

IN VITRO - IN VIVO EVALUATION OF A NOVEL DRUG DELIVERY SYSTEM FOR COLONIC TARGETING

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Abstract

A novel experimental pH- and time-dependent colonic drug delivery system was prepared and its *in vitro* and *in vivo* performances were evaluated. The colonic delivery system consists of minitablets with a big swelling capacity coated with an inlet retardant layer insoluble at all pH ranges but permeable (Eudragit RS 30D) and an outlet layer, insoluble and impermeable at acidic pH but easily soluble at a pH value higher than 5.5 (Eudragit L 55 30D). *In vitro* results showed that this pharmaceutical system is able to prevent drug release in acidic medium for 2 h but to release its drug content in neutral medium (phosphate buffer, pH 6.8), after 3 h lag-time. *In vivo* data obtained on 8 healthy volunteers show that the absorption of drug begins after a lag time (t_{lag}) of 2.5 - 3 hours and the maximum plasma level (t_{max}) was realised after 5.7 hours, suggesting that the release of drug occurs in the colon.

Rezumat

A fost preparat un nou sistem farmaceutic cu cedare colonică, iar performanțele sale au fost evaluate *in vitro* și *in vivo*. Sistemul farmaceutic este format din minitablete cu mare capacitate de umflare, acoperit cu un strat de retardare interior, insolubil indiferent de pH dar permeabil (Eudragit RS 30D) și un strat exterior insolubil și impermeabil la pH acid, dar ușor solubil la pH mai mare de 5.5 (Eudragit L55 30D). Rezultatele *in vitro* au arătat că acest sistem farmaceutic este capabil să prevină eliberarea substanței medicamentoase în mediu acid pentru o perioadă de timp de 2h și să o elibereze în mediu neutru (tampon fosfat pH 6.8) după o perioadă de latență de 3h. Datele *in vivo*, obținute pe 8 voluntari sănătoși, au arătat că absorbția substanței medicamentoase începe după o perioadă de latență (t_{lag}) de 2,5 - 3 ore, iar concentrația maximă plasmatică (t_{max}) se atinge după 5,7 h, ceea ce sugerează că cedarea substanței medicamentoase are loc în colon.

Keywords: colonic delivery, minitablets, indomethacin, time and pH dependent delivery

Introduction

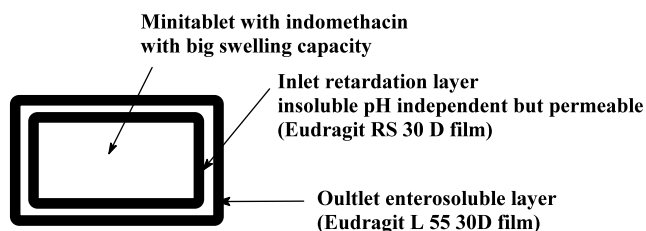
The development and the design of colon-specific drug formulations represent a technological challenge as these dosage forms must pass through the upper gastrointestinal (GI) tract in intact form before delivering the drug to the colon. To achieve a successful colonic delivery, a drug needs to be protected from absorption and /or the environment of the upper gastrointestinal tract (GIT) and then be abruptly released into the proximal colon, which is considered the optimum site for colon-targeted delivery of drugs [1, 2, 3]. During the past decade, a large number of delivery systems have been developed with the intention of colon – targeted drug delivery. The various pharmaceutical approaches which have been used for targeting drugs to the colon are mainly based on pH-dependent, time-dependent and/or bacterially degradable polymers [4, 5].

The aim of this work was the preparation and *in vitro* and *in vivo* evaluation of a novel experimental colonic drug delivery system with pH- and time-control. The developed colonic delivery system was designed to avoid the inherent problems associated with only pH – delivery systems and only time – delivery systems. The developed colonic delivery system exploited the advantage of the relatively consistent transit time through the small bowel coupled with the pH difference between the stomach and the small intestine.

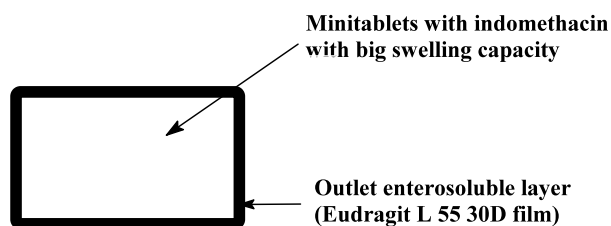
Materials and methods

The colonic delivery system and enteric formulations preparation

The design of the colonic delivery system is shown in Figure 1 and that of the enterosoluble formulation is shown in Figure 2. Both formulations (colonic delivery system and enterosoluble formulation) were obtained starting from the same minitables, as cores. The core minitables contain an active pharmaceutical ingredient (API), one excipient with a big swelling capacity (sodium starch glycolate) and other requisite excipients. The configuration of colonic delivery system consists of a core minitab with a big swelling capacity coated with two layers of polymer coating. The first coating (next to the core) is a retardation layer insoluble at any pH range but permeable (Eudragit RS 30D) and the other coating is an enteric layer, insoluble and impermeable at acidic pH but easily soluble at a pH higher than 5.5 (Eudragit L55 30D). The development of the colonic delivery system minitables type using a central experimental design was reported previously [6].

**Figure 1**

The structure of the colonic delivery system minitabets type

**Figure 2**

The structure of enterosoluble formulation

Minitabets were obtained via direct compressing using a Korsch EK 0 (Korsch, Germany) eccentric press equipped with die and punches of 5 mm. The composition, both qualitative and quantitative, of the minitabets is presented in Table I. The machine was adjusted to obtain minitabets of 50 mg weight. The minitabets were coated with polymeric films (Eudragit RS 30D, Eudragit L 55 30D), in a fluidized bed coating device Strea 1 (Aeromatic AG, Switzerland). The composition of the coating formulas (inlet retardation layer – based on Eudragit RS and outlet enterosoluble layer – based on Eudragit L 55) is presented in Table II.

Table I
Minitabets preparation formula (qualitative and quantitative)

Raw materials	mg/tablet	%	g/laboratory batch
Indomethacin (S&DChemicals, UK),	5.000	10.00	50.00
Sodium starch glycolate (JRS Pharma, Germany)	2.000	4.00	20.00
Polyvinylpyrrolidone K ₂₅ (BASF, Germany)	2.500	5.00	25.00
Lactose monohydrate (Meggle, Germany)	2.500	5.00	25.00
Microcrystalline cellulose PH102 (JRS Pharma, Germany)	37.625	75.25	376.25
Magnesium stearate (Union Derivan, Spain)	0.250	0.50	2.50
Silicon dioxide (Rohm Pharma, Germany)	0.125	0.25	1.25
Total	50	100.00	500.00

Table II

Coating formulas (colonic delivery system and enterosoluble formulation)

Raw materials		colonic delivery system			enterosoluble formulation		
		g/batch	mg/tablet	%	g/batch	mg/tablet	%
Core retardation layer	Minitablets with indomethacin	180	50	86.52	-	-	-
	Eudragit RS 30D	18.54	5.15	8.91	-	-	-
	Triethyl citrate	2.039	0.567	0.98	-	-	-
	HPMC E 15 LV	0.612	0.17	0.29	-	-	-
	Talcum	5.562	1.545	2.67	-	-	-
	Titan dioxide	0.927	0.258	0.45	-	-	-
	Pigment	0.371	0.103	0.18	-	-	-
Minitablets with indomethacin coated with Eudragit RS 30D		180	57	92.81	-	-	-
Core enterosoluble layer	Minitablets with indomethacin	-	-	-	180	50	86.52
	Eudragit L 55 30D	9	2.850	4.64	9	2.89	4.64
	Triethyl citrate	1.35	0.428	0.70	1.35	0.434	0.7
	Talcum	2.70	0.855	1.39	2.7	0.867	1.39
	Titan dioxide	0.45	0.143	0.23	0.45	0.145	0.23
	Pigment	0.45	0.143	0.23	0.45	0.145	0.23

where: HPMC – hydroxypropylmethylcellulose

In vitro release studies. In order to simulate the GI tract conditions, dissolution studies were carried out in acidic media pH 1.2 (HCl 0.1N) for 2 hours and then in neutral media pH 6.8 (phosphate buffer) for 22 hours. The samples were tested separately in each medium. Dissolution studies of coated minitables were carried out in an European Pharmacopoeia dissolution apparatus I (Pharmatest, PTWS, Germany) at a rotation speed of 50 rpm in 900 mL medium at 37 ± 0.5 °C.

In vivo studies. Three administrations with indomethacin oral dosage forms were performed to each subject with 200 mL of water following an overnight fast for at least 10 h in an open randomized crossover study, separated by a 7 days drug-free period; one treatment with immediate release dosage forms (2 immediate release capsules contained 25 mg indomethacin/capsule from Terapia, Romania), one treatment with enterosoluble dosage forms (10 enteric release minitables contained 5mg indomethacin/minitab) and colonic release dosage forms (10 colonic release minitables contained 5 mg indomethacin/minitab). The *in vivo* study was conducted in accordance with the recommendations of the

Declaration of Helsinki (World Medical Assembly 1964) as revised in Tokyo (1975). The study protocol has been approved by the ethical University committee. The volunteers were informed about possible risks and adverse effects of taking the drug, and an informed written consent was obtained from each one. The study included 8 healthy male volunteers aged 22-30 years, 55-93 kg body weight. Blood samples were collected at 0 - 1 - 2 - 2.5 - 3 - 3.5 - 4 - 4.5 - 5 - 5.5 - 6 - 7 - 8 - 9 - 10 - 12 hours after drug administration. The indomethacin assay was performed with a HPLC-MS validated method [7, 9]. The pharmacokinetic parameters were assessed using the Kinetica 4.4 pharmacokinetic data analysis software (Thermo Electron's, SUA).

Results and discussion

The pharmacotechnical properties results of the minitabets cores

The minitabets were analysed using standard pharmacotechnical methods for tablets in European Pharmacopoeia. Because the minitabets would be coated in fluidised bed, lower acceptance criteria as in European Pharmacopoeia for hardness and friability were established. The pharmacotechnical results obtained with cores preparations are presented in Table III. The minitabets accomplish all the established parameters; they have good mass uniformity, very good hardness, very low friability and short disintegration time.

The pharmacotechnical properties results of the enterosoluble formulation

The pharmacotechnical results obtained after minitabets (cores) were coated with Eudragit L 55 and resulted enterosoluble formulation are presented in Table III. In order to evaluate coating efficiency via weight gain, the average mass and uniformity of mass were determined before and after coating. According to the obtained results coating efficiency was very good. The enterosoluble formulation meets the disintegration time requirement for enterosoluble preparation: all the minitabets resist in acid medium for 2 hours and in neutral medium the disintegration time is 6 minutes.

The pharmacotechnical properties results of the colonic delivery system

The pharmacotechnical results obtained after minitabets (cores) were coated with Eudragit RS and Eudragit L 55 and resulted the colonic delivery system are presented in Table III. In order to evaluate coating efficiency via weight gain, the average mass and uniformity of mass were determined after both coating steps, with Eudragit RS and with Eudragit L

55. According to the results obtained, coating efficiency was very good for both coating steps.

Table III
Pharmaceutical characteristics of minitables cores,
enterosoluble formulation and colonic delivery system

Test		Acceptance criteria	Results
Uncoated minitables			
Minitables Uncoated (cores)	Average mass	50mg $\pm$ 7.5%	50.10 mg
	Uniformity of mass	Between 53.75 mg – 46.25 mg	50 mg (+0.2%)
		NMT* 2/20 deviate from mean by more than $\pm$ 7.5% and none more than $\pm$ 15%	Min 48 mg Max 52 mg
	Hardness	NLT** 6 kg/cm ²	7.9 kg/cm ² Min 7; Max 8 kg/cm ²
	Friability	NMT* 0.5 %	0.098%
	Disintegration time	NMT* 10 minutes	1.2 minutes
Assay of indomethacin			
mg/tablet		4.75 – 5.25 mg/comp.	5.024 g
%		95.00 – 105.00% (5 mg $\pm$ 5%)	100.48%
Enterosoluble formulation (after coating with Eudragit L 55)			
Minitables Coated with Eudragit L	Average mass	54mg $\pm$ 7.5%	54.11 mg
	Uniformity of mass	Between 49.95 mg – 58.05 mg	54 mg (+0.11%)
		NMT* 2/20 deviate from mean by more than $\pm$ 7.5% and none more than $\pm$ 15%	Min 52 mg Max 56 mg
	Hardness	NLT** 6 kg/cm ²	7.4kg/cm ² Min 6.5; Max 10 kg/cm ²
	Disintegration time:		
- in acid medium HCl 0.1N		No tablets in 2 hours	0 tablets
- in phosphate buffer pH 6.8 medium		NMT* 15 minutes	6 minutes
Colonic delivery system minitables type (after coating with Eudragit RS)			
Minitables Coated with Eudragit RS	Average mass	57mg $\pm$ 7.5%	56.00 mg
	Uniformity of mass	Between 52.73 mg – 61.28 mg	57 mg (-2.62%)
		NMT* 2/20 deviate from mean by more than $\pm$ 7.5% and none more than $\pm$ 15%	Min 54 mg Max 58 mg
	Hardness	NLT** 6 kg/cm ²	7. 2 kg/cm ² Min 7; Max 9 kg/cm ²
Minitables Coated with Eudragit RS + Eudragit L 55	(after coating with Eudragit L 55)		
	Average mass	61mg $\pm$ 7.5%	61.7 mg
	Uniformity of mass	Between 56.43 mg – 65.58 mg	61 mg (+0.82%)
		NMT* 2/20 deviate from mean by more than $\pm$ 7.5% and none more than $\pm$ 15%	Min 58 mg Max 66 mg
	Hardness	NLT** 6 kg/cm ²	7. 6 kg/cm ² Min 7; Max 9 kg/cm ²

* NMT = no more than

** NLT = no less than

Results from *in vitro* release evaluation

Dissolution testing of colon delivery systems with the conventional basket method has usually been conducted in different buffers for different transit times that colon specific delivery system might encounter *in vivo* [10, 11, 12, 13]. Thus, the *in vitro* release dissolution behavior was evaluated using a pH change method, involving dissolution in 0.1 N hydrochloric acid (pH 1.2) for 2 h followed by 22 h in 0.05 M phosphate buffer (pH 6.8). The dissolution profiles of indomethacin from the colonic delivery system (coated with Eudragit RS and Eudragit L) and enteric release formulation (coated only with Eudragit L) in 0.1 N hydrochloric (pH 1.2) are shown in Figure 3. From all coated minitabets with Eudragit L 55 30D (colonic delivery system and enteric release formulation) no indomethacin was released after 2h at pH 1.2. Furthermore, the coated minitabets maintained their conformation and weight after being kept in acidic medium for 2h. The results confirm the robustness of enteric coating films and the good compatibility with the minitabets cores. The dissolution profiles of indomethacin from the colonic delivery system (coated with Eudragit RS and Eudragit L), enteric release formulation (coated only with Eudragit L) and immediate release (IR) capsules in 0.05 M phosphate buffer (pH 6.8) are shown in Figure 3. In neutral medium (phosphate buffer, pH 6.8) from the immediate release capsule (IR capsules) and from the enterosoluble formulation, drug release occurs fast (more that 88 % after 1 h). From colonic delivery minitabets, drug release occurs after a lag period of 3-4h and 88% is reached in 12h.

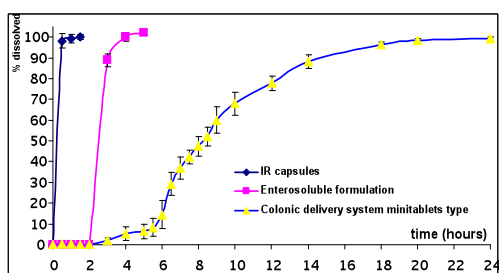


Figure 3

Drug release from immediate release (IR) capsules, enterosoluble formulation and colonic delivery system, in acid medium pH 1.2 for the first 2 hours and phosphate buffer pH 6.8 for the next 22 hours

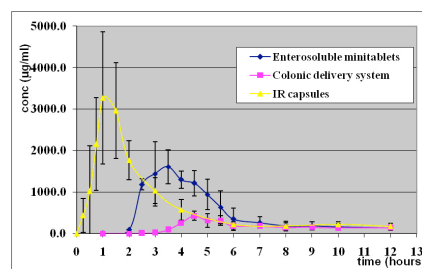


Figure 4

The mean plasma values of indomethacin after oral administration of 50 mg indomethacin (as immediate release (IR) capsules, enterosoluble minitabets and colonic delivery systems)

Results from *in vivo* evaluation

When a drug delivery system is conceived and a prototype

formulation with acceptable *in vitro* characteristics is obtained, *in vivo* studies are usually conducted to evaluate the site specificity of the drug release and to obtain relevant pharmacokinetic information of the drug delivery systems. *In vivo* performances of colonic delivery systems minitables type was evaluated on 8 human subjects using indomethacin as model drug. Figure 4 shows the plasma concentration profile of indomethacin of colonic delivery systems minitables, enteric coated core minitables and immediate release capsules. The results obtained on enteric coated minitables show that the release of drug from this dosage forms occurs after 1 – 2.5 h and the peak time of plasma level was reached after approximately 3.5 h. The colon delivery systems results show that the release of drug from this dosage forms occurs late (after 3.5 – 4.5 h lag time) and the peak of plasma level was reached after approximately 5.5 h. Compared with enteric coated minitables and immediate release capsules, the onset of indomethacin from colonic delivery system was delayed for more than 3 h (table IV).

Table IV
Pharmacokinetic parameters of indomethacin from studied formulations (*mean, SD, n = 8*)

Parameters	Immediate release capsules	Enterosoluble formulation	Colonic delivery system
	Mean, SD	Mean, SD	Mean, SD
T _{max} (h)	0.75 ± 0.56	3.4 ± 0.65	5.7 ± 1.92
T _{lag} (h)	0	1.2 ± 0.8	2.8 ± 1.2
C _{max} (ng/mL)	3272.22 ± 1465.49	1861.46 ± 306.67	114.28 ± 107.89
ASC (ng/mL*h)	6472.57 ± 2306.97	5451.02 ± 477.97	325.23 ± 245.31

where: AUC – area under the curve, SD – standard deviation.

First plasma indomethacin concentration at 0.25h from immediate release capsules and 2h from enteric coated minitables after oral administration suggest a stomach residence time of approximately 2 – 2.5 h. The transit time of the tablet in the small intestine of humans has been characterised to be about 2.13 – 2.75 h [14]. Therefore, taking into consideration an average lag time of 2.5 h after indomethacin appearance in plasma from colonic delivery system in comparison with enteric coated minitables, it can be inferred that onset of drug release from colonic delivery system took place in the proximal colon. It is believed that both formulations (enteric coated minitables and colonic delivery system) are intact in stomach and do not release the drug into the stomach. In the small intestine the outlet Eudragit L 55 30D film is dissolved fast and the drug release occurred only from enteric coated minitables. The inlet Eudragit RS 30D film delayed a lag time in release of indomethacin for 2.5 - 3h after stomach emptying for colonic delivery formulation. This means that breaking of the Eudragit RS film occurs late, after approximately 2.5 - 3 h after

minitables arrived in duodenum, time needed for the minitables to pass through the small intestine and arrive into the colon. So, the disintegration of the system and the release of indomethacin occurred at the target site of the gastrointestinal tract (proximal colon). The low bioavailability and incomplete absorption may be due in part to low water quantity for drug dissolution in colonic content, and in part due to the small surface area available for drug absorption. Similar results, with low bioavailability of drug in colon were obtained on *in vivo* studies in dogs [10] and humans [15].

Conclusions

In this work, *in vitro* and *in vivo* performance of an experimental colonic drug delivery system was evaluated. *In vitro* studies showed that this pharmaceutical system is able to prevent drug release in acid medium (0.1N HCl solution, pH 1.2) for 2 h but in neutral medium (phosphate buffer, pH 6.8) the drug release starts after 3 h and occurs in approximately 8 h. According to *in vivo* data obtained, the absorption of indomethacin from minitables with colon release occurs after a lag time of 2.5-3 h (that means the release of drug begins in the last part of the ileum or in the colon), and the maximum plasma level was reached after 5.7 hours (meaning that the greatest part of the drug was released in the large intestine). Thus, this new colonic drug delivery system is potentially useful for oral site-specific drug delivery including colon targeting.

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Manuscript received: September 1st 2009