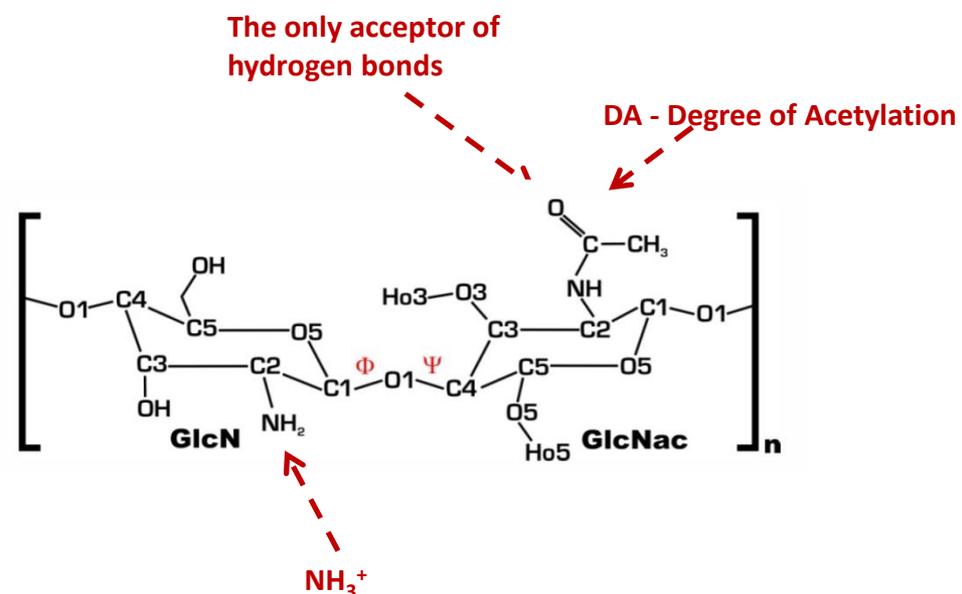


# Structure and Properties of Chitosan/Chitin-Nanofibrils Based Materials

Jindřich Hašek, IBT AV ČR Praha

**Chitosan** – random co-polymer  
GlcN/GlcNac with content of GlcNac (i.e. DA - Degree of Acetylation) lower than 50 %.

In our case, we used the HMC produced commercial chitosan,  $M_w=374$  kDa, degree of acetylation DA = 11%. The average length of a single chain several hundreds monomers (~ 1 nm). Flexible polycations well soluble in acidic water.

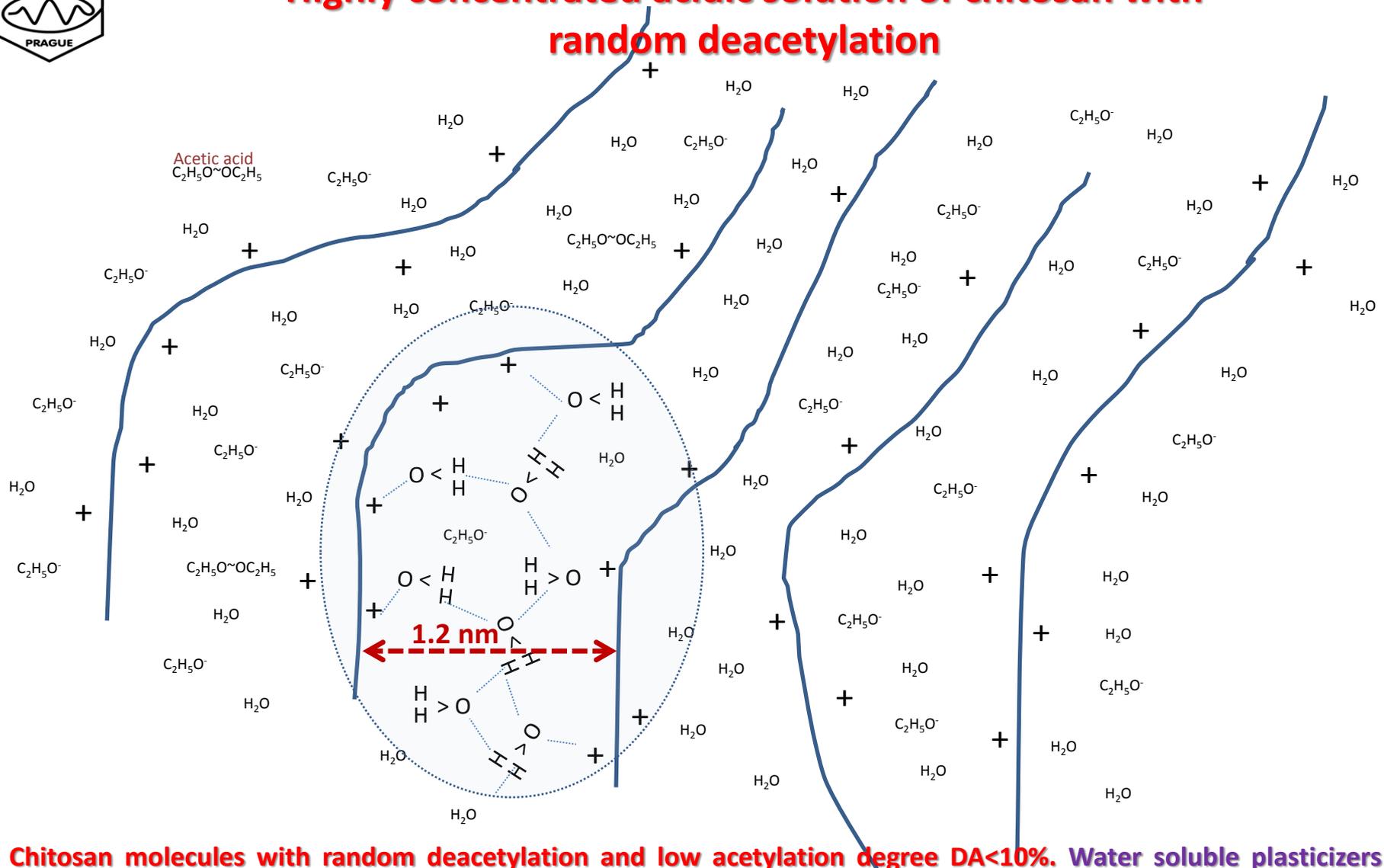




# Properties of chitosan

Degree of acetylation, pH, tacticity, block or random co-polymer

# Highly concentrated acidic solution of chitosan with random deacetylation

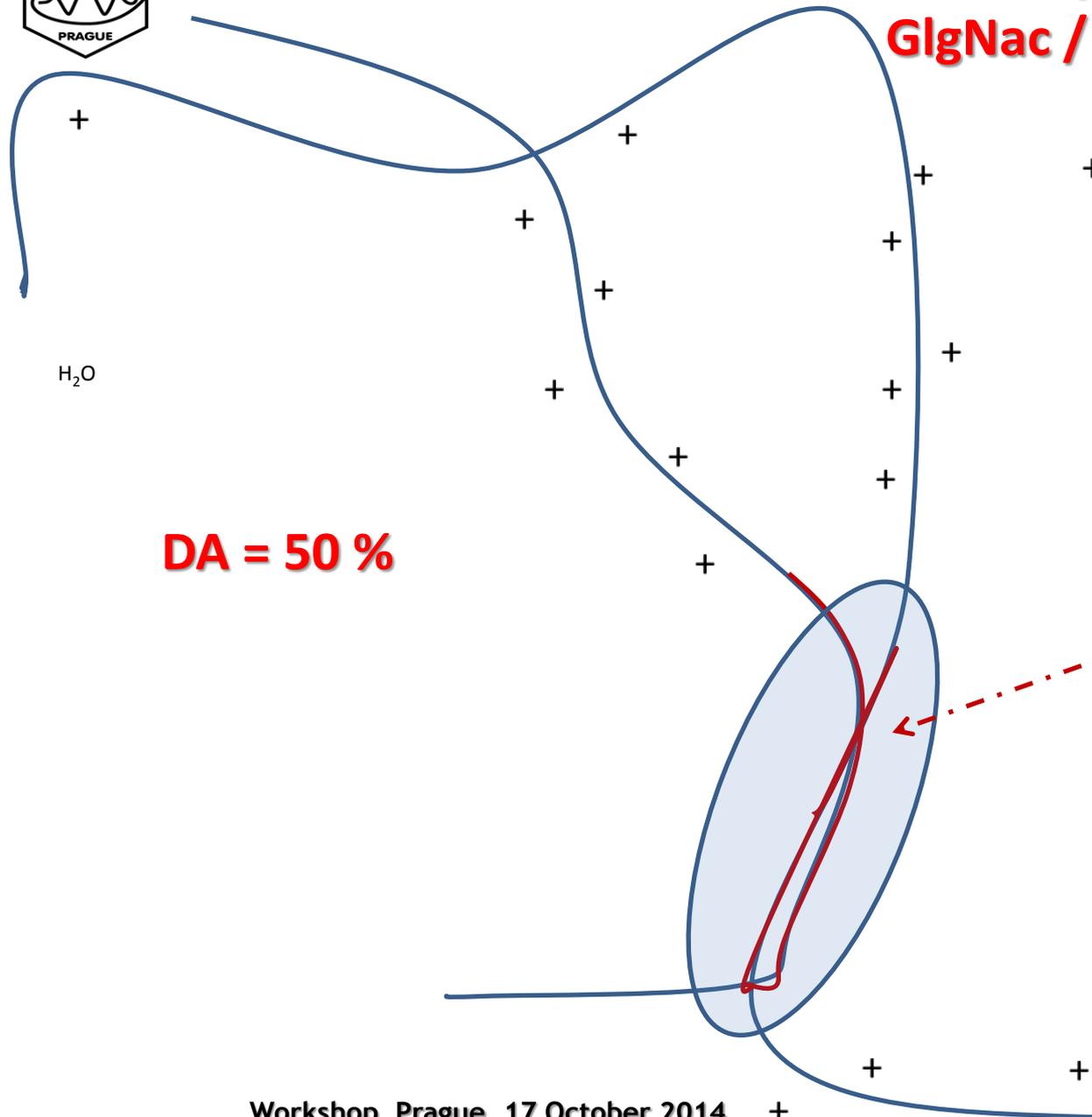


**Chitosan molecules with random deacetylation and low acetylation degree  $DA < 10\%$ . Water soluble plasticizers penetrate between chitosan chains and change dielectric constant and diminish polarization of water layers.**



# Acidic solution of a block co-polymer

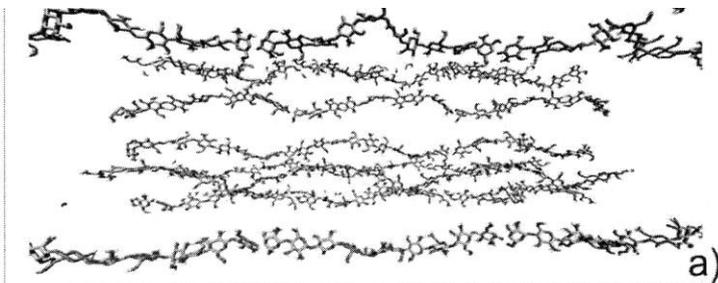
**GlgNac / GlgN (chitin / chitosan)**



**DA = 50 %**

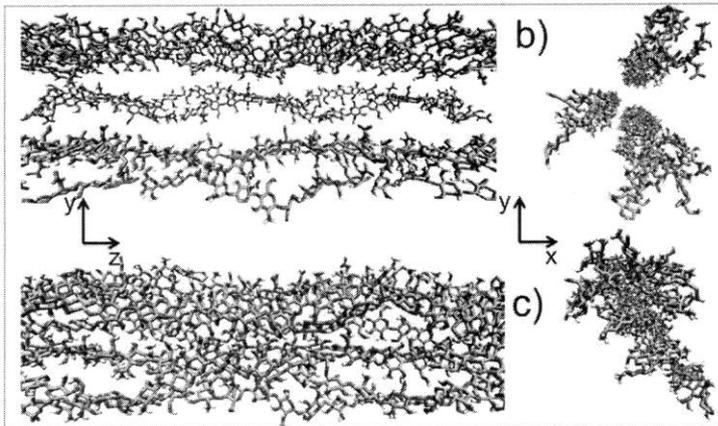
*Direct entanglements  
due to continuous  
GlgNac blocks*

# Solubility of chitosans with different degree of acetylation, different stacking (random x block co-polymer), and different tacticity (atactic x syndiotactic)



$DA = 0\%$

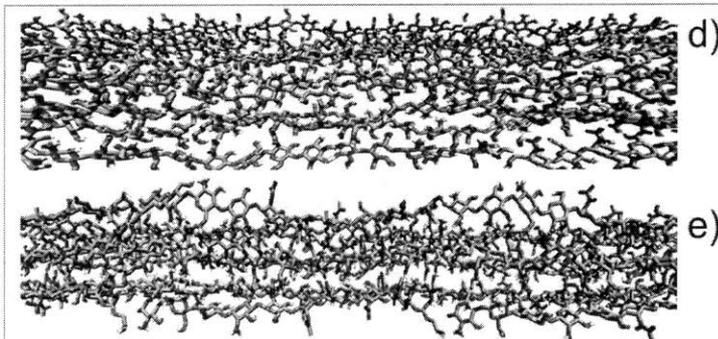
**100 % chitosan**



*40% uniformly*

**40 % block GlcNac**

*40% block*



*60% uniformly*

**60 % block GlcNac**

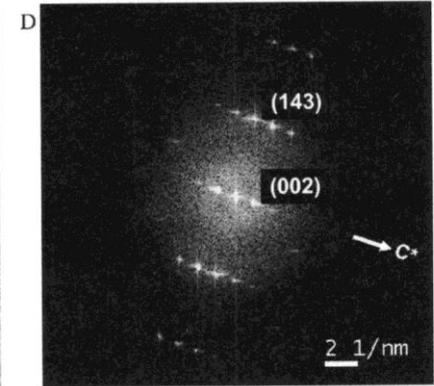
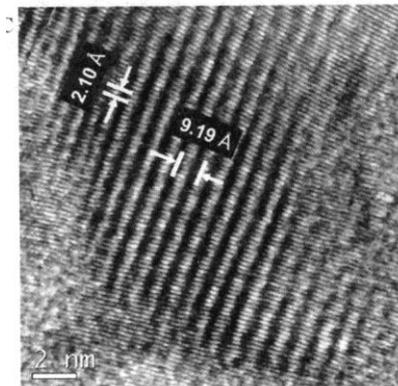
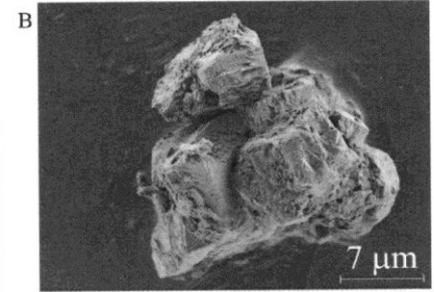
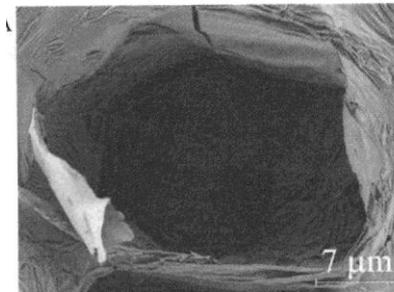
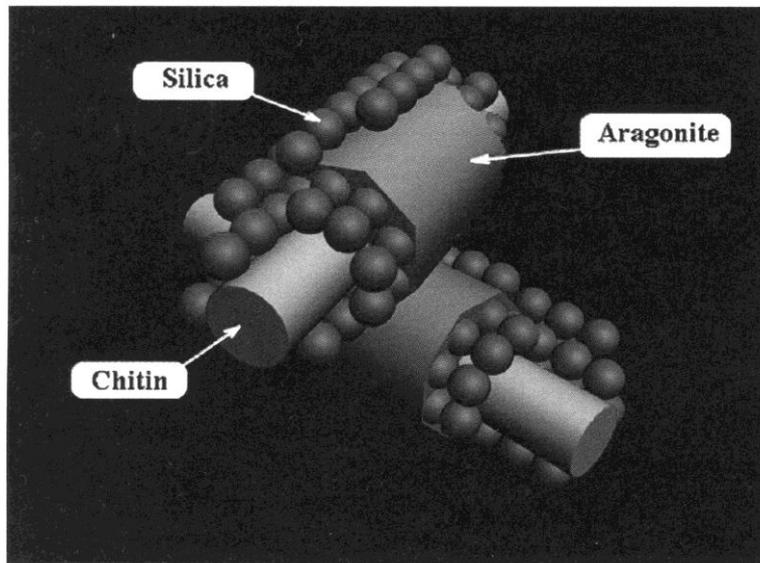
*60% block*

*DA*

# Properties of chitin nanofibrils

Crystal polymorph, rigidity, surface modification of nanofibrils

# Chitin nanofibrils are constructs of biological origin



# SEM of CN-filled films dried in the frozen state

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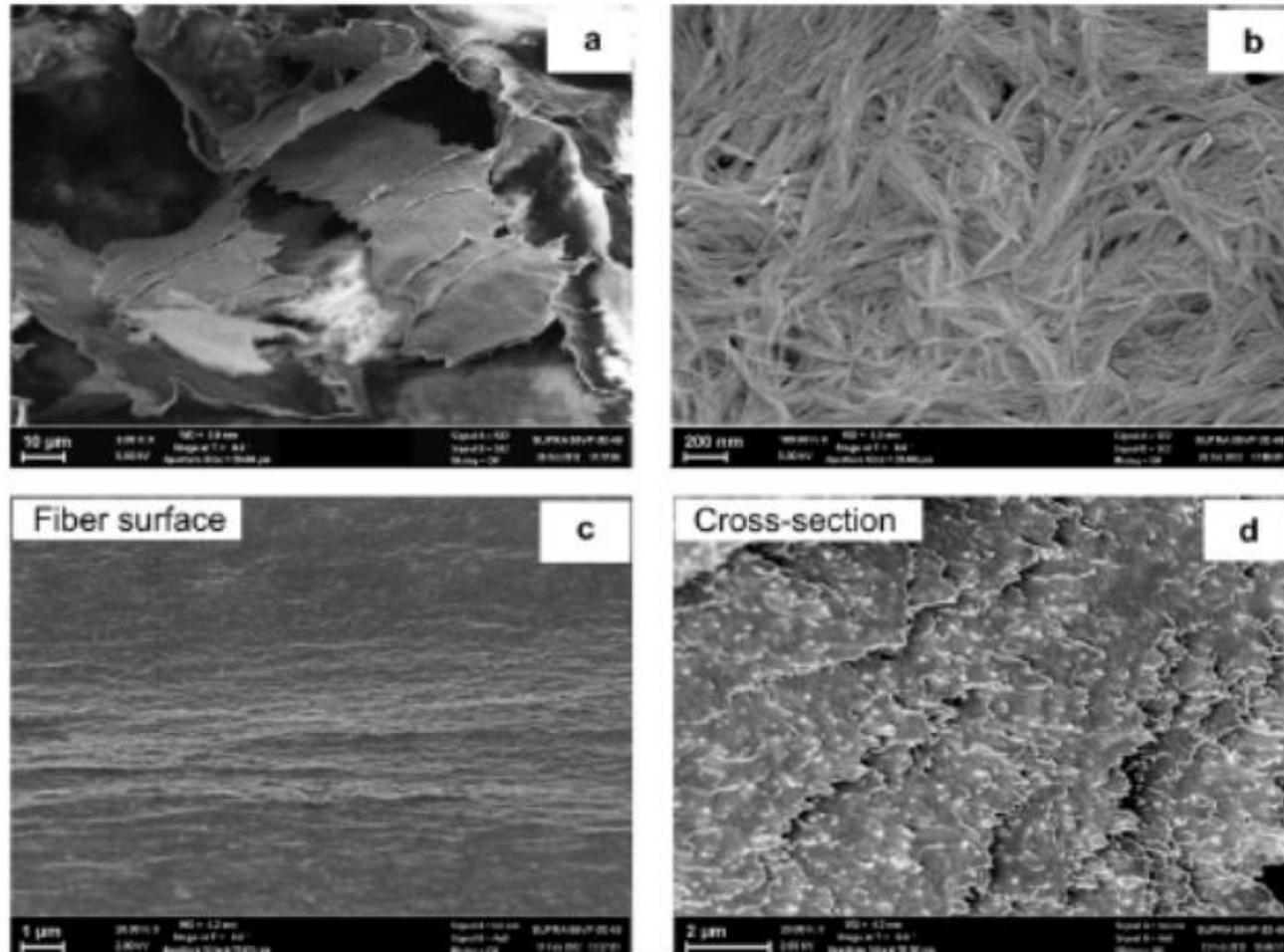
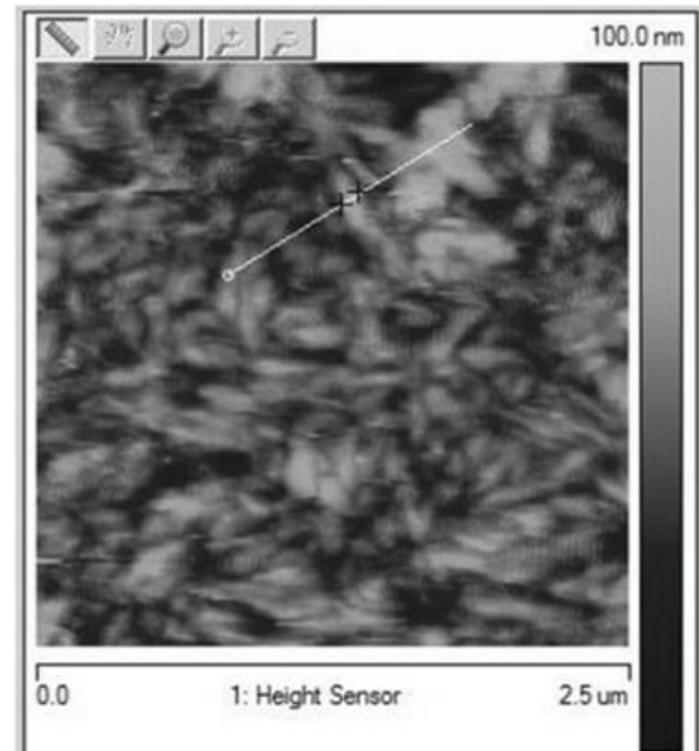
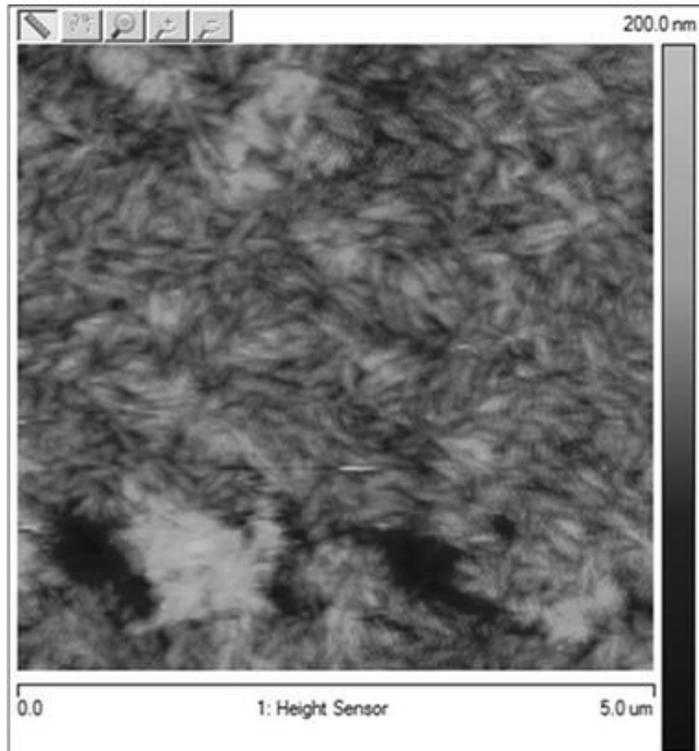


Fig. 1. Scanning electron micrographs of the film obtained from chitin nanofibril water dispersion by freeze drying (a and b); cross section of the composite monofilament chipped in a liquid nitrogen, fiber contains 1 wt.% of chitin nanofibrils (c and d).

## AFM images of swollen chitin nanofibrils in an aqueous commercial dispersion (MAVI SUD S.r.L., Italy)

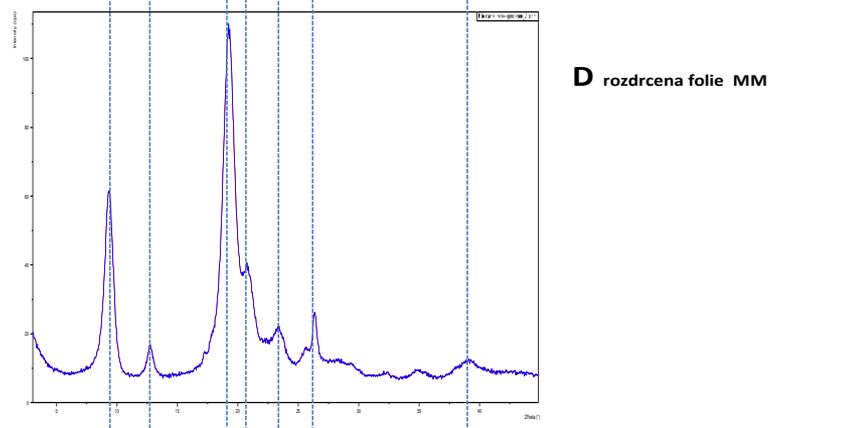
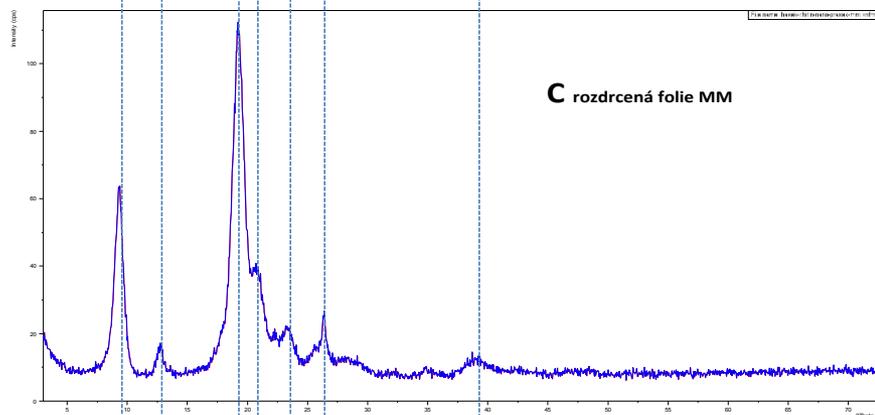
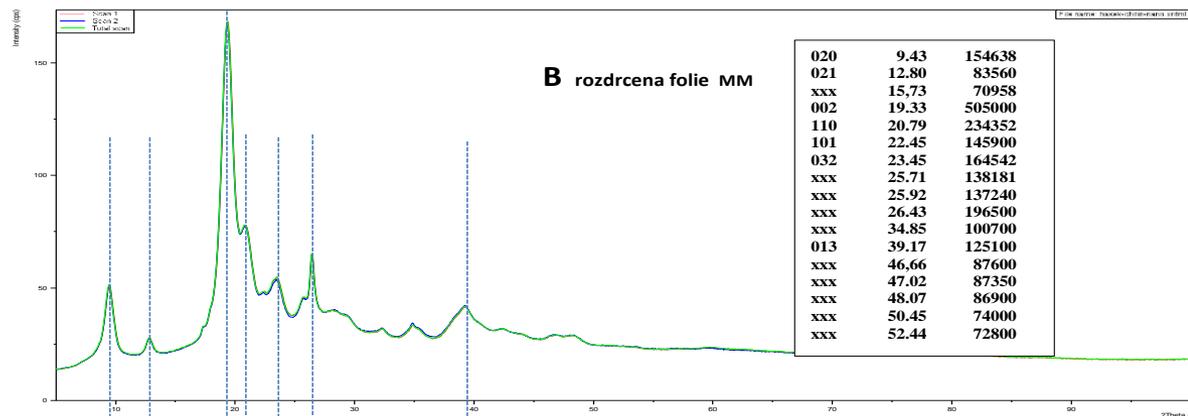


Average diameter of chitin nanofibrils in an aqueous dispersion was 75-80 nm. Analysis was carried out under water.

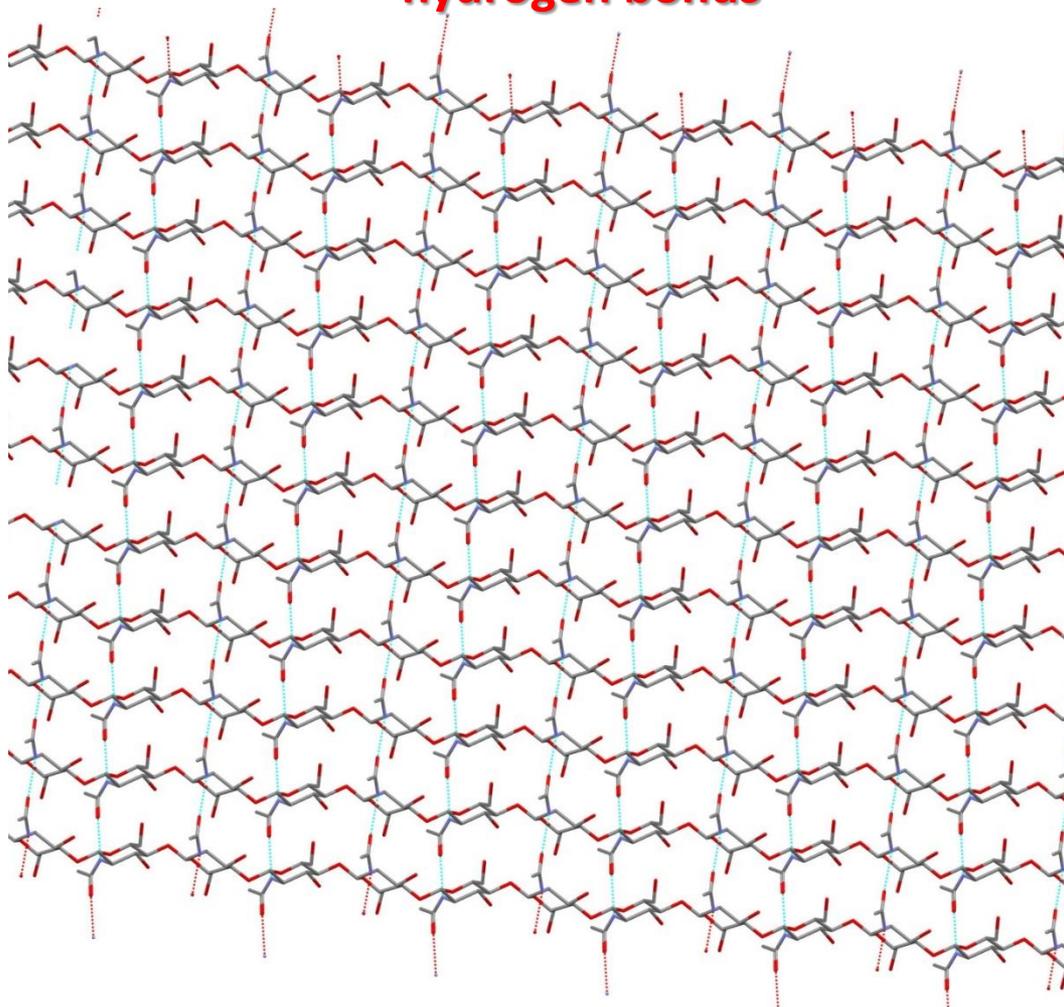


**Unique  
identification of  
chitin in its  
insoluble  
 $\alpha$ -crystalline  
phase in the  
nanofibrils**

**$\beta$ -crystalline  
phase of chitin  
can intercalate  
solvent and  
other small  
molecules**



**Chitin  $\alpha$ -polymorph of the crystalline whisker  
(antiparallel polymer chains) is insoluble because of a network of strong  
hydrogen bonds**





# Commercial chitin nanofibrils

## show degree of deacetylation in range 5–12 %

It corresponds to deacetylation of 1 or 2 layers of chitin macromolecules exposed on the surface of nanofibril.

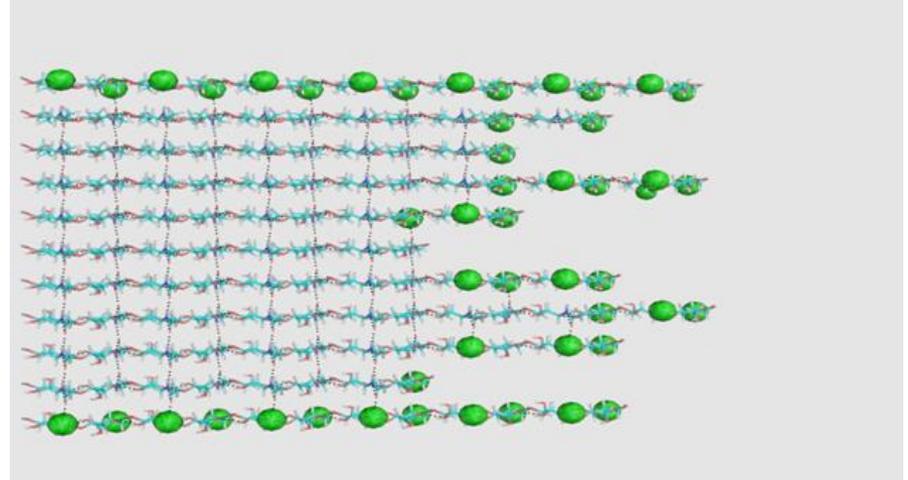
### It fits well:

1. Nanofibril is composed ~400 chitin molecules, 40 of them exposed on the surface. Smaller nanofibrils with diameter ~ 20 nm thick have DD ~ 12 %, large nanofibrils with diameter ~40 nm have DD ~ 8 %.
2. No solvent can penetrate into crystalline chitin unless the crystal is irreversibly broken. Thus, it is natural that only the chitin molecules on the surface of nanofibrils are deacetylated.
3. The molecules on the nanofibrils surface are not chitosan molecules – chitosan would dissolve immediately.

**Chitin nanofibrils ( $M_w = 10^{10}$  Da) are huge, elongated  $\alpha$ -chitin crystalline rods with highly deacetylated surface responsible for perfect homogeneous distribution in acidic water and formation of stable colloidal dispersion**

## **Conclusion:**

- 1. An average nanofibril has similar length as the length of a single chitin molecule.**
- 2. The nanofibrils are chitin crystal whiskers with deacetylated surface.**
- 3. Surface molecules are co-polymers of syndiotactically deacetylated chitin with randomly deacetylated chitosan at terminals.**

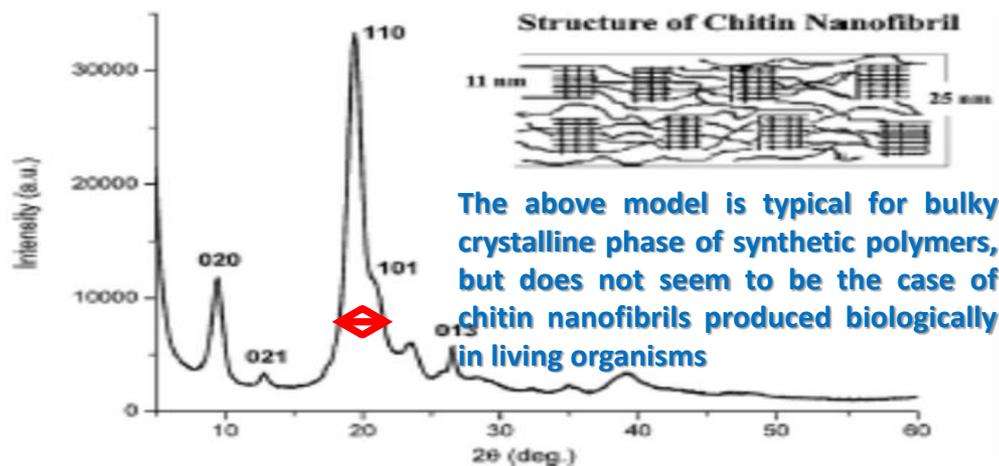


Model of a small  $\alpha$ -chitin nanofibril

## Wet spinning of fibers made of chitosan and chitin nanofibrils

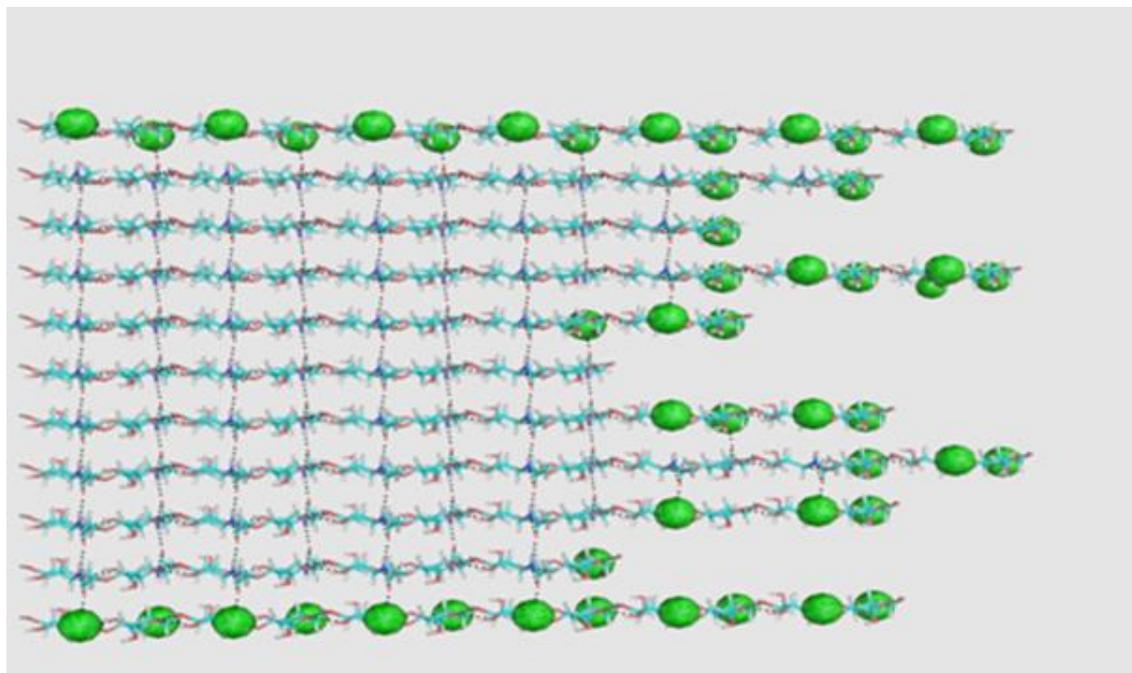
Vladimir E. Yudin<sup>a,b</sup>, Irina P. Dobrovolskaya<sup>a,\*</sup>, Igor M. Neelov<sup>a,c</sup>, Elena N. Dresvyanina<sup>a</sup>, Pavel V. Popryadukhin<sup>a</sup>, Elena M. Ivan'kova<sup>a</sup>, Vladimir Yu. Elokhovskii<sup>a</sup>, Igor A. Kasatkin<sup>d</sup>, Boris M. Okrugin<sup>d</sup>, Pierfrancesco Morganti<sup>e</sup>

The flat fibril-like structures include the chitin nanofibrils, (width about 25–30 nm) oriented along the fiber axis. This value is somewhat larger than the size determined for the freeze-dried suspension of chitin nanofibrils (20 nm), that suggests a good adhesion of chitosan to chitin nanofibril or existence of thin (~5–10 nm) chitosan layer on the chitin nanofibril surface. A good adhesion of chitosan to chitin nanofibrils is caused by the fact that chitosan macromolecules contain lateral groups of both  $\text{NH}_2$  and  $-\text{NH}-\text{CO}-\text{CH}_3$ , the latter are characteristic of the chitin macromolecules as well.



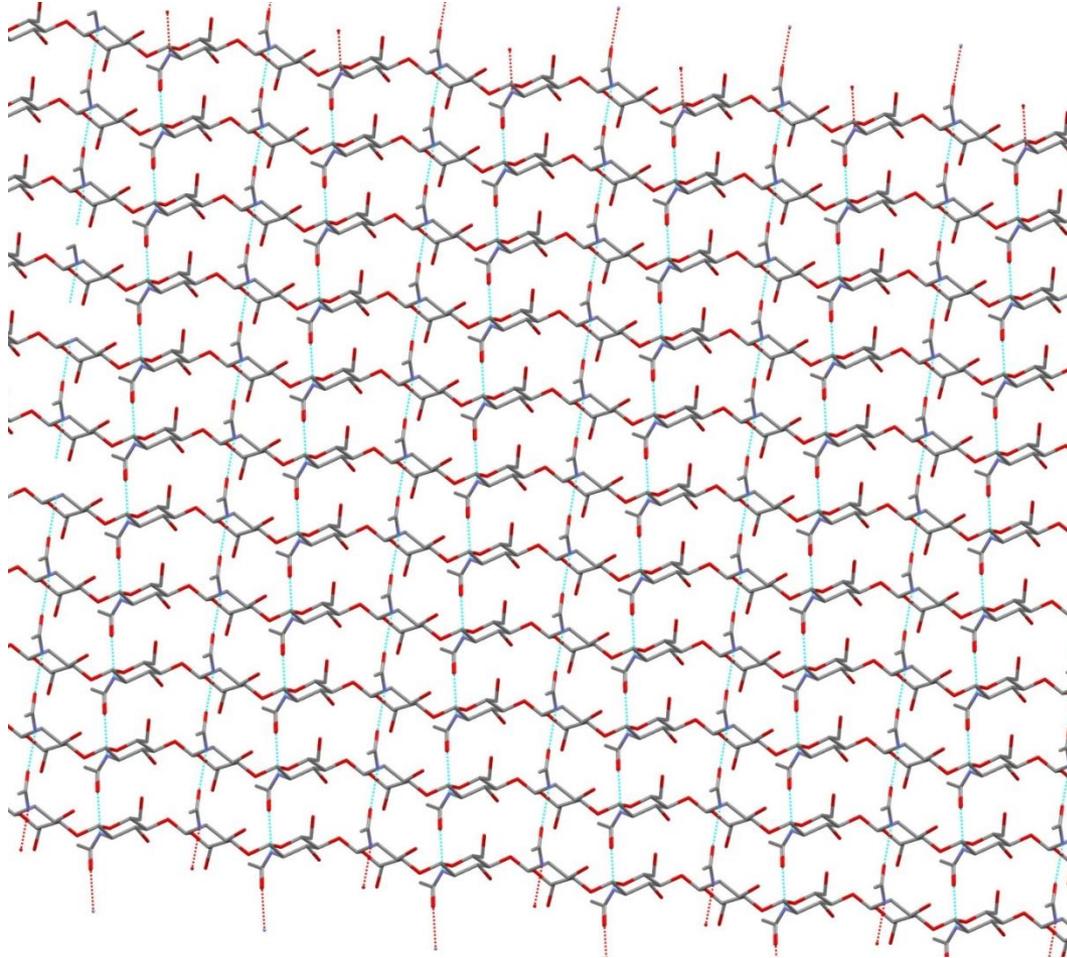
The model of nanofibrils proposed by Yudin ignores the fact that reflection width is influenced not only by crystallite size but also by the innate disorder observed in all polymer structures. It lead them to artificially small crystallite sizes and the model of two layers of small crystals connected by amorphous regions.

Fig. 2. X-ray diffraction pattern of the chitin nanofibril powder obtained by lyophilization of its water dispersion.



**Surface molecules are co-polymers of syndiotactically deacetylated chitin with randomly deacetylated chitosan at terminals**

**Chitin  $\alpha$ -polymorph of the crystalline whisker  
(antiparallel polymer chains) is insoluble because of a strong network  
of hydrogen bonds**

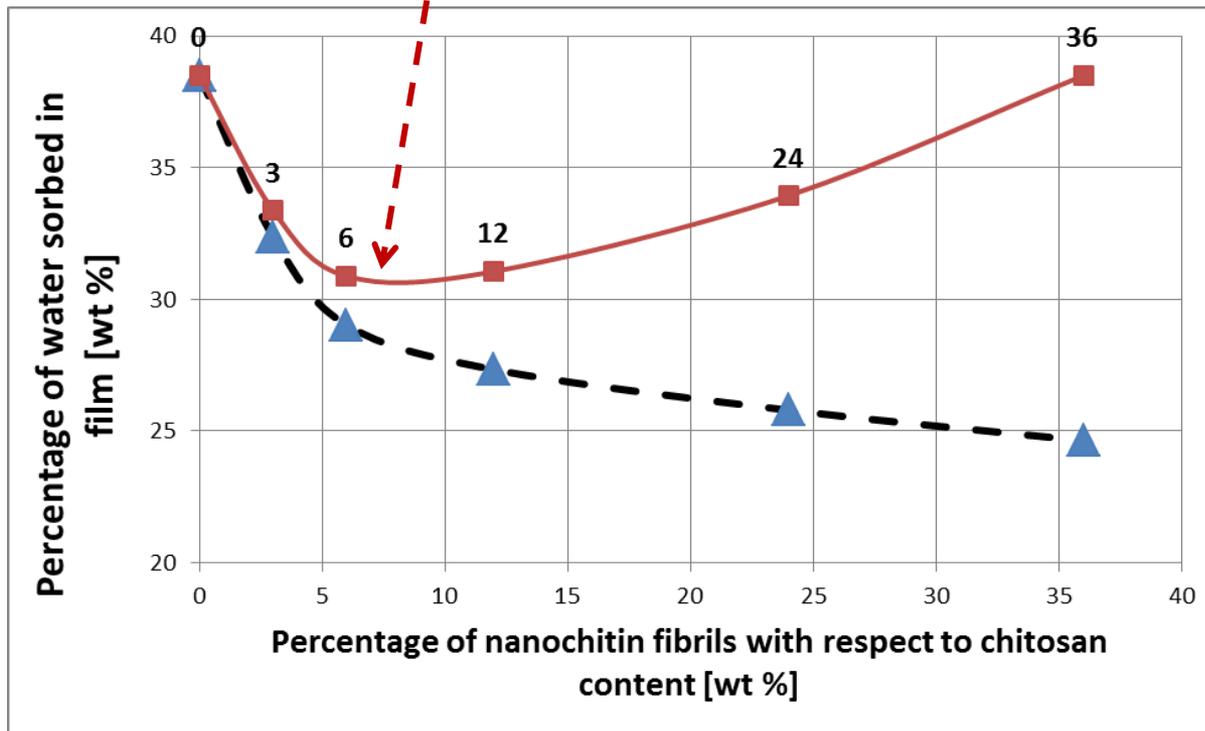




# Orientation of polycation molecules (chitosan) along the positively charged surface of chitin nanofibrils

Parallel orientation of chitosan molecules increase local density of solution

**The lowest concentration of water in the chitosan matrix (red curve) corresponds to the most ordered parallel chitosan chains, where the domains fill the whole space but do not penetrate yet**



Addition of  $\alpha$ -chitin nanofibrils into chitosan film decreases a percentage of water adsorbed in the film. The blue triangles show that the percentage of water soaked into the chitosan film under the water vapor pressure 3 kPa. The red curve (squares) shows the water concentration in the chitosan matrix (under supposition that water do not penetrate into the  $\alpha$ -chitin crystalline phase). Minimum water concentration (at about 8 wt% of nanofibrils) corresponds to the state when the nanofibrils uniformly distributed in sample fill the whole volume of the sample.



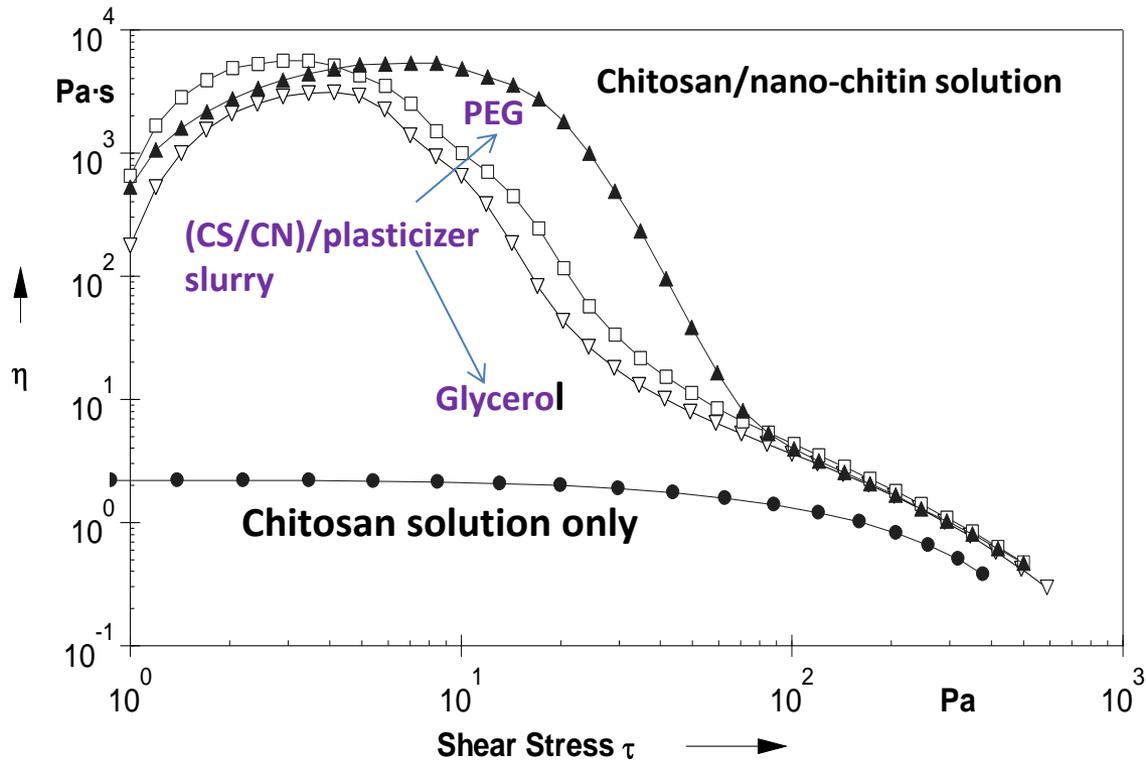
**High content of water in polycation based materials is an obstacle. Water can diffuse or evaporate and change thus the material properties.**

**We propose that for special purposes water solvent could be replaced by anionic liquids of higher molecular weight and higher temperature of evaporation.**



# Rheological properties of the film casting solutions

- 1. Addition of nanofibrils induce well-organized chitosan domains** around each  $\alpha$ -chitin nanofibril
- 2. Parallelization of chitosan molecules squeeze some water** soaked in the chitosan matrix
- 3. Plasticizers diminish the organized domains, lower viscosity** of chitosan matrix, and **lower the energy loss** necessary for reorientation of the  $\alpha$ -chitin nanofibril domains
- 4. Kinetic energy necessary for movement of whole domains is 4 orders higher** than in the case of single chitosan molecules.





# Rheological study of chitosan acetate solutions containing chitin nanofibrils

Rheological properties of chitosan acetate solutions containing chitin nanofibrils (nano-chitin) and biocompatible plasticizers intended for preparation of biodegradable films are reported in the steady, oscillatory and transient shear flow. Based on our results of **mechanical properties and absorption of water vapor in the films, experiments were carried on solutions with an optimum CS/CN proportion 65/35 wt % in the films.**

The time-dependent dynamic experiments revealed the **CN as an effective “gelling agent” of chitosan phase.** The phenomenon is explained by a chitosan-like surface of CN and by the interactions inducing **orientational cooperativity of thousands of chitosan molecules dissolved in close neighborhood of the anisotropic chitin nanoparticle.**

Additions of glycerol or poly(ethylene glycol), improving mechanical properties of the films, **delay significantly the beginning of gelation of CS/CN slurries. The effect is induced by an increase in viscosity of the slurries and by their more chaotropic character.**

**Rheological properties are important for technological processes to get anisotropically characteristic products – strong and tiny fibers, oriented films, films possessing unexpected color effects, etc.**

*VE. Yadin et al. / Carbohydrate Polymers 108 (2014) 176–182*

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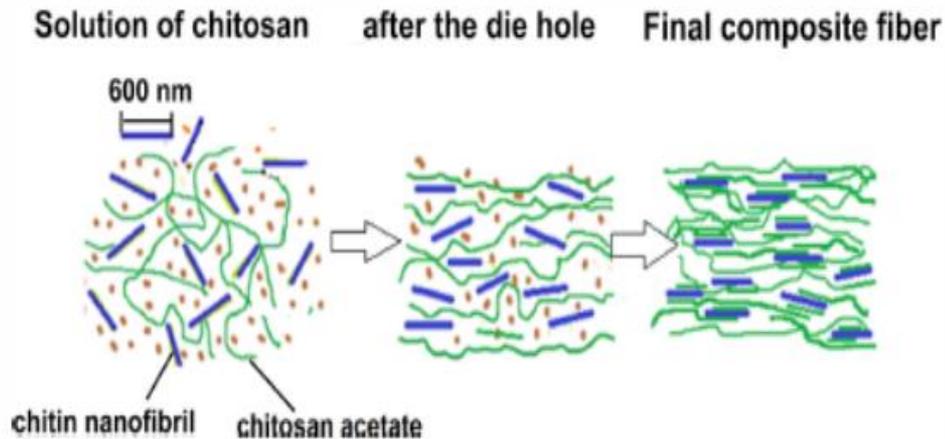


Fig. 4. Formation of the chitosan (green) composite fiber structure containing chitin nanofibrils (blue) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

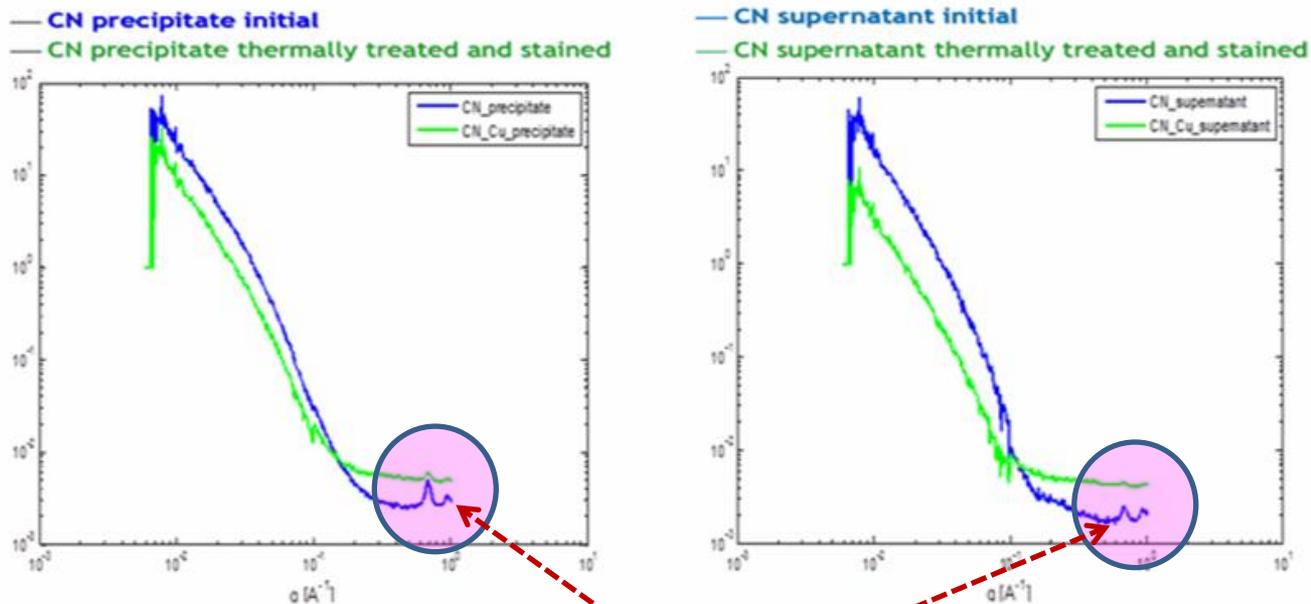


# Degradation of chitin nanofibrils by transition metal ions

# Destruction of nanofibrils by salts of transition metals

## Crystallinity of the thermally treated CN suspension containing glycerol

CN suspension was centrifuged at 5000 rpm for 1 hour to separate large and small nanofibrils. CN precipitate and CN supernatant were mixed with glycerol (70/30 wt%) and heated at 60 °C during 1 hour. After cooling some drops of saturated copper acetate solution was added to stain CN.



Diffraction peaks completely disappeared after addition of copper acetate



# Properties of the plasticized CS/CN films



# Casting CS/CN slurries

<b>Content in the slurry, wt%</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>
	<b>Chitosan (CS) solution</b>	<b>CS/n-chitin</b>	<b>CS/n-chitin/glycerol</b>	<b>CS/n-chitin/PEG-600</b>
chitosan	1.9	1.5	1.5	1.5
chitin nanofibres	-	0.8	0.8	0.8
glycerol	-	-	1.0	-
PEG-600	-	-	-	1.1
acetic acid	2.0	1.6	1.6	1.6
water	96.1	96.0	95.0	95.0
pH	3.97	3.99	3.99	3.99
<b>TOTAL %</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>

<b>Content in the film, wt%</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>
	<b>chitosan (CS)</b>	<b>CS/n-chitin</b>	<b>CS/n-chitin/glycerol</b>	<b>CS/n-chitin/PEG-600</b>
chitosan	90	59	41	40
chitin nanofibres	-	31	22	21
glycerol (maximum*)	-	-	27	-
PEG-600	-	-	-	29
acetic acid (estimate*)	5	5	5	5
water (estimate*)	5	5	5	5
<b>TOTAL %</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>

# Dried chitin nanofibrils (CN)

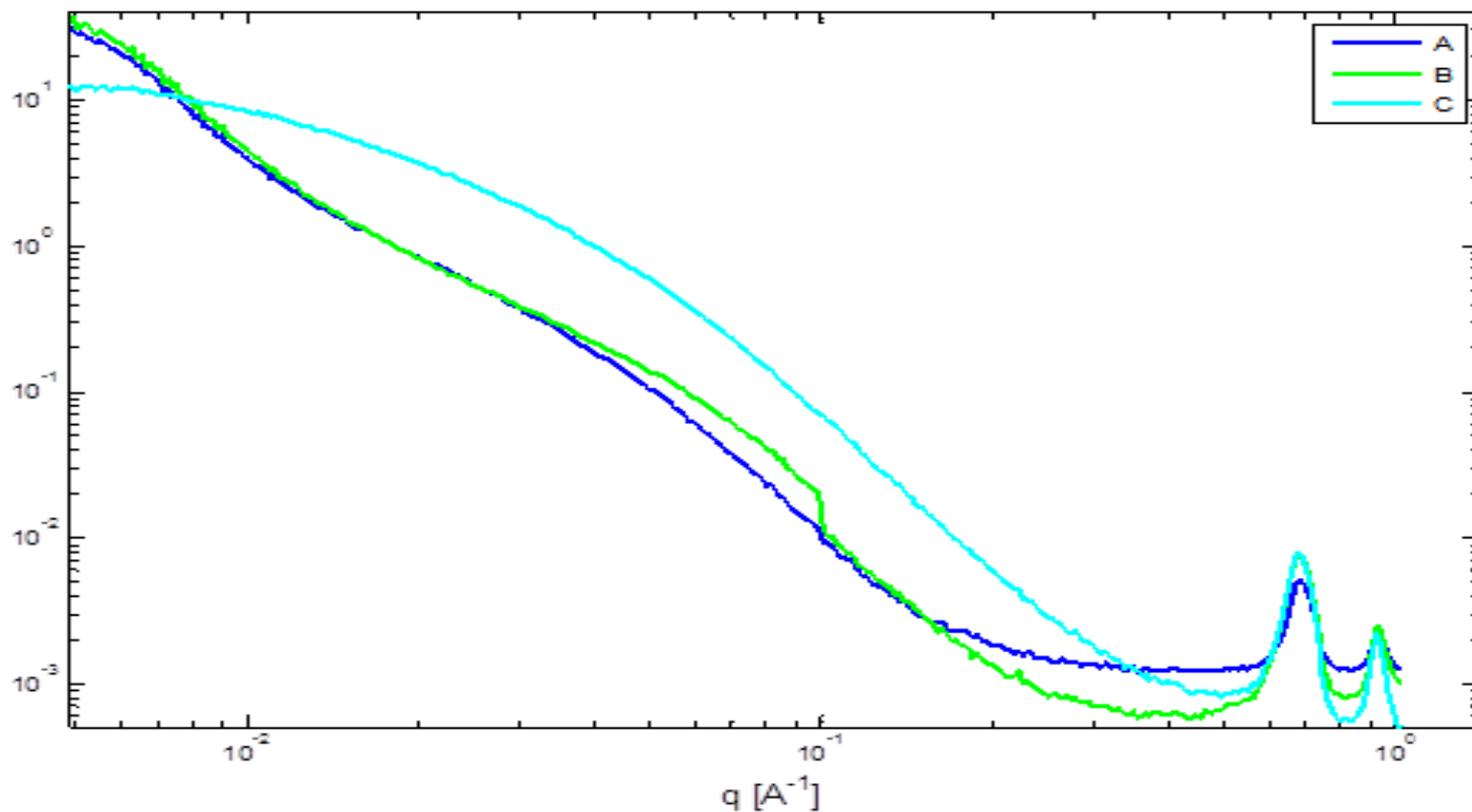


Fig. 1. Diffraction patterns of films prepared by evaporation of CN dispersion.

- A. Dark brown and **fragile CN film** formed by evaporation of the **raw CN dispersion** (with 0,5 wt% sodium benzoate and other salts at pH ~ 1.8, MAVI SUD S.r.L.),
- B. Matt beige **plastic CN film** formed by evaporation of water from the slurry consisting of 70 % of dialyzed CN dispersion and 30 % of glycerol, pH ~ 5,
- C. Beige, transparent and **fragile CN film** formed by evaporation of the **dialyzed CN dispersion**, pH ~ 5.

# Chitosan powder and chitosan films

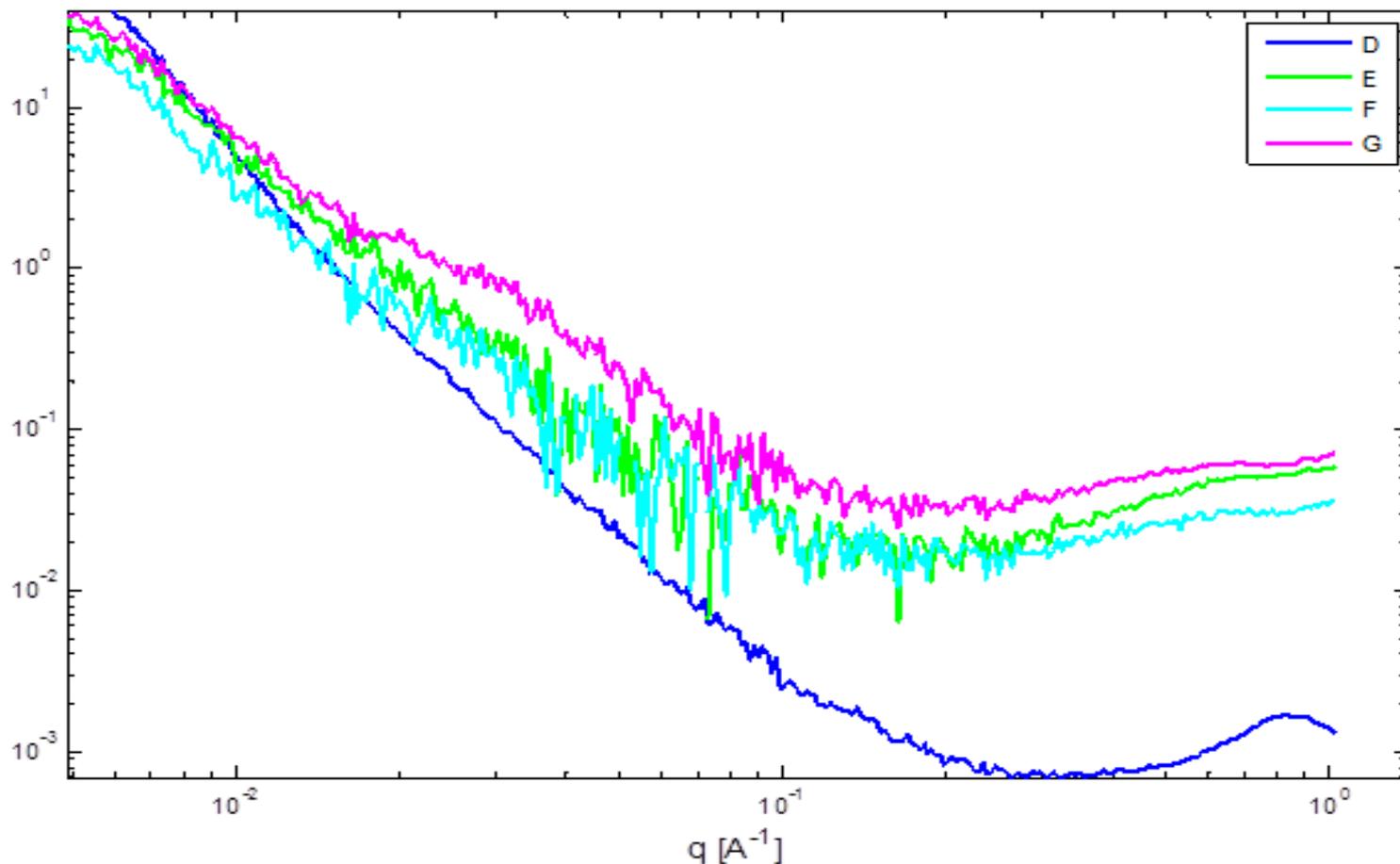


Fig. 2. Chitosan films prepared from chitosan solution without CN nanofibrils.

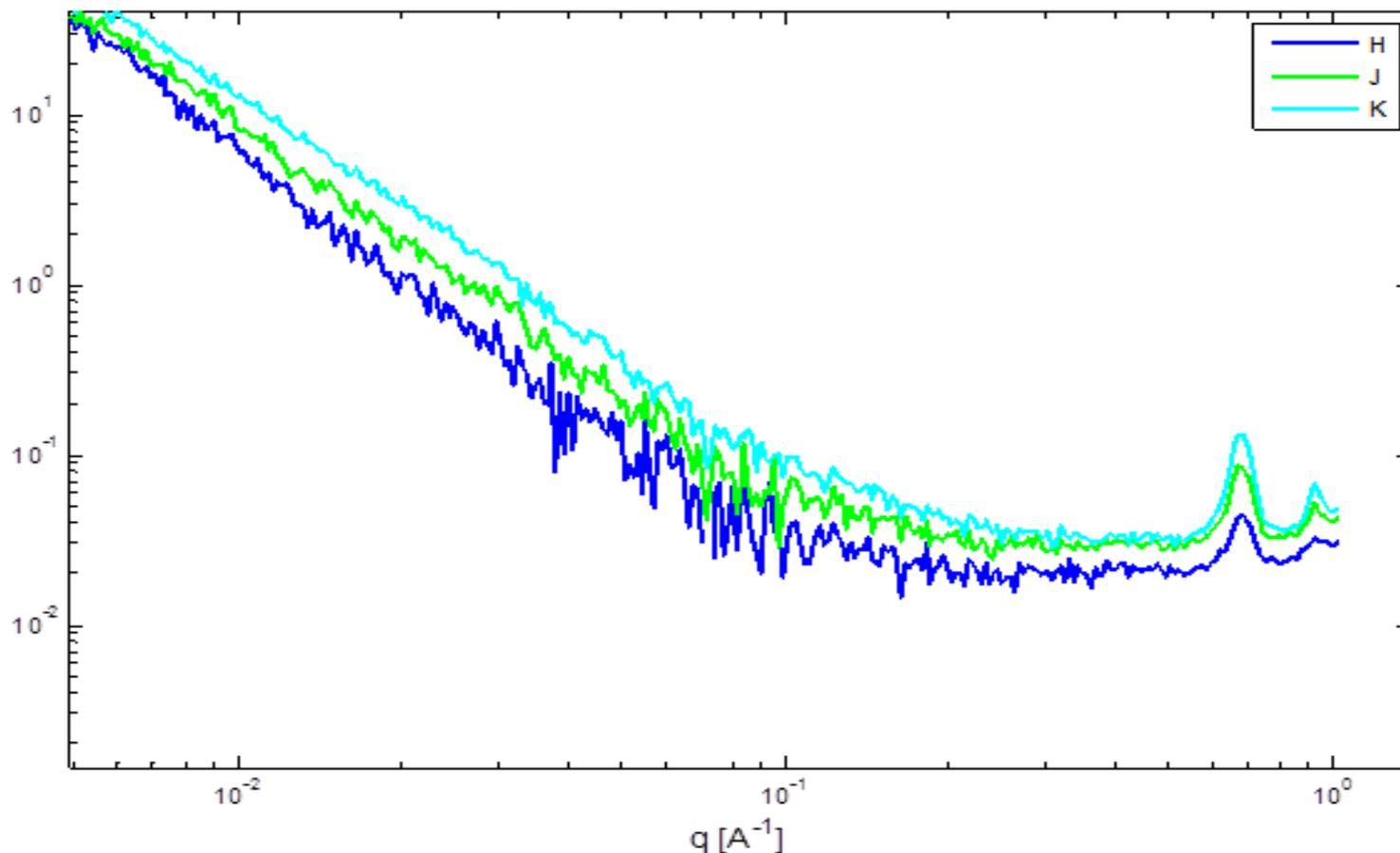
**D.** Pure chitosan powder 80-140  $\mu\text{m}$  “Giusto Faravelli” - slightly creamy color.

**E.** CS film formed by evaporation of water from CS solution containing 1,9 % acetic acid, pH ~ 4.2.

**F.** CS film formed by evaporation of water from the slurry containing of CS (70 %), acetic acid (0,9 %), and glycerol (30 %).

**G.** CS film formed by evaporation of water from the slurry containing of CS (70 %), acetic acid (1,9 %) and glycerol (30 %).

## Chitosan films filled by chitin nanofibrils



*Fig. 3. Chitosan films filled by chitin nanofibrils. The films were prepared by evaporation of water from the slurry containing of acetic acid (1.9 %) and mixture of CS and CN with proportions of: 85/15, 75/25 and 65 /35% .*

*H. 15% of CN nanoparticles,*

*J. 25 % of CN nanoparticles,*

*K. 35 % of CN nanoparticles.*



**Properties of composite materials prepared from cationic co-polymers, nanoparticles and additives can be predicted with high reliability, however in some cases, many mutually correlated parameters can make the controlled experimental preparation somewhat difficult.**

**The chitosan-like surface of nanofibrils clearly explains high stability of chitin nanofibrils** as huge elongated rigid particles in acidic solutions without any observed sedimentation for many months. Each nanofibril is composed of thousands macromolecules safely anchored by their chitin segments in the crystalline core of particle. But the **deacetylated parts of all these molecules near the nanofibril surface (forming of course the whole surface of the fibril)** form in acidic solution eagerly numerous hydrogen bonds with water via their charged amine groups.

**Addition of the chitin nanofibrils (rigid objects composed of several tenth thousands of rather long linear chitin chains) into the chitosan solution results in a gel formation.** In the absence of an external force, the system is in a deep potential minimum, because the intermolecular interactions between chitosan chains and chitosan-nanofibrils are strong. The strong interactions of each highly anisotropic chitin nanofibrils with many thousands of chitosan molecules uniformly distributed in a large volume of solution ensure its stability and solid-like behavior under static conditions. Thus, the following model can be used to explain the observed rheological behavior of the system. **The homogeneous matrix of chitosan polycations homogenously occupies the whole volume of the solution. It is connected by strong attractive and repulsive forces with a network of also uniformly distributed chitin nanofibrils with high capacity for accumulation of kinetic energy.**

The plasticizers PEG-600 or glycerol added to the CN-filled CS solutions decreased the yield stress value by about one half. The plasticizing of the slurries with glycerol or PEG reduced their yield stresses similarly to the values 3.9 and 3.1 MPa, respectively. **Effect of the plasticizers at their concentration comparable with the CN-filled slurry is explained by shielding the electrostatic interactions between positively charged segments, shifting a character of solvent to the chaotropic state and by lowering a number of free water molecules in the CS/CN slurries.**



## Conclusions

1. **Excellent solubility** of chitosan and chitin nanofibrils and their uniform dispersion in the casted films is ensured **by polarization between the  $\text{NH}_3^+$  surface groups and the acidic solvent** present even in the highly hydroscopic solid phase.
2. Effect of nanofibrils on formation of the chitin-chitosan thixotropic gels lies in the **big mass and rigidity** of elongated chitin crystalline nanofibrils with **almost fully deacetylated surface**.
3. The deacetylated **surface of nanofibrils organizes thousands surrounding chitosan macromolecules in domains** about  $\sim 10$  nm far from each nanofibril. In dependence on the technology used the effect of domains can be useful in preparation of solid phase materials with anisotropic properties (fibers, films,...).
4. **The organizational effect of domains penetrates through the whole volume already in 5 % content of nanofibrils. At concentrations of nanofibrils over 35 %, the domains already interpenetrate** introducing a new disorder in the molecular system.
5. Diffraction patterns (SAX-WAXS) showed, that the **plasticizers used [glycerol and poly(ethyleneglycol) analogs], did not changed primarily the film structure** (space arrangement of molecules in system). Their effect is due to the **changes of dielectric properties and viscosity of the chitosan/solvent matrix** present everywhere in between chitin nanofibrils.
6. **Chitin nanofibrils remained intact in all prepared mixtures** with exception of the use of heavy metal ingredients (as can be seen from Fig.1).
7. **Addition of even small amounts of copper acetate added to chitin nanofibril solutions completely destroyed the crystallinity of nanofibrils.** The effect is explained by a catalytic attack of the complexation ion on the frayed ends of chitin nanofibrils leading to the splitting fibrils into thinner filaments (see Fig.2).