IN VITRO PREPARATION OF SELF-ASSEMBLED SUPER-SWOLLEN HYDROGELS FROM SOLUTIONS OF LIGNOCELLULOSE IN N,N-DIMETHYLACETAMIDE/LITHIUM CHLORIDE

NINA KOTELNIKOVA,[*] ALEKSANDRA MIKHAILIDI,[**] YULIYA MARTAKOVA[***] and SEPPO ANDERSSON[****]

[*]*Institute of Macromolecular Compounds, Russian Academy of Sciences, 199004 St. Petersburg, Russia*
[**]*St. Petersburg State University of Technology and Design, 191186 St. Petersburg, Russia*
[***]*Institute of Chemistry, Ural Branch of Russian Academy of Sciences, 167982 Siktivkar, Russia*
[****]*Deparment of Physics, University of Helsinki, FI-00014 Helsinki, Finland*
✉ *Corresponding author: Nina Kotelnikova, nkotel@mail.ru*

Dedicated to the memory of Acad. Prof. Cr. I. Simionescu

Stable super-swollen hydrogels were prepared *in vitro* from powder lignocelluloses of various origins via direct dissolution in DMAc/LiCl, followed by subsequent regeneration from the solutions. The main properties of the swollen hydrogels, such as equilibrium solvent content, porosity and dye uptake, were determined. The hydrogels retained large amounts of water (up to 2500 wt%), had high porosity and specific surface areas. The superabsorbance of the hydrogels was confirmed by WAXS results. The hydrogels seem to be "smart" matters due to their pH-dependent behaviour, as demonstrated by swelling experiments. The formation of hydrogels occured via spontaneous self-assembly from the solutions and was due to the reconstruction of a new hydrogen bond web between the lignocellulose chains and the water. The hydrogels were stable, but crucially changed their morphology after drying, because of water removal and collapse of the H-bonds formed. These findings demonstrate that the lignocellulose hydrogels are novel attractive materials that will find potential applications in a wide range of fields.

Keywords: lignocelluloses, solutions, DMAc/LiCl, self-assembly, hydrogels

INTRODUCTION

Hydrogels are currently viewed as three-dimensional (3D) materials with the ability to absorb large amounts of water, which fills the voids between polymer chains and maintains their dimensional stability. The 3D integrity of hydrogels in their swollen state is sustained by either physical or chemical crosslinking.[1–4]

Hydrogels contain mostly water (the mass fraction of water is much greater than that of polymer). The ability of a hydrogel to hold a significant amount of water implies that the polymer chains must have at least moderate hydrophilic character.[5] Many authors have defined the hydrogels and gelation mechanisms.[6,7] Shortly, gelation can take place either by physical linking (physical gelation) or by chemical linking (chemical gelation). Examples of weak physical bonds are hydrogen bonds, block copolymer micelles, and ionic associations. On the other hand, chemical gelation involves formation of covalent bonds and always results in a strong gel.

Hydrogels can be classified into different categories depending on various parameters, including the preparation method, the charge, and the mechanical and structural characteristics. Permanent or chemical gels are covalently cross-linked (replacing the hydrogen bond by stronger and more stable covalent bonds) networks.[8] They attain an equilibrium swelling state, which depends on the polymer-water interaction parameter and the crosslink density.[9]

Chemical crosslinking is a highly versatile method to create hydrogels with good mechanical stability. However, the crosslinking agents used are often toxic compounds, which need to be extracted from the gels before they can be applied.[8]

Physical gels are reversible and the networks made with physically crosslinkable junctions are held together by molecular entanglements, and/or secondary forces including ionic, hydrogen bonding or hydrophobic interactions. In physically cross-linked gels, dissolution is prevented by physical

interactions, which exist between different polymer chains.[8] All of these interactions are reversible, and can be disrupted by changes in physical conditions or by application of stress.[9]

Hydrogels derived from biopolymers have been widely studied for years and are still stirring up interest as polymeric networks. They could be used in medical practice, in pharmacy, as scaffolds for drug delivery in biotechnology, etc.[1,9,10] The cellulose biopolymer makes up the principal structure of many plants. Cellulose-based hydrogels have attracted much attention, due to the numerous advantages that this biodegradable polymer presents. Plant-derived cellulose has been broadly applied in paper, textile and food industry as well as a biomaterial in cosmetics and medicine, as reinforcing agent in composite materials,[11-13] due to its structure and good biocompatibility.[14] The cellulose-based hydrogels prepared from suspensions of nanofibrillar cellulose have been studied extensively.[15] They can also be formed from solutions of cellulose derivatives (hydroxypropylcellulose, carboxymethycellulose, methylcellulose and others),[16] from cellulose-based composites or cellulose/synthetic hybrid polymers,[1-3,17-19] and many others. The composites have included various types of natural (chitosan, chitin, agarosa, gelatin, collagen, proteins, DNA)[17,20,21] and synthetic components [poly(vinyl alcohol), poly(ethylene glycol, poly(acrylic acid) (PAA) or poly(methacrylic acid) (PMAA)],[3,11] besides the cellulose, and exhibit a wide range of properties.

The majority of studies have been carried out on bacterial cellulose, which forms the hydrogels, due to its peculiarities of synthesis and shape.[11] Plant-derived cellulose has been rarely used to prepare hydrogels. The main reason is the fact that natural cellulose has poor solubility, because of the strong hydrogen bonding between the hydroxyl groups of cellulose involved within the cellulose chains.[6] This problem can be overcome by various treatments of cellulose fibres, including their direct dissolution in complex solvents to decrease the hydrogen bonding. Numerous solvent systems have been applied to dissolve the cellulose fibres directly. Some of them are NaOH/urea aqueous solution,[22] ionic liquids,[23] lithium chloride (LiCl)/N-methyl-2-pyrrolidinone, LiCl/N,N-dimethylacetamide,[24] tetrabutylammonium fluoride/dimethyl sulfoxide and others. Recently M. Kostag *et al*. have found that the combination of ammonium salt/aprotic organic liquid can be prospective as a solvent for cellulose samples, taking microcrystalline cellulose as an example. Thus, acetone containing the well-soluble salt triethyloctylammonium chloride appeared to be an efficient solvent for cellulose. The addition of an amount of 10 mol% of triethyloctylammonium chloride promoted cellulose dissolution. The cellulose solutions in this solvent had low viscosity making it a promising system for shaping processes and homogeneous chemical modification of the cellulose.[25]

The N,N-dimethylacetamide/lithium chloride (DMAc/LiCl) solvent system for cellulose is one of the most popular. The dissolution of cellulose in this solvent can be made at room temperature and is easily performed.[26,27] This is also due to the fact that the hydrogels may be shaped from solutions. Saito *et al.* have prepared transparent cellulose hydrogels from various solutions of cellulose samples, including DMAc/LiCl, regenerated in organic and inorganic media and studied the impact of the regeneration conditions on the physical properties of cellulose hydrogels. The cellulose solution was cast on a glass plate and was immediatedly coagulated in a mixed water/organic solution. The hydrogel obtained was washed with water and contained up to 80% of water. However, the obtained gels were very thin and could be described as films rather than gels.[28] Ishii *et al.* studied the dissolution of cellulose in a DMAc/LiCl solution and its aggregation behavior from the DMAc/LiCl solution with SAXS measurements and with polarized optical microscopy. The authors prepared gels in the form of spherical particles. The formation of these particles is easier to implement than preparing monolithic samples and this form limits their characterisation and application. It has been shown that gel formation depends on the concentration of cellulose and on the gelation method.[29] The gel prepared by the addition of water was turbid, the one prepared by ion exchange was colorless, transparent, and optically anisotropic. On the basis of the results of the SAXS measurements, the relation between the structure of cellulose in dissolved and gelled states has been determined as originating in large-scale fluctuations of the cellulose chain density.

J. Obradovic *et al.* have prepared three-dimensionally shaped cellulosic materials without chemical modification via a two-step procedure: swelling of softwood pulp in DMAc/LiCl followed by moulding. Swollen cellulose pulp in the form of gel was precipitated with two different anti-solvents: distilled water and a combination of 2-propanol and deionized water, which resulted in various X-ray structure, mechanical properties of the final products and number of lithium cations therein. SEM studies showed that the samples exhibited a change in morphology: the surface of cellulose after the

mechanochemical treatment was rough and did not contain any fibres. The possibilities of moulding preswollen cellulose materials without derivatisation appeared to be successful, due to disrupting the intermolecular bonds of cellulose.[24]

Generally, the processing of cellulose gels involves several steps: pretreatment of starting cellulose samples to activate them, dissolution of the samples (various solvents and cellulose source materials can be used), precipitation of cellulose, and solvent removal, while avoiding cellulose agglomeration. This can be done by supercritical drying, freeze-drying, rapid decompression, etc.[30] The solvent system N,N-dimethylacetamide/lithium chloride (DMAc/LiCl) has been extensively studied for the dissolution of cellulose samples and their regeneration from the solutions in various forms. However, detailed studies on the formation of hydrogels from these solutions and the properties of the hydrogels are scarce.[26,28,29]

The present study has aimed to explore the formation of self-assembled hydrogels occurring in solutions of lignocellulosic samples of various origins in DMAc/LiCl. The assessment of the impact of various factors, i.e. the regeneration mode and the method of drying, on the gelation behavior is another objective of the study. In this paper, we focused mainly on the stages providing the regeneration from the dissolved state, the spontaneous gelation accompanied by the formation of gels and hydrogels and the subsequent drying of hydrogels. The main chemical properties of the hydrogels were evaluated based on various experimental methods. Furthermore, we included preliminary results obtained using WAXS to clarify changes in the supermolecular structure occurring within pristine powder lignocelluloses during dissolution and regeneration.

EXPERIMENTAL

Materials

Samples isolated from short-length flax fibres, bleached sulphite hardwood pulp and cotton cellulose were subjected to hydrolytic degradation followed by dissolution. The pristine flax fibres were linseed waste. The hardwood pulp was purchased from Syktyvkar Timber Plant ("Mondi", Syktyvkar, Russia). Cotton cellulose was purchased from Vladimir Chemical plant (Vladimir, Russia).

Analytical grade anhydrous LiCl, purum p.a., was purchased from Fluka. LiCl was annealed at 200 °C for 4 h, then stored in a desiccator over P_2O_5 before use and employed without further purification. Analytical grade N,N-dimethylacetamide was purchased from Sigma-Aldrich and used as received. All other chemicals were of analytical grade and used without further purification.

Hydrolysis of pristine samples

The wood pulp and the flax fibres were hydrolyzed with 10 wt% aqueous solutions of nitric acid at 100 °C and at a solid-to-liquid ratio of 1:10 for 2 h, as described elsewhere.[31] The hydrolyzed samples were rinsed with water, dried, and primarily characterized as to their intrinsic viscosity and average degree of polymerization (DP_v), as already described elsewhere.[32,33] The content of α-cellulose was determined by dissolving the samples in a 17.5 wt% aqueous solution of NaOH, and the lignin content was determined with the sulfuric acid method, according to Komarov's modification.[27] The hydrolysis yielded lignocellulose samples in a powder form. Microcrystalline cellulose (MCC) was prepared from cotton cellulose at a laboratory scale as described elsewhere[34] and was characterized by the same methods.

Dissolution of powder samples

The powder lignocellulose samples from flax fibres (PF), hardwood pulp (PH) and MCC were submitted to dissolving in DMAc/LiCl. For each sample, at least three sub-samples were studied. The dissolution was performed according to a procedure described elsewhere[35,36] and was adapted to the powder samples prepared.[27] The solutions were prepared in several steps, since the dissolution of lignocellulosics in DMAc/LiCl strongly required a pretreatment of the samples.

Sample pretreatment

The air-dried sub-sample (1 g) was suspended in 200 ml of deionised water with continuous stirring for 2 h at an ambient temperature. The excess water was filtered through a glass filter (10 μm pore size), the sample was rinsed twice with DMAc, then was placed in a flask with 10 ml of DMAc and left overnight. Then, the excess DMAc was removed by filtration through a glass filter, and the sample was ready for the dissolution.

Procedure

100 cm^3 of DMAc was poured into a round-bottom three-neck flask and the flask was heated to 165 °C. A portion of the pretreated sample was added to DMAc to give a solution with the concentration of 1.0-5.0 wt%

and was stirred continuously with a glass impeller for 2 h. The resulting suspension was refluxed for 30 min, then LiCl was added to obtain an 8.0 wt% solution. The mixture was kept under stirring for 4 h, yielding a clear or semi-clear solution. The solution was cooled to room temperature and filtered through a glass filter in order to separate the insoluble solids.

Regeneration of samples from DMAc/LiCl solutions. Aggregation of solutions, spontaneous gelation and formation of hydrogels

To obtain the hydrogels, a special procedure was developed for the regeneration of the samples from the solutions. The shape and properties of the regenerated samples strongly depend on a number of parameters, such as concentration of the solutions, regenerating mode during the absorption of the solvent and/or subsequent aggregation, time for gel formation and drying procedure. To monitor the gelation behavior of the samples, we altered their concentrations in the solutions of DMAc/LiCl from 0.5 to 5.0 wt%.

The multistage experimental processing to prepare the hydrogels is depicted in Scheme 1. Digital images of the processing are shown in Fig. 1. Every solution was poured in a Petri dish or in a plastic vessel and left under ambient conditions for 5-7 days. In order to avoid contaminations, we used a special chamber with a coating covering the dishes completely, besides the free space for the dishes. Air access to the chamber was free. Every sample was weighed on the dish at the beginning of the casting and then twice a day. The gel was formed spontaneously by absorbing the solvent and finally the weight of the swollen sample did not change any more.

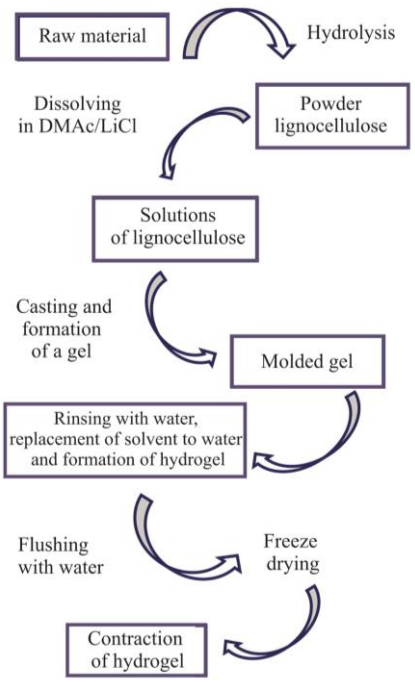
To release the rest of the solvent from the dish with the prepared gel, the gel was pulled out from the solution and thoroughly rinsed successively with cold and hot water several times. Finally, the gel was kept in cold water for several days, and the water was changed every day. This led to a replacement of the solvent by the water. The obtained hydrogel was stored in water and was stable for as long as needed.

Depending on the objectives, the hydrogels were air-dried under ambient conditions, dried in a vacuum oven at 40 °C or freeze-dried.

Characterizations

Dissolving ability of powder samples

The dissolving ability was monitored gravimetrically by estimating the decrease in weight of the powder sample and the weight of the rinsed and dried residue after filtration. The dissolving ability (D_a, %) was calculated from the equation: $D_a = [(W_p - W_r) \cdot W_p^{-1}] \cdot 100$, where W_p is the weight of the powder sample and W_r is the weight of the insoluble residue. The measurements of D_a were made in duplicate.



Scheme 1: Multi-step hydrogel processing

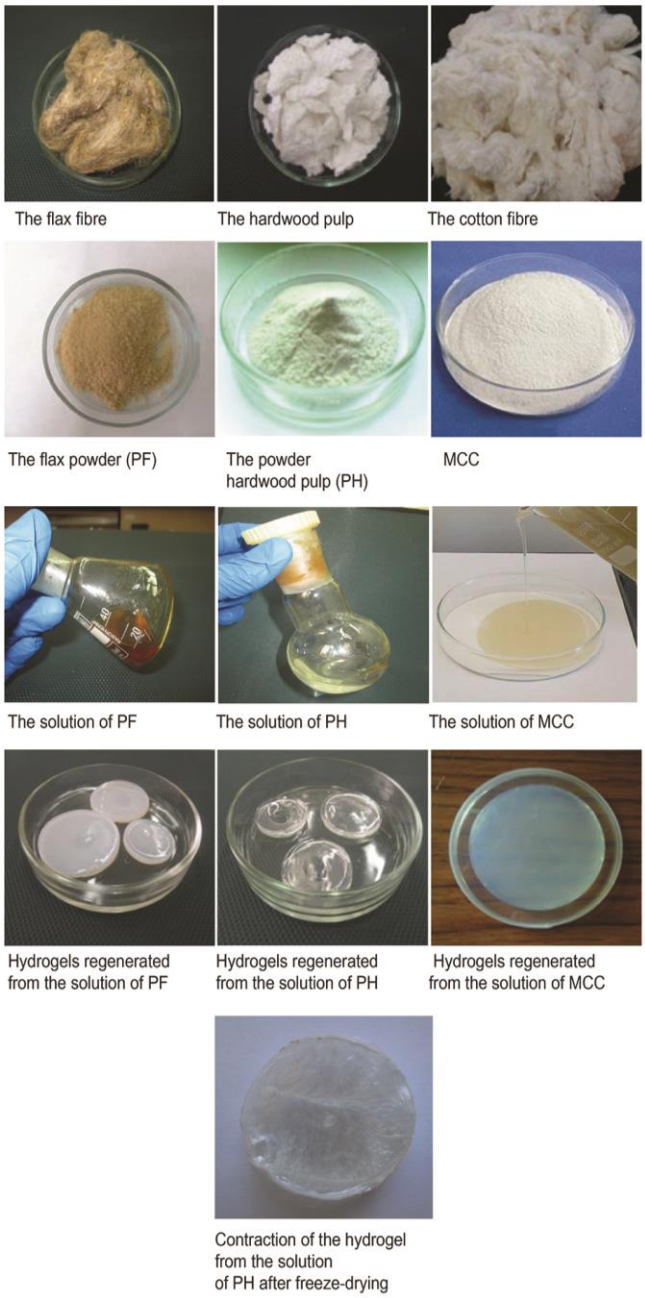


Figure 1: Digital images of hydrogel processing
(the concentration of samples in the solutions of DMAc/LiCl was 1 wt%)

Equilibrium solvent content in gels and water content in hydrogels

The solvent absorbed by the gels or the water absorbed by the hydrogels are quantitatively represented by the equilibrium solvent content (*ESC*) or equilibrium water content (*EWC*), by using the equation below:[37]

$$ESC \text{ or } EWC = (m_s - m_0) \cdot m_s^{-1}$$

where m_s is the mass of the swollen gel or hydrogel at time t at the equilibrium, and m_0 is the mass of the gel dried to a constant weight in vacuum at 40 °C. The *ESC* and *EWC* values were expressed as an average of three measurements.

Gel porosity

The overall porosity of the hydrogels, i.e. proportion of sample volume occupied by pores, was calculated, according to the following equation:

$$P_t = (1 - m_0) \cdot P^{-1} \cdot L^{-1} \cdot \rho^{-1}$$

where m_0 is the weight of the hydrogel dried as described in the previous section, P is the square of the surface of the hydrogel, cm^2, L is the thickness of the hydrogel, cm, and ρ is the density of cellulose (ρ=1.561 kg m^{-3}).[38] The hydrogels were regenerated from the solutions of PF, PH or MCC with the concentration 3 wt%. The P_t values were calculated as an average of two measurements.

Dye sorption equilibrium experiment

Batch sorption studies were applied in all sorption experiments. The powder samples PF, PH and MCC, the hydrogels and the freeze-dried hydrogels were studied. Direct dye, Methylene Blue (MB), used in sorption studies was purchased from Mosrektiv, Russia. Solutions of the dye with a concentration 10^{-4} M were prepared in distilled water by diluting the concentrated stock solution. The mass of the absorbent and the volume of the solution were kept constant for each set of experiments. The time required for the absorption to reach equilibrium under ambient conditions was 24 h. The hydrogels were used in a known volume of dye solution until the equilibrium was reached. After the sorption, the dye solution was separated from the hydrogels by decantation. The photocolorimetric method was applied for determining the concentrations of the dye solutions in the hydrogels. The measurements were carried out with a SF-2000 photocolorimeter (FEK OKB "Spektr", Russia) at the ambient temperature. The absorbances of the dye solutions were read at 607 nm, using a 3 mm thickness of the absorbing layer. Distilled water was chosen as a reference. The equilibrium concentrations of the dye solutions were determined with precalibrated scales. The maximum value of the MB uptake, i.e. maximum absorption capacity of the hydrogels (A_{max}, $mg\ g^{-1}$), was calculated as the difference in the concentration of MB before and after absorption, according to the equation:[39]

$$A_{max} = (c_0 - c_{eq}) \cdot V \cdot W_p^{-1}$$

where c_0 is the concentration of the solution of MB before the absorption, mg l^{-1}, c_{eq} is the concentration of the solution of MB after the absorption, mg l^{-1}, V is the volume of the solution of MB taken to the absorption, l, W_p is the mass of the powder, hydrogel or freeze-dried hydrogel, g. A_{max} was expressed as an average of two measurements.

Specific surface area of hydrogels

The specific surface area (SSA) of the hydrogels was calculated as the total surface of the sample that was in contact with the solution of MB. It was expressed as SSA ($g \cdot m^{-2}$) of the sample. The mean area occupied at the solid-liquid interface by a molecule of MB corresponding to a monomolecular layer on the surface was used as 130 Å (X_m). SSA was calculated with the equation below:[38,40]

$$SSA = X_m \cdot N \cdot A_{max} \cdot M_w^{-1}$$

where X_m is the monolayer capacity of MB (mole g^{-1}), N is Avogadro's number, mol^{-1}, A_{max} is the maximum absorption capacity of the hydrogel, M_w is the molecular weight of MB. SSA was expressed as an average of three measurements.

Solubility of dried hydrogels

The solubility of the freeze-dried hydrogels was examined qualitatively in water, 17.5% aqueous solution of NaOH, ethanol, acetone, toluene, 86% solution of H_3PO_4 and 72% solution of H_2SO_4. The samples were kept in the media under ambient conditions for 24 h. To determine the solubility in cadoxen, which is usually used to determine the viscosity of the cellulosics, the samples were kept for dissolving at 0 °C for 24 h.

Swelling study of dried hydrogels

The tablet shaped freeze-dried hydrogels were immersed in distilled water with pH 6.0, using water as a standard solution and solutions with the pH ranging from 2.0 to 10.0 at room temperature for 24 hours. The pH was adjusted to 2.0 using a NaOH stock solution and to 10.0 using a HCl stock solution by diluting the stock solutions with distilled water. The pH value was controlled with a pH meter pH-150 MI ("IT", Russia). Swollen hydrogels were removed from the water or the solutions with different pH by filtration through a glass filter under constant vacuum, were wiped superficially with filter paper and weighed. The weight equilibrium swelling ratios Q were calculated using the following equation:

$$Q = (m_g - m_r) \cdot m_r^{-1}$$

where Q is the swelling ratio, $g \cdot g^{-1}$, m_r is the mass of the removed hydrogel and m_g is the mass of the swollen hydrogel at equilibrium at a given pH. Experiments were run in duplicate.

WAXS measurements

WAXS measurements were performed on a Huber 420/511 four-circle goniometer. A ground and bent germanium monochromator (reflection 111) was used to select the $CuK_{\alpha 1}$ radiation (λ=1.541Å). The diffractometer was used in the symmetrical transmission mode. The scattered photons were detected by a NaI (Tl) scintillation counter. The intensity was measured as a function of the scattering angle 2θ by θ-2θ scan.

Scans were obtained from 5 to 50°. For better comparison of the samples, the pristine powder samples were also subjected to WAXS characterization, along with the hydrogels regenerated from the solutions. The powder samples (100 mg) were pressed in an ad hoc mold to form pellets. The swollen samples were measured between two thin Mylar films with a thickness of 0.00610 mm (DuPont Teijin Hopewell, VA, China).

Macroscopic digital images

Digital images of the samples were obtained with Canon Power Shot A3400 IS and Canon Power Shot SX20 IS digital cameras (both manufactured in Japan). The samples were photographed in normal light.

RESULTS AND DISCUSSION

Stages of gelation

The preparation of the gels was a multistage process (Fig. 1, Scheme 1), and each stage required an appropriate method. The main stages were as follows:

- the hydrolysis of raw materials and the preparation of the powder samples;
- the pretreatment of the powder samples and their dissolution in a solution of DMAc/LiCl;
- aging of the solutions at ambient atmosphere; slow aggregation accompanied by spontaneous gelation; formation of the gels;
- removal of the solvent by rinsing with water and replacement of the solvent by water, formation of the hydrogels;
- drying or freeze-drying of the hydrogels.

Each stage in turn also consisted of several steps. However, the entire process was adequate and feasible.

Some characteristics of powder samples and dissolving values

The main characteristics of the powder samples subjected to dissolution in DMAc/LiCl have been described elsewhere.[27] Shortly, the properties are listed in Table 1. The characteristics of the samples differed, i.e. MCC was chemically "purer" and had a lower DP_v compared to PF and PH. However, those samples exhibited higher dissolving ability, while MCC dissolved to a lesser extent. Obviously, the ordered supramolecular structure and the high crystallinity of MCC essentially impacted the dissolving ability. Nevertheless, all the samples dissolved to 94.8-98.7 wt%.

After the dissolution, the solution of PH was optically clear and colorless, the solution of PF was dark-yellow colored and slightly opalescent, and the solution of MCC was somewhat turbid (Table 1).

Equilibrium solvent content in the gels and water content in the hydrogels

ESC is an important characteristic of the swollen gels. Table 2 shows the data for the just received gels containing the solvent (*ESC*) and for the hydrogels containing water after the solvent was replaced by water (*EWC*). The gels and the hydrogels seemed to be super-swollen systems and retained large amounts of the solvent or water. The *EWC* for the hydrogel prepared from the solution of PF was 2500 wt% and for the hydrogel prepared from the solution of PH was 2800 wt%. MCC had lower *EWC* (2000 wt%).

Table 1
Characteristics of powder samples and solubility in DMAc/LiCl

Samples	Content of main components, %		DP_v	D_a, %
	Lignin	α-Cellulose		
PF	3.4	88.4	270	97.7
PH	4.0	79.5	240	98.7
PC	0	98.7	170	94.8

Table 2
Maximum values of *ESC* in gels and *EWC* in hydrogels and porosity (P_t) of hydrogels

Samples	*ESC*, wt%	*EWC*, wt%	P_t, %
PF	2700	2500	98.9
PH	3300	2800	97.4
MCC	2150	2000	86.8

Table 3
SSA of powder samples, swollen hydrogels, freeze-dried hydrogels, A_{max} of swollen hydrogels and *Q* of freeze-dried hydrogels

Source	*SSA*, $m^2 \cdot g^{-1}$			A_{max}, $mg \cdot g^{-1}$	*Q*, $g \cdot g^{-1}$		
	Powders	Swollen hydrogels	Freeze-dried hydrogels		pH 2.0	pH 6.0	pH 10.0
PF	4.7	41	5.5	4.1	4.2	3.1	5.7
PH	5.7	45	6.2	4.8	4.4	3.4	5.5
MCC	12.7	38	4.8	3.9	3.8	2.8	4.9

The absorbance of the samples was calculated as the ratio of absorbed water (mol) to one hydroxyl group of the anhydroglucose unit of cellulose (AGU), varied from 75 to 85 H_2O_{mol}/OH_{group}, depending on the concentration of the hydrogel. At first sight, these values of water retention seem to be unusually high. However, they are similar to those of bacterial cellulose.[42] This makes possible the actual application of hydrogels from solutions of lignocelluloses in chemical, pharmaceutical and medical domains.

Note that all the samples retained small amounts of water after drying, irrespectively of the method of drying. The freeze-dried hydrogel of PH retained 0.27 g $H_2O \cdot AGU^{-1}$ and the air dried hydrogel retained 4.2 g $H_2O \cdot AGU^{-1}$. The freeze-dried hydrogel of PF retained 2.1 g $H_2O \cdot AGU^{-1}$ and the air dried hydrogel retained 5.5 g $H_2O \cdot AGU^{-1}$, respectively. The dried hydrogels of MCC retained 2.4 and 5.7 g $H_2O \cdot AGU^{-1}$, respectively. The results obtained reveal that the drying strongly impacted the water retention of the hydrogels and the so-called tightly bound water was retained differently in the hydrogels of various origins.

Porosity of hydrogels

The overall porosity values for the hydrogels are shown in Table 2. The concentration of the solutions was 3 wt%. The porosity values were rather high. The highest P_t was that of the hydrogel of PH, the values being lower for the hydrogels of PF and MCC. The P_t values correspond to those for bacterial cellulose and are higher than those for bead cellulose and for some modified or crosslinked cellulosics.[19,43,44]

Specific surface area of powders and hydrogels on the basis of dye sorption equilibrium experiments

The results obtained by determination of P_t of the samples were confirmed with the dye sorption equilibrium experiments. The *SSA* of the powder PF, PH and MCC, the hydrogels and the freeze-dried hydrogels, A_{max}, due to MB uptake by the swollen hydrogels, are listed in Table 3. The *SSA* values for the powders were arranged in the following series: MCC>PH>PF. The highest *SSA* was that of MCC. However, the series of *SSA* for the hydrogels and for the dried hydrogels looks different: PH>PF>MCC. The hydrogel of PH had the highest *SSA* both in the swollen state and in the dried one.

It is known that cellulose belongs to a class of capillary-porous systems presenting a highly branched inner and outer surface of macro- and micropores, and of dead-end pore voids. The values of *EWS*, P_t, *SSA* and A_{max} showed that the hydrogels, due to the porous system, can be successfuly applicable for medical purposes when the absorption of significant contents of liquids is necessary.

Dissolving capacity of dried hydrogels

While drying, the swollen hydrogels collapsed. Drying crucially impacts the properties of the hydrogels, especially, their dissolving capacity. The dissolution study of the freeze-dried hydrogels in

different media showed that they did not dissolve in water, 17.5% aqueous solution of NaOH, ethanol, acetone, partly hydrolysed in the solution of H_2SO_4 and partly dissolved (to 80-90 wt%) in the solution of H_3PO_4. They swelled poorely in toluene. Moreover, the dried hydrogels did not dissolve in the same solution of DMAc/LiCl that was applied for the dissolution of the pristine lignocellulose powders. This means that strong bonds among the structural links or macromolecules of the regenerated samples are formed. When a three-dimensional structure is formed, the solubility of the samples would be low, but the swelling capacity may be maintained. To confirm this assumption, the solubility of the freeze-dried hydrogels in cadoxen solution was assessed. It appeared that the samples left to dissolve at 0 °C for 24 h swelled partly but did not dissolve.

Equilibrium swelling of dried hydrogels in solutions of different pH

Equilibrium swelling studies were performed with the objective of determining the effect of pH on the swelling capacity of the hydrogels. It appeared that *Q* values depend on the pH of the solutions. The highest *Q* values were observed for all hydrogels at pH 10.0, *Q* values were lower at pH 2.0 and the lowest *Q* values were recorded for water with pH 6.0 (Table 3). Since chemical processing is usually accompanied by swelling, hydrogels can be refered to "smart" substances, due to their pH-dependent chemical properties. This specifity of the hydrogels allowed basically their application for wide range of various practical purposes.

Crystalline structure of reference powder lignocelluloses and regenerated samples studied with WAXS measurements

X-ray diffraction intensity profiles (XDP) of the reference powder lignocelluloses PF and PH are shown in Fig. 2. The X-ray patterns presented peaks characteristic of the cellulose I crystal structure, which is the native cellulose allomorph,[45] although the peak widths are similar among the samples. The relative intensity of the peaks was slightly different. The degree of crystallinity, according to Segal's method,[46] was 0.68 for PH and 0.64 for PF, and the peak width, according to Scherer's equation,[45] was 4.15 and 4.10, respectively. Fig. 3 presents the XDP of the super-swollen hydrogel from the solution of PH and for comparison the XDP of water. The X-ray pattern of the hydrogel did not reveal any reflections of cellulose lattice. Both patterns contained smear halos in a 2θ range from 20° to 45°, although they revealed no difference in the XDPs. This means that the concentration of HP in the hydrogel was very low and lignocellulose held a great amount of the water. Therefore, it was not possible to estimate any crystalline parameters with the measuring using WAXS. Obradovic *et al*.[24] studied swelling of softwood pulp followed by moulding and regeneration with two different anti-solvents. The results showed that the crystalline structure of softwood pulp was lost after swelling in DMAc/LiCl and solidifying with distilled water. Duchemin *et al*.[47] studied the transformation of microcrystalline cellulose due to partial dissolution in 8% DMAc/LiCl and explained the disappearance of the crystalline diffraction in specimens solidified in distilled water, indicating that slow solidification produced crystallites of small size or with imperfections in the crystalline structure.

Thus, the described operation was a multi-step process, as schematically shown in Scheme 2. The first stage was the dissolution of the reference powder lignocelluloses in the DMAc/LiCl solution. The web of intra- and intermolecular H-bonds in the lignocellulose chains were ruptured in the solutions and free cellulose chains were released. The next stage was gelation and finally the formation of the hydrogels. These stages were performed by spontaneous self-assembly and aggregation of the lignocellulose chains under ambient conditions. The prepared swollen gels were subjected to rinsing with water, followed by the replacement of the solvent by water. The super-swollen hydrogels retained large amounts of water and were stable as long as needed. Obviously, a new web of H-bonds between OH-groups and the water was reconstructed. However, the stable matters crucially collapsed while drying, and formed superhard and poorly accessible structures.

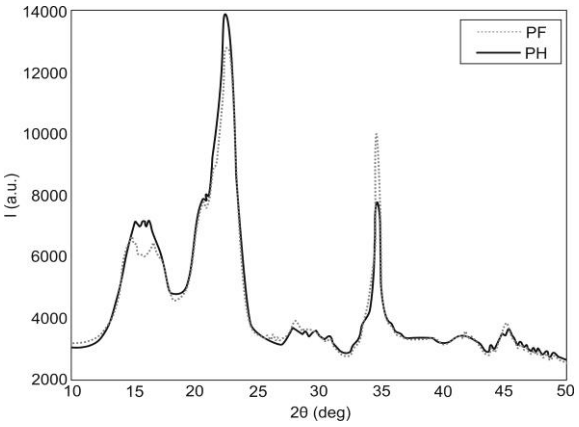


Figure 2: X-ray diffraction intensity profiles of powder lignocelluloses PF and PH

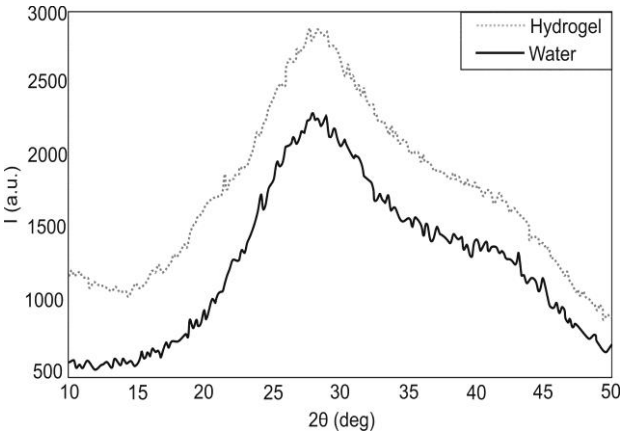
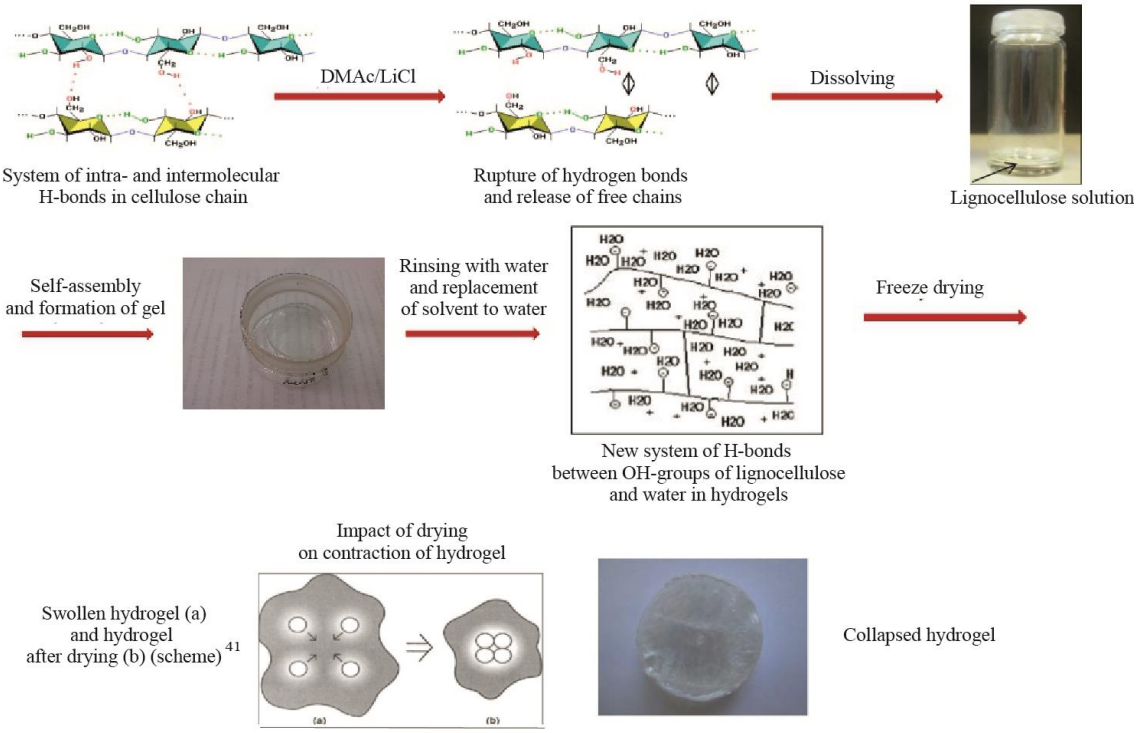


Figure 3: X-ray diffraction intensity profiles of hydrogel of PH and water



Scheme 2: Detailed scheme and visualization of hydrogel processing

CONCLUSION

We have successfully obtained *in vitro* stable super-swollen hydrogels. The hydrogels were prepared from powder lignocelluloses of various origins via direct dissolution in DMAc/LiCl, followed by subsequent regeneration from the solutions. The main properties of the swollen hydrogels, such as equilibrium solvent content, porosity and dye uptake, were determined. The hydrogels retained large amounts of water (up to 2500 wt%), had high porosity and specific surface areas. The superabsorbance of the hydrogels was confirmed by WAXS results. The hydrogels seem to be "smart" matters due to their pH-dependent behaviour, as demonstrated by the swelling experiments. The formation of the hydrogels occured via spontaneous self-assembly from the solutions, due to the reconstruction of a new hydrogen bond web between the lignocellulose chains and the water. The hydrogels were stable, but crucially changed their morphology after drying, due to water removal and collapse of the H-bonds formed. These findings demonstrate the potential of lignocellulose hydrogels as novel attractive materials with possible applications in a wide range of domains. The behavior of the hydrogels needs further studies due to its complexity.

REFERENCES

[1] A. Sannino, C. Demitri and M. Madaghiele, *Materials*, **2**, 353 (2009).
[2] H. Kono, S. Fujita and I. Oeda, *J. Appl. Polym. Sci.*, **127**, 478 (2013).
[3] W. B. Wang, J. X. Xu and A. Q. Wang, *eXPRESS Polym. Lett.*, **5**, 385 (2011).
[4] M. Ebara, Y. Kotsuchibashi, R. Narain, N. Idota, Y.-J. Kim *et al*., "Smart Biomaterials", NIMS Monographs, National Institute for Materials Science, Springer, Japan, 2014, pp. 9-65.
[5] G. A. Paleos, "What Are Hydrogels?" Pittsburgh Plastics Manufacturing, Butler, PA Copyright Pittsburgh Plastics Manufacturing Inc., 2012.
[6] S. K. H. Gulrez, S. Al-Assaf and G. O Phillips, "Hydrogels: Methods of Preparation, Characterisation and Applications. Progress in Molecular and Environmental Bioengineering – From Analysis and Modeling to Technology Applications", edited by A. Carpi, InTech, 2011, available at <www.intechopen.com>.
[7] O. Okay, "General Properties of Hydrogels, Hydrogel Sensors and Actuators", edited by G. Gerlach and K.-F. Arndt, Springer Series on Chemical Sensors and Biosensors, Springer-Verlag, Berlin Heidelberg, 2009, pp. 1-14.
[8] W. E. Hennink and C. F. van Nostrum, *Adv. Drug Deliv. Rev.*, **54**, 13 (2002).
[9] J. M. Rosiak and F. Yoshii, *Nucl. Instrum. Meth. B*, **151**, 56 (1999).
[10] J. Zhu and R. E. Marchant, *Expert. Rev. Med. Devices*, **8**, 607 (2011).
[11] Y. Sekiguchia, C. Sawataria and T. Kondo, *Carbohyd. Polym.*, **53**, 145 (2003).
[12] Y. Qing, Y. Wu, Z. Cai and X. Li, *J. Nanomater.*, **2013**, Article ID 594734, (2013), doi: 10.1155/2013/594734.
[13] J. Watanabe, Y. Kiritoshi, K. W. Nam and K. Ishihara, in "Encyclopedia of Biomaterials and Biomedical Engineering", Marcel Dekker, Inc., 2004, pp. 790-801.
[14] W. Wang, J. Wang, Y. Kang and A. Wang, *Compos.: Part B-Eng.*, **42**, 809 (2011).
[15] S. Beck-Candanedo, M. Roman and D. G. Gray, *Biomacromolecules*, **6**, 1048 (2005).
[16] D. Pasqui, M. De Cagna and R. Barbucci, *Polymers*, **4**, 1517 (2012).
[17] T. A. Salah El-Din, A. A. Elzatahry, S. S. Aldeyab and E. A. Kamoun, *Afr. J. Biotechnol.*, **11**, 13058 (2012), available at <http://www.academicjournals.org/AJB>.
[18] Y. Chen, Y. Liu and H.-M. Tan, *BioResources*, **3**, 247 (2008).
[19] M. Pandey, M. C. I. M. Amin, N. Ahmad and M. M. Abeer, *Int. J. Polym. Sci.*, (2013) Article ID 905471, available at <http://dx.doi.org/10.1155/2013/905471>.
[20] B. Wickleinab and G. Salazar-Alvarez, *J. Mater. Chem. A*, **1**, 5469 (2013).
[21] H. Kono and M. Zakimi, *J. Appl. Polym. Sci.*, **128**, 572 (2013).
[22] J. Zhou, C. Chang, R. Zhang and L. Zhang, *Macromol. Biosci.*, **7**, 804 (2007).
[23] R. Ariño, M. Brodin, A. Boldizar and G. Westman, *BioResources*, **8**, 2 (2013).
[24] J. Obradovic, H. Wondraczek, P. Fardim, L. Lassila and P. Navard, *Cellulose*, **21**, 4029 (2014).
[25] M. Kostag, T. Liebert and T. Heinze, *Macromol. Rapid Commun.*, **35**, 1419 (2014).
[26] H. Aono, D. Tatsumi and T. Matsumoto, *J. Polym. Sci. B: Pol. Phys.*, **44**, 2155 (2006).
[27] N. E. Kotelnikova, Yu. V. Bykhovtsova, A. M. Mikhailidi, M. V. Mokeev, N. N. Saprykina *et al*., *Cellulose Chem. Technol.*, **48**, 643 (2014).
[28] H. Saito, A. Sakurai, M. Sakakibara and H. Saga, *J. Appl. Polym. Sci.*, **90**, 3020 (2003).
[29] D. Ishii, D. Tatsumi, T. Matsumoto, K. Murata, H. Hayashi *et al*., *Bioscience*, **6**, 293 (2006).
[30] R. J. Moon, A. Martini, J. Nairn, J. Simonsen and J. Youngblood, *Chem. Soc. Rev.*, **40**, 3941 (2011).
[31] T. P. Shcherbakova, N. E. Kotelnikova and Yu. V. Bichovtzova, *Russ. J. Bioorg. Chem.*, **38**, 689 (2012).
[32] N. E. Kotelnikova, G. A. Petropavlovskii, V. A. Shevelev, L. A. Volkova and G. G. Vasil'eva, *Cellulose Chem. Technol.*, **10**, 391 (1976).
[33] A. V. Obolenskaya, Z. P. El'nitskaya and A. A. Leonovich, "Laboratornye raboty po khimii drevesiny i tsellyulozy" (Laboratory Works on Wood and Cellulose Chemistry), Moscow, Ekologiya, 1991.
[34] G. A. Petropavlovsky and N. E. Kotelnikova, *Acta Polym.*, **36**, 118 (1985).
[35] U. Henniges, M. Kostic, A. Borgards, T. Rosenau and A. Potthast, *Biomacromolecules*, **12**, 871 (2011).
[36] A. Potthast, Th. Rosenau, R. Buchner, Th. Röder, G. Ebner *et al*., *Cellulose*, **9**, 41 (2002).
[37] S. Kundakçi, E. Karadağ and Ö. B. Üzüm, *J. Encapsul. Ads. Sci.*, **1**, 7 (2011).
[38] S. J. Gregg and K. S. W. Sing, "Adsorption, Surface Area and Porosity", Academic Press, London and New York, 1967.
[39] B. B. Tewari and C. O. Thornton, *Rev. Soc. Quím. Perú*, **76**, 4 (2010).
[40] F. Asghari, M. Jahanshahi and A. A. Ghoreyshi, *J. Chromatogr. A*, **1242**, 35 (2012).
[41] A. K. Bajpai, S. K. Shukla, S. Bhanu and S. Kankane, *Progress Polym. Sci.*, **33**, 1088 (2008).
[42] L. Fu, Y. Zhang, J. Zhang and G. Yang, in: "Bacterial Cellulose for Skin Repair Materials, Biomedical Engineering – Frontiers and Challenges", edited by R. Fazel, InTech, 2011, pp. 249-275.
[43] J. Huo, Y. Zheng, S. Pang and Q. Wang, *Cellulose*, **20**, 841 (2013).
[44] K. Leppanen S. Andersson, M. Torkkeli, M. Knaapila, N. Kotelnikova *et al*., *Cellulose*, **16**, 999 (2009).
[45] L. Segal, J. J. Creely, A. E. Martin Jr. and C. M. Conrad, *Text. Res. J.*, **29**, 786 (1962).
[46] P. Scherrer, *Göttinger Nachrichten Gesell.*, **2**, 98 (1918).

[47] B. J.-C. Z. Duchemin, R. H. Newman and M. P. Staiger, *Cellulose*, **14**, 311 (2007).