Development of a magnetic system for the treatment of *Helicobacter pylori* infections

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ABSTRACT

We report a study to develop a magnetic system for local delivery of amoxicillin. Magnetite microparticles produced by coprecipitation were coated with a solution of amoxicillin and Eudragit®S100 by spray drying. Scanning electron microscopy, optical microscopy, X-ray powder diffraction and vibrating sample magnetometry revealed that the particles were superparamagnetic, with an average diameter of 17.2 μm , and an initial susceptibility controllable by the magnetite content in the suspension feeding the sprayer. Our results suggest a possible way to treat *Helicobacter pylori* infections, using an oral drug delivery system, and open prospects to coat magnetic microparticles by spray drying for biomedical applications.

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The development of technologies for oral delivery of drugs has been an area of extensive investigation. This route of administration is preferred because oral drug delivery results in improved patient compliance and comfort compared to the parenteral route of administration [1].

One of the major problems in oral pharmacotherapy is the delivery of drugs to a specific location, and keeping the drug at the target for the desired length of time. In order to reach an acceptable therapeutic level at the desired site, large amounts of drug must be administered, but only a small fraction of the dose will actually reach the intended organ or disease.

Helicobacter pylori are spiral-shaped gram-negative bacteria with polar flagella that live near the surface of the human gastric mucosa. It is the only known organism capable of colonizing the harsh environment of the human stomach, and is the most common chronic bacterial infection. Although asymptomatic in the majority of infected subjects, it is also the cause of significant human disease [2].

Over the past twenty years, a large research effort has been dedicated to find suitable treatment regimens for healing *H. pylori* infection. The difficulty in treating the infection is largely due to its localization on the surface of the gastric mucosa and in the layer of mucus protecting it. The human immune system does not contribute significantly to the healing of the infection, so these

compartments need to be penetrated by anti-*H. pylori* agents at a concentration capable of killing the bacterium [3]. Furthermore, the stability of the drug in the low pH of gastric fluid, and a minimum residence time of the antibiotic in the stomach are required. A number of antibacterial agents, such as amoxicillin and tetracycline, have very low minimum inhibitory concentration (MIC) values against *H. pylori* in culture. However, single antibiotic therapy is not effective in the eradication of *H. pylori* infection in vivo, due to the low concentration of the antibiotic reaching the bacteria under the mucosa and the short residence time of the drug on the site of the infection [4]. A combination of more than one antibiotic and anti-secretory agent has been commonly used for the complete eradication of *H. pylori*.

Two key issues are better stability of the drug in the low pH of the stomach and longer residence time. These factors together will allow a better chance for the antibiotic to penetrate through the gastric mucus layer to act on *H. pylori* [5]. One possible way of achieving both requirements is the magnetic vectorization of antibiotics in polymerized magnetic particles, with low-gastric dissolution rate. Magnetic drug delivery by particulate carriers is a very efficient way of delivering a drug to a localized disease site at the gastrointestinal (GI) tract. The speed of travel through the stomach and intestines can be slowed down at specific positions by an external magnet, thus changing the timing and/or extent of drug absorption in stomach or intestines [6].

The kinetics of particle uptake in GI tract depends on diffusion and accessibility through mucus, initial contact with enterocytes, cellular trafficking and post-translocation events. The smaller the

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particle diameter is, the faster they can diffuse through the GI secretion to reach the colonic enterocytes [7]. Particles in the micrometer size range pass between the epithelial cells into the subepithelial layer. From there they are transmitted both by the lymph vessels and by the mesenteric veins into the circulation [8]. Furthermore, earlier studies described a mechanism of persorption in epithelial cells of the GI tract by which even large particles are taken up into lymphatic and blood circulation and translocate to the liver and other organs. Also, the general opinion is that the smaller the particles, the more reactive and toxic are their effects, since any intrinsic properties of particles will likely be emphasized with the increase in surface area per unit mass [9].

Microparticles are likely to be safer than nanoparticles for local drug delivery in the GI tract, since they probably have lower rate of diffusion as well as a lower reactivity and weaker interaction with the immune system. Regarding biomedical applications, the iron oxides magnetite (Fe_3O_4) and maghemite ($\gamma\text{-Fe}_2\text{O}_3$) are amongst the most studied magnetic particles to date, because of their generally appropriate magnetic properties and biological compatibility [10–13]. These magnetite particles dissolve in acid media [14] and a proper protection against gastric dissolution is an essential step to enable their use for local drug delivery in the GI tract. Furthermore, some drugs are also unstable in low pH [15]. Protecting compounds from gastric environment is a key issue in pharmaceutical technology. A number of different approaches have been proposed so far, including coating with pH-sensitive polymers, time-dependent delivery systems, and the use of biodegradable polymers [16]. The most commonly used pH-responsive polymer to facilitate drug delivery to the ileo-colonic region is the methacrylic acid and methyl methacrylate ester copolymer marketed as Eudragit[®]S100 which is soluble at pH > 7.0 (Rohm Pharma, Darmstadt, Germany).

In this paper, we focus on the development of a stomach-specific formulation of amoxicillin for eradication of *H. pylori* infection. We have prepared drug-containing Eudragit[®]S100 microparticles with a magnetite core. The method of fabrication is a two-step process taking advantage of the well-known technique of coprecipitation of iron salts to prepare iron oxides [17], and the advantages of the spray-drying technique, a single-step process to prepare microcapsules, widely used in the industry [18].

Solutions of ferric chloride and ferrous chloride were prepared as a source of iron by dissolving the respective chemicals in a 0.1 M HCl solution under vigorous stirring using a mechanical stirrer (IKA RW-20, Germany) at 960 rpm at room temperature (25 °C). We used 0.1 M ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) and 0.05 M ferrous chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$) to prepare a homogeneous mixture containing the iron ions. In a typical experimental procedure, 9 ml of the mixture of ferrous and ferric salts was added drop-wise into 450 ml of 1 M NaOH under sonication (Unique USC 1800, 40 kHz, Brazil) and vigorous mechanical stirring at 960 rpm (IKA RW-20, Germany) for 30 min at room temperature (25 °C). The solution color changed from orange to black, leading to a black precipitate. The supernatant was discarded by decantation. Distilled water was then added to wash the precipitates. This procedure was repeated typically three or four times to remove excess ions and salt in the suspension. The washed precipitates were dispersed in distilled water.

The second step consisted in the production of Eudragit[®]S100 polymeric magnetic microparticles with amoxicillin by the spray-drying technique. Spray drying is a one-step process by which a liquid product is atomized in a hot gas current to instantaneously obtain a powder. Most often air is used as the atomizer gas, and more rarely an inert gas such as nitrogen. The initial liquid feeding the sprayer can be a solution, an emulsion or a suspension. Among the advantages of the spray-drying technique is the possibility of modulating the characteristics of the powder by proper selection

of the drier parameters. By changing instrumental parameters (e.g., feed pump, air flow and drying temperature), different results may be obtained in terms of morphology, mean diameters (from micrometer size to millimeter size particles), and percentage of recovery of the microparticles [19]. The spray-drying process to prepare amoxicillin polymeric particles was performed with a Mini Spray dryer (Büchi B191, Germany) using a 0.7 mm diameter nozzle. A precise volume of magnetite microparticles suspension according to the suspension mass content, was measured and mixed to a beforehand dispersed Eudragit[®]S100 in distilled water by mechanical stirring. To this suspension an amoxicillin solution was added and then adjusted to the desired final volume with additional water. Ninety milliliters of the preparation magnetite/Eudragit[®]S100/amoxicillin 2.5:2 (w/w) were fed at 1.2 ml/min (inlet temperature 120 °C) by means of a peristaltic pump and sprayed into the drying chamber of the instrument by means of a flow of compressed air. We prepared two samples starting with suspensions with different amounts of magnetite. The Amoxicillin Mag12% was prepared by feeding the sprayer with a suspension containing 50 mg of magnetite, 100 mg of amoxicillin and 250 mg of Eudragit[®]S100. Amoxicillin Mag22% was prepared with a larger content of magnetite, using a suspension of 100 mg of magnetite, 100 mg of amoxicillin and 250 mg of Eudragit[®]S100. The original mass composition (in grams) of the suspensions to prepare Amoxicillin Mag12% and Amoxicillin Mag22% corresponded to 11% and 22% of magnetite, respectively. The amoxicillin polymeric particles were separated in a cyclone and settled down into a collector. The dried powders were recovered, weighed and stored in a well-closed glass vessel at room temperature.

The quantitative composition of the composite particles was performed by measuring the amoxicillin and magnetite content in a two-stage process. First, ethanol was used to dissolve the polymeric material (Eudragit[®]S100) [20] of known amounts of spray dried microparticles powder, and the magnetite microparticles were separated by centrifugation, at 3500 rpm, for 10 min. The amoxicillin content was determined by the spectrophotometer analysis at a wavelength of 233 nm [21] from the supernatant. The absorbance results were correlated with amoxicillin concentration through a calibration curve previously determined. Second, in order to determine the magnetite content, the precipitate was dissolved in an acid chloride 0.1 N solution [14], stirring during nine days to ensure total dissolution. The indirect determination of magnetite content was performed by quantification of iron, using the complexation with sulfosalicylic acid and measuring the absorbance at wavelength of 420 nm [22]. We found the following compositions: Amoxicillin Mag22% (4% amoxicillin, 87% Eudragit and 9% magnetite) and Amoxicillin Mag12% (3.9% amoxicillin, 92.1% Eudragit and 4% magnetite). We note that the amoxicillin content in both formulations may be higher, due to possible adsorption to the magnetite particles during the first stage of the process, leaving the supernatant poorer in amoxicillin than in the particles.

The samples were sonicated for 10 min to avoid agglomeration of the particles, and placed on glass slides and size measurements of 1500 microparticles of each formulation sample were performed according to the Ferret's diameter principle using an optical microscope (Leitz/Leica BIOMED microscope, Germany) calibrated with a stage micrometer scale [23]. Morphology analysis was conducted by microscopy on a scanning electron microscope (XL 30 ESEM, Philips, The Netherlands).

As shown in Fig. 1, the size of the particles was not uniformly distributed around the median value. Instead, the size distribution of the magnetite particles, as well as the size distribution of the amoxicillin polymeric particle samples, showed a tendency towards a bimodal distribution with a left-sided tail, and larger weight for

small particle diameters. For the magnetite microparticles a mean diameter of $11.8\ \mu\text{m}$ was found. It was also determined that 90%, 50% and 10% of the sample was smaller than 20.4 , 10.0 and $6.0\ \mu\text{m}$, respectively (Fig. 1a). The mean diameter of the polymeric magnetic microparticles with amoxicillin was found to be $17.2\ \mu\text{m}$. It was also determined that 90%, 50% and 10% of the sample was smaller than 27.6 , 16.4 and $9.2\ \mu\text{m}$, respectively (Fig. 1b). The increase in the average particle size distribution, as well as the changes seen in the

Fig. 1. Size distribution of (a) magnetite particles and (b) Amoxicillin Mag22%.

surface of the particles, as seen in the scanning electron microscopy pictures in Fig. 2 indicates that the magnetic particles were successfully coated by Eudragit[®] S100.

Powder X-ray diffraction (XRD) analysis was used to investigate the structural properties of the amoxicillin polymeric particles and the magnetite particles. It is an efficient means of accessing the crystallization of amorphous polymers used in drug formulations [24]. In Fig. 3a, we show a typical result for the magnetite powder samples. It consists of wide peaks, corresponding to a volume of elastic coherence with dimensions around $5\ \text{nm}$. This is a rather intriguing feature of magnetite microparticles produced by coprecipitation in the absence of surfactants or other agglomeration inhibitor agents such as polymers.

As reported previously [14,25], the microparticles consist of a large number of aggregated nanometer size crystallites that form micrometer-sized particles that are superparamagnetic, as appropriate for most biomedical applications. The width of the peaks in the XRD data (see also [25]) revealed that the coherence length in the crystal lattice was of the order of $5.8\ \text{nm}$. Two factors may contribute to the formation of micrometric particles consisting of the assembly of a large number of superparamagnetic nanometric crystallites. No surfactant was used and no peptization was carried out. Therefore, there is a good chance of neutralization of the nanometer-sized crystallites, during the initial stage of the coprecipitation process, allowing their agglomeration into micrometer-sized particles. It may look intriguing that micrometric magnetite particles turn out to be superparamagnetic. However, the boundaries of the nanometric crystallites composing the microparticle are very likely to contain lattice defects that impose a constraint on the propagation of the magnetic order, leading to uncorrelated superparamagnetic crystallites. This has been reported to occur in thin magnetite films [26]. Our samples consist of stable micrometer size particles. We are currently investigating the effect of using external fields of the order of $100\ \text{mT}$, during

Fig. 2. Scanning electron microscopy image (a) and (b) of magnetite particles (c) Amoxicillin Mag12% and (d) Amoxicillin Mag22%.

Fig. 3. X-ray powder diffraction patterns of (a) magnetite particles, (b) Eudragit[®]S100 after and before (in the inset) the spray-drying process, (c) Amoxicillin Mag12% and (d) Amoxicillin Mag22%.

the coprecipitation, in the arrangement of the nanocrystallites within the microparticles. Our preliminary results indicated that the magnetization curves of particles grown in the absence, and in the presence, of external field are reproducible. Also, the initial static susceptibility is larger for samples grown in the presence of the external field [27], indicating that the microparticles consist of stable assembly of a huge number of nanometer-sized crystallites.

We have also found that the large change in magnetite content between Amoxicillin Mag12% and Amoxicillin Mag22% did not affect the structural properties of the amoxicillin polymeric particles. As shown in Fig. 3, there is no significant difference in the XRD patterns of the amoxicillin samples. Both display narrow lines, corresponding to volumes of larger crystalline dimensions, compared with the original magnetite samples.

We notice that the crystallinity of the amoxicillin polymeric particles, as seen in XRD spectra in Fig. 3, may come both from the original amoxicillin structure [28], or the crystallization of the Eudragit[®]S100 polymer in the spray drier process (Fig. 3).

We have found that the magnetic properties of the amoxicillin polymeric particles may be easily tailored by the original composition of the suspension feeding the spray drier. As shown in Fig. 4, Amoxicillin Mag22%, with 22% (in mass) of magnetite in the original suspension, has an initial susceptibility and saturation magnetization values twice as large as those of the Amoxicillin Mag12%, prepared with half magnetite content. We have also found, from magnetization measurements that with the current parameters, spray drying leads to a reduction in the magnetite content of the amoxicillin polymeric particles. As shown in Fig. 4, the saturation magnetization of the amoxicillin polymeric samples is reduced by a factor of two, compared with the original composition of the magnetite/Eudragit[®]S100/amoxicillin suspension used to feed the spray drier. This is due to differences in the physical properties of the components of the suspension, which control the impact of the operating parameters of the spray drier on the final yield [29].

Magnetic force has been used to increase the GI tract residence time of protein drugs [30], acyclovir and cinnarizine [31]. Peristaltic waves, which vary from subject to subject, may be so effective as to

Fig. 4. Magnetization curves of (a) magnetite microparticles, (b) Amoxicillin Mag12% and (c) Amoxicillin Mag22%.

interfere significantly in the efficiency of the magnetic force, even in experiments, based on the use of extracorporeal magnets to control gastrointestinal transit time [31], which have a simpler goal than magnetic targeting.

We note that the successful application of magnetic vectorization to the treatment of *H. pylori* infections depends on a number of factors. In this paper we deal with the starting point, which is the fabrication of polymeric particles, which protects the drug

(such as amoxicillin) from degradation in the gastric pH, and can be directed by an external field to the site of the infection. These particles can be manipulated in formulations such as capsules or tablets in order to enhance the magnetic force. There are other important questions, such as how far deep in the stomach can an external magnetic field reach, and drive the drug to the site of action. This is a rather difficult question to answer and a practical clinical device should focus in the region near the infected site (a few centimeters from the abdomen surface).

In summary, we have prepared polymeric amoxicillin microparticles, with a mean diameter of 17.2 μm , and a magnetic core of magnetite superparamagnetic particles. We have shown that the spray-drying process allows easy control of the initial susceptibility and saturation magnetization of the amoxicillin microparticles. The increase by a factor of about two in the magnetite content of the formulations (from Amoxicillin Mag12% to Amoxicillin Mag22%), leads to a corresponding change in the initial susceptibility of the amoxicillin particles. One might think of increasing the initial susceptibility by preparing formulations with larger magnetite content. This, however, would lead to decreasing the drug content of the particles. We are currently investigating other amoxicillin formulations by changing the parameters used in the coprecipitation process to produce the magnetite microparticles, as well as by adjusting the composition of the feed and parameters of spray drier, such as the gas flow rate, pressure and temperature. To our best knowledge, the present work is the first attempt to prepare a magnetic system for local delivery of antibiotics, in particular for the treatment of *H. pylori* infections.

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