Intercalation of the Microbial Biopolymers Welan Gum and EPS I into Layered Double Hydroxides

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Three microbial polysaccharides, namely welan gum, scleroglucan, and EPS I, a novel polysaccharide obtained from a newly isolated bacillus species with structural similarities to xanthan gum, were employed in the fabrication of bio-nanocomposites based on layered double hydroxides (LDH). Synthesis was performed by direct co-precipitation of $Zn(NO_3)_2$ and $Al(NO_3)_3$ in the polysaccharide solutions at pH $\sim$ 8.5. The reaction products were characterized by powder X-ray diffraction (XRD), elemental and thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), and scanning and transmission electron microscopy (SEM and TEM). It was found that welan gum is successfully intercalated into the Zn–Al–LDH structure, giving a *d*-spacing of 2.38 nm for the interlayer distance, while neutral scleroglucan failed to be intercalated. Instead, this biopolymer was only surface-adsorbed on inorganic CaAl–OH–LDH platelets, as was evidenced by de-washing experiments. These results indicate that the anionic functionality of the polysaccharides presents a main driving force behind their intercalation. In contrast to regular xanthan gum, EPS I was intercalated into the LDH structure to give a sharp X-ray reflection representing a *d*-spacing of 2.77 nm. This behavior proves that slight modifications of the polysaccharide can greatly improve its intercalation ability.

Key words: Polysaccharide, Layered Double Hydroxide, Intercalation, Welan Gum, Scleroglucan

Introduction

Polysaccharides are widely used auxiliaries in a variety of industrial applications which include food preparations [1], cosmetics and health products, building materials and petroleum recovery systems. There, they are employed as viscosifiers, gelation agents and stabilizers for aqueous systems. Polysaccharides, or gums as they are also referred to, can produce gels or act as emulsion stabilizers [2], flocculants [3], film formers [4, 5], binders [6], lubricants [7], and friction reducers [8]. This makes them useful in many different areas such as functional ingredients in food products [9], thickeners for paints and coatings [10], health and cosmetic [11] products, and even oilwell cementing [12]. Early on, most of these polysaccharides were extracted from higher plants or marine algae [13]. Since the 1960's, however, there has been an increased interest and industrial success in the utilization of extracellular polysaccharides produced by fermentation employing microorganisms such as bacteria or fungi. Increasing demand has cast the focus on this class of polysaccharides, and recently, much research has been invested in genetically modified gums with enhanced performances.

With the improvement in technology, researchers have developed systems whereby these polysaccharides are entrapped *e. g.* by intercalation into layered compounds, and can be released on demand. Such systems have become quite popular in the medical field such as in drug delivery [14] or where a controlled change in viscosity of the system is demanded. One prominent host matrix to enable such timed release is presented by the group of layered double hydroxides (LDHs). There, intercalated biopolymers can be released promptly under designated conditions, *e. g.* through anion exchange [15].

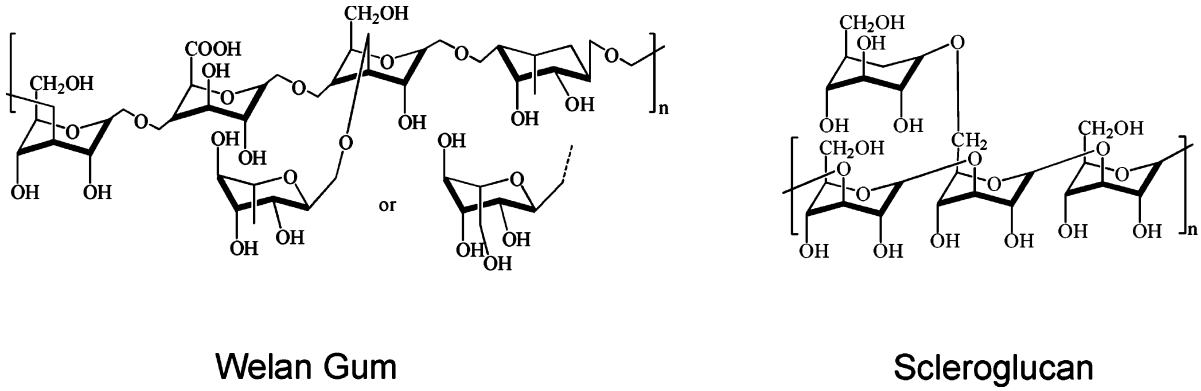

Fig. 1. Chemical structure of the microbial polysaccharides welan gum and scleroglucan.

In our study, three different microbial polysaccharides, welan gum, scleroglucan and a novel polysaccharide with structural similarities to xanthan gum named EPS I were selected for intercalation experiments utilizing the Zn–Al–LDH system as host structure. The chemical formulae of welan gum and scleroglucan are presented in Fig. 1. The polysaccharides differ in their chemical structures, and thus in their charge and molecular arrangement (secondary and tertiary structures) which they adopt in solution. These fundamental differences determine the variation in solubility and the tolerance against divalent ions, especially Ca^{2+}, and the rheological behavior in aqueous solution [16].

Welan gum is a biopolymer produced by bacteria from the *Alcaligenes* species (ATCC-31555). It possesses a main chain which is made up of repeating units of (1→4)-linked glucose, glucuronic acid, glucose and L-rhamnose. A side chain per repeating unit is (1→3) linked to the second component of the glucose repeating unit [17, 18]. The side chain consists of only one monosaccharide, either L-rhamnose or L-mannose (Fig. 1). Due to the carboxyl groups present in the glucuronic acid, welan gum has an anionic backbone and under alkaline pH possesses a negative charge. In aqueous solution, welan gum exists as a stiff double helix whereby the side chains form hydrogen bonds with the carboxylic groups along the trunk of the biopolymer [19]. Scleroglucan, on the other hand, is a branched, neutral homopolysaccharide. The polymer consists of a linear main chain of β-D-(1→3)-glucopyranosyl units and β-D-glucopyranosyl units which are (1→6) linked to every third unit [20]. The chemical structure of the tetrasaccharide repeating unit of scleroglucan is displayed in Fig. 1. In aqueous solution, scleroglucan forms a triple helix, whereby the glucose side groups protrude and prevent the helices from aggregating [21]. EPS I represents a new biopolymer with structural similarities to xanthan gum. It was isolated from a fermentation broth of a newly isolated bacillus sp. and possesses a linear backbone with protruding side chains. The major sugar components are glucose, mannose, galactose, and glucuronic acid (3 : 2 : 1 : 1 resp.). The backbone contains glucuronic acid, while the side chains consist of neutral sugars which are partially ketalized with pyruvate so that in average there is one carboxylic acid group in four carbohydrate monomer residues. Thus, its anionic charge density is half that of xanthan gum. However, there is a significant difference between EPS I and xanthan gum in that in the latter molecule, the uronic acids bearing the anionic charge are located in the side chains whereas in EPS I, they are situated in the trunk chain.

For the LDH host structure, the Zn–Al–LDH system was selected because of the relatively low pH of 8.5 $\sim$ 9 required for its synthesis. Under those pH conditions, the biopolymer samples employed here are sufficiently stable. Feasibility of incorporation of the three biopolymers into the $[Zn_2Al]$-LDH structure was explored through the co-precipitation synthesis method. The resulting reaction products were then characterized by XRD, elemental analysis, TG/DSC, SEM and TEM techniques. The intercalation ability of these biopolymers was compared, and a conclusion was drawn regarding the impact of their anionic charge and molecular structure (steric position of the carboxylate group) on their intercalation tendency.

Table 1. Specific anionic charge of the biopolymers.

Biopolymer	Specific anionic charge, q (μeq g^{-1})
Scleroglucan	67
Welan gum	726
EPS I	745

Results and Discussion

Properties of the biopolymers

All biopolymers exhibited a molecular weight of $\sim 10^5$ g mol^{-1}. Due to the similarity in molecular weight of the biopolymers, this property was excluded as an influencing factor in the ability of these biopolymers to intercalate.

Next, the specific anionic charge of the biopolymers was measured as the ease of intercalation often correlates with the charge of the substrate. The results are presented in Table 1. Scleroglucan displayed the lowest anionic charge of 67 μeq g^{-1} only, which identifies it as an almost neutral polysaccharide. The residual negative charge presumably is owed to partial deprontonation of -OH groups. On the other hand, welan gum and EPS I showed a high and almost comparable anionic charge of $\sim 730\,\mu$eq g^{-1}. Both polysaccharides possess only one carboxylate group in their repeating unit. Compared to xanthan gum which possesses two carboxylate groups per repeating unit (anionic charge measured $= 1530\,\mu$eq g^{-1}), EPS I carries only half the charge. And, unlike xanthan gum where the uronic groups are present in the side chains, the anionic charge of EPS I is located along the backbone as in welan gum.

The results instigate that welan gum and EPS I, because of their prominent anionic character, appear to be well dispositioned for successful intercalation, whereas for scleroglucan there may be difficulties to interact with the cationic sheets of the LDH.

Co-precipitation experiments

Co-precipitation is a useful method to incorporate anionic polymers possessing high molecular weight into layered double hydroxides [22]. This method is often carried out under conditions of supersaturation, so as to facilitate optimal 'co-organized assembly' of the LDH sheets in the presence of the polymer to be intercalated. In previous work, successful intercalation of anionic biopolymers including alginate, pectin and carrageenan into Zn–Al–LDH structures has been demonstrated [23, 24]. Surprisingly, in these experiments, intercalation of xanthan gum was almost negligible inspite of its high anionic charge, and the authors attributed this effect to a shielding of the carboxylate groups located in the side chain by the helical structure of dissolved xanthan gum [25]. In our study, two anionic biopolymers, welan gum and EPS I, and charge-neutral scleroglucan were probed to determine the effect of structural modification and anionic charge on the intercalation ability of these biopolymers. The tendency of EPS I to intercalate will be compared to that of a regular xanthan gum sample, which is known to intercalate poorly in [Zn_2Al]–LDH systems, producing precipitates of extremely low crystallinity [25]. All intercalation products were prepared by the co-precipitation method and compared to pristine [Zn_2Al]NO_3–LDH.

X-Ray diffraction analysis

The XRD patterns of [Zn_2Al]NO_3–LDH as well as those of the products obtained in the presence of biopolymers are shown in Fig. 2. For welan gum and EPS I, the increase in the basal spacings confirms successful intercalation into the LDH structure. The d_{00l} spacings were obtained using the first rational orders corresponding to the 00l reflections, with values of 2.38 and 2.77 nm for the welan gum and EPS I intercalates, respectively.

For the [Zn_2Al]–EPS I–LDH nanocomposite, the gallery height (interlayer distance) was 2.29 nm, in contrast to that of xanthan gum which according to the literature exhibits a value of 1.44 nm only [25]. This polysaccharide intercalates so well that no [Zn_2Al]NO_3–LDH is observed as a by-product, and only a minor amount of [Zn_2Al]$CO_3 \cdot n H_2O$ as a result of contamination with CO_2 during analysis was present. For the EPS I nanocomposite, the characteristic low angle reflection was broad, indicating poor crystallinity. This confirms that intercalation of EPS I between the [Zn_2Al]–LDH layers occurs in a quite disordered manner, as can be seen from the high signal to noise ratio. Overall, the successful intercalation of EPS I indicates that a slight genetic modification of the polysaccharide can result in a substantial change in its intercalating ability. Despite the lower anionic charge

of EPS I (only ∼ 50 % of that of xanthan gum), incorporation is drastically higher than with xanthan gum. This signifies that the ease in intercalation of such helical polysaccharides is dependent on the steric position of the anionic functionality. Apparently, intercalation occurs preferentially when the anionic charges are present along the backbone, instead of the side chain.

Likewise, welan gum with its anionic charge present along its backbone, also shows successful formation of a [Zn_2Al]–welan gum–LDH nanocomposite with a gallery height of 1.9 nm. In aqueous solutions loaded with electrolytes, welan gum exists as a half-staggered, parallel double helix, similar to that of gellan [26]. At high pH, the side chains fold back onto the main chain to form hydrogen bonds with the carboxylate groups, thus enhancing the stiffness and stability of the double helix. The welan gum helix possesses a width of 2.08 nm indicating that upon intercalation, it is slightly compressed to be accommodated at an average interlayer distance of 1.9 nm. The presence of a strong reflection representing a d value at 0.87 nm indicates that substantial amounts of [Zn_2Al]NO_3–LDH are formed as a by-product.

In the case of neutral scleroglucan, no reflection at very low 2θ angles was observed which would have indicated a successful incorporation of this biopolymer (Fig. 2). Instead, the characteristic peak for $[Zn_2Al]\cdot(NO_3)\cdot nH_2O$–LDH was detected, accompanied by a second reflection possessing a d_{001} spacing of 0.79 nm, which is characteristic for $[Zn_2Al(OH)_6]\cdot(CO_3)_{0.5}$–LDH, resulting from partial contamination and anion exchange of the sample with CO_2. In aqueous solution, scleroglucan forms a triple helix whereby the lateral glucose units protrude and prevent helices from aggregating. Due to the compactness of the triple helix, this biopolymer exhibits a width of 1.79 nm only, which from its steric size would disposition it well to intercalate between the brucite-like layers. Thus, we attribute the difficulty of scleroglucan to intercalate to the low anionic charge

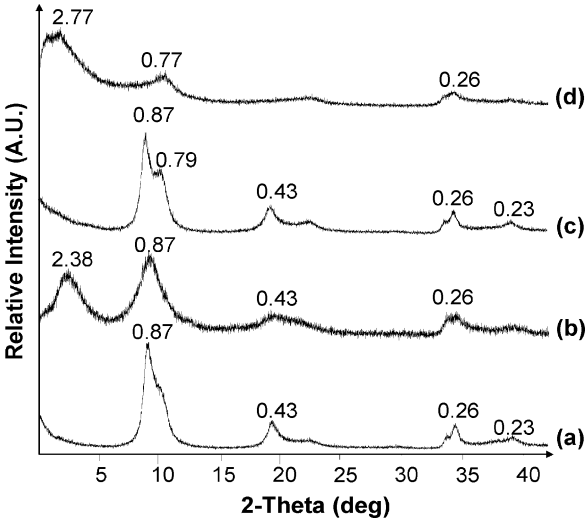

Fig. 2. X-Ray diffraction patterns of (a) $[Zn_2Al(OH)_6]NO_3$–LDH and of reaction products obtained from Zn/Al nitrate and (b) welan gum, (c) scleroglucan, and (d) EPS I (d_{00l} values in nm).

of this biopolymer, confirming that the assembly process for LDH depends on the electrostatic attraction between the host structure and the guest molecules.

In the XRD diagrams, also the (110) reflections representing the inorganic LDH frame are observed at $2\theta \sim 34^\circ$ at small intensity. Sometimes, they are not observed in the XRD pattern as occurs for example with the intercalation of large molecules such as DNA [27, 28]. In the present cases, the (110) signal is clearly observed for all samples.

Elemental analysis

Carbon analysis of the reaction products indicates the degree of incorporation of the biopolymers into the LDH structure. Based on the carbon contents found (Table 2), intercalation of significant amounts (∼ 20 – 25 wt.-%) of biopolymers was confirmed for

Table 2. Chemical analysis of reaction products obtained from co-precipitation of Zn/Al nitrate with the biopolymers (Al/Zn by ICP/AES; biopolymer content from CHNS analysis; water content from TG analysis).

Added polymer	Zn (%)	Al (%)	C (%)	H (%)	N (%)	Zn/Al ratio	Biopolymer (%)	Biopolymer[a] (%)	H_2O (%)	[Zn_2Al]NO_3–LDH (%)
Welan gum	25.7	5.8	12.9	4.3	1.9	1.83	24.4	47.1	16.2	48.2
EPS I	26.6	5.8	8.8	3.9	1.1	1.89	19.5[b]	27.4[b]	16.3	28.7
Scleroglucan	26.4	5.8	11.5	3.6	2.2	1.88	25.2	56.1	15.2	55.1

[a] Amount of pure [Zn_2Al]biopolymer–LDH, free of nitrates; [b] calculation based on chemical composition of xanthan gum.

both welan gum and EPS I. Any significant retention of the polysaccharides from solution was excluded based on a parallel experiment, where a maximum adsorbed amount of 5 wt.-% biopolymer was found when $[Zn_2Al]NO_3$–LDH particles were immerged in aqueous solutions of the biopolymers. From the N analysis, it became evident that all reaction products contain more or less NO_3^- which presumably is bound in the $[Zn_2Al]NO_3$–LDH by-product. Taking these N contents into account, the amounts of $[Zn_2Al]NO_3$–LDH present and the portion of pure $[Zn_2Al]$biopolymers–LDH were calculated (Table 2). From the results it became obvious that the welan gum reaction product was more contaminated with $[Zn_2Al]NO_3$–LDH than the EPS I precipitate, as also derived from the XRD diagram (Fig. 2).

From the anionic charge of the biopolymers (Table 1) and the amounts of polysaccharide present in the products, the contributing charge of the guest molecules was calculated as 356, 204 and 38 *m*eq per 100 g LDH for welan gum, EPS I and scleroglucan, respectively. The anion exchange capacity (AEC) of a LDH possessing a ratio of Zn to Al of 2 is approx. 340 *m*eq per 100 g LDH. Thus, the amount of welan gum intercalated into this LDH structure fully compensates the cationic charge. In contrast to welan gum, the charge contribution from intercalated EPS I was found to be much less at 204 *m*eq per 100 g LDH, indicating that additional charge compensation is required from inorganic anions, OH^- and in less amount of NO_3^-, as evidenced by elemental analysis and XRD data. Accordingly, it can be assumed that concomitant incorporation of the biopolymer with OH^- and NO_3^- occurs.

The XRD analysis has suggested that scleroglucan had failed to intercalate into the LDH structures, presumably because of its very low anionic charge (Table 1). However, from elemental analysis, the amount of scleroglucan found in the co-precipitation product was in the same range as that for the intercalates from welan gum and EPS I. This can be attributed to the

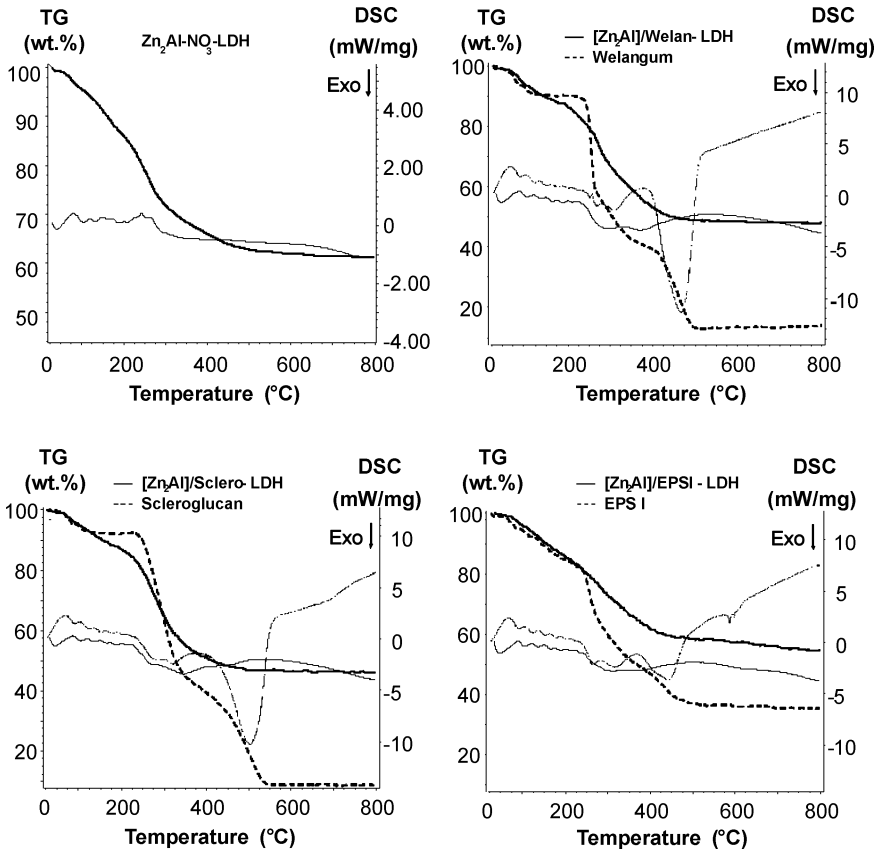

Fig. 3. TG/DSC curves obtained for $[Zn_2Al]NO_3$–LDH and co-precipitation products with welan gum, EPS I and scleroglucan (dashed lines correspond to pure biopolymers while the solid lines refer to the precipitates).

high amount of scleroglucan adsorbed on the surface of the inorganic layers. In a separate experiment, the reaction products with scleroglucan, EPS I and welan gum were washed several times (0.04 g precipitate with 3 g water per wash), and the amount of desorbed polymers was measured in order to eliminate the amount of biopolymers sorbed physically on the surfaces of the inorganic [Zn_2Al]–LDH platelets. After eight washes, only 14 % and 9.9 % of EPS I and welan gum was desorbed, whereas $\sim$ 80 % of the scleroglucan were removed during the wash. This allows to calculate that the co-precipitation products contain 22.0 wt.-% of welan gum, 16.8 wt.-% of EPS I and 5.0 wt.-% only of scleroglucan. The low content of scleroglucan in the final product, as seconded by XRD analysis, distinguishes this biopolymer from the former two. It indicates that surface adsorption is the prevalent mode of interaction for scleroglucan, whereas for welan gum and EPS I, the biopolymers intercalate.

Thermal stability

The TG curve of pristine [Zn_2Al]NO_3–LDH (Fig. 3) shows a mass loss of 16 wt.-% between 25 to 200 °C, which is attributed to the removal of physically sorbed water located at the exterior surfaces of the LDH particles and in the interlayer space. This mass loss is accompanied by a series of endothermic peaks. A second mass loss (9.4 wt.-%) between 200 and 264 °C corresponds to partial dehydroxylation of the brucite-type layers. The remaining mass loss up to 800 °C can be attributed to the elimination of nitrate and of a minor amount of carbonate. At 800 °C, the residual inorganic material possesses a mass of 61.3 wt.-%.

Fig. 3 also exhibits the TG/DSC curves of the co-precipitates with welan gum, EPS I and scleroglucan. In all cases, a weight loss is observed between 25 and 220 °C (16.2 wt.-% for welan gum; 15.5 wt.-% for scleroglucan; and 16.3 wt.-% for EPS I). Similar to [Zn_2Al]NO_3–LDH, this weight loss can be attributed to the overall water loss from the LDH. Further heating at temperatures $>$ 220 °C produces two ranges of substantial weight loss for all pure biopolymers, which is much less pronounced for the co-precipitates, indicating an improvement in thermal stability when the biopolymers are shielded, *e. g.* from oxygen, by the inorganic layers. For all co-precipitates, the weight losses between 220 °C and 430 °C were 33.4 wt.-% for welan gum; 35.9 wt.-% for scleroglucan; and 23.5 wt.-% for EPS I. For scleroglucan,

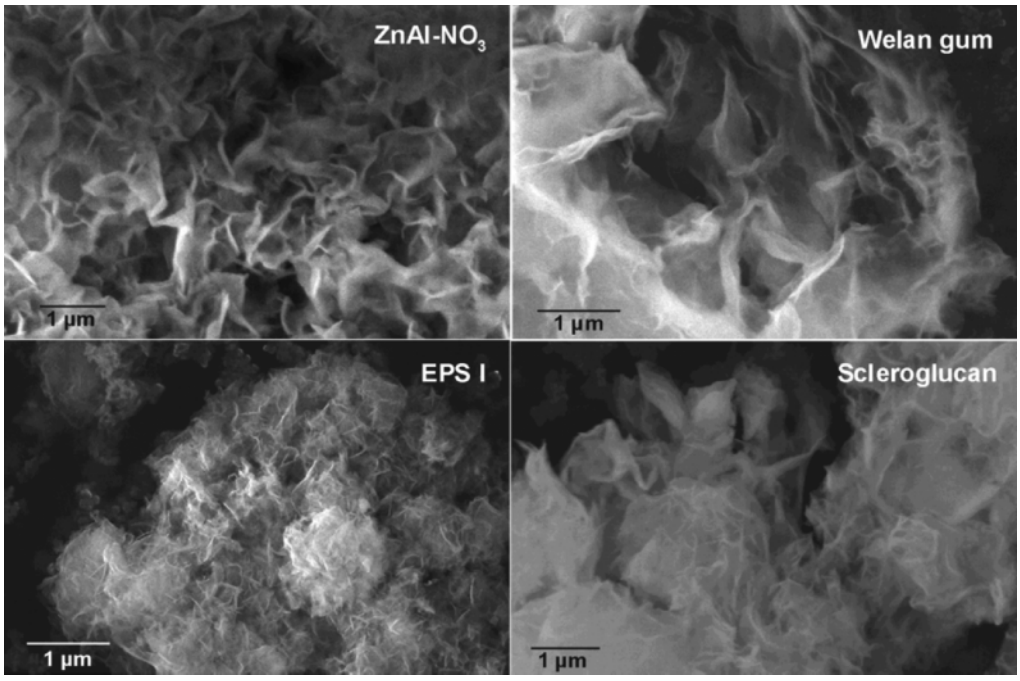

Fig. 4. SEM images of the pristine [Zn_2Al]NO_3–LDH and of the co-precipitates from welan gum, EPS I and scleroglucan.

a higher decomposition rate between 200 and 300 °C was found when compared to the precipitates with welan gum and EPS I. This confirms that scleroglucan is merely physically adsorbed onto the surfaces of LDH particles and thus more prone to degradation, whereas EPS I and welan gum are protected by intercalation.

TEM and SEM imaging

SEM images of pristine $[Zn_2Al]NO_3$–LDH (Fig. 4) show the characteristic morphology. This appearance of intergrown platelets arranged like a 'sand rose' differs from that observed for the precipitates obtained in the presence of the biopolymers. The intercalates of welan gum and ESP I exhibit the morphology of thin nanofoils (thickness $\sim$ 10 nm). This lower stacking arrangement can explain the broad reflections and the lack of d_{00l} reflections of higher order in the XRD patterns (Fig. 2). Additionally, the particles of $[Zn_2Al]$–EPS I–LDH nanocomposites were found to be much smaller in size than those of the others, which confirms its lower crystallinity as observed in the XRD diagram. In the case of the scleroglucan precipitates, the layered particles appeared to be more similar to those in the $[ZnAl]NO_3$–LDH sample, with a slight change in morphology. Such effect stemming from surface adsorption has been reported before [29, 30].

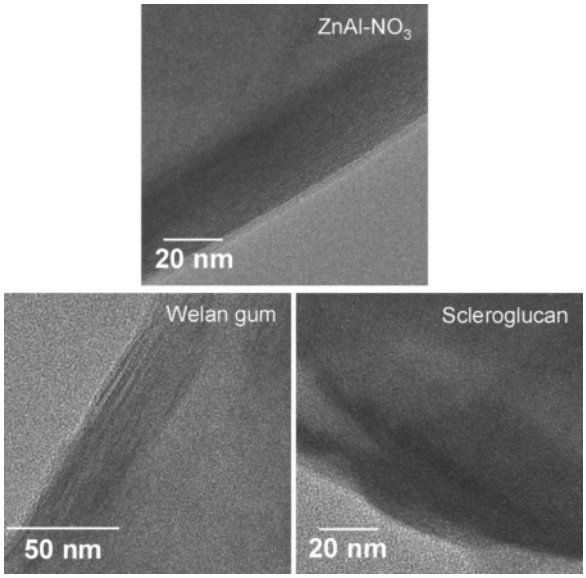

Fig. 5. TEM images of the pure $[Zn_2Al]NO_3$–LDH and of co-precipitates from welan gum and scleroglucan.

From TEM analysis (Fig. 5), well ordered layered structures with an average d-spacing of $\sim$ 0.9 nm can be observed for pure $[ZnAl]NO_3$–LDH, whereas more disordered layers at much higher interlayer distances are found for the sample incorporating welan gum. These results correlate well with the XRD analysis. By contrast, samples from co-precipitation with scleroglucan showed only layered structures characteristic of $[Zn_2Al]NO_3$–LDH, confirming that no intercalation into the LDH framework occurs with this biopolymer.

Conclusion

Three different microbial polysaccharides were investigated for their ability to intercalate into LDHs. It was found that the intercalation ability of these biopolymers depends on two main factors; the charge and the steric position of the anionic functions in the biomolecule. Successful incorporation is only possible when the biopolymer possesses an anionic charge which is sufficient to compensate a significant portion of the positive charge in the inorganic frame. Additionally, intercalation is more favored when the anionic charges are present on the backbone of the biopolymer, instead of its side chains.

Therefore, when LDHs are utilized for chemical encapsulation of such biopolymers *e. g.* for medical applications to provide a time-controlled release, these two factors should be taken into account. Additionally, specific structural modifications of such polysaccharides can bring about a drastic change in their intercalation properties.

Experimental Section

Starting materials and reagents

Industrial samples of welan gum (Biozan®, Kelco Oil Field Group, Houston, TX/USA) and scleroglucan (Biovis®, BASF Construction Polymers GmbH, Trostberg/Germany) were used as received. EPS I was obtained *via* fermentation employing a newly isolated bacillus species. It was synthesized in the laboratories of Volker Sieber, Chair for Chemistry of Biogenic Resources, Technische Universität München, Straubing/Germany.

The following reagents were of analytical grade: NaOH (> 98 %, Merck, KGaA Darmstadt/Germany); $Ca(NO_3)_2 \cdot 4H_2O$, $Mg(NO_3)_2 \cdot 6H_2O$, $Zn(NO_3)_2 \cdot 4H_2O$ and $Al(NO_3)_3 \cdot 9H_2O$, K_2SO_4 (all with purity > 99 %, Merck KGaA, Darmstadt/Germany).

Biopolymer characterization

The biopolymers were characterized by size exclusion chromatography (SEC) performed on an Alliance 2695 separation module (Waters, Eschborn/Germany) equipped with a PL aquagel-OH guard 15 μm precolumn and two PL aqua gel-OH 60 μm columns (Polymer Laboratories, distributed by Varian, Darmstadt/Germany). Aqueous 0.2 M $NaNO_3$ solution adjusted to pH = 9 w/NaOH was used as eluent at a flow rate of 1.0 mL min^{-1}. Prior to application on the columns, the 2 g L^{-1} biopolymer solutions were filtered through a 5 μm filter. Molar masses (M_w and M_n) as well as the polydispersity index (PDI) were determined using an 18 angle static light scattering detector ("DAWN EOS" from Wyatt Technology Corp., Santa Barbara, CA/USA). Polymer concentrations were determined using a differential refractive index detector (RI-2414 from Waters, Eschborn/Germany) while the hydrodynamic radius (R_h) was measured using a dynamic light scattering detector ("QELS" from Wyatt Technology Corp., Santa Barbara, CA/USA). The value of dn/dc which is needed for calculating the molar masses was 0.155 mL g^{-1} (value for polygalacturonate) [31].

Specific anionic charges of the biopolymers were determined employing a particle charge detector (PCD 03 pH from BTG Mütek GmbH, Herrsching/Germany). Solutions containing 0.02 wt.-% of the biopolymers dissolved in millipore water were prepared and titrated against a 0.001 N solution of cationic polydiallyl dimethyl ammonium chloride (polyDADMAC) as counter polyelectrolyte until charge neutralization was attained. The amount of negative charge per gram of polymer was calculated from the consumption of the cationic polyelectrolyte.

Co-precipitation synthesis

0.30 g of an individual biopolymer was dissolved in 80 mL of degassed millipore water under a nitrogen blanket. To this solution, 0.1 M $Zn(NO_3)_2$ and 0.05 M $Al(NO_3)_3$ were fed dropwise over a period of 40 min utilizing a rate of 1 mL min^{-1} with a peristaltic pump (MCP 360, ISMATEC®, Wertheim-Mondfeld/Germany). The pH value was maintained between 8.5 and 9 by intermittent addition of 0.1 M NaOH solution. The precipitate was kept unagitated in the mother liquid for 24 h, centrifuged for 15 min at 8,500 rpm and washed thrice with millipore water. The final product was lyophilized after decanting.

As reference sample, $[Zn_2Al(OH)_6]\cdot NO_3\cdot xH_2O$ LDH (in short $[Zn_2Al]NO_3$–LDH) was synthesized following the same procedure as above but replacing the biopolymer solution with millipore water.

Product characterization

The synthesized $[Zn_2Al]NO_3$–LDH and the reaction products obtained in presence of the biopolymers were characterized by X-ray diffractometry at room temperature employing a D8 Advance, Bruker AXS instrument (Bruker, Karlsruhe/Germany) with Bragg-Bretano geometry. Samples were prepared on a front-mount plastic holder and analyzed in a scanning range from 0.6 to 40° in 2θ with a step size of 0.15 sec per step, a spin revolution time of 4 sec, an aperture slit of 0.1°, and a nickel filter for the incident beam. The elemental composition was determined on an Elementar vario EL instrument (Elementar Analysensysteme GmbH, Hanau/Germany) utilizing the CHNS method. Thermogravimetric analysis was performed on a Netzsch STA 409 apparatus (Selb/Germany) in the range from 300 to 1373 K in air flow of 30 cm^3 min^{-1} at a heating rate of 10 °C min^{-1} using α-Al_2O_3 as standard. An amount of $\sim$ 40 mg of the sample was placed in platinum crucibles per analysis. SEM and TEM images were taken with an XL30 ESEM FEG instrument (FEI Company, Eindhoven/The Netherlands) and a JEOL JEM-2100 microscope (JEOL Company, Tokyo/Japan), respectively.

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