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METHODS USED TO DEVELOP MUCOADHESIVE DRUG DELIVERY SYSTEMS: BIOADHESION IN THE GASTROINTESTINAL TRACT

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Introduction

The term "adhesion" is defined by the physicist as "the molecular force of attraction in the area of contact between unlike bodies that acts to hold them together" (Webster, 1989). "*Bio*adhesion" refers to adhesive phenomena where at least one of the adherents is of biological nature (Kaelbe and Moacanin, 1977). The materials are attached to each other by interfacial forces for an "extended" period of time (Gu, Robinson and Leung, 1988; Duchêne, Touchard and Peppas, 1988). The term "mucoadhesion" is often employed when the biological substrate is a mucosal surface (Robinson, 1990).

In nature, the adhesion of living beings to surfaces is often an imperative for survival, keeping the organism at a favourable site and avoiding its being transferred to a less favourable environment. The numerous forms of symbiosis generally require some sort of permanent attachment of one organism to the other. The attachment of micro-organisms to the gut is one good example of biological adhesion between two organisms, either symbiotic or infectious. These interactions are often mediated by bacterial adhesins, lectin-like, carbohydrate-binding proteins, expressed on the bacterial surface which bind specifically to sugar residues of mucins or other carbohydrates of the host cell surface (Beachey, 1980; Boedecker, 1984; Mergenhagen and Rosan, 1985; Hörstedt, Danielsson, Nyhlin, Stenling and Suhr, 1989). Although these are all examples of "biological adhesion" the term bioadhesion has been used mainly to describe adhesive phenomena related to the ability of some synthetic and biological macromolecules as well as hydrocolloids to adhere to biological tissues for therapeutic purposes in medicine (Kaelbe and Moacanin, 1977; Peppas and Buri, 1985). The adhesion mechanisms involved are usually a combination of physical and chemical phenomena.

The present review deals with bioadhesion aimed at improving drug therapy using man-made materials as adhesives, focusing on oral drug administration. Bioadhesive systems have been used for many years for medical applications other than drug delivery in the area of dentistry for denture adhesives (Wright, 1981; Hollingsbee and Timmins, 1990), stoma adhesives such as karaya gum in Stomahesive® or synthetic polypectins (Winkler, 1986) and for surgical applications such as the cyanoacrylates used as "surgical glue" (Wang, 1974, Harper and Ralson, 1983). It is only about 15 years ago that pharmaceutical researchers began to explore more intensively the possible applications of bioadhesion with the aim of improving drug treatment.

Oral administration of drugs

Local treatment of diseases can be unsatisfactory, because the drug may not stay at the site of action long enough for the desired effect (typically eye, mouth or vaginal cavity). Similarly systemic treatment via the oral route can be hampered because the drug may not stay at the site of absorption long enough. Oral administration of drugs is the most popular route of administration for both health care personnel and patients. Taking pharmaceutical dosage forms via the mouth is generally well accepted. It can easily be undertaken almost anywhere and it is generally safe. In contrast, invasive methods (e.g. injection) usually require the assistance of trained personnel and the procedure always involves certain risks.

Oral drug administration begins with ingestion of the dosage form through the mouth. From there it passes down the oesophagus and into the stomach. Once released, little drug is absorbed from the stomach because of its relatively small surface area, particularly in the case of cationic drugs which will be mainly ionised in the acidic conditions of the stomach. It has been recognized that the major site of drug absorption is the small intestine. Its large surface area (approximately 100 m^2) makes it very efficient for the uptake of solutes (Bowman and Rand, 1980). Theoretically, drug absorption can occur along the entire length of the small intestine, nevertheless the majority of drugs are actually absorbed from the proximal small intestine (Booth, 1967). However, if the drug is poorly soluble or is in the form of a controlled release dosage form, significant absorption of the drug may also occur in the large intestine (Davis, 1989), despite its limited surface area. More recent studies with a once-a-day preparation (e.g. theophylline) have shown that therapeutic drug levels can be maintained for periods of up to 24 h (Gruber, Longer and Robinson, 1987) even though these systems are expected to have emptied from the stomach, passed through the small intestine and have arrived at the ileocaecal junction after 4–12 h (Davis, 1985). Oral drug delivery ceases eventually with faecal excretion of any unabsorbed drug.

Absorption enhancement in Oral Drug Delivery Systems

Low appearance of the drug in the systemic circulation (bioavailability) can be due to (i) rapid transit of the drug-containing delivery system past the ideal absorption site, (ii) rapid degradation of the drug in the gastrointestinal tract once it has been released (peptide drugs) and, (iii) low transmucosal permeability due to size, ionisation, solubility or other characteristics of the drug molecule. There are several strategies that have been proposed in an attempt to overcome some of these problems, for example delaying the transit of a pharmaceutical dosage form within the gastrointestinal tract. This approach will be beneficial for drugs which are only poorly absorbed from selected regions of the small intestine and whose bioavailability is believed to be dependent on the residence time of the dosage form at or upstream of its small intestinal absorption window. Such a drug is hydrochlorothiazide (Beerman, Groschinsky-Grind and Rosen, 1976; Lynch, Pownall and Taylor, 1987). It is particularly important in the case of controlled release drug delivery systems (DDS), designed to release drugs over extended periods of time (e.g. 12–24 h). Once in the colon, these DDS may be delivering a proportion of drug to a non-optimal site for absorption (Davis, 1985). An ideal oral sustained release dosage form should be comparable to an intravenous infusion, which continuously supplies the amount of drug needed to maintain constant plasma levels once steady state is reached (Förster and Lippold, 1982)

Proposed strategies to delay gastrointestinal transit

Various attempts have been made to delay gastrointestinal transit. These have involved pharmacological and physiological as well as pharmaceutical approaches. Pharmacological means involve the co-administration or incorporation into the dosage form of a drug that delays gastrointestinal emptying such as antimuscarinics, e.g. propantheline, which are relaxants of the smooth muscle (Beermann and Groschinsky-Grind, 1978; Manninen, Apajalahti, Melin and Karesoja, 1973) or a drug that changes motility, e.g opiate analgesics or derivatives such as loperamide, (Minami and McCallum, 1984). However, the potential side effects that may occur from such treatments on a routine basis would not be acceptable for regulatory approval.

The use of passage-delaying excipients which activate a physiological mechanism to delay gastrointestinal transit has also been proposed. This may involve stimulation of the duodenal or jejunal receptors using a fat derivative like triethanolamine myristate (Gröning and Heun, 1984, 1989) or the use of large amounts of a polymer like polycarbophil that may induce a volume related fed-like state (Harris, Fell,

Sharma and Taylor, 1990) and delay gastric emptying due to a blocking effect. In fact, in some of the studies involving so called bioadhesive polymers it is not quite clear whether the gastric retention shown in animal models is due to this, or due to true adhesion, or a combination of phenomena (Russel and Bass, 1985; Harris, Fell, Taylor, Lynch and Sharma, 1989, 1990; Harris *et al.*, 1990).

Pharmacological and physiological approaches set out to delay gastrointestinal transit by modification of the rate of gastric emptying with passage-delaying agents. The pharmaceutical strategies instead attempt to achieve the same objective by actually retaining the dosage form at or upstream of its absorption site for as long as possible by virtue of its special physical or physicochemical characteristics.

Size-related retention of a dosage form in the stomach has been studied with various systems. If large enough, the formulation would not be expelled from the stomach even when the pyloric sphincter is in its non-contracted state. Their size has to increase after ingestion to such an extent that gastric emptying is totally inhibited (Moës, 1993). According to Moës uncommon delivery systems such as swelling balloon hydrogels (Park and Park, 1987) or unfolding stratified medicated polymer sheets (BE Patent No. 867, 692) or non-erodible or erodible tetrahedron shaped devices (Cargill, Caldwell, Engle, Fix, Porter, and Gardner, 1988; Cargill, Engle, Gardner, Porter, Sparer and Fix, 1989) have never passed beyond the experimental stage and clinical data are unavailable. In any case these gastric retention devices may not be safe. The hazard of lodging in the oesophagus (Kikendall, Friedman, Oyewole, Fleischer and Johnson, 1983; Al-Dujaili, Salole and Florence, 1983; Wilson, 1990) or permanent retention in the stomach with cumulative effects (Brahams, 1984; Vere, 1984) could lead to life-threatening problems.

Another approach uses dosage forms of moderately high density, based on the premise that high density formulations remain in the stomach longer than conventional formulations since they would be localised in the lower part of the antrum provided the density exceeds that of the normal stomach contents, i.e. > 1.4 g/cm^3 (Bechgaard and Ladefoged, 1978). The effectiveness of this approach has not been confirmed on a broad basis and the evidence remains controversial (Moës, 1993).

A further retention principle is based on the use of buoyant dosage forms which float on the gastric contents as a result of their relatively low density. Floating dosage forms have been discussed extensively by Moës (1993) in his recent review, of which we will give a summary here. The first floating dosage forms (F forms) were described by Sheth and Tossounian (1984). These F forms, called "hydrodynamically balanced systems" (HBS), maintain their low density while the polymer hydrates and builds a gelled barrier at the outer surface. Research at Hoffmann-LaRoche has led to various patents for floating drug delivery systems and *in vivo* studies on diazepam HBS capsules such as Valium® CR and Valrelease®

and the L-dopa plus benserazide containing formulation Madopar® HBS (Prolopa® HBS). Numerous other rather sophisticated buoyancy mechanisms have been invented too. In his critical review Moës clarified the conflicting views on the gastric retention capabilities of floating systems resulting from a number of *in vivo* trials by different authors (Müller-Lissner and Blum, 1981; Davis, Stockwell, Taylor, Hardy, Whalley, Wilson, Bechgaard and Christensen, 1986; Timmermans and Moës, 1990; Timmermans, 1991; Kaus, 1987; Sangekar, Vadino, Chaudry, Parr, Beihn and Digenis, 1987; Lippold and Günther, 1991). It was concluded that amongst the various strategies proposed for prolongation of gastric residence time of oral DDS, floating delivery systems offer the best protection against early and random gastric emptying to date. In order to achieve adequate floating of the dosage form, it should be administered after a meal and the patient should not lie down after dosing.

The retention of a formulation via bioadhesion has been another strategy aiming at retention of dosage forms at or upstream of their absorption site via attachment to the mucous surfaces lining the gastrointestinal tract ("indirect bioadhesion"). It has been proposed that a mucoadhesive polymer could act as drug carrier and adhesive, thus retaining the delivery system either in the stomach or in the small intestine.

The greatest challenge for the concept of bioadhesion would be the successful delivery of orally administered proteins and peptide drugs. These endogenous compounds regulate numerous endocrine and other physiological processes in the human body. They are highly active drugs with a low toxicity, if dosed correctly. Recent advances in recombinant DNA-technology and production technology have made it possible to produce oligopeptides on a large scale. Insulin, growth hormones and interferons, amongst others can be used broadly for therapeutic and diagnostic purposes. Despite the advances and achievements in gene technology, therapeutic use of these substances still remains difficult (with a few exceptions, e.g. insulin). Due to their low absorption characteristics and instability (enzymatic degradation and biotransformation) parenteral application of peptides is usually unavoidable (Junginger and Verhoef, 1992).

We can talk of "direct bioadhesion" when very intimate contact to the absorbing epithelial tissue occurs and an increased concentration gradient is achieved. A decrease in diffusion path from the oral DDS to the absorbing biological membrane is an additional advantage for improving absorption particularly in intestinal delivery of peptide drugs. At the same time this would prevent previous dilution and possible degradation in the luminal fluids (Hayton, 1980). The addition of penetration enhancers to an adhering dosage form could enable alteration of membrane permeability. Inclusion of specific enzyme inhibitors could prevent early degradation of the peptide (Wearly, 1991; Junginger and Verhoef, 1992) and consequently increase bioavailability.

The development of gastrointestinal bioadhesive drug delivery systems

Gastrointestinal retention of dosage forms through adhesion to the mucosa has been studied for over a decade now, mainly *in vitro* or *ex vivo* with few *in situ* or *in vivo* studies and even fewer trials in man (Table 18.1). Despite the fact that bioadhesion, or more specifically mucoadhesion, has led to some success in drug delivery for ocular, buccal, nasal, [transdermal[1]], vaginal and cervical applications (Chen and Cyr, 1970; Schor, Davis, Nigalaye and Bolton, 1983; Nagai, Nishimoto, Nambu, Suzuki and Sekine, 1984; Nagai, 1986; Duchêne *et al.*, 1988; Greaves and Wilson, 1993; Smart, 1993; Bouckaert, Temmerman, Dhont and Remon, 1994), gastrointestinal mucoadhesive drug delivery systems have yet to be established (Helliwell, 1993). There follows below

(i) A summary of the physiological aspects of the gastrointestinal tract that have to be considered in an attempt to evaluate the potential of bioadhesion; and
(ii) A general overview of the different methods that have been used to study mucoadhesion outlining the particular difficulties in finding a suitable method capable of giving meaningful and relevant data.

THE SUBSTRATE: ADHERENT MUCUS AND ITS MUCINS

Mucus, known for its viscoelastic properties, its stickiness and the characteristic *Spinnbarkeit*[2] contains between 95%–99.5% water. Its most important polymeric, gel-forming component is the mucus glycoprotein or mucin (0.5%–5%) (Carlstedt and Sheehan, 1988; Neutra and Forstner, 1987; Gibbons, 1972). In the gastrointestinal tract, mucus is secreted by specialized cells as a polymer of high molecular weight. Unlike other gastrointestinal secretions, it adheres to the mucosal epithelial surfaces as a water insoluble gel until degradation and erosion takes place (Allen, 1989). Chemical analysis of the mucus gives evidence of a rather heterogeneous material which also contains small amounts of a variety of proteins, lipids, bacteria, sloughed-off epithelial cells and in some cases nucleic acids (Creeth, 1978). It becomes clear that mucoadhesion is a process that involves large amounts of water, or more vividly, it could be seen as "adhesion to water in a semisolid form" where the mucins play a key role in maintaining the gel-like properties of

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[1] The transdermal drug delivery systems such as the skin adhesives are bioadhesive systems in a wider sense and not discussed here. Currently commercially available products are Estraderm® TTS (estradiol), Scopoderm® TTS (scopolamine), Nicotinell® TTS (nicotine) and Deponit T® (glyceroltrinitrate)

[2] Spinnbarkeit: the inherent ability of the material to form a thread

the substrate for a potential drug delivery platform. Mucin displays considerable heterogeneity that has been well described (e.g. Carlstedt and Sheehan, 1984; Neutra and Forstner, 1987; Allen, 1989; Sheehan and Carlstedt, 1989; Harding 1989). The size and shape of the molecules (macrostructure) as well as their biochemical composition and assembly (microstructure) may vary considerably, however, there are some common features. Mucins are large molecules with molecular weights ranging from 0.5×10^6 to over 4×10^6 g/mol. They contain high amounts of carbohydrate (for gastrointestinal mucins 70%–80% carbohydrate, 12%–25% protein and up to ~5% ester sulphate). Undegraded mucins are made up of multiples of a basic unit (M~400,000–500,00), linked together to give linear arrays (Silberberg and Mayer, 1982). The basic unit is made from a single chain polypeptide backbone with two distinct regions: (i) a heavily glycosylated polypeptide chain rich in serine, threonine and proline, to which a large number of carbohydrate side chains are attached, followed by (ii) one or two terminal peptide segments that bear very little or no carbohydrate side chains and are therefore often referred to as "naked protein sections". The carbohydrate chains may contain up to five different monosaccharides, namely D-galactose, L-fucose, N-acetylglucosamine, N-acetylgalactosamine and sialic acid. As multi-branched oligosaccharides they are covalently attached via O-glycosidic linkages from N-acetylgalactosamine to serine and threonine residues of the protein core. The absence of uronic acid and only trace amounts of mannose (<1%) distinguish mucin glycoproteins from the proteoglycans of connective tissue and serum glycoproteins, respectively. Sialic acid residues which belong to a family of acidic sugars (Schauer, 1992) (in gastrointestinal mucins usually either N-acetyl or N-glycollyl-neuraminic acid) are usually in a terminal position on the carbohydrate chain, whereas ester sulphate residues occur in a more internal position, e.g. as N-acetylglucosamine-6-sulphate in pig gastric mucus (Allen, 1978; Slomiany and Meyer, 1972). They both contribute in giving the molecule a net negative charge, thought to be of importance in interactions with polycationic materials (Lehr, Bouwstra, Schacht and Junginger, 1992b; Fiebrig, Harding and Davis, 1994). The heavily glycosylated regions ("T-domains"), resistant to proteolysis, have often been referred to as "bottle brush regions" in analogy to the bristles (carbohydrate side chains) attached to a wire (central protein core). They are linked linearly to one another via the "naked" regions into "subunits" ($M \sim 2.5\times10^6$) and then further by disulphide bridging into the mucin macrostructure, which is able to form a gel.

Secretion and Function of Mucus

The adherent mucus layer in the gastrointestinal tract is secreted by specialised cells. They are the surface epithelial cells found mostly in the stomach but also in other parts of the gut and the goblet cells of the small and large intestine, glands in

the stomach as well as Brunner's glands in the duodenum (Neutra and Forstner, 1987; Allen, 1989; Ito, 1981). We distinguish two kinds of mucus relevant to the concept of mucoadhesion. Firstly the aforementioned water insoluble mucus gel lining the mucosa of the gastrointestinal tract which forms the target substrate and has a variable thickness, 50–450 μm, in man and about half that in the rat (Allen, 1978; Kerss, Allen and Garner, 1982). Secondly the soluble, often viscous mucus found in the luminal contents (Allen and Carroll, 1985). Soluble mucus may result from direct secretion, erosion of the adherent gel by proteases or through mechanical shear (Allen, 1989). It is believed that the adherent mucus layer plays a major role in protection of the delicate underlying epithelium against the various endogenous and exogenous insults, such as acidic pH (providing an "unstirred boundary layer"), digestive enzymes (pepsin), pathogens (bacteria) and abrasion, while the soluble mucus may play an important role in acting as a lubricant for ingested food. The requirement for such a protective adherent gel layer becomes obvious since from a physiological point of view the luminal side of the gastrointestinal tract can still be considered as the outer side of the body. These and other aspects regarding the function of mucus have been extensively described by various authors e.g. Allen (1981, 1983, 1989), Silberberg and Meyer (1982) and Bhaskar, Garik, Turner, Bradley, Bansil, Stanley and Lamont (1992).

THE ADHESIVE: ANIONIC, CATIONIC AND NEUTRAL POLYMERS

The general properties a mucoadhesive polymer for oral drug delivery should possess are:

- High molecular weight;
- Good wettability and spreading;
- No undesirable pharmacological or physiological actions or toxicity;
- Capacity for high drug loading;
- Economical.

For bioadhesion to occur, an intimate contact between the adhesive and the substrate (mucus) is a prerequisite, where factors like good wettability and spreading as well as hydration are important (Huntsberger, 1967; Chen and Cyr, 1970; Peppas and Buri, 1985). During the establishment of the adhesive bond the total surface energy between the two materials is diminished, destroying two free surfaces and creating a new interface. Intimate contact is achieved when the spreading coefficient (S) for one of the two phases is positive and wetting of the substrate by the adhesive has occurred (Kaelbe and Maocanin, 1977; Leung and Robinson, 1988; Boddé, 1990). The spreading coefficient (S) represents the difference between the "work of adhesion" (W_a) and the "work of cohesion" (W_c) of one of the two phases

$$S = W_a - W_c \quad (1)$$

and $S \geq 0$ favours wetting of the substrate by the adhesive (Junginger, 1991).This first step is believed to be followed by physical or mechanical bond formation obtained by deposition and inclusion of the adhesive material in the crevices of the mucus and chain entanglement between polymer chains of both phases (also referred to as inter-diffusion) (Boddé, 1990; Jabbari, Wisniewski and Peppas, 1993). Lehr, Bouwstra, Speis, Onderwater, van het Noordeinde, Vermeij-Keers, van Munsteren and Junginger (1992c) have used electron microscopy in an attempt to visualize intermixing between a polyacrylic acid derivative (polycarbophil) and mucus. They were unable to observe intermixing in the micron range but did not exclude this phenomenon for the nanometre range. Sufficient chain flexibility is required to form secondary chemical bonds such as van der Waals forces as well as hydrogen bonding (Leung and Robinson, 1988; Duchêne *et al.*, 1988). The formation of primary (covalent) chemical bonds is important in hard tissue adhesion in orthopaedics and dentistry. However, for mucoadhesion, chemical reactions of this type have not been considered so far, since a long term attachment is not required (Peppas and Buri, 1985).

Polymers with hydroxyl or carboxyl groups on their surface had been recognised as the most desirable candidates for bioadhesion, rather than polymers with other functional groups or cations (Peppas and Buri, 1985). The polyacrylic acid derivatives polycarbophil (Carbopol® EX-55) and carbomer (Carbopol® 934) have been by far the most studied mucoadhesive polymers to date (Table 18.3). Both materials are polyanionic and interaction with mucus has largely been attributed to chain entanglement of the polymer chains with mucin as a result of swelling in water and hydrogen bonding due to the carboxyl groups in their unionised state at low pH (Robinson, Longer and Veillard, 1987; Leung and Robinson, 1988; Ponchel, Touchard, Duchêne and Peppas, 1988; Jabbari *et al.*, 1993). Polycarbophil is described as a water insoluble but swellable polymer of polyacrylic acid crosslinked with divinylglycol used in the treatment of diarrhoea and as a bulk laxative. Carbomer is a water soluble polymer of acrylic acid loosely crosslinked with allylsucrose.

According to Lehr *et al.* (1992b), the need for hydrogen-bonding capabilities and negative charge in bioadhesive materials should not be generalized. They suggested that polycationic polymers might interact with the anionic sites on the mucins more favourably due to their opposite charges providing additional molecular attraction forces. For example, interactions between charged polymeric molecules have been employed in colloid titration (Terayama, 1952; Senju, 1969). The method is based on the principle that positively charged macromolecules will react with negatively charged macromolecules. The neutralisation reaction will proceed stoichiometrically, allowing an estimation of either material if a standard colloid solution is used. Katayama, Takai, Kariyama and Kanemasa (1978) used the method

for the titration of heparin using cat-floc (polydiallyldimethyl ammonium chloride) as standard polycation. Van Damme, Blackwell, Murphy and Preston (1992) measured the negative charge content in cartilage using cat-floc as well. Interactions between alginates and pectins with cationic polypeptides such as poly(L-lysine) and poly(Lys-Lys-Ala) have been studied using circular dichroism (Bystricky, Malovíková and Sticzay, 1990). Differences in interaction efficiency between the polymers were attributed to differences in conformational flexibility of the polyanionic chains in solution. Takahashi, Takayama, Machida and Nagai (1990) studied the characteristics of polyion complexes of chitosan with sodium alginate and sodium polyacrylate using viscometry and Fourier transform infra-red spectroscopy (FT-IR). They found that chitosan and alginate reacted with a defined binding ratio which was found to be relatively constant in media of various pH values. In contrast, for polyacrylate–chitosan interactions the unit molecular binding ratio was greatly affected by the pH.

Chitosan appears to be an ideal candidate as a mucoadhesive polycationic polymer — it is being produced on a large scale (Jeuniaux, Voss-Fouchart, Poulicek and Bussers, 1989; Alimuniar and Zainuddin, 1992). Although chitosan has not received regulatory approval by the Food and Drug Administration (FDA) for pharmaceutical and alimentary use, chitosan containing material obtained from the treatment of the waste streams of food processing plants, may be used as livestock feed in the U.S.A. as long as the level of chitosan does not exceed 0.1% (Weiner, 1992).

Chitosan has been approved as a food additive in Japan since 1983 and has been placed on the Japanese Natural Additive List. It is used as a thickener and stabilizer (Weiner, 1992). It is a food ingredient in some dietary cookies and noodles from Hihon Kayaku Inc. and Tanami Foods Inc. as well as in vinegars of Nakano Inc., making use of the hypocholesterolaemic properties (Hirano, 1989). The food industry has also exploited the chelating properties of chitosan for the clarification of beverages such as apple and carrot juices (Imeri and Knorr, 1988; Soto Peralta, Müller and Knoll, 1989).

The lack of acute oral toxicity of chitosan has been supported by experiments in mice (Arai, Kinumaki and Fujita, 1968) who determined an LD50 of > 10g/kg. However the literature lacks adequate scientific studies on long term and widespread human exposure through food and pharmaceutical products (McCurdy, 1992).

Variations in molecular weight and degree of deacetylation together with the ability to form gels and films allow flexibility in formulation design (Acatürk, 1989; Miyayaki, Yamaguchi, Takada, Hou, Takeichi and Yasabuchi, 1990; Errington, Harding, Vårum and Illum, 1993). Chitosan has been shown to possess mucoadhesive properties almost as strong as poly(acrylic acid) (Lehr *et al.*, 1992b). Various types of chitosan have been screened for their mucoadhesive properties together with some other polymers (see Table 18.1). Sedimentation velocity

experiments on an analytical ultracentrifuge revealed a complex formation between chitosan (Sea Cure +210) and purified pig gastric mucin (Fiebrig *et al.*, 1994), Table 18.2.

Table 18.1 SURVEY OF MUCOADHESIVE PROPERTIES OF VARIOUS POLYMERS REPRODUCED FROM LEHR *ET AL.* (1992b). MUCOADHESION WAS DETERMINED AS THE FORCE OF DETACHMENT FOR POLYMER-COATED COVER GLASSES FROM PIG INTESTINAL MUCOSA IN ISOTONIC SALINE. THE DRY FILMS WERE SWOLLEN IN THE TEST MEDIUM FOR 5 min AND THEN BROUGHT INTO CONTACT WITH THE TISSUE UNDER VERY SLIGHT PRESSURE (~ 10 mN) AND KEPT IN THIS POSITION FOR AN ADDITIONAL 1 min. A VERTICAL FORCE WAS SLOWLY INCREASED UNTIL THE POLYMER BECAME DETACHED. INDICATED IS THE MEAN (SD) FORCE OF DETACHMENT OF 2 TO 6 MEASUREMENTS.

Polymer	*Force of detachment (mN/cm^2)*
Cationic polymers	
Chitosan (Wella 'low viscosity')	3.9 (1.2)
Chitosan (Wella 'high viscosity')	6.7 (0.7)
Chitosan (Dr. Knapezyk)	5.7 (1.1)
Daichitosan H	8.0 (5.7)
Daichitosan VH	9.5 (2.4)
Sea Cure 240	4.1 (2.9)
Sea Cure 210 +	9.5 (2.5)
Chitosan (Sigma)	6.6 (3.0)
Polycarbophil/Daichitosan VH blend	11.9 (2.5)
DEAE-dextran	0
Aminodextran	0
Non-ionic polymers	
Steroglucan	2.8 (2.8)
HE-starch	0.6 (0.8)
HPC	0
Anionic polymers	
CMC (low viscosity)	1.8 (1.1)
CMC (medium viscosity)	0.3 (0.3)
CMC (high viscosity)	1.3 (1.0)
Pectin	0
Xanthan gum	0
Polycarbophil	17.6 (3.6)

TABLE 18.2 SEDIMENTATION COEFFICIENTS ($s_{20°C,BUFFER}$ ± STANDARD DEVIATION) IN SVEDBERG UNITS, S ARE GIVEN FOR MUCIN AT RESPECTIVE CONCENTRATIONS OF 0.2 mg/ml AND 1 mg/ml IN ACETATE BUFFER pH 4.5, I=0.1 AS WELL AS FOR THEIR COMPLEX RESULTING FROM ADMIXTURE OF THE TWO COMPONENTS. INCREASES IN s-VALUES ARE INDICATIVE OF CONCOMITANT INCREASE IN MOLECULAR WEIGHTS AS A RESULT OF ASSOCIATION.

Mixture ($s_{20,b}$ *for complex)*	*Control* ($s_{20,b}$ *for mucin)*	*Control* ($s_{20,b}$ *for chitosan)*
780.00 (24.00)	52.50 (1.50)	2.10 (0.01)

Is mucus an appropriate target?

There are three physiological aspects which remain critical for the concept of gastrointestinal mucoadhesion: (i) turnover of the adherent mucus layer, (ii) interactions of the formulation with soluble, i.e. non-adherent mucus prior to adhesion and (iii) gastrointestinal motility.

TURNOVER OF THE ADHERENT MUCUS LAYER

Adherent mucus is continuously lost into the gastrointestinal lumen by proteolysis and mechanical sloughing (e.g. Allen, 1981; Allen and Caroll, 1985). The latter, caused by the ingested food and its digestion, is thought to be the major cause of loss of gastric mucin (Waldron-Edward, 1977). A dynamic balance exists at the mucosal surface *in vivo*, between mucus secretion and mucus erosion. Mucus erosion, either by pepsin or by abrasion, must be replenished by the mucosal secretion of new material in order to maintain a protective function (Allen, Flemström, Garner and Kivilaakso, 1993). The difficulties in measuring mucus secretions *in vivo* has been outlined by Allen (1989). Studies on the turnover time of intestinal mucus gel layer in the rat *in situ* loop (Poelma and Tukker, 1987) by Lehr, Poelma, Junginger and Tukker (1991) have attempted to shed some light on the limitations to gastrointestinal mucoadhesion. In view of these authors the maximal residence time of a bioadhesive DDS at the site of adhesion would be limited by the time it takes for the mucus gel layer to be renewed as determined by the steady state of synthesis, secretion and degradation of the mucins (Allen, 1981). Although their estimate for the mucus turnover time is relatively crude (47–270 min), it is interesting to find that this time scale is similar to the mean residence time found for mucoadhesive microspheres (94±18 min) in earlier experiments using the same

animal model (rat). Further to this it had been observed that stimulating the mucus output by perfusion with 10 mM sodium taurocholate led to a significant shortening of the mean residence time of microspheres. Of even greater interest is the observation that the microspheres did not become detached from dead mucosal tissue *in vitro* when the system was stirred for more than 18 h. This leads to a further consideration; that of choosing the appropriate model substrate. This is discussed below. Although mucus turnover in an *in situ* isolated gut loop in the rat (which has undergone surgery and has been removed from its normal function) might be quite different from mucus turnover in healthy humans or patients, this physiological factor could substantially limit potential adhesion to the adherent mucus in the gastrointestinal tract.

INHIBITORY INTERACTIONS WITH SOLUBLE MUCINS

Any formulation entering the gastrointestinal tract is likely to interact with soluble mucins of the lumen. This in turn might render the device incapable of adhering to the target surface. That this might indeed be a serious problem has been shown recently by Lehr, Bouwstra, Kok, Noach, de Boer and Junginger (1992d). Tomato lectin, that specifically binds to isolated pig enterocytes and monolayers of human Caco-2 cell cultures, was proposed as a favourable candidate for specific bioadhesion to epithelial cells of the gastrointestinal tract. However, binding was also noticed to occur with crude pig gastric mucus. This would mean that *in vivo* the adhesion of lectins to the mucosal surface may be inhibited by mucus. Fiebrig *et al.* (1994) showed marked interaction between dilute solutions of highly purified pig gastric mucin and the potentially bioadhesive polymer chitosan (SeaCure+210, characterized by Errington *et al.*, 1993), using analytical ultracentrifugation.

In a further study, human gastric mucin, purified from gastric aspirates, showed a similar interaction. The mucin with a weight average molecular weight of $M=(1.8\pm0.1)\times10^6$ g/mol as determined by GPC/MALLS and a sialic acid content of 3.2% (for methods see Fiebrig *et al.*, 1994) at a concentration of 0.3 mg/ml sedimented at a rate of (11.0±0.18) S in an acetate buffer of pH 4.0 and at a temperature of 20°C (Figure 18.1). When mixed with chitosan at a concentration of 1.0 mg/ml a sedimenting species of (222.0±1.8) S was detected (Figure 18.2), corresponding to a complex between mucin and chitosan. Using chitosan alone as control, under the same conditions of concentration, solvent and temperature, a sedimentation coefficient of (2.1±0.03) S was obtained (Table 18.2), a value close to that expected for this macromolecule.

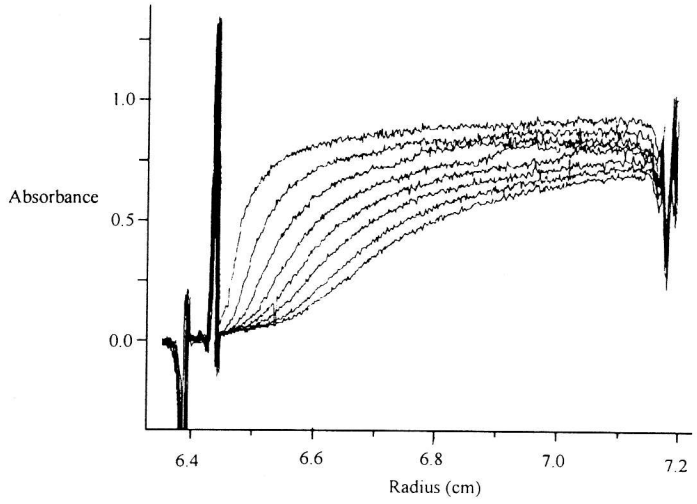

Figure 18.1 Human gastric mucin (control) at a concentration of 0.3 mg/ml in acetate buffer pH 4.5. Scans taken at 20°C, 20,000 rpm and a scanning interval of 11 min with absorption optics at a wavelength of λ = 226 nm using an XL-A Beckman Analytical Ultracentrifuge. Sedimentation coefficient $s_{20^{\circ}C,\,buffer}$ = (11.0±0.18) S

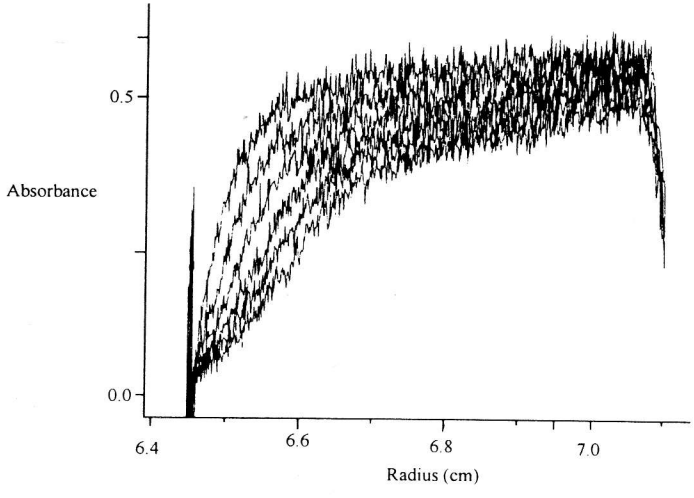

Figure 18.2 Human gastric mucin/chitosan mixture (0.3/1.0 mg/ml) in acetate buffer pH 4.5. Scans taken at 20°C, 7,000 rpm and a scanning interval of 6 min with absorption optics at a wavelength of λ = 256 nm using an XL-A Beckman Analytical Ultracentrifuge. Sedimentation coefficient $s_{20^{\circ}C,\,buffer}$ = (222.0±1.8) S

GASTROINTESTINAL MOTILITY

Gastrointestinal motility patterns and in particular the so called "housekeeper wave" which involves strong gastrointestinal contractions, serves as a cleaning

mechanism to clear all indigestible materials, including non-disintegrating dosage forms, from the stomach or proximal intestine (Code and Marlett, 1975; Grundy, 1985; Leung and Robinson, 1988). Thus, a good oral mucoadhesive drug delivery system needs to overcome the cleaning action of the "housekeeper wave" and remain in the stomach or proximal small intestine.

Experimental approaches used to study mucoadhesion

MODEL MUCUS' AND MUCINS FOR *IN VITRO* EXPERIMENTATION

In vitro test methods can be divided into (i) those that employ freshly excised tissue from various animals (frog, rat, rabbit, pig, cow, etc.) used either immediately as live or dead tissue or stored frozen and defrosted prior to use and (ii) those methods using mucus or mucin at various degrees of degradation and purity either solubilised or as gel usually from pig stomach or bovine submaxillary glands (Table 18.3). Whatever model material is used, its relevance to the human mucus, whether in health or disease state, has to be considered (MacAdam, 1993). Dead mucosal tissue may not produce any new mucus while degradation of existing mucus will still take place. This will have a marked effect on the rheological characteristics of the substrate, considered to be highly relevant to adhesion phenomena. Mucus thickness may vary from species to species and intersubject as well as intrasubject variability of the mucosal tissue poses problems in terms of reproducibility. Mucin carbohydrate composition varies within the gastrointestinal tract (Allen, 1989). Pig gastric mucin appears to be a good model mucus glycoprotein for the human because its carbohydrate composition is comparable. The content of sialic acid is similar to that of the small intestine in humans and pig mucus is readily available in reasonable quantities from freshly slaughtered pigs. Purification methods allow the removal of other components present in mucus in order to obtain purified mucin which still shows the gel-forming characteristics of native mucus (Bell, Sellers, Allen, Morris, Ross-Murphy, 1985; Allen, 1989).

Commercially available pig gastric mucins or mucus (Table 18.3) are quite different in their composition when compared with freshly prepared and purified material and they may be rather degraded or the freeze drying procedure may have altered the structure in such a way that it becomes difficult to redissolve them completely. Commercially available submaxillary mucins are quite different from the mucins secreted in the gastrointestinal tract. They are secreted in a viscous soluble form rather than as water-insoluble gels (for differences see Gottschalk, Bhargava and Murty, 1972). Nevertheless highly purified mucins can give more accurate information on the actual interaction with the main mucin-forming component. The use of dilute mucin solutions allows the study of mucin–bioadhesive polymer interactions on a fundamental level.

It has been recognized that the degree of hydration of the bioadhesive DDS as well as the amount of water available plays an important role in determining the strength of adhesion or whether adhesion can take place at all (Leung and Robinson, 1988; Chen and Cyr, 1970). The hydration aspect can be controlled in local applications such as mouth or vagina by drying excess water immediately prior to application (Deasy and O'Neill, 1989). The amounts of fluid available are also limited. In the gastrointestinal tract, however, excess water at the site of adhesion as well as excess in the amount of surrounding liquid cannot be controlled. Lehr, Bouwstra, Schacht and Junginger, (1992b) pointed out that numerous so-called mucoadhesive polymers adhere only under conditions where the amount of interstitial liquid is limited. This kind of dry-to-wet adhesion or "blotting adhesion" is due to the capillary forces drawing liquid from the mucus into the delivery system (Huntsberger, 1967; Lehr, Bouwstra, Schacht and Junginger, 1992b; Mortazavi and Smart, 1993). If the polymer involved offers no intrinsic ability to form a bond with the substrate (e.g. some cellulose derivatives), the initial adhesive forces although high at the beginning may become negligible as soon as the material is fully hydrated (Junginger and Lehr, 1990). Therefore adhesion measurements in fully hydrated systems and over a period of time are necessary to avoid attributing a high adhesive force to intrinsic mucoadhesive properties. The adhesion mechanism of capillary attraction between a dry, water-absorbing polymer and a wet, mucosal surface being dehydrated is quite different to the interactions between two hydrogels (polymer and mucus) in equilibrium with a third liquid phase (Mortazavi and Smart, 1993).

IN VITRO METHODS

Tensiometry

The main method used in *in vitro* testing of mucoadhesion is tensiometry (Table 18.3: Entry Numbers 1, 2, 14, 15, 17–32). The method employs putative mucoadhesive polymers that are usually in the form of tablets made by direct compression of the polymer or polymer coated surfaces made by casting of polymer solutions. These are consequently put in contact with a mucus surface usually with a given force applied to the system for a given period of time after which the adhesive joint is destroyed by applying a vertical force in the opposite direction or a shear force in the horizontal direction. The force required to destroy the bond is taken as a qualitative and quantitative parameter for adhesion. If the experiment is done under full hydration of the polymer and in an aqueous environment the likelihood of mimicking *in vivo* conditions is higher, given that a potential formulation, which is usually swallowed with liquid would not arrive at the target site in a totally dry

state. However, this method neglects the fact that a swallowed formulation does not make intimate contact with the mucus gel spontaneously. Furthermore, the cohesion of mucus is also related to its thickness and rheological features. The experimental setup does not allow to appreciate if the bonding failure is adhesive or cohesive in nature (Huntsberger, 1967; Lehr *et al.*, 1992c). Although information on the screening of polymers can easily be obtained, the method appears unsuitable for assessing adhesive behaviour of formulations intended for gastrointestinal application. Similar comments apply to the Wilhelmy plate method used by e.g. Smart, Kellaway and Worthington, (1984) (Table 18.3, Entry Numbers 3–5). Tensiometry seems more useful for buccal, vaginal or other applications where liquid is controllable and more limited.

Flow through systems

Flow through systems appears an appropriate alternative in assessing the various aspects of mucoadhesion with a pharmaceutical formulation in the gastrointestinal tract. A flow channel device was first described by Mikos and Peppas (1990). The channel had a length of approximately 30 cm, a width of 4 cm and a height of approximately 0.5 cm, it was thermostatted by a jacket connected to a constant temperature water bath. A cavity inside the channel allowed placement of a mucin gel or the mucosal of a tissue and the placement of a single polymer microparticle on top of it. The channel was connected through a set of valves to a gas cylinder. The volumetric flow rate was gradually increased until the particle, which was observed by an optical microscope, was detached from the mucous surface. The system is suitable as a model for studying nasal mucoadhesion. For gastrointestinal models a fluid should be substituted for the air. Liquid flow methods were developed later (Table 18.3, Entry Numbers 33–35) and a similar technique employed live tissue to monitor intestinal drug absorption at the same time (Junginger, Lehr, Bouwstra, Tukker and Verhoef, 1990). The observation of adhesion of a formulation (which ought to be insoluble so as to avoid dilution and rapid wash-off as well as rapid drug leaching) from a flow of solution directly onto a mucus tissue would be most desirable. A swellable but insoluble formulation is usually achieved by crosslinking of the polymer chains. This would lead to chain rigidity which in turn can limit mucoadhesion since the proposed "interpenetration" or "interdiffusion" mechanism would be restricted.

Colloidal gold staining

A method presented by Park (1989) is particularly interesting because unlike tensiometric methods it does not monitor a destruction process. Instead of measuring

the adhesion strength or the duration of adhesion, the "adhesion number" is determined as a direct function of adhesion. Furthermore the technique is claimed to give precise and reproducible values, is simple to perform and the experimental cost is fairly low.

The adherent material (or substrate) consisted of colloidal gold particles of an approximate diameter of 18 nm that carried mucin adsorbed onto their surface. The mucin used was a solution of bovine submaxillary mucin Type I. Colloidal gold (cAu) sols are usually prepared by reducing $HAuCl_4$ with reducing agents like sodium citrate. Particle sizes vary depending on the reducing agent as well as on the preparation procedure.

The bioadhesive material used by Park was a copolymer made from acrylic acid and acrylamide cross-linked with N,N'-methylene-bis-acrylamide [P(AA-co-AM)]. The transparent hydrogels made of this material were cut into rectangular shapes of varying thickness. The polymer strips were incubated with the cAu-mucin conjugates and after a rinsing procedure, the absorbance of the strip was measured at 525 nm with a spectrophotometer using a transparent control polymer strip as a blank. The values obtained were a function of the amount of cAu adsorbed onto the surface. Alternatively the absorbance of the cAu-mucin preparation was measured before and after incubation. In this case, the magnitude of the decrease in the absorbance value from the initial value (ΔAbs) was used as a quantitative parameter indicating the interaction between cAu-mucin and hydrogel. The author also used an image analyser to quantify the intensity of red colour on the polymer surfaces. This alternative is necessary for mucoadhesive polymers which are not transparent.

The cAu-mucin conjugates prepared by Park required the addition of albumin to stabilise the preparation further. Albumin molecules are believed to adsorb onto small bare spots on the cAu particle where mucin molecules do not cover (Horisberger and Rosset, 1977; De Mey, 1984). It can be argued, however, whether any interaction phenomenon observed is due solely to the properties of the mucin. Besides, if the affinity of albumin to the cAu is higher than that of mucin, a displacement of mucin from the cAu is also possible.

As outlined by Park (1989) an alternative approach using the colloidal gold staining technique is that of developing cAu-(bioadhesive polymer) conjugates instead of cAu-mucin conjugates. The polymer coated cAu particles acting as the adhesive this time could be directly applied to the surface of target tissues. In this case the cAu-polymer conjugate would act as a model drug delivery system. The possibility of additional adsorption of drugs, e.g. peptide drugs onto these "nanospheres" would be interesting. Chitosan-stabilised cAu has been successfully prepared by Horisberger and Clerc (1988) to use as a marker for anionic sites on various micro-organisms and by Fiebrig, Harding, Stokke, Vårum, Jordan and Davis (1994) to visualize the sites of interaction of chitosan in a mucin-chitosan complex.

Miscellaneous methods

Viscometry, rheometry, photon correlation spectroscopy and nuclear magnetic resonance as well as analytical ultracentrifugation (Table 18.3, Entry Numbers 7–9, 11–13) are methods that can give information on [bioadhesive polymer]–[glycoprotein] interactions. The use of a standardised material throughout the experiments allows the comparison of results and avoids inter-sample variations. Such methods also allow us to study the influence of factors that may influence interaction (ionic strength, bile salts, temperature, proteins) and hence the elucidation of interaction mechanisms. It is well appreciated that the experimental conditions bear no relation to *in vivo* situation. Concentrations may be very low, as in the case of analytical ultracentrifugation and dynamic light scattering.

IN VIVO

When studying bioadhesion, with the aim of assessing the performance of a bioadhesive DDS, it is usually best to work with an animal model, human volunteers or patients. For buccal, vaginal, cervical or nasal applications (Table 18.3) the residence time of the device can be inspected visually, while the subject can give direct information on aspects of tolerance (discomfort, usefulness, etc.) (Nagai, 1986; Bottenberg, Cleymaet, de Muynck, Remon, Coomans, Michotte and Slop, 1991; Smid-Corbar, Kristl, Cop and Groselj, 1991). Plasma levels of drug or pharmacodynamic effect (for e.g. delivery of insulin) can give direct evidence for bioequivalence or enhanced bioavailability. Aspects of intersubject variability and disease condition may have to be taken into account.

With regards to gastrointestinal bioadhesive DDS we are faced with major experimental difficulties. Once swallowed, the device has to reach the adherent mucus layer. As has been outlined earlier, the process of bioadhesion requires *intimate contact* in its first step. There is little experimental evidence for this prerequisite actually taking place in gastrointestinal bioadhesion. Lodged on the target surface a delivery system has to resist the dislodging forces of gastrointestinal motility. In humans this particular aspect has been examined for the first time in a double blind study by Anderson (1991) using coloured tablets made from DEAE-dextran and ethylcellulose (1:1) with ethylcellulose tablets as controls. Seven patients undergoing routine gastroscopy examination swallowed both tablets with approximately 20 ml of water immediately before endoscopy. Mucoadhesion or the lack of it was assessed using a finger controlled water jet attachment. The clinician sprayed the tablet with water, for a fixed time period and at a constant rate, and assessed adhesion in terms of the number of sprays required to dislodge the tablet. The results did not suggest any significant adhesion of the test formulation

to the gastric mucosa. In 50% of the patients examined at longer time intervals (up until 65 min post dose) neither the control nor the test tablet could be found in the stomach. For those patients where tablets could be observed, no significant differences in adhesive behaviour between control and test tablet with the gastric mucosa could be detected when judged using the finger controlled water jet attachment. There was no actual measurement of the water spray properties. However, the results illustrate the probable lack of adhesion for both tablet formulations as well as the intersubject variation in gastric emptying times for tablet formulations in the fasted state as observed by other authors.

The rat as *in vivo* model (Table 18.3) has a mucus layer about half the thickness of that in man (Allen, 1978; Kerss *et al.*, 1982), while little is known about mucus turnover compared to man. It has been suggested that in the rat there is very little soluble gastric mucus when compared with the dog where there are considerable amounts of this material (Robinson *et al.*, 1987). Although the mucin of pig gastrointestine is similar to that of humans with regards to its carbohydrate and protein composition (Allen, 1989), gastric emptying in pigs has been shown to be slower than in man (Aoyagi, Ogata, Kaniwa, Uchiyama, Yasuda and Tanioka, 1992) and consequently this animal may not be the appropriate *in vivo* model.

TABLE 18.1 SUMMARY OF METHODS USED TO STUDY BIOADHESION FOR DRUG DELIVERY SYSTEMS

Entry Number	*Substrate*	*Adhesive*	*Drug*	*Preparation*	*Test*	*Application*	*Reference*
In vitro studies using various model substances (mucin, mucus or other) as substrate							
1	Plexiglas®	PE gel (5% in petrolatum) + Na-CMC or gelatin	—	Viscous pastes hydrated with artificial saliva	Tensiometry		Gurny, Meyer and Peppas, 1984
Instron 1114 tensile tester, the paste is placed between two Plexiglas® discs, discs are pulled apart and stress—strain curves recorded measuring "adhesive strength".							
2	Filter paper discs + 15% PGM_n solution	PAA, HA, HAE polymers	—	Hydrated circular matrices, 12 mm diameter	Tensiometry	Ocular	Saettone, Chetoni, Torracca, Burgalassi and Giannaccini, 1989
Modified tensile tester of Ch'ng *et al.* is used to determine the force required to separate two mucin coated surfaces; the relevance of mucin-soaked filter papers as mucous substrate can be argued. PGM_n from Biochemo SpA.							
3	Homogenized mucus from guinea-pig intestine	Numerous polymers	—	Polymer-coated glass plate	Wilhelmy plate method	—	Smart, Kellaway and Worthington, 1984
The method measures the maximum force required to detach the plate from the mucus gel with a microforce balance.							
4	Crude PGM_s (Sigma)	PAA and PMAA with various cross-linkers	—	Polymer-coated rexin sheet	Wilhelmey plate method	—	Chitnis, Malshe and Lalla, 1991
A horizontal polymer-coated rexin sheet is pulled by means of a "small crane" from a 5% mucin solution kept in a beaker on a top-loading balance, the minimum weight is recorded; the polymer was allowed to hydrate for 5 minutes.							

5	Pig small intestinal mucus, native and homogenised	CaPC, NaCMC, HPMCP, HPMC Eudr. RS100, AP	—	Polymer-coated glass plates, either as film or as powder	Wilhelmey plate method	—	Sam, van den Heuij and Tukker, 1992

Based on the method described by Smart *et al.*, polymer powders (non film-forming sample) are adhered to partially dried Eudragit RS100 and force—time diagrams are recorded.

6	Bovine submaxillary mucin TI (Sigma)	P(AA-co-AM)	—	—	Colloidal gold staining	—	Park, 1989
			—	—		—	

Colloidal gold is stabilised with mucin and hence acts as a marker for light as well as electron microscopy. Determination of the "adhesion number" measures bond formation rather than a destruction process as a function of staining intensity.

7	PGM_s	PAA, low molecular weight	—	Polymer solutions at pH 7.4 and 4.0	Photon correlation spectroscopy (PCS)	—	Kerr, Kellaway, Lewis, Kelly and Parr 1989

Malvern RR144 with a multibit correlator, K7025 and a He—Ne 35 mW laser was used to measure the diffusion coefficients.

8	PGM_s	PAA, low molecular weight	—	Polymer solutions at pH 7.4 and 4.0	Nuclear magnetic resonance (^{13}C-NMR)	—	Kerr *et al.*, 1989

Bruker WM360 FT spectrometer operating at 90 MHz and RT.

9	PGM_n, purified	DEAE—dextran	—	Dilute polymer solutions	Analytical ultracentrifugation	(Gastrointestinal)	Anderson, Harding and Davis, 1989

Based on the assumption that any marked interaction between the two components would lead to co-sedimentation, shown by an increase in the sedimentation coefficient of the sedimenting boundaries.

10	Bovine sub-maxillary mucin gel (Sigma)	PHEMA	—	Microspheres ~156 μm diameter	Flow channel method (air flow method)	—	Mikos and Peppas, 1990

The method observes the adhesive behaviour of a microparticle under the microscope connected to a photo or video camera as air is blown through a channel containing the preparation.

11	PGM_s (Sigma)	Various neutral, anionic and cationic polymers including chitosan	—	Polymer solutions in 0.1 N HCl and acetate buffer pH 5.5	Viscometry	—	Hassan and Gallo, 1990

Brookfield viscometer Model RTV and Ostwald viscometer were used to measure the solutions of individual polymers and mixtures thereof.

12	Crude and homogenised PGM_s; PGM_n	C-934P	—	C-934P gel (5 mg/g)	Rheometry	—	Mortazavi, Carpenter and Smart, 1992, 1993

Determination of mean storage modulus and loss modulus of mucus or mucin—polymer mixtures.

13	PGM_n, purified	Chitosan	—	Dilute polymer solutions	Analytical ultracentrifugation	(Gastrointestinal)	Fiebrig, Harding and Davis, 1994

Same as Anderson *et al.* 1989

Ex vivo techniques (using animal mucosal tissue as substrate in most models)

14	Mouse peritoneal membrane	HPC:C-934, mixing ratios 3:1, 2:1, 1:1 or 1:2	(Insulin)	Tablets made by direct compression of polymer mixtures	Tensiometry	(Buccal)	Ishida, Machida, Nambu and Nagai, 1981

"Stickiness test" measuring "sticking force". Instead of a tensile tester a simple spring balance is use, the tablet is applied dry and the force measured until detachment occurs.

15	Pig oesophagus	Various gelatin capsules and tablets	Not relevant	Various gelatin capsules and tablets	Tensiometry	Oesophagus	Marvola, Vahervuo, Sothmann, Marttila and Rajaniemi, 1982

The is used to study the tendency of formulations to adhere to the oesophagus, measuring the weight required until detachment of formulation.

16	Fresh rabbit stomach tissue	Polymers from AA and MA with various cross-linking agents	—	Prehydrated, insoluble polymer	Tensiometry	—	Ch'ng, Park, Kelly and Robinson, 1985

Measures the force required to detach mucus from bioadhesive polymer i.e. the destruction process of a mucosa--polymer—mucosa "sandwich" in a fully hydrated environment.

17	Fresh rabbit conjunctival membrane (eyelid)	Polymers of AA cross-linked with dvG and dmH	^{14}C-labelled progesterone	Prehydrated, insoluble polymer	Tensiometry	Ocular	Hui and Robinson, 1985

Same as Ch'ng *et al.* (1985).

18	Bovine sublingual mucus tissue	C-934 0%—90% mixed with HPMC	—	12 mm diameter tablets prepared by direct compression	Tensiometry	—	Ponchel, Touchard, Duchêne and Peppas, 1987

Based on the methods of Gurny *et al.* and Ch'ng *et al.* using Instron 1026 tensile apparatus, tablet is prehydrated with a defined amount of water before being placed on mucus tissue, glued to the lower support, relative humidity of 60% in the environment, measurement of detachment vs. elongation.

19	Bovine sublingual mucus tissue	C-934 0%—90% mixed with HPMC	—	12 mm diameter tablets prepared by direct compression	Tensiometry	—	Peppas, Ponchel and Duchêne, 1987

Experimental setup of Peppas *et al.* (1987), repeated straining followed by relaxation measuring stress relaxation.

20	Bovine sublingual mucus tissue	C-934 0%—90% mixed with HPMC	50% metronidazole	12 mm diameter tablets prepared by direct compression	Tensiometry	—	Ponchel, Touchard, Wouessidjewe, Duchêne and Peppas, 1987

Experimental setup of Peppas *et al.* (1987), measuring detachment force vs. elongation.

21	Fresh rabbit stomach tissue	Copolymers from AA and MM with divinyl glycol as cross-linking agent	—	Prehydrated, insoluble polymer	Tensiometry	—	Leung and Robinson, 1988

Same as Hui and Robinson (1985), with shear stress measurement additional to the tensile stress testing.

22	Frog oesophagus with and without mucus layer	PNVP, PHEMA, PMMA, NaCMC, C-941, ALA, NaAL	—	Polymer powder stuck onto hydrated PHEMA disc	Tensiometry	—	Robert, Buri and Peppas, 1988

Du Noüy tensiometer measuring detachment force, not conducted in liquid medium, otherwise polymer is removed, reference disc made from PTFE.

23	Pig oral mucosal tissue	Copolymers of AA and BuAA XL:EDEMA	—	Hydrogel strip	Tensiometry	—	de Vries, Bodde, Busscher and Junginger, 1988

Instron 1122 test unit to determine the peel force pulling at 90° away from the surface.

24	Pig oral mucosal tissue	Copolymers of AA and BuAA XL:EDEMA	—	Hydrogel strip	Tensiometry and determination of "shear time"	—	de Vries *et al.*, 1988

The epithelial flap was attached vertically with pins to a rigid surface, a hydrogel strip laid on top of the mucosa surface by applying a known force. Upon attaching a 50 g mass to the bottom end of the test strip, the time needed for the test strip to come off the substrate was measured at 34° and 100% humidity.

25	Bovine vaginal and sublingual mucosa	HPMC and C-934P	Metronidazole	Tablet made by direct compression	Tensiometry	Vaginal and oral	Lejoyeux, Ponchel, Wouessidjewe, Peppas and Duchêne, 1989

Method of Peppas *et al.*, 1987, system dipped (or not) in a liquid medium of variable composition (pH, ionic strength, divalent cations), determination of adhesion work.

26	Bovine vaginal mucosa	C-934P, NaCMC	$ZnSO_4$	Tablets made by direct compression	Tensiometry	Vaginal	Gürsoy, Sohtorik, Uyanik and Peppas, 1989

Tensilon, UTM11, Japan used for measuring detachment force.

27	Rat small intestine	C-934, PC, PEG, HPC, HPMC, gelatin	See comments	Discs made from compressed powder	Tensiometry	—	Smart, 1991

Experimental setup of Ch'ng *et al.*, sample surrounded by buffer pH 6.8, commercial buccal preparations were tested as well: Adcortyl in Orabase® (triamcinolone acetonide), Buccastem® (prochlorperazine maleate) and Suscard Buccal® (glyceryl trinitrate), instead of "sandwich" one mucosal surface only.

28	Pig attached gingiva	Modified starch, C-934, PEG and NaCMC	Sodium fluoride	7 mm diameter flat tablets by direct compression	Tensiometry	Buccal (dental)	Bottenberg, Cleymaet, de Muynck, Remon, Coomans, Michotte and Slop, 1991

Same as Ponchel *et al.*, 1987

29	Ox sublingual mucosa	C-934	—	Tablet made by direct compression	Tensiometry	—	Jaques and Buri, 1992

Tablet diameter: 10 mm, Schenk—Trebel RM 50 tensile tester, and force and displacement transducer. Adhesion of the connective side to the underlying support: also made of C-934, tablet applied in a dry state, work of elongation of the mucosa is measured.

30	Healthy human uterine cervix tissue	HPC, HEC, MC C-934P, NaCMC and mixtures thereof	—	Flexible films: casting polymer solutions followed by cold air stream drying	Tensiometry with a LVDT sensor	—	Woolfson, McCafferty, Gorman, McCarron and Price, 1992

Experimental similar to that of Peppas *et al.*, 1987, sensor output: force vs. vertical displacement, both adherents are glued to the respective mounting pedestal, polymers prehydrated as well as dry.

31	Pig buccal mucosa	HPC—PAA mixtures	Propranolol	Direct compressed discs, 9.5 mm in diameter	Tensiometry	Buccal (systemic)	Chen and Hwang, 1992

Modified tensile test of Peppas *et al.*, 1987 and Leung and Robinson, 1988

32	Rat jejunum or stomach	PC, NaCMC, HPMC, MC PT	—	Polymer-coated glass spheres or drug crystals	Liquid flow method	—	Ranga-Rao and Buri, 1989

Diameter of glass beads: 0.45 mm — 0.50 mm, crystals of drug > 630 μm, particles are applied in a non-hydrated, dry state; particles adhered to the tissue after washing procedure are counted.

33	Rat ileal segments	HPMA	Tritiated ostradiol as marker	Polymer nanospheres	Liquid flow method	—	Pimienta, Lenaerts, Cadieux, Raymond, Juhasz, Simard and Jolicoeur, 1990

Ileal segments secured on glass tube (cut lengthwise and inclined at an angle of 12°), then perfused and perfusate fractions analysed (2,4,6,7-^{3}H-estradiol radioactive marker) with â-counter, control: glass tube without mucus tissue.

34	Rat ileal segments	Poloxamer (407, 238, 403), poloxamine (908)	—	IHCA nanospheres coated with polymers	Liquid flow method	—	Pimienta, Chouinard, Labib and Lenaerts, 1992

Liquid flow method as Pimienta *et al.*, 1992, radioactivity of the collected samples (ZnPC-$^{125}I_4$) label) was measured by γ-scintigraphy.

35	Rabbit small intestinal tissue	AA or MMAA grafted onto starch	Isosorbide dinitrate	Polymer grafted starch microspheres	Liquid flow method	Buccal (systemic)	Vyas and Jain, 1992

Method of Pimienta *et al.* 1990, ratio of applied to adhered micro-spheres.

36	Cultured human conjunctival epithelial cells	Numerous anionic, cationic and neutral polymers	—	Polymer solutions	Excimer fluorescence of pyrene	—	Park and Robinson, 1984

Addition of polymer binding to the cell membrane results in compression of the pyrene-containing lipid bilayer of the cell, causing a change in fluorescence measured by a fluorescence spectrophotometer.

37	Rat intestine	HPMA copolymers with various residues	—	Polymer solutions	Electron microscopy	Gastrointesti-nal	Bridges, Woodley, Duncan, Kopeckova and Kopecek, 1987

Samples prepared for light and electron microscopy after interaction with colloidal-gold—HPMA copolymer conjugates.

38	Rat intestine	HPMA copolymers with various residues	--	Polymer solutions	Incubation method	Gastrointestinal	Bridges, Woodley, Duncan and Kopecek, 1988

Intestinal rings were incubated with ^{125}I-labelled copolymers with carbohydrate residues such as galactosamine, mannosamine, glucosamine, fucosylamine or glactose or cationic groups (quaternary ammonium groups) and assayed for radioactivity.

In vivo testing in animal models (rats, rabbits, dogs)

39	Rat	HPC:C-934 ratio 1:2	^{3}H-TAA	Double layered tablet	Microautora diogram of tongue tissue	Buccal	Kubo, Yamamoto, Yamaguchi, Hashimoto, Ikura and Suzuki, 1982

Ointment used as control.

40	Fasted rat	PC, P(MA-dvB)	--	Polymers labelled with ^{51}Cr in gelatin capsule	Determination of radioactivity in gut segments	--	Ch'ng, Park, Kelly and Robinson, 1985

Surgical insertion of No. 3 gelatin capsules containing the polymer into rat stomach under anaesthesia.

41	Fasted rat	PC	Chlorothiazide	PC-albumin beads in gelatin capsule	Plasma level of the drug via HPLC	Gastrointestinal	Longer, Ch'ng and Robinson, 1985

PC particles and albumin beads mixed at a ratio of 7:3 (w/w) were loaded into gelatin No. 4 capsules, mean particle diameter 505 μm, surgical insertion of gelatin capsules containing the polymer into rat stomach under anaesthesia.

42	Rat	C-934	Hydrochlorothiazide	Polymer solutions containing the dispersed drug	HPLC analysis of urine	Gastrointestinal	Harris, Fell, Taylor, Lynch and Sharma, 1989

Formulations were delivered with a syringe directly into the stomach of the conscious rat.

43	Rat	PC, C-934, HA, PSSA, HEC	--	Polymer solutions with ^{51}Cr-labelled microspheres	Intestinal segments assayed for radio activity	Gastrointestinal	Harris, Fell, Taylor, Lynch and Sharma, 1990

Formulations were delivered directly into the stomach by passing a gavage tube down the oesophagus. Where the formulation was too viscous to be dosed by gavage, gelatin capsules were filled with the suspension immediately prior to administration. Radioactivity determined with γ-counter.

44	Rat	PC, C934P	DGAVP	PHEMA microspheres coated with PC	DGAVP plasma concentration by RIA	Gastrointestinal	Lehr, Bouwstra, Tukker and Junginger, 1990; Lehr, Bouwstra, Kok, de Boer, Tukker, Verhoef, Breimer and Junginger, 1992

Chronically isolated intestinal loop (*in situ*) as described by Poelma and Tukker, 1987, microspheres 315 μm -- 400 μm as well as direct injection of the formulation into the small intestine via catheter.

45	Rabbit	Polymer of AA cross-linked with dmH	Progesterone ^{14}C- labelled	Polymer--drug suspension	Determination of radioactivity in humor samples	Ocular	Hui and Robinson, 1985

Application into the lower cul-de-sac of the rabbit.

46	Rabbit	Na-hyaluronate	Pilocarpine	Aqueous solutions of drug and polymer labelled with ^{99m}Tc	Miotic response and γ-scintigraphy	Ocular	Gurny, Meyer and Peppas, 1987

Saline solutions as well as pH-sensitive latex preparation and temperature setting Pluronic preparation as controls.

47	Rabbit	C-934P PVA (control)	--	Mucoadhesive polymer solution radiolabelled with ^{113m}In	γ-scintigraphy	Ocular	Davies, Farr, Hadgraft and Kellaway, 1988a

Equiviscous control polymer PVA as well as radiolabelled PBS using ^{113m}DTPA were used as controls.

48	Rabbit	Same as Gurny *et al.*, 1987	Pilocarpine	Mucoadhesive polymer solution with 1% pilocarpine	Miotic response	Ocular	Davies, Farr, Hadgraft and Kellaway, 1988b
49	Rabbit	PAA, HPMC, CMC	--	$BaSO_4$ microcapsule coated with polymer, packed into hard gelatin capsule	Radio-opaque material shown on repeated X-ray photographic imaging	Gastrointestinal	Chattaraj, Sudip and Gupta, 1988

Time taken for over 50% microcapsules to empty from stomach and travel to the small intestine. $BaSO_4$ tablets used as control.

50	Rabbit	PAA, HA, HAE polymers	Pilocarpine and tropicamide	4 mm diameter circular inserts	Miotic and mydriatic activity tests	Ocular	Saettone, Chetoni, Torracca, Burgalassi and Giannaccini, 1989

Inserts were made from films (slow evaporation of polymer solution) or by directly compressing polymer, commercial aqueous eyedrops of pilocarpine and tropicamide were used as reference vehicles.

51	Rabbit	PAA, HA, HAE polymers	--	50 µl of gels or inserts containing fluorescin	Inspection under long-wave fluorescent lamp	Ocular	Saettone *et al.*, 1989

Measurement of time during which a fluorescent layer was present over the corneal surface.

52	Rabbit	Solubilised fresh pig gastric mucus	--	^{99m}Tc-labelled PGM_s solution	γ-scintigraphy	Ocular	Greaves and Wilson, 1993

^{99m}Tc-MDP as control solution, preparations instilled into lower fornix.

53	Beagle dog	HPC:C-934 ratio 1:2	Insulin	Two base tablet -- core: insulin; periphery: bioadhesive polymer	Plasma insulin level and blood sugar level	Buccal	Ishida, Machida, Nambu and Nagai, 1981

The tablet was applied on the oral mucosa of the dog. In order to stick the tablet to the oral mucosa, it was necessary to wipe away the saliva with absorbent cotton previous to application.

54	Beagle dog	MCC, HPC, C-934	Insulin	Powder dosage form	Plasma glucose level	Nasal	Nagai, Nishimoto, Nambu, Suzuki and Sekine, 1984

For application, a polythene tube was inserted in the dog's nose about 2 cm from the nostril and then was sprayed by a special sprayer, liquid controls were administered by dropping the sample solution in the nostril.

Human Trials

55	Female volunteers	HPC:C-934 ratio 1:2	Bleomycin	Disc-type preparation made by direct compression	Number of cancerous foci found after 2--3 weeks of treatment	Cervical (uterine cancer)	Machida, Masuda, Fujiyama, Ito, Iwata and Nagai, 1979

13 mm in diameter prepared by using the die and punch which is used in preparing KBr tablets samples for infra-red spectroscopy, preparation placed on cervix.

56	Female volunteers	HPC:C-934 ratio 1:2	Bleomycin, carbazilquinone, 5-fluorouracil	Rod-shaped preparation made by direct compression	Number of cancerous foci found after 2--3 weeks of treatment	Cervical (uterine cancer)	Machida, Masuda, Fujiyama, Iwata and Nagai, 1980

40 mm in length prepared with specially designed dies and punches, for application in the cervical canal.

57	Human volunteers	HPC:C-934 ratio 1:2	Triamcinolone acetonide	Double-layered tablet	"Improvement" and "usefulness"	Buccal	Nagai, Machida, Suzuki and Ikura, patent

Upper supporting layer: coloured lactose; lower adhesive layer: contains drug, commercial name: Aphtach®.

58	Human volunteers	HPC:C-934 ratio 1:2	Lidocaine	Two base tablet -- core: lidocaine; periphery: bioadhesive polymer and cap layer	Gas chromatography on lidocaine	Buccal (toothache)	Ishida, Nambu and Nagai, 1982

Application to the human gingiva, the percentage of lidocaine absorbed was calculated from the amount remaining in the dosage form after removing it completely from the gingiva.

59	Human volunteers	PVA	Protirelin or salicylate as	Same as Anders and Merkle, 1989	Amount of residual polymer	Buccal (systemic)	Anders and Merkle, 1983

Polymer and drug remaining on the patch after a given time were assayed, protirelin by RIA or HPLC, sodium salicylate by UV spectrometry, PVP was analysed using a colorimetric method.

60	Human volunteers	Na-hyaluronate	Pilocarpine	Aqueous solutions of drug and polymer	Miotic response	Ocular	Gurny, Ibrahim, Aebi, Buri, Wilson, Washington, Edman and Camber, 1987

Saline solutions with drug as controls.

61	Human volunteers	PC	--	Pellets in hard gelatin capsules	γ-scintigraphy	Gastrointestinal	Khosla and Davis, 1987

Pellets of Amberlite IRA410 anionic resin (0.5 mm -- 1.0 mm), ^{99m}Tc-labelled, mixed in size 0 hard gelatin capsules with 100 mg polycarbophil (0.5 mm -- 1.0 mm), three fasted subjects swallowed one capsule with 100 ml of water, control formulation without PC.

62	Human volunteers	HEC, HPC, PVP, PVA	--	Two-ply laminates (adhesive patches)	Time until patch lost its adhesive contact with the mucosa	Buccal	Anders and Merkle, 1989

Laminates consisting of an impermeable backing layer and hydrocolloid polymer layer containing the drug made by casting and drying.

63	Mongrel dogs and human volunteers	HPC:C-934, 2:1	Timolol base	Two base tablet -- core: drug; periphery: bioadhesive polymer and cap layer	Residual drug content assayed by spectrophotometer after periodical removal	Buccal (systemic)	Deasy and O'Neill, 1989

Sedated dogs: the oral mucosa adjacent to the upper first molar was dried with absorbent tissue prior to application of a compact; human volunteers: after drying the gingival mucosa below the first molar, a compact was located there by applying finger pressure to the cap for approximately 20 s. Sodium lauryl sulphate used as a penetration enhancer in some cases.

64	Male volunteers	PC, C-934P	--	Amberlite resin beads and polymer mixture in hard gelatin capsule	γ-scintigraphy with ^{99m}Tc	Gastrointestinal	Harris, Fell, Sharma and Taylor, 1990

Formulations similar to those of Longer *et al.*, 1985, size 0 hard gelatin capsule filled with radiolabelled resin beads (Amberlite, particle size 20 μm) in admixture with bioadhesive powder or lactose (control).

65	Healthy volunteers	Modified starch, C-934, PEG and NaCMC	--	7 mm diameter flat tablets made by direct compression	Self-assessment by subject	Buccal (dental)	Bottenberg, Cleymaet, de Muynck, Remon, Coomans, Michotte and Slop, 1991

Assessment of irritation, hindrance, taste alteration, etc. and end of adhesion after application of one tablet on the attached gingiva in the region of the upper canine pressed directly onto the mucosa for 30 s.

66	Human volunteers	Eudispert®	Metronidazole	Films prepared by casting and drying	Duration of adhesion until complete wash-off from the buccal mucosa	Buccal (local)	Smid-Corbar, Kristl, Cop and Groselj, 1991

Plasticizers incorporated in films, such as glycerol, PEG or sorbitol; in addition to the duration of adhesion assessment, metronidazole in saliva was determined via HPLC.

67	Female patients with bacterial vaginosis	C-934	Metronidazole	Tablet containing 5% bioadhesive polymer; remainder: maize starch and 10% drug	Bacterial vaginosis evaluated clinically, by microscopy and bacteriologically	Vaginal	Bouckaert, Temmerman, Dhont and Remon, 1994

Control: placebo and Flagyl® conventional oral tablets, it does not show the effect of the bioadhesive itself.

Abbreviations

AA	acrylic acid
ALA	alginic acid
AO	absorptive ointment
AP	cross-linked amylopectin
BuAA	butyl acrylate
C934	Carbopol 934
C934P	Carbopol 934 pharmaceutical grade
C941	Carbopol 941
CaPC	calcium polycarbophil
CMC	carboxymethyl cellulose
DGAVP	9-desglycinamide, 8-arginine vasopressin
dmH	2,5-dimethyl-1,5-hexadiene
DTPA	diethylenetriaminepentaacetic acid
dvG	divinyl glycol
EDEMA	ethylene glycol dimethacrylate
Eudispert	copolymer of poly(methylmethacrylic) acid and its methylester
HA	hyaluronic acid
HAE	HA ethyl ester
HEC	hydroxyethyl cellulose
HP	hydrophilic petrolatum
HPC	hydroxypropyl cellulose
HPLC	high performance liquid chromatography
HPMA	polyhydroxypropylmethacrylate
HPMC	hydroxypropylmethyl cellulose
HPMCP	hydroxypropylmethylcellulose phthalate
^{3}H-TTA	tritium-labelled triamcinolone acetonide
IHCA	isohexylcyanoacrylate
LVDT	linear variable differential transformer
MA	methacrylic acid
MC	Methocel
MCC	microcrystalline cellulose
MDP	methylene diphosphate
MM	methyl methacrylate
MMAA	methyl methacrylic acid
NaAL	sodium alginate
NaCMC	sodium carboxymethylcellulose
PAA	polyacrylic acid
P(AA-co-AM)	copolymer of acrylic acid and acrylamide cross-linked with N,N'methylene-bis-acrylamide
PBS	phosphate-buffered saline
PC	polycarbophil (Carbopol EX55)
PE	polyethylene
PEG	polyethylene glycol
PGM_n	pig gastric mucin
PGM_s	pig gastric mucus
PHEMA	poly(2-hydroxyethylmethacrylate)
PMAA	poly(methacrylic acid)
P(MA-dvB)	poly(methacrylic acid-divinylbenzene)
PNVP	poly(N-vinyl-2-pyrrolidone)
PSSA	poly(styrenesulphonic) acid
PT	pectin
PTFE	poly(tetrafluoroethylene)
PVA	polyvinyl alcohol
PVP	polyvinyl pyrrolidone
RIA	radioimmunoassay
RT	room temperature
WP	white petrolatum
XL	cross-linker
ZnPC-$^{125}I_4$	^{125}I-phthalocyanine-Zn complex

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