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Review

The use of polysaccharides to target drugs to the colon

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Abstract

Targeting pharmaceutical drugs to the colon makes it possible to guarantee local or systemic drug delivery to this site. To deliver the compounds in a non-degraded form to the last part of the gastrointestinal tract, they must first of all pass through the stomach, the upper part of the intestine and must use the characteristics of the colon to specifically release the drugs in this part of the digestive tract. Usual methods for the specific delivery of drugs to the colon are based on the chemical or technological modification of excipients. Among these, pH-dependent coatings or those degraded specifically by the colonic microflora make it possible to create dosage forms containing high levels of drugs compared to matrix or hydrogel systems. Nevertheless, inter- and intra-individual variations in gut pH and in transit time along the gastrointestinal tract can stand in the way of specific drug delivery.

To improve the specificity of drug release, certain types of polysaccharides can be used to create the dosage forms. These excipients are specifically degraded by the colonic microflora and have been used as polymer drug conjugates, coatings and matrix agents. However, most of these compounds are strongly hydrophilic leading to premature release. For these reasons, some polysaccharides, such as inulin, amylose, guar gum and pectins, have been chemically modified to increase their hydrophobicity or have been combined with other conventional hydrophobic polymers. This article reviews the potential uses of polysaccharides, the limits and the future developments in this field with these natural polymers.

Keywords: Colon; Polysaccharide; Pectin; Polymer; Specific drug delivery systems

1. Introduction

The colon, especially the first part of the lower intestine, can be liable to numerous pathological conditions, such as constipation, Crohn's disease, ulcerative colitis, carcinomas and infections. Recommended treatments include the administration of anti-inflammatory drugs, chemotherapy drugs and/or antibiotics, which must be released in the colon (Ashford & Fell, 1994). From a medical point of view, it is also useful to have dosage forms that are able to specifically release drugs, such as peptides, proteins (Bai, Chang & Guo, 1995; Watts & Illum, 1997), vermifuges and diagnostic agents, in the colon, due to the capacity of this part of the gastrointestinal tract to absorb these drugs. To achieve an optimum pharmacological action of these drugs, it is necessary to transport them in adequate concentrations and without any release before they reach the upper part of the colon. Since the early eighties, studies have been carried out by several companies and research groups in order to create dosage forms capable of specific drug delivery to this part of the gastrointestinal tract. Unfortunately, a lot of research developments have led to dosage forms that release the drugs before they reach the colon (Ashford & Fell, 1994).

With regard to the rectal route, the drugs do not always reach the specific sites of the colonic diseases and the sites of colonic absorption (Hardy, Lee, Clark & Reynolds, 1986; Wood, Wilson & Hardy, 1985). To reach the colon and to be able to specifically deliver and absorb the drug there, the dosage forms must be formulated taking into account the obstacles of the gastrointestinal tract. The various strategies developed to achieve this goal have used the specific characteristics of this organ, i.e. pH, microflora, enzymes, reducing medium and transit time. Nevertheless, these parameters can vary from one individual to the next and also according to the pathological condition and diet.

Before reaching the colon, the dosage forms must pass through the stomach (pH ~ 1.5–3.5), the duodenum (pH ~ 6), the intestine (pH ~ 5.5–6.8) and the caecum (pH 6.8–7.3) (Fig. 1, Table 1). In the colon, the pH ranges from 6.4 in the ascending colon to 7.0 in the descending colon (Fig. 2). The colon presents a reducing medium with a mean redox potential of −200 mV (Hovgaard & Brøndsted,

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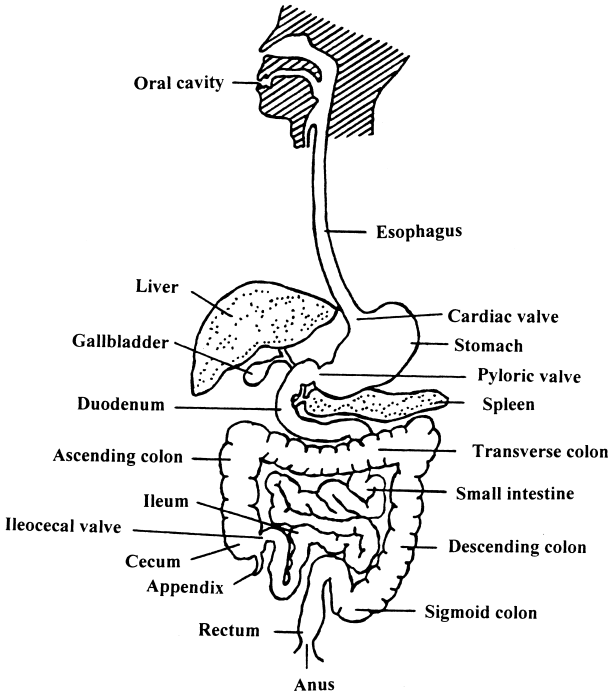


Fig. 1. Anatomy of the human gastrointestinal tract (Friend & Tozer, 1992).

Table 1
Summary of anatomical and physiological features of the small intestine and colon

Region of the gastrointestinal tract	Characteristic
Length (cm)	
Entire gastrointestinal tract	500–700
Small intestine	
Duodenum	20–30
Jejunum	150–250
Ileum	200–350
Large intestine	
Cecum	6–7
Ascending colon	20
Tranverse colon	45
Desending colon	30
Sigmoid colon	40
Rectum	12
Anal canal	3
Internal diameter (cm)	
Small intestine	3–4
Large intestine	6
pH	
Stomach	
Fasted	1.5–3
Fed	2–5
Small intestine	
Duodenum (fasted state)	≈ 6.1
Duodenum (fed state)	≈ 5.4
Ileum	≈ 7–8
Large intestine	
Cecum and colon	5.5–7
Rectum	≈ 7

1996). Taking inter- and intra-individual variations into account, these redox potentials can range from −100 down to −400 mV (Friend & Tozer, 1992; Schroder, Lewkonia & Price-Evans, 1973; Van der Mooter & Kinget, 1995). The relatively high value of the pH before and in the colon has led to the development and synthesis of polymers that must dissolve at pH > 7. These consist of copolymers of methacrylic acid, methylmethacrylate and ethylacrylate, such as Eudragit® (Fig. 3).

The colon is also characterised by the existence of microflora living in anaerobic conditions and the proliferation of *Clostridium*, *Bacteroïdes fecalis*, *Lactobacillus anaerobic* and *Bifidobacteria*. The colonic flora and its nutritional sources remain qualitatively similar from one individual to another but can change quantitatively from one individual to another (Table 2). The different factors influencing the gastrointestinal microflora are listed in Table 3. Oxygen is a limiting factor for the growth of colonic microflora (Friend & Tozer, 1992; Van der Mooter & Kinget, 1995), the metabolic activity of which can be affected by factors such as age, gastrointestinal diseases, the administration of drugs, the fermentation of food residues. All of these factors can lead to the inactivation of drugs or to the development of certain side effects. The bacterial colonic microflora is able to ferment a wide variety of non-absorbable disaccharides, oligosaccharides and polysaccharides. Moreover, non α-glucan polymers from natural sources, such as cellulose, hemicellulose and pectic compounds, can be fermented in the small intestine. The enzymes involved in the metabolism of the drugs or other compounds are, exclusively, certain types of reducing enzymes or enzymes involved in degradation by hydrolysis (Rubinstein & Radai, 1995). The presence and capacities of such enzymes have been used to specifically cleave certain types of drugs attached to another molecule or a polymer and then to develop the concept of prodrugs or conjugated polymers (Van der Mooter & Maris, 1997). The drugs are generally attached by a nitrogenous double bond. Cleavage of the drugs is achieved in the anaerobic and reducing medium by the azo-reductase enzyme and certain biomolecules, such as NADPH (Peppercorn & Goldman, 1972). The same concept has been also used to synthesise polymers with nitrogenous double bonds or disulfide bonds in the backbone (Schacht and Wilding, 1993; Saffran, Kumar, Savariar, Burnham, Williams & Neckers, 1986; Sintov, Ankol, Levy & Rubinstein, 1992). Using these polymers as coatings for dosage forms, they must also be specifically disintegrated by the action of the enzymes and the microflora. With respect to transit time in the colon, some trials conducted in humans have demonstrated that this time can range from 0.8 to more than 20 h. Based on the fact that the colon is the last part of the intestine, some authors (McNeil & Stevens, 1990) have developed dosage forms (Targit®, Time-Clock®, Pulsincap®) in which the release of the drug is time-dependent (Fig. 4).

All of these developments are subject to the risks related

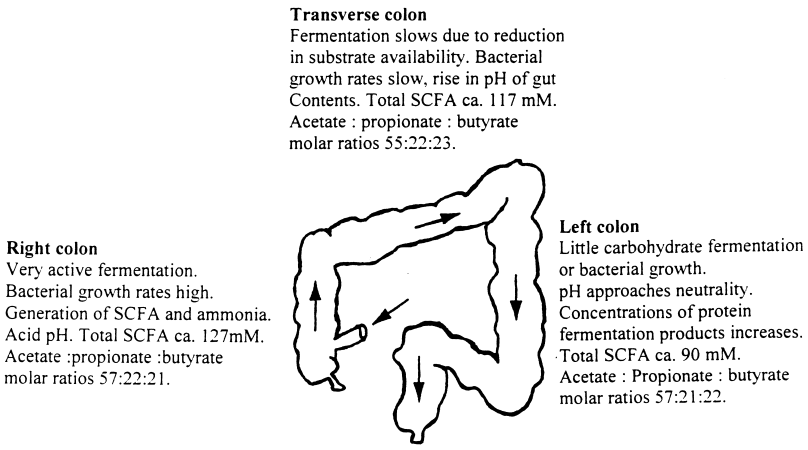


Fig. 2. Anatomy of the three parts of the colon (Macfarlane & Cummings, 1991).

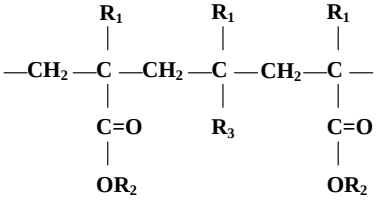
to the variability in parameters from one sick person to another. pH values, the reducing medium and the transit time can all lead to premature and non-specific drug delivery in the colon. Furthermore, azo-polymers have demonstrated some toxicity. All of these considerations have led current research developments to use polysaccharides, which are non-toxic and for which degradation is colon-specific.

2. Dosage forms based on the specific degradation of polysaccharides in the colon

2.1. The use of glycosides as excipients for colon drug delivery

Polysaccharides are hydrophilic, hydrosoluble and can form gels. A large number of them, along with conjugated glucuronides, are resistant to gastric and intestinal bacteria and are specifically hydrolysed by the colonic bacteria (Ashford & Fell, 1994; Cummings, Soutgate, Branch, Wiggins, Houston, Jenkins et al., 1979; Hovgaard and Brøndstedt, 1996; Scheline, 1973). Pectins (Englyst, Hay & Macfarlane, 1987; Salyers, West, Vercellotti & Wilkins, 1977a; Salyers, Vercellotti, West & Wilkins, 1977b), chitosan (Tozaki, Komioke, Tada, Maruyama, Terabe & Suzuki, 1997) and inulin are fermented by *Bifidobacteria* and *Bacteroïdes* (Salyers et al., 1977a,b). These polysaccharides show promising potential for specific drug delivery to the colon (Rubinstein, 1990; Rubinstein, Radai, Erza, Pathak & Rokem, 1993). Moreover, their intrinsic film-forming properties (Coffin and Fishman, 1993, 1994) mean that they can be used as coatings for pharmaceutical dosage forms. In addition to conventional dosage forms, such as tablets and capsules, Berliner and Nacht (1997a,b) have used coated delivery vehicles to encapsulate drugs, such as non-porous microspheres, microcapsules, non-crosslinked porous beads, rigid crosslinked polymeric beads or liposomes, for the effective delivery of an active substance to the colon. They suggested coating these dosage forms with a polysaccharide such as pectin. Van Loo and Frippiat (1998) also noticed that inulin (with a polymerisation degree of at least 20) can be used both as an active ingredient, and as a carrier with drugs or even prodrugs, for the prevention and/or treatment of colon cancer in humans. On the basis of their widespread use in the pharmaceutical industry as excipients and their non-toxic character, they can be investigated in this specific application.

For colonic administration, polysaccharides are used in



R_1 = -CH_3, R_2 = -CH_3 and R_3 = -COOH (Eudragit® L and S)
R_1 = -CH_3, R_2= -CH_2-CH_3 and R_3= -COOH (Eudragit® L100-55 and L30D-55)
R_1 = -CH_3, R_2 = -CH_3 and R_3 = -$COOCH_3$ (Eudragit® NE30D)
R_1 = -CH_3, R_2 = -CH_3 and R_3 = -$COOCH_2CH_2N^+(CH_3)_3Cl^-$ (Eudragit® RL and RS)

Fig. 3. Chemical structure of Eudragit®.

Table 2
The human gastrointestinal flora (Simon & Gorbach, 1983, 1984)

	Stomach	Jejunum	Ileum	Feces
Total bacterial count	$0–10^3$	$0–10^5$	$10^3–10^7$	$10^{12}–10^{12}$
Aerobic or facultative bacteria				
Enterobacteria	$0–10^2$	$0–10^3$	$10^2–10^6$	$10^4–10^{10}$
Streptococci	$0–10^3$	$0–10^4$	$10^2–10^6$	$10^5–10^{10}$
Staphylococci	$0–10^2$	$0–10^3$	$10^2–10^5$	$10^4–10^7$
Lactobacilli	$0–10^3$	$0–10^4$	$10^2–10^5$	$10^6–10^{10}$
Fungi	$0–10^2$	$0–10^2$	$10^2–10^3$	$10^2–10^6$
Anaerobic bacteria				
Bacteroides	Rare	$0–10^2$	$10^3–10^7$	$10^{10}–10^{12}$
Bifidobacteria	Rare	$0–10^3$	$10^3–10^5$	$10^8–10^{12}$
Gram-positive cocci	Rare	$0–10^3$	$10^2–10^5$	$10^8–10^{11}$
Clostridia	Rare	Rare	$10^2–10^4$	$10^6–10^{11}$
Eubacteria	Rare	Rare	Rare	$10^9–10^{12}$

dosage forms as (i) prodrugs, (ii) matrix systems and dry coatings by direct tableting and (iii) conventional coating agents (Edman, Kristensen & Wideholt, 1992).

2.2. Prodrugs

Polysaccharides are used as glucuronic prodrugs, which are specifically degraded by colonic β-glucuronidases (Haeberlin, Empey, Fedorak, Nolen & Friend, 1993), and glycosidic prodrugs, which are specifically degraded by colonic glycosidases (Friend & Chang, 1984a; 1985). The most widely used polysaccharide of this type is dextran (Hovgaard & Brøndstedt, 1996). The action of bacterial glycosidase enzymes on the glycosidic bond permits the release of the attached drug, then triggering its pharmacological activity (Ashford & Fell, 1994). Biolabile prodrug compounds are prepared from a polysaccharide with a molecular weight (M_w) ranging from 40,000 up to 5,000,000, selected from dextran, carboxymethyl dextran, diethylaminoethyl dextran, starch, hydroxyethyl starch, alginates, glycogen, pullullan, agarose, cellulose, chitosan, chitin and carrageenan. Following the oral administration of these prodrugs, the parent drug is selectively released in the terminal ileum and the colon over an extended period of time (Larsen, Johansen, Harboe, Kurtzhals & Oleser, 1989). Using β-cyclodextrins as carriers for 5-amino salicylic acid (5-ASA) in the form of prodrugs makes it possible to prevent the release of more than 2% of 5-ASA in simulated gastric and intestinal fluids. The active ingredient is released, in particular, by degradation of the prodrug by the colonic microflora (Bonsignore Loy, Fadda & Garau, 2000). For oligopeptides, this approach can only be investigated when they are not degraded by intestinal bacteria (Haeberlin et al., 1993). High levels of short chain fatty acids (SCFA), such as acetate, propionate and butyrate, can be targeted to have beneficial effects in the prevention of colonic disorders (rectal cancer, diverticulitis, colitis, diarrhoea and constipation) by using an ester covalently linking the SCFA to a carrier that is preferably a form of carbohydrate. The SCFA were protected by their link with the carbohydrate as they passed through the small intestine. The carbohydrates chosen for this application were digestible in the small intestine, such as digestible starch, which

Table 3
Factors influencing the gastrointestinal microflora (Rowland, 1988)

1. Host factors
a. Species, strain and individual differences due to:
Acid and alkali secretion
Intestinal motility
Intestinal structure
Levels of endogenous nutrients (mucin, gut proteins, bile secretions, sloughed mucosal cells)
Redox potential
Bile salts
Antibodies
b. Age
c. Gastrointestinal disorders

2. Environmental factors
a. Drugs
b. Diet
c. Xenobiotics

3. Bacterial factors
a. Bacterial metabolites (short chain fatty acids, bacteriocins)
b. Bacterial interactions (competition)
c. pH

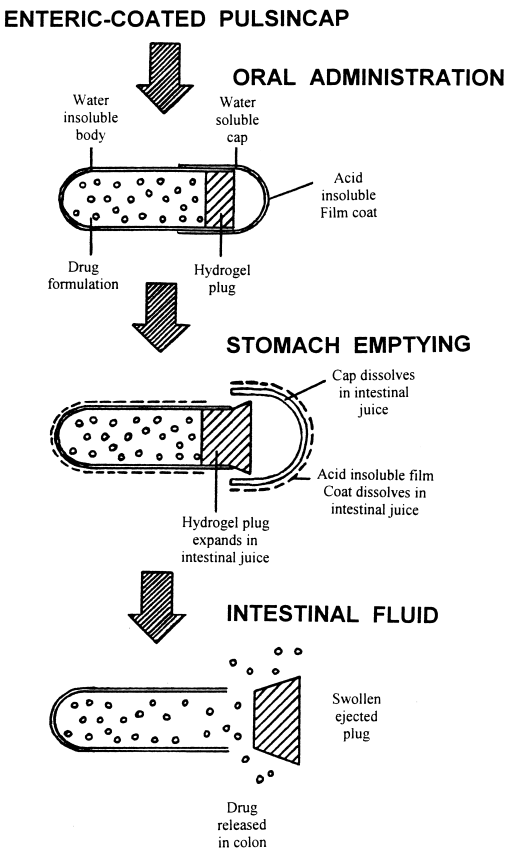


Fig. 4. Schematic representation of the Pulsincap™ system.

can also be protected from digestion in the small intestine by substitution (Anisson, Topping & Illman, 1995).

To permit the colon-specific drug delivery of corticosteroids, Friend and Chang (1984b) and Friend and Fedorak (1993) synthesised prodrugs. The sugar was linked to the corticosteroid through an ether or thioether linkage at the 21-position of the corticosteroid. This linkage is specifically degraded by the colonic bacteria to release the 'parent' corticosteroid.

2.3. Matrix systems and dry coatings by direct tableting

Sintov and Rubinstein (1991) and Sintov, Ankol, Levy and Rubinstein, (1993) produced matrices made of oligosaccharide, containing polymers such as chondroitin, modified chondroitin products (resulting from an equimolar reaction between chondroitin sulfate and 1,12 diaminododecane), pectic salts, acrylic oligosaccharide and raffinase crosslinked methacrylic copolymer, in which indomethacin was incorporated.

Friend and Wong (1996, 1997) prepared tablets containing a powdered mixture of drug, such as a corticosteroid, 5-ASA, a peptide or a stimulant laxative and a hydrocolloid gum obtainable from higher plants like locust bean gum, tragacanth gum or karaya gum. These tablets were able to guarantee the therapeutically effective release of the drug in the lower gastrointestinal tract, particularly the colon, following oral administration, without significant release of the drug in the upper gastrointestinal tract.

2.3.1. Pectins and guar gums

Pectin is a polygalacturonic acid and the chain molecule is negatively charged at neutral pH. The pK-value of pectin is approximately 3.5. The overall distribution of hydrophilic and hydrophobic groups on the pectin molecule determines the solubility (tendency to gel) of a particular pectin. Dry coatings using pectins have been investigated in several studies (Ashford, Fell, Attwood, Sharma & Woodhead, 1993, 1994). The degree of esterification of a high ester pectin influences the gelling properties. High methyl ester (methoxylation degree $> 50\%$) pectins, which are less soluble than low methyl ester (methoxylation degree $< 50\%$) pectins due to their low number of methyl ester groups, are more interesting (Ashford et al., 1993). The gelation of pectins makes it possible to reduce the penetration of water into the dosage form and hence the dissolution of the drug incorporated into it.

These dry coated dosage forms can reach the upper ascending colon 6–8 h after ingestion (Ashford & Fell, 1994). In vitro, the degradation of such coatings by labelled pectinolitic enzymes is faster than by *Bacteroïdes ovatus* (Wakerly, Fell & Attwood, 1996). To have sufficient protection, the coatings must be in the range of 700 mg–1 g for tablets weighing 120 mg before the coating process (Ashford et al., 1993).

Similar results have been observed with guar gum using indomethacin as a tracer in in vitro release studies and in human trials (Krishnaiah, Satyanarayana, Rama Prasad & Narasimha, 1998; Rubinstein & Gliko-Kabir, 1995). In these studies, 180–230 mg of dry coating was required to permit the specific delivery of the contents of an 80 mg tablet without coating to the colon (Krishnaiah et al., 1998). Although the application of a dry polysaccharide coating to a tablet makes it possible to produce dosage forms containing a large quantity of drug, the industrial application of this process remains difficult.

2.3.2. Polysaccharide mixtures

Pectins and chitosan, used as a mixture to be applied as a dry coating to pellets, demonstrate better protection of the drugs in the upper part of the digestive tract than pectins used alone. Chitosan is ionised at pH 1.1 and soluble at this pH, like pectins. The carboxylic groups of the pectins and the amino groups of the chitosan interact (optimal interaction for a weight of pectins/weight of chitosan ratio of 10:1 (Meshali & Gabr, 1993)) and reduce release of the drug. For ratios of 1:5 (weight of pellets/weight of coating), the in vitro release of paracetamol after 5 h is complete using pectins alone and is in the range of 20% using the mixture of both polysaccharides (Fernendez-Hervas & Fell, 1998). However, the drug is often released in vitro at pH 6, even in the absence of enzymes. It is likely that these dry coated dosage forms with a mixture of these polysaccharides cannot reach the colon in whole form.

A drug delivery system made with gelatin and polysaccharide degraded by the colonic microflora (for example: soluble pectinate, pectate, alginate, chondroitin sulfate, polygalacturonic acid, tragacanth gum, gum arabic and a mixture thereof) and with an aldehyde and/or a polyvalent metal and/or an additional polysaccharide (not degraded in the upper gut, such as dextran, amylose, arabinogalactan, arabinoxylan, cellulose, guar gum, pectin, starch, xylan and a mixture thereof) also makes it possible to deliver drugs to the colon (Lee, Lim, Lee & Pai, 1999).

2.3.3. Chemical modifications to polysaccharides to be used as coatings and matrix systems

To make polysaccharides less hydrophilic, hemisynthesis operations (acetylation and methylation) have been conducted on inulin (Damian, Van den Mooter, Samyn & Kignet, 1999), on chondroitin sulfate and these have been tested using indomethacin as the tracer in release studies (Rubinstein, Nakar & Sintov, 1992), on guar gum (Gliko-Kabir, Yagen, Penhasi & Rubinstein, 2000a,b; Vervoort, Vinckier, Moldenaers, Van den Mooter, Angustijns & Kignet, 1999) and also on pectins to create macromolecules with a lower solubility. All of these macromolecules can be crosslinked to reduce the release kinetics of the drug contained in the dosage forms.

2.3.3.1. Calcium pectinate. When the degree of esterification is less than 50%, pectins form rigid gels by

reacting with calcium salts or multivalent cations (Edman et al., 1992), which crosslink the galacturonic acids of the main polymer chains. Calcium pectinate is obtained by the formation of an ionic bond between the carboxylic acid groups of the pectin molecules and the calcium ions. The resulting structure has the form of an 'eggbox' (Grant, Morris, Rees, Smith & Thom, 1973). Stable in solutions with a low pH, they swell in solutions that are slightly basic. Calcium pectinate has been studied (Rubinstein and Gliko-Kabir, 1995; Rubinstein et al., 1993) and has been indexed as a hydrophilic coating agent which is insoluble when prepared according to the interfacial complexation process (Sriamornsak, Puttipatkhachorn & Prakongpan et al., 1997b). In this case, calcium pectinate prevents the encapsulated drug from being prematurely released.

In vitro studies on matrices created with calcium pectate have shown that, both in the case of pectinolytic enzymes and in experiments involving rat caecal content, indomethacin was released specifically and significantly more quickly in comparison with control studies. From these studies, it was also concluded that, by using various calcium (or other metal) pectic salts with different solubility properties as matrices, the rate of drug release could be controlled and adjusted (Sintov & Rubinstein, 1991).

Recently, droplets of gels made of calcium pectinate, prepared by ionic gelation, have been tested as carriers for the controlled delivery of indomethacin. These solid dosage forms released about 80% of the drug after 4 h (Sriamornsak, Prakongpan, Puttipatkhachorn & Kennedy, 1997a; Sriamornsak et al., 1997b) whereas the transit time from the mouth to the rectum is much longer (Davis, Hardy, Taylor, Whalley & Wilson, 1984; Davis, Hardy & Fara, 1986).

The number of methyl ester groups on the pectins and the calcium ion content inside the pectin layer influence the solubilization of the layer and the release kinetics of the drug (Sriamornsak & Nunthanid, 1999). Increasing the calcium content led to a higher degree of crosslinking and aggregation; however, an excess of calcium ions results in a phenomenon of pre-gelation (Wakerly, Fell, Attwood & Parkins, 1997) and can increase the release kinetics of the drug.

Using these kinds of systems, the release kinetics of a drug like paracetamol are of zero order. For matrix systems without calcium ions, the release of the drug is faster when the matrix is composed of pectins with a high degree of amidation. 90% of drug was released from this system within 6 h using amidated pectins with a substitution degree of 24% whereas only 8 h are required to release the same concentration of drug using pectins with a substitution degree of 13%. The presence of calcium ions decreased the release of the drug using low methyl ester pectins (90% of drug released within 6 h without calcium and 80% of drug released within 6 h with calcium) but increased the release of the drug with high methyl ester pectins (85% of drug released within 8 h when the pectins did not contain any calcium ions whereas 95% of drug released within 4 h in the presence of calcium ions (Wakerly et al., 1997)).

Another factor influencing the release of a drug is hydration of the matrix system. The release of the drug is due to two phenomena: erosion of the coating and diffusion. When the gel layer is thick, the diffusional pathway is important, which reduces the drug release. In addition, the calcium pectinate coating is even more sensitive to the erosion phenomenon if the ionic strength of the gel is low. While a gel resulting from low methyl ester pectins crosslinked with calcium ions is not eroded, high methyl ester pectins become hydrated in the presence of calcium ions and the matrix is eroded. However, the pre-gelation of high methyl ester and low methyl ester pectins can lead to the matrix breaking and erosion. In those cases, the drug before it can reach the colon.

According to several authors (Semdé, 1999; Wakerly et al., 1997), the optimum calcium ions ranges from 15 to 30 mg/g of pectin for low methyl ester pectins. The inclusion of amylose in calcium pectinate systems makes it possible to reduce the release kinetics of some drugs in comparison with calcium systems composed of calcium pectinate alone. When amylose is incorporated in a matrix constituted of a tracer like indomethacin and calcium pectinate, the release of indomethacin decreases from 55 to 40% after 5 h at pH 7.4. Conversely, the inclusion of dextran led to more rapid release of the tracer over the first 3 h than when the amylose was incorporated into the calcium pectinate. After 5 h, the incorporation of dextran and amylose led to similar levels of tracer release (Munjeri, Collect & Fell, 1997a,b). From this study, it was also concluded that tracer release is even greater when it is more soluble. The more rapid release of the tracer from this system in the presence of pectinolytic enzymes proves that the polysaccharides are still accessible and degraded by the enzymes (Munjeri et al., 1997a,b).

2.3.3.2. Calcium alginate. Some dosage forms made with alginate (Haug, Larsen & Smidsord, 1963) and calcium alginate (Murata, Nakada, Miyamoto, Kawashima & Seo, 1993) have been studied. Like pectins, alginic acid used in the form of calcium alginate is hydrolysed by colonic bacteria.

In vitro, dosage forms made using calcium pectinate released a tracer more rapidly than the same dosage forms made with calcium alginate and a mixture of calcium pectinate–alginate. In vitro, the latter began to release the tracer from a pH 5.4 and released 100% of the tracer between 4 and 10 h after ingestion. Studies on the degree of crosslinking would also appear to be useful to target the release of the drug contained in the dosage forms (Pillay & Fassihi, 1999a,b; Pillay, Dangor, Govender, Moopanar & Hurbans, 1998a,b).

2.3.3.3. Hydrogels based on polysaccharides. Like pectins and alginate, dextran and amylose are crosslinked by

calcium ions (Shefer, Kost & Langer, 1992). If treated by hemisynthesis, dextran can also form hydrogels (Larsen, Jensen & Olesen, 1991). The degree of crosslinking and swelling of hydrogels made of dextran are the two parameters governing the degradation kinetics (Brøndsted, Hovgaard & Simonsen, 1995a; Chiu, Hsiue, Lee & Huang, 1999).

In vitro, dextran with a molecular weight of 70,000 Da delayed the release of hydrophobic drugs at pH 5.4 (Brøndsted, Hovgaard & Simonsen, 1995b; Brøndsted, Andersen & Hovgaard, 1998) and also of proteins (Hennink, Franssen, Van Dijk Wolthuis & Talsma, 1997). Brøndsted and Hovgaard (1994) created drug delivery systems composed of a polymer matrix and a drug contained in or surrounded by the matrix. The specific characteristic of these systems was that the polymer matrix was made of dextranase degradable polymer (dextran or modified dextran) and a crosslinking agent providing network linkage between the polymer chains. For hydrocortisone, for example, at pH 5.4 and in the presence of dextranase, drug release from a hydrogel made of dry dextran went up from 100% after 1 h (crosslinking: 4 mol%) to 100% after 2.5 h (crosslinking: 11 mol%) (Brøndsted et al., 1995b). Due to the fact that dextranases are only present in the colon, the polymer matrix can only be degraded by dextranases once the dosage forms reach the colon.

Studies conducted in vitro have demonstrated the potential value of hydrogels based on dextran to guarantee the colonic release of a large number of drugs (Brøndsted et al., 1998). Similar results have been observed with guar gum crosslinked with borate ions (Rubinstein and Gliko-Kabir, 1995). When the dissolution medium was deprived of enzymes, crosslinking guar gum with trisodium trimetaphosphate enabled the release of only 20% of hydrocortisone. On the contrary, when it contained enzymes, hydrocortisone was entirely released. Studies on the formation of hydrogels based on inulin are currently under way (Maris, Van den Mooter, Samyn & Kignet, 2000; Vervoort, Van den Mooter, Augustijns, Busson, Toppet & Kinget, 1997; Vervoort et al., 1999).

In vitro studies have demonstrated that the release rate of indomethacin from a crosslinked chondroitin was lower than that of plain chondroitin used as a control. When examined in the presence of caecal homogenate, 54% of the drug was released from the untreated carrier. Comparable studies using a medium, which did not contain any homogenate, demonstrated a different release profile. When the treated chondroitin was examined, 32.5% of indomethacin was released after 60 min in a buffer. In a parallel experiment, 67.8% of indomethacin was released from non-treated chondroitin in the same buffer (Sintov & Rubinstein, 1991).

In order to transport probiotic micro-organisms, such as *Bifidobacterium* to the large bowel, Brown, Mc Naught, Granly, Conway, Evans, Topping et al. (1996), proposed using a modified or unmodified resistant starch (hydroxy propylated, acetylated, octenyl succinated, carboxymethylated or succinated starch) as a carrier, particularly a high amylose starch, which acts as a growth or maintenance medium for micro-organisms in the large bowel.

2.4. Conventional coatings and matrices: glycoside–polymer combinations

Watanabe, Kawai, Katsuma and Fukui (1995) described a drug delivery system consisting of a drug coated with an organic acid-soluble macromolecular substance and a saccharide, which rapidly generated an organic acid due to the action of enteric bacteria in the lower gastrointestinal tract. These dosage forms can be coated with an enteric macromolecular substance to enhance colon specificity. Busetti and Olgitati (1998) patented a multilayer coating system composed of a layer of a swellable pH-dependent polymer (comprising a hydrophilic gelling polymer selected from methylcellulose and its derivatives and also methacrylate copolymers, polysaccharides and mixtures thereof), surrounded by an outer enteric layer that is gastro-resistant and dissolves at pH levels above 4.5.

To improve the poor mechanical properties and film forming of polygalacturonic chains, Lehmann and Dreher (1991) added polymethacrylate copolymers to glycosides used as coating agents. For this reason, Eudragit® RS 30D has been added to β-cyclodextrins (Siefke, Weckenmann & Bauer, 1993).

The incorporation of pectins in aqueous dispersions of ethylcellulose has been suggested to target the release of drugs in the colon (Vervoort & Kinget, 1996; Villar-Lopez, Otero-Espinar & Blanco-Mendez, 2000). The integrity of such coatings can be better controlled by preventing excessively rapid swelling and the solubilization of pectins during transit of the dosage form from the mouth to the caecum. The drug is released due to bacterial enzymatic degradation of the polysaccharides making up the coatings, leading to the creation of pores or even to the disintegration of the film coatings during colonic transit. Due to the fact that the permeability of the coating increases with the proportion of the polysaccharides, it is essential to add low concentrations of these compounds. These combinations can be made of polysaccharide–polymer mixtures but also of multilayer systems.

2.4.1. Amylose–ethylcellulose aqueous dispersion coatings

Amylose is degraded by colonic bacteria and is resistant to, among others things, pancreatic enzymes. At body temperature, the amorphous amylose is in the vitreous state and is insoluble in gastric and duodenal juices. The use of amylose as macromolecules to construct matrix systems or coatings led to swelling of the systems and, hence, to premature drug release (Ring, Archer, Allwood & Newton, 1991). Combination with ethylcellulose makes it possible to control this swelling and release of the drug (Milojevic, Newton, Cummings, Gibson, Botham, Ring et al., 1993). Delayed-release dosage forms have been

prepared using an active substance mixed with amorphous amylose, the mixture being coated with an inner coating of amorphous amylose and an outer coating of a film-forming cellulose or acrylic polymeric material or a mixed coating composed of amylose and a film-forming cellulose or acrylic polymeric material (Ring et al., 1991). Allwood, Archer and Ring (1989) also suggested applying the same concept using vitreous amylose as matrix and coating agents. A combination of a complex such as butan-1-ol amylose and an aqueous dispersion of ethylcellulose (Ethocel®) at a ratio of 1:4 (w/w) has been used to coat pellets containing salicylic acid (Milojevic, Newton, Cummings, Gibson, Botham, Ring et al., 1996a,b) and glucose with Eudragit® RS and RL. Some in vitro studies and others conducted in humans (Cummings, Milojevic, Harding, Coward, Gibson, Botham et al., 1996) have demonstrated that glucose was released in the colon using this kind of coating. Recently, Newton and Siew (1999) developed a coating system made of amylose and a water-insoluble film-forming polymer (in the weight ratios 3:2 and 2:3) using an organic solvent, while Newton and Leong (1999) patented a similar system, using a mixture of an amylose-alcohol complex and ethylcellulose, to coat tablets. The interesting in vitro results need to be confirmed in vivo.

2.4.2. Inulin–Eudragit® RS coatings

Coatings made of inulin with a mean polymerization degree of 24 mixed with Eudragit® RS (Fig. 3) are resistant to gastric and intestinal juices and are effectively degraded by the colonic flora (Vervoort & Kinget, 1996). Inulin can therefore be used to lead to specific release of drugs in the colon. The bacterial degradation of films led to a fall in pH in the micro-environment due to the generation of lactic and acetic acids and other volatile fatty acids (Rasic & Kurmann, 1983). The plasticizers used to make these coatings are dibutylphthalate and acetyltriethylcitrate at a concentration of 20% (w/w) of the dry mixture. The hydrophilic character of the plasticizer increases the accessibility of the inulin for the colonic flora. Improvements still need to be made to this composition for better control of drug release at this site (Vervoort & Kinget, 1996).

2.4.3. Multilayer coatings of chitosan–Eudragit® L and S

Pellets containing chitosan and sodium diclofenac and coated with Eudragit® L or S (Fig. 3) make it possible to release the drug at pH 7.4 with zero order kinetics. The slope of the straight line for release is dependent on the solid dosage form/Eudragit® mass ratio and the type of Eudragit® used. In similar proportions (mass of pellets/mass of Eudragit®: 1:5), in vitro, Eudragit® S makes it possible to release 80% of the drug within 16 h while Eudragit® L releases 100% within 12 h (Lorenzo-Lamosa & Remuñán-Lopez, 1998). In vivo, a film coating made of chitosan/Eudragit® L makes it possible to release the drug in the colon of beagles (Suzuki, Hashiudo, Matsumoto & Fujii, 1991).

2.4.4. Guar gum–Eudragit® L and RL coatings

Guar gum is a gelling polysaccharide that is degraded by the colonic bacteria. However, the release of drugs coated with guar gum begins slowly in the small intestine. The gums have been mixed with methacrylate copolymers (Eudragit® L or Eudragit® RL) in order to coat the tablets. In vitro studies on drug release in the presence of glycosidic enzymes (Watts & Illum, 1997) did not reveal any specific drug release.

2.4.5. Combination of pectins with polymers

2.4.5.1. Pectin/hydroxypropylmethylcellulose matrices. Matrix tablets have been prepared by direct tableting from mixtures of high methyl ester pectins/hydroxypropylmethylcellulose (Kim & Fassihi, 1997a,b) and from mixtures of high methyl ester pectins/hydroxypropylmethylcellulose/gelatin (Kim & Fassihi, 1997c). Thick layers of coatings made of high methyl ester pectins by direct tableting have made it possible to considerably delay the release of tracers from the tablets in dissolution media.

Studies on conventional coatings have been conducted using pectin/chitosan/hydroxypropylmethylcellulose mixtures. Used as tablet coatings, these are also able to delay drug release before it is completely degraded in the colon. However, it has been demonstrated that the release of the drugs from these formulations begins in the small intestine in humans (Macleod, Fell & Collett, 1999a; Macleod, Fell, Collett, Sharma & Smith, 1999b).

2.4.5.2. Pectin–ethylcellulose aqueous dispersion coatings. The first studies have been conducted with pectin/ethylcellulose aqueous dispersion mixtures (Surelease® — formulations containing 40–60% pectins) to coat paracetamol pellets. With this type of coating, 5–30% of the paracetamol was released within 6 h at pH 7.4 in a dissolution medium without enzymes. In the presence of pectinolytic enzymes, the release of the drug increased (Macleod, Fell & Collett, 1997; Wakerly, Fell, Attwood & Parkins, 1996) and this was even more marked when the coatings contained high proportions of pectins. In vitro trials have confirmed that increasing the coating layer reduced drug release (Wakerly et al., 1996). Thus, ethylcellulose reduces the hydrosolubility of a coating containing pectins and increases the impermeability of the coating. Stability studies have shown that coalescence of the film coating no longer occurred following the coating process. The techniques used to apply the film coating to the dosage forms on the one hand affect the release kinetics of the drug and, on the other, the stability of this type of dosage form (Arwidsson, Hjelstuen, Ingasen & Graffner, 1991; Lippold, Lippold, Sutter & Gunder, 1990; Parikh, Portery & Rohera, 1993).

Finally, the high methyl ester pectin concentrations in coatings that also contain Aquacoat® ECD 30 must be

between 0 and 20% (w/w) (Bodmeier & Paeratakul, 1994; Hutchings, Clarson & Sakr, 1994; Obara & Mc Ginity, 1994, 1995) to give the film coatings certain interesting mechanical properties (Macleod et al., 1997). On the basis of previously obtained results, studies have been conducted with high methyl ester pectins and calcium pectinate combined with Aquacoat® ECD 30 or Surelease®. These film coatings contained 5, 10 and 15% (w/w) of pectins or 10% (w/w) of calcium pectinate and dibutyl sebacate and triacetine as plasticizers (Semdé, 1999; Semdé, Amighi, Pierre, Devleeschouwer & Moës, 1998). The film coatings containing high methyl ester pectins and Aquacoat® ECD 30 released at least 80% of the pectins within 1 h in a dissolution medium without pectinolytic enzymes at pH 7.5, whatever the pectin concentration and irrespective of the presence or absence of calcium ions. On the basis of the results obtained with these film-coating compositions, it was concluded that these were not suitable for targeting drugs to the colon.

The nature of the ethylcellulose aqueous dispersion also affects the release kinetics of pectins (Semdé, 1999; Semdé et al., 1998). For film coatings containing Surelease®, pectin release in a dissolution medium without pectinolytic enzymes depends on the high methyl ester pectin concentration. Film coatings containing 5% pectins released about 50% of the total pectin quantity after 4 h at pH 4.5. Film coatings containing 10% pectins released 80% of the total quantity after 1 h. Semdé (1999) and Semdé et al. (1998) have also suggested that the pectin concentration released by a film-coating composition containing 5 and 10% probably depends on the porosity of the film coating, which is lower in a composition containing only 5%.

When high methyl ester pectins or calcium pectinate are dispersed in a molecular state in a polymeric film coating, these can also diffuse through the film coating (Semdé, 1999). The diffusion rate depends on the molecular size of the pectins or on the calcium pectinate and the mesh size of the other polymer(s) (ethylcellulose or acrylic polymers) composing the film coating and the type of aqueous dispersion. The calcium pectinate molecules (low methyl ester pectins + calcium) are less hydrophilic and bigger than the low methyl ester pectins not crosslinked with calcium. Consequently, they are released more slowly by the various film coatings.

2.4.5.3. Pectin–Eudragit® coatings. Matrix systems containing calcium pectinate (mixture of calcium pectinate/pectins and calcium pectinate/guar gum) have been evaluated in vivo (Adkin, Kenyon, Lerner, Landau, Strauss, Caron et al., 1997). The resulting tablets have been coated with an enteric acrylic polymer such as Eudragit® L. The disintegration of all the tablets started in the last part of the ileum and continued in the colon. These dosage forms were not suitable for the specific release of hydrophilic drugs in the colon, since they are released before reaching the colon.

In the absence of pectinolytic enzymes, a reduction in the release kinetics of pectins from film coatings made of Eudragit® NE 30 D and 10% calcium pectinate was observed. Comparison of the same film coatings containing 5–10% (w/w) low methyl ester or high methyl ester pectins without calcium made it possible to conclude that this can be attributed to the presence of calcium (30–60 mg Ca/g of pectin). The release delay depends on the calcium/pectin ratio and appeared to be optimal when Eudragit® NE 30D is combined with calcium pectinate at a concentration of 30 mg Ca/g of pectins (Semdé, 1999; Semdé et al., 1998). Macromolecules of pectins leave the polymer coating via a diffusion mechanism and create pores. Similarly, drugs of low molecular weight are known to diffuse through Aquacoat® ECD 30, Eudragit® RS 30D and RL 30D polymeric film coatings (Semdé, 1999).

With respect to films containing Eudragit® RS, pectin release was slow and remained constant for about 8 h (~10% of pectins released) for films containing 5 and 10% high methyl ester pectins and 10% low methyl ester pectins. Release was more rapid when the film coatings contained 15% high methyl ester pectins (40% of pectins released after 8 h) and almost complete in less than 1 h with calcium pectinate (low methyl ester pectins + calcium). These results can be attributed to the fact that the quaternary ammonium groups are able to interact with carboxylic groups of high methyl ester pectins to form a pectin–Eudragit® RS complex that prevents the release of pectins. The existence of these interactions is also demonstrated with a film coating composed of a mixture of calcium pectinate and Eudragit® RS. In this case, almost all the pectins were released in under an hour. The addition of 30 mg Ca/g to low methyl ester pectins seemed sufficient to prevent any interaction between the pectins and Eudragit® RS 30D. As far as pectin release is concerned, film coatings made of Eudragit® RS 30D and calcium pectinate with a ratio of 30 mg Ca/g of pectins demonstrated similar performances as those of film coatings composed of high methyl ester pectins or calcium pectinate with Aquacoat® ECD 30, Surelease® or Eudragit® NE 30D.

The release kinetics of pectin from isolated film coatings showed that only combinations of Eudragit® RS 30D with high methyl ester pectins or low methyl ester pectins (10% (w/w)) were able to possess the two essential properties (gastrointestinal resistance and degradation/dissolution in the colon) to guarantee specific drug delivery to the colon.

Study of the release kinetics of pectins from film coatings containing Eudragit® RS 30D in a buffer solution containing pectinolytic enzymes has shown that the release kinetics of pectins and their release-rate are governed by the pectin content of the film coatings. Film coatings containing 5% high methyl ester pectins are not permeable enough to the pectinolytic enzymes, leading to a significant reduction in pectin release from film coatings incubated with and without pectinolytic enzymes. Combination of high methyl ester pectins or

calcium pectinate with insoluble polymers did not lead to specific drug release in the colon. However, a pectin that is an anionic polymer is able to form an insoluble complex with a cationic polymer such as Eudragit® RL, which is not degraded by the enzymes of the colonic flora. The best solute release kinetics in the presence or in the absence of enzymes are obtained for film coatings composed of 10–15% (w/w) pectins and Eudragit® RL. The incorporation of a sufficiently high ratio of this type of complex in film coatings that also contain insoluble and flexible polymers such as Eudragit® NE 30 D makes it possible to obtain an increase in drug release following the action of pectinolytic enzymes. Indeed, since the pectin–Eudragit® RL complex incorporated is insoluble, the film coating will be less permeable to the drug in the absence of pectinolytic enzymes. Enzymatic degradation and the consecutive release of pectin from the film coating will lead to the destruction of the complex, allowing the Eudragit® RL to regain its original physicochemical properties. The polymer will then be able to solvate, to swell and to increase release of the drug (Semdé et al., 1999; Semdé, Amighi, Pierre, Devleeschouwer & Moës, 2000a). With respect to isolated films made of either pectins + Eudragit® RS 30D or pectins + Eudragit® NE 30D, the action of pectinolytic enzymes made it possible to decrease the release kinetics of theophylline through the isolated films compared with the release kinetics without enzymes. Indeed, spaces created by enzymatic degradation are filled due to reorganisation of the polymer macromolecules (Semdé, Amighi, Pierre, Devleeschouwer & Moës, 2000b).

When Eudragit® RL or Eudragit® RS and high methyl ester pectins are mixed together, the addition of pectin led to flocculation and caking of the latex even occurred when the pectin concentration reached 5% (w/w).

The viscosity of the mixture increased with the addition of 0–5% high methyl ester pectins. Beyond a concentration of 5% (w/w) of high methyl ester pectins, the viscosity of the mixture decreased. This phenomenon can be explained by the gradual neutralisation of the positive charges present on the surface of particles of Eudragit® RL 30D. The polymer is probably in a flocculated state, with the formation of weak aggregates, responsible for the large increase in viscosity.

3. Conclusion and prospects

Dosage forms designed to specifically release drugs in the colon have been the subject of numerous studies. Coated forms are easier to produce than matrix systems. The toxicity of the degradation products of azo-polymers restricts their use. On the one hand, dosage forms based on a single degradation method do not permit targeted release. Indeed, the pH and transit time can vary depending on the individual and the particular disease. Drug release can be premature or even non-existent in these cases.

On the other hand, dosage forms based on the use of polysaccharides would appear to be promising. Firstly, the polysaccharides described above and their degradation products are non-toxic and are already used as pharmaceutical excipients. Secondly, the colonic flora does not appear to present many modifications and remains qualitatively similar from one individual to another. Chemical modifications made to polysaccharides make it possible to reduce release of the drugs in the gut. However, the kinetics of degradation and of solute release from hydrogels depends on numerous parameters and on the nature of the drugs. For these reasons, the formulation of a hydrogel designed to permit specific drug delivery to the colon, is dependent on the physicochemical characteristics of the drugs incorporated into the dosage forms.

Combinations of polysaccharides and polymers that are either insoluble or soluble at colonic pH have been tested. These combinations are based on the erosion and swelling of film coatings all along the gastrointestinal tract and degradation of polysaccharides in the colon. Whilst some in vitro studies with Eudragit® would appear to be promising, more in-depth investigation is still required. Moreover, in vivo studies must be carried out to confirm the interesting results obtained in vitro with these combined polymers.

It would appear that the mechanisms of degradation of film coatings designed for specific drug delivery in the colon must be multiple if this aim is to be achieved. The combination of degradation mechanisms based on pH, flora and the reducing medium for a single film coating are the broad directions to be taken by future research when looking at ways of creating film coatings guaranteeing optimum release of the content of dosage forms.

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