A NEW ROUTE TOWARDS THE PREPARATION OF
A BIODEGRADABLE NANOCOMPOSITE BASED ON CHITIN
WHISKERS FROM EXOSKELETON OF SHRIMP AND
POLYCAPROLACTONE

V. Castillo¹, M. Matos¹, A. J. Müller²


Abstract

The main objective of this work was to explore the possibility of future preparation of nanocomposites using á-chitin whiskers as a reinforcing agent and poly(e-caprolactone), PCL, as the matrix polymer. Whiskers were successfully obtained as a result of the acid hydrolysis of purified chitin extracted previously from the exoskeleton of shrimp. These whiskers had a form factor of 15, with an average length of 300 nm. Once an aqueous suspension of chitin whiskers was produced, we developed a method in this paper to change the solvent (first to methanol and then to toluene), in order to incorporate the nano-fibers in non-water soluble polymers like PCL. A non flocculating suspension of the whiskers in toluene was prepared by means of the incorporation of a suitable surfactant. The dispersion of the whiskers was better in the aqueous medium than in toluene, indicating that the dispersion in the latter medium could be improved if a more efficient surfactant is found. Finally, preliminary experiments showed that PCL/chitin whiskers films can be prepared by first dissolving the PCL in the toluene suspension and later evaporating the solvent. Differential Scanning Calorimetry experiments demonstrated that a nucleating effect of the whiskers on the PCL was induced as well as a small increase in crystallinity. The efficiency of the chitin whiskers as nucleating agents for PCL was estimated by comparing the increased in crystallization temperature obtained with that of ideally self-nucleated material. When 5% chitin was employed a nucleating efficiency closed to 40% was achieved as well as 20% increase in crystallinity.

Keywords: Chitin, whiskers, polycaprolactone, nanocomposites, biodegradation.

Resúmen

El objetivo principal de este trabajo fue explorar la posibilidad de elaborar materiales nanocompuestos utilizando whiskers de á-quitina como agente reforzante y poli(e-caprolactona), PCL, como matriz polimérica. Los whiskers fueron obtenidos a partir de la hidrólisis ácida de quitina purificada extraída a partir del exoesqueleto del camarón. Estos whiskers presentaron un factor de forma igual a 15, con una longitud promedio de 300 nm. Una vez obtenida la suspensión acuosa de whiskers de quitina, se desarrolló un método en nuestro laboratorio para cambiar el solvente (primero a metanol y luego a tolueno), con el fin de incorporar las nanofibras en suspensiones de polímeros no solubles en agua tales como PCL. Se preparó entonces una suspensión no flocculante de whiskers de quitina en tolueno mediante la incorporación de un surfactante adecuado. La dispersión de los whiskers fue mejor en medio acuoso que en tolueno, indicando que la dispersión en este último medio podría mejorarse con un surfactante más eficiente que el empleado en este trabajo. Finalmente, experimentos preliminares mostraron que las películas de PCL/whiskers de quitina pueden ser preparadas disolviendo primero la PCL en la suspensión de tolueno y luego evaporando el solvente. Los resultados obtenidos mediante calorimetría diferencial de barrido demostraron el efecto nucleante de los whiskers de quitina en la PCL así como un ligero incremento en la cristalinidad. La eficiencia de los whiskers como agentes de nucleación de la PCL fue estimada mediante la comparación del incremento en la temperatura de cristalización obtenida con la del material autonucleado idealmente. Se encontró un valor de eficiencia cercano a 40% así como un aumento de 20% en la cristalinidad con un 5% de quitina empleado.

Palabras Clave: whiskers á-quitina, policaprolactona, nanocompuestos, biodegradación.
1. Introduction

Polymers, whether synthetic or natural, represent the most abundant and diverse group of biomaterials due to their great versatility, combination of structures and due to the fact that depending on the preparation method, they can show a wide range of properties [1]. This offers great possibilities, because by an adequate structure selection, biologically inert or totally biodegradable devices, showing excellent tolerance due to their biocompatibility, can be prepared [2].

Poly(e-caprolactone), PCL, is a biocompatible polymer which has a slow in vivo biodegradation, slower than the one of poly(L-lactic acid) (PLLA), due to its high crystallinity and hydrophobic character [1]. Furthermore, it has a relatively low melting point and low melt viscosity, making it easy to process. According to this, PCL can be considered a good alternative to be used as a matrix polymer for the preparation of fiber reinforced compounds [1].

Chitin is a high molecular weight linear polysaccharide, specifically β-(1, 4)(N-acetyl-D-glucosamine). There has been a growing interest in this material due to its abundance and unusual combination of properties that include toughness, bioactivity, biocompatibility, and biodegradability [3]. The shellfish processing industry generates great amounts of waste from shells, which represent about 30 weight % in chitin. Worldwide, about 105 metric tons of chitin coming from waste are available yearly for industrial uses, the main sources being shrimp and crab waste material. Additionally, chitin is the second most abundant natural polymer after cellulose [4].

Considering the bio-absorbable properties and the physico-chemical characteristics of both materials, besides their wide availability, chitin and PCL could be prepared as a compound capable of meeting the requirements of a new bio-absorbable material [1] using chitin as a natural filler that may be able to improve mechanical properties of the matrix without affecting its biodegradability. Further, unlike chitin, PCL molecules do not have any other functional group available for chemical modifications of the material besides hydroxyl and carboxyl groups on their chain ends. As a consequence, the blending of these materials could produce a good candidate for a new biomaterial with improved mechanical properties, controlled biodegradability, and with the availability of chemical functionalities to carry out subsequent modifications [5].

However, although there are concrete examples where the use of reinforced materials or compounds allows obtaining a product with improved characteristics, this is often not enough to reach the desired performance; in other cases, this performance is only achieved by incorporating high filler contents, which can later result unfavorable. For this reason, alternatives able to resolve these drawbacks have been proposed in order to achieve higher performance materials.

One of these alternatives is the use of nanocomposites, the main characteristics of which is a substantial improvement in the mechanical properties by the use of very low filler contents [6,7,8].

The so-called whiskers (almost perfect monocrystals) which are characterized by a regular form, high form factor and monocrystalline nature [9] are among the different kind of nanocomposites. These materials have been obtained mainly from inorganic compounds (coal [8], ceramics, etc.) and, more recently, from organic compounds such as cellulose and chitin. The latter have been prepared in order to reinforce a synthetic polymer matrix, and, in most of the cases, a substantial increase in mechanical properties of the matrix above the glass transition temperature of the material has been found [10-17].

Based on the aforementioned reasons, it is interesting to study the feasibility to prepare a new composite based on poly(e-caprolactone and chitin whiskers in order to develop a biocompatible and biodegradable material with improved mechanical behavior.

For the future preparation of nanocomposites, chitin whiskers were extracted from shrimp shells obtaining an aqueous suspension of the microcrystals. This suspension was further "transferred" to an organic solvent, specifically toluene, where the addition of a surfactant was necessary in order to keep the suspension in a stable colloidal state.

2. Experimental

2.1 Materials

For the development of this research work, the following materials were used: poly(e-caprolactone), chitin extracted from shrimps, and a commercial chitin purchased from Sigma for comparative purposes. Some relevant characteristics of the materials employed are shown in Table 1.

3. Experimental Procedure

3.1 Chitin Extraction from Shrimp Shells:

Shrimp shells of the *plecticus robustus* species, selected from the waste material provided by a local restaurant, were used as the source of polysaccharide. The shells used corresponded to the abdominal region and the carapace of shrimp; the rest of the skeleton was discarded. Once chosen, they were washed under running water and their size reduced by using a kitchen aid (mixer). The final size obtained ranged between 2x3 and 3x5 mm, approximately. Finally, they were placed in an oven at 50°C for 12 hours, in order to determine the initial weight of the sample. Later, chitin was extracted, based on procedures reported in literature [4, 15-18], following the steps outlined in Figure 1.
Table 1. Characteristics of the employed materials.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Acronim</th>
<th>Source</th>
<th>(T_g) ((\degree C))</th>
<th>(T_m) ((\degree C))</th>
<th>(\rho) ((g/cm^3))</th>
<th>Mw ((g/mol))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly((\varepsilon)-caprolactone)</td>
<td>PCL</td>
<td>Union Carbide</td>
<td>-61</td>
<td>60</td>
<td>1,15</td>
<td>120,000</td>
</tr>
<tr>
<td>Comercial Chitin</td>
<td>QA_c</td>
<td>Aldrich Chemical</td>
<td></td>
<td></td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Natural Chitin</td>
<td>QA_n</td>
<td>Schrimp Shells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cleaning and Washing of shells in running water

Size Reduction

Boiling in KOH 5% for 6h

12h in 5% KOH at 25°C

Boiling in KOH 5% for 6h

Bleaching 6h at 80°C in a solution of NaClO Solution + Na Acetate buffer

Acid Hydrolysis Boiling with a slow addition of 3N HCl (30mL/gr)

Dialysis - Centrifugation - Decanting until pH=2

Removal of residues Dialysis until pH=6

Fig. 1. Scheme of mechanical and chemical treatments applied to shrimp shell to obtain chitin whiskers.

3.2 Change of Solvent: Water – Toluene

Once the aqueous suspension of chitin whiskers is obtained, microcrystals are generally incorporated into a polymeric matrix in one of two ways. The first consists in using a water soluble polymer and the second, in preparing a polymer latex. Considering there are few water soluble polymers, the use of latex has been the most widely used procedure. However, in this study we have developed an alternative method for the preparation of the nanocomposite, based on obtaining a stable colloidal suspension of the small crystals in an organic solvent (toluene). This allows for the incorporation of the whiskers not only in a PCL matrix, but also in any polymer soluble in this medium. To change the solvent, the dialysis in a “bridge” solvent (methanol) was used.

After obtaining a suspension of 0.5 weight % of chitin in toluene, a Beycostat NA (mono-ester and di-ester of phosphoric acid) (BNA) surfactant supplied by CECA was added using a 1.6:1 surfactant-chitin proportion. The procedure that was followed is shown in Figure 2.

After the preparation of the toluene suspension, the PCL was dissolved in by heating and continuous stirring of the solution. PCL films were prepared by solvent evaporation at room temperature for 5 days with 2 and 5 weight % chitin whiskers. Finally, the solution was poured in Petri dishes after about 1/3 of the solvent had evaporated. Calculations were made to obtain approximately 0.2 mm thick films. These films were submitted to characterization tests like Differential Scanning Calorimetry (DSC), Infrared Spectroscopy (IR).

Fig. 2. Schematic representation of the preparation of the chitin whiskers suspension in toluene.

3.3 Fourier Transform Infrared Spectroscopy (FTIR)

Chitin samples were prepared by evaporating a suspension on an AgBr substrate, then FTIR spectra were recorded in a Nicolet Magna Spectrometer, model 750 in a 4000-3000 cm\(^{-1}\) range. The resolution employed was 4 cm\(^{-1}\) and 32 scans.
3.4 Differential Scanning Calorimetry

A Perkin Elmer DSC-7 Calorimeter was employed. The equipment was calibrated with hexatriacontane and indium under an ultra high purity nitrogen atmosphere. Samples weighing approximately 10 mg were encapsulated in Aluminium pans. The samples were first heated to 120°C to erase any previous thermal history. Then, cooling scans were recorded at 10°C/min from 140 down to -20°C followed by subsequent heating scans at the same rate.

3.5 Transmission Electron Microscopy

One drop of chitin suspension in water and also toluene (diluted 100 times) was deposited on a carbon covered surface. A JEOL JEM-1220 transmission electron microscope (TEM) was used at 100Kv.

4. Results and Discussion

4.1 Purification Method:

The extraction method described by Paillet and Dufresne [16], which was developed and improved from other research works [4, 17, 18], was used. However, it was necessary to introduce some modifications in terms of the source of extraction and the limitations in the availability of certain materials. In the first place, KOH was used during the purification to eliminate proteins. Most part of these proteins are physically associated with chitin; the rest is bonded in a conjugated form [19, 20].

After the deproteinization, it was necessary to eliminate pigment residues of the material, which was accomplished by using a chlorinated base bleaching solution in a sodium acetate buffer solution at pH = 4.5. Bleaching reactions are oxidative reactions allowing the elimination of organic deposits from the wall of the material [21] (demineralization). The formation rate of the oxidized material is influenced by the pH of the medium, the temperature and the concentration and nature of the oxidizing agent [22]; hence, the importance of carrying out the process in abuffer solution, to allow a greater control of the reaction rate. In moderately acid solutions (pH = 3-5), as those used in this study, the effective oxidant is the non-dissociated hypochlorous acid and the reaction proceeds slowly [23]. Due to the high protein and mineral content present in the shells, the weight loss was significant in each step of the process. Finally, after the aforementioned steps, the α(1→4)-N-acetyl-D-glucosamine was obtained. The structure of the obtained material was confirmed by comparing the FTIR spectrum of the purified extracted chitin and that of a crab shell chitin produced by Sigma. The comparison is shown in Figure 3.

Both FTIR spectra presented in Figure 3 include bands which can be observed at approximately 3450, 3265, 3102, 1666, 1622, 1574, 1430, 1361, 1315, 1020, 951 and 887 cm⁻¹ that are consistent with the structure of α-chitin [24].

No characteristic bands corresponding to β chitin were found (i.e., 972 or 632 cm⁻¹), an expected result in view of the chitin source employed here [24]. According to these results, the extraction of the polysaccharide from the exoskeleton of shrimp was successful. On the other hand, the absence of a peak at 1540 cm⁻¹, corresponding to proteins, demonstrates that the successive treatments were strong enough to eliminate all the proteins and obtain pure chitin [17].

4.2 Production of Chitin Whiskers:

It is well known that, as well as cellulose, chitin forms microfibrilar arrays in living organisms [25]. These microfibrils are in turn aligned in larger dimension fibers. The presence of these arrays could be verified using polarized light optical microscopy. Not only the presence of fibrils was observed, but also their high degree of orientation could be demonstrated by the birefringence exhibited by them when observed through crossed polarizers.

In order to obtain chitin microcrystals, an acid hydrolysis was carried out slowly, to guarantee the effective termination of the process avoiding a possible excessive degradation, considering that in hydrolysis the reaction rate is controlled by the ability of the acid to penetrate the complex interconnected crystalline lattice and the amorphous and mesomorphic regions conforming the fiber [26]. Acid hydrolysis leads to the hydrolytic scission of the α-glycosidic bonds of chitin, which generates compounds with identical chemical structure, but differing from the original in their molecular size and the distribution of the degree of polymerization [26].

Unlike what happens in the case of cellulose, acid hydrolysis of chitin does not promote crystal aggregation in clusters. To the contrary, as the hydrolysis proceeds, the free amino groups are exposed and in their protonated state they provide electrostatic repulsion, which stabilizes the suspension in the aqueous hydrolysis medium.
Figure 4a shows a micrograph of the chitin whiskers suspension obtained by TEM. The lengths of whiskers is almost constant. Similar results have been reported earlier [9-12, 13-18] and are explained as follows: crystal lengths are exponentially distributed and the attack of the acid occurs in the ends of those particles; therefore, the fiber average size remains unchanged. The calculated average length was around 300 nm. The average form factor (L/D, where L is length and D diameter) of those whiskers was about 15 and their surface area was close to 140 m²/g.

![Micrograph](image)

Fig. 4. Micrograph obtained by means of a Transmission Electron Microscope of: (a) aqueous suspension of chitin whiskers diluted 100 times; (b) suspension of chitin whiskers in toluene.

The acetylation degree of the material was determined through by FTIR following Brugnerotto et al. [27] and was 93% after acid hydrolysis (64% before acid hydrolysis, idem for the commercial chitin). This shows that acid hydrolysis preferentially removes the units corresponding to non-acetylated blocks, especially if they are on the surface of the crystals.

This work intended to find an alternative method to prepare a nanocomposite with a good dispersion of the reinforcing material. In this sense, the preparation of a suspension of the filler in an organic solvent, specifically toluene, was studied. An adequate suspension of whiskers in toluene would allow not only the use of PCL as the matrix polymer, but also the use of any other polymer soluble in this medium, like, for example, polyethylene (PE), polypropylene (PP) (both at high temperatures), polystyrene (PS), poly(vinyl chloride) (PVC), poly(vinyl acetate) (PVA), polyacrylates, etc., significantly increasing the application field of the filler that is being studied.

It is important to point out that the preparation of chitin whiskers suspensions in non-polar organic solvents has not been described so far in the open literature and the only analogous work in this matter was carried out by Bonini [28] who obtained a cellulose whiskers suspension in toluene using a different technique than the one used in this research work. In order to change the suspension environment, we proposed a technique that, unlike the one proposed by Bonini [28], avoids any kind of aggregation before toluene is added. The method consists in changing the suspension media from methanol to toluene by dialysis. The suspension keeps its non-flocculating character due to the polarity of the solvent. The selection of methanol as a “bridge” solvent was established as a consequence of its miscibility with water as well as with toluene, in view of its alcohol nature. On the other hand, among the available alcohols, methanol is the one with the lowest boiling point (67°C), which helps the subsequent removal and avoids possible structural or degradative changes in chitin due to high temperatures. Once this stage has been achieved, and granting that there is no water in the suspension media, toluene is added and subsequently methanol is eliminated by differences in boiling temperatures.

The spontaneous colloidal character exhibited by aqueous suspensions of the chitin whiskers is a consequence of electrostatic repulsions of the amino groups which become protonated after the acid hydrolysis. On the contrary, in non-polar solvents, like toluene, ions do not dissociate [28]; therefore, the charge is not preserved. Accordingly, once the fibers are in toluene, they form large aggregates difficult to break up, due to strong hydrogen bonding interactions existing among crystals. In order to ensure a good dispersion of whiskers in toluene, it was necessary to incorporate a surfactant (amount of surfactant added in a 1.6:1 surfactant : chitin relationship because of the higher specific area of chitin whiskers) in order to promote and ensure compatibility of fibers with the organic solvent. In this way, the type of repulsion would be transformed from electrostatic repulsion in water to steric repulsion in toluene [28]. In this work, a di-ester of phosphoric acid (BNA) anionic surfactant was selected. BNA has polar groups that are able to interact with chitin and has an additional advantage: the presence of long aliphatic chains in each phosphate group, which could increase its efficiency. The steric repulsion between fibers is produced assuming that the adsorption mechanism (physical mechanism) between the solid-liquid interface occurred by hydrogen bonding (by analogy with the aqueous medium) between hydroxyl and acetamide groups of chitin and the polar groups (ester, etc.) of the surfactant.
Considering that micelle formation in non-polar solvents is limited, we have assumed that micelles are not formed regardless of the high amount of surfactant added to the solvent and their disposition along fibers is similar to that shown in Figure 5.

Bonini [28] carried out a study on the type of array that BNA surfactant adopts on the surface of cellulose whiskers in a toluene suspension using Small Angle Neutron Scattering (SANS). He found that whiskers kept their original form and that the radius of gyration (about 15 Å) increased when they were modified by the surfactant, reinforcing the hypothesis that whiskers were uniformly coated by the surface-active agent (as shown in figure 5) in the form of a brush.

The micrograph in figure 4b shows that the whiskers suspension does not have exactly the same dispersion quality than whiskers in water (figure 4a), because a greater amount of aggregates can be observed. However, whiskers keep their fibrillar appearance, which was expected according to the preceding explanations. The physical stability of the films did not change with time. The use of a different surfactant may improve the dispersion that can be obtained.

Once the suspension of chitin whiskers in toluene was ready, preliminary film samples with 2 and 5% of chitin whiskers were prepared. The resulting films were qualitatively homogeneous and more translucent than their homologous neat PCL films.

4.3 Differential Scanning Calorimetry (DSC):

Results obtained by DSC are shown in Table 2. It can be observed that the studied PCL shows a crystallinity degree of 47% and melting and crystallization peak temperatures of 55.5 °C and 30.6 °C, respectively. After incorporating 2 and 5% chitin whiskers to the PCL matrix, the most relevant changes observed were an increase in the crystallization peak temperature (up to 3.5°C for PCL.5%) and a moderate increase in the crystallinity degree. These changes along with the increase in translucency as compared to neat PCL indicate that the chitin whiskers are able to nucleate the PCL matrix.

The efficiency of chitin as a nucleating agent can be ascertained by comparing the change in crystallization temperature obtained with that produced by self-seeds of PCL. We have followed the procedure proposed by Fillon et al. [29], to first self-nucleate neat PCL in order to obtain the maximum possible shift of the crystallization temperature for this polymer. For the PCL employed here our self-nucleation studies indicated that \( T_{C_{\text{MAX}}} = 35.4^\circ\text{C} \). The nucleation efficiency (\%NE) can be calculated by the following simple equation:

\[
\% NE = \left( \frac{T_{C_{\text{MAX}}}-T_C}{T_{C_{\text{MAX}}}-T_C} \right) \times 100\%
\]

<table>
<thead>
<tr>
<th>Sample</th>
<th>Chitin Content (weight %)*</th>
<th>( \Delta H_c ) (J/g)</th>
<th>( T_{c_e} ) (°C)</th>
<th>( T_{c_p} ) (°C)</th>
<th>( \Delta H_m ) (J/g)</th>
<th>( T_{m_e} ) (°C)</th>
<th>( T_{m_p} ) (°C)</th>
<th>( %^b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL</td>
<td>0</td>
<td>-58</td>
<td>30.6</td>
<td>26.5</td>
<td>64</td>
<td>53.0</td>
<td>55.5</td>
<td>47</td>
</tr>
<tr>
<td>PCL2%</td>
<td>2</td>
<td>-65</td>
<td>32.4</td>
<td>29.6</td>
<td>72</td>
<td>51.8</td>
<td>54.9</td>
<td>53</td>
</tr>
<tr>
<td>PCL5%</td>
<td>5</td>
<td>-74</td>
<td>31.2</td>
<td>30.0</td>
<td>80</td>
<td>51.0</td>
<td>54.0</td>
<td>59</td>
</tr>
</tbody>
</table>

*Expressed as weight percentage of PCL.

*Degree of crystallinity calculated taking \( H_g = 136 \text{ J/g} \).
where $T_{c, NA}$ and $T_c$ are the crystallization temperature of the polymer with and without nucleation agent, respectively, and $T_{c, max}$ is the maximum crystallization temperature obtained during the self-nucleation procedure in the absence of annealing [29].

Table 3 shows the nucleation efficiency of the chitin whiskers as nucleating agents for PCL and for the two nanocomposites prepared. From data shown in Table 3, it is clear that the presence of chitin whiskers favors the crystallization of the polymer through a nucleation phenomenon, acting with a nucleation efficiency close to 40% for 5% whiskers.

Table 3. Nucleation Efficiency (%NE) of chitin whiskers as nucleation agents for PCL, for the different compositions studied

<table>
<thead>
<tr>
<th>Sample</th>
<th>$T_{c, max}$ (°C)</th>
<th>%NE</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL</td>
<td>29.6</td>
<td>34.8</td>
</tr>
<tr>
<td>PCL2%</td>
<td>29.6</td>
<td>34.8</td>
</tr>
<tr>
<td>PCL5%</td>
<td>30.0</td>
<td>39.3</td>
</tr>
</tbody>
</table>

In an ongoing work, we are trying to improve the degree of dispersion of the whiskers in the organic solvent and hence in the polymer matrix, besides studying the effect of a higher percentage of chitin in the nanocomposites, in order to evaluate in the near future the change in the mechanical properties of the composite before and after surpassing the percolation threshold of the whiskers.

5. Conclusions

The source of chitin (shells from the *pleoticus robustus* species shrimp) had an approximate 20% chitin content, the acetylation degree of which increased considerably after an acid hydrolysis process. Through a hydrolytic process, chitin whiskers were obtained from the shrimp exoskeleton, having an approximate 300 nm length and 7.0 nm diameter. A non-flocculating chitin whiskers suspension in toluene was obtained by incorporating a commercial surfactant, Beycostat BNA, by means of an experimental procedure that has not been previously reported in literature.

The chitin whiskers are able to nucleate the PCL as indicated by improved translucency in the films prepared as compared to neat PCL, and by an increase in both crystallization temperature and crystallinity. The nucleation efficiency was evaluated to be close to 40% for the PCL sample with 5% chitin when compared to an ideally self-nucleated neat PCL.

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