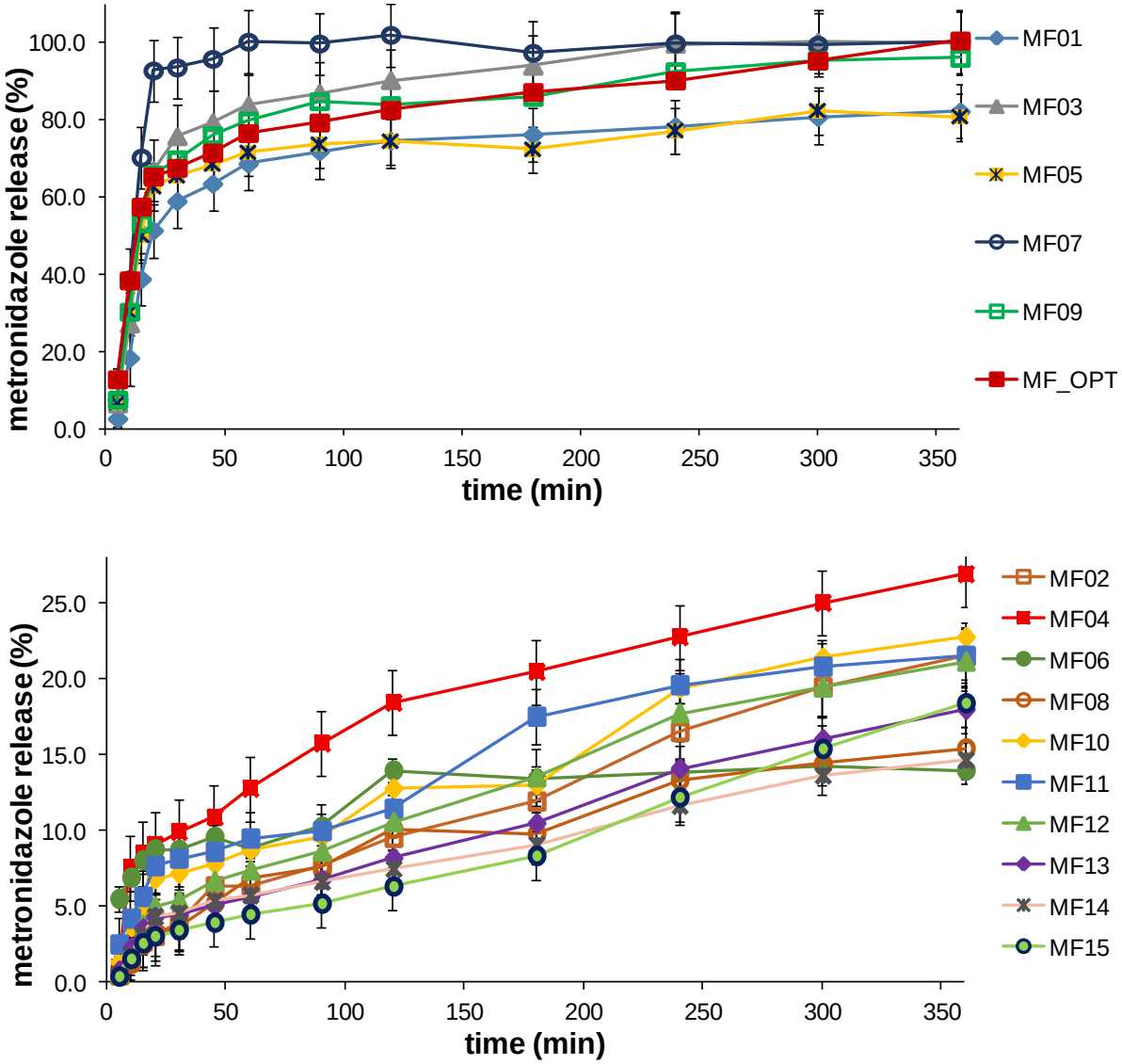

Fig. 17. Dissolution profiles of floating tablets (optimization project)

Quadratic model could be fitted (p<0.01) on dissolution data. Sodium alginate (X_1) concentration was significant for dissolution at all sampling times (p<0.0001), L-HPC B1 (X_2) quantity was significant only data after 30 minutes (p<0.05), and sodium bicarbonate (X_3) showed only tendency (p<0.10) until the first 10 minutes. This phenomenon may be due to the disintegrative effect (CO_2 generation) of sodium bicarbonate on the surface of the tablets resulting in increased dissolution. ANOVA pointed out a possible interaction between sodium alginate and L-HPC B1, which negatively affected the metronidazole dissolution.

The most rapid dissolution could be observed at MF07 (sodium alginate: 5.0 %, L-HPC B1: 45.0 %, sodium bicarbonate: 13.0 %) having total dissolution after 60 minutes. Compositions with more than 5.0 % sodium alginate (10.0 or 15.0 %) could not produce more than 26.87±1.05

% dissolution after 6 hours. In the case of 5.0 % sodium alginate, the least released amount of metronidazole was circa 80-82 % (MF01, MF05).
Standard deviations (SD) of all dissolution data were also analyzed with ANOVA and sodium alginate was found as a significant factor influencing SD values (p<0.05). The more sodium alginate quantity the tablets had the less SD values were observed, which consequently could influence manufacturing reproducibility.

5.2.2.3. Optimization of technological and biopharmaceutical parameters

In order to determine a composition of a desired floating tablet, optimization was carried out based on the evaluation of floating behavior and release experimental parameters. One of the aims was to minimize amount of excipients, since less excipients amount are applied, the more active substance(s) can be loaded into tablets. Floating parameters were adjusted so that the formulation would have the best floating properties within this concentration interval manifesting in minimization of t_{lag} and maximization of F_{max} and $F_{max/100mg}$. The proportion of release until the first 30 minutes was minimized, after 30 minutes it was maximized.
Based on the optimization criteria, an optimal composition (MF_OPT) was determined having 5.0 % sodium alginate, 38.63 % L-HPC B1 and 8.45 % sodium bicarbonate by Design Expert 7.0.0 software. Floating parameters (Table 9) were also ascertained. Dissolutions of two commercially available non-floating metronidazole generics were tested, in order to compare them with the optimized formulation. The comparative dissolution is depicted in Fig. 18.

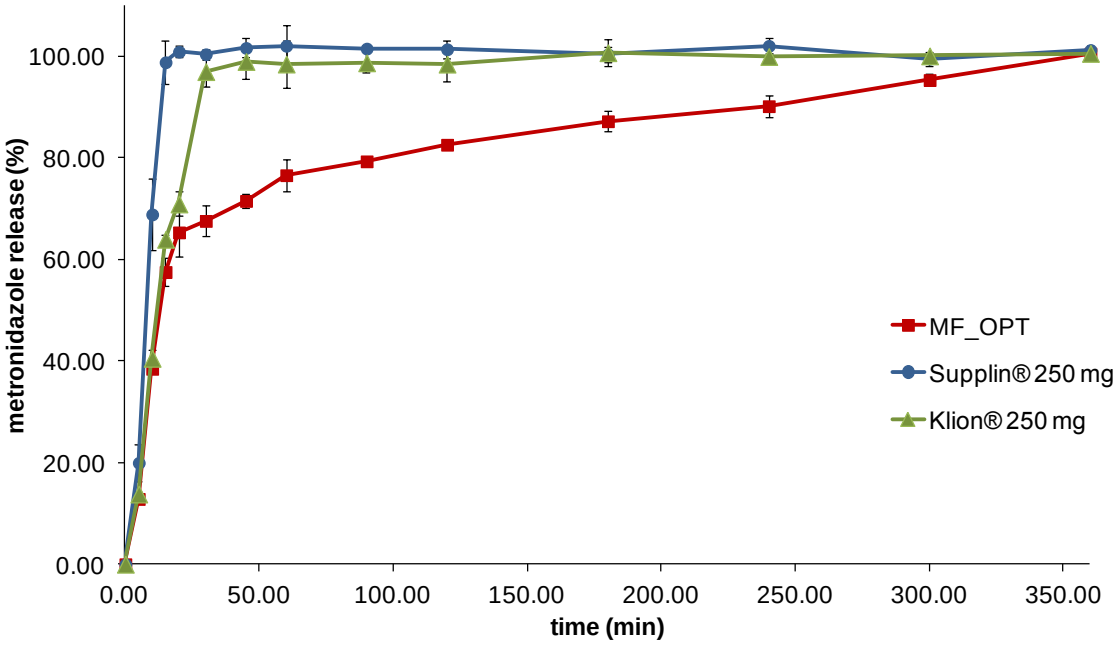

Fig. 18. Comparison of dissolution profiles of two commercially approved non-floating metronidazole tablets and the optimized formulation (MF_OPT)

Results showed that dissolution of MF_OPT could be regarded to be biphasic release involving an initiative rapid - (~60 %) and a prolonged release section (~40 %). MF_OPT tablets had several advantages including a biphasic release and remarkable floating parameters compared to the rapidly released metronidazole from the two approved tablets.

Optimized floating tablets were studied with further tests and evaluations in order to identify their further possibilities and advantages, thus microbiologically detected dissolution -, physical interaction -, *ex vivo* mucoadhesive studies and *in vivo* imaging evaluation were carried out.

5.2.2.4. Drug release kinetics

Evaluation of metronidazole release revealed that tablets having sustained dissolution (MF2, MF4, MF6, MF8, MF10, MF11, MF12, MF13, MF14, MF15) can be characterized by Higuchi model, which refers to drug diffusion from polymer matrices. These floating tablets contained 10.0 or 15.0 % sodium alginate. In the case of low sodium alginate concentration (5.0 %), none of the applied models fitted significantly. Result of kinetics analysis is shown in Table 10.

The best fitted release model of MF_OPT was first order kinetics, but due to the biphasic dissolution coefficient of determination was only R^2=0.820.

Table 10. Result of model dependent evaluation of drug release (optimization project)

	Zero order model			First order model			Higuchi model			Weibull model		
Sample	*m*	*n*	R^2	*m*	*n*	R^2	*m*	*n*	R^2	*m*	*n*	R^2
MF1	0.23	37.64	0.509	-0.05	4.08	0.678	4.81	18.53	0.693	0.83	-3.54	0.728
MF2	0.06	1.82	0.964	0.00	4.59	0.969	1.09	-1.92	0.980	0.82	-6.12	0.939
MF3	0.28	48.88	0.518	-0.02	4.20	0.896	5.65	26.63	0.693	0.90	-3.20	0.860
MF4	0.08	6.75	0.857	0.00	4.54	0.883	1.43	1.64	0.944	0.63	-4.58	0.758
MF5	0.18	46.33	0.373	0.00	3.91	0.500	3.77	30.87	0.546	0.60	-2.44	0.664
MF6	0.03	7.34	0.808	0.00	4.53	0.812	0.61	5.15	0.888	0.24	-3.20	0.917
MF7	2.00	24.71	0.658	-0.08	4.55	0.817	19.96	-19.83	0.789	1.72	-4.82	0.891
MF8	0.05	2.03	0.900	0.00	4.59	0.909	0.93	-1.26	0.972	0.84	-6.32	0.919
MF9	0.24	48.80	0.481	0.00	3.89	0.771	4.92	29.24	0.657	0.72	-2.66	0.785
MF10	0.06	3.99	0.911	0.00	4.57	0.919	1.12	0.13	0.940	0.58	-4.76	0.906
MF11	0.07	4.69	0.934	0.00	4.56	0.942	1.16	0.75	0.946	0.49	-4.26	0.942
MF12	0.06	2.64	0.954	0.00	4.58	0.963	1.15	-1.28	0.977	0.75	-5.66	0.900
MF13	0.05	2.36	0.958	0.00	4.58	0.963	0.86	-0.55	0.962	0.63	-5.35	0.909
MF14	0.04	2.53	0.897	0.00	4.58	0.907	0.72	0.00	0.959	0.67	-5.63	0.858
MF15	0.04	1.53	0.960	0.00	4.59	0.961	0.74	-0.96	0.946	0.72	-6.03	0.887
MF_OPT	0.22	50.30	0.524	0.00	3.89	0.820	4.40	33.06	0.690	0.59	-2.13	0.816
Klion®	0.56	51.16	0.497	-0.04	3.59	0.618	8.66	23.56	0.671	1.07	-3.07	0.866
Supplin®	6.07	-7.26	0.833	-0.34	6.24	0.897	42.31	-76.73	0.899	3.07	-7.23	0.953

5.2.2.5. Microbiologically detected dissolution studies

Microbiological inhibition of metronidazole released by MF_OPT floating tablets were evaluated in order to show its *in vitro* pharmacological effect as a function of time. Dissolution result of MF_OPT with spectrophotometric detection was compared with the microbiologically detected dissolution (Fig. 19). This graph shows a great similarity between assay based on pharmacological effect and UV absorbance assay. Microbiological inhibition zone of MF_OPT dissolution (at 4 hour) is shown in Fig. 20.

Difference (f_1) and similarity factors (f_2) were calculated and used for qualitative, model independent comparison of dissolution profiles [1, 92, 114] endorsed by Food and Drug Administration [115]. These evaluation methods used generally to compare dissolution profiles in studying generics have several criteria such as three or more dissolution media have to be applied. In these studies, dissolutions detected by two different methods were assessed thus not all criteria were fulfilled, since the aim was different.

Lower value than 15 at f_1 and value between 50 and 100 at f_2 classified the dissolution profiles to be similar. Difference factor (f_1) 5.23 and similarity factor (f_2) 66.61 were found to be statistically significant, which showed similar dissolution profiles of spectrophotometric and microbiological detection methods.

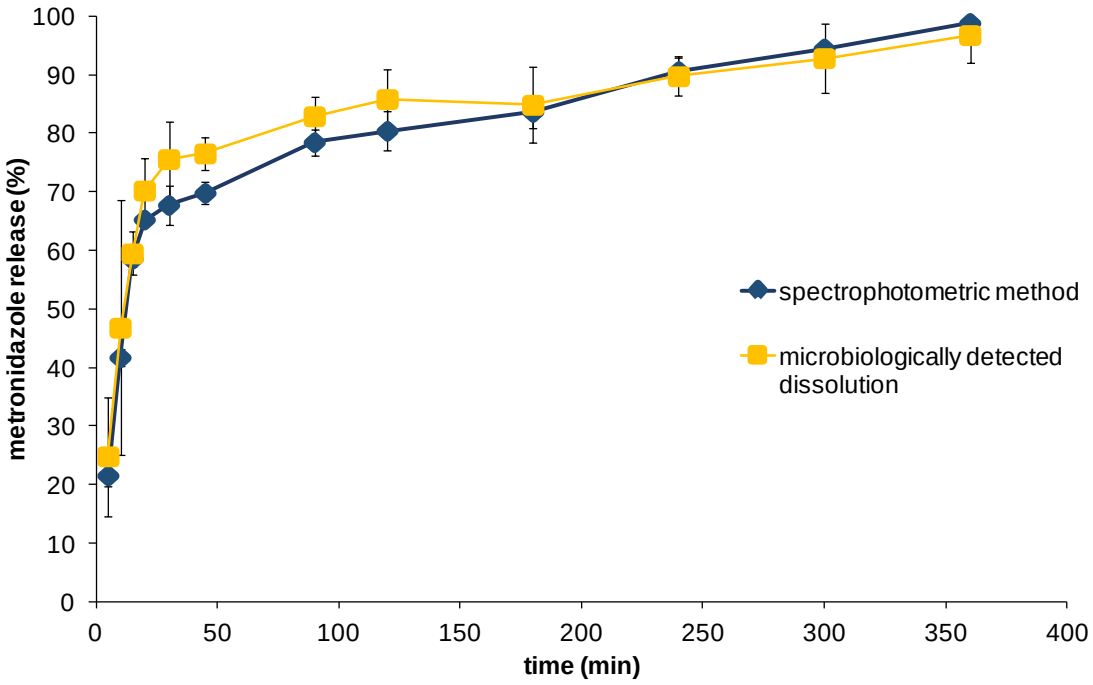

Fig. 19. Comparison of spectrophotometric and microbiologically detected dissolution of MF_OPT

Fig. 20. Inhibition zone of microbiologically detected dissolution of MF_OPT at 4 h

5.2.2.6. Drug-excipients interaction studies

Result of the thermoanalytical method showed a sharp endothermic peak of pure metronidazole at 159.9 °C, which was considered as the melting point. Based on the composition of MF_OPT, all the excipients were blended with pure metronidazole separately. The samples did not show significant alteration of metronidazole melting point, which is between 159 and 163 °C according to the literature [116]. DTA thermograms of samples are shown in Fig. 21. Consequently, results of this study could not indicate any API-excipient interactions.

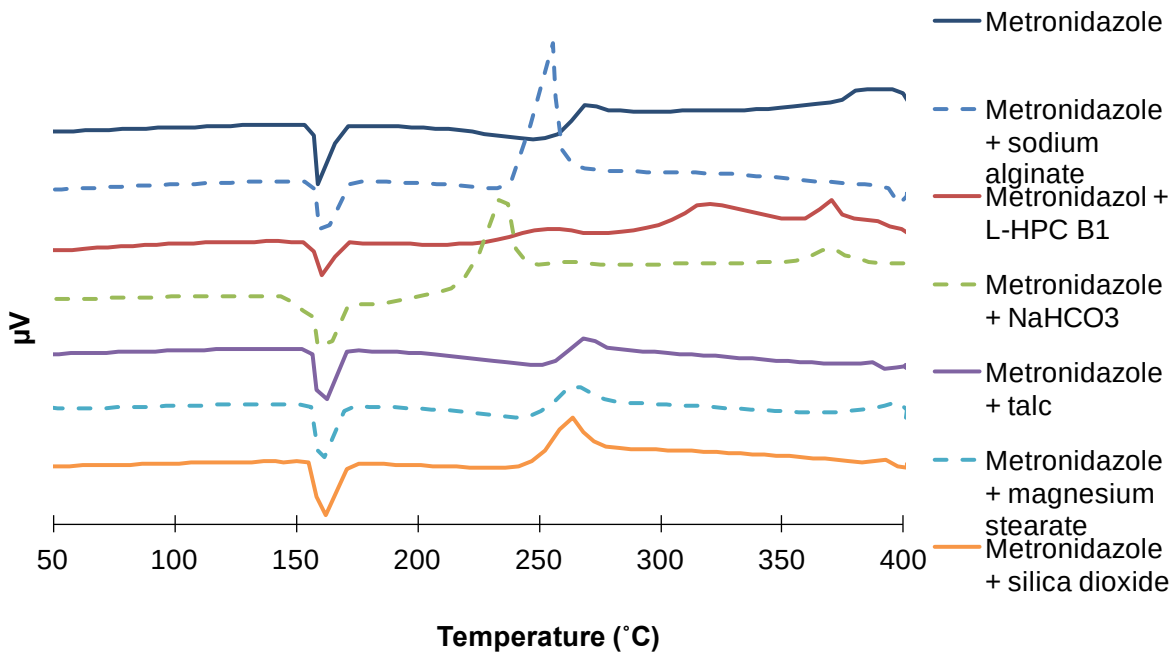

Fig. 21. DTA thermograms of metronidazole and metronidazole-excipient blends according to MF_OPT composition

In isothermal stress test studies, periodical visual control of the samples did not show any color change or alteration in appearance compared to control samples. After 3 weeks in 50 °C, metronidazole assays were performed by HPLC method. Retention times of samples were around 7 minutes and no additional peaks could be found. Recovered quantities of stressed and control samples are shown in Table 11.

Table 11. Result of isothermal stress testing of metronidazole after 3 weeks stressed storage

Samples	Drug : Excipient ratio	Control samples (%)	Stressed samples (%)
Metronidazole	-	97.25±1.83	95.26±4.95
Metronidazole + sodium alginate	1:0.1116	95.61±2.42	96.04±2.38
Metronidazole + L-HPC B1	1:0.8619	98.74±4.07	99.32±0.58
Metronidazole + $NaHCO_3$	1:0.1885	97.34±2.68	95.65±0.82
Metronidazole + talc	1:0.0446	99.74±3.92	98.14±1.67
Metronidazole + magnesium stearate	1:0.0223	96.23±1.61	97.06±1.80
Metronidazole + silica dioxide	1:0.0022	99.20±3.49	96.52±1.82

In order to determine separation capability of the HPLC method, we have performed a measurement of pure metronidazole exposed to UV light (2x8W, Ser.: 1407, Gamag, Switzerland) at 254 nm for 30 minutes. In this experiment an additional peak (decomposition product) was found (Fig. 22).

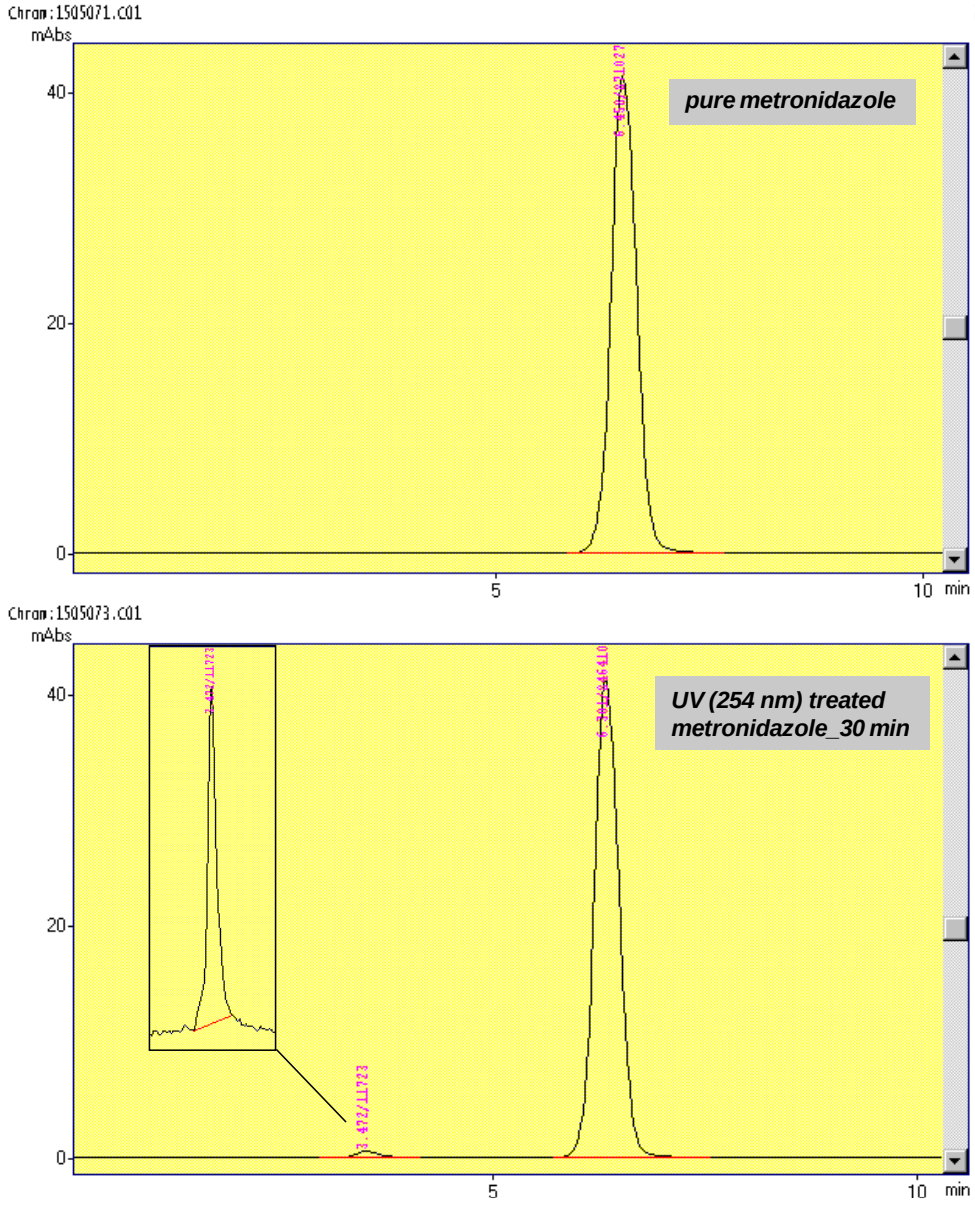

Fig. 22. Chromatograms of pure metronidazole and metronidazole exposed to UV (at 254 nm for 30 minutes)

5.2.2.7. *Ex vivo* mucoadhesion studies

Mucoadhesion is one of the approaches of gastroretentive systems, which can be to combine with other gastroretentive technologies. Their combination may result in more prolonged

gastric retention, with which location of drug release can be more predictable. This synergic effect is more desirable for drug delivery systems with APIs aimed at the site of gastric mucosa. In this optimization project, the aim was to design and develop a floating tablet containing metronidazole for *Helicobacter pylori* eradication from gastric mucosa.

In the *ex vivo* mucoadhesive studies, the two most frequently performed mucoadhesion measurements were done in order to present the potential in mucoadhesive properties of MF_OPT tablets: detachment force and rheological mucoadhesion studies.

Ex vivo mucoadhesion studies evaluated the mucoadhesion of floating tablets in different ways. Detachment force study evaluates the possible mucoadhesion in a relatively dry status. Mucoadhesion is based on the dehydration theory [57]. In contrast with the rheological method, which indirectly measures mucoadhesion based on macromolecular interpenetration [117]. Rheological method interprets the viscosity changes, when the tablet components are in dissolved and swollen form.

Detachment force studies were carried with MF_OPT and three other tablets with modified composition as reference having sodium alginate, L-HPC B1 (MF_OPT_L-HPC) or both excipient (MF_OPT_EXC) absences from composition. The result of detachment force study is shown in Fig. 23. MF_OPT tablets have resulted in remarkably higher detachment force (505.49±45.62 mN) compared to MF_OPT_L-HPC (314.91±37.88 mN) and MF_OPT_EXC (264.68±15.42 mN). Tablets without L-HPC B1 had higher detachment force than the reference without both excipients. This study may show the potential in the possible physical synergistic effect between the applied gel forming polymer and disintegrant affected with rapid water absorption.

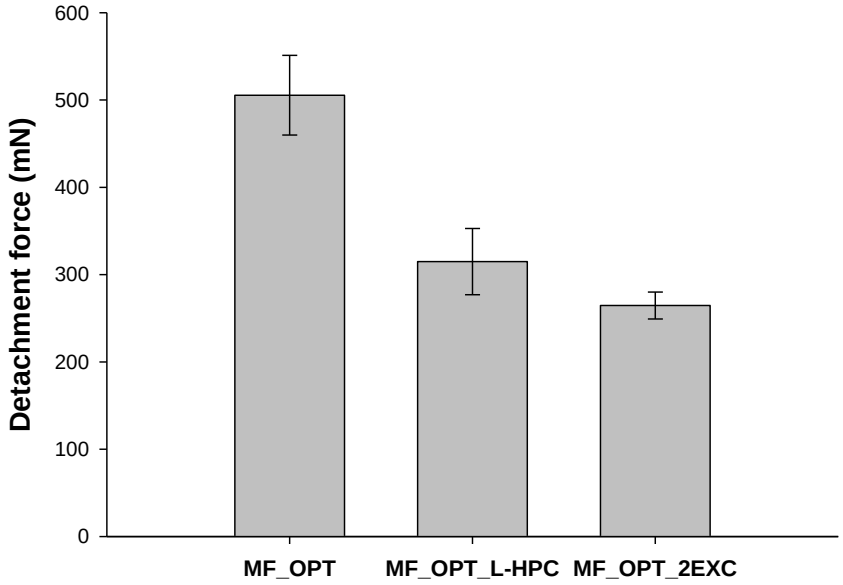

Fig. 23. Result of the detachment force study of MF_OPT (5.0 % sodium alginate, 38.63 % L-HPC B1, 8.45 % sodium bicarbonate) and reference tablets without sodium alginate and/ or L-HPC B1 (MF_OPT – optimized composition, MF_OPT_L-HPC – MF_OPT without L-HPC B1, MF_OPT_2EXC – MF_OPT without sodium alginate and L_HPC)

Result of MF_OPT tablets with the absence of sodium alginate led to splitting of tablets due to rapid hydration effect of L-HPC B1. Less coherence of the tablet structure was observed at tablets without sodium alginate in the presence of acidic medium, therefore these tablets were not evaluated.

Low viscosities were measured at 3 % mucus (7.63±1.24 mPas) and at MF_OPT tablet dispersion (27.57±23.22 mPas). Mixture of tablet and mucus showed significant rise of viscosity (846.89±78.25 mPas at 2.63 1/s). At low shear rates having the greatest interest [102], eightfold increase could be observed. Flow curve of mixture of tablet and mucus showed plastic flow behavior. Result of rheological method is shown in Fig. 24.

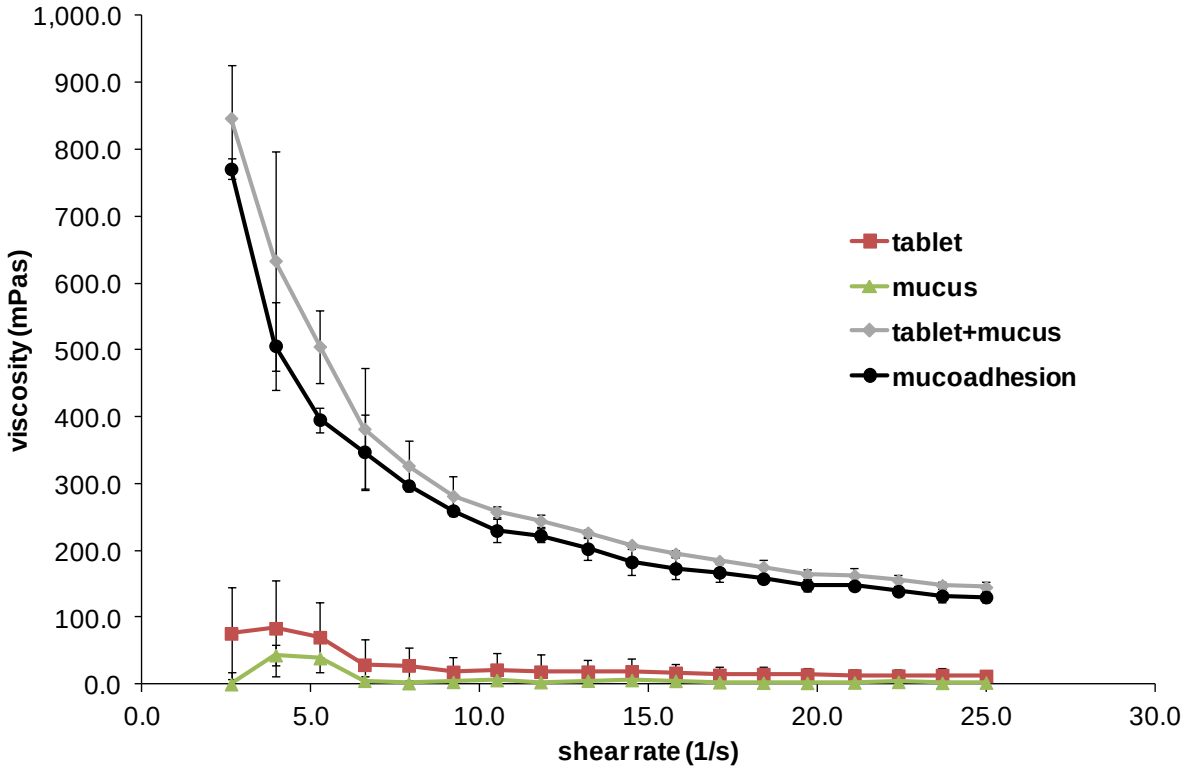

Fig. 24. Result of *ex vivo* rheological mucoadhesion studies of 3 % mucus, MF_OPT equilibrated to 3 % L-HPC and sodium alginate (tablet), their mixture (tablet+mucus) and calculated viscosity increase signed as 'mucoadhesion'

5.2.2.8. *In vivo* X-ray CT evaluation of floating tablets in rat

In vivo gastric retention of MF_OPT tablets with 10 % $BaSO_4$ were examined using rat model, which was performed as a correlation to human model in a cost-effective way in order to gain valuable preliminary information. *HU* values of tablets at sampling times showed (Table 12) that 10% $BaSO_4$ content in tablets may result in significantly ($p<0.0001$) higher *HU* value in comparison to the liver's *HU* as reference ($HU_{liver} = 800$). Thus, tablets could be distinguished from tissues interfering with the view of the tablets.

Table 12. Mean Hounsfield Units and quantities of voxels inside VOIs of MF_OPT tablets with 10% $BaSO_4$ at sampling times

t	Mean Hounsfield unit (*HU*)	Quantities of voxels
5 min	1482.35±304.03	1735
1 h	1165.00±103.23	1132
2 h	1152.38±106.90	1755
3 h	1202.83±155.36	1949
4 h	1194.20±146.70	1815
6 h	1151.43±104.99	1341
8 h	1151.27±106.23	1324
48 h	1197.29±112.15	870

Images (Fig. 25) showed the fact that MF_OPT tablets could remain in stomach for at least 8 hours. The period of gastric retention could be enough regarding the fact that more than 96 % of API was released within 6 hours based on the results of *in vitro* spectrophotometric and microbiologically detected dissolution studies (Fig. 19). After 48 hours, tablets could be identified in the intestinal tract. The prolonged residence time in intestinal region may be due to the effect of anesthesia, which effect is published by Torjman *et al.* [118].

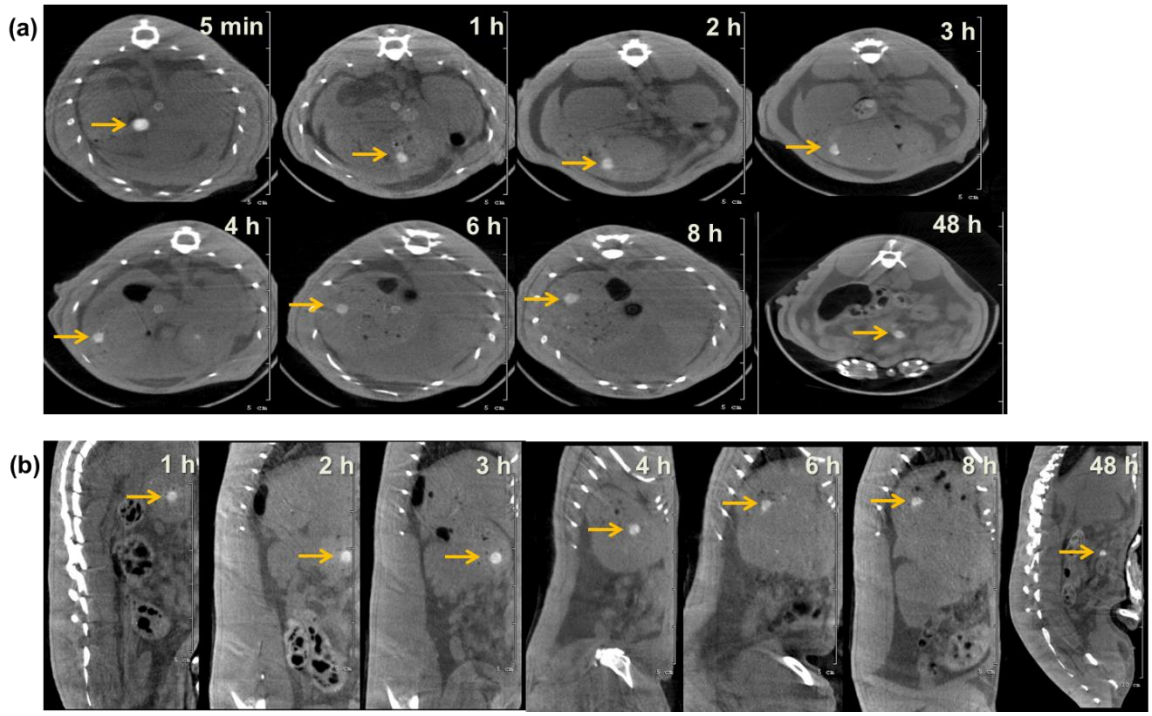

Fig. 25. X-Ray CT images of MF_OPT tablets loaded with 10 % barium sulfate at different time periods in transverse (a) and in sagittal plane (b) (location of tablets indicated with arrows)

The position of MF_OPT tablet in gastrointestinal tract at 8 hours is shown with yellow in Fig. 26 and Fig. 27. The homogeneity parameter of the tablet could be visualized in a spectacular manner. In the Fig. 26/A, identifications of voxels were performed in wide range, with which the location of tablets could be visualized. In Fig. 26/B, tablet structure may be viewed by the imaging of the dispersion of $BaSO_4$ particles. This fine resolution X-Ray CT image visualized the voxels for the VOIs of tablet (indicated with yellow colors) and for VOIs of background (a conventional grey scale). With the application of this technique, valuable information could be gained related to the *in situ* behavior of tablets including disintegration, swelling, gas creation etc.

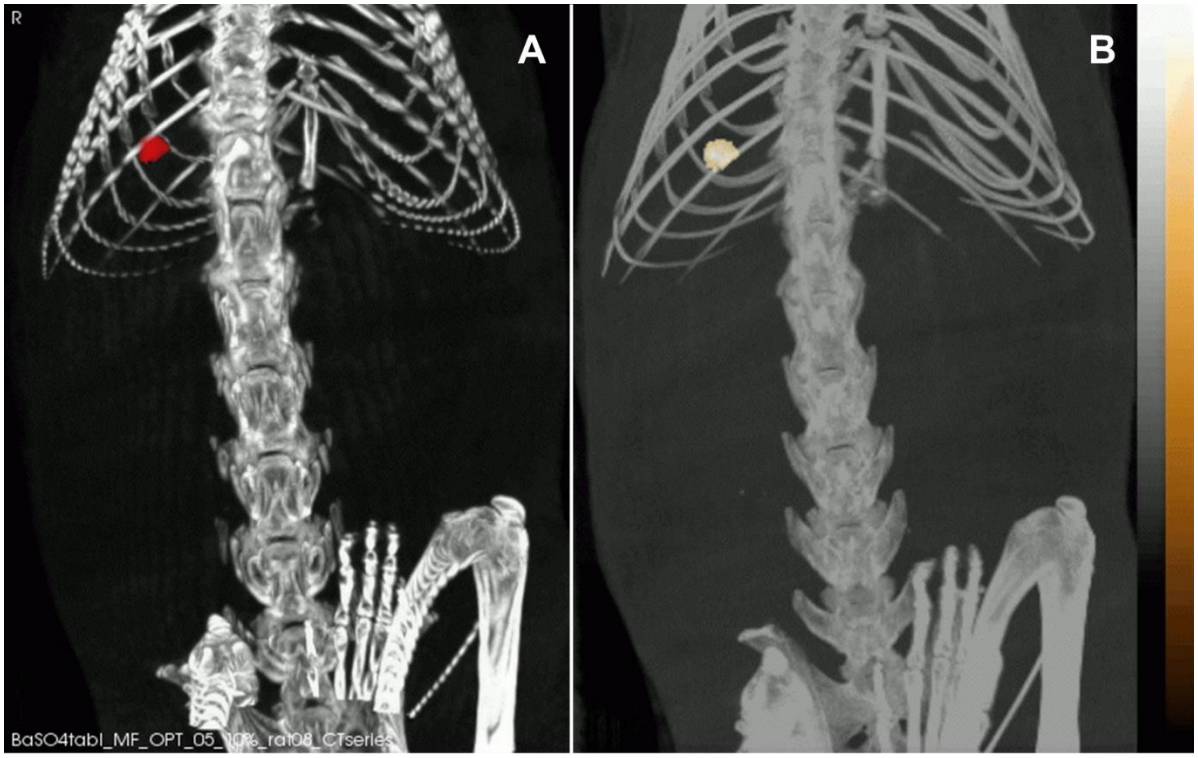

Fig. 26. Position of MF_OFT tablets in rat at 8 hours A: Identification of tablets with simple X-Ray CT evaluation; B: Identification and quantification of tablets with fine resolution X-Ray CT technique applying two different lookup tables

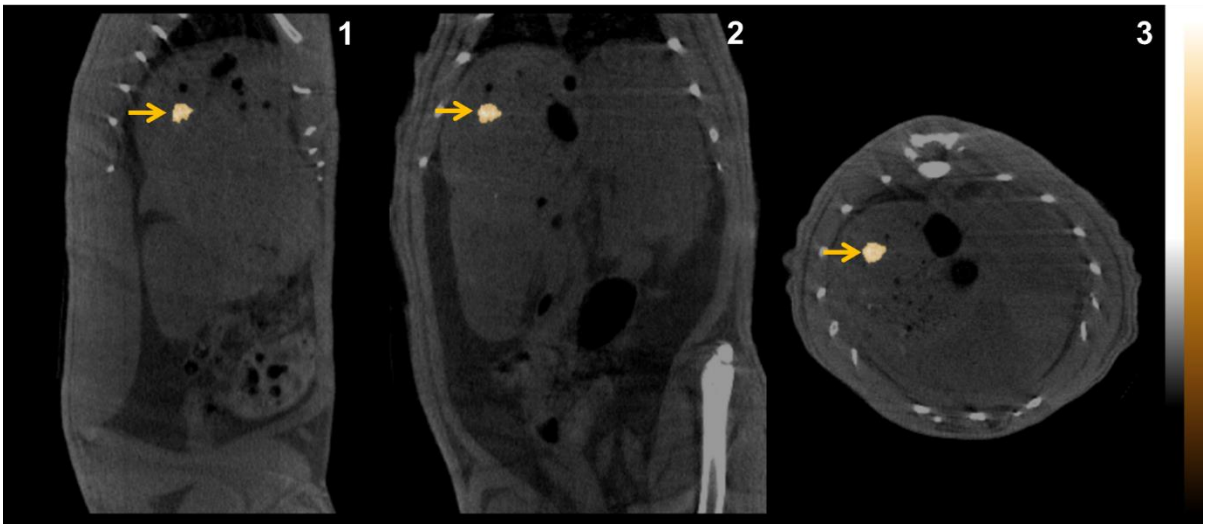

Fig. 27. *In vivo* images of the position and structure of MF_OPT tablet at 8 hour after administration in different planes (1 - axial, 2 - coronal, 3 - sagittal)

5.3. Summary of new results

Based on the evaluation of preliminary and optimization studies, new results of my research are the following:

1. Sodium alginate (high viscosity grade) was considered as a suitable matrix polymer in development of floating drug delivery tablets and its application resulted in rapid or sustained drug release depending on its concentration.
2. Differences between the types of L-HPC 11 and B1 were identified and were not remarkable at formulation studies. In the case of *in vitro* preformulation studies, more significant differences were observed at the evaluation of microscopic shape and size. In addition to L-HPC B1 showed better flowability, more intense wettability, thus this type was used in optimization project.
3. Summaries of statistically significant influences are shown in Table 13 and Table 14. The mark of ✔ indicates significance (p<0.05), ! is used when factor showed only trend (p<0.10) and ✘ presents absence of significance.

Table 13. Summary of significant influences in preliminary project

Preliminary project - significant influences	Applied model	Sodium alginate (%, X_1)	L-HPC (%, X_2)	L-HPC types (%, X_3)	Interaction ($X_1 X_2$)
floating lag time (t_{lag})	linear	✓	✗	✗	✗
total floating time ($t_{floating}$)	quadratic	✓	✓	✗	✓
maximal floating force (F_{max})	interaction (2FI)	✓	✓	✗	✓
time needed for maximal floating force (t_{Fmax})	quadratic	✓	✗	✗	✗
swelling capability (S_i)					
● from 30 min to 1 h	linear	✓	✗	✗	✗
● from 2 h to 4 h	linear	✓	✓	✗	✗
dissolution					
● from 5 to 45 min	quadratic	✓	✓	✗	✓
● from 45 min to 4 h	quadratic	✓	✗	✓	✗

4. From the result of preliminary project, several conclusions were drawn, which could be used to navigate the optimization project (vid. 5.2.1.4 Conclusion of the preliminary project).

Table 14. Summary of significant influences in optimization project

Optimization project - significant influences	Applied model	Sodium alginate (%, X_1)	L-HPC B1 (%, X_2)	$NaHCO_3$ (%, X_3)	Interaction
floating lag time (t_{lag})	quadratic	✓	✓	✗	$X_2 X_3$
maximal floating force (F_{max})	linear	✓	✓	✓	✗
maximal floating force calculated for 100 mg ($F_{max/100mg}$)	linear	✓	✓	!	✗
time needed for maximal floating force (t_{Fmax})	-	-	-	-	-
dissolution					
● from 5 to 10 min	quadratic	✓	✗	!	✗
● from 30 min to 6 h	quadratic	✓	✓	✗	✗
SD values of dissolution data	linear	✓	✗	✗	✗

5. Remarkably high floating forces and fast start of buoyancy could be measured with the floating tablets in optimization project.

6. Metronidazole dissolution kinetic results showed various release behavior. Trend could be observed that best fitting model of releases from floating tablets with 10.0 or 15.0 % sodium alginate was Higuchi model.
7. Optimization of factors could be done by the use of optimization criteria. The optimized composition contained:
    - 5.0 % sodium alginate (X_1),
    - 38.63 % L-HPC B1 (X_2),
    - 8.45 % sodium bicarbonate (X_3).
8. The optimized formulation showed promising properties including low floating lag time (t_{lag}=13.25±0.50 s), high floating force (F_{max}=12.75±1.87 mN) and biphasic drug dissolution.
9. Great similarity could be found in metronidazole dissolution detected by spectrophotometric and microbiological method (f_1=5.231; f_2=66.613).
10. Between drug and excipients, studies did not reveal any interactions.
11. Two frequently applied *ex vivo* mucoadhesion studies were performed, and optimized tablets have shown mucoadhesive properties. This result will promote our research team to examine *in vivo* mucoadhesion of this or similar floating tablets.
12. Detachment force *ex vivo* mucoadhesion results showed that L-HPC B1 could significantly improve the *ex vivo* mucoadhesion of sodium alginate. This phenomenon may open new possibilities to increase mucoadhesion properties of well know polymers without chemical modification.
13. X-Ray CT imaging result showed a prolonged *in vivo* retention of floating tablets in gastric region.
14. Fine resolution images could be captured with the use of a special X-Ray CT technique. The application of this imaging method can have very important role in continuously monitoring of the drug delivery system. This technique with well-adjusted and defined parameters could allow not only tracking of the dosage form, but also possessing data about the *in situ* behavior of dosage form involving swelling, disintegration, and gas generation.

6. Conclusion

The aim of the PhD work was to summarize the principals, the mechanisms and technological approaches of floating drug delivery systems in general. The literature survey presented the physiological properties and motions of stomach mentioning the influencing factors on gastric motility and absorption. This section highlighted the fact that in designing of floating drug delivery systems, evaluation of biopharmaceutical and physiological parameters has prominent role. These can affect the choice of API, excipients and the applied technologies. Several research papers have proven that the shape or size of the preparation or even the consumed nutrition can have a remarkable effect on the floating kinetics and dissolution of floating drug delivery systems. The theoretical base and possible mechanisms of mucoadhesion were also detailed, since mucoadhesive examinations of optimized tablets were carried out.

Experimental aim of the PhD work was to design, study and to develop a floating drug delivery system having appropriate floating and dissolution properties. Two experimental designs were created: the first as preliminary project, the second as optimization project.
The preliminary project included the evaluation of 27 floating tablets, in which two numerical and one categorical factors were applied. The conclusions of this project have been utilized in creation of optimization experimental matrix. Face centered central composite design have been applied in the optimization phase having three numerical factors (15 floating tablet samples). Through the optimization, a suitable composition was found having remarkable floating behavior and biphasic release compared to two commercially available metronidazole products. Experimental results revealed significant *ex vivo* mucoadhesion of the optimized composition. Mucoadhesion may cooperate with the floating mechanism to achieve better gastroretention. *In vivo* studies represented 8 hours gastric retention of tablets.

With the use of experimental design, navigation of the concentration intervals could lead to the design and development of suitable drug delivery systems. In this PhD work, a promising controlled-release floating tablet containing metronidazole was developed, which highlights the possibility to improve the local effect of anti-*Helicobacter pylori* agents via a gastroretentive system based on floating and mucoadhesion.

List of Figures

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