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Design, characterization and preliminary *in vitro* evaluation of a mucoadhesive polymer based on modified pectin and acrylic monomers with potential use as a pharmaceutical excipient

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ABSTRACT

Bio-based polymers have been reported to have applications in biomedical and pharmaceutical areas. Polysaccharides, especially prepared from plant sources, have served a variety of uses. This work aims to prepare a polymer for use as a pharmaceutical excipient containing a functionalized carbohydrate (pectin) and acrylic monomers (methyl methacrylate, butyl methacrylate and ethyl acrylate) *via* an emulsion polymerization technique. Carbon double bonds were incorporated into the pectin by reacting this natural polymer with glycidyl methacrylate. The methacrylated pectin was then polymerized with acrylic monomers by emulsion polymerization. The mucoadhesive performance of the materials was investigated by *in vitro* preliminary assays based on viscometric studies, texture analysis and film wettability. The obtained results showed that the synergistic viscosity increase with greater concentrations of modified pectin. The contact angle decreased, suggesting an increase in the wettability for polymers with large amounts of methacrylated pectin. The addition of mucin in lattices caused an increase in the intermolecular forces and in the work of adhesion. This corroborates the use of pectin as a mucoadhesive excipient for mucoadhesive drug delivery systems.

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1. Introduction

Advances in polymer science have led to the development of novel delivery systems. There is currently a growing interest regarding the use of carbohydrates that are individually modified and/or associated with synthetic polymers in the field of materials science for pharmaceutical, dental, biomedical and food applications. In recent years, there has also been increasing interest in the development of plant-derived composite materials, which are sometimes called "green composites" or "bio-based polymers". They combine the advantages of synthetic polymers, such as processability, stability and low hydrophilicity, with unique properties of natural materials, such as low toxicity, low cost, biodegradability and biocompatibility. Green composites are materials with ecofriendly properties primarily due to the renewability and sustainability of their sources (Abdul Khalil, Bhat, & IreanaYusra, 2012; Beneke, Viljoen, & Hamman, 2009; Carneiro-da-Cunha et al., 2012; Coviello, Matricardi, Marianecci, & Alhaique, 2007; Deshmukh, Setty, Badiger, & Muralikrishna, 2012; Kadajji & Betageri, 2011; Klein, 2009; Kumar et al., 2012; Rodrigues & Emeje, 2012; Sandolo, Coviello, Matricardi, & Alhaique, 2007; Sharma, Dhuldhoya, Merchant, & Merchant, 2006; Šimkovic, Gedeon, Uhliariková, Mendichi, & Kirschnerová, 2011). However, very high concentrations of carbohydrates in their putative form are required to successfully function as dose-dependent drug release modifiers due to their high swellability/solubility in gastric medium. Hence, such carbohydrates must be modified to alter their physicochemical properties (Rana et al., 2011).

Pectin is a cell wall structural carbohydrate present in all higher plants. Its heterogeneous and complex chemical structure has a linear backbone consisting of D-galacturonic acid units linked by α-(1-4) linkages that are randomly interrupted by L-rhamnose α-(1-2) linkages. Other neutral sugars such as D-galactose, L-arabinose, D-xylose, L-rhamnose, L-fucose and traces of 2-O-methyl fucose may be present in the side chains. Its molar mass is high, ranging between 50,000 and 180,000 g/mol. Galacturonic acid has carboxyl groups that can be naturally esterified by methyl groups (CH_3) or that can react with ammonia to produce

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carboxyamide. The degree of esterification (DE) or amidation (DA) can be expressed as a percentage and provides a basis for the classification of the pectin. In general, pectins with a degree of esterification higher than 50% are considered high-ester pectins (HM), and those with a DE lower than 50% are considered low-ester pectins (LM) (Chen et al., 2014; Sharma et al., 2006; Sriamornsak, Wattanakorn, & Takeuchi, 2010; Sriamornsak, 2003).

This polysaccharide has been extensively studied due to its particular characteristics: its application in targeted drug delivery systems (DDS) for the colonic region and its adhesive properties. One of the main advantages associated with the use of pectin in drug delivery systems is its resistance to passage through the proximal portion of the gastrointestinal tract (GIT), as it is specifically biodegraded by the anaerobic bacteria resident in the colon. Thus, the use of pectin is associated with the development of delivery systems for colon-specific drugs used for local or systemic treatment of ulcerative colitis, irritable bowel syndrome, Crohn's disease, colon carcinoma and other pathologies (Jain, Gupta, & Jain, 2007; Raj, Rubila, Jayabalan, & Ranganathan, 2012; Shukla & Tiwari, 2012; Sinha & Kumria, 2001; Souto-Maior, Reis, Pedreiro, & Cavalcanti, 2009).

Pectin is rich in carboxylic groups and capable of interacting with functional groups in the mucus layer. Another advantage attributed to the use of pectin as a pharmaceutical excipient is its adhesive property, derived from the formation of hydrogen bonds between its polysaccharide carboxyl groups and mucus hydroxyl groups (Abruzzo et al., 2012; Charlton, Davis, & Illum, 2007; Hagesaether, Hiorth, & Sande, 2009; Hagesaether & Sande, 2008a, 2008b; Muzzarelli et al., 2012; Sharma & Ahuja, 2011; Srivastava & Malvyia, 2011; Thirawong, Kennedy, & Sriamornsak, 2008; Thirawong, Nunthanid, Puttipipatkhachorn, & Sriamornsak, 2007). Bioadhesion is a pharmaceutical strategy that has been explored to increase the residence time of DDS in the body. Another advantage is the possibility of greater contact between the drug and mucous membranes, reducing the required concentration of the drug administered and the number of doses per day (Dodou, Breedveld, & Wieringa, 2005; Hagesaether et al., 2009; Lai, Wang, & Hanes, 2009; Smart, 2005).

Bioadhesive polymers can be classified as mucoadhesives (those that adhere to the mucus layer) or cytoadhesives (those that adhere specifically to the cell membrane), most of which have a mucoadhesive property. Mucoadhesion can be achieved by the use of materials capable of interacting with mucin by forming non-covalent bonds, hydrogen bonds, ionic bonds or van der Waals interactions and, thus, promote polymer/mucus entanglement. Polymers that have bioadhesive properties are usually hydrogel-forming macromolecules with a large number of groups that form hydrogen bonds (carboxyls, hydroxyls, amides or sulfates) (Dodou et al., 2005; Streubel, Siepmann, & Bodmeier, 2006).

There are three sub-families of mucins: (a) gel-forming secreted mucin, (b) cell surface mucin, and (c) non-gel-forming secreted mucin. Gel-forming mucins are the major constituents of mucus, which are responsible for mucus viscoelasticity. They are rich in cysteines, an amino acid that has an N-terminal and a C-terminal, which have a role in intermolecular disulfide interactions. Mucin is an oligosaccharide composed of galactose, fucose, N-acetylglucosamine, N-acetylgalactosamine and sialic acid residues. At a pH above 3, the sialic acid and terminal sulfate groups are ionized, giving the molecule a negative charge. From the polymeric standpoint, mucin can be considered a polymer that has polyelectronic alternating domains and grafted sugars, connected by flexible regions showing a small tendency of glycosylation. The molecule has hydrophilic and hydrophobic regions, with the ability to form hydrogen bonds and electrostatic interactions (Bansil & Turner, 2006; Duchene, Touchard, & Peppas, 1988; Harding, Davis, Deacon, & Fiebrig, 1999; Linden, Sutton, Karlsson, Korolik, & Mcguckin, 2008; Svensson & Asnebrant, 2010).

It is believed that the following events are involved in bioadhesive phenomena: adsorption, spreading of the bioadhesive material on the mucosal membrane, an increase in contact area, and interpenetration between the mucosal membrane and polymer chains. The stages involved in the mucoadhesion and the possible interactions that may occur with mucin are shown in Fig. 1. Initially, an intimate contact between the layer and the mucoadhesive DDS occurs due to either a good wetting of the bioadhesive surface or swelling of the mucoadhesive polymer. Contact is then established, and the interpenetration of the mucoadhesive polymer chain on the mucus occurs. Weak chemical bonds are formed during the final stage. Numerous theories have been proposed to explain bio/mucoadhesion. The most prevalent theories are the same as those that describe adhesion events for metallic and polymeric materials. These include electronic, adsorption, wetting, diffusion and fracture theories (Bansil & Turner, 2006; Hagesaether et al., 2009; Huang, Leobandung, Foss, & Peppas, 2000; Sriamornsak et al., 2010).

During the development of adhesive materials and/or DDS, the determination of the bio/mucoadhesive capacity is necessary to quantify the adhesive strength, to compare different bioadhesive materials, and to allow for quality control tests. A simple procedure to assess the absolute force of bioadhesion *in vitro* is based on the viscous behavior of mucin-polymer blends. The flow of liquids and quasi-solids can be described by the shear viscosity, or simply viscosity, which is defined as the resistance of a material to flow. In other words, viscosity is a measurement of the internal friction of a fluid, which becomes apparent when a layer of the fluid moves over another layer. The amount of force required to cause this movement is called shear. Shear forces occur when a fluid is physically moved or distributed. Highly viscous fluids require a greater force to move a layer over another than less viscous materials. The reciprocal of viscosity is fluidity, an indicator of the "mobility" of a material. Stress and shear forces can be defined in terms of time, direction, and the extent of deformation. Because of their peculiar properties, polymers with high molar masses and entangled conformations show viscoelastic behavior, that is, they have elastic and viscous deformation components. In general, the higher the viscosity of a material, the greater is the resistance to flow (Chen, Wen, Janmey, Crocker, & Yodh, 2010; Macosko, 1994; Swarbrick, 2007). The results obtained by Awasthi (2011) suggested that dilute pectin solutions show Newtonian behavior, but at a moderate concentration, they exhibit non-Newtonian behavior, and this pseudoplastic nature was found to increase with concentration. As the pH of the polymer solution was lowered, an increase in the viscosity of the system has been observed, which may be due to the charges acquired by carboxyl acid groups.

Another important *in vitro* method used by various authors to measure the adhesion in materials, food and pharmaceutical products is texture analysis using texture analyzers. The test is performed by applying controlled forces to the sample and recording the resulting force, deformation and time. In a typical test, the probe touches the surface of the sample and progresses into the inside of the sample at a predetermined speed. For example, the maximum penetration resistance force and the performed work can be measured and associated with the work of cohesion of the sample or the work of adhesion to the probe (Jones, Lowlor, & Woolfson, 2002; Thirawong et al., 2007). Tamburic and Craig (1995) and other authors (Jones, Brown, & Woolfson, 2001; Jones et al., 2000) have described the relationship between the viscoelastic properties of pharmaceutical systems, in particular the elastic properties, and adhesion to mucin.

Another parameter that interferes with bioadhesion is the wettability of a material. The event occurring at the solid/liquid interface

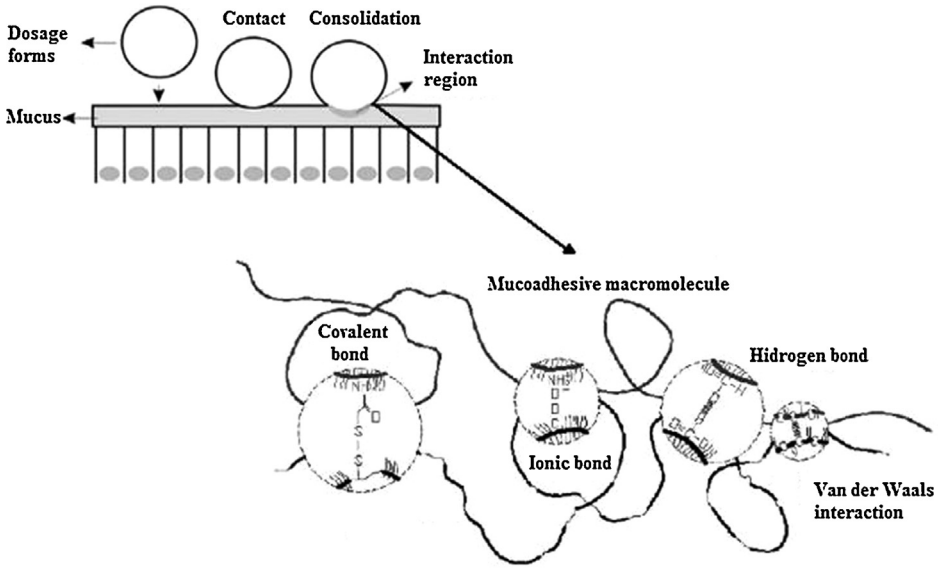

Fig. 1. Schematic representation of events related to mucoadhesion.
Adapted from Smart (2005).

can be studied by measurements of the contact angle (ϕ). The ϕ can be correlated with the mucoadhesive property of polymers, as more hydrophilic materials are associated with a greater possibility of adhering to mucus for a given wettability (Bansil & Turner, 2006; Huang et al., 2000; Karbowiak, Debeaufort, & Voilley, 2006). The contact angle is a quantitative measurement of the wettability of a material, defined by the intersection of two planes tangent to the liquid: the solid surface of the contact and a third phase, usually air or water vapor. Formally, the ϕ between a drop of liquid with a known surface tension and a solid surface depends on the relationship between the adhesive forces (surface) and cohesive forces (liquid) (Florence & Attwood, 2005; Karbowiak et al., 2006; Sinko, 2006). The wetting theory, which is one of the general theories of adhesion, has been adapted for the investigation of mucoadhesion. It involves the ability of a liquid to spread spontaneously onto a surface as a prerequisite for the development of adhesion (Białopiotrowicz, 2003; Smart, 2005; Sriamornsak, Wattanakorn, Nunthanid, & Puttipipatkhachorn, 2008).

The uncontrolled rates of hydration, pH-dependent solubility, thickening, the drop in viscosity during storage, and the possibility of microbial contamination are problems associated with the use of natural polymers. The main disadvantage of the use of pectin in drug delivery systems is its high solubility in water, which leads to limitations in the protection of drugs during passage through the stomach and small intestine. Another problem is that pectin shows resistance to consolidation due to its high elastic recovery capacity during the processes of compression and ejection of tablets. It is suggested that the mechanism of compaction is fragmentation with little plastic deformation. Chemical modifications of natural problems not only minimize these drawbacks but also enable their use for specific drug delivery purposes (Ahrabi, Madsen, Dyrstad, Sande, & Graffner, 2000; Kim, Venkatesh, & Fassihi, 1998; Rana et al., 2011). An approach to modify mechanical properties and reduce the high water solubility of pectin is to chemically modify the polysaccharide so that the modified pectin can be combined with other monomers and polymers. This type of chemical modification can be performed by reacting natural macromolecules with glycidyl methacrylate (GMA) to incorporate polymerizable units (carbon double bonds) into natural macromolecules. The chemical modification of low-methoxyl pectin with glycidyl methacrylate (Pec_GMA) aimed to introduce double bonds in pectin, such that it can be polymerized in the presence of acrylic and methacrylic monomers. Then, its solubility in water can be decreased, resulting in a material with properties suitable for pharmaceutical use.

The aim of this study was to prepare a material based on pectin and acrylic monomers, such as methyl methacrylate, butyl methacrylate and ethyl acrylate (MMA, BMA and EA), synthesized by emulsion. The emulsion polymerization method allows the preparation of lattices in aqueous medium in the absence of organic solvents. These latexes can be used as pharmaceutical excipients as a coating agent or as a matrix agent in the preparation of solid dosage forms. The selection of monomers was based on the composition of commercial pharmaceutical polymer dispersions, included in monographs of the Pharmacopoeias and the Handbook of Pharmaceutical Excipients. The Pharmacopoeias contain descriptions of the polymers used in the preparation of matrix tablets prepared from EA, MMA, and BMA (Rowe, Sheskey, & Owen, 2011; USP, 2014) monomers. The adhesive capacity of these materials was tested *in vitro* by preliminary assays based on the change in viscosity, texture analysis and the wettability capacity of the material. All of the chosen methods are fast and can assist in the design of new materials. The present study aimed to prepare and to evaluate potentially useful materials such as excipients for pharmaceutical use that could display bioadhesive behavior.

2. Materials and methods

2.1. Materials

A low-ester citric pectin (GENU®, LM 104) was kindly provided by CP Kelco (Brazil). The pectin was standardized resulting in an LMP with Mw = 30,000 g/mol and a 35% degree of esterification. Partially purified powder of mucin from porcine stomach, type II, was purchased from Sigma Chemical Co., Ltd. (USA) and used as received. HCl 0.1 N solution was purchased from Synth (Brazil). Ethanol, ferrous sulfate, ammonium persulfate, and sodium dithionite of analytical purity were purchased from Synth and Vetec (Brazil). Sodium dodecyl sulfate (SLS) and Renex® 300 (obtained through the reaction of nonylphenol and ethylene oxide with a degree of ethoxylation of 30) were kindly donated by Cognis

Table 1
Composition of EPEC acrylic lattices obtained by emulsion polymerization.

Components (%, w/v)	EPEC 1	EPEC 2	EPEC 3	EPEC 4
Pec_GMA	2.00	3.5	5.00	6.00
BMA	23.20	23.20	23.20	23.20
MMA	8.40	8.40	8.40	8.40
EA	8.40	8.40	8.40	8.40
SDS (25%, w/v)	0.99	1.03	1.07	1.08
Renex® (70%, w/v)	0.26	0.27	0.28	0.29
Ferrous sulfate	0.036	0.036	0.036	0.036
Ammonium persulfate	0.162	0.171	0.175	0.182
Sodium dithionite	0.162	0.171	0.175	0.182
Purified water (enough amount)	100.00	100.00	100.00	100.00

(Brazil) and Oxiteno (Brazil), respectively. The EA, MMA and BMA monomers were purchased from Sigma Chemical Co., Ltd. (USA) and used without further purification.

2.2. Modification of pectin by GMA

Initially, the moisture content of the pectin was determined by loss on drying. Two (2) grams of polysaccharide were weighed on a filter weighing scale and left in an oven at 105 °C for 3 h. The containers were removed, placed in a desiccator at room temperature and weighed again. This process was repeated until a constant weight was obtained. Analyses were carried out in triplicate, and the mean and the standard deviation were calculated using MS Excel. The results were used to adjust the pectin content in the formulations.

The modification of the pectin was performed according to the method described by Souto-Maior, Reis, Muniz, and Cavalcanti (2008). First, a colloidal solution of pectin was prepared in water at a concentration of 2.5% (w/v) under mechanical stirring until completely dispersed. The pH was checked and adjusted to 3.5 with a 0.1 N HCl solution. Then, 4.8 mL of GMA was added, and the mixture was mechanically stirred for 24 h at 50 °C. The ratio of GMA to pectin used was 0.067 g/mol to 0.34 g/mol, respectively, based on the repeat unit of the pectin backbone ($C_{13}O_{13}H_8$). After the reaction time, the GMA-modified pectin was precipitated in ethanol and separated by vacuum filtration. To evaluate the polymerization ability of the functionalized polysaccharide, it was polymerized using ammonium persulfate as an initiator. To this end, 40 mL of freshly prepared dispersion was magnetically stirred in the presence of the initiator, which was added at a concentration of 1% (w/w) monomer mass. After 1 h, casting films were prepared, which were dried in an oven at 75 °C for 36 h.

2.3. Preparation of polymers containing Pec_GMA by emulsion polymerization

Lattices containing Pec_GMA were prepared by emulsion polymerization. The compositions of the systems are shown in Table 1, and the polymers were called EPEC. To perform the synthesis, a three-neck 250-mL round bottom flask with a digital mechanical stirrer, a thermometer and a nitrogen intake system were used to control the microenvironment of polymerization. A heating/cooling mantle was also used to control the temperature during polymerization. Initially, the newly prepared Pec_GMA was dispersed in 50 mL of purified water, under slow mechanical stirring (250 rpm) to avoid the formation of bubbles and heating. This process took several hours, depending on the concentration of Pec_GMA, which ranged from 2% (w/v) to 6% (w/v) according to the volume of the formulation. After complete homogenization, the temperature was increased to 60 °C, and the mixture of monomers was added. Initiators were previously solubilized in the remainder of the purified water and added to the system, which was kept under slow stirring for 4 h at a controlled temperature (below 85 °C). The EA, MMA and BMA monomers were used, and the total percentage of initiators in each polymer was 0.88% (w/w) (monomers mass). The films prepared by casting were subjected to FTIR analysis under the same conditions mentioned above to confirm polymerization. For emulsion polymerization, anionic surfactants have been the most commonly used because they produce lattices with a relatively small particle size, whereas nonionic surfactants generally provide lattices with good stability but a large particle size. In this work, a mixture of anionic surfactant (sodium dodecyl sulfate – SDS) and nonionic surfactants (Renex® 300) was used (Villanova, Ayres, Reis, & Oréfice, 2012).

2.4. Characterization of the pectin and modified pectin

2.4.1. ^1H NMR and ^{13}C NMR evaluations

Nuclear magnetic resonance spectra were recorded using a Bruker 400 MHz spectrometer with a 5-mm dual probe of hydrogen and carbon at room temperature. Deuterium oxide (D_2O) solutions of pectin, GMA and Pec-GMA were prepared to acquire the spectra, and the overall duration acquisition was 3.95 s. The chemical shift was reported as δ values (ppm).

2.4.2. Fourier transform infrared spectroscopy (FTIR)

Pectin and Pec_GMA EPEC were also analyzed by Fourier transform infrared (FTIR). For this purpose, casting films from the pure pectin were prepared by dispersion in water (2.5%, w/v). For the other polymers, casting films were prepared, washed with purified water to remove products that did not react and left again in an oven at 40 °C overnight. Prepared as such, films were analyzed by FTIR in a Thermo Scientific Nicolet 6700 spectrometer provided with a Centaurus microscope to characterize the materials. FTIR-ATR spectra of the films in the region of 650–4000 cm^{-1} with a resolution of 4 cm^{-1} using germanium crystal were obtained with dry nitrogen purge and 64 scans were recorded and averaged per spectrum.

2.5. Determination of total solids by gravimetric method

The total solid content of the polymer lattices was determined by gravimetric method through moisture weight loss (Riddle, 1954). Samples (200 μL) were loaded onto a glass vial and the weight determined. Triplicate measurements were obtained for each sample that were put into an oven at 105 °C for 1 h to remove all volatiles. At the end of this time, the glass vials were removed from the oven, cooled until room temperature in a desiccator, and reweighed. Total solids were then calculated, and the results from these triplicate determinations must agree within 0.5% relative.

2.6. pH determination

The pH of the polymer lattices was checked by direct measurement in a digital pH meter Marconi, Model MA PA 200.

2.7. Wetting study via determination of the contact angle

Water contact angles (ϕ) were measured at the surface of the polymer films using a contact angle goniometer (model Digidrop DI, France) at 25 ± 2 °C. Ten microliters of water was gently dropped on the surface of the EPEC films using a microsyringe. All of the droplets were released from 1 cm above the surface to minimize the inconsistency between each measurement. The angle between the tangent line and the polymer surface from goniometric scale, 30 s after the release of each droplet onto the surface, was measured. Three consecutive measurements were performed, using

the Surface Energy mode of the software, which enables a direct measurement of the contact angle (in degrees).

2.8. Viscometric studies

Various *in vitro* and *ex vivo* methods are used for testing the efficacy of mucoadhesive polymers. Commonly used methods include tensile strength measurements, shear strength measurements *via* rotatory and oscillatory techniques, chip based systems, and imaging techniques. In this work, the adhesive property of the materials was preliminarily evaluated using a viscometric technique. This method is based on comparing measurements of viscosity (η) from dispersions containing pure mucin and pure polymers and from mixtures of mucin and polymers. Some authors observed a synergistic increase in η when mucin and adhesive polymers, such as chitosan, poly(acrylic acid) and pectin, were mixed with mucin (Hassan & Gallo, 1990; Thirawong et al., 2008). To this end, aqueous dispersions of non-purified Type-II mucin derived from the stomach of pigs were prepared. Because dry mucin contains many hydroxyl groups in branched chains, amine groups in the backbone, and terminal carboxyl or sulfate groups, it can be dispersed in water (Peppas & Huang, 2004). The powder was dispersed under magnetic stirring for 3 h at concentrations of 5 and 7.5% (w/v). The same procedure was used to disperse the mucin in the polymeric lattices (EPEC 1, EPEC 2, EPEC 3 and EPC 4). Subsequently, the apparent viscosity of the dispersions was determined using a Brookfield viscometer, Model RV-DVII + Pro. Analyses were performed using adapters for small quantities (Sample Adapter, model 13R and UL Adapter), maintained at 25 °C and using a spindle SC4-28. The rotation speed varied depending on the viscosity of the system to obtain a torque within the equipment accuracy range (between 10 and 90%) between 100 and 200 rpm. The software was programmed to perform 5 readings every 5 s for 60 s (at each speed) at equilibrium intervals of 120 s. Eqs. (1)–(3) were used to determinate the viscosities.

$$\eta_{calc} = \eta_{cop} + \eta_m \quad (1)$$

$$\eta_s = \eta_{exp} - \eta_{esp} \quad (2)$$

$$\eta_r = \frac{\eta_{exp}}{\eta_{esp}} \quad (3)$$

where η_s = synergistic viscosity; η_{exp} = experimental viscosity; η_{esp} = expected viscosity; η_{cop} = viscosity of the polymer; η_m = viscosity of the dispersion containing pure mucin; and, η_r = relative viscosity.

2.9. Texture analysis

The adhesive texture was evaluated according to Tamburic and Graig (1997) using a TA-XT2i texturometer (Stable Micro Systems Ltd., Surrey, UK) with a 5-kg load cell. Analyses were performed on pure EPEC aqueous lattices and in the presence of 5% (w/w) aqueous mucin gel, used in compression mode, by measuring the resistance to the penetration and removal of a steel probe (diameter of 10 mm). The probe was brought in contact with the semisolid samples and was inserted at a defined rate (1 mm/s) into each formulation to a defined depth (15 mm). The study was carried out at room temperature (25 °C) with at least three repeats obtained for each sample. The peak force of the first cycle represents the force necessary to compress the sample. The maximum force required to separate the sample from the probe during the probe extraction is the adhesive force.

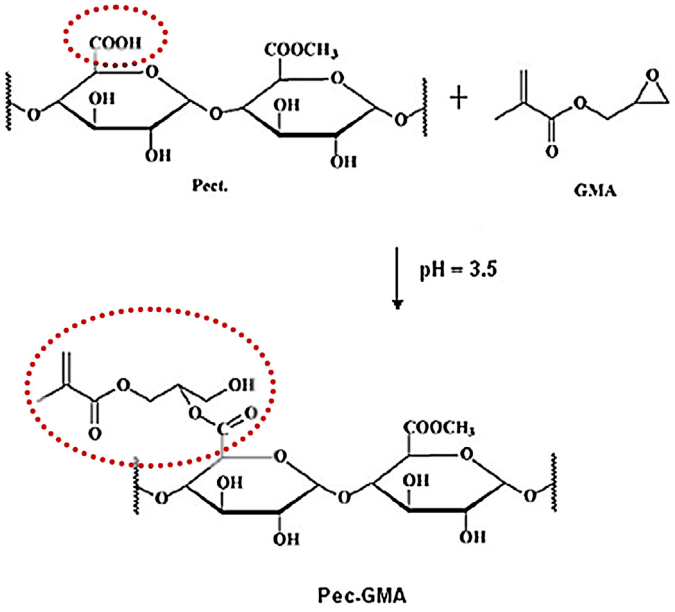

Fig. 2. Reaction scheme of the formation of Pec-GMA at pH 3.5.

3. Results and discussion

3.1. Modification of pectin

The moisture content measured for the pectin was 9.8% (±0.15), which is considered typical for commercially available pectins (Kurita, Fujiwara, & Yamazaki, 2008; Thirawong et al., 2008). To prepare solutions of pectin, the mass was weighed according to adjustments of the water content.

GMA is a hydrophilic monomer that is soluble in water at a concentration of 2.3% (w/v) and miscible with most organic solvents. It has two reaction sites, namely an epoxy group on one side of the molecule and a methacrylate on the other side, which makes it useful for the functionalization of different materials. From GMA, double bonds (C=C) can be introduced into the molecule to be modified to allow it to polymerize with other monomers (Elizalde-Peña et al., 2007; Erol, Poyraz, Koroğlu, & Cifci, 2009; Han, Kumar, Rozman, & Noor, 2003; Li, Wang, & Elisseeff, 2003; Reis, Guilherme, Cavalcanti, Rubira, & Muniz, 2006). GMA is able to undergo additional polymerization reactions, and homopolymers and polymers based on GMA form a class of potentially functional polymers. (Cañamero, De la Fuente, Madruga, & Fernández-García, 2004; Gohsh & Krishnamurti, 2000; París, Mosquera, & Fuente, 2008; Shanthi & Panduranga, 2001; Tarducci, Kinmond, & Badyal, 2000).

The high reactivity of the epoxy group compared to a wide variety of reagents provides new routes for preparing numerous multifunctional polymers *via* chemical modifications. Epoxy groups can be used for the functionalization of amino acids, proteins, sugars and polysaccharides. GMA chemical reactions may occur with acidic (COOH) and basic (OH) groups of different molecules in aqueous solution, affected by the pH of the medium. At low pH values (approximately 3.5), the reaction occurs by the hydrolysis of GMA with the opening of the epoxide ring. At alkaline pH (above 8), GMA reacts with OH groups either by ring opening or transesterification (Reis, Cavalcanti, Rubira, & Muniz, 2003; Reis et al., 2009; Souto-Maior et al., 2008, 2009). In this work, the ring opening mechanism is the preferred route, and the expected reaction is shown in Fig. 2. The formation of the modified pectin was monitored using FTIR and NMR.

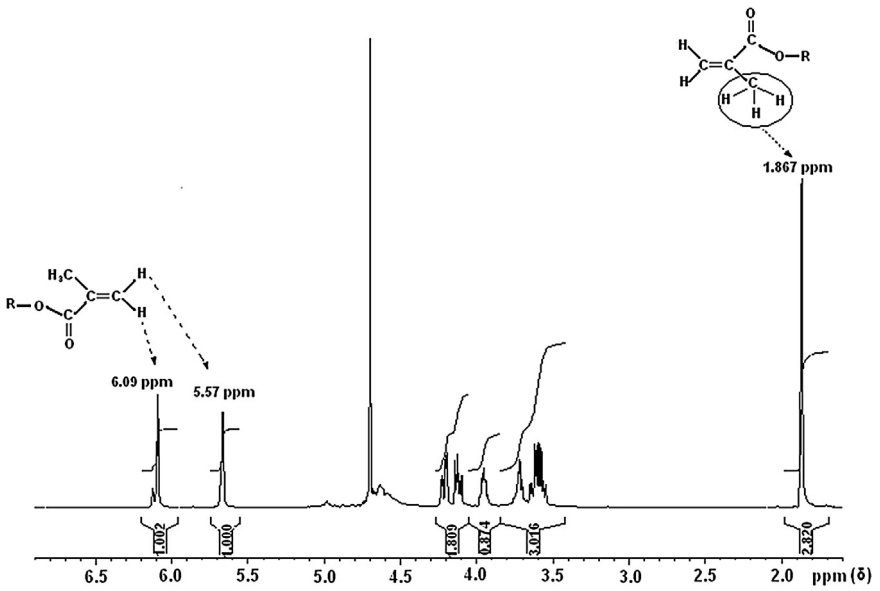

Fig. 3. ^{1}H NMR spectrum of Pec-GMA.

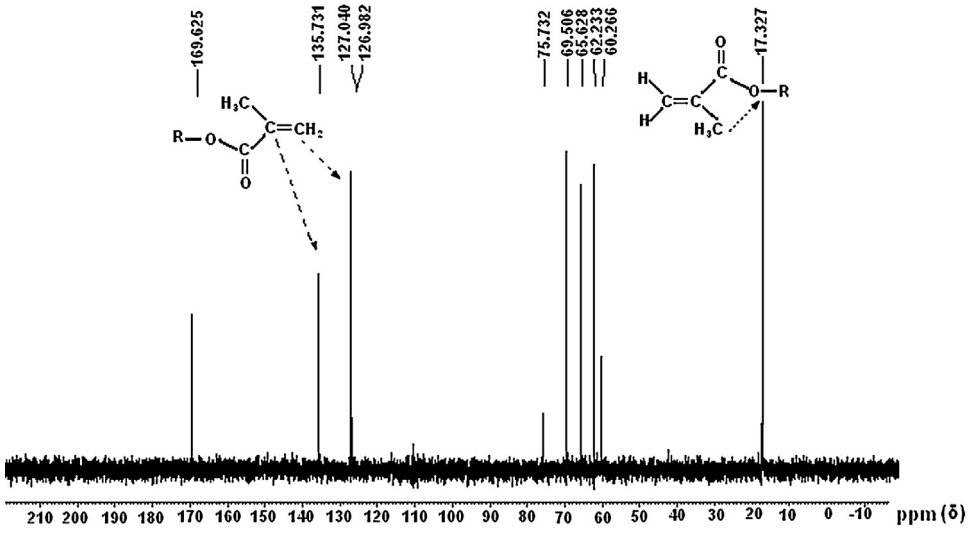

Fig. 4. ^{13}C NMR spectrum of Pec-GMA.

The ^{1}H spectrum of Pec-GMA is shown in Fig. 3. The signals appearing at $\delta = 6.09$ and 5.57 ppm were assigned to hydrogen linked to vinyl carbons. The high-intensity signal at $\delta = 1.867$ ppm, detected in the same spectrum, was attributed to the hydrogen of methyl groups linked to the vinyl carbons (Guilherme et al., 2009).

The ^{13}C NMR spectrum of Pec-GMA highlights the vinyl-carbon signals corresponding to the methacryloyl groups attached to the pectin. The signals at $\delta = 135.7$ ppm and at $\delta = 127.0$ ppm were attributed to the vinyl carbon groups (C=C), and the signal at $\delta = 17.4$ ppm was assigned to methyl carbons liked to the vinyl carbons (Guilherme et al., 2009). The ^{13}C NMR spectrum of Pec-GMA is shown in Fig. 4 and also suggested the success of pectin modification.

Several characteristic infrared absorption bands have been reported in the literature for pectin because it is a natural product with a composition that can vary according to its source (Manrique & Lajolo, 2002; Mishra, Anis, Mondal, Dutt, & Banthia, 2009; Synytsya, Čopíková, Matějka, & Machovič, 2003). The main peaks of the commercial GENU® pectin type LM-104 can be observed in Fig. 5. The peaks reported for GMA are 3060 cm^{-1}

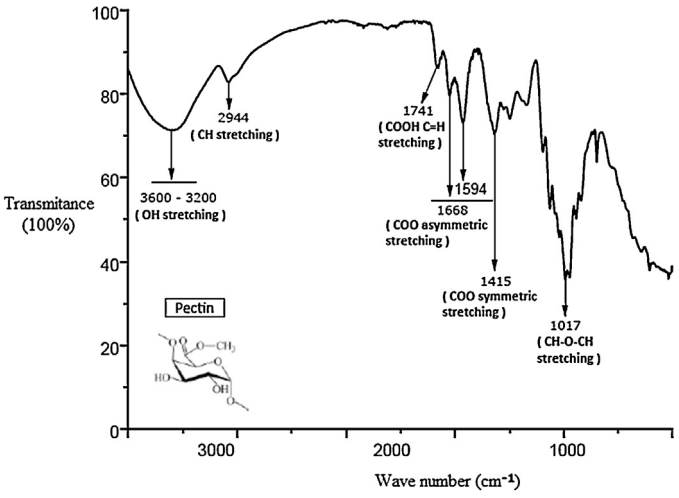

Fig. 5. FTIR spectrum of the low methoxyl GENU® pectin LM 104.

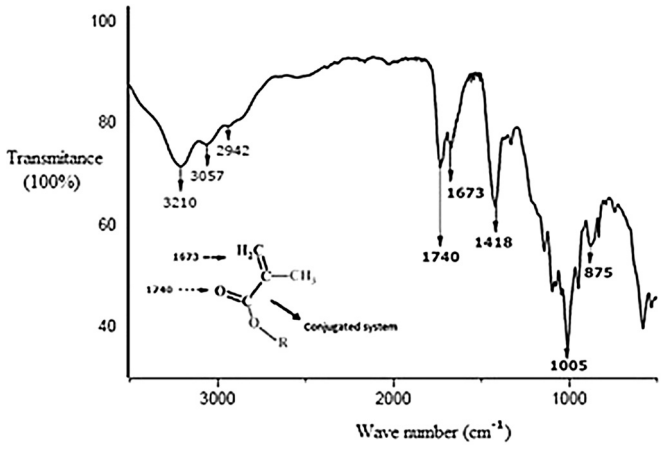

Fig. 6. FTIR spectrum of the GMA-modified pectin.

(C—H stretching of C located in the epoxide ring), 1720 cm^{-1} (axial deformation of the conjugated ester C=O bond), 1630 cm^{-1} (axial deformation of the C=C double bond), and 910 and 842 cm^{-1} (asymmetric and symmetric deformation of the epoxy ring in GMA, respectively) (Tarducci et al., 2000). The modified pectin (Pec_GMA) spectrum showed displaced peaks with different intensities compared to those of the pure polysaccharide (Fig. 6). Peaks at 1740 cm^{-1} and 1673 cm^{-1} and peak suppression at 1594 cm^{-1} suggest a coupling with GMA, as previously indicated by the NMR analysis. The conservation of signals at 1418 cm^{-1} and 1010 cm^{-1} in the spectrum of the modified product indicates the persistence of carboxyl groups and the maintenance of the integrity of the —O—CH—CH— bond present in the pectin. Hydroxyl peaks can be observed at 3210 cm^{-1}, in addition to peaks related to —CH stretching between 3057 and 2942 cm^{-1} (Reis et al., 2003, 2006; Souto-Maior et al., 2008).

3.2. Preparation and characterization of EPEC polymers

The freshly prepared Pec_GMA was used in the process of polymerization with acrylic monomers *via* the emulsion polymerization technique. The proportions of Pec_GMA in the polymers EPEC 1, EPEC 2, EPEC 3 and EPEC 4 were 2% (w/w) (EPEC 1), 3.5% (w/w) (EPEC 2), 5% (w/w) (EPEC 3) and 6% (w/w) (EPEC 4), respectively. The process used for the preparation of emulsions containing Pec_GMA resulted in white, milky, homogeneous, monomeric, odor-free and stable lattices. By visually observing the flow of the dispersions within glass vials, the viscosity of the EPEC dispersions increased as the Pec_GMA concentration increased. The pH was verified by direct measurement as approximately 4. The solids content was gravimetrically determined and changed according to the concentration of the modified pectin. The results are shown in Table 2. The films from the EPEC samples obtained *via* casting were translucent and slightly yellowish due to the presence of modified polysaccharides.

3.3. Determination of contact angle

The mucoadhesive process involved in the formation of bioadhesive bonds has been described in three steps: (i) wetting and swelling of the polymer; (ii) interpenetration of the bioadhesive polymer chain and entanglement of the polymer and the mucin chains; and (iii) formation of weak chemical bonds between entangled chains. From the success for this process, the wetting of dose formulations has a great impact on their adhesive properties. Several techniques have been used in wettability studies including contact angle measurements (Sriamornsak et al., 2008). When the surface is completely wetted by a liquid, the contact angle (ϕ) is < 30°. In contrast, if 30° < ϕ < 90°, the surface can be treated as partially wettable. When $\phi \geq 90°$, the surface is hydrophobic and is not wetted by the liquid. As expected, the increase in modified pectin content in the polymer caused a reduction in the contact angle. For films containing pure acrylic, 3.5 and 5% (w/w) Pec_GMA were partially water-wettable, and the contact angles were measured as 74.20° (not shown in table), 70.80° (EPEC 2) and 58.2° (EPEC 3). The film containing 6% (w/w) Pec_GMA (EPEC 4) exhibited a different behavior. The contact angle of ϕ of 24.2° suggests that the film is hydrophilic and is completely wetted by water. The results of wettability tests are shown in Table 2 and in Fig. 7.

Table 2
Values obtained in determining the solids content, pH and contact angle for EPEC polymers.

Parameters	EPEC 1	EPEC 2	EPEC 3	EPEC 4
Solids content (%)	9.84	14.23	18.24	22.30
pH	4.14	4.12	3.97	4.00
Contact angle (°)	–	70.8	58.2	24.2

The first step of the bioadhesion process, characterized by the stage of contact between the mucous membrane and the dosage form, is more effective for highly hydrophilic materials (Mortazavi & Smart, 1995; Smart, 2005; Sriamornsak et al., 2008, 2010). Therefore, the polymers prepared with higher concentrations of modified pectin exhibited higher wettability (greater hydrophilicity) and, consequently, a greater possibility of adhering to the mucus.

3.4. Determination of the in vitro mucoadhesion

Many methods based on the interaction of materials with mucin can be used to evaluate the adhesive capacity of materials to characterize their mucoadhesion properties. Thirawong et al. (2007) used the texture method to evaluate the *in vitro* mucoadhesive properties of various pectins on gastrointestinal mucosa. Moreover, rheological parameters, investigated using a viscometer or a dynamic oscillatory rheometer, increased after mixing the pectin and mucin, which indicates that the interaction between them is due to physical entanglement. Others have measured the zeta potential and particle size of mucoadhesive materials and mucin particles to study bioadhesion (Takeuchi et al., 2005). These methods have been used to explain the mucoadhesive behavior of pectin and mucin according to electrical theories and have been used to design optimal mucoadhesive polymers. Sriamornsak et al. (2010) employed atomic force microscopy (AFM) to investigate the interaction between porcine stomach mucin and pectin and to predict the mucoadhesive capacity.

The method proposed by Hassan and Gallo (1990) to evaluate the preliminary mucoadhesion capacity has been used by several authors and is based on the increase in viscosity (η) that mucin provides to a polymer. It is generally accepted that the entanglement of chains, conformational changes and chemical interactions between mucoadhesive polymers and mucin are capable of producing changes in the rheological behavior of the two macromolecular species involved (Hassan & Gallo, 1990; Rossi, Ferrari, Bonferoni, & Caramella, 2001; Thirawong et al., 2008). The technique assumes that the rheological response of the polymer–mucin system must be greater than the contributions of the formulation and mucin alone. Thus, the synergistic viscosity, also known as the rheological synergy parameter or the interaction parameter, can be calculated from the experimentally measured viscosities ($\eta_s = \eta_{exp} - \eta_{calc}$) (Callens, Ceulemans, Ludwig, Foreman, & Remon, 2003; Marschütz & Bernkop-Schnürch, 2002; Thirawong et al., 2008).

Measurements for viscosity were taken for pure mucin dispersed in water (η_m), for EPEC polymers in the absence of mucin and for the EPEC–mucin mixture. The results are shown in Table 3.

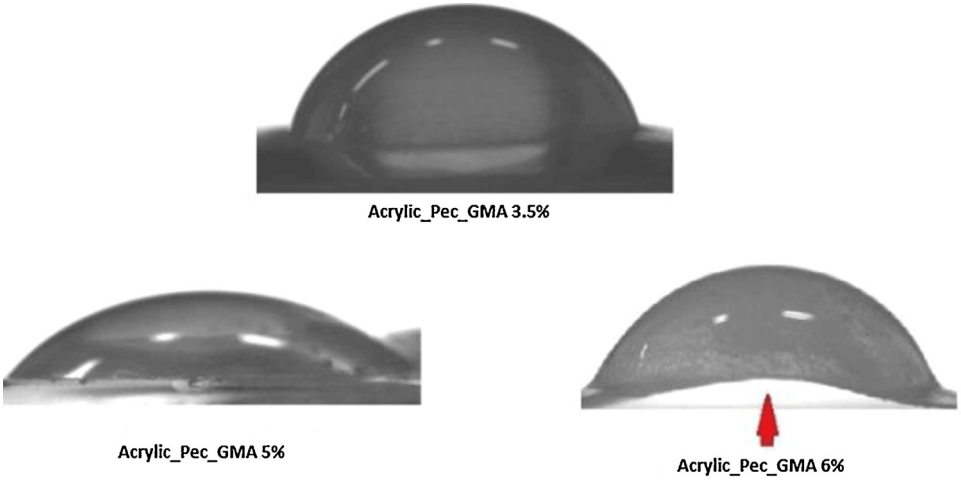

Fig. 7. Spreading of the water drop on surfaces of films formed from EPEC copolymers produced.

Table 3
Values of experimental and calculated viscosity for systems containing 5% (w/v) mucin.

Viscosity				
Measured		Calculated		
Pure	(EPEC + mucin)	Expected ($\eta_{cop} + \eta_m$)	Synergistic ($\eta_{exp} - \eta_{esp}$)	Relative (η_{exp}/η_{esp})
η_{mucin} = 45 cP				
η_{EPEC2} = 5 cP (SD = 1.50)	η_2 = 759 cP (SD = 1.88)	η_2 = 50 cP	η_2 = 709 cP	η_2 = 14.18 cP
η_{EPEC3} = 10 cP (SD = 0.00)	η_3 = 1440 cP (SD = 1.56)	η_3 = 55 cP	η_3 = 1385 cP	η_3 = 25.18 cP
η_{EPEC4} = 240 cP (SD = 1.60)	η_4 = 2958 cP (SD = 2.37)	η_4 = 285 cP	η_4 = 2673 cP	η_4 = 9.38 cP

SD = standard deviation.

Table 4
Peak force, work adhesion and debonding distance for the pure EPEC and for the EPEC/mucin mixtures.

	Peak force (N) ($\times 10^{-2}$)	Work of adhesion (N s) ($\times 10^{-2}$)	Debonding distance (mm)
EPEC 2	1.309 ± 2.89	0.0545 ± 3.57	5.135 ± 2.95
EPEC 2 + mucin	1.623 ± 3.18	0.426 ± 6.15	2.185 ± 5.15
EPEC 3	2.131 ± 4.79	0.0798 ± 5.50	4.878 ± 5.72
EPEC 3 + mucin	2.212 ± 7.77	0.182 ± 3.39	2.225 ± 4.07
EPEC 4	2.218 ± 0.37	0.092 ± 3.50	2.763 ± 3.31
EPEC 4 + mucin	2.633 ± 5.63	0.126 ± 4.11	1.912 ± 4.50

The viscosity of the polymers in the absence of mucin was low and exhibited a non-linear relationship to the amount of modified pectin. The results obtained during the measurements of the apparent viscosity showed that it increased approximately 100-fold after the addition of mucin.

Thirawong et al. (2007) showed that the η of pectin and pectin–mucin system was higher in acidic medium, due to the greater ability to form hydrogen bonds, resulting in labile intermolecular cross-links. At a higher pH, the ionization can increase, leading to a loss of the ability to form hydrogen bonds. The pH of the EPEC polymers was approximately 4, contributing to the appropriate measurements of viscosity. In all cases, the apparent viscosity obtained experimentally was higher than the calculated viscosity and increased as the concentration of the functionalized polysaccharide increased. Studies carried out by authors who used viscometric and rheometric techniques for the evaluation of mucoadhesion suggest that the increase in viscosity of the system–mucin mixture in the presence of pectin is due to the entanglement of chains and to the adsorptive phenomena, although hydrogen bonds or van der Waals interactions can be determined. Thus, the resistance to flow increases due to the formation of a greater number of interactions between mucin and the free hydroxyl and carboxyl groups of the polysaccharide (Sriamornsak & Wattanakorn, 2008; Sriamornsak et al., 2010; Thirawong et al., 2007).

3.5. Texture analysis

Texture analysis studies indicated that the addition of aqueous mucin gel to EPEC lattices caused an increase in both the cohesion and adhesive forces. The values of the maximum force for samples of pure EPEC and EPEC/mucin mixtures were similar. Instead, the parameters work of adhesion and debonding distance were different. EPEC/mucin mixture has the lowest penetration resistance and, the largest debonding distance in the sample suggests the existence of a cohesive force in these materials. Interaction with mucin also resulted in increased work of adhesion for all lattices. Table 4 presents the values of the texture analysis for the pure EPEC and for the EPEC/mucin mixtures.

4. Conclusions

According to the FTIR and NMR analyses, the functionalization of pectin by glycidyl methacrylate occurred successfully in the presence of heat and a low pH. The modified polysaccharide was used in the preparation of acrylic polymers containing EA, MMA and

BMA monomers using the emulsion polymerization process. An increase in the concentration of the functionalized pectin in lattices increased the wettability of the obtained polymers, as well as the synergistic viscosity, in the systems containing mucin, indicating an increase in the formation of intermolecular interactions. The addition of mucin in lattices caused an increase in the intermolecular forces and in the work of adhesion. One important criterion for designing and predicting the behavior of excipients toward adhesiveness is their ability to exhibit mucoadhesion. Although there have been several models used to assess the mucoadhesive properties of materials, the methods used in this study were successfully used to identify mucoadhesive excipients. According to the obtained results, a positive correlation was observed between different *in vitro* methods. Preliminary evaluations of the adhesive capacity of acrylic polymers containing modified pectin indicated that they can be useful in the development of bioadhesive materials for pharmaceutical systems. Studies should continue to develop a solid multiparticulate mucoadhesive DDS using EPEC polymers.

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