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Design and properties of biopolymer–silica hybrid materials: The example of pectin-based biodegradable hydrogels*

Nouria Agoudjil[1,2], Clémence Sicard[1], Vincent Jaouen[1],
Catherine Garnier[3], Estelle Bonnin[3], Nathalie Steunou[1],
and Thibaud Coradin[1,‡]

[1]UPMC Université Paris 06, CNRS, Chimie de la Matière Condensée de Paris, 75005 Paris, France; [2]Laboratoire d'Etude Physico-Chimique des Matériaux et Application à l'Environnement, USTHB, 16111 Alger, Algeria; [3]INRA, UR 1268 Biopolymères, Interactions, Assemblages, 44316 Nantes, France

Abstract: The association of natural polymers with silica is a fruitful strategy to design novel hybrid structures with potential applications in the fields of biotechnology, medicine, and environmental sciences. Here we illustrate the principles of formation, the structure, and the properties of such biohybrid systems by the specific example of silica–pectin porous materials. The influence of the silica source, i.e., aqueous silicates and tetraethoxysilane (TEOS), was more specifically addressed. The alkoxide precursor may be associated with the polysaccharide in a wide range of concentrations. In contrast, the high initial viscosity and fast condensation rate of silicates limits the range of accessible compositions. Owing to weak interactions between the mineral and organic components, silicification does not improve the thermal stability of the polymer but has a strong impact on the water stability of the materials, which depends on the silica source. It is shown that the stability of the silica–pectin hybrid materials during enzymatic degradation is optimum for low carbohydrate content and high silica content, independently of the inorganic precursor.

Keywords: biodegradation; biohybrid materials; carbohydrates; materials chemistry; pectin; silica.

INTRODUCTION

A large variety of biohybrid materials formed by the association of biopolymers with polymerizable sources of silica have been developed over the last few years [1], with main applications in (bio)catalysis [2], the biomedical field [3], and the textile industry [4]. Two main approaches have been described [5]: (i) addition of the silica source to a biopolymer solution that has either intrinsic gelling properties or forms a gel due to its interactions with silica materials; (ii) addition of the silica source to a preformed biopolymer network, resulting either in a coating of the outer surface or full impregnation and in situ condensation. The biopolymer–silica interface can be controlled by intrinsic charge matching (i.e., using cationic polymers that activate the condensation of anionic silica species) [6] or by introducing

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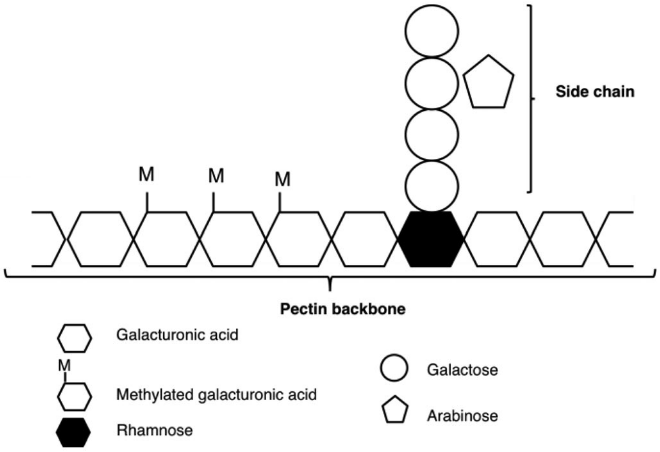
[‡] Corresponding author

organosilanes, creating electrostatic or covalent bond between the bioorganic and inorganic networks [7]. Biohybrid structures can also be formed with neutral polymers, such as cellulose derivatives [8], relying on hydrogen-bond formation and even with negatively charged macromolecules, with a possible localization of cationic counterions at the interface [9].

So far, a large majority of these biohybrid materials have been obtained using alkoxysilanes, but these molecules are obtained by high-energy process and/or using unsafe chemicals [10]. Alternatively, aqueous silicates prepared via solvent-free routes exhibit low toxicity and environmental impact and can be used for biopolymer silicification [11]. However, at a given concentration, they form more viscous solutions and gel much faster than alkoxides, especially near neutral pH. Silicates are also more sensitive to ionic strength and presence of cationic additives.

The present work was undertaken with the purpose of identifying the conditions for the association of a given biopolymer with different siloxane sources to obtain biohybrid materials that may find environmental applications. For this purpose, we selected pectin, a common polysaccharide. From a chemical point of view, pectins are polyanionic polymers at neutral pH with a backbone mainly composed of α(1-4)-linked galacturonic acids (Scheme 1) [12]. The extent to which the carboxylic acid moieties can undergo methylation is defined by the "degree of methylation" (DM). This backbone also contains side chains composed of neutral sugars, mainly rhamnose, galactose, and arabinose. Pectins are water-soluble down to pH ≈ 4 but insoluble in most organic solvents. Because both pectins and silica are negatively charged at neutral pH, no attractive electrostatic interactions are expected to arise between the polymer and the silicon sources. This should favor the formation of homogeneous hybrid material rather than precipitation. Noticeably, although it is a very common biopolymer, pectin has been only sparingly associated with silica within hybrid materials, always using silicon alkoxides as precursors and with a limited polymer content (5 wt % to SiO_2) [13].



Scheme 1 Schematic structure of pectin (adapted with permission from the original version in ref. [14], copyright © 2007, John Wiley).

Here the preparation and characterization of homogeneous pectin–silica hybrid gels from organic (tetraethoxysilane, TEOS) and mineral (sodium silicate) precursors are reported. The water stability and, for the first time to our knowledge, the enzymatic degradation of such polysaccharide–silica hybrid systems are presented, showing noticeable variations with silica sources, carbohydrate content, and silicification rate.

RESULTS

Conditions for material preparation

In a first step, a phase diagram of the pectin–silicate and pectin–TEOS systems in water was established after 24 h of reaction (Fig. 1). Preliminary experiments on the dissolution of pectin in deionized water show that solutions obtained at polymer concentrations higher than 0.4 wt % are too viscous to allow a homogeneous mixing with silica precursors. The maximum concentration in silicate and TEOS precursors, allowing a suitable mixture with pectin before silica gel formation, are 0.3 and 0.7 M, respectively, except for a 0.4 wt % polymer concentration where the highest silicate concentration leading to a homogeneous gel is 0.2 M. As far as minimum concentrations are concerned, no gel is obtained for a 0.1 wt % pectin concentration, whatever the silicate or TEOS concentration. For both precursors, no gel is obtained upon mixing 0.1 M silica solution with a 0.2 wt % polymer solution. The composition of the different materials studied in this work is gathered in Table 1.

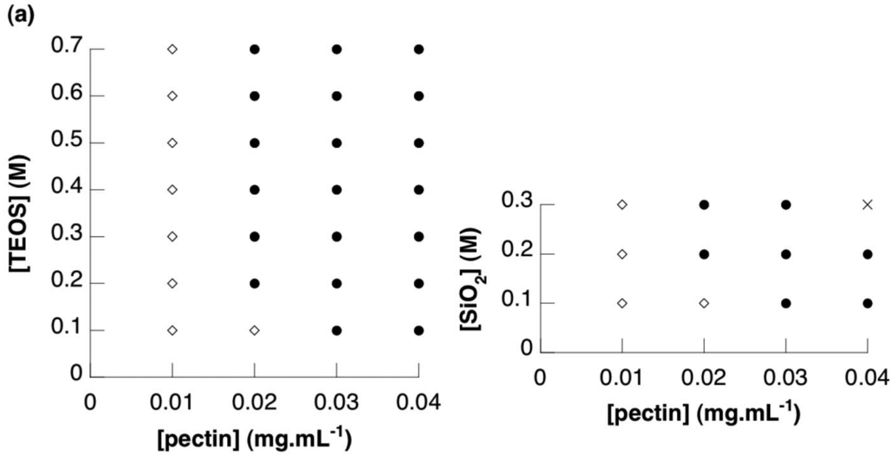


Fig. 1 Phase diagrams of pectin with (a) TEOS and (b) silicates after 24 h: no gel formation (diamonds), gel formation (dark dots), inhomogeneous gels, or precipitates (crosses).

Table 1 Name, silica source, silica concentration $[SiO_2]$, and pectin concentration [pectin] of materials.

Sample	Silica source	$[SiO_2]$ (M)	[pectin] (mg·mL^{-1})
P3Si3	$Na_2Si_3O_7$	0.3	0.03
P4Si1	$Na_2Si_3O_7$	0.1	0.04
P4Si3	$Na_2Si_3O_7$	0.3	0.04
P2T7	$Si(OC_2H_5)_4$	0.7	0.02
P3T3	$Si(OC_2H_5)_4$	0.3	0.03
P3T7	$Si(OC_2H_5)_4$	0.7	0.03
P4T1	$Si(OC_2H_5)_4$	0.1	0.04
P4T3	$Si(OC_2H_5)_4$	0.3	0.04
P4T5	$Si(OC_2H_5)_4$	0.5	0.04
P4T7	$Si(OC_2H_5)_4$	0.7	0.04

Chemical, physical, and structural characterizations

TGA analysis of the initial pectin powder indicates that it undergoes thermal degradation mainly in the 200–600 °C range, and that about 50 wt % of the polymer is lost between 200 and 400 °C (Fig. 2). In the same temperature range, the selected material obtained with TEOS (**P3T3**) undergoes a weight loss corresponding to the expected pectin content. For the composites obtained with silicates (**P3Si3**, **P4Si3**), the weight loss in the same temperature range is found relatively lower. This is due to the presence of sodium ions from the initial silicate ($Na_2Si_3O_7$) solution together with added HCl for neutralization [9]. Noticeably, no difference is observed in the onset temperature of degradation of the materials, suggesting that silica has no significant influence on the thermal stability of the biopolymer.

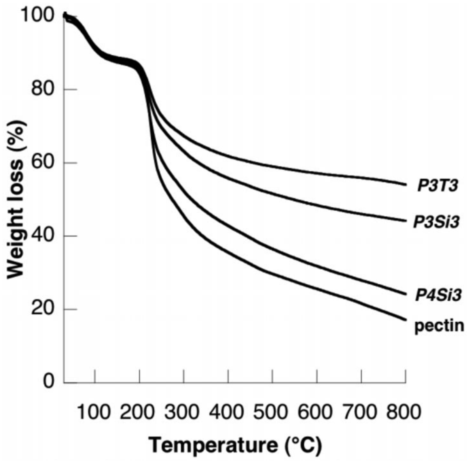


Fig. 2 TGAs of selected pectin–silica materials.

In the high wavenumber domain, Fourier transform infrared (FTIR) spectra of the original pectin display a broad band at ca. 3550 cm^{-1} corresponding to ν(OH) stretching of the polymer hydroxyl groups and a series of overlapping peaks at ca. 2960, 2920, and 2850 cm^{-1} corresponding to the symmetric and asymetric stretching vibration $\nu(CH_2)$ and $\nu(CH_3)$ (Fig. 3) [15]. A band at 1750 cm^{-1} is also apparent corresponding to the carbonyl vibration band of acetyl groups. The vibration band at 1650 cm^{-1} can be attributed to $\delta(H_2O)$ of adsorbed water. Two groups of overlapping bands are observed between 1450 and 1250 cm^{-1} and between 1200 and 1000 cm^{-1}, corresponding to the so-called fingerprint region of the pectin. The former includes the bending vibration of CH_2 and CH_3 groups as well as the ν(COC) of the methyl ester groups [16]. The latter includes the ν(COC) of the polymer backbone as well as the ν(CC)(CO) bands. The two bands at 950 and 830 cm^{-1} correspond to the bending vibration γ(OH) of the hydroxyl groups. In the presence of a high amount of TEOS (**P3T7**), the 1200–1000 cm^{-1} region is occupied by the stretching vibration modes of the Si–O bonds [17]. A more intense band also appears at ca. 950 cm^{-1} corresponding to the Si–OH group. The Si–O–Si bending vibration mode that is an indicator of the extent of silica condensation is found at 800 cm^{-1}. The 470 cm^{-1} band corresponds to the bending vibration modes of the SiO_4 tetrahedron. Keeping the amount of pectin constant and decreasing the TEOS amount (**P3T3**), or increasing pectin content at similar TEOS amount (**P4T3**), the fingerprint region of the polymer in the 1450–1250 cm^{-1} domain becomes slightly more apparent. No significant difference is observed between silicate- and TEOS-based materials at similar pectin content (compare **P3Si3** with **P3T3**).

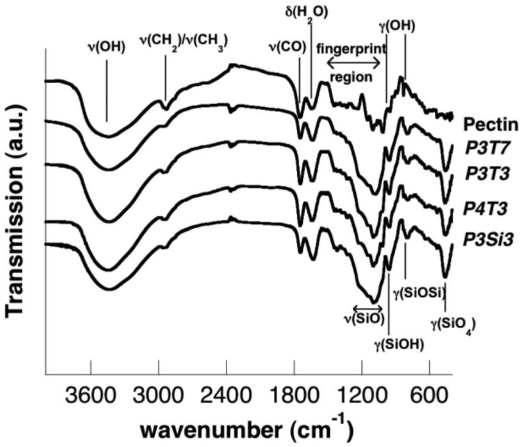


Fig. 3 FTIR spectra of selected pectin–silica materials.

Observations by scanning electron microscopy (SEM) show that an increasing amount of TEOS in the pectin solution turns a dense network (**P4T1**) into a fragmented material consisting of thin platelets (**P4T7**) (Figs. 4a,b). At intermediate silica–pectin ratio, cellular porous materials are obtained (**P2T7**) (Fig. 4c). Noticeably, the presence of silicates seems to perturb the material structure to a greater extent than TEOS (compare **P4Si**, Fig. 4d with **P4T1**, Fig. 4a).

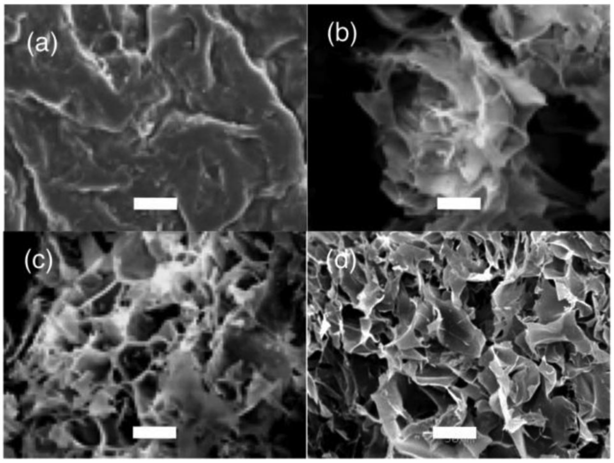


Fig. 4 SEM images of selected pectin–silica materials: (a) **P4T1**, (b) **P4T7**, (c) **P2T7**, (d) **P4Si1** (scale bar = 20 μm).

Water stability and enzymatic degradation

The stability of pectin–TEOS and pectin–silicate wet gels was first evaluated in water. The percentage of dissolved–redispersed gel as a function of time is shown in Fig. 5. Over three weeks at room temperature, **P4Si3** is extensively degraded and the initial gel phase is difficult to distinguish from the redispersed matter. From dry weight measurements, more than 50 wt % of the hybrid system is found in the supernatant. Over the same period of time, at a similar pectin and silica content, **P4T3** undergoes a more limited degradation and can still be visualized in the reaction vessel. Dry weight measurements indicate

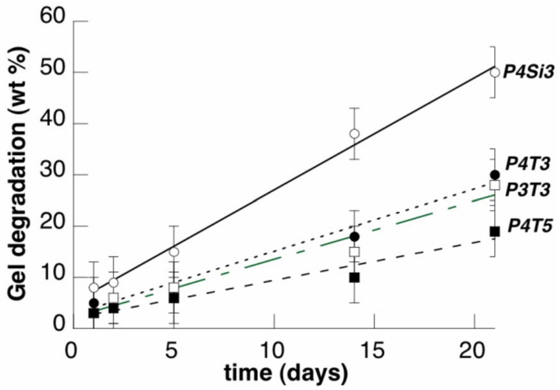


Fig. 5 Water stability of selected pectin–silica gels.

that only ca. 30 wt % of the initial gel is resuspended–dissolved. Decreasing the pectin content at fixed silica concentration does not significantly influence the degradation of the material, as shown for **P3T3**. In contrast, when TEOS content is increased at fixed pectin concentration, only ca. 15–20 wt % of the **P4T5** gel is resuspended–redissolved after three weeks.

Enzymatic degradation occurs in a different manner. Over the first two days, an important weight loss is observed for all samples that continue more slowly afterwards (Fig. 6). When the different samples are compared, **P4Si3** and **P4T3** show significantly higher degradation (>80 wt % after 10 days) compared to **P3T3** and **P4T5** (65 and 55 wt %, respectively). It is worth noting that such amounts are greater than (or equal to, for **P4T5**) the initial pectin weight content, suggesting that both polymer and silica degradation occur. Control experiments performed at pH 4 but without enzymes show either no significant or very limited degradation (<5 % over 10 days) for all samples.

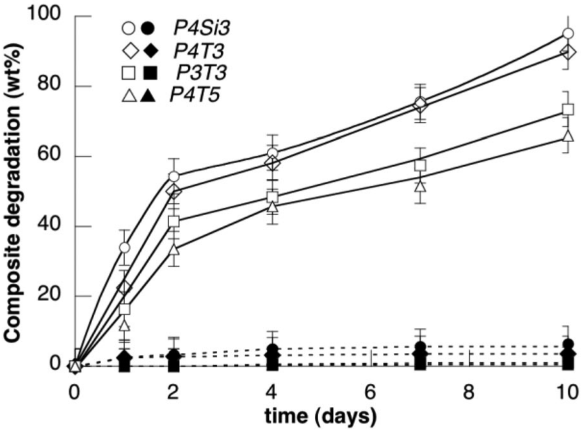
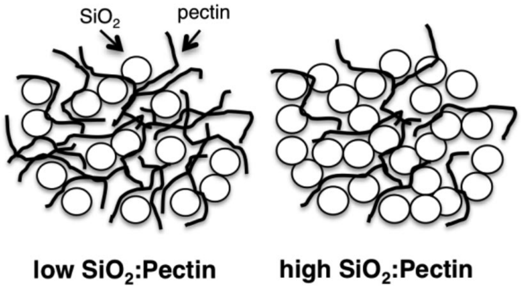


Fig. 6 Degradation of selected pectin–silica gels in acidic conditions (pH = 4) without (dark symbols) or with added enzyme (open symbols).

DISCUSSION

The first aim of this work was to elucidate the conditions to form hybrid polysaccharide–silica materials based on the in situ condensation of molecular precursors in the aqueous biopolymer solution. It is shown here, that it is possible to obtain homogeneous materials whose composition range depends on the precursors. Because no strong interaction is expected to arise between the inorganic and biological components, limitations are mainly due to intrinsic properties of the systems: viscosity of the precursors and polysaccharide and gelation time of the silica sources. As a result, the silicon alkoxide source that consists of prehydrolyzed silicon monomers, allows the formation of hybrid materials with much higher silica concentration than silicates that contain a mixture of reactive oligosilicic acids.

Examination of the materials by SEM shows that the introduction of silica in the polymer solution leads to porous network formation at the micrometer scale. This morphology is similar to the expected one for silicate-based gels. In contrast, alkoxide-based xerogels are usually obtained as dense materials [18]. Therefore, it can be proposed that pectin acts as a dispersion agent: it does not prevent silica gel formation but it locally limits its 3D extension (Scheme 2). Within this hybrid network, the pectin–silica interface is expected to be weak, as confirmed by thermogravimetric analyses (TGA) showing no variation in the degradation temperature of pectin with silica content.



Scheme 2 Proposed structure of silica–pectin hybrid materials at low and high SiO_2:pectin ratio.

Water stability and enzymatic degradation tests are in good agreement with the proposed structure. For pectin–silica at pH 7, a continuous degradation is observed, due to the fact that both pectin and silica are soluble in these conditions. In contrast, at pH 4 where silica and pectin are much less soluble, no significant redispersion is observed. However, when the enzyme is present at the same pH, a fast degradation is observed over two days, that then proceeds more slowly. In all cases, the extent of degradation is more limited when the relative amount of pectin is smaller. In parallel, the amount of redispersed material is larger than the initial pectin content. Therefore, it can be proposed that the hydrolysis of pectin first occurs, inducing a loss of cohesion of the whole materials and therefore favoring redispersion. Noticeably, mineral silicate precursors are less advantageous than organosilanes in terms of composition range and water stability, but the source of silica does not impact the mechanism of enzymatic degradation.

CONCLUSION

The here-described system allows the identification of some key features of biopolymer–silica materials obtained by an in situ silica condensation process. In terms of synthesis, the maximum content of each component is dependent on the viscosity of the macromolecule solutions and the kinetics of silica formation, respectively, both parameters increasing significantly with initial concentrations. Considering the structure of the resulting hybrid systems, silicification appears to introduce porosity in the biopolymer network. Noticeably, it was recently demonstrated that it is possible to take advantage of this phenomena in the field of biomaterials [19]. Finally, the processes of degradation of these materials in vivo or in the environment can occur via bioresorption (i.e., spontaneous dissolution in the presence of fluids) and biodegradation (i.e., biological fragmentation) phenomena. The former is applicable to both silica and biopolymers while the latter is, in most cases, only observed for the biological macromolecules [20]. This opens the possibility to use such materials for the controlled release of active molecules, both in the medical field [21] but also for soil treatment [22].

EXPERIMENTAL SECTION

Preparation of pectin–silica hybrid materials

The citrus pectin sample was provided by Cargill Texturizing Systems (Baupte, France) with a DM = 46. Silicate-based materials were prepared by neutralization of a 10-mL diluted sodium silicate solution ($Na_2Si_3O_7$, 10 % NaOH, 27 % SiO_2) of concentration [SiO_2] in the 0.1–0.4 M range by HCl, followed by slow addition of the pectin powder under stirring to reach a final pectin concentration [Pect] in the 1–5 wt % range. In the following text, these materials are denoted **P[Pect]Si[SiO$_2$]**. TEOS-based materials were prepared by the addition of the pectin powder to 10 mL of a diluted TEOS [$Si(OC_2H_5)_4$] solution of concentration [TEOS] in the 0.1–0.8 M range prehydrolyzed for 1 h in acidic conditions (HCl, pH = 3) and neutralized with NaOH. In the following text, these materials are denoted **P[Pect]T[TEOS]**. For both systems, a phase diagram was established after 24 h. When homogeneous gels were recovered, they were freeze-dried before further analysis, leading to porous xerogel monoliths.

Stability and enzymatic degradation studies

For water-stability evaluation, as-prepared (i.e., wet) materials were covered with 10 mL of deionized water and kept at room temperature. At selected sampling time, 5 mL of the solution was withdrawn and replaced by 5 mL of deionized water. The sampled suspension was put in an oven for 3 days at 105 °C for dry weight measurements. For enzymatic degradation tests, endo-polygalacturonase from *Aspergillus aculeatus* (E.C. 3.2.1.15) was selected, allowing the endohydrolysis of (1–>4)-α-D-galactosiduronic linkages in pectin. One unit of pectinase should liberate 1.0 µmol of galacturonic acid per min at pH 4.0 at 25 °C. Xerogels obtained after-freeze drying were cut into pieces of ca. 20 × 10 mm (300–400 mg). They were placed in 10-mL plastic vessels pierced with holes that were immersed in glasswares filled with 65 mL acidified water (pH 4) containing or not 100 enzyme units per mL. At sampling time, the immersion solution was recovered and placed in an oven for 5 days at 105 °C for dry weight measurements. It was replaced by a freshly prepared solution in which the remaining gel was immersed for further reaction.

Characterizations

TGAs were performed on a NETSCH STA 409PC apparatus under Ar with a 5 °C/min heating ramp. FTIR spectroscopy was performed on a Perkin Elmer Spectra 400 equipment. SEM was performed on a JEOL JSM-5510LV working at 20 kV after gold coating of the samples.

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