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### Highlights

#### **Dispersion of halloysite loaded with natural antimicrobials into pectins: Characterization and controlled release analysis**

*Carbohydrate Polymers xxx (2015) pp. xxx–xxx*

Giuliana Gorrasi\*

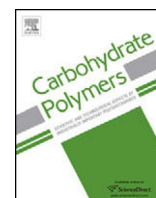
- Green composites pectins/halloysite nanotubes, loaded with rosemary oil, were prepared.
- Composite were produced using ball milling technology in presence of water.
- Structural organization and physical properties of cast films were analyzed.
- The release kinetic of the rosmarinic acid, chosen as model molecule, was also performed.
- Systems enlighten very good perspectives in the active packaging field.

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## Carbohydrate Polymers

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# Dispersion of halloysite loaded with natural antimicrobials into pectins: Characterization and controlled release analysis

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## ARTICLE INFO

## Article history:

Received 7 May 2014

Received in revised form 12 March 2015

Accepted 15 March 2015

Available online xxx

## Keywords:

Pectins

Halloysite

Green composites

Active packaging

Controlled release

## ABSTRACT

This paper reports the preparation and characterization of green composites based on pectins and nano-hybrids composed of halloysite nanotubes (HNTs) loaded with rosemary essential oil. Different hybrid percentages were mixed into a pectin matrix, by ball milling in the presence of water. Cast films were obtained and analyzed. Structural organization and physical properties (thermal, mechanical, barrier to water vapor) were correlated to the nano-hybrid content. A preliminary study on the kinetics of release of the rosmarinic acid, chosen as a model molecule, was also performed. This work showed the potential of these systems in the active packaging field where controlled release of active species is required.

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## 1. Introduction

In the last few years, the possibility of using products from the agriculture and food industry to develop new biodegradable materials for packaging applications is rapidly growing. Furthermore, in food packaging, extending the shelf life of packed products is a critical issue. The development of innovative packaging materials where different functionalities are included in a single film layer is of considerable interest. Nanotechnology is a very promising strategy for improving physical and barrier properties of polymeric materials. The introduction of nanofillers into biopolymers has been shown to be a good strategy to overcome some critical issues, such as a poor barrier to water vapor and low mechanical properties (Sorrentino, Gorrasi, & Vittoria, 2007; Lagaron & Lopez-Rubio, 2011). Natural pectins are green emerging materials for food packaging applications. They can be found in the primary walls of the cells and show a complex hetero-polysaccharide structure, containing linear and branched regions (Siew & Williams, 2008). The degree of esterification, determining the solubility of pectins, their gelling ability and film forming properties is fundamental for industrial applicability to a large extent. The degree of methyl esterification depends on the origin of the source and the processing conditions e.g. storage, extraction, isolation and purification (Kirby, MacDougall, & Morris, 2008; Vincken et al.,

2003; Zsivánovits, Marudova, & Ring, 2005; Espitia, Dub, de Jesús Avena-Bustillos, de Fátima Ferreira Soares, & Mc Hugh, 2014). Very recently a new class of inorganic fillers, halloysite nanotubes (HNTs), has attracted considerable interest. These are green materials, not hazardous for the environment, and available inexpensively in thousands of tons from natural deposits. HNTs are aluminosilicate clays with external diameter of about 50–80 nm, an internal one (lumen) of 10–15 nm and a length of about 1000 nm. From the chemical point of view, halloysite is two-layered aluminosilicate clay,  $\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4 \cdot n\text{H}_2\text{O}$ , which exhibits a predominant form of hollow tubes, and it is similar to kaolin, but its aluminosilicate sheets are rolled into tubes (Joussein et al., 2005; Bates, Hilderbrand, & Swineford, 1950; Lvov, Shchukin, Möhwald, & Price, 2008; Du, Guo, & Jia, 2010; Price, Gaber, & Lvov, 2001; Abdullayev & Lvov, 2011; Yelleswarapu, Gu, Abdullayev, Lvov, & Rao, 2010; Abdullayev, Price, Shchukin, & Lvov, 2009; Kirkman, 1981; Singh, 1996; Singh & Mackinnon, 1996; Abdullayev, Shchukin, & Lvov, 2008; Churchman & Carr, 1975; Bergaya, Theng, & Lagaly, 2006; Vergaro, Abdullayev, Cingolan, Lvov, & Leporatti, 2010; Tazaki, 2005; Carr, Chaikum, & Patterson, 1978). The HNT external surface is composed of Si–O–Si groups, whereas the internal surface consists of a gibbsite-like array of Al–OH groups (Lvov et al., 2008). Due to the tubular shape and less abundant –OH groups on the surface, HNTs can be easily dispersed in polymers without any need for exfoliation, as required for a good dispersion of platy clays, kaolin and montmorillonite. They are able to host a great variety of active molecules, which can be released in a controlled way in specific environments. Polymeric materials can be filled with these tiny

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tubular containers (Lvov & Price, 2008; Guo et al., 2009; Lvov, Price, Gaber, & Ichinose, 2002; Liu, Guo, Du, & Jia, 2007; Wei, Abdullayev, Hollister, Mills, & Lvov, 2012; Cavallaro, Lazzara, & Milioto, 2011; Zheng & Wang, 2010; Du, Guo, & Jia, 2006; Du, Guo, Liu, & Jia, 2007; Liu, Jiab, Jiab, & Zhou, 2014) that release specific active molecules (antimicrobial, drugs, flame retardant, self-healing, anti-corrosion, etc.) in specific environments (Joussein et al., 2005; Bates et al., 1950; Lvov et al., 2008; Du et al., 2010; Price et al., 2001; Q3 Abdullayev and Lvov, 2010; Abdullayev & Lvov, 2011; Yelleswarapu et al., 2010). The combination of pectins and halloysite nanotubes may generate novel materials with excellent and unique properties combining the advantages of macromolecules, derived from renewable resources, and nanoparticles, environmentally friendly. Both components being available at low cost. In this paper we report the preparation and characterization of green composites based on pectins and nano-hybrids of HNTs loaded with rosemary essential oil, as an antimicrobial agent, for potential application in packaging field. The antibacterial activity of several essential oils over a wide range of microorganisms has been extensively studied both in vitro and applied to foods (Liu, Guo, Du, & Jia, 2008; Deans & Q4 Ritchie, 1987; Hammer, Carson, & Riley, 1999; Dorman & Deans, 2000; Burt, 2004; Gómez-Estaca, López de Lacey, López-Caballero, Gómez-Guillén, & Montero, 2010). The nano-hybrid percentage in the composites was 5, 10, 20 wt%. Structural organization and physical properties (thermal, mechanical, barrier) were analyzed and correlated to the nano-hybrid loading and preliminary studies of in-vitro release of rosmarinic acid carried out.

## 2. Experimental

### 2.1. Materials

HNTs with a surface area of 20 m<sup>2</sup>/g were kindly supplied by Imerys (Tableware, New Zealand). Food grade rosemary essential oil (*Rosmarinus officinalis*) made by Specchiasol (Italy) was purchased from a specialized local market. Pectin from apples was purchased from Sigma Aldrich (Italy) in powder form. The molecular weight of pectin was in the range 30,000–100,000 and the degree of esterification was about 70–75%, on a dry basis, total impurities <10% water, CAS number: 9000-69-5. Glycerol was purchased from Sigma Aldrich (Italy).

### 2.2. Nano-hybrid preparation

A nano-hybrid composed of halloysite and rosemary essential oil was prepared by mixing 27 wt% of HNTs and 73 wt% of essential rosemary oil. The suspension was kept under vacuum for 20 min. Slight fizzing of the suspension under vacuum indicated that air was removed from the tubes. Once the vacuum was broken, essential oil molecules were allowed to enter into the lumen. This procedure was repeated 3 times to increase the loading efficiency.

### 2.3. Composites nano-hybrid/pectin preparation

Composites pectin/nano-hybrid were prepared by the following procedure. 1 g of pectin was dissolved in 20 mL of distilled water and 2 mL of glycerol, as plasticizer. The obtained solution was stirred for 3 h at 70 °C. The nano-hybrid (a viscous suspension made by HNTs and Essential oil) was added to the pectin/glycerol/water solution at different amounts, in order to obtain nano-hybrid concentrations equal to 5, 10 and 20 wt%. To allow the best mixing, after stirring the composite solution for 30 min, each composition was submitted to high energy ball milling at ambient temperature for 20 min. The apparatus used was a Retsch (Germany) centrifugal ball mill (model S 100), with a cylindrical steel jar of 50 cm<sup>3</sup> containing 5 tungsten carbide balls of 10 mm of diameter. The rotation

speed was 580 rpm. The mixtures obtained were poured into Petri dishes and dried in oven at 70 °C for 7 days. Films of pure pectin were obtained using the same experimental conditions. All films, having the thickness of about 200 μm, were analyzed. Composite samples will be coded as follows: NH<sub>x</sub>, where x is the nano-hybrid amount (wt%).

### 2.4. Methods of analysis

Thermogravimetric analyses (TG-DTA) were carried out from 25 to 1000 °C (heating rate of 10 °C/min) under air flow, using a Mettler TC-10 thermo-balance (Italy). X-ray powder diffraction measurements (XRD) were performed by a Bruker diffractometer (Germany), equipped with a continuous scan attachment and a proportional counter, with Ni-filtered Cu Kα radiation (λ = 1.54050 Å).

The mechanical properties of the samples were evaluated at room temperature and ambient humidity (about 50%) using a dynamometric apparatus INSTRON 4301 (UK). Experiments were conducted with a deformation rate of 2 mm/min. The initial length of the samples was 10 mm. The elastic modulus was calculated from the linear part of the stress-strain curves, which extended to a deformation of 0.1%. Data were averaged for five samples.

Diffusion of water vapor was evaluated using a conventional Mc Bain spring balance system, which consists of a glass water-jacketed chamber serviced by a high vacuum line for sample degassing and vapor removal. Inside the chamber, samples were suspended from a helical quartz spring supplied by Ruska Industries (Houston, TX) having a spring constant of 1.62 cm/mg. The temperature was controlled to 30 ± 0.1 °C by a constant temperature water bath. Samples were exposed to the water vapor at fixed pressures, P, giving different water activities a = P/P<sub>0</sub>, where P<sub>0</sub> is the saturation water pressure at the experimental temperature. The spring position was recorded as a function of time using a cathetometer. The spring position data were converted to mass uptake data using the spring constant, and the process was followed to a constant value of sorption for at least 24 h. Data were averaged on three samples (Felder & Huvard, 1980).

The release kinetics of the rosmarinic acid in a fixed volume of methanol were obtained by ultraviolet spectrometric measurement at ambient temperature, using a Spectrometer UV-2401 PC Shimadzu (Japan).

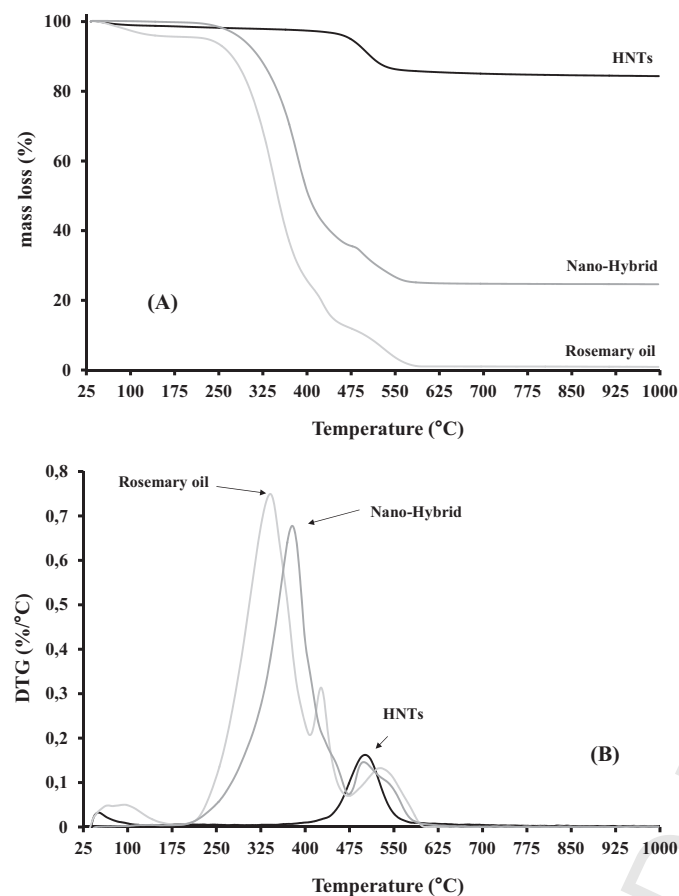
### 2.5. Preliminary antimicrobial analysis

Preliminary antimicrobial analysis was evaluated on unfilled plasticized pectin and on pectin loaded with 20 wt% of nano-hybrid. Films were stored at room temperature (i.e. 25–30 °C) and environmental humidity (i.e. about 60%) for three months. The formation of mould on films was followed visually

## 3. Results and discussion

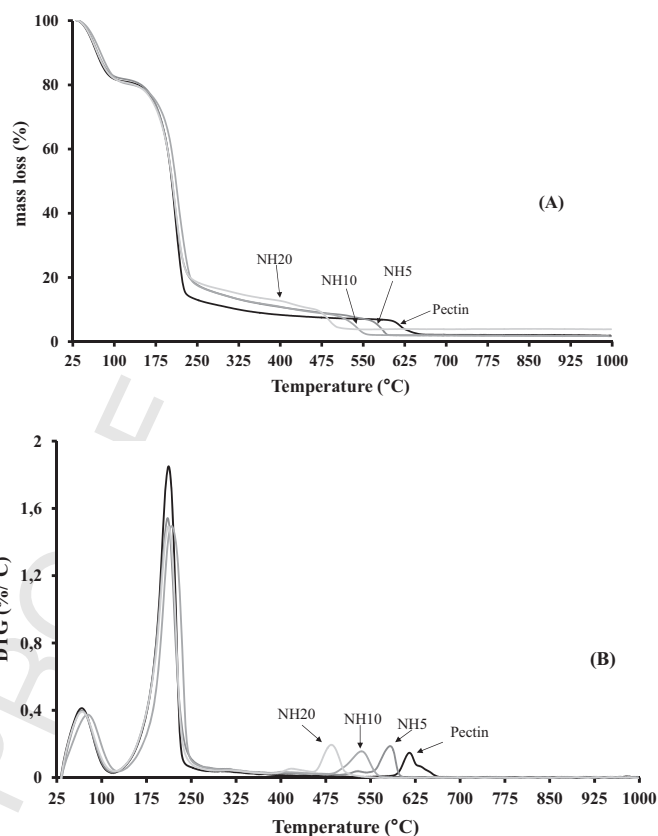
### 3.1. TG-DTG analysis

The distribution of essential oil molecules inside and/or outside HNTs is too complex to be completely determined. The degradation temperatures of single components and the nano-hybrid can give an indication about the molecules' intercalation. Fig. 1 reports the mass loss (%) (TG) (A) and DTG (B) analysis on HNTs, essential oil, and the prepared nano-hybrid. Halloysite shows one degradation step at about 500 °C, that is attributed to the dehydroxylation of the matrix (Horvath, Frost, Mako, Kristof, & Cseh, 2003). Thermal degradation of rosemary oil is very complex, because it is a mixture of many organic compounds. It presents three steps, well evidenced from DTG curves: the first one centered at 343 °C, the second one at 430 °C, a third one at about 533 °C. The first event is attributed



**Fig. 1.** Mass loss (%) (A) and DTG (B) analysis on HNTs, essential oil, and the nano-hybrid.

to the thermal decomposition of saturated and unsaturated molecular compounds. The second one is due to a fast decomposition of volatile products generated in the first step. The third one is generated from the oxidation of carbon residues. A shift toward higher values for the three degradation temperatures of essential oil, centered at 380 °C, 452 °C and 544 °C is evident for the nano-hybrid. The last two events are present as shoulders in the DTG of the nano-hybrid. The degradation of the inorganic halloysite does not change going from the unfilled sample to the nano-hybrid. Such result allows to hypothesize that the oil molecules undergo a delay in the degradation because most molecules were successfully entrapped into the nanotubes and their degradation can probably occur only after a spillage. Fig. 2 reports the TG (A) and DTG (B) results for pectin film and composites with 5, 10 and 20 wt% of nano-hybrid. Thermo-oxidative degradation of pectins and composites is a very complex phenomenon that results from several chemical and physical variables. All samples, either pure pectin or composites, display a characteristic three-step thermal degradation, as shown in Fig. 2(A). The first step, occurring at about 80 °C, corresponds to the water loss. It is followed by the second step, between about 150 °C and 250 °C. In this temperature range it was reported that the degradation is primarily from pyrolytic decomposition consisting of a primary and secondary decarboxylation involving the acid side group and a carbon in the ring (Shim, Hajaligol, & Baliga, 2004; Waymack, Belobe, Baliga, & Hajaligol, 2004; Mangiacapra, Gorrasi, Sorrentino, & Vittoria, 2006). The third step between about 600 °C and 650 °C corresponds to the oxidation region. The second degradation stage is very similar for both the pure pectin and the composite samples, whereas the third degradation phenomenon is present at lower temperatures, as clearly



**Fig. 2.** Mass loss (A) and DTG (B) results for pectin and composites at different nano-hybrid loading.

evidenced by DTG analysis (Fig. 2 (B)). The temperature decreases on increasing the nano-hybrid percentage into the composites. It has been generally found that HNTs are flame retardant for polymers and bio-polymers (Lvov & Abdullayev, 2013). In this case we can hypothesize that the decomposition of volatile products and the oxidation of carbon residues observed in the second and third step of oil degradation occur in this temperature range, and also have a catalytic effect promoting pectin oxidation.

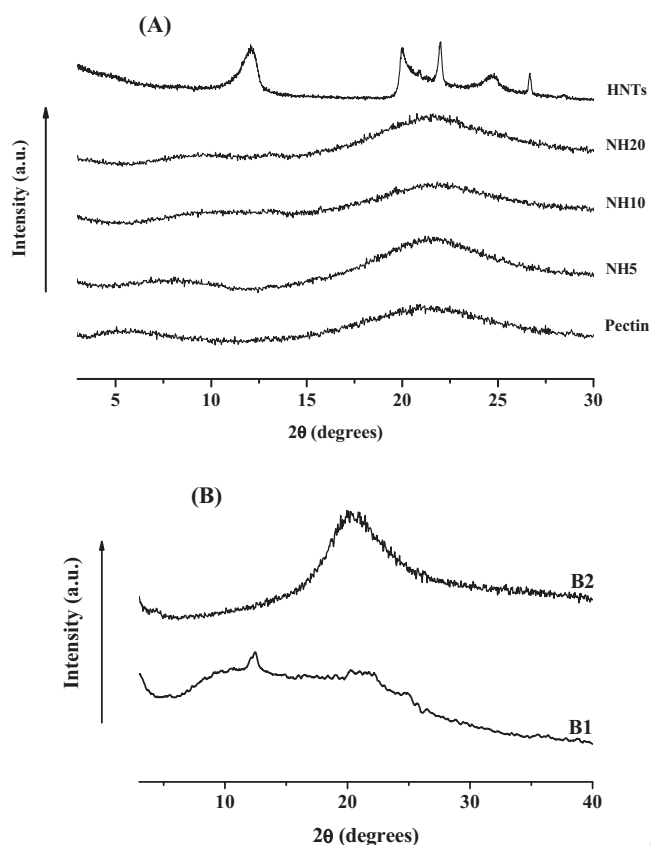
### 3.2. XRD analysis

Fig. 3(A) reports the XRD of HNTs and composites at different nano-hybrid loading. The dehydrated HNTs shows a characteristic  $2\theta \sim 12.2^\circ$  (7.3 Å), that is representative of the first order (001) basal reflection (Tazaki, 2005). Such spectrum is typical of halloysite extracted from Matauri Bay (New Zealand) (Nicolini, Budziak Fukamachi, Wypych, & Mangrich, 2009). None of HNT peak can be seen in the composite diffractograms. This could mean an expansion after the intercalation of essential oil, and the mechanical assisted mixing with pectin in presence of water. The amorphous structure with a broad peak located at about  $22^\circ$  of  $2\theta$  is typical of amorphous pectin plasticized with glycerol. Such a structure is retained in all the composites.

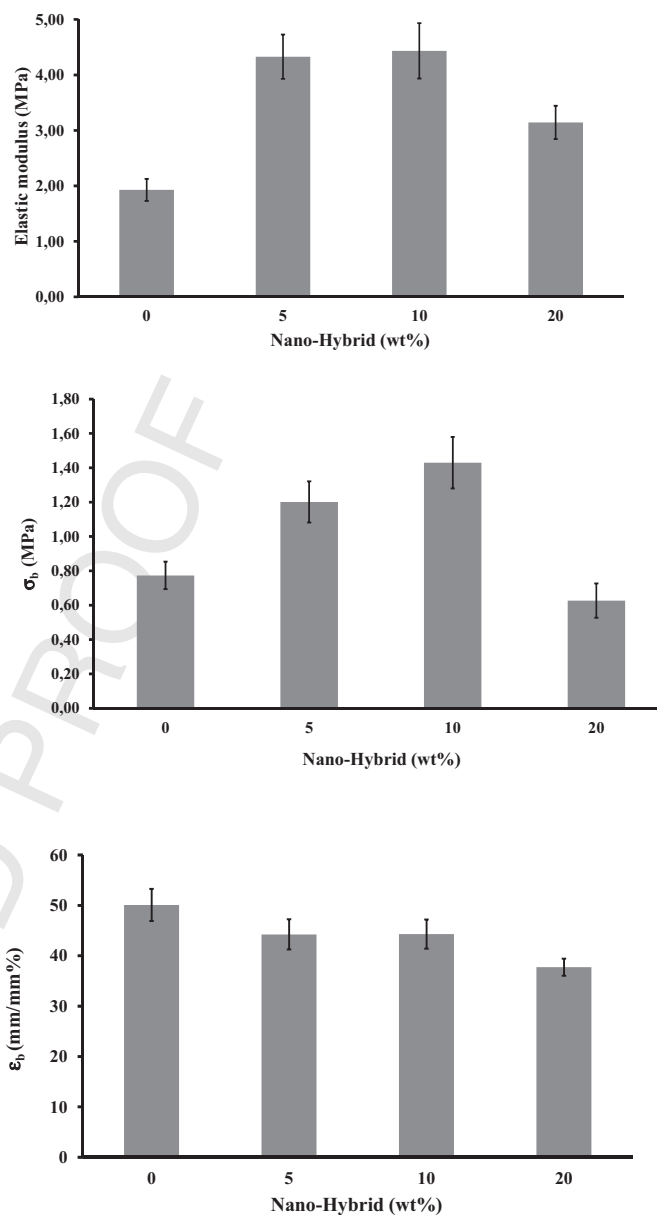
### 3.3. Mechanical properties

Fig. 4 reports the stress-strain curves of pectin and composites. From these curves the elastic modulus,  $E$  (MPa), the stress at break point,  $\sigma_b$  (MPa), and the elongation at break,  $\epsilon_b$  (mm/mm%) were evaluated. The mechanical parameters are reported in Fig. 5. The elastic modulus is lower than the one detected for the pure pectin films (Gorrasi, Bugatti, & Vittoria, 2012) for the plasticizing effect

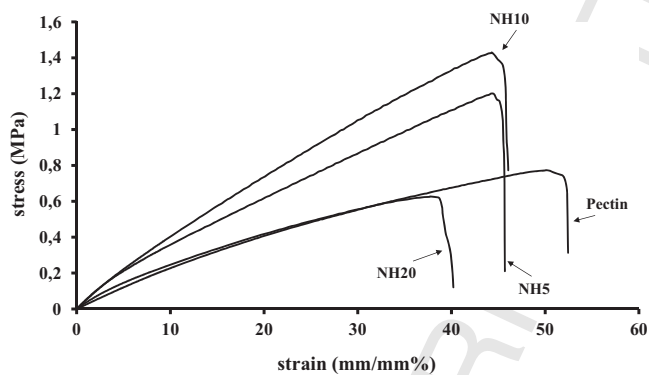




**Fig. 3.** XRD of HNTs and composites at different nano-hybrid loading (A); of sample HN20 after 28 days of contact time with methanol (B1) and of plasticized unfilled pectin after 24 h of contact with methanol (B2).



**Fig. 5.** Mechanical parameters evaluated from stress–strain curves for pectin and composites at different nano-hybrid loading.



**Fig. 4.** Stress–strain curves of pectin and composites at different nano-hybrid loading.

of glycerol. The elastic modulus is significantly improved for composites with 5 and 10 wt% of nano-hybrid, due to the reinforcing effect of HNTs, and slightly lower for composite with 20 wt% of hybrid filler. However, its value remains higher than the modulus of unfilled pectin. The reinforcing effect of HNTs is evident also for the stress at break,  $\sigma_b$  (MPa), especially at low nano-hybrid content. For higher loading (i.e. 20 wt%) we observe a decrease of  $\sigma_b$ . The lower the nano-hybrid percentage, the better is the dispersion of the filler into the organic phase, with a consequent improved mechanical parameters at low concentration. Increasing the percentage of filler in the composites (i.e. 20%), the formation of “clusters” can occur, thus determining a deterioration of the mechanical parameters. Such decrease of mechanical properties is due either to the

nano-hybrid or to the “free” oil loading. The oil molecules will tend to self-aggregate and create phase separation between the polymer matrix and the fillers. Despite this, such mechanical parameter is not much worse than the unfilled pectin. Glycerol, in turn, has a positive effect on elongation at break. Pure un-plasticized pectin can be elongated less than 2%. It is evident that pectin film, plasticized with glycerol, can be elongated up to 50%. This parameter remains almost unchanged for the composites filled with 5 and 10 wt% of nano-hybrid, and slightly lower for the composite filled with 20 wt% of filler.

### 3.4. Transport properties

The transport properties of a liquid or a vapor in a polymeric matrix are defined as the sorption and diffusion of a solvent molecule across the amorphous phase. The crystalline regions normally are considered to be impermeable. In the case of composite materials, the presence of domains of inorganic phases makes the systems more complicated. It has been demonstrated that the

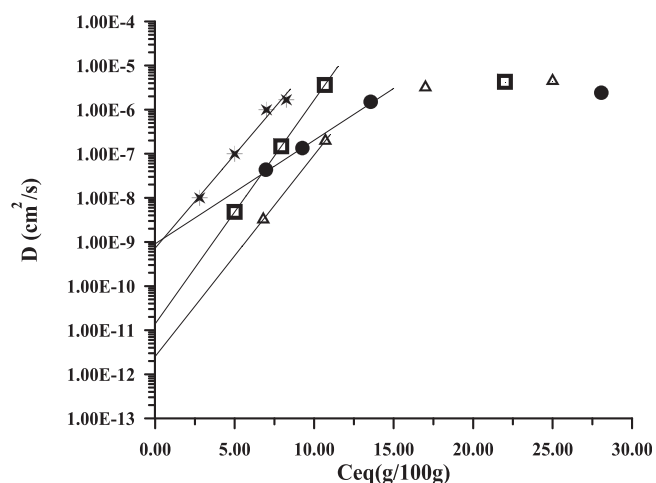


Fig. 6. Diffusion coefficients,  $D$  ( $\text{cm}^2/\text{s}$ ), as a function of the sorbed concentration of water vapor,  $C_{\text{eq}}$  ( $\text{g}/100\text{g}$ ), for: pectin (●), NH5 (\*), NH10 (□), NH20 (Δ).

morphology and the microstructure of such multi-phase systems play a very important role in determining the transport phenomena (Gorrasi et al., 2003, 2008, 2012; Gorrasi, Anastasio, Bassi, & Pantani, 2013). Fig. 6 reports the diffusion coefficients in pectin and composite samples,  $D$  ( $\text{cm}^2/\text{s}$ ), as a function of the sorbed concentration of water vapor,  $C_{\text{eq}}$  ( $\text{g}/100\text{g}$ ). Two regions of the curves are clearly recognizable: at low vapor concentrations, the diffusion coefficient increases steeply and linearly with  $C_{\text{eq}}$ , whereas a transition can be observed for concentrations of sorbed vapor higher than about 10%. The strong interaction with the penetrating molecules, leading to a high mobility of polymer chains, can induce structural transformations such as clustering of solvent molecules, crazing or partial dissolution. The systems lose their compactness and diffusion becomes independent of the amount of vapor adsorbed, as can be observed by the second part of the curves. In the first linear part of the curves we applied the following empirical equation, valid for polymer-solvent systems can be applied (Gorrasi et al., 2003, 2008):

$$D = D_0 \exp(\gamma C_{\text{eq}}) \quad (1)$$

and the zero diffusion coefficient,  $D_0$  ( $\text{cm}^2/\text{s}$ ), can be obtained for all the samples. The logarithm of  $D_0$  is reported in Fig. 7(A), as function of the nano-hybrid content (wt%). The improvement of barrier properties, in terms of decrease of diffusion, increases with the nano-hybrid loading, in the investigated composition range. On discussing the X-ray measurements it was evidenced that the pectin matrix in the composites and in the pure polymer shows the same amorphous structure. As a consequence of an unchanged polymer morphology for the composite samples, compared to pure pectin, the decrease of the values of  $D_0$  must be ascribed to the presence of the filler. On the other hand, the presence of the filler increases to a large extent the tortuosity of the system, leading to an expected large decrease in the value of the diffusion coefficient (Gorrasi et al., 2003, 2008). It is worth noticing that  $D_0$  ( $\text{cm}^2/\text{s}$ ) is a thermodynamic parameter differently from the diffusion coefficients evaluated at each vapor activity, which can be correlated to the use of the materials in interactive environments.  $D_0$  provides information related to the starting structure of the samples (i.e. fractional free volume and tortuosity of the path) strictly correlated to the morphological texture. Fig. 7(B) reports the  $\gamma$  values extracted from Fig. 6, following Eq. (1). They increase quite linearly up to nano-hybrids content of 10%, with a small decrease for sample HN20. Such a decrease could be related to the deterioration of the mechanical parameter probably due to some cluster formation at high filler content. This is also a confirmation that the presence of the nano-hybrid filler

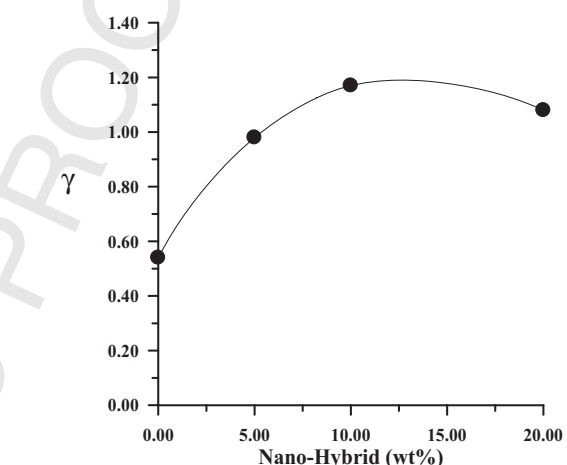
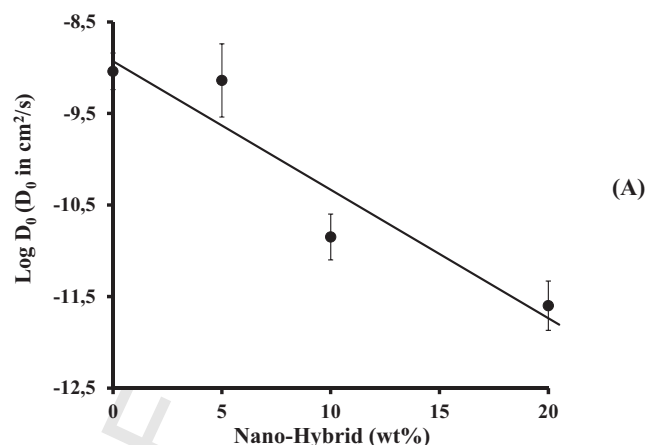


Fig. 7. Logarithm of  $D_0$ ,  $D_0$  ( $\text{cm}^2/\text{s}$ ), as function of the nano-hybrid content (wt%) (A);  $\gamma$  values, evaluated according to from Eq. (1), as function of nano-hybrid content (wt%) (B).

would introduce constraints on molecular mobility of the pectin macromolecular chains.

### 3.5. Release of rosmarinic acid

Rosemary essential oil, like all natural essential oils, is a complex mixture of several organic compounds (Luis & Johnson, 2005). In order to test the prepared composites as controlled release systems, we followed the release of one of the major component: rosmarinic acid, using UV spectrometry. The solvent used was methanol. This solvent was used because it is a good extracting solvent for rosmarinic acid, and the composites did not lose their dimensional stability during the release. A preliminary study of rosmarinic acid release from the composite filled with 20 wt% of nano-hybrid was conducted. For comparison, a composite having the same essential oil percentage (about 15 wt%), directly dispersed into the polymeric matrix was prepared. Fig. 8 shows released fraction (wt/wt%), defined as the amount of rosmarinic acid released divided by the total amount of rosmarinic acid present into the composite, as a function of the square root of the contact time in hours. It is clearly evident that the release of rosmarinic acid from the composite filled with the nano-hybrid is much slower than the release of the same molecule simply blended to the pectin. All the rosmarinic acid molecules dispersed into pectins are released in about 4 h, whereas only about 25% is released in the same time from the nano-hybrid into pectin. In addition, the release of the active molecule from the



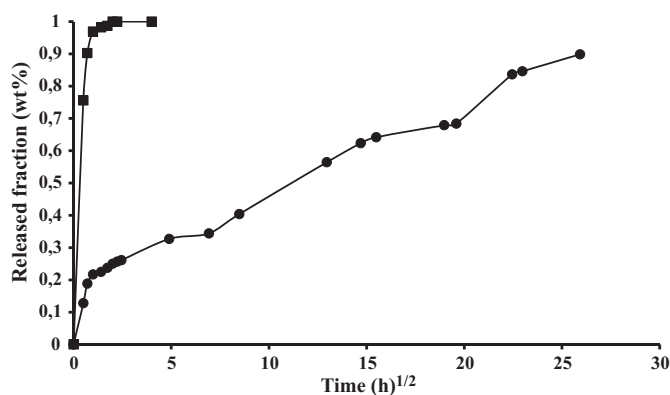
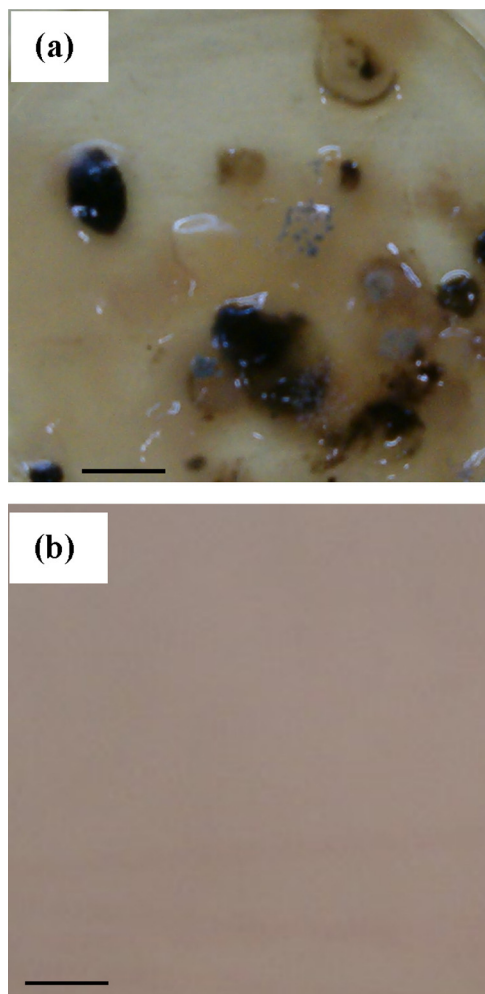


Fig. 8. Rosmarinic acid released fraction (wt/wt%) as function of contact time (h)<sup>1/2</sup> for: pectin and 15 wt% of rosemary oil (■), and sample NH20 (●).

composite filled with nano-hybrid shows different steps. In the first step we observe a rapid diffusion of the rosmarinic acid molecules. This is probably due the fraction of essential oil not intercalated into the HNTs, but located around the nanotubes and/or free dispersed into the pectin matrix. After a rapid diffusion in the first stage, the release rate decreases and presents different stages. After about 28 days the 90% of the molecule was released. The XRD spectra of the



1 cm

Fig. 9. (a) Plasticized unfilled pectin; (b) composite NH20.

sample NH20 after 28 days of contact with solvent. It is reported in Fig. 3(B1). The presence of the basal peak of HNTs at  $2\theta \sim 12.2^\circ$  is representative of a reorganization of the filler structure, after the release of the loaded molecules, in the pristine state. In Fig. 3(B2) the spectra of pure plasticized pectin, after 24 h of contact with methanol are also reported. It is evident that after contact with this solvent pectin retains its amorphous structure.

### 3.6. Evaluation of antimicrobial activity

A preliminary study on the antimicrobial activity of the prepared composites was conducted. We observed the mould formation on the unfilled pectin film and on the NH20 composite. Samples were stored at room temperature and humidity conditions. Fig. 9 shows pictures taken on pectin (a) and NH20 (b) films after 3 months of storage at room temperature (i.e.  $25\text{--}30^\circ\text{C}$ ) and environmental humidity (i.e. 60%). Mould formation was observed in the pectin films after 2 weeks of storage, but it was not detected in the nanocomposite films even after 3 months. These results clearly suggest the potential of utilizing pectin films enriched with antimicrobials as novel packaging materials. Therefore, such systems appear very promising in the active packaging field, where the antimicrobial drug should not be easily released, but “entrapped” into the polymer matrix as long as possible. Work is in progress to test the bacterial inhibition growth, in order to evaluate a threshold antimicrobial value of inhibitory concentration.

## 4. Concluding remarks

This paper reported the development of new green biodegradable nanocomposites based on pectins and a nano-hybrid composed of natural halloysite (HNTs) and rosemary oil. Essential oils, applied to the formulation of edible films, provide them additional antioxidant and/or antimicrobial properties.

- TG-DTG analysis allowed to hypothesize that the essential oil molecules into the nano-hybrid delay the thermo-oxidation, probably because some molecules were successfully entrapped into the nanotubes. The degradation of the pectin matrix was not greatly influenced from the nano-hybrid filler, except for the oxidation stage at high temperatures, that resulted anticipated.
- X-ray analysis on the composites showed that pure pectins present an amorphous structure, which is kept in all the composites. The basal peak of dehydrated HNTs tends to disappear and/or to expand after the intercalation of essential oil and the ball milling assisted mixing with pectin in presence of water.
- Mechanical properties showed an improvement, or at least a preservation, of all the mechanical parameters. In particular, the elongation at break point reached values up to 50%.
- Barrier properties to water vapor, improved. The presence of the filler increased the tortuosity of the system, leading to a decrease in the value of the diffusion coefficient.
- A comparison of the molecular release of the rosmarinic acid molecules directly dispersed in pectins and in the nano-hybrid was reported, indicating a much slower release in the latter case.
- Preliminary evaluation of antimicrobial activity indicated the potential application of the prepared composites in the packaging field, opening new perspectives for pectins-antimicrobials as coating agents for a wide number of packaging polymers.

## Acknowledgements

Author wish to thank Miss Valentina Senatore for carrying out part of the experiments during her work of thesis for bachelor in Chemical Engineering at University of Salerno, Dr Luigi Vertuccio

for technical support, Prof Roberto Pantani for many fruitful discussions.

## References

- Abdullayev, E., & Lvov, Y. (2011). Clay nanotubes for corrosion inhibitor encapsulation: Release control with end stoppers. *Journal of Materials Chemistry*, *20*, 6681–6687.
- Abdullayev, E., Price, R., Shchukin, D., & Lvov, Y. (2009). Halloysite tubes as nanocontainers for anticorrosion coating with benzotriazole. *Applied Materials & Interfaces*, *2*, 1437–1442.
- Abdullayev, E., Shchukin, D., & Lvov, Y. (2008). Halloysite clay nanotubes as a reservoir for corrosion inhibitors and template for layer-by-layer encapsulation. *Polymer Materials Science & Engineering*, *99*, 331–332.
- Bates, T., Hilderbrand, F., & Swineford, A. (1950). Morphology and structure of endellite and halloysite. *American Mineralogist*, *35*, 463–485.
- Bergaya, F., Theng, B. K. G., & Lagaly, G. (Eds.). (2006). *Handbook of clay science*. (p. 1224). Amsterdam: Elsevier Science & Technology.
- Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods—A review. *International Journal of Food Microbiology*, *94*, 223–253.
- Carr, R. M., Chaikūm, N., & Patterson, N. (1978). Intercalation of salts in halloysite. *Clays and Clay Minerals*, *26*, 144–152.
- Cavallaro, G., Lazzara, G., & Milioto, S. (2011). Dispersions of nanoclays of different shapes into aqueous and solid biopolymeric matrices. *Extended Physicochemical Study Langmuir*, *27*, 1158–1163.
- Churchman, G. J., & Carr, R. M. (1975). The definition and nomenclature of halloysites. *Clays and Clay Minerals*, *23*, 382–388.
- Deans, S. G., & Ritchie, G. (1987). Antibacterial properties of plant essential oils. *International Journal of Food Microbiology*, *5*, 165–180.
- Dorman, H. J. D., & Deans, S. G. (2000). Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, *88*, 308–316.
- Du, M., Guo, B., & Jia, D. (2010). Newly emerging applications of halloysite nanotubes: A review. *Polymer International*, *59*, 574–582. <http://dx.doi.org/10.1002/pi.2754>
- Du, M., Guo, B., & Jia, D. (2006). Thermal stability and flame retardant effects of halloysite nanotubes on poly(propylene). *European Polymer Journal*, *42*, 1362–1369.
- Du, M., Guo, B., Liu, M., & Jia, D. (2007). Thermal decomposition and oxidation ageing behaviour of polypropylene/halloysite nanotube nanocomposites. *Polymers and Polymer Composites*, *15*, 321–328.
- Espitia, P. J. P., Dub, W. X., de Jesús Avena-Bustillos, R., de Fátima Ferreira Soares, N., & Mc Hugh, T. H. (2014). Edible films from pectin: Physical–mechanical and antimicrobial properties—A review. *Food Hydrocolloids*, 287–296.
- Felder, R. M., & Huvard, G. S. (1980). C. R Fava (Ed.), *Methods of experimental physics* (vol. 16) (pp. 315–318). New York, NY: Academic Press.
- Gómez-Estaca, J., López de Lacey, A., López-Caballero, M. E., Gómez-Guillén, M. C., & Montero, P. (2010). Biodegradable gelatin–chitosan films incorporated with essential oils as antimicrobial agents for fish preservation. *Food Microbiology*, *27*, 889–896.
- Gorrasi, G., Bugatti, V., & Vittoria, V. (2012). Pectins filled with LDH-antimicrobial molecules: Preparation, characterization and physical properties. *Carbohydrate Polymers*, *89*, 132–137.
- Gorrasi, G., Tortora, M., Vittoria, V., Pollet, E., Lepoittevin, B., Alexandre, M., et al. (2003). Vapor barrier properties of polycaprolactone montmorillonite nanocomposites: Effect of clay dispersion. *Polymer*, *44*, 2271–2279.
- Gorrasi, G., Vittoria, V., Murariu, M., Da Silva Ferreira, A., Alexandre, M., & Dubois, P. (2008). Effect of filler content and size on transport properties of water vapor in PLA/calcium sulfate composites. *Biomacromolecules*, *9*(3), 984–990.
- Gorrasi, G., Anastasio, R., Bassi, L., & Pantani, R. (2013). Barrier properties of PLA to water vapour: Effect of temperature and morphology. *Macromolecular Research*, *21*, 1110–1117.
- Guo, B., Zou, Q., Lei, Y., Du, M., Liu, M., & Jia, D. (2009). Crystallization behavior of polyamide 6/halloysite nanotubes nanocomposites. *Thermochimica Acta*, *484*, 48–56.
- Hammer, K. A., Carson, C. F., & Riley, T. V. (1999). The antimicrobial activity of plant oils. *Journal of Applied Microbiology*, *86*, 985–990.
- Horvath, E., Frost, R. L., Mako, E., Kristof, J., & Cseh, T. (2003). Thermal treatment of mechanochemically activated kaolinite. *Thermochimica Acta*, *404*, 227–235.
- Joussein, E., Petit, S., Churchman, J., Theng, B., Righi, D., & Delvaux, B. (2005). Halloysite clay minerals—A review. *Clay Minerals*, *40*, 383–426.
- Kirby, A., MacDougall, A., & Morris, V. (2008). Atomic force microscopy of tomato and sugar beet pectin molecules. *Carbohydrate Polymers*, *71*, 640–647.
- Kirkman, J. H. (1981). Morphology and structure of halloysite in New Zealand tephros. *Clays and Clay Minerals*, *29*, 1–9.
- Lagaron, J. M., & Lopez-Rubio, A. (2011). Nanotechnology for bioplastics: Opportunities, challenges and strategies. *Trends in Food Science & Technology*, *22*, 611–617.
- Liu, M., Guo, B., Du, M., & Jia, D. (2008). The role of interactions between halloysite nanotubes and 2,2'-(1,2-ethenediyl-di-4,1-phenylene) bisbenzoxazole in halloysite reinforced polypropylene composites. *Polymer Journal*, *40*, 1087–1093.
- Liu, M., Guo, B., Du, M., & Jia, D. (2007). Drying induced aggregation of halloysite nanotubes in polyvinyl alcohol/halloysite nanotubes solution and its effect on properties of composite film. *Applied Physics A: Materials Science & Processing*, *88*, 391–395.
- Luis, J. C., & Johnson, C. B. (2005). Seasonal variations of rosmarinic and carnosic acids in rosemary extracts, Analysis of their in vitro antiradical activity. *Spanish Journal of Agricultural Research*, *3*(1), 106–112.
- Liu, M., Jiab, Z., Jiab, D., & Zhou, C. (2014). Recent advance in research on halloysite nanotubes-polymer nanocomposite. <http://dx.doi.org/10.1016/j.progpolymsci.2014.04.004>.
- Lvov, Y., & Abdullayev, E. (2013). Green and functional polymer–Clay nanotube composites with sustained release of chemical agents. *Progress in Polymer Science*, <http://dx.doi.org/10.1016/j.progpolymsci.2013.05.009>.
- Lvov, Y., & Price, R. (2008). Halloysite nanotubes a novel substrate for the controlled delivery of bioactive molecules. In E. Ruiz-Hitzky, K. Ariga, & Y. Lvov (Eds.), *Bio-inorganic hybrid nanomaterials* (pp. 440–453). London, Berlin: Wiley.
- Lvov, Y., Price, R., Gaber, B., & Ichinose, I. (2002). Thin film nanofabrication via layer-by-layer adsorption of tubule halloysite, spherical silica, proteins and polycations. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *198–200*, 375–382.
- Lvov, Y., Shchukin, D., Möhwald, H., & Price, R. (2008). Halloysite clay nanotubes for controlled release of protective agents. *ACS Nano*, *2*, 814–820.
- Nicolini, K. P., Budziak Fukamachi, C. R., Wypych, F., & Mangrich, A. S. (2009). Dehydrated halloysite intercalated mechanochemically with urea: Thermal behavior and structural aspects. *Journal of Colloid and Interface Science*, *338*, 474–479.
- Price, R., Gaber, B., & Lvov, Y. (2001). In-vitro release characteristics of tetracycline HCl, khellin and nicotinamide adenine dinucleotide from halloysite; a cylindrical mineral. *Journal of Microencapsulation*, *18*, 713–723.
- Shim, H.-S., Hajaligol, M. R., & Baliga, V. L. (2004). Oxidation behavior of biomass chars: Pectin and Populus deltoides. *Fuel*, *83*, 1495–1503.
- Siew, C. K., & Williams, P. A. (2008). Role of protein and ferulic acid in the emulsification properties of sugar beet pectin. *Journal of Agricultural and Food Chemistry*, *56*, 4164–4171.
- Singh, B., & Mackinnon, I. (1996). Experimental transformation of kaolinite to halloysite. *Clays and Clay Minerals*, *44*, 825–834.
- Singh, B. (1996). Why does halloysite roll?—A new model. *Clays and Clay Minerals*, *44*, 191–196.
- Sorrentino, A., Gorrasi, G., & Vittoria, V. (2007). Potential perspectives of bio-nanocomposites for food packaging applications. *Trends in Food Science & Technology*, *18*, 84–95.
- Tazaki, K. (2005). Microbial formation of a halloysite-like mineral. *Clays and Clay Minerals*, *53*, 224–233.
- Vergaro, V., Abdullayev, E., Cingolan, R., Lvov, Y., & Leporatti, S. (2010). Biomacromolecules, *11*, 820–828.
- Vincken, J.-P., Schols, H., Oomen, M., McCann, M., Ulvskov, P., Voragen, A., et al. (2003). If homogalacturonan were a side chain of rhamnogalacturonan I. Implications for cell wall architecture. *Plant Physiology*, *132*, 1781–1789.
- Waymack, B. E., Belobe, J. L., Baliga, V. L., & Hajaligol, M. R. (2004). Effects of metal salts on char oxidation in pectins/uronic acids and other acid derivative carbohydrates. *Fuel*, *83*(11–12), 1505–1518.
- Wei, W., Abdullayev, E., Hollister, A., Mills, D., & Lvov, Y. (2012). Clay nanotube/poly(methyl methacrylate) bone cement composites with sustained antibiotic release. *Macromolecular Materials and Engineering*, *297*, 645–653.
- Yelleswarapu, C., Gu, G., Abdullayev, E., Lvov, Y., & Rao, D. (2010). Nonlinear optics of nontoxic nanomaterials. *Optics Communications*, *283*, 438–441.
- Zheng, Y., & Wang, A. (2010). Enhanced adsorption of ammonium using hydrogel composites based on chitosan and halloysite. *Journal of Macromolecular Science, A: Pure and Applied Chemistry*, *47*, 33–38.
- Zsivánovits, G., Marudova, M., & Ring, S. (2005). Influence of mechanical properties of pectin films on charge density and charge density distribution in pectin macromolecule. *Colloid & Polymer Science*, *284*, 301–308.