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RESEARCH ARTICLE

In vitro dissolution of pH sensitive microparticles for colon-specific drug delivery

Anna Angela Barba[1], Annalisa Dalmoro[1,2], Matteo d'Amore[1], and Gaetano Lamberti[2]

[1]*Dipartimento di Scienze Farmaceutiche e Biomediche and* [2]*Dipartimento di Ingegneria Industriale, Università degli Studi di Salerno, Fisciano (SA), Italy*

Abstract

Objective: The objective of this work is to prepare oral dosage systems based on enteric materials in order to verify their possible use as Colon-Specific Drug Delivery Systems (CSDDSs).

Methodology: In particular, three different copolymers of methyl-methacrylate (MMA) - acrylic acid (AA) are synthesized with increasing percentage of MMA (from 70% to 73%) and they are used to produce microparticles by the double-emulsion solvent evaporation method. The microparticles, loaded using theophylline as model drug, are then tested for drug release under varying pH to reproduce what happens in the human GI tract.

Results: All the investigated systems have shown an effective pH sensitiveness: they show a good gastro-resistance, releasing the model drug only at higher pH, small intestine or colon, depending on the kind of used copolymer.

Conclusion: The results confirm the usefulness of both the materials and the methods proposed in this study for colon-specific delivery applications.

Keywords: Colon delivery, enteric materials, double-emulsion solvent evaporation method

Introduction

Targeted delivery of drugs is a very important issue in current studies in pharmaceutical sciences. In particular, targeted delivery to colon is of great interest for two main reasons: 1) it is a useful approach in the treatment of colon local diseases (inflammatory bowel diseases (IBDs) including Crohn's disease and ulcerative colitis, as well as colon cancer); 2) it could be an efficient way to deliver drugs such as proteins and peptides, which are degradable in the stomach and in the small intestine, and which could be well absorbed in the colon region, thus maximizing their overall systemic absorption.[1–5]

An ideal colon-specific drug delivery system (CSDDS) should minimize the drug release/degradation during its transit through the GI tract, in the stomach and in the small intestine and, then, it should produce a fast release into the proximal colon.[1,6] In some cases, release in the colon could be required to be an extended type, because of the large transit time in the colon itself. However, along the GI tract physiological changes are practically continuous, consisting in an increase of pH (from acid values in the stomach, roughly 1–2, to neutral values in the small intestine, roughly 6–7, to higher values in the colon, roughly 7–7.5), a decrease in fluid content and in the mobility, an increase in fluid viscosity, and an increase of concentration of bacterial flora, together with a change in its composition, and of the related enzymatic kind and concentrations.[7] Enzymes, in particular, change from digestive to hydrolytic and reductive,[6] allowing the degradation of non-starch polysaccharides, which pass undisturbed through the small intestine.[1] The most common approaches to design and to produce colon-specific drug delivery systems (CSDDS) are based on the change in pH, and in bacterial flora/enzymatic activity, on time-dependent systems and on pressure-activated systems. Furthermore, CSDDSs have been proposed based on combination of some of these approaches.

The CSDDSs based on pH change are the most common, firstly proposed using Eudragit-S to coat 5-amino salycilic acid (5-ASA) or steroids (drugs with high anti-inflammatory

Address for Correspondence: Annalisa Dalmoro, Dipartimento di Scienze Farmaceutiche e Biomediche, Università degli Studi di Salerno, via Ponte Don Melillo, Fisciano, 84084 Italy. Tel: +39089968291. Fax +39089969602. E-mail: adalmoro@unisa.it

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activity).[8] These CSDDSs are formulated using synthetic polymers (mainly Eudragit)[3,9,10] or natural polymers (chitosan, pectin),[4,11–14] and their combinations.[6,7,15] These systems are based on conventional technologies such as enteric-coated tablets[7,11] or capsules.[6,10] However, due to the variable transit time through the colon, the treatment based on these systems could be ineffective because of a delayed drug release (slow disaggregation/dissolution). A size reduction of the drug carrier could be of aid to overcome this problem. Thus a number of systems were proposed, based on micro or nanoparticles.[3,4,9,12,15] The main disadvantage of these latter is that the inter-subject pH variation can make unpredictable the site-specificity of the drug release and, furthermore, since the pH in the small intestine and in the colon are not very different, the dissolution behavior of the polymers used as excipients could not be sensitive to small pH differences. However, advantages of multiparticulate formulations are to be pointed out: less influence by food, more consistent absorption compared to single unit systems, possibility to insert them in capsules in order to obtain a more controlled release.[16]

The bacterial/enzymatic activated CSDDSs have attracted great attention, starting from the pioneering works on azo-polymers.[17] The reason for this interest is that the microflora changes abruptly in both quantity (from 10^2 CFU/mL (Colony Forming Units) to 10^{11}–10^{12} CFU/mL passing from terminal ileum to ascending colon) and quality, since two class of enzymes (azoreductases and polysaccharidases) are present in an adequate quantity only in the colon.[1,2,4,7] Drawbacks of this approach are: (i) use of some of the proposed polymers is limited by concerns about their safety[2,4]; (ii) flora of bacteria is individually variable[2]; (iii) enzymatic degradation is a slow process, sometimes requiring more than 12 h to be completed.[1]

The time-dependent CSDDSs are designed to release the drug after a given time interval after swallowing. Most of them are based on core/coating(s) systems,[18] being the coating able to tune the time interval needed to start the drug release, either due to the different blends of polymers used as in the Pulsincap™ System,[19] or to the different coating thicknesses as in the Time Clock® System,[20] or to the swelling of an intermediate coating that can cause the rupture of an external insoluble coating.[21] The main drawback of these systems is that, while the transit time in small intestine is practically inter-individually invariable, the retention time in the stomach is highly inter-individually variable with a consequent spreading in the initial release sites in the distal GI tract.

The pressure-activated CSDDSs are based on physiological effects. According to them, the reduced water content brings to pressure higher in the colon than in the small intestine, inducing intestinal wall movements (peristalsis). The increase in pressure could cause the system rupture, and then the drug release, such as in PCDCs (Pressure-Controlled Colon Delivery Capsules), obtained by coating tablets, made of drug and polymer (PEG), a mixture which liquefies at body temperature, by a water insoluble polymer, ethyl cellulose.[22] Even if some preliminary confirmation of their validity have been reported,[23,24] these systems are still scarcely characterized *in-vivo*.

Many CSDDSs were developed, basing their behavior on a combination of the previously seen mechanisms of action. For example, a three layer system (an inner core with a swellable agent, an intermediate elastic semi-permeable membrane with a defined elongation limit and an outer enteric coating) has been proposed by, Shah et al.,[2] being based on the enteric and on the time-dependent mechanisms of action. Another multilayer system, COlon-targeted DElivery System (CODES™), was proposed by Watanabe et al.[25] and tested by Katsuma et al.[5] CODES™ is based on enteric and on bacterial-activated mechanisms of action. Even if, in principle, layered systems seem to overcome all the previously mentioned drawbacks, it is worth to note that their manufacture is hard to be precisely carried out on a large process scale, and their performance is very closely related to the precision of the realization process (i.e. the required thickness of the layer has to be strictly respected).

Last but not least, there is the need for standardized protocols to test the CSDDSs in a precise and repetitive manner, since the Pharmacopeias do not codify for such tests.[1] For example, the USP[26] suggests two methods to test the enteric systems (<Chap. 724> methods A and B for testing of delayed-release (enteric-coated) dosage forms), being both methods designed to reproduce what happens to the pH in the stomach (Acid stage, pH 1.0 for 2 h) and in the small-intestine (Buffer stage, pH 6.8 until the end of the test), neglecting what happens through the colon.

On the basis of the literature analysis and of the reviews of commercial products performed above, it can be concluded that the last word on the colonic delivery is far from being uttered. However, among the presented systems, the advantage for the enteric ones is in terms of cost and ease of manufacturing.[16] Moreover the pH increase is a more repetitive feature along the GI tract (against the transit time, nature of the bacterial flora and pressure waves). A system (intending both the constitutive material and the preparative method) which would be able to carefully tune the drug release in response to a change in pH is thus highly desirable. Furthermore, a properly defined, simple and inexpensive testing method is highly desirable.

Aims of this work are to point out a suitable preparation method and to test some materials candidate to produce effective CSDDS, also defining an effective testing protocol.

Materials and methods

Materials

Methyl-methacrylate (MMA) and Acrylic acid (AA), Theophylline (TP, CAS Number: 58-55-9), Dichloromethane (DCM, CAS Number: 75-09-2),

Ethanol (ET, CAS Number: 64-17-5), Isopropanol (IS, CAS Number: 67-63-0), Span 80 (CAS Number: 1338-43-8), Polyvinyl alcohol (PVA, CAS Number: 9002-89-5), Hydrochloric acid (CAS Number: 7647-01-0), and Sodium phosphate tribasic dodecahydrate (CAS Number: 10101-89-0) were all supplied by Sigma Aldrich (Milan, Italy). HydroxyPropyl-MethylCellulose (HPMC, Methocel K15M Premium Grade, was kindly supplied by Colorcon).

The enteric polymers used for microparticles preparation were synthesized, as described in a previous work,[27] using different percentages of the two starting monomers: MMA and AA. Co-polymers used in this work were poly(MMA-AA) with 70%, 72%, or 73% (v/v) of MMA. Theophylline was selected as model drug. It is well-known that theophylline is not used for colon delivery, however it is a little (low molecular weight), water soluble, and easily detectable molecule. Therefore, theophylline is a good model molecule to get some idea about the effectiveness of proposed materials and methods to obtain specific drug delivery systems (the attention is focused now on the ability of enteric polymers to tune the release of a model molecule, theophylline, that in future will be replaced by a colon specific drug). DCM, ET and IS were used as solvents, Span 80 was used as surfactant, PVA was used as emulsion stabilizer, HPMC was used as stabilizer of the internal aqueous phase. Deionized water was used for all the experiments. Solutions at different pH, simulating the gastrointestinal conditions, were obtained using hydrochloric acid, sodium phosphate, and deionized water.

Methods

Microparticles preparation

The double emulsion ($W_1/O/W_2$) solvent evaporation method to produce microparticles was previously described.[28] Briefly, the internal aqueous phase (W_1) was obtained by mixing at room temperature 200 mg of TP in 40 mL of deionized water and then adding 400 mg of HPMC. The organic phase (O) was composed of 200 mg of poly(MMA-AA) solubilized by a mixture of DCM/IS/ET (20 mL/4 mL/3 mL). A drop of Span 80 was mixed with the oil phase, as a surfactant. The external water phase (W_2) was 100 mL of water with 1% (w/v) of PVA. The oil phase, 5 mL, was emulsified at room temperature with the internal aqueous phase, 1 mL, using an ultrasonic mixing in order to obtain the emulsion W_1/O. The primary emulsion (6 mL) was poured in 100 mL of W_2 and the resulting $W_1/O/W_2$ emulsion was mixed with a magnetic stirrer for about 1 h. $W_1/O/W_2$ was placed in a Rotavapor (Laborota 4002 control, Heidolph), under vacuum, with a temperature increase from 20°C to 50°C at a speed of 18 rpm, to allow solvent evaporation. The suspended microparticles were washed thrice with distilled water by centrifugation at 6000 rpm for 5 min; then they were analysed by optical microscope (Leica DM LP). The final product was obtained by evaporation of residual water in an oven at 40°C. The microparticles were collected, weighed and stored at room temperature.

Theophylline assay

Three different media, at pH 1.0, pH 6.8 and pH 7.4, were prepared. The acid solution (medium at pH 1.0) was 0.1 N hydrochloric acid; the buffer solutions were obtained adding to the acid solution a given volume of 0.2 M tribasic sodium phosphate. Then, further adding of some 0.2 M tribasic sodium phosphate solution caused the pH to increase up to 7.4.

Afterwards, solutions with different concentrations of TP (namely 1, 2, 3, 5, 10, 15, 20 mg/L), were prepared for the three different media, with the aim of analytical method tuning. Measurements were made by UV-visible spectrometer (Lambda 25 by Perkin Elmer) collecting the full absorption spectra in a wavelength range from 200 to 400 nm, and obtaining the height of the peak closest to 273 nm, to avoid incorrect measurements due to shift in λ_{max}. The procedure of spectra fitting instead of the simple reading of the absorbance at a given wavelength has been proved to be much more effective and – in principle – to be used to eliminate the interferences due to polymers or other substances, in a previous work.[29] The relationship between concentration and absorbance follow the well-known Lambert-Beer law, with unity correlation coefficients very close to one (>0.999) for each medium. This implies that the analytical medium is very precise, sensitive and accurate. In particular, A = (0.056)C at pH 1.0; A = (0.064)C at pH 6.8 and A = (0.058)C at pH 7.4, the concentration being expressed in mg/L. Using these relationship, during the release tests the TP concentration was easily measured analyzing samples of 3 mL, taken at predetermined times.

Image analysis

Size control of particles was performed by image analysis (software Image J 1.40g) on optical microscope pictures of an acid suspension of the microparticles. For each suspension, several snapshots were taken, in order to get a significant number of particles (no less than one hundred). Then, their size was measured by Image J (Feret's diameters) and then the average values and the standard deviations were calculated. These values are reported in Table 1.

Three steps dissolution test

The dissolution model was chosen in order to be enough similar to existing dissolution system USP 2. *In vitro* drug release studies were performed putting a known amount of microparticles (10–20 mg) in 75 mL of 0.1 N hydrochloric acid (pH 1) at 37°C, stirred at 140 rpm using a magnetic anchor/rotor (slightly higher than common used speeds of 100 rpm, however more disadvantageous conditions than common ones), following a pH progression method i.e. pH 1.0 for first 2 h, pH 6.8 for next 3 h (obtained adding 25 mL of a 0.2 M tribasic sodium phosphate solution) and pH 7.4 (obtained adding further 5 mL of a 0.2 M tribasic sodium phosphate solution) for the remaining of the experiment. Samples of 3 mL were

taken at predetermined times and assayed for TP release by UV-visible spectrometer (Lambda 25 by Perkin Elmer), as described in previous section. The samples were then put back in the dissolution vessel in order to avoid a wrong measurement. In fact, especially in medium at pH 1, where the microparticles are undissolved and a more heterogeneous system is present, the 3 mL sample could not contain a microparticles concentration just corresponding to 1/25 (3 mL of 75 mL) of dissolution medium: replacing it with fresh medium causes a loss of an unknown amount of microparticles and consequently of drug, which alters the actual release. The release percentage was expressed in terms of released drug over the amount of microparticles inserted in the dissolution vessel. All the experiments were performed in triplicate.

In principle, the measurements in presence of pH gradients could be effectively carried out in USP apparatus 3, the "Bio-Dis" apparatus; or in different fluid-dynamic conditions, using the USP 4, the "flow-through" cell apparatus. However, these apparatuses are uncommon in research laboratories and, furthermore, they usually works better with higher amount of powders than 20 mg (since the dissolution medium volume involved is of the order of 1 L, then the drug concentrations are very low). Therefore, a simple and effective protocol, based on apparatuses commonly available in all the laboratories, and useful in testing small amount of pharmaceuticals (since the preparation protocols usually produce little quantities), is highly desirable.

Results and discussion

Definition of the *in-vitro* testing conditions

Studies to evaluate the performance of enteric pharmaceutical systems, prepared to be orally administered, require the definition of a protocol to reproduce *in-vitro*, as close as possible, what will happen after the administration *in-vivo*.[30] The United States Pharmacopea test for enteric systems[26] requires 2 h in a pH 1.0 solution (by HCl 0.1 M), followed by another time interval, defined differently for each system to be tested, during which this latter is soaked in a pH 6.8 solution. By this way, the pH values usually encountered in stomach and in the small intestine are reproduced with a good accuracy, even if there is no recommendation for the time interval in the "small intestine" compartment. On the other hand, physiological studies confirmed[31] that the transit time in the small intestine in humans is practically constant on the value of 3 h. Therefore, the protocol to test systems devoted to the colonic delivery should account for the transit in the stomach (pH 1.0, retention time about 2 h, actually it is a strong function of the nature and of the quantity of the food assumed), then in the small intestine (pH 6.8, retention time close to 3 h), finally in the large intestine (in its entrance region, the ileo-cecal region, the pH raises to 7.4, in the distal colon the pH could decrease up to 5.5, and then it could raise again to 7). The transit time is very variable: trials reported values from less than 1 h up to 20 h.[32] Since no protocol has been established, different research groups define their own protocols, testing the systems by soaking them in parallel, in different pH media[12,15] (1.0 or 1.2, 6.8, 7.4 mimicking an unrealistic situation in which the system was directly administered in the different GI tracts); or by soaking them in two subsequent media with different pH[6,11] (the first medium being strongly acidic 1.0 or 1.2, to mimic the stomach; and the second medium simulating the intestine, according to the USP recommendation, but also using media with pH higher than the 6.8 value suggested by USP, to better simulate the large intestine environment); or soaking them in three subsequent media with different pH values. This is the most useful approach: Makhlof et al.[9] proposed the gradient pH-h (1.2–2, 6.8–2, 7.4–20) and Yehia et al.[7] proposed (1.0–2, 6.8–3, 7.4–19). This last mimics the real physiological conditions in the best way (since the real transit time in the small intestine is 3 h).[31] Further refinements, i.e. the use of a four-level pH gradient, as suggested by Han et al.[6] (1.2–2, 6.8–1, 7.4–2, 6.8–13) and Schellekens et al.[10] (1.2–2, 6.8–2, 7.5–0.5, 6.0–1.5) are unnecessary and could be misleading, since the real physiology is not defined and reproducible to this level. On the other hands, further improvements of the testing methods could be obtained by adding to the dissolution media a properly designed blend of enzymes. In the present work there is no need of such adding, since the polymers used are not polysaccharides, whose degradation rate is influenced by the colonic enzymes, but acrylic polymers, whose dissolution is governed only by the pH level. In conclusion, for the purpose of the present study, the testing protocol was defined as reported in the "Materials and methods" section, i.e. the pH-time gradient was 1.0–2, 6.8–3, 7.4–19 (the same used by Yehia et al.[7]).On the basis of selected protocol, theophylline assays were carried out at pH 1.0, pH 6.8 and pH 7.4 for analytical method tuning, as described in the "Theophylline assay" section.

Release tests

Three different copolymers were used in the frame of this work to assess their ability to be used as CSDDS: the one synthesized using a blend with 70% MMA monomer and 30% AA monomer, the one with 72% of MMA, the one with 73% of MMA in the monomers blend. According to Barba et al.[27] the polymer based on 70% of MMA shows a significant swelling at pH of 6.5 and dissolves at pH of 6.8 (Figure 5 in the reference 27). The two novel copolymers are expected to swell and dissolve at higher pH. After the synthesis, they swell at pH 7.0–7.4 (the 72% and the 73%, respectively) and dissolve at pH 7.2–7.5 (respectively)). Therefore, the microparticles prepared using the 72% MMA polymer could be useful for enteric delivery and the 73% MMA is the best choice for colonic delivery.

Microparticles loaded with TP were prepared using the three copolymers. A fraction of the powders for each sample was totally dissolved to assay the amount of drug loaded (and thus the loading ratio or encapsulation

efficiency). The theoretical loading ratio was 9.61% w/w (0.0961 mg TP/(mg TP + mg HPMC + mg acrylic polymer)). The actual drug loadings were reported in Table 1. They range between 1.6% and 2.3% w/w, which correspond to encapsulation efficiencies or loading ratios (with respect to the theoretical value) ranging between 17% and 24%, in agreement with the results previously reported by Dalmoro et al.[28] and similar to the drug loading reported for similar systems in literature. Indeed Lorenzo-Lamosa et al.[4] report, for sodium diclofenac microencapsulated with chitosan in Eudragit L and S, an average drug loading of 5% w/w, and Oosegi et al.[15] report, for succinyl-prednisolone conjugate with chitosan microencapsulated with Eudragit L and S, an average drug loading of 3%. On the other hand, more consolidated preparation techniques such as the compression coating can lead to even lower drug content. For example, using budesonide and lactose (3 mg + 57 mg) to produce the core and then coating it by different coating mixtures (400 mg), tablets are obtained with drug content up to 0.6% w/w (mg budesonide/(mg budesonide + mg lactose + mg coating mixtures)).[7] However low encapsulation efficiencies could be attributed to the oil phase used for particles preparation (phase O in $W_1/O/W_2$), which is partially soluble in water owing to the presence of ethanol and isopropyl alcohol. Therefore, the water soluble theophylline can easily diffuse from internal phase W_1 to the external environment during preparation, causing a decrease in drug content in final product.

Figure 1 reports the release profiles, in terms of the ratio between drug released and drug loading, with time, for the three systems investigated, i.e. the microparticles obtained using the 70% MMA copolymer (graph a), the microparticles obtained using the 72% MMA copolymer (graph b), and the microparticles obtained using the 73% MMA copolymer (graph c). In Figure 2, it is reported a microphotograph of an acid suspension of the microparticles, prior of the dissolution test, which confirms that the microparticles produced are spherical in shape. Crushed microparticles (extraneous matter visible in the pictures) can be present in low amount due to the harvesting method. Optical microscope pictures were subjected to image analysis in order to measure particles size. The medium size diameter for the three kinds of microparticles ranges from 60 to 80 μm (the larger of them having diameters of some hundreds of micrometers), as shown in Table 1. As expected, no effect of the polymer nature (70, 72 or 73% of MMA) on the particle size has been observed (Figure 2).

All the microparticles prepared, despite the copolymer used, release low amount of drug during the acid stage (the first two hours at pH 1.0, mimicking the stomach). The microparticles remains practically undisturbed at low pH, since they do not swell neither they do dissolve. The small amount of released drug could arise from the molecules still attached to the microparticles surface, which were not leached during the microparticles washing.

Table 1. Particles size, drug loading and loading ratio (encapsulation efficiency), with relative standard deviations (on three experiments), obtained in the production of microparticles based on different enteric polymers (theoretical drug loading ratio 9.61%).

Run	%MMA	Particles size, μm	Drug loading %	Loading ratio %
# 1	70	76 ± 46	2.21 ± 0.27	23.0 ± 2.8
# 2	72	61 ± 32	1.62 ± 0.01	16.8 ± 0.1
# 3	73	72 ± 31	2.30 ± 0.91	23.9 ± 9.5

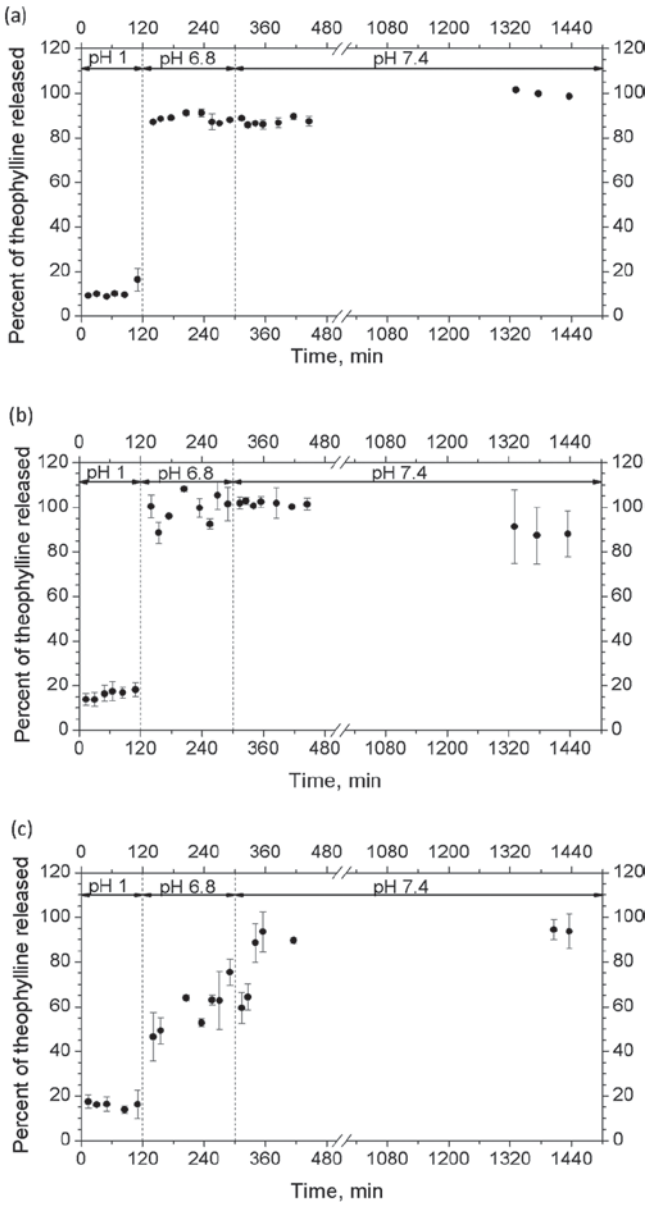

Figure 1. Release evolution with time from microparticles based on (a) poly(MMA-AA) with 70% MMA; (b) poly(MMA-AA) with 72% MMA; (c) poly(MMA-AA) with 73% MMA.

The microparticles obtained using the copolymer based on 70% MMA monomer blend dissolved very fast once the pH was raised to 6.8, i.e. during the simulation of their pass from the stomach to the small intestine. The dissolution, which was confirmed by visual observation (the dissolution volume no longer contains a

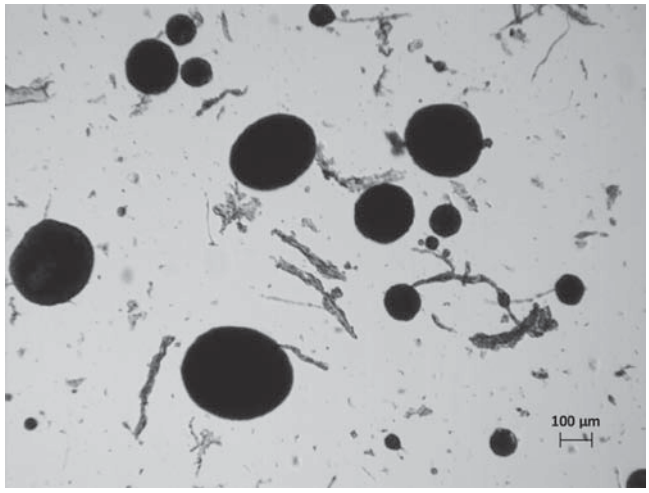

Figure 2. Optical microscopy image of the particle suspension (example, poly(MMA-AA) with 72% MMA).

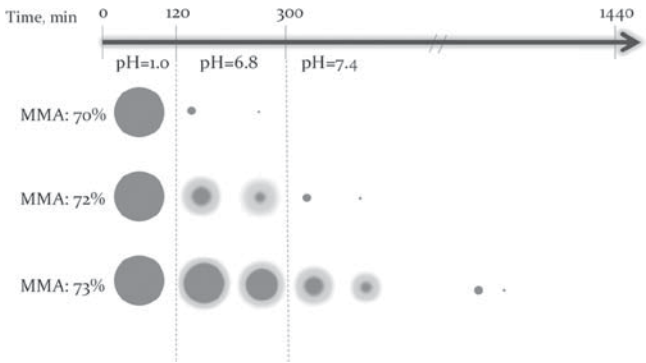

Figure 3. Mechanisms involved in drug release from microparticles based on different MMA-AA copolymers, subjected to pH medium change.

suspension but it is clear), leads to the immediate release of the drug content, which is evident from graph (a) in Figure 1. Three hours later, when the pH was further raised to 7.4 to mimic the pass from the small intestine to the large intestine, there were no other phenomena observed. The microparticles were already dissolved, and the drug level in the vessel does not change. This means that the full drug content would be already released in the small intestine, and thus a pharmaceutical system based on 70% copolymer is suitable for the drug release in the proximal tract of GI.

The microparticles based on the 72% copolymer swell very fast once the pH was raised to 6.8 (the formation of a gel layer around the central core of particles was observed), and they also start to dissolve. This leads to a fast release of the drug content, as graph (b) in Figure 1 confirms. Once the pH is raised to 7.4 (three hours later), the dissolution process is rapidly completed, but no further increase in drug release is observed. Therefore, even if the microparticles are not completely dissolved in the small intestine, systems based on this copolymer should not be useful for the purpose of colonic delivery of theophylline. It is worth to note that the drug used for this study, the theophylline, is a small molecule (MM 180.16 g/mol, Stokes radius 0.37 nm), therefore once the polymer of the matrix swells, the theophylline easily diffuses through the gel network and then it is released. On the other hand, a drug with similar solubility, but with larger molecular mass and Stokes radius in principle would require large time to diffuse through the swelled gel and then to be released. It means that systems based on 72% copolymers could be useful for the controlled release of larger molecules.

The microparticles based on 73% MMA show a limited swelling once subjected to the raise in pH to 6.8 (from visual observation, making a comparison with 72% system), and they rapidly release a certain amount of drug (roughly, 50% of the drug loading). Once the pH is raised to 7.4, they undergo a further swelling and then start to dissolve, releasing the remaining about 30% of the drug loading (a little more than 10% being released in the acidic stage). The depicted behaviour is reported in graph (c) in Figure 1. Therefore, the system based on this polymer could be effectively used in the drug targeting in the colonic region. Once more, a larger molecule will be released slowly in the small intestine region (where the polymer starts to swells, allowing the fast release of the small molecule of theophylline).

The levels (and the trends) of the drug released have been measured for each time and they were expressed as average values (the error bars being the standard deviations of the measurements). Since the observed behaviors (step-shaped for 70% and 72% MMA; gradually increasing for 73% MMA) falls outside of the error bars, they were clearly due to difference in release mechanisms and not to potential measurements errors.

It is worth to note, in conclusion, that the microparticles proposed in this work, based on the 73% MMA copolymer, are a system easy to be prepared (no coating step is required), effective in the controlled release, potentially useful for release targeted to the colon region, eventually for drugs of large molecular size.

Mechanisms involved

In Figure 3 are schematically reported the experimentally observed behaviours of the three kind of microparticles during their dissolution tests. It is a summary of the observations already mentioned during the discussion of the release tests:

1. For the 70% MMA copolymer based microparticles (first row in Figure 3), the first pH step (from 1.0 to 6.8) causes the microparticles to rapidly dissolve, as confirmed by visual observation, and then the drug can be released.
2. For the 72% MMA copolymer based microparticles (second row in Figure 3), the first pH step (from 1.0. to 6.8) causes significant swelling and limited dissolution of the microparticles. This phenomena is clearly evident by microparticle size increasing, keeping essentially their shape (not shown photographs). The

high swelling causes a large drug release, by diffusion through the gel network (larger molecules are expected to be released with a slower kinetics). The second pH step (from 6.8 to 7.4) causes a fast dissolution of the microparticles, without any further drug release (since it has been already released in the 6.8 pH phase).

3. For the 73% MMA copolymer based microparticles (third row in Figure 3), the first pH step (from 1.0 to 6.8) causes a limited swelling and very limited dissolution (supported by observation about a reduced increase in microparticles size compared to the previous sample, based on 72% copolymer), consistent with a certain release of drug. The second pH step (from 6.8 to 7.4) causes a larger swelling and a slow dissolution, bringing to the release of the remaining drug.

The tests carried out confirmed the usefulness of materials and methods proposed in this work, and also allowed some interpretations of the phenomena which take place (swelling and dissolution). These could be rules to be used in the design of novel pharmaceutical systems for the drug release in the colonic region.

Conclusions

In this work microparticles containing theophylline were obtained by a W/O/W solvent evaporation method, using several copolymers of methyl-methacrylate (MMA) and acrylic acid (AA), with increasing percentage of MMA in the starting monomers mixture (from 70% to 73%). The microparticles, tested by exposure to environments with pH mimicking the values encountered in the human gastro-intestinal tract, produced different pattern of release. In particular, high content of MMA in the copolymer synthesis mixture causes the release to be directed in the colonic region (where the pH is higher).

The study demonstrated thus the usefulness of the materials (poly(MMA-AA) with different MMA/AA ratio) and of the method (W/O/W solvent evaporation) to produce microparticles able to release a drug in a localized site along the GI tract. Therefore these tools (materials and methods) could be useful for the targeted release (for example in the colonic environment) in the oral drug delivery.

Declaration of interest

The authors report no conflicts of interest.

References

1. Yang L, Chu JS, Fix JA. Colon-specific drug delivery: new approaches and *in vitro*/*in vivo* evaluation. Int J Pharm 2002;235:1–15.
2. Shah NH, Railkar AM, Phuapradit W, inventors; Google Patents, assignee. Colon targeted delivery system. U.S. patent 6,039,975. 2000.
3. Lamprecht A, Yamamoto H, Takeuchi H, Kawashima Y. Microsphere design for the colonic delivery of 5-fluorouracil. J Control Release 2003;90:313–322.
4. Lorenzo-Lamosa ML, Remuñán-López C, Vila-Jato JL, Alonso MJ. Design of microencapsulated chitosan microspheres for colonic drug delivery. J Control Release 1998;52:109–118.
5. Katsuma M, Watanabe S, Takemura S, Sako K, Sawada T, Masuda Y et al. Scintigraphic evaluation of a novel colon-targeted delivery system (CODES) in healthy volunteers. J Pharm Sci 2004;93:1287–1299.
6. Han M, Fang QL, Zhan HW, Luo T, Liang WQ, Gao JQ. *In vitro* and *in vivo* evaluation of a novel capsule for colon-specific drug delivery. J Pharm Sci 2009;98:2626–2635.
7. Yehia SA, Elshafeey AH, Sayed I, Shehata AH. Optimization of budesonide compression-coated tablets for colonic delivery. AAPS PharmSciTech 2009;10:147–157.
8. Dew MJ, Hughes PJ, Lee MG, Evans BK, Rhodes J. An oral preparation to release drugs in the human colon. Br J Clin Pharmacol 1982;14:405–408.
9. Makhlof A, Tozuka Y, Takeuchi H. pH-Sensitive nanospheres for colon-specific drug delivery in experimentally induced colitis rat model. Eur J Pharm Biopharm 2009;72:1–8.
10. Schellekens RC, Stellaard F, Mitrovic D, Stuurman FE, Kosterink JG, Frijlink HW. Pulsatile drug delivery to ileo-colonic segments by structured incorporation of disintegrants in pH-responsive polymer coatings. J Control Release 2008;132:91–98.
11. Fernandez-Hervas M, Fell J. Pectin/chitosan mixtures as coatings for colon-specific drug delivery: an in vitro evaluation. Int J Pharm 1998;169:115–119.
12. Bigucci F, Luppi B, Cerchiara T, Sorrenti M, Bettinetti G, Rodriguez L et al. Chitosan/pectin polyelectrolyte complexes: selection of suitable preparative conditions for colon-specific delivery of vancomycin. Eur J Pharm Sci 2008;35:435–441.
13. Dalmoro A, Barba AA, Lamberti G, d'Amore M. Intensifying the microencapsulation process: ultrasonic atomization as an innovative approach. Eur J Pharm Biopharm 2012;80:471–477.
14. Dalmoro A, Barba AA, Lamberti G, Grassi M, d'Amore M. Pharmaceutical applications of biocompatible polymer blends containing sodium alginate. Adv Poly Tech 2012;31:219–230.
15. Oosegi T, Onishi H, Machida Y. Novel preparation of enteric-coated chitosan-prednisolone conjugate microspheres and *in vitro* evaluation of their potential as a colonic delivery system. Eur J Pharm Biopharm 2008;68:260–266.
16. Singh BN. Modified-release solid formulations for colonic delivery. Recent Pat Drug Deliv Formul 2007;1:53–63.
17. Saffran M, Kumar GS, Savariar C, Burnham JC, Williams F, Neckers DC. A new approach to the oral administration of insulin and other peptide drugs. Science 1986;233:1081–1084.
18. Ueda Y, Hata T, Yamaguchi H, Ueda S, Kodani M, inventors; Google Patents, assignee. Time-controlled explosion systems and processes for preparing the same. U.S. patent 4,871,549. 1989.
19. Hebden JM, Gilchrist PJ, Perkins AC, Wilson CG, Spiller RC. Stool water content and colonic drug absorption: contrasting effects of lactulose and codeine. Pharm Res 1999;16:1254–1259.
20. Steed K, Hooper G, Monti N, Strolin Benedetti M, Fornasini G, Wilding I. The use of pharmacoscintigraphy to focus the development strategy for a novel 5-ASA colon targeting system. J Control Release 1997;49:115–122.
21. Bussemer T, Dashevsky A, Bodmeier R. A pulsatile drug delivery system based on rupturable coated hard gelatin capsules. J Control Release 2003;93:331–339.
22. Hu Z, Kimura G, Mawatari S, Shimokawa T, Yoshikawa Y, Takada K. New preparation method of intestinal pressure-controlled colon delivery capsules by coating machine and evaluation in beagle dogs. J Control Release 1998;56:293–302.
23. Hu Z, Mawatari S, Shibata N, Takada K, Yoshikawa H, Arakawa A et al. Application of a biomagnetic measurement system (BMS) to the evaluation of gastrointestinal transit of intestinal pressure-controlled colon delivery capsules (PCDCs) in human subjects. Pharm Res 2000;17:160–167.
24. Hu Z, Mawatari S, Shimokawa T, Kimura G, Yoshikawa Y, Shibata N et al. Colon delivery efficiencies of intestinal pressure-controlled colon delivery capsules prepared by a coating machine in human subjects. J Pharm Pharmacol 2000;52:1187–1193.

25. Watanabe S, Kawai H, Katsuma M, Fukui M, inventors; Google Patents, assignee. Colon-specific drug release system. U.S. patent 6,368,629. 2002.
26. Cascone S, De Santis F, Lamberti G, Titomanlio G. The influence of dissolution conditions on the drug ADME phenomena. Eur J Pharm Biopharm 2011;79:382–391.
27. Barba A, Dalmoro A, Santis F, Lamberti G. Synthesis and characterization of P(MMA-AA) copolymers for targeted oral drug delivery. Polym Bull 2009;62:679–688.
28. Dalmoro A, Lamberti G, Titomanlio G, Barba AA, d'Amore M. Enteric Micro-Particles for Targeted Oral Drug Delivery. AAPS Pharm Sci Tech 2010;11:1–8.
29. Barba AA, Chirico S, Dalmoro A, Lamberti G. Simultaneous measurement of theophylline and cellulose acetate phthalate in phosphate buffer by UV analysis. Can J Anal Sci Spect 2008;53:249–253.
30. Grassi M, Lamberti G, Cascone S, Grassi G. Mathematical modeling of simultaneous drug release and *in vivo* absorption. Int J Pharm 2011;418:130–141.
31. Davis SS, Hardy JG, Fara JW. Transit of pharmaceutical dosage forms through the small intestine. Gut 1986;27:886–892.
32. Vandamme TF, Lenourry A, Charrueau C, Chaumeil J. The use of polysaccharides to target drugs to the colon. Carbohydr Polymer 2002;48:219–231.