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Abstract

Release modules of amoxicillin and clarithromycin combined in a single dosage form designed to float in the gastric content and to sustain the intra-gastric concentrations of these two antibiotics used for the eradication of *Helicobacter pylori* have been studied.

The modules having a disc shape with curved bases were formulated as hydrophilic matrices. Two modules of clarithromycin were assembled by sticking the concave base of one module to the concave base of the other, creating an internal void chamber. The final dosage form was a floating assembly of three modules of clarithromycin and two of amoxicillin in which the drug release mechanism did not interfere with the floatation mechanism.

The assembled system showed immediate *in vitro* floatation at pH 1.2, lasting 5 hours. The *in vitro* antibiotics release profiles from individual modules and assembled systems exhibited linear release rate during buoyancy for at least eight hours. The predicted antibiotic concentrations in the stomach maintained for a long time levels significantly higher than the respective Minimum Inhibitory Concentrations. In addition, an *in vivo* absorption study performed on beagle dogs confirmed the slow release of clarithromycin and amoxicillin from the assembled system during the assembly's permanence in the stomach for at least 4 hours.

Introduction

Helicobacter pylori *(H. pylori)* infection is an important factor in the development of gastritis, gastric ulcer and gastric carcinoma[1]. More than 50% of people worldwide[2] are infected with this pathogen. Factors such as age and socio-economic conditions affect its prevalence[3]. *H. pylori*~~*Helicobacter pylori*~~ mainly resides in the gastric mucosa or at the interface between the mucous layer and the epithelial cells in the antral region of the stomach. The first-line pharmacological treatment for *H. pylori* ~~*Helicobacter pylori*~~ eradication is one week of oral therapy with high doses of two antibiotics (clarithromycin and amoxicillin or metronidazole) combined with a proton-pump inhibitor[4-5], often exposing the patient to adverse effects. The complexity of an administration schedule, combining different dosage forms taken together, may lead to non-adherence to the therapeutic regimen, giving rise to the appearance of resistant strains. Therefore, the systemic treatment failure is dependent on the difficulty to reach and maintain an effective antimicrobial concentration on the gastric epithelial surface[6].

Because of these problems, medicines should be provided that are based on drug release control enabling to sustain effective local drug concentration, thus simplifying the regimen. Given that the bacterium lives in the gastric mucosa, a logical way to improve the therapy would be to administer a gastro-retentive dosage form capable of releasing the antibiotics for as long as possible in the bacterium niche. This approach could provide effective and prolonged local levels of antibiotic even by administering lower doses of drug.

Several described gastro-retentive dosage forms are floating preparations[7-11] but most of them did not show a prompt floatation. Moreover, the release of the drug is often disturbed by the floatation mechanism adopted. For example, frequently

described floating drug delivery systems are swellable matrices, in which the floatation is determined by the bubbles, produced by a gas-generating agent, entrapped in the gel layer[12-13]. Since the bubbles develop in the gel layer, their presence interferes with the release of drug due to the fact that the gel layer controls the drug release. A drug dosage form based on module assembly technology could provide a modular delivery system for prolonged drug release in gastro-retentive conditions in which the adopted floatation mechanism does not interfere with the release control mechanism[14-16]. The release module having a disc shape with a convex and a concave base, has been formulated as a tableted hydrophilic matrix for floatation and drug release control. Two of these release modules assembled by sticking the concave base of one to the concave base of the other form an assembly with an internal void chamber that showed immediate floatation and gastro-retention in humans[17]. To the floating assembly, additional modules can be stacked to obtain multi-kinetics and multi-drug delivery systems[18]. In summary, module assemblage could allow for the delivery of more than one drug in a single dosage form at a specific time, proper rate and duration of release. Moreover, according to the number of modules assembled, the dose administered could be adjusted.

Therefore, the aim of this work was thus to study the drug release mechanism and kinetics of a floating system designed to sustain the intra-gastric concentrations of amoxicillin and clarithromycin in combination. By applying the module assembly technology[14] for the construction of a floating prolonged release dosage form, it was postulated that the drug delivery system could float on gastric fluid without disturbing the drug release control. The objective would be to keep the concentration of the two antibiotics above the respective minimum inhibitory concentration (MIC) in the stomach for 4-5 hours. Different modules of clarithromycin and amoxicillin prepared

as controlled release formulations were assembled in one single drug delivery system (Figure 1). The *in vitro* antibiotic release rate and mechanisms from individual modules and assembled systems, together with the buoyancy performance of the assembly, were investigated. The drug concentration attainable in the stomach was predicted applying a pharmacokinetic model. Furthermore, to assess clarithromycin and amoxicillin release rate and site from the assembled system, a pilot *in vivo* study using dogs was conducted.

Materials and methods

Materials

Clarithromycin was supplied by Special Product's Lines (Rome, Italy) and amoxicillin purchased from Sandoz GmBH (Kundl, Austria). Hydroxypropylmethylcellulose polymers (HPMC K15M; HPMC K100M) were obtained from Colorcon Limited (Orpington, U.K.); polyvinylpyrrolidone (PVP K30) was purchased from BASF SE (Ludwigshafen, Germany) and polyethylene glycol (PEG 6000) was obtained from Lisapharma S.p.A. (Erba (CO), Italy). All other chemicals were standard pharmaceutical grade.

Methods

Preparation of prolonged release clarithromycin modules

Clarithromycin modules were prepared by compression of clarithromycin granules prepared by kneading 100 g of clarithromycin and 10 g of HPMC K15M using 40 ml of a 50:50 hydroalcoholic solution (v/v) containing 5 % w/v of PEG 6000 and 5 % w/v of PVP K30. Granules were obtained by using an oscillating arm granulator (Erweka AR400, Düsseldorf, Germany), equipped with a 0.8 mm mesh. Granules were dried

in an oven for about 5 hours at 45 °C. Then, granules were blended with a 3 % w/w of talc and 1 % w/w of magnesium stearate in a Turbula® blender for 25 minutes. The blend was compressed at a tablet weight of 120 mg using a single-punch eccentric tableting machine (EKO Korsch, Berlin, Germany) equipped with 7.4 mm diameter cylindrical punches having a tip surface suitable for manufacturing convex or concave bases. Two different module shapes were compressed (see Figure 1). The male modules had an average thickness of 4.8 ± 0.2 mm and the female ones of 4.4 ± 0.2 mm.

Preparation of prolonged release amoxicillin modules

Amoxicillin female modules were prepared by direct compression of a mixture of 100 g of amoxicillin, 46.2 g of HPMC K100M, 6.2 g of talc and 1.6 g of magnesium stearate powders, using the same single-punch eccentric tableting machine equipped with 7.4 mm cylindrical punches. The female modules (see Figure 1d) were tableted at 154 mg. The average thickness was 5.8 ± 0.2 mm.

Assembly of Dome Matrix modules

The assembled systems were obtained by "clicking" together the modules by hand in the following way: one male (see Figure 1a) and one female module of clarithromycin (see Figure 1b) were interlocked concave to concave face. This assembly, named void configuration, ensured the floatation of the release system to be exploited *in vivo* for localizing and maintaining the drug release in the stomach. Additional female modules of clarithromycin or amoxicillin were stacked on the convex base of the female module of the void configuration assembly (see Figure 1).

Assay for clarithromycin content

The content of clarithromycin was determined following the method reported in the USP 34 ed. monograph "Clarithromycin Extended-Release Tablets". The HPLC apparatus and conditions were the following: Shimadzu Liquid Chromatograph LC-10AT (Shimadzu Europe GmbH, Duisburg, Germany); UV-VIS detector SPD-10A at 210 nm; Luna C18 column 4.6 x 150 mm, 5 um (Phenomenex, Torrance, CA, USA) maintained at 50 °C; mobile phase: methanol:phosphate buffer pH 4.0 (65:35 (v/v)); flow rate: 1 ml/min; injection volume: 50 μl (Autosampler Model 542, ESA Inc., Chelmsford, USA). The system suitability gave the following results: theoretical plates 2184; peak symmetry 1.01; RSD 1.7%.

Assay for amoxicillin content

The content of amoxicillin was determined following the method reported in USP 34 ed. monograph "Amoxicillin Tablets". The HPLC apparatus and conditions were the following: Shimadzu Liquid Chromatograph LC-10AT (Shimadzu Europe GmbH, Duisburg, Germany); UV-VIS detector SPD-10A at 230 nm; μBondapack C18 column 3.9 x 300 mm, 5 um (Waters Corp., Milford, MA, USA) maintained at 40 °C; mobile phase: acetonitrile:phosphate buffer pH 5.0 (2.5:97.5 (v/v)); flow rate: 0.7 ml/min; injection volume: 10 μl (Autosampler Model 542, ESA Inc., Chelmsford, USA). The system suitability gave the following results: theoretical plates 2253; peak symmetry 2.25; RSD 0.5%.

In vitro drug release of individual modules and assembled system

Clarithromycin

The USP dissolution apparatus II with paddle rotating at 75 rpm was employed. The

dissolution medium was Britton-Robinson buffer solution pH 3.0[19] at 37 °C. 500 ml and 900 ml were used for dissolving the individual modules and the assembled systems respectively. Samples collected at fixed time points were filtered through a 0.45 μm membrane (CA 0.45 μm, LabService Analytica, Bologna, Italy) and analyzed using the HPLC assay method.

Amoxicillin

The USP dissolution apparatus IV, equipped with a 12 mm diameter cell, was employed. Simulated gastric fluid without enzymes pH 1.2 at 37 °C was used as dissolution medium. The flow rate was set at 8 ml/min. Samples of 8 ml collected at fixed times were filtered through a 0.45 μm membrane and analyzed using the HPLC assay method.

Determination of floatation characteristics

As previously described[20-21], the buoyancy of the assembly was determined by measuring the resultant weight of the system made of three modules of clarithromycin and two modules of amoxicillin, submerged in simulated gastric fluid without enzymes at 37 ± 0.5 °C.

Simulation of clarithromycin and amoxicillin concentrations in the stomach

The intra-gastric concentrations of clarithromycin and amoxicillin were simulated and predicted using STELLA® software (isee systems, Lebanon, NH, USA). The slope of the amount released vs time linear profiles for the assembled system was elected as the intra-gastric release rate. The initial volume of fluid in the stomach in fed state was set at 1.5 L[22]; the emptying rate followed a first order kinetic with $t_{50\%}$ of 0.20 h[23].

The gastric residence of the dosage form was fixed at 5 h[17].

Oral absorption study in dogs

An *in vivo* study to measure drug absorption upon oral administration was carried out using six male beagle dogs, weighing 15-20 kg. The study was performed at the Faculty of Health Sciences, Fernando Pessoa University (Portugal), approved by the competent ethics and scientific committee and conducted in accordance with EU Legislation[24]. The dogs were fed before drug administration with a standard meal and water was left *ad libitum.* A hard gelatin capsule (Coni-Snap® size 00, Capsugel, Colmar, France) containing the assembly of Figure 1 (three modules of clarithromycin and two of amoxicillin) was administered. In order to check by X-ray the location in the stomach of the assembled system during *in vivo* drug release, 10 mg of barium sulfate powder were introduced in the empty chamber of the clarithromycin void configuration assembly.

Blood samples were collected immediately before and after 60, 120, 240, 480, 720 min from drug administration. 200 µl of plasma was added to 200 µl of chilled acetonitrile containing the internal standards (oxacillin and erythromycin for amoxicillin and clarithromycin, respectively; 50 µl of each at concentration of 10 µg/ml). The sample was mixed for at least 5 min to allow complete protein precipitation. After centrifugation at 16,000 X g for 10 min, 200 µl of the supernatant was transferred to a clean vial. The solvent was evaporated to dryness under nitrogen gas flow. The residue was reconstituted in 100 ml of 0.1% (v/v) aqueous formic acid and left to mix for 5 min. Samples were centrifuged again at 16,000 X g for 10 min. The supernatant was transferred to a polypropylene auto-sampler vial and stored at 5 °C until analysis by UPLC-MS/MS. The analytical method consisted

of a Waters Acquity UPLC instrument coupled to a Quattro Premier XE tandem-quadrupole mass spectrometer (Waters Corp., Milford, MA). The analytical column was an Acquity UPLC BEH C18 (2.1 mm x 50 mm, 1.7 µm; Waters Ltd., Dublin, Ireland), to which a 0.2 µm pre-column filter unit was added. The mobile phase was a gradient of solution A (0.1% FA in water) and solution B (0.1% FA in methanol), with an initial composition of 10% solution B. The mobile phase composition changed linearly from 10% B at 1 min to 80% B at 2 min and onward to 100% B at 4 min. The composition was switched back to 10% B at 5 min and maintained until 6 min. The flow rate was 0.3 ml/min, with a column temperature of 40 °C. From each sample, 10 µl was injected in duplicate onto the column. The analysed compounds were detected via MS with an electrospray ionization interface in positive multiple reaction monitoring mode. Optimized multiple reaction monitoring settings for the individual drugs, including cone voltage and collision energy are shown in Table 1. The acquisition settings were as follows: capillary voltage, 3.4 kV; source temperature, 120 °C; desolvation temperature, 300 °C; desolvation gas flow, 600 L/h; cone gas flow, 50 L/h; and dwell time, 80 ms.

Data were acquired using Masslynx V4.1 software. For all compounds, calibration curves were obtained by plotting the peak area ratios of drug versus internal standard against the theoretical concentration. The LOQ values were 0.015 µg/ml for amoxicillin and 0.024 µg/ml for clarithromycin and the LOD values were 0.004 µg/ml and 0.006 µg/ml, respectively. The precision in terms of relative standard deviation was 4.87 % for amoxicillin and 3.11 % for clarithromycin.

Statistics

Graphing was performed by means of KaleidaGraph software (version 4.5.2,

Synergy Software, Reading, PA, USA). Confidence limits of the power equation exponent were determined from the slope of the log/log plot of data at 95% probability level.

Results and discussion

Formulation and manufacturing of modules and assembled systems

Clarithromycin and amoxicillin modules were formulated taking into account the biopharmaceutical characteristics of the active substances and the therapeutic program for *H. pylori*~~*Helicobacter pylori*~~ eradication. The high doses of these antibiotics, required in therapy, restricted the formulation design space, but at the same time simplified the module composition. Clarithromycin and amoxicillin oral recommended doses for *Helicobacter* eradication are 500 mg and 1000 mg respectively, twice a day for 7 days of treatment[3-6]. Given such high drug amounts and the need to construct a dosage form in a size convenient for swallowing, the modules used in this study had a diameter of 7.4 mm and a thickness between 4.4 and 5.8 mm. With this size, each module could accommodate between 100 and 200 mg of powder formulation and, for administration purposes, up to six assembled modules could be introduced into a Coni-Snap® size 00 capsule. The modules were prepared as hydrophilic matrices for prolonged release. Amoxicillin modules contained 100 mg of drug and HPMC K100M. The clarithromycin modules included 100 mg of clarithromycin, HPMC K15M, PVP K30 and PEG 6000. The choice of excipients was mainly guided by the solubility of drugs at the relevant pH values. For instance, the modules of clarithromycin, which was the less soluble drug substance (6.9 g/L at pH 3.0[25]), contained more soluble polymers compared to HPMC used in amoxicillin modules. Both module formulation exhibited compressibility

characteristics favorable to module tableting. Their friability was lower than 1% and the diametric crushing strength was between 3.9 and 10.7 kg (Monsanto hardness tester).

The modules of amoxicillin and clarithromycin were then assembled in different numbers and configurations. A total of five assembled modules formed the amoxicillin-clarithromycin site-specific delivery system used for floatation and release studies, as illustrated in Figure 1.

In vitro drug release rate of single modules and assembled systems

The individual modules and various assembled configurations, all substantially hydrophilic matrices, were studied with respect to drug release rate and kinetics. The release rate of the individual modules was tested in a flow-through apparatus at pH 1.2 for amoxicillin[26] and in a paddle apparatus at pH 3.0 for clarithromycin[19]. The choice of different conditions was made in order to overcome the solubility and instability problems of clarithromycin and amoxicillin at acidic pH values[19, 26-27]. The obtained release profiles (Figure 2) were analyzed using the power equation in which the value of time exponent gives indication on the mechanism of drug release[28]. The analysis was extended up to 80% values of fraction released in consideration of the quasi-linear profile in the measured range. In the case of the clarithromycin modules (male and female), a quasi-constant release rate up to 80% of drug released was observed, with a non-significant difference between male and female in terms of rate. However, the exponent *n* value was significantly higher for the female module, indicating an effect of the module geometry on the release kinetics (see *n* values and 95% confidence limits in Figure 2 caption). The quasi-linearity of the curve supported an erosive release mechanism of these matrices. In fact, in 4-5 hours clarithromycin

modules were completely dissolved. Thus, clarithromycin modules in the medium at pH 3.0 exhibited a behavior more erosive than diffusive. While assuring the maximum stability for clarithromycin, a value of 3.0 for the pH is in line with the pH values found in humans in fed conditions[29].

The release rate of amoxicillin from the female module was slower than that of clarithromycin female module, likely owing to the presence (30% w/w) of the high molecular weight hydrophilic HPMC polymer in the formulation. Moreover, *in vitro* release was measured at pH 1.2 using the flow through apparatus instead of the paddle employed for clarithromycin. This apparatus was used to limit the amoxicillin *in vitro* degradation at pH 1.2 medium[26]. With this matrix the kinetics appeared linear up to 80% of amoxicillin released, as reflected by the value of exponent *n* (0.93 ± 0.11). Amoxicillin was totally released within 6 hours from a gelled system that completely dissolved in 24 hours. Tested at pH 3.0, where amoxicillin is less soluble[27], the module showed a lower drug release (40% in 8 hours).

The release rate and duration for both drugs from individual modules was judged adequate relatively to the assumed residence time in the stomach of the modules when assembled. In fact, in a previous *in vivo* study[17] a period of gastro-retention for an assembled system in void configuration was determined between 4 and 5 hours.

Two clarithromycin modules having the same formulation and mass, but male and female geometry, were then assembled in void configuration by interlocking their concave bases. To increase the total unit dose, additional clarithromycin female modules were stacked onto the convex female face of this void assemblage, giving rise to void/stacked assembled systems made of two, three or five clarithromycin modules. Thus, these assemblies were investigated for *in vitro* drug release at pH 3.0 in paddle apparatus during floatation. Clarithromycin release profiles are

reproduced in Figure 3 as amount released versus time.

The profiles of amount released versus time show that the clarithromycin release rate increased with the number of modules from two to five, owing to the increased area exposed to dissolution medium. The release area exposed was 183.7 mm^2, 247.9 mm^2 and 376.4 mm^2 for the two modules, three modules and five modules, respectively.

In the case of the two-module void assembly, the clarithromycin release rate was slowed down compared to the cumulative drug released from the male and female modules individually. This was attributed to the fact that, upon assembly by "clicking" together the male and female module, the internal surface was no more accessible to the solvent. After a small initial burst, clarithromycin was released at a quasi-constant rate as the system visibly underwent dissolution/erosion.

The release profiles up to 80% of drug released were also fitted to the power equation and the release kinetics were identified from the values of the exponent n. The n values obtained with these assembled hydrophilic matrices (see caption to Figure 4) were significantly different from 0.5. This supported the dissolution/erosion mechanism of the drug/polymer matrix, as observed with several other hydrophilic matrices containing small amounts of polymer or soluble drugs and polymers[30].

The addition of clarithromycin modules on the basic void assembly, pushed the release kinetics towards higher linearity (see the exponent *n* values in Figure 4 caption), but the significance of the increase remained at $p \geq 0.2$. Since there was no change in module composition, this kinetics shift has to be assigned to the modification of the aspect ratio of the delivery system. In fact, the release from the lateral surface of the assembled pile augmented with additional modules, whereas the base contribution in the pile was not changed. It was previously observed with a

soluble drug that increasing the length of the assembly by module addition, shifted the release kinetics towards higher linearity[31]. The release from the lateral side of the compressed disc was found to be kinetically different from that of the axial side[32] and the lateral side contribution to clarithromycin release in the assembly multiplied with the number of stacked modules.

In the delivery system prepared for the *in vivo* study, two female amoxicillin modules were stacked on a three-clarithromycin module assembled system, as indicated in Figure 1. The *in vitro* amoxicillin and clarithromycin release profiles of the combined assembled systems were determined by dissolution at pH 1.2 in flow-through apparatus for amoxicillin and at pH 3.0 in paddle for clarithromycin. The release profiles are illustrated in Figure 5.

Amoxicillin and clarithromycin release profiles from the combined system remained quasi-linear. Individually, two assembled modules of amoxicillin (Figure 5) or three assembled modules of clarithromycin (see Figure 3) showed a release rate higher than that of amoxicillin and clarithromycin from the assembly. The assembly of three modules of clarithromycin and two of amoxicillin reduced the exposed release area compared to the independent drug assemblies. However, the release of the two combined drugs in the assembled system at steady state remained very close to linearity.

Simulation of intra-gastric drug concentration

In order to estimate the maintenance of clarithromycin concentration in the stomach above the MIC~~Minimum Inhibitory Concentration~~ of *Helicobacter* (2 µg/mL)[33-34], the release rates of clarithromycin assembled systems made of three (300 mg) and five modules (500 mg) (see Figure 3) were used for predicting intra-gastric

concentrations by the STELLA software[35].

The simulated intra-gastric concentration of clarithromycin (Figure 6) from the assembled systems containing three or five modules remained higher than the clarithromycin MIC. The simulation supports the possibility of a steady drug concentration maintained for the expected residence time of the system within the stomach. The five-module system of clarithromycin could maintain the drug concentration largely above the MIC for the time of residence considered. In the case of the system with three clarithromycin modules, containing a dose of only 300 mg, the steady gastric drug concentration was threefold higher than the MIC. Literature pharmacokinetics data of clarithromycin show that 2 hours after administration of 500 mg of clarithromycin, the stomach tissue concentration was 10.5 µg/g in the antrum, 20.8 µg/g in the fundus and 4.2 µg/g in the mucus[36].

In humans, the gastro-retention could allow for a longer effective concentration of clarithromycin directly in contact with *H. pylori*~~*Helicobacter pylori*~~ at a lower dose than the usual systemic administration. The number of clarithromycin modules could then be reduced from five to three, and the two eliminated clarithromycin modules could be replaced by two amoxicillin modules, each containing 100 mg of drug.

From the combination of two amoxicillin modules with three clarithromycin modules, the simulated concentration profile of clarithromycin was slightly reduced compared to the corresponding three-module system in Figure 6. The concentration profile of amoxicillin maintained for 5 hours steady values sixfold higher than the MIC that is 0.5 µg/mL[37]. Although the amount of amoxicillin in the assembled system (200 mg) was significantly lower than the usually prescribed dose (1000 mg), the simulation suggested that this dose could maintain amoxicillin concentration in the stomach higher than the MIC value for a prolonged time.

Floatation assessment of assembled system

The male and female modules interlocked concave to concave base (void assembly), in simulated gastric fluid displayed an immediate floating lasting more than 5 hours. Increasing the number of modules added on the void assembly, a non-immediate floatation was expected. However, the system with five assembled modules, i.e., three clarithromycin and two amoxicillin, immediately floated on the fluid as well, with an initial resultant weight value of 80 mg. The buoyancy force of the assembled system measured over time increased to 100 mg, maintaining this value for more than 5 hours, i.e., the time designed for completing drug release. This indicates that the floating property of the delivery system is strengthened by the presence of the swollen polymer. As~~In~~ shown in Figure 7, ~~showing the system during the floatation measurement,~~ the left side of the swollen system during the floatation measurement corresponds to amoxicillin modules and the right to clarithromycin modules. ~~The picture of Figure 7 shows that t~~The system has two portions with different physical behavior during release: amoxicillin is released from a more highly swollen gel, whereas clarithromycin from a portion of the system that jellified less and eroded more. Moreover, it can be observed that the external gel layer of the matrix was not disturbed by the presence of air bubbles as expected from the mechanism adopted for floatation.

Oral absorption in dogs (bioavailability pilot study)

The drug release rate and the system gastro-retention were investigated by an oral absorption test in dogs. Dogs were used primarily for assessing the drug release kinetics, concomitantly to the system localization in the gastrointestinal (GI) tract. The administered product consisted of three clarithromycin modules (two in void

configuration) and two amoxicillin modules stacked on them. The clarithromycin maximum plasma concentration (C_{max}, 10.56 ± 3.66 µg/mL) was achieved after 8 hours, with a profile indicating a prolonged absorption of drug (Figure 8). The maximum amoxicillin plasma concentration (C_{max}, 1.53 ± 0.44 µg/mL) was achieved after two hours. Then, the plasma levels were steadily maintained between 1.25 and 1.15 µg/mL until twelve hours. ~~The plasma levels were lower than other values in dog reported in the literature[38-39] for immediate release tablets of the same drugs.~~ The shape of the curves suggested that both drugs were slowly released over a long period of time. The plasma levels were lower than other values in dog reported in the literature[38-39] for immediate release tablets of the same drugs.

During the blood sampling, the dog was examined by X-rays in order to concomitantly localize the position of the system in the animal GI. The X-ray pictures revealed that the assembled system was still in the dog stomach at 4 hours (Figure 9). The in vitro release profiles ~~behavior reflected in~~ correlated with the measured plasma levels and the permanence of the delivery system inside the stomach. ~~demonstrated that t~~ The goal of sustaining the antibiotic concentration in the stomach using swellable release matrices assembled to form a floating delivery system ~~was realistic~~ is affordable.

Conclusion

A modular single unit dosage form containing clarithromycin and amoxicillin for *H. pylori*~~*Helicobacter pylori*~~ therapy was constructed by interlocking five drug modules. The final dosage form included three assembled clarithromycin modules and two amoxicillin modules stacked on them. The assembled system floated *in vitro* for more than 5 hours on the surface of a dissolution medium at pH 1.2. The simulated

drug intra-gastric concentrations over time were significantly higher than the MIC values of both antibiotics. An oral absorption experiment in dogs showed prolonged plasma concentrations of clarithromycin and amoxicillin after administration of the combined system. The X-ray examinations confirmed that the system was still in the stomach 4 hours after administration.

The ability of the assembled system to float in the stomach content does not interfere with the drug release control mechanism since the floating mechanism is determined by the internal void of the assembly and the drug release control by the walls of the system. This is different and new compared to other technologies in which floatation depends on the same mechanism that controls drug release (e.g. hydrophilic matrices entrapping air/gas bubbles in the swollen polymer). By maintaining a prolonged direct contact between *H. pylori*~~*Helicobacter pylori*~~ and the antibiotic molecules, the assembled system has the potential to reduce the drug doses and the number of dosage forms administered daily.

The assembly of modules having different drug content and/or different kinetics in a single dosage unit leads to the assumption of an increase in patient compliance and adherence to the therapeutic regimen. Indeed, the actual size of the present unit dose (5-module assembly inside a Coni-Snap 00 type capsule, comprising the two drugs) is not significantly different from the average size (22 mm) of commercially available capsules and tablets containing only amoxicillin (i.e., not combined with clarithromycin) at dosages of 500 mg or higher[40].

Declaration of interest

The authors report no declaration of interest.

References

[1] Kusters JG, van Vliet AHM, Kuipers EJ. Pathogenesis of Helicobacter pylori infection. Clin Microbiol Rev 2006; 19: 449-90.

[2] Helicobacter pylori in developing countries. World Gastroenterology Organisation Global Guidelines. 2010.

[3] Scarpignato C, Pelosini C. Antisecretory drugs for eradication of Helicobacter pylori: antibacterial activity and synergism with antimicrobial agents. In: Scarpignato C, Bianchi G Porro eds. Clinical pharmacology and therapy of Helicobacter pylori infection. Basel: Karger Medical and Scientific Publishers, 1999: 136-80.

[4] Wolle K, Malfertheiner P. Treatment of Helicobacter pylori. Best Pract Res Clin Gastroenterol 2007; 21: 315-24.

[5] Malfertheiner P., Mégraud F, O'Morain C, Hungin APS, Jones R, Axon A, Graham DY, Tytgat G, Current concepts in the management of Helicobacter pylori infection – The Maastricht 2-2000 Consensus Report. Aliment Pharmacol Ther 2002; 16: 167-180.

[6] Graham DY, Lu H, Yamaoka Y. Therapy for Helicobacter pylori infection can be improved: sequential therapy and beyond. Drugs 2008; 68: 725-36.

[7] Raijnkanth PS, Balasubramaniam J, Mishra B. Development and evaluation of a novel floating in situ gelling system of amoxicillin for eradication of Helicobacter pylori. Int J Pharm 2007; 335: 114-22.

[8] Whitehead L, Collett JH, Fell JT. Amoxicillin release from a floating form based on alginates. Int J Pharm 2000; 210: 45-9.

[9] Gòmez-Burgaz M, Garcià-Ochoa B, Torrado-Santiago S. Chitosan-carboxymethylcellulose inter-polymer complexes for gastric-specific delivery of

clarithromycin. Int J Pharm 2008; 359: 135-43.

[10] Nama M, Gonugunta CSR, Veerareddy PR. Formulation and evaluation of gastro-retentive dosage forms of clarithromycin. AAPS PharmSciTech 2008; 9: 231-7.

[11] Patel SS, Ray S, Thakur RS. Formulation and evaluation of floating drug delivery system containing clarithromycin for Helicobacter pylori. Acta Pol Pharm 2006; 63: 53-61.

[12] Thakar K, Joshi G, Sawant KK. Bioavailability enhancement of baclofen by gastroretentive floating formulation: statistical optimization, in vitro and in vivo pharmacokinetic studies. Drug Dev Ind Pharm 2013; 39: 880-888.

[13] Rapolu K, Sanka K, Vemula PK, Aatipamula V, Mohd AB, Diwan PV. Optimization and characterization of gastroretentive floating drug delivery system using Box-Behnken design. Drug Dev Ind Pharm 2013; 39: 1928-1935.

[14] Losi E, Bettini R, Santi P, Sonvico F, Colombo G, Lofthus K, Colombo P, Peppas NA. Assemblage of novel release modules for the development of adaptable drug delivery systems. J Control Rel 2006; 111: 212-218.

[15] Losi E, Peppas NA, Ketcham RA, Colombo G, Bettini R, Sonvico F, Colombo P. Investigation of the swelling behavior of Dome Matrix drug delivery modules by high-resolution X-ray computed tomography. J Drug Del Sci Tech 2013; 23: 165-70.

[16] Colombo P, Santi P, Bettini R, Strusi OL, Sonvico F, Colombo G. Modules, new assemblage kits and new assembles for the controlled release of substances, PCT WO 2008/067829 A1, 2008.

[17] Strusi OL, Sonvico F, Bettini R, Santi P, Colombo G, Barata P, Oliveira A, Santos D, Colombo P. Module assemblage technology for floating system: in

vitro flotation and in vivo gastro-retention. J Control Rel 2008; 129: 88-92.

[18] Hascicek C, Rossi A, Colombo P, Massimo G, Strusi OL, Colombo G. Assemblage of drug release modules: effect of module shape and position in the assembled systems on floating behavior and release rate. Eur J Pharm Biopharm 2011; 77: 116-21.

[19] Ishii K, Katayama Y, Itai S, Ito Y, Hayashi H. In vitro dissolution tests corresponding to the in vivo dissolution of clarithromycin tablets in the stomach and intestine. Chem Pharm Bull 1995; 43: 1943-8.

[20] Strusi OL, Barata P, Traini D, Young PM, Mercuri S, Colombo G, Sonvico F, Bettini R, Colombo P. Artesunate-clindamycin multi-kinetics and site-specific oral delivery system for antimalaric combination products. J Control Rel 2010; 146: 54-60.

[21] Timmermans J, Moës AJ. Measuring the resultant weight of an immersed test material. Part I. Validation of an apparatus and a method dedicated to pharmaceutical applications. Acta Pharm Tech 1990; 36: 171–5.

[22] Goetze O, Steingoetter A, Menne D, van der Voort IR, Kwiatek MA, Boesiger P, Weishaupt D, Thumshirn M, Fried M, Schwizer W. The effect of macronutrients on gastric volume responses and gastric emptying in humans: a magnetic resonance imaging study. Am J Physiol Liver Physiol 2007; 229: G11-7.

[23] Kortejarvi H, Urtti A, Yliperttula M. Pharmacokinetic simulation for biowaiver criteria: the effect of gastric emptying, dissolution, absorption and elimination rates. Eur J Pharm Sci 2007; 30: 155-66.

[24] Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and scientific purposes. Official

Journal of the Europeen Comunities, L 358/1986, p. 1-28.

[25] Nakagawa Y, Itai S, Yoshida T, Nagai T. Physicochemical properties and stability in the acidic solution of a new macrolide antibiotic, clarithromycin, in comparison with erythromycin. Chem Pharm Bull 1992; 40: 725-8.

[26] Emara LH, Abdou AR, El-Ashmawy AA, Badr RM, Taha NF, Mursi NM. In vitro release evaluation of gastroretentive amoxicillin floating tablets employing a specific design of the flow-through cell. Diss Tech 2013; 20: 27-33.

[27] Tsuji A, Nakashima E, Hamano S, Yamana T. Physicochemical properties of amphoteric β-lactam antibiotics I: stability, solubility, and dissolution behavior of amino penicillins as a function of pH. J Pharm Sci 1978; 67: 1059-66.

[28] Peppas NA. Analysis of Fickian and non-Fickian drug release in polymers. Pharm Acta Helv 1985; 60: 110-1.

[29] Simonian HP, Vo L, Doma S, Fisher RS, Parkman HP. Regional postprandial differences in pH within the stomach and gastroesophageal junction. Digest Dis Sci 2005; 50: 2276-85.

[30] Zuleger S, Lippold BC. Polymer particle erosion controlling drug release. I. Factors influencing drug release and characterization of the release mechanism. Int J Pharm 2001; 217: 1139-52.

[31] Colombo P, Sonvico F, Colombo G, Bettini R. Novel platforms for oral drug delivery. Pharm Res 2009; 26: 601-11.

[32] Colombo P, Catellani PL, Peppas NA, Maggi L, Conte U. Swelling characteristics of hydrophilic matrices for controlled release. New dimensionless number to describe the swelling and release behavior. Int J Pharm 1992; 88: 99-109.

[33] De Francesco V, Zullo A, Ierardi E, Vaira D. Minimal inhibitory concentration

(MIC) values and different point mutations in the 23S rRNA gene for clarithromycin resistance in Helicobacter pylori. Dig Liver Dis 2009; 41: 610-1.

[34] Boyanova L, Koumanova R, Gergova G, Popova M, Mitov I, Kovacheva Y, Derejian S, Katsarov N, Nikolov R, Krastev Z. Prevalence of resistant Helicobacter pylori isolates in Bulgarian children. J Med Microbiol 2002; 51: 786-90.

[35] http://www.iseesystems.com. Accessed on 2 September 2014.

[36] Gustavson LE, Kaiser JF, Edmonds AL, Locke CS, DeBartolo ML, Schneck DW. Effect of omeprazole on concentrations of clarithromycin in plasma and gastric tissue at steady state. Antimicrob Agents Chemother 1995; 39: 2078-83.

[37] Wu H, Shi XD, Wang HT. Resistance of Helicobacter pylori to metronidazole, tetracycline and amoxicillin. J Antimicrob Chemother 2000; 46: 121-3.

[38] Zhang X, Zou M, Sanming L, Chen X, Zhomg D. Bioavailability of clarithromycin cyclodextrin ternary complexes upon oral administration to healthy beagle dogs. Drug Dev Ind Pharm 2008; 34: 1048-53.

[39] Vree TB, Dammers E, van Duuren E. Variable absorption of clavulanic acid after an oral dose of 25 mg/kg of clavubactin and synulox in healthy dogs. J Vet Pharmcol Therap. 2003; 26: 165-71.

[40] Pillbox website by US National Library of Medicine, NIH. Available at: http://pillbox.nlm.nih.gov/pillimage/search.php. Accessed on 16 November 2014.

Table 1. Parameters of UPLC-MS/MS system: Q1, parent ion mass; Q3, daughter ion mass; CV, cone voltage; CE, collision energy; Rt, retention time. Oxacillin is the internal standard for amoxicillin, while erythromycin is the internal standard for clarithromycin.

Antibiotic	Q1 (m/z)	Q3 (m/z)	CV (V)	CE (eV)	Rt (min)
Amoxicillin	366.6	349.5	18	10	2.9
Oxacillin	402.6	243.4	20	14	2.6
Clarithromycin	748.9	558.8	30	30	2.7
Erythromycin	734.9	576.6	30	25	2.6

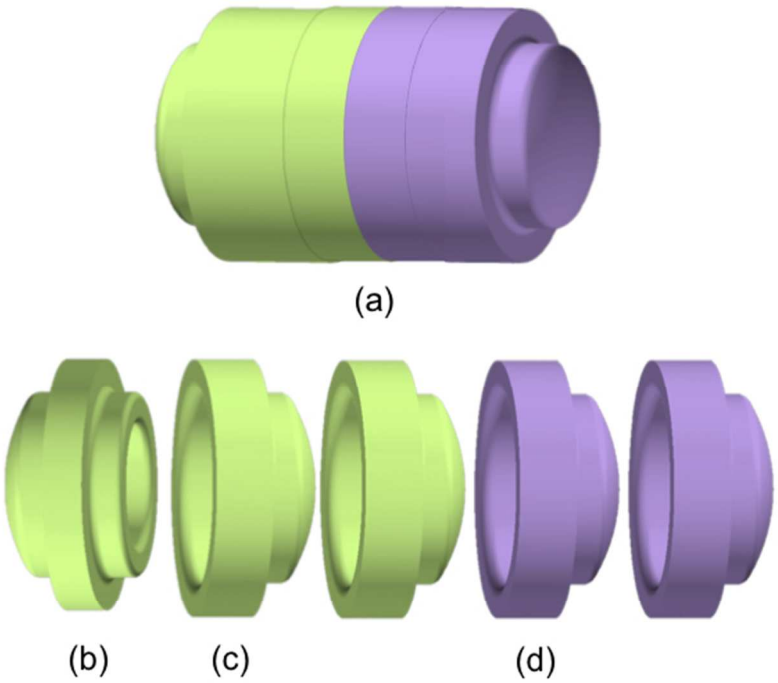

Figure 1. Modular assembled system for HP treatment: (a) clarithromycin controlled release modules (yellow: male (b) and female (c)) and amoxicillin controlled release female modules (purple, (d)).
60x45mm (300 x 300 DPI)

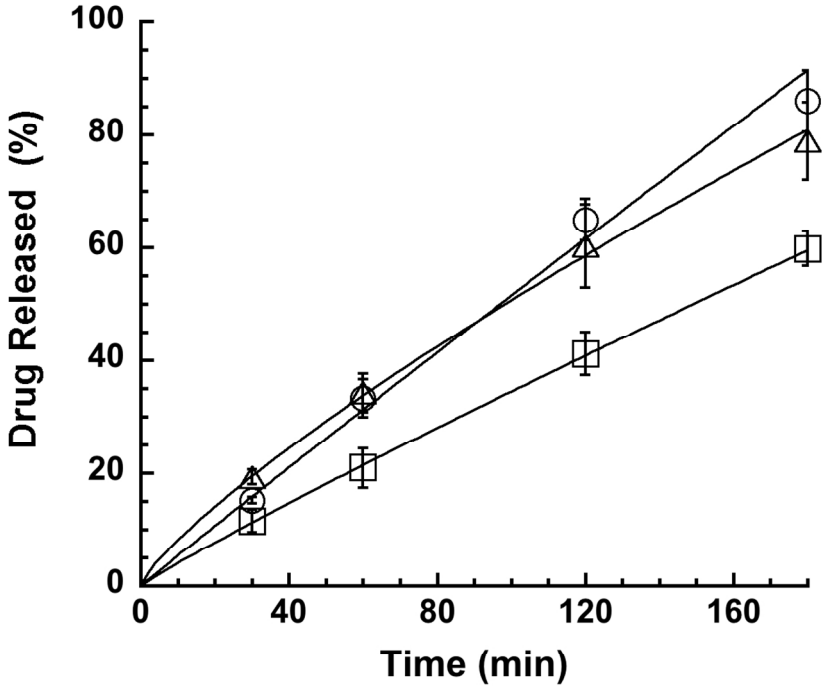

Figure 2. Release profiles of clarithromycin and amoxicillin individual modules (mean ± S.D.; n=3). Lines represent the power equation fitting: (○) clarithromycin female module, drug released (%) = $0.573 \cdot min^{0.977 \pm 0.116}$; (△) clarithromycin male module, drug released (%) = $1.309 \cdot min^{0.794 \pm 0.063}$; (□) amoxicillin female module, drug released (%) = $0.478 \cdot min^{0.929 \pm 0.105}$.
120x101mm (300 x 300 DPI)

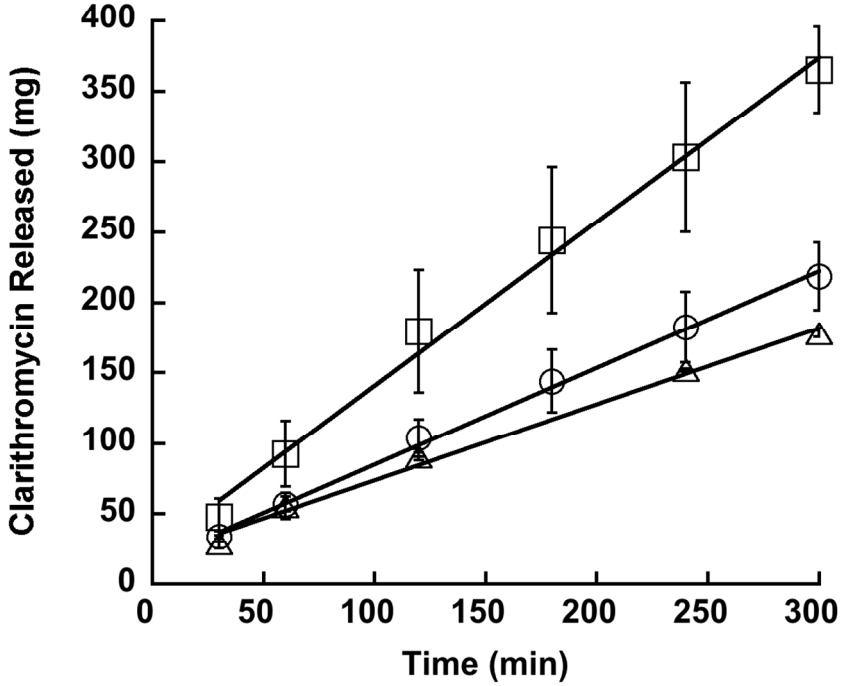

Figure 3. Amount of clarithromycin released from the assembled system (mean ± S.D.; n=3). Lines represent the fitting to linear equation: (△) two-clarithromycin modules in void configuration, amount (mg) = 19.94 + 0.539 min; (○) three-clarithromycin modules, amount (mg) = 16.99 + 0.685 min; (□) five-clarithromycin modules, amount (mg) = 24.83 + 1.164 min.
120x101mm (300 x 300 DPI)

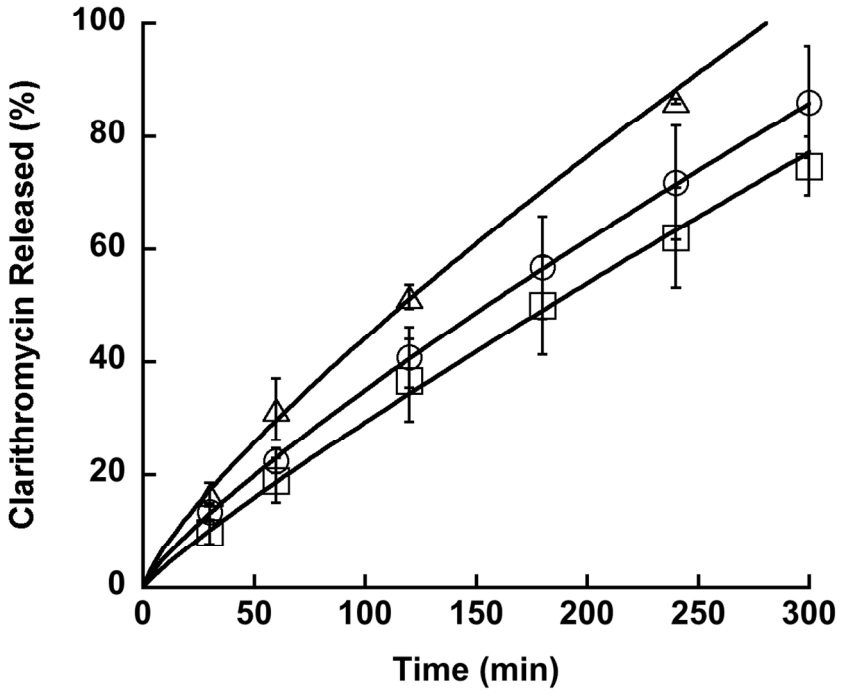

Figure 4. Percent release profiles of clarithromycin (mean ± S.D.; n=3) from assembled system. Lines represent the fitting to the power equation: (△) two-clarithromycin modules in void configuration, drug released (%) = 1.174 $min^{0.788 \pm 0.085}$; (○) three-clarithromycin modules, drug released (%) = 0.812 $min^{0.817 \pm 0.073}$; (□) five-clarithromycin modules, drug released (%) = 0.506 $min^{0.881 \pm 0.128}$.
120x101mm (300 x 300 DPI)

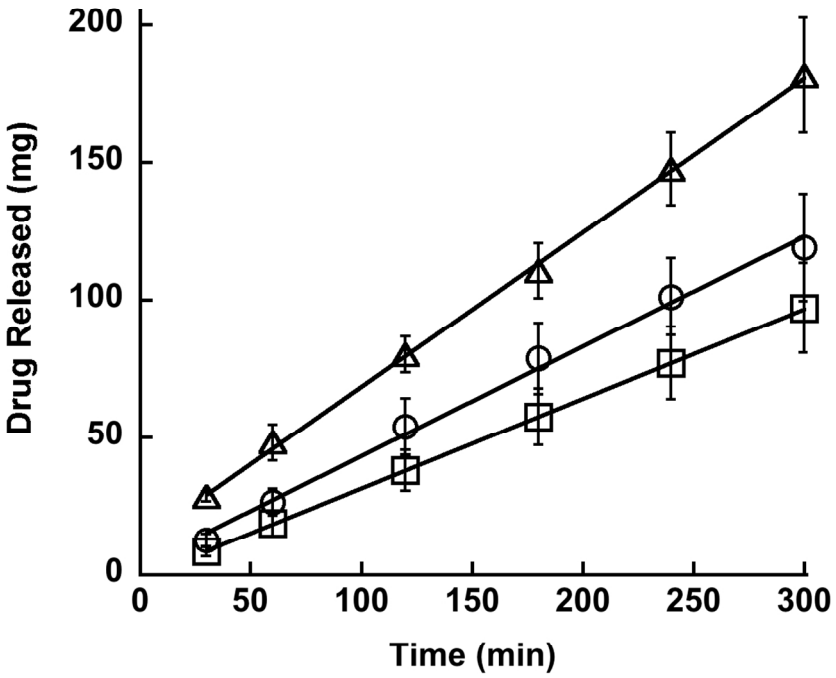

Figure 5. Amount released profiles (mean ± S.D.; n=3). Lines represent the fitting to linear equation: (○) two-amoxicillin assembled modules, amount (mg) = 3.346 + 0.399 min; (□) amoxicillin from three-clarithromycin and two- amoxicillin module assembled system, amount (mg) = -1.149 + 0.326 min; (△) clarithromycin from three-clarithromycin and two-amoxicillin module assembled system, amount (mg) = 12.447 + 0.561 min.
120x101mm (300 x 300 DPI)

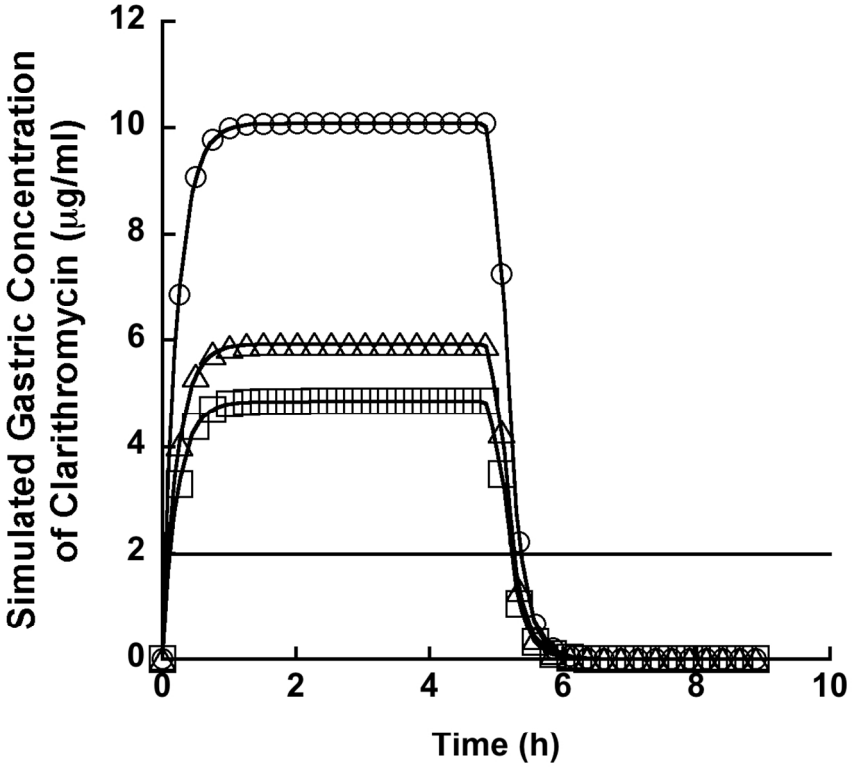

Figure 6. Simulated gastric concentration of clarithromycin from assembled system: (○) five-clarithromycin modules; (△) three-clarithromycin modules; (□) three-clarithromycin modules and two-amoxicillin modules. Straight line indicates clarithromycin MIC (2 µg/ml).
120x114mm (300 x 300 DPI)

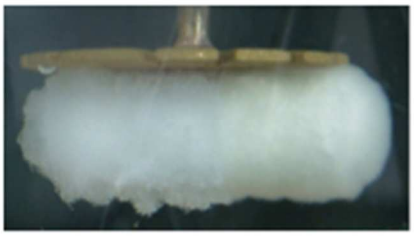

Figure 7. Five-module assembled system of clarithromycin and amoxicillin after 60 minutes of floatation.
18x11mm (300 x 300 DPI)

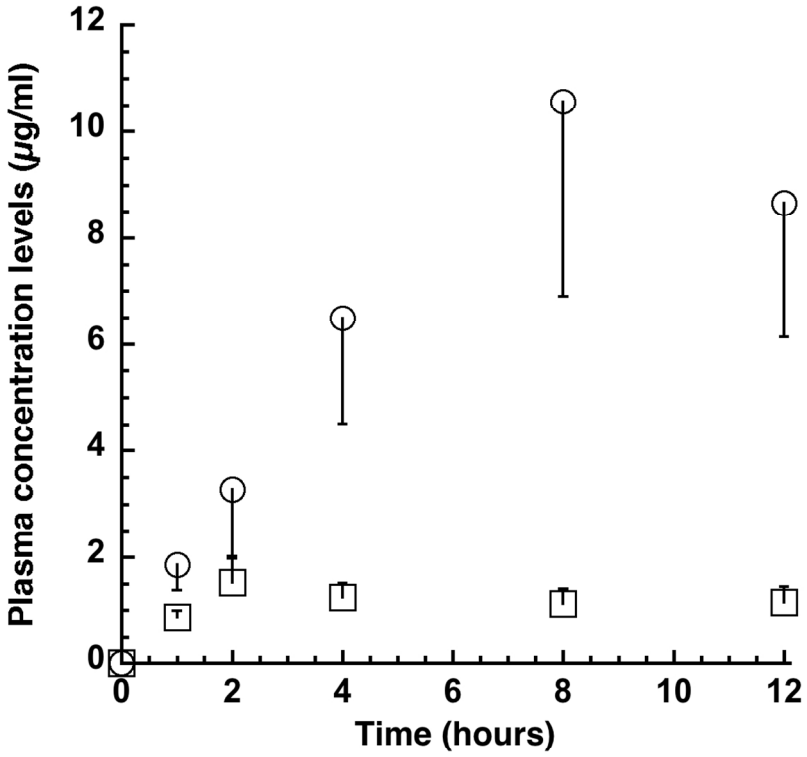

Figure 8. Plasma concentration levels after oral administration of the assembled system to dogs: (○) clarithromycin and (□) amoxicillin.
120x114mm (300 x 300 DPI)

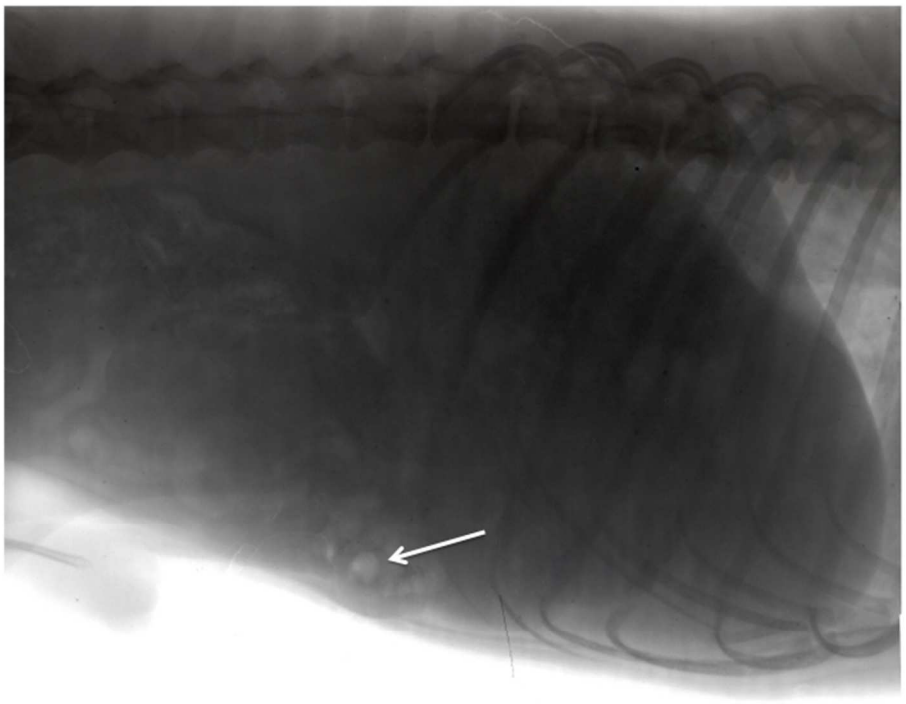

Figure 9. X-ray picture of beagle stomach 4 h from the administration.
60x45mm (300 x 300 DPI)