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Pectin gelation with chlorhexidine: Physico-chemical studies in dilute solutions

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ABSTRACT

Low methoxyl pectin is known to gel with divalent cations (e.g. Ca^{2+}, Zn^{2+}). In this study, a new way of pectin gelation in the presence of an active pharmaceutical ingredient, chlorhexidine (CX), was highlighted. Thus chlorhexidine interactions with pectin were investigated and compared with the well-known pectin/Ca^{2+} binding model. Gelation mechanisms were studied by several physico-chemical methods such as zeta potential, viscosity, size measurements and binding isotherm was determined by Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR). The binding process exhibited similar first two steps for both divalent ions: a stoichiometric monocomplexation of the polymer followed by a dimerization step. However, stronger interactions were observed between pectin and chlorhexidine. Moreover, the dimerization step occurred under stoichiometric conditions with chlorhexidine whereas non-stoichiometric conditions were involved with calcium ions. In the case of chlorhexidine, an additional intermolecular binding occurred in a third step.

1. Introduction

Pectin is an anionic polysaccharide contained in cell walls and mainly extracted from citrus or apple fruits. Its structure consists primarily of a succession of D-galacturonic acid and L-rhamnose units. Pectin is used as gelling or thickening agent not only in food industries but also in the pharmaceutical field due to its biocompatibility and biodegrability.

Pectins are classified in two groups depending on their degree of methoxylation (DM): Low-Methoxyl pectin (LM) with a DM <45% and High-Methoxyl (HM) pectin with DM >45%. LM pectin has the ability to form gels in the presence of divalent cations such as calcium, zinc or copper ions. This gelation results from a strong and specific interactions between calcium ions and galacturonate blocks (Axelos & Thibault, 1991). The mechanism involves junction zones created between calcium ions and the oxygen functions of galacturonic acid of two adjacent LM pectin chains. This mechanism based on electrostatic and ionic binding is called the "egg box" model (Fig. 1). This model was first described for alginate gelation in presence of dications and then transposed to pectin due to the similarities in structure and behaviour of these polymers (Grant, Morris, Rees, Smith, & Thom, 1973; Powell, Morris, Gidley, & Rees, 1982).

Nevertheless, many studies highlighted differences in the gelation mechanism of pectin and alginate. For example, Fang et al. (2008) compared the binding behaviour of Ca^{2+} ions with LM pectin and alginate. Both mechanisms exhibit a monocomplexation step followed by a dimerization step: Ca^{2+} ions link to one polymer chain inducing intermolecular binding between Free Galacturonic Acid (FGA) units.

Then, for alginate, a lateral association of dimers occurs. However, this phenomenon is not observed for LM pectin, certainly due to the structural difference of alginate and pectin exhibiting blockwise or random distribution of free acid units, respectively (Fang et al., 2008). A theoretical study based on molecular modelling (Braccini & Pérez, 2001) showed significant differences in the way of polymer chains interact. These results thus challenge the "egg-box" model for an appropriate description of polygalacturonic acid units' cross-linking.

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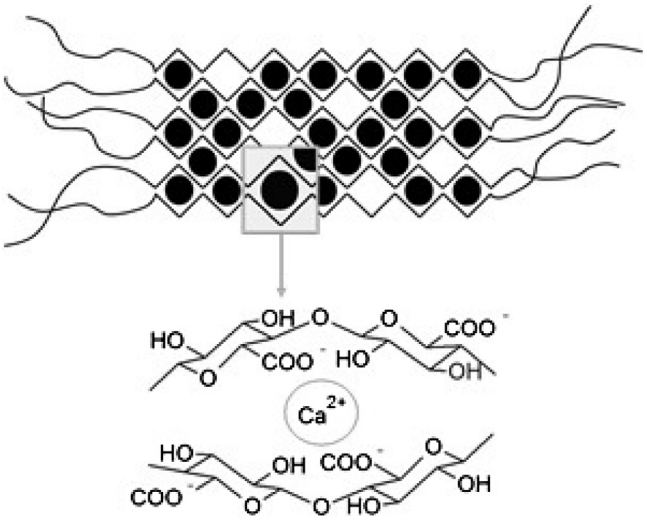

Fig. 1. Schematic representation of alginate gelation: the "egg box" model (Dupuis, Chambin, & Génelot, 2006).

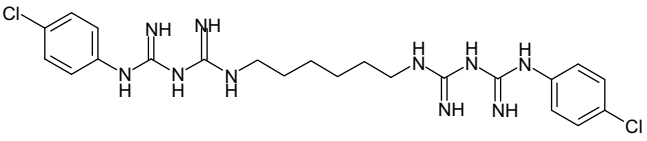

Fig. 2. Chlorhexidine structure.

Most of the pectin gelation studies were performed with calcium. However, pectin gelation has been reported with numerous divalent cations according to specific affinity. Thom, Grant, Morris, and Rees (1982) showed by circular dichroism that Cu^{2+} ions have a less specific binding mechanism than Cd^{2+}, Ni^{2+}, Pb^{2+} and Ca^{2+} ions. Based on light scattering measurements, Thibault and Rinaudo demonstrated that the pectin gelation selectivity depends on dication radius: $Ba^{2+} > Sr^{2+} > Ca^{2} > » Mg^{2+}$ (where there is no chain–chain interactions) (Thibault & Rinaudo, 1986). On the other hand, Dronnet and co-workers have experimentally defined a scale of selectivity ($Pb^{2+} \sim Cu^{2+} > > Zn^{2+} > Cd^{2+} \sim Ni^{2+} > Ca^{2+}$) which is not in agreement with the previous study (Dronnet, Renard, Axelos, & Thibault, 1996).

Chlorhexidine (CX) is a bisbiguanide antiseptic with a broad-spectrum efficacy against Gram-positive, Gram-negative bacteria and fungi (Fig. 2). This dicationic substance is commonly used as salt (e.g., diacetate or digluconate) in different fields such as urology, gynecology, dentistry and periodontal therapy or the treatment of buccal injuries such as sores or ulcers (for example, in mouth rinses). Its therapeutic efficacy as buccal antiseptic is limited by the short retention time in the buccal cavity due to the presence of saliva in association with swallowing and chewing. The CX biodisponibility could be improved by using a mucoadhesive drug delivery system which should extend the contact between the active substance and the buccal mucosa. For this purpose, the development of CX-loaded pectin mucoadhesive particles was considered in order to improve local CX efficiency during buccal administrations. For the CX encapsulation, an emulsification/gelation method based on Ca^{2+}/pectin interactions and previously described by Pliszczak et al. (2011) was considered. However, the first assays highlighted interactions between CX and the polymer leading to pectin gelation without the presence of Ca^{2+} dications. Hence, the aim of this study was to investigate gelation mechanism of pectin in presence of CX. For this purpose, pectin/CX interactions were characterised by several physico-chemical studies in the dilute regime: relative viscosity, size and zeta potential measurements, binding isotherm determined by ^{1}H NMR. Binding behaviour of Ca^{2+} and CX with pectin were compared.

2. Material and methods

2.1. Material

Unipectine OF400 SB was obtained from Arlès (France). Aqueous solution of chlorhexidine digluconate (20%w/v), alcohol oxidase *from Pichia Pastoris*, 2–4 pentadione and 3-phenylphenol (85%w/v) were purchased from Sigma Aldrich (France). Calcium chloride dihydrate and D-(+)-galacturonic acid monohydrate (97%w/w) were products of Fluka Analytical. Pullulan polysaccharides standards (with molecular weight: 11 100, 47 100, 107 000, 200 000, 375 000 and 708 000 g mol^{-1}) were purchased from Agilent.

2.2. Pectin characterisation

2.2.1. Determination of molecular weight

The apparent molecular weight of pectin (MW) was determined by size exclusion chromatography (SEC) on an Agilent 1200 Series system equipped with a guard column (Agilent Polymer Labs PolarGel M 50 × 7.5 mm) and two columns (Agilent Polymer Labs PolarGel M 300 × 7.5 mm and PolarGel L 300 × 7.5 mm). The detection was done with a refractive index detector (Waters 2414). 20 μL of pectin solution were injected at a concentration of 1 mg mL^{-1}. Elution was achieved at a flow rate of 1.5 mL min^{-1} with an aqueous solution of 0.025 M Na_2SO_4 at 30 °C. Molecular weight calculations were done with Polymer Laboratories Cirrus software by using a calibration curve obtained with polysaccharide standards.

2.2.2. Determination of galacturonic acid content

In pectin chains, carboxyl groups of galaturonic acid (GalA) units could remain as FGA or be esterified. The determination of GalA content allows to get information about chemical properties of pectin.

In the literature, the quantification of uronic acid is performed by using the well-known colorimetric method of Blumenkrantz and Asboe-Hansen. Briefly, the dehydration of galacturonic acid units is achieved leading to the formation of 2-Furancarboxylic acid. The latter is then esterified by using meta-hydroxydiphenyl to form a chromophoric compound that absorbs at 520 nm (Blumenkrantz & Asboehansen, 1973; Brian-jaisson, 2014).

In this study, the procedure of the Kintner and Van Buren (Kintner & Van Buren, 1982), based on the Blumenkrantz and Asboe-Hansen method, was slightly modified. 1 mL of standards containing 15 up to 75 μg mL^{-1} galacturonic acid were prepared and placed in Pyrex tubes. Then 6 mL of 0.0125 M sodium tetraborate in concentrated sulfuric acid were added. After stirring, samples were heated at 100 °C in a water bath for 10 min, and immediately cooled in ice water for 10 min. After addition of 0.1 mL of 0.15% 3-phenylphenol in 0.5% NaOH, tubes were vortexed for 30 min in the dark at room temperature. Then absorbance at 520 nm was measured with a Perkin Elmer Lambda XLS spectrophotometer, leading to the determination of a calibration curve.

The same protocol was applied to 1 mL pectin samples (from 50 to 150 μg/mL) in order to determine, via the calibration curve, their GalA content.

The galacturonic acid content (%GalA) is expressed by the equation:

$$\%GalA = \left(m_{GalA}/m_{pectin}\right) \times 100 \quad (1)$$

where m_{GalA} is the mass of galacturonic acid and m_{pectin} is the mass of pectin.