SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Formulation of gastroretentive solid dosage forms

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1. Introduction

Today the pharmaceutical industry is one of the largest sectors of the world economy, accounting for a significant share of global economic turnover, with annual sales of nearly US$1,250 billion in 2019 alone. The strong market presence of solid dosage forms is demonstrated by the fact that, according to FDA statistics, 54% of new oral dosage forms were solid products in 2019.

Drug Delivery Systems (DDS) can be divided into five generations. The first generation consists of conventional medicines such as capsules, tablets and granules, while coated versions of these are in the second generation. The third generation includes controlled drug delivery systems, while the fourth and fifth generations are targeted therapeutic systems: monoclonal antibodies, nanorobots or gene therapy.

The drawbacks of conventional carriers are increasingly being highlighted for old and newly synthesized active substances. Some active substances require the development of more specific carriers due to their pharmacokinetic properties. One such regulated delivery system of interest to us is the gastroretentive formulation. These formulations remain in the stomach where they release their active ingredient in a prolonged manner. Pharmacons with an absorption window in the dudodenum or jejunum, being less stable at more alkaline pH or having poor solubility, are suitable for enhancing their bioavailability by gastroretentive (GR) formulations.

A good knowledge of the gastrointestinal tract is essential for the design of gastroretentive formulations. The stomach is in the left side of the abdominal cavity, below the diaphragm. It is the widest part of the digestive tract and can expand to several times its resting volume. The stomach is separated from the oesophagus and the duodenum by the lower esophageal muscle and the pyloric sphincter. The physiological motor function of the stomach has three main functions: storage, grinding and transfer of gastric contents to the intestinal tract. The proximal stomach, the fundus and corpus together have the main function of receiving food. The antrum is involved in grinding, mixing and chyme transmission. The reason of the different functions of the two parts is the different smooth muscle structure and the different nerves regulation. The periodic depolarization at a frequency of 3/min is called basal electrical rhythm (BER). The BER is transmitted from Cajal cells to electrically coupled smooth muscle cells, causing the muscle to contract in the direction of the pylorus. The movements characteristic of the fasting state occur every 2-3 hours and are characterized by four parts,

migrating myoelectric complex (MMC), according to Wilson and Washington: Phase I (basal phase): 40-60 minutes, characterized by infrequent contractions, Phase II: 40-60 minutes, characterized by increasing and denser action potentials and contractions, Phase III: 4-6 minutes, consisting of strong and frequent contractions, during which the food is transmitted to the duodenum, Phase IV: 0-5 minutes, leading from Phase III to Phase I. The well-fed state is similar to phase II, which promotes proper grinding and homogenization of the food with the digestive juices, but MMC is not experienced, and gastric motility slows down.

Gastroretentive dosage forms have a prolonged gastric residence time, their emptying from the stomach is inhibited, and are capable of prolonged release of the active substance. There are many possibilities for the creation of GR carriers in the literature.

In the case of external magnetic field-based GR dosage forms, the formulation is held in place by a magnetic field device. In addition to the active substance, the drug formulation also contains magnetizable materials (neodymium-iron-boron alloy (NdFeB), iron or iron oxide particles). Due to the disadvantages of this technology, their use has not been widespread, despite the fact that better bioavailability has been achieved with dipeptidyl peptidase IV inhibitors, fluorophores, aminophen or acyclovir.

The first bioadhesive/mucoadhesive GR system was described by Park and Robinson in 1984. In mucoadhesion, the mucin-containing mucosa is used to bind the drugs in place. Both natural and artificial polymers can be used as excipients for example: polyacrylates, hydroxypropyl methyl cellulose (HPMC), sucralfate, dextran, tragacanth, tamarind, or Na-alginate.

GR systems based on size increase can be divided into two groups, those that swell in gastric acid and those that expand. The 3 basic rules for GRs are that they must be small enough to be easily swallowed by patients, change shape rapidly in contact with gastric juice and, after drug releasing, shrink or erode to a size that can be emitted from the stomach. According to the literature, the size of the open pylorus can be up to 12.8 mm. Formulations larger than this will remain in the stomach throughout their degradation, thus increasing their gastroretention time. The typical polymers used are polylactic acid, glycolic acid copolymer or cellulose for the expanding form and acrylamide, guar gum, Na-alginate or polyvinyl alcohol for the swelling form.

The last major group includes GR systems which, due to their density, are able to remain in the stomach for a longer time. The low-density formulations increase retention time by floating on the surface of the gastric contents, while the heavier formulations increase retention time by anchoring on the bottom of the stomach. Clarke et al. described that the retention time of preparations with a density above 2.5 g/cm^3 in the stomach is increased due to descent, which makes it more difficult to pass into the duodenum. In the formulation of preparations, high density excipients are used, the most suitable are barium sulphate, aluminium or other heavy inert excipients (zinc oxide, titanium dioxide, iron powder).

Low-density formulations can be divided into two groups, those based on gas formation and those not based on gas formation. In gas trapping systems, a gas-forming excipient is mixed into a swellable matrix, so that the resulting gas is trapped in the matrix, reducing its apparent density. In almost 100% of cases, the formulations are based on the formation of carbon dioxide, mostly using sodium bicarbonate and combined with Na-alginate, ethyl cellulose, chitosan or hydroxypropyl methyl cellulose (HPMC) polymer. The main advantages of non-gaseous GR systems over gaseous systems are that they show zero lag-floating time and usually have a harder structure that resists the grinding movements of the stomach. Today, newest technologies for the design of low-density gastroretentive drug carriers are achieved by melt-based extrusion or foaming of melts. In melt-based extrusion, an excipient is added to the molten matrix in liquid or solid form, which, when it exudes from the extruder, inflates the formulation. The finished extrudate is cut to size and filled into capsules or pressed into tablets with excipients to achieve the final pharmaceutical form.

Previously at the Department, we have developed a new technology to produce a gastroretentive formulation by foaming molten suspensions under atmospheric pressure. Using this method, we can produce floating, low-density, solid, dosage forms. Our formulations containing a blend of stearic acid (SA) and polyethylene glycol 4000 (PEG 4000) achieved densities below 1 g/cm^3 after the foaming protocol. The gastroretention of the formulations was confirmed by *in vitro* studies. In the light of these results, the feasibility of industrial manufacturability was raised, which inspired the topic of my dissertation.

2. Aims

The aim of my PhD dissertation was to prepare a gastroretentive dosage form that is more advantageous than other gastroretentive preparations described in the literature, according to the following:

- To transfer the previously developed and patented new production technology to a device suitable for continuous production
- To design and build innovative production apparatus that is suitable to scale up to industrial production
- To optimize the critical parameters of the production
- Development of contrast agent containing GR drug carriers
    - *in vitro* and *in vivo* investigations
- Production of low-density foam capsules containing verapamil HCl
    - *in vitro* characterization
    - *in vivo* bioavailability study

3. Materials and methods

Materials and experimental animals

Polyethylene glycol 4000 (PEG 4000), stearic acid, type 50 (SA), lactose monohydrate, barium sulphate ($BaSO_4$) and verapamil-HCl (VER) were Ph. Eur. grade and purchased from Molar Chemicals Ltd. (Halásztelek, Hungary). Other reagents were analytical grade and purchased from Sigma Aldrich Ltd. (Budapest, Hungary). The hard gelatine capsules (Coni-Snap, size 00) were gifted by Capsugel (Morristown, New Jersey, USA). Fischer-344 rats were bred by Animalab Ltd (Budapest, Hungary). Beagle dogs (six, female) were bred by WOBE Ltd. (Budapest, Hungary). To anaesthetize the animals, CP-Ketamine (ketamine hydrochloride 10%; Produlab Pharma BV, The Netherlands), CP-Xylazine (2%; Produlab Pharma BV, The Netherlands), Seduxen inj. (diazepam, 0.5%; Richter Gedeon Nyrt., Hungary) and Forane (Isoflurane, Abbott Laboratories Ltd. Hungary) were used as a medicine.

Development of the foam cell device

The QUICKfoamcell Lab® was designed and built in the frame of a cooperation (QUICK 2000 Ltd., Tiszavasvári, Hungary). The apparatus can be divided in two main parts. The melt container (600 mL) is temperate and has 8 mm wide drainpipe at the bottom. IKA EURO-ST D overhead stirrer with 4-bladed propeller stirrer continuously mix the melt in the container. A Watson-Marlow 114 ST peristaltic pump transfers the homogeneous melt into foam cell (second main part). The temperate foam cell capacity is 30 mL with 3 openings: inlet for the dispersion, gas inlet and outlet for foamed product. The agitation is done by IKA® ULTRA-TURRAX® T-25 Digital disperser equipped with a dispersing tool (S25 N -10G). Gas is introduced to the melt by another Watson-Marlow 114 ST peristaltic pump. Dosing is controlled by TAKASAGO PK–6405–NC pinch valve.

150 grams of melt was foamed by the following method. PEG 4000 and SA were measured and melted in melt container whit gentle stirring. Then API was dispersed in the melted mixture. Firstly, the foam cell was filled with the molten suspension. The continuous foaming was carried out in cycles, while having high agitation by IKA® ULTRA-TURRAX® T-25 Digital disperser. After every cycle, the hot foam was filled into 00 size hard gel capsules and allowed to cool to room temperature.

Optimization of production temperature

To determine the effect of temperature on the density of foams, 20 capsules were produced at different temperature, while the other parameters were left unchanged. Temperature of foam cell was set to 60, 58, or 56 °C, the volume of gas was kept at 2 mL with 0.25 mL/s gas injection rate. The agitator shaft was kept at 15000 rpm speed. Control samples were prepared without foaming process and the samples contained only the matrix of foams without lactose.

The density of the produced foams was determined by the total weight of filling and the volume of capsule (0.91 mL). Every capsule was completely filled with melt, after the solidification, the capsules were sealed with cap and their density was defined.

Optimization of critical parameters

The continuous production was optimized using a Box–Behnken experimental design. The independent variables were the volume of gas (mL), gas injection rate (mL/s), and agitator shaft speed (rpm), and considered as the critical parameters in the production process with an effect on product density. These three experimental factors were varied in the design, at 3 levels in 12 runs, that presented on Table 1. The volume of gas was changed between 0.1 to 4 mL, while the gas injection rate from 0.02 to 0.5 mL/s and the speed of agitator was set from 3000 to 25000 rpm. This design was employed to investigate the quadratic response surface and to construct a second-order polynomial model using TIBCO Statistica® 13.4 (Statsoft Hungary, Budapest, Hungary).

The 3D response surface plots for density were plotted according to the regression model by keeping one variable at the center level. For statistical analysis, GraphPad Prism® (Version 6.01, GraphPad Software Inc.) was used. Unpaired t-tests were performed when two groups were compared, and one-way ANOVA was chosen when comparison of multiple groups was performed. Differences were considered significant at $p < 0.05$.

Contrast agent, barium-sulphate, loaded sample formulation

Preparation of sample

The barium-sulphate loaded samples, that contained 30% $BaSO_4$, 50% PEG 4000 and 20% stearic acid, were foamed based on the method mentioned above. The independent variables were set to 2.5 mL of gas and injected with 0.25 mL/s rate, at 15000 rpm agitator shaft

speed. The hot foam was filled into plastic tubes (d:5 mm, h:50 mm). After cooling to room temperature, the rods were cut to uniform size (d:5 mm, h:5 mm).

Determination of the density

The following method was used to determine the density of the solid compositions, (unfoamed or foamed). The shape of $BaSO_4$-loaded samples was cylindrical with a diameter of 5 mm and a height of 5 mm, the top and bottom were perpendicular to the mantle, thus the method of calculating the volume of a the cylinder was suitable. The volume of cylinder-shaped final preparation was 0.0981 ml and the total weight was checked by analytical balance and the density of each sample was determined by the quotient of weight and volume.

Micro-CT investigations

The following method was used to determine the solid foam structure. The tablet was fixed into the sample holder. A SkyScan 1272 compact desktop micro-CT system was used for the measurement. Scanning parameters were the following: image pixel size: 5 microns, matrix size: 1344x2016 (rows x columns), Source Voltage: 50 kV; Source Current: 200 μA, Flat Field Correction and Geometrical Correction were used. After scanning SkyScan NRecon package (Version: 2.0.4.2) was used to reconstruct cross-section images from tomography projection images. Post-alignment, Beam-hardening correction, Ring artefact correction and Smoothing were done. The output formats were DICOM and BPM images.

In 2D/3D analysis we used CTAn software. Based on density analysis, used Thresholding, ROI shrink-wrap, Reload, 2D and 3D Analysis plugins. The gray threshold values of air bubbles were between 0-40, and with ROI shrink-wrap we eliminated background before analysis. The 3D visualisation can be seen in CTVox software with colour coding.

Texture analysis

The mechanical properties and structure of the dry and wetted foamed compositions were characterised by texture analysis. Dry samples were tested at room temperature without immersing them into dissolution media. The wetted samples were carefully removed from the pH 1.2 media after 0.5, 1, 2, and 4 h later and excess water was carefully removed by soft tissues from the samples. Wet and dry samples were analysed by Brookfield CT3 texture analyser. An acrylic cylinder, TA25/1000, (d: 50.8 mm) compressed the samples with constant speed (0.50 mm/s) until 4500 g of load. The load (g) values were plotted in the function of time (s) to present the changes in the texture in real time.

In vivo gastroretentive study

In vivo gastroretentive study was performed to confirm the retention of the $BaSO_4$ loading samples in the stomach. For experiment, 16-week-old, 250-300 g weighted male Fischer-344 rats (n=3; Animalab Ltd, Budapest, Hungary) were used. Animals were housed under conventional conditions (23±2°C, 50±10% humidity and 12 h circadian cycle). The semi-synthetic diet (VRF1; Akronom Ltd., Budapest, Hungary) and drinking water were available *ad libitum* to all animals. The animal experiments did not apply invasive techniques, ethical permission was not required to the investigations. Laboratory animals were kept and treated in compliance with all applicable sections of the Hungarian Laws and animal welfare directions and regulations of the European Union. During *in vivo* CT, rats (n=3) were anaesthetized by 3% isoflurane (Forane) with a dedicated small animal anaesthesia device and barium containing mini tablet were administrated *per os* directly into the stomach of the animals. For the anatomical localization whole body CT scans were acquired after 0.5 and 2 hours using the nanoScan SPECT/CT (Mediso Ltd, Hungary) scanner. The following acquisition parameters were used: X-ray tube voltage 60 kVp, current 86 mA; exposure time 170 ms per projection; voxel size: 1x1 mm.

Verapamil loaded capsule

Preparation of the foamed capsule

Three different compositions of verapamil-HCl containing capsules were produced by continuous foaming process that was mentioned above. The production temperature (foam cell) was set to 56 °C. Three formulations were prepared by increasing stearic acid content.

Determination of density

The method of determination of density was described before in the section of Optimization of parameters. The size, weight and the volume of the capsules were the same.

Scanning electron microscope and chemical analysis

Hitachi Tabletop microscope (TM3030 Plus) was used to characterise the solid foams. Samples were split in halves and were attached to a fixture with a double-sided adhesive tape containing graphite. Before SEM examination gold-sputtered coating was not deposited on the surface of the samples. The measurement requires vacuum and low accelerating voltage 5kV.

Chemical element analysis (oxygen or chlorine) was done on the fractured surface by Bruker EDX 70 detector.

Dissolution test

900 mL of hydrochloric acid media, pH: 1.2 without pepsin was selected for dissolution tests. Rotating paddle method with the rotation speed of 75 rpm and 37 °C was set up in a dissolution tester (Erweka DT 800). Samples of 3 mL were removed after 5 min, 15 min, 30 min, 1, 2, 3, 4, 5, 6, 7, 8 and 10 hours. The samples were filtered through a 0.22 μm PES membrane syringe filter and diluted with the dissolution buffer. The released amount of VER was determined by UV/VIS spectrophotometer (Shimadzu UV 1601, Shimadzu Corp. Kyoto, Japan) at 278 nm. Three random samples were selected for the tests from every composition. Dissolution data was fitted to zero-order, first-order and Korsmeyer-Peppas model in MS Excel.

Validation of production

During the process validation, 3 batches of V2 composition were produced by foam cell at 3 different time, following the parameters of standard protocol which has been described in section of Preparation of foam capsule. The densities, API contents and dissolution profiles of the batches were compared. To compare the dissolution data of validation products, similarity and difference factors were calculated, as a model independent approach: similarity, f2 and difference, f1 factor was calculated for each one.

Micro-CT

The same micro-CT method was used as describe above in case of $BaSO_4$ samples.

Floating strength determination

Based on the work of Simons and Wagner, we built an apparatus capable of detecting the buoyancy force of a sample. A net holder was directly mounted to the tensiometer (Attension). Thus, the rising force was calculated directly from the weight changes of the net. Measurements were performed in 500 mL of pH 1.2 buffer at 37 °C while stirred continuously to create comparable conditions to those of *in vitro* release studies.

Texture analysis

Similar texture analysis method was used as described above in case of $BaSO_4$ samples, according to the following amendments. The dissolution medium was 900 ml and 20 drops of 5% w/w Sicovit® Tartrazine (BASF, Ludwigshafen, Germany) were added to follow the

wetting of foamed capsules. Samples were taken at 0, 60, 180, 300, 420 and 600 minutes and photo were taken of the samples.

Water uptake and matrix erosion studies

Wetting and erosion properties of the formulations were determined by the following method. The initial weights of the samples were recorded before the experiment, then they were placed into the dissolution vessels as described in section of dissolution test. After 1-, 3-, 5-, 7- and 10-hours samples were carefully removed from the dissolution media and the weight of the wet samples were measured after blotting the excess water. Then the samples were dried in an oven (Memmert SFE 550, Memmert GmbH, Germany) at 45 °C until they reached a constant weight.

Dissolution test after long term and accelerated storage conditions

To evaluate the formulation stability, stability studies were carried out according to ICH guidelines. Ten capsules stored in airtight glass container. Samples were placed in climate chamber (ICH110, Memmert GmbH + Co. KG, Schwabach, Germany) under accelerated storage conditions (40 ± 2°C, 75 ± 5% RH) for 3 months. Another ten capsules were also stored in an airtight container and keep at room temperature for two years. At the end of studies, samples were evaluated for appearance and *in vitro* drug release. The *in vitro* drug releases were compared with the initial release profiles, the dissolution data of samples by similarity, f2 and difference, f1 factors.

In vivo pharmacokinetic study

The pharmacokinetic study was conducted according to regulations of the Hungarian Scientific Ethical Committee on Animal Experimentation (approval number: HB/06-ÉLB/1657-4/2019). Six female dogs (10±0.5 kg, WOBE Ltd. Budapest, Hungary) were involved in the experiment. Animals were kept between 15-21 °C and relative humidity of 50±10 %, providing 12 hours of daily light and 12 hours of dark period. All the dogs were provided to have free access to water, food was provided *ad libitum*. Acclimatization was allowed for 21 days prior to experiments. The experiments were carried out in two steps leaving a 7-day wash-out period between them. 12 hours before each experiment food was withdrawn allowing only the free access to water. Blood sample collection was done by intravenous cannulation on the forearms. After sample collection, sterile saline (3 ml) was injected for rinsing and sodium heparin solution was used as anticoagulant.

Firstly 50 mg of Verapamil HCl dissolved in 10 ml of purified water was administered orally to the animals (n=6). Blood samples (2-3 ml) were collected 0.5, 1, 2, 4, 6, 8 and 24 hours after administration. Blood samples were centrifuged immediately (3500 rpm for 10 min), plasma samples were kept at -80°C until further analysis. After the 6 hours' blood sample collections food was given to all dogs.

Following 1 week as a wash-out period, 120 mg Verapamil HCl foamed capsules were given orally to all dogs (n=6). The procedure (sampling time, sample amounts, plasma separation and storage) was as described above. Food was also given 6 hours after capsule administration.

Gastroscopy

After 2 and 4 hours to ensure that the solid drug carrier presents in the stomach using a video fiberscope (Ultrasound Video Fiberscope, HOYA Corporation, Shinjuku-ku, Tokyo, Japan) the gastric content was examined and short videos were captured. Before the examination, the animals were anesthetized with intramuscular ketamine and diazepam.

Quantitative determination of verapamil in plasma

Liquid–liquid extraction method was used for sample preparation. pH 6.0 phosphate buffer was prepared according to the European Pharmacopoeia. Briefly, 6.8 gram of sodium dihydrogen phosphate was dissolved in 1 L of purified water, pH was adjusted with sodium-hydroxide. 250 µL of dog plasma samples were mixed with 0.5 mL of phosphate buffer (pH 6.0). Then it was extracted with 1.5 mL of a mixture of TBME–ethyl acetate (1:1, v:v). After centrifugation (4000 g for 8 min), the organic phase was separated and evaporated completely with a stream of nitrogen at 40 °C. The residue was dissolved in 500 µL of LC mobile phase and was measured by Thermo Accela +LTQ XL LCMS instrument. Chromatographic separation was achieved by a Kinetex XBC18 column (100 × 2.1 mm, 2.6 µm). The mobile phase for elution comprised methanol + 0.1% formic acid (A) and water + 0.1% formic acid (B). The gradient conditions were 0.00 min 70% B, 2.00 min 0% B, 3.10 min 70% B, and 5.50 min 70% B. The column temperature was maintained at 40 °C with the flow rate set at 0.3 ml/min, and a 10.0 µL sample was injected. The optimal ESI ionization parameters were as follows: heater temperature: 200 °C; sheath gas: N_2; flow rate: 10 arbitrary units (arb); aux gas flow rate: 5 arb; spray voltage: 5 kV; capillary temperature: 275 °C; and capillary voltage: 23.50 V. Sample measurements were run in positive ion mode (MS) The verapamil $[M+H]^+$ ion mass

(455m/z) was detected in SIM mode. For calibration verapamil was dissolved in LC mobile phase. The results were analysed by GraphPad Prism 6.1 software and relative bioavailability was calculated.

Statistical analysis

GraphPad Prism® (version 6.01, GraphPad Software Inc.) was used for statistical analysis. When comparing two samples, T-test was performed, One-way ANOVA and Tukey's or Dunnett's post hoc test were chosen to compare more samples. Differences were considered significant at $p < 0.05$.

4. Results

Optimization of process parameters

The relationship of viscosity and gas entrapment efficacy was described in our previous publication by Vasvári G. et al. It was revealed that during the precise cooling, the viscosity values increased as the molten dispersion became semi-solid from its liquid state. The freezing range of molten mixture of PEG 4000 and SA was around 53–55 °C. The production temperature was a key parameter to maximise gas entrapment efficacy additionally avoiding the freezing during the foaming process. The effect of the temperature was determined by manufacturing at 60, 58 and 56 °C. 56 °C was found to possess the lowest density, namely 817.14 mg/cm^3.

The effect of the volume of gas (ml), gas injection rate (ml/s), and agitator shaft speed (rpm) on density of samples was also investigated. In this study the temperature was set at 56 °C. Increasing the speed of agitator shaft decreased the density of samples in every case. 14500 rpm was enough to reach optimal density (< 1000 mg/cm3) with all settings. In case of volume of gas and speed of gas, it was found that the degree of foaming decreases at the extreme values. As a result of rapid introduction of higher levels of gas, the rate of foaming was reduced. On the other hand, at lower ranges of gas injection rate or at low levels of gas, the rate of foaming decreased. The optimal parameters were the following: volume of gas: 2.5 – 3.25 ml; the injected speed of gas: 0.25-0.35 ml/s; and the speed of agitator: 14500 rpm. 14500 rpm was enough in every case to reach target density range, which is lower than 1000 mg/cm^3.

Barium-sulphate containing sample

Density of samples

The density values of the dispersions before and after foaming were significantly different. It was found that the composition reached 507± 45.48 mg/cm^3 after the foaming process. The initial density was 1107 ± 108.18 mg/cm^3. This means a 54% decrease in the mass, due to the dispersed gas. The foamed compositions showed zero floating lag time with continuous floating until complete disintegration (over 4h) in pH 1.2 puffer.

Micro-CT

The image of microCT scans performed on $BaSO_4$-loaded composition. The foaming process dispersed gas bubbles into the molten suspension confirmed by microCT images. On

the other hand, the unfoamed composition also showed bubbles, but the number was negligible and the size was significantly higher than the foamed preparation. The distribution of the bubbles was random. The reconstructed micro-CT scans showed a closed spheroid cell structure. The size distribution was homogenous, 93.2% of bubbles were in 0-100 μm range of diameter and the average size of bubble was around 40 μm.

Texture analysis

The results of the texture analysis showed that a hard structure was presented in spite of the air entrapment, but applying 4500 g of compression load on the foam results cracks in the dry state at 25°C. During the erosion test the hardness of matrix decreased and the cracks disappeared from the texture curve at the same time. Wetting caused the gelling of the outer layer of the samples, thus the compression test probe reached the solid resistant core later during the measurement. The complete wetting was detected after 120 min.

In vivo gastroretentive study

The formulation appeared in a well-defined way in the stomach 30 min after the administration. After 2 hours the retention of the formulation was confirmed in the stomach in x-ray taken, in spite of the erosion. This study proved the ability of the foam to remain in the stomach for prolonged period of time to satisfy the desired needs of such formulations.

Verapamil containing sample

Density of compositions

Three compositions were produced by continuous production, and the final densities were calculated. The densities of all samples were below 1000 mg/cm^3, which is necessary to achieve gastric retention. From V1 to V3, the compositions contained increasing amounts of stearic acid as a lipophilic agent. The lowest density was measured for V2. Interestingly, we obtained higher values for V3 despite increasing the lipophilic agent content in the formulation.

SEM analysis

SEM analysis showed different properties of the compositions, exactly V3 shows totally different broken surface from V1 and V2. API accumulated on the surface of the bubbles in general, however in the case of V3 the white verapamil-HCl crystals can be found on the inner surface of the cavities, while in the case of V1 and V2 the crystals are covered by the matrix on

the outer surface of the bubbles. In all of 3 samples, high porosity was observed, which can be detected in the form of holes on the fractured surface. The chemical analysis of V3 proved that the API can be found on the wall of pores. The oxygen rich component of the matrix surrounds the VER crystals.

Dissolution test

The dissolution test showed prolonged drug release up to ten hours, in all cases. Flotation was checked visually and none of them sunk before the end of the test. V1 composition reached the highest dissolution rate, 85.3%, V2 and V3 reached lower values, 79.4% and 77.4%. When the dissolution efficiencies were determined, it was found that V1 showed the fastest release with the value of 70.03%, while for V3, the value of DE was only 68.43%. All compositions fitted the best to first-order kinetic, however, V2 fitted best with the target zero-order kinetics (the correlation coefficient was 0.7572). As V2 showed the best properties, in further experiments mainly this composition was examined.

Validation of production

Production's validation was then performed with sample V2, and this composition was further analysed as a final product. Three batches were made by the method that was described above. The average weight of the product was 890.7 ± 26.7 mg, and none of the samples had higher deviation from the average weight than the allowed in the pharmacopoeia. The dissolution profiles of batches were compared with the V2 profile, and none was different form that.

Microtomography investigation

The foaming process dispersed air into the molten suspension containing verapamil and the creation of cavities was confirmed by micro-CT images. The distribution of cavities in the matrix and the size distribution of the bubbles were homogenous. 89.2% of them were in the 20–120 μm range of diameter, and the average diameter of cavities was around 78 μm. The reconstructed model of the foam structure showed a closed spheroid cell structure, but some of the bubbles could be observed at the edges opening to the surface. Some of them formed a cavity system in the matrix, although their number was few compared to the whole matrix.

Texture analysis

The results of the texture analysis showed a hard structure, despite of the high porosity. Using 4500 g compression load on the foam, any cracks, fractures, or any other injuries were not detected on the dry sample at 25°C. During the drug release the initial hardness of the samples decreased. After 60 and 180 min of dissolution, a soft layer could be found around the hard core, which was easily removable, the two parts easily separated by micro-CT scans. At 300 and 420 min of dissolution some cracking were seen, the hard core became fragile, the core was broken from 3810 grams (on 300 min sample) and 3770 grams (on 420 min sample). The thorough wetting was detected after 600 min, the breakable hard core disappeared.

Water uptake and matrix erosion studies

During dissolution, the mass of matrix decreased continuously. The matrix did not show swelling, the sum of remaining matrix and the water content was constant. Only the 30% of the sample remained after the test.

Floating strength determination

At the zero-time point, the sample could float on the top of the acidic media and generated 1.5 mN buoyancy force. During the swelling and erosion of the capsule shell, the buoyancy decreased to 1.2 mN, then started to increase and took up a plateau phase at around 2mN, until the API was completely released.

Dissolution test after long term and accelerated storage conditions

Three parallel dissolution studies were performed to prove appropriate drug liberation after 3 months and 2 years, respectively. The dissolution profile of the samples was compared by similarity and difference factors. The difference factor was less than 5.00 in all cases and the similarity factor was greater than 50.00.

In vivo study

The animals were orally administered with solutions containing verapamil-HCl (50 mg) and a solid retard gastroretentive foam in the bioavailability test. The verapamil plasma concentrations were compared. Gastroscopy was performed and proved the gastric retention after two hours and after 4 hours the capsule was eliminated from the stomach. The capsule reached the maximum concentration in plasma after 4 hours of administration, while the solution reached earlier, already after 0.5 hours. In the case of the foam, the plasma

concentration was above 50 ng/ml for 5 times longer than in the case of the solution, while the initial concentration was just 2.5 times higher. The relative bioavailability was 99.3%.

5. Discussion

Our aim was to design, build and optimise a novel foaming apparatus that is suitable for continuous production of foamed molten dispersions. Our device is suitable for continuous production with an approximate capacity up to 300-500 capsules/hour. The QUICKfoamcell can be divided into two basic units. The melt container with a size of 600 mL, but the capacity can be easily multiplied as needed. The vessel can be heated to 70 °C, which makes it suitable for various mouldable polymers and materials. The main unit of foaming is the foam cell, where gas is dispersed in the molten dispersion with a high agitation speed. The final dosage form is hard capsule since capsule shell is used as mould in which the foamed composition can solidify. 00 size capsule has a volume of 0.91 ml. Depending on the density of the product, it can contain up to 300 mg of active ingredient, which broadly cover the therapeutic dose of most API whose bioavailability can be enhanced by gastroretention. During optimization of process parameters, the ideal production temperature was determined between 54-56 °C, but the optimal temperature depends on the composition. The API might change the solidification temperature and viscosity of the melt, which affects the production temperature. We found that increasing the speed of agitation causes significantly higher gas dispersion efficiency, while the volume of the introduced gas and the gas injection rate has an optimal range. We hypothesize that a bubble plug formed around the dispersing tool at a higher range of parameters, while at lower values of parameters the foaming is unsatisfactory. $BaSO_4$ increased the melt viscosity and thus increased foaming by higher gas entrapment with a density decrease of almost 54%. Due to the density of 507 mg/cm^3, the composition shows zero floating lag time with continuous floating without gas generation. The microCT image clearly shows the white $BaSO_4$ particles, which have a high X-ray absorption. The bubbles in the composition show homogeneous distribution with the average diameter of 40 μm. Gas bubbles are not open to the outer environment creating closed-cell structure in the whole matrix. $BaSO_4$ formulation is hard at room temperature but can be crushed with a compressive force of 15 N, this force is lower than the average friability of tablet or pellet with similar sizes, but it is strong enough to resist the grinding motions of the stomach, until complete wetting. Texture analysis showed that the complete wetting of the samples occurs in 120 minutes. $BaSO_4$ samples remained in one piece, longer than we expected. Since $BaSO_4$ has negligible solubility in aqueous media, its dissolution does not propagate matrix erosion and may contribute to preserve the hardness or texture of the sample. During compression of the sample in the texture analysis, small amount of water was pressed out from the samples immersed into the acidic media for 4 hours. It can

be explained by the poor wetting angle of $BaSO_4$, as well. Incorporation of $BaSO_4$ into test formulations is often used to check gastric retention *in vivo*. Samples were detected and identified easily in the *in vivo* test. In the light of the study, we confirmed that the $BaSO_4$ containing samples own at least a 2-hour long gastric residence time. The preparations were not emptied from the stomach despite, we have noticed slight decrease in sample sizes. Due to its low density, the current foamed dosage form is suitable for gastroretention, which has been demonstrated *in vitro* and *in vivo*, as well.

Our aim was also to increase the bioavailability of verapamil-HCl by applying the above mentioned solid-foam formulation. Verapamil formulations must be administered frequently, but this can be reduced by GR formulations. From the technological point of view the thermal stability of verapamil is also favorable for the formulation with the technology developed by our research team previously. During the production of verapamil solid foam the foam cell temperature was higher with 2 °C than was previously described with $BaSO_4$ composition, due to higher melting point of the verapamil mixture. Three compositions were produced with 15% verapamil-HCl, containing 80-120 mg of verapamil per capsule, which corresponds to the active substance content available in the literature and of the marketed preparations. The increase of stearic acid content from 10% (V1) to 12,5% (V2) helped to form the foam structure as Vasvári et al. described earlier, but it is important to mention that excessive stearic acid content reduced foaming. In case of V3, the increased density can be the reason of the changes in the wetting of the API particles by the molten matrix. The SEM images support the altered solid particle location. In the case of V3, verapamil crystals are found in the inner surface of the cavity, while in the case of V1 and V2, they are dispersed in the matrix, localization in the cavities are not specific. The foaming efficacy does not depend on only the hydrophilicity of the matrix, and according to our experiences the particle size of the solid phase also significantly affects the degree of foaming. Earlier we successfully produced a lower density product using smaller drug particles (314 nm ± 115), $BaSO_4$, with the above-mentioned technology and using verapamil particles with the average particle size (13.4 μm ± 11.2) resulted a higher density. The drug dissolution test showed prolonged drug release in all of the compositions. Compared with marketed preparations, the drug release is slower than from Isoptin SR, but shows similar kinetics compared to the Calaptin SR. During the validation three parallel productions were performed and compared, the validation results comply with FDA and GMP regulations. The micro-CT scans show high porosity, the distribution of bubbles in the matrix is homogeneous, and also the size distribution of cavities is monodispersed. In general, open pores could

accelerate the rate of dissolution, our capsule has few open pores, so their presence does not contribute significantly to the erosion of the preparation. The texture analysis during the dissolution showed that, the samples remained hard until 300 min. At the end of dissolution, 30 % of the initial weight was still presented and the matrix became plastic which was easily removed by the grinding or churning motions of the stomach. The PEG and API dissolved from the matrix while SA remained undissolved during the dissolution test. The composition did not show swelling ability. In a dissolution study performed after storage on accelerated conditions, the formulation was compared to initial formulation with factors f1 and f2, which met expectations. A similar result was observed for capsules stored under an extended 2-year standard conditions. The results of dissolution studies showed that no major changes of the verapamil containing solid foam matrices occurred, which influence the drug dissolution. *In vivo* pharmacokinetics study proved that the GR system was able to significantly change the pharmacokinetics parameters of verapamil. In case of verapamil solution, the *in vivo* plasma concentration of verapamil is correlated with the published conventional verapamil-HCl pill, Staveran. In this study verapamil solution was used to reveal the absorption properties and pharmacokinetics of pure verapamil from the GI tract and avoid the influence of excipients or the liberation of verapamil from a formulation. Comparing the results of the solid foam capsule with a retard pill, Isoptin SR, the t_{max} shift can be observed. Instead of a sharp peak, a plateau was observed, and plasma levels could be further maintained in the therapeutic range. 99,3 % relative bioavailability was reached. The dissolution results of verapamil foams are reflected in *in vivo* data. The *in vitro* - *in vivo* correlation predicts well the *in vivo* absorption by the *in vitro* tests. With the application of high porosity verapamil foam, the gastroretentive purposes of controlled release and stomach retention are achieved simultaneously. In terms of our results, it can be said that our preparation is suitable for gastroretention and can reduce the frequency of administration of the preparation, thus achieving better adherence. Our results were confirmed by both *in vitro* and *in vivo* experiments.

6. Conclusion

During my PhD work, a novel apparatus was designed and built to produce low density GR carrier that based on continuous foaming of molten dispersions. The foaming process was optimized by a Box–Behnken experimental design to determine the most effective setup to create solid foams with high porosity. We developed $BaSO_4$ containing samples with low density, namely 507 mg/cm3. The high porosity was showed in micro-CT scan. In acidic media, texture analysis was performed to characterize the *in vitro* wetting of the samples. The contrast agent and PET/CT were used to prove the *in vivo* gastroretention of the foam formulation in a rat model. We have produced verapamil-loaded gastroretentive capsules and investegated the *in vitro* and *in vivo* behavior. After optimizing the apparent density of the product by considering the SA contents, production runs were validated, as well. Micro-CT scans revealed a closed cell structure where the main fraction of the voids is smaller than 120 microns. Texture analysis results confirmed hard structure, even after 5 hours of dissolution. Despite continuous erosion of the PEG matrix, floating strengths of the samples remained stable during dissolution. Our studies confirmed that the first order drug release was preserved, even after 2 years of storage. *In vivo* pharmacokinetic study verified the prolonged release of the API from the matrix, maximum drug plasma concentration was reached after 4 hours of administration. Compared to the verapamil oral solution, a relative bioavailability of 99,3 % was reached. Summarizing our results, we state that our foaming device can be successfully used to produce low density molded capsule by continuous operation with *in vivo* gastroretentive properties.

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List of publications related to the dissertation

1. **Haimhoffer, Á.**, Vasvári, G., Budai, I., Béres, M., Deák, Á., Németh, N., Váradi, J., Sinka, D. Z., Bácskay, I., Vecsernyés, M., Fenyvesi, F.: In Vitro and In Vivo Studies of a Verapamil-Containing Gastroretentive Solid Foam Capsule.
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2. **Haimhoffer, Á.**, Vasvári, G., Trencsényi, G., Béres, M., Budai, I., Czomba, Z., Rusznyák, Á., Váradi, J., Bácskay, I., Ujhelyi, Z., Fehér, P., Vecsernyés, M., Fenyvesi, F.: Process Optimization for the Continuous Production of a Gastroretentive Dosage Form Based on Melt Foaming.
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3. Rusznyák, Á., Malanga, M., Fenyvesi, É., Szente, L., Váradi, J., Bácskay, I., Vecsernyés, M., Vasvári, G., **Haimhoffer, Á.**, Fehér, P., Ujhelyi, Z., Nagy, B. J., Fejes, Z., Fenyvesi, F.: Investigation of the Cellular Effects of Beta- Cyclodextrin Derivatives on Caco-2 Intestinal Epithelial Cells.
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