CHAPTER 2

Influence of critical process parameters on the release characteristics of ColoPulse tablets: a quality by design approach

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Abstract

ColoPulse tablets are characterized by a pulsatile and colon-specific drug release. Release starts in the ileo-colonic region after pH 7.0 has been reached. The influence of the critical process parameters pKa of active substance, coating thickness, exposure to pH 6.8 and type of coating solvent on drug release from a ColoPulse tablet was investigated using a quality by design approach. Release parameters lag time, pulse time and total release were determined using an abbreviated dissolution test. The results indicate that acetone is the preferred coating solvent and that pKa of the model substance and coating thickness affect drug release. Application of a 2.7 mg/cm^2 hydroxypropylmethylcellulose seal coating on the tablet core did not solve the problem of poor release for strongly acidic substances. Substances with a pKa from 6 up to approximately 11 combined with a coating thickness of 11 - 17.5 mg/cm^2 will display the desired release profile. Substances with pKa < 3 and > 11 are in general not suitable for use in the current ColoPulse formulation. The knowledge obtained will facilitate the selection of suitable active substances to be formulated in a ColoPulse tablet and will make further rational development of ColoPulse tablets more feasible and efficient.

1. Introduction

Colon-specific delivery of drugs is particularly of interest in the (local) treatment of inflammatory bowel diseases. Drugs may have a poor bioavailability in the ileo-colonic region due to destabilization by gastric acid, degradation by digestive enzymes or inactivation by binding to bile salts when formulated in immediate release solid dosage forms. Moreover, (unwanted) absorption in a higher part of the gastrointestinal tract will reduce the amount of drug substance available for a local effect in more distal parts of the intestine.

The colonal environment exhibits a low proteolytic activity compared to both stomach and small intestine and, combined with a long residence time, makes it a potentially suitable delivery site for (proteinaceous) drugs intended for topical treatment [1].

In the literature several strategies for colon-specific delivery have been described. They are mainly based on physiological parameters like pH, gastric emptying, residence time, intraluminal pressure and microflora [1,2]. The ColoPulse technology is an example of a pH responsive system. ColoPulse tablets contain a coating consisting of Eudragit S100 in which the super-disintegrant croscarmellose is dispersed in a non-percolating manner yielding a high and pulsatile release of the active substance in the ileocolonic region. Release from a ColoPulse tablet is triggered by the physiologically occurring increase in pH from 5.5 to 6.8 in the upper small intestine to 7.5 in the ileo-colonic region and starts at a pH over 7.0 [3,4].

A recent *in vivo* study showed no difference in bioavailability and site of release from a ColoPulse tablet between healthy volunteers and patients with Crohn's disease in remission [5]. That study was done with ColoPulse tablets containing stable isotopes of urea by which it was possible to measure release in different parts of the intestine. A logical next step in the development and application of ColoPulse technology is the formulation of tablets containing an active drug substance used in daily patient care. Infliximab and a combination of mesalazine and budesonide are considered as good candidates in this context [6,7].

From a pharmacotherapeutic point of view it is desirable that the ColoPulse technology can be applied as platform technology enabling rapid development of oral formulations with different active substances. However, it was found in an earlier study that the composition of the tablet core influences the *in vitro* release pattern of active substances from a ColoPulse tablet. When using citric acid as model substance the release was < 10% compared to tablets containing the neutral or alkaline substances sodium benzoate or sodium bicarbonate. Furthermore, it was observed that coating thickness influences the release

pattern from a ColoPulse tablet [8]. To overcome this problem the application of an additional seal coating layer of material that does not affect release, (i.e. hydroxypropylmethylcellulose (HPMC)) was suggested.

Knowledge about critical process parameters (CPP) influencing degradation of the coating will be helpful in the selection of suitable active substances to be formulated in a ColoPulse tablet. This provides insight in and understanding of the design space of possible ColoPulse formulations and will make rational development and formulation of ColoPulse tablets more feasible and efficient.

The aim of the present study was to obtain more insight in the influence of critical process parameters on the release pattern of ColoPulse tablets based on a quality by design approach [9]. Furthermore the influence of application of a 2.7 mg/cm^2 HPMC coating to prevent stabilization / destabilization of the coating by the tablet core was investigated.

2. Materials and methods

2.1. Materials

Polyethylene glycol (PEG) 6000, acetone, caffeine, magnesium stearate, lactose monohydrate, silicon dioxide, primojel, talc, citric acid, monobasic sodium phosphate, dibasic sodium phosphate, sodium hydroxide, HPMC 4000 mPa.s (BUFA, the Netherlands), microcrystalline cellulose (Avicel PH102, FMC Biopolymer, USA), croscarmellose sodium (Ac-di-sol, FMC Biopolymer, USA), methacrylic acid-methyl methacrylate copolymer 1:2 (Eudragit S100, Röhm, Germany) were obtained via a certified wholesaler (Spruyt-Hillen, the Netherlands). Ethanol 96% and water for injections were obtained from Fresenius Kabi (Germany). Oxalic acid was obtained from Sigma Aldrich (Germany). All ingredients were of pharmacopoeial grade (Ph Eur and/or USP) except oxalic acid (chemical grade).

2.2. Methods

2.2.1. Experimental design

The pKa of active substance (X1), coating thickness (X2), exposure to H 6.8 (before switch to pH 7.5) (X3) and coating solvent (X4) were identified by an expert team as critical process parameters (CPP) highly influencing the drug release from a ColoPulse tablet. The design space was calculated using Design-Expert® software (version 8.4.4.1 Statease®) for Windows. A central composite design was applied for the response surface methodology (RMS). The numeric process variables X1-X3 were varied over five levels. For each parameter a

minimum and a maximum was determined (table 1). Factor X4 was tested as a categorical factor level (acetone - ethanol). This yielded a total of 2 x 20 runs including 6 replicates of the center points as controls. Furthermore for each coating solvent 6 axial and 8 factorial points were assigned. Runs were performed randomly to prevent bias. For each run, tablets were prepared with the assigned model substance, coating thickness Eudragit S and coating solvent. In table 2 a summary of the combination and levels of CPP, the order of runs and type of design points is shown.

Table 1: Critical Process Parameters (CPP) for ColoPulse coating

Factor	Description	Units	Type	Subtype	Min	Max
X1	Model substance	pKa	Numeric	Continuous	1.3	15.7
X2	Coating thickness	mg/cm^2	Numeric	Continuous	4.0	21.0
X3	Time exposed to pH 6.8	min	Numeric	Continuous	30	450
X4	Coat Solvent	n.a.	Categoric	Continuous	Acetone	Ethanol

2.2.2. Tablet core

Five different core tablet formulations (A-E) were prepared (table 3) based on five different model substances (instead of active substances) chosen on the basis of their pKa. All tablets contained caffeine as a marker substance for dissolution testing. The mixture was prepared by blending caffeine, the model substance and excipients, except magnesium stearate, in a Braun blender for two minutes. After adding magnesium stearate blending was continued for one minute. Subsequently the powder was compacted using an eccentric tablet press (HOKO KJ 2) to 9 mm biconvex tablets with a weight of 350 mg. Tablets met all quality control criteria except for content of caffeine due to disturbance of the analysis caused by extreme high pH (table 4) of dibasic sodium phosphate and sodium hydroxide. However, for both substances, no problems with flowability of the powder mixture were noticed and all other parameters showed a relative small standard deviation. Therefore it was assumed that the caffeine content would be within the requested limits of 90-110%.

Table 2: Summary of experimental design: combination and levels of CPP, order of runs and type of design points

Combination	Run	CPP				Type design point
		pKa model substance	Coating thickness (mg/cm^2)	Time pH 6.8 (min)	Coating Solvent	
1	11	7.1	12.5	240	Ethanol	Center
2	31	12.3	7.5	360	Acetone	Factorial
3	3	3.1	17.5	120	Ethanol	Factorial
4	39	15.7	12.5	240	Acetone	Axial
5	12	7.1	12.5	240	Acetone	Center
6	13	7.1	12.5	240	Ethanol	Center
7	32	12.3	17.5	360	Ethanol	Factorial
8	4	3.1	17.5	360	Ethanol	Factorial
9	14	7.1	12.5	240	Acetone	Center
10	15	7.1	20.9	240	Ethanol	Axial
11	40	15.7	12.5	240	Ethanol	Axial
12	33	12.3	17.5	120	Acetone	Factorial
13	34	12.3	17.5	360	Acetone	Factorial
14	35	12.3	7.5	360	Ethanol	Factorial
15	5	3.1	17.5	120	Acetone	Factorial
16	36	12.3	7.5	120	Ethanol	Factorial
17	16	7.1	12.5	36	Ethanol	Axial
18	17	7.1	12.5	240	Acetone	Center
19	18	7.1	20.9	240	Acetone	Axial
20	19	7.1	4.1	240	Acetone	Axial
21	37	12.3	7.5	120	Acetone	Factorial
22	6	3.1	7.5	360	Ethanol	Factorial
23	20	7.1	12.5	240	Ethanol	Center
24	38	12.3	17.5	120	Ethanol	Factorial
25	21	7.1	12.5	444	Ethanol	Axial
26	7	3.1	17.5	360	Acetone	Factorial
27	8	3.1	7.5	360	Acetone	Factorial
28	1	1.3	12.5	240	Ethanol	Axial
29	22	7.1	12.5	240	Ethanol	Center
30	23	7.1	12.5	240	Acetone	Center
31	24	7.1	12.5	240	Ethanol	Center
32	2	1.3	12.5	240	Acetone	Axial
33	25	7.1	12.5	240	Acetone	Center
34	26	7.1	4.1	240	Ethanol	Axial
35	27	7.1	12.5	36	Acetone	Axial
36	28	7.1	12.5	444	Acetone	Axial
37	29	7.1	12.5	240	Ethanol	Center
38	9	3.1	7.5	120	Acetone	Factorial
39	10	3.1	7.5	120	Ethanol	Factorial
40	30	7.1	12.5	240	Acetone	Center

Table 3: Composition of the tablet cores

		A oxalic acid	B citric acid	C monobasic sodium phosphate	D dibasic sodium phosphate	E sodium hydroxide
pKa Model substance		1.3	3.1	7.1	12.3	15.7
Model substance		100 mg	100 mg	100 mg	100 mg	100 mg
Marker substance	Caffeine	25 mg	25 mg	25 mg	25 mg	25 mg
Excipients	Avicel PH 102	85 mg	85 mg	85 mg	85 mg	85 mg
	Lactose	123 mg	123 mg	123 mg	123 mg	123 mg
	Primojel	14 mg	14 mg	14 mg	14 mg	14 mg
	Silicon dioxide	0.7 mg	0.7 mg	0.7 mg	0.7 mg	0.7 mg
	Magnesium stearate	2.5 mg	2.5 mg	2.5 mg	2.5 mg	2.5 mg
Batch size		500	500	500	500	500

Table 4: Quality control data of the tablet cores presented as means and (standard deviation) where applicable

Parameter	Specification	A oxalic acid	B citric acid	C monobasic sodium phosphate	D dibasic sodium phosphate	E sodium hydroxide
Tablet weight	350 mg (n = 20)	348.6 mg (3.4)	348.0 mg (5.5)	351.4 mg (5.9)	351.2 mg (5.4)	353.3 mg (4.9)
Weight variation	RSD < 4.0% (n = 20)	1.0%	1.6%	1.7%	1.6%	1.4%
Friability	< 1.0% after 100 rotations (n = 1)	0.2%	0.3%	0.2%	0.3%	0.0%
Resistance to crushing	80-150 N (n = 20)	91.3 N (9.4)	107.7 N[a] (7.2)	82.7 N (8.5)	122.8 N (7.8)	87.2 N (16.8)
Disintegration time	All < 15 min (n = 6)	All < 15 min	All < 15 min	All < 15 min	All < 15 min	All < 15 min
Content (caffeine)	90-110% (n = 10)	104.6 (7.4)	97.3 (5.5)	109.9 (10.2)	111.9 (10.5)	46.4 (12.1)

[a] n = 3

2.2.3. Tablet coating

HPMC coating

The tablet core was coated with a HPMC seal coating with the intention to act as a barrier between the core and the subsequently applied ColoPulse coating. HPMC was dissolved in a mixture of water for injections and ethanol 96% in a ratio of 85:15 (v/v) yielding a 5% (w/v) solution. The coating was applied on the different tablet cores using a conventional continuous spray-coating process performed in an in-house build mini rotating drum until a weight gain of approximately 7 mg per tablet was reached. This corresponded with a coating of 2.7 mg/cm^2 HPMC which was proven not to influence the dissolution behavior

of the tablet core (data not shown). Coating conditions were: rotation speed of 60 rpm, temperature of approximately 35°C, continuous coating.

ColoPulse coating

After the HPMC coating had dried, a ColoPulse coating was applied according to the schedule in table 2 (4.1, 7.5, 12.5, 17.5, 20.9 mg/cm^2) using the above described spray-coating equipment. Coating conditions were: rotation speed of 60 rpm, temperature of approximately 35°C, discontinuous coating. The coating suspension was composed of a mixture of Eudragit S-100:PEG 6000:Ac-di-sol:talc in a ratio of 7:1:3:2 (w/w/w/w). The solvent was a water/acetone or a water/ethanol 3:97 mixture (w/v) according to the schedule in table 2. During each run 70 randomly taken tablets were coated followed by curing for 2 hours at 40°C. Coating thickness was determined and expressed as the amount of Eudragit S100 applied per cm^2 using the following formula

$$Coating\ thickness = \frac{weight\ coated\ tablet - weight\ uncoated\ tablet}{surface\ uncoated\ tablet}\ x\ fraction\ eudragit\ S100$$

2.2.4. Release characteristics

The release characteristics of a ColoPulse tablet are reflected by the lag time ($t_{5\%}$) and the pulse time. Therefore lag time (Y1), pulse time (Y2) and total release (Y3) were identified as most suitable critical quality attributes (CQAs) (table 5). The lag time is the time point at which the tablet starts to release the active substance and is defined as the time at which 5% of the marker substance caffeine is released. The pulse time reflects the pulsatile release characteristics and is defined as the period between the lag time ($t_{5\%\ release}$) and the time 70% is released ($t_{70\%\ release}$). These parameters were measured in an abbreviated dissolution test with a total duration 2.5 – 9.5 hours (depending on the time at phase II (pH 6.8). In this dissolution test, known as the Gastro-Intestinal Simulation System (GISS) which was described previously by Schellekens et al. [10], the pH is varied over time to simulate the different stages during passage of the gastrointestinal tract. In the current study an abbreviated GISS version was applied that existed only of the phases II (jejunum) and III (distal ileum). Phase I (stomach) and phase IV (proximal colon) were excluded. The specifications of the abbreviated test are given in table 6. In this study only the phases II and III were used, since the ColoPulse system is expected to show its typical performance characteristics in these phases and not in the first of fourth phase. The release of caffeine from the tablets was measured with in-line UV

spectroscopy at a wavelength of 273 nm. For each run n = 3 tablets from the same batch were tested simultaneously in three separate vessels of the abbreviated GISS.

Table 5: Critical Quality Attributes (CQAs) for release from a ColoPulse tablet

Factor	Description	Units	Specification
Y1	Lag time after switch to phase III	Minutes	> 240 minutes
Y2	Pulse time	Minutes	≤ 60 minutes
Y3	Total release	Percentage	> 80%

Table 6: Specifications of the dissolution test (GISS)

Phase	Gastrointestinal Segment	Volume (ml)	Residence time (min)	pH	Osmolality (mosmol/kg)
I	Stomach	500	not applicable[a]	1.2 ± 0.10	150 ± 25
II	Jejunum	629	36-444	6.8 ± 0.20	250 ± 50
III	Ileum (distal)	940	2.0	7.5 ± 0.25	250 ± 50
IV	Colon (proximal)	1000	not applicable[a]	6.0 ± 0.25	250 ± 60

[a]Phase I (stomach) and phase IV (proximal colon) were excluded in this study

2.2.5. Statistical analysis

The Design-Expert® Software indicated for each CQAS which model was favorable to use for the analysis (i.e linear or quadratic) based on their significance using an analysis of variance (ANOVA) F-test. A Box-Cox transformation was performed when suggested by the software. A model was considered suitable for analysis when R-squared was > 0.80, when the predicted R-squared was in reasonable agreement (difference < 0.2) with the adjusted R-squared and when the normal probability plot of residuals contained appeared to be randomly. Non-significant model terms were removed using backward elimination.

3. Results

The study was performed according to the study design summarized in table 2. A summary of the results of the combination of CPPs tested and their measured CQAs is given in table 7. In table 7 the CPPs are subsequently sorted on the basis of the pKa of the model substance and coating thickness for readability. An example of a typical dissolution profile for tablets containing different model substances obtained with the described abbreviated GISS (only phase II and III) is shown in figure 1. The coating thickness was 12.5 mg/cm^2 for all tablets. This figure shows that the release pattern of a ColoPulse differs when tablets

containing three model substances covering a wide range of pKa's are compared.

Table 7: CQAs lag time, pulse time and total release (mean and (SD), n = 3)

Run	Model sub-stance (pKa)	Coating thickness (mg/cm2)	Time exposed to pH 6.8 (min)	Coating solvent	Lag time (min)	Pulse time (min)	Total release (%)
28	1.3	12.5	240	Ethanol	-[a]	-[a]	4 (1.8)
32	1.3	12.5	240	Acetone	-[a]	-[a]	3 (0.4)
38	3.1	7.5	120	Acetone	155 (32.0)	-[a]	47 (2.8)
39	3.1	7.5	120	Ethanol	130 (7.2)	119 (15.1)	84 (3.1)
22	3.1	7.5	360	Ethanol	109 (26.7)	339 (25.8)	89 (6.4)
27	3.1	7.5	360	Acetone	94 (10.8)	324 (16.7)	108 (10.4)
3	3.1	17.5	120	Ethanol	212 (10.2)	-[a]	35 (5.5)
15	3.1	17.5	120	Acetone	194 (1.2)	-[a]	28 (2.7)
8	3.1	17.5	360	Ethanol	428 (2.3)	-[a]	22 (2.1)
26	3.1	17.5	360	Acetone	436 (11.4)	-[a]	27 (5.8)
20	7.1	4.1	240	Acetone	168 (4.6)	100 (5.7)	100 (10.7)
34	7.1	4.1	240	Ethanol	52 (3.1)	198 (1.5)	107 (7.5)
17	7.1	12.5	36	Ethanol	87 (7.2)	35 (6.2)	102 (6.1)
35	7.1	12.5	36	Acetone	74 (3.1)	17 (2.5)	103 (2.3)
1	7.1	12.5	240	Ethanol	269 (7.5)	69 (8.3)	89 (6.3)
5	7.1	12.5	240	Acetone	278 (7.5)	29 (20.1)	112 (22.8)
6	7.1	12.5	240	Ethanol	250 (6.0)	80 (8.1)	112 (3.3)
9	7.1	12.5	240	Acetone	274 (8.0)	25 (17.2)	111 (25.7)
18	7.1	12.5	240	Acetone	272 (3.5)	42 (5.5)	96 (6.1)
23	7.1	12.5	240	Ethanol	248 (7.1)	79 (2.3)	100 (7.8)
29	7.1	12.5	240	Ethanol	235[b] (7.1)	75[b] (1.7)	102[b] (7.8)
30	7.1	12.5	240	Acetone	270 (4.5)	45 (24.0)	97 (5.7)
31	7.1	12.5	240	Ethanol	222 (9.3)	97 (6.6)	113 (24.1)
33	7.1	12.5	240	Acetone	283 (2.0)	11 (1.5)	101 (4.3)
37	7.1	12.5	240	Ethanol	232 (0.6)	84 (14.5)	99 (9.1)
40	7.1	12.5	240	Acetone	278 (6.4)	39 (28.0)	97 (9.7)
25	7.1	12.5	444	Ethanol	374 (15.1)	103 (19.2)	112 (7.4)
36	7.1	12.5	444	Acetone	221 (33.8)	242 (46.0)	110 (9.4)
10	7.1	20.9	240	Ethanol	303 (1.2)	51 (0.7)	81 (18.2)
19	7.1	20.9	240	Acetone	298 (3.5)	13 (4.4)	104 (10.8)
16	12.3	7.5	120	Ethanol	54 (2.3)	20 (2.5)	85 (6.2)
21	12.3	7.5	120	Acetone	116 (18.9)	34 (2.3)	103 (9.1)
2	12.3	7.5	360	Acetone	195 (28.4)	115 (43.0)	106 (13.7)
14	12.3	7.5	360	Ethanol	48 (5.3)	30 (4.9)	100 (5.6)
12	12.3	17.5	120	Acetone	167 (1.0)	21 (1.0)	108 (9.6)
24	12.3	17.5	120	Ethanol	156 (4.6)	23 (10.1)	95 (2.6
7	12.3	17.5	360	Ethanol	175 (3.1)	31 (15.2)	113 (8.6)
13	12.3	17.5	360	Acetone	352 (28.8)	58 (26.0)	117 (20.2)
4	15.7	12.5	240	Acetone	41 (6.4)	42 (11.0)	100 (1.3)
11	15.7	12.5	240	Ethanol	20 (1.2)	60 (12.7)	93 (3.5)

[a] not measurable

[b] n = 2

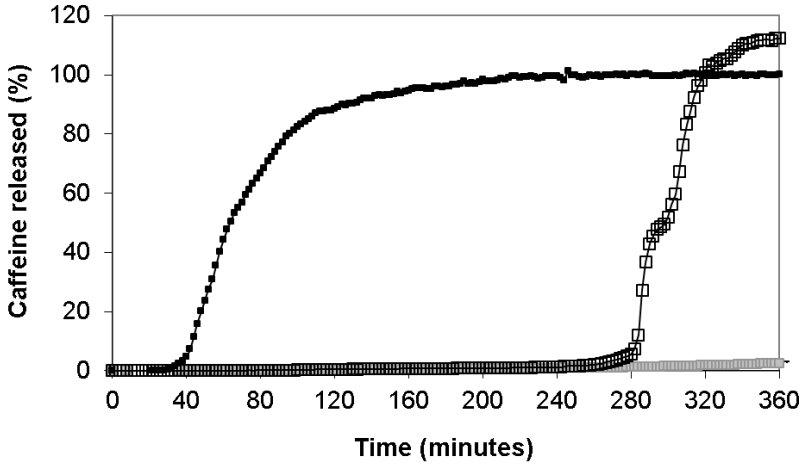


Figure 1: Example of dissolution profiles of ColoPulse tablets containing different model substances. Coating thickness is 12.5 mg/cm^2, time at pH below 6.8 is 4 hours, solvent is acetone

Oxalic acid (pKa 1.3)
Monobasic sodium phosphate (pKa 7.1)
Sodium hydroxide (pKa 15.7)

3.1. Model building

To study the influence of the CCPs on the CQAs a model was constructed for each CQA using the Design-Expert® software. All models had an R-squared > 0.80 (0.842, 0.837 and 0.885 for lag time, pulse time and total release, respectively). For all models the difference between the adjusted R-square and the predicted R-square was < 0.2 and the normal probability plot of residuals appeared to be random. However, models could not be build if the substance with lowest pKa of 1.3 (oxalic acid) was included and only partially for the substance with pKa of 3.1 (citric acid) because in 7 out of 10 runs no lag and/or pulse time could be observed (table 7). Therefore the suitability of the models for substances with an acid nature remains to be investigated, because the experimental design used in our study did not comprise enough data points in this area.

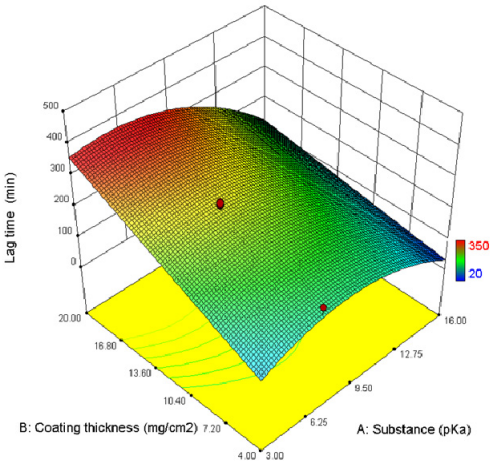
For the presented figures the time exposed to pH 6.8 was set at 4.0 hours because this corresponds to normal physiological conditions (oral to cecal transit time = 240 ± 88 min [11]) and the combined time at phase I and II in the original GISS [10].

3.2. Lag time

The lag time was determined using the abbreviated GISS. For tablets with oxalic acid as model substance no lag time and no pulse-time could be measured because the coating did not disintegrate and the tablets remained intact.

The 3D plot and the corresponding contour plot in figure 2 indicate that the lag time is influenced by the pKa of the model substance and the coating thickness. The lag time increased with increasing coating thickness and the lag time decreased with increasing pKa. In general, substances with a pKa > 6 and a coating thickness > 11 mg/cm ^{2} will yield an adequate lag time. Lag time has to be > the CPP "exposure to pH 6.8". This depends on both coating thickness and model substance. There was no difference in lag time between tablets coated with coating fluid containing acetone or ethanol.

(A)



(B)

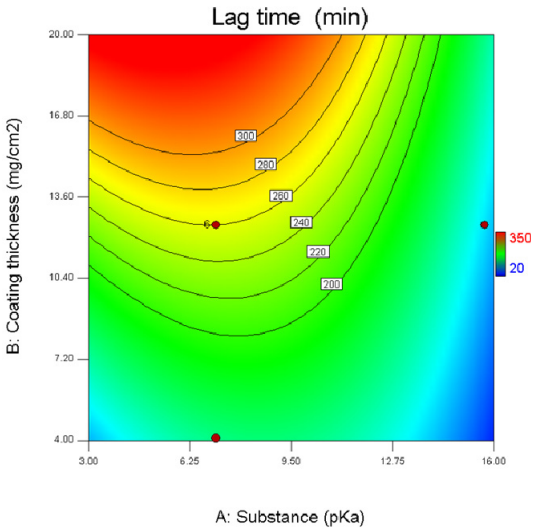


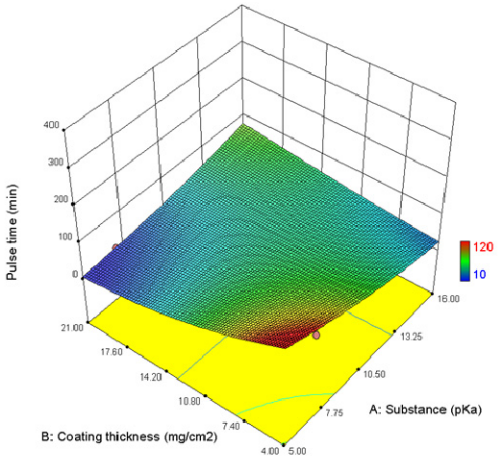
Figure 2: Example of 3D surface plot (A) and contour plot (B) showing the influence of pKa and coating thickness on lag time (solvent acetone, time exposed to pH 6.8: 240 minutes). The red dots represent design points.

3.3. Pulse time

The pulse time of the different ColoPulse tablets was determined in the abbreviated GISS. As described above hardly any release was observed for tablets with oxalic acid as model substance. For tablets with citric acid as model substance, pulse time could not be determined due to incomplete total release (< 47%) in three out of four runs. In the other run a pulse time > 60 minutes (324 minutes) was measured.

The 3D plot and corresponding contour plot in figure 3 indicate that the pulse time is out of specification for substances with pKa approximately < 6, but, as explained, the model does not comprise enough design points between pKa 3 and 6. Because a coating thickness of 4 (very low) and 21 mg/cm^2 (very high) were both tested only once, results at the outside limits of the model should be interpreted with caution. In general, figure 3 shows that the pulse time increased with decreasing pKa. The pulse time increased with increasing coating thickness.

(A)



(B)

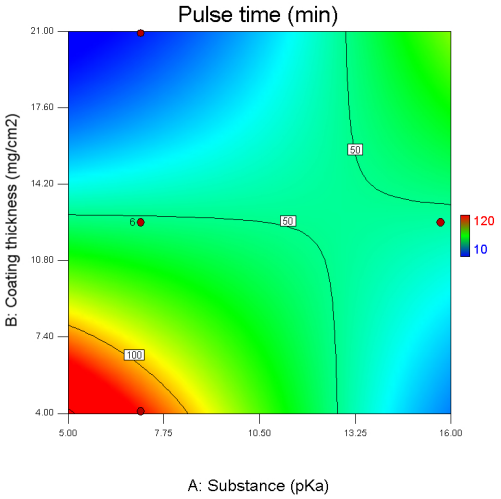


Figure 3: Example of 3D surface plot (A) and contour plot (B) showing the influence of pKa and coating thickness on pulse time (solvent acetone, time exposed to pH 6.8: 240 minutes). The red dots represent design points.

Regarding pulse time, a difference was observed between the coating solvents ethanol and acetone. For example, for a marker substance with pKa 7, a coating thickness of 12.5 mg/cm^2 and 4 hours exposed to pH 6.8, pulse time appeared to be out of specification, i.e. > 60 minutes, for 6 out of 6 runs for tablets when ethanol was used as coating solvent. Under these conditions all tablets coated with acetone as coating solvent had a pulse time < 60 minutes. The difference

between ethanol and acetone, with longer pulse times for ethanol, is elegantly illustrated with the cube plots for both solvents (figure 4).

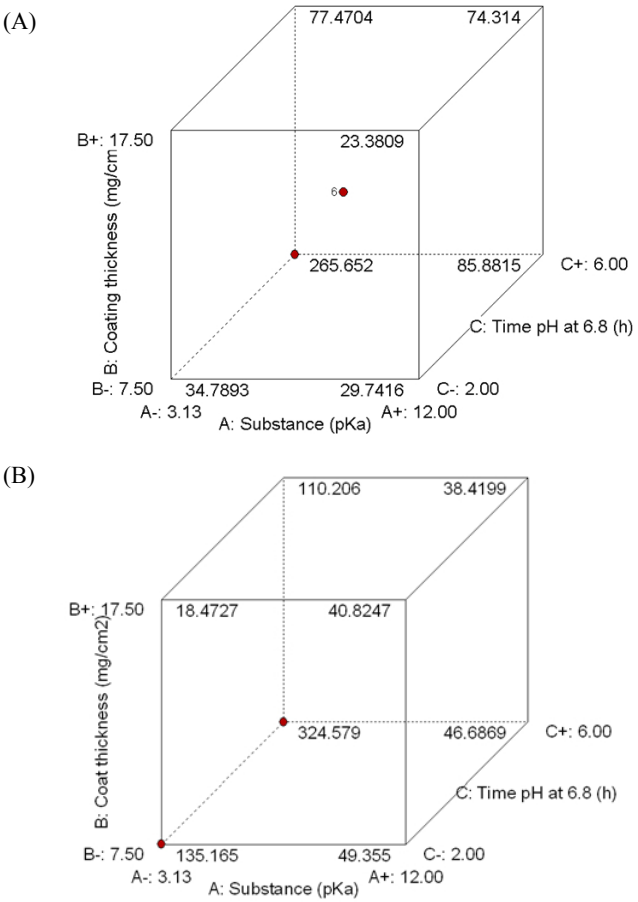


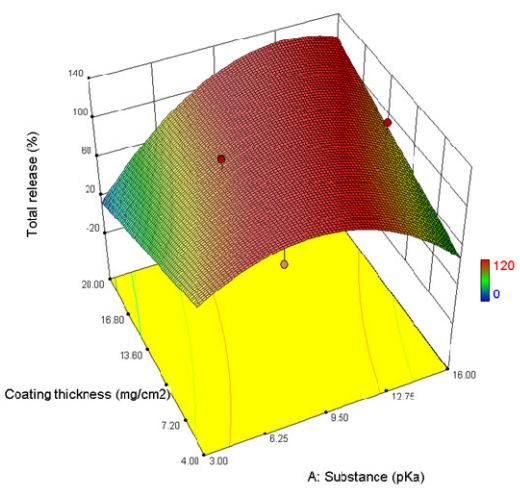
Figure 4: Cube plot of the effect of pKa, time exposed to pH 6.8 and coating thickness on pulse time for ColoPulse tablets coated with acetone (A) and ethanol (B).

3.4. Total release

Total release was determined as the percentage of caffeine released at the end of the dissolution test. The 3D plot and the corresponding contour plot in figure 5 show that the total release from a ColoPulse tablet with a model substance of pKa > 6 was influenced mainly by the model substance and not by coating thickness. Total release increased from 0% at pKa 1.3 to > 80% at pKa > 6.0.

The time exposed to pH 6.8 in the dissolution test had no influence on total release. However, coating thickness did have influence on release for substances with a pKa < 3. For these substances the total release increases when coating thickness decreased. There was no difference regarding lag time between tablets coated with acetone or ethanol.

(A)



(B)

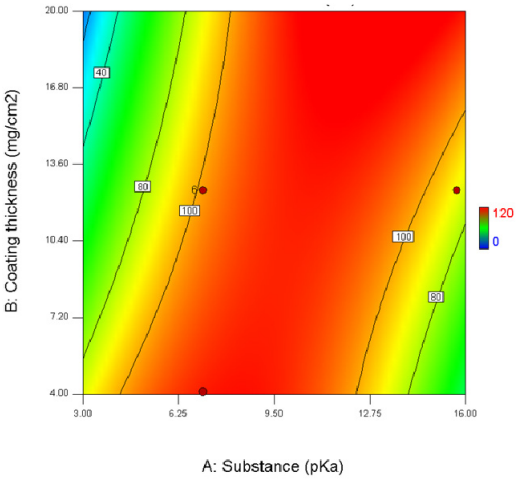


Figure 5: Example of 3D surface plot (A) and contour plot (B) showing the influence of pKa and coating thickness on total release (solvent acetone, time exposed to pH 6.8: 240 minutes). The red dots represent design points.

4. Discussion

This is the first study in which the influence of selected critical factors (the CPP) on the performance of ColoPulse tablets is systematically studied using a quality by design approach. The results are in agreement with our previous findings [8] that the composition of the core as well as the coating thickness influence the release pattern of an active substance from a ColoPulse tablet, but the current study provides more insight in the extent of these phenomena. Moreover, information was generated on the effect of process and testing variables.

The application of a 2.7 mg/cm^2 HPMC coating between tablet core and ColoPulse coating did not act as the desired barrier to prevent any effect of the core on the coating performance. Neutral to weakly acidic and slightly alkaline drug substances appear to be excellent candidates for formulation in a ColoPulse tablet. In contrast, substances with low (< 3) and high pKa (> 11) were less or not suitable to be formulated into a ColoPulse tablet with the current formulation. A low pKa strongly reduced total drug release combined with extreme long pulse times to an unacceptable level. The suitability of a ColoPulse coating for substances with their pKa between 3 and 6 remains to be investigated. These results can be relevant for future development of ColoPulse tablets. Manallack describes that among the WHO essential medicines 78% of them has an ionizable group with a pKa in the range of 2-12 and in another dataset 50% of all substances containing a single acid group had a pKa between 3 and 6 [12].

4.1. Ethanol vs acetone

No influence of the coating solvent, ethanol or acetone, on the model parameters lag time and total release was found. However pulse time was > 60 min for a substantial number of the results when tablets were coated with ethanol as solvent. This indicates that acetone is the preferred coating solvent for application of a ColoPulse coating. The difference can possibly be explained by acetone being more volatile than ethanol (vapor pressure approximately 25 kPa versus 8 kPa, respectively, at 25ºC). This causes acetone to evaporate faster than ethanol during the coating process and subsequent curing of the tablets which results in less residual solvent in the coating. Therefore more research on coating and curing conditions has to be done before ethanol can be used as coating solvent to apply ColoPulse coating.

4.2. Lag time

For interpretation of the results regarding lag time, it has to be realized that *in vivo* the pH rises along the small intestine, will reach a value above pH 7.0 in the terminal ileum and drops relatively fast to a value around 6 after passage of the ileocecal valve occurs. After this pH drop (phase IV in the original GISS) (pulsatile) release of active substance from the ColoPulse tablet will be limited [4]. Furthermore it has to be taken into account that, from a technical point of view, application of a coating with a thickness up to 17.5 mg/cm^2 is practically feasible and results in a smooth coating combined with an acceptable coating time. A higher coating thickness bears practical difficulties, although the CQAs are within specifications. From the presented data it can be concluded that substances with a pKa from 6 up to approximately 11 in combination with a feasible coating thickness of 11 - 17.5 mg/cm^2 will result in an adequate lag time (250-300 minutes). For oxalic acid with a pKa of 1.3 only a 12.5 mg/cm^2 coating thickness was studied. From a previous study [8] it is known that substances with low pKa show relatively long lag times and poor total release. Therefore additional research to optimize the model for low pKa values was considered unuseful.

4.3. Pulse time

An *in vitro* pulse time of ≤ 60 minutes is relevant, because when tablets meet this specification it is known that the *in vivo* pulse time of a ColoPulse tablet is approximately 220 minutes in healthy volunteers and in Crohn's patients [5]. A longer *in vitro* pulse time is therefore not desirable. From the presented data regarding to pulse time, it can be concluded that substances with pKa < 3 are not suitable to be formulated in a ColoPulse tablet because of long pulse times and that coating thickness has only limited influence on pulse time for substances with pKa > 6.

4.4. Total Release

As expected total release was ≥ 80% in almost all runs with a measurable pulse time. For the marker substances with pKa 1.3 and 3.1 only limited release of caffeine was found in the majority of the runs. This is in agreement with the results published by Schellekens et al. [8]. In this paper it was described that substances with low pKa showed little total release of caffeine. It was hypothesized that attrition of tablets during the initial phase of the coating process produces some powder that is incorporated into coating. This creates a micro-environment with the uptake of a small amount of water in the coating resulting in stabilization or destabilization of the coating in case the core tablet contains an acidic or a basic compound, respectively. For this reason an HPMC

coating between core and ColoPulse coating was suggested as possible solution for this problem.

In our study tablets containing oxalic acid were clearly swollen at the end of the experiment and the appearance of the core could be described as somewhat granulated. The coating remained intact, except for the formation of some small pores that could also be visually observed during the GISS. For tablets based on citric acid more pores were seen, but again the coating remained merely intact. At the end of the experiment all tablets containing citric acid were clearly swollen, while the structure of the core could be described as a granulated gel. From these findings it can be concluded that the applied 2.7 mg/cm^2 HPMC coating did not exhibit the desired barrier function and did not prevent the over-stabilization of the coating. Possible explanations for this observation may be found in either the occurrence of minor amounts of acid in the HPMC layer due to attrition or in the permeation of minor liquid amounts through the ColoPulse coating in the early stages of the test, which led to diffusion of acid into the coating before phase III.

4.5. Future research

More research has to be done on the formulation of substances with high and low pKa in a ColoPulse tablet. Strategies could be to find a suitable other sealcoating that does not affect tablet dissolution, to increase the thickness of the HPM seal coating or to make the coating less porous for example by lowering the amount of PEG 6000 or a combination of these. Furthermore the influence of the percentage of the drug substance on the total tablet weight can influence this interaction. In the current study this percentage was 28.6%, which is relatively high. It is not unlikely that the described interaction is less relevant when this percentage is reduced by decreasing the amount of active substance or by increasing the amount of filler material. Another option for future research could be to “titrate” the tablet core i.e. add acidic excipients when the active compound is strongly basic and add basic excipients when the active compound is strongly acidic. However, this might influence the biopharmaceutical properties of the active substance.

5. Conclusion

The aim of the current study was to investigate the influence of the CPP pKa of active substance, coating thickness, time exposed to pH 6.8 and type of coating solvent on the performance of a ColoPulse tablet using a quality by design approach. The application of a 2.7 mg/cm^2 HPMC seal coating between tablet core and ColoPulse coating did not act as the desired barrier to prevent influence of active substances on the ColoPulse coating. All CPPs influenced the lag time,

pulse time and/or total release of a ColoPulse tablet. Acetone was the preferred coating solvent with the current settings and the pKa of the model substance and coating thickness both influenced release from a ColoPulse tablet. Based on this, active substances with a pKa from 6 up to approximately 11 in combination with a coating thickness of 11 - 17.5 mg/cm^2 will result in an adequate release profile.

Active substances with pKa < 3 are in general not suitable to be formulated in a ColoPulse tablet, because the coating is stabilized by the tablet core which prevents release from the tablet. Substances with pKa > 11 are in general not suitable, because destabilization of the coating by the tablet core leads to preliminary release of the content. The suitability of the models remains to be investigated in more detail for substances with a pKa between approximately 3 and 6, because the experimental design used in our study did not comprise enough data points in this area. The results of this study can be used in the formulation of new ColoPulse tablets and will make further rational development more feasible and efficient.

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