

Synthesis and Characterization of Nanosized Pectin-Based Formulations

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Summary: Pectin conjugated magnetic nanocomposites were prepared by chemical coprecipitation method *in situ*. The FTIR, acoustic, Mössbauer spectrometry, XRD measurements confirmed the nanoscaled structure of Fe_3O_4 -pectin composite. The synthesized magnetite nanoparticles were uniform with a mean size of $\sim 14-17$ nm for low pectin concentration as 10 wt.%.

Keywords: biomaterials; magnetite; nanocomposites; pectin; stabilization

Introduction

Micro- and nanosized particles based on pectin substances have attracted particular attention in research on the technologies of medical, material science, and pharmaceutical chemistry, in view that these types of structures can imprint new properties into a polymer formulation.^[1,2,3,4,5] The concept of using a pectin-made hydrogel, as a potential strategy for synthesis of either nano- or microparticles^[6,7] is based on its biodegradable nature and flexible structural networks allowing the device to be designed to a specific shape. Moreover, a variety of pharmacological properties of pectins suggest them as a potential source of new drugs.^[8,9,10]

Higher biological ability as antimicrobial, anticancer, and chemosensitizing agents was observed for complexes of pectin substances with biocompatible metals.^[10,11,12,13,14] It has been demon-

strated that the pectin metal complexes possessed equal or higher antitumor potential in relation to some kind of tumors. Pectin-based metallocomplexes were capable of enhancing the antitumor effect of 5-Fluorouracil and cisplatin while decreasing their toxic and side effects.

Our previous studies^[11,12,13] suggest that for the production of biomaterials with enhanced effect the most promising seems to be the production of nanoscale derivatives of pectin biopolymers with multifunctional pharmaceutical properties. In this work, our attention has been focused on fabrication of coating Fe_3O_4 nanoparticles with different pectin (Pec) concentration to be ultimately applied as antitumor substances. Sugar beet pulp was the object for the isolation of the pectin. The physical and chemical characterization of the synthesized nanoparticles (Fe_3O_4 -Pec) was conducted, and effect of pectin concentrations was evaluated.

Experimental Part

Pectin (Pec) was isolated from sugar beet pulp according to.^[8] Sample of native pectin was pre-dispersed in the high-energy grinder (SPEX SamplePrep, speed of dispersion is 1425 rtm, material for balls and grinding/mixing vial is wolfram carbide) within

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1–30 min in order to produce the nanosized fraction. Sample of pectin with 50 nm in size was used as an organic precursor for synthesis of Fe_3O_4 -Pec nanocomposites. Content of D-galacturonic acid in this pectin sample is 83.0%, amount of carboxyl and methoxyl groups made 14.8 and 4.6 wt. %, accordingly; $M_w = 15$ kD determined by size exclusion chromatography.

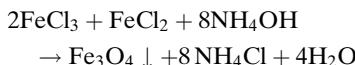
Protocol of Synthesis of Crystalline

Hydrate $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$

To 10 mL of water and 17 mL of HCl (density 1.19 g/mL) in china 6.0 g of Fe powder was gradually added. The intense evolution of hydrogen was observed. After cooling the solution was cooled, the blue crystals formed was filtered and dried in vacuum.

Protocol for Synthesis of Fe_3O_4 Nanoparticles

High-dispersion magnetite was prepared according to Elmore reaction.^[16] The $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (Sigma-Aldrich, 98%) (9.82 g) and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (12 g) salts (the aqueous solutions of 10 wt. % concentration) were mixed at $\text{Fe}^{3+}/\text{Fe}^{2+} = 2/1$ in inert atmosphere. Then NH_4OH (25 wt. %) was added at vigorous stirring (1200 rot/min). The reaction was carried out as follows:



The dark precipitate was observed. The co-precipitated NH_4Cl was removed by repeated washing with hot distilled water. The solid product was placed in a desiccator over concentrated H_2SO_4 for two days under argon. Such a process for synthesis of nanoparticles of iron oxides is so-called two-stage growth process, which involves a fast nucleation of amorphous primary particles followed by a slow aggregation and crystallization of primary particles.

Protocol for Synthesis of Iron-Containing Nanoscale Pectin-Based Composites

Various amounts of pectin (0.075, 0.15, 0.3, 0.6, 1.5 and 2.4 g) were added accordingly to

magnetite water suspension (2.4, 1.5, 0.6, 0.3, 0.15, 0.075 g) in order to achieve different concentration levels of pectin. Amount of pectin in Fe_3O_4 -Pec was varied from 2.5 to 80 wt.%, name of samples are referred as Fe_3O_4 -Pec2.5, Fe_3O_4 -Pec5, Fe_3O_4 -Pec10, etc. accordingly. Synthesis and treatment of as-prepared Fe_3O_4 -Pec precipitate was performed by a similar way as magnetite.

Physical-Chemical Analysis of Pectin-Based Formulations

The particle size distribution was analyzed regarding their attenuation spectra using the acoustic spectrometry (Ultrasound spectrometer DT-1201, Quantachrome, USA). Fourier transform infrared (FTIR) spectra were obtained by pressing the pectin samples into KBr pellet and analyzing with a FTIR spectrometer IR-200 (ThermoNicolet, USA) on spectral range of $400\text{--}4000\text{ cm}^{-1}$ (4 cm^{-1} resolution, 64 scans per spectrum). Mössbauer spectra were recorded on a spectrometer (MS-1101-E, Mostec) with a $\text{Co}^{57}(\text{Rh})$ source in the constant acceleration regime. The calibration by rates was performed relative to the spectrum of metallic α -Fe. The phase composition analysis of the nanocomposites obtained was performed using X-ray diffractometry on a DRON-UM-2 diffractometer using $\text{Cu}(\text{K}_\alpha)$ radiation.

Results and Discussion

Results of the FTIR spectra of Pec and the Fe_3O_4 -Pec nanocomposites (Figure 1) indicated that the final product is a true composite of pectin and magnetite. FTIR spectra demonstrated the absorption peaks at near 1388 and 1633 cm^{-1} , which are related to stretching bands of carboxylate (COO^-) groups of Fe_3O_4 -Pec. It is found that the intensity of peak at 1743 cm^{-1} (induced by COOH groups of Pec) for Fe_3O_4 -Pec10 is obviously weaker than that for Pec. The broad band in the region of $3000\text{--}3600\text{ cm}^{-1}$ corresponds to -OH stretching vibration of iron pectate.

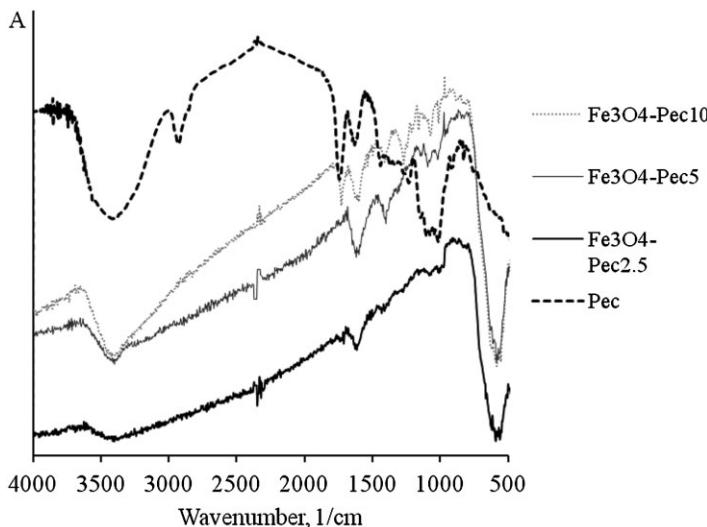


Figure 1.
FTIR spectra of Pec and Fe_3O_4 -Pec nanocomposites.

According to X-ray diffractometry studies, the major phase formed during a chemical coprecipitation in the presence of pectin is a magnetite Fe_3O_4 (Figure 2). XRD results indicate that the addition of pectin to Fe_3O_4 nanoparticles have not changed the crystal structure of nanoparticles, the intensity of the peaks is reduced with using pectin. The average crystal size of the nanoparticles calculated from the diffraction peak half-

widths according to Scherrer's equation for the Fe_3O_4 nanoparticles stabilized by pectin in concentrations of 2.5–10 Macc.% decreased from 17 nm for magnetite to 14 nm for nanocomposites. The pectin macromolecule binds to the particles just after nucleation of the Fe_3O_4 nanoparticles preventing further growth.

Pectin has a strong affinity to the surface of metal oxide particles.^[17] The absorption

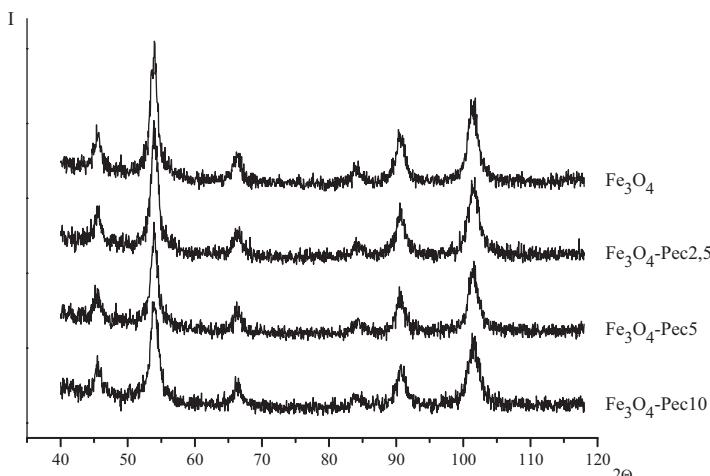


Figure 2.
XRD patterns of the synthesized nanoparticles.

of pectin on the magnetic particle's surface leads to an enhanced electrostatic and steric stabilization of the particles due to the absorbed layer of highly charged macromolecular compounds. According to^[18] Fe is present inside the ionic structures as Fe(III)-hydroxy polymer, which interacts electrostatically with both the carboxylate and -OH alcoholic groups as well as the oxygen of the glycosidic bond.

Data of the Mössbauer spectra of the magnetite (zero test) measured at 300 K confirmed XRD data (Figure 3). All spectra represent a sextet with wide asymmetric lines. The Mössbauer spectra of the investigated samples are characteristic for small particles magneto-ordered phases. Presence of quadrupole doublet allows to draw a conclusion about presence in the sample of small superparamagnetic particles. The analysis of the obtained spectra and the restored distribution has shown that sextet lines became narrower. Modeling decoding of a spectrum has shown that the synthesized substance, apparently is a mix maghemite and magnetite.

As it can be seen from the Figure 3, pectin addition leads to the slight reduction of magnetic particles size. More uniform

particle size distribution was observed for Fe₃O₄-Pec2.5. The effect of pectin content on the size of iron oxide nanoparticles and particle size distribution was found insufficiently for low concentrations. But, according to data of ultrasound spectrometry (Figure 4), with increasing pectin concentration (i.e. above 10% w/w) an increase in the mean size of Fe₃O₄ was detected. Results of the particle size distribution are summarized in Figure 4 (insertion).

This behavior is observed only at higher pectin concentrations, probably because above a critical concentration a self-gelation of pectin occurs, and therefore stabilization of nanoparticles of iron oxides is not favorable due to steric factor.^[18] Moreover, increasing of pectin concentration leads to increase of particle size distribution.

It is known,^[9] that pectins exhibit a high gelation capacity. Apparently, increase of the size of magnetite nanoparticles and consequently inefficient stabilization of nanoparticles by a high concentration of pectin are caused by increase of viscosity of pectin solution due to the swelling and gelation of pectin. The slow kinetics of dissolution process at high concentration of pectin (up to 1 hour) causes its uncompetitiveness with a high rate of growth of the particles. The various kinetics of processes of dissolution of pectin and growth of nanoparticles is shown also in wide particle size distribution.

Conclusion

The present work confirms the development of pectin based nanosized formulations *via* introduction of nanosized iron oxides ions into pectin backbone. The formation of magnetite nanoparticles within pectin using the coprecipitation route was found to be dependent on the concentration of this polymer. Changing pectin content in the pectin-based formulations could modify mean size for Fe₃O₄ nanoparticles. The synthesis *in situ* allows a more uniformly dispersion of the nanopar-

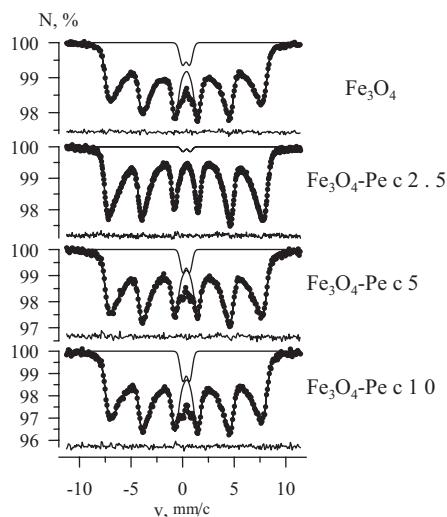


Figure 3.

⁵⁷Fe Mössbauer spectra (recorded at 300 K) for synthesized formulations: (1) - Fe₃O₄; (2) Fe₃O₄-Pec2.5; (3) Fe₃O₄-Pec5; (4) Fe₃O₄-Pec10.

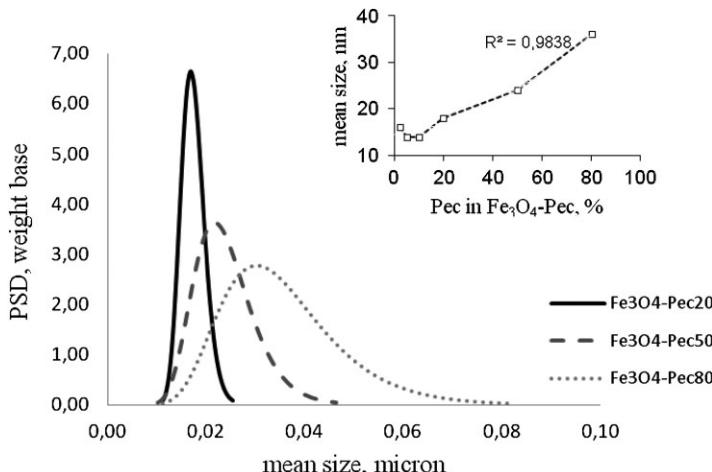


Figure 4.
Dependence of mean size of Fe_3O_4 on Pec content.

ticles within the polymer matrix. The results from this study enable us to state that the complexes containing pectin and iron oxide are an interesting way of preparing controlled-size formulations.

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