



# Chitosan Films Reinforced with Chitin Nanofibrils:

## Effect of Composition and Preparation Conditions on Films Properties

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*The idea of preparing the films by reinforcing chitosan phase with chitin nanofibrils was based on high chemical and structural similarity of both components that ensure their excellent compatibility. Therefore, CS phase can accept 40 wt% and more of chitin nanofibrils without phase separation. This gives unprobable opportunity for preparing water-insoluble chitosan films „crosslinked“ with chitin nanofibrils without necessity of using any chemical crosslinkers.*



# Raw Materials quality

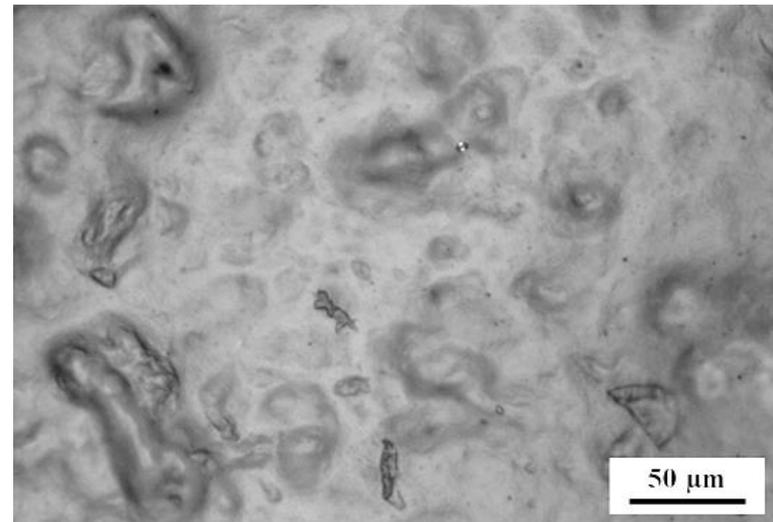
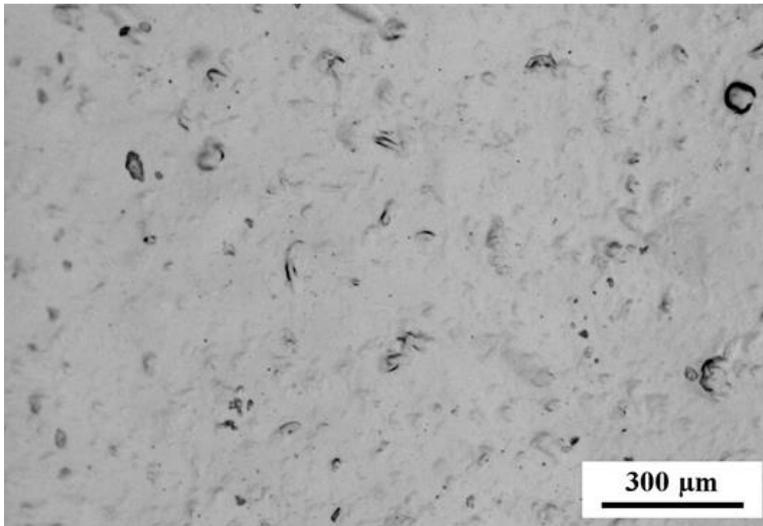
## Commercial Chitosan

(Giusto Faravelli X.p.A., Italy)

$M_w = 1425 \pm 35$  kDa, DA = 21%

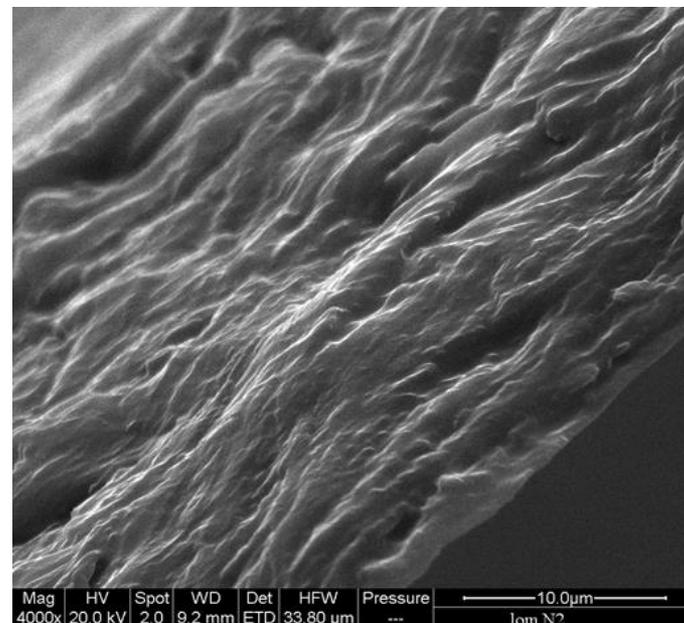
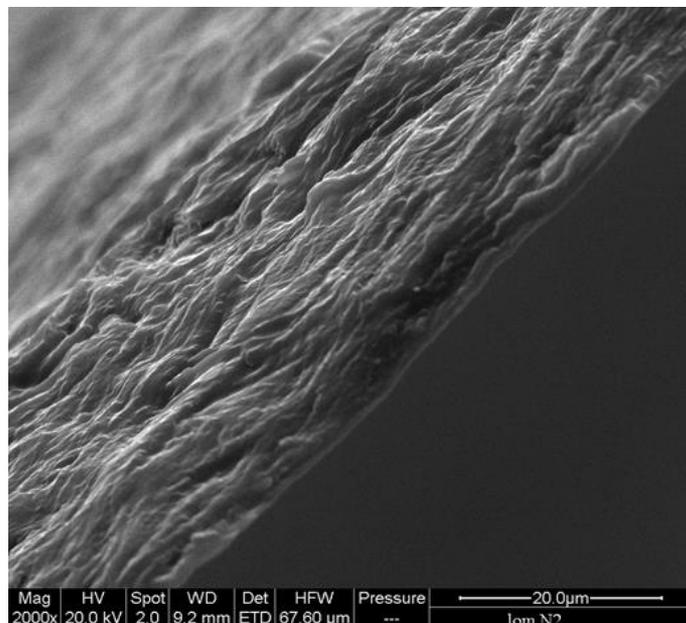
*The quality and properties of all products including films prepared by casting technique from nano-chitin (NC) in the form of nanofibrils or whiskers and chitosan (CS) depend, first of all, on quality of raw materials. In our study, the powder of commercial CS and aqueous dispersion of NC produced both by the Italian companies Giusto Faravelli X.p.A. and Mavi Sud S.r.L, respectively, were used. For evaluating the effect of quality of the raw CS and NC on properties of the CS/NC composite films, the test samples of films from commercial CS powder and NC aqueous dispersion were prepared and characterized.*

# SEM of CS/CN films from unfractionated commercial chitosan



*The composite CS/NC (85/15 wt%) films plasticized with glycerol (25 wt% of total CS and NC content) looked commercially unattractive because of high heterogeneity and roughness of their surfaces.*

# SEM of fractures of CS/CN films from unfractionated commercial chitosan



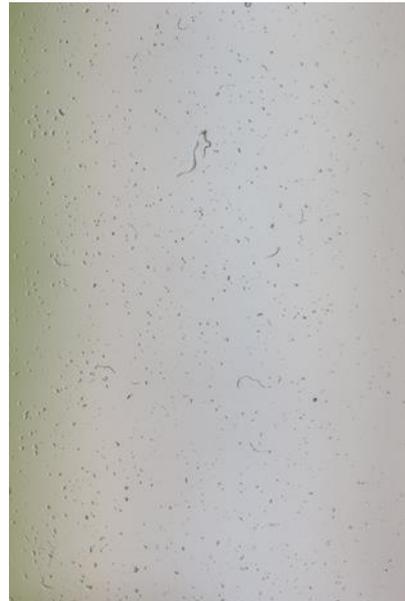
*Scanning electron micrographs (SEM) of the fracture of a film prepared from commercial materials with CS/CN proportion 85/15 wt%.*

# Optical view of CS/CN films from chitosan fraction (80-140 $\mu\text{m}$ )

1. Dissolution of CS
2. Mixing CS solution with CN dispersion and glycerol
3. Concentrating the slurry by vacuum evaporation



1. 25 °C, 6 hours
2. 25 °C, 1 hour
3. 60 °C, 30 min



1. 25 °C, 12 hours
2. 60 °C, 0,5 hour
3. 60 °C, 30 min

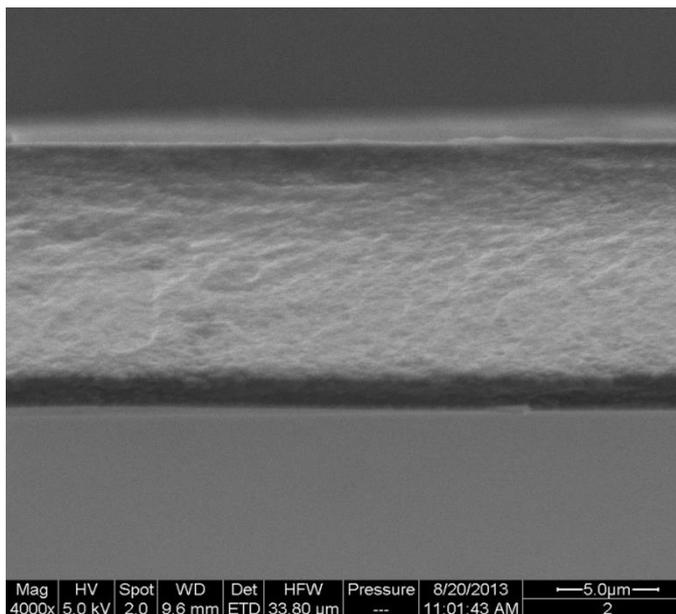


1. 25 °C, 12 hours
2. 60 °C, 1 hour
3. 60 °C, 30 min

Therefore, the clarification procedure of commercial CS was developed. It consisted of sewing the CS powder through two sieves with pore sizes of 140 and 80  $\mu\text{m}$ . Further, the only CS fraction (80-140  $\mu\text{m}$ ) was used in a three-step preparation procedure of the slurry.

# SEM of Gelatin-containing CS/CN film

Composition: [CS/CN]/Glycerol (70/30)/30 wt%  
Gelatin content: 10 wt% of CS



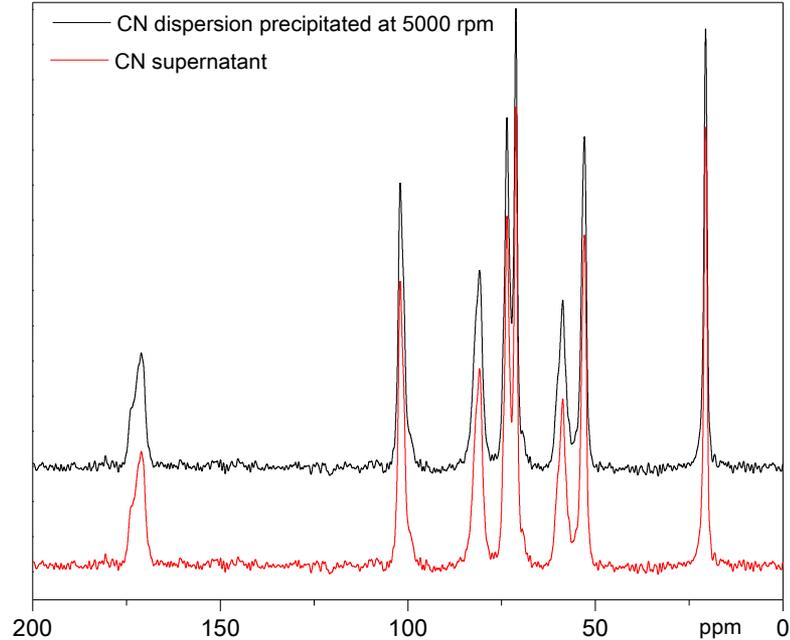
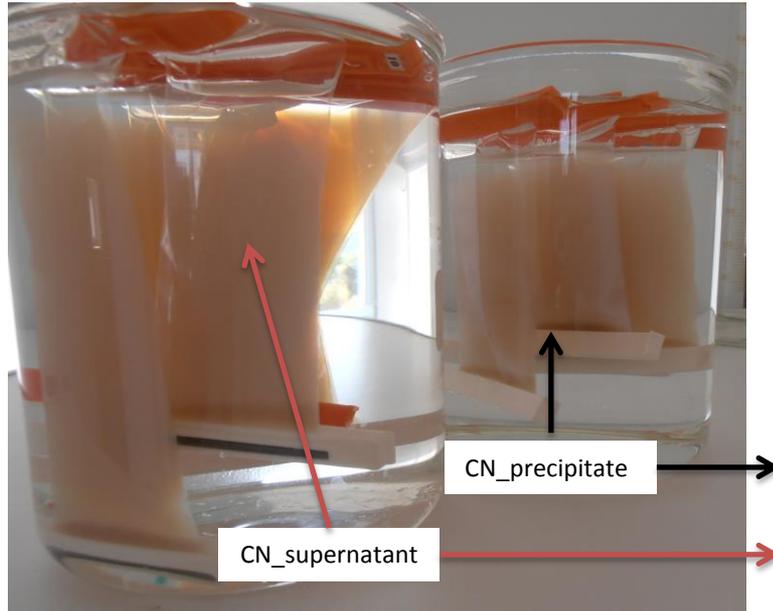
*The CS/CN films became commercially attractive when the slurry was prepared following by the optimized conditions. They looked similarly to cellulosic Cellophane ones. In comparison with films made from commercial powder, the films from clarified CS were transparent and did not contain large undissolved particles when prepared in accordance with optimal conditions. The internal inhomogeneity of these films decreased considerably.*



# Chitin nanofibrils (MAVI Sud S.r.L., Italy)

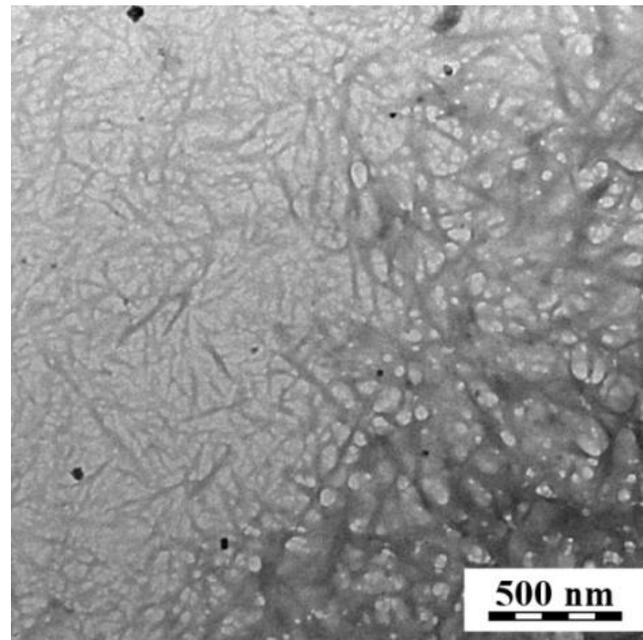
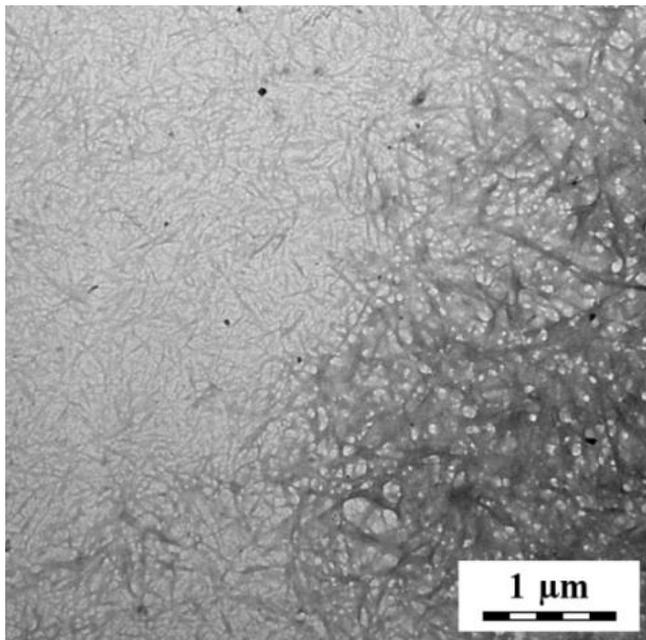
*The commercial aqueous NC dispersion contained low-molecular-weight (LMW) additives such as sodium benzoate (about 0.5 wt%) and an excess of acid. The former is usually added to suppress the microbial growth under storage of NC dispersion. Since the degradation of CS is enhanced in acidic media, it is obvious that the stability of dried CS/NC films will be kept better if their pH is close to neutral.*

# Fractionation of Chitin Nanofibrils



*For removal of acidic substances, NC dispersions were undergone dialysis against water. In the course of dialysis through 3.5-kDa SpectraPor films, the LMW additives were successfully removed: pH increased from about 1.7 to 5-5.2. Finally, NC dispersion was fractionated by centrifugation at 5000 rpm for 30 min. The obtained fractions: NC-precipitate and NC-supernatant had practically the same DA (about 97%). For ensuring higher homogeneity of the CS/NC films, they were prepared by using the NC-supernatant only after its previous concentration (about 8-10 folds) by vacuum evaporation.*

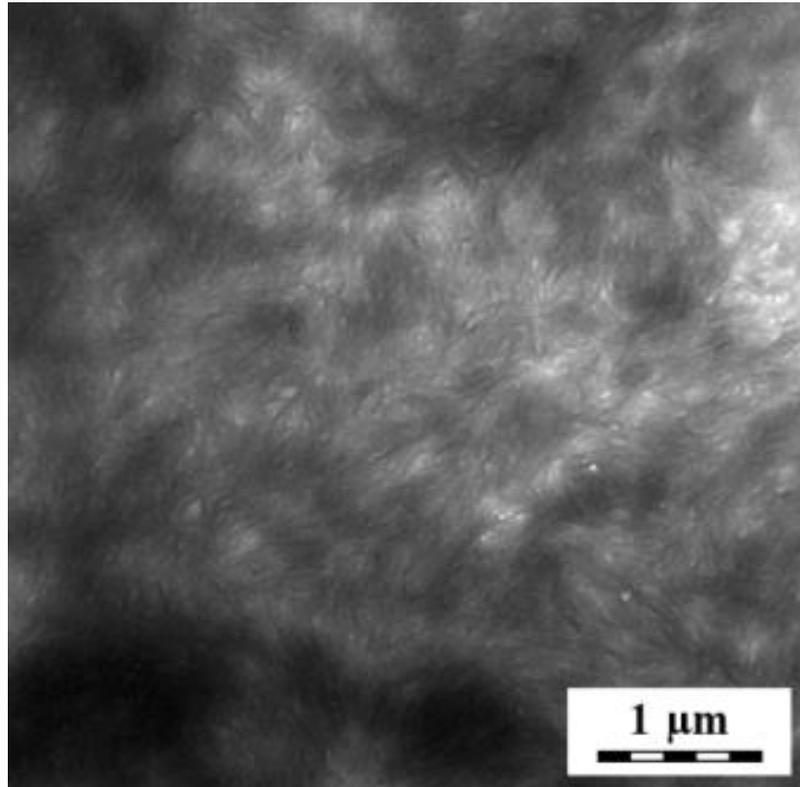
# TEM images of CN\_precipitate and CN\_supernatant



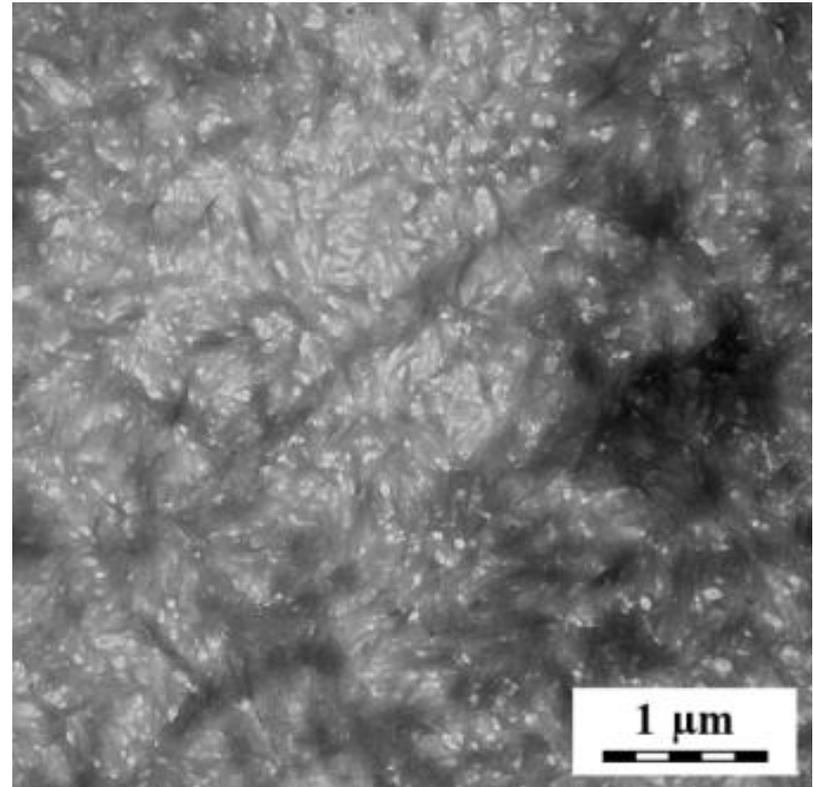
**CN/Glycerol (70/30 wt%) mixture was heated at 60 °C for 1 h**

*The morphology of NC fibrils in the dried state was investigated by scanning (SEM) and transmission (TEM) electron microscopy. The fibrils in both NC-precipitate and NC-supernatant were a needle- or spindle- shaped.*

# TEM images of CN\_precipitate



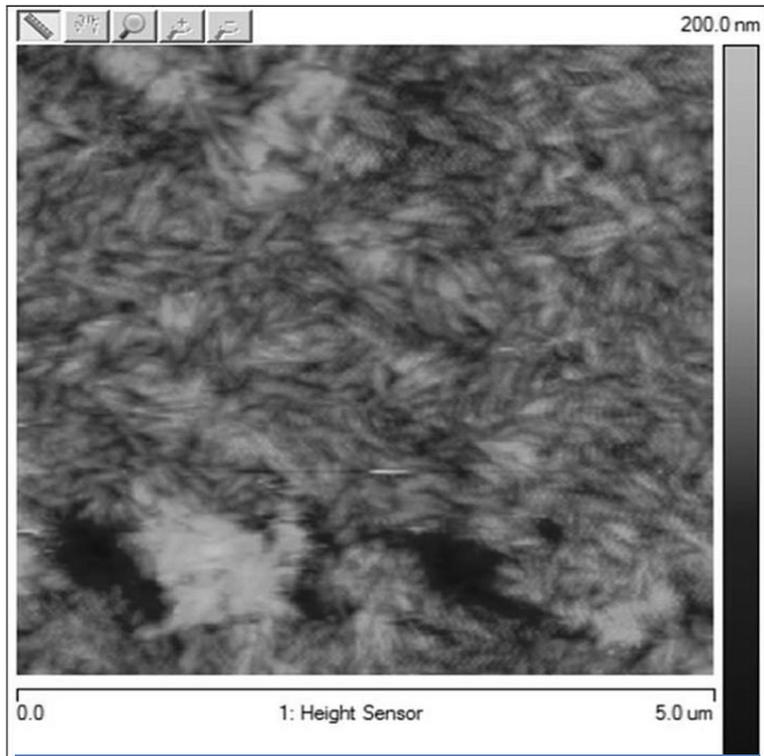
**with sorbed copper acetate**



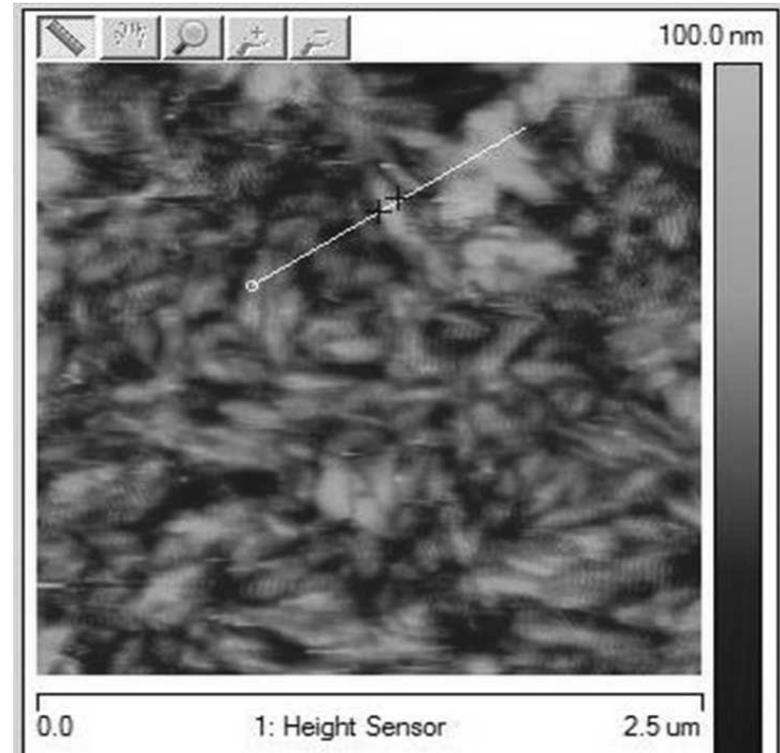
**with sorbed bromphenol blue**

*It was found that copper ions or bromphenol blue molecules, being added to NC fibrils aqueous dispersion for better visualization by TEM, were absorbed on their surface. This observation allowed us to suggest that some N-acetyl-D-glucosamine groups present on the surface of NC fibrils. Their origin is disputable. They could appear due to deacetylating some N-acetyl-D-glucosamine groups on the surface of chitin nanofibrils. They could also belong to chitosan chains, which were tightly captured to the surface of some chitin nanofibrils. Namely these glucosamine groups participated in sorption of copper ions through formation of coordination bonds between amine groups and transition metal ions.*

# AFM images of chitin nanofibrils



**(5 × 5  $\mu\text{m}$ ) Z-scale 200 nm**



**(2,5 × 2,5  $\mu\text{m}$ ) Z-scale 100 nm**

*The most valuable information about the real morphology of NC fibrils in the swollen state was obtained by the atomic-force microscopy (AFM), which was performed in the presence of water. An average diameter of NC fibrils was estimated to be equal to about 75-80 nm. The aspect ratio of swollen NC was about 8, i.e. the length of chitin nanofibrils was equal to about 500-600 nm in NC-supernatant.*



# Choice of the support for casting the CS/CN slurry

*For successful preparation of films by using the casting technique, compatibility of the slurry with a support is the necessary but not the only requirement. The possibility of taking off the dried films from the chosen support without any efforts has to be ensured without fail.*

*Hydrophobic supports from poly(ethylene), poly(propylene), poly(carbonate), poly(vinyl chloride-transparent) and the silanized paper with the values of contact angle higher than 90 °, were unsuitable because of incompatibility of the aqueous CS/NC slurry with these supports. Support based on poly(vinyl acetate), e. g. Acetate Clear (Grafix, USA) was compatible with the slurry but it was deformed due to penetration of acetic acid from the slurry inside this support during drying the film.*

*The best compatibility of CS/NC slurry was observed with poly(vinyl terephthalate), Dura Lar, DL) films (Graphics, USA). The contact angle of DL support was  $77\pm 1$  °. Another support based on poly(vinyl chloride) (PVC) can be also used in spite of its higher contact angle ( $90\pm 1$ °); probably higher heterogeneity of its surface ensures this possibility. In this study, the stainless steel (SS) plates were also used for casting the CS/NC plasticized slurries because this type of supports gave more opportunities than polymeric ones for both the variation of drying conditions and repeated use.*

# Commercial polymeric supports used in casting technique

PE	PP	PVC d-c-fix (Germany)	Dura Lar (Grafix, USA)	PVA Acetate Clear (Grafix, USA)	PMMA	Teflon	PC
Poly(ethylene)	Poly(propylene)	Poly(vinyl chloride)	Poly(vinyl terephthalate)	Poly(vinyl acetate)	Poly(methyl methacrylate)	Teflon	Poly(carbonate)

## Contact angle

$$\Theta = \gg 90$$

$$\Theta = \gg 90$$

$$\Theta_{\text{grey}} = 90 \pm 1$$

$$\Theta_{\text{transparent}} = 106 \pm 4$$

$$\Theta = 77 \pm 1$$

$$\Theta = 66 \pm 2$$

$$\Theta = 70 \pm 2$$

$$\Theta \gg 90$$

$$\Theta \gg 90$$

## Behavior of (CS/CN)/glycerol slurry on the supports:

**silanized paper**



**Acetate Clear**



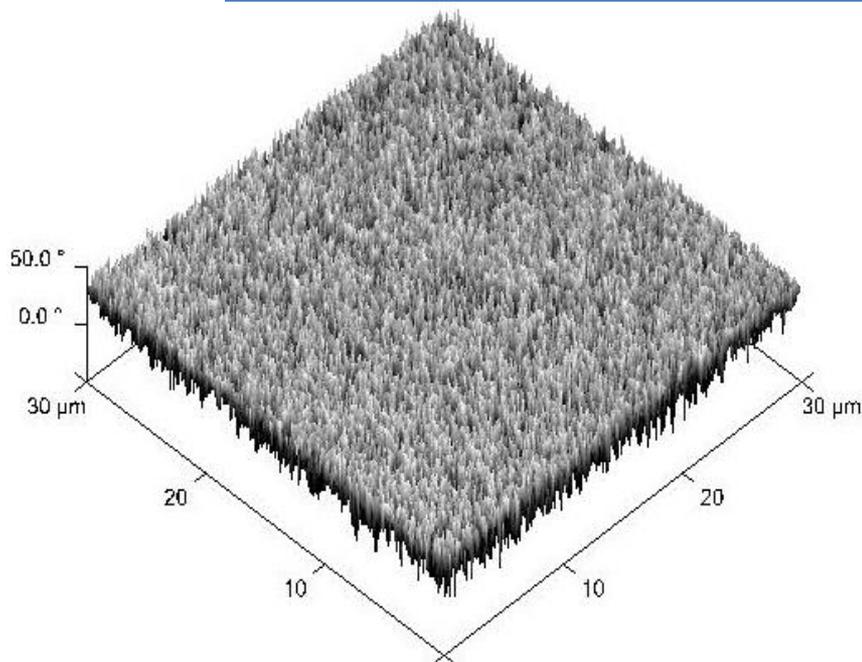


# Morphology of surfaces of CS/CN films

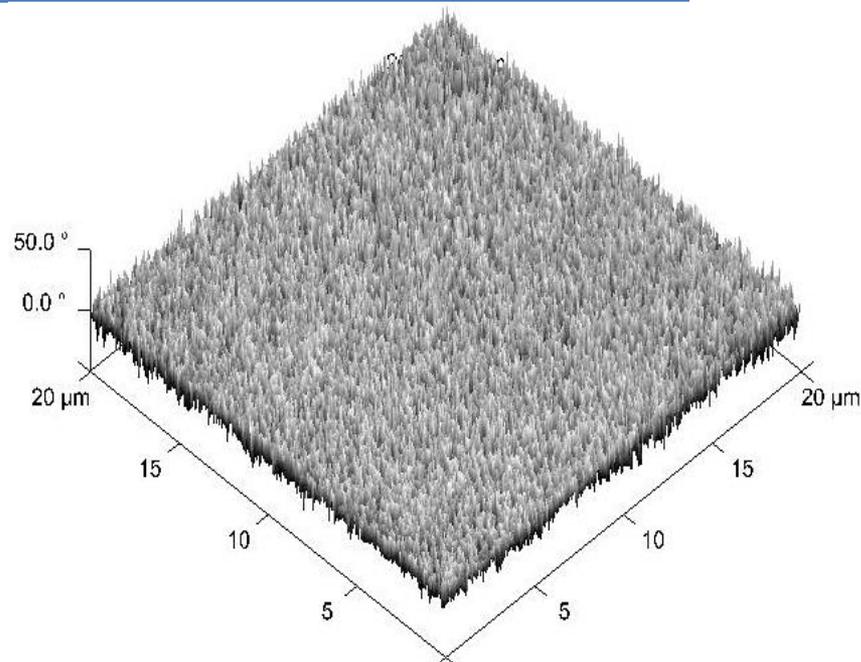
Workshop\_Prague, 17 October 2014

# AFM images of the film with CS/CN ratio (40/60 wt%)

Film was plasticized with Glycerol (50 wt%)



Surface faced to air

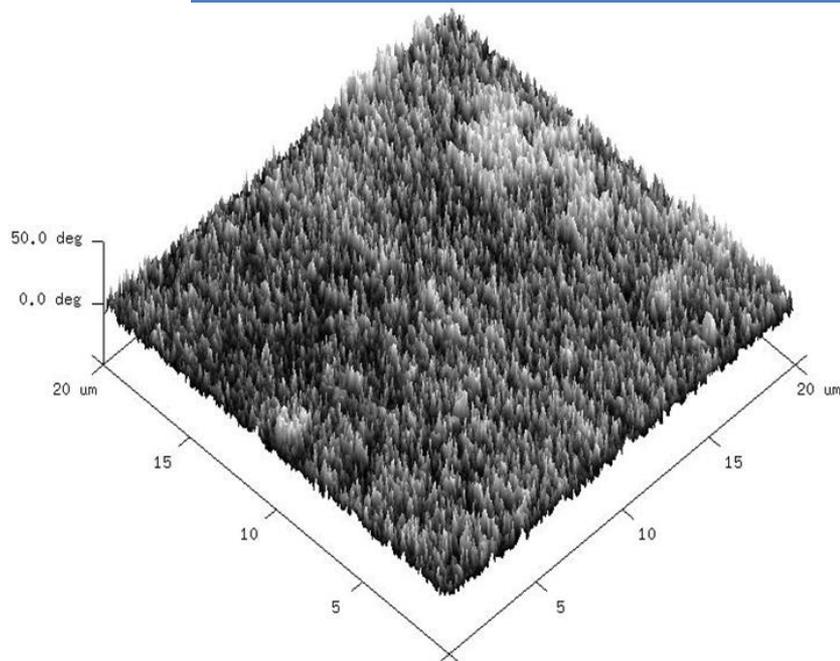


Surface faced to silanized glass support

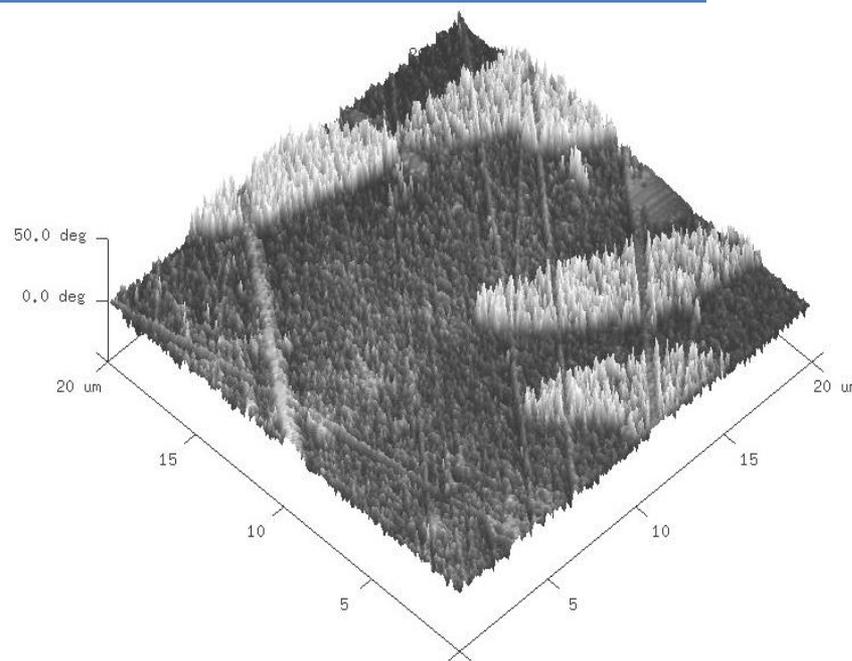
*AFM analysis of the dry CS and CS/CN films has shown that both the top and bottom surfaces had heterogeneous structure on the nano-level. The heterogeneity of the top (faced to air) and bottom (faced to the support) surfaces of CS/CN films can be seen.*

# AFM images of CHITOSAN film

Film was plasticized with Glycerol (30 wt%)



Surface faced to air

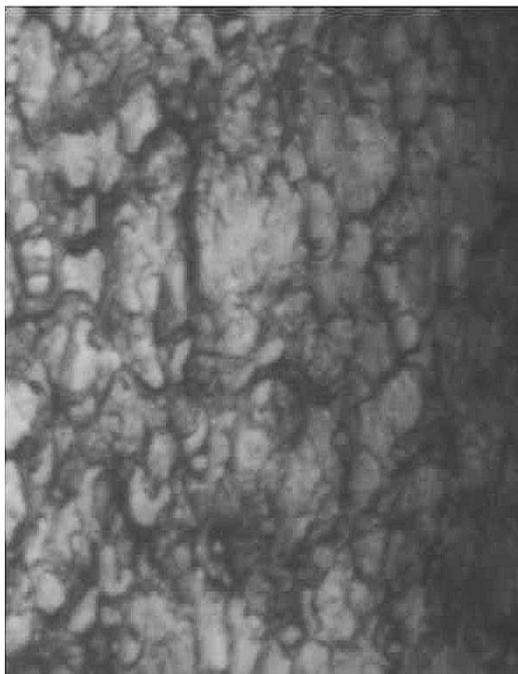


Surface faced to stainless steel support

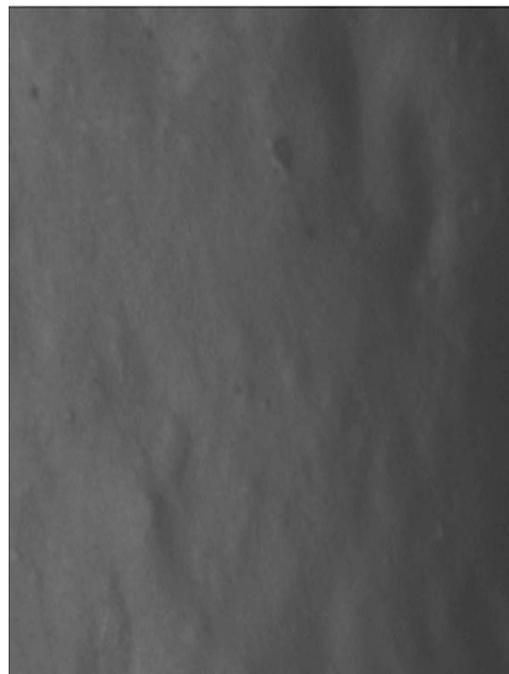
*The films were prepared by casting the slurry with various component proportions on different supports. Quality of CS/NC films depended on the chemical nature of a support, on which the slurry was cast.*

# Optical view of CS/CN film prepared on Stainless Steel support

Composition: [CS/CN]/Glycerol (75/25)/25 wt%



**Surface faced to SS support**



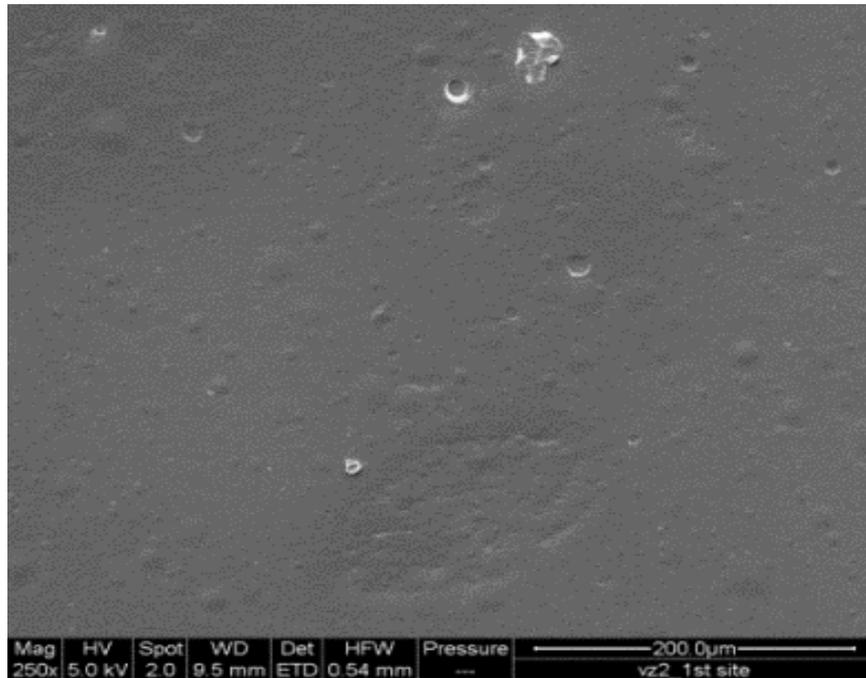
**Surface faced to air**

*Effect of the support type on heterogeneity of the bottom surface of both CS and CS/NC films was more pronounced if the slurry was cast on a stainless steel support.*

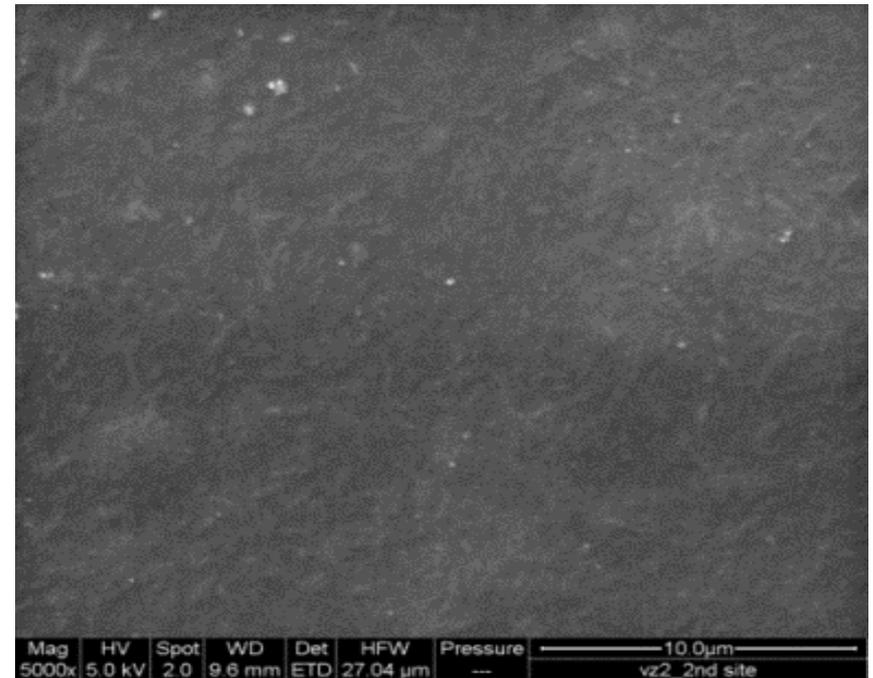


# SEM of CS (Italian)/CN films

Composition: (CS/CN)/Glycerol (75/25)/25 wt%



**Surface faced to SS support**



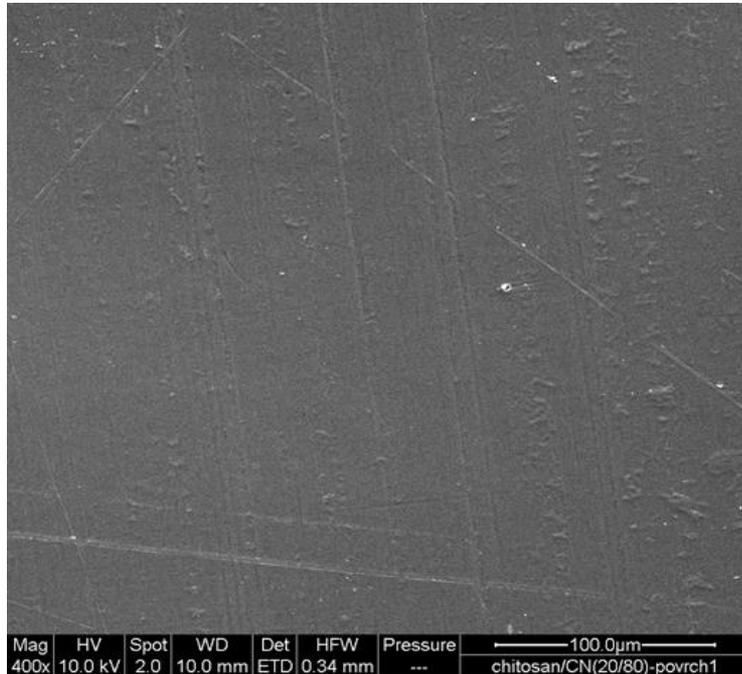
**Surface faced to air**

*The bottom surface was opaque whereas the top one was shiny when the film was prepared by casting the slurry on a stainless steel support.*

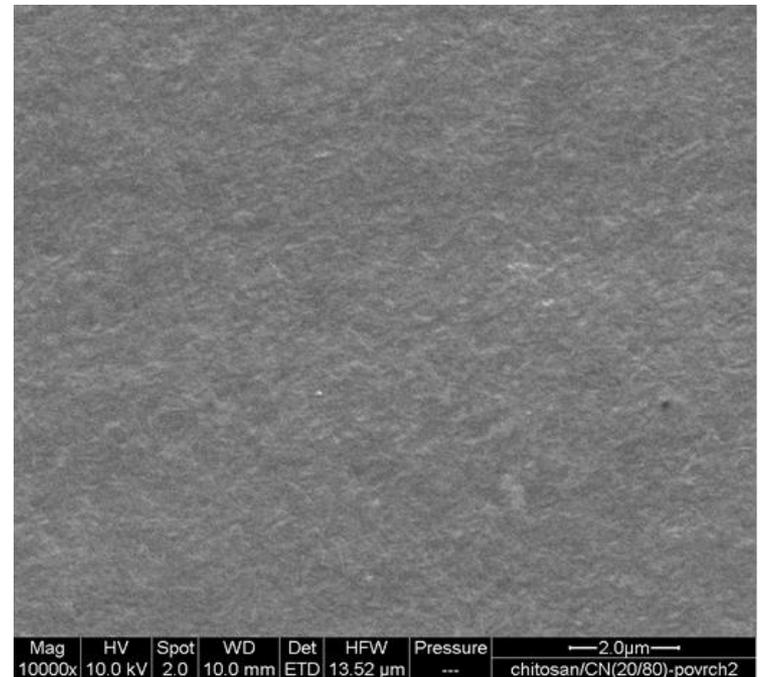


# SEM of CS (Norwegian) /CN films

Composition: [CS/CN]/Glycerol [20/80]/50 wt%



**Surface faced to Dura Lar support**



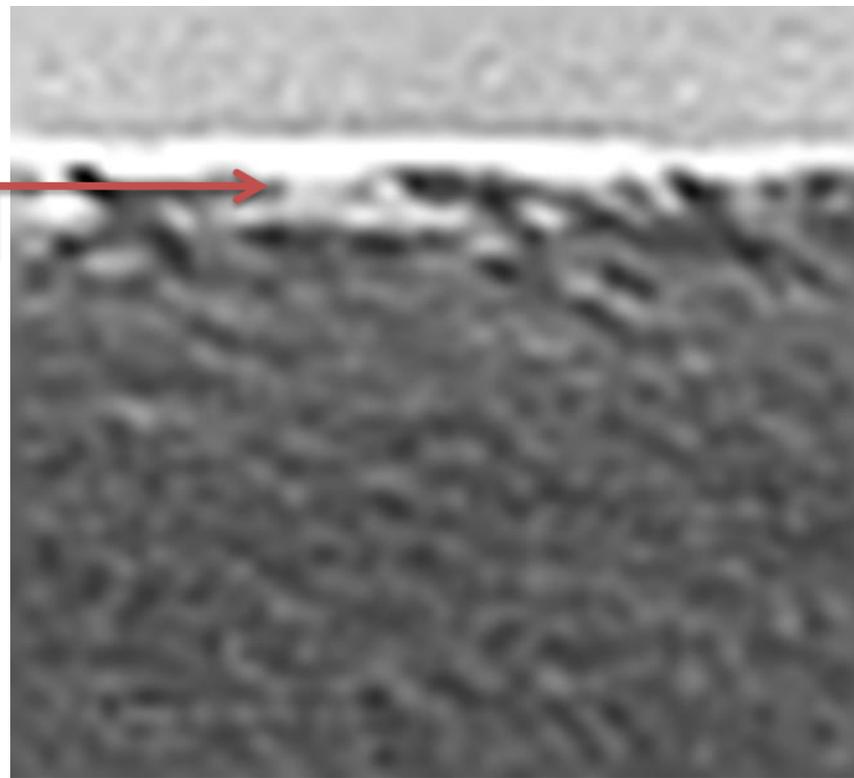
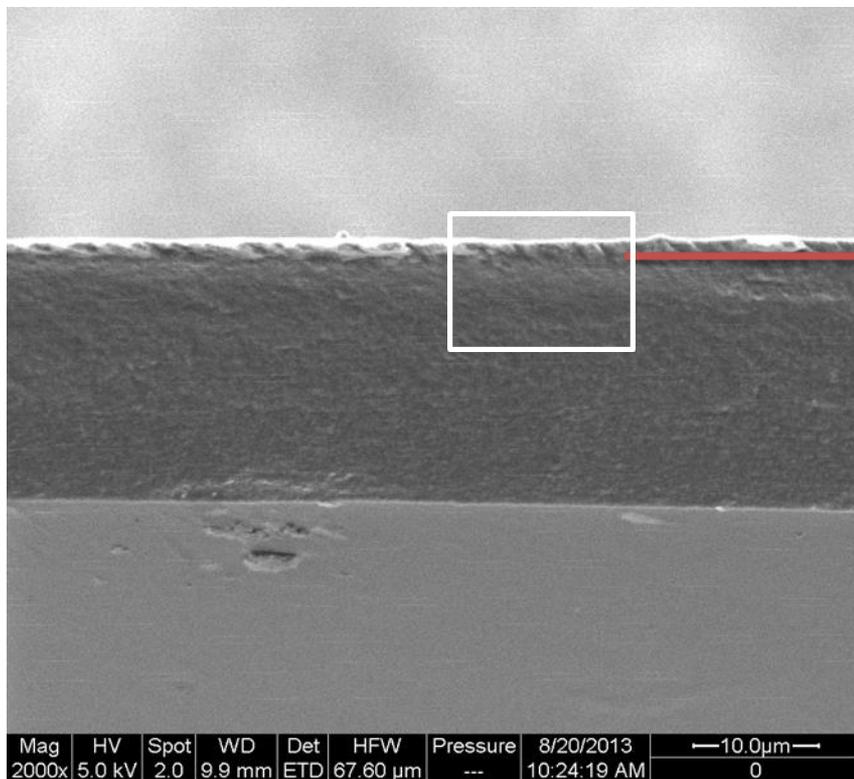
**Surface faced to air**

*The film's surfaces looked different even at low magnification.*



# SEM of fracture of CS/CN film

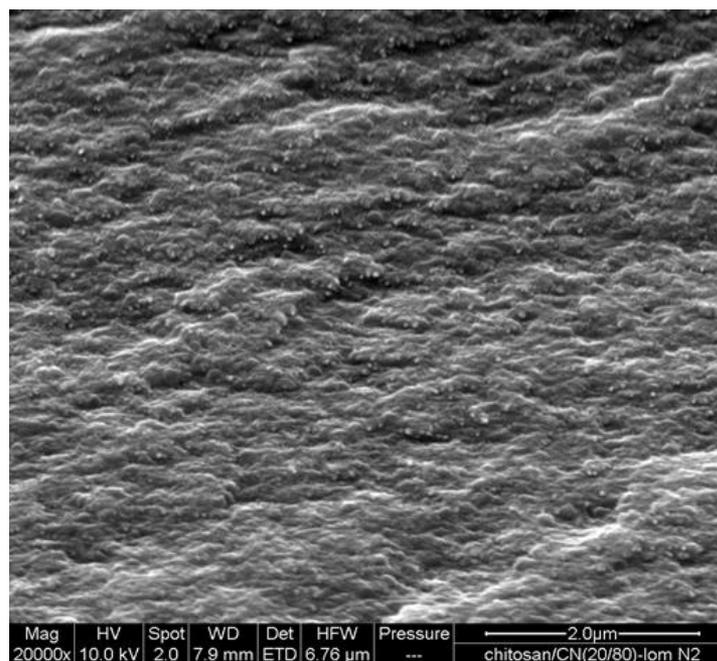
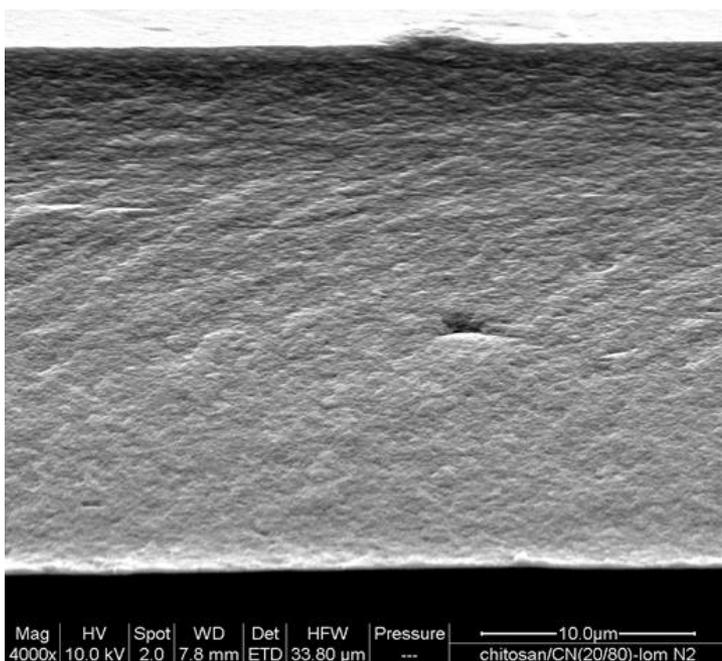
Composition: (CS/CN)/Glycerol (70/30)/30 wt%



*In SEM images of CS/NC films prepared not only on the stainless steel support but also on polymeric ones (PVC or Dura Lar), a thin layer on the top surface of films was observed. The morphology of this layer seemed to be different than that of the inner part of films. This layer was observed if the NC content was high (about 25-30 wt%).*

# SEM of fracture of CS (Norwegian)/CN film

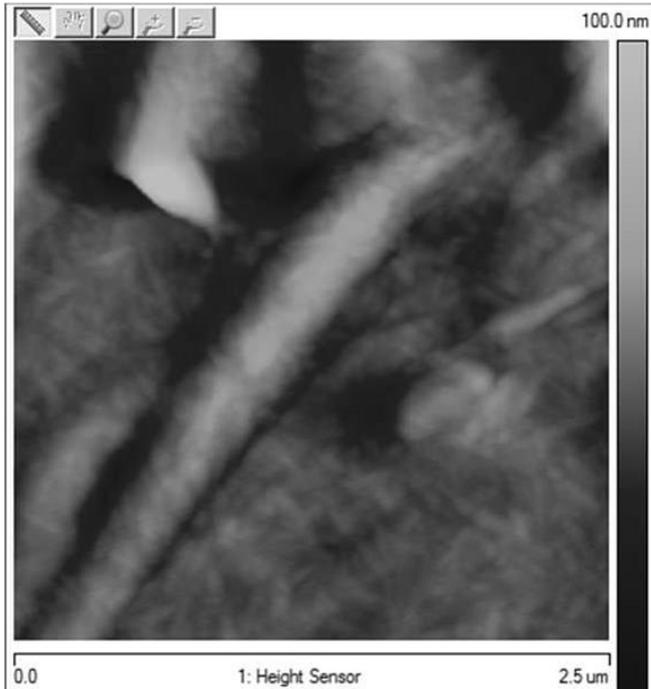
Composition: (CS/CN)/Glycerol (20/80)/50 wt%



*The distribution of NC fibrils inside the CS phase was rather uniform due to high compatibility of both components.*

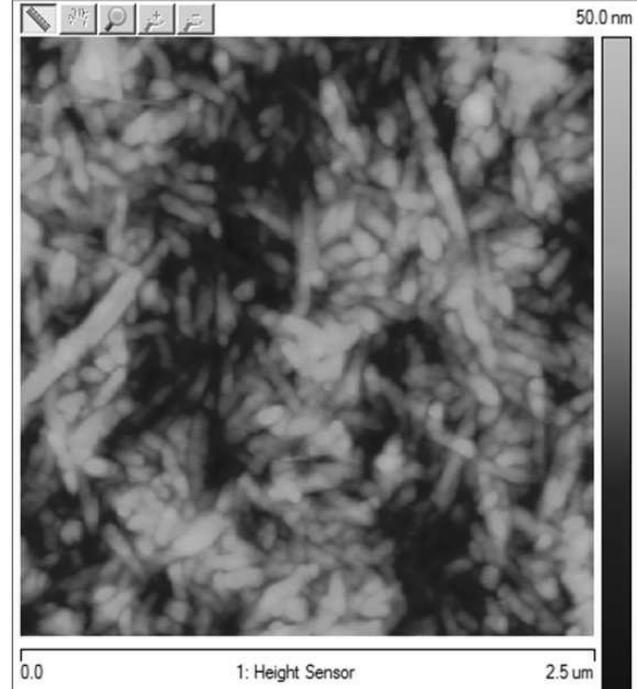
# AFM of CS/CN film in water

## Surface faced to SS support



$(10 \times 10 \mu\text{m})$  Z-scale 100 nm

## Surface faced to air



$(2,5 \times 2,5 \mu\text{m})$  Z-scale 50 nm

AFM analysis of this film in the swollen state has shown that the bottom layer looked dense but spotted with numerous cracks, which can be seen under lower magnification as well. Very likely, rather high adhesion of CS/NC slurry to the heterogeneous surface of the stainless steel support was the reason of appearing these defects because some efforts were required for taking off the dried film from the support. In AFM image, the shine top surface of the CS/NC film looked rather loosely-packed at high magnification. It was enriched with chitin nanofibrils stacked together with chitosan chains. Some part of chitin nanofibrils were pushed out from CS phase on the top surface of the film.

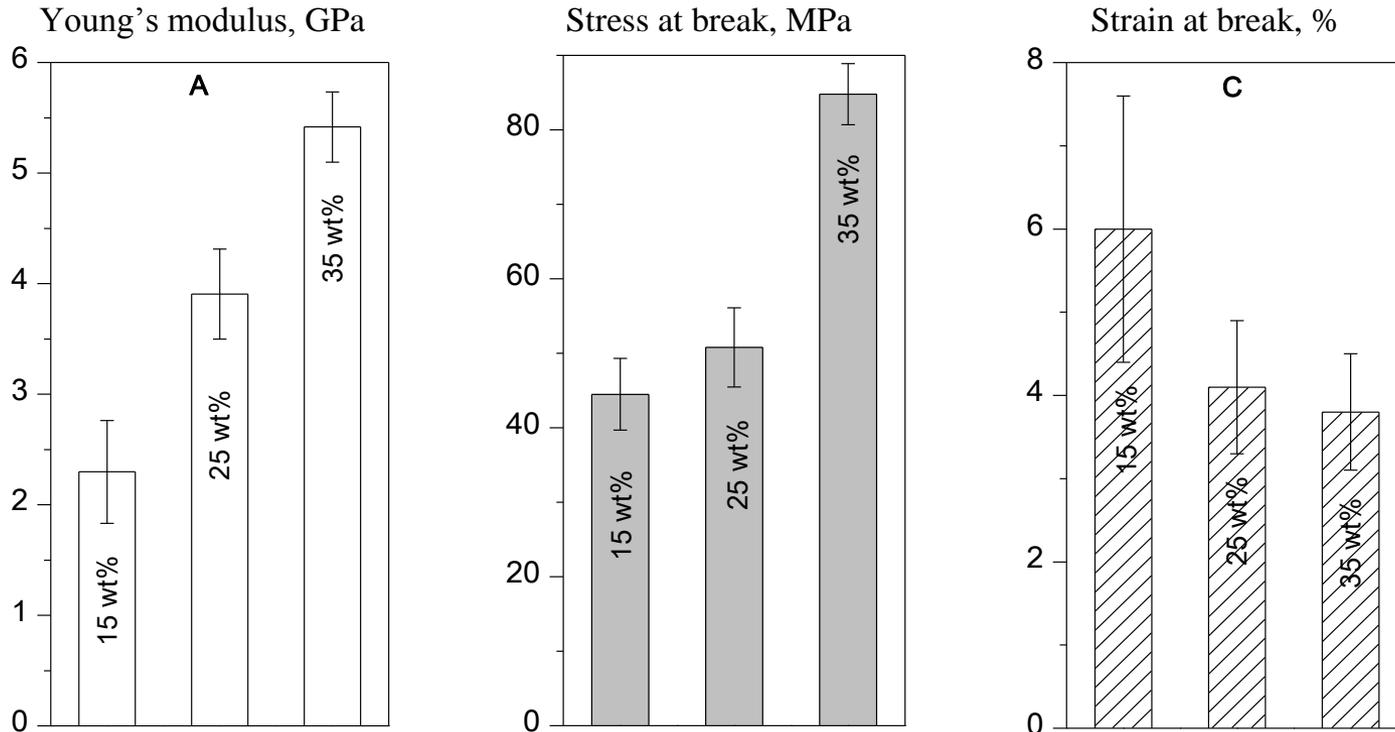


# Effect of component proportion on mechanical properties of CS/CN films

*Crustacean's exoskeletons are one of numerous nanocomposites designed by Nature, in which the components form rather mechanically stable product owing to multiple physical bonds controlling the perfect spatial adjustment of all components to each other. They consist of polysaccharide (chitin), proteins and mineral salts. Being the waste of fish industry, they are accumulated daily in huge quantities. The aim of our study consisted in preparing the completely biodegradable films by reinforcing the deacetylated chitin (chitosan, CS) with nanofibrils of chitin (nano-chitin, NC) insoluble in acidified water in contrast to CS. These components are both the copolymers of D-glucosamine and N-acetyl-D-glucosamine connected together through  $\beta$ -(1 $\rightarrow$ 4) glycosidic bonds in linear chains. From chemical point of view, CS and NC are the same („single“) polymer, the polysaccharide chains of which differ in content of acetylated glucosamine rings. Acetylation degree was low for CS (21%) and high for NC (97%), giving to the former the excellent film-forming properties and to the latter the ability to reinforce CS phase owing to high energy of chitin nanofibrils having highly developed surface at the solid-liquid interface. Similarly to the origin nanocomposites, in which low- and high-molecular-weight (HMW) additives contribute to mechanical properties of crustaceans' exoskeletons, some both LMW (plasticizers such as glycerol, polyglycerol-3, calcium, magnesium or barium ions, potassium phosphate, sodium salt of monolignols or ethylenediaminetetraacetic acid) and HMW substances (nano-lignin, starch or gelatin) were added in the slurries to enhance the hydrophobicity, decrease swelling in water and ensure the optimum balance between rigidity and elasticity of the composite CS/NC films.*

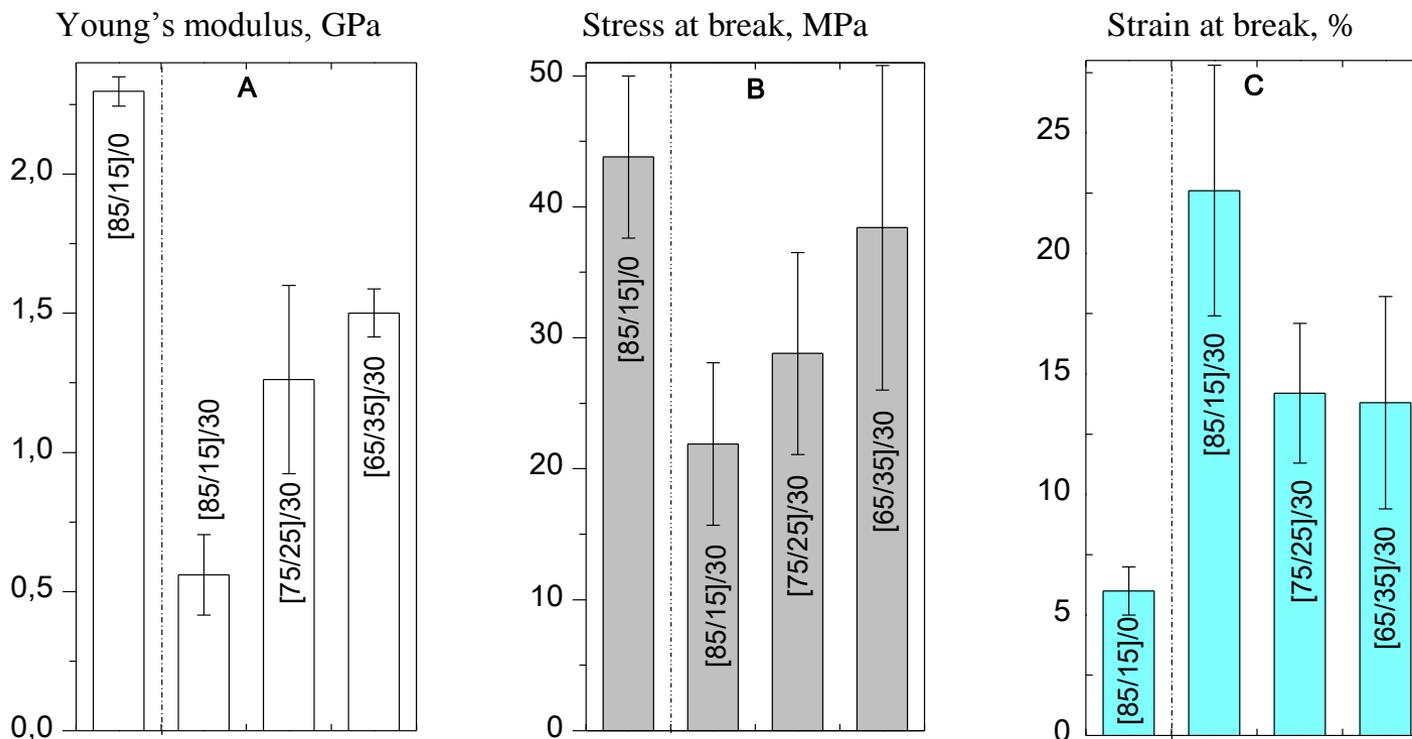


# Dependence of mechanical properties of nonplasticized CS/CN films on CN content



*Nano-chitin themselves exhibited excellent reinforcing effect on CS phase. The ability of nonplasticized CS/CN film to withstand against the applied tensile forces increased twice with increasing NC content from 15 wt% to 35 wt%. The stretching of the film with 35%-NC content decreased about 60%.*

# Dependence of mechanical properties of CS/CN films plasticized with polyglycerol-3 on CN content



*The elasticity of films prepared from CS and NC only is not sufficient for their using as packaging films. The films were too brittle. For increasing their elasticity, glycerol and polyglycerol-2, -3, or -4 (Solvay, Belgium), which were permitted for application in food industry, were used in our study.*



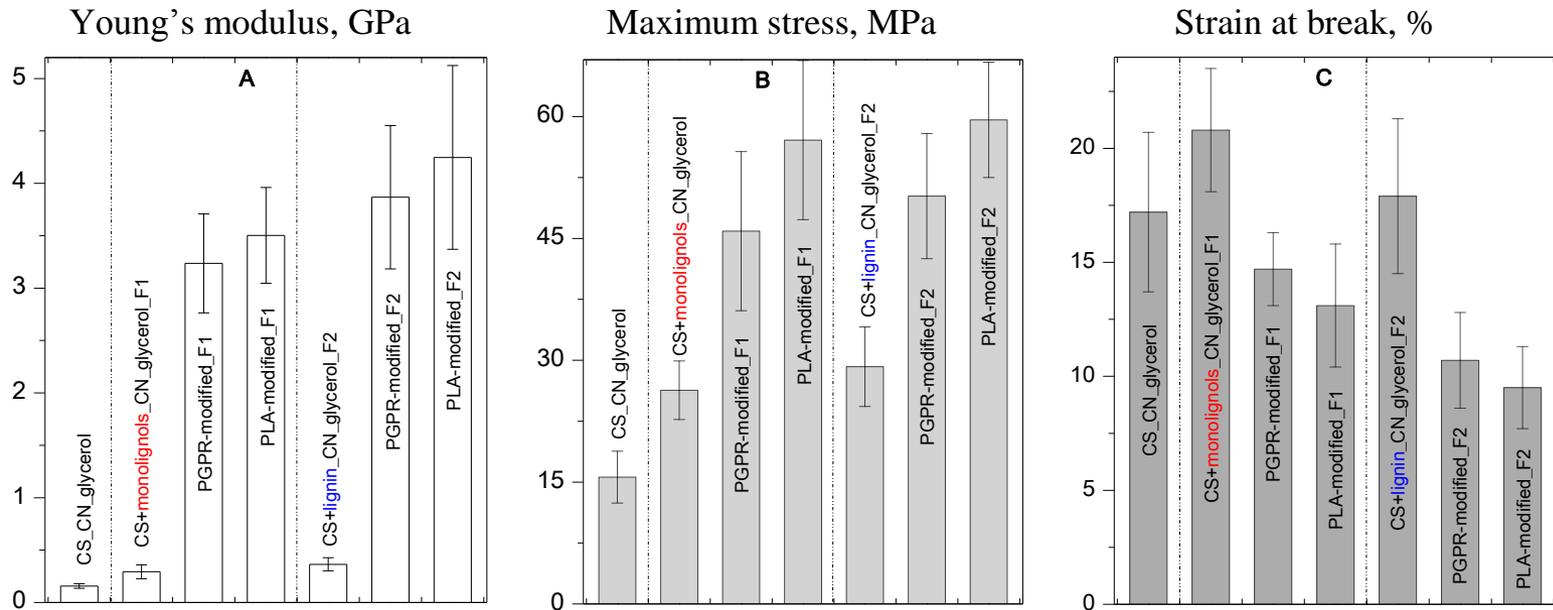
# Changes of the tensile stress and strain of CS/CN films in dependence on CN and pG-3 content

pG-3, wt% CN, wt%	0	30	Decrease	0	30	Increase
	Tensile stress, MPa			Tensile strain, %		
15	43.8	21.9	2.0	6.0	22.6	3.8
25	50.0	28.8	1.7	4.1	14.2	3.5
35	84.3	38.4	2.2	3.8	13.8	3.6

*If the films with the same CS/NC proportions were plasticized with polyglycerol-3 (30 wt% of CS and NC content), their rigidity decreased approximately twice in comparison with nonplasticized films, whereas their elongation increased about four times.*

# Dependence of mechanical properties of CS/CN films on monolignols and nano-lignin content

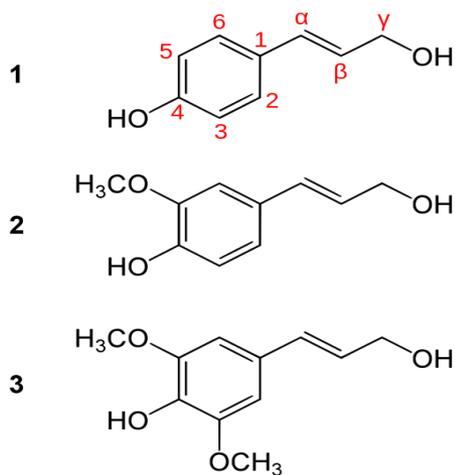
Content of: monolignols = 5 wt%, nano-lignin = 10 wt%



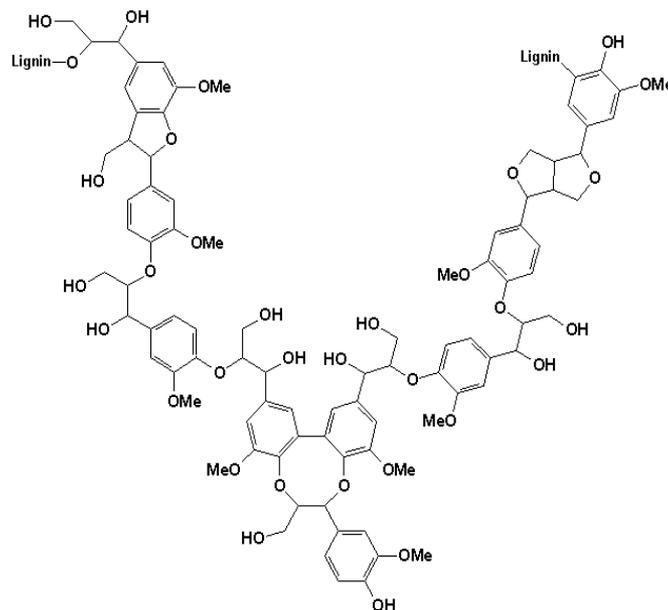
Composition: (CS/CN)/Glycerol = (70/30)/30 wt%

Both monolignols and nano-lignin behaved as reinforcing agents of chitosan matrix and that the former was less effective than the latter one.

# Structure of monolignols and lignin



1. p-coumaryl alcohol
2. coniferil alcohol
3. sinapyl alcohol

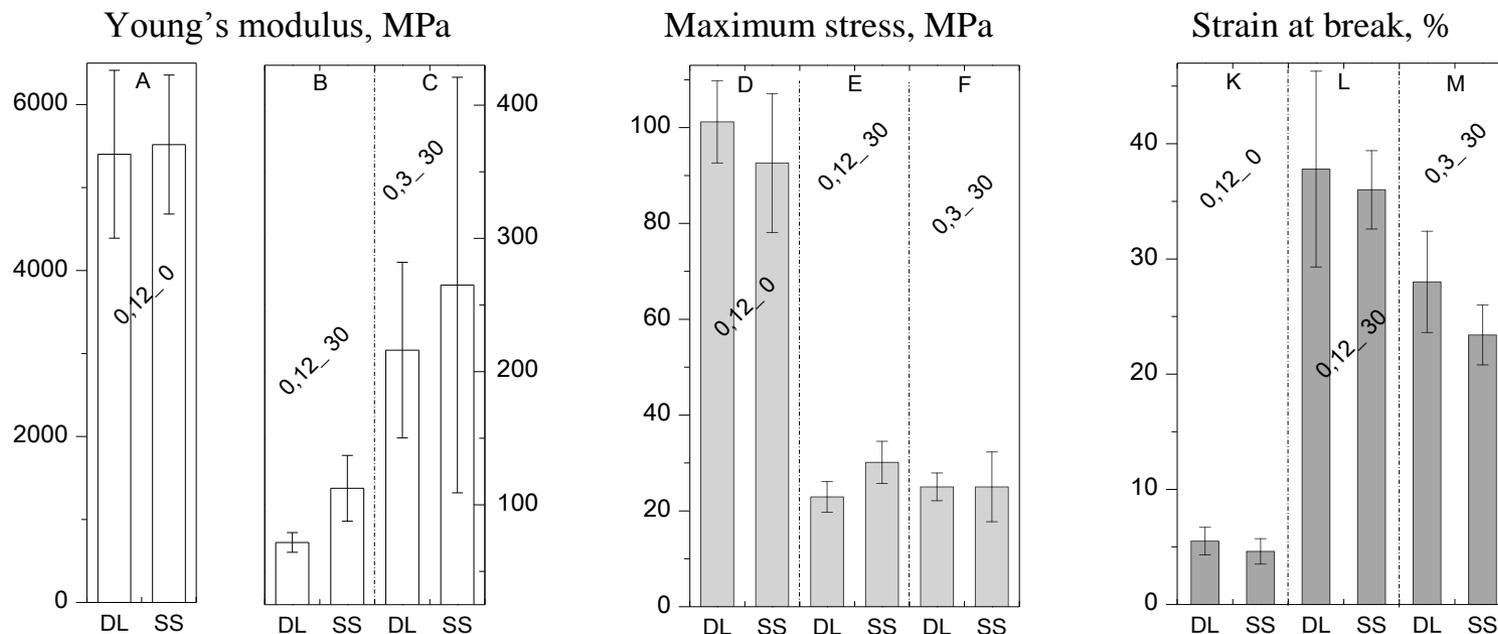


A piece of lignin

*It was suggested that lignin being chemically linked to carbohydrates and possibly proteins in the cell wall of wood participates in formation of large macromolecules and controls their water permeability.*

# Dependence of mechanical properties of CS/CN films on $\text{Ca}(\text{OH})_2$ and glycerol content

Content of  $\text{Ca}(\text{OH})_2$ , mM: 0.12 (A, B, D, E, K, L); 0.3 (C, F, M)



CS/CN = 70/30 wt%. Content of tri-glycerol, wt%: 0 (A, D, K); 30 (B, C, E, F, L, M).

In CS/CN films plasticized with polyglycerol-3 their rigidity increased negligibly at increase in content of calcium ions from 0.12 to 0.3 mmol but the elasticity decreased confirming the reinforcing effect of Ca ions.

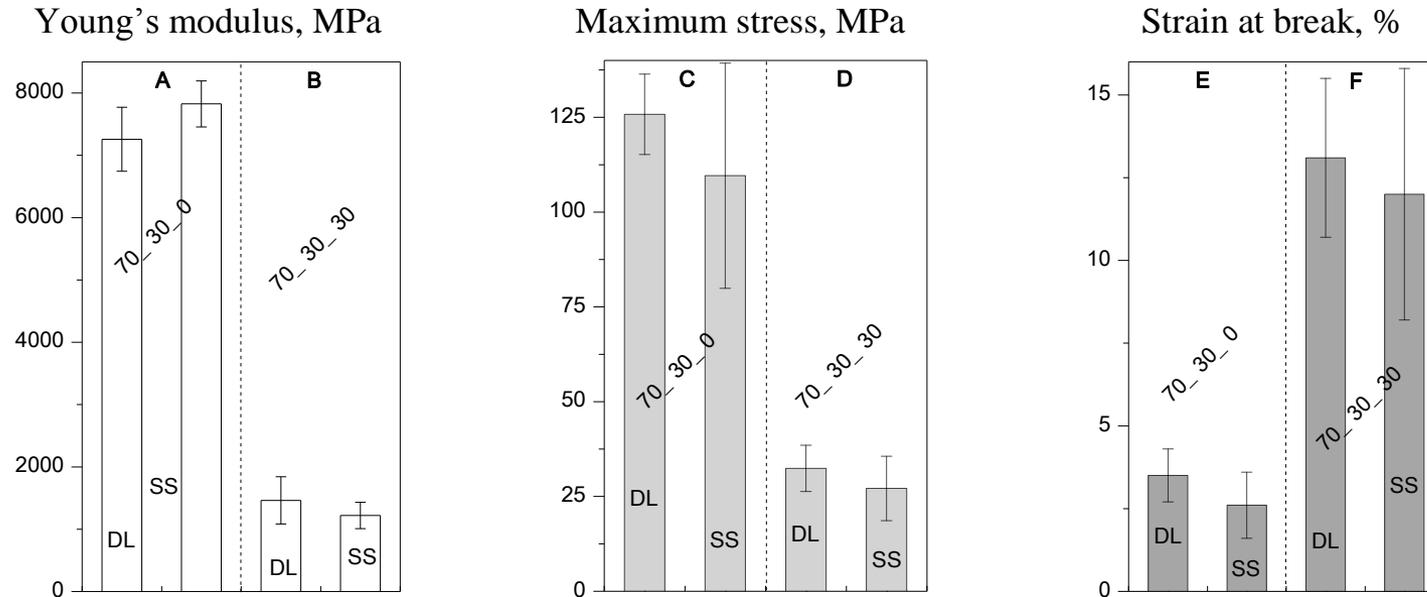
# Sorption of water vapors within CS/CN films

	(CS/CN)/plasticizer wt%	Plasticizer, wt%	Moisture content, %	Additive or modifier	Support	Water vapors sorption capacity [%] at 25 bars
1	[70/30]/25	PEG-600	7.6		DL	25.0
6	[70/30]/25	Glycerol	42.5	Ca (0.3 mmol)	— " —	37.0
7	[70/30]/25	— " —	43.9	Ca (0.3 mmol)	SS	37.0
2	[70/25]/30	— " —	5.3		— " —	25.0
3	[65/35]/30	pG-3	5.5		— " —	33.0
4	[75/25]/30	— " —	6.8		— " —	34.0
5	[85/15]/30	— " —	7.4		— " —	20.0
8	[70/30]/10	Glycerol	8.3		— " —	22.0
9	[70/30]/10 Glycerol	— " —	6.0		SS	24.0
11	[70/30]/30 Glycerol	— " —	7.2	PGPR	DL	21.0
12	[70/30]/30 Glycerol	— " —	6.8	PGPR	SS	25.0
13	[70/30]/30 Glycerol	— " —	8.4	PLA	DL	22.0
14	[70/30]/30 Glycerol	— " —	10.3	PLA	SS	22.0
	KFC					13.0

The CS/NC films containing calcium ions (0.3 mmol/g CS) had twice higher moisture content in the dry state .

# Dependence of mechanical properties of CS/CN films on the support type

DL: poly(vinylterephthalate); SS: stainless steel support



Content of glycerol, wt%: 0 (A, C, E); 30 (B, D, F).

The strength and stretching of the films with CS/CN proportion (70/30 wt%) was always somewhat lower if they were prepared by casting on the stainless steel support.



# Metal ions in CS/CN films prepared on stainless steel or Dura Lar supports

Metal	Content of metal ions in films, mg/kg										
Support	Al	Fe	Cr	Cu	Pb	Zn	Ni	Co	V	Cd	Mn
DL	1.5	16.0	1.7	2.6	3.3	4.2	<1	<1	<1	<0,5	<1
SS	9.9	16.8	2.0	6.3	1.8	5.1	<1	<1	<1	<0,5	<1

Comparison of the content of metal ions in CS/NC films prepared by casting the slurry on Dura Lar and stainless steel supports has shown that the latter contained about 7 and 2.5 times higher amount of Al and Cu ions, respectively.

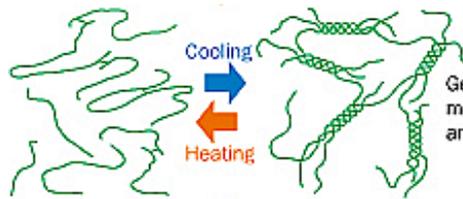


# Contact angle of CS/CN films prepared on the stainless steel and Dura Lar supports

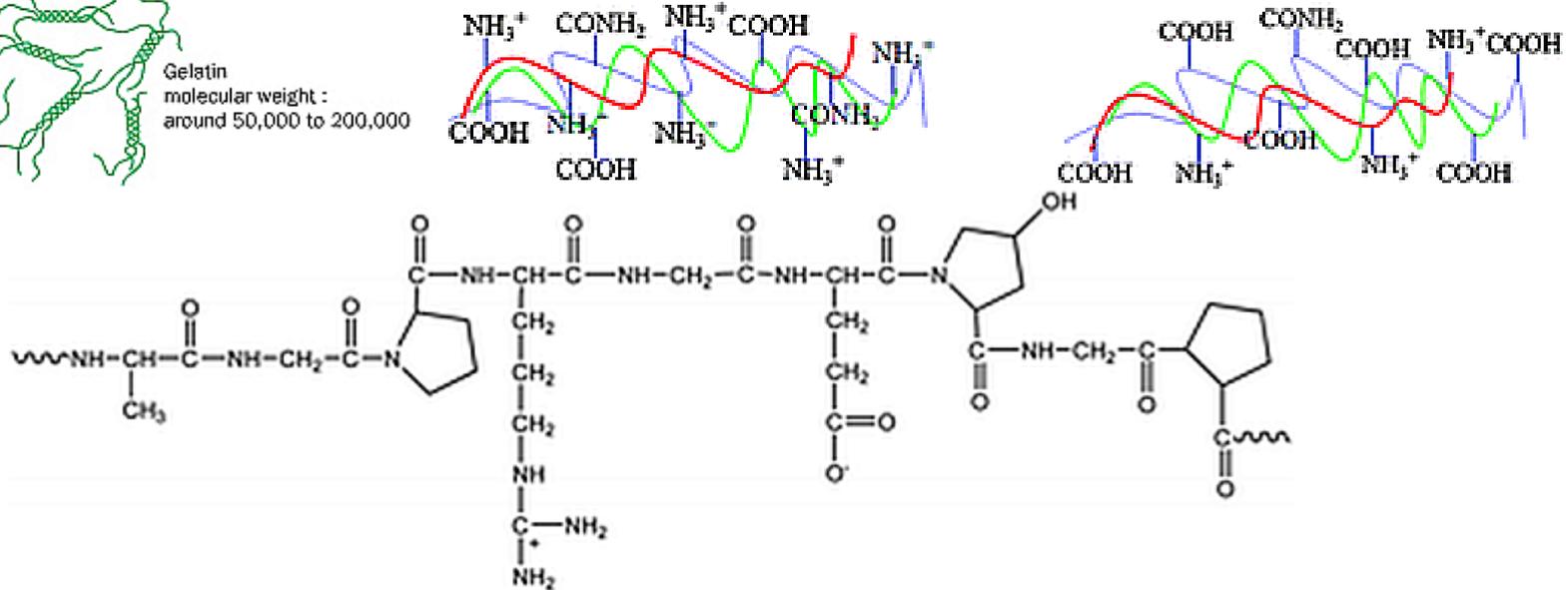
Support \ Film surface	Glycerol, wt%		Polyglycerol-3, wt%	
	Top	Bottom	Top	Bottom
<b>Glycerol, wt%</b>	0		30	
<b>DL</b>	<b>106,9±4,5</b>	<b>107,4±3,4</b>	<b>105,2±5,1</b>	<b>107,0±3,8</b>
<b>SS</b>	<b>109,2±3,9</b>	<b>119,5±2,8</b>	<b>106,5±3,5</b>	<b>115,4±2,6</b>
<b>Polyglycerol-3, wt%</b>	0		30	
<b>DL</b>	<b>102,2±4,4</b>	<b>107,1±3,8</b>	<b>103,2±4,1</b>	<b>106,5±3,9</b>
<b>SS</b>	<b>105,6±2,7</b>	<b>125,2±4,8</b>	<b>104,7±1,8</b>	<b>109,7±0,6</b>

For both nonplasticized and plasticized films prepared on Dura Lar support, the difference between contact angles of the top and bottom surfaces was negligible independently on the plasticizer used. In contrast, the contact angles of the bottom surface of nonplasticized films prepared on the stainless steel supports were about 10-20 degrees higher than that of the top surface.

# Structure of Gelatin

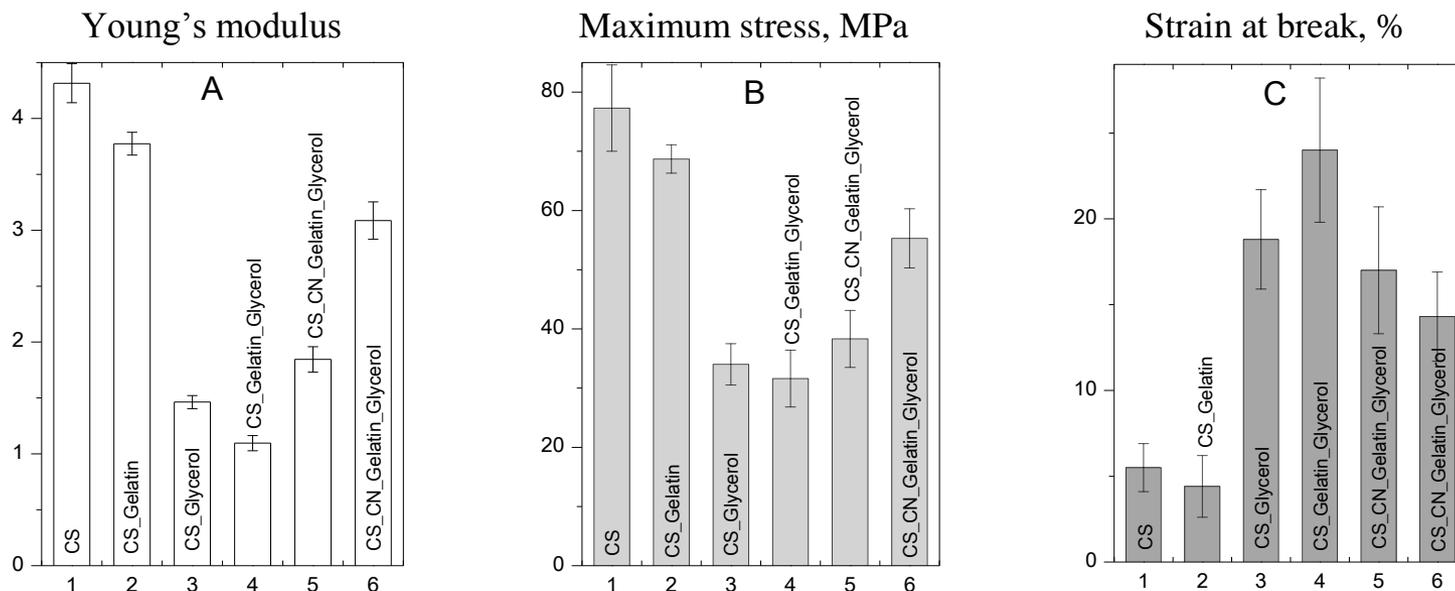


Gelatin  
molecular weight :  
around 50,000 to 200,000



*In crustaceans' exoskeletons, chitin chains forms molecular complexes with proteins and mineral salts giving to the natural composite the required mechanical stability and elasticity. In our study, gelatin (the hydrolyzed collagen) was incorporated in CS/NC films with the aim of improving their properties. Gelatin being the food protein is widely applied in food industry, pharmacy and medicine. The ionized polypeptide chains of gelatin bearing positively and negatively charged residues of amino acids will interact with chitosan chains and influence on their spatial arrangement in CS/CN films. This could affect the rigidity and elasticity of the composite CS/CN films.*

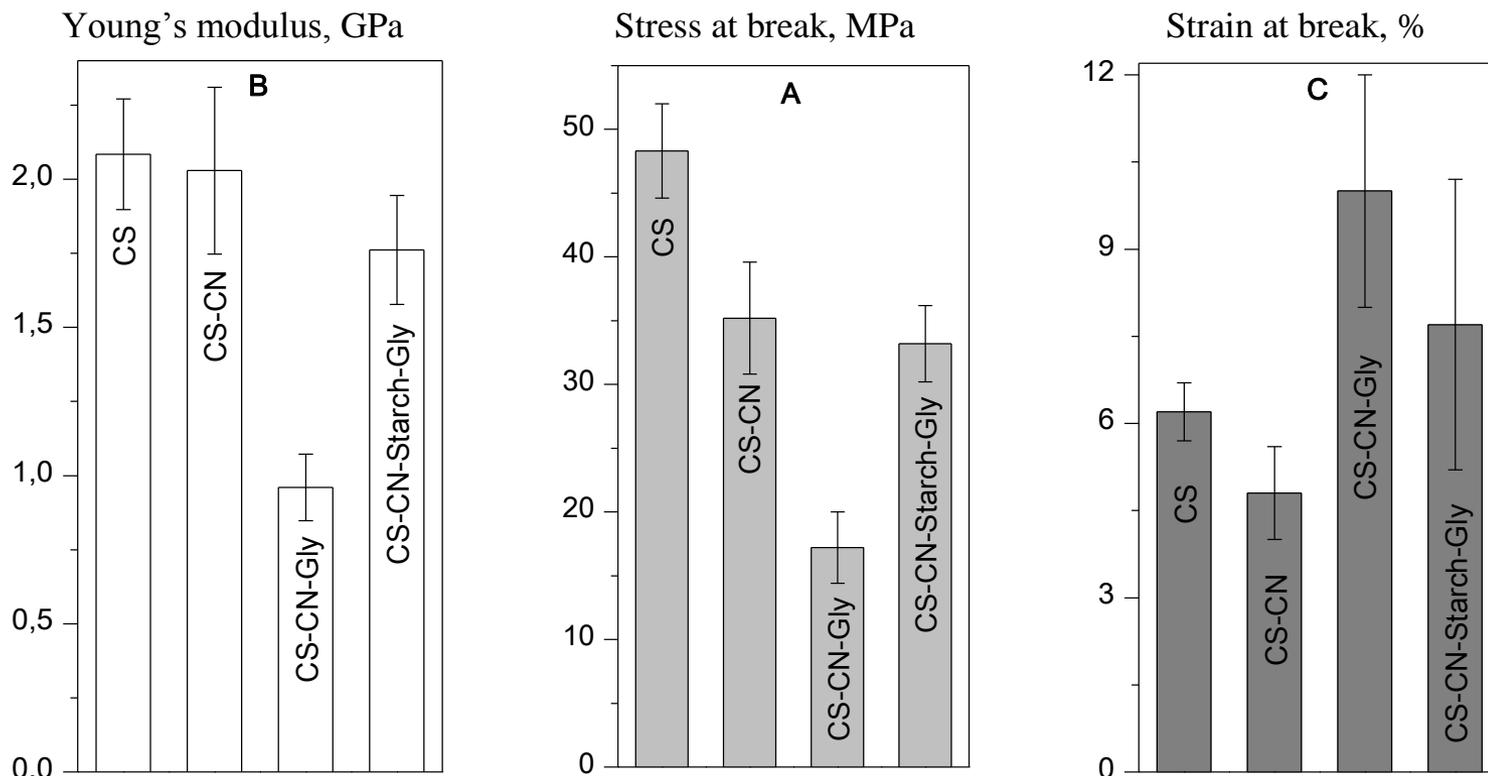
# Mechanical properties of CS/CN films in dependence on Gelatin and glycerol content



**CS\_Gelatin, wt%: 87,5\_12,5**  
**CS\_Glycerol, wt%: 70\_30**  
**[CS\_Gelatin]\_Glycerol, wt%: [87,5\_12,5]\_30**  
**[CS/CN\_Gelatin\_Glycerol, wt%: [75/25]\_12,5\_30**  
**[CS/CN\_Gelatin\_Glycerol, wt%: [85/15]\_12,5\_20**

Complex interactions between chitosan chains, chitin nanofibrils, glycerol and gelatin were responsible for changes in mechanical properties of the composite films containing this protein component

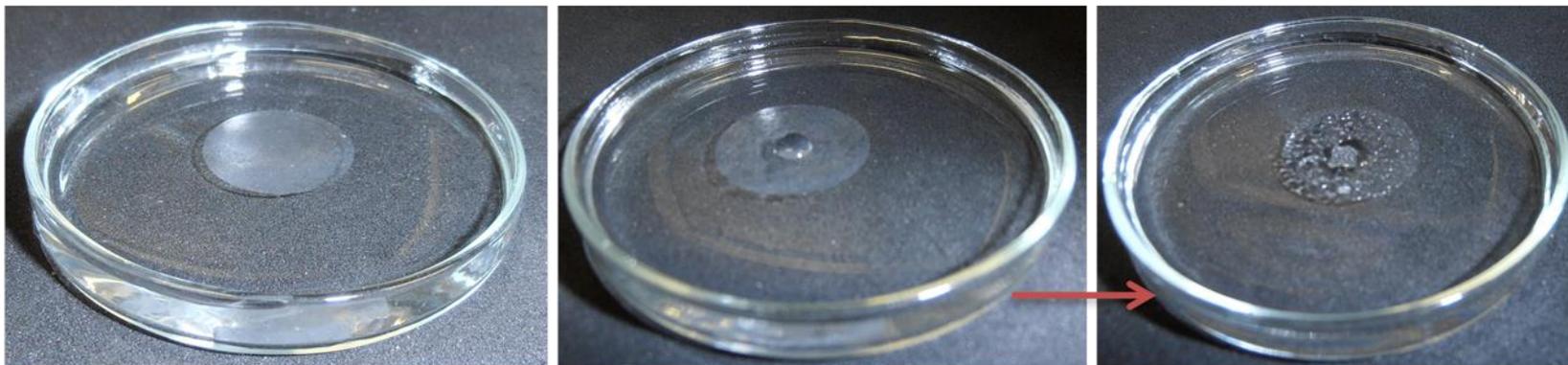
# Mechanical properties of CS/CN films in dependence on Starch and glycerol content



If the films with CS/CN proportion (70/30 wt%) plastisized with glycerol (25 wt%) contained also starch (25 wt% of CS content), the mechanical strength of this composite film increased and it became less elastic.

# Behavior of CS/CN films in contact with water

(CS/CN)/glycerol film = (75/25)/30 wt%



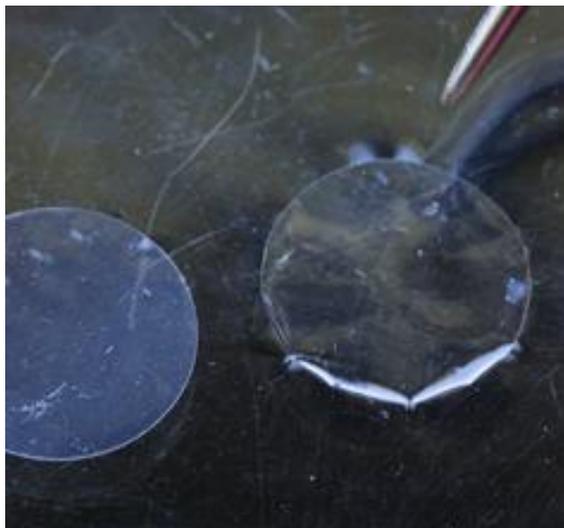
A film on the surface of water

A water drop on the film surface

Next day

*The idea of surface modification of plasticized CS/CN films has arisen after analysis of both the morphology of the films' surfaces of their behavior in contact with water.*

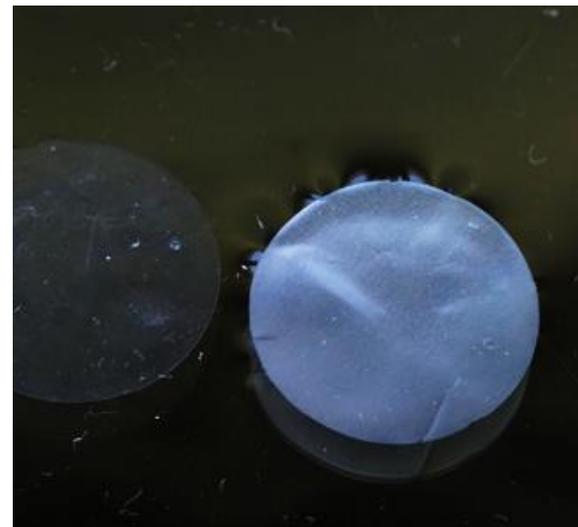
# Dependence of swelling in water of CS/CN films on the support type



**Dura Lar**



**PVC**



**Stainless steel**

The type of the support, which was used for casting the slurry, influenced considerably on hydrophobicity of the films.

# Swelling in water of PLA-modified CS/CN films in time

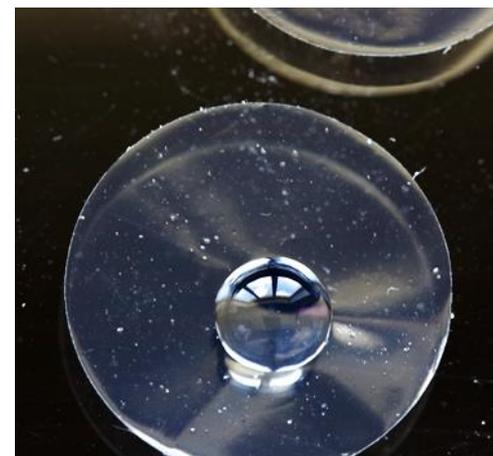
PLA-modified on the water surface during:



5 minutes



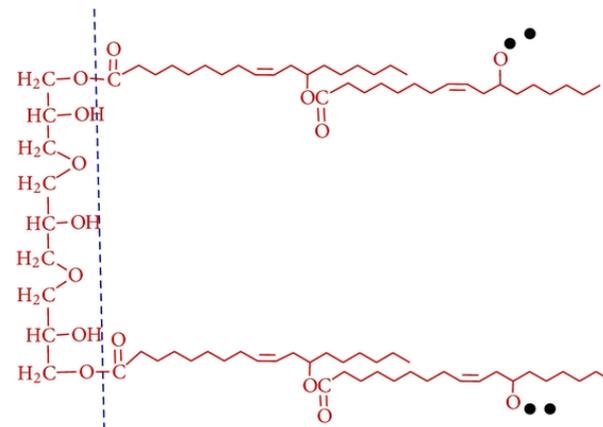
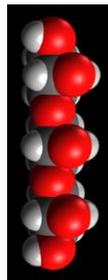
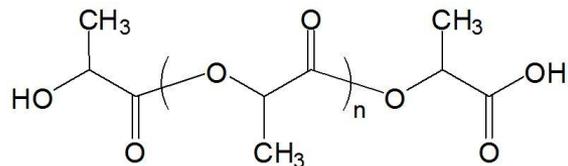
10 days



a drop of water

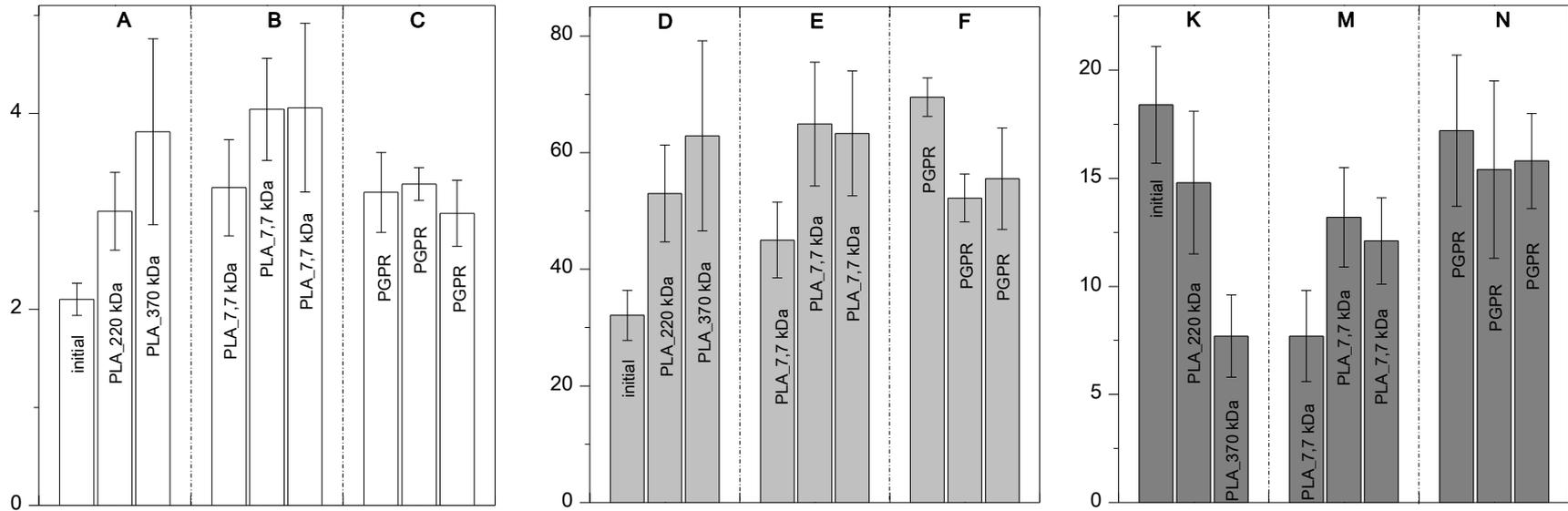
The hydrophobicity and mechanical properties of (CS/CN)/glycerol films have improved by using PLA with higher molecular weight, which can be still dissolved in 1,4-dioxane.

# Surface modifiers of CS/CN films: PLA or PGPR



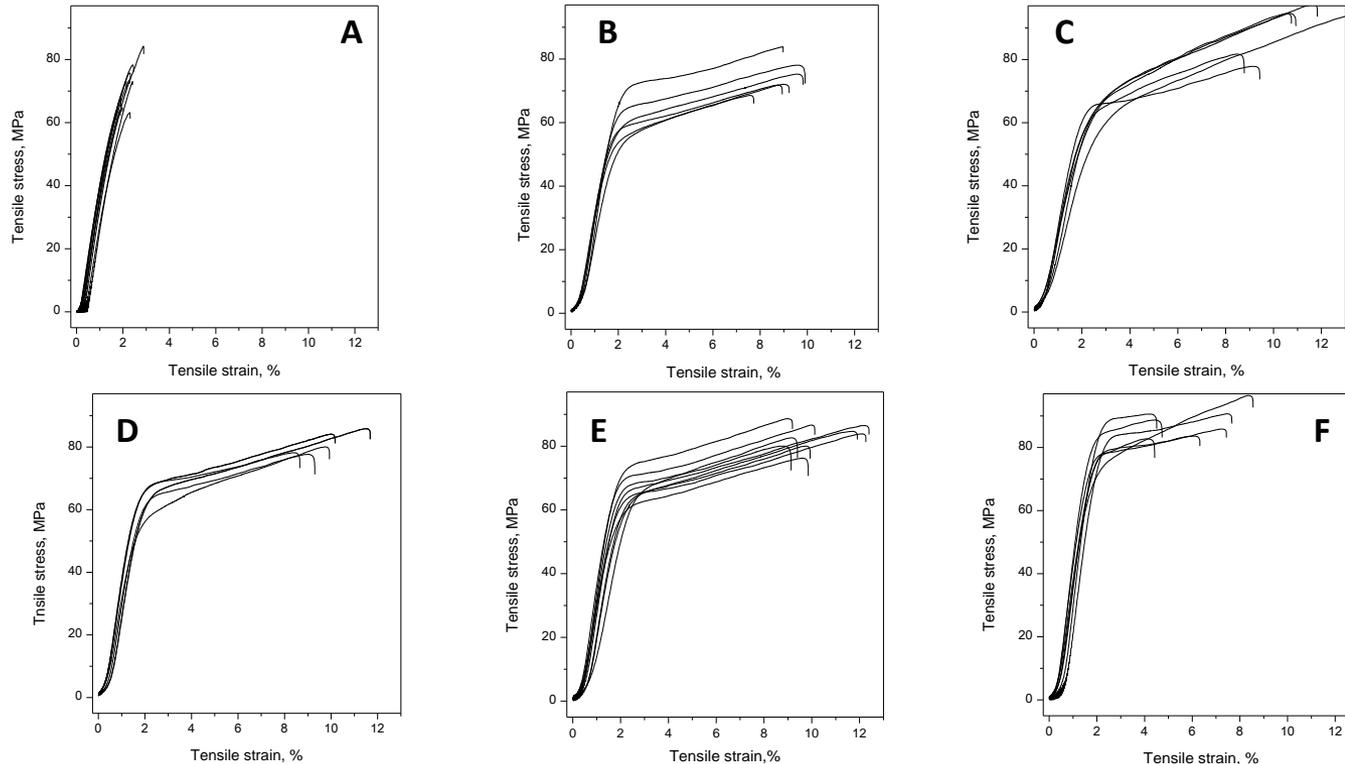
*Since the CS/NC films are intended for food packaging, the choice of a suitable modifier with the aim of enhancing the film's surface hydrophobicity was limited only by those, which have been already permitted for application in food industry. In our study, polylactides (PLA) with Mn 7.7 kDa, 220 kDa and 337 kDa and polyglycerol polyricinoleate (PGPR) known by their hydrophobic properties were used. They made a good showing in food industry. The bags from PLA networks or nonwoven tissue are widely used for tea and coffee packaging. PGPR being a powerful water-in-oil emulsifier is routinely used in bakery and confectioner's industry for improving homogenization of the components with different nature.*

# Dependence of mechanical properties of CS/CN films on surface modification with PLA or PGPR



The hydrophobicity and mechanical properties of (CS/NC)/glycerol films have improved by using PLA, which can be still dissolved in 1,4-dioxane (i.e. with higher molecular weight).

# Comparison of mechanical properties of CS/CN films and cellulosic paper



The stress vs strain dependences of cellulosic KFC paper (A) and the films with (CS/CN)/polyglycerol-3 proportion (70/30)/25 wt% (B – F); B, C modified with PLA (7.7 kDa

In contrast to cellulosic paper, the created prototype of CS/CN films had more than three times higher elasticity (about 9-12%) at comparable or somewhat higher mechanical strength, especially after their surface modification with PLA

# Conclusions

1. The most Low- and high-molecular-weight additives are the reinforcing agents of chitosan matrix with considerably lower efficiency than nano-chitin.
2. The right correlation between contents of CN and plasticizer ensures preparation of the CS/CN films with balanced strength and stretching.
3. The surface modification of the film's surfaces by sorption immobilization of polylactides or polyglycerol polyricinoleate enhanced the water repellence considerably.
4. The prototype CS/CN films with suitable properties for packaging of food products with reduced moisture content was prepared.
5. Expansion of the food product range for packaging in CS/CN films requires the optimization of the modification step, drying conditions of the films and the type of the support.

**We thank for your kind attention**

