Polymer Gels as Pharmaceutical Dosage Forms

Rheological Performance and Physicochemical Interactions at the Gel-Mucus Interface for Formulations Intended for Mucosal Drug Delivery

BY

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ABSTRACT


Drug delivery to the nasal and ocular mucosa faces several obstacles. One of these is from the effective clearance mechanisms present in the nose and eye. Polymer gels with suitable rheological properties can facilitate the absorption of poorly absorbed drugs by increasing the contact time of the drug with the mucosa. This has been attributed to the rheological and mucoadhesive properties of the gel. The main objective of this thesis was to investigate the importance of these features for the anticipated in vivo contact time, here exemplified by the ocular and nasal routes of administration.

The in situ gelling polymer gellan gum was found to have a favourable rheological and in vivo performance. When administered in the nasal cavity of rats, a gel was formed that could remain at the site of administration for up to 4 hours. In addition, the epithelial uptake and transfer of a 3 kDa fluorescein dextran was higher than for a mannitol solution. Therefore, it was concluded that a gellan gum formulation should be a promising strategy for nasal drug delivery.

The potential mucoadhesive properties of a variety of polymer gels were investigated using a rheological method and by measuring the tensile force required to detach the gel from a mucosa. With both methods the rheological properties of the gel were a determining factor for the results obtained. The rheological method was found to have several limitations. One of these was that a positive response, interpreted as mucoadhesion, was only seen with weak gels. The tensile method could, in contrast, detect strengthening of the mucus only for strong gels. However, this method reflects the in vivo performance of the gel better than the rheological method.

Finally, dielectric spectroscopy was explored as a tool for investigating the likelihood of intimate surface contact between the gel and the mucus layer. This novel approach involved determining the ease with which a charged particle can pass the gel-mucus interface layer, and may enable the study of the events at the interface closer to the molecular level, than is possible with the rheological and tensile strength methods.

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Till min familj
Det finns alltid en tredje utväg bara man är i stånd att finna den.

*Selma Lagerlöf*
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This thesis is based on the following papers, which will be referred to by their roman numerals in the text:


VII. Jansson, B., Hägerström, H., Edsman, K. and Björk, E. Gellan gum increases the uptake and transfer of fluorescein dextran in rat nasal epithelium. Submitted.
Abbreviations

B7HF  Blanose 7HF, sodium carboxymethylcellulose
C907  Carbopol 907, linear polyacrylic acid
C934  Carbopol 934, cross-linked polyacrylic acid
C940  Carbopol 940, cross-linked polyacrylic acid
C981  Carbopol 981, cross-linked polyacrylic acid
EHEC  ethyl(hydroxyethyl)cellulose
HPMC  hydroxypropyl(methyl)cellulose
P127  Pluronic F-127, poloxamer,
      polyoxyethylene:polyoxypropylene block copolymer
SC211 Seacure CL 211, chitosan hydrochloride

Glc  glucose
GlcA glucuronic acid
Rhap rhamnose
NaCl sodium chloride
FD3 fluorescein dextran, molecular weight 3 kDa
BSMG mucin from bovine submaxillary glands
PS mucin from porcine stomach

AFM  atomic force microscopy
CNS  central nervous system
PCA  principal components analysis
PLS  partial least square projection to latent structures
TFR  tear fluid ratio

\[ G' \]  elastic (storage) modulus
\[ G'' \]  viscous (loss) modulus
\[ \delta \]  phase angle
TW  tensile work
PF  peak force
DF  deformation to failure
CF  compatibility factor
C  capacitance
\[ \varepsilon \]  permittivity
Z  impedance
\[ R_{hf} \]  high frequency resistance
\[ R_b \]  barrier resistance
\[ C_b \]  barrier capacitance
\[ T_d \]  diffusion parameter
1. Introduction

1.1 A gel or not a gel?

The soft, resilient and sometimes wobbly materials known as jellies are usually made from fruit juice cooked with sugar or from cooked-down meat juices, and have been used in households for several centuries. In the 1860s, a scientific interest was taken in this kind of material by Thomas Graham, who reported on the unusual diffusion properties of jellies [1]. Later, he also introduced the term hydrogel for hydrates of silicic acid with gelatinous properties [2]. Since then it has been difficult for chemists, physicists and medical researchers to reach a consensus as to what constitutes a gel. This was already recognized in 1926 by Dorothy Jordan Lloyd [3], who stated:

“The colloidal condition, the “gel”, is one which it is easier to recognize than to define”

and later on in the same paper she wrote:

“There is no need to assume that all gels have the same molecular architecture. There is little doubt, however, that they all possess a solid phase...”

As pointed out by Flory almost 50 years later [4], the one feature identified almost universally as an essential characteristic of a gel, is its solid-like behaviour. And the word solid, or solid-like, does frequently recur in the gel definitions found in encyclopedias and dictionaries, along with less formal descriptions such as

“gel: a thick, wet substance that is used in various bath or beauty products: hair gel” (Longman Dictionary of Contemporary English 3rd Ed. [5])

The phenomenological definition proposed in 1993 by Almdal et al [6], states that a gel is a soft, solid or solid-like material which consists of at least two components, one of which is a liquid present in abundance. The elastic and resilient character should be observable by the human eye and, as a consequence, on a time scale of seconds, a gel should not flow under the influence of its own weight. The solid-like characteristics of a gel are defined in terms of two dynamic mechanical properties: an elastic (or storage) modulus, $G'(\omega)$, which, when plotted against time (or frequency), exhibits a pronounced plateau extending to times at least the order of seconds, and a viscous (or loss) modulus, $G''(\omega)$, which is considerably smaller than the elastic modulus in the plateau region (Figure 1a).
Polymer gels are produced through the cross-linking of polymer chains, by the formation of either covalent bonds (chemical cross-linking) or non-covalent bonds (physical cross-linking) (Figure 2). Non-covalent bonds, can, for example, be hydrogen bonds and ion-bridges, the latter being common in the gelation of polyelectrolytes [7].

The term hydrogel has been extended since its introduction by Thomas Graham, and it now includes three-dimensional cross-linked polymeric networks that are capable of swelling in aqueous media. Thus, a hydrogel in its swollen state is described by the definition proposed by Almdal et al.

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1.2 Gels for drug delivery purposes

The first use of gels for medical applications was presented by Wichterle and Lim in 1960 [8], and involved the manufacturing of soft contact lenses and implant materials from hydroxyethyl methacrylate polymers. Since these early uses, gels have been used as vehicles for the delivery of drugs for both local treatment and systemic effects, see the review by Peppas et al. [9]. Many different administration routes have been explored, including, for example, cutaneous [10-14] and subcutaneous [15-19] delivery, buccal delivery [20-22], delivery to the periodontal pocket [23-27], esophagus [28], stomach [29-32], colon [33-35], rectum [36-39] and vagina [40-43].

This thesis concentrates on gel formulations intended for mucosal drug delivery, exemplified by the ocular and nasal routes. Both of these routes have substantial clearance mechanisms to protect the eye and the respiratory tract from unwanted “intruders”, such as particles, bacteria and irritants. Unfortunately, these mechanisms are just as effective for the clearance of drugs.

1.3 Clearance and residence time

A drug solution instilled in the eye is eliminated within 5–10 min because of the blinking and the associated rapid tear fluid turnover (16% per minute) (Figure 3a). In combination with the low drug permeability of the cornea, this leads to a low bioavailability, typically 1% or less [44]. The duration of the therapeutic effect is often short, and hence, frequent dosing is necessary.

The nasal mucociliary clearance arises from the coordinated movements of cilia (Figure 3b), transporting the mucus layer towards the throat, where the mucus and particles trapped in the mucus are swallowed. In humans, the mucus flow rate is of the order of 5 mm/min, resulting in a residence time of around 10–20 min in the nasal cavity [45]. Lipophilic low-molecular weight drugs are absorbed quite efficiently across the nasal epithelium, whereas larger, hydrophilic drugs, such as peptides and proteins have substantially lower bioavailabilities, of about 10% and less than 1%, respectively [46].

![Figure 3](image)

**Figure 3.** The short residence time of a drug solution in the eye arises mainly from the blinking and the rapid tear fluid turnover (a). In the nasal cavity, the short residence time is primarily caused by the movements of the cilia, transporting the mucus towards the throat (b).
To prolong the residence time at the absorption site and thereby facilitate the uptake of the drug, a number of strategies have been investigated. For example, the use of ointments, powders, and microspheres has been reported to increase the contact time with the mucosa, in comparison to a solution. Furthermore, gels have been studied for the ophthalmic administration of several drugs, including pilocarpine [47-50], tropicamide [50, 51], timolol [52-54] and other β-receptor antagonists [54], methylprednisolone [55], and oligonucleotides [56], and for nasal administration of, e.g., insulin [57], calcitonin [57, 58], roxithromycin [59], nifedipine [60] and a tetanus toxoid vaccine [61].

Gel formulations with suitable rheological properties can increase the contact time with the mucosa at the site of absorption (see, for example, references [52, 62-69]). The prolonged contact time has been attributed to the rheological properties of the formulation, which reduce or delay its clearance from the mucosa, and to specific interactions of the polymer in the gel with mucus components, which have been named mucoadhesion.

### 1.3.1 Drug release from gels

To take full advantage of the residence time, the drug should be released in adequate amounts throughout the entire period of time. Most gels that are used in pharmaceutical applications consist of typically 1% polymer and 99% water. The viscosity can be substantial owing to the presence of the polymer, but the transport conditions for a small drug molecule can be expected to be approximately the same as they are in water [70]. The polymer network is of little hindrance and the drug is likely to diffuse out of the gel rather rapidly. There are several ways of achieving sustained release, e.g., by suspending the drug in the gel (at a concentration exceeding the solubility) [10, 17], by formulating the drug as micro- [71] or nanospheres [72], by distributing the drug to liposomes [11, 73, 74] or surfactant aggregates [75-78], or by utilizing interactions between the drug and the polymer [79-81].

### 1.4 Environmentally responsive polymers

Hydrogels that change their swelling behaviour upon exposure to an external stimulus, such as, e.g., a change in the pH [82, 83], temperature [84], light [85] or electric field [86], are known as “environmentally responsive polymers”, or “smart hydrogels”. They have recently attracted considerable interest within the field of drug delivery [87, 88] as a means of providing an on-off release [89] by swelling and shrinking in response to the presence and absence of, for example, glucose [90-92] or antigens [93, 94]. In the long term perspective it is hoped that sensor-actuator systems could be developed from these hydrogels.

The term “in situ” gelling polymers” also describes a stimulus-induced response, but is generally used more narrowly to denote formulations that gel upon contact with the mucosa, that is, they gel once in position. The most prominent advantage of such formulations is that they are fluid-like prior to contact with the mucosa,
and can thus easily be administered as a drop or by a spray device. This is in contrast to ordinary gels, which may be difficult to administer with spray devices, especially if the solid-like features are salient. *In situ* gelling formulations have been evaluated for several administration routes, including the ophthalmic and nasal routes [62-64, 67, 69, 95], and have shown to increase the residence time and improve drug absorption. The gelation can be induced by a shift in pH (as, e.g., for cellulose acetate phtalate [96]), a shift in temperature (as for the thermogelling poloxamers [27, 97], xyloglucans [48] and EHEC/ionic surfactant mixtures [26, 53]), or by the presence of cations (as for deacetylated gellan gum [52] and alginates [49]).

The rheological performance of deacetylated gellan gum and its effects on nasal contact time and uptake *in vivo* have been investigated in this thesis. They will be discussed in Chapter 3.

### 1.5 Bioadhesion and Mucoadhesion

The idea of using bioadhesive polymers to prolong the contact time in the mucosal routes of drug delivery was introduced in the early 1980s and, since then, it has attracted considerable attention from pharmaceutical scientists. The potential of a drug delivery system to localize a drug at the site of absorption for an extended period of time, and to promote intimate contact between the formulation and the underlying absorbing tissue has great appeal for both local and systemic effects.

#### 1.5.1 Definitions

Good [98] considered bioadhesion to be the phenomenon in which two materials, at least one being of biological nature, are held together for extended periods of time by interfacial forces. The term has also been defined as the ability of a synthetic or natural macromolecule to adhere to a biological tissue [99], which can be either an epithelial surface or the mucus layer covering a tissue. In the latter case, the phenomenon is generally referred to as mucoadhesion [100]. However, in parts of the extensive literature available on the subject, the two terms seem to be used interchangeably. A suggestion has been put forward that bioadhesion be regarded as an all-inclusive term to describe adhesive interactions with any biological or biologically derived substance, and that mucoadhesion only be used when describing a bond involving mucus or a mucosal surface [101].

#### 1.5.2 From bioadhesive tablets to lectin-mediated binding

Amongst the early pioneering work on bioadhesive systems is that of Nagai and coworkers, who showed that the local treatment of aphthae in the oral mucosa was improved by using an adhesive tablet [102]. In addition, they observed increased systemic bioavailability of insulin when given intranasally to beagle dogs as a
powder dosage form [103]. For application to the oral mucosa, novel mucoadhesive ointments were also introduced; these were in fact polymer gels based on polyacrylic acid [20] and polymethyl methacrylate [21]. Bremercker and coworkers stated that the latter system could “be utilized for other drugs, other symptoms, and all mucous membranes…”!

Indeed, the new bioadhesion concept rapidly lead to the idea that bioadhesion could be used advantageously to improve absorption through several administration routes. Over the years, bioadhesive systems have been used for nasal, ocular, buccal, vaginal, rectal and oral drug delivery.

Most of the early work on bioadhesive polymers was performed with “off-the-shelf” polymers, such as the polyacrylic acids in the dry state, often in the form of powders [104, 105], tablets [106], coated spheres [107] or dried films [108]. From these studies, rankings of polymers were made and general conclusions were drawn about the physicochemical characteristics of good bioadhesives, with respect to, e.g., molecular weight, cross-linking density and charged groups. Theories of mucoadhesion began to appear at this time, generally adapted from those of adhesion between other surfaces, as will be discussed below.

However, in the 1990s, as the interest in polymer gels as pharmaceutical dosage forms increased, it was realized that different mechanisms involved in mucoadhesion would be important compared with those of dry dosage forms. It was pointed out that adhesion observed with dry dosage forms may, to large extent, arise from water transfer and dehydration of the mucus layer [109, 110]. This is not likely to be important for gels since they are already fully hydrated. Thus, it should not be presumed that conclusions drawn about the potential mucoadhesion of a dry polymer dosage form are valid for a gel prepared from the same polymer.

Originally, the advantages of mucoadhesive drug delivery systems were considered to lie in their potential to prolong the residence time at the site of absorption, and to provide an intensified contact with the underlying mucosal epithelial barrier (to enhance the absorption of drugs that are usually poorly absorbed). Later, it was discovered that some mucoadhesive polymers, such as polyacrylic acids and chitosan, possess multifunctional properties, and can, for example, modulate the permeability of the epithelial tissues by partially opening the tight junctions [111, 112]. The polyacrylic acids have also shown to inhibit proteolitical enzymes [113], probably by depleting the enzymes of Ca$^{2+}$ and Zn$^{2+}$ ions [114, 115].

Despite this multifunctionality, such mucoadhesive polymers are of limited interest for oral drug delivery since they cannot distinguish between adherent or shed-off mucus, or the surfaces of other gut contents. Instead, for oral drug delivery, the potential of the more specific interactions of plant and bacterial lectins or lectin-like molecules with epithelial cell surfaces is being explored [116]. These molecules can be regarded as cytoadhesives, since they are capable of specifically recognizing and binding to sugar moieties present on the epithelial cell membranes. Owing to this property, they are considered to be promising targeting agents for mucosal delivery of drugs and vaccines [117]. It has been reported that they can be coupled to, for example, microspheres, resulting in an increased residence time in the gastrointestinal tract [118].
The work of this thesis is concerned with the original mucoadhesion concept, as defined above. Polyacrylic acids, also mentioned earlier in this chapter, were included as model polymers in the mucoadhesion studies described in Papers II-VI.

1.5.3 Mechanisms involved in the mucoadhesion process

A complete understanding of how and why certain macromolecules attach to a mucous surface is not yet available, but a few steps involved in the process are generally accepted, at least for solid systems:

1. Spreading, wetting and swelling of the dosage form at the mucous surface, initiates intimate contact between the polymer and the mucus layer.
2. Interdiffusion and interpenetration take place between the chains of the mucoadhesive polymer and the mucus gel network, creating a greater area of contact.
3. Entanglements and secondary chemical bonds are formed between the polymer chains and mucin molecules.

It can be noted that, for polymer gels that are already in equilibrium swelling, the wetting and swelling step is unlikely to be involved.

The components of the mucus involved in interactions are the mucin molecules. These are glycoproteins of high molecular weight (in the range 1–50 \( \cdot \) \( 10^6 \) Da) present in a concentration of 0.5–5% [119], which are also responsible for the viscoelastic properties of the mucus. The mucins are negatively charged at physiological pH because of sialic acid residues in the oligosaccharide units. Hydrogen bonds are often considered to be the most important of the types of secondary chemical bonds that can be formed in the mucoadhesion process [100, 120, 121]. Other types of bonds that might be involved include ionic bonds and van der Waals interactions.

1.5.4 Theories of mucoadhesion

A complete and comprehensive theory that can predict adhesion based on the chemical and/or physical nature of a polymer is not yet available. Five theories of adhesion that were originally developed to explain the performance of such diverse materials as glues, adhesives and paints, have been adapted to the study of mucoadhesion [101, 120, 122]:

1. The electronic theory assumes that a double layer of electrical charge is formed at the interface as a result of the different electronic characteristics of the mucoadhesive polymer and the mucus, and that attractive forces develop from electron transfer across the electrical double layer.
2. The adsorption theory states that a mucoadhesive polymer adheres to mucus because of van der Waals interactions, hydrogen bonds, electrostatic attractions, or hydrophobic interactions.

3. The wetting theory emphasizes the intimate contact between the mucoadhesive polymer and the mucus, and, primarily in liquid systems, it uses interfacial tensions to predict spreading, and subsequent adhesion.

4. The diffusion theory states that the chains of the mucoadhesive polymer and the mucin interpenetrate to a sufficient depth (in the range of 0.2–0.5 µm) to create a semipermanent bond through entanglement. The interpenetration is governed by the diffusion coefficients, which are in turn dependent on the molecular weight and the flexibility of the chains.

5. The fracture theory analyzes the force that is required for separation of two surfaces after adhesion. It is considered to be appropriate for the calculation of fracture strengths of adhesive bonds involving rigid mucoadhesive materials [101], and has frequently been applied to the analysis of tensile strength measurements on, for example, microspheres [123] and powder specimens [124].

These general theories are not particularly useful in establishing a mechanistic base to bioadhesives, but they do identify variables that are important to the bioadhesion process [125].

1.5.5 Methods for measuring mucoadhesion

The first method involving the study of putative bioadhesive polymers was described by Park and Robinson in 1984 [126]. With this method the polymer interaction with a conjunctival epithelial cell membrane was investigated by using fluorescent probes. In recent years, molecular interactions at cell surfaces have been examined by using, for example, force microscopy techniques, such as AFM [127, 128].

The majority of the bio- and mucoadhesion methods found in the literature are based on measuring the force required to break the adhesive bond between the model membrane and the adhesive. Depending on the direction in which the adhesive is being separated from the substrate, peel, shear, and tensile forces can be measured (Figure 4). The peel adhesion tests are mainly used for buccal [129] and transdermal patches [130], whereas the shear and tensile tests have been widely employed in mucoadhesion studies on a variety of polymer preparations.

Among the most common shear strength tests is the Wilhelmy plate method, which was described by Smart and coworkers [108], used for investigating the adhesion of polymer films to mucin solutions. A vast number of tensile strength methods are found in the literature, most of which determine the force required to detach tablets [106, 131-133], disks [134-136], and powder specimens [104, 105, 124] from excised mucous tissue.
Methods such as washing tests based on measuring the retention on mucous tissue have been described for polymer coated particles [107, 137] and liquid formulations [138, 139]. Individual microspheres have been investigated by using an electrobalance [123], a flow channel method [140], and contact angle measurements [141].

Colloidal gold staining was proposed in 1989 for bioadhesive hydrogels [142]. Interactions with mucin-gold conjugates resulted in the development of a red colour on the hydrogel surface. A direct-staining method to evaluate polymer adhesion to human buccal cells, following exposure to aqueous polymer dispersions, was recently reported [143].

For polymer gels a simple viscometric method was described by Hassan and Gallo in 1990 [144]. During recent years dynamic rheological measurements have been used and changes in viscoelastic properties have been studied. This approach has become by far the most widely used method for gels and polymer solutions, and will be further discussed in Chapter 4 and in Papers II-III.

A few tensile strength methods have also been reported for gels, based on measuring the detachment from mucin tablets or mucin solutions [145-147]. The development of a tensile strength method using freshly excised porcine nasal mucosa and a texture analyzer was the subject of Paper IV, and will be further discussed in Chapter 5.

In vivo methods for measuring mucoadhesion are relatively scarce. Some of the reported methods involve the use of gamma-scintigraphy [68, 148, 149] or dyes [150] to assess the residence time at the application site, while others involve measuring the gastrointestinal transit by using radioisotopes [105, 151]. These approaches lack the precision to be able to distinguish between those effects that are attributable to mucoadhesive interactions and those arising from other causes that can contribute to the residence time or transit.
2. Aims of the thesis

The overall objective of this thesis was to investigate the importance of the rheological and the mucoadhesive properties of a gel for the contact time that can be expected in vivo. This is exemplified by the ocular and nasal routes of administration, however the principles discussed may also apply to other mucosal routes.

Principally, the work included in the thesis may be divided into two parts:

(I) Rheological and in vivo performance of gellan gum where the more specific aims were:

- to study how the polymer concentration and salt content influence gellan gum formulations by evaluating viscoelastic properties (Paper I).
- to investigate the effects of a gellan gum formulation on the in vivo uptake and transfer in rat nasal epithelium (Paper VII).

(II) Mucoadhesion methods for gel formulations where the more specific aims were:

- to evaluate the use of rheological measurements as a means of investigating mucoadhesive interactions between gels and mucin (Papers II-III).
- to develop and evaluate a tensile strength method adapted for mucoadhesion measurements on polymer gels and freshly excised nasal mucosa (Papers IV-V).
- to explore whether low frequency dielectric spectroscopy could be used as a tool for investigating the compatibility between pharmaceutical gels and mucous tissue (Paper VI).
3. **In situ** gelling performance of deacetylated gellan gum

Gellan gum is a linear anionic microbial polysaccharide that is secreted by the strain *Sphingomonas paucimobilis* (formerly known as *Pseudomonas elodea*). The polymer backbone is comprised of a tetrasaccharide repeat unit of glucose, glucuronic acid and rhamnose in the molar ratio 2:1:1 [152, 153] (Figure 5). In its native form the polysaccharide partially carries O-acetyl and O-glyceryl substituents, which inhibit crystallization of localized regions of the chains [154] and suppress intermolecular aggregation [155]. Deacetylation of the polysaccharide enables extensive intermolecular association to take place and the formation of strong brittle gels with cations to occur [155, 156]. Marketed as Gelrite or Kelcogel, the deacetylated form of gellan gum is approved in the USA and EU for use in food as a gelling, stabilizing and suspending agent [157].

![Figure 5. The structure of gellan gum from *Sphingomonas paucimobilis*. The native polysaccharide partially carries O-acetyl and O-glyceryl substituents, whereas the commercial product is completely deacetylated.](image)

Because of its ability to form strong clear gels at physiological ion concentration, gellan gum has been widely investigated for use as an *in situ* gelling agent in ocular formulations. It has been reported that it can provide a significantly prolonged corneal contact time [52, 63, 64, 67] and it is currently marketed in a controlled-release glaucoma formulation called Blocadren® Depot (Timoptic-XE®). It has also been suggested that gellan gum is a promising polymer for use in nasal formulations [158]. However, prior to the work conducted for this thesis, only one *in vivo* study has been published on this subject [159], where a gellan gum formulation was shown to moderately enhance the antibody response after nasal administration of viral antigens. Other *in situ* gelling systems, such as temperature and pH responsive gels, have, on the other hand, appeared more frequently in nasal drug delivery studies, and have been shown to increase the residence time and improve drug absorption (see, for example, references [62, 69, 160-162]).
The most prominent advantage of using an *in situ* gelling formulation is that, owing to its low viscosity, it can be readily administered as a drop or a spray. After gelling is induced by some physiological stimulus at the site of administration, the formulation attains semisolid properties.

The *in situ* gelling properties of gellan gum are attributed to its responsiveness to cations present in physiological conditions. Several models have been put forward to explain the gelation [163, 164], and the model proposed by Robinson et al (Figure 6) will be discussed in more detail here. In an ion-free aqueous medium at room temperature, the polymer chains form double helices, resulting in a fluid that has a viscosity close to that of water. Upon contact with gel-promoting cations (Na⁺, K⁺, Ca²⁺) present in tear fluid and nasal secretion a portion of the helices associate and cation-mediated aggregates are formed, acting as cross-links in the gel network [164]. However, the gelation of gellan gum is also affected by temperature. On heating a gellan sample in an ion-free medium, the polymer chains adopt a disordered coil conformation. Two transitions are seen on heating a sample with cations present: firstly, the non-aggregated helices melt out and, secondly, the aggregated helices melt out at a higher temperature.

![Figure 6](image.png)

**Figure 6.** The model for the gelation of gellan gum on addition of cations (●), proposed by Robinson et al. Modified from reference [164].
A simple illustration of the rheological differences between gellan gum samples made in the absence and presence of ions, is shown in Figure 7. A gellan sample made in water, such as the formulation used in the *in vivo* experiments in Paper VII, exhibits the properties of a polymer solution, i.e., the $G'$ and $G''$ are relatively low and frequency dependent. On the other hand, a sample that is prepared in 0.9% NaCl to simulate physiological conditions, has a frequency-independent $G'$ that is considerably higher than $G''$ over a large frequency range. That is, it exhibits the rheological behavior of a strong cross-linked gel [6, 165].

![Figure 7. The frequency dependence of the elastic modulus, $G'$ (circles), and the viscous modulus, $G''$ (triangles), for a 0.5% gellan sample made in water (open symbols) and for a 0.5% gellan preparation in 0.9% NaCl (filled symbols), simulating the *in vivo* gelation.](image)

In Paper I rheology was used to examine the capacities of the different cations to cross-link gellan gum, thereby forming a gel. The effects of the polymer concentration and ionic content on the gel strength were also investigated. The total ionic content in the gellan preparations was varied, while the proportions of the different cations (Na$^+$, K$^+$, Ca$^{2+}$) were kept constant. The standard medium was simulated tear fluid, containing 142 mM Na$^+$, 19 mM K$^+$ and 0.6 mM Ca$^{2+}$ [166]. The tear fluid ratio, TFR, was defined as:

$$TFR = \frac{[\text{ions present in sample}]}{[\text{ions present in tear fluid}]} \quad (1)$$

It can be noted that the composition of nasal secretion is similar to that of tear fluid but the ion content is somewhat higher: 150±32 mM Na$^+$, 41±18 mM K$^+$, 8±4 mM Ca$^{2+}$ [167]. Thus, to a certain extent, the discussion of important parameters in the following sections is also relevant to nasal drug delivery.
3.1 The influence of the polymer concentration and the ionic content

Figure 8 shows how the gel strength (described by the elastic modulus, \( G' \)) depends on the polymer concentration. Here, all gels were prepared in simulated tear fluid (TFR=1). It can be seen that, even at the lowest polymer concentration tested (0.1%), a gel was formed – albeit a very weak one – with a frequency independent \( G' \) (phase angle, \( \delta \), <10°). On increasing the concentration to 0.25% a very firm gel was produced with \( G' = 1500 \) Pa.

![Figure 8. The elastic modulus \( G' \), obtained at the frequency 1 Hz, for gellan gum samples prepared in simulated tear fluid as a function of polymer concentration.](image)

In Figure 9 the elastic modulus, \( G' \), and the phase angle, \( \delta \), for 0.5% gellan gum are shown as functions of the ion concentration (described by TFR). The elastic modulus can be seen to increase substantially as the ionic content increases, but reaches a plateau at a tear fluid ratio of 0.5, i.e., when the amount of ions present is half of that in the tear fluid. Between a tear fluid ratio of 1 and 2, the elastic modulus and the phase angle are almost constant. At tear fluid ratios above two, the gel strength decreases and the gels become turbid. This is consistent with the previous observations made by, for example, Morris and coworkers [168]. At high ionic content the polysaccharide is less soluble even at high temperatures, and the authors suggested that insoluble polysaccharide aggregates (that scatter light) can act as heterogeneous nuclei, leading to the growth of microgels that interconnect to form a weakened gel network.
Figure 9. The elastic modulus, $G'$ (●), and the phase angle, $\delta$ (□), obtained at the frequency 1 Hz, for 0.5% gellan gum samples as functions of the ion concentration (described by TFR).

In Paper I it was also observed that the $G'$ of 0.5% gellan gum in a solution containing only Na$^+$ ions decreased at high Na$^+$ concentrations. With a Na$^+$ concentration of 426 mM, the $G'$ was only 20% of that of a solution containing 142 mM Na$^+$ (corresponding to the Na$^+$ concentration of tear fluid). The turbidity of the 426 mM sample was high and the melted sample was granular, indicating that microgel aggregates were present.

Divalent cations, such as Ca$^{2+}$, are known to produce stronger gellan gum gels than monovalent ions such as K$^+$ and Na$^+$ [169-171]. This has been attributed to the different strengths of the interactions that interconnect the gellan double helices. In the presence of monovalent cations, such as K$^+$, the helices are connected by the carboxylate – K$^+$ – water – K$^+$ – carboxylate interactions. On the other hand, with divalent cations, such as Ca$^{2+}$, each K$^+$ – water – K$^+$ bridge is expected to be replaced by a single Ca$^{2+}$ ion, so that the helices are linked by carboxylate – Ca$^{2+}$ – carboxylate interactions, which are obviously stronger than the K$^+$/water bridge [172].

Moreover, K$^+$ ions have been shown to be more efficient gel-promoters than Na$^+$ ions [170, 171], an effect that has been ascribed to the smaller size of the hydrated K$^+$ ion, as compared to the hydrated Na$^+$ ion [169]. For example, 1.0% gellan gels with an elastic modulus of 100 Pa were found to be formed in the presence of 75 mM NaCl, 50 mM KCl or 4 mM CaCl$_2$, in the study by Miyoshi et al [170]. Both tear fluid and nasal secretions contain a relatively small proportion of the most potent gel-promoting divalent cations and a relatively large proportion of monovalent cations, in particular Na$^+$ ions. In Paper I it was concluded that the Na$^+$ ion is the most important gel-promoting cation in the in vivo situation, since the elastic modulus of a gel prepared with only Na$^+$ (in the concentration corresponding to that in tear fluid) was approximately equal to that obtained in standard tear fluid, see Table 1. At the temperature of the eye, 35°C, the contributions of K$^+$ and Ca$^{2+}$ to the gel strength were almost negligible, which
could obviously be attributable to the relatively small proportions of these ions present in tear fluid.

**Table 1.** The elastic modulus, \( G' \), and the phase angle, \( \delta \), for 0.5% gellan gels prepared with single ions in the concentration corresponding to that in tear fluid (Conc\(_i\)). For comparison, the values for a gel prepared in tear fluid are also shown.

<table>
<thead>
<tr>
<th>Ion present</th>
<th>Conc(_i) (mM)</th>
<th>( G' ) (Pa)</th>
<th>( \delta ) (degrees)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K(^+)</td>
<td>19</td>
<td>0.34±0.05</td>
<td>25±4.5</td>
</tr>
<tr>
<td>Na(^+)</td>
<td>142</td>
<td>8 800±750</td>
<td>2.3±1.0</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>0.6</td>
<td>21±12</td>
<td>5.5±4.0</td>
</tr>
<tr>
<td>Tear fluid</td>
<td>-</td>
<td>10 600</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Mean value±standard deviation, n=3.

### 3.2 Rheological thermal scans

In Paper I the gellan gum gels were formed in the rheometer cup during a controlled cooling process. In this way no shearing took place before the rheological measurement and the characteristics of the gels were highly reproducible. The cooling rate should be kept constant, and it was recently reported that the cooling rate chosen can affect both the gel strength and the turbidity [173]. This approach was also used for preparation of the gellan gum gels and mucin mixtures that were investigated in Paper II, and will be discussed in Chapter 4.

Rheological thermal scans have been frequently described in the literature, as a means of characterizing the gelation of gellan gum [155, 170, 174]. Such scans can also be useful for investigating transitions such as melting in this material when it is being heated or cooled at different rates. From the scans performed in Paper I, thermal hysteresis was evident, as the heating and cooling curves were not superimposed (Figure 10). This has been observed in previous studies [155, 170, 175]. The heating curve also shows that the gel starts to melt in the same temperature interval as it was formed during cooling (around 45ºC), indicating a helix to coil transition of the non-aggregated helices. The melting of the cation-aggregated helices began at a higher temperature, around 85ºC, but was not complete. However, another, more unexpected transition was observed at 60ºC. The elastic modulus increased, indicating the formation of some other structures in the gel. Such structural increase phenomena have also been reported by others [175]. The two-step changes seen during heating have been attributed to an initial dissociation of unaggregated helices, accompanied by an enhanced sol fraction of “dangling ends” that can contribute to an increased gel strength, which is then followed by dissociation of the cation-mediated aggregates [176].

From Paper I it was concluded that gellan gels are formed in tear fluid even when the polymer concentration is only 0.1%, and that gellan preparations of the order 0.5–1% do not require more than 10–25% of the quantity of ions in tear fluid to form gels. Furthermore, it was shown that the most important gel-promoting ion *in vivo* is Na\(^+\). Because of these findings it can be expected that
rapid gelation takes place in vivo, and that potential dilution effects at the administration site would not pose a problem.

![Figure 10](image)

**Figure 10.** The temperature dependence of the elastic modulus, $G'$, at the frequency 1 Hz during cooling (filled symbols) and heating (open symbols) for 0.5% gellan gum gels, prepared in 426 mM NaCl (corresponding to the concentration of Na⁺ in TFR=3). ($\blacksquare$, $\square$) First temperature scan cycle. ($\bullet$, $\circ$) Second temperature scan cycle.

### 3.3 Intranasal administration

Traditionally, the nasal route has been used for delivery of drugs for local treatment of diseases such as nasal congestion, allergy and infections. More recently, nasal administration has been put forward as one of the most convenient alternatives for systemic delivery when oral administration of a drug gives an undesirably slow effect, or when a drug is highly metabolized or incompletely absorbed in the gastrointestinal tract. Nasal sprays may also be preferred to injections because of the better patient compliance, and may have applications in, e.g., pain management [177] and vaccinations [178]. Moreover, several animal studies show that some substances are transferred directly to the CNS after nasal administration, as reviewed by Mathison et al [179] and Illum [180]. Some studies indicate that this transfer along the olfactory nerves is also present in man [181, 182], and may become important for, e.g., the delivery of short peptides (around 1 kDa) for neuroprotection, as discussed by Gozes [183].

In Paper VII the nasal uptake of a 3 kDa fluorescein dextran from a gellan gum formulation was investigated. This model substance is in the same molecular weight range as, for example, salmon calcitonin, which is currently marketed for intranasal administration.
For systemic delivery, powder formulations and microspheres have frequently been used as a means of increasing the nasal absorption of drugs, see, for example, the reviews by Dondeti et al [184] and Illum [46]. Enhanced bioavailability has also been reported after administration of viscous polymer solutions (e.g., [57, 185]), but it has not been clearly established whether this is caused by a prolonged residence time in the nasal cavity. Harris et al [186, 187] reported quite small differences in residence time and bioavailability when various concentrations of methylcellulose was utilized. Pennington et al [188] reported clearance half times of 1 hour and 2.2 hours for 0.6% HPMC and 1.25% HPMC, respectively, although the difference between the two concentrations was not statistically significant.

The dependence of the bioavailability and residence time on viscosity may be a consequence of the larger droplet size caused by the higher viscosity of the formulation, rather than of the higher viscosity itself [186]. The nasal spray pumps used today primarily result in deposition in the anterior nasal cavity, and Harris et al [186, 187] reported that this pattern was further amplified with increasing viscosity. As already pointed out, the main advantage of using an in situ gelling formulation is that, owing to its low viscosity, it can be readily administered as a drop or a spray. The formulation adopts semisolid properties when gelling is induced by some physiological stimulus at the site of administration. Such a stimulus might be, for example, the presence of ions, as in the case of gellan gum. Based on the results of Paper I, rapid gelling could be expected upon contact with the mucosa since small quantities of ions were shown to be sufficient for the formation of a strong gel.

The aim of Paper VII was to investigate whether the use of a gellan gum formulation would change the extent or the time profile of the uptake and transfer of fluorescein dextran (FD3) across nasal epithelia in comparison to a plain isotonic water solution. Furthermore, the distribution of fluorescence and histological changes in the mucosa were to be evaluated.

### 3.4 Outline of the in vivo experiments

The gellan formulation chosen (0.5% FD3, 0.5% gellan gum, 4% mannitol) had a low viscosity, around 7.5 mPas. Thus, it was feasible to administer the formulation by using a polyethylene tube, which was inserted into the right nostril of the anesthetized rats, attached to a micropipette. A plain isotonic mannitol solution (4%) of fluorescein dextran (0.5%) was used as the control formulation. A more detailed description of the methods can be found in Jansson & Björk [189], but briefly, the experiments were conducted as follows: At times 15, 60, 120 and 240 min after administration, the rats were perfusion fixed via the heart, and the nasal cavity, including the olfactory bulbs, was isolated. After decalcification, the cavity was cut in slices approximately 2–3 mm thick, which were assigned to the levels I–V, according to the schedule depicted in Figure 11. Following dehydration and embedding, the samples were sectioned using a microtome, and the sections were viewed in a fluorescence microscope. A number of areas of the nasal cavity were defined: the upper and lower septum;
nasoturbinate and maxilloturbinate (only present in the anterior nasal cavity); and
endoturbinates and ectoturbinates (only present in the posterior nasal cavity). The
epithelium and lamina propria in every region were evaluated in terms of the
occurrence of FD3 fluorescent cells and were given scores in the range 1–5, where
1 indicates fluorescence in sporadic cells and 5 in all cells. Each area was given
two scores, one maximum and one minimum. In the respiratory epithelium,
individual scores were given to columnar cells and goblet cells and in the
olfactory epithelium to olfactory cells and supporting cells.

Figure 11. Cuts (1–7) were made perpendicular to the hard palate and to the plane of the
nasal septum. The roman numerals I–V denote the various levels of the nasal cavity.
endo- and ectoturbinates, maxilloturbinate, nasoturbinate, olfactory bulb

3.5 Deposition and gelation in the nasal cavity

No general adverse effects, related to the intranasal administration of the mannitol
solution or the gellan formulation (such as bleeding from the nose, or respiratory
dysfunctions) were observed in the rats. Furthermore, there were no obvious
histological changes such as swelling, cilia loss or loss of epithelial cells.

The FD3 gellan formulation was mainly located in the anterior nasal cavity at
all time points studied. When investigating the different levels of the nasal cavity,
gel was observed in large areas of levels I and II in all animals. More posterior, at
level III, the gel was frequently observed in discrete regions whilst at level IV it
was only occasionally observed. This localization pattern indicates a rapid
gelation upon contact with the mucosa, which was expected from the results of
Paper I. In humans, the surface area of the nasal cavity is more than 10 times
larger than in rats, while the volume of the formulation would only be 2–3 times
larger. Therefore, the gelling is likely to occur rapidly in humans as well. Less
spreading of the dose would be expected than after administration of a plain
solution because of the rapid gelling so the demands made on the deposition from
a spray pump would increase as a consequence.

From the micrographs in the following section (Figures 13 and 14) it is evident
that the gel formed in vivo was strong enough to remain in the nasal cavity for the
whole time interval studied.
3.6 Increase of the uptake and transfer in rat nasal epithelium

Figure 12 illustrates the fluorescence scorings of the two formulations at different times after administration, for the most anterior (I) and the most posterior (IV) levels investigated.

At level I, the scores were higher after administration of the FD3 gellan formulation than they were for the FD3 mannitol solution. There was a clear tendency for the extent of the fluorescence to decrease with time after administration of the FD3 mannitol solution, a trend that was less pronounced after administration of the gellan gum formulation. At level IV, the scores were higher for the mannitol solution. For the levels in between, the difference between the scores for the two formulations was less pronounced (data not shown).

![Figure 12](image)

**Figure 12.** The average range of fluorescence scoring in: (a) the respiratory epithelium at level I, (b) the lamina propria at level I, and (c) the olfactory epithelium and lamina propria at level IV. Dashed bars indicate the scoring after administration of the FD3 mannitol solution and solid bars the scoring after administration of the FD3 gellan formulation.

Since the localization of the gel seemed to influence the uptake, two regions in which there was a high probability of deposition of both formulations were identified, one comprising respiratory and the other olfactory epithelium. The respiratory region selected was the middle part of the septum at level I.
(Figure 13a), and the olfactory region was the superior, medial aspect of ectoturbinate 1' at level III (Figure 14a).

In the respiratory region defined, the extent of FD3 fluorescence was approximately equal for the two formulations 15 min after the administration. 60, 120 and 240 min after administration of the gellan formulation, the degree of FD3 fluorescence exceeded that observed after administration of the mannitol solution. This is illustrated by Figure 13b-d, showing micrographs from this region in animals euthanized 120 min after administration of the formulations.

Figure 13.
(a) At level I, the middle part of the septum (square) was identified as a region with a high probability of deposition of both the mannitol solution and the gellan formulation.
s septum, n nasoturbinate, m maxilloturbinate.
In (b-d) representative micrographs are shown for the respiratory region defined in (a), from rats euthanized 120 min after administration of the formulations. The cell nuclei are red (propidium iodide).
(b) FD3 gellan formulation, scale bar 50 \( \mu \)m. (c) Higher magnification of (b), scale bar 10 \( \mu \)m. (d) FD3 mannitol solution, scale bar 50 \( \mu \)m.
The extent of fluorescence in the epithelium and lamina propria was higher after administration of the FD3 gellan formulation than after the FD3 mannitol solution. There was intracellular as well as some paracellular (arrows) fluorescence.
At level III in the defined olfactory region, gel was visible in the lumen of the nasal cavity in 5 out of 8 studied images from rats having obtained the FD3 gellan formulation. In all of these cases, there was a higher degree of FD3 fluorescence than in the corresponding images from rats having received the FD3 mannitol solution, which is exemplified by the micrographs in Figure 14b-d.

Figure 14.
(a) At level III, the olfactory region selected was the superior, medial part of ectoturbinate 1' (square). 
s septum, 1’, 2’ ectoturbinates, 1, 2, 3 endoturbinates, np nasopharynx.
In (b-d) representative micrographs are shown for the olfactory region defined in (a), from rats euthanized 120 min after administration of the formulations. The cell nuclei are red (propidium iodide).
(b) FD3 gellan formulation, scale bar 50 µm. (c) Higher magnification of (b), scale bar 10 µm. (d) FD3 mannitol solution, scale bar 50 µm.
The extent of fluorescence in the epithelium and lamina propria was higher after administration of the FD3 gellan formulation than after the FD3 mannitol solution. Some point-shaped intracellular (arrows) fluorescence could be seen, but no paracellular fluorescence was evident in the olfactory epithelium.
In the respiratory epithelium, both intracellular and paracellular FD3 fluorescence was observed, whereas in the olfactory epithelium, only intracellular FD3 fluorescence could be discerned. No other differences, such as variations in FD3 fluorescent cell types, were found.

FD3 fluorescence in the olfactory bulb was also observed. Here the degree of fluorescence was closely correlated to the fluorescence observed in the adjacent olfactory mucosa.

Both the semi quantitative scoring and the micrographs clearly illustrate that there was a more extensive localization of FD3 fluorescence in the anterior nasal cavity after administration of the gellan gum formulation than after administration of the mannitol solution. This was true for both the respiratory epithelium and the underlying lamina propria. The reason for the higher degree of fluorescence after administration of the gellan gum formulation was not investigated, but it is most likely related to the high local concentrations caused by slower clearance of the gel from the nasal cavity. This is confirmed by the observation that only the degree of fluorescence was different, whereas the distribution of the fluorescence in the epithelial cells was the same, irrespective of whether the gellan formulation or the mannitol solution had been administered.

The lower scoring for the gellan formulation in the posterior nasal cavity is attributable to lower deposition in these areas. The micrographs clearly show that where the gel had been deposited, the degree of FD3 fluorescence in the olfactory epithelium and lamina propria was high. When intending to target drugs to the olfactory epithelium in humans, the design of the spray pump will become very important. Spray pumps that are able to deposit spray in the olfactory region will need to be designed since the spray pumps used today mostly deposit the spray in the vestibule of the nasal cavity, and do not reach the olfactory epithelium at all.

In conclusion, Paper VII shows that a gellan gum formulation can have a residence time of at least 4 h in the rat nasal cavity without having visible harmful effects on the mucosa. The epithelial uptake of the model substance, a 3 kDa fluorescein dextran, and its transfer across the epithelium was improved by using the gellan gum formulation rather than a mannitol solution. This increase was not accompanied by any qualitative changes in the epithelial FD3 distribution. Indeed, this study shows that nasal drug delivery, whether systemic or olfactory, could benefit from using an in situ gelling polymer such as gellan gum.
4. Rheology as a means of evaluating polymer-mucin interactions

In 1990, Hassan and Gallo [144] proposed a simple rheological test for the assessment of the strength of polymer-mucin interactions. The idea put forward by the authors was that when a putative mucoadhesive polymer is mixed with a mucin solution, there should be a synergistic increase in viscosity. The approach is, to some extent, based on the interdiffusion-interpenetration theory of the mucoadhesion process and, as such, aims to simulate the interpenetration layer between the gel and the mucus layer (Figure 15).

Hassan and Gallo suggested that the viscosity of a polymer-mucin mixture should be considered to be the result of the contributions from the separate components, the polymer and the mucin, and from a viscosity component arising from mechanical interactions (entanglements) and chemical interactions between the polymer and the mucin. For mucoadhesive polymers it is believed that the rheological response of a polymer-mucin mixture should be larger than the sum of the contributions from the gel and the mucin, a phenomenon that is commonly described as “rheological synergism”. It can be noted that Hassan and Gallo did not publish any further studies on the subject. Instead, their approach was adopted by several other researchers, and the rheological method has been used extensively over the last 10 years, presumably because of its simplicity. During recent years, dynamic measurements have been used, and the rheological response has mostly been evaluated in terms of the elastic and the viscous moduli.

Several attempts have been made to screen and rank a number of different polymers (e.g., [121, 145, 190, 191]). However, a wide variation in results is
found in the literature, which has been attributed mainly to differences in the mucin type and concentrations used [192-194], as well as to the different polymer concentrations [191, 195].

There has been some debate concerning the use of commercial freeze-dried mucins as a substitute for fresh mucus. When freeze-dried mucins are redispersed in water, they do not obtain the same viscoelastic properties as freshly isolated mucus [193, 194]. However, the use of fresh mucus gels is not without its problems either, since it requires reproducible isolation and purification procedures. In Papers II and III two commercially available mucins were used, the PS and the BSMG mucins. The ion contents of these mucins are presented in Table 2, and may be important if the samples are prepared in water, especially if an ion-sensitive polymer is being studied. The polymers used in Papers II and III, gellan gum and cross-linked polyacrylic acids are indeed ion-sensitive, but the investigations were performed under simulated physiological conditions, i.e., in the presence of salts, where the small quantities of ions originating from the mucins were not expected to have a significant influence on the results.

Table 2. The ion content of the 4% mucin samples used in Papers II and III. For comparison, the ion content of simulated tear fluid is also given.

<table>
<thead>
<tr>
<th>Ion content (mg/kg)</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Ca²⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>4% PS mucin</td>
<td>76</td>
<td>11</td>
<td>34</td>
</tr>
<tr>
<td>4% BSMG mucin</td>
<td>318</td>
<td>6</td>
<td>171</td>
</tr>
<tr>
<td>Simulated tear fluid</td>
<td>3 270</td>
<td>724</td>
<td>23</td>
</tr>
</tbody>
</table>

4.1 The synergism parameters

The most commonly used synergism parameter, $\Delta G'$, also called the interaction term, is the elastic component that is calculated from

$$G_{\text{mix}}' = G_p' + G_m' + \Delta G'$$  \hspace{1cm} (2)

where $G_{\text{mix}}'$ is the elastic modulus of the polymer-mucin mixture and $G_p'$ and $G_m'$ represent the elastic modulus of polymer and mucin, respectively. In Papers II and III this equation was simplified [147, 196] because of the negligibly small elastic modulus of the mucin solutions used in these studies and, instead, $\Delta G'$ was calculated from

$$G_{\text{mix}}' = G_p' + \Delta G'$$  \hspace{1cm} (3)

Furthermore, the relative synergism parameter, which has been put forward as an alternative to the absolute synergism parameter [192], was calculated from

$$\text{Relative } \Delta G' = \frac{\Delta G'}{G_p'} = \frac{(G_{\text{mix}}' - G_p')}{G_p'}$$  \hspace{1cm} (4)
In Paper III the choice of synergism parameter was found to have a bearing on the results obtained. This is discussed in section 4.6.

### 4.2 Mucin interactions with gellan gum

In Paper II the potential interactions of gellan gum with the PS and the BSMG mucins were investigated. As discussed in Chapter 3 (and in Paper I), the gelling process of gellan gum is highly temperature dependent, with a distinct sol-gel transition occurring at around 45°C. In Paper II, the gellan-mucin mixtures were prepared at 65°C and subjected to controlled cooling, which ensured that homogeneous samples were obtained and that the rheological data would be reproducible without noticeably degrading the mucin.

The gellan mixtures with PS mucin (4% w/w) in simulated tear fluid showed positive interaction terms ($\Delta G'$) for gellan concentrations of 0.25% and 0.5%, whereas a concentration of 0.75% gave an interaction term that was not significantly different from zero (Figure 16a). The gellan gum gel produced in tear fluid at 0.75% was very strong ($G'$ around 14 000 Pa). Therefore, the effects of adding PS mucin were probably too small to be seen.

In contrast, the effect of adding BSMG mucin was large; the gel structure was almost totally destroyed (Figure 16b). This could be caused by strong interactions or it could be the result of incompatibility between the BSMG mucin and gellan gum. Another alternative is that BSMG mucin affects the gelling process, for example by binding divalent cations to the mucus sialic acid groups [104], and in so doing, deprives the gellan gum of the necessary cross-linkers.

*Figure 16.* The elastic modulus of gellan gum gels of various concentration, $G'_p$ (white), the corresponding mixtures with mucin, $G'_{mix}$ (black), and the calculated interaction terms, $\Delta G'$ (striped). All samples were prepared in simulated tear fluid. Values shown were obtained at a frequency of 1 Hz. Mean values±standard deviation, $n=3$, except for mixtures with BSMG mucin where $n=1$.

(a) Gellan mixtures with 4% PS mucin. (b) Gellan mixtures with 4% BSMG mucin.
4.3 Mucin interactions with cross-linked polyacrylic acids (Carbopol polymers)

In Papers II and III a series of Carbopol polymers were used as model polymers; these are well-known commercially available polyacrylic acid polymers (Figure 17). They are generally considered to be mucoadhesive, merely on the basis of early studies on dry formulations such as tablets and compacts, and have been frequently included in rheological mucoadhesion studies (e.g., [145, 191, 195, 197, 198]). This makes them suitable for methodological evaluation, the subject of Paper III in particular.

![Figure 17. Schematic structure of the cross-linked polyacrylic acid (Carbopol) polymers.](image)

The Carbopol polymers were delivered from the manufacturer as dry flocculated powders, with the average size of each floccule being around 2–7 µm. The floccules consist of a number of inseparable primary particles containing a network of cross-linked polymer chains. In contact with water and exposed to a neutral pH, the floccules will swell up to 1000 times their volume and can then be regarded as swollen gel particles. If the concentration is sufficiently high, the gel particles will come into contact with each other, forming a continuous gel network in the sample. This is seen as a distinct increase in $G'$ at low concentrations, whilst at higher concentrations the preparations have typical gel properties (Figure 18). In the presence of ions, the carboxylic acid groups will be shielded and the polymer chains then adopt a less expanded structure. Thus a higher polymer concentration is required for gel formation in the presence of salts than for an ion-free medium.
Figure 18. The elastic modulus, $G'$, as a function of polymer concentration for three cross-linked Carbopol polymers (C934, C940, C981) and one linear Carbopol polymer (C907). All samples were prepared in 0.9% NaCl. Values shown correspond to the frequency 1 Hz. For the cross-linked polymers a distinct increase in $G'$ was seen at low concentration, whereas the linear polymer had a flatter curve.

The most evident effect seen in Papers II and III was that positive values of the synergism parameters were obtained at low polymer concentrations. At higher concentrations the cross-linked polymers gave negative values, whereas the only linear polymer included (C907) gave positive values with both of the mucins over the entire concentration range tested (Table 3).

In contrast to the results obtained with gellan gum, the BSMG mucin gave larger positive values of $\Delta G'$ than the PS at the low polymer concentrations, and smaller negative values at higher concentrations.

4.4 The effect of gap width and the choice of frequency

With particulate samples such as the cross-linked Carbopol gels, it is important to ensure that the rheological experiments measure the bulk properties of the sample. As a rule of thumb, the gap width of the measuring geometry should be at least 10 times greater than the diameter of the particles. With smaller gaps, there is a risk that the measured rheological properties are correlated to the number of gel particles within the gap and the properties within each particle instead of reflecting the bulk properties. This problem was demonstrated in Paper II by using a parallel plate measuring geometry for which the gap width can be varied. For a mixture of 0.75% C934 and 4% PS mucin the sign of the calculated synergism parameter switched from negative to positive when the gap width was less than or
equal to 0.5 mm (Figure 19). For the BSMG mixture this was not observed, the interaction term was independent of the gap width. This may mean that the BSMG mixture has a more continuous gel structure without exhibiting evident particulate behaviour.

Table 3. Rheological parameters for the Carbopol gels and the Carbopol-mucin mixtures. All samples were prepared in 0.9% NaCl.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Conc (%)</th>
<th>$G_p^\prime$ (Pa)</th>
<th>$G_{mix}^\prime$ (Pa)</th>
<th>$\Delta G^\prime$ (Pa)</th>
<th>Rel. $\Delta G^\prime$</th>
<th>$G_{mix}^\prime$ (Pa)</th>
<th>$G_p^\prime$ (Pa)</th>
<th>$\Delta G^\prime$ (Pa)</th>
<th>Rel. $\Delta G^\prime$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C907</td>
<td>2</td>
<td>0.103 (0.014)</td>
<td>0.37 (0.11)</td>
<td>0.27 (0.11) s.</td>
<td>2.6</td>
<td>1.28 (0.21)</td>
<td>0.05 (0.32)</td>
<td>1.17 (0.21) s.</td>
<td>11.4</td>
</tr>
<tr>
<td>C907</td>
<td>2.2</td>
<td>0.153 (0.026)</td>
<td>0.605 (0.042)</td>
<td>0.452 (0.049) s.</td>
<td>2.95</td>
<td>1.64 (0.47)</td>
<td>0.07 (0.1)</td>
<td>1.48 (0.47) s.</td>
<td>9.67</td>
</tr>
<tr>
<td>C907</td>
<td>7.4</td>
<td>9.93 (0.32)</td>
<td>21.5 (1.0)</td>
<td>11.6 (1.1) s.</td>
<td>1.2</td>
<td>37.2 (2.4)</td>
<td>0.14 (0.1)</td>
<td>27.3 (2.4) s.</td>
<td>2.7</td>
</tr>
<tr>
<td>C981</td>
<td>0.25</td>
<td>0.182 (0.076)</td>
<td>0.309 (0.069)</td>
<td>0.13 (0.10) n.s.</td>
<td>0.70</td>
<td>0.215 (0.025)</td>
<td>0.034 (0.228) n.s.</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>C981</td>
<td>0.5</td>
<td>10.40 (0.40)</td>
<td>2.44 (0.80)</td>
<td>-7.96 (0.89) s.</td>
<td>-0.77</td>
<td>3.58 (0.22)</td>
<td>-6.62 (0.46) s.</td>
<td>-0.66</td>
<td></td>
</tr>
<tr>
<td>C981</td>
<td>2</td>
<td>123.3 (7.2)</td>
<td>42.3 (4.2)</td>
<td>-81.0 (8.3) s.</td>
<td>-0.66</td>
<td>98.5 (25.1)</td>
<td>-24.8 (26.1) n.s.</td>
<td>-0.20</td>
<td></td>
</tr>
<tr>
<td>C934</td>
<td>0.5</td>
<td>0.147 (0.065)</td>
<td>0.53 (0.21)</td>
<td>0.39 (0.22) n.s.</td>
<td>2.6</td>
<td>3.61 (0.42)</td>
<td>3.46 (0.42) s.</td>
<td>23.6</td>
<td></td>
</tr>
<tr>
<td>C934</td>
<td>0.75</td>
<td>12.9 (1.2)</td>
<td>3.73 (0.63)</td>
<td>-9.1 (1.3) s.</td>
<td>-0.71</td>
<td>15.0 (2.4)</td>
<td>2.1 (2.6) n.s.</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>C934</td>
<td>2</td>
<td>532 (17)</td>
<td>147.3 (3.5)</td>
<td>-385 (17) s.</td>
<td>-0.72</td>
<td>271.0 (9.9)</td>
<td>-261 (20) n.s.</td>
<td>-0.49</td>
<td></td>
</tr>
<tr>
<td>C940</td>
<td>0.3</td>
<td>0.21 (0.11)</td>
<td>0.82 (0.42)</td>
<td>0.61 (0.84) n.s.</td>
<td>2.9</td>
<td>1.78 (0.38)</td>
<td>1.57 (0.44) s.</td>
<td>7.47</td>
<td></td>
</tr>
<tr>
<td>C940</td>
<td>0.45</td>
<td>10.47 (0.058)</td>
<td>4.15 (0.51)</td>
<td>-6.32 (0.52) s.</td>
<td>-0.60</td>
<td>6.5 (1.8)</td>
<td>-4.0 (1.8) s.</td>
<td>-0.38</td>
<td></td>
</tr>
<tr>
<td>C940</td>
<td>0.5</td>
<td>16.0 (3.7)</td>
<td>4.52 (0.39)</td>
<td>-11.5 (3.7) s.</td>
<td>-0.72</td>
<td>12.5 (1.6)</td>
<td>-3.5 (4.0) n.s.</td>
<td>-0.22</td>
<td></td>
</tr>
<tr>
<td>C940</td>
<td>2</td>
<td>635.7 (4.6)</td>
<td>206 (10)</td>
<td>-429 (11) s.</td>
<td>-0.68</td>
<td>293.3 (5.1)</td>
<td>-342.3 (6.9) s.</td>
<td>-0.54</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Mean values (standard deviation), n=3. Values shown correspond to the frequency 1 Hz. The notations s. and n.s. indicate whether $\Delta G^\prime$ is significantly (s.) or not significantly (n.s.) different from zero ($\alpha=0.05$).

Figure 19. Dependence of the elastic modulus on gap width for 0.75% C934 ($G_p^\prime$, white), mixtures with 4% mucin ($G_{mix}^\prime$, black), and the calculated interaction terms ($\Delta G^\prime$, striped). All samples were prepared in simulated tear fluid, n=2. (a) Mixtures with PS mucin. (b) Mixtures with BSMG mucin.
Another factor that may affect the results is the choice of frequency at which the rheological data are obtained. This is especially true for linear polymers (entangled polymer solutions), where $G'$ and $G''$ are frequency dependent. In Paper III a clear frequency dependence was only observed for those preparations that contained the linear polymer C907, whereas the cross-linked polymers showed very little or no frequency dependence (Figure 20). Thus, for the cross-linked gels the synergism parameter $\Delta G'$ was not affected by the choice of frequency. For the C907 preparations, on the other hand, the size of the synergism parameter $\Delta G'$ increased as the frequency increased. However, the sign of the synergism parameter was not affected by the choice of frequency.

![Figure 20. The frequency dependence of the elastic modulus ($G'$, filled symbols) and the viscous modulus ($G''$, open symbols), shown for a few polymer preparations (a) and their corresponding mixtures with BSMG mucin (b). The frequency dependence of the synergism parameter $\Delta G'$ is shown in (c) for a number of preparations of various polymer concentration.](image)

### 4.5 The effect of the comparison strategy

One specific problem with comparative and ranking studies is that, so far, there has been no consensus regarding the conditions under which the comparison should be made. Should one, for example, compare the polymers at the same concentration? Or maybe one should adjust the concentration to make a
comparison between polymer preparations with the same rheological properties. These two different strategies were used in Paper III with the aim of evaluating the reliability of the rheological method. By using the chemically similar Carbopol polymers we expected to lower the risk of artifacts and errors which might occur if polymers with very diverse chemical properties were used.

Two series were chosen where all the polymers either had a concentration of 0.5 or 2%, but different values of \( G' \). Two further series were chosen where the preparations had approximately the same elastic properties, i.e., the same value of \( G' \) (0.2 and 10 Pa), but different polymer concentrations. It was evident that the method gave different results and led to a different ranking order depending on the rationale for the comparison. However, within the group of cross-linked polymers, the results obtained in the 0.2 and 10 Pa series were more similar than the results in the 0.5 and 2% series where the synergism parameters even had different signs. As it is obvious that the rheological properties of the gel are important for the results obtained with this method, it seems logical to compare polymer preparations having approximately the same elastic properties, since the rheological properties can be very different even though the polymers have the same concentration.

4.6 The effect of the choice of synergism parameter

In Figure 21 the absolute \((\Delta G')\) and the relative \((\Delta G'/G'_p)\) synergism parameters obtained with BSMG are plotted against the elastic modulus of the gel \((G'_p)\). The absolute parameter exhibits large negative values at high values of \( G'_p \) and moderate positive ones at low \( G'_p \). The relative parameter, on the other hand, has large positive values at low \( G'_p \), but approaches its negative limit of \(-1\) (see Eq 4) at high \( G'_p \). Consequently, the results obtained are not only sensitive to where the study is performed in the “rheological range”, i.e., at what value of \( G'_p \), but also to which synergism parameter is being considered.

The “relative approach” to synergism seems to be the most reasonable one. With this approach, one considers how many times stronger (or weaker) the polymer-mucin mixture is compared to the gel itself. However, the relative parameter \( \Delta G'/G'_p \) is far from ideal because it has a negative limit of \(-1\), whereas positive values approach infinity when \( G'_p \) approaches zero. From this it follows that the magnitude of positive values cannot be compared to the magnitude of negative ones. In Paper III, we suggested a new “relative” parameter, called the log \( G' \) ratio. This is calculated as

\[
\log G' \text{ ratio} = \log \left( \frac{G'_\text{mix}}{G'_p} \right)
\]

With this parameter a value of 1 means that the \( G' \) of the polymer-mucin mixture is 10 times higher than that of the gel. Further, a value of 0 indicates that the addition of mucin has brought about no change in \( G' \), and a value of \(-1\) represents a decrease in \( G' \) by a factor of 10, etc. The use of the log ratio instead of the conventional relative parameter \((\Delta G'/G'_p)\) has the advantage that the magnitude of
positive and negative values is fully comparable, since the log ratio does not
approach a fixed limit on the negative scale as does the conventional parameter.
But, of course, the introduction of the log ratio does not solve the problem that the
method itself gives results that are strongly influenced by the rheology of the gel.

Figure 21.
(a) The absolute ($\Delta G'$) and (b) the relative ($\Delta G' / G'_p$) synergism parameters obtained with
BSMG mucin, as functions of the elastic modulus of the Carbopol gels ($G'_p$).

4.7 Issues associated with the interpretation model

The existence of negative synergism values observed with the cross-linked
Carbopol gels and with gellan gum, complicates the interpretation of the results.
However, the concept of rheological synergism is not restricted to polymers
interacting with mucin, but has also been observed and discussed in previous
studies with, e.g., polymer-polymer mixtures [199-202]. From these studies, it is
not obvious that an interaction automatically results in a positive value for the
rheological synergism parameter. An interaction could equally well result in
weaker properties of the mixture than the sum of the individual components. Thus
one must conclude that the soundness of the conventional model used to interpret
the results obtained with this method is questionable.

Negative synergism values have previously been observed with cross-linked
Carbopol polymers and commercial mucins [147, 192], and it has been suggested
that ions remaining in the mucin samples could cause weakening of the gels. This
is probably not the only reason why the gels become weaker, since the BSMG
mucin actually contains more ions than the PS (see Table 2), but gives larger
positive values of $\Delta G'$ than the PS at the low polymer concentrations and smaller
negative values at higher concentrations, as discussed in section 4.3. Moreover, in
Paper III the samples were prepared in 0.9% NaCl to simulate physiological
conditions, and at this salt content, the relatively small number of ions present in
the mucins is negligible. Instead, the values of the synergism parameters may be a consequence of the particulate properties of the cross-linked gels:

At high polymer concentrations the gel particles are in strong contact, forming a continuous gel network. When mucin is added to such a gel, the individual gel particles will be surrounded by mucin solution, resulting in a weaker contact between the particles and thus weaker bulk properties. Correspondingly, at low polymer concentrations, the gel particles are not in contact with each other, but surrounded by pure medium. Upon addition of mucin to such a preparation, the gel particles will be surrounded by mucin solution instead. This may enhance the contact between the particles, or at least make the bulk properties somewhat stronger because of the higher viscosity of the mucin solution than the pure medium.

The question can be raised whether negative values of the synergism parameter really do imply that the gel would perform poorly in a conceivable in vivo situation, and vice versa. With this method, the interpenetration layer is simulated by mixing the gel with mucin. A gel is considered to be advantageous if the interpenetration layer (i.e., the gel-mucin mixture) is stronger than the gel itself. But does such an interpretation apply to the situation at a gel-mucosa interface? Consider the general situation where a gel is in contact with a mucous tissue. The mucoadhesive joint can be considered to consist of three regions: the gel, the mucus layer, and the interface, the interpenetration layer. It has been put forward that the residence time of the gel on a mucous tissue would be associated with the failure of the weakest region and, therefore, the cohesive properties of each region are important [110]. From this it can be deduced that the gel should have a sufficient level of cohesiveness to ensure that the gel has a long residence time on the mucous tissue, and that a strengthening of the mucus layer would be advantageous, as would a strong interpenetration layer. But it cannot be deduced that the interpenetration layer necessarily has to be stronger than the gel dosage form itself. In this respect, the basis of the interpretation model that is used with the method is questionable and does not readily transfer to a potential in vivo situation.

In Paper III the results obtained with the rheological method were also compared to those from tensile strength measurements, a more in vivo-like approach that will be further discussed in Chapter 5. From these measurements it was concluded that increased gel strength results in stronger adhesion. This is in contrast to the results from the rheological method, where positive values of the synergism parameters are found for weak gels only. This lack of correlation gives rise to even more questions regarding the appropriateness of the rheological approach to the situation at the gel-mucosa interface.

On the basis of the limitations of the method demonstrated and discussed in Papers II and III, it was concluded that the rheological method should not be used as a stand-alone method for the study of mucoadhesive properties of polymer gels.
5. Tensile strength methods for measuring the mucoadhesion of gels

Most of the *in vitro* methods for measuring mucoadhesion that have been reported during the last 20 years are based on tensile and shear strength measurements [122, 203]. The tensile strength methods have been used extensively to study the mucoadhesion of solid formulations such as tablets and disks [106, 131-136]. However, the inconsistencies between the equipment adopted and instrumental parameters have been pointed out, notably by Tobyn et al [204], and could provide an explanation for the wide variation in results and conclusions found in the literature.

As described in Chapter 1, several theories have been forwarded to explain the mucoadhesion process. For example, the fracture theory of adhesion [205] has been applied in the analysis of tensile strength measurements on polymer microspheres [123] and powder specimens [124]. However, it is likely that different mechanisms are important for dry dosage forms and for fully hydrated systems such as gels. For example, adhesion arising from water transfer and dehydration of mucus is important mainly when dry or partially hydrated dosage forms are concerned [109].

For gels, in particular, it is important to consider the possible regions where the failure of the mucoadhesive joint can take place, and this has been thoroughly discussed by Smart [110]. Which region is the weakest when the dosage form is in contact with a mucous membrane: the dosage form, the mucus layer or the interface layer? For solid dosage forms, the dosage form itself is seldom the weakest region, but in the case of gels it might very well be. With a tensile strength method, the different regions of the mucoadhesive joint can be assessed, thereby providing a means of assessing whether the mucoadhesion measurement reflects a genuine interaction between the dosage form and the mucus layer or just a cohesive failure of the dosage form. However, tensile strength methods developed for solid formulations cannot be employed straight off for polymer gels, since much smaller forces are involved for gels and, in addition, greater deformation is likely.

5.1 Development of a tensile strength method for polymer gels

Paper IV describes the development of a tensile strength method suitable for studying the mucoadhesive properties of polymer gels using freshly excised porcine nasal mucosa and a texture analyzer. Previously, a few similar methods have been used for polymer gels [145-147], but with very different experimental
configurations and mainly utilizing compressed mucin disks or mucin solutions. The measurement configuration adopted in Paper IV involved one piece of mucosa and a large volume (70 mL) of gel (Figure 22a). In Paper V this configuration was compared to that shown in Figure 22b, in which a relatively small volume of gel (100 µL) was situated in between two pieces of mucosa.

**Figure 22.** Experimental configurations used in the mucoadhesion measurements. (a) Large volume configuration (b) Small volume configuration

**Figure 23.** Force-distance curve defining the tensile work, the peak force and the deformation to failure.

The measurement started by lowering the upper mucosa until contact was made with the gel. After a certain contact time the upper mucosa was slowly withdrawn upwards at a constant speed until detachment occurred. During the entire measurement a force-distance curve was recorded from which the tensile work (i.e., the area under the curve during the withdrawal phase), the peak force and the deformation to failure were determined. These parameters were defined according to Figure 23, and have previously been put forward as measures of mucoadhesion [123].

The influence of the withdrawal speed was most pronounced for highly entangled polymer solutions such as sodium hyaluronate preparations. However, the precision was best at 0.1 mm/s, which is consistent with previous studies [106, 145, 204]. This was the speed used in the subsequent measurements.

The contact time was not perceived to have a significant influence on the results over the time intervals tested (2 to 20 min). This indicates that any influence the formation of molecular entanglements and secondary chemical bonds has is rapid and occurs within 2 min. Therefore, a contact time of 2 min was used throughout the study presented in Paper V.

### 5.2 Interpretation of data

As the mucosa is separated from the gel, failure will occur in the weakest of the three regions of the mucoadhesive complex: in the gel, in the mucus or in the interface layer between the gel and the mucus where it is possible that interactions strengthen the mucus layer. Consequently, the force-distance curve recorded in the measurement gives a measure of the strength of the bonds in the weakest
region. In Paper IV, we proposed that to interpret the results and to determine the region in which failure occurs (i.e., which bonds are reflected in the acquired data), the cohesiveness of the individual components should also be measured. A comparison of the cohesiveness of these components with the results from the mucoadhesion measurement should help to identify which region is the weakest. It was concluded in Paper IV that this procedure offers a good basis from which to assess whether the measured tensile work reflects a genuine interaction of the gel preparation with the mucus layer or the cohesive failure of the gel. For preparations that do not have a sufficient level of cohesiveness, no information is gained about possible interactions between the mucus and the polymer, but then the question is whether such a weak dosage form could really give a long residence time at the mucosa.

5.3 A multivariate data analysis approach

The use of multivariate techniques in different pharmaceutical applications is steadily increasing and its usefulness has been reviewed recently [206]. Paper V describes the evaluation of the mucoadhesive properties of a series of 24 gel preparations using tensile strength measurements and the interpretation procedure discussed above. In addition, multivariate data analysis in the form of principal components analysis (PCA) [207, 208] and partial least square projection to latent structures (PLS) [208, 209] was applied to extract useful information from the rather large quantities of data obtained.

The rheological diversity of the selected series of gels was analysed with PCA (Figure 24). The dataset was found to be heterogeneous, which is important if conclusions are to be drawn that are valid for gels with a variety of consistencies.

![Figure 24](image)

**Figure 24.** Rheological heterogeneity of the selected gels investigated by PCA. The scores of the first two principal components (t1 and t2) describing 98% of the diversity of the descriptor space are shown. The elastic modulus, the viscous modulus and the phase angle, each of which was obtained at 0.05 and 1 Hz, were used as the input matrix. The dataset covered all four quadrants of the PCA plot, showing that the selected series of gels was heterogeneous with respect to the rheological properties. None of the gels were identified as outliers.
Figure 25. Plots of the measurements made for the interpretation of mucoadhesive properties of the gel preparations. The tensile work (a-b), the peak force (c-d) and the deformation to failure (e-f) obtained from mucoadhesion measurements (black, n=3–12) and from measurements of the cohesiveness of the gel preparations (grey, n=3–12) and the mucus layer (white, n=25). Data from the large volume configuration are shown in (a), (c) and (e), and data from the small volume configuration in (b), (d) and (f).
5.3.1 Interpretation of the mucoadhesion measurements

Figure 25 shows the mucoadhesion parameters and the cohesiveness parameters of the gel and the mucus obtained with each of the configurations depicted in Figure 22. In the following, the results for the tensile work will be interpreted. From Figure 25a-b it can be seen that for some preparations the mucoadhesion work did not differ significantly from either the cohesive work of the gel or that of the mucus. For other gels, the TW\text{gel} and the TW\text{mucoad} were approximately the same, but they were significantly higher than the TW\text{muc}, implying that a strengthening of the mucus had taken place. Furthermore, for some gels the TW\text{mucoad} was not only higher than the TW\text{muc}, but it was also lower than the TW\text{gel}, indicating that a strengthening of the mucus had taken place, and that the mucoadhesion measurement reflected the strength of the interface or the mucus, and not the cohesive properties of the gel. In the PCA score plot these gels were located in the right half, whereas the ones giving no strengthening of the mucus were located in the left half. In this regard, the results were consistent irrespective of the configuration used. The gels giving rise to strengthening of the mucus included linear and cross-linked preparations, characterized by having substantially higher values of $G'$ and $G''$ compared to the weaker preparations that did not give any strengthening of the mucus.

5.3.2 PLS analysis of the cohesiveness data

The shape of the force-distance curve obtained for polymeric microspheres has been discussed in detail by Chickering & Mathiowitz [123]. However, because of their flexible network structure, polymer gels can be deformed to a greater extent than microspheres. Hence the force-distance curve would not only reflect the deformation of the tissue and the mucoadhesive bonds, but also the deformation and the rheological behaviour of the gel. Dyvik & Graffner [210] discussed this in terms of the high or low viscosity of the samples. Similar observations were made in Papers IV and V, where, for example, the cross-linked gels with pronounced elastic properties seemed to exhibit high peak forces, whereas linear low-concentration preparations with a pronounced viscous character showed a considerable amount of deformation.

PLS analysis was used in Paper V to clarify the relation between the rheological properties and the cohesiveness parameters. This multivariate method takes PCA a step further as it deals with both descriptive (X) and response (Y) data, and it can be used for predicting Y from X data [206]. In Table 4 the PLS models for the cohesiveness parameters are shown. For the peak force of the gel (PF\text{gel}) the most important rheological descriptor was the elastic modulus ($G'$), while the deformation parameter (DF\text{gel}) was principally described by the viscous modulus ($G''$), even though the models did not have a high predictive power. The cohesive work of the gel (TW\text{gel}) was best described by several rheological descriptors, which was not unexpected since the TW\text{gel} is obtained as the area under the force-distance curve, and thus incorporates both the PF and the DF.
Table 4. PLS models for the gel cohesiveness parameters

<table>
<thead>
<tr>
<th></th>
<th>Large volume configuration</th>
<th>Small volume configuration</th>
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<tbody>
<tr>
<td></td>
<td>TW&lt;sub&gt;gel&lt;/sub&gt;</td>
<td>PF&lt;sub&gt;gel&lt;/sub&gt;</td>
</tr>
<tr>
<td>R&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.65</td>
<td>0.75</td>
</tr>
<tr>
<td>Q&lt;sup&gt;2&lt;/sup&gt;</td>
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<td>0.73</td>
</tr>
<tr>
<td>Rheological descriptors&lt;sup&gt;a&lt;/sup&gt;</td>
<td>all</td>
<td>log G′&lt;sub&gt;(1 Hz)&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>log G′&lt;sub&gt;(0.05 Hz)&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> The descriptors are given in order of importance.

5.3.3 PLS analysis of the mucoadhesion data

With the intention of identifying important descriptors and comparing the two measurement configurations, PLS analysis was also performed for the mucoadhesion parameters using the rheological and the cohesiveness parameters as the input matrix (X variables). The results from the analysis are presented in Table 5.

Table 5. PLS models for the mucoadhesion parameters

<table>
<thead>
<tr>
<th></th>
<th>Large volume configuration</th>
<th>Small volume configuration</th>
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<tbody>
<tr>
<td></td>
<td>TW&lt;sub&gt;mucoad&lt;/sub&gt;</td>
<td>PF&lt;sub&gt;mucoad&lt;/sub&gt;</td>
</tr>
<tr>
<td>R&lt;sup&gt;2a&lt;/sup&gt;</td>
<td>0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.79&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Q&lt;sup&gt;2a&lt;/sup&gt;</td>
<td>0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.78&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rheological descriptors&lt;sup&gt;a&lt;/sup&gt;</td>
<td>log G′&lt;sub&gt;(1 Hz)&lt;/sub&gt;</td>
<td>log G′&lt;sub&gt;(1 Hz)&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>log G″&lt;sub&gt;(0.05 Hz)&lt;/sub&gt;</td>
<td>log G″&lt;sub&gt;(0.05 Hz)&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Only the rheological parameters were used as the input (X) matrix. The descriptors are given in order of importance.

<sup>b</sup> The rheological descriptors and the cohesiveness parameters were used as the input (X) matrix. The descriptors are given in order of importance.

<sup>c</sup> Skewed data, could not be modelled.

For the large volume configuration the model obtained for PF<sub>mucoad</sub> was similar to that for PF<sub>gel</sub> when using only rheological data as the input matrix. Including the cohesiveness parameters in the input matrix showed that PF<sub>gel</sub> also contributed to the model for PF<sub>mucoad</sub> and the predictive power was increased markedly. From these results it can be deduced that, with this configuration, the PF<sub>mucoad</sub> mainly
reflected the gel properties. This could also be anticipated from Figure 25c where very little difference is observed between the PF\textsubscript{gel} and the PF\textsubscript{mucoad} for most of the gels. In principal, the presence of a piece of mucosa in the configuration would make no difference to the results obtained because the gel properties determined the result. On the other hand, one should expect the gel properties to give at least some capacity to predict the mucoadhesive interactions. This is because the rheological properties of the gel are related to features that are generally considered to be important for the formation of entanglements during the mucoadhesion process, e.g., physicochemical factors such as the molecular weight, cross-linking density and molecular flexibility [100, 120, 211].

For the small volume configuration, the models for PF\textsubscript{gel} and PF\textsubscript{mucoad} had a lower predictive power than those obtained with the large volume configuration and different descriptors were important. Here the mucoadhesion measurement did not solely reflect the gel properties since other information than that included in the input matrix was found to be necessary to describe the PF\textsubscript{mucoad}. Consequently, the small volume configuration seemed to be more suitable than the large volume one.

The interpretation of the results from the PLS analysis of the TW was not as straightforward because the models did not have such a high predictive power as the peak force models and, in addition, there were different descriptors included in the models for TW\textsubscript{gel} and TW\textsubscript{mucoad}. It could, however, be concluded that the small volume configuration seemed to be a better choice than the large volume configuration, even though the difference was not as apparent as it was for the peak force.

In Paper IV the tensile work seemed to be a more suitable mucoadhesion parameter than the peak force, something which has also been noted by other authors [212]. In Paper V this seemed to be valid for the large volume configuration since the gel properties did not have such a significant impact on the tensile work as they had on the peak force. However, the same observation cannot be made from the models obtained with the small volume configuration. Thus a general conclusion concerning which mucoadhesion parameter is the most appropriate could not be drawn since which parameter is preferable is largely dependent on the way the measurements are conducted.
Dielectric spectroscopy is a well established technique that is frequently used in physics and electrochemistry. It involves the study of the response of a material to an applied electric field, and can be used to derive structural information. More specifically, a sinusoidal voltage is applied across the sample and the current response is measured as a function of frequency. From the current response, the complex impedance or permittivity of the sample is obtained which can be related to properties determined by the charges moving in the system [213, 214].

Two main fields of application can be identified. Firstly, dielectric data gives information about the electrical properties of a sample. This has practical applications within the electronics industry, for example, in the development of semiconductors. Secondly, dielectric spectroscopy can be used as an analytical tool, where the dielectric spectrum is interpreted in terms of the structure and behaviour of the material under study, and related to other structural and physical properties such as changes in crystal structure or gel morphology [215]. Owing to the latter application, the interest in this technique from areas outside its traditional sphere has increased markedly in the last decade, and dielectric spectroscopy is now used more or less routinely within biotechnology, medicine and pharmaceutics [215-218].

Within the field of pharmaceutical sciences there have been a few reports recommending the use of dielectric spectroscopy for quality control purposes. As the dielectric data may be regarded as a fingerprint, samples prepared under or exposed to different conditions may be compared [215]. For example, the detection of interbatch variation of common pharmaceutical excipients such as lactose has been reported [219].

Other, more widespread, pharmaceutical applications of this technique include the analysis of disperse systems such as liquid emulsions (e.g. [220-223]), semisolid creams (e.g. [224, 225]), liposomes (e.g. [226-228]), micellar systems (e.g. [229-231]) and liquid crystalline structures (e.g. [232-234]). Furthermore, a number of publications on the dielectric response of gels have appeared [190, 235-240]. In these studies, the high frequency conductance through the gels (the bulk response) and a polymer barrier layer appearing at low frequencies have been analyzed, on the basis of the theory outlined by Hill & Pickup [241]. To our knowledge, however, dielectric spectroscopy has never been used for evaluating the mucoadhesive properties of polymer gels, or, for that matter, of any other pharmaceutical systems.
In Paper VI, our hypothesis was that dielectric spectroscopy could be used as a versatile tool not only to extract information about separate gels and mucosae, but also to assess the likelihood of intimate surface contact between the mucus and the gel, i.e., their “compatibility”. Since dielectric spectroscopy evaluates the movement of charged particles, the compatibility is obtained as a measure of the ease with which a charged particle passes a barrier between the gel and the mucosa. A low barrier indicates a high degree of compatibility between the gel and the mucus layer and vice versa. In the measurements presented in Paper VI, freshly excised porcine nasal mucosa (as described in Paper IV and Chapter 5) was used with five different pharmaceutical gels: 0.75 and 2.0% Carbopol 934 (C934), 2.0% sodium carboxymethylcellulose (B7HF), 7.0% chitosan hydrochloride (SC211) and 25.0% poloxamer (P127). The dielectric measurements provided information about the gels and the mucous tissue, separately, and about the interface between the gel and the mucus layer. The results were then used to create a measure of the compatibility between the gel and the mucus layer. As stated above, to our knowledge, this was the first time dielectric measurements were used in this way. Furthermore, the results obtained from dielectric spectroscopy were compared to mucoadhesion data obtained with the tensile strength method described in Paper IV (and Chapter 5). To facilitate comparison, the setups used resembled one another as closely as possible.

6.1 Dielectric spectroscopy measurements

The dielectric spectroscopy technique has been reviewed in several books and articles, see for example the books of Macdonald [214] and Craig [215] and references therein. Since a theoretical treatment of the technique is beyond the scope of this thesis, the parameters that can be measured and the relations between them will only be briefly described.

When a sinusoidal voltage is applied across a material, one obtains information about the complex frequency dependent capacitance

\[ C(\omega) = C_{\text{Real}}(\omega) + jC_{\text{Im}}(\omega) \]  \hspace{1cm} (6)

from the measured current response. In the equation above and in the text that follows, \( j \) denotes the imaginary number \( \sqrt{-1} \) and \( \omega \) is the angular frequency. The indices \( \text{Real} \) and \( \text{Im} \) denote the real and the imaginary part of a quantity, respectively. From the measured capacitance, the relative permittivity \( \varepsilon \) can easily be extracted from

\[ \varepsilon(\omega) = \varepsilon_{\text{Real}}(\omega) + j\varepsilon_{\text{Im}}(\omega) = \frac{d}{A \varepsilon_0} \left[ C_{\text{Real}}(\omega) + jC_{\text{Im}}(\omega) \right] \]  \hspace{1cm} (7)

where \( d \) and \( A \) are the sample thickness and the cross-sectional area, respectively, and \( \varepsilon_0 \) is the permittivity of free space \( (8.854 \cdot 10^{-12} \text{ F/m}) \). The permittivity describes how the free and bound charges in a material respond to an applied electric signal.
Another common way to extract information about the dielectric behavior of a material is to analyze its complex total impedance \( Z \) instead of the complex permittivity. \( Z \) is related to the measured capacitance by

\[
Z(\omega) = Z_{\text{Real}}(\omega) + jZ_{\text{Im}}(\omega) = \frac{1}{j\omega\left[C_{\text{Real}}(\omega) + jC_{\text{Im}}(\omega)\right]}
\]

where \( Z_{\text{Real}} \) describes the frequency dependent resistance of the system, from which the conductance of the material can be extracted as \( 1/Z_{\text{Real}} \). In Paper VI, the measured response was analyzed mostly in terms of impedances instead of permittivities.

As shown in Figure 26, the samples of interest were situated between the cylindrical electrodes in three configurations. For each gel, three successive series of measurements were made in the following manner: A fresh gel sample was analyzed using the configuration of Figure 26b. Thereafter, fresh pieces of mucosa were analyzed using the setup in Fig. 26c. Immediately after this measurement, a new gel sample was placed in between the mucosa pieces, as demonstrated by Figure 26d, and the dielectric measurement was repeated. This procedure was followed to ensure that a measure of the compatibility between the gels and the mucosa could be extracted, and for this measure to be independent of the specific piece of mucosa used.

**Figure 26.** Experimental setups used in the dielectric measurements. (a) Cross-section of the electrode configuration. (b) Setup for the measurements on gels. (c) Setup for the measurements on mucosa pieces where one piece of mucosa was attached to each of the two electrodes with the mucus layer facing away from the electrode surface. (d) Setup for the measurements on combined systems of gel and mucosa.
6.2 Dielectric response

Figure 27 shows the permittivity as a function of frequency on a log-log scale as well as the imaginary part of the total impedance ($Z_{\text{Im}}$) as a function of the real part ($Z_{\text{Real}}$) on a linear scale for all three types of experiment in Figure 26. In this figure, one of the series of measurements on the P127 gel is displayed, to show the typical response of a gel and a combined gel-mucosa system.

**Figure 27.** The permittivity as a function of frequency and the imaginary part $Z_{\text{Im}}$ of the impedance plotted as a function of the real part $Z_{\text{Real}}$ for the three different types of measurement. The panels display one of the series of measurements made on the P127 gel. A few explicit frequency readings are marked in the plots of the impedance.
A common way to characterize the dielectric response is to compare the sample to a resistor/capacitor circuit that would give an identical response [215]. This approach was used in Paper VI, where all three series of experiments could be modeled by an equivalent circuit similar to the Randles circuit [242], viz that of Figure 28. The responses consisted of a high frequency resistance $R_{hf}$, which could be extracted from the graphs as the point where $-Z_{lm} = 0$, in a series circuit with $R_b$ and $C_b$, modeling a barrier and an element $Z_d$ representing diffusion.

Figure 28. The equivalent circuit, where $R_{hf}$ is the high frequency resistance, $R_b$ and $C_b$ are the barrier resistance and capacitance, respectively, and $Z_d$ is the diffusion impedance.

The observed dielectric response is fairly easily interpreted for the gels. The presence of a high frequency response, $R_{hf}$, in series with a barrier response has been observed in previous studies on pharmaceutical gels [190, 239]. In these studies, the high frequency resistance was interpreted as arising from charges moving in the bulk of the system, i.e., through the gel network, while the barrier response was caused by the interfaces, i.e., by a polymer layer that was built up at the electrode surfaces and that presented a greater or lesser degree of resistance to the movement of the charged species. The present findings are very much in line with the former analyses on gels [190, 239].

For the mucosa and the systems of gel and mucosa, the interpretation of the circuit elements is a little bit more complicated since these systems consist of more than just one layer of material. However in general, the high frequency resistance $R_{hf}$ represents charges moving over a very short distance while the parallel elements dominating the response at lower frequencies are attributed to charges moving over longer distances and thus involve passing one or more barriers. In the mucosa, the charged species may be exposed to several types of barrier. Just as for the gels, one type is the barrier between the electrode and the mucosa. The other barriers would naturally be the different layers of which the mucosa is constituted: the submucosa, the epithelial cells and the mucus layer that covers the epithelium. In this respect, it is important to note that the interface between the two touching mucus layers of the mucosa does not create a barrier since the material on each side of the interface is the same. Furthermore, because of the glycoprotein content and the physiological ion concentration of the mucus layer, this is the part of the mucosa that is most likely to give the highest contribution to the measured conductance ($1/R_{hf}$) in the high frequency region when using the configuration shown in Figure 26c. One can transfer this interpretation to the system of gel and mucosa, where one has a situation
analogous to that of the two touching mucosae, but with the addition of the gel-mucosa interfaces. For this system it can be argued that it is the series configuration of the mucus layers and the gel in which the major contribution to the high frequency conduction occurs.

6.3 Circuit parameters of the gels and the mucosa

The real part $Z_{\text{Real}}$ of the measured impedance was fitted to the real part of the circuit impedance to enable $R_{\text{hf}}$, $R_b$, $C_b$, and the parameter $T_d$ describing $Z_d$ to be extracted. The curves and fitting parameters obtained for the measurements in Figure 27 are displayed in Figure 29.

The circuit parameters extracted for the gels are shown in Table 6. Some of the data could be attributed to the polyelectrolyte character of the polymers. The two negatively charged polymers, C934 and B7HF, and the only positively charged polymer (SC211) were found to have approximately the same level of conductance (described by $1/R_{\text{hf}}$), whereas the conductance of the only non-ionic polymer (P127) was considerably poorer. Furthermore, the value of the 2.0% C934 gel was of the same order of magnitude as that reported elsewhere [239] and we observed that the conductance decreases ($R_{\text{hf}}$ increases) for the 0.75% C934 gel because of the decrease in the number of charge carriers, just as was found in the previous study.

The barrier resistance $R_b$ is a measure of the difficulty the charge carriers in the gel have to penetrate the polymer barrier created close to the electrodes. In the low conductance P127 we found that $R_b$ was much lower than for the other gels. This indicates that the free carriers move in almost the same manner as in the bulk gel, i.e., they do not experience any significant changes in the gel structure close to the electrodes. This may be
attributed to the homogeneous micellar cubic liquid crystalline phase that is formed at this concentration by self assembling of the polymer [243]. For the other gels, a barrier resistance of between 11 and 40 Ω was observed, indicating that a polymer network different from that inside the bulk of the gel was built up close to the electrodes, as previously described for polyacrylic acid gels [190, 239].

Table 6. Dielectric data for gel layers of 1 mm thickness.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Concentration (% w/w)</th>
<th>High frequency resistance $R_{hf}$ (Ω)</th>
<th>Barrier resistance $R_b$ (Ω)</th>
<th>Barrier capacitance $C_b$ (µF)</th>
<th>Diffusion parameter $T_d$ ($10^{-3}$ s$^{1/2}$/Ω)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C934</td>
<td>0.75</td>
<td>15.0 (0.7)</td>
<td>40.3 (1.2)</td>
<td>42.3 (2.3)</td>
<td>1.45 (0.08)</td>
</tr>
<tr>
<td>C934</td>
<td>2.0</td>
<td>10.3 (0.3)</td>
<td>19.2 (0.6)</td>
<td>30.7 (2.0)</td>
<td>1.57 (0.12)</td>
</tr>
<tr>
<td>B7HF</td>
<td>2.0</td>
<td>9.31 (0.4)</td>
<td>39.1 (1.4)</td>
<td>24.8 (2.2)</td>
<td>0.910 (0.05)</td>
</tr>
<tr>
<td>SC211</td>
<td>7.0</td>
<td>11.8 (0.4)</td>
<td>11.5 (0.8)</td>
<td>24.6 (1.9)</td>
<td>1.20 (0.11)</td>
</tr>
<tr>
<td>P127</td>
<td>25.0</td>
<td>28.7 (0.2)</td>
<td>2.31 (0.06)</td>
<td>28.7 (1.2)</td>
<td>1.19 (0.07)</td>
</tr>
</tbody>
</table>

$^a$ Mean values (absolute deviations), n=3

From the measurements on pieces of mucosa in the configuration of Figure 26c, we observed that only $R_{hf}$ correlated significantly to the thickness of the mucosa in the sense that a thicker mucosa tended to have a lower conductance (higher $R_{hf}$) than a thinner one. This is, of course, to be expected since there is an inverse correlation between conductance and sample thickness. In this respect it is interesting to note that, while the total thickness of the two pieces of mucosa (the electrode gap) increased by a factor of 9, $R_{hf}$ only increased by a factor of 2. This indicates that it is the mucus layer of the mucosa that gives the major contribution to $R_{hf}$, i.e., that the conduction in the mucus layer governs the high frequency response. The variation in the thickness of the pieces of mucosa is mainly attributable to the varying thickness of the submucosa since this is the thickest layer of the mucosa. Furthermore, one could expect the mucus layer to contribute only slightly to the thickness variations because the mucus layer in the nasal cavity is generally very thin (about 5 μm) [46, 244].

The barrier and diffusion parameters showed no clear dependence on mucosa thickness, which is as expected, too, since the barriers are located at interfaces between different layers of materials, and interfaces are generally not affected by bulk thickness. When comparing the magnitude of the circuit elements for the gels and the mucosa, we saw that the resistances measured for the mucosa were much larger than those for the gels, while the opposite was true for the capacitance.

6.4 A measure of the compatibility between the gel and the mucus layer

In the following, the creation of a measure of the compatibility between the different gels and the mucus layer, which we call the Compatibility Factor (CF), is described. CF provides a measure of the likelihood of intimate surface contact. A high compatibility between a gel and the mucus layer, i.e., a high CF value, is
equivalent to a low barrier between the two media, i.e., a low resistance experienced by the mobile ions.

Successive measurement and the subsequent extraction of circuit parameters provide the high frequency resistance for the gel, \( R_{hf}^{gel} \), for the two pieces of mucosa, \( R_{hf}^{mucosa} \), and for the system consisting of the two pieces of mucosa with the gel in between them, \( R_{hf}^{gel+mucosa} \). From Figure 30 it is evident that the only differences between the sum \( R_{hf}^{gel} + R_{hf}^{mucosa} \), and the value \( R_{hf}^{gel+mucosa} \), are that the sum contains a contribution from the interface between the two touching mucus layers, while \( R_{hf}^{gel+mucosa} \) incorporates a contribution from the two interfaces between the gel and the mucus layers. This is the key to finding a parameter describing the resistance between the gel and the mucus layers, and thus, an expression for \( CF \). Since resistances connected to conduction processes increase linearly with the thickness of the conduction path, normalization must be performed. Instead of using the measured \( R_{hf}^{gel} \) in our calculations we will use the normalized value, \( \bar{R}_{hf}^{gel} \), given by

\[
\bar{R}_{hf}^{gel} = R_{hf}^{gel} \frac{L_{gel}^{gel+mucosa}}{L_{gel}^{gel}}
\]  

Here \( L_{gel}^{gel+mucosa} \) is the thickness of the gel layer in the experiment shown in Figure 26d. This thickness is obtained from the difference between the electrode gap in the configuration for Figure 26d and Figure 26c. \( L_{gel}^{gel} \) is the gel thickness in the configuration in Figure 26b, which was always equal to 1 mm. In principle, the difference \( \bar{R}_{hf}^{gel} + R_{hf}^{mucosa} - R_{hf}^{gel+mucosa} \) should be a good measure of the magnitude of the compatibility: the larger the difference, the smaller the resistance between the gel and the mucus layer and the higher the compatibility. However, this expression is not independent of the actual piece of mucosa used. A mucosa-independent compatibility factor is obtained by dividing the expression by the high frequency resistance of the specific piece of mucosa used in the measurement series, i.e.,

\[
CF = \frac{\bar{R}_{hf}^{gel} + R_{hf}^{mucosa} - R_{hf}^{gel+mucosa}}{R_{hf}^{mucosa}}
\]
The measurement series presented in Figure 29 will now be used to demonstrate how CF is calculated:

From the figure it is found that

\[ R_{hf}^{gel} = 28.5 \, \Omega, \quad R_{hf}^{mucosa} = 99.0 \, \Omega, \quad \text{and} \quad R_{hf}^{gel+mucosa} = 116 \, \Omega. \]

In this particular measurement series, \( L_{gel}^{gel+mucosa} \) equals \( L_{gel}^{gel} \), so \( \bar{R}_{hf}^{gel} = R_{hf}^{gel} \).

This gives a value for CF of

\[
CF = \left[ (28.5 \, \Omega + 99.0 \, \Omega) - 116 \, \Omega \right] / 99.0 \, \Omega = 0.116
\]

The two other series that were measured using the P127 gel gave CF values of 0.107 and 0.134, respectively. The calculated CF values for all gel systems under study are shown in Figure 31a. It can be noted that the variation between the three measurement series performed on each gel was very small considering the quite large variation in the thickness of the mucosa.

![Figure 31](image.png)

**Figure 31.**
(a) The compatibility factor CF for the gels under study. The error bars indicate the absolute variation between three series of measurements.
(b) The measured tensile work from mucoadhesion measurements (striped, n=3–4) and from measurements of the cohesiveness of the gels (n=3–4) and the mucus (n=25) (white).

Mean values±standard deviation.
* Denotes significant difference between the \( TW_{mucoad} \) and \( TW_{muc} \) (\( p<0.05 \)), whereas † denotes significant difference between the \( TW_{mucoad} \) and the \( TW_{gel} \) (\( p<0.05 \)).
6.5 Comparison to tensile strength measurements

In Figure 31b we show the tensile work obtained from mucoadhesion measurements and from measurements of the cohesiveness of the gels and the mucus layer, using the small volume configuration described in Paper V (and Chapter 5). This setup closely resembles the one used in the dielectric measurements.

When interpreted as outlined in Chapter 5, it was found that a strengthening of the mucus layer seemed to have occurred for the 2.0% C934, the SC211 and the P127 gels, and that the mucoadhesion measurement did not reflect a cohesive failure of the gel. For the other two gels, on the other hand, no strengthening of the mucus could be detected and the region reflected in the mucoadhesion measurement may be either the mucus layer or the gel.

From Figure 31b it is evident that the 2.0% C934 and 7.0% SC211 gels had the highest values for the mucoadhesion work. This is in excellent agreement with the present findings for the compatibility factor (Figure 31a). However, the third highest CF was for the 0.75% C934 gel, whereas the mucoadhesion measurement on this gel did not allow one to determine whether there had been a strengthening of the mucus or just a cohesive failure of the gel. This is not unexpected because this gel has rather weak rheological properties, and the gel could very well be the weakest part of the system. In a previous study by Jones and coworkers [146] and in Paper IV it was observed that with a tensile strength mucoadhesion method, stronger rheological properties result in stronger adhesion to the mucosa.

What kind of information is obtained from the dielectric measurements in comparison with that obtained from the tensile strength measurements can be debated. We do not believe that the dielectric measurements can give direct information about the mucoadhesive bond formation, i.e., the possible physicochemical interactions between the polymer molecules and the mucus glycoproteins that take place at the interface. Rather, the compatibility provides an assessment of the likelihood of intimate surface contact between the gel and the mucus, which is generally considered to be the step preceding the bond formation in the mucoadhesion process. One may, therefore, not expect the compatibility factor to correlate perfectly with the results from the tensile strength measurements since molecular factors other than for the possibilities of intimate surface contact could govern the strengthening of the mucus and the formation of secondary chemical bonds. However, it could be noted that the two gels with the highest CFs in this study were also those that showed the most significant strengthening of the mucus layer as measured with the tensile strength method. Further work is needed to evaluate the versatility of the dielectric measurements and the compatibility factor, and also to clarify how these investigations can provide information complementary to that from other studies on mucoadhesive polymer gels.
7. Concluding remarks

The potential of a gel formulation to prolong the residence time at the site of absorption has been attributed to its rheological and mucoadhesive properties. The work conducted for this thesis has included studies of both of these features, with the overall objective having been to investigate their importance for the anticipated in vivo residence time.

In the rheological study of the in situ gelling polymer gellan gum described in Paper I, it was found that small quantities of cations were sufficient to induce the formation of a strong gel, and thus rapid gelation could be expected in vivo. It was intriguing to see that the expectation that this polymer would gel rapidly was fulfilled when the formulation was administered to the nasal cavity of rats, as described in Paper VII. On contact with the mucosa a gel was rapidly formed that remained in the nasal cavity for as long as 4 hours, without having any visible harmful effects on the mucosa. In addition, the epithelial uptake and transfer of the model substance, a 3 kDa fluorescein dextran, was increased, in comparison with that seen after administration of a mannitol solution. It was concluded that the use of an in situ gelling polymer, such as gellan gum, represents a promising strategy for nasal drug delivery.

Gellan gum formulations were also included in two of the mucoadhesion studies (Papers II and V). However, the findings, with respect to its mucoadhesive properties, were inconsistent, and did not enable a general conclusion to be drawn.

For an in situ gelling formulation capable of forming such strong gels as gellan gum, it is very likely that the rheological performance of the formulation is more important for achieving a long contact time than the possible interactions with the mucin molecules at the gel-mucus interface. However, the importance of the mucoadhesive interactions will, presumably, be dependent upon the administration route under consideration. Moreover, and maybe somewhat provocatively, it can be argued that strong bonds between the gel and the mucus might not actually be advantageous, especially not in the nasal cavity. The rapid mucus turnover is one of the most important protective mechanisms in the nose, and if the dosage form is extensively bound to the mucus it might be cleared from the mucosa at the same speed as the mucus.

In my opinion, the rheological properties, or perhaps it would be more appropriate to say, the cohesive properties, are likely to be more important for the contact time than the mucoadhesive interactions, not only for gels congealing in situ, but for ordinary preformed gels as well. This is partly supported by the finding that the rheological properties were a determining factor for the results obtained in the mucoadhesion studies, irrespective of whether the rheological (Papers II and III) or the tensile strength (Papers IV and V) method was used. If one compares the two mucoadhesion methods, it still remains unclear which one gives the most accurate information. One of the limitations of the rheological method was that a positive response, interpreted as mucoadhesion, was only seen
with weak gels. The tensile method could, in contrast, detect strengthening of the mucus only for strong gels, and failed to distinguish a genuine interaction from a cohesive failure of the gel if the gel was weak or liquid-like. However, this method reflected the *in vivo* situation better than the rheological method.

A general problem with mucoadhesion methods is that the interactions are expected to occur at a microscopic or molecular level, whereas the response is usually measured on a macroscopic level. In this context it can be noted that researchers in the field have, so far, failed to visualize the presence of an interpenetration layer and molecular interactions using microscopic techniques [110]. The use of dielectric spectroscopy for determining the ease with which a charged particle can pass the gel-mucus interface layer (Paper VI) was an attempt to study the events at the gel-mucus interface closer to the molecular level. It was an entirely new approach to the study of mucoadhesion of polymer gels, and represents an interesting subject for future investigations. The question can, however, be raised whether or not a molecular method will be more useful than a macroscopic one. I believe this will depend upon what one is trying to establish: Whether there are molecular interactions, or, would the possible interactions be important for the *in vivo* situation? For gels one can also discuss whether molecular interactions would be advantageous for providing a long contact time if the cohesive forces within the gel are insufficient.
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References


A doctoral dissertation from the Faculty of Pharmacy, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series *Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy*. (Prior to July, 1985, the series was published under the title “Abstracts of Uppsala Dissertations from the Faculty of Pharmacy”.)