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# Novel polysaccharide Nanowires; Synthesized from Pectin-Modified Methacrylate

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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**Abstract. Pectin as polysaccharide polymer can be used in biomedical engineering and food industry due to its prominent properties such as biocompatibility and bio-degradability. Here, we first time reporting the preparation of nanowires and nanoparticles by water/ oil emulsion method using methacrlayted pectin (Ma-pectin). We have envisioned that these new materials can be considered as a new generation of biomaterials, which would pave the way for various biomedical engineering applications.**

Nowadays, nanoparticles play an important role in the drug delivery systems by entrapping a drug in their interior structure, adsorbing to the surface, or covalently attaching to the precursor materials. In view of this, polysaccharides nanoparticles, particularly pectin, are considered as promising materials for the pharmaceutical industry due to their outstanding properties such as versatility, biodegradability, non-toxicity, easy procedures and biocompatibility.[1-3]

Pectin is a class of complex polysaccharides with negative charge, which can be found in the primary cell wall of plants, and is also widely used in the food industry as gelling and thickening agents. In addition to this gelling property, pectin can also be considered as a potential carrier in drug delivery systems due to its specific degradation by colonic enzymes. However, due to the rather complex structure of pectin, a few promising studies can be found in the literatures about pectin nanoparticle.[4-6]

In an earlier study, Cheng and Lim[7] used the pectinate spherical nanoparticle by ionotropic gelation with calcium ions as a potential colonic delivery system for insulin. Later, Opanasopit et al.[8] prepared pectin nanoparticles (in spherical shape) by the iontropic gelation method and found that the size of the nanoparticle varied with the cation type. Furthermore, they showed that the pectin nanoparticles can be also used as safe and effective gene delivery carriers. In another study, Sharma et al. [9] found that the pectin spherical nanoparticles which wee synthesized by the iontropic gelation technique, can also be considered as potential candidates for such as ocular drug delivery for cornea diseases instead of the conventional solution drug. Furthermore, Adriano V. Reis et al.[10] reported the synthesis of hollow-structured nanospheres from pectin by the self-assembly technique for the first time. They used the modified pectin for undertaking the cross-linking reaction in a water-in-benzyl alcohol nanodroplet emulsion. They also mentioned that this hollow nanoparticle structure has the potential to be used in different fields such as catalysis and chromatography. Recently, Jonassen et al.[11] reported the preparation of spherical nanoparticles based on the chemically modified pectin, amidated low-methoxylated pectin (AM-Pec) and low-methoxylated pectin (LM-Pec), by the ionotropic gelation method. They found that the AM-Pec nanoparticles are much less polydisperse and more stable in storage condition than the LM-Pec nanoparticles. Therefore, AM-Pec nanoparticles can be considered more suitable for drug delivery systems due to their muco-adhesive properties and targeting abilities.

Although the spherical nanoparticles offer new possibilities in the search for a suitable drug delivery system, more recent study showed that nanorod/ wire-shaped nanoparticles (nanorods/nanowires) compared to spherical nanoparticles appear to adhere more effectively to the surface of endothelial cells lining the inside of blood vessels, allowing for drug-targeting to specific type of cells.[12] However, to the best of our knowledge, there is no available report in the literature to focus on this matter. In this respect, this work focuses on synthesis of the nanorod/ nanowire based on the modified pectin by glycidyl methacrylate (GMA) with a combination of the two techniques; inotropic gelation and covalent cross-linking. Preparation of organic polymer nanowires is challenging in particular carbydrates polymers because of the chemical structure. Here, we first time reporting the synthesis and preparation of nanowires and nanoparticles and pellets

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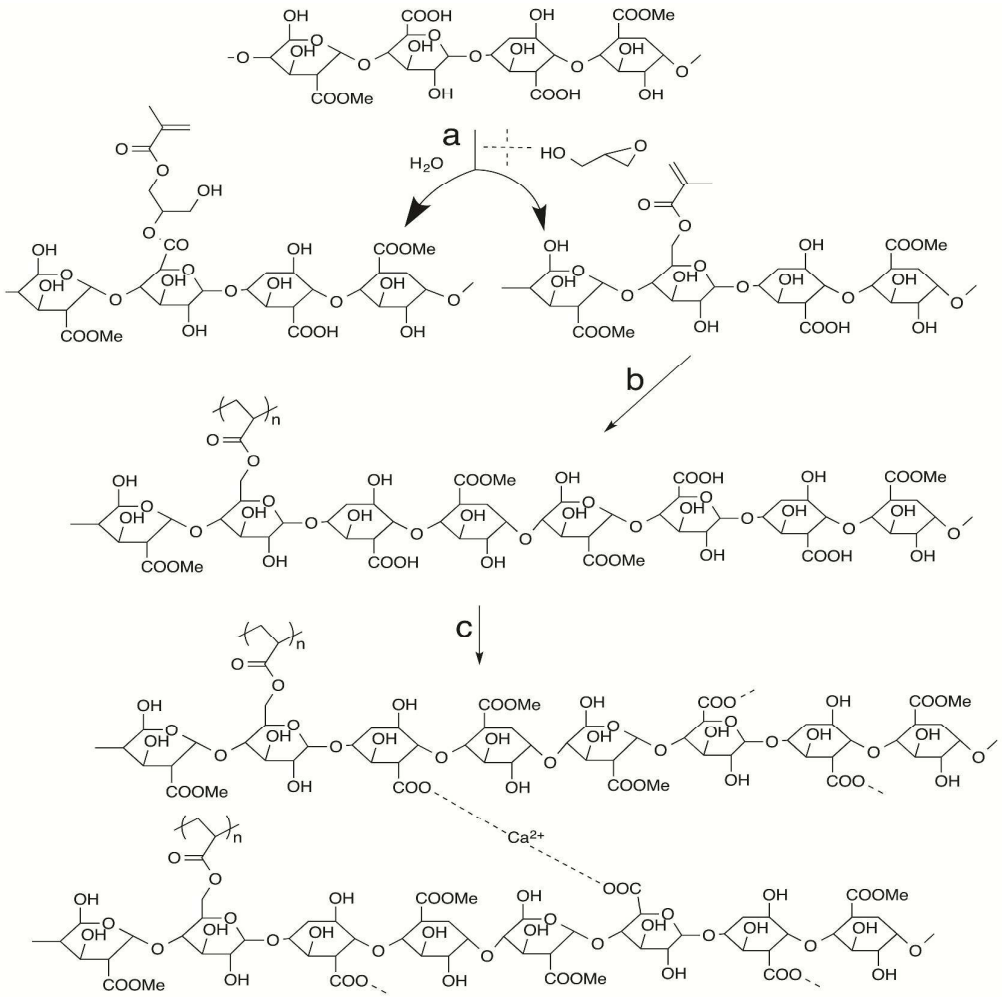
Electronic Supplementary Information (ESI) available




**Scheme 1**. Modification of pectin polysaccharide. Reaction conditions. a) 4.8 ml of GMA at pH3.5 for 24 hours at 50 $^{O}$C. b) 0.1 mM sodium persulfate at 60 $^{O}$C for 6 hours and c) 100 mg $CaCl_2$ at 50 $^{O}$C for 1 hour.

by using modified methacrlayed pectin (Ma-pectin) by oil/water emulsion method. We synthesized pectin nanowires with different concentrations of calcium chloride with carbonyl groups of pectin. The final product is also characterized with the aid of a scanning electron microscope (SEM) and Nuclear magnetic resonance spectroscopy (H-NMR). As a result, this work introduces for the first time the unique shape of the pectin nanoparticle, nanorods/nanowires, which can be potentially used in various industrial fields such as pharmaceutical for a drug delivery system and the cosmetic industry. These are primary result of our research work, however we are still working on pectin morphology and their application studies. Firstly, methacrylate was functionalized on pectin by following transesterfication reaction at pH 3.5. See scheme 1.

The NMR confirmed that methacrylation of vinyl protons was confirmed at 5.6 and 6.1 ppm and methyl protons (-CH3) appeared at 1.9 ppm as shown in Supplementary Information Figure. S1.

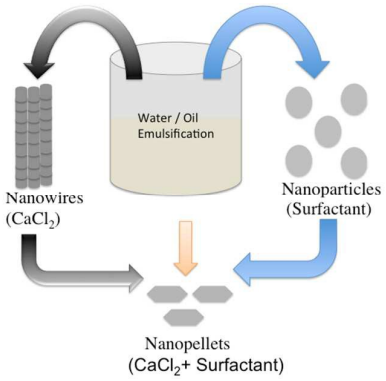



**Figure 1**. Graphical representation preparation of pectin nanowires, particles and pellets synthesis by water/ oil emulsion method.

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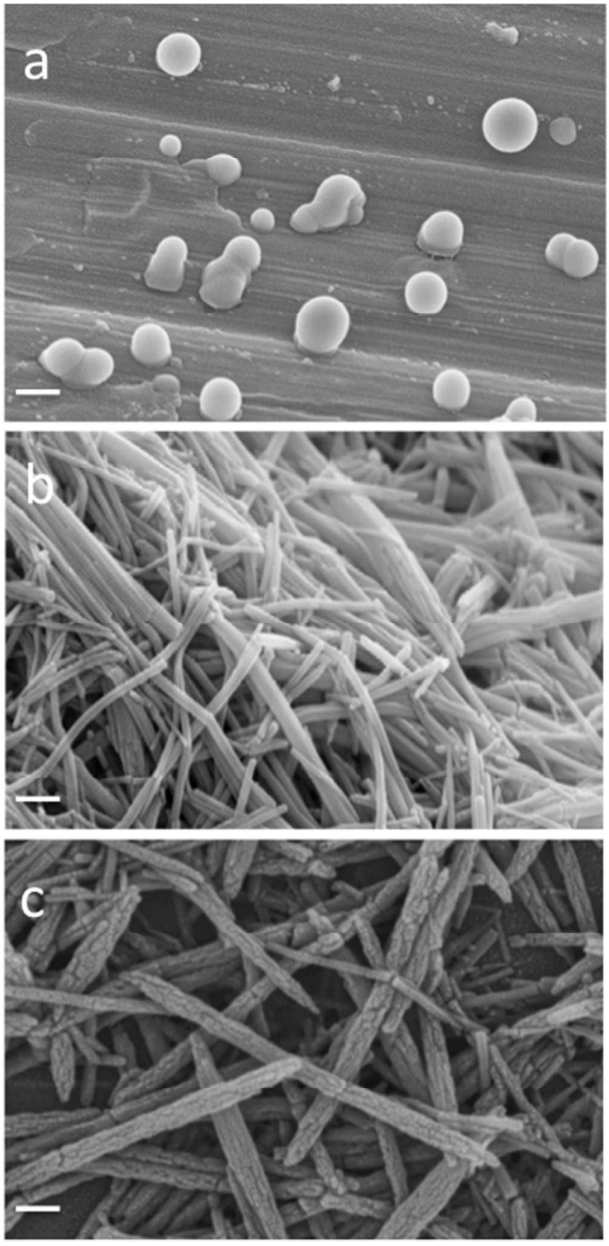


**Figure 2**. SEM images of modified Ma-pectin nanoparticles (a) sonication with dodecane, b) Nanorods/fibers of Ma-pectin and c) nanowires was prepared by treating with $CaCl_2$ in toluene. Scale bar 100 nm.

The hydrogel network was formed *via* radical polymerization/ cross-linked reaction of the Ma-pectin in the presence of sodium persulfate. The modification of the pectin with GMA leads to considerable changes in the polysaccharide morphology. The pectin-modified reaction was performed in a heterogeneous system due to the interfacial reaction with methacrylate (Ma)-water systems. A high-speed stirring (6000 rpm) was used, which helps to have better agitation in the mixture that leads to produce the particle and also prevents the falling of heavy droplets at higher mixing speed. In order to achieve a satisfactory emulsion volume to produce the particles, the reagent volumes and mixing times were doubled. As the 10% molar pectin was added, the emulsion became thicker and when it was added, the solid precipitate was obtained (data not shown). The Ma-pectin was reduced to 5 molar percentages to produce the spherically shaped particles (Figure S2b).

The spherical nanoparticles were prepared by using the water/oil emulsification method with the aid of surfactant. The Ma-pectin was polymerized in aqueous medium and tetrabutyl bromide (TBABr) was used as surfactant in dodecane to emulsify with the water. The SEM images (Figure. 2a) show that the size of pectin nanoparticles varied from 100 to 150 nm and the nanoparticles were spherical in shape. We change the condition by varying the concentration of Ma-pectin and the cross-linking agent (here calcium chloride was used), to reach our ultimate goal, which was to change the morphology of pectin fiber into nanorods and nanowires as schematically presented all the morphologies of modified pectin in Figure 1.

It was hypothesized that the pectin droplets would not completely crosslink in the short-time mixing period once the calcium chloride was added. When the aqueous solution of pectin was dropped into a divalent cation solution ($CaCl_2$, $MgCl_2$ or $MnCl_2$), pectinate particles were instantaneously produced by ionotropic gelation. In this process, intermolecular cross-links were formed between the negatively charged carboxyl groups of pectin and the positively charged divalent cations. The calcium chloride function was to act as the cross-linker between the pectin carbonyl group and the calcium ions forming the solid particles with egg-box structures see supplementary Information Figure S3.

Although Ma-pectin was cross-linked via radical polymerization, the number of acid groups in the backbone of the pectin was free enough to crosslink with calcium chloride. When 3% (molar) of Ma-polymer solution was added dropwise into $CaCl_2$ solution the final product has the nanorod-shaped morphology as was shown in Figure 2b. Furthermore, by increasing the percentage of Ma-pectin to 5% (molar) of Ma-pectin polymer solution, the morphology was transformed into nanowires (see supplementary Information for the reaction

condition and morphological structures are presented in Table S1).

It can be seen from the above results that the addition of 15% pectin leads to a decrease in the amount of the free acid with pectin backbone. Also, due to low concentration, the pectin particle cannot be well spread throughout mixture; therefore the individual nanoparticles stick together and form rod-shaped nanoparticles. On the other hand, increasing the concentration of Ma-pectin during the mixture sonication for 90 min leads to the pre-polymerized reaction with sodium persulfate due to the large number of free acid groups. Hence, they cross-linked heavily with pectin and form egg-box shape (see supplementary Information Table S1). However, increasing the sonication time to 120 minutes leads to a change in the pectin morphology from egg-box to nanowires particles, as shown in Figure S3 (see Supplementary Information), which are actually produced from the growth of the egg-box shape. The lengths of the nanowires were 700 nm and their diameters were 80 nm.

The important argument here is how to control the structure of the nanorods and change their morphologies. To change the morphology we increased sonication time and concentration of the modified pectin but when we increase the concentration, it can be noticed that there is a formation of white lumps in the mixture, and an increase in the time of sonication yields aggregation structure (data are not shown here). Hence, in this situation, we introduced the use of the surfactant for changing the morphology. In this case, tetrabutyl ammonium bromide as a surfactant was suggested to be used. In this respect, by addition of the different percentages of surfactant, it is possible to change the morphology of pectin from nanowires or nanorods into small pellets as presented in Figure S3 b see in Supplementary Information.

In summary, by using radical polymerization methods Ma-pectin morphology can be changed into spherical nanoparticles and also by using the calcium chloride sonication method, it is possible to pepare the pectin nanorods or nanowires and also form pectin pellets.

All these types of fabrication at the nanoscale and its applications are feasible due to the unique properties of this material of the pectin may open up new possibilities for biomedical research and the application.[13] For example, such as pectin polysaccharides act as a joint lubricant and scaffolding in tissue engineering, polysaccharides on the surface of the cell are involved in various biological functions, including the accession, the recognition and the metastases. Since then, the pectin is not toxic biomaterial, could strongly considered in the delivery of medicines, sensors and drugs other biochemical work.

In our work, we designed and modified pectin by methacrylation and the Ma-Pectin was polymerized by radical polymerization. The nanoparticles were prepared by using the water/oil emulsification method with the aid of surfactant as presented in Figure 1. Furthermore, we introduced a new method of preparing nanorods and nanowires by using calcium chloride under sonication. In this respect, it is expected that this new morphology of pectin nanoparticle, such as rod and wire shapes, would establish new biomaterials for use in different biomedical applications. However, we are still working more on the pectin morphologies and their application.

Acknowledgement.

Thanks to Mrs. Soorya Kizhakke Vettil for helping to conclude the results. I acknowledge Dr. Hojat Madadi and Mrs. Fatemeh Shiravand.

For more synthetic procedure and methods see Supplementary Materials.

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Preparation of nanowires and nanoparticles of water/ oil emulsion method using methacrlayted pectin (Ma-pectin).