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Rheological behaviour and microstructure of microfibrillated cellulose suspensions/low-methoxyl pectin mixed systems. Effect of calcium ions

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ABSTRACT

In this study, the influence of the presence of low-methoxyl pectin (LM pectin) on the rheological and microstructural properties of microfibrillated cellulose suspensions was elucidated in order to create new structures with new and interesting textures. For that purpose, the rheological properties of the cellulose/LM pectin mixtures in variable proportions were compared with those of the individual biopolymers. The influence of the presence of calcium and/or sodium ions on the properties of the mixed systems was studied. The microstructure of the resulting system was studied by transmission electron microscopy and confocal laser scanning microscopy. It was found that, in the presence of LM pectin, a synergistic effect was observed when calcium ions were also present, leading to increased rheological properties of the composites. Indeed, addition of calcium to the mixtures induced LM pectin gelation, which was favoured in the presence of sodium, the pectin network contributing to the formation of a stronger cellulose/LM pectin composite gel. The presence of LM pectin alone in the microfibrillated cellulose suspensions does not significantly modify the viscoelastic and microstructural properties of microfibrillated cellulose suspensions. Whether calcium was added to the mixtures or not in water, the viscoelastic properties of the mixtures are mainly controlled by cellulose. The same behaviour was observed for the mixtures in NaCl without added calcium. Contrary to this observation, it was noticed that in presence of both sodium and calcium ions, the viscoelastic properties of the mixtures are largely governed by LM pectin. On the other hand, it was showed that the flow behaviour of microfibrillated cellulose suspensions is modified in the presence of LM pectin with an increase in thixotropic character shear-thinning behaviour, which was more pronounced in the presence of NaCl. It was also shown, from TEM observations, that an interpenetrating network formed in cellulose/LM pectin composites gel in the presence of calcium ions. In the same way, the CLSM observations allowed the separate localization of cellulose and LM pectin within the composite systems to be highlighted. The results obtained suggests that it is possible to thus create new structures with new interesting textures, by mixing microfibrillated cellulose suspensions and LM pectin in suitable proportions in the simultaneous presence of both sodium and calcium ions.

1. Introduction

Polysaccharides and mixed polysaccharide systems are used as raw materials in a variety of food or non-food applications, either to modify flow behaviour, enhance mechanical strength, or improve adhesion performance (Pérez & Mazeau, 2005). Due to this extensive use, many academic and industrial scientists wish to better understand the behaviour of mixed polysaccharide systems in order to optimize their use and find new structures or potentially novel applications.

Cellulose and pectins, the major components of most plant primary cell walls, are polysaccharides having different structures and very diverse properties (Carpita & Gibeaut, 1993; Talbott & Ray, 1992). Cellulose is biosynthesized not only by higher plants but also by a wide variety of bacteriae, algae and fungi and by some animals (tunicates for instance) (Attala, 1999; Horii, 2000; Salmon & Hudson, 1997). It is a linear homobiopolymer with high molecular weight composed of long chains of D-glucose units joined together by β-1,4-glucosidic bonds, which are tightly linked by hydrogen bonds to form cellulose microfibrils (Kirk & Othmer, 1967). By using high-pressure homogenization, a process commonly used in the food industry, it is possible to disintegrate the cellulose structure into suspension of myofibrils to obtain what is known as microfibrillated cellulose (MFC) (Herrick, Casebier, Hamilton, & Sandberg, 1983; Turbak, Snyder, & Sandberg, 1983). Cellulose nanofibers

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materials have excellent mechanical and thermal properties. They have a high Young's modulus of 138 GPa for the crystalline regions, in the longitudinal direction (Sakurada, Nukushina, & Ito, 1962) and a very low thermal expansion coefficient (10^{-7} K^{-1} in the longitudinal direction) (Nishino, Matsuda, & Hirao, 2004). Therefore cellulosic nanofibers were used in many applications as a reinforcing phase in nanocomposite films (Azizi Samir, Alloin, & Dufresne, 2005). In addition to their exceptional mechanical and thermal properties, when properly dispersed in water, cellulosic nanofibers interact with each other to build a strong network that provides a gel-like structure to the aqueous medium, even at low concentration (Agoda-Tandjawa et al., 2010; Pääkkö et al., 2007). These interesting mechanical properties of MFC suspensions make them a promising nanofiber for use in future materials. Besides cellulose, pectins, the other major component of primary cell wall, are anionic polysaccharides, which can be commercially extracted from fruit industry waste such as citrus peels and apple pomace. These polysaccharides are composed of a backbone of $(1 \rightarrow 4)$-linked α-D-galacturonic acid units that can be methyl esterified and acetylated. This linear chain is interspersed with α-$(1 \rightarrow 2)$-linked L-rhamnopyranosyl residues, to which are linked neutral sugar side-chains, composed mostly of arabinose and galactose (Ralet & Thibault, 2002; Thakur, Singh, & Handa, 1997; Voragen et al., 2005). The degree of methylation (DM), i.e., the proportion of galacturonic acids esterified by methyl groups (on the carboxylic acid) is of great importance for the use of pectins as gelling agents. Depending on the degree of methyl esterification (DM), they are classified into high methoxyl (HM) pectin (DM > 50%) and low methoxyl (LM) pectin (DM < 50%). LM pectins are well-known to form gels in the presence of calcium ions over a wide range of pH values (Axelos & Thibault, 1991). It was established that the affinity of pectins towards calcium ions becomes greater when the degree of methylation of pectins and the ionic strength of the systems decreased (Garnier, Axelos, & Thibault, 1994). On the other hand, the affinity of pectic chains towards calcium ions also increased with polymer concentration. According to Garnier et al. (1994), pectin-calcium interactions are anti-cooperative in water and become cooperative in the presence of 0.1 M NaCl.

Recently, Zykwinska, Ralet, Garnier, & Thibault (2005) showed that pectins can bind *in vitro* to cellulose via their neutral sugar side chains. On the other hand, Chanliaud and Gidley (1999) reported that commercial citrus pectins which are well-known to contain very limited amounts of neutral sugar side chains due to their extraction under harsh acidic conditions, do not bind to cellulose. Even though pectins low in neutral sugar side chains do not bind to cellulose, their viscosifier effect or gelling properties could be used in order to find new structures.

Great attention has been given to the possibility of using mixtures of polysaccharides to improve gel strength. Up to now, research into the assembly of cellulose and pectins has mainly focused on the way in which they interact in the plant primary cell walls. However, although LM pectins and cellulose suspensions display interesting gel-like behaviour, the rheological behaviour of cellulose/LM pectins mixed systems in presence or not of NaCl and/or $CaCl_2$ has not been a topic of previous studies and little information on their microstructure in relation with rheological properties exists.

This study focuses on a better understanding of the properties of model systems composed of microfibrillated cellulose, and an anionic polysaccharide, a low methoxyl pectin (LM pectin) in order to propose controlled "cellulose/pectins" model systems, having interesting texturing properties. The rheological and microstructural properties of the mixtures were studied in water or in 0.1 M NaCl, in the presence or not of 3 mM $CaCl_2$ at pH 6.8 and were compared to the properties of each biopolymer.

2. Materials and methods

2.1. Materials

Sugar-beet cellulose was extracted from dried sugar-beet pulp provided by S.I.D.E.S.U.P (Sugar factory in Engenville, France), as described elsewhere (Agoda-Tandjawa et al., 2010). The obtained cellulosic residue contained 80.3% of glucose and a low amount of galacturonic acid (1.2%) (Agoda-Tandjawa et al., 2010).

The commercial citrus LM pectin sample (LMNA DE 28, no. 13827), kindly given by Cargill Texturant systems France SAS (Baupte, France), had a degree of etherification of 28.5% and 80.6% of galacturonic acid content. Pectin powder was purified by washing with acidic ethanol (EtOH/HCl: 5%/70%) in order to eliminate ions in excess and to obtain the polysaccharide under an acidic form.

Calcium content of both biopolymers was determined by atomic absorption spectroscopy. Results were 3.75×10^{-2} mM in purified cellulosic residue and 3.31×10^{-2} mM in purified LM pectin powder.

2.2. Samples preparation and biopolymer mixtures

The aqueous suspensions of cellulose microfibrils (microfibrillated cellulose suspensions) have been obtained by treating aqueous cellulose suspension through a Rannie two-stages Homogenizer (5 passes at 300 bars and 5 other passes from 380 to 400 bar) as described elsewhere (Agoda-Tandjawa et al., 2010).

The purified LM pectin powder was dispersed in deionized water (2.5%, w/w) under gentle stirring at room temperature for 4 h. The pH of the solution was adjusted at 6.8 with NaOH, then left overnight at 4 °C under gentle magnetic stirring to carry on the solubilization. After this process, it was verified that the pH was still 6.8 and the solution was filtered respectively through a 8, 3, 0.8 and 0.45 μm filter to remove the non-dissolved particles and aggregates. Sodium azide (0.02%) was added to the cellulose suspension and the LM pectin solution to prevent from bacterial contamination. Concentrations were calculated from the determination of the dry matter.

The suspensions of cellulose microfibrils and LM pectin solutions were mixed in variable proportions (0.25/0.75, 0.5/0.5 and 0.75/0.25) at room temperature in water or in 0.1 M NaCl to obtain 1% (w/w) of total biopolymer concentration while $CaCl_2$ would be added or not. It was verified that the pH after mixing was still 6.8. All the samples were then homogenized with the high intensity mixer (Polytron PT 45/80, Kinematica, Switzerland) at 20 000 rpm for 5 min. The mixtures were then stirred 25 min at 80 °C before addition of a hot $CaCl_2$ solution and then let to equilibrate at this temperature for 5 min. $CaCl_2$ solution was added to obtain 3 mM $CaCl_2$ in the medium at a total biopolymer concentration of 1% (w/w). All the prepared systems are shown in Table 1.

2.3. Rheological measurements

The rheological measurements were carried out using an ARES (TA Instruments) controlled strain rheometer equipped with a 40 mm teflon plate-and-plate geometry and a Peltier temperature controller. The gap was fixed at 1 mm. The cellulose/LM pectin system or the individual component, prepared as described above, was poured onto the ARES plate heated at 80 °C. Samples were covered with paraffin oil to prevent evaporation during measurements, and the system was cooled to 20 °C. The kinetics of gel formation at 20 °C was monitored by measuring the storage modulus (G') and the loss modulus (G'') at 1 rad s^{-1}. When the moduli reached a plateau (after ~20 h), the system was considered to be at equilibrium and mechanical spectra were recorded over the frequency range 100–0.01 rad s^{-1}. In all experiments, the measurements were

Table 1
Composition and labels of the different samples of microfibrillated cellulose, LM pectin, and their mixtures.

Label of the samples	In water			Label of the samples	In 0.1 M NaCl		
	Cellulose (%, w/w)	LM pectin (%, w/w)	CaCl$_2$ (mM)		Cellulose (%, w/w)	LM pectin (%, w/w)	CaCl$_2$ (mM)
C1	1	0	0	C1-Na$^+$	1	0	0
C1-Ca^{2+}	1	0	3	C1-Na$^+$-Ca^{2+}	1	0	3
C0.75	0.75	0	0	C0.75-Na$^+$	0.75	0	0
C0.5	0.5	0	0	C0.5-Na$^+$	0.5	0	0
C0.25	0.25	0	0	C0.25-Na$^+$	0.25	0	0
LM.P1	–	–	–	LM.P1-Na$^+$	0	1	0
LM.P1-Ca^{2+}	0	1	3	LM.P1-Na$^+$–Ca^{2+}	0	1	3
LM.P0.75-Ca^{2+}	0	0.75	3	LM.P0.75-Na$^+$–Ca^{2+}	0	0.75	3
LM.P0.5-Ca^{2+}	0	0.5	3	LM.P0.5-Na$^+$–Ca^{2+}	0	0.5	3
LM.P0.25-Ca^{2+}	0	0.25	3	LM.P0.25-Na$^+$–Ca^{2+}	0	0.25	3
C0.25-LM.P0.75	0.25	0.75	0	C0.25-LM.P0.75-Na$^+$	0.25	0.75	0
C0.5-LM.P0.5	0.5	0.5	0	C0.5-LM.P0.5-Na$^+$	0.5	0.5	0
C0.75-LM.P0.25	0.75	0.25	0	C0.75-LM.P0.25-Na$^+$	0.75	0.25	0
C0.15-LM.P0.85-Ca^{2+}	–	–	–	C0.15-LM.P0.85-Na$^+$–Ca^{2+}	0.15	0.85	3
C0.25-LM.P0.75-Ca^{2+}	0.25	0.75	3	C0.25-LM.P0.75-Na$^+$–Ca^{2+}	0.25	0.75	3
C0.3-LM.P0.7-Ca^{2+}	–	–	–	C0.3-LM.P0.7-Na$^+$–Ca^{2+}	0.3	0.7	3
C0.5-LM.P0.5-Ca^{2+}	0.5	0.5	3	C0.5-LM.P0.5-Na$^+$–Ca^{2+}	0.5	0.5	3
C0.75-LM.P0.25-Ca^{2+}	0.75	0.25	3	C0.75-LM.P0.25-Na$^+$–Ca^{2+}	0.75	0.25	3

performed at a 0.7% strain, which was in the linear viscoelasticity domain. At the end of these viscoelastic measurements, flow measurements were performed at 20 °C. First, the thixotropic behaviour was estimated by programming a shear rate cycle between 0 and 100 s^{-1} for 2 × 2 min to detect an eventual hysteresis. Then apparent viscosity was monitored by decreasing the shear rate from 100 to 0.01 s^{-1} to describe the flow behaviour. Each measurement was performed in triplicate ($n = 3$).

2.4. *Microscopic observations*

2.4.1. *Observation by TEM (transmission electron microscopy)*

For these observations, the mixtures were prepared as described above. The samples were cooled from 80 to 20 °C and left at room temperature for 20 h to gel formation. Each system was diluted in deionized water or in 0.1 M NaCl according to such medium. A drop of each diluted aqueous dispersion was first placed on a carbon-coated TEM copper grid (Quantifoil, Germany) and let to air-dry. The sample was then negatively stained with uranyl acetate (Merck, Germany). For that, the sample-coated TEM grid was successively placed on a drop of an aqueous solution of uranyl acetate (2%, w/w) and on a drop of distilled water. The grid was then air-dried before introducing it in the electron microscope. The samples were viewed using a JEOL JEM-1230 TEM operating at 80 kV.

2.4.2. *Observation by CLSM (confocal laser scanning microscopy)*

CLSM was used in the fluorescence mode. Observations were made with a Nikon A1 microscope with 20× objective. As both microfibrillated cellulose and LM pectin do not exhibit intrinsic fluorescence, it was necessary to label them.

Cellulose was covalently labelled with Rhodamine B isothiocyanate (RITC), according to the slightly modified method of de Belder and Granath (1973). Microfibrillated cellulose suspension (45 g at ∼5%, w/w) whose pH was adjusted beforehand to 6.8, was dispersed in 60 mL of Dimethyl sulphoxide (DMSO) for 15 min under gentle magnetic stirring at room temperature. 10 drops of pyridine, 0.02 g of Rhodamine B isothiocyanate (RITC) and 60 mg of Dibutylin dilaurate (DBTDL) were added to the DMSO containing cellulose. A two hours reaction time was given at a temperature of 30 °C. The labelled cellulose was precipitated with absolute ethanol, washed with ethanol 95% and acetone, filtered with a fine nylon mesh cloth (approx 250 number mesh size British sieve standards). The RITC-cellulose was dried overnight in an air oven at 35 °C, then dispersed in deionized water at a concentration of 1% (w/w) and dialyzed against deionized water containing 0.02% sodium azide until no detection of free dye. Finally, the RITC-cellulose was kept at 4 °C.

LM pectin was covalently labelled with Fluoresceinamine (FA). LM pectin (1 g) was dissolved in deionized water (150 mL) under magnetic stirring at room temperature. The pH of the solution was adjusted at 6.8 with NaOH. Nearly 0.03 g of FA was dissolved in 5 mL of DMSO and the solution was added to the pectin solution under gentle magnetic stirring at room temperature. Then 30 μL of cyclohexyl isocyanide and 30 μL of acetaldehyde were added. A 5 h reaction time was given to the mixture at ambient temperature. Afterwards, the precipitation of the labelled pectin was carried out using absolute ethanol and ether. The precipitated labelled pectin was washed and filtered as mentioned above. The FA-LM pectin was dried overnight in an air oven at 35 °C, then solubilized in deionised water at a concentration of 1% (w/w) and dialyzed as described above until no detection of free dye. Finally, the FA-LM pectin was kept at 4 °C.

The warm labelled samples (80 °C), prepared as for rheological measurements, were poured between a preheated concave slide and coverslip, then hermetically sealed to avoid dehydration. It was verified that labelling did not change the rheological behaviour of the systems (results not shown). The systems were cooled from 80 to 20 °C and the CLSM observations were performed at 20 °C after 20 h. A laser beam at the wavelengths of 561 nm and 488 nm were used to excite RITC and FA, respectively, and the emissions of fluorescence were recorded between 570–620 nm, and 500–550 nm, respectively. CLSM allowed to adjust the *xy* plane and take images at different *z*-values into the sample. In this work, all observations reported were taken at the same depth into the sample (approximately 40 μm under the top surface).

3. Results and discussion

3.1. *Viscoelastic behaviour of cellulose/LM pectin systems*

3.1.1. *Gelation kinetics and viscoelastic properties of LM pectin*

Variations of storage modulus (G') as a function of time at 20 °C are shown in Fig. 1 for LM pectin at various concentrations (0.25–1%, w/w), in the presence of 3 mM CaCl$_2$ in water (Fig. 1a) or in 0.1 M NaCl (Fig. 1b).

In water without NaCl (Fig. 1a), the values of the storage moduli increased slowly and remained constant after 10 h for the LM pectin concentrations of 0.5, 0.75 and 1% (w/w), G' plateauing to ∼3, 4 and

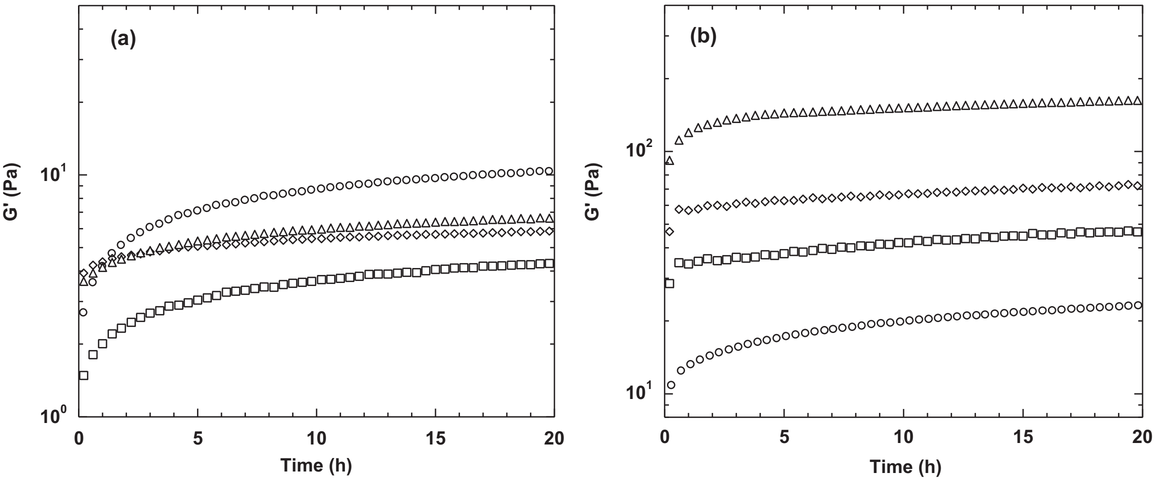

Fig. 1. G' variations as a function of time for LM pectin in the presence of 3 mM $CaCl_2$ at various concentrations: 0.25% (w/w) (○), 0.5% (w/w) (□), 0.75% (w/w) (◊) and 1% (w/w) (Δ) in water (a) or in 0.1 M NaCl (b), at pH 6.8. Measurement conditions: 20 °C, 1 rad s^{-1} angular frequency, 0.7% strain.

~6 Pa, respectively. In contrast, for 0.25% (w/w) LM pectin, the value of G' continuously increased and it required around 15 h to reach the plateau, with G' being of the order of 9 Pa at 20 h, showing a slow organization of 0.25% (w/w) LM pectin gel. At 0.25% (w/w), calcium and carboxylate contents are close to each other, meaning that all ions can be involved in junctions zones. Furthermore, the dilution results in a high accessibility of carboxylate groups, facilitating calcium binding and thus leading to a stronger gel. When pectin concentration increases, calcium have first to screen the electrostatic repulsions between polymer chains before acting as binding agent. However, the increase in polymer concentration offers more facility to establish junctions, thus explaining the increase in G' (from 0.5% to 1% in pectins) and the decrease in gelation time. On the other hand, these results support the conclusions of Garnier et al. (1994) who reported that in water, at low LM pectin concentration, the lower the DM, the higher was the affinity of LM pectin chains towards calcium ions and that this effect decreased when the polymer concentration increased.

In 0.1 M NaCl (Fig. 1b), the beginning of the kinetics showed a more rapidly increased of G' and reached a plateau after 5 h whatever the LM pectin concentration. After 5 h, G' tented to plateauing to ~20, 50, 70 and 160 Pa, respectively at 0.25, 0.50, 0.75 and 1% (w/w). The phenomenon observed in the case of 0.25% (w/w) LM pectin gel in water, was not seen anymore when NaCl was added, meaning that electrostatic screening hindered this particular behaviour. The storage moduli G' of LM pectin in water were always lower than those obtained in the case of 0.1 M NaCl, as described elsewhere (Garnier et al., 1994; Gigli, Garnier, & Piazza, 2009).

For examples example on Fig. 2 shows the mechanical spectrum at the end of the gelation kinetics of 0.5% (w/w) LM pectin in presence of 3 mM $CaCl_2$ in water (Fig. 2a) or in 0.1 M NaCl (Fig. 2b). The variations of G' (the storage or elastic modulus) and of G'' (the loss or viscous modulus) as a function of angular frequency are plotted in the angular frequency range 10^2–10^{-2} rad s^{-1}.

In 0.1 M NaCl, the viscoelastic behaviour of LM pectin was dominated by the elastic component G' with $G' > 10G''$ and a flat frequency independence of G' typical of true gel (Clark & Ross-Murphy, 1987). On the other hand, in water without NaCl, a tendency towards a slight angular frequency dependent profile was observed for LM pectin, which exhibits a weak solid-like behaviour with $G' > G''$ and a plateau towards the low angular frequency range.

From these results, it is clear that the higher the LM pectin concentration, the higher the G' values, in presence of 3 mM $CaCl_2$ except for very dilute systems in pure water. This can be supported by the fact that increasing LM pectin concentration for a constant calcium concentration increased gel strength due to more numerous linkages between LM pectin chains via calcium ions (Axelos &

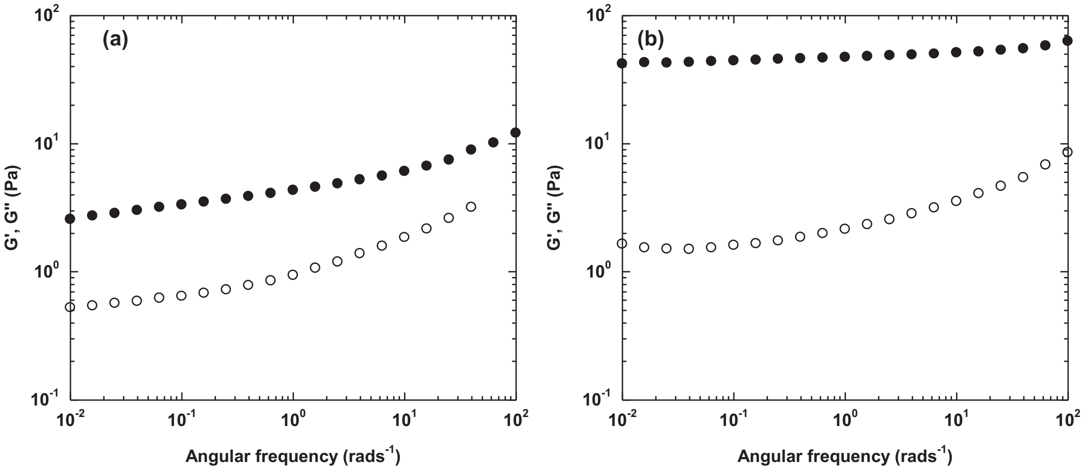

Fig. 2. Mechanical spectra of 0.5% (w/w) LM pectin in the presence of 3 mM $CaCl_2$, in water (a) or in 0.1 M NaCl (b), at pH 6.8. Measurement temperature, 20 °C; strain, 0.7%. G', filled symbols; G'', empty symbols.

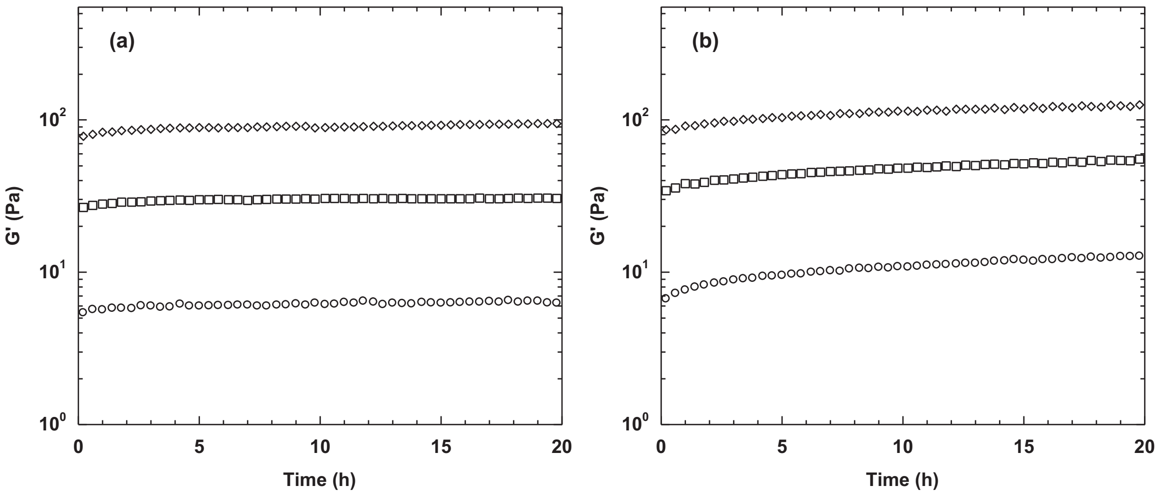

Fig. 3. G' variations as a function of time for cellulose/LM pectin mixtures at various mixing ratios: 0.25/0.75 (○), 0.5/0.5 (□), and 0.75/0.25 (◇), in water (a) or in 0.1 M NaCl (b), at pH 6.8. Measurement conditions: 20 °C, 1 rad s^{-1} angular frequency, 0.7% strain.

Thibault, 1991). It was also seen that the moduli G' and G'' of LM pectin in water were always lower than those obtained in case of 0.1 M NaCl. These results could be explained by the fact that pectin-calcium interactions are anti-cooperative in water and cooperative in the presence of 0.1 M NaCl, as suggested by Garnier et al. (1994). Therefore, in water without salt, electrostatic repulsion does not allow the pectic chains to be close enough to induce strong gels, whereas in NaCl the reinforcement of the pectic gel can be related to the decrease of electrostatic repulsions between LM pectins chains by screening charges.

3.1.2. *Gelation kinetics and viscoelastic properties of cellulose/LM pectin systems*

3.1.2.1. Cellulose/LM pectin systems without added calcium ions. In these conditions, the gelation of LM pectin cannot occur. The mixture will be thus a system made up of cellulose microfibrils dispersed in a continuous phase containing pectins in solution.

Fig. 3 shows the variations of storage modulus (G') as a function of time at 20 °C for cellulose/LM pectin mixtures at mixing ratios of 0.25/0.75, 0.5/0.5 and 0.75/0.25 and at a total biopolymer concentration of 1% (w/w), in the absence of $CaCl_2$ in water or in 0.1 M NaCl.

In water without NaCl (Fig. 3a), it appeared that no time dependence of the storage moduli was observed after the first hour whatever the concentration of each component. G' increased with the cellulose concentration within the mixtures. The same behaviour was observed for the mixed systems in 0.1 M NaCl (Fig. 3b), with slightly higher moduli due to the effect of Na$^+$ ions on cellulose microfibrils as observed previously (Agoda-Tandjawa et al., 2010).

In example on Fig. 4 are shown the mechanical spectra at the end of the gelation kinetics of the mixtures at the mixing ratio of 0.5/0.5 in water without added salts (Fig. 4a) and in 0.1 M NaCl (Fig. 4b), together with those of pure cellulose suspensions (0.5%, w/w), taken as references.

It is evident from Fig. 4 that the mixture displayed a typical solid-like behaviour with $G' > 10G''$ and relatively frequency-independent moduli as for pure cellulose suspension. In water without added salts and in 0.1 M NaCl, the mechanical spectra of the mixtures are closed to those of pure cellulose suspensions at the given concentration. The same behaviour was observed for the mixtures at the mixing ratios of 0.25/0.75 and 0.75/0.25 (results not shown). It was also seen that the higher the cellulose concentration, the higher the G' and G'' values for the mixtures (results not shown), as well as for

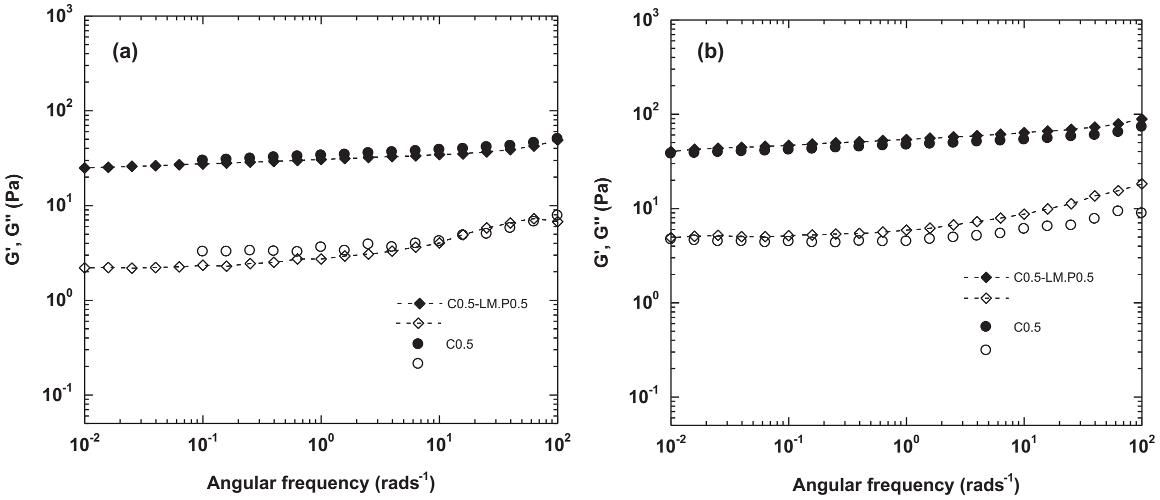

Fig. 4. Mechanical spectra of cellulose/LM pectin mixture at a mixing ratio of 0.5/0.5 and those of cellulose suspension at the concentration of 0.5% (w/w), taken as references, in water (a) or in 0.1 M NaCl (b) at pH 6.8. Measurement temperature, 20 °C; strain, 0.7%. G', filled symbols; G'', empty symbols.

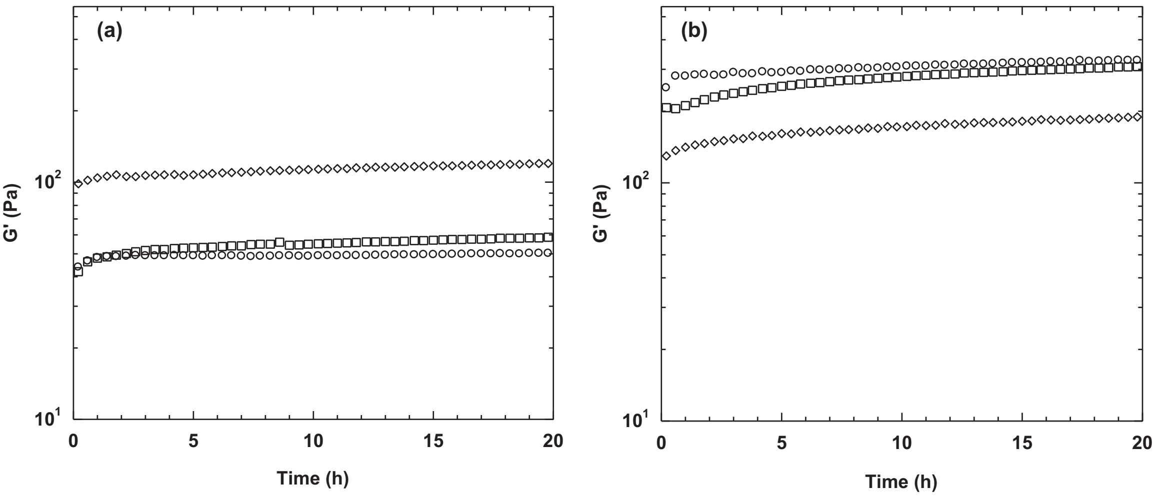

Fig. 5. *G*′ variations as a function of time for cellulose/LM pectin mixtures in the presence of 3 mM $CaCl_2$ at various mixing ratios: 0.25/0.75 (○), 0.5/0.5 (□), and 0.75/0.25 (◊), in water (a) or in 0.1 M NaCl (b), at pH 6.8. Measurement conditions: 20 °C, 1 rad s^{-1} angular frequency, 0.7% strain.

pure cellulose suspensions what was previously observed (Agoda-Tandjawa et al., 2010). Cellulose is likely to govern the viscoelastic properties of the cellulose/LM pectin mixed system in water or in 0.1 M NaCl in absence of calcium ions. On the other hand, cellulose suspensions and cellulose/LM pectin mixtures without calcium displayed quite similar *G*″/*G*′ ratio (of the order of 0.1), suggesting the same organization. The presence of LM pectin within the cellulose network had little effect on the viscoelastic properties of cellulose network. These observations show that, under these conditions and whatever the cellulose/LM pectin mixing ratio, pectins in solution do not intervene in the viscoelastic properties of the mixed system; only cellulose microfibrils contribute to the rheology of the system. This is of course related to the fact that LM pectins do not gel under these conditions. It is probable that the viscoelasticity of the continuous medium – where pectins are – is too low so that they cannot play a role in the properties of the system.

Contrary to this result, Tatsumi, Kourogi, Chen, & Matsumoto (2008) showed that the presence of polysaccharide such as guar gum or xylan in cellulose fiber disperse systems, reduces the viscoelastic properties of the mixed systems and the authors explained this behaviour to be a consequence of the fact that the addition of guar gum or xylan reduces the friction at the fiber contact points; moreover, it makes the fibers lie in the better order and laminate more regularly. Similarly, Lowys, Desbrieres, & Rinaudo (2001) observed the same behaviour when cellulose derivatives such as carboxymethylcelluloses were added to cellulose suspensions.

3.1.2.2. Cellulose/LM pectin systems with added calcium ions. Contrary to the previous systems, the gelation of LM pectin occurs in these conditions, and the mixture will thus be a composite gel.

Variations of storage modulus (*G*′) as a function of time at 20 °C are shown in Fig. 5 for cellulose/LM pectin mixtures at the same mixing ratios with 3 mM $CaCl_2$ in water or in 0.1 M NaCl.

In water without NaCl (Fig. 5a), the gelation profiles of the mixtures were qualitatively similar to those obtained for the corresponding systems without $CaCl_2$ (see Fig. 3). *G*′ values at 20 h were of the order of 50, 58 and 120 Pa for cellulose/LM pectin mixing ratios of 0.25/0.75, 0.5/0.5 and 0.75/0.25, respectively. As observed for the corresponding systems without $CaCl_2$, *G*′ increased as the cellulose concentration was increased within the mixtures. The only difference was in the *G*′ values, the mixture without calcium being less rigid while in the presence of calcium the gel strength of the mixture was higher. Contribution of LM pectin gel to final gel strength could explain this result.

In 0.1 M NaCl (Fig. 5b), a quite similar gelation profiles was observed for the mixtures. Moreover, a spectacular change is noted as well in the order of the moduli variations as in their values. For the example, *G*′ values at 20 h are of ∼330, 310 and 190 Pa for cellulose/LM pectin mixing ratios of 0.25/0.75, 0.5/0.5 and 0.75/0.25, respectively. Clearly, the composite gels were strengthened in 0.1 M NaCl compared to in water without NaCl. In these conditions, *G*′ of cellulose/LM pectin mixtures increased with LM pectin concentration within the mixtures. This result is rather coherent with the fact that the presence of NaCl reinforces the rigidity of LMpectin/Ca^{2+} network resulting in stronger composite gels.

The mechanical spectra at the end of the gelation kinetics of the mixtures at a mixing ratio of 0.5/0.5 in the presence of 3 mM $CaCl_2$ are illustrated in Fig. 6 together with those of pure cellulose suspensions (0.5%, w/w) and LM pectin (0.5%, w/w) with 3 mM $CaCl_2$ in water or in 0.1 M NaCl, taken as references.

In water without NaCl (Fig. 6a) and in 0.1 M NaCl (Fig. 6b), the profiles of the mechanical spectra of cellulose/LM pectin mixtures, were qualitatively similar to those obtained for pure cellulose suspension, evidencing again the solid-like behaviour. The same behaviour was observed for the mixtures at the mixing ratios of 0.25/0.75 and 0.75/0.25 (results not shown). In water without NaCl (Fig. 6a), when LM pectin was added to cellulose suspensions in the presence of calcium ions, the moduli were shifted to higher values throughout the overall mechanical spectra meaning that the viscoelastic properties of the mixed cellulose/LM pectin gels were reinforced. The moduli (*G*′ and *G*″) were higher for the cellulose/LM pectin mixtures in presence of calcium ions than the mixtures without calcium ions and the individual components. This effect was most pronounced when increasing the cellulose concentration. The *G*″/*G*′ ratios of cellulose/LM pectin mixtures in the presence of calcium did not differ very much to those obtained without calcium ions (of the order of 0.1) meaning that both systems are structured on the same way like cellulose suspension network.

On the other hand, adding 3 mM $CaCl_2$ to cellulose/LM pectin in 0.1 M NaCl, resulted in an important increase of the moduli, *G*′ and *G*″, throughout the overall mechanical spectra (Fig. 6b), meaning that the viscoelastic properties of cellulose/LM pectin composite gels were also reinforced. The moduli (*G*′ and *G*″) observed for the mixtures in these conditions are higher than those obtained for the corresponding mixtures without NaCl. This suggests that the addition of calcium to the mixtures induced pectin gelation, which was favoured in the presence of sodium, and thus contributes to the final strength of the composite gels.

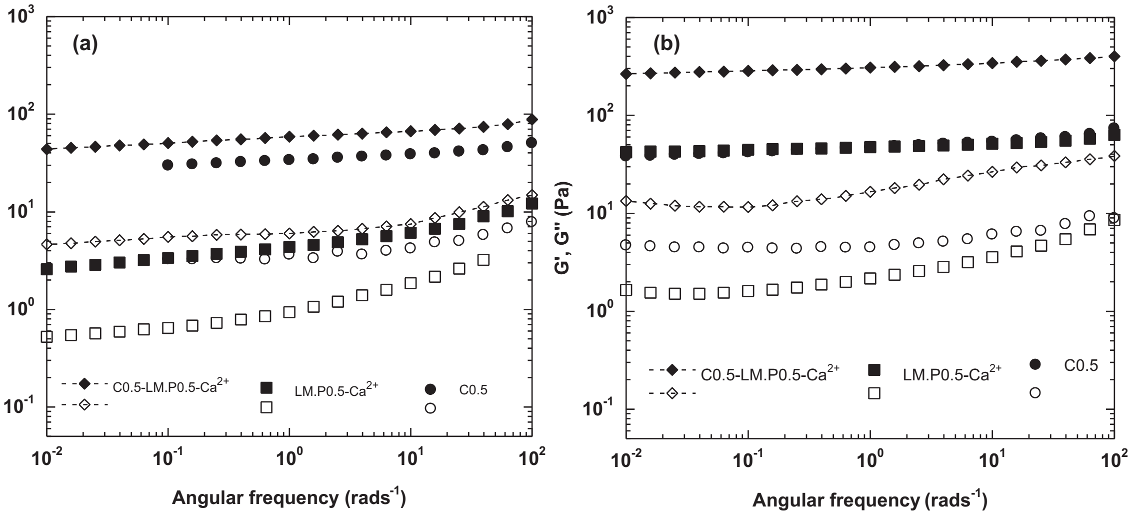

Fig. 6. Mechanical spectra of cellulose/LM pectin mixtures in presence of 3 mM $CaCl_2$ at a mixing ratio of 0.5/0.5 (b), and those of the individual component (0.5%, w/w LM pectin with 3 mM $CaCl_2$ and 0.5%, w/w pure cellulose suspensions), taken as references, in water (a) or in 0.1 M NaCl (b) at pH 6.8. Measurement temperature, 20 °C; strain, 0.7%. G', filled symbols; G'', empty symbols.

Contrary to the former cases, the reinforcement of the mixed cellulose/LM pectin or cellulose network was most pronounced with the increase of LM pectin concentration.

In 0.1 M NaCl, the G''/G' ratios of cellulose/LM pectin mixtures with added calcium ions, which are ~0.04 at the mixing ratios of 0.25/0.75 and 0.5/0.5, and ~0.08 at 0.75/0.25, are very low compared to those obtained in the absence of calcium ions (of the order of 0.1) meaning that the elastic character of the mixtures was reinforced under both salts conditions. In the same conditions, cellulose suspensions at 0.25–0.75% (w/w) were characterized by G''/G' values around 0.1, whereas LM pectin at 0.25–0.75% (w/w) with 3 mM $CaCl_2$ are characterized by G''/G' values around 0.04. This means that, cellulose suspensions have lower elastic character than LM pectin gels in 0.1 M NaCl.

In addition, it should be noted that the presence of 3 mM $CaCl_2$ in the cellulose suspensions does not importantly modify their viscoelastic properties. For example G' at cellulose concentration of 0.5% (w/w) being of 25 Pa ($G''/G' = 0.09$) in the presence of calcium ions and 28 Pa ($G''/G' = 0.11$) in absence of added calcium ions (results not shown).

3.2. Rheological synergism of cellulose and LM pectin mixtures

In order to get better understanding of the rheological synergism and the effects of the two different ions (Na^+ and/or Ca^{2+}) on the properties of cellulose/LM pectin mixtures, the values of the storage modulus, G', obtained at 0.1 rad s^{-1} from mechanical spectra at 20 °C, were plotted versus the cellulose concentration and LM pectin concentration, for the different mixtures studied, at a total polymer concentration of 1% (w/w) (Fig. 7).

For cellulose/LM pectin mixtures in water with 3 mM $CaCl_2$, G' decreased as the LM pectin concentration increased within the mixtures. The same profile was observed for cellulose/LM pectin mixtures in water or in 0.1 M NaCl without added $CaCl_2$ (result not shown). These results suggest that, in these conditions, microfibrillated cellulose in suspension govern the viscoelastic properties of the cellulose/LM pectin composite gels. In contrast, with added $CaCl_2$ in the presence of 0.1 M NaCl, G' of cellulose/LM pectin mixtures increased when increasing the LM pectin concentration from 0.25% (w/w) to 0.75% (w/w) and then decrease with further increasing of LM pectin concentration. This indicates that the viscoelastic properties of the cellulose/LM pectin composite gels were governed by LM pectin.

In water without NaCl, the addition of $CaCl_2$ to cellulose/LM pectin mixtures at mixing ratios of 0.75/0.25, 0.5/0.5 and 0.25/0.75 increased the G' 1.5-fold, 1.8-fold and 6.5-fold, respectively, in comparison to pure cellulose suspensions in water at the given concentrations. Similarly, for cellulose/LM pectin mixtures in 0.1 M NaCl at the same ratios, the presence of $CaCl_2$ leads to an increase of the G' of about 2.4-fold, 10-fold and 37-fold, respectively, in comparison to pure cellulose suspensions in water.

In addition, for cellulose/LM pectin mixtures at the ratios of 0.5/0.5 and 0.25/0.75 in 0.1 M NaCl in the presence of 3 mM $CaCl_2$, G' values were about 1.5 times higher than those obtained for 1% (w/w) cellulose suspension under the same salts conditions. Similarly for the same mixtures, G' values were about 2 times higher than those obtained for 1% (w/w) LM pectin under the same salts conditions.

From these results, it appears that a strong synergism was noted for the mixed system in the presence of both Ca^{2+} and Na^+. This effect is most pronounced at LM pectin component concentration

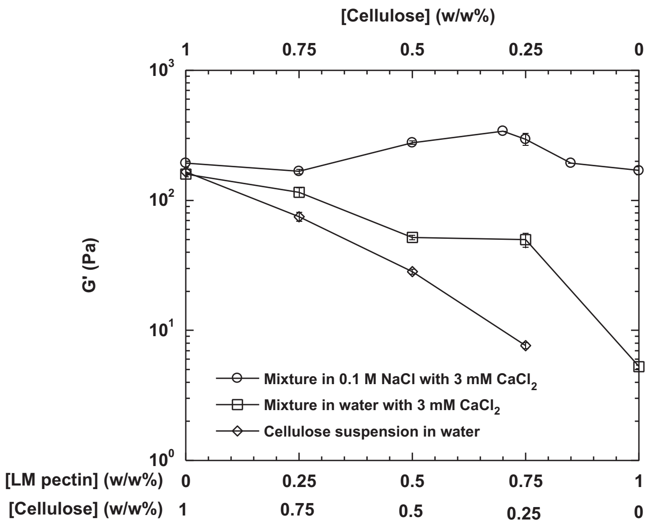

Fig. 7. Variations of G' as a function of LM pectin/cellulose mixing ratio at a total polymer concentration of 1% (w/w) in the presence of 3 mM $CaCl_2$ in water or in 0.1 M NaCl, and those of G' obtained for cellulose suspensions alone in water (from 0.25 to 1%, w/w), taken as references, at 20 °C and at an angular frequency of 0.1 rad s^{-1}.

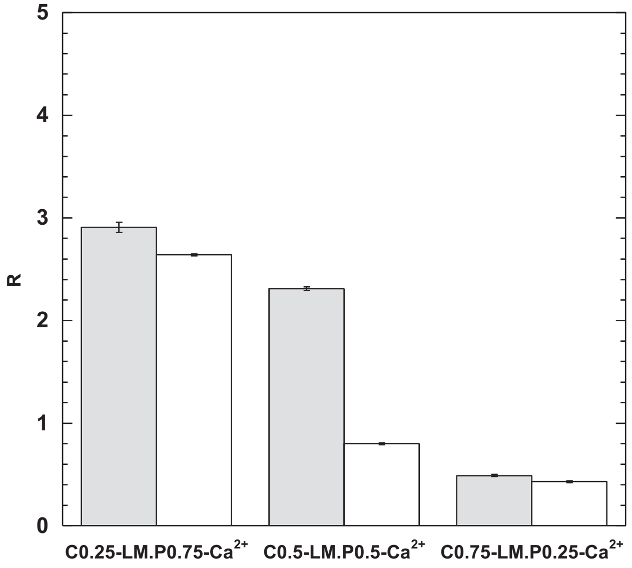

Fig. 8. Rheological synergism of cellulose/LM pectin mixtures with 3 mM $CaCl_2$ in 0.1 M NaCl (gray column) or in water (empty column) $R = (G'_{\text{mixture-Ca}^{2+}} - (G'_{\text{cellulose}} + G'_{\text{LM pectin-Ca}^{2+}}))/(G'_{\text{cellulose}} + G'_{\text{LM pectin-Ca}^{2+}})$.

situated between 0.5 and 0.75% (w/w). The most reinforced composite gels obtained in this case can be related to the effect of both salts as described above. This allows for example to use less quantities of each material without losses of the rheological properties.

Furthermore, Fig. 8 showed that in the mixed systems a synergistic effect on G' can be observed. Indeed, we have quantified the synergistic effect by calculating R as followed:

$$R = \frac{G'_{\text{mixture Ca}^{2+}} - (G'_{\text{cellulose}} + G'_{\text{LM pectin-Ca}^{2+}})}{G'_{\text{cellulose}} + G'_{\text{LM pectin-Ca}^{2+}}}.$$

The fact that R values are positive evidence that added LM pectin to cellulose suspensions in presence of calcium ions reinforced cellulose gel structure. In the presence of calcium ions, the 0.25/0.75, 0.5/0.5 and 0.75/0.25 cellulose/LM pectin mixtures in 0.1 M NaCl induced higher rheological synergism values than those from the corresponding mixtures in water. Whether the mixtures were prepared in NaCl or not, the rheological synergism values decreased as the cellulose concentration was increased within the mixtures. The mixtures at the mixing ratios of 0.25/0.75 and 0.5/0.5, exhibited a more pronounced rheological synergism values than that obtained from the 0.75/0.25 cellulose/LM pectin mixtures. These differences confirm that more ordered gel networks were formed by addition of 0.75% (w/w) and 0.5% (w/w) LM pectin to cellulose suspension at the total polymer concentration of 1% (w/w) in presence of calcium ions with and without 0.1 M NaCl rather than the use of 0.25% (w/w) LM pectin.

3.3. Influence of the presence of LM pectin on the flow behaviour of cellulose suspensions

Aqueous cellulose suspension exhibited a slightly thixotropic behaviour (Agoda-Tandjawa et al., 2010). For all the investigated cellulose/LM pectin systems, in the two solvent conditions, an hysteresis loop was also seen, the up curve being above the down curve between 0 and 100 s^{-1} (results not shown). LM pectin addition lead to a more pronounced thixotropic character in 0.1 M NaCl than in water without NaCl (results not shown). From a general point of view, it appeared that the thixotropic character was more pronounced when cellulose concentrations increased in the mixtures.

The equivalent flow curves plotted in logarithmic scales, apparent viscosity (η_A) versus shear rate ($\dot{\gamma}$), are reported in Fig. 9 for the different systems. The shear rate dependence of the apparent viscosity of cellulose suspension with and without LM pectin in presence or not of 0.1 M NaCl exhibited shear-thinning behaviour (Fig. 9a–c). For cellulose concentration of 0.5% (w/w) and 0.75% (w/w), a noticeable plateau region was seen in the curves between 15 and 40 s^{-1} indicating probably that some specific structures were formed in the suspensions on this range of shear rate and that the suspensions do not uniformly flow.

The addition of LM pectin to cellulose suspension in presence or not of 0.1 M NaCl, contributes to an increase of the apparent viscosity of cellulose suspensions with a more pronounced shear-thinning behaviour. However, the plateau, slightly visible at high shear rates with 0.5% (w/w) and 0.75% (w/w) cellulose suspension alone, was not seen anymore when LM pectin was added, meaning that LM pectin hindered the formation of particular structure under shear. From these results, it appears that, contrary to the viscoelastic properties, the flow behaviour of cellulose suspensions was modified in presence of LM pectin. Indeed, the fact that the presence of LM pectin in the mixture involved an increase in the thixotropic character and the shear-thinning behaviour of cellulose suspensions can be related to the viscosifier effect of LM pectin. The flow behaviour seems more sensitive to the presence of pectins than the viscoelastic properties.

3.4. Microscopic structures in cellulose/LM pectin mixtures

The large variations in viscoelastic behaviour of cellulose/LM pectin mixtures due to Ca^{2+} and/or Na^+ addition together with the mixing ratios of both polymers raised the question about microstructural variations among the mixed gels. In order to understand these structural differences evidenced with the rheological characterization, transmission electron microscopy (TEM) and confocal laser scanning microscopy (CLSM) observations were performed to visualize the ultrastructural arrangement within cellulose/LM pectin mixed systems networks compared with the structure of the individual components, depending on the salts conditions.

3.4.1. Microscopic structures by TEM in cellulose/LM pectin mixtures

3.4.1.1. Microstructure of cellulose/LM pectin mixtures in water with or without Ca^{2+}. TEM Micrographs of LM pectin, cellulose suspension and cellulose/LM pectin mixtures at a mixing ratio of 0.5/0.5 in water with and without 3 mM $CaCl_2$, prepared at the corresponding conditions of gels formation as described above are shown in Fig. 10. Panels a and b of Fig. 10 show the microstructure of LM pectin in water without salts and with $CaCl_2$, respectively. In water without salts (Fig. 10a), LM pectin does not gel, and then any network structure could be seen by TEM observation. With added $CaCl_2$ to LM pectin in water, the gel (Fig. 10b) was composed of an apparently homogeneous network that appeared artificially dense as observed previously (Chanliaud & Gidley, 1999) for 2% DM 30 pectin/12.5 mM $CaCl_2$ gel in water with many pores giving the aspect of microgels networks. Micrographs obtained from cellulose suspension alone (Fig. 10c), revealed a network structure of well-defined and packed fibrils randomly dispersed with a diameter of 2–15 nm and a length of up to 10 μm, as observed previously (Agoda-Tandjawa et al., 2010). This network structure of cellulose microfibrils clearly observable is very different from those of LM pectin. This difference in morphology observed will allow to recognize the components in the mixtures. In presence of calcium ions (Fig. 10d), the network structure of cellulose suspension does not change a lot in nature, confirming that calcium does not disturb the organization of the cellulose microfibrils. In

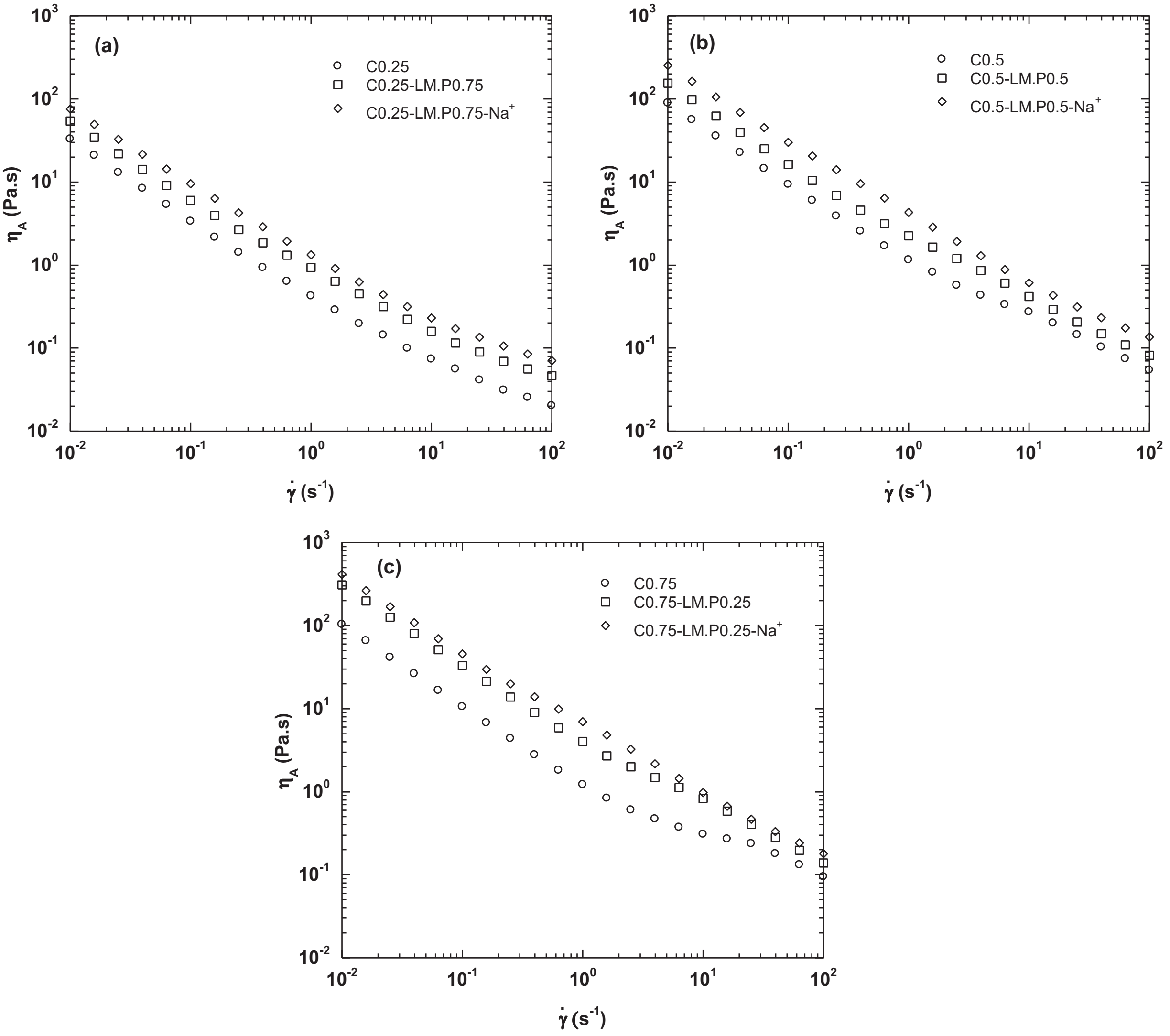

Fig. 9. Flow curves, apparent viscosity as a function of shear rate, of cellulose suspensions at the concentration of 0.25% (w/w) (a), 0.5% (w/w) (b), 0.75% (w/w) (c) and of cellulose/LM pectin mixtures at the concentration ratios of 0.25/0.75 (a), 0.5/0.5 (b), and 0.75/0.25 (c), in water and in 0.1 M NaCl at pH 6.8. Measurement temperature: 20 °C.

the absence of calcium ions, the addition of LM pectin to cellulose suspension at a mixing ratio of 0.5/0.5 reveals the same network structure as well as cellulose alone, as shown in Fig. 10e. The same microscopic structure was seen at the corresponding conditions at the mixing ratios of 0.25/0.75 and 0.75/0.25 (results not shown). With added calcium ions to cellulose/LM pectin mixture, a drastic change was observed in the mixture network. The 0.5/0.5 mixed system structure became denser (Fig. 10f). Indeed, the cellulose microfibrils are partially masked by a dense pectin network. As in the former cases, the same microscopic structure was seen at the corresponding conditions for 0.25/0.75 and 0.75/0.25 mixed systems (results not shown). It was also seen that, in presence of calcium ions, the mixed system becomes denser with the increase in cellulose concentration (results not shown).

In water, cellulose/LM pectin composite formed in the presence or not of calcium ions present a very different architecture. Cellulose/LM pectin mixed systems without calcium and single cellulose suspensions were composed of open porous networks, in which clusters of entanglements microfibrils were embedded, even if these systems had different structural composition, containing more bundles of aligned microfibrils and densely packed fiber networks. No major differences in the network structure were seen between pure cellulose suspensions and mixed cellulose/LM pectin systems without calcium ions evidenced by equivalent G' and G'' values observed for both systems. This has to be ascribed to the fact that LM pectin does not gel without calcium ions. Moreover, it does not chemically interact with cellulose because it contains very limited amounts of neutral sugar side chains as shown by the chemical characterization described elsewhere (Agoda-Tandjawa et al., 2010). In presence of calcium ions, the networks structures showed very dense regions in which cellulose microfibrils are recovered by a gelled LM pectin network. The mixed system network became denser and exhibited more elasticity with increase in cellulose concentration as showed by the rheological parameters G' and $\tan\delta$. Therefore, the fact that the solid-like viscoelastic properties of cellulose suspensions were reinforced with the addition of LM pectin and calcium ions originates from the gelation of LM pectin in presence of calcium ions. The open porous networks in the former case disappeared with the presence of calcium ions. This suggests that LM pectin gel is trapped within the mesh of cellulose network indicating probably the formation of interpenetrating cellulose/LM pectin composite gel network. On the other hand, in presence of calcium ions, the network structure of cellulose suspension does not change a lot in nature in agreement with the values of G' and G'' moduli which are not very different.

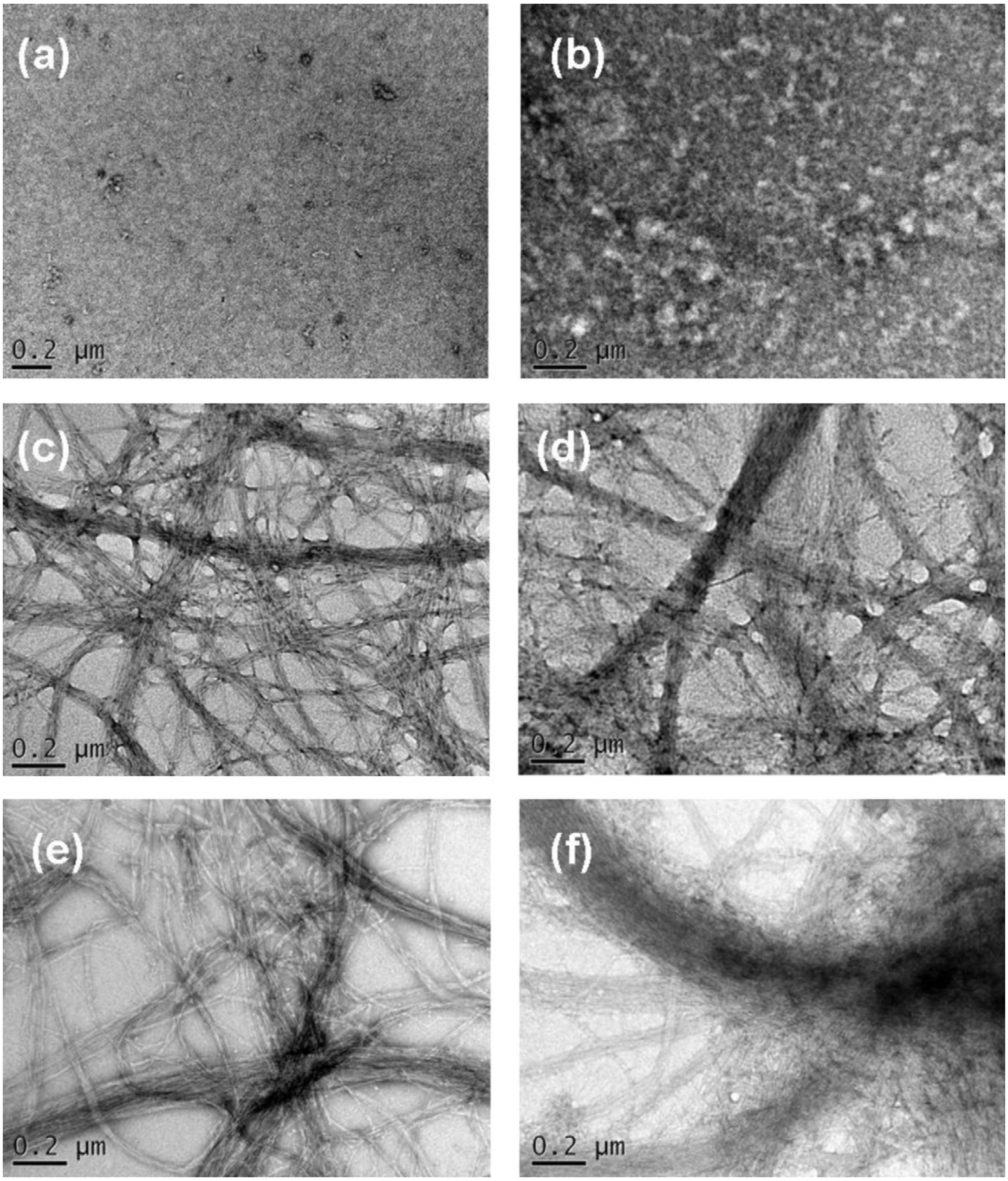

Fig. 10. TEM micrographs of LM pectin (a and b), cellulose suspension (c and d), and of cellulose/LM pectin mixtures (e and f) at a mixing ratio of 0.5/0.5 without added salts (first row) and with 3 mM $CaCl_2$ (second row) in water at pH 6.8. Scale bar = 0.2 µm.

3.4.1.2. *Microstructure of cellulose/LM pectin mixtures in 0.1 M NaCl with or without Ca^{2+}.*

Fig. 11 shows TEM micrographs of LM pectin, cellulose suspension and cellulose/LM pectin mixtures at the same corresponding mixing ratio (0.5/0.5) in 0.1 M NaCl with and without 3 mM $CaCl_2$, prepared at the corresponding conditions of gels formation as described above.

In Fig. 11, parts a and b show the microstructure of LM pectin in 0.1 M NaCl in the absence and the presence of $CaCl_2$, respectively. Fig. 11a clearly shows isolated structures being able to correspond to pectic chains more or less aggregate in the presence of 0.1 M NaCl, with many pores less than 0.2 µm in size. With addition of calcium ions, a homogeneous and denser network structure is seen, even if some few variations in the network density can be seen (Fig. 11b).

The microstructure of pure cellulose in 0.1 M NaCl (Fig. 11c) reveals a dense entanglement-like structure composed of apparently randomly oriented cellulose fibers; it appears to be similar to that of pure cellulose with 0.1 M NaCl and 3 mM $CaCl_2$ (Fig. 11d). These single cellulose microstructures were slightly denser than the corresponding micrographs obtained in water, indicating that the more rigid structure was favoured in NaCl, due to NaCl electrostatic screening.

As in the former case, calcium ions addition affects the microstructure of cellulose/LM pectin mixtures. The microstructure of cellulose/LM pectin mixture at the mixing ratio of 0.5/0.5 in presence of 0.1 M NaCl and 3 mM $CaCl_2$ was much denser than the structure of the corresponding mixture without calcium ions (Fig. 11e–f). These microstructures were also much denser in comparison to the corresponding micrographs obtained in water, indicating that a more rigid structure was favoured in NaCl, in agreement with the values of G' modulus. The same microscopic structures were seen at the other mixing ratios (results not shown). As described previously, the network structures of the mixed systems without added

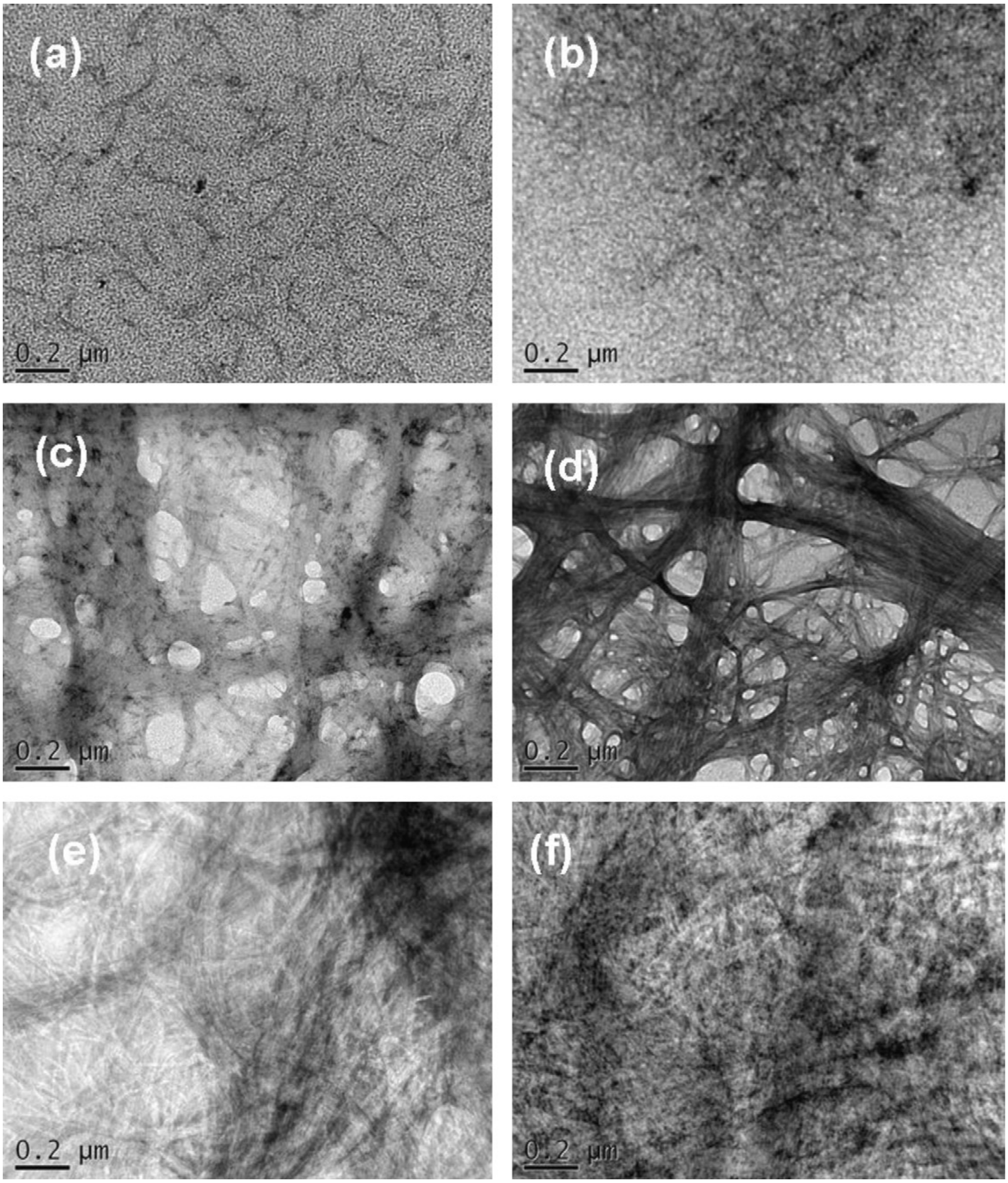

Fig. 11. TEM micrographs of LM pectin (a and b), cellulose suspension (c and d), and of cellulose/LM pectin mixtures at a mixing ration of 0.5/0.5 (e and f) without added $CaCl_2$ (first row) and with 3 mM $CaCl_2$ (second row) in 0.1 M NaCl at pH 6.8. Scale bar = 0.2 μm.

calcium are not very different for those of cellulose alone in 0.1 M NaCl, which is in good agreement with the similarity in viscoelastic properties. In contrast to the former case, the mixed systems network became denser with increase in LM pectin concentration (results not shown). That was confirmed by the viscoelastic properties, where the values of G' modulus increased with LM pectin concentration. The open porous networks seen in cellulose/LM pectin mixed systems without calcium ions disappear with the presence of calcium ions. This confirms that LM pectin gel is trapped within the mesh of cellulose network indicating probably the formation of interpenetrating cellulose/LM pectin composite gel network.

3.4.2. Microscopic structures by CLSM in cellulose/LM pectin mixtures

To visualize by CLSM the spatial relatives distributions and assess ultrastructural organization at larger distance scales of cellulose microfibrils and LM pectin in the mixtures, we chose specifically to examine the mixture at the mixing ratio of 0.5/0.5 and the individual components at the given concentrations in presence or not of NaCl and/or $CaCl_2$.

CLSM micrographs of cellulose/FA-LM pectin mixtures in water or in NaCl with and without 3 mM $CaCl_2$, prepared at the corresponding conditions of gels formation as described above are shown in Fig. 12. This figure highlights a spectacular difference in the organization of the systems according to the salts condition of each medium. For the systems without calcium in water or in 0.1 M NaCl, inhomogeneities in the distribution of non-labelled cellulose were evidenced by the presence of dark cotton-like zones, more or less aggregated, embedded in a continuous fluorescent phase of homogeneous LM pectin. This type of structure observed was also observed for double localization experiments using cellulose labelled with RITC and LM pectin labelled with FA, and the

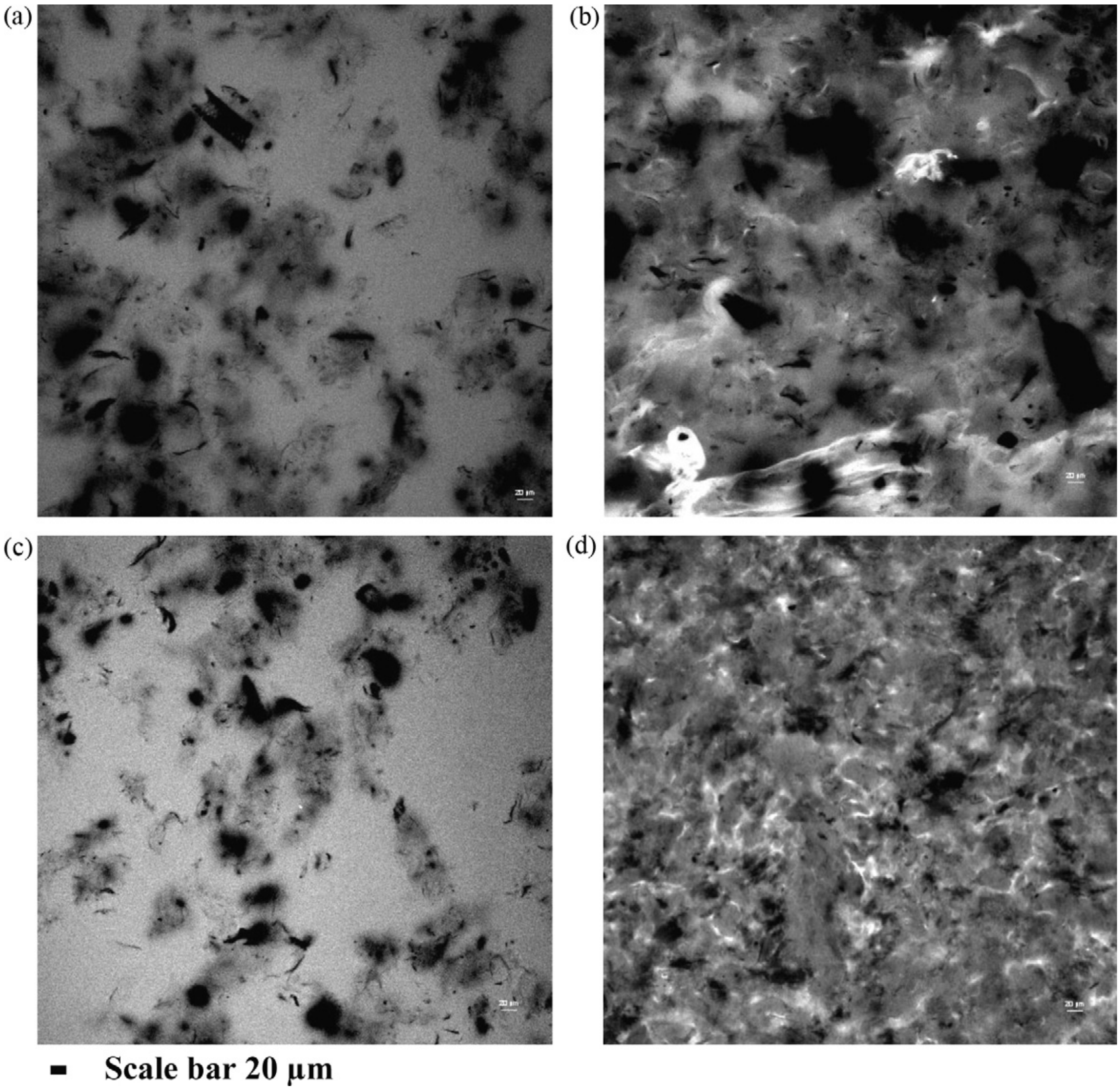

Fig. 12. CLSM micrographs of cellulose/FA-LM pectin mixtures at a mixing ratio of 0.5/0.5: in water without added salts (a), in water with 3 mM $CaCl_2$ (b), in 0.1 M NaCl (c), and in 0.1 M NaCl with 3 mM $CaCl_2$ (d), at pH 6.8. Scale bar = 20 μm.

mixtures made of RITC-cellulose and LM pectin, in the same salts conditions (results not shown). When calcium was present in the mixture, a major change in the texture of the image was observed, with an inhomogenous distribution of the fluorescence which can be related to the formation of the pectic network. Indeed, in the presence of calcium, the network structure of the mixture appears denser; this dense structure is much more pronounced and highly overlapped in the presence of the two cations. That is compatible with the higher values of G' and G'' moduli observed for the corresponding systems.

From these overall CLSM observations, it appears that, whether cellulose or LM pectin is labelled or not, the two components are clearly distinguished with a relatively similar distribution. Of course, these observations provide evidence that in the mixture, cellulose microfibrils and LM pectin are localized in different phases.

3.5. Viscoelastic properties and microscopic observations

Without added calcium, no major differences in the viscoelastic properties and microscopic observations were seen between cellulose suspension alone and cellulose/LM pectin mixed systems. We might therefore conclude that in the absence of calcium ions, the viscoelastic and structural properties of cellulose suspension are relatively unaffected (certainly in rheological and microstructural terms) by the LM pectin presence.

With added calcium, a dramatic change in the viscoelastic properties and microscopic observations of the systems were observed. According to TEM microscopic observations, the formation of interpenetrating cellulose/LM pectin composite gel network was evidenced in presence of calcium ions. As a mimic of cell wall assembly, Chanliaud and Gidley (1999) also make evidence of the formation of interpenetrating cellulose/pectin composites networks by deposition of cellulose from *Acetobacter xylinus* into a preformed pectin/calcium systems. Moreover, whether calcium was added to the mixture or not, CLSM observations allowed to highlight a separate localization of cellulose and LM pectin. In this circumstance, interpenetrating networks can be formed when the biopolymers gel (Amici, Clark, Normand, & Johnson, 2000).

The spectacular synergistic effect observed for the composite in presence of Ca^{2+} and Na^{+} is to be related to the combined effect of the two cations as described above and to the very overlapping aspect of the two polysaccharide networks.

4. Conclusion

The influence of the presence of low-methoxyl pectin (LM pectin) on the rheological and microstructural properties of microfibrillated cellulose suspensions was studied in order to propose controlled "cellulose/pectins" model systems, having interesting texturing properties. Through this study, we showed that the presence of LM pectin alone in the microfibrillated cellulose suspensions does not importantly modify the viscoelastic and microstructural properties of microfibrillated cellulose suspensions. On the other hand, the flow behaviour is modified in the presence of LM pectin with an increase of the thixotropic character and the shear-thinning behaviour of cellulose suspensions which can be related to the viscosifier effect of LM pectin.

Addition of calcium to the mixtures induced LM pectin gelation, which was favoured in the presence of sodium, the pectin network contributing to the formation of a stronger cellulose/LM pectin composite gel. Whether calcium was added to the mixtures or not in water, the viscoelastic properties of the mixtures are mainly controlled by cellulose. The same behaviour was observed for the mixtures in NaCl without added calcium. Contrary to this observation, it was noticed that in presence of both sodium and calcium ions, the viscoelastic properties of the mixtures are rather governed by LM pectin. According to cellulose and LM pectin quantities in the mixture, it appears that, there is a balance between the contribution of both biopolymers and the presence of cations for the final rigidity of the mixed system.

In addition, it can be distinguished the formation of two types of systems: a cellulose/LM pectin composite gel with a network of cellulose microfibrils showing the gelling characteristics and a gelled LM pectin network in the presence of calcium in water or in NaCl, and a system made up of a network of cellulose microfibrils showing the gelling characteristics and not gelled LM pectin in absence of calcium. It was also highlighted, from TEM observations, the formation of an interpenetrating cellulose/LM pectin structure in the presence of calcium ions. In the same way, the CLSM observations allowed to highlight a separate localization of cellulose and LM pectin within the composite systems. The whole of the results obtained suggests that it is however possible to thus create new structures with new interesting texturing properties, by mixing cellulose suspensions and LM pectin in suitable proportions in the simultaneous presence of both sodium and calcium ions.

Our present knowledge of these systems encourages the use of cellulose in new markets like new materials, either to substitute the products usually used because it presents better properties for lower costs, or to be advantageously mixed to LM pectin to decrease the content of each biopolymer without losses of the rheological properties.

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