**In vitro Evaluation of a Multiple-Unit Formulation for Oral Colonic Release of Insulin**

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**SUMMARY**

A multiple-unit formulation for time-dependent colonic release of insulin was prepared and evaluated. Minitablet cores containing the protein drug along with a permeation enhancer, sodium glycoalcholate, in a 1:10 ratio were in turn spray-coated with i) Methocel®E50, ii) blended Eudragit®NE / Explotab®V17 (5:1 as solids) and iii) Aqoat®AS, in order to obtain, respectively, a swellable/erodible layer, a flexible and moderately permeable coat able to slow down water penetration into the underlying functional layer and an enteric film intended to overcome the issue of variable gastric residence. As shown by release testing, the pursued performance was obtained in vitro. Indeed, no drug liberation took place in the acid stage, and a complete release of both compounds occurred, after consistent delay phases, in phosphate buffer. Three-month storage at 4°C did not result in significant changes in the release profiles.

**INTRODUCTION**

Oral colon delivery is under extensive investigation as a possible strategy to improve the oral bioavailability of peptide and protein drugs. Although the large bowel fails to be ideally suited for absorption, it may indeed offer a number of advantages over the small intestine, including a long transit time, lower levels of peptidases and a higher responsiveness to permeation enhancers [1,2]. A swellable/erodible delivery platform (Chronotopic™) based on a low-viscosity hydroxypropyl methylcellulose (HPMC) coating, which was demonstrated to provide the desired in vitro and in vivo release behaviour when conveying small molecules, was accordingly proposed for colonic release of insulin and selected adjuvants, such as protease inhibitor and absorption enhancer compounds [3-6]. For this purpose, the influence of all the involved manufacturing steps on the protein integrity was explored, thereby ruling out the occurrence of any significant degradation during the preparation of the delivery system. More recently, the design of the Chronotopic™ device, originally presented in single-unit configurations, was modified to comply with the size requirements of multiple-unit dosage forms in view of the relevant benefits in terms of consistent gastrointestinal transit and drug absorption patterns [7]. In particular, an insoluble, flexible film composed of the neutral polyacrylate Eudragit®NE and the superdisintegrant sodium starch glycylate, added to act as a pore former, was applied to HPMC-coated minitablet cores in order to improve the efficiency of the hydrophilic layer in delaying the drug liberation without altering the typical release-controlling performance [8,9]. Such two-layer formulations were shown to give rise to a prompt in vitro release after lag phases of programmable duration and to the pursued in vivo behaviour. Moreover, they were proved physically stable while stored under ambient conditions for 3.5 years.

On the basis of these premises, the aim of the present work was to evaluate the above-described two-layer multiple-unit system as a possible colon delivery carrier for bovine insulin and the permeation enhancer sodium glycoalcholate.

**EXPERIMENTAL METHODS**

**Materials:** bovine insulin (Sigma-Aldrich, US-MO); copovidone (Kollidon®VA64, BASF, G); HPMC (Methocel®E50, Colorcon, I); hydroxypropyl methylcellulose acetate succinate (HPMCAS, Aqoat®AS-LG, Shin-Etsu, Tokio, J, a gift from Seppic Italia, I); magnesium stearate (Carlo Erba Reagenti, I); microcrystalline cellulose (Avicol®PH200, FMC Europe, B); poly(ethylcarboxylate, methylmethacrylate) aqueous dispersion (Eudragit®NE30D, Evonik Röhm, G, a gift from Rofarma, I); polyethylene glycol (PEG 400, ACEF, I); sodium glycoalcholate (NaGly, Sigma-Aldrich, US-MO); sodium starch glycoalcholate (Explotab® and Explotab®V17, Mendell, UK).

**Methods:** a 4% bovine insulin, 40.0%, sodium glycoalcholate, 51.0% microcrystalline cellulose, 4.5% sodium starch glycoalcholate and 0.5% magnesium stearate powder mixture was compacted in a rotary machine (AM-8S, Officine Ronchi, I) equipped with concave punches (2.5 mm diameter, 3 mm curvature radius). Minitablets, checked for weight (12.00±0.40 mg), height (2.20±0.40 mm), breaking force (46.8±10.50 N), friability (<1%) and disintegration time (<1 min; USP35 disintegration apparatus, DT3, Sotax, CH), were coated up to a film thickness of 250 µm by rotary fluid bed (GPCG1.1, Glatt, D) with an 8% HPMC-0.8% PEG 400 aqueous solution [10,11]. HPMC-coated cores were in turn coated up to 20 µm film thickness by bottom-spray fluid bed with Eudragit®NE30D containing 20% w/w (on dry polymer) of Explotab®V17. Curing was then carried out at 40°C for 24 h. The two-layer system was finally coated with a 6.0% HPMCAS hydroalcoholic (ethanol 75% w/w) solution in a ventilated coating pan (GS, I). Inlet and product temperatures were 60°C and 30°C, while nebulizing and pattern pressures were 0.75 and 0.50 bar, respectively. The enteric coating level was 7.5 mg/cm².

**In vitro** release test (n=3) was performed by a modified disintegration apparatus (37±1°C, phosphate buffer pH=6.8, 31 cycles/min, 160 ml) [12]. Gastroresistant systems were preliminarily tested in HCl 0.1M for 2h. Fluid samples were withdrawn at successive time points, and bovine insulin as well as NaGly were assayed with previously established RP-HPLC methods [5]. The release test was repeated after three-month
storage in glass vials at 4°C. From the release curves, in vitro lag time ($t_{10\%}$) was calculated as the time to 10% drug release. Statistical analysis was performed by means of two-tail t-student test accounting for heteroscedasticity, and the differences were considered significant for $p<0.05$.

RESULTS AND DISCUSSION

In order to prepare the two-layer multiple-unit device under examination, bovine insulin and NaGly, previously selected as a permeation enhancer, were incorporated into the minitablet core in order to obtain a concurrent release thereof [5]. The drug-containing minitablet was subjected to three successive spray-coating steps, making use of an aqueous HPMC solution, a water dispersion of Eudragit®NE and Explotab®V17 and, finally, of a hydroalcoholic dispersion of HPMCAS. The latter polymer was applied to overcome the influence of variable gastric residence time and thus achieve colon delivery based on the time-dependent approach. When evaluated for release, the gastroresistant system showed no drug liberation during the acid stage of the test, which confirmed the proper deposition of HPMCAS to the substrate and the formation of a continuous film. On exposure of the system to simulated intestinal pH conditions, a pulsatile release of insulin and NaGly was observed after lag phases of reproducible and comparable duration (Fig.1). $t_{10\%}$ of 62.7±11.2 and 67.0±1.1 min were indeed obtained with the two compounds. The possibility of a concomitant liberation of the protein and the absorption enhancer was thus assessed. The release profiles yielded by the entericoated formulation in the phosphate buffer medium showed no major changes with respect to a non-gastroresistant reference system tested in the same medium, and no significant difference was found in insulin $t_{10\%}$ ($p>0.05$). This indicated that the coating process aimed at the application of HPMCAS would not bring about any alteration of the underlying functional layers. Both the drug and the adjuvant were entirely released, with a slightly lower rate in the case of the protein. A 100% assay of the latter proved that the manufacturing process, despite a number of potentially challenging steps involved, would not impact on its integrity.

Release testing was carried out again after 3 months of storage at 4°C, in order to preliminarily study the stability characteristics of the formulation. No major differences were noticed in the release patterns. In addition, $t_{10\%}$ remained practically unchanged ($p>0.05$) for both insulin and NaGly.

CONCLUSION

The feasibility of a previously described multiple-unit time-dependent colon delivery system as an insulin carrier was explored. Such a formulation included a minitablet core, containing the protein drug and the absorption enhancer sodium glycocholate, a swellable/erodible low-viscosity HPMC layer, a Eudragit®NE/Explotab®V17 coat and an outermost HPMCAS gastroresistant film. Tested in vitro, the proposed system showed the desired pulsatile release performance, which was not altered following three-month storage of the formulation under refrigerated conditions.

REFERENCES