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In vitro degradation by colonic bacteria of inulinHP incorporated in Eudragit RS films

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Abstract

Inulin is a naturally-occurring gluco-fructan, which can resist hydrolysis and digestion in the upper gastro-intestinal tract. In the colon it is fermented by the colonic microflora. Therefore inulinHP (inulin with a high degree of polymerization) was formulated as a biodegradable colon-specific coating by suspending it in Eudragit RS films. The in vitro degradability of the prepared isolated films was studied by incubating them in a faecal degradation medium. Measurements of the pH of the degradation medium and determination of the permeability coefficients of the incubated films as a function of time, indicated that inulinHP was indeed degraded by the faecal bacteria, even when it was suspended in Eudragit RS films. Films with different amounts of incorporated inulinHP and with different plasticizers were evaluated. The isolated films could also withstand gastric and intestinal fluid.

Keywords: Inulin; Biodegradable coating; Colon-specific drug delivery form; Colon targeting; Permeability; Diffusion

1. Introduction

Colon-specific drug delivery is of value in the local treatment of colonic disorders such as ulcerative colitis, Crohn's disease and colon carcinomas. It has also gained interest in the oral administration of protein and peptide drugs, which are normally degraded by the enzymes of the upper gastro-intestinal tract.

Site-specific delivery of drugs to the colon can be obtained by coating drugs with pH-sensitive polymers (Lehmann, 1975; Peeters, 1990) or by using a time-controlled release dosage form (Pozzi et al., 1994).

The high enzymatic activity of the microbial flora of the colon can also be exploited to achieve delivery of drugs to the colon. This is accomplished by the development of coating-materials based on bacterial degradable polymers (Saffran et al., 1986), bacterial degradable matrices and hydrogels (Brondsted and Kopecek, 1991, 1992; Rubinstein et al., 1992, 1993) and prodrugs (Friend and Chang, 1984, 1985; Kopeckova and Kopecek, 1990; Haeberlin et al., 1993).

In the present study isolated films consisting of Eudragit RS and inulinHP were prepared and evaluated for their in vitro biodegradation by human faecal bacteria.

Inulin is a naturally-occuring storage carbohydrate, found in many plants such as onion, garlic,

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artichoke and chicory. Chemically, inulin belongs to the group of the gluco-fructans. It consists of a mixture of oligomers and polymers containing from 2 up to 60 or more D-fructose molecules, which are linked by β 2–1 bonds. A D-glucose molecule can be linked at the end of the chain.

It is generally accepted that inulin can resist hydrolysis and digestion in the upper gastro-intestinal tract. In the colon however, it is fermented by the colonic microflora, more specifically by *Bifidobacteria* and *Bacteroides*, bacterial species comprising the majority of the colon micro-organisms (McCane and Lawrence, 1929; Nilsson and Björck, 1988; Roberfroid, 1993; Wang and Gibson, 1993).

In order to formulate inulin as a biodegradable coating-material, it was incorporated as a suspension in Eudragit RS films, since inulin itself has no film-forming properties. Eudragit RS is a copolymer of acrylic and methacrylic acid esters with a low content of quaternary ammonium groups. It was chosen as film-former because it gives water-insoluble, pH-independent, low permeable films which are inert to endogenous digestive secretions and enzymes.

To prevent dissolution of inulin out of the film, inulin with a high degree of polymerization, thus with a low solubility, was used.

2. Materials and methods

2.1. Materials

Inulin with a high degree of polymerization (inulinHP with an average degree of polymerization of 23) was kindly donated by Tiense Suikerraffinaderij (Tienen, Belgium). Schaedler Broth was purchased from Gibco BRL (Life Technologies Inc., Gaithersburg, MD, USA). Eudragit RS 100 was supplied by Röhm Pharma (Darmstadt, Germany), dibutyl phtalate by Merck (Hohenbrunn, Germany) and acetyltriethyl citrate by Pfizer (New York, USA). Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared according to The US Pharmacopeia XXII (1990).

2.2. Methods

2.2.1. Preparation of isolated films

To a solution containing 5.00 g Eudragit RS in 25 ml chloroform, either 2.50 g inulinHP (FI, FIII) or 5.00 g inulinHP (FII) was added. Dibutyl phtalate (FI, FII) or acetyltriethyl citrate (FIII) was used as plasticizer (20% w/w of the dried Eudragit RS).

Of this suspension 1.5 ml was cast on a teflon-coated glass plate. To prevent too rapid solvent removal by convection at room temperature, the films were covered with a funnel. After complete evaporation of the solvent, the films were removed from the glass plate, dried to constant weight at 40°C and stored in a desiccator until use.

The weight of the isolated films was 0.415 g ($\pm$ 0.004) for film FI, 0.530 g ($\pm$ 0.020) for film FII and 0.442 g ($\pm$ 0.011) for film FIII. The thickness, measured with a micrometer (Lorentzen and Wetters, Van der Heyden, Brussels, Belgium) was 0.142 mm ($\pm$ 0.005) for film FI, 0.186 mm ($\pm$ 0.010) for film FII and 0.151 mm ($\pm$ 0.008) for film FIII. Composition and characteristics of the different films are summarized in Table 1.

2.2.2. In vitro bacterial degradation study (Van den Mooter et al., 1992)

A human faecal degradation medium was prepared by inoculating Schaedler Broth with freshly voided human faeces (50 g faeces in 100 ml medium). Schaedler Broth is a nutritious medium, especially employed in anaerobic microbiology. Before inoculation it was sterilized by autoclaving and purged with CO_2:H_2:N_2 (10:10:80). The faecal flora is representative of the flora of the large bowel.

The prepared films, as well as inulinHP powder, were incubated in the degradation medium. After 24 h, utilization of inulinHP was assessed by pH measurements (Sentron, Roden, The Netherlands) (Müller and Lier, 1994).

The permeability coefficients of the films were determined before and after incubation in the faecal medium for 8, 16 and 24 h. The control films were incubated in Schaedler Broth only.

Table 1
Composition and characteristics of the prepared films

Film	Composition film-forming suspension	Weight of the film (g)	Thickness of the film (mm)
FI	5.00 g Eudragit RS	0.415	0.142
	2.50 g InulinHP		
	1.00 g Dibutyl phtalate in 25 ml $CHCl_3$	(0.004)	(0.005)
FII	5.00 g Eudragit RS	0.530	0.186
	5.00 g InulinHP		
	1.00 g Dibutyl phtalate in 25 ml $CHCl_3$	(0.020)	(0.010)
FIII	5.00 g Eudragit RS	0.442	0.151
	2.50 g InulinHP		
	1.00 g Acetyltriethyl citrate in 25 ml $CHCl_3$	(0.011)	(0.008)

The preparation of the degradation medium and the degradation studies took place in the Anaerobic Workstation (DW Scientific, West Yorkshire, UK) at 37°C.

2.2.3. Permeability study of the isolated films

The permeability coefficient of the films was determined by measuring spectrophotometrically at 272 nm (HP8452A diode array spectrophotometer, Hewlett Packard Co., CA, USA) the amount of caffeine diffusing from the donor to the acceptor compartment, through the films pinched between the two compartments. The permeability coefficient was calculated using the quasisteady state conditions as discussed by Flynn et al. (1974):

$$\frac{2PS}{V}t = -\ln\frac{(C_o - 2C_a)}{C_o}$$

where P is the permeability coefficient, S is the surface area of the film through which diffusion takes place, V is the volume of the acceptor or donor compartment, t denotes time, C_o is the initial concentration of caffeine in the donor compartment and C_a is the concentration of caffeine in the acceptor compartment. P can be calculated from a plot of $-\ln(C_o - 2C_a)/C_o$ vs. time.

2.2.4. Gastro-intestinal resistance of the isolated films

A colon-specific coating-material has to resist the gastro-intestinal fluids to protect its load succesfully till the colon is reached. To investigate this resistance, the donor and acceptor compartment of the diffusion cell were composed of SGF, SIF and demineralized water. In each case the permeability coefficients for caffeine were determined.

2.3. Statistical analysis

In order to establish the significance of differences either a two- tailed, unpaired *t*-test or analysis of variance was used when appropriate.

Table 2
pH values of the human faecal degradation medium after an incubation time of 24 h. The pH of the medium at the start of the experiment was 6.51. The standard deviation is given in parentheses

A	B	C	D	E	F
6.06 (0.0058)	6.08 (0.017)	5.67 (0.015)	5.40 (0.021)	5.28 (0.017)	5.04 (0.015)

A = Blanc human faecal degradation medium.
B = Degradation medium + film without inulinHP.
C = Degradation medium + film containing 110 mg inulinHP (FI).
D = Degradation medium + 110 mg inulinHP powder.
E = Degradation medium + film containing 200 mg inulinHP (FII).
F = Degradation medium + 200 mg inulinHP powder.

Table 3
Permeability coefficients (P) for caffeine of the isolated films FI and FII after incubation in the degradation medium (test) and in Schaedler Broth (control). The standard deviation is given in parentheses. P is expressed in 10^{-6} cm/s

Film	Incubation time (h)	P control	P test
FI	0	0.713 (0.077)	
	8	1.376 (0.122)	1.972 (1.027)
	16	1.257 (0.164)	15.222 (7.321)
	24	1.566 (0.332)	49.585 (44.59)
FII	0	6.727 (0.732)	
	8	8.321 (0.520)	39.984 (15.98)
	16	13.717 (0.032)	94.684 (43.56)
	24	14.359 (2.072)	—

3. Results and discussion

3.1. pH measurements

The results of the pH measurements are given in Table 2. From these data it can be concluded that inulinHP is indeed degraded by the used human faecal medium, even when it is incorporated in a Eudragit RS film. Decrease of pH indicates the presence of degradation products such as lactic acid, acetic acid and other volatile fatty acids (Rasic and Kurmann, 1983).

Although inulinHP, incorporated in a film, is utilized to a lesser extent than the same amount of inulinHP powder, it still gives a significant ($P < 0.05$) lower pH value than the blanc degradation medium or the degradation medium containing a blanc film. A higher amount of inulinHP incorporated in the film, results in an increased degradation: when film FII is incubated in the faecal medium, a significant ($P < 0.05$) lower pH is obtained in comparison with film FI.

3.2. Permeability study

Table 3 shows the permeability coefficients for caffeine of the isolated films FI and FII after incubation in the faecal degradation medium (test) and in Schaedler Broth (control).

A significant increase ($P < 0.05$) in the permeability coefficient is detected after incubation in the faecal degradation medium, in comparison with the control medium, except for an incubation time of 8 h for film FI. This time period doesn't seem to be long enough for a significant degradation of the incorporated inulinHP.

Increasing the amount of inulinHP in the film, gives rise to an increase in P, not only for the test condition, but also for the control condition. Diffusion of inulinHP out of the film seems to be more pronounced in the case of film FII.

After 24 h in the faecal medium, inulinHP of film FII is degraded to such an extent that no permeability coefficient could be calculated anymore.

In a next step, isolated films (FIII) were prepared, containing the more hydrophilic plasticizer acetyltriethyl citrate instead of dibutyl phtalate. It was believed that the more hydrophilic films would make the inulinHP more accessible to bacterial degradation (Van den Mooter et al., 1993). The results of the permeability study of these films are summarized in Table 4. A significant increase in P is now not only detected after an incubation time of 16 or 24 h, but already after 8 hours. A comparison of the P-values of the test condition for film FIII with the P-values of the test condition for film FI indicates that FIII films tend to give higher P-values. Although it can not be proved statistically for all data points (because of the rather large standard deviations), it seems that the hydrophilicity of the films tends to influence the accessibility of the inulinHP to bacterial degradation.

Table 4
Permeability coefficients (P) for caffeine of the isolated film FIII after incubation in the degradation medium (test) and in Schaedler Broth (control). The standard deviation is given in parentheses. P is expressed in 10^{-6} cm/s

Incubation time (h)	P control	P test
0	0.881 (0.100)	
8	0.959 (0.0685)	5.962 (2.794)
16	1.024 (0.205)	20.222 (17.680)
24	1.154 (0.164)	72.566 (48.966)

Table 5
Permeability coefficients (P) for caffeine of the isolated films FI and FII when donor and acceptor compartment are composed of SGF, SIF and demineralized water. The standard deviation is given in parentheses. P is expressed in 10^{-6} cm/s

Film	SGF	SIF	Water
FI	0.670	0.697	0.713
	(0.0613)	(0.0811)	(0.0770)
FII	5.653	6.128	6.727
	(0.969)	(0.759)	(0.732)

3.3. Gastro-intestinal resistance of the isolated films

The permeability coefficients for caffeine of film FI and film FII, determined when donor and acceptor compartment were composed of SGF, SIF and demineralized water, are gathered in Table 5. The isolated films appear to withstand gastric and intestinal fluid, since there is no significant ($P < 0.05$) difference in the permeability coefficients of the three experimental conditions. The developed coatings can thus be considered as being pH-independent, as expected.

4. Conclusion

The results of this study indicate that inulinHP, incorporated in Eudragit RS films, can be degraded by the faecal microflora. Increasing the amount of incorporated inulinHP renders the films more permeable. When a more hydrophilic plasticizer is used, the accessibility of inulinHP to bacterial degradation seems to be increased. The films also resist gastric and intestinal fluid. These are all promising results in the evaluation of inulinHP as a candidate for the development of a colon-specific drug delivery form. Further improvement of the formulation is needed.

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