FORMATION OF FIBRES AND SPHERES FROM CHITIN SOLUTION

Dorota Biniaś*, Włodzimierz Biniaś, Jarosław Janicki

Faculty of Materials, Civil and Environmental Engineering,
University of Bielsko-Biała
Bielsko-Biała, ul. Willowa 2, Poland;
e-mail: dbinias@ath.bielsko.pl

Abstract

The applications of chitin are limited due to its insolubility in most organic solvents. The chemical modification of chitin to generate new bio-functional materials can bring more desirable properties. A concentration of 5% can be obtained. We have successfully prepared chitin fibres and spheres by dissolving chitin in 85% phosphoric acid in various coagulating agents and then regenerating it in 10% sodium hydroxide.

The change in molecular structure was studied by Fourier Transform Infrared Spectroscopy (FTIR). The surface morphology of different biomaterials was observed using scanning electron microscopy (SEM) and optical microscopy (OM).

Key words: *fibres, spheres, chitin, phosphoric acid, regeneration*

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1. Introduction

Chitin is the second most important natural polymer in the world; the main sources exploited are two marine crustaceans, shrimp and crabs [1]. Chitin has found widespread use in biomedical pursuits and has been used in drug delivery systems and tissue engineering [2].

Natural chitin is, however, a highly crystalline biopolymer with poor solubility in water and common organic solvents; thus, its direct application is rather limited [3]. Chitin is soluble in concentrated mineral acids or in a number of anhydrous carboxylic acids. Concentrated phosphoric acid has been used for a long time as a solvent for cellulose [4], a polysaccharide which has a structure and properties close to those of chitin. Compared to other solvents, such as ionic liquid [5, 6] and organic solvent systems, phosphoric acid is inexpensive and is a food additive with no toxicity. Concentrated phosphoric acid is shown to be a useful solvent for the preparations of regenerated chitins [7,8].

The literature has reported that the phosphorylation of chitosan could be achieved through different reaction routes [9].

2. Materials and methods

2.1. Materials

Chitin from shrimp shells, practical grade powder $(C_8H_{13}NO_5)_n$, was purchased from Sigma-Aldrich, 85 wt% phosphoric acid (H_3PO_4) was purchased from CHEMPUR, acetone (C_3H_6O) and sodium hydroxide (NaOH) was purchased from Avantor Performance Materials Poland S.A, 1-propanol (C_3H_8O) (99% extra pure) from Sigma-Aldrich. Distilled water was used throughout the experiment unless specified. All other materials used were of analytical grade.

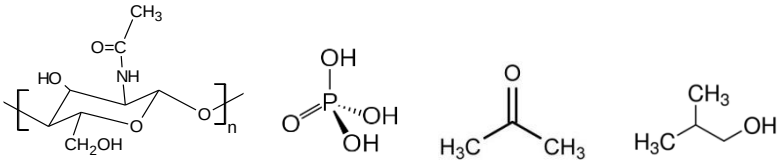

Figure 1. Chemical structure of the materials used: a) chitin, b) phosphoric acid, c) acetone and d) 1-propanol

2.2 Methods

2.2.1. Preparation of chitin biomaterials

Chitin solutions were prepared by stirring the chitin powder (5.00 g) at high speed in 85% phosphoric acid at room temperature after complete dissolution.

The chitin phosphate solution was injected in the coagulating agents by using a syringe with a needle diameter of 0.7 mm. Various coagulation solutions were used: water, acetone and 1-propanol. Figure 2 shows images of the samples obtained after injecting the 5% chitin solution with phosphoric acid in various coagulating agents. After coagulation, there was a regeneration process for chitin with 10% NaOH solution over 24 hours. The regenerated chitin was thoroughly washed with distilled water and then neutralised NaOH, before being dried at room temperature.

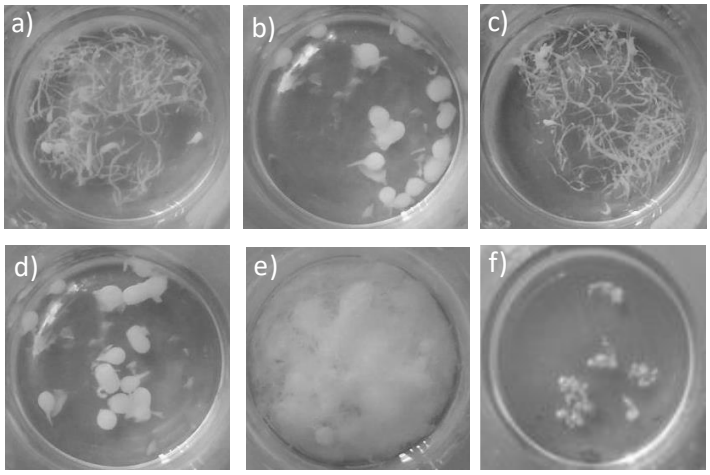

Figure 2. Photos of the chitin fibres and spheres prepared in the several coagulant baths: a) and b) water; c) and d) acetone, and e) and f) 1-propanol.

2.2.2. Characterisation of regenerated chitins

2.2.2.1. Spectroscopic measurements

The PAS-FTIR technique represents a potent tool to analyse solid materials. Its main advantage is the fact that the morphology of the samples does not interfere with the spectral characteristics. Spectra were obtained without treatment of the materials and low quantities of the samples are required [10].

The FTIR-PAS spectra were generated using a Nicolet 6700 (Thermo Scientific, USA) spectrometer equipped with an MTEC model 300 photoacoustic detector (Thermo Scientific, USA). A helium gas purging flow was used to reduce the noise produced by the evaporation of moisture from samples. The samples were packed in small ring cups with a diameter of 10mm.

The spectral region was as follows: 4000–500 cm^{-1}, resolution: 8 cm^{-1}, number of scans: 64 of the solid samples using the OMNIC software (Thermo Scientific). Samples for photoacoustic testing were placed in a special snap holder.

2.2.3. Analysis of morphologies

2.2.3.1. Scanning Electron Microscopy (SEM)

The morphology and structure of the microspheres and fibres was observed by scanning electron microscopy (SEM) using the JSM 5500 LV by JEOL (Japan), equipped with a wolfram cathode at an accelerating voltage of 10 kV. Before SEM observations, the samples were sputter-coated with gold.

2.2.3.2. Optical Microscopy

The morphologies of the obtained materials before drying were observed by means of an optical microscope (PZO, Warszawa) equipped with an ARTCAM CCD camera.

3. Results and Discussion

The FTIR–PAS spectra of chitin from shrimp shells, and chitin obtained in individual coagulation baths were recorded as shown in Figure 3.

The major spectral peaks observed in this range, as well as the corresponding peak assignments, are shown in Table 1 [11-16].

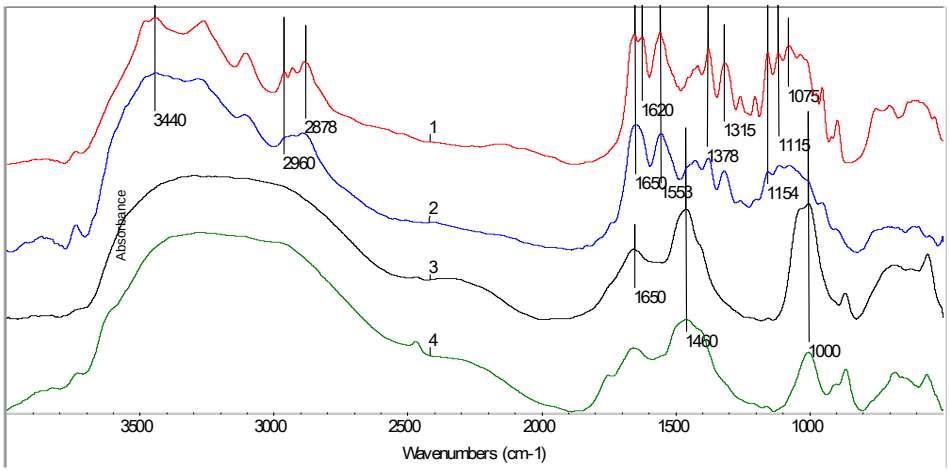

Figure 3. FTIR-PAS spectra of native chitin from shrimp shells and chitin regenerated in different individual coagulation baths (the sample labelled 1 is native chitin from shrimp shells, 2 - is chitin coagulation in water, 3 - is chitin coagulation in acetone, 4 - is chitin coagulation in 1-propanol).

Table 1. The most important absorption bands of mid-infrared spectra of the chitin [11–16]

Wavenumber (cm^{-1})	**Vibration**
3600–3000	O-H stretching
3250–3100	N-H stretching
2960–2920	Asymmetric C-H stretching
2880–2850	Symmetric C-H stretching
1680–1640	C=O stretching, Amide I band
1625–1620	C=O stretching, Amide I band
1635	O=H bending
1570–1540	N-H in-plane; C-N stretching; Amides II band
1450–1430	C-H bending
1380–1375	Symmetric C-H bending,
1315–1308	CH_2 wagging, Amides III band
1155–1153	Asymmetric bridge oxygen bending,
1115–1110	Asymmetric in-phase ring stretching mode,
1075–1050	C-O-C asymmetric stretching in-phase ring,
1045–1000	C-O asymmetric stretching in-phase ring,
950	C-H wagging
895–877	C-H ring stretching

The FTIR spectra of chitin from shrimp shells and chitins after regeneration were recorded in the wavenumber region (4000–500cm^{-1}) as shown in Figure 3.
The band at about 3440cm^{-1} is the characteristic OH group. The two bands which appeared at about 3256cm^{-1} and 3105cm^{-1} were assigned to N–H symmetric and asymmetric stretching vibration of the amide groups. An absorption band at 2960cm^{-1} and 2878cm^{-1} is due to the asymmetric and symmetric C-H stretching [17].

The α-chitins can be distinguished by FTIR spectroscopy through the vibration modes of amide I in the spectral region 1680–1620cm^{-1}. FTIR-PAS spectra of chitin from shrimp shells show two absorptions at about 1650 and 1620cm^{-1} (C=O stretching), which are characteristic of α-chitin (Fig. 3), while in β-chitin, only one band is present at 1656 cm^{-1}. The amide II band at 1558cm^{-1} is characteristic of α-chitin. Peaks at 1378cm^{-1} and 1315cm^{-1} may be assigned to the O–H deformation of –CH_2–OH and –CH–OH, although they overlap with –CH_3 deformation and –CH_2 wagging [9].
The band at 1315cm^{-1} corresponded to the C-N bond, and the peak at 1154cm^{-1} is the asymmetric bridge oxygen (C–O–C) stretching. A prominent band is observed around 1115cm^{-1} corresponding to asymmetric C-O-C and asymmetric stretching of a hydroglucose ring. The bands at 1075 and 1032cm^{-1} were due to the asymmetric stretching of both C-O-C and pyranose groups.

Figure 4 a-b shows SEM photographs of the native chitin from shrimp.

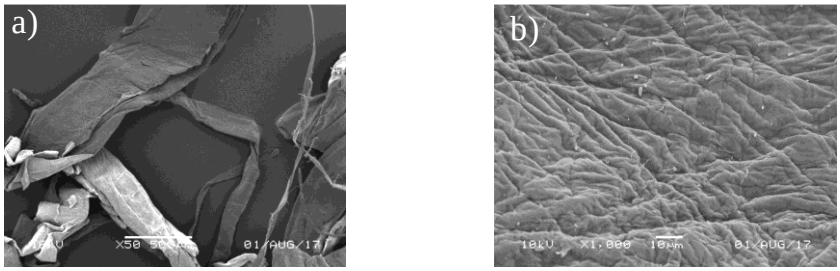

Figure 4a-b. SEM photographs of the chitin from shrimp with different magnifications: a) 500μm scale, and b) 10μm scale.

Figure 5a-c shows the photographs obtained by means of an optical microscope and scanning electron microscope of the coagulation spheres in water (H_2O) and regenerated in 10% NaOH.

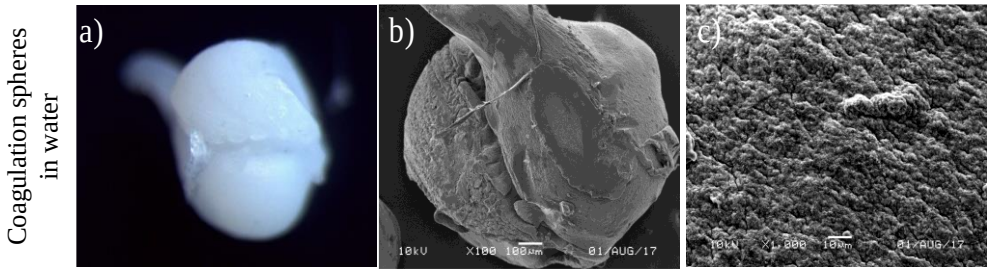

Figure 5a-c. Morphology of chitin spheres: optical microscope images (a), SEM images (b) and SEM images of the surface (c).

The size distribution of the coagulation spheres in water determined by optical microscope is presented in Fig. 5a; the average diameter of chitin spheres was about 1.0 mm.

Figure 6a-c shows the photographs obtained by means of an optical microscope and scanning electron microscope of the coagulation fibres in water (H_2O) and regenerated in 10% NaOH.

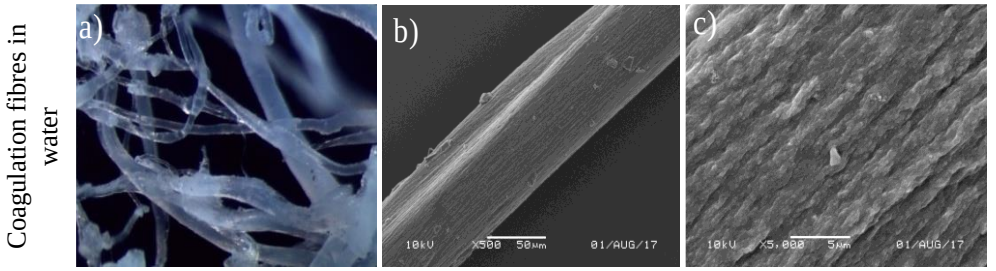

Figure 6a-c. Morphology of chitin fibres: optical microscope images (a), SEM images (b) and SEM images of the surface (c).

The diameter of the chitin fibres is about 82μm.

Figure 7a-c shows the photographs obtained by means of an optical microscope and scanning electron microscope of the coagulation spheres in acetone (C_3H_6O) and regenerated in 10% NaOH.

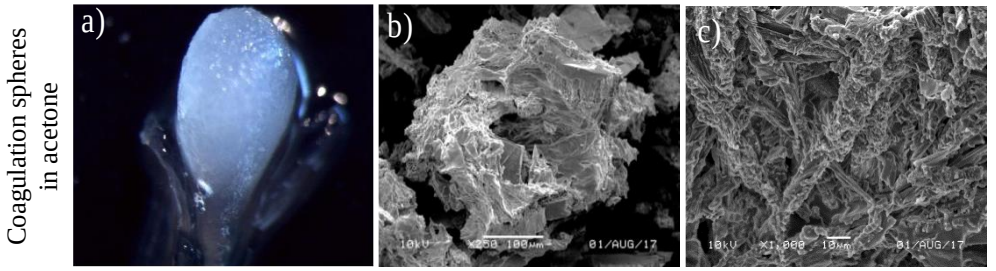

Figure 7a-c. Morphology of chitin spheres: optical microscope images (a), SEM images (b) and SEM images of the surface (c).

The size distribution of the coagulation spheres in acetone determined by optical microscope is presented in Fig. 7a and the average diameter of chitin spheres was about 0.80 mm.

Figure 8a-c shows the photographs obtained by means of an optical microscope and scanning electron microscope of the coagulation fibres in acetone (C_3H_6O) and regenerated in 10% NaOH.

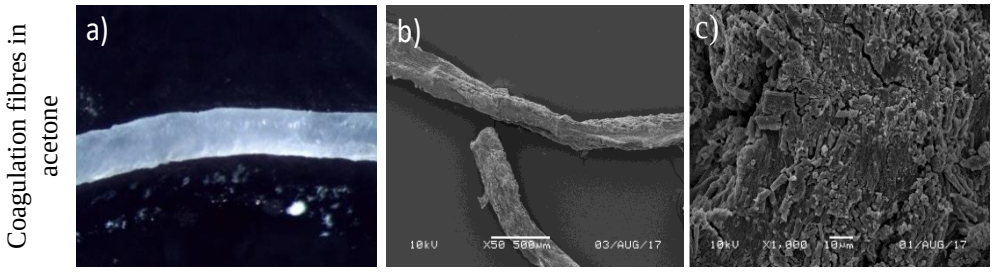

Figure 8a-c. Morphology of chitin fibres: optical microscope images (a), SEM images (b) and SEM images of the surface (c).

The diameter of the chitin fibres is about 250μm.

Figure 9a-c shows the photographs obtained by means of an optical microscope and scanning electron microscope of the coagulation spheres in 1-propanol (C_3H_8O) and regenerated in 10% NaOH.

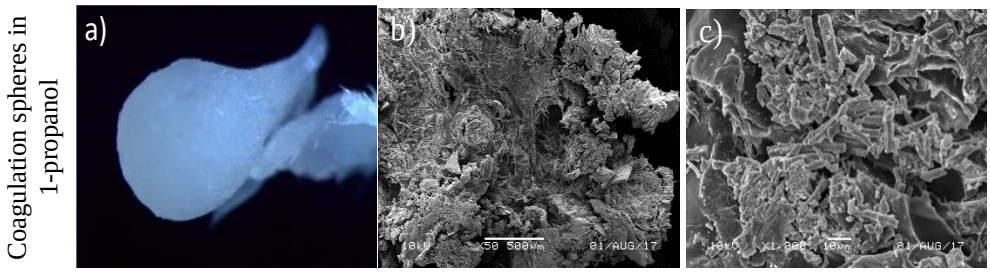

Figure 9a-c. Morphology of chitin spheres: optical microscope images (a), SEM images (b) and SEM images of the surface (c).

The size distribution of the coagulation spheres in 1-propanol determined by optical microscope is presented in Fig. 9a and the average diameter of chitin spheres was about 0.60 mm.

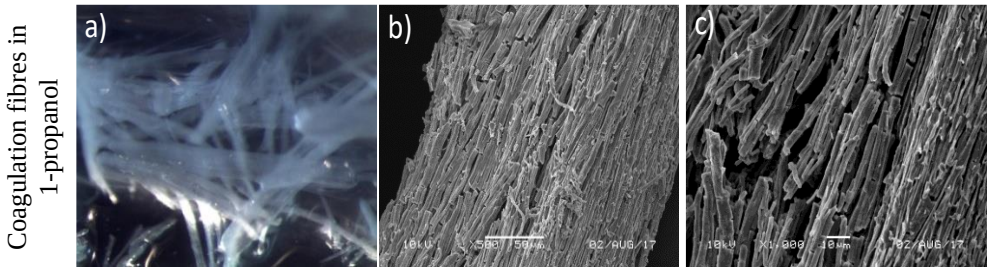

Figure 10a-c. Morphology of chitin fibres: optical microscope images (a), SEM images (b) and SEM images of the surface (c).

The SEM images of chitin fibres coagulated in 1-propanol exhibited microfibril and porous structures. The diameter of the microfibril chitin fibres is about 1-2μm.
SEM micrographs demonstrated a clear difference in the morphology of regenerated chitins when the coagulation bath treatments were varied.

4. Conclusions

In the present work, a simple and versatile method was demonstrated for the fabrication of biomaterials from chitin solution. It was found that phosphoric acid is an excellent direct solvent for chitin.
Concentrated phosphoric acid is shown to be a useful solvent for the preparation of regenerated chitins. Regeneration in water led to the self-assembly of structured chitin. Chitin fibres or spheres can be obtained at varied conditions.
The morphological structure depends to a large extent on the composition of the dissolution mixture and on the coagulation environment.

As a solvent, a controlled-substance mixture is used that is strongly active against hydrogen bonds which maintain the native chitin structure and affect its solubility. An important factor affecting the formation of chitin regenerated from the solution is the composition and parameters of the coagulation bath. Coagulation baths have specific effects on the rebuilding of hydrogen bonds responsible for the physicochemical properties of the products.

5. References

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