Università degli Studi di Padova

Sede Amministrativa: Università degli Studi di Padova

Dipartimento di Scienze Chimiche

SCUOLA DI DOTTORATO DI RICERCA IN SCIENZE MOLECOLARI

INDIRIZZO SCIENZE FARMACEUTICHE

CICLO XXVII

SCREENING OF POLYMERS FOR THE DEVELOPMENT OF MUCOADHESIVE TABLETS

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Abstract

Mucoadhesive dosage forms are delivery systems able to adhere to a particular region of the body for extended periods of time. This can lead to several advantages, such as a reduction of the administration frequency and an enhancement of drug bioavailability. For this reason, the phenomenon of mucoadhesion is widely studied, despite not fully understood.

The mucoadhesive properties come from polymers, especially hydrophilic polymers becoming adhesive once activated by moistening. The mucoadhesive polymers play the key role in determining the mucoadhesive ability of a dosage form. Hence, it is necessary to deepen the study of the polymers properties.

The present research mainly focuses on the screening of different mucoadhesive polymers for the development of mucoadhesive tablets with intestinal target. Particularly, this research aims to study different factors affecting mucoadhesion in order to identify the most important one that might predict the mucoadhesive ability of the final solid dosage form.

Results of research activities are summarized in five chapters:

- Chapter 1 gives an overview on the phenomenon of mucoadhesion, and the methods for the detection of the mucoadhesive properties;
- in Chapter 2 the methods developed for the study of tablets mucoadhesive properties are presented;
- the influence of the amount of polymer on the mucoadhesive properties and on the release rate of a model drug (sodium butyrate) is analyzed in Chapter 3;
- in Chapter 4 the Design of Experiment (DoE) techniques are used to develop tablets with good mucoadhesive properties and an extended-release, containing sodium butyrate or mesalazine as active ingredients;
- Conclusions and proposals for the future work can be found in Chapter 5.

Riassunto

Le formulazioni mucoadesive sono sistemi in grado di aderire ad una particolare regione del corpo per un tempo prolungato. Numerosi sono i vantaggi che ne derivano, tra cui la riduzione della frequenza di somministrazione del farmaco ed anche un possibile aumento della biodisponibilità. Per questo motivo, il fenomeno di mucoadesione è ampiamente studiato in campo scientifico. Nonostante ciò, a causa della sua complessità non è stato ancora compreso del tutto.
Le proprietà mucoadesive di una formulazione derivano dalla presenza di polimeri, generalmente idrofilici, in grado di aderire alle mucose in seguito ad idratazione. I polimeri mucoadesivi, quindi, ricoprono un ruolo chiave nel determinare le capacità mucoadesive di una formulazione e risulta fondamentale studiare in maniera approfondita le proprietà del polimero.

Il *focus* della presente ricerca è lo *screening* di diversi polimeri, al fine di sviluppare compresse mucoadesive che abbiano come *target* la mucosa intestinale. In particolare, sono stati studiati diversi fattori in grado di influenzare le proprietà mucoadesive di una formulazione allo scopo di individuare la proprietà più importante che potrebbe fornire un'informazione di tipo predittivo sulla capacità mucoadesiva del prodotto finito.

I risultati di questo studio sono riassunti in cinque capitoli:

- il Capitolo 1 fornisce una panoramica sul processo di mucoadesione e sui metodi per valutare le proprietà mucoadesive;
- il Capitolo 2 presenta i metodi, che sono stati sviluppati in questo lavoro di ricerca, per lo studio delle proprietà mucoadesive delle compresse;
- nel Capitolo 3 viene analizzata l'influenza della quantità di polimero sulle proprietà mucoadesive e sulla velocità di rilascio di un farmaco modello (sodio butirrato);

- nel Capitolo 4 vengono impiegate tecniche di Disegno Sperimentale al fine di sviluppare compresse mucoadesive a rilascio prolungato contenenti sodio butirrato o mesalazina come principi attivi;
- Conclusioni e prospettive future sono esposte nel Capitolo 5.

To my family

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Chapter 1

Introduction

1.1 Definition of adhesion, bioadhesion and mucoadhesion

Adhesion, bioadhesion and mucoadhesion are three terms which refer to the same process taking place in different environments.

Adhesion is defined as an interfacial phenomenon in which two materials are held together for extended periods of time by interfacial forces (Chowdary & Srinivasa Rao, 2004; Smart, 2005). When adhesion occurs in a biological setting, and at least one of the two materials is biological, it is termed "bioadhesion" (Andrews, et al., 2009). The attachment could be between an artificial material such as a polymer and a biological substrate (Chowdary & Srinivasa Rao, 2004). When this substrate is represented by a mucous membrane the term "mucoadhesion" is used (Andrews, et al., 2009). In the pharmaceutical sciences this concept is referred to pharmaceutical dosage forms called "mucoadhesives" since they are able to adhere to the mucus layer of a mucosal tissue (figure 1.1).

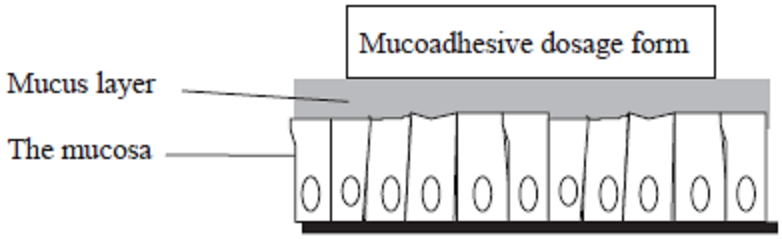


Figure 1.1. *The mucoadhesive joint between a mucoadhesive dosage form and a mucosal tissue (Smart, 2005).*

1.2 Mucoadhesive dosage forms

Mucoadhesive dosage forms are delivery systems in which the bioadhesive properties of polymers allow to target a drug to a particular region of the body for extended periods of time (Chowdary & Srinivasa Rao, 2004).

Mucoadhesive drug delivery systems may be formulated in different types of dosage form (e.g. tablets, films, gels, micro- and nano-particulate suspensions, in situ gelling systems and sprays) for various administration routes (e.g. ocular, nasal, buccal, gastrointestinal, vaginal and rectal) (Khutoryanskiy, 2011). As a consequence, they seem to be very smart and several studies reported in literature prove their great potential.

1.2.1 Advantages of mucoadhesive dosage forms

Compared to conventional dosage forms, the mucoadhesive drug delivery systems show various advantages:

(i) they prolong residence time of the dosage form at the site of application and absorption, with a reduction of the administration frequency;

(ii) a more intimate contact of the dosage form with the underlying absorption surface is facilitated; this may also allow a change of tissue permeability by modifying the tight junctions between the cells and hence the absorption of macromolecules, such as peptides and proteins; moreover, it may also lead to a possible improvement and enhancement of drugs bioavailability;

(iii) possibility of site-specific drug delivery (Khutoryanskiy, 2011; Chowdary & Srinivasa Rao, 2004).

1.3 Structure and function of mucus

Mucus is a complex viscous adherent secretion synthesized by specialized goblet cells in the columnar epithelium that lines the walls of all the body cavities that are exposed to the external environment, such as the gastrointestinal, respiratory and reproductive tracts and also oculo-rhino-otolaryngeal tracts (Chowdary & Srinivasa Rao, 2004; Smart, 2005; Kharenko, et al., 2009; Bansil & Turner, 2006).

In those locations it serves many functions, such as the lubrication for the passage of substances, the maintenance of a hydrated layer over the epithelium, the action as a barrier to infectious agents and noxious substances and as a permeable gel layer for the exchange of gases and nutrients with the underlying epithelium (Bansil & Turner, 2006). Specifically, in the gastro-intestinal tract the mucus facilitates the movement of food boluses along the digestive canal and helps shield the epithelium from proteolytic enzymes and shear forces induced by peristaltic waves (Kharenko, et al., 2009; Peppas & Sahlin, 1996). Mucus lost due to degradation and turbulence is replaced by the constant secretion of mucus (Peppas & Sahlin, 1996).

Mucus is composed mainly of water (95%), but also contains salts, lipids such as fatty acids, phospholipids and cholesterol, proteins with a defensive role such as lysozyme, immunoglobulins, defensins, growth factors and trefoil factors (Bansil & Turner, 2006). However, the main component responsible for its viscous and elastic gel-like properties is the glycoprotein mucin (Bansil & Turner, 2006).

The mucous gel covering the epithelium varies in thickness. In the human stomach the mean thickness is 192 μm, while in the duodenum the thickness ranges from 10 to 400 μm. Cohesion of the gel is dependent upon the glycoprotein concentration (Peppas & Sahlin, 1996).

Mucus may be secreted either constantly or intermittently. The amount of mucus secreted changes under the influence of external and internal factors (Kharenko, et al., 2009).

1.3.1 Mucin

The term “mucin” (MUC for human) refers to members of a glycoproteins family representing the major structural components of the mucus and responsible for mucus gelatinous structure, cohesion, and antiadhesive properties (Andrianifahanana, et al., 2006; Kharenko, et al., 2009).

Currently, at least 19 human mucins have been identified: MUC1, -2, -3A, -3B, -4, -5AC, -5B, -6, -7, -8, -9, -11, -12, -13, -15, -16, -17, -19, and -20 (Andrianifahanana, et al., 2006; Bansil & Turner, 2006). These mucins may be classified in two main groups: the “secreted (gel-forming and non-gel-forming) mucins” and the

"membrane-bound mucins" which anchor to the plasmalemma by a transmembrane domain; they differ for structural characteristics and physiological fates (figure 1.2) (Andrianifahanana, et al., 2006).

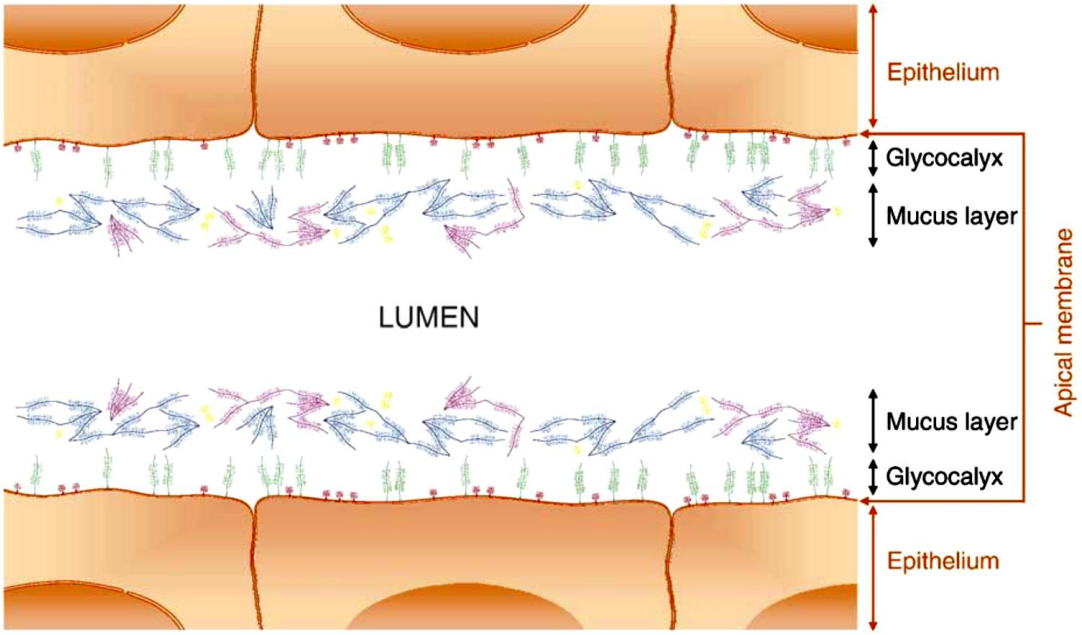


Figure 1.2. *Typical expression of mucins at the epithelium–lumen interface: membrane-bound mucins form the glycocalyx, whereas secreted mucins are the major components of the gel-like mucus layer. Examples shown include MUC1 (red) and MUC4 (green) for membrane-bound mucins, MUC2 (blue) and MUC5AC (pink) for secreted gel-forming mucins, and MUC7 (yellow) for secreted non-gel-forming mucins (Andrianifahanana, et al., 2006).*

Mucin is produced by epithelial cells of various organs belonging to respiratory, digestive, reproductive, otologic, ocular, and urinary systems (Andrianifahanana, et al., 2006).

Despite the type and the body site, glycoproteins usually have similar structure and are highly glycosylated protein molecules with molecular weights ranging from 0.5 to 20 MDa. The sugar moieties consist of about 80% of mucin molecular mass, while the remaining 20% is represented by the protein core, termed "apomucin" (Bansil & Turner, 2006; Andrianifahanana, et al., 2006; Kharenko, et al., 2009).

Glycoproteins form a branched three-dimensional network with large numbers of loops (Kharenko, et al., 2009). The macromolecules associate with one another through non-covalent bonds forming a highly entangled network: this molecular association is central to the structure of mucus and is responsible for its rheological properties (Andrews, et al., 2009).

Mucin glycoproteins may be described as consisting of a basic unit made from a single-chain polypeptide backbone (protein core) characterized by two types of area (figure 1.3): (1) heavily glycosylated regions where many large carbohydrate side chains are attached, predominantly via O-glycosidic linkages, and (2) terminal "naked proteins regions" where there is little glycosylation (Andrews, et al., 2009; Kharenko, et al., 2009). Glycosylation increases the resistance of the molecules to proteolytic hydrolysis (Kharenko, et al., 2009).

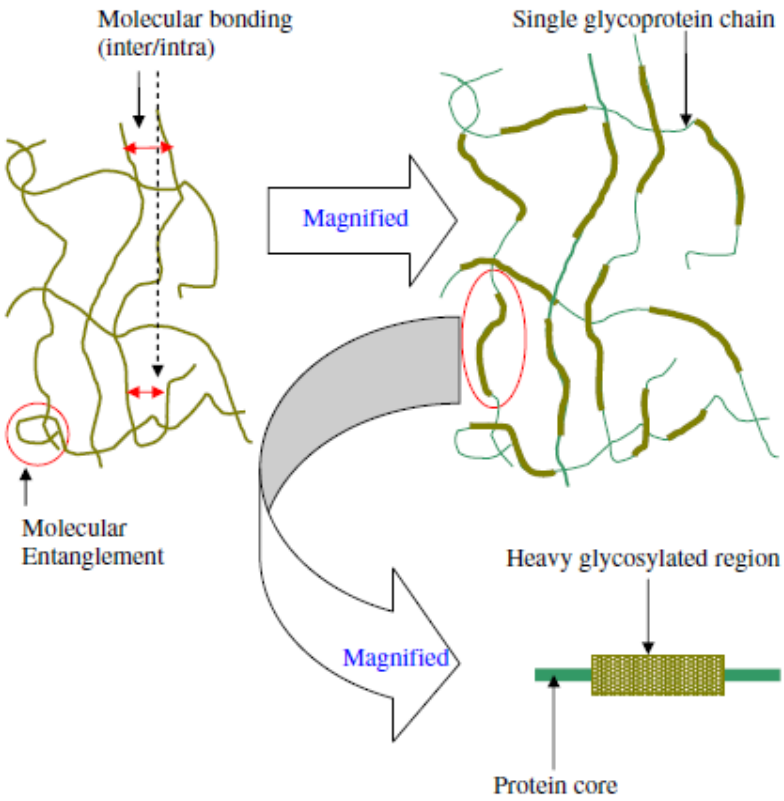


Figure 1.3. *Mucin structure (Andrews, et al., 2009).*

The polypeptide chain consists of 800-4500 amino acid residues (Andrianifahanana, et al., 2006; Kharenko, et al., 2009). The glycoprotein C- and N-terminal domains contain more than 10% of cysteine which is responsible for the formation of large mucin oligomers via disulfide bonds (Kharenko, et al., 2009). The greater part of the protein backbone consists of a repeating sequence of serine, threonine, and proline residues (STP tandem repeats) (Kharenko, et al., 2009).

Oligosaccharide branches are attached to 63% of the protein core, at about every three residues within the glycosylated regions, with the result that there are approximately 200 carbohydrate side chains per glycoprotein molecule; sugar side chains are linked to the hydroxyl side chains of serine and threonines by O-glycosidic bonds and arranged in a "bottle brush" configuration about the protein core. Each side chain contains between 2 and 20 sugar residues, primarily *N*-acetylgalactosamine, *N*-acetylglucosamine, fucose, galactose, sialic acid and traces of mannose and sulfate (Peppas & Sahlin, 1996; Kharenko, et al., 2009; Bansil & Turner, 2006). As chains usually terminate with either fucose or sialic acid (*N*-acetylneuraminic acid, pK_a = 2.6), the glycoproteins are negatively charged at physiological pH values (Kharenko, et al., 2009).

Mucin is stored in both submucosal and goblet cells, where calcium ions provide to shield the negative charges of the molecule, allowing the compact packing of such molecules. When mucin molecules are released into lumen, the outflux of calcium determines the exposition of negative charges which repulse each other leading to the expansion of the molecule. This is followed by the entanglement of mucin chains and the formation of non-covalent interactions such as hydrogen, electrostatic, and hydrophobic bonds, with the subsequent development of a viscoelastic gel. In the presence of water, these mucin chains overlap, interpenetrate and form a structured network that mechanically functions as mucus. The rheological behavior of mucus is a result of flow resistance of individual chains, entanglement and non-covalent intermolecular bonding (Andrews, et al., 2009).

The main function of mucin consists in the protection, lubrication and hydration of the external surfaces of epithelial tissue layers lining human body ducts and lumen. Moreover, certain types of mucin are involved also in more sophisticated biological

processes such as epithelial cell renewal and differentiation, cell signaling, and cell adhesion. Since mucin serves several functions, an alteration of its production and/or a change of its biochemical characteristics may have a negative effect on cell behavior. The deregulated expression and/or aberrant glycosylation of mucins have indeed been associated with various pathological conditions, including malignant and inflammatory disorders (Andrianifahanana, et al., 2006).

1.4 The mucoadhesive/mucosa interaction

In order to develop a mucoadhesive dosage form it is necessary to understand the mucoadhesion phenomenon, the forces and mechanisms that lead to an effective bond between the polymer and the mucus layer (Serra, et al., 2009).

1.4.1 Bio/mucoadhesive forces

For mucoadhesion to occur, different kinds of interfacial phenomena and forces arise at the interface mucoadhesive/mucosa, including:

(i) *mechanical and physical interactions* such as tangling of polymer and mucin chains;

(ii) *hydrogen bonds* formed by hydroxyls, carboxyls, sulfate and amino groups and generally weaker than ionic or covalent bonds;

(iii) *van der Waals bonds* which are probably the weakest form of interaction;

(iv) *hydrophobic bonds* which are indirect bonds occurring when non polar-groups are present in aqueous solutions; these groups associate with each other to minimize the effect produced by water molecules;

(v) *ionic bonds* formed by electrostatic interaction of two oppositely charged ions (Smart, 2005);

(vi) *covalent bonds* which are strong bonds like the previous (v) and are attained by the chemical reaction of the polymer and the substrate (Serra, et al., 2009); an example of covalent bond is represented by the disulfide bridge S-S arising from the oxidation of two sulfhydryl (-SH) groups (Sudhakar, et al., 2006);

(vii) *recognition of specific ligands* (lectins-sugars, etc.) (Kharenko, et al., 2009).

Hence, three main types of interaction between a polymer and the mucus layer exist: mechanical or physical bonds (i), secondary chemical bonds (ii, iii, iv) and primary chemical bonds (v,vi) (Serra, et al., 2009).

Although van der Waals interactions and hydrogen bonds are weaker than covalent or ionic bonds, quite strong adhesion can also be achieved with this kind of forces by the formation of large numbers of interaction sites (Kharenko, et al., 2009). For example, anionic polyelectrolytes, characterized by high molecular weight and high polar group contents (such as carboxyl and hydroxyl groups), may exhibit great mucoadhesive properties with a minimum of toxic effects (Kharenko, et al., 2009).

Nevertheless, even with covalent bonds which are permanent, the effectiveness of the mucoadhesive dosage form should be evaluated in light of mucus turnover and epithelial desquamation (Serra, et al., 2009).

Moreover, it must be considered that the interaction between two molecules is composed not only of attraction but also of repulsion. Indeed, besides the attractive forces previously listed, also repulsive interactions, such as electrostatic and steric repulsion, exist. While attractive forces favor adhesion, repulsive ones oppose it. Hence both forces must be considered in the development of a mucoadhesive dosage form (Sudhakar, et al., 2006).

1.4.2 Types of mucoadhesive/mucosa interactions

Considering the mechanism of mucoadhesion, different kinds of interaction can arise, depending on the type of the mucoadhesive dosage form and the type of mucosal surface:

(i) dry or partially hydrated mucoadhesive dosage forms coming in contact with considerable and continuous mucus layers, as shown in section a) figure 1.4;

(ii) fully hydrated mucoadhesive dosage forms coming in contact with considerable and continuous mucus layers (section b) figure 1.4);

(iii) dry or partially hydrated mucoadhesive dosage forms coming in contact with thin and discontinuous mucus layers (section c) figure 1.4);

(iv) fully hydrated mucoadhesive dosage forms coming in contact with thin and discontinuous mucus layers (section d) figure 1.4) (Kharenko, et al., 2009; Smart, 2005).

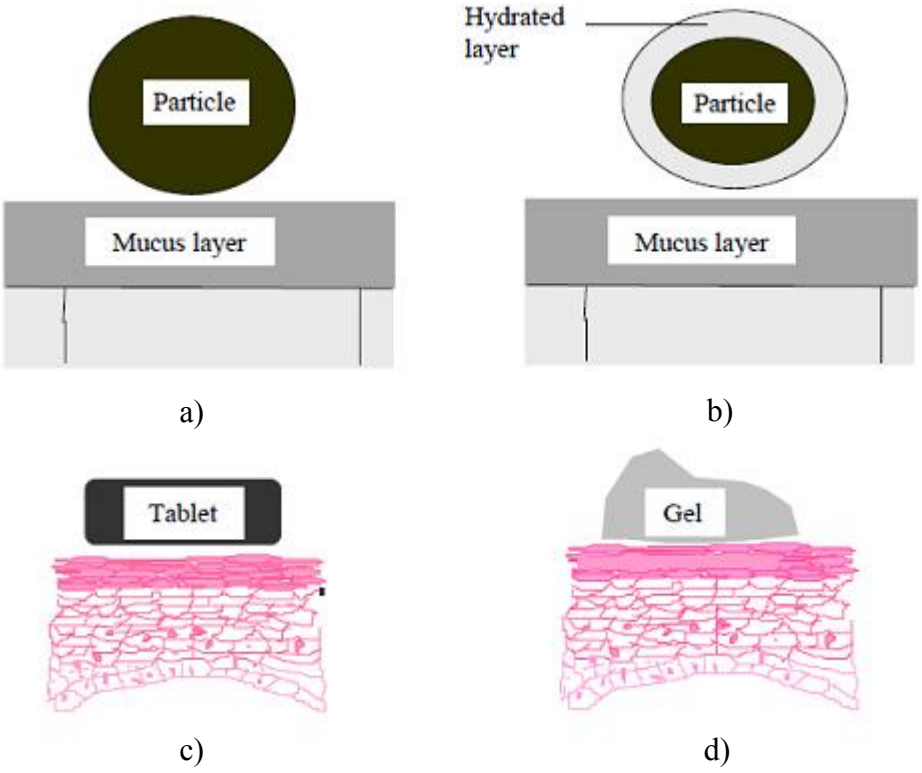


Figure 1.4. *Examples of different kinds of mucoadhesive/mucosa interaction: a) aerosolized particles on the nasal mucus layer; b) particle suspensions on the gastrointestinal mucus layer; c) tablets or patches on the buccal or vaginal mucus layers; d) liquids or aqueous semisolids as gels administered into esophagus, eye or for vaginal delivery (modified from Smart, 2005).*

1.4.3 Theories of Mucoadhesion

Mucoadhesion is a complex phenomenon that has not been fully understood. So far, several general theories of adhesion based on different kind of physical or chemical interactions have been used to explain the process (Khutoryanskiy, 2011). Indeed, as

seen previously, mucoadhesion can occur between different types of mucous membranes and drug delivery systems, which may be solid, viscous, or liquid. As a consequence, there is not a single universal theory able to explain all of these different situations but mucoadhesion probably results from a combination of the following theories (Khutoryanskiy, 2011; Kharenko, et al., 2009):

(i) the *electronic theory* suggests that an electronic transfer occurs between mucoadhesive polymer and mucus when these two surfaces exhibit different electronic characteristics. This results in the formation of a double layer of electrical charges at the mucus and mucoadhesive interface with subsequent adhesion due to electrostatic attraction between oppositely charged surfaces (Smart, 2005; Andrews, et al., 2009; Khutoryanskiy, 2011).

(ii) The *adsorption theory* considers adhesion as the result of various chemical interactions (primary and secondary bonding) between the adhesive polymer and the mucous substrate. As seen previously, primary bonds consist in ionic, covalent and metallic bonding, while secondary bonds consist in hydrogen bonds, van der Waals forces and hydrophobic interactions. The last one may also play an important role, especially when the mucoadhesive polymers have an amphiphilic nature; hydrophobic interactions can also explain the bioadhesivity of hydrophobic substrates (Andrews, et al., 2009; Khutoryanskiy, 2011; Lee, et al., 2000). On the other hand, for a bioadhesive polymer with a carboxyl group, hydrogen bonding is considered to be the dominant force at the interface (Lee, et al., 2000).

(iii) The *wetting theory* correlates the surface tension of mucus/mucoadhesive polymer and their interfacial energy with the polymer ability to spread on the mucus layer, considering such ability as a prerequisite for the development of adhesion. Therefore, polymers able to spread spontaneously onto the mucus surface, show greater mucoadhesive performances (Khutoryanskiy, 2011; Smart, 2005). This theory is mainly

applicable to liquid or low viscosity mucoadhesive dosage forms (Andrews, et al., 2009).

(iv) The *diffusion-interlocking theory* proposes the time-dependent diffusion of mucoadhesive polymer chains into gaps, loops and pores of the glycoprotein chain network (of the mucus layer) and the diffusion of glycoprotein mucin chains into the polymer matrix until an equilibrium penetration depth is achieved (figure 1.5). Hence, it consists of a two-way diffusion process driven by the concentration gradients of the two materials. The penetration rate and the depth of interpenetration depend upon the diffusion coefficients of both interacting layers and the contact time (Andrews, et al., 2009; Khutoryanskiy, 2011; Shaikh, et al., 2011; Jiménez-Castellanos, et al., 1993; Kharenko, et al., 2009).

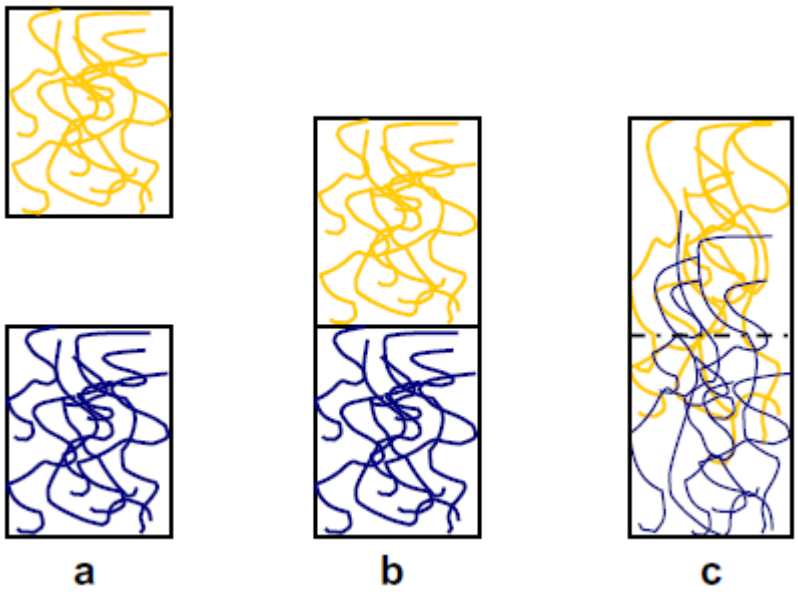


Figure 1.5. *The diffusion-interlocking theory of adhesion. a) Yellow (polymer) layer and blue (mucus) layer before contact; b) upon contact; c) diffusion after contact for a period of time and creation of a semipermanent adhesive bond (Andrews, et al., 2009).*

The mean diffusional depth of the bioadhesive polymer segments, s, may be represented by the following equation:

$$s = \sqrt{2tD} \quad (1.1)$$

where D is the diffusion coefficient and t is the contact time (Shaikh, et al., 2011).

Efficient adhesion is normally achieved when the thickness of interpenetration layer reaches 0.2–0.5 μm (Khutoryanskiy, 2011).

This process is also influenced by the molecular weight of mucoadhesive macromolecules, their hydrodynamic size and cross-linking density, chain mobility/flexibility and expansion capacity of both networks (Andrews, et al., 2009; Khutoryanskiy, 2011).

(v) The *fracture theory* relates the force required for polymer detachment from the mucus to the strength of their adhesive bond (Andrews, et al., 2009). This force is related to the mucoadhesive capabilities of the polymer (Serra, et al., 2009). The fracture theory is considered to be appropriate to describe the adhesion process involving rigid mucoadhesive materials (Khutoryanskiy, 2011; Shaikh, et al., 2011).

(vi) The *mechanical theory* involves rough and porous materials and suggests that surface roughness favors adhesion due to an increase in contact area (Khutoryanskiy, 2011).

1.4.4 The mucoadhesion process

Considering the different types of interaction that can occur between the dosage form and the mucus layer, the mucoadhesion phenomenon could be seen as a process composed of sequential phases, associated with different theories and mechanisms. The model considers two steps, illustrated in figure 1.6:

(i) the *contact stage* (step 1), when an intimate contact occurs between the mucous membrane and the mucoadhesive dosage form, which spreads over the substrate, wets and swells (wetting theory);

(ii) the *consolidation stage* (step 2), when various physicochemical interactions occur to consolidate and strengthen the mucoadhesive joint

(electronic, adsorption and diffusion-interlocking theories); the first bonds to be created are non-covalent, then further non-covalent and covalent bonds are formed, due to the interpenetration of the polymer and mucin chains (Smart, 2005; Khutoryanskiy, 2011).

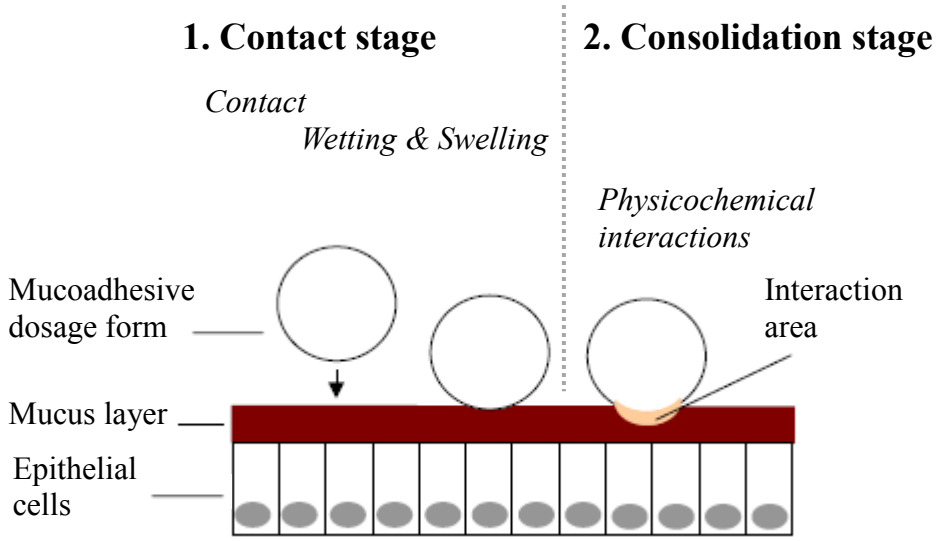


Figure 1.6. *The two steps of the mucoadhesion process (modified from Smart, 2005).*

The initial contact could be induced mechanically, e.g. placing the dosage form in the buccal cavity, eye or vagina. Alternatively, the deposition of the dosage form could happen exploiting the aerodynamics of the organ such as in the respiratory tract or peristalsis and other movements of the gastrointestinal tract (Smart, 2005). Obviously, an increase in the applied pressure favors the intimate contact because it causes a viscoelastic deformation at the interface (Lee, et al., 2000).

Smart (Smart, 2005) applied the DLVO theory, developed in the 1940s by Derjaguin and Landau (Derjaguin & Landau, 1941) and by Verwey and Overbeek (Vervey & Overbeek, 1948), in order to describe the adsorption process of the dosage form. In case of small particles their movement within the body depends on Brownian motion, the flow of liquids within body cavities and body movements like peristalsis. As mentioned in Section 1.4.1, when a particle comes in close contact with a surface both repulsive and attractive forces arise (Smart, 2005). The relative strength of these opposing forces depends on the nature of the particle, the aqueous environment and

the distance between the particle and the surface. In particular, smaller particles have a greater surface area-volume ratio, and as a result, the attractive forces may be greater too (Smart, 2005). Regarding the distance, as shown in figure 1.7, at a certain distance of about 10 nm (secondary minimum) particles can be weakly held because the attractive forces are balanced by the repulsive ones. In order to obtain a stronger adsorption, particles must overcome a repulsive barrier (energy barrier in the graph) and after that the primary minimum (around 1 nm) can be achieved (Smart, 2005).

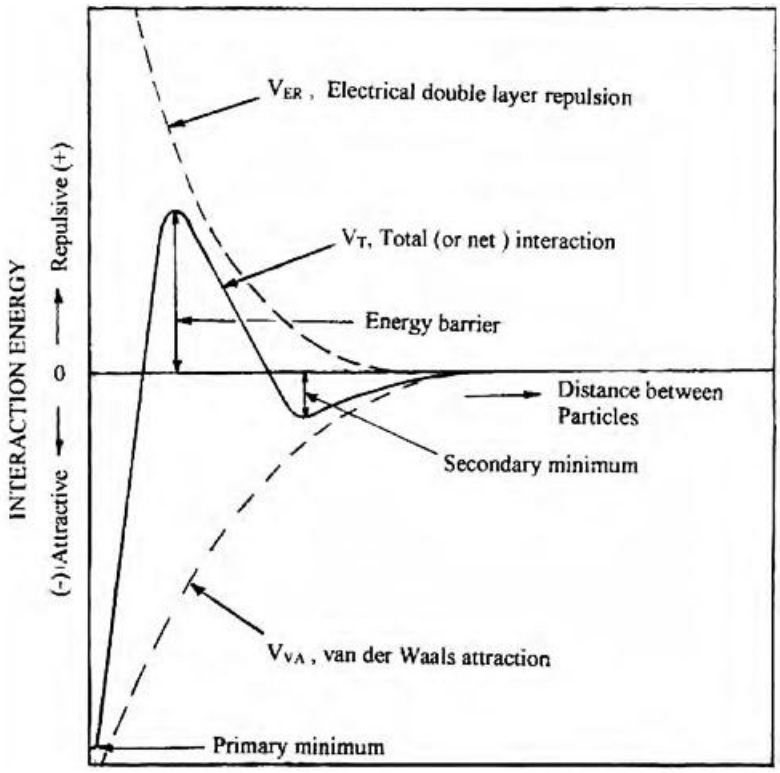


Figure 1.7. *Repulsive and attractive forces as a function of distance of separation on the bases of DLVO theory, where Vi are the potential energies (modified from Florence & Attwood, 1998).*

However, it must be considered that *in-vivo* the surface with which the particles come in contact is not a solid but a mucus gel. Moreover, the particles may be subjected to processes of hydration or coating with biomolecules, with, as a result, a possible change of their physicochemical properties (Smart, 2005).

The presence of folds and crevasses on the mucous membranes of the gastrointestinal tract and of an unstirred water layer at the surface permits the retention of the dosage form at that level with only weak adhesive forces (Smart, 2005).

On the other hand, if strong or prolonged adhesion is required, for example in case of larger formulations exposed to stresses such as blinking or mouth movements, then a second consolidation stage is necessary (Smart, 2005). In order to adhere to the surface, mucoadhesive materials must be activated by the presence of moisture, which acts as a plasticizer. In these conditions the mucoadhesive molecules become free, conform to the shape of the surface, and bond predominantly by weaker van der Waals and hydrogen bonding but also, in the case of cationic materials, by electrostatic interactions with the mucin negatively charged groups (such as carboxyl or sulphate) (Smart, 2005).

In relation to the mucus characteristics a dosage form can establish the adhesive joint more or less easily. Indeed, in case of surfaces with only limited amounts of mucus, a dry mucoadhesive polymer dehydrate without difficulty the mucus gel by extracting its water component, allowing the polymer molecules the freedom to form hydrogen bonds with the epithelial surface (Smart, 2005).

On the other hand, in presence of a substantial mucus layer, the formation of the adhesive joint may be reached less easily because there is the need to overcome the anti-adherent properties of mucus and hence a change in the physical properties of the mucus layer is necessary (Smart, 2005).

Considering the adhesive joint as composed of three regions, the mucoadhesive material, the mucosa and an interfacial region, two theories for the consolidation process may be developed (Smart, 2005).

The first theory is based largely on the diffusion-interlocking theory and considers the interpenetration of the mucoadhesive and mucin macromolecules and, subsequently, the formation of secondary interactions (Smart, 2005).

In the case of dry or partially hydrated formulations come into contact with a substantial mucus gel, a second theory could be used to explain the adhesion mechanism. In this case a water movement occurs until the equilibrium is reached (dehydration process) (figure 1.8) (Smart, 2005). In particular, in the case of

polyelectrolyte gels, characterized by a marked affinity for water, a high osmotic pressure is established with a significant swelling of the dosage form. When the swollen dosage form comes into contact with the mucus gel, the process of dehydration will occur rapidly and a consolidation of the mucus joint will be achieved (Smart, 2005).

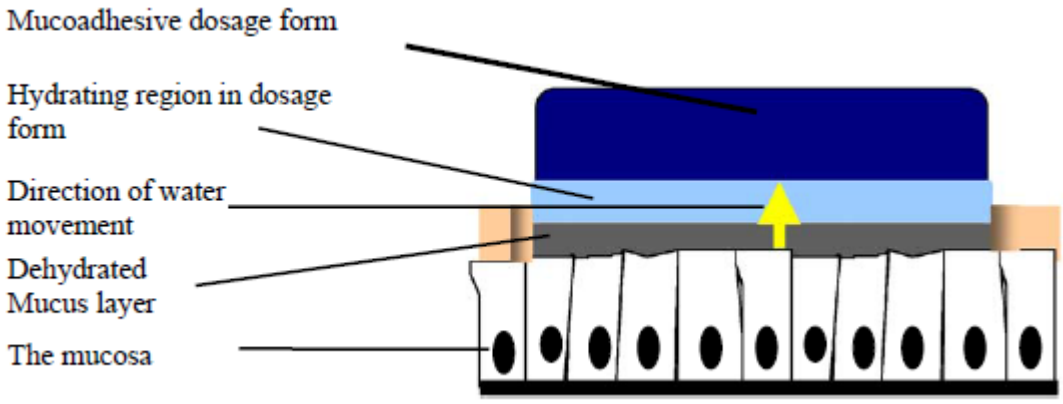


Figure 1.8. *The dehydration theory of mucoadhesion (Smart, 2005).*

Due to dehydration, the mucus gel becomes adhesive (Mortazavi & Smart, 1993).

1.5 Factors affecting mucoadhesion

Since mucoadhesion is a very complex phenomenon, the strength of the adhesive joint may be influenced by different factors related to the characteristics of the mucoadhesive material and the mucosa but also the environment.

1.5.1 Properties of the mucoadhesive polymer

Numerous are the properties of the polymer involve in its ability to adhere to a mucosal tissue. Among these are included the following:

(i) *hydrophilicity* or the presence of hydrophilic functional groups (such as hydroxyl, carboxyl, amide, sulphate), which are able to form hydrogen bonds with the substrate and lead to the swelling of the polymer in aqueous medium. In the swollen polymer the chains are at the maximum distance; this leads to an increase of their flexibility and a more efficient

interpenetration with the mucin glycoproteins and thus the maximum exposure of potential docking sites is achieved. However, when hydration and swelling are too high a slimy mucilage, which can be easily removed from the substrate, is obtained. Depending on the type of polymer, the degree of hydration which corresponds to the maximum adhesion varies.

(ii) *Molecular weight (Mw) and spatial conformation*. Low-molecular-weight polymers penetrate the mucus layer better than high-molecular-weight polymers which, on the contrary, promote physical entanglement. The optimum molecular weight for the maximum mucoadhesion depends on the type of polymer used, but generally it ranges between 10 kDa and 4000 kDa. Polymers with a Mw higher than 4000 kDa will not moisten easily and thus the exposure of the free group is limited, while polymers with a Mw lower than 10 kDa form weak gels or dissolve quickly. In general it is observed that for linear polymers, the bioadhesive forces increase with increasing molecular weight up to 100 kDa and beyond this level there is not much effect. But it must be considered that, although a critical length of the molecules is necessary for interpenetration and molecular entanglement, also size and spatial conformation of the adhesive macromolecules could affect the mucoadhesive capability. For example, dextran with very high molecular weight (about 20000 kDa) shows adhesive strength similar to that of polyethylene glycol (PEG) with a molecular weight of 200 kDa; this is due to the fact that the helical conformation of dextran may shield many adhesively active groups while PEG is linear.

(iii) *Cross-linking and swelling* which are inversely proportional. The lower the cross-linking density, the higher the polymer chain flexibility, the hydration rate and, hence, the degree of swelling. Indeed, polymer chain flexibility and swelling are required for the diffusion of polymer chains and the exposure of sites for the formation of bonds and the mechanical entangling with mucin. Therefore the exposure of a larger surface area determines better mucoadhesive properties. On the other hand, for

polymers with the tendency to overhydrate, cross-linking could have a positive effect.

(iv) *Concentration*. The optimum concentration for the maximum mucoadhesion depends on the type of polymer. Considering liquid formulations or similar, when the polymer concentration is too high the solution becomes solvent-poor and the chains available for interpenetration are not numerous so the mucoadhesive properties decrease. In the case of solid dosage forms, such as tablets, the strength of the adhesive joint increases with increasing of the polymer concentration.

(v) *Charge density* of macromolecules. The presence of surface charges permits the formation of electrostatic interactions between polymer and the negative charges of mucin glycoproteins (Shaikh, et al., 2011; Smart, 2005; Jiménez-Castellanos, et al., 1993).

1.5.2 Environmental factors

Besides polymer properties, the environment can also influence the mucoadhesive ability of the dosage form in different ways:

(i) *pH changes* which can lead to differences in the dissociation degree of ionizable functional groups of both glycoprotein and polymer chains and, hence, can modify the charge density of the macromolecules. As a consequence, for example, at high pH values the carboxyl functional groups are in the dissociated form and thus a change in the spatial conformation of the macromolecule from a coiled state to a rod-like structure more suitable for chain interpenetration could be achieved (Andrews, et al., 2009; Jiménez-Castellanos, et al., 1993). However, the negative charges due to the dissociated functional groups could produce also repulsion forces.

(ii) *Initial contact time* between the mucus layer and the dosage form, which is directly proportional with the mucoadhesive strength of the dosage form because the initial contact time influences the swelling degree of the

dosage form and the extent of interpenetration of polymer and mucin chains (Lee, et al., 2000; Kharenko, et al., 2009).

(iii) *Contact force* which is directly proportional with the depth of diffusion of the chains (Kharenko, et al., 2009).

(iv) *Ionic strength* of the surrounding medium which influence the mucoadhesion strength because metal ions may shield chains functional sites reducing swelling and mucoadhesive force (Andrews, et al., 2009); on the other hand the presence of divalent cations may induce gel formation, as in the case of sodium alginate and calcium salt.

(v) *Moistening* which is required to allow the expansion and mobility of polymer chains and, hence, create a "macromolecular network" of sufficient size for the interpenetration of polymer and mucin molecules (Kharenko, et al., 2009).

1.5.3 Physiological factors

Physiological variables can also affect mucoadhesion:

(i) *mucus turnover*, i.e. the time required to replenish the mucus layer, which varies from a few hours to a day depending on the body sites. It increases in presence of pathogens and may limit the retention of the dosage form at the site of action and hence its effectiveness. This is less important in the case of mucosal tissue with a relatively low mucus turnover (e.g. mouth or vagina) while in areas of markedly high mucus turnover (e.g. intestines), adherence time probably don't overcome 2 hours.

(ii) *Mucus viscosity* which varies depending on the body sites. The viscosity should be not too low but also not too high, because in the first case the polymer/mucus bond would be weak and easily detachable, in the other case the thick mucus layer would function as a barrier and the interpenetration and diffusion processes are limited.

(iii) *Concomitant diseases* (e.g. ulcer disease, colitis, allergic rhinitis, bacterial or fungal infection) which can modify the amount of secreted mucus and its physicochemical properties.

(iv) *Tissue movement* which can affect the mucoadhesive/mucosa contact and the retention time of the dosage form at the target site (e.g. peristalsis, blinking).

1.6 Mucoadhesive polymers

1.6.1 Polymer ideal characteristics

An ideal mucoadhesive polymer should have the following characteristics:

- hydrophilicity;
- presence of strong anionic or cationic charges;
- sufficient chain mobility to allow diffusion and interpenetration;
- surface energy properties favoring the spreading onto mucus;
- good swelling;
- optimum molecular weight, spatial conformation and concentration for mucoadhesion;
- an appropriate cross-linking degree in order to prevent overhydration unless suppression of bond forming groups;
- fast adhesion to mucosa, ability to form a strong bond and possession of some site specificity;
- presence of adhesively active groups;
- sufficient mechanical strength;
- biocompatibility and biodegradability;
- polymer and its degradation products should be non-toxic, non-irritant;
- easily available at low cost;
- polymer must not decompose on storage or during the shelf life of the dosage form;
- polymer should allow easy incorporation of the drug and offer no hindrance to its release (Kharenko, et al., 2009; Shaikh, et al., 2011; Sudhakar, et al., 2006; Lee, et al., 2000; Khutoryanskiy, 2011).

1.6.2 Classification of mucoadhesive polymers

Mucoadhesive polymers are generally hydrophilic macromolecules, also called "wet adhesives" since they are activated by moistening. They can be divided into three main subsets, namely anionic, cationic and non-ionic polymers. Of these, anionic and cationic polymers have been shown to exhibit the greatest mucoadhesive strength (Smart, 2005).

The mucoadhesiveness of anionic polymers, such as poly(acrylic acid), carboxymethylcellulose and sodium alginate, is related to the ability of the carboxylic groups to form hydrogen bonds with oligosaccharide chains of mucin while mucoadhesive properties of cationic polymers, e.g. chitosan, are mainly based on the electrostatic interaction occurring between their positive charges and the mucin negative charges (Khutoryanskiy, 2011).

Beside wet adhesives, which represent traditional non-specific first-generation mucoadhesive polymers, in recent years a novel second-generation of mucoadhesive polymers has also been developed, including, lectins and thiolated polymers. Lectins are generally defined as proteins or glycoprotein complexes able to bind sugars selectively in a noncovalent manner. The thiolated polymers, also named thiomers, are hydrophilic macromolecules exhibiting free thiol groups on the polymeric backbone (Shaikh, et al., 2011). The presence of thiol groups in the polymer allows the formation of stable covalent bonds (disulfide bridges) with cysteine-rich subdomains of mucus glycoproteins. This can lead to an increase in the residence time and bioavailability (Khutoryanskiy, 2011; Shaikh, et al., 2011).

1.7 Methods to study mucoadhesion

In order to design and develop a mucoadhesive delivery system, it is fundamental the assessment of the mucoadhesive properties of materials and dosage forms. The methods developed to assess mucoadhesion include *in vitro* and *in vivo* techniques. The last ones always follow a screening, realized using *in vitro* techniques and aims to highlight the most promising mucoadhesive materials (Lee, et al., 2000). Nevertheless, there is only a limited number of *in vivo* studies in literature because of time, cost and ethical constrains (Shaikh, et al., 2011).

Several *in vitro* methods for the evaluation of mucoadhesive properties of different dosage forms, are reported in literature. The most common methods are those based on the measure of the force needed to break the adhesive joint, i.e. Atomic Force Microscopy and tensile methods using modified balances or tensile testers as Texture Analyser. Beside these methods, there are others based on particle interactions measurements which include mucin particle method and BIACORE, proposed by Takeuchi et al. (Takeuchi, et al., 2005), rheology. ellipsometry, and flow channel method (Woertz, et al., 2013; Khutoryanskiy, 2011).

The main *in vivo* techniques for the evaluation of the mucoadhesive properties include gamma scintigraphy and magnetic resonance imaging (MRI), two non-invasive techniques able to perform gastrointestinal transit studies (Lee, et al., 2000; Shaikh, et al., 2011).

Chapter 2

Development of methods to study mucoadhesion

2.1 Introduction

Over the last three decades mucoadhesion has become of interest for its potentiality to increase the residence time of the dosage form at the site of action (local action, e.g. within the gastrointestinal tract) or absorption (systemic delivery, e.g. via the nasal cavity), improving drug bioavailability and reducing administration frequency (Khutoryanskiy, 2011; Smart, 2005). Furthermore the development of these systems is very flexible since mucoadhesive drug delivery systems may be formulated in different dosage forms (e.g. tablets, films, gels) and administered by various routes, such as ocular, nasal, buccal and gingival, gastrointestinal (oral), vaginal and rectal (Khutoryanskiy, 2011). All of these advantages have contributed to the expansion of the research and the market for this kind of products.

These systems owed their mucoadhesive properties to materials, especially polymers, capable of adhere to a mucosal tissue. The main group of mucoadhesives is represented by hydrophilic macromolecules, containing groups (e.g. hydroxyl, carboxyl or amine groups) able to form numerous hydrogen bonds with the mucus layer. They are called “wet” adhesives because they are activated by moisture, which plasticizes the system allowing mucoadhesive molecules to become free, conform to the shape of the surface, and able to form van der Waals and hydrogen bonds with the mucus layer (Smart, 2005). This is a simplification since mucoadhesion is a complex

phenomenon, not yet fully understood, but certainly consisting of a combination of different interaction mechanisms (Khutoryanskiy, 2011).

The degree of mucoadhesion is influenced by various polymer-based physicochemical properties, including molecular weight, chain flexibility, hydrophilicity, ability to form hydrogen bonds, concentration and swelling extent (Andrews, et al., 2009; Kharenko, et al., 2009). Moreover, there are also environmental and physiological factors, such as changing in pH or presence of concomitant diseases, which can influence the strength and duration of the mucoadhesive interaction (Kharenko, et al., 2009).

In the design of a mucoadhesive drug delivery system all of these factors should be considered, first of all polymer properties. Therefore, in the development of these systems the choice of the polymer plays a key role in determining the success and the effective mucoadhesiveness of the final product. Hence, the importance of making a screening of different materials throughout the development of techniques for the detection of polymer properties related to its mucoadhesion capacity.

2.1.1 Aim

The research started with the screening of four anionic and natural polymers: sodium alginate (SA), tragacanth gum (TG), xanthan gum (XG) and k-carrageenan (KC).

These polymers were used to realize tablets whose mucoadhesive properties were studied directly by means of a tensile test using a Texture Analyser and indirectly throughout the comparison of the results obtained from the tensile test and the ones derived from the evaluation of certain properties influencing mucoadhesion: water uptake and swelling of the dosage form and polymer molecular weight. This kind of "two-way approach" has been chosen to point out which is the best mucoadhesive polymer and how the mucoadhesive capacity is affected by polymer properties.

2.2 Materials

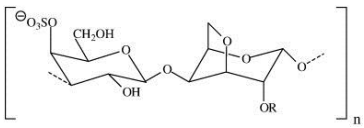
The following were used: sodium alginate E401 (Satialgine S1100), xanthan gum Ph. Eur.-USP, tragacanth gum powder NF18, talc PHARMA USP Ph.Eur., magnesium stearate FU-Ph.Eur., microcrystalline cellulose T1 Ph.Eur., sodium chloride, all seven

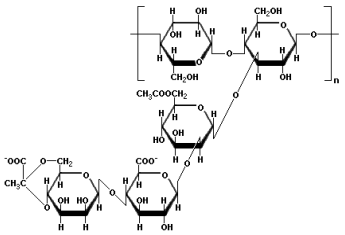
supplied by A.C.E.F. S.p.A. (Italy); Ludipress® and mucin (from porcine stomach, type II) purchased from BASF The Chemical Company (Germany) and Sigma-Aldrich (USA), respectively; Gelcarin GP 911NF K-Carrageenan supplied by IMCD UK LTD (United Kingdom).

In all solutions, deionized water was used.

Characteristics, properties and applications of the mucoadhesive polymers are described in detail in table 2.1.

Table 2.1. *Properties and application of the polymers used in the study.*

Polymers	Properties	Applications
Gelcarin GP 911NF (K-Carrageenan) High molecular weight polysaccharide extracted from red seaweed of the class Rhodophyceae (especially from Eucheuma, Chondrus e Gigartina species). It consists chiefly of potassium, sodium, calcium, magnesium, and ammonium sulfate esters of galactose and 3,6-anhydrogalactose copolymers. These hexoses are alternately linked at the α-1,3 and β-1,4 sites in the polymer.  R=H (Rowe, et al., 2006; FMC Corporation, 1993)	• K-Carrageenan is a strongly gelling polymer which has a helical tertiary structure (formed with potassium ions) that allows gelling. It contains 25% ester sulfate by weight and approximately 34% 3,6-anhydrogalactose. • Hygroscopic polymer, soluble in water at 80°C and partially soluble in cold water. • Potassium salts form in water a firm gel structure which becomes tightly aggregated as the level of potassium is increased. Moreover the presence of divalent cations may cause helices to aggregate and the gel to contract. • Carrageenan is thermally reversible, so at high temperatures it will impart minimal viscosity to the system, while upon cooling it will thicken.	• It is used in the manufacture of stable gels, creams, lotions, eye drops, suppositories, tablets, and capsules. • It stabilizes existing emulsions and suspensions thanks to its thickening and thixotropic properties. • Incorporation of carrageenan into tablet matrices together with calcium or potassium salts leads to the formation of a gel which fosters drug sustained-release. • Carrageenan has mucoadhesive properties and it can be used to produce mucoadhesive formulations for oral and buccal drug delivery.
Xanthan gum (Ph. Eur.-USP) High molecular weight extracellular heteropolysaccharide, produced by fermentation with the gram-negative bacterium, Xanthamonas campestris.	• It contains a cellulosic backbone (β-D-glucose residues) and a trisaccharide side chain of β-D-mannose-β-D-glucuronic acid-α-D-mannose attached with alternate glucose residues of the main chain. The terminal D-mannose residue may carry a	• It is used as a suspending, gelling, stabilizing, thickening, emulsifying, viscosity-increasing agent and a binder in oral and topical pharmaceutical formulations, cosmetics, and foods. • It is also used to prepare



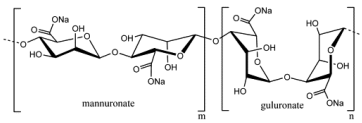
(Rowe, et al., 2006; Talukdar & Kinget, 1995)

pyruvate function, while the non-terminal D-mannose unit in the side chain contains an acetyl function.
- The rigid polymer chain may exist in solution as a single, double, or triple helix that interacts with other xanthan gum molecules to form complex, loosely bound networks.
- It is prepared as the sodium, potassium, or calcium salt.
- It is soluble in water while practically insoluble in ethanol and ether.
- It is nontoxic, and it has good stability and viscosity properties over a wide pH and temperature ranges.
- Xanthan gum gels show a pseudoplastic behavior.

sustained-release formulations, such as matrix tablets.
- It has been incorporated in an ophthalmic liquid dosage form, which interacts with mucin, thereby helping in the prolonged retention of the dosage form in the precorneal area.
- It may be used to increase the bioadhesive strength in vaginal formulations and as a mucoadhesive controlled-release excipient for buccal drug delivery.
- Moreover it can be used as an excipient for controlled colonic drug delivery.

Sodium alginate (E401)

Sodium salt of alginic acid, extracted from brown seaweed.



(Rowe, et al., 2006)

- Alginic acid is a linear copolymer composed of two monomeric units, D-mannuronic acid and L-guluronic acid.
- A 1% [w/v] aqueous solution exhibits a pH of about 7.2.
- It is a hygroscopic polymer, slowly soluble in water, where it forms a viscous colloidal solution.
- Practically insoluble in most organic solvents and in aqueous acidic solutions in which the pH is less than 3.
- Various grades of sodium alginate are commercially available that yield aqueous solutions of varying viscosity. Typically a 1% [w/v] aqueous solution, at 20°C, will have a viscosity of 20-400 mPa*s. Viscosity may vary depending upon concentration, pH, temperature, or the presence of metal ions. Above pH 10, viscosity decreases.

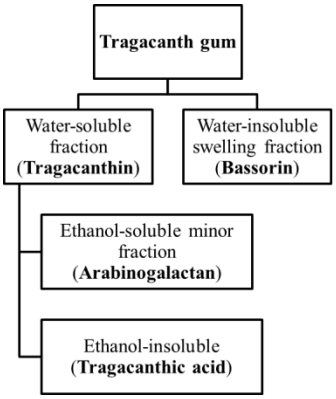
- It is used in oral and topical pharmaceutical formulations as suspending agent, stabilizing agent, tablet and capsule disintegrant, tablet binder, diluent for capsules and viscosity increasing agent.
- It is also used in the preparation of sustained-release oral formulations since it can delay the dissolution of a drug from tablets, capsules, and aqueous suspensions
- Finally, it is used in the preparation of mucoadhesive dosage forms or ophthalmic solutions that form a gel *in situ* after administration.

Tragacanth gum (powder NF18)

Anionic polysaccharide of high

- Sugar composition: galacturonic acid, xylose, fucose, arabinose,

- Emulsifying, stabilizing, suspending and viscosity-

molecular weight, obtained as a dried exudate from the stems and branches of *Astragalus gummifer* Labillardière and other species of Astragalus grown in Western Asia. It consists of two main fractions: a water-insoluble component (bassorin), which has the capacity to swell and form a gel, and a water-soluble component (tragacanthin).  (Rowe, et al., 2006; Balaghi, et al., 2011)	galactose, glucose, and traces of rhamnose. The proportions of each sugar vary among the gums from various species of Astragalus. • Molecular weight of about 840kDa. • The viscosity of tragacanth dispersions varies according to the grade and source of the material. Typically, 1% [w/v] dispersions may range in viscosity from 100-4000 mPa*s at 20°C. Viscosity increases with increasing temperature and concentration, and decreases with increasing pH. Maximum initial viscosity occurs at pH 8, although the greatest stability of tragacanth dispersions occurs at about pH 5. • Practically insoluble in water, ethanol (95%) and other organic solvents. In water it swells rapidly forming viscous colloidal solutions or gels. • A 1% [w/v] aqueous dispersion has a pH of 5-6. • Highly acid-resistant hydrocolloid.	increasing agent. • Diluent in tablet formulations.

2.3 Methods

2.3.1 Determination of intrinsic viscosity and Viscosity Average Molecular Weight of polymers

Intrinsic viscosity determination was carried out with a 0.46 mm diameter Ubbelohde capillary viscosimeter (Schott-Geräte GmbH, Germany) immersed in a heated circulating water bath to maintain a constant temperature of 25°C for all polymers.

For each polymer, solutions with decreasing concentration in the range of 0.25-0.03g*dL^{-1} were prepared. Elution time of each sample was measured five times and the average elution time was then calculated. The corresponding reduced viscosities were obtained by the following equation:

$$\eta_{red} = \frac{t - t_s}{C \cdot t_s} \quad (2.1)$$

where η_{red} is the reduced viscosity, t is the average elution time, t_s is the average elution time of the solvent and C is the solution concentration.

Reduced viscosity versus concentration curves were then constructed and intrinsic viscosity was estimated by extrapolating the reduced viscosity value when the concentration tends toward 0 by means of a linear regression.

The Viscosity Average Molecular Weight of polymers was estimated from intrinsic viscosity by the Mark-Houwink-Sakurada equation:

$$[\eta] = \mathrm{KM}^{\alpha} \quad (2.2)$$

where $[\eta]$ is the intrinsic viscosity, M is the average molecular weight, K and α two constants which depend on the solvent and the temperature used (Brandrup , et al., 1999).

The operating conditions, i.e. the solvent and K and α values, for the different polymers are reported in table 2.2. The molecular weight of the polymers not listed in the table was derived from literature data.

Table 2.2. *Operating conditions used for each kind of polymer.*

Polymer	Solvent	K (x10^3) [dL*g^{-1}]	α [-]	References
SA	NaCl 0.1 M	0.1228	0.963	(Mancini, et al., 1996)
XG	NaCl 0.1 M	0.0017	1.140	(Brandrup , et al., 1999)
KC	NaCl 0.1 M	0.0310	0.950	(Rochas, et al., 1990)

2.3.2 Powder flowability measures

Flow properties of powders were evaluated determining the Compressibility Index and the Hausner Index, which were calculated as follows:

$$Compressibility\ Index = 100 \times \left[\left(V_0 - V_f\right)/V_0\right] \quad (2.3)$$

$$Hausner\ Index = \left(V_0/V_f\right) \quad (2.4)$$

where V_0 and V_f are, respectively, the unsettled apparent volume and the final tapped volume of the powder.

V_0 and V_f were measured according to a FUI XII ed. (F.U.I., 2008) modified method: 10 g of powder were let flow into a volumetric cylinder leading to V_0 value; then the cylinder was tapped 20 times from a specific height (1 cm) and V_f was calculated.

Powders of polymers and mixtures polymer/excipients blend used for tablets preparation were subjected to the test. Each sample was analyzed three times.

The flow properties of the powders was evaluated using the scale of flowability reported in FUI XII ed (F.U.I., 2008) (table 2.3).

Table 2.3. *Scale of flowability* (F.U.I., 2008).

Compressibility Index (CI) [%]	Flowability	Hausner Index (HI) [-]
1-10	Excellent	1.00 – 1.11
11-15	Good	1.12 – 1.18
16-20	Discrete	1.19 – 1.25
21-25	Passable	1.26 – 1.34
26-31	Poor	1.35 – 1.45
32-37	Very poor	1.46 – 1.59
>38	Extremely poor	> 1.60

2.3.3 Preparation of mucoadhesive tablets

Tablets were prepared by direct compression of mixtures composed by 60% [w/w] of polymer and 40% [w/w] of an excipients blend. The excipients blend was added in order to increase the flow properties of the polymers and its composition is reported in table 2.4.

Tablets were prepared using a single punch tablet press (COSALT type, Officina CO.STA. S.r.l., Italy) fitted with a flat-faced circular punch (5 mm diameter) (figure

2.1). The weight of the tablets ranges from 76 mg to 136 mg and the thickness ranges from 3 to 4 mm.

Table 2.4. *Excipients blend composition.*

Components	**Amount [%]**
Ludipress®	85
Microcrystalline Cellulose T1	10
Magnesium Stearate	3
Talc	2

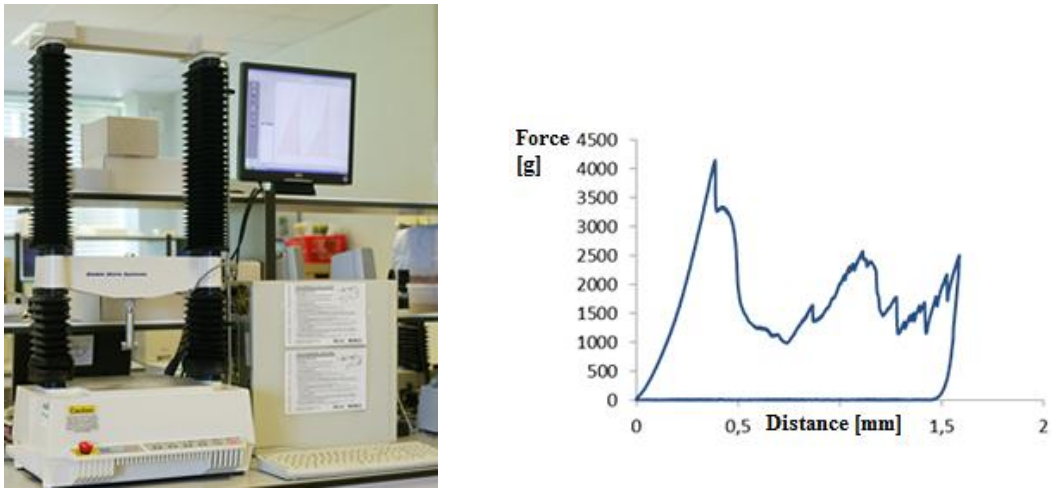
Figure 2.1. *Single punch tablet press.*

2.3.4 Tablets crushing strength

The evaluation of the tablets breaking force or crushing strength was carried out by means of a T.A.HDi®/250 Texture Analyser (Stable Micro System Ltd, UK) (figure 2.2) equipped with a cutting probe. During the test, the cutting probe moves with a downward rate of 0.1 mm*s^{-1}.The instrument starts to acquire data once the trigger

force of 0.1 N is reached. The compression force needed for breaking the tablet was registered. At least 3 tablets for each type were analysed.

Data processing was performed by using Texture Expert® software.



(A) **(B)**

Figure 2.2. *T.A.HDi®/250 Texture Analyser (A) with an example of graph obtained from the crushing strength test (B).*

2.3.5 Evaluation of tablets behavior in aqueous medium

Since these wet adhesive polymers exhibit their mucoadhesive properties once hydrated, the behavior of tablets in aqueous medium has been studied through the evaluation of the water uptake capacity and the swelling degree.

- *Water Uptake capacity*

The Water Uptake capacity of tablets is the ability of tablets to absorb water and it is related to the hydrophilicity of tablets components, especially the polymer. Water Uptake capacity of tablets was determined by a gravimetric method. Tablets were fixed on a plastic support with cyanoacrylate glue (section (A), figure 2.3) and accurately weighed. Tablets were then immersed in a becher containing 30 mL of water at room temperature (section (B), figure 2.3). At intervals of 5 minutes for 1 hour, the tablets were taken out of the incubation medium and accurately weighed after removing the excess of water (section (C), figure 2.3). The amount of Water

Uptake (WU), expressed as a percentage, was calculated according to the following equation:

$$WU = \left(\frac{W_t - W_0}{W_0}\right) \times 100 \quad (2.5)$$

where W_t is the weight of the wet tablet at time *t* and W_0 is the initial weight of the dry tablet.

The analysis was repeated three times for each formulation.

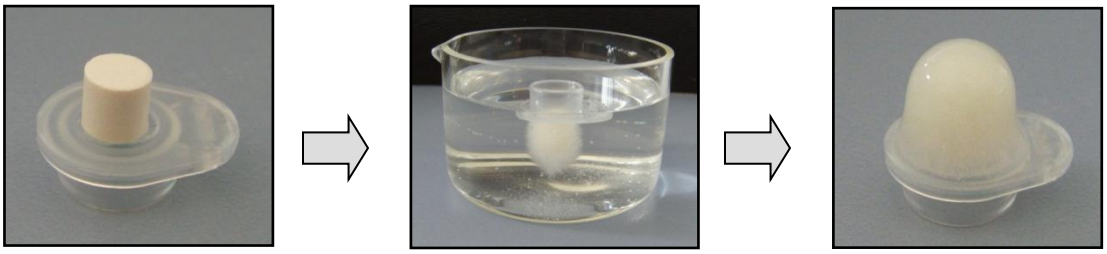
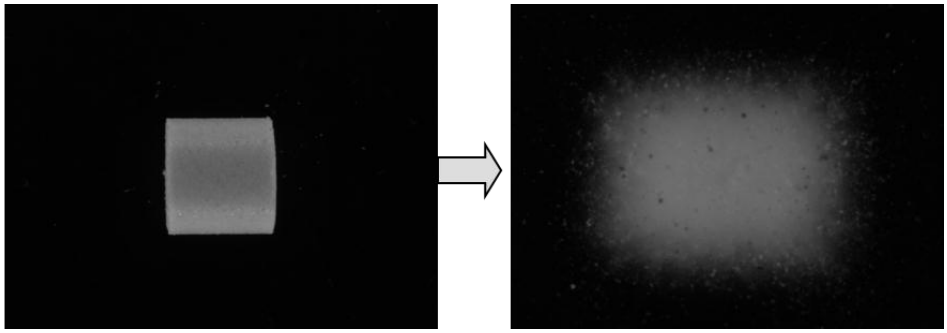


Figure 2.3. *Schematic representation of the gravimetric method used to measure tablets WU: (A) the dry tablet was weighed and fixed on a plastic support; then the tablet was immersed in water (B) and every 5 minutes for 1 hour, it was taken out and accurately weighed after removing the excess of water (C).*

- *Swelling studies*

Swelling studies were performed by means of an image analysis realized by using a CMOS Bayer Camera (DBK-61BUC02) with a resolution of 2048x1536, purchased from The Imaging Source Europe GmbH (Germany). IC Capture 2.1 (The Imaging Source) and Matlab 2010 (The MathWorks Inc.) software were used for images acquisition and mathematical analysis, respectively.

For the analysis, tablets were fixed with a double-sided tape on the black bottom of a plastic cubic cell, which was filled with 30 mL of water (section (A), figure 2.4). Then tablets were allowed to swell for 1 hour at room temperature and at scheduled time intervals (1 minute) an image was captured (section (B), figure 2.4).



(A) **(B)**

Figure 2.4. *Schematic representation of the image analysis used to measure tablets swelling: (A) dry tablet at time zero and (B) swollen tablet after immersion in water for 60 minutes.*

This image was elaborated by the software in order to measure the approximate volume of the dosage form using the equation that express the volume of a solid of revolution:

$$V = \Pi \int_C^B r_x^2 \, \delta x \tag{2.6}$$

where V is the volume of the solid of revolution, r_x is the radius of the solid circumference and δx is the solid height, as explained in figure 2.5.

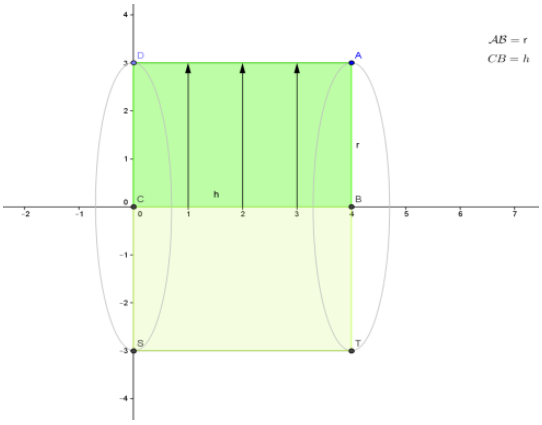


Figure 2.5. *Graphical representation of a solid of revolution.*

The Swelling Index (SI) of tablets, expressed as a percentage, was calculated according to the following equation:

$$SI = \left(\frac{V_t - V_0}{V_0}\right) \times 100 \quad (2.7)$$

where V_t is the volume of the swollen tablet at time t and V_0 is the initial volume of the dry tablet.

The analysis was repeated three times for each formulation.

2.3.6 Tensile Test for the detection of tablets mucoadhesive properties

The mucoadhesive performance of tablets was measured by means of a T.A.HDi®/250 Texture Analyser (Stable Micro System Ltd, UK) equipped with a load cell of 250 kg, using the "adhesive test" mode. The tablet was fixed with cyanoacrylate glue on the mobile metallic cylindrical probe (6 mm diameter) of the instrument, covered with an aluminum foil. The tablet was immersed in 30 mL of water for five minutes, the excess of water was removed and then the tablet was brought in contact with the mucus substitute fixed on the mucus sliding lower platform. The two materials were held in contact for a specific time with a specific force and then the probe was removed vertically at a constant upward speed (figure 2.6).

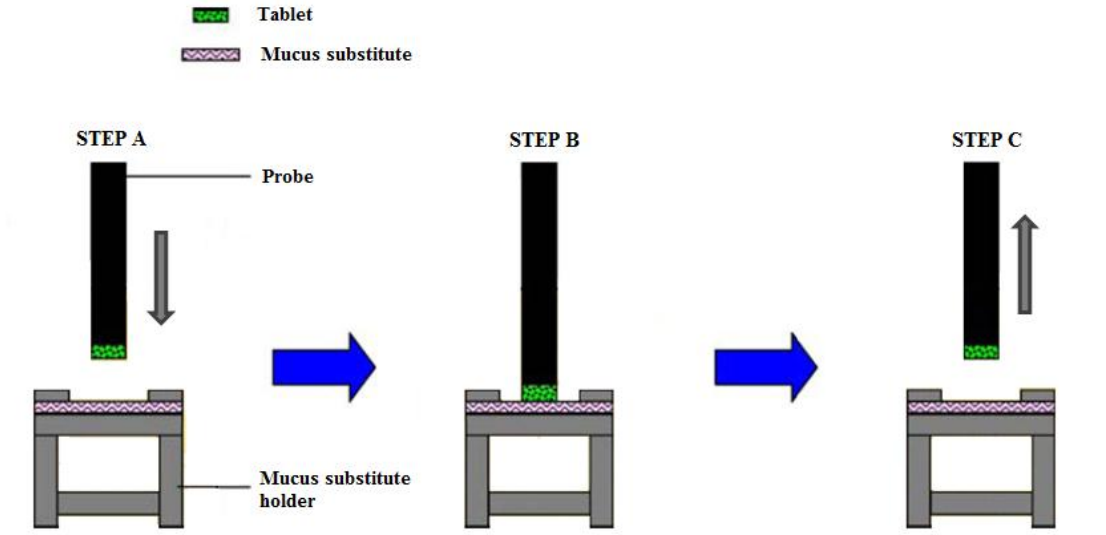


Figure 2.6. *Schematic representation of the tensile test performed in this study: probe with hydrated tablet was moved downward (STEP A); hydrated tablet was held in contact with the mucus substitute with specified time and force (STEP B); probe was withdrawn at a specified rate and the two materials were separated (STEP C) (modified from Thirawong, et al., 2007).*

The study was carried out at room temperature (25°C) with at least five repeats obtained for each sample.

Data processing was performed by using Texture Expert® software. In particular, the force required to detach the tablet from the mucus substitute (maximum detachment force) was measured as the peak value (F_{max}, [mN]) in the force-time plot, while the work of adhesion (W_{ad}, [mN*mm]) was calculated as the area under the force-distance plot (figure 2.7).

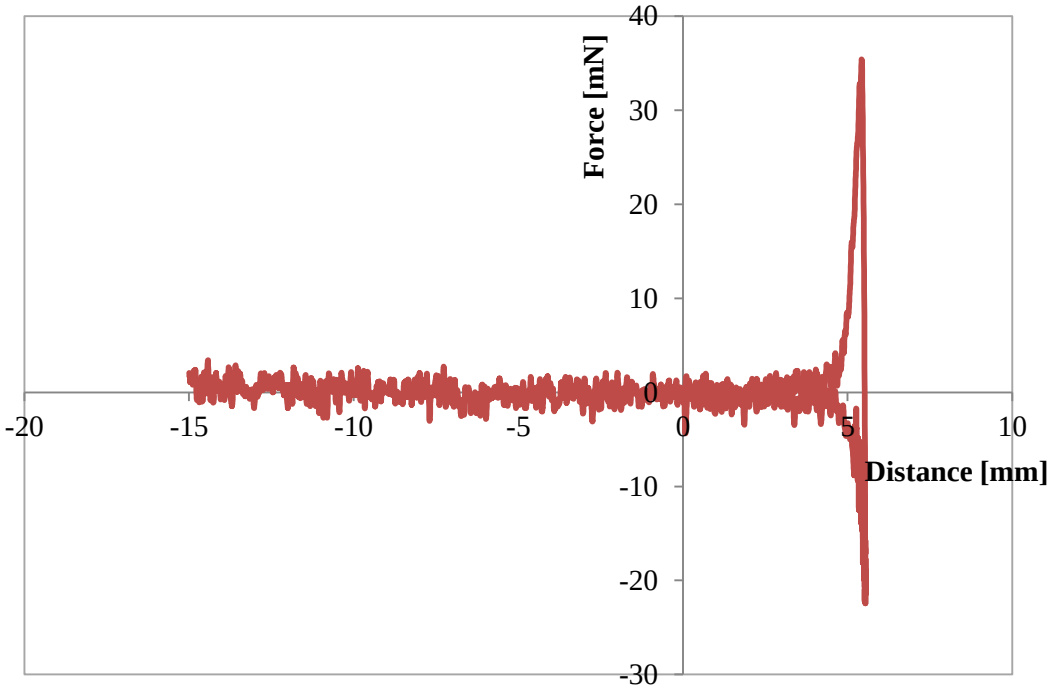


Figure 2.7. *A typical plot of force [mN] versus distance [mm] obtained from the mucoadhesive test using texture analyser and used for the determination of* W_{ad}.

The operating conditions of the procedure used to perform the tensile test are the following:

- tablet pre-hydration time of 5 min;
- mucus substitute consisting of 30% [w/w] aqueous mucin gel settled in a cylindrical cell with a depth of 2 mm and 36 mm diameter;
- probe speed of 0.2 mm*s^{-1};

- contact force between tablet and mucus substitute of 0.1 N;
- contact time between tablet and mucus substitute of 60 sec;
- data acquisition rate, i.e. the rate at which data is stored into the computer memory, equal to 50 points*s^{-1}.

2.4 Results and Discussion

The study started by selecting four ionic natural polymers, having well-known mucoadhesive properties: sodium alginate (SA), xanthan gum (XG), tragacanth gum (TG) and k-carrageenan (KC). Powders were characterized by flowability test in order to assess their suitability for compression.

Results are reported in table 2.5, which shows that all polymers have discrete or good flow properties and therefore they may be compressed without the necessity to add other excipients. However, their compression in pure form was not feasible due to the high speed of the single punch tablet press and the high adhesion of polymers to the punches.

Table 2.5. *Flow properties of polymer powders in terms of Hausner Index, Compressibility Index and Flowability, according to the classification of FUI XII ed (F.U.I., 2008).*

Polymer	Hausner Index (HI) [-]	Compressibility Index (CI) [%]	Flowability
SA	1.24±0.00	19.35±0.00	Discrete
XG	1.16±0.02	13.85±1.49	Good
TG	1.18±0.02	15.00±1.50	Good
KC	1.20±0.02	16.38±1.37	Discrete

For this reason, an excipients blend (Ludipress®, microcrystalline cellulose T1, magnesium stearate and talc) was added to each polymer. The final composition of powder mixtures used to realize tablets is shown in table 2.6.

The high percentage of the mucoadhesive polymer was chosen to better discriminate the differences between polymers.

Table 2.6. *Quali-quantitative composition of the mixtures used to prepare tablets.*

Components	**Amount [%]**
Polymer	60.0
Ludipress®	34.0
Microcrystalline Cellulose T1	4.0
Magnesium Stearate	1.2
Talc	0.8

The resulting mixtures were characterized by flowability test, whose data are reported in table 2.7: the addition of the excipients blend further improves or does not alter the flow properties of polymer powders, with the exception of KC (figure 2.8). In this case flow properties get worse, presumably due to the development of cohesive forces during the mixing.

Table 2.7. *Flow properties of the different mixtures in terms of Hausner Index, Compressibility Index and Flowability, according to the classification of FUI XII ed (F.U.I., 2008).*

Mixture	**Hausner Index (HI) [-]**	**Compressibility Index (CI) [%]**	**Flowability**
SA-Excipients blend	1.19±0.01	16.34±0.88	Discrete
XG-Excipients blend	1.16±0.01	13.93±0.73	Good
TG-Excipients blend	1.15±0.01	12.93±0.76	Good
KC-Excipients blend	1.27±0.02	21.36±1.52	Passable
Excipients blend	1.08±0.03	7.77±2.28	Excellent

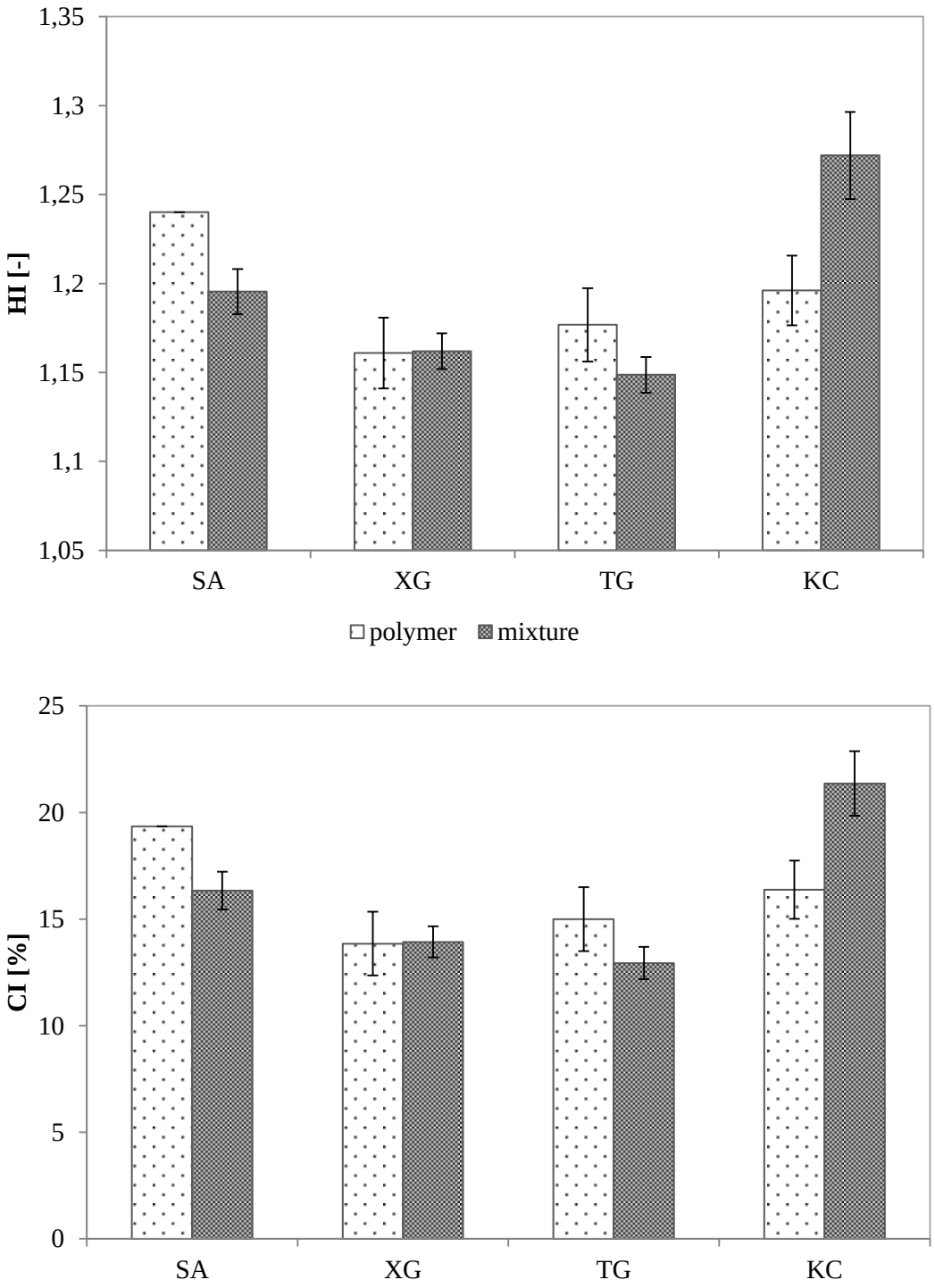
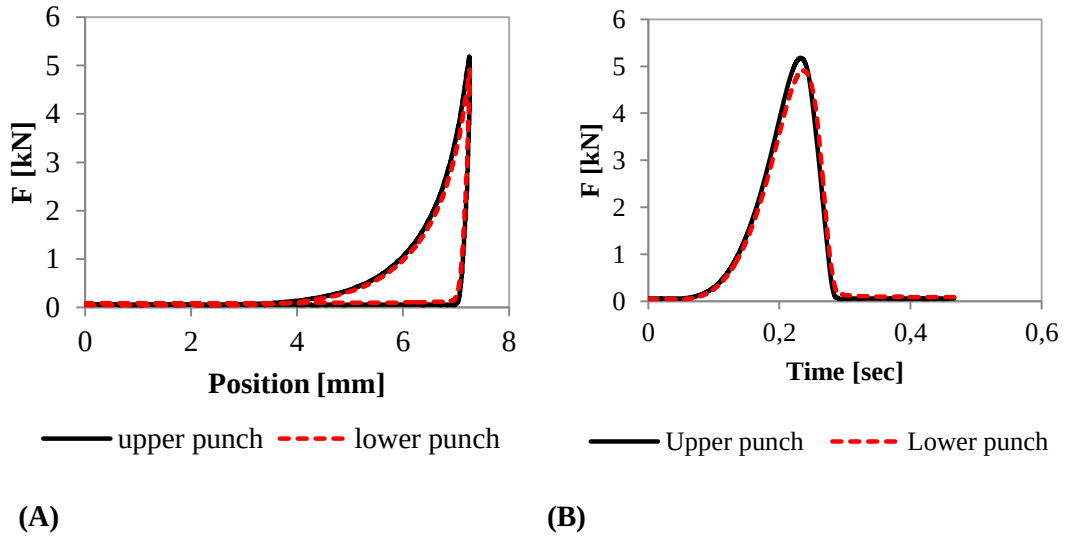


Figure 2.8. *HI [-] and CI [%] values of the polymers in pure form and in mixture with the excipients blend.*

Polymer mixtures were hence compressed using a single punch tablet press with a circular flat punch of 5 mm diameter. The instrument is equipped with sensors for the measurement of forces. Therefore it was possible to record the forces involved in the process, as shown in figure 2.9.



(A) **(B)**

Figure 2.9. *Example of graphs obtained from data recorded during the compression process of the mixture containing SA; graphs report the force [kN] versus the displacement of the punches [mm] (A) and the force [kN] versus time [sec] (B).*

For each mixture it is possible to obtain the value of the total work of the upper punch (Wtot [J]) necessary to compress the mass, which can be considered as the sum of three different contributions: work dissipated in the elastic return of the compressed mass (Wel [J]), work dissipated in frictional forces (Wf [J]) and net work of compression (Wcomp [J]). Transforming the various contributions as percentages of the total work (100%) it is possible to make a comparison of the values obtained with the four mixtures. The resulting Wf and Wcomp values are reported in figures 2.10 and 2.11. In particular, the higher Wf value and the lower Wcomp value obtained for mixture containing KC, confirm its poor attitude to be compressed.

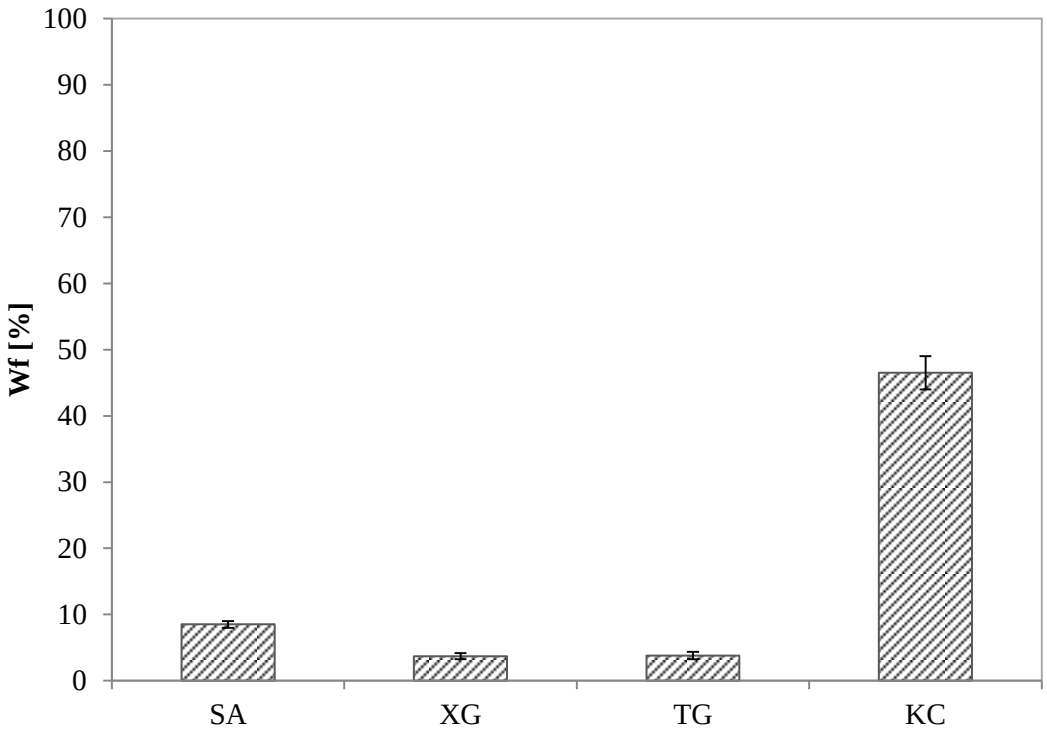


Figure 2.10. *Wf values [%] of the four mixtures.*

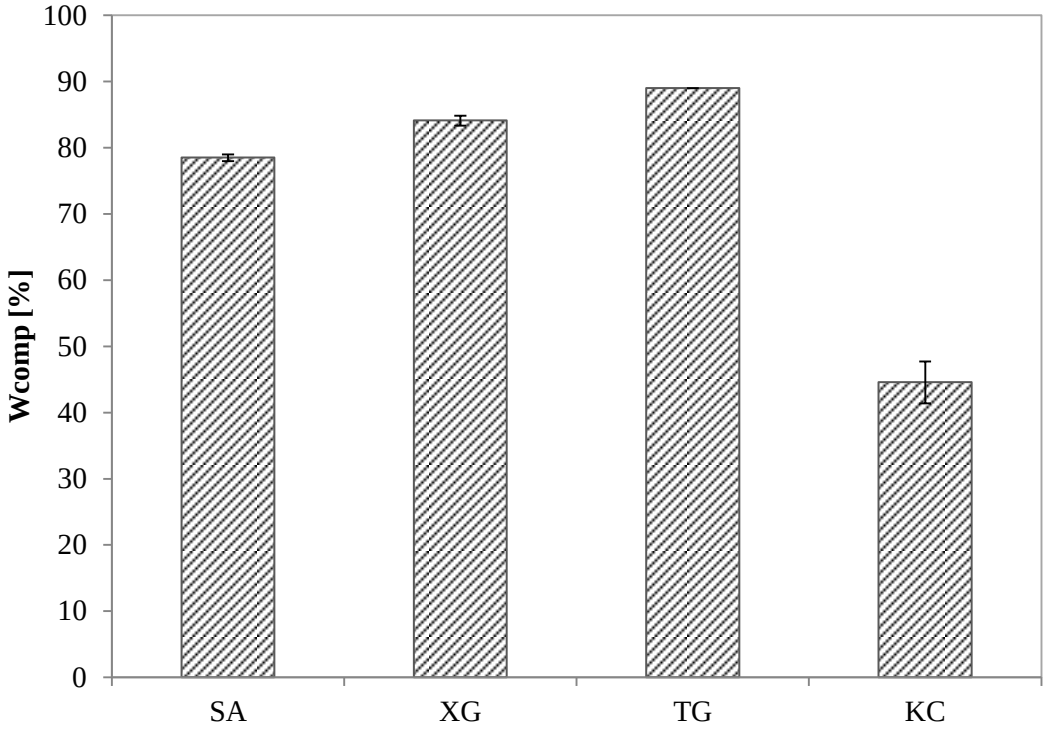


Figure 2.11. *Wcomp values [%] of the four mixtures.*

Tablets were subsequently subjected to technological characterization in order to evaluate tablets crushing strength, whose data are reported in figure 2.12.

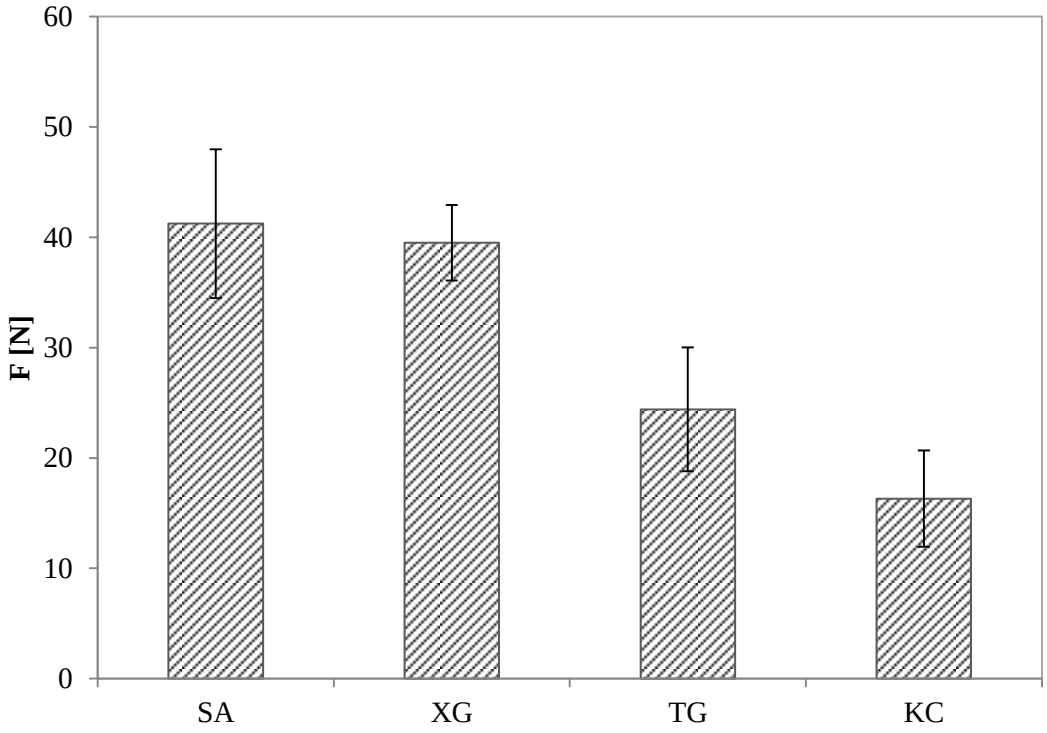


Figure 2.12. *Crushing strength (F [N]) of the different tablets.*

To compare crushing strength values of the different tablets, it must be considered that tablets containing SA, XG and TG present similar weight of 134±4 mg, while the weight of KC tablets is equal to 78±2 mg. Thus only a comparison between SA, XG and TG tablets may be made and it reveals that tablets containing SA and XG are the most resistant.

Nevertheless, all the tablets prove to be resistant enough to possible subsequent manipulations and to the destructive forces present in the gastrointestinal tract (Kamba, et al., 2002). Indeed, as reported by Kamba M. et al. (Kamba, et al., 2002), the maximum mechanical destructive force of the human stomach is 1.9 N while that of the small intestine is 1.2 N.

The phenomenon of mucoadhesion is closely related to the extent and rate of polymer hydration and swelling in aqueous medium (Thirawong, et al., 2007). As a result, in order to obtain the maximum mucoadhesive force it is necessary to reach the optimum values of dosage form hydration and swelling, which ensure maximum exposure of the docking sites for the bond with mucin and chains interpenetration. However, an excessive hydration and swelling can lead to a drastic drop in the adhesive strength and cracking of the outer cap of the tablet with unwanted drug loss (Baloğlu, et al., 2003).

In order to investigate tablets behavior in aqueous medium, two characterizations were developed: a gravimetric test to assess the water uptake capacity of the dosage form and an image analysis test to assess its swelling extent or swelling index. Water was chosen as hydration medium since it represents the basic medium for the development of a new method and does not involve other possible influencing factors.

Results obtained from the two tests are reported in figures 2.13 and 2.14.

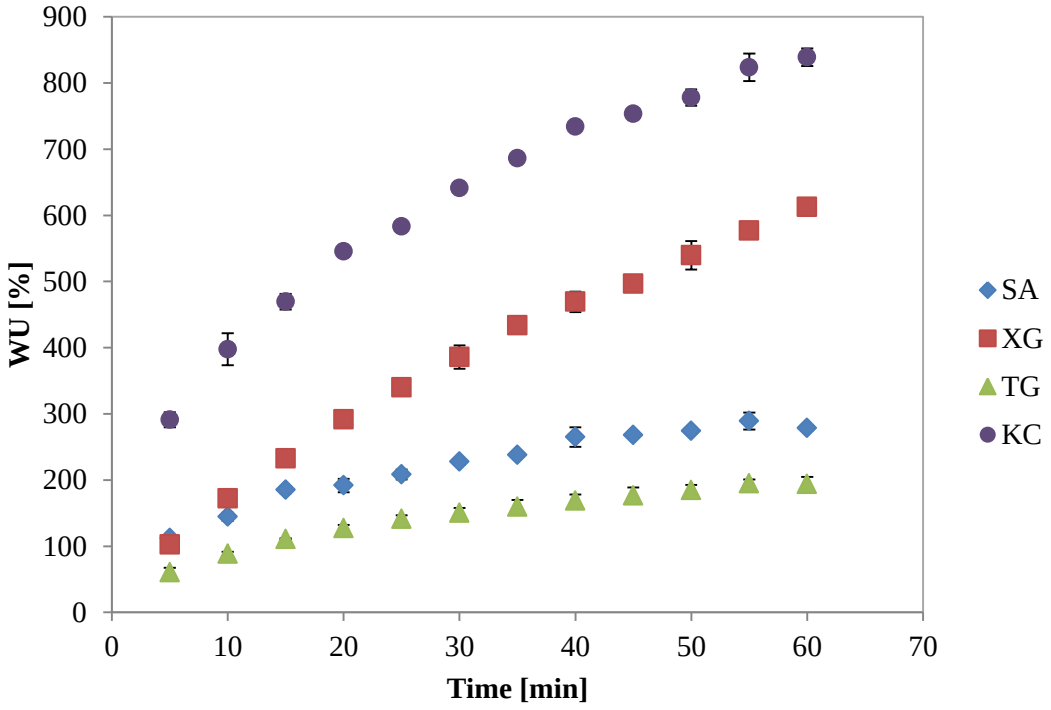


Figure 2.13. *Water Uptake (WU [%]) values of the different tablets in water.*

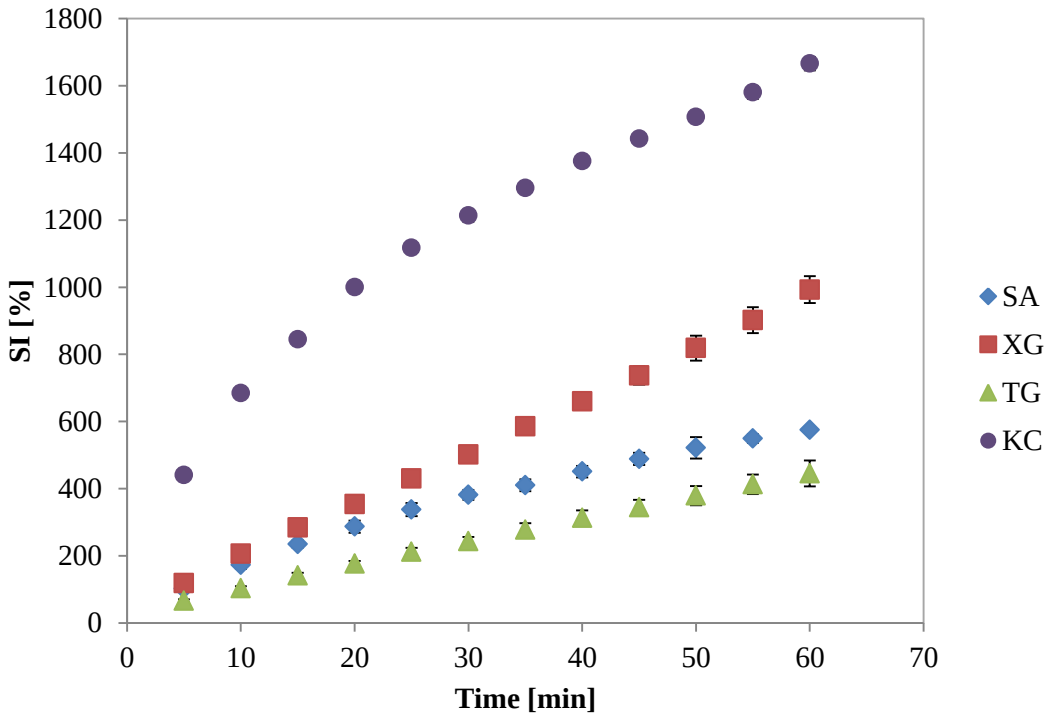


Figure 2.14. *Swelling Index (SI [%]) values of the different tablets in water.*

The graphs show an analogy between the profiles of water uptake and swelling index, for each polymer.

In particular the figures highlight that:

- tablets containing KC and XG are able to swell and absorb water to a greater extent than the other tablets;
- comparing tablets containing XG with those containing KC, the kinetics is initially faster for KC: weight and volume significantly increase at 5 minutes, then it slows reaching a plateau; on the other hand, the kinetics of tablets containing XG is quite linear for the entire test;
- tablets containing TG absorb a lower amount of water than the others. This may be due to the chemical structure of TG, composed by a water soluble component and a water insoluble one. The presence of the water insoluble portion reduces the ability of the polymer to hydrate.

To compare the swelling index with the water uptake values the graph shown in figure 2.15 was constructed.

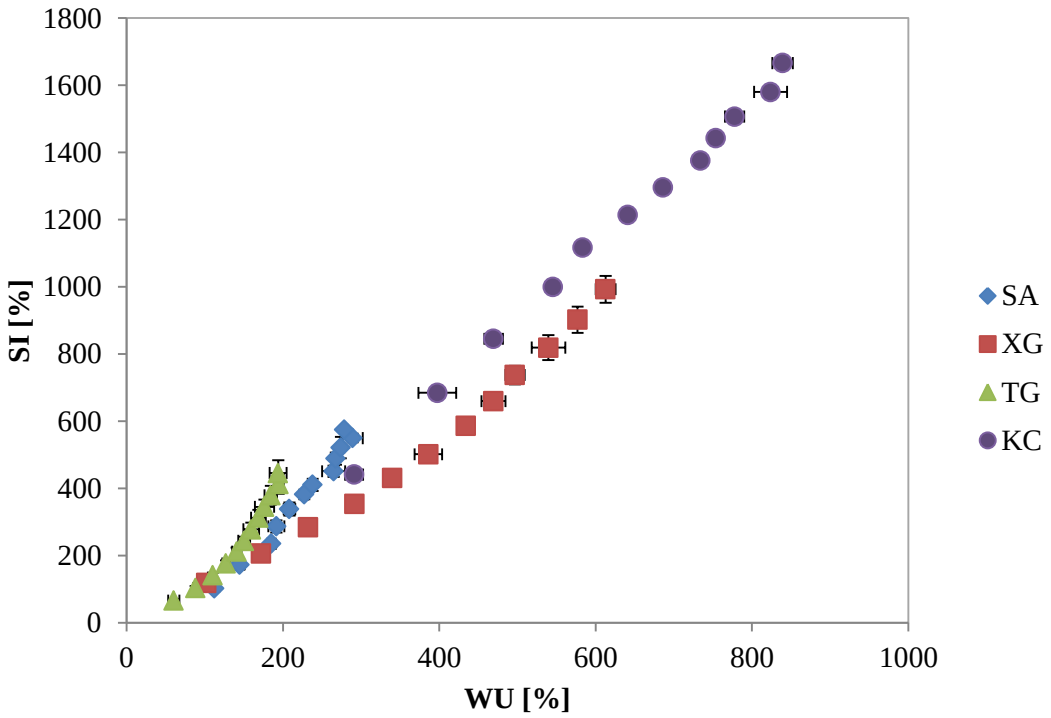


Figure 2.15. *WU % and SI % values at the same time t_i of the four formulations.*

The graph highlights that there is a relationship between WU and SI: an increase in weight corresponds to an increase in volume. Moreover, it shows that tablets containing TG, even if they reach a final degree of swelling lower than tablets containing XG and KC, they swell faster. This can be explained by the chemical structure of tragacanth gum.

In figure 2.16 is represented a graph of the water uptake/swelling index ratio versus time which highlights the relationship between the variation in weight and volume of the four tablets once placed in aqueous medium. Indeed swelling may be due not only to the absorption of water molecules but also to the expansion of the polymer chains, which is certainly favored by hydration but not necessarily related to it in a linear manner.

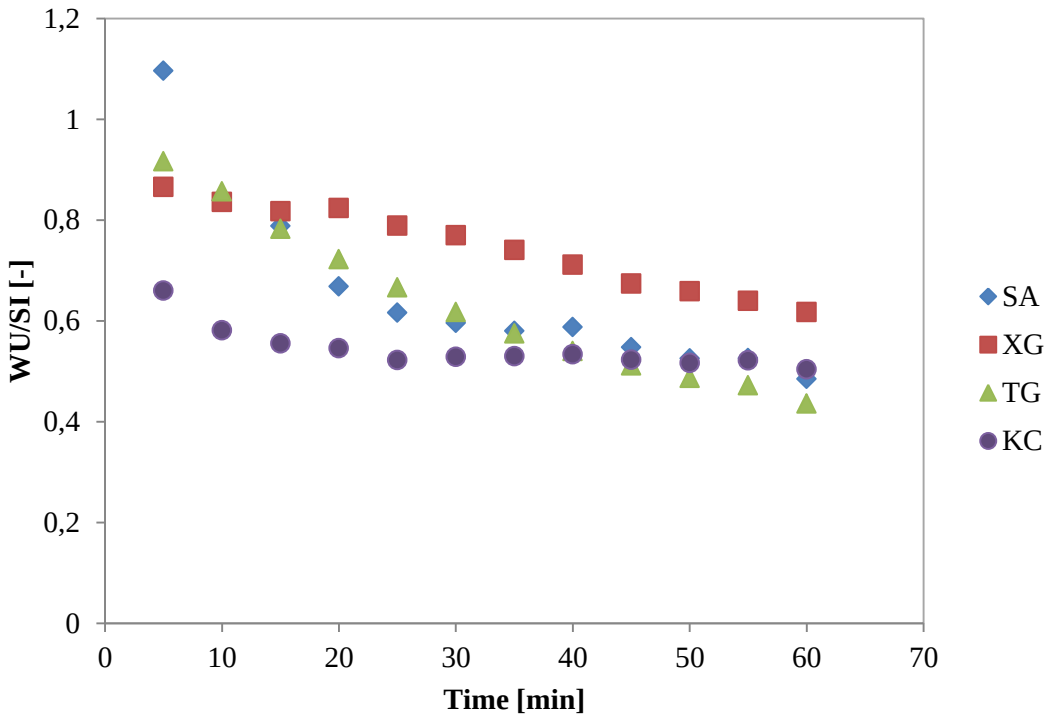


Figure 2.16. *Values of WU/SI ratio versus time for the four formulations.*

For tablets containing TG and SA, the time seems to play a very important role in determining the degree of swelling and WU since the ratio WU/SI decreases markedly over time. This means that SI increases more than WU, and hence the polymer chains tend to expand absorbing a relatively small amount of water.

Subsequently, the mucoadhesive properties of the tablets were evaluated by tensile test with Texture Analyser® using water as hydration medium.

Several factors could influence the results of the test, such as experimental variables (time of pre-hydration, force applied, etc.) or the type of mucous substitute. The use of biological substrates derived from animals can lead to poorly reproducible data due to the inherent variability of tissues of different animals. Consequently, for a simple screening of different mucoadhesive capacity of various polymers may be more appropriate to use a standardized and easily available substrate, which also avoids animals sacrifice. This substrate is represented by mucin which can be employed in form of discs or gel (Khutoryanskiy, 2011; Tamburic & Craig, 1997; Thirawong, et al., 2007).

To perform the test, a layer of 30% [w/w] aqueous mucin gel, loaded on a Perspex® cylindrical cell, was used as biological substrate.

The other experimental variables include: tablet pre-hydration time in aqueous medium, pre-test, test and post-test speed of the probe, the contact force of the tablet with the mucous substrate and their contact time.

The values of these parameters were selected on the basis of data reported in literature (Thirawong, et al., 2007) and some preliminary analysis. To ensure adhesion of the pharmaceutical dosage form, the contact force was set at 0.1 N. This value is lower than the mechanical force resulting from peristalsis (Kamba, et al., 2002) and, at the same time, it guarantees a good sensitivity of the instrument. Regarding the contact time, literature reports that an increase of this parameter generally leads to an increase in both force and work necessaries to produce the detachment of the mucoadhesive system from the substrate. However, some studies have shown that an increase in contact time higher than 60 seconds not always entails a further increase of the force and work of adhesion. Hence, a contact time of 60 seconds was adopted for the analysis. The probe pre-test, test and post-test speed have been fixed at 0.2 $mm*s^{-1}$. The last very important parameter is the pre-hydration time of the tablet on which depends the degree of hydration of the polymer (Thirawong, et al., 2007). After some preliminary analysis, a pre-hydration time of 5 minutes was adopted because it allowed to better discriminate the different behavior of the polymers. The experimental conditions are summarized in table 2.8.

Table 2.8. *Operating conditions used for the development of the mucoadhesive test.*

PARAMETER	VALUE
CONTACT FORCE	0.1 N
CONTACT TIME	60 sec
PROBE SPEED	0.2 $mm*s^{-1}$
PRE-HYDRATION TIME	5 min

The mucoadhesive performance of the dosage form was evaluated considering the maximum detachment force (F_{max}) and the work of adhesion (area under the curve of

detachment, W_{ad}) (Khutoryanskiy, 2011). Figure 2.17 shows the graphs of F_{max} and W_{ad} for the four formulations, measured using two different batches of mucin.

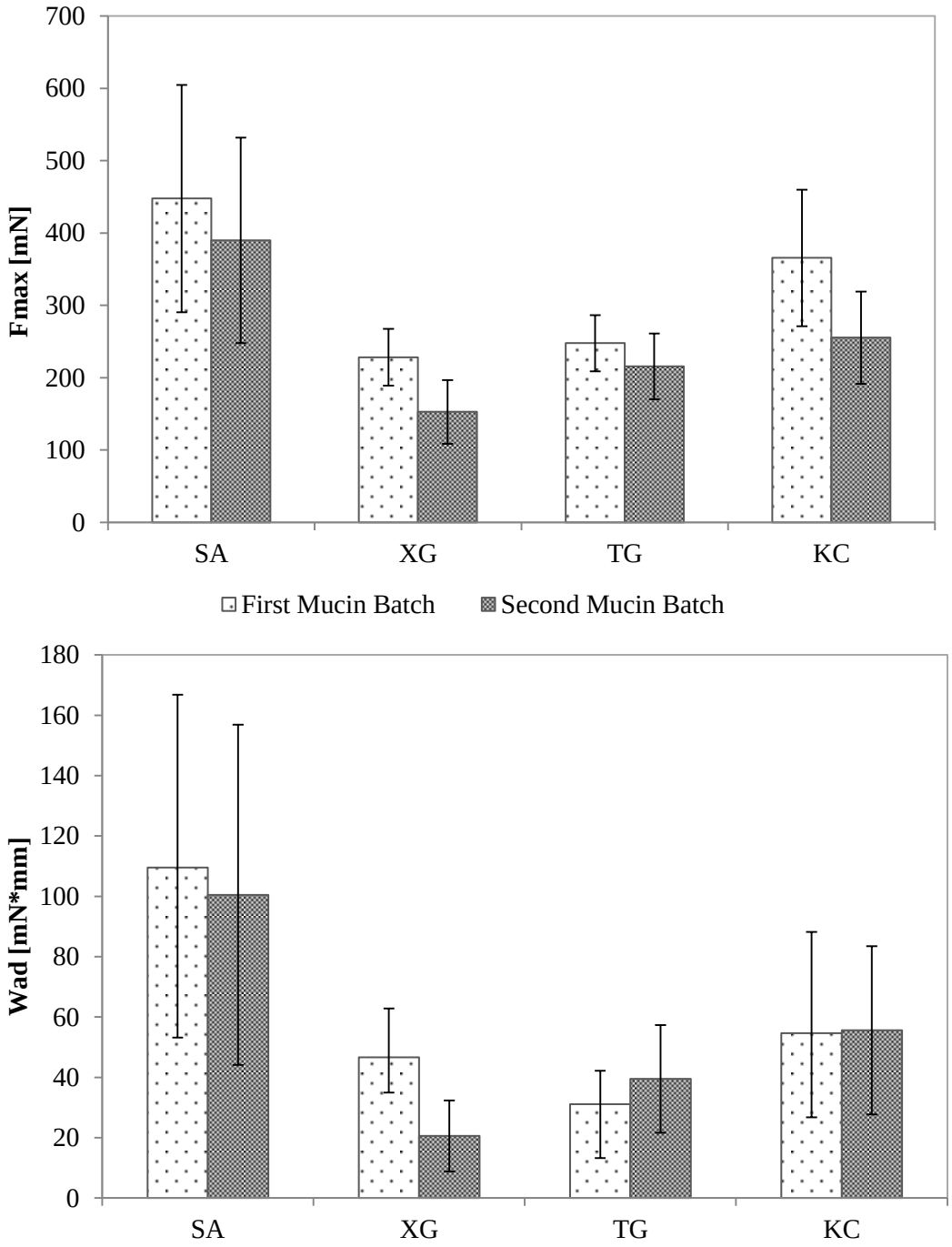


Figure 2.17. *Values of F_{max} (top) and W_{ad} (bottom) of the four systems, considering two batches of mucin.*

The change of the batch of mucin led to a decrease of the absolute value of F_{max} and W_{ad} for all four systems, except the work of adhesion of the formulation containing TG. Nevertheless, the classification of the four polymers according to their mucoadhesive ability seems not to change with the second mucin batch. SA seems to exhibit the best mucoadhesive properties despite showing the greatest variability. SA is followed by KC, and hence TG and XG, which prove to be quite similar.
Another important factor affecting the mucoadhesive properties of a dosage form was determined: the molecular weight of the polymers. It was measured by means of a viscosimetric method and the subsequent application of the Mark-Houwink-Sakurada equation (Brandrup , et al., 1999). Data are reported in table 2.9.

Table 2.9. *Viscosity Average Molecular Weight (M [kDa]) of the polymers; TG molecular weight was extracted from literature data (Belitz, et al., 2009).*

	SA	**XG**	**TG**	**KC**
M [kDa]	132	1341	840	407

Finally, a comparison of the mucoadhesive properties of the tablets with the other mucoadhesion influencing factors (tablets water uptake and swelling index and polymers molecular weight), was made. In table 2.10 are reported the values of the average molecular weight (M [kDa]), the water uptake and swelling index at 5 minutes (pre-hydration time) and 60 minutes and the maximum detachment force (F_{max} [mN]) and work of adhesion (W_{ad} [mN*mm]) measured using the second mucin batch.

Table 2.10. *Comparison of the mucoadhesive properties with the influencing factors.*

Polymer	**M [kDa]**	**WU_{5min} [%]**	**SI_{5min} [%]**	**WU_{60min} [%]**	**SI_{60min} [%]**	**F_{max} [mN]**	**W_{ad} [mN*mm]**
SA	132	112	102	278	575	390	100
XG	1341	102	118	613	993	153	20
TG	840	60	66	194	445	215	39
KC	407	291	441	839	1666	255	56

Results suggest the presence of an inverse proportionality between polymer molecular weight and mucoadhesive properties of the dosage form: the lower the average molecular weight, the higher the mucoadhesive properties (figure 2.18).

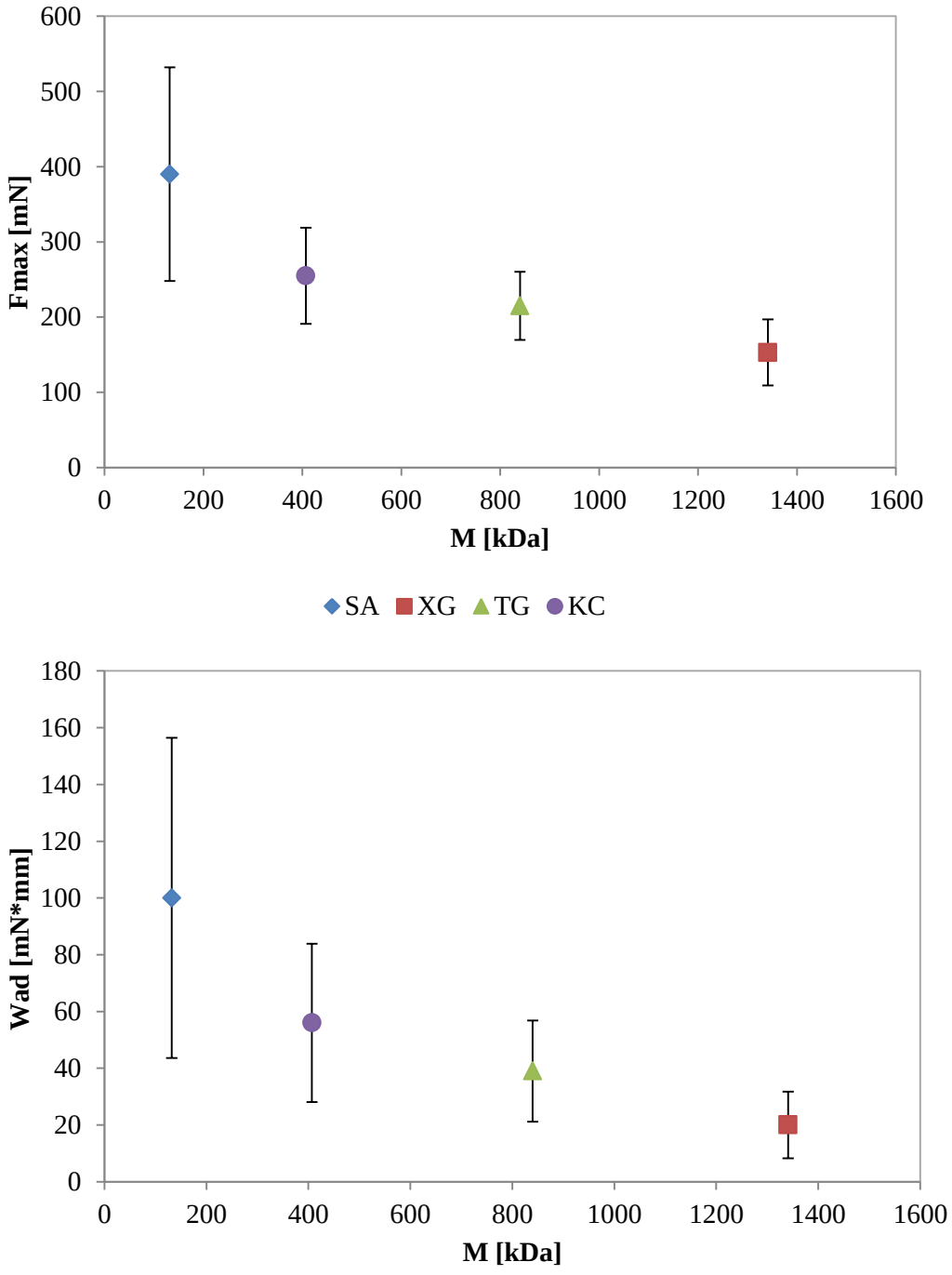


Figure 2.18. *Relationship between polymer average molecular weight (M [kDa]) and F_{max} [mN] (graph on the top) or W_{ad} [mN*mm] (graph on the bottom) for the four formulations.*

For the other parameters, no match was identified.

Nevertheless, there is a correspondence between the results of WU and SI obtained at 5 and 60 minutes. This evidence justifies the use of the values of WU and SI at 60 minutes in the comparison between WU or SI and mucoadhesive properties.

2.6 Conclusions

In this study different methods for the screening of mucoadhesive polymers were developed:

- a tensile test to detect the mucoadhesive properties of the tablet;
- a gravimetric method to study the ability of the polymer to adsorb water;
- an image analysis to detect the ability of the polymer to swell;
- a viscosimetric method to determine the polymer molecular weight.

The screening suggests that SA owns the best mucoadhesive properties. Moreover results highlight that the higher the molecular weight of the polymer, the lower the mucoadhesive properties of the dosage form, while the degree of swelling and water uptake of the dosage form seems to be not correlated to mucoadhesive properties.

Chapter 3

Formulation of mucoadhesive tablets containing a model drug

3.1 Introduction

Oral delivery is the preferred route for drug administration because it is natural, not invasive and painless than other traditional routes, first of all intravenous and intramuscular injection, which also requires specialized personnel for the administration.

Moreover, the oral route has a large mucosal surface available for drug absorption and then for its access to the systemic circulation.

This feature can be exploited by developing mucoadhesive oral formulations, which, adhering to the mucosal surface of the gastrointestinal tract, prolong and improve the contact between the active molecule and the mucosal surface and allow to realize a drug extended-release. This proves to be very advantageous in the case for example of drugs characterized by a narrow absorption window in the intestine, because in this way it is possible to prolong the residence time at or before this absorption window.

However, a lot of drugs are inactivated in the gastro-intestinal tract, due to e.g. the stomach pH, the presence of proteolytic or peptidolytic enzymes, and the hepatic first-pass effect. From this standpoint, it would be interesting to target a drug directly to the intestine, allowing it to circumvent most of the previous drawbacks (Duchêne & Ponchel, 1997).

3.1.1 Structure and function of the gastrointestinal tract

The digestive system include the gastrointestinal (GI) tract or alimentary canal, which in adults measure 10-12 meters, and the accessory organs of digestion including the salivary glands, liver, gallbladder, and exocrine pancreas. In particular, the alimentary canal is constituted by a set of hollow organs in communication between them that begin with the oral cavity, which, through the isthmus of the fauces, is followed by the pharynx, esophagus, stomach and intestines. The latter is divided into two portions: small intestine, formed by duodenum, jejunum and ileum, and large intestine, consisting of cecum, colon and rectum (Reed & Wickham, 2009; Celotti, 2002; Pasqualino & Panattoni, 2002).

The digestive system presents the following functions: to ingest and digest food, absorb essential nutrients (carbohydrates, proteins, fats, minerals and vitamins), and eliminate waste. Digestion occurs by mechanical and chemical processes. Mechanical digestion includes chewing, swallowing, and peristalsis (the method by which food moves through the entire gut), and defecation. Chemical digestion is the enzymatic breakdown of food in the mouth, stomach, and small intestine. When the partially digested food and fluid enter the small intestine, biochemical and enzymes secreted by the liver and exocrine pancreas break it down into absorbable monosaccharides, amino acids, and fatty acids. These nutrients pass through the small intestine wall into blood and lymphatic vessels and are transported to the liver for storage or further processing (Reed & Wickham, 2009).

The wall of the gastrointestinal tract is made by four distinct concentric layers: the mucosa, the submucosa, the muscularis externa, and either the adventitia or the serosa (figure 3.1) (Reed & Wickham, 2009).

The mucosa, the innermost layer of the gut wall, lines the entire GI tract and consists of epithelium, lamina propria, and muscularis mucosa. The mucosal epithelium is differentiated along the GI tract; tissue specialization correlates with the regional function of the tract. At the upper and lower ends of the GI tract (the mouth, esophagus, and anal canal) the mucosal epithelium is protective and composed of stratified squamous epithelial cells. On the other hand, the mucosal epithelium in the stomach, small intestine, and colon are composed of simple columnar or glandular

epithelial cells. The cells in these regions secrete mucous, enzymes, and other biochemicals that either protect the mucosa or aid the digestion (Reed & Wickham, 2009; Pasqualino & Panattoni, 2002).

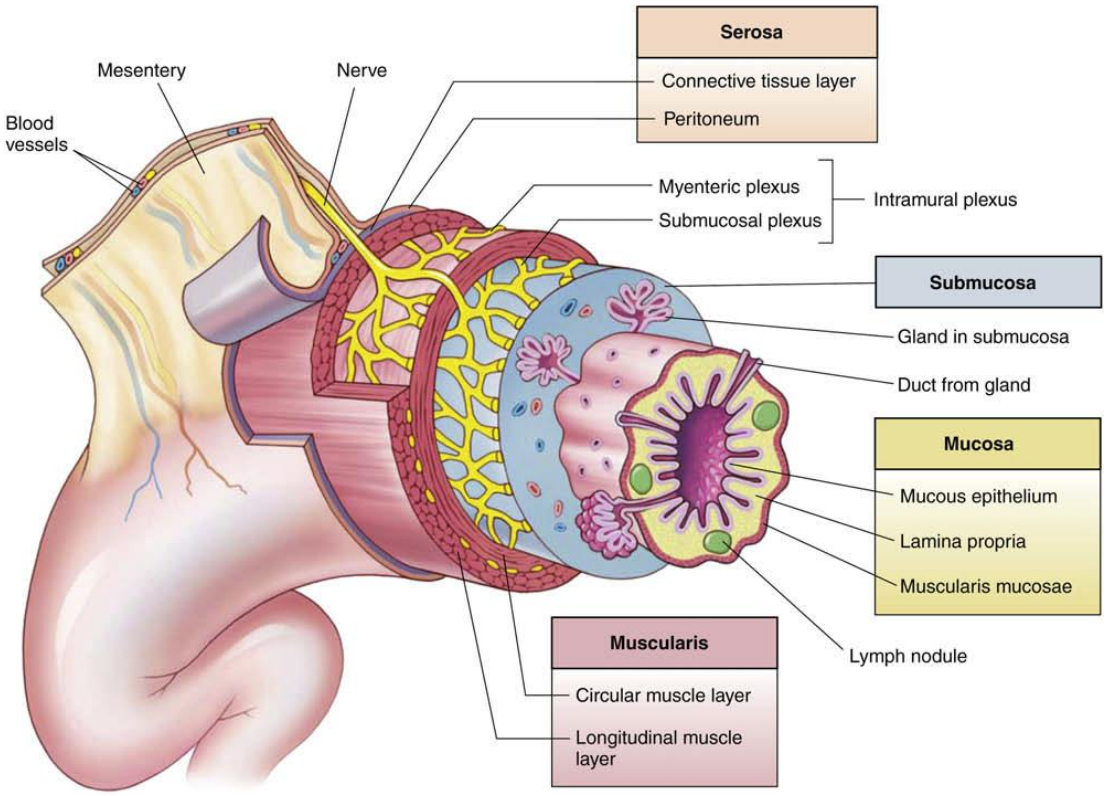


Figure 3.1. *Segment of the GI tract illustrating the 4 layers of the GI wall (Reed & Wickham, 2009).*

The epithelial cells are highly dynamic, with a quick turnover (24-72 hours) which ensures an effective restoration of the mucosa integrity, and have functions of absorption and secretion (mainly mucus); in particular, the secretion of mucus ensures the flow of luminal contents and the protection from abrasive agents and pathogens. Such protection is also supported by the presence of lymph nodes (Peyer's patches) and an abundant population of immune cells habiting the mucosa of the GI tract. Throughout the small intestine, at the level of the glandular crypts, Paneth cells are also involved in the defense mechanisms of the mucous membrane as they produce antibacterial proteins (Celotti, 2002).

At the gastric level, the epithelium is lined by mucous cells that produce mucus and bicarbonate to avoid destruction by hydrochloric acid. The maintenance of a layer of bicarbonate and mucus is essential to protect the gastric wall by the action of the proteolytic gastric juice. The activity of mucous cells is controlled by cholinergic and mechanical stimulations and by the presence of prostaglandin E, which is produced by different type of cells. Prostaglandins also act by increasing the mucosal blood flow (which is essential for the continuous production of mucus), by maintaining the integrity of intercellular junctions and by stimulating the turnover of epithelial cells in response to damage to the mucosa. Several factors inhibit the mucus formation, such as the intake of NSAIDs which block the production of prostaglandins (Celotti, 2002).

3.1.2 Aim

In this Chapter a mucoadhesive formulation, containing a model drug (sodium butyrate) with the intestinal tract as target, has been developed.

The research started by expanding the range of polymer to be screened, considering also two cellulose derivatives with different ionic character: sodium carboxymethylcellulose (NaCMC), anionic, while hydroxyethylcellulose (HEC), nonionic. The properties of the new polymers were compared to those of the polymers previously studied, in order to identify the polymer with the best mucoadhesive properties. In this phase the tensile test was optimized in order to produce more robust results and get closer to the intestinal physiological conditions by changing the hydration medium.

The polymers with the best mucoadhesive properties were used to prepare tablets containing sodium butyrate. Finally, the influence of the amount of polymer on the mucoadhesive properties and the drug release was studied.

3.2 Materials

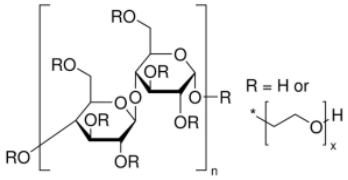
The following were used: sodium alginate E401 (Satialgine S1100), xanthan gum Ph. Eur.-USP, tragacanth gum powder NF18, talc PHARMA USP Ph.Eur., magnesium stearate FU-Ph.Eur., microcrystalline cellulose T1 Ph.Eur., calcium phosphate

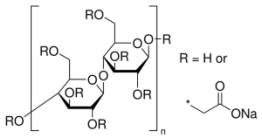
tribasic E341, mannitol for direct compression, potassium phosphate monobasic, sodium hydroxide, sodium chloride, all eleven supplied by A.C.E.F. S.p.A. (Italy); Ludipress® and Gelcarin GP 911NF K-Carrageenan purchased from BASF The Chemical Company (Germany) and IMCD UK LTD (United Kingdom), respectively; mucin (from porcine stomach, type II), sodium butyrate 98% and acetonitrile supplied by Sigma-Aldrich (USA); hydroxyethylcellulose (Tylose® H 4000 G4 PHA) and sodium carboxymethylcellulose (Blanose® cellulose gum 7H3SF, degree of substitution 0.80-0.95) supplied by Clariant GmbH (Germany) and Hercules Inc. (USA), respectively; phosphoric acid and methanol HPLC Gradient Grade purchased from Acros Organics (Belgium) and J.T. Baker® (Netherlands), respectively.

In all preparations of solutions and buffers, deionized water was used.

Characteristics, properties and applications of the new mucoadhesive polymers are described in detail in table 3.1.

Table 3.1. *Properties and applications of the new polymers used in the study.*

Polymers	Properties	Applications
Hydroxyethylcellulose (Tylose® H 4000 G4 PHA) Partially substituted poly(hydroxyethyl) ether of cellulose.  (Rowe, et al., 2006)	• Nonionic, water-soluble and hygroscopic polymer, available in several grades that vary in viscosity and degree of substitution (2-20000 mPa*s for a 2% [w/v] aqueous solution). • A 1% [w/v] aqueous solution owns a pH of 5.5-8.5. • Practically insoluble in most organic solvents.	• It is used as a thickening agent in ophthalmic and topical formulations, as a binder and film-coating agent for tablets. • Hydroxyethylcellulose hydrogels may also be used in various delivery systems.
Sodium Carboxymethylcellulose (Blanose® Cellulose Gum 7H3SF) Sodium salt of a polycarboxymethyl ether of cellulose.	• Hygroscopic polymer, practically insoluble in acetone, ethanol (95%), ether, and toluene; the aqueous solubility varies with the degree of substitution (DS). • Molecular weight ranges from 90-700 kDa. • DS of 0.80-0.95; viscosity of a	• It is widely used in oral and topical pharmaceutical formulations, mainly for its viscosity-increasing properties. • It may also be used as a tablet binder and disintegrant, and to stabilize emulsions. • Its mucoadhesive properties are used in various pharmaceutical

 (Rowe, et al., 2006)	1% aqueous solution of 1000-2800 mPa*s at 25°C (Brookfield LVF, spindle n°3, 30 rpm). • High concentrations, usually 3-6%, of the medium-viscosity grade are used to produce gels.	formulations to localize and modify the release kinetics of active principles applied to mucous membranes. • Moreover, it can be used to prevent post-surgical tissue adhesions, for bone repair and to realize dermatological patches.

3.2.1 Sodium Butyrate

Sodium butyrate (SB) was chosen as a model drug since it acts locally in the gastro-intestinal tract and it is therefore suitable for the administration through this type of pharmaceutical dosage form.

Sodium butyrate is the sodium salt of butyric acid ($CH_3CH_2CH_2COO^-$ Na^+), a short-chain fatty acid characterized by a solubility in water of 100 mg/mL, measured at 20°C. It is present in nature as a component of the milk and its derivatives fat fractions. In humans it is a metabolite of intestinal bacteria, an important energy source for the intestinal epithelial cells and it plays a key role in the homeostasis of the gastrointestinal tract. The largest resource of sodium butyrate in the human colon derives from carbohydrates introduced into the body with food.

The functions of the butyric acid in the intestine are:

- stimulation of the turnover and the physiological maturity of colonocytes and key role in maintaining the mucosa integrity and in repairing the intestinal lesions;
- stimulation of the reabsorption of water and sodium in the colon (useful in presence of diarrhea of infectious origin or induced by antibiotics);
- aid in lowering the intestinal pH and hence creation of an unfavorable environment for the development of pathogenic bacteria;
- stimulation of the repairing and healing processes of the rectal mucosa, thus representing a potential effective approach in the prevention of acute and chronic damages resulting from radiotherapy.

Consequently, products containing sodium butyrate are indicated in the treatment of some disorders of the gastrointestinal tract as the following:

- spastic, infectious or antibiotic-associated colitis;
- irritable bowel syndrome, diarrhea, diverticulitis;
- chronic inflammatory diseases, such as Crohn's disease (Cummings, 1981).

3.3 Methods

3.3.1 Determination of intrinsic viscosity and Viscosity Average Molecular Weight of polymers

Viscosity average molecular weights of NaCMC and HEC were measured using the method described in Section 2.3.1.

The operating conditions, i.e. the solvent used and K and α values, for the new polymers are reported in table 3.2.

Table 3.2. *Operating conditions used for HEC and NaCMC.*

Polymer	Solvent	K ($x10^3$) [$dL*g^{-1}$]	α [-]	References
HEC	Water	0.0953	0.870	(Brandrup , et al., 1999)
NaCMC	NaOH 0.5 M	0.5370	0.730	(Eremeeva & Bykova, 1998)

3.3.2 Powder flowability measures

Flow properties of powders (polymers in pure form and in mixture) were evaluated by means of the method described in Section 2.3.2.

3.3.3 Preparation of mucoadhesive tablets

Tablets were prepared by direct compression of the powders, using a single punch tablet press (COSALT type, Officina CO.STA. S.r.l., Italy) fitted with a flat-faced circular punch (5 mm diameter). The weight of the tablets ranges from 78 mg to 136 mg and the thickness ranges from 3 to 4 mm.

The composition of the placebo tablets is reported in table 3.3.

Table 3.3. *Quali-quantitative composition of the mixtures used to prepare the placebo tablets.*

Components	Amount [%]
Polymer	60.0
Ludipress®	34.0
Microcrystalline Cellulose T1	4.0
Magnesium Stearate	1.2
Talc	0.8

The formulation of tablets containing sodium butyrate consists in 30, 45, 60% [w/w] of polymer (SA or NaCMC), 20% [w/w] of sodium butyrate, 15% [w/w] of an excipients blend (table 2.4, Section 2.3.3); the formulation was completed with the addition of varying amounts of a water-soluble excipient (mannitol, MA) or a water-insoluble excipient (calcium phosphate, CP).

3.3.4 Technological characterization of tablets

Tablets were characterized by uniformity of mass test and tablet crushing strength determination.

The evaluation of the uniformity of mass of single-dose preparations was performed according to F.U.I XII ed (F.U.I., 2008): weigh individually 20 units taken at random, and determine the average mass. Not more than 2 of the individual masses deviate from the average mass by more than the percentage deviation shown in table 3.4 and none deviates by more than twice that percentage.

Table 3.4. *Values of the uniformity of mass of single-dose preparations assay for tablets* (F.U.I., 2008).

Dosage form	Average mass	Percentage deviation
Tablets (uncoated and film-coated)	80 mg or less	10
	More than 80 mg and less than 250 mg	7.5
	250 mg or more	5

The evaluation of tablets breaking force or crushing strength was carried out by means of the same method reported in Section 2.3.4.

3.3.5 Evaluation of tablets behavior in aqueous medium

The evaluation of tablets behavior in aqueous medium was performed by measuring the water uptake and swelling of tablets with the methods described in Section 2.3.5. Subsequently, these methods were optimized by changing the aqueous medium moving from deionized water to phosphate buffer pH 6.8 (according to F.U.I., 2008), in order to simulate the intestinal conditions.

Moreover, for placebo tablets another parameter was measured: tablets wettability, which represents a necessary condition for mucoadhesion to occur. This parameter was evaluated by means of the determination of solid-liquid contact angle.

- *Wettability and Contact Angle*

The wettability assessment of tablets was based on the determination of the solid-liquid contact angle (Lazghab, et al., 2005).

The wettability of a solid may be defined as the tendency more or less marked of a solid to be wetted by a liquid; hence, it can be expressed as a function of the contact angle arising between solid and liquid after deposition of a liquid drop on a solid surface, such as the base of a tablet: the higher the affinity between solid and liquid, the smaller the contact angle between them (Colombo, et al., 2004). In particular, in the case of angles between 0° and 90°, the solid is readily wettable by the liquid, while in the case of angles between 90° and 180° is hardly wettable (Colombo, et al., 2004).

The measure of the contact angle between tablet and water was realized using an image analysis called "*drop shape analysis*": the contact angle (θ) was obtained from the image of a sessile drop and it corresponds to the angle arising from the intersection point (3-phase solid-liquid-vapor contact point) between the drop base line and the drop shape line (figure 3.2).

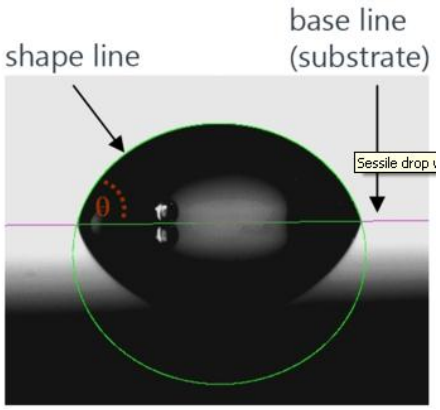


Figure 3.2. *Example of image of a sessile drop (the drop profile fitting [Tangent-1] is highlighted in green while the base line of the drop on the solid surface is represented in pink).*

For the analysis, the Drop Shape Analyser - DSA 30S (KRŰSS GmbH, Germany) was used, as represented in figure 3.3.

Figure 3.3. *DSA 30S, the instrument used for contact angle measures.*

The instrument is equipped with a dosing system and a sample holder placed between a halogen lamp and a camera. Using the dosing syringe a drop of water of 5 μL was created and after deposited on the flat portion of the tablet (base). The camera recorded a video of the whole process and then the processing of the individual frames was performed by means of the software DSA4 2.0.: once the operator defines the baseline, the software recognizes and outlines the drop profile which is subsequently fitted using a selected mathematical model. In this study a geometrical asymmetrical model (ellipse method "Tangent-1") was selected. This method

approximates the drop shape to an ellipse, as shown in figure 3.2. The processing of the video frames was limited to the first 3.5 seconds from the deposition of the droplet on the tablet. For each frame the software measured both left and right contact angles and calculated the average value of the two. Results represent the mean contact angle values obtained analyzing six placebo tablets for type of polymer.

3.3.6 Optimization of the tensile test

The assessment of mucoadhesive properties is fundamental for the production of novel drug delivery systems (Khutoryanskiy, 2011). Although many methods have been developed for studying mucoadhesion, pharmacopoeial methods and standard apparatus are not available so far; consequently, an inevitable lack of uniformity between test methods has arisen (Shaikh, et al., 2011; Woertz, et al., 2013). Therefore the development of at least robust and reproducible detection methods must assume a central role in this kind of research.

To pursue this goal we decided to optimize the tensile test in order to get closer to physiological conditions and verify precision and accuracy of the single data.

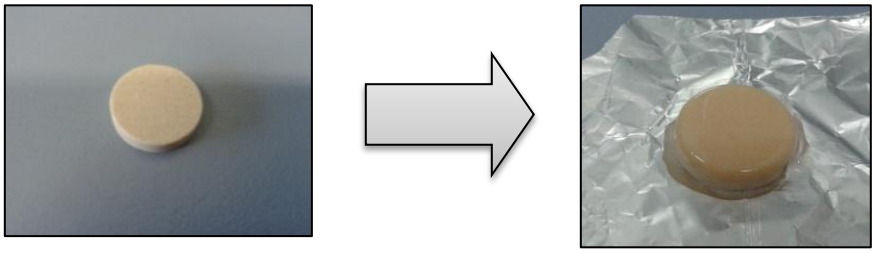
The optimization of the tensile test (described in Section 2.3.6) was carried out firstly replacing water as tablet hydration medium with phosphate buffer pH 6.8 to simulate the physiological conditions of the intestine.

Then the following operating parameters were changed:

- contact force applied between the mucus substitute and the tablet of 0.4 N, instead of 0.1 N;
- contact time between the mucus substitute and the tablet equal to 15 sec, instead of 60 sec;
- data acquisition rate, i.e. the rate of data storage into the computer memory, equal to 200 points*s^{-1} instead of 50.

Finally, the mucus substitute consisting of a 30% [w/w] aqueous mucin gel was replaced with mucin discs (Baloglu, et al., 2011) (13 mm diameter and 3 mm depth) prepared by the compression of 500 mg mucin in a single punch press (Atlas Manual 15T Hydraulic Press, SPECAC LTD., UK) using a compression force of 10 tons. For

the tensile test the mucin disc was fixed with cyanoacrylate glue on an aluminum foil and it was hydrated in the same way of tablets (phosphate buffer 6.8 for 5 minutes before starting the tensile test) (figure 3.4).



(A) **(B)**

Figure 3.4. *Dry mucin disc (A) and mucin disc after 5 minutes of hydration (B).*

The tensile test was performed using the three different procedures described in detail in table 3.5.

Table 3.5. *Three different procedures of the adhesive test (all performed with phosphate buffer pH 6.8 as hydration medium).*

Procedure	Mucus substitute	Contact Force [N]	Contact Time [sec]	Data aquisition rate [points*s^{-1}]
1	mucin gel	0.1	60	50
2	mucin gel	0.4	15	200
3	mucin disc	0.4	15	200

In this way it was possible to verify the correspondence of the results obtained using the three different procedures and hence the accuracy of the experimental data.
For the other technical specifications (temperature, etc.), see Section 2.3.6. For each polymer were performed five repetitions.
The mucoadhesive properties of tablets containing sodium butyrate were evaluated using the procedure 3.

3.3.7 Dissolution test

Tablets containing sodium butyrate were subjected to *in vitro* drug dissolution tests, performed according to FUI XII ed. (F.U.I., 2008), with a dissolution apparatus 2 (Sotax AT7 Smart, Sotax, Switzerland) at 100 rpm. The dissolution tests were carried out at 37 ± 0.5 °C in 900 mL of simulated intestinal fluid (phosphate buffer pH 6.8) as dissolution medium. During the release studies, 1 mL of dissolution medium sample was removed and filtered; SB quantification was performed using the HPLC technique reported in Section 3.3.8. The volume removed was replaced each time with fresh medium. Results are averaged from three replicated experiments.

3.3.8 Analytical method for the determination of sodium butyrate

The quantitative determination of sodium butyrate was made by HPLC analysis (model: 1220 Infinity LC, Agilent Technologies, USA) using a UV/VIS detector. For the analysis, a mixture of phosphoric acid pH 2.38 aqueous solution and acetonitrile (ratio 90:10) was used as mobile phase.

The analytical conditions of the method are the following:

- Agilent ZORBAX RX-C18 column (5 µm, 4.6*250 mm, 80 Å);
- flow rate of the mobile phase 1 mL/min;
- detection wavelength of 210 nm.

Using these conditions the sodium butyrate retention time is about 9.50 minutes. Figure 3.5 reports an example of sodium butyrate chromatogram.

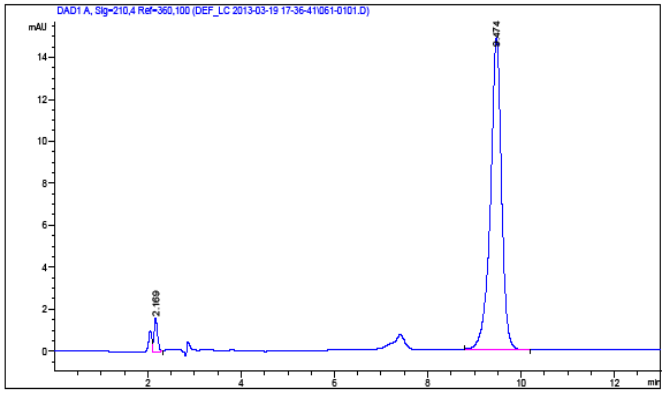


Figure 3.5. *Example of sodium butyrate chromatogram.*

3.4 Results and Discussion

The aim of this part of the study was the development of mucoadhesive tablet containing SB as model drug. In order to achieve this purpose the range of polymers considered in the previous chapter was further increased. In particular, two semisynthetic cellulose derivatives with different ionic characteristic were included: hydroxyethylcellulose (HEC), nonionic, and sodium carboxymethylcellulose (NaCMC), anionic.

The new polymers were subjected to the flowability test reported in Chapter 1. The Hausner Index and the Compressibility Index of the polymers in pure form and in mixture (excipients blend: Ludipress®, microcrystalline cellulose T1, magnesium stearate and talc) were evaluated according to FUI XII. ed. (F.U.I., 2008). The flowability test showed that the addition of the excipients blend to the new polymers further improves or does not change the powder flowability (table 3.6).

Table 3.6. *Results of the flowability test of the new polymers.*

Powder	Hausner Index (HI) [-]	Compressibility Index (CI) [-]	Flowability
HEC	1.30±0.01	23.16±0.76	Passable
HEC-Excipients blend	1.16±0.02	13.71±1.69	Good
NaCMC	1.18±0.01	15.53±0.46	Discrete
NaCMC-Excipients blend	1.19±0.02	16.18±1.47	Discrete

Mixtures containing 60% [w/w] of polymer and 40% [w/w] of excipients blend are compressed in order to produce tablets which were then characterized by the test of uniformity of mass (F.U.I., 2008) and the crushing strength test.

The force necessary to break the tablets is equal to 42.96±2.93 N for HEC and 68.01±4.24 N for NaCMC. These values confirm that tablets are enough resistant to possible subsequent manipulations and to the destructive forces present in the gastrointestinal tract, which are equal to 1.9 N in the stomach and 1.2 N in the small intestine (Kamba, et al., 2002).

The next step was to evaluate the ability of the new polymers to hydrate and swell in water in terms of water uptake (WU) and swelling index (SI), as shown in figure 3.6.

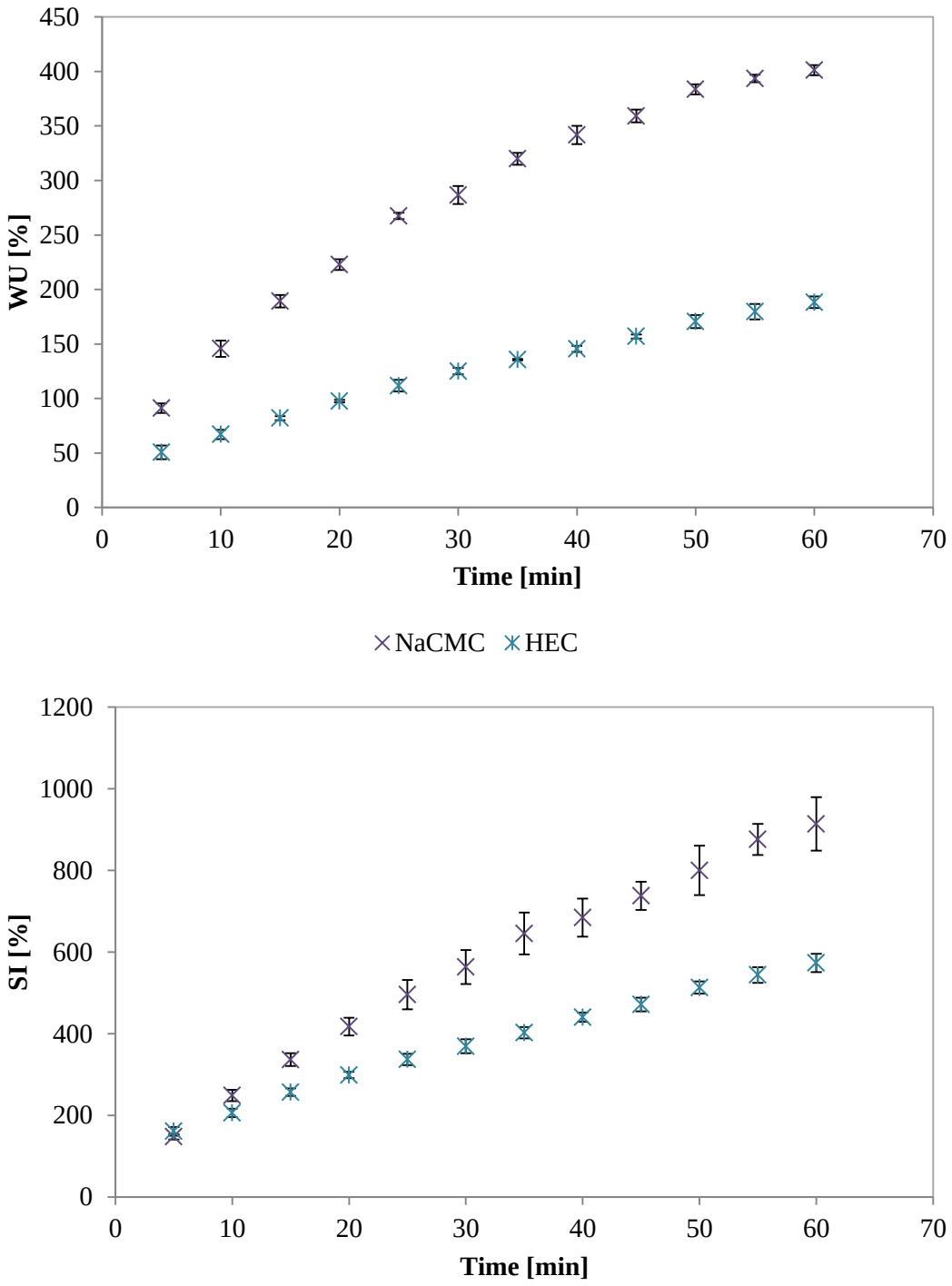


Figure 3.6. *Values of WU [%] (top) and SI [%] (bottom) of the new polymers in water.*

The graphs show that tablets containing NaCMC swell and absorb water in larger amounts compared to those of HEC and also in this case a good linearity exists

between WU and SI: an increase in weight corresponds to an increase in volume, as highlighted in figure 3.7.

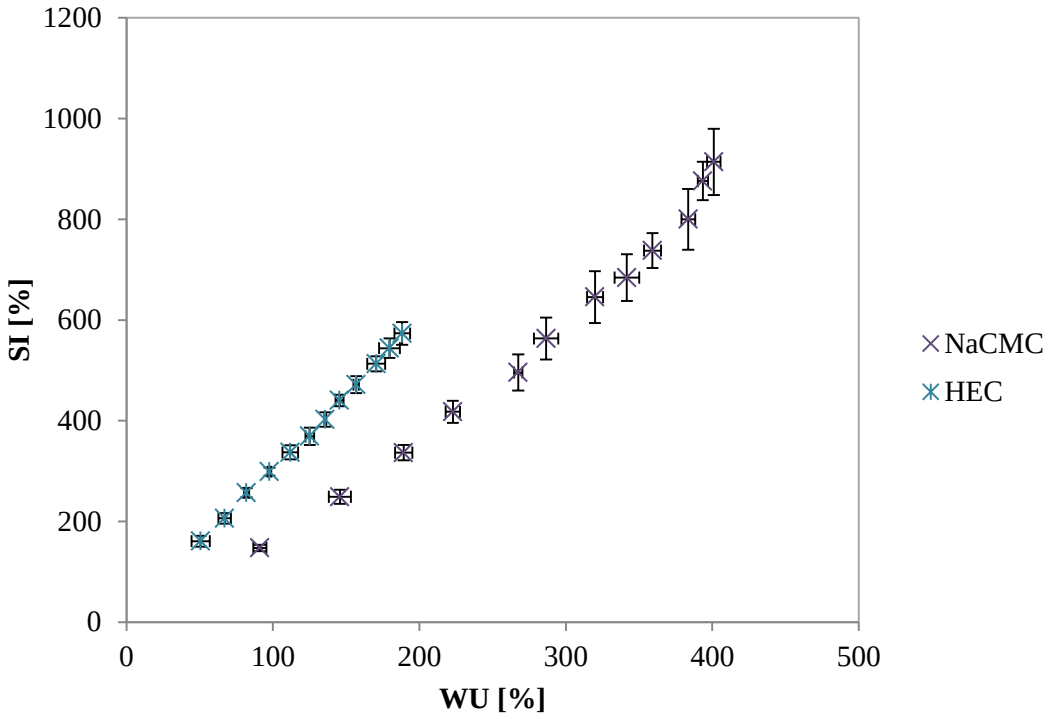


Figure 3.7. *Relationship between water uptake (WU [%]) and swelling index (SI [%]) for the tablets containing NaCMC and HEC.*

The viscosity average molecular weight of NaCMC and HEC was determined by means of the same method used for the other polymers. NaCMC exhibits an average molecular weight of 519 kDa while HEC of 467 kDa.

The mucoadhesive properties of the tablets containing NaCMC and HEC were evaluated in terms of maximum detachment force (F_{max}) and work of adhesion (W_{ad}) using the tensile test (procedure 1 of table 3.5). Results reported in figures 3.8 and 3.9 show that tablets containing HEC and NaCMC seem to exhibit similar mucoadhesive properties. However, comparing results obtained for all the polymers, sodium alginate remains the polymer with the best mucoadhesive properties.

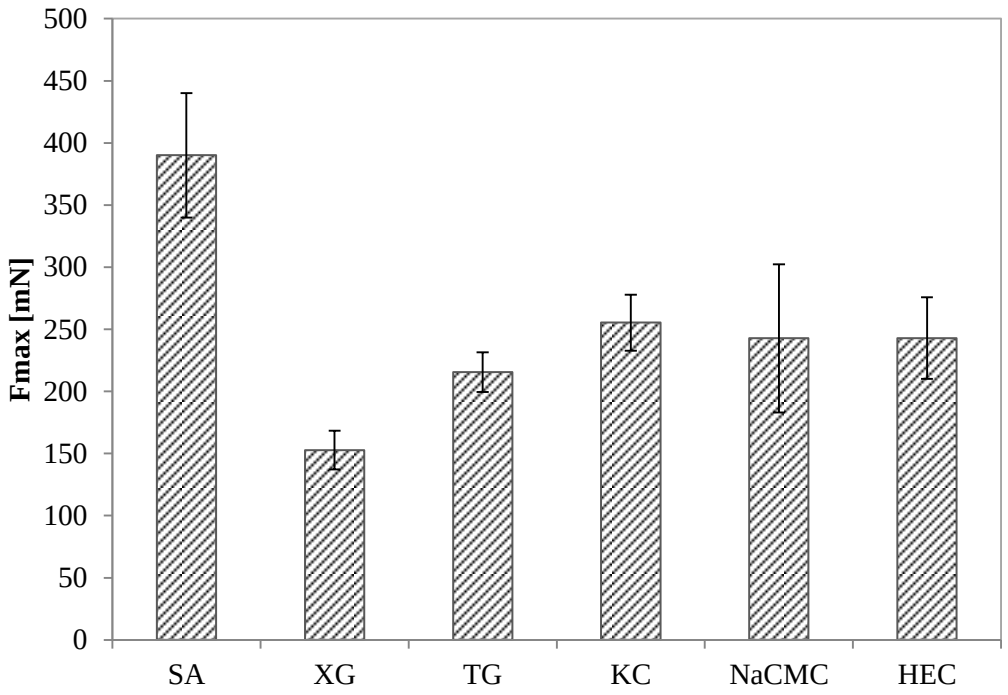


Figure 3.8. *Values of F_{max} of the all the tablets (for SA, XG, TG and KC the results corresponding to the second mucin batch were considered).*

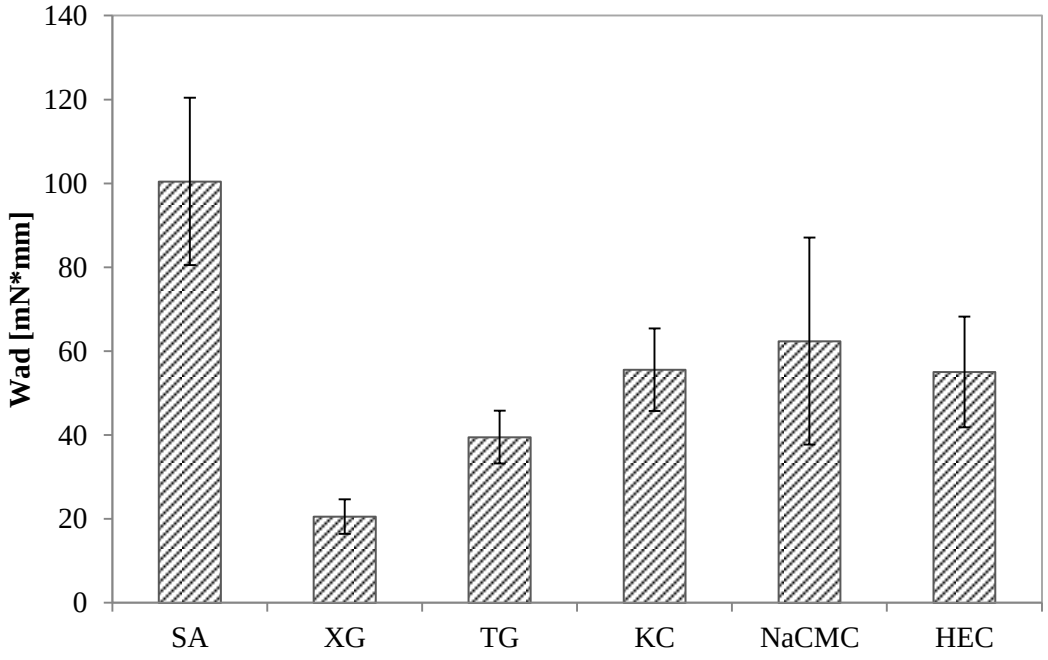


Figure 3.9. *Values of W_{ad} of all the tablets (for SA, XG, TG and KC the results corresponding to the second mucin batch were considered).*

In order to highlight the presence of a relationship between the mucoadhesive properties and the other evaluated polymer properties, some comparison were performed (table 3.7).

Table 3.7. *Comparison of molecular weight (M), water uptake (WU) and swelling index (SI) at 5 and 60 min, maximum detachment force (F_{max}) and work of adhesion (W_{ad}) for all the tablets containing different polymers (the mucoadhesive properties of tablets containing SA, XG, TG and KC are referred to the second mucin batch).*

Polymer	**M** [kDa]	**WU_{5min}** [%]	**SI_{5min}** [%]	**WU_{60min}** [%]	**SI_{60min}** [%]	**F_{max}** [mN]	**W_{ad}** [mN*mm]
SA	132	112	102	278	575	390	100
XG	1341	102	118	613	993	153	20
TG	840	60	66	194	445	215	39
KC	407	291	441	839	1666	255	56
NaCMC	519	91	147	401	914	243	62
HEC	467	51	161	188	573	243	55

Data confirm the presence, as seen in Chapter 2, of an inversely proportional relationship between polymer molecular weight (M) and mucoadhesive properties (F_{max} and W_{ad}) of the tablets (figures 3.10 and 3.11): the lower the average molecular weight, the higher the mucoadhesive properties.

As highlighted previously, for the other parameters no evident match was found.

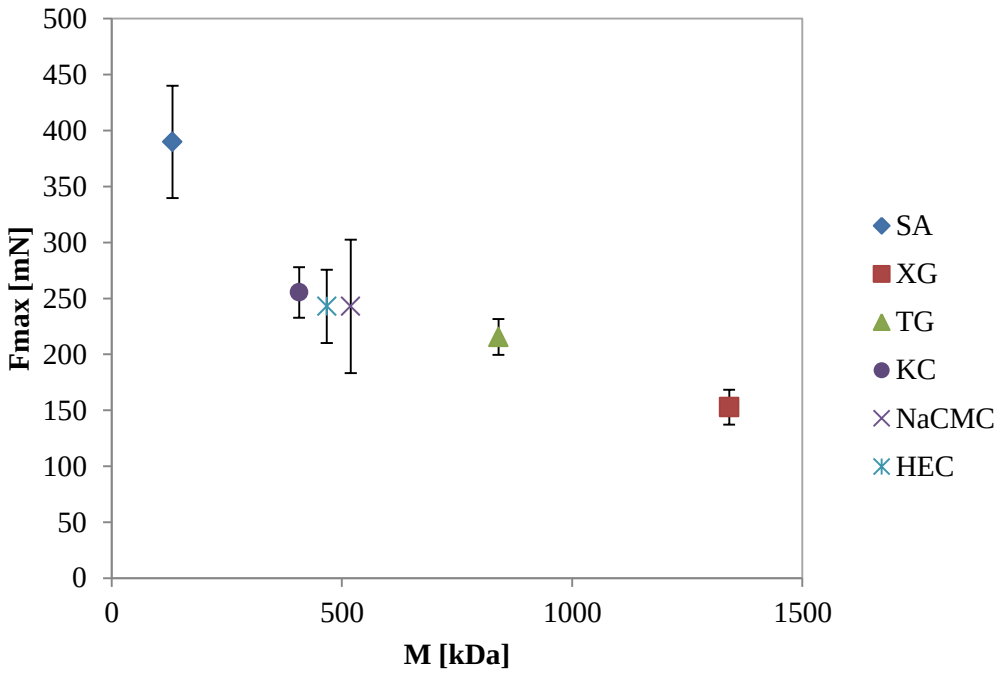


Figure 3.10. *Relationship between polymers molecular weight (M [kDa]) and the mucoadhesive properties (F_{max} [mN]) of the tablets in water.*

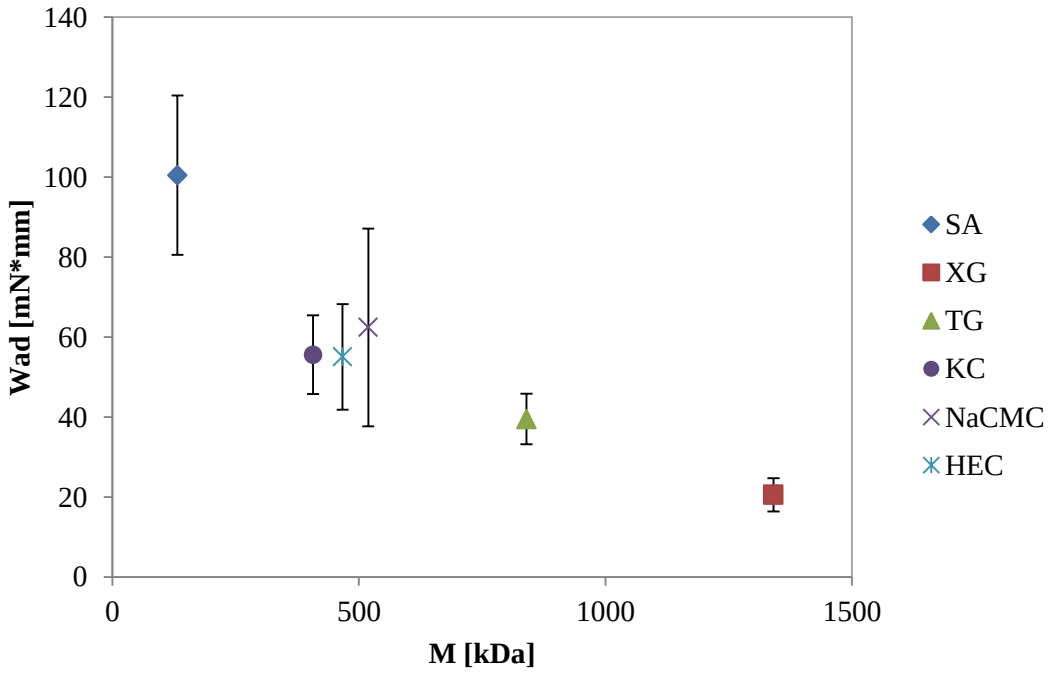


Figure 3.11. *Relationship between polymers molecular weight (M [kDa]) and the mucoadhesive properties (W_{ad} [mN*mm]) of the tablets in water.*

In order to develop a dosage form with intestinal target, the hydration medium was changed to get closer to the physiological conditions: water was replaced with phosphate buffer pH 6.8 and the three characterizations (water uptake, swelling index and tensile test) were repeated for all the tablets, except those containing KC. These tablets, indeed, disintegrate rapidly in phosphate buffer as κ-carrageenan dissolves in aqueous medium in presence of sodium ions (Rowe, et al., 2006). This hypothesis was confirmed by the immediate disintegration of a tablet containing KC placed in a 0.9% sodium chloride solution.

Results of water uptake and swelling index pointed out that there were no significant differences in polymers behavior in the two solvents, with the exception of tablets containing XG. Indeed, in this case, WU and SI in buffer are considerably lower than those obtained in water (figures 3.12 and 3.13). This behavior may be correlated with the fact that the solubility of XG is influenced by presence of salts (Rowe, et al., 2006).

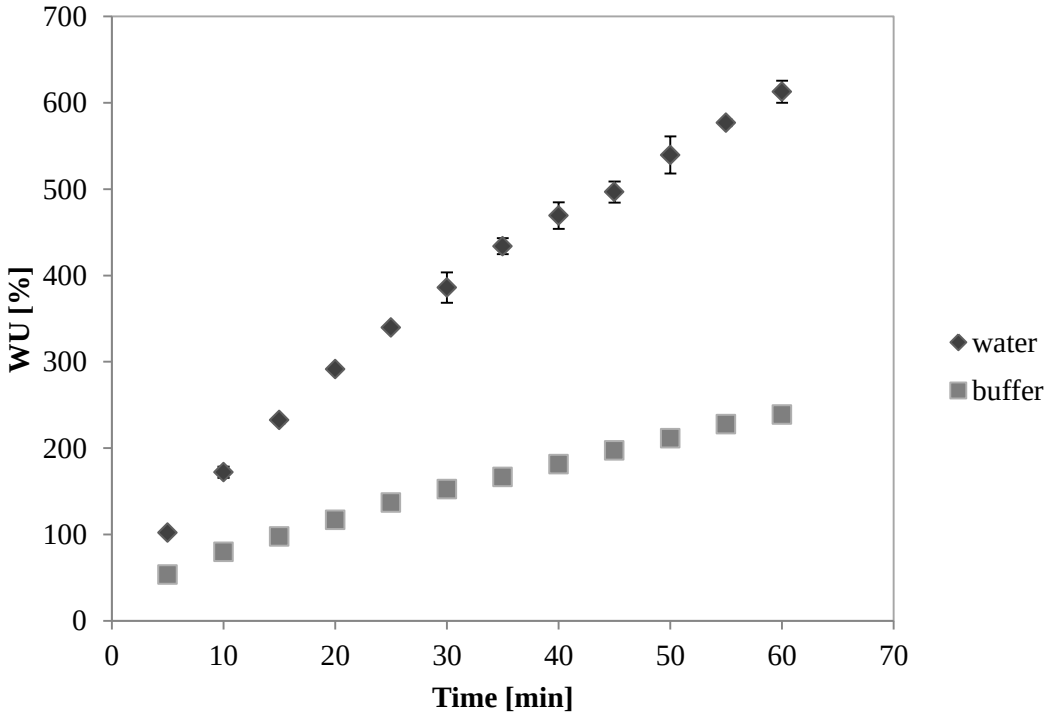


Figure 3.12. *WU [%] profiles of tablets containing XG in water and in phosphate buffer pH 6.8.*

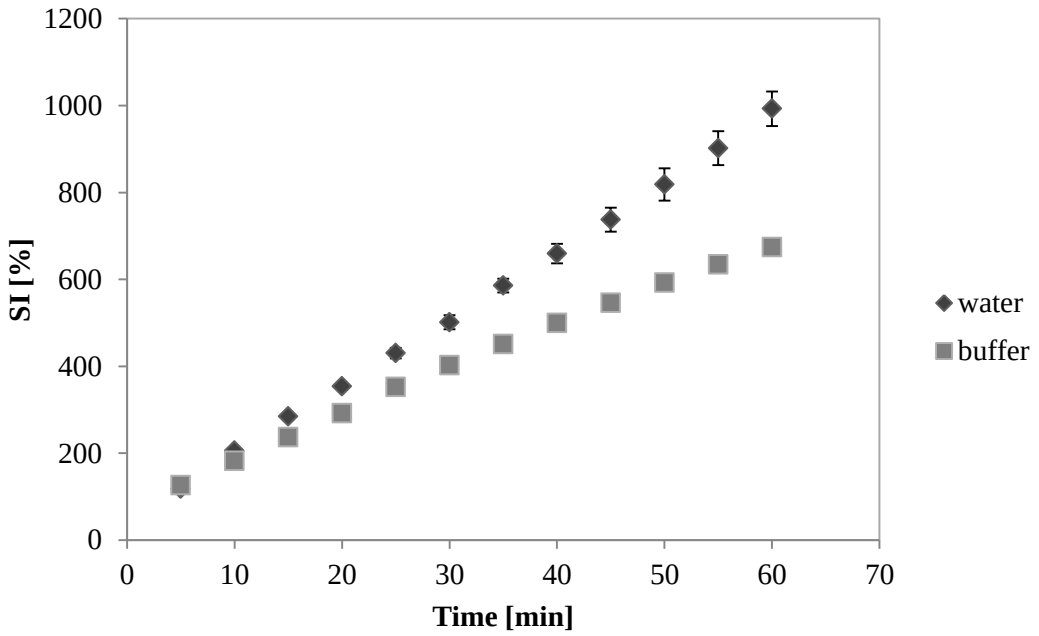


Figure 3.13. *SI [%] profiles of tablets containing XG in water and in phosphate buffer pH 6.8.*

Swelling index and water uptake profiles of all the polymers are reported in figures 3.14 and 3.15.

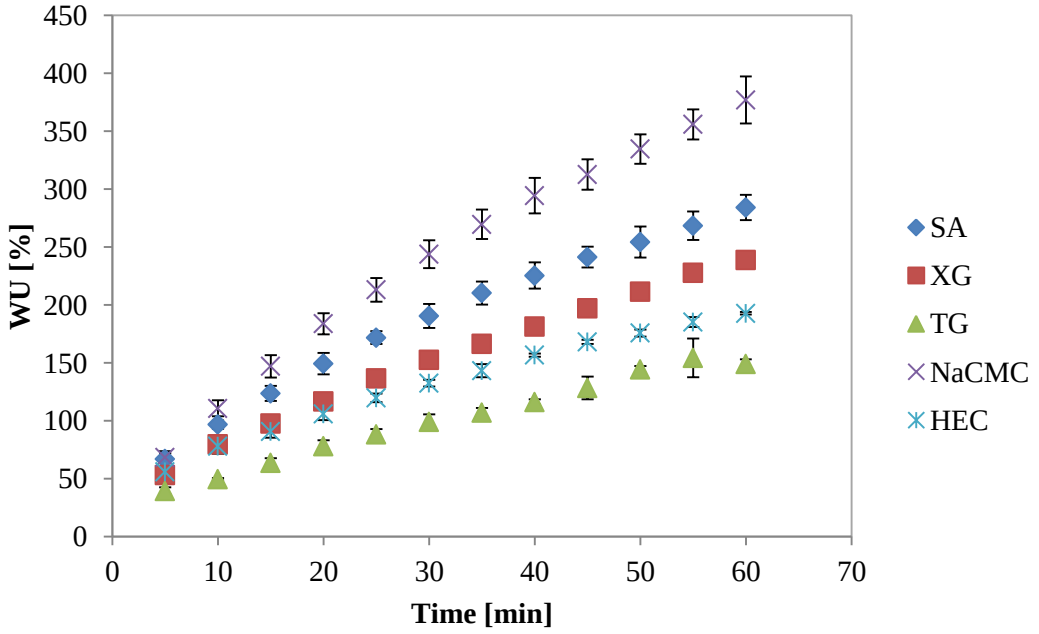


Figure 3.14. *WU [%] profiles of all the polymers in phosphate buffer pH 6.8.*

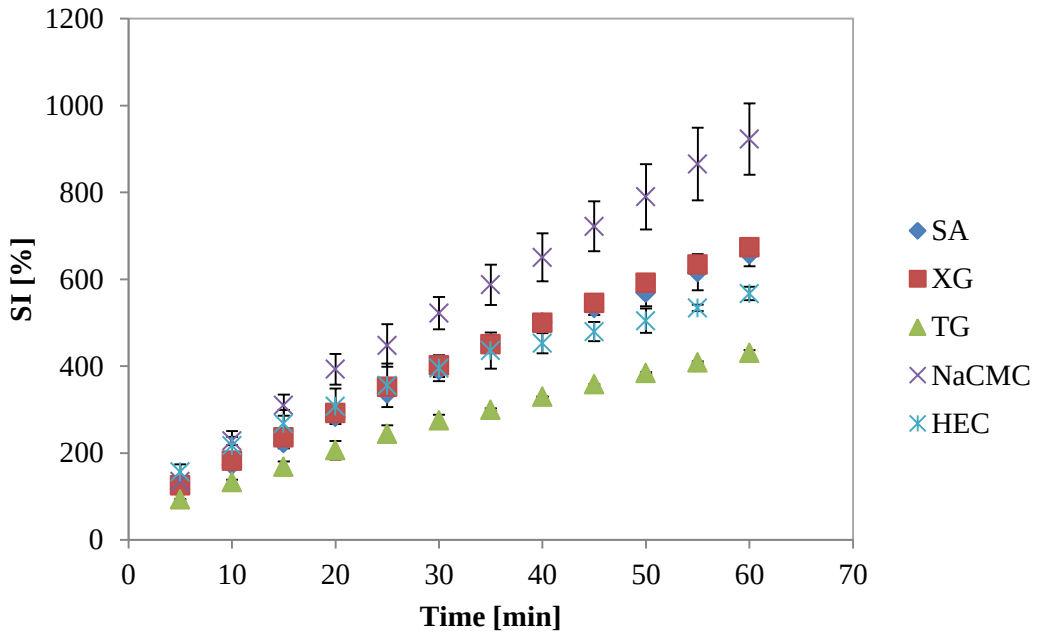


Figure 3.15. *SI [%] profiles of all the polymers in phosphate buffer pH 6.8.*

Comparing the WU and SI values obtained using phosphate buffer as medium, it is possible to note that also in this case an increase in water uptake produces an increase in volume, as shows in figure 3.16.

From figure 3.16 polymers can be divided into two groups depending on their ability to adsorb water and swell. In particular, the ratio between swelling index and water uptake can be used to express the swelling ability of a polymer: the lower the swelling ability, the lower the swelling of the polymer corresponding to a certain level of water uptake. Consequently, SA and NaCMC exhibit the lower swelling ability.

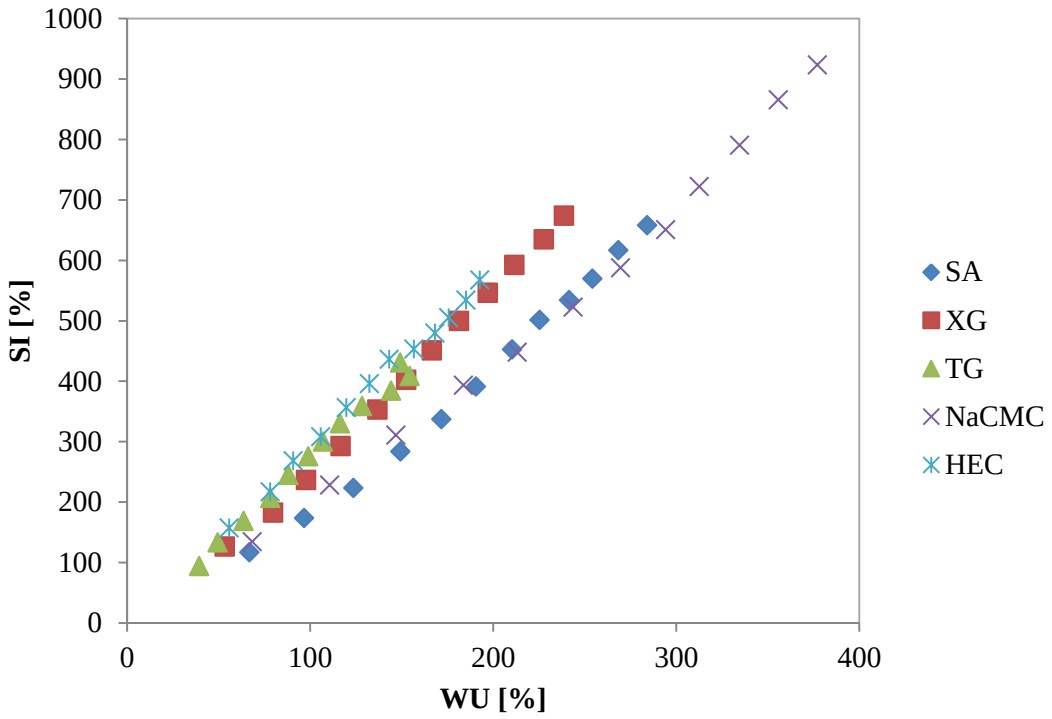


Figure 3.16. *Relationship between WU [%] and SI [%] in phosphate buffer 6.8 of the different systems.*

The mucoadhesive test performed in buffer revealed that the polymers having the higher mucoadhesive properties in terms of F_{max} and W_{ad} are those presenting the lower swelling ability: SA and NaCMC (figures 3.17-3.18). It therefore seems to exist a relationship between mucoadhesion and water uptake/swelling, not observed before with the results obtained in water.

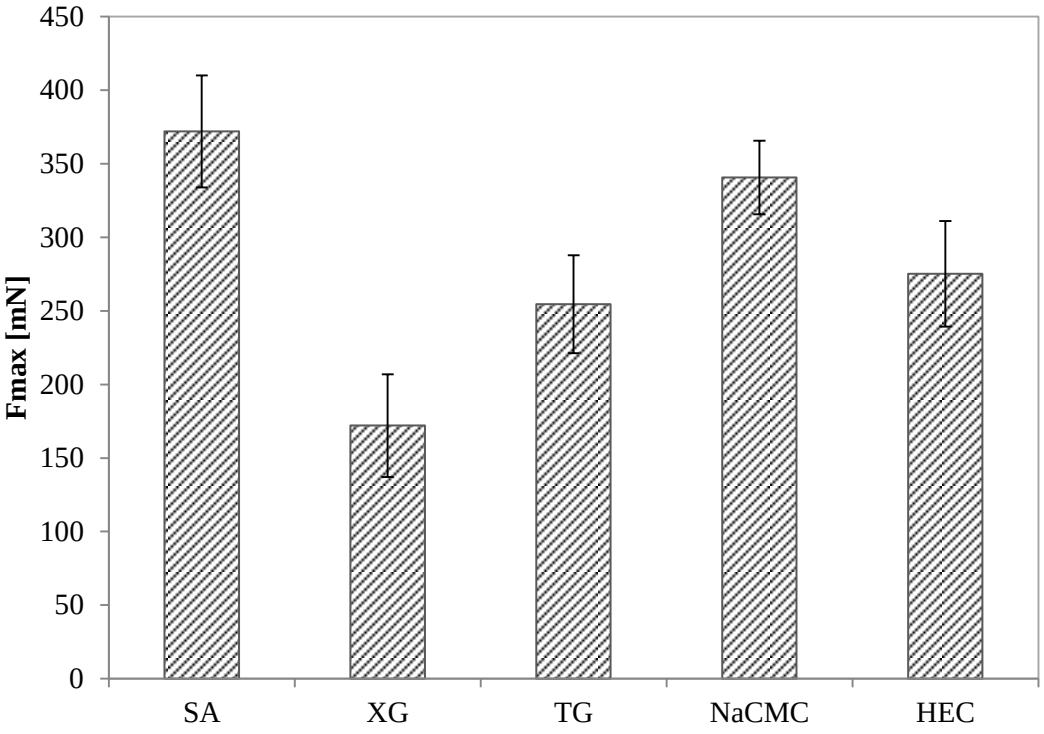


Figure 3.17. *F_{max} [mN] values of all tablets in phosphate buffer pH 6.8.*

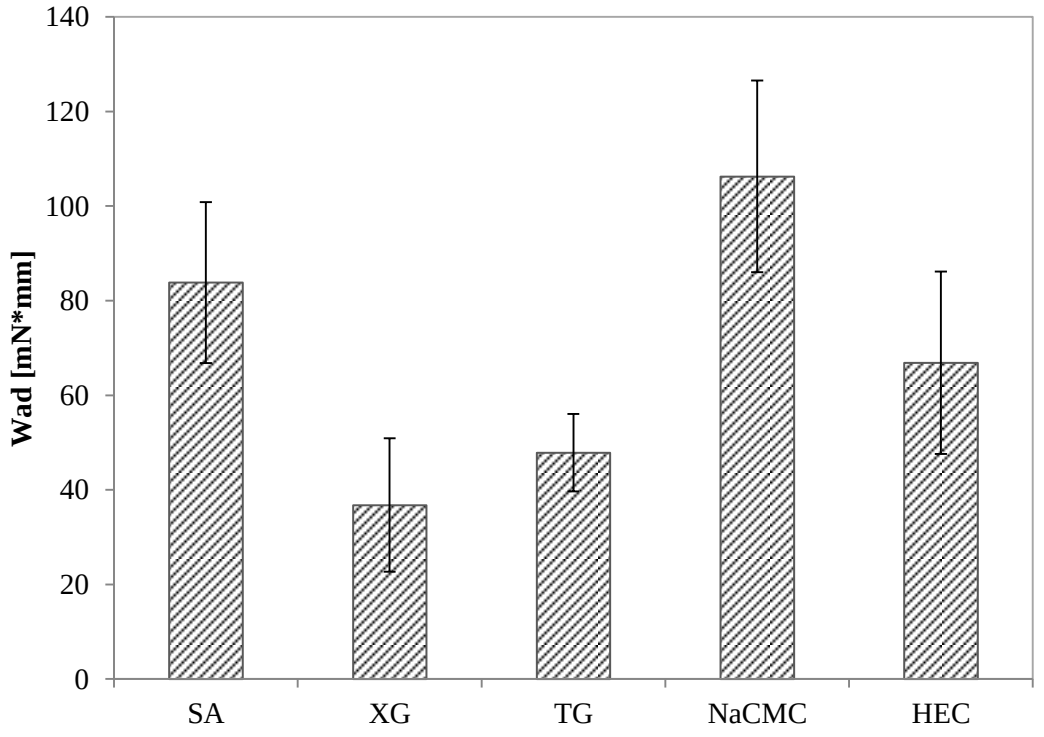


Figure 3.18. *W_{ad} [mN*mm] values of all tablets in phosphate buffer pH 6.8.*

In order to confirm these observations it was decided to further optimize the tensile test to make data more robust. The wettability of the tablets was also investigated in order to clear the behavior of the tablets in aqueous medium.

For the tensile test some operating parameters were changed in order to obtain more precise results and decrease the background noise of the instrument:

- contact force of 0.4 N;
- contact time of 15 sec;
- data acquisition rate of 200 points*s^{-1}.

The experiments in buffer were then repeated for all systems considered (procedure 2 of table 3.5).

Subsequently, it was decided to introduce a further modification to the method: the mucin gel was replaced with mucin discs (Baloglu, et al., 2011), realized by means of direct compression of powders. The experiments in buffer were then repeated for all the tablets, using the new experimental parameters and the mucin disc (procedure 3 of table 3.5).

The comparison between the results obtained with the three different procedures is reported in figure 3.19.

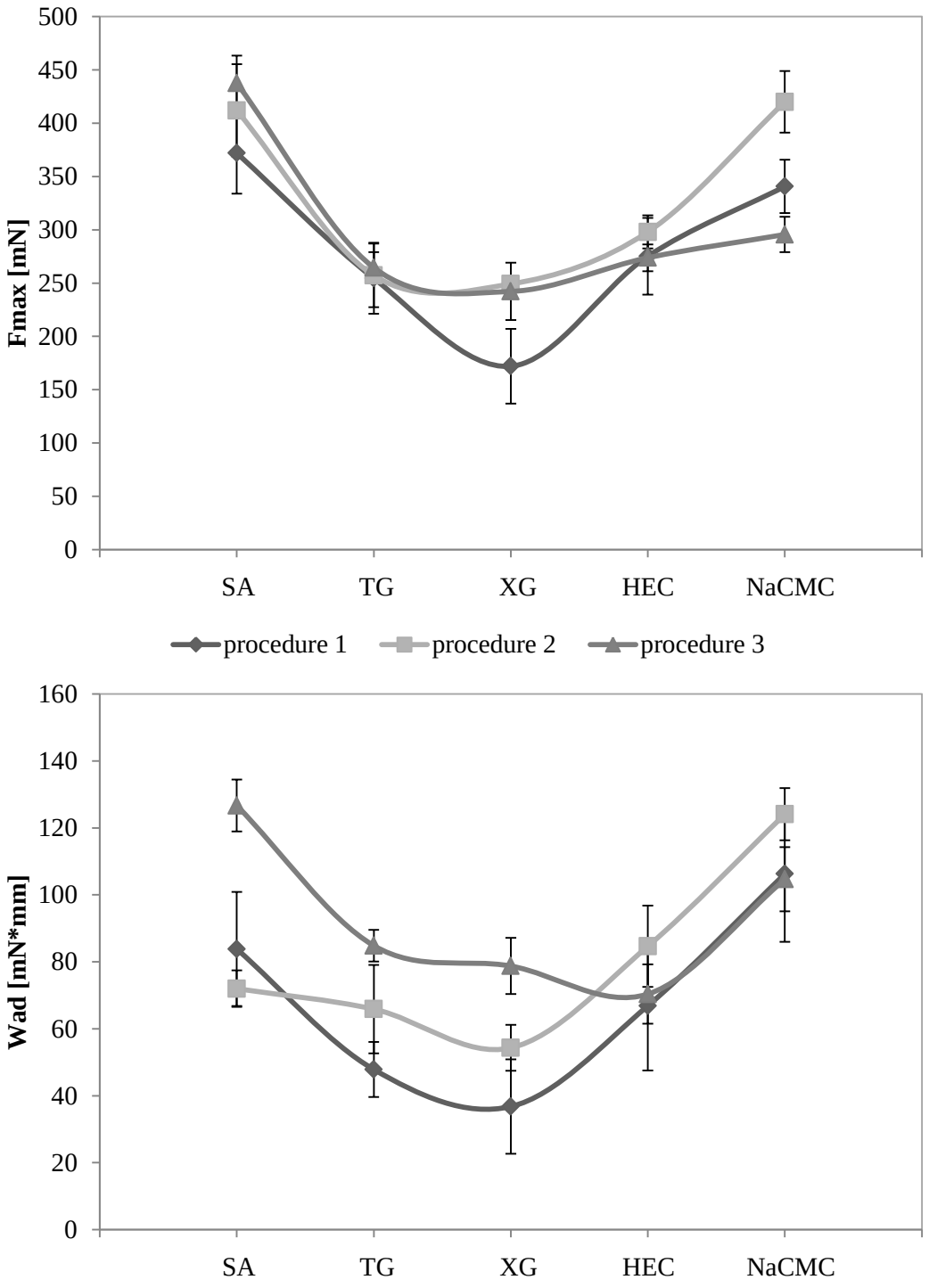


Figure 3.19. *Results of the tensile test, in terms of F_{max} and W_{ad}, performed using the three different procedures.*

The graphs show that the values of maximum detachment force and work of adhesion assume the same trend with all three procedures: this observation confirm the accuracy of the data. Since the results obtained with the different procedures are comparable, the choice of the substrate to continue the study and the suitable operating conditions has been made considering the standard deviation and the working time. In particular, the procedure 3 allows a reduction of both the working time necessary to prepare the substrate and the data standard deviation; consequently, it was chosen to continue the study.

According to studies reported in literature (Colombo, et al., 2004; Lazghab, et al., 2005), the water wettability of the tablets has been studied by measuring the contact angle arising from the deposition of a drop of water on the tablet.

Figure 3.20 shows the contact angle values of the first 3.5 seconds after the deposition of the drop of water on tablets containing the various polymers. A horizontal line divides the values of angle less than 90°, expression of good wettability, from the values of angle between 90° and 180°, expression of poor wettability.

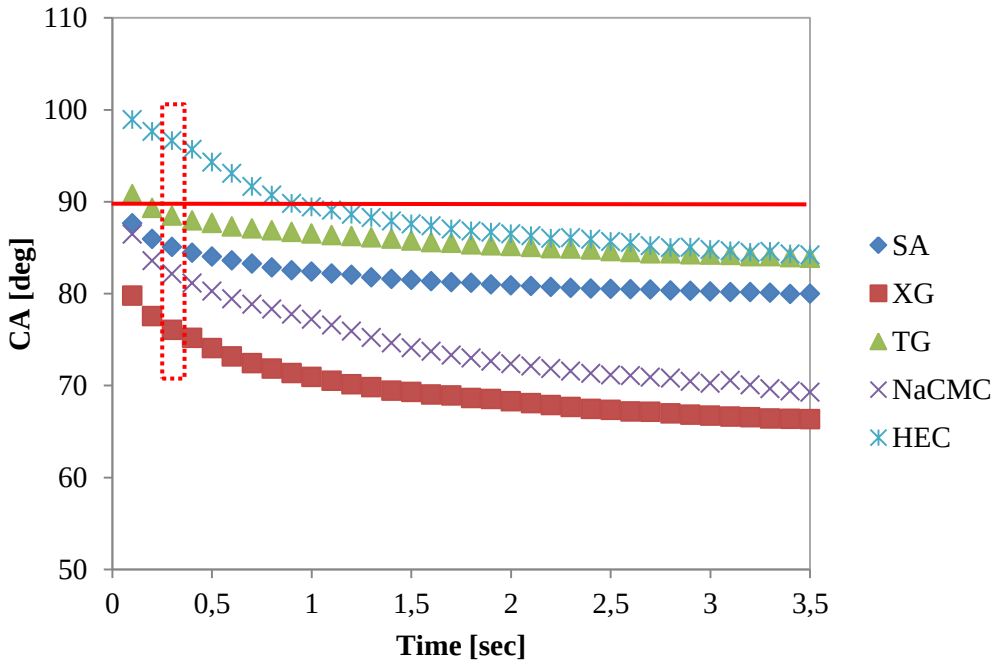


Figure 3.20. *Average contact angle (CA [deg]) between tablets and water over time [sec].*

To make a comparison of the contact angle values of the various tablets, it is necessary to make some assumptions.

The contact angle is defined as the angle that arises when a balance between the cohesive force, that holds together the particles of liquid, and the adhesive strength between the liquid molecules and the solid surface, is established. However, in the time elapsed between drop deposition and measurement of the angle, a series of unwanted time-dependent phenomena, such as absorption or erosion, may grow.

Thus, considering the contact angle values corresponding to 0.3 seconds after drop deposition (sufficient time for the equilibrium to be established in the absence of time-dependent phenomena), the non-ionic polymer HEC shows a value of contact angle higher than 90°, unlike other polymers; this is justified by its nature (nonionic). HEC is followed by TG, probably due to its water-insoluble component, and then by the other ionic polymers, presenting lower values of contact angles. Nevertheless, results are consistent with the values of WU and SI in water at 60 minutes, equal to those performed in buffer, except XG.

In order to confirm that the different behavior of XG in buffer is time-dependent, the buffer wettability of XG was studied and results concerning both solvents are compared in figure 3.21.

Since contact angle values obtained with the two solvents are comparable, it is possible to state that the influence of the presence of salts on XG behavior is time-dependent and hence does not affect XG wettability.

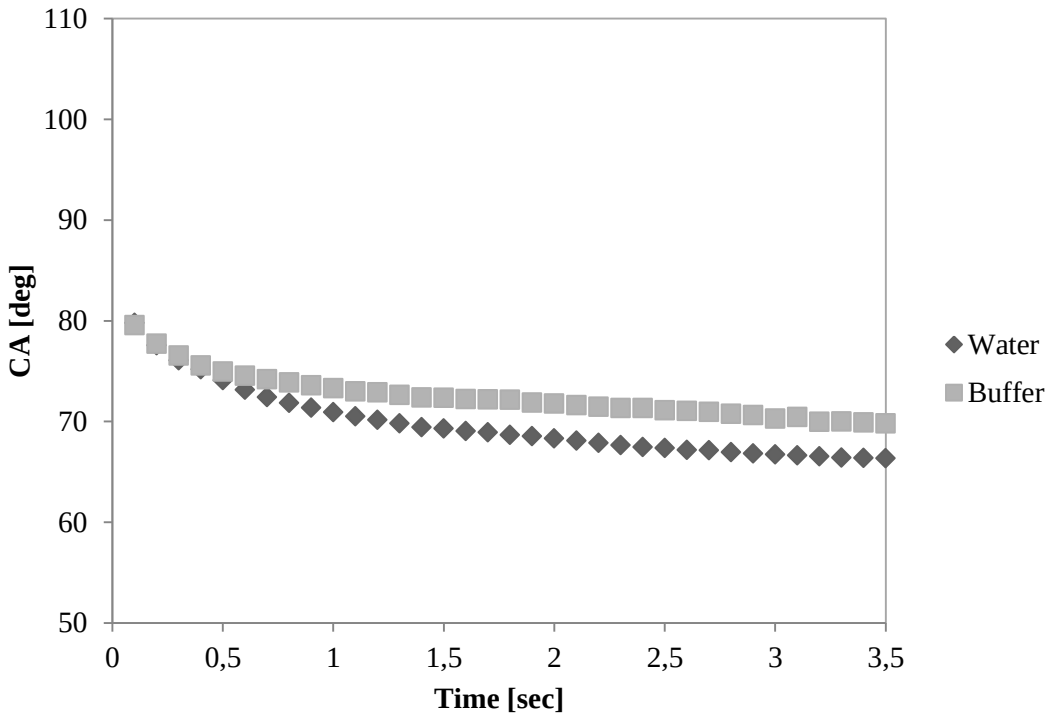


Figure 3.21. *Average contact angle (CA [deg]) between XG tablets and water or buffer, over time [sec]*.

Finally, comparing the molecular weights of all polymer with their mucoadhesive properties (F_{max} and W_{ad}) in phosphate buffer 6.8 (figures 3.22) it is possible to confirm the inversely proportional relationship previously observed: the higher the average molecular weight of the polymer the lower the mucoadhesive properties (F_{max} and W_{ad}).

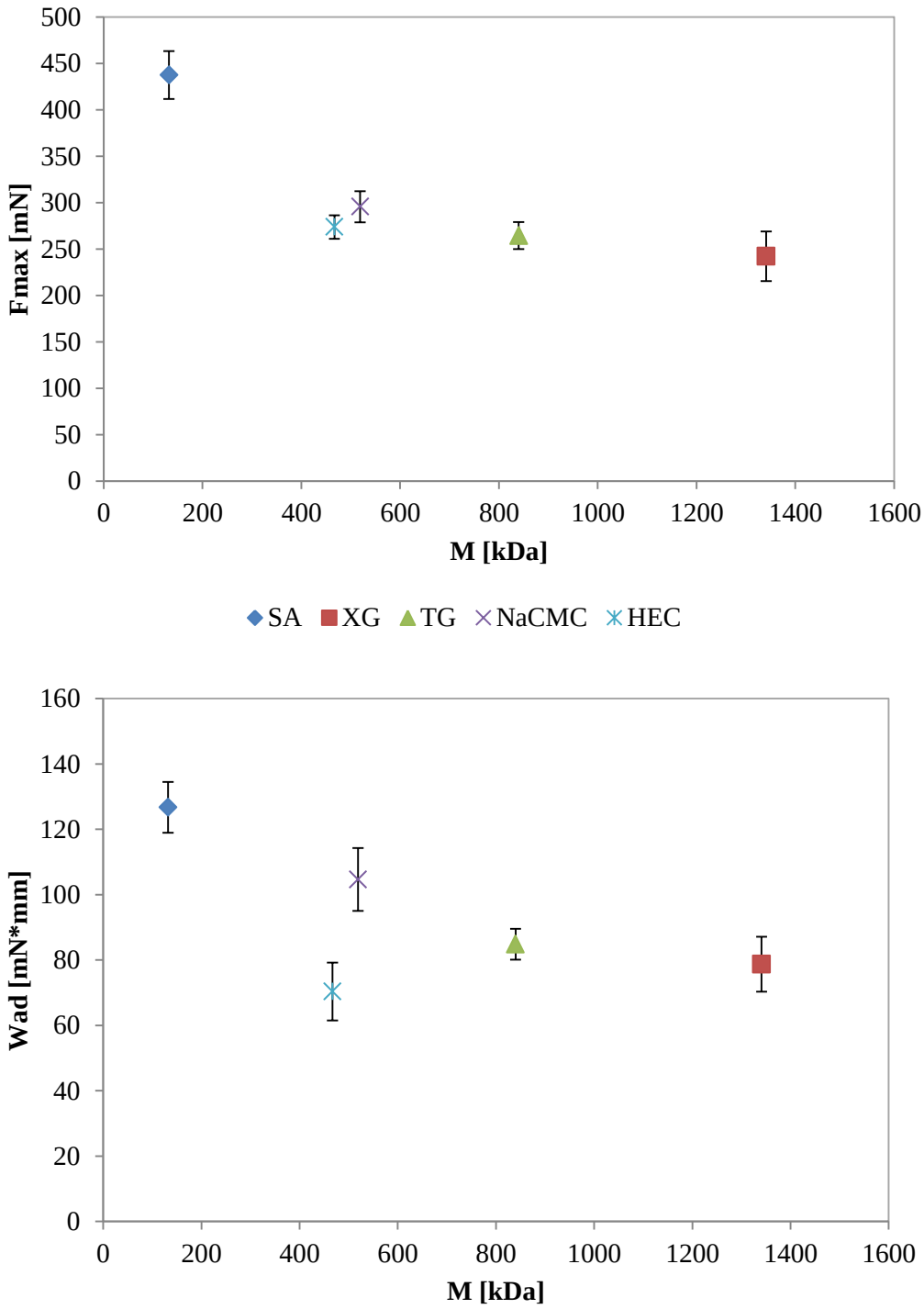


Figure 3.22. *Relationship between polymers molecular weight (M [kDa]) and the mucoadhesive properties (F_{max} [mN] or W_{ad} [mN*mm]) of the tablets evaluated using procedure 3 and phosphate buffer pH 6.8.*

From the results, two polymers were selected in order to continue the study: SA and NaCMC. These polymers were used to formulate tablets containing a model active pharmaceutical ingredient (API) and assess how these polymeric matrices could influence the API release. Sodium butyrate (SB) was chosen as model drug. It presents a water solubility of 100 mg/mL and it is used as adjuvant in the treatment of intestinal disorders, for which the formulation in mucoadhesive tablets can be advantageous. Tablets with increasing amounts of polymer were prepared in order to evaluate the influence of the different amount of polymer on the mucoadhesive properties of the dosage form and on the API release.

The formulations evaluated consist in 30, 45, 60% [w/w] of polymer (SA or NaCMC), 20% [w/w] of sodium butyrate, 15% [w/w] of excipients blend; the formulations were completed with the addition of a water-soluble excipient (mannitol, MA) or a water-insoluble excipient (calcium phosphate, CP). Table 3.8 shows the formulations composition and the related flowability results.

Table 3.8. *Hausner Index, Compressibility Index and flowability of the various mixtures formulated, according to FUI XII ed.* (F.U.I., 2008).

Polymer	Amount of polymer [%]	Excipient	Hausner Index [-]	Compressibility Index [%]	Flowability
NaCMC	30	MA	1.18±0.01	15.15±0.10	Good
NaCMC	45	MA	1.21±0.01	17.65±0.10	Discrete
NaCMC	60	MA	1.21±0.01	17.14±0.10	Discrete
SA	30	MA	1.23±0.01	18.56±0.30	Discrete
SA	45	MA	1.24±0.02	19.79±1.34	Discrete
SA	60	MA	1.29±0.03	22.55±1.70	Passable
NaCMC	30	CP	1.22±0.04	18.02±2.65	Discrete
NaCMC	45	CP	1.31±0.05	23.48±2.65	Passable
NaCMC	60	CP	1.25±0.04	20.15±2.32	Discrete
SA	30	CP	1.26±0.05	20.74±3.31	Discrete
SA	45	CP	1.16±0.04	13.89±3.47	Good
SA	60	CP	1.19±0.03	16.03±2.28	Discrete

As highlighted in the table, mixtures possessed an almost discrete flowability; it was therefore possible to realize the tablets, which have been characterized in terms of crushing strength and uniformity of mass. Results showed that tablets presented good

strength (values higher than 20 N) (table 3.9) and they complied with the Uniformity of mass requirements (F.U.I., 2008).

Table 3.9. *Average Mass and Percentage Deviation of the tablets.*

Polymer	Amount of polymer [%]	Excipient	Average Mass [mg]	Percentage Deviation	Crushing Strength [N]
NaCMC	30	MA	118.6±1.8	1.52	21.88±2.51
NaCMC	45	MA	119.2±0.9	0.76	22.52±2.36
NaCMC	60	MA	114.8±1.4	1.22	23.03±2.10
SA	30	MA	118.5±1.9	1.60	31.64±2.33
SA	45	MA	115.3±2.4	2.08	34.73±2.48
SA	60	MA	115.3±1.8	1.56	38.78±2.65
NaCMC	30	CP	110.8±4.2	3.79	23.67±5.10
NaCMC	45	CP	131.7±1.6	1.21	47.06±7.29
NaCMC	60	CP	121.5±0.6	0.49	51.19±7.57
SA	30	CP	108.3±2.7	2.49	44.83±8.74
SA	45	CP	129.3±2.7	2.09	61.86±8.35
SA	60	CP	132.3±4.5	3.40	78.44±6.63

Subsequently, the tablets water uptake and swelling index were measured in phosphate buffer pH 6.8. Results of the water uptake test are reported in figures 3.23 and 3.24, which show that an increase of the amount of polymer corresponds to an increase of water uptake. This matches the fact that the higher the amount of polymer, the higher the number of functional groups available to form hydrogen bonds and thus the system hydrophilicity.

Furthermore, the tablets containing NaCMC absorb a higher amount of medium with those containing SA. It is also possible to note that tablets containing CP absorb larger amount of water than those containing MA.

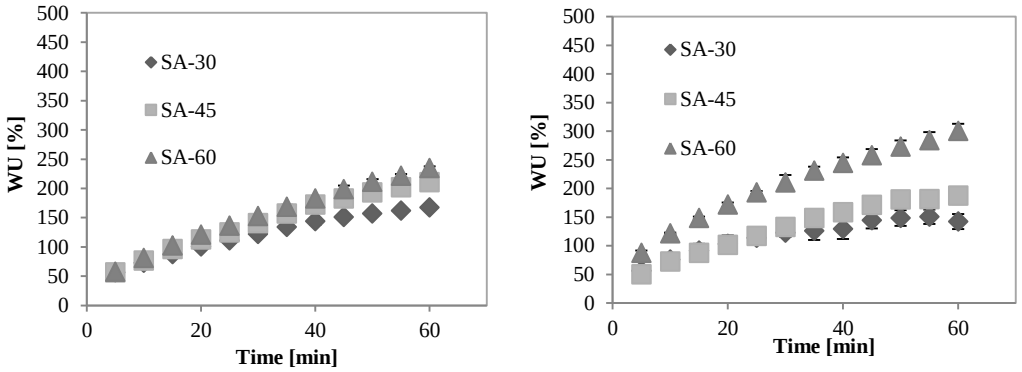


Figure 3.23. *Water Uptake profiles of the tablets containing SA 30, 45, 60% and MA (A) or CP (B).*

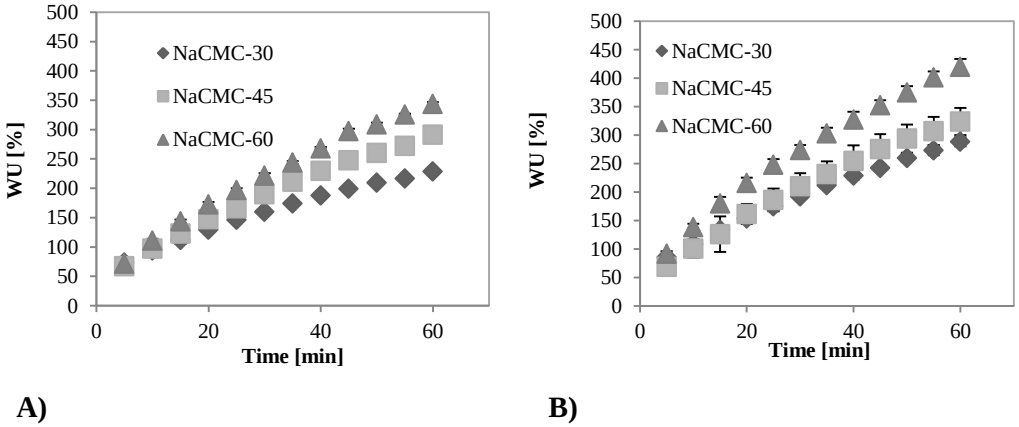


Figure 3.24. *Water Uptake profiles of the tablets containing NaCMC 30, 45, 60% and MA (A) or CP (B)*

For tablets containing MA as excipient, the swelling index depends on the amount of polymer (figures 3.25 A and 3.26 A); in particular, the greater the amount of polymer, the higher the amount of chains available to form the network.

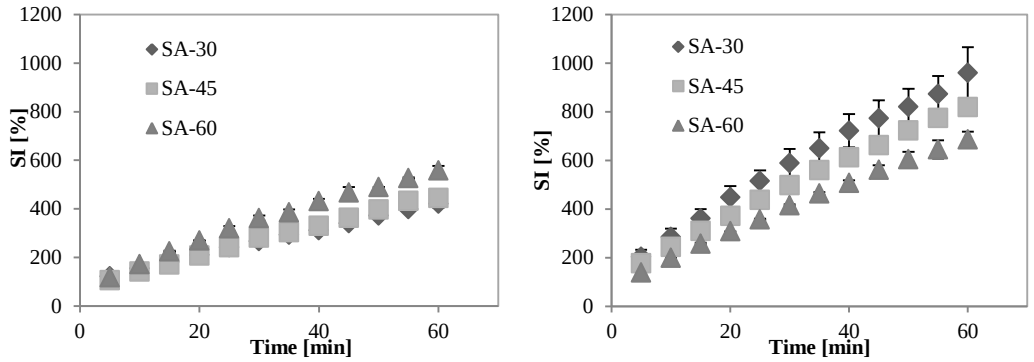


Figure 3.25. *Swelling Index profiles of the tablets containing SA 30, 45, 60% and MA (A) or CP (B)*

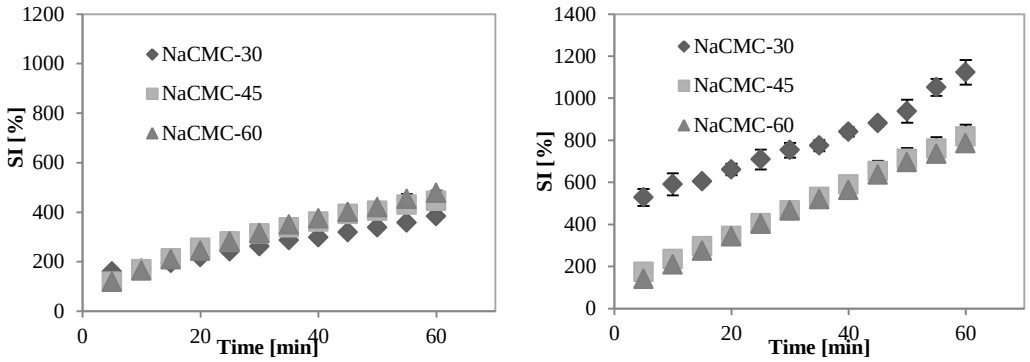


Figure 3.26. *Swelling Index profiles of the tablets containing NaCMC 30, 45, 60% and MA (A) or CP (B)*

Tablets containing CP show an opposite trend (figures 3.25 B and 3.26 B): the lower the polymer percentage, the higher the swelling and thus the larger is the mesh network. As a consequence, in presence of large mesh size the CP is not retained in the polymer network and water further penetrates into the structure promoting the swelling.

Nevertheless, tablets containing CP swell more than those containing MA. In the case of MA, a lower amount of water is available for the polymer swelling due to the solubilization of the water-soluble excipients.

Subsequently, the mucoadhesive properties in phosphate buffer pH 6.8 were evaluated by means of the procedure 3 of the tensile test (described in table 3.5). Data reported in figures 3.27 and 3.28 highlight that there are no significant differences on the mucoadhesive ability of the two polymers. Results show that, with both polymers, the tablets containing CP exhibit better mucoadhesive properties than those with MA; the presence of MA may reduce the hydration of the polymer, which is an important condition for mucoadhesion. It is evident, instead, the difference between the tablets containing increasing amounts of polymer: with increasing the percentage of polymer in the formulation, a gradual increase of the mucoadhesive capacity occurs.

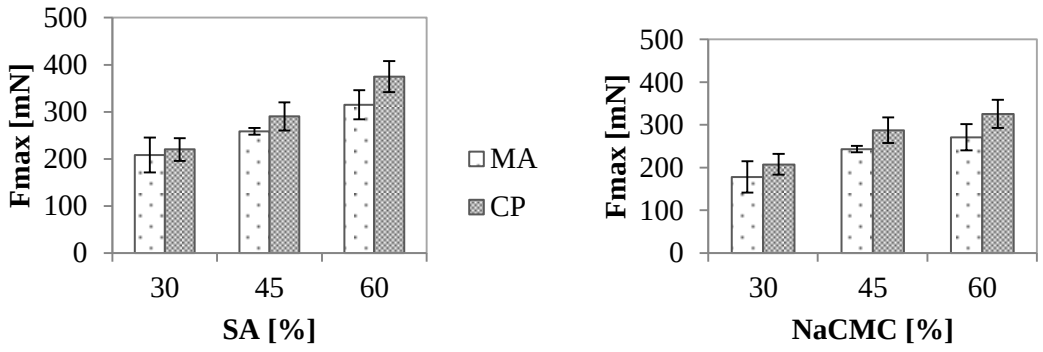


Figure 3.27. *Results of the tensile test in terms of F_{max} [mN].*

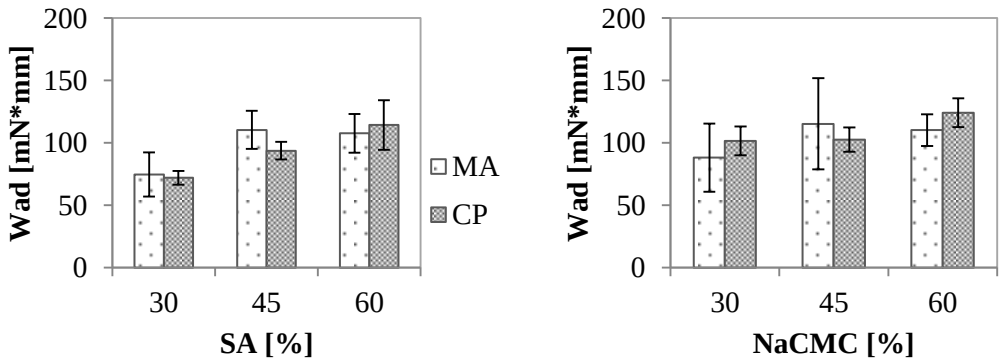


Figure 3.28. *Results of the tensile test in terms of W_{ad} [mN*mm].*

In order to verify how the type and amount of polymer may influence the release of SB from the tablets, dissolution tests in simulated intestinal fluid (phosphate buffer pH 6.8) were performed.

The release profiles are shown in figures 3.29-3.32. Data show that there are no significant differences between the tablets realized with increasing concentrations of polymer.

This may be due to the high solubility of the drug (approximately 100 mg/mL at 20°C); in the case of molecules very soluble in water, the release is mainly controlled by the diffusion of the molecule through the polymer gel layer, while for poorly soluble drugs it mainly depends on the dissolution and the relaxation of the polymer chains.

Comparing the two polymers, tablets with NaCMC gave a slower release than those containing SA. This behavior may be attributed to the capacity of NaCMC to form a gel layer more viscous than SA. This agrees with the viscosity values of 0.5% [w/w] water dispersions of the two polymers, which are equal to 199 mPa*s for NaCMC and 100 mPa*s for SA (measurements carried out at 20°C with a Brookfield viscosimeter VT7R, impeller R2, 30 rpm).

Moreover, for tablets containing NaCMC and CP, the release rate depends on the amount of polymer: the lower the amount of polymer the faster the drug release. These data fit with the results obtained in the swelling study.

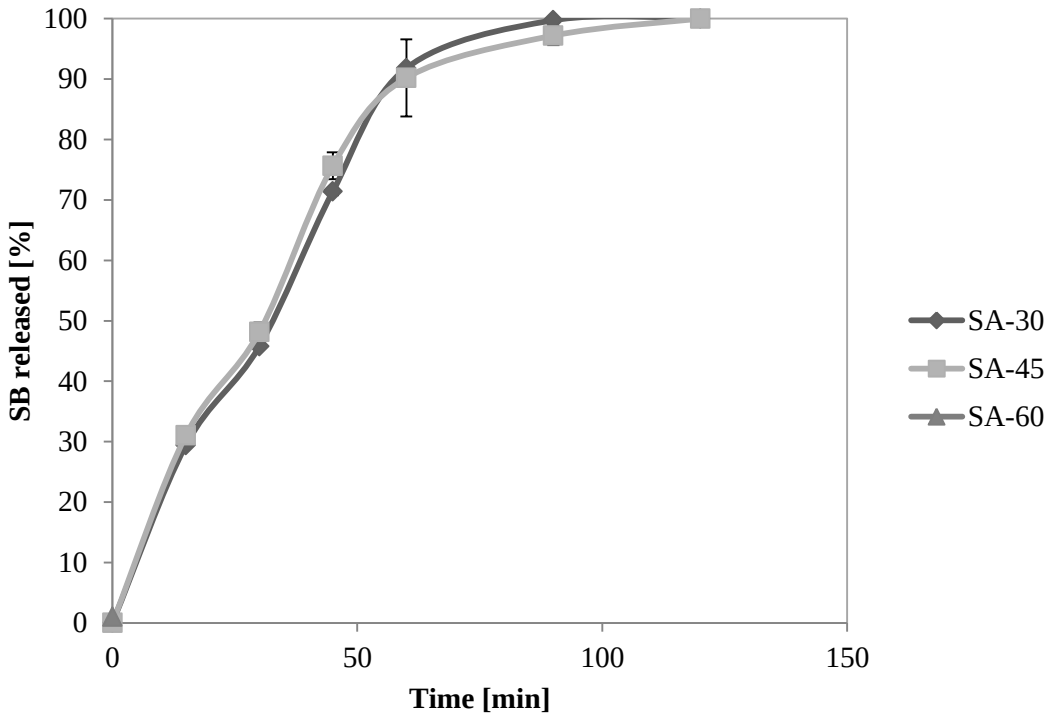


Figure 3.29. *Dissolution profiles of tablets containing MA and SA.*

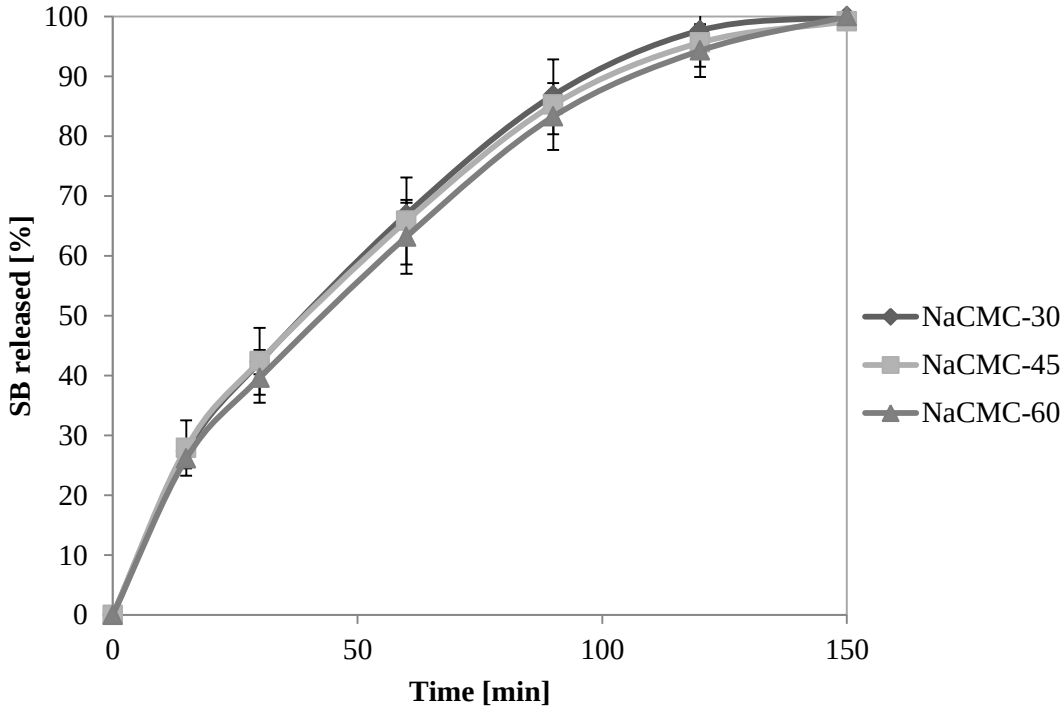


Figure 3.30. *Dissolution profiles of tablets containing MA and NaCMC.*

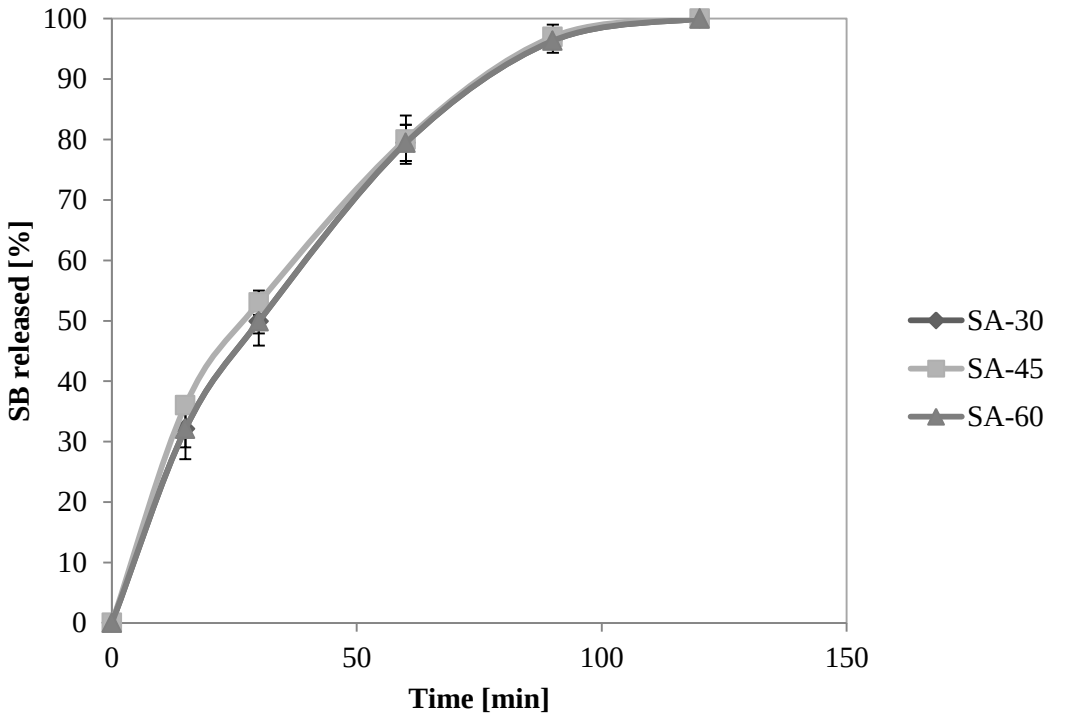


Figure 3.31. *Dissolution profiles of tablets containing CP and SA.*

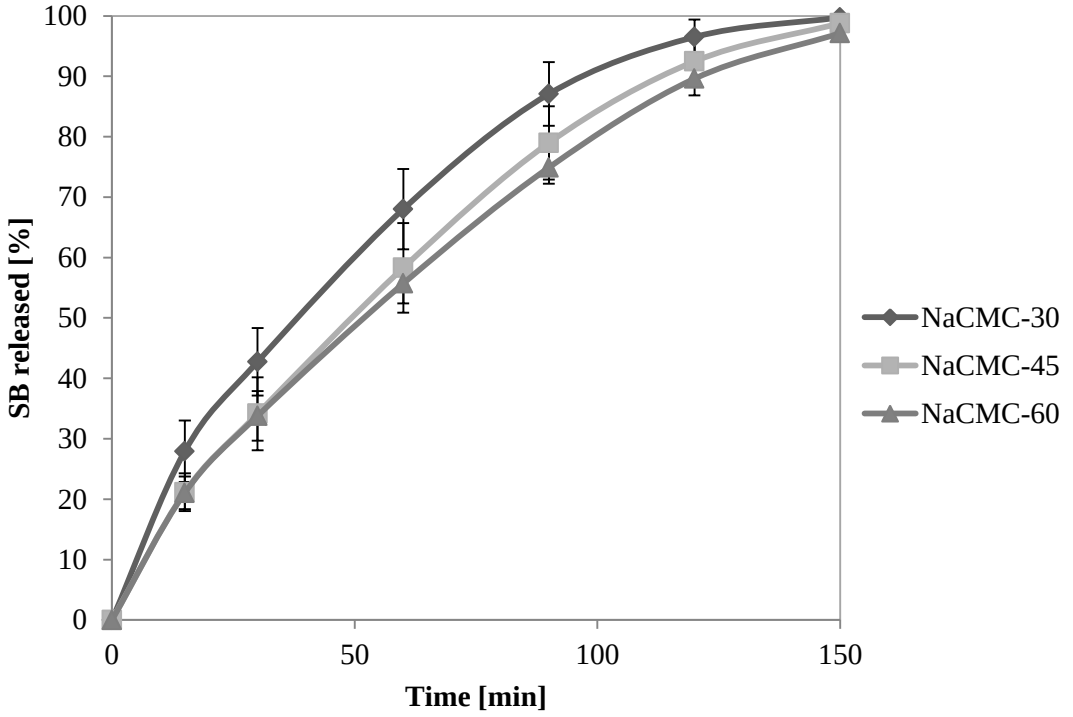


Figure 3.32. *Dissolution profiles of tablets containing CP and NaCMC.*

3.5 Conclusions

In this phase of the research the number of polymers studied was expanded and the tensile test was optimized.

Among the studied properties of the polymer and the dosage form, the most important one for mucoadhesion seems to be the polymer molecular weight.

Two polymers having the best mucoadhesive properties (SA and NaCMC) were selected to continue the study and sodium butyrate was chosen as model drug.

Formulations containing different amounts of polymers were tested in order to identify the relationship between polymer concentration and mucoadhesion.

The results showed that the higher the amount of polymer, the greater the mucoadhesive properties. The dissolution profiles of SB seem to be not significantly influenced by the formulation variables since the drug is very soluble in water.

Chapter 4

Development of sustained-release mucoadhesive tablets

4.1 Introduction

4.1.1 Aim

The aim of this study was to develop sustained-release mucoadhesive tablets containing sodium butyrate or mesalazine using the Design of Experiments techniques (DoE).

For this purpose the range of polymers was further expanded including a polymer with well-known extended-release properties, i.e. hydropylmethylcellulose.

4.1.2 Design of Experiments (DoE)

A process can be represented as a combination of operations which transform inputs (e.g. raw materials) in outputs (e.g. finished product). It may be influenced by controllable and measurable factors (e.g. temperature, concentration and pH), and non-controllable factors (e.g. impurities), both able to affect the characteristics of the experimental response. Thus, the knowledge of these factors permits to control the process and the final product characteristics.

The Design of Experiments (DoE) considers the experiment as a system composed of independent variables (experimental factors) and dependent variables (experimental responses). DoE measures and analyzes the effects of the changes in the parameters affecting the system properties (experimental responses).

The term “experimental factor” identifies a parameter supposed to influence the tested phenomenon and whose variation causes a more or less intense variation of the experimental responses, i.e. data obtained experimentally (Phan-Tan-Luu & Cela, 2009).

The experimental factors can be qualitative or quantitative and the alternatives in which they occur are defined levels that identify the experimental domain, or the area of interest of the study.

In the development of the DoE it is necessary to:

1. recognize and state the contest;
2. select the variables and their levels;
3. choose the experimental responses;
4. choose the experimental plan (DoE);
5. perform the experiments;
6. point out data statistical analysis.

In order to obtain an equation expressing the influence of the experimental factors on the response, it is necessary to postulate a mathematical model suitable for the description of the studied phenomenon. The main model used for the study of many systems is a polynomial model of the first, second, or third degree (Phan-Tan-Luu & Cela, 2009).

The number of the model coefficients increases with increasing the degree of the polynomial and, after the third degree, the number of experiments to be carried out becomes extremely high. However, a polynomial of second or third degree generally represents a phenomenon (Phan-Tan-Luu & Cela, 2009).

Once chosen the mathematical model, it is necessary to define the experiments to be performed in order to calculate the model coefficients and to evaluate the effect of the variables on the experimental response.

A set of experiments can be represented by means of the experimental matrices, or "tables" constituted by N lines, corresponding to N experiments, and k columns, corresponding to k variables studied.

The variables are the parameters that will potentially affect the characteristics of the system and they may be qualitative (e.g. the type of excipient) or quantitative (e.g. the pH value). In order to assess the interaction between the variables and the responses, variables must be made comparable to each other by transforming them into codified or normalized variables, according to the equation 4.1:

$$x_i = \frac{U_i - U_i^0}{\Delta U_i} \quad (4.1)$$

where x_i is the value of the normalized variable, U_i is the value of the natural variable, U_i^0 is the value of the natural variable in the middle of the experimental domain, ΔU_i is the range of the natural variable.

Experimental matrices are constructed in terms of normalized values and their choice depends on the postulated model. The experimental plan, which describes the experiments to be performed, is obtained by transforming the normalized values in experimental values.

Once performed the experiments and obtained the experimental responses, it is possible to calculate the coefficients of the postulated model (Phan-Tan-Luu & Cela, 2009).

Screening of independent variables

A system can be influenced by a large number of variables. The screening technique allows to assess whether a particular variable can influence the system by analyzing the change that this variable induces to a certain parameter, assumed to characterize the system of interest (experimental response).

In the screening technique it is assumed that a linear relationship between variables and responses exists, and the model employed will be a first degree polynomial.

To obtain the experimental plan with the minimum number of experiments, the appropriate matrix to the experimental domain must be selected.

Finally, the Analysis of Variance (ANOVA) was performed in order to evaluate whether the experimental response varies significantly in relation to the considered variables. With the study of screening the variables able to influence the system may

be identified since they determine a significant variation of the experimental responses.

Variables resulting not significant for the system may be arbitrarily fixed since their variation, within the experimental domain, does not determine a change of the experimental responses (Cela, et al., 2009).

Study of the effects of variables

The DoE could be also used to study how some selected variables affect the experimental responses and the presence of interactions between them.

In this case the mathematical model that is assumed to describe adequately the system is represented by a second degree polynomial at least. Furthermore, a matrix, able to give a good estimation of the coefficients, must be chosen, e.g. a full factorial matrix involving all combinations between variables.

Also in this case ANOVA and the estimation of the coefficients significance must be carried out.

Finally, the validity of the mathematical model is evaluated by calculating the multiple R-squared (R^2) and Adjusted R-squared (R^2_A).

Study of mixtures

In many product development areas, the application of experiments involving mixtures or blends is quite common. Generally, in mixture studies the interest is in developing better or innovative formulations with optimum characteristics (responses) able to satisfy determined requirements (Voinovich, et al., 2009).

In the case of mixtures, the variables are quantitative and continuous and they show two important properties:

1. they are dependent being their sum equal to 1 or 100% of the mixture composition;
2. they are dimensionless.

Shape and size of the experimental domain depend on the number of formulation variables considered in the study. For *k* variables, a *k-1* dimensions domain will be obtained.

When *k*=3 the experimental domain is represented by an equilateral triangle, whose vertices correspond to the pure components, the sides to the binary mixtures and the interior points to the ternary mixtures (figure 4.1).

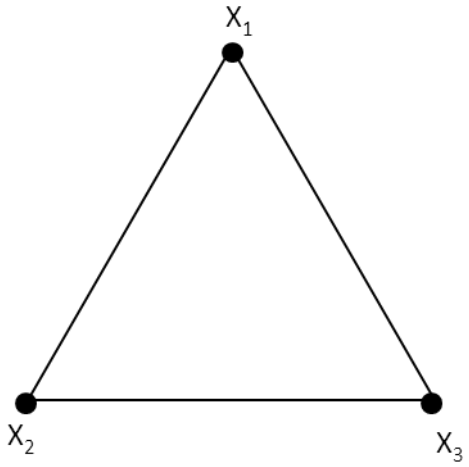


Figure 4.1. *Representation of experimental domain for 3 factors mixtures without constraint.*

It is also possible to limit the experimental domain by introducing quantitative constraints and relational limits between variables. Once defined the experimental domain, a mathematical model, able to describe the system, must be postulated and a matrix suitable to calculate the model coefficients must be chosen.

The ability of the model to describe the system and to predict the experimental response is assessed by calculating R^2 and R^2_A coefficients and by performing some additional experiments (test points).

The choice of the test points is fundamental in order to have correct information about the quality of the predictive capacity of the model (Cornell, 1990). These points should be placed where the variance of the measured value is higher. If at the test points the experimental values are very similar to those estimated by using the model, it can be concluded that the mathematical model is appropriate to describe the system and to predict the experimental responses. Otherwise, if the difference between experimental and calculated values is too high, this means that probably coefficients

have not been estimated with sufficient accuracy and that the model does not fit well the system.

In this case a model of higher degree must be chosen to describe the complexity of the system and a higher number of experiments must be performed in order to have a more accurate measure of the coefficients.

If the model provides a good fitting of data it will be possible to create the isoresponse surfaces describing the variation of the response as a function of the composition of the mixture (figure 4.2). The isoresponse surface could be used to choose the mixture having the desired response.

For systems including several experimental responses, the overlap of the isoresponse surfaces, allows to identify an area of "*optimum*", which contains the mixture composition able to give the best experimental responses (Voinovich, et al., 2009).

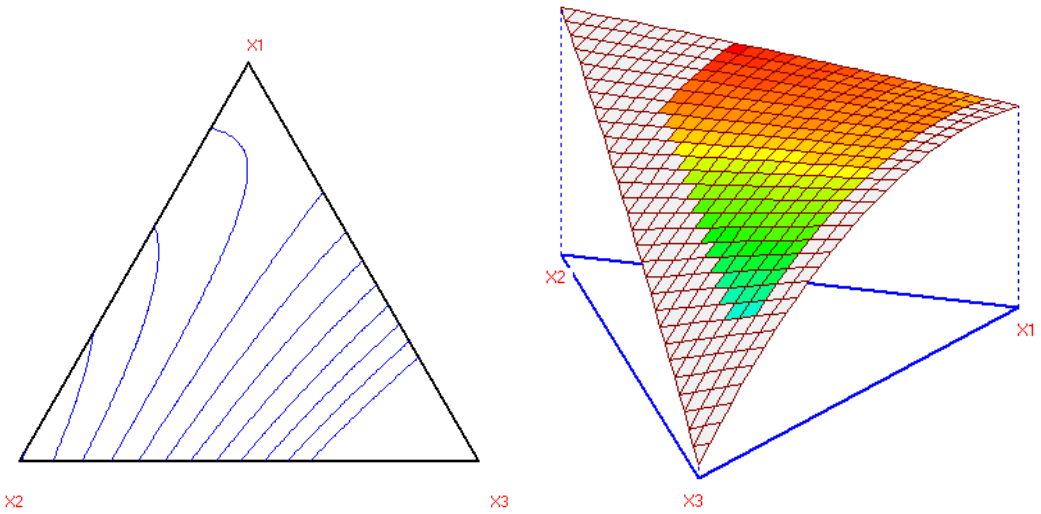


Figure 4.2. *Examples of two-dimensional (left) and three-dimensional (right) isoresponse surfaces.*

4.2 Materials

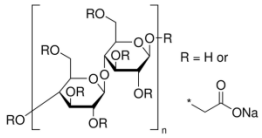
The following were used: sodium carboxymethylcellulose E466 medium viscosity, talc PHARMA USP Ph.Eur., magnesium stearate FU-Ph.Eur., microcrystalline cellulose T1 Ph.Eur., calcium phosphate tribasic E341, mannitol for direct compression, potassium phosphate monobasic, sodium hydroxide and sodium

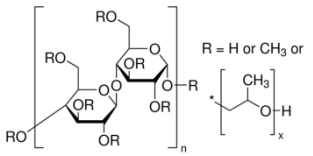
chloride, all nine supplied by A.C.E.F. S.p.A. (Italy); Ludipress® purchased from BASF The Chemical Company (Germany); mucin (from porcine stomach, type II), sodium butyrate 98%, acetonitrile and 5-aminosalicylic acid 95% (Mesalazine), all four supplied by Sigma-Aldrich (USA); hydroxypropylmethylcellulose (Metolose, hypromellose-USP, grade 90SH-100000SR, substitution type 2208, viscosity 100000 mPa*s) purchased from Shin-Etsu Chemical Co., Ltd., (Japan); phosphoric acid and methanol HPLC Gradient Grade supplied by Acros Organics (Belgium) and J.T. Baker® (Netherlands), respectively.

In all preparations of solutions and buffers, deionized water was used.

Characteristics, properties and applications of the new mucoadhesive polymers are described in detail in table 4.1.

Table 4.1. *Properties and applications of the new polymers used in the study.*

Polymers	Properties	Applications
Sodium Carboxymethylcellulose (Sodium Carboxymethylcellulose E466 medium viscosity) Sodium salt of a polycarboxymethyl ether of cellulose.  (Rowe, et al., 2006)	• Hygroscopic polymer, practically insoluble in acetone, ethanol (95%), ether, and toluene; the aqueous solubility varies with the degree of substitution (DS). • Molecular weight ranges from 90-700 kDa. • DS of 0.80; viscosity of a 2% aqueous solution of 470 mPa*s; the pH of a 1% aqueous solution is 7. • High concentrations, usually 3-6%, of the medium-viscosity grade are used to produce gels.	• It is widely used in oral and topical pharmaceutical formulations, mainly for its viscosity-increasing properties. • It may also be used as a tablet binder and disintegrant, and to stabilize emulsions. • Its mucoadhesive properties are used in various pharmaceutical formulations to localize and modify the release kinetics of active principles applied to mucous membranes. • Moreover it can be used to prevent post-surgical tissue adhesions, for bone repair and to realize dermatological patches.
Hydroxypropylmethylcellulose (Metolose, Hypromellose-USP) Partially O-methylated and O-(2-hydroxypropylated) cellulose.	• Nonionic, hygroscopic polymer; soluble in cold water, forming a viscous colloidal solution; practically insoluble in chloroform, ethanol (95%), and ether, but soluble in mixtures of methanol and dichloromethane, and mixtures of water and alcohol.	• It is widely used in oral, ophtalmic and topical pharmaceutical formulations. • In oral products, it is primarily used as a tablet binder, in film-coating, and as a matrix for use in extended-release tablet formulations. • It is also used as an emulsifier,



(Rowe, et al., 2006)

- A 1% [w/w] aqueous solution exhibits a pH of 6.8.
- A 2% aqueous solution shows a viscosity of 100000 mPa*s at 20°C.
- Methoxy content: 23.4%; hydroxypropoxy content: 9.5%.
- The molecular weight is approximately 10-1500 kDa.

suspending agent, thickening agent and stabilizing agent in topical formulations.

- Moreover, it is used in the manufacture of films, capsules, as an adhesive in plastic bandages, and as a wetting agent for hard contact lenses.

4.2.1 5-aminosalicylic acid (Mesalazine)

5-aminosalicylic acid or Mesalazine (ME) (figure 4.3) is a nonsteroidal anti-inflammatory drug (NSAID), belonging to the broader category of amino salicylic acids, with a selective action on the intestinal mucosa. ME exhibits a solubility of 0.965 mg/mL and 3.2 mg/mL in phosphate buffer pH 6.8, both measured at 20°C. It is used for the treatment of inflammatory diseases of the gastro-intestinal tract (Bondesen, et al., 1987). ME presents a topical anti-inflammatory action on the intestinal mucosa thanks to its very slow absorption. Its mechanism of action involves the inhibition of the production of chemical mediators of inflammation such as arachidonic acid metabolites (prostaglandins, thromboxanes and leukotrienes).

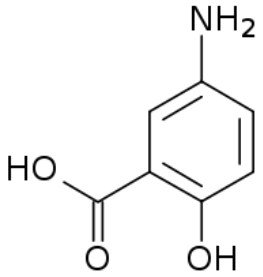


Figure 4.3. *Structural formula of Mesalazine.*

4.3 Methods

4.3.1 Determination of intrinsic viscosity and Viscosity Average Molecular Weight of polymers

Viscosity average molecular weights of sodium carboxymethylcellulose and hydroxypropylmethylcellulose were measured using the method described in Section 2.3.1, at 20°C for sodium carboxymethylcellulose and at 25°C for hydroxypropylmethylcellulose.

The operative conditions, i.e. the solvent and K and α values used for the two polymers are reported in table 4.2.

Table 4.2. *Operating conditions used for each polymer.*

Polymer	Solvent	K ($x10^3$) [$dL*g^{-1}$]	α [-]	References
Sodium carboxymethylcellulose	NaOH 0.5 M	0.5370	0.730	(Eremeeva & Bykova, 1998)
Hydroxypropylmethylcellulose	Water	0.3390	0.880	(Vázquez, et al., 1996)

4.3.2 Powder flowability measures

Powder flowability (polymers in pure form and in mixture) was evaluated by means of the method described in Section 2.3.2.

4.3.3 Preparation of mucoadhesive tablets

Tablets were prepared by direct compression of the powders, using a single punch tablet press (COSALT type, Officina CO.STA. S.r.l., Italy) fitted with a flat-faced circular punch (5 mm diameter). The weight of the tablets ranges from 49 to 114 mg and the thickness ranges from 3 to 4 mm.

4.3.4 Technological characterization of tablets

The tests of uniformity of mass and tablet crushing strength were performed according to the methods described in Sections 3.3.4 and 2.3.4, respectively.

4.3.5 Evaluation of tablets behavior in aqueous medium

The evaluation of tablets behavior in aqueous medium was performed by measuring the water uptake and the swelling of tablets with the method described in Section 2.3.5 using phosphate buffer as medium. Moreover, the wettability of placebo tablets was measured according to the method described in Section 3.3.5 (Wettability and Contact Angle).

4.3.6 Tensile Test for the detection of tablets mucoadhesive properties

The assessment of the mucoadhesive properties of the tablets was performed according to the method indicates as procedure 3 and described in Section 3.3.6.

4.3.7 Dissolution test

Tablets containing sodium butyrate and mesalazine were subjected to dissolution test, performed according to FUI XII ed. (F.U.I., 2008), with a dissolution apparatus 2 (Sotax AT7 Smart, Sotax, Switzerland) at 100 rpm. The dissolution tests were carried out at 37±0.5°C in 900 mL of simulated intestinal fluid (phosphate buffer pH 6.8) as dissolution medium. During the release studies, 1 mL of dissolution medium sample was removed and filtered; SB e ME quantifications were performed using the methods reported in Sections 3.3.8 and 4.3.8, respectively. The volume removed was replaced each time with fresh medium. Results are averaged from three replicated experiments.

4.3.8 Analytical method for the determination of mesalazine

The quantitative determination of mesalazine was realized by UV-Vis spectrophotometric analysis (UV-Vis spectrophotometer, Varian Cary 50 Scan, Agilent Technologies, USA) using a detection wavelength of 327 nm. In figure 4.4 is reported an example of the spectrum.

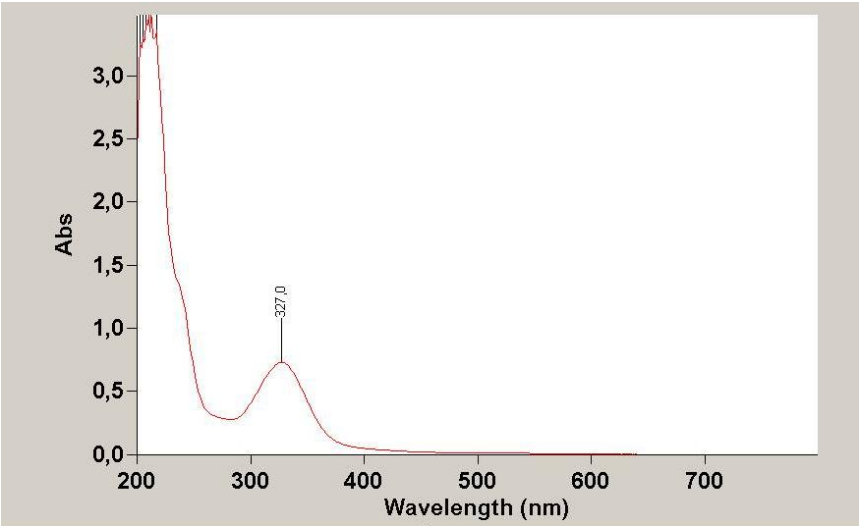


Figure 4.4. *Example of the absorption spectrum of mesalazine in phosphate buffer pH 6.8.*

4.3.9 Planning of experiments and data analysis

Experiments were planned using the software Nemrodw (NewrodW software version 2000-D, D. Mathieu, J. Nony, R. Phan-Tan-Luu, , LPRAI Marseille France).

4.4 Results and Discussion

In order to develop sustained-release mucoadhesive tablets, hydroxypropylmethylcellulose (HPMC), a non-ionic polymer well-known for its extended-release properties, was tested (Rahman, et al., 2010).

The properties of HPMC have been compared with those of the other polymers previously studied.

In this phase of the study a different type of sodium carboxymethylcellulose was used because of the necessity to change the supplier. In particular, the new NaCMC, indicated as NaCMC-B, presents different degree of substitution and viscosity and consequently it needed to be characterized again.

HPMC and NaCMC-B were subjected to the technological characterizations already planned for the other mucoadhesive polymers.

Mixtures consisting in 60% [w/w] of polymer and 40% [w/w] of excipients blend (Ludipress®, microcrystalline cellulose T1, magnesium stearate and talc) were

prepared and subjected to flowability test. The results suggest that the powders have a discrete flowability and thus they provide a uniform filling of the compression chamber (table 4.3).

Table 4.3. *Results of the flowability test of the new polymers.*

Powder	Hausner Index (HI) [-]	Compressibility Index (CI) [-]	Flowability
HPMC-Excipients blend	1.25±0.04	19.83±2.80	Discrete
NaCMC-B-Excipients blend	1.24±0.02	19.41±1.56	Discrete

Therefore, it was possible to realize tablets with a good crushing strength value which ensures their resistance (94.75±12.85 N for HPMC and 63.57±4.28 for NaCMC-B).

Afterward the abilities of hydration and swelling of the new polymers in phosphate buffer pH 6.8 were evaluated in terms of water uptake (WU) and swelling index (SI). The results have been compared with those previously obtained with the other polymers (figure 4.5).

Graphs show that tablets containing polymers with ionic character (SA, NaCMC, NaCMC-B and XG) swell and absorb water in larger amounts than those containing non-ionic polymers (HEC and HPMC). The lower SI of tablets containing TG is presumably due to the chemical characteristics of the polymer. Indeed it is characterized by a very complex structure and by the presence of fractions having different solubility.

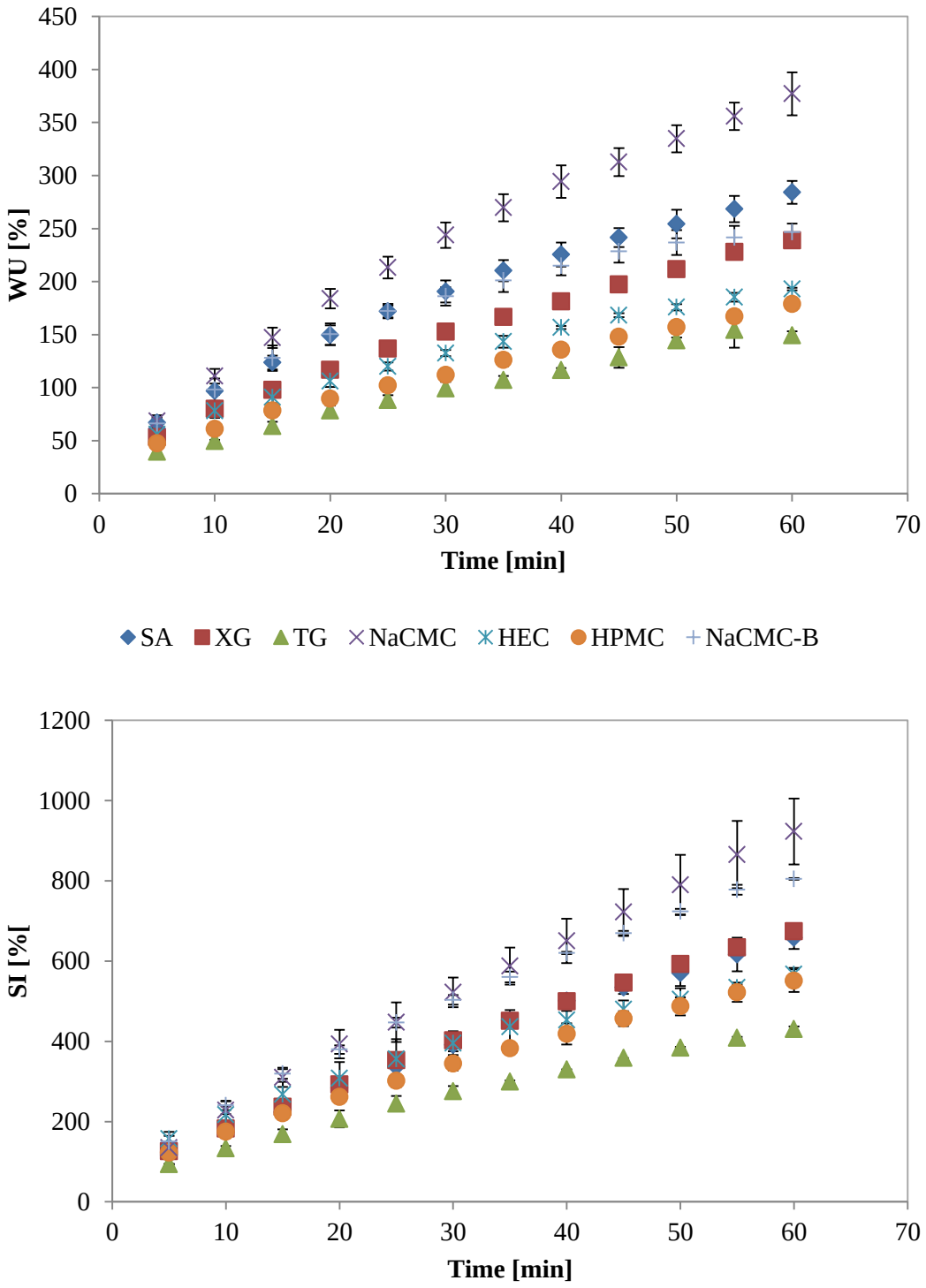


Figure 4.5. *Water uptake [%] and swelling index [%] profiles of the tablets in phosphate buffer pH 6.8: comparison between different polymers.*

The wettability of the new tablets was evaluated by measuring the contact angle between water and tablets.

Figure 4.6 shows the contact angle values of the first 3.5 seconds after the deposition of the drop of water on tablets containing the various polymers.

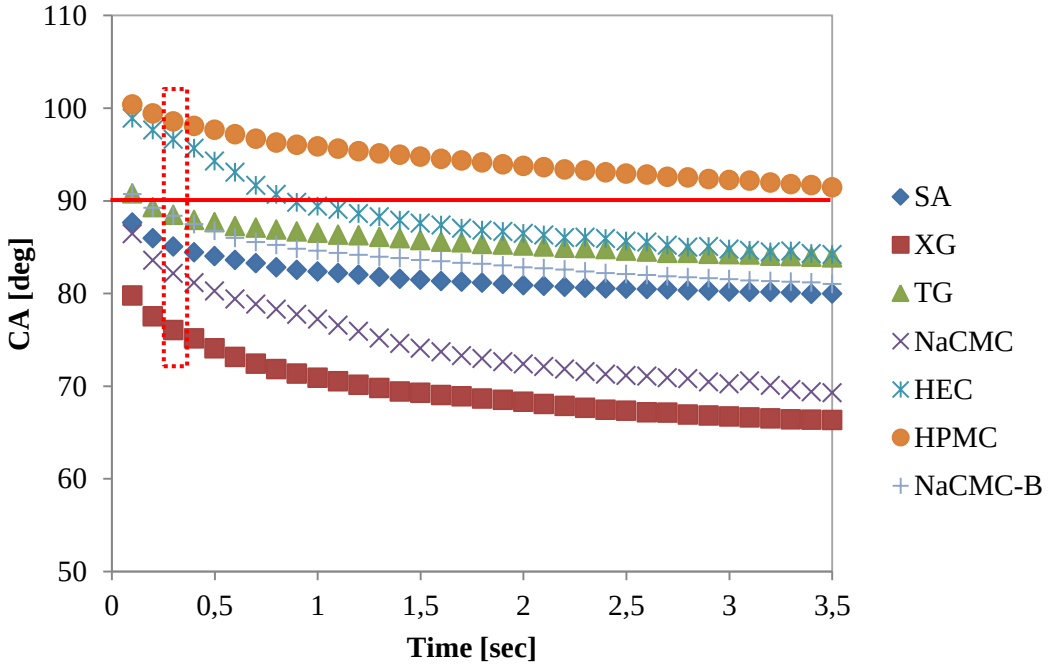


Figure 4.6. *Tablets contact angles (CA [deg]). The horizontal line divides the values of angle less than 90°, expression of good wettability, from the values of angle between 90° and 180°, expression of poor wettability.*

Considering the average contact angles corresponding to 0.3 seconds after drop deposition (red dotted box), the non-ionic polymers (HEC and HPMC) show values of contact angle higher than 90°, unlike the other polymers. These results match the water uptake and swelling index data. Therefore, the assessment of tablets wettability may represent a complementary method for the evaluation of the polymer behavior in aqueous medium.

The molecular weights of the two new polymers were determined by viscosimetric method and they resulted 117 kDa for NaCMC-B, and 96 kDa for HPMC.

Then mucoadhesive properties of tablets containing NaCMC-B and HPMC were measured in terms of maximum detachment force (F_{max}) and work of adhesion (W_{ad}) using the procedure 3 (table 3.5, Section 3.3.6). Figure 4.7 shows the values of F_{max} and W_{ad} of all seven kinds of tablets.

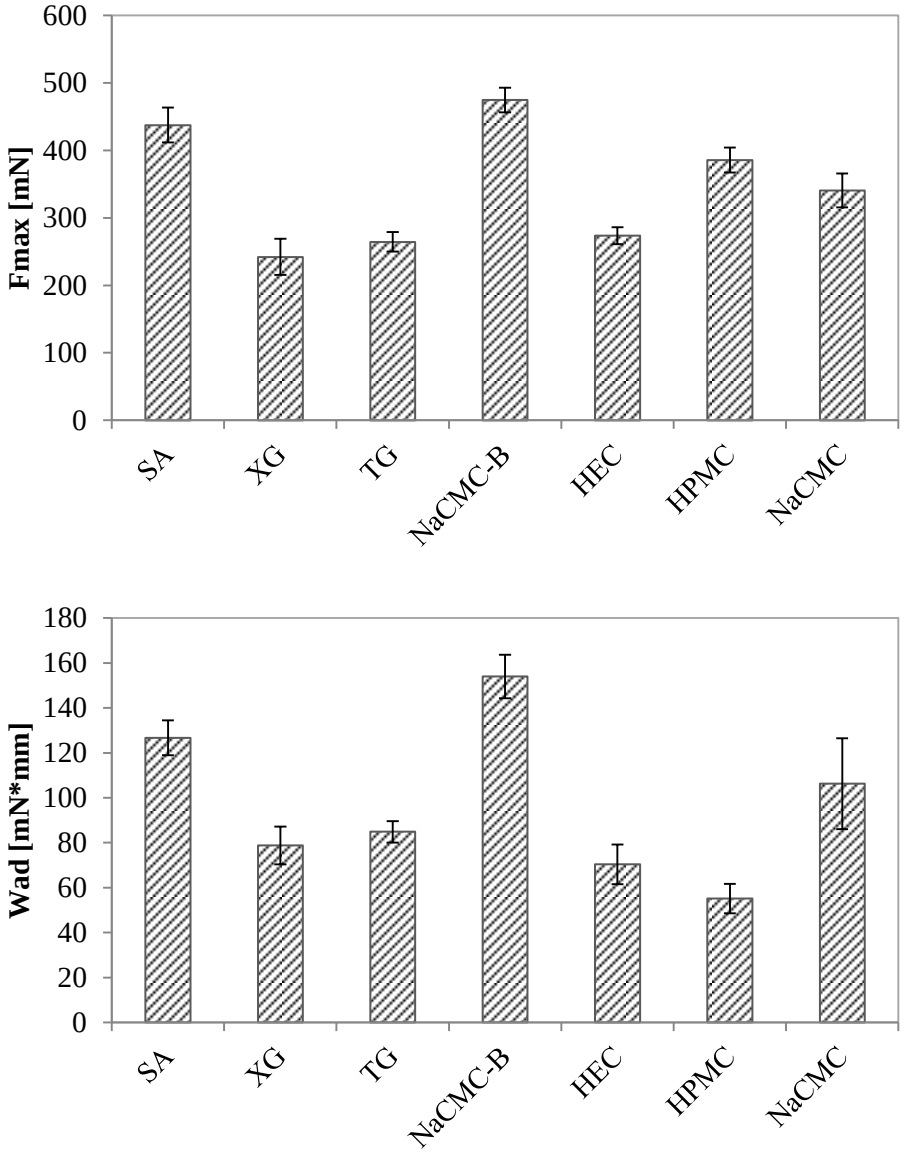


Figure 4.7. *Fmax [mN] (top) and Wad [mN*mm] (bottom) values of the tablets. The tensile test is performed using procedure 3.*

Results confirmed previous data: polymers having the best mucoadhesive properties (highest F_{max} and W_{ad}) are SA and sodium carboxymethylcellulose (NaCMC,

NaCMC-B). In addition, HPMC presented high F_{max} but the W_{ad} value was lower than expected.

The Texture analyzer used for the tensile strength measurements produces very narrow peaks, which can make the measurement of the area under the curve unreliable. This could explain the lack of consistency between the W_{ad} and F_{max} results (Ivarsson & Wahlgren, 2012). Thus only the F_{max} measurements are included in the following discussion.

The mucoadhesive properties of the tablets do not match the WU and SI data because the phenomenon of mucoadhesion is complex and influenced by numerous parameters. Hence, probably the hydration and swelling of the polymer are not the key factors in determining the mucoadhesion.

Comparing the molecular weights of polymers with their mucoadhesive properties (F_{max}) it is possible to confirm the trend previously observed: the lower the molecular weight the higher the mucoadhesive properties (figure 4.8). In particular the best mucoadhesive properties are obtained with polymers having a molecular weight around 100 kDa.

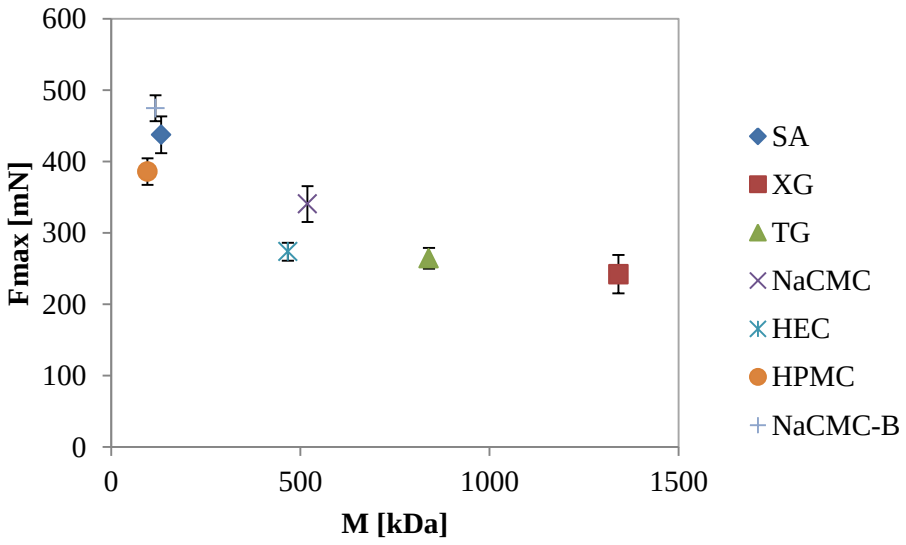


Figure 4.8. *Relationship between polymers molecular weight (M [kDa]) and the mucoadhesive properties (F_{max} [mN]) of the tablets in phosphate buffer pH 6.8 (procedure 3).*

To carry out the study, two polymers with different ionic character, NaCMC-B and HPMC, were selected. The choice was determined according to the results concerning the mucoadhesive properties (NaCMC-B), and with the aim to obtain a sustained-release formulation (HPMC).

In order to assess if variables of formulation and the type of production process (direct compression or granulation-compression) influence the mucoadhesive properties and the release rate of the drug (experimental responses), the Design of Experiments techniques were employed.

The formulation variables considered in the study are the following:

- type of polymer (NaCMC-B or HPMC);
- type of API (sodium butyrate, SB or mesalazine, ME);
- type of diluent (calcium phosphate, CP or mannitol, MA).

The variables (x_i) selected for the study and their levels are reported in table 4.4.

Table 4.4. *Qualitative independent variables considered in the study of screening.*

VARIABLE	DESCRIPTION	ASSUMED LEVELS	NORMALIZED LEVELS
X1	TYPE OF API	SB	-1
		ME	+1
X2	TYPE OF POLYMER	NaCMC-B	-1
		HPMC	+1
X3	TYPE OF EXCIPIENT	MA	-1
		CP	+1
X4	TYPE OF PRODUCTION PROCESS	DIRECT COMPRESSION	-1
		GRANULATION-COMPRESSION	+1

In order to evaluate if the selected variables were able to influence the two experimental responses, a DoE for the screening of the independent variables was used. The two selected experimental responses were: the maximum detachment force

(F_{max} – Y1) to describe the mucoadhesive properties of the tablet and the time necessary to obtain the release of 50% of the drug (T_{50} – Y2) to describe drug release kinetics.

During the screening, quantitative composition of the tablets were maintained constant. All the formulations contain: active ingredient 20% [w/w], polymer 45% [w/w], excipient 33% [w/w]. The formulation was completed with a mixture of talc and magnesium stearate (1:1) 2% [w/w], as lubricant for the compression process.

Figure 4.9 shows the graphical representation of the screening.

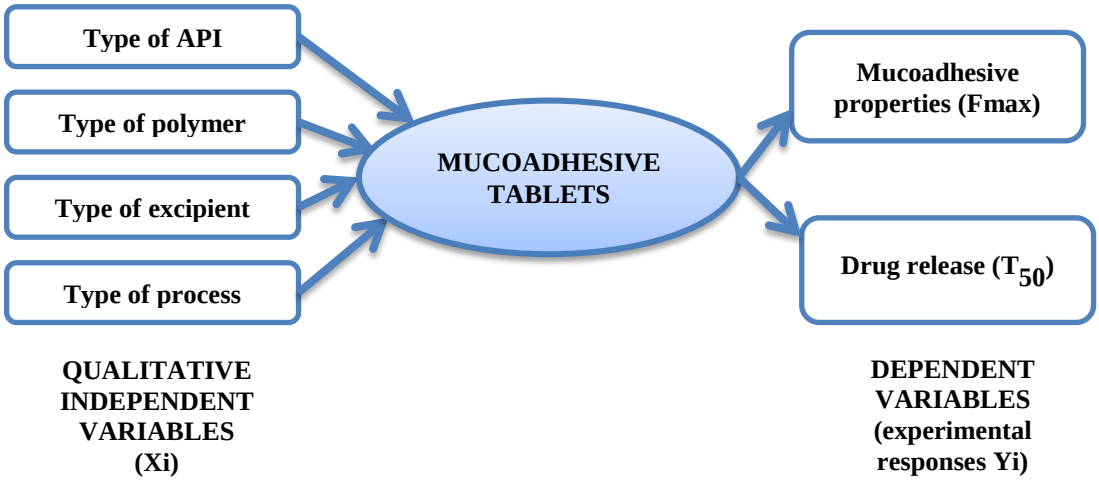


Figure 4.9. *Graphical representation of the variables selected for the screening.*

The mathematical model postulated for the screening of the four experimental variables is:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 \quad (4.2)$$

The experiments necessary to estimate the coefficients (*bi*) of the mathematical model were designed by employing a Hadamard matrix.

The Hadamard matrix for four variables at 2 levels is reported in table 4.5.

Table 4.5. *Hadamard matrix for the analysis of 4 variables at 2 levels.*

N°Exp.	X_1	X_2	X_3	X_4
1	1	1	1	-1
2	-1	1	1	1
3	-1	-1	1	1
4	1	-1	-1	1
5	-1	1	-1	-1
6	1	-1	1	-1
7	1	1	-1	1
8	-1	-1	-1	-1

The matrix expressed in terms of normalized levels is then converted in the experimental plan (table 4.6) that describes the experiments required to estimate the mathematical coefficients.

Table 4.6. *Experimental plan for the screening.*

N°Exp	TYPE OF API	TYPE OF POLYMER	TYPE OF EXCIPIENT	TYPE OF PRODUCTION PROCESS
1	ME	NaCMC-B	CP	DIRECT COMPRESSION
2	ME	HPMC	CP	DIRECT COMPRESSION
3	SB	NaCMC-B	MA	DIRECT COMPRESSION
4	SB	HPMC	MA	DIRECT COMPRESSION
5	SB	NaCMC-B	CP	GRANULATION-COMPRESSION
6	SB	HPMC	CP	GRANULATION-COMPRESSION
7	ME	NaCMC-B	MA	GRANULATION-COMPRESSION
8	ME	HPMC	MA	GRANULATION-COMPRESSION

All the experiments were performed and some repetitions were carried out in order to estimate the variance.

Tablets were characterized by mucoadhesive test (procedure 3) and dissolution test and the results are reported in table 4.7.

The experimental responses were used to estimate the coefficient of the mathematical model, instead the ANOVA was performed in order to validate the analysis.

Table 4.7. *Experimental responses obtained for each experiment.*

N°Exp	X1	X2	X3	X4	Y1	Y2
	TYPE OF API	**TYPE OF POLYMER**	**TYPE OF EXCIPIENT**	**TYPE OF PRODUCTION PROCESS**	**Fmax [mN]**	**T_{50} [min]**
1	ME	NaCMC-B	CP	DIRECT COMPRESSION	422±55	65±1
2	ME	HPMC	CP	DIRECT COMPRESSION	450±50	300±2
3	SB	NaCMC-B	MA	DIRECT COMPRESSION	254±9	45±1
4	SB	HPMC	MA	DIRECT COMPRESSION	215±57	45±1
5	SB	NaCMC-B	CP	GRANULATION -COMPRESSION	304±53	45±1
5*	SB	NaCMC-B	CP	GRANULATION -COMPRESSION	300±50	40±2
6	SB	HPMC	CP	GRANULATION -COMPRESSION	360±52	30±1
7	ME	NaCMC-B	MA	GRANULATION -COMPRESSION	423±23	50±2
8	ME	HPMC	MA	GRANULATION -COMPRESSION	444±44	210±3

* the test was repeated for the calculation of the standard deviation

The analysis revealed that the variables able to influence the maximum detachment force (Y1) are the type of active ingredient and the type of excipient (figure 4.10).

This means that the mucoadhesive properties vary significantly depending on the delivered drug and the type of the excipient.

On the other hand, variables able to significantly influence the drug release (Y2) are the type of active ingredient and the type of polymer (figure 4.10).

Results show that the type of production process has no influence on both the experimental responses; for this reason, this variable has been fixed and all tablets were then produced by direct compression.

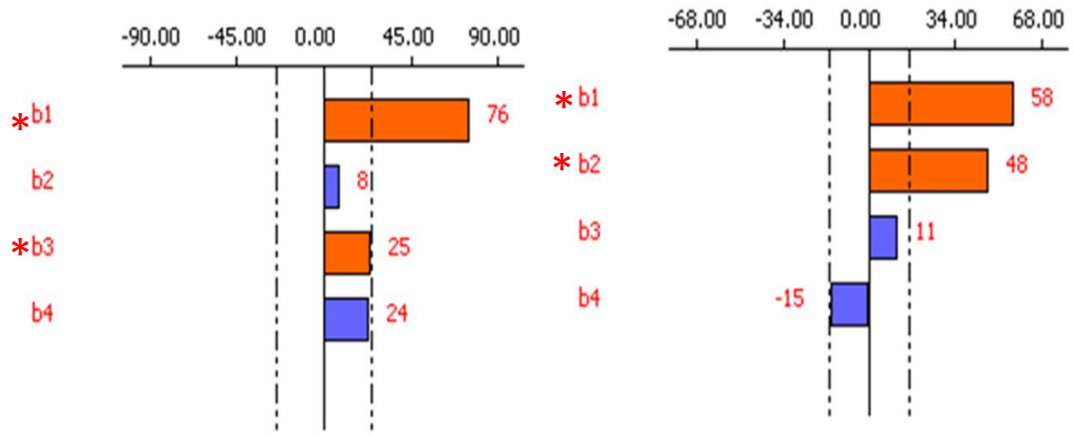


Figure 4.10. Graphical representation of the significance of the estimated coefficients for the responses Y1 (left) and Y2 (right). The asterisk (*) marks the significant variables.

In order to assess the type of the effect exerted by the formulation variables on the experimental responses, a DoE for the study of the effects of qualitative variables was used (table 4.8).

Table 4.8. *Variables for the study of effects.*

VARIABLES	DESCRIPTION	ASSUMED LEVELS	NORMALIZED LEVELS
X1	TYPE OF API	SB	-1
		ME	+1
X2	TYPE OF POLYMER	NaCMC-B	-1
		HPMC	+1
X3	TYPE OF EXCIPIENT	MA	-1
		CP	+1

For this purpose, a second degree polynomial model was postulated:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 \quad (4.3)$$

This model takes into account not only the effect of the single experimental variables but the presence of the interactions, too.

The experiments necessary to estimate the 7 coefficients of the mathematical model are designed using a full factorial matrix (2^3//8), which considers all the possible combinations between the variables and corresponds to the experimental plan shown in table 4.9.

Table 4.9. *Experimental plan for the study of the effect of variables.*

N°Exp.	**X1**	**X2**	**X3**
	TYPE OF API	**TYPE OF POLYMER**	**TYPE OF EXCIPIENT**
1	SB	NaCMC-B	MA
2	ME	NaCMC-B	MA
3	SB	HPMC	MA
4	ME	HPMC	MA
5	SB	NaCMC-B	CP
6	ME	NaCMC-B	CP
7	SB	HPMC	CP
8	ME	HPMC	CP

The composition of the tablets was maintained constant during the study. All the formulations contain: active ingredient 20% [w/w], polymer 45% [w/w], excipient 33% [w/w], and a mixture of talc and magnesium stearate (1:1) 2% [w/w]. All the experiments were performed and the experimental responses (F_{max} - Y1 and T_{50} - Y2) were evaluated. Results are reported in table 4.10.

The experimental responses were used to estimate the coefficient of the mathematical model, while the ANOVA was performed in order to validate the analysis.

Table 4.10. *Experimental responses obtained from each experiment.*

N°Exp.	X1	X2	X3	Y1	Y2
	TYPE OF API	TYPE OF POLYMER	TYPE OF EXCIPIENT	Fmax [mN]	T_{50} [min]
1	SB	NaCMC-B	MA	254±10	45±2
2	ME	NaCMC-B	MA	415±23	40±5
3	SB	HPMC	MA	215±42	45±1
4	ME	HPMC	MA	420±27	240±10
5	SB	NaCMC-B	CP	255±25	25±1
5*	SB	NaCMC-B	CP	220±34	20±3
6	ME	NaCMC-B	CP	422±56	65±2
7	SB	HPMC	CP	372±25	25±1
8	ME	HPMC	CP	450±52	300±5

* the test was repeated for the calculation of the standard deviation

Data highlighted that Y1 or maximum detachment force is influenced by the type of the drug, while the kinetics of release (Y2) is influenced by the type of drug, type of polymer, and by the interactions API-polymer and API-excipient (figure 4.11).

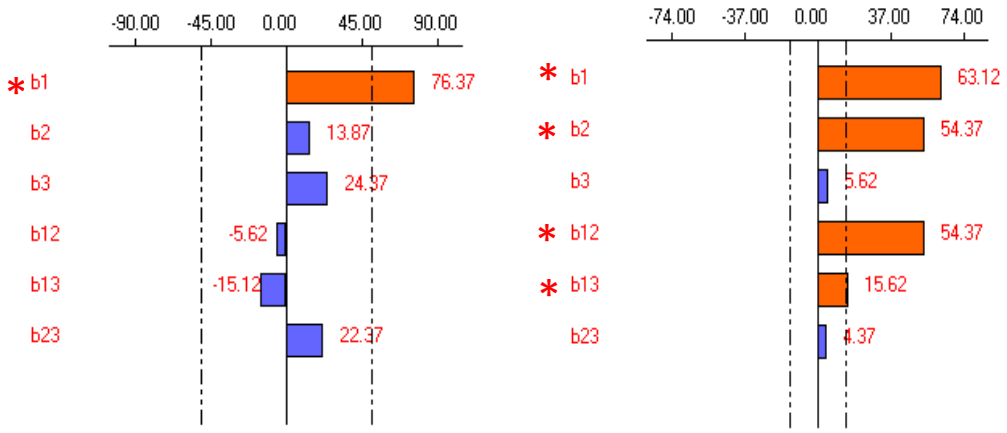


Figure 4.11. *Graphical representation of the significance of the estimated coefficients for the responses Y1 (left) and Y2 (right). The asterisk (*) marks the significant variables.*

The graphical analysis of the effects shows that, for the same polymer used, the replacement of sodium butyrate with mesalazine leads to an increase in the maximum detachment force (figure 4.12).

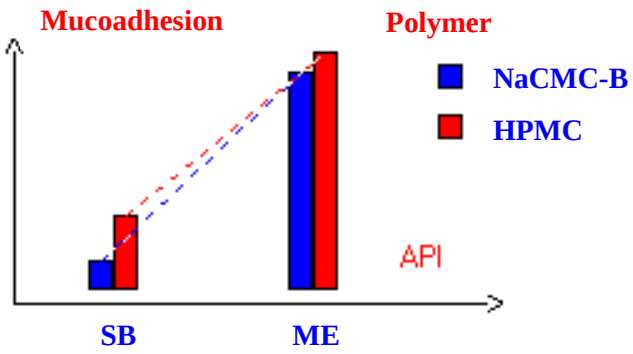


Figure 4.12. *Graphical representation of the influence of the type of API on the mucoadhesive properties.*

Figure 4.13 shows the graphical representation of the effect of the type of active ingredient and the type of polymer on T_{50} values.

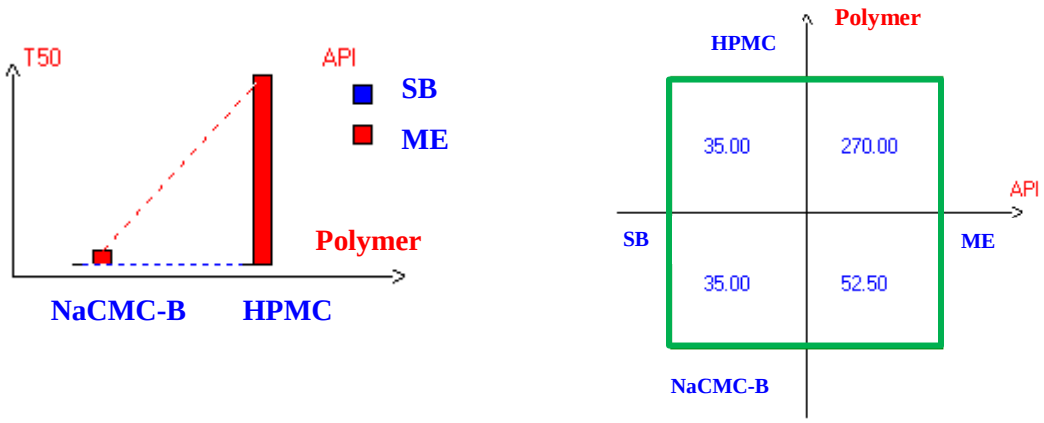


Figure 4.13. *Graphical representation of the effect of the type of API and the type of polymer on T_{50} values.*

In presence of sodium butyrate, drug release rate is always fast, due to its high solubility, instead for mesalazine, a poor water soluble drug, the release rate is markedly affected by the type of polymer.

In this case the slower release rate is obtained in presence of HPMC probably due to the creation of a more viscous gel layer. The co-presence of a water insoluble excipient can lead to a further slowdown in drug release rate.

Results highlight that (figure 4.14):

- the replacement of sodium butyrate with mesalazine leads to an increase in the mucoadhesive properties and in the time required to obtain the drug release;
- the replacement of NaCMC-B with HPMC leads to a significant increase in the T_{50} in the case of mesalazine;
- the API-polymer and API-excipient interactions are relevant in determining the drug release rate. In particular, the poorer the water solubility of the API and the excipient, the slower the drug release.

Consequently, the formulations that allow obtaining the better mucoadhesive properties and the slower dissolution rate of the drug are those containing HPMC as polymer and CP as diluent.

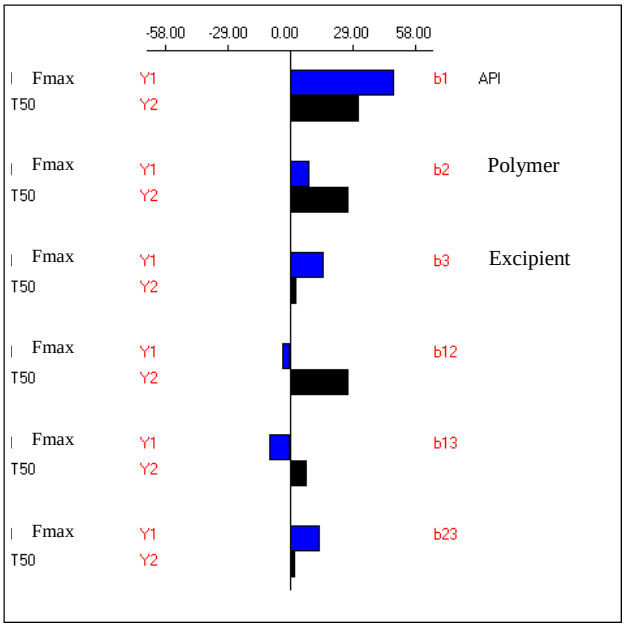


Figure 4.14. *Effects of variables and their interactions on the two experimental responses.*

Finally, a study of mixtures was performed using DoE technique in order to evaluate the influence of the amount of the three formulation variables (SB or ME, CP and HPMC) on the two experimental responses. For this purpose each API was studied separately. To carry out this study the quantitative limits for the components of the mixture were initially fixed (table 4.11), because the final dosage form must contain at least 20% [w/w] of drug and must present a good mucoadhesiveness and slow release.

Table 4.11. *Variables and quantitative limits selected for the study of mixtures.*

VARIABLE	CODE	LOWER LIMIT [%]	HIGHER LIMIT [%]
Amount of API	X1	20	100
Amount of HPMC	X2	10	60
Amount of CP	X3	0	100
Mixture of talc and magnesium stearate			fixed 2%

The quantitative limits of the three variables define the experimental domain shown in figure 4.15.

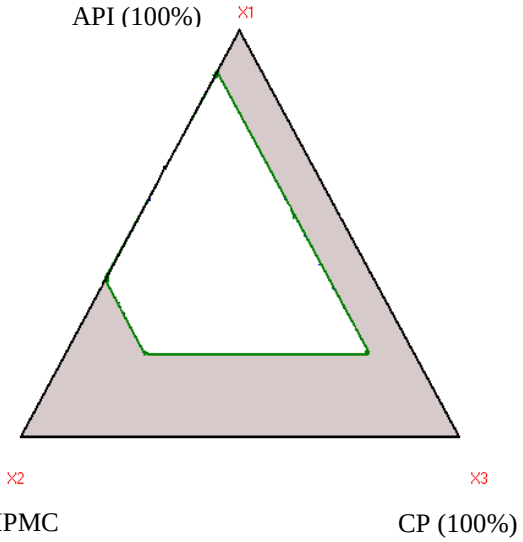


Figure 4.15. *Experimental domain for the study of mixtures.*

The mathematical model selected to describe the relationship between variables and experimental responses is a polynomial equation of the second degree:

$$Y = b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 \quad (4.4)$$

A matrix consisting of 13 experiments is used to estimate the coefficients. In order to reduce the number of the experiments the exchange algorithm was used. The experiments were selected on the basis of three criteria: D-criterion (Det(M)**1/p), the A-criterion o trace criterion (Trace(X'X)-1) and variance function (dMax). The best combination of these three criteria is called optimal and the corresponding design matrix is called optimal design matrix (Cornell, 1990; de Aguiar, et al., 1995).

The optimal design matrix is that having the maximum Det(M)**1/p value, the minimum Trace(X'X)-1 value and the dMax close to 1.

The values of the three criteria are reported in figure 4.16.

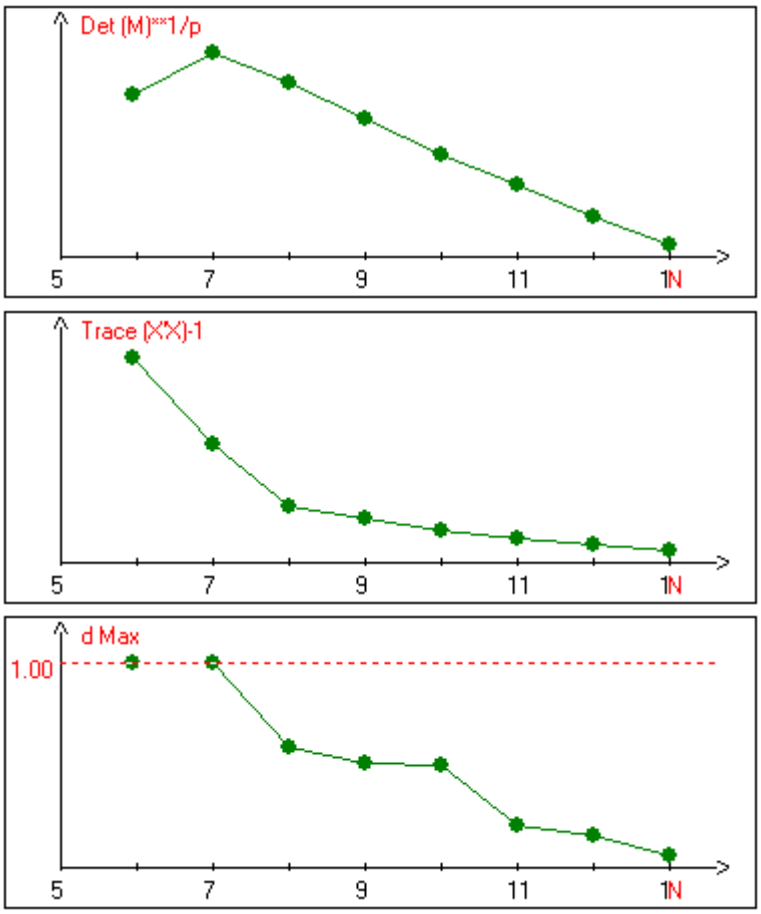


Figure 4.16. *Values of the three criteria considered in the choice of the optimal design matrix.*

On the basis of Det(M)**1/p value, Trace(X'X)-1 and the dMax values the matrix consisting of 7 experiments was selected.
The experiments are resumed in table 4.12.

Table 4.12. *Experimental plan consisting of 7 experiments selected to carry on the study of mixtures.*

N°Exp.	X1	X2	X3
	API [%]	HPMC [%]	CP [%]
1	88	10	0
2	38	60	0
3	20	10	68
4	20	60	18
5	63	35	0
6	54	10	34
7	20	35	43

The experimental plan was realized for both type of API. All the experiments were performed and tablets were subjected to mucoadhesion and dissolution tests.
Results obtained are discussed in the following sections.

- **Tablets containing SB**

Results of tablets containing sodium butyrate are reported in table 4.13.
ANOVA was used to verify the capability of the model to describe the phenomenon and thus its predictive ability.

Table 4.13. *Experimental responses obtained for tablets containing SB.*

N°Exp.	X1	X2	X3	Y1	Y2
	SB [%]	**HPMC [%]**	**CP [%]**	**Fmax [mN]**	**T_{50} [min]**
1	88	10	0	556±48	7±0
2	38	60	0	319±27	35±2
3	20	10	68	204±33	20±1
4	20	60	18	355±33	45±3
5	63	35	0	306±46	30±2
5*	63	35	0	320±37	35±2
6	54	10	34	373±40	7±1
7	20	35	43	306±55	32±2

* the test was repeated for the calculation of the standard deviation

For an easier analysis of the response behavior over the whole experimental domain in function of the three quantitative variables, the isoresponse surfaces were drawn using the software NemrodW (NewrodW software version 2000-D, D. Mathieu, J. Nony, R. Phan-Tan-Luu, , LPRAI Marseille France) (figures 4.17 and 4.18).

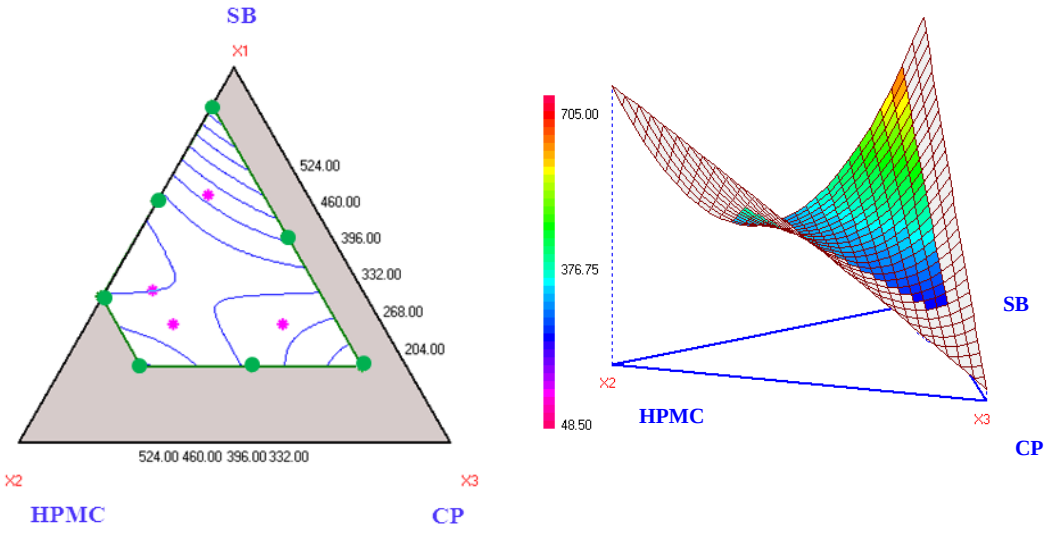


Figure 4.17. *Isoresponse surfaces regarding Y1 (F_{max}), obtained for tablets containing SB (green symbols represent the initial experiments, pink symbols represent the test points).*

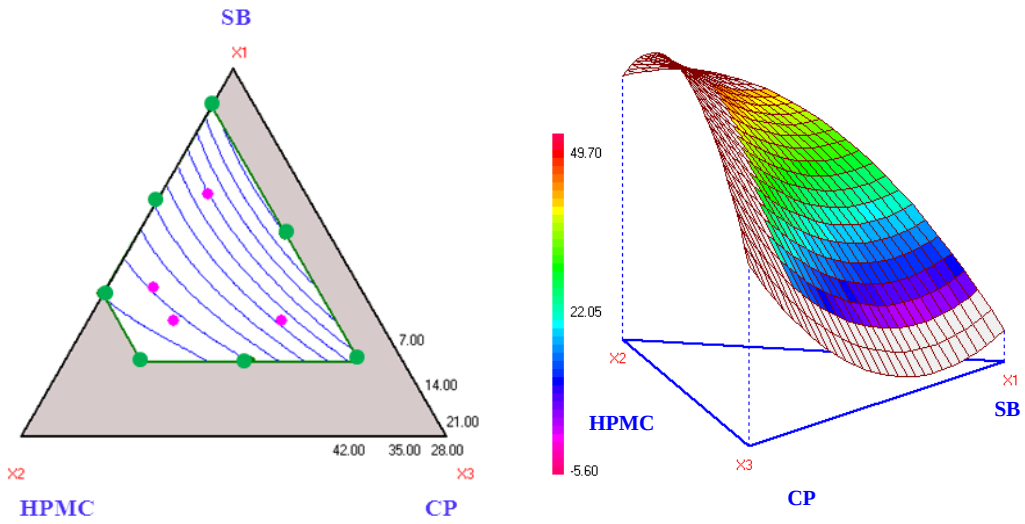


Figure 4.18. *Isoresponse surfaces regarding Y2 (T_{50}), obtained for tablets containing SB (green symbols represent the initial experiments, pink symbols represent the test points).*

Each line of the isoresponse surface represents a specific value of F_{max} or T_{50}.
The obtained surfaces have a curvilinear shape that indicates the presence of a complex system where all variables can influence the responses.

To test if the postulated model is predictive, some additional tests called test points (T000N) have been performed. Experimental values of F_{max} and T_{50} were compared with the values calculated by using the estimated coefficients (table 4.14). The smaller the difference between experimental values and calculated values, the better the predictive ability of the model.
The analysis of the test points demonstrates that the model has a good predictive capacity.

Table 4.14. *Experimental responses of the test points for SB.*

TEST POINTS		EXPERIMENTAL VALUES		CALCULATED VALUES		DIFFERENCE BETWEEN EXPERIMENTAL AND CALCULATED VALUES	
		Y_1 [mN]	Y_2 [min]	Y_1 [mN]	Y_2 [min]	Y_1 [mN]	Y_2 [min]
SB	**T0001**	370 ±62	25 ±1	379	21	9	4
	T0002	319 ±40	40 ±3	306	37	13	3
	T0003	292 ±35	30 ±2	284	26	8	4
	T0004	321 ±48	35 ±2	320	38	1	3

The overlap of the two isoresponse surfaces, obtained for both the experimental responses, allows to identify an area (a combination of the three variables) of the experimental domain, called "optimum" corresponding to formulations with good mucoadhesion, extended-release and high amount of drug (figure 4.19).

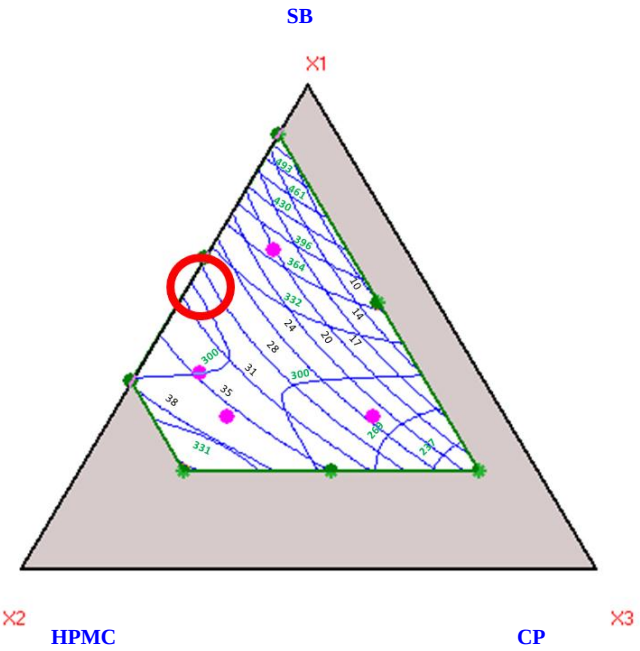


Figure 4.19. *Overlapping of the two isoresponse surfaces for the tablets containing SB: the red circle identifies the optimum (green numbers are the F_{max} values and black numbers are the T_{50} values).*

In the case of SB that area corresponds to a T_{50} value of about 30 minutes, a F_{max} value of about 320 mN and an amount of sodium butyrate of about 55% [w/w].

- **Tablets containing mesalazine**

Results of tablets containing mesalazine are reported in table 4.15.

ANOVA was used to verify the capability of the model to describe the phenomenon and thus its predictive ability.

Results show that the second-degree polynomial model is suitable to describe the system for the two experimental responses.

Table 4.15. *Experimental responses obtained for tablets containing mesalazine.*

N°Exp.	X1	X2	X3	Y1	Y2
	ME [%]	**HPMC [%]**	**CP [%]**	**Fmax [mN]**	**T_{50} [min]**
1	88	10	0	433±53	8±2
2	38	60	0	466±55	360±3
3	20	10	68	342±53	45±5
4	20	60	18	421±71	300±4
5	63	35	0	430±58	450±8
5*	63	35	0	427±48	420±1
6	54	10	34	417±45	7±2
7	20	35	43	405±62	270±5

* the test was repeated for the calculation of the standard deviation

For an easier analysis of the response behavior over the whole experimental domain in function of the three quantitative variables, the isoresponse surfaces were drawn using the software NemrodW (figures 4.20 and 4.21).

The obtained surfaces have a curvilinear shape that indicates the presence of a complex system where all variables can influence the responses.

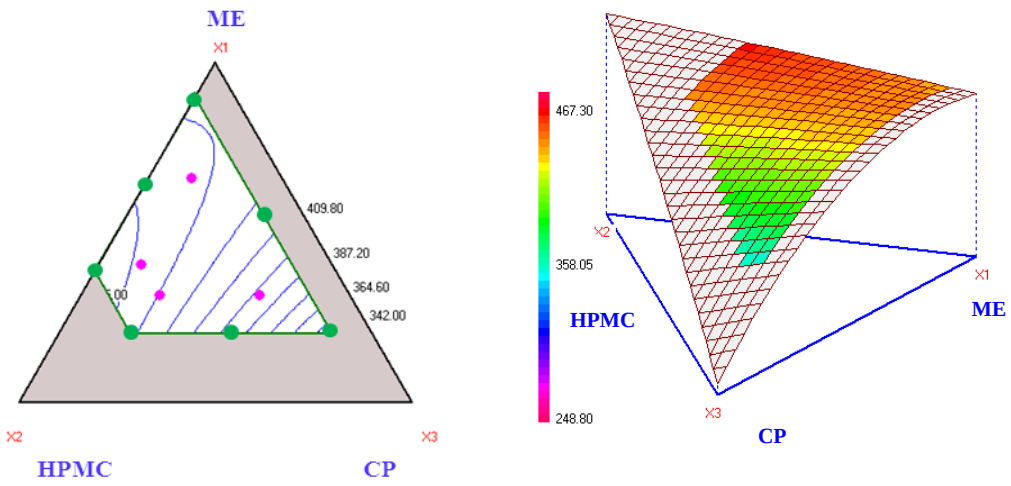


Figure 4.20. *Isoresponse surfaces regarding Y1 (F_{max}), obtained for tablets containing ME (green symbols represent the initial experiments, pink symbols represent the test points).*

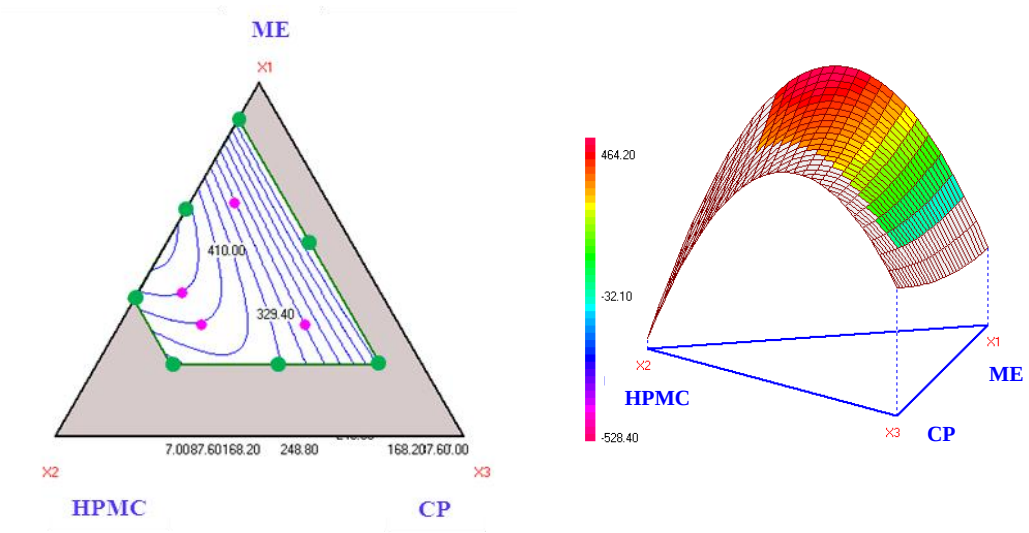


Figure 4.21. *Isoresponse surfaces regarding Y2 (release rate of API), obtained for tablets containing ME (green symbols represent the initial experiments, pink symbols represent the test points).*

Even for tablets containing ME, four test points (T000N) have been performed to verify the predictive ability of the model. Experimental values of F_{max} and T_{50} were compared with the values calculated by using the estimated coefficients (table 4.16). The analysis of the test points demonstrates that the model has a good predictive capacity.

Table 4.16. *Experimental responses of the test points for ME.*

TEST POINTS		EXPERIMENTAL VALUES		CALCULATED VALUES		DIFFERENCE BETWEEN EXPERIMENTAL AND CALCULATED VALUES	
		Y1 [mN]	**Y2 [min]**	**Y1 [mN]**	**Y2 [min]**	**Y1 [mN]**	**Y2 [min]**
ME	**T0001**	434±59	320±5	451	287	17	33
	T0002	454±22	42±7	471	429	17	9
	T0003	410±13	300±2	421	280	11	20
	T0004	488±33	380±3	470	388	18	8

The overlap of the two isoresponse surfaces, obtained for both the experimental responses, allows to identify an area of optimum within the experimental domain, corresponding to formulations with good mucoadhesion, extended-release and high amount of drug (figure 4.22).

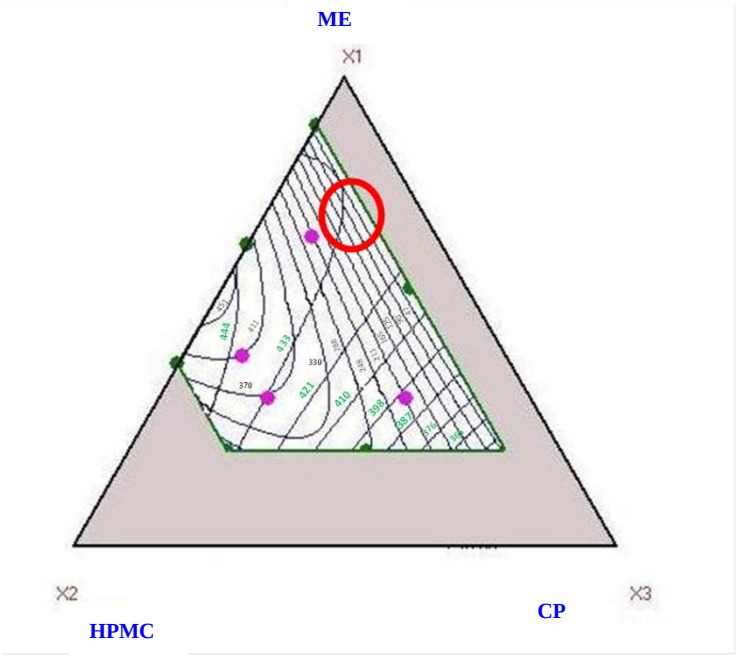


Figure 4.22. *Overlapping of the two isoresponse surfaces for the tablets containing ME: the red circle identifies the optimum (green numbers are the F_{max} values and black numbers are the T_{50} values).*

In the case of ME that area corresponds to a T_{50} value of about 90 minutes, a F_{max} value of about 420 mN and an amount of mesalazine higher than 50% [w/w].
For tablets containing ME, areas with higher values of T_{50} could be selected. However, the preferred value of T_{50} is about 90 minutes, since mucin turnover ranges from 4 to 6 hours in the intestine.

Since polymers used to develop mucoadhesive tablets are able to form a swellable matrix and produce a sustained-release dosage form, some consideration about the drug release can be made.

- **Drug release from hydrophilic swellable matrices**

Swelling-controlled systems, also known as hydrogel matrices, polymeric matrices, hydrocolloid matrices or hydrophilic matrices, can be utilized to modify the drug release rate. Among the different types of swelling-controlled systems, the free-swellable matrices, in which the matrix can swell unhindered, are the most common.
When a swellable matrix is immersed in water, water molecules interact with the hydrophilic groups of the polymer. As the water is further soaked into the matrix, the spaces inside the polymer network are filled and hence the drug particles are dissolved. Water acts as a plasticizer and reduces the polymer glass transition temperature, Tg, until it reaches the temperature of the system; as a consequence, the polymer chains relax, become more flexible and the polymer swells. For example, in the case of HPMC, the glass transition temperature decreases from 184°C to 37°C when the dry form of the polymer is immersed in water (Lofthus, 2005).
The swelling causes great changes in the matrix with regard to the structural organization of the polymer and the mobility of its chains, affecting in this way the drug release.
The most important key factors determining the drug release from a hydrophilic matrix are the following:

- polymer content
- drug:polymer ratio
- drug solubility
- viscosity of polymer

- solubility of excipients
- structure and hydrophilicity of polymer (Lofthus, 2005).

When a swelling matrix is immersed in water it is possible to identify two or three different fronts (see figure 4.23).

Erosion front: is the interface between the outermost edge of the matrix and the water; at this interface the polymer can reach a level of hydration that allows it to disentangle and dissolve, and hence, to erode.

Swelling front: is the front where the polymer swells; the swelling and dissolution properties of the polymer are important in determining the matrix dimensions and the diffusion pathways that the drug may take to leave the system. This front always moves inwards towards the core of the system.

Diffusion front: is present only if the delivered drug has a low solubility or a slow dissolution rate. It is located between the swelling and the erosion fronts. The diffusion front in the rubbery phase of the matrix represents the boundary where the drug becomes dissolved. As the swelling front does, also the diffusion front moves inwards towards the center of the matrix. The diffusion front is present only if the drug dissolves after the polymer has swelled. Since the polymer swells, the drug diffusivity increases as a consequence of the increased water content. When the water concentration exceeds the solubility of the drug, complete dissolution occurs. The drug can then diffuse out of the matrix. As the swelling of the matrix advances inwards towards the center, the diffusional pathway of the drug increases, and so the release rate of the drug will gradually diminish (Lofthus, 2005; Siepmann & Siepmann, 2008).

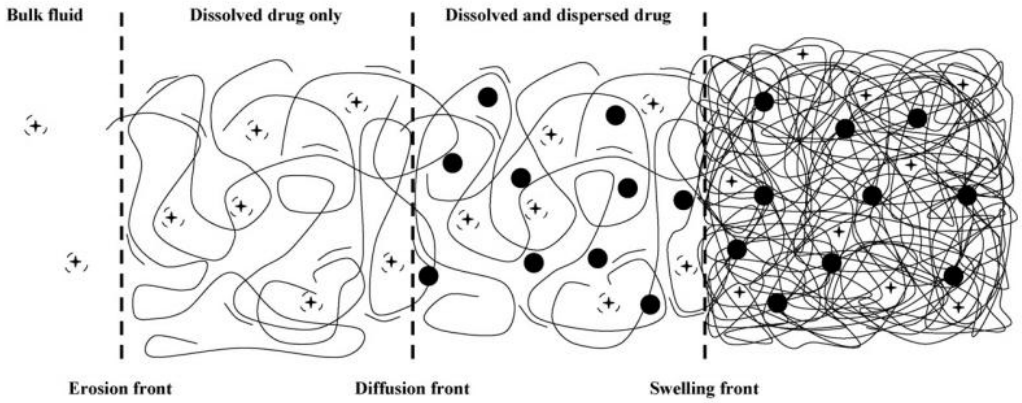


Figure 4.23. *Representation of the three fronts present in a swelling-controlled drug delivery (Siepmann & Siepmann, 2008).*

After the polymer swelled, the drug can be released from the matrix by diffusional mechanisms, (Fickian mechanism) or other mechanisms, such as erosion or convective release. The release of the drug is controlled by the interaction between the solvent, the polymer and the drug, and the kinetics depends on the development of drug gradient in the gel layer. Therefore the thickness of the gel, the drug loading and solubility are the major factors that determine the drug release kinetics. For a non-swellable polymer the drug release is almost solely dependent on diffusion.

Time-independent, non-Fickian or case II transport of the drug can be observed in a two-dimensional film of hydrophilic polymer when polymer dissolution is equal to the polymer swelling. More common, in hydrophilic matrices is the occurrence of a transport mechanism intermediate between Fickian and non-Fickian, namely anomalous transport where the polymer relaxation and erosion of the polymer chains contribute to non-Fickian drug release (Lofthus, 2005; Fu & Kao, 2010).

Models for the description of release mechanisms

Many different mathematical models have been proposed to describe the drug release mechanisms from hydrophilic matrices. The use of an appropriate equation may allow to calculate and to predict these processes. However, at the present the most common equations have limitations to their use, as it is necessary to make certain assumptions about the models.

The Ritger-Peppas equation

The Ritger-Peppas equation is a semi-empirical model for the analysis of release data.

$$\frac{M_t}{M_\infty} = K\, t^n \qquad (4.5)$$

Where M_t is the amount of drug released at time t, M_∞ is the amount of solute released after infinite time, M_t/M_∞ is the fractional solute release, t is the release time, k is a constant incorporating structural and geometrical characteristics of the system, and n is the release exponent which might be indicative of the mechanism of drug release (Lofthus, 2005; Siepmann & Siepmann, 2008).

This equation is used to study the mechanism of release, because it has favorable aspects as regards limitations and assumptions. One assumption that must be made is that there are perfect sink conditions during the swelling, and that diffusion is concentration independent. The Ritger-Peppas equation can only be applied to the first 60% of fractional drug release (Lofthus, 2005).

The release of drug from the matrices depends mainly on diffusion through the matrix, swelling of the polymer and erosion of the swollen polymer. Diffusional release shows first order kinetics or Fickian kinetics. In the case of Fickian mechanism the rate of drug diffusion is much less than that of polymer relaxation. Thus the release will be determined chiefly by the drug diffusion in such a system (Fu & Kao, 2010). In the case of Fickian release the release kinetics are therefore proportional to the square root of time. With a pure diffusional drug release, the diffusional coefficient n is equal to 0.50 if the swellable device is a thin film or 0.45 and 0.43 if the system has a cylindrical or spherical shape, respectively (see table 4.17).

For Case II system, the reverse is true. The rate of drug diffusion is much larger than that of polymer relaxation. A characteristic of Case II mechanism is that the rate of interface movement is constant, so that the released amount is directly proportional to time. In the anomalous case, the rates of drug diffusion and polymer relaxation are about the same size (Fu & Kao, 2010).

Table 4.17. *Values of the diffusional exponent n and mechanism of diffusional release for controlled-release systems.*

Diffusional exponent			Drug release mechanism
Thin film	**Cylindrical shape**	**Spherical shape**	
0.50	0.45	0.43	Fickian diffusion
0.50<n<1.00	0.45<n<1.00	0.43<n<1.00	Anomalous
1.00	1.00	1.00	Zero-order release

In order to describe the drug release mechanisms from hydrophilic matrices constituted by HPMC, the dissolution profiles reported in figures 4.24 and 4.25 were fitted using the exponential equation proposed by Ritger and Peppas.

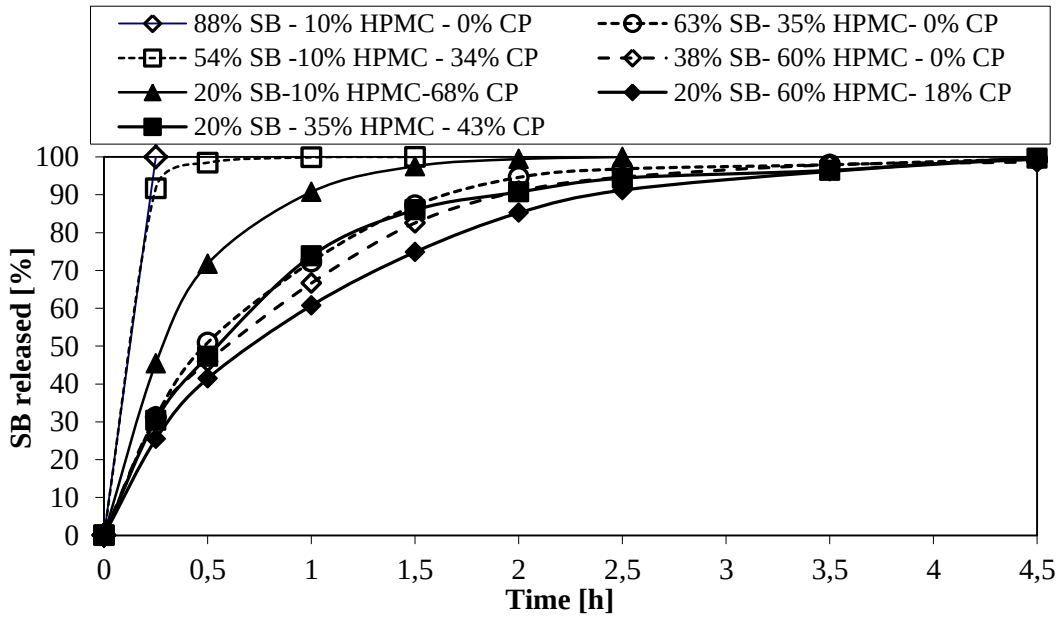


Figure 4.24. *Dissolution profiles of all the formulations containing SB.*

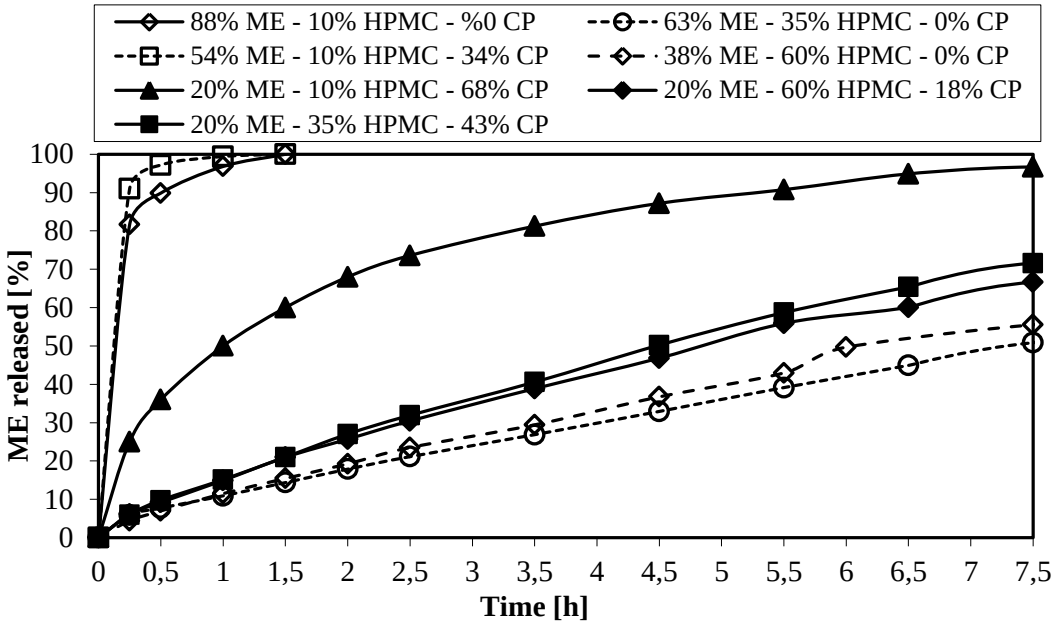


Figure 4.25. *Dissolution profiles of all the formulations containing ME.*

The fitting has permitted to evaluate the value of the exponent *n* for all the different formulations and thus to determine the drug release mechanism. Results are reported in table 4.18.

Results show that, with both types of drugs, the dissolution profiles of the formulations number 1 and 6 are very fast and it is not possible to use the equation proposed by Ritger and Peppas. In the other cases the release exponent assumed a value ranging from 0.480 to 0.813 and thus the release mechanism is anomalous. This means that the drug release is a function of both dissolution and diffusion mechanisms. However, when the *n* value is closed to 0.45 the drug release is mainly due to the diffusion.

Table 4.18. *Fitting parameters of the Ritger and Peppas exponential equation calculated for all the dissolution profiles.*

Formulation				Fitting parameters		
N°	**SB [%]**	**HPMC [%]**	**CP [%]**	**n [-]**	**K [-]**	**R2 [-]**
1	88	10	0	-	-	-
2	38	60	0	0.544	66.698	0.998
3	20	10	68	0.655	112.665	0.999
4	20	60	18	0.606	61.142	0.999
5	63	35	0	0.813	90.456	0.996
6	54	10	34	-	-	-
7	20	35	43	0.694	74.538	0.994

Formulation				Fitting parameters		
N°	**ME [%]**	**HPMC [%]**	**CP [%]**	**n [-]**	**K [-]**	**R2 [-]**
1	88	10	0	-	-	-
2	38	60	0	0.801	11.199	0.997
3	20	10	68	0.480	49.646	0.999
4	20	60	18	0.734	15.518	0.999
5	63	35	0	0.764	10.690	0.997
6	54	10	34	-	-	-
7	20	35	43	0.773	15.648	0.999

k is a constant incorporating structural and geometrical characteristics of the system and thus its value is a function of numerous variables such as form and dimension of the system, type of polymer, type of diluent, and nature of the active. In this case the geometrical characteristics of the matrices are similar and, as a consequence, its value is mainly affected by the formulation variables. Comparing the *k* values of the different formulations, it is possible to note that the higher the amount of polymer, the lower the *k* values and the higher the consistency of the gel layer (figure 4.26).

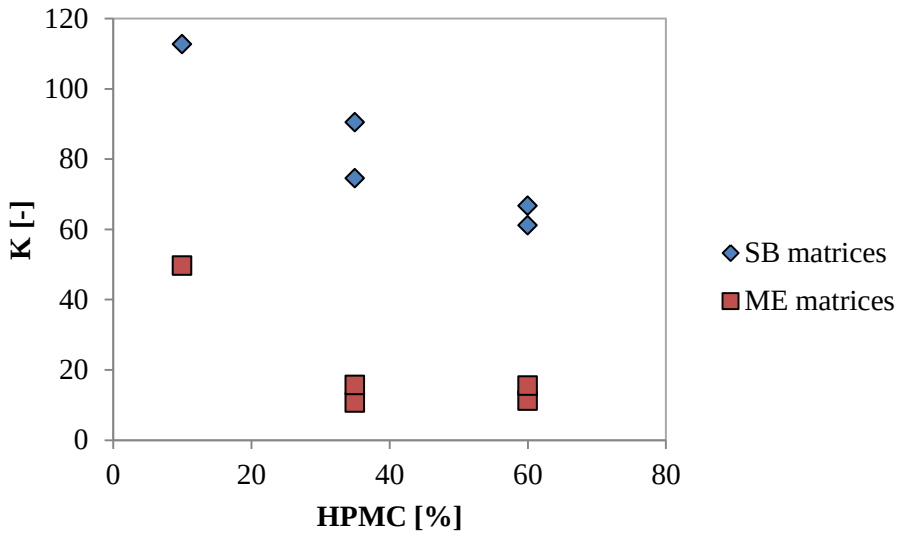


Figure 4.26. *Relationship between the amount of polymer and k value.*

From figure 4.26 it is also possible to note that formulations containing ME present lower *k* values than those with SB and this is in agreement with the fact that ME, having a poor water solubility, concurs to produce a more compact and dense gelled matrix. These observations are consistent with the fact that the systems containing ME present the lower drug release rate.

4.5 Conclusions

The purpose of this Chapter was to develop sustained-release mucoadhesive tablets containing two drugs characterized by different water solubility (sodium butyrate and mesalazine), and having the intestinal mucosa as target.

With this aim the range of polymers was further expanded by including HPMC.

On the basis of mucoadhesion measurements and literature data, HPMC and NaCMC (type B) were selected to carry on this study.

The DoE techniques were first used to evaluate the effects of the type of production process and the types of polymer, excipient and drug on mucoadhesion and drug release rate. Results revealed that mucoadhesion and the drug release rate are not affected by the type of the production process; however, the mucoadhesive properties depend on the type of excipient and active ingredient. On the other hand, variables

able to significantly influence the drug release are the type of active ingredient and the type of polymer.

Particularly, the best results were obtained with HPMC and calcium phosphate.

In order to develop a sustained-release dosage form for both the selected drugs, the DoE techniques were used again.

The DoE was used to identify mixtures, containing drug, HPMC and calcium phosphate, and characterized by good mucoadhesion, extended-release of the active ingredient and high amount of drug.

Chapter 5

Conclusions

The screening of polymers showing different physicochemical and mucoadhesive properties allowed to identify the parameter mainly influencing mucoadhesion.

A good hydration of the polymer is fundamental for the activation of the mucoadhesion process. However, lower values of water uptake and swelling of the dosage form do not always correspond to lower values of mucoadhesive properties, as in the case of HPMC.

Polymer molecular weight exhibits a good linear relation to mucoadhesion: the lower the polymer molecular weight, the higher the mucoadhesive properties of the dosage form. Hence, results suggest that polymer molecular weight is the most critical factor affecting mucoadhesion.

To confirm these remarks, three standards of sodium carboxymethylcellulose with different molecular weight (90 – 250 – 700 kDa) were purchased from Sigma-Aldrich (USA). The three standards were characterized by the determination of the Viscosity Average Molecular Weight. Results are reported in table 5.1.

Table 5.1. *Molecular Weight and Viscosity Average Molecular Weight of the three standards of NaCMC.*

Polymer	**Molecular Weight [kDa]**	**Viscosity Average Molecular Weight [kDa]**
NaCMC-90	90	42
NaCMC-250	250	170
NaCMC-700	700	607

Standard polymers were then used to prepare tablets containing 60% [w/w] of polymer and 40% [w/w] of excipients blend. However, due to the high cohesiveness and poor flowability and compressibility of the mixture containing NaCMC-700, this type of tablets was not produced.

Nevertheless, tablets containing the other two standards were prepared and their mucoadhesive properties were evaluated using the procedure 3 (table 3.5, Section 3.3.6) of the tensile test.

In figure 5.1 the new results were compared with those found with the other polymers.

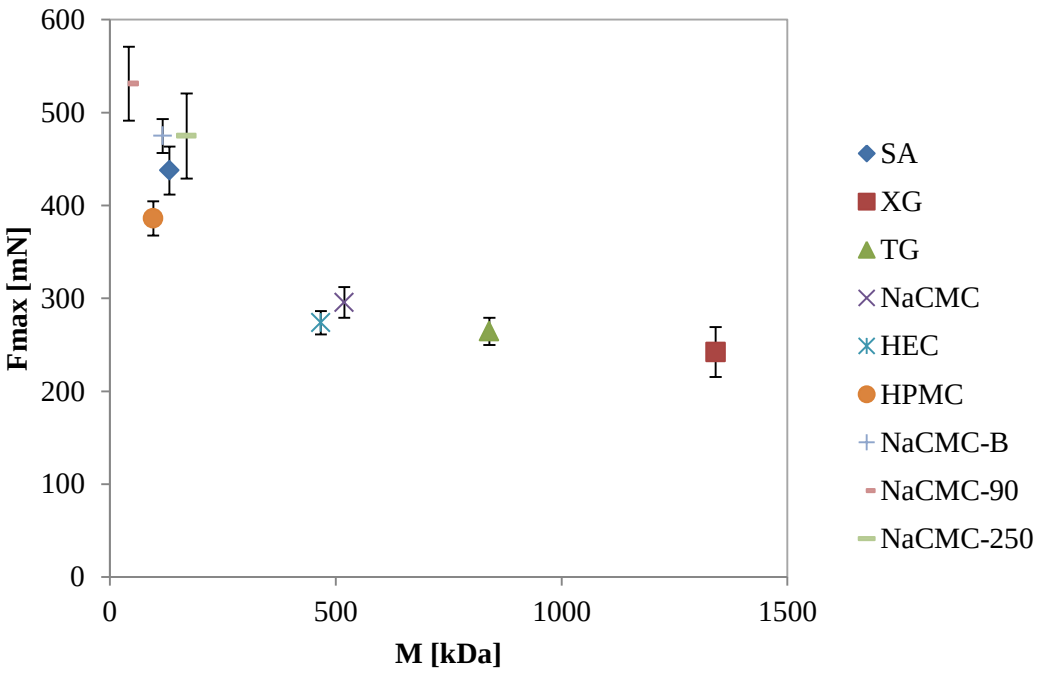


Figure 5.1. *Relation between molecular weight (M [kDa]) and mucoadhesive properties (Fmax [mN]).*

Data highlight that the mucoadhesive properties of the NaCMC standards match the results previously obtained and this means that the molecular weight is a key factor in determining the mucoadhesive properties.

However, the choice of the mucoadhesive polymer must be made taking into account also the chemical nature of the polymer.

It is hence possible to make a few remarks:

- generally, natural polymers with complex structure such as xanthan gum and tragacanth gum can present lower mucoadhesion due to a reduction of the interaction between polymer and mucin;
- mucoadhesion of nonionic polymers is facilitated by the formation of a viscous gel layer;
- mucoadhesion of tablets is influenced by the nature of the excipient and drug;
- mucoadhesion of tablets is influenced by the amount of polymer, the higher the amount of polymer the higher the mucoadhesion;
- during the formulation of mucoadhesive tablets it is important also consider tha thydrophilic polymers having mucoadhesive properties could reduce the release rate of the drug.

The study of the polymer conformation in aqueous medium could represent a future goal in order to further investigate the mucoadhesion phenomenon.

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