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Preparation of micro and nanoparticles from corn cobs xylan

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Summary

Xylan, a hemicelllulose extracted from corn cobs, was used to prepare micro and nanoparticles. First, a chemical evaluation of xylan extract was performed. Then, particles were prepared by a coacervation method based on neutralization of an alkaline solution with an acid solution. The influence of polymer content (2.85 to 100 mg/ml) and surfactant presence (0.6 to 1.8%$_{(v/v)}$) on the manufacturing process was evaluated. It was demonstrated that neutralization of the xylan solution with HCl or acetic acid was able to generate micro and nanoparticles and that surfactant concentration influences both the particle size stability and morphology. Therefore, the optimal concentration of surfactant was 1.5%$_{(v/v)}$.

Introduction

In spite of the abundance of natural renewable biomass resources in the world, which could be used especially for the production of polymeric materials and chemicals, the use of these resources is still negligible in comparison to products derived from petroleum. Nevertheless, some important polymers considered as biomass derived, like poly (lactic acid) and copolymers, polyhydroxyalkanoates, and cellulose derivatives, are commercial products with successful application in the biomedical area such as the production of resorbable sutures, prosthetic devices, bioactive agents and drug delivery carriers [1].
An interesting group of polymers with potential properties for use in the biomedical area are the hemicelluloses [2-4]. Hemicelluloses are plant polysaccharides readily available, especially from annual plants (agriculture crop residues such as corn cobs, corn grain, wheat stems, seed coats and sugar cane stalks). These polysaccharides, associated with cellulose, pectin and lignin, constitute the cell wall of land plants [5,6].

Xylan, the most common hemicellulose, represents more than 60% of the polysaccharides existing in the cell walls of corn cobs [7]. It is considered the second most abundant biopolymer in the plant kingdom. Its chemical structure is mainly composed of D-glucuronic acid, L-arabinose and D-xylose in the approximate ratio of 2 : 7 : 19 [8]. Some years ago Olson and co-workers [9] evaluated the effect in the human digestive process on this polymer, and they showed the ability of xylan to pass through the digestive tract unchanged. This resistance to digestion makes it eligible as a potential excipient that could be used in the pharmaceutical industry. Recently, the xylan component from corn cobs has been shown to possess valuable properties such as capacity to form thixotropic aqueous dispersion, to exhibit shear-thinning behavior (typical for pseudo-plastic polymer) and behave as plastic material at higher concentration, to present viscoelastic properties, to exhibit tensioactive properties and to show some biological properties [4]. Those properties make it with potential application in the pharmaceutical field [3,10-14].

On the other hand, micro and nanoparticles as drug delivery carriers are gaining success by achieving enhanced efficacy and reduced toxicity of potent drugs. They are able to protect, to vectorize and to control drug diffusion into the body. The improvement of pharmaceutical grade polymers permitted the development of such therapeutic systems [15]. The aim of this work was to evaluate the use of xylan from corn cobs to prepare micro and nanoparticles.

Experimental

Materials

Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were respectively from Reagen, Rio de Janeiro-RJ, and Q.E.E.L., São Paulo-SP. Polyoxyethylene (20) sorbitan mono-laurate (Tween® 20) and acetic acid were purchased from Galena, São Paulo, Brazil. Methyl alcohol, sodium hypochlorite 4-6% and sulfuric acid were from VETEC, Rio de Janeiro, Brazil.

Extraction of xylan component

The hemicellulose from corn cobs was obtained after neutral extraction of organic and inorganic products of low molecular weight with water and de-lignification with aqueous sodium hypochlorite (1.3%$_{(w/v)}$ available chlorine) containing 25 g/L detergent; the xylan was then extracted from the pulpy mass with aqueous 4%$_{(w/v)}$ sodium hydroxide. During extraction with sodium hydroxide, the system was maintained under moderate stirring at room temperature for one hour. Except for the de-lignification step, this procedure was the same as described previously [16,17]. After neutralization to precipitate the xylan, the product was retained on a filter, exhaustively washed with water and then dried under vacuum.

Determination of xylan's composition

The hemicellulose sample was hydrolyzed with 1M trifluoracetic acid solution for 4h at 100°C. The hydrolysate was reduced with sodium borohydride, followed by acetylation with pyridine-acetic acid 1:1, for 16h at room temperature. The alditol acetates obtained were analyzed by Gas-Liquid Chromatograph 5890 S II, equipped with a DB-210 capillary column (30m x 0.25 mm), at 220°C. The temperature of the flame ionization detector and injector was 250°C, and nitrogen was the carrier gas.
The content of uronic acid was estimated by the m-hydroxyphenyl method [18].
The lignin content was determined by ASTM D1106-56 method [19].

Size-exclusion chromatography

The average molar mass and polydispersity of the hemicellulose were obtained from Size-exclusion chromatography, in a VARIAN 9002-SDS liquid chromatograph equipped with a Rheodyne 7125-injector and TSK-Gel GMPWXL column (Toyo Soda, Tokyo, Japan), 300 x 7.8 mm. The eluent used was 0.5M NaOH, and the calibration curve was obtained from dextran standards, DXT6K, DXT27K, DXT97K, DXT165K and DXTT5000K, with weight-average molar mass (M_w) 5700, 27000, 97000, 165500, 4900000, respectively. The elution was carried out at 0.5 mL/min and monitored by a refractive index detector, model Star 9040.

Preparation of micro and nanoparticles

An original method based on a reaction of neutralization between the NaOH and HCl or acetic acid was used in the preparation of micro and nanoparticles. With gentle stirring, a solution of xylan in 1N NaOH was prepared. The microparticles were formed spontaneously as the acid solution was added to the xylan alkaline solution, and a neutral solution was achieved. The influence of three factors was evaluated in the formation of nano and microparticles: (1) the polymeric concentration (2 - 100 mg/ml), (2) the concentration of HCl, which was studied in a range among 1:1 to 2.5:1 mole/mole in HCl and NaOH, respectively, and (3) the concentration of Tween® 20 (from 0.6 to 1.8 $\%_{(v/v)}$). In the latter case, the particles were prepared with a 1N acetic acid solution. The composition of all formulations is shown in **Table 1**.

Characterization of micro and nanoparticles

The particles were characterized by their morphology, diameter, pH and long term stability. The color and homogeneity of the suspensions as well as the presence of phase separation were scored after visual examination at room temperature. The homogeneity and aspect of the dispersion were also examined by microscopy (Studar Lab, São Paulo-SP, Brazil) at x1250 with an oil immersion objective equipped with a micrometre grid. Pictures were taken by a camera connected to the microscope (Studar Lab, São Paulo-SP, Brazil). The mean diameter of the nanoparticle suspensions was estimated by photon

correlation spectroscopy using a Nanosizer (Supernanosiser® N4MD, Coultronics, Paris, France). The preparations, previously filtered, were diluted 1/100 in water and analyzed at 20 ± 1°C. The Nanosizer was calibrated with a suspension of latex particles of 0.3 µm. All analyses were done in triplicate.

Table 1: Composition of particle preparations

Weight of xylan (mg)	Vol. of 1N NaOH solution (ml)	Vol. of acid (ml)	Polymer content (mg/ml)	Conc. of Tween® 20 [%(v/v)]	Sample identification
20	2	5*	2.85	-	XP_1
20	5	5*	2	-	XP_2
40	5	5*	4	-	XP_3
160	5	5*	16	-	XP_4
320	5	5*	32	-	XP_5
640	5	5**	64	-	XP_6
1000	5	5**	100	-	XP_7
1000	5	5**	100	0.6	XP_8
1000	5	5**	100	0.9	XP_9
1000	5	5**	100	1.2	XP_{10}
1000	5	5**	100	1.5	XP_{11}
1000	5	5**	100	1.8	XP_{12}

* HCl 1.0N; **1.0N acetic acid 1.0N; XP = Xylan's particle

Results and discussion

Xylan characterization

Table 2 presents the analytical results of the hemicellulose sample extracted. The xylan's composition was quite similar to that published by Ebringerová and co-workers [2,8] for xylans from corn cobs. The polysaccharide obtained is mainly constituted of xylose (84%) and arabinose (10%), but small quantities of others neutral sugars (mainly glucose - 3.7% and galactose - 2.0%) are also present. The uronic acid amounted 3.0%. The lignin content (3%) was considered low, taking into account its initial concentration of 18.8% in the raw material. It is known that lignin is closely associated to the cellulose and hemicelluloses in the cell wall, by physical or chemical linkages. There are evidences that lignin is present even in water soluble fractions of some xylans [20]. The complete lignin removal needs critical treatments, that can provoke a significant polysaccharides degradation. The values of average molar mass and polydispersity for the xylan studied (**Table 2**) are in accordance with the results of another samples [2,8] and indicate that no significant depolymerization occurred.

Table 2: Sugar composition, lignin content, average molar mass and polydispersion of the hemicellulose examined.

Sugar composition (mole%)					Uronic acid (%)
xylose	arabinose	glucose	galactose	mannose	
84	10	3.7	2.0	0.3	3.0

Lignin content (%m/m) = 3; M_w (g/mole) = 13.15 x 10^4; M_n (g/mole) = 8.51 x 10^4; Polydispersity (M_w/M_n) = 1.54

Characterization of micro and nanoparticles

The neutralization of alkaline xylan solution with HCl initiated a coacervation process and promoted the formation of micro and nanoparticles (**Table 3**). Nanoparticles were formed only when small amounts of xylan were present (**Samples XP_1-XP_4**). In fact, while smaller concentrations generated nanoparticles (100 - 900 nm), the increase of xylan's concentration produced microparticles (> 10 µm).

The particle size distribution of all preparations was observed first by optical microscopy with a 100x objective amplification. For the samples containing less than 32 mg/ml of polymer (**XP_1-XP_4**) nothing could be seen because of the smaller particle size. For these preparations the Nanosizer® was used to measure the particle size distribution. Besides that, particles made with an excess of HCL (**XP_1**) became unstable after one day of preparation and a brown sediment was observed (**Table 4**).

Table 3: Microparticles characterization

Sample identification	Visual Aspect	pH	Particle Size (nm)
XP_1	homogeneous	0.50	1790 ± 264
XP_2	homogeneous	7.00	371 ± 106
XP_3	homogeneous	7.00	985 ± 260
XP_4	homogeneous	7.00	677 ± 120
XP_5	homogeneous	7.00	ND
XP_6	homogeneous	6.00	ND
XP_7	homogeneous	7.00	ND
XP_8	homogeneous	7.00	ND
XP_9	homogeneous	7.00	ND
XP_{10}	homogeneous	7.00	ND
XP_{11}	homogeneous	7.00	ND
XP_{12}	homogeneous	7.00	ND

ND = not determined because particle size was above 10µm

Long term stability

The long-term stability results throughout 60 days are shown in **Table 4** and **Figure 1**. As in the manufacturing process, the final pH had a significant effect on the stability during 60 days. The particles were more stable at similar HCl/NaOH molar ratio (neutral

pH) than at high HCl molar concentration; above a range of 2:1 mole/mole HCl and NaOH, respectively, the particles (**XP_1**) settled quickly and a dispersible flocculate was formed. Probably this phenomenon was due to the enhanced xylan phase separation at acid pH [17]. Consequently, the polymer studied does not support acid medium.

In addition to this visual instability, the **XP_1** preparation presented a particle size distribution that was reduced following the 60 days (**Figure 1**). We believe that this was due to the HCl excess, which breaks down the particles formed and hydrolyzes the polymer. This fact permitted us to conclude that the use of similar acid and base amounts to produce a neutral solution was quite important to achieve the particle preparation.

Table 4: Visual aspect of particles during 60 days

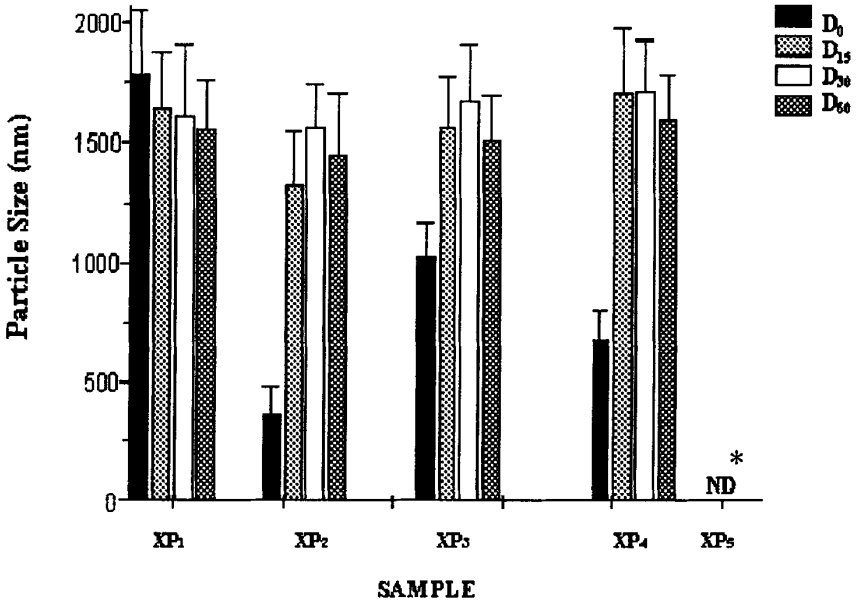
Sample identification	D_0	D_1	D_2	D_4	D_8	D_{15}	D_{30}	D_{60}
XP_1	homog.	DS	DS	DS	DS	DS	DS	DS
XP_2	homog.	homog.	homog.	homog.	DS	DS	DS	DS
XP_3	homog.	homog.	homog.	homog.	DS	DS	DS	DS
XP_4	homog.	homog.	homog.	homog.	DS	DS	DS	DS
XP_5	homog.	homog.	homog.	homog.	DS	DS	DS	DS
XP_6	homog.	homog.	homog.	homog.	homog.	homog.	DS	DS
XP_7	homog.	homog.	DS	DS	DS	DS	caking	caking
XP_8	homog.	homog.	homog.	homog.	homog.	homog.	homog.	homog.
XP_9	homog.	homog.	homog.	homog.	homog.	homog.	homog.	homog.
XP_{10}	homog.	homog.	homog.	homog.	homog.	homog.	homog.	homog.
XP_{11}	homog.	homog.	homog.	homog.	homog.	homog.	homog.	homog.
XP_{12}	homog.	homog.	homog.	homog.	homog.	homog.	CSL	CSL

D = Day; homog. = homogeneous DS = Dispersible flocculate; CSL = Clear supernatant layer

The average size of the particles prepared with low polymer concentration and similar acid/base content (**XP_2 - XP_4**) was proportional to the concentration in polymer. While particles with 2 mg/ml (**XP_2**) of polymer gave a particle size of 371 ± 106 nm at day 0 (**D_0**), particles with 16 mg/ml (**XP_4**) presented a particle size of 677 ± 120 nm (**Figure 1**). When the polymer content was between 2-35 mg/ml, preparations using acetic acid were completely homogeneous during 180 days (results not shown). Therefore, we have adopted acetic acid to prepare **XP_6 - XP_{12}**. Although the high polymeric content of **XP_6 - XP_7** preparations (64-100 mg/ml), the systems remained quite stable. Only when the concentration was too high (100mg/ml) a cake was formed after 30 days.

To evaluate the influence of surfactant in the microparticles manufacture, five formulations were developed (**XP_8 - XP_{12}**). This phenomenon was evaluated by optical microscopy analysis and by visual aspect. The latter one showed that particles prepared with surfactant were more stable, and the dispersible flocculate never appeared. On the other hand, an optimal surfactant concentration was $1.5\%_{(v/v)}$ (**XP_{11}**). Above this amount a clear supernatant layer, due probably to the surfactant excess, was observed after 30 days

(Table 4, sample $\mathbf{XP_{12}}$). Conversely, below 1.5%$_{(v/v)}$ surfactant concentration, some flocculation was observed after 120 days (results not shown).



* Particles prepared with more than 16mg/ml of polymer ($\mathbf{XP_5}$ - $\mathbf{XP_{12}}$) were too big, and they were not measured by the Nanosizer®.

Figure 1: Particle size variation during 60 days

The optical microscopy analyses showed that only particles made with 100 mg/ml of polymer could be visualized. In addition, the influence of surfactant on the morphology of particles was evident. As could be seen in **Figure 2**, made from the $\mathbf{XP_7}$ sample, which was prepared without surfactant, the microparticles were surrounded and aggregated by several small ones. In contrast, the optimal microparticle preparation, $\mathbf{XP_{11}}$, presents a quite spherical shape and no small surrounding particles were observed (**Figure 3**).

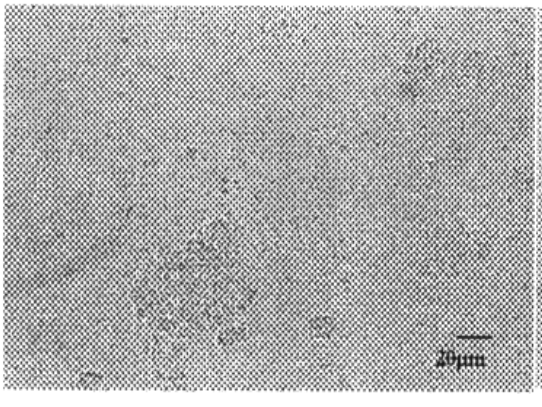


Figure 2: Microparticle of xylan without surfactant ($\mathbf{XP_7}$ sample)

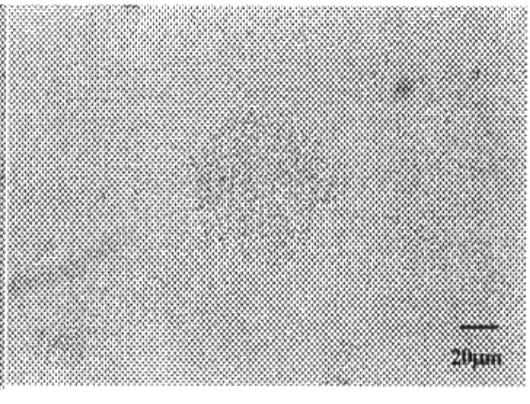


Figure 3: Microparticle of xylan with surfactant ($\mathbf{XP_{11}}$ sample)

Conclusions

It was demonstrated that the neutralization of alkaline xylan solution with HCl or acetic acid is able to generate a coacervation process and promotes the formation of micro and nanoparticles. The size of xylan particles obtained is concentration dependent. At high concentrations, large particles were obtained (microparticles), and the inverse occurs at low concentration. To prepare microparticles, the ideal polymer concentration was 100mg/ml. This concentration permitted us to achieve microparticles which are easily observed by the optical microscopy.

The surfactant influences both the particle size stability and its morphology. The concentration of 1.5%$_{(v/v)}$ presented the best results.

We believe that the micro and nanoparticles generated could be used to drug delivery systems. It will be the next step to take in our scientific investigation.

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