Proceedings of 15th Agricultural Research Symposium (2016) 164-168

Fabrication of Magnetic Nanoparticles Coated with Chitosan

J.M. SAFREEN[1], J.G. SHANTHASIRI[2], S.N.T. DE SILVA[3] and C.A.N. FERNANDO[3]

[1]*Department of Biotechnology, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP), 60170, Sri Lanka*

[2]*National Science Foundation, Maitland Place, Colombo 07*

[3]*Nano Technology Degree Programme, Department of Electronics, Faculty of Applied Sciences, Wayamba University of Sri Lanka, Kanadulla, Kuliyapitiya, 60200,Sri Lanka*

ABSTRACT

Functionalized magnetic nanoparticles (MNPs) have numerous novel applications. Chitosan coated magnetic nanoparticles (CsMNPs) are able to bind to diverse chemical groups and ions which lead to a number of applications such as protein and metal adsorption, guided drug and gene delivery, magnetic resonance imaging, tissue engineering and enzyme immobilization. In this study, chitosan (Cs) was prepared by a deacetylation reaction from shrimp-shell waste and was characterized by Fourier Transform Infrared Spectroscopy (FTIR). Conditions were optimized to synthesize MNPs, by coprecipitation method, from aqueous Fe^{2+} and Fe^{3+} salt solutions by the addition of a base under an inert atmosphere, followed by modification of the surface of MNPs with chitosan (CsMNPs) with and without sonication. The particle size was analyzed by the FRITSCH particle analyzer. -N-H stretching of chitosan and -C-O-C- stretching and -C-H- stretching of chitin had exact stretching vibration patterns similar to the standards while other characteristic band stretching vibrations showed slight deviation from the standards. The MNPs and CsMNPs of 90 nm appeared at temperature 70 °C and the NH_4OH_{aq} adding rate of 0.5 mL/ 1-2 min.

KEYWORDS: Coprecipitation, Enzyme immobilization, Magnetic nanoparticles, Natural polymers

INTRODUCTION

The recent developments of nanotechnology in synthesizing biocompatible and functionalized magnetic nanoparticles (MNPs) have numerous novel applications in biomedicine. Especially, Fe_3O_4 Ferrite MNPs have been rising as a significant useful material due to their specific properties such as supper paramagnetism, nontoxic nature, small size, *etc.* The studies of utilizing various biocompatible and biodegradable polymers which can functionalize and modify the surface of the Fe_3O_4 nanoparticles (NPs) have been carried out broadly. MNPs are usually composed of the magnetic cores and a polymeric shell having favorable functional groups and features for various applications (Dung *et al.*, 2009).

Fe_3O_4 MNPs was treated with various materials to improve their biocompatibility and chemical stability. Modifications of Fe_3O_4 NPs have been carried out by carbon, precious metals, silica and biopolymers, such as chitosan and cellulose that have been applied as support in many heterogeneous catalytic systems (Safari and Javadian, 2014).

Chitosan is a partially deacetylated polymer of N-acetyl glucosamine that can be obtained through alkaline deacetylation of chitin. It should be noted that chitosan, as a polyamine, is the second most abundant natural polysaccharide in the world after cellulose. The amine and -OH groups endow chitosan with many special properties, making it applicable in many areas and easily available for chemical reactions (Zhao *et al.*, 2011; Figure 1).

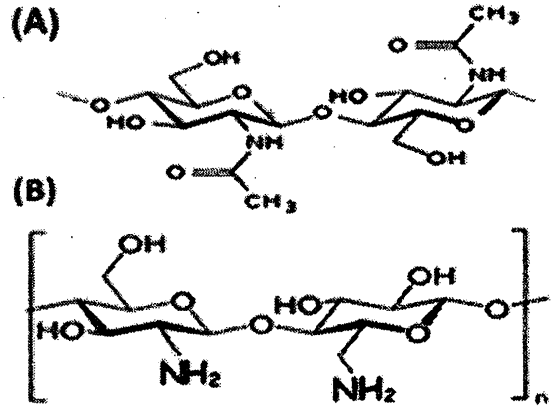

Figure 1. Chemical structure of chitin (A) chitosan (B)

Chitosan-coated magnetic nanoparticles (CsMNPs) constitute a very interesting material which has a number of actual and potential applications. This type of NPs containing a core of a magnetic material, usually a mixture of magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3), owe their functionality to the free amino and hydroxyl groups that chitosan contains. Through these groups, CsMNPs are able to bind to a diverse chemical groups and ions, which lead to a number of applications such as protein and metal adsorption, guided drug and gene delivery, magnetic resonance imaging, tissue

engineering and enzyme immobilization (Osuna *et al.*, 2012; Figure 2).

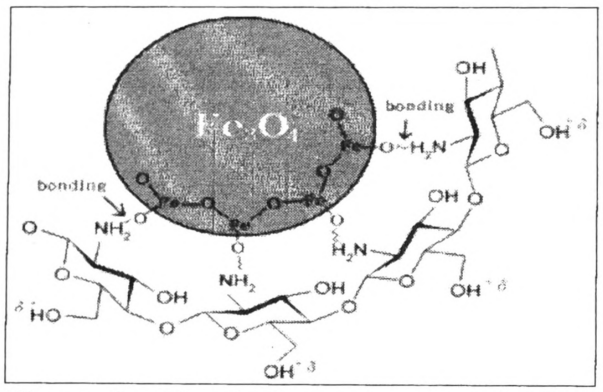

Figure 2. Chemical structure of chitosan coated. (Hong *et al.*, 2010).

Immobilized enzymes are widely used for a variety of applications. Based on the type of application, the method of immobilization and support material can be selected. The immobilized enzymes can be separated from the reaction mixture and unimmobilized enzymes can be reused. Also, immobilized enzymes are more stable than their native counter parts, as they can function in harsh conditions such as, high temperature, surfactants, and oxidizing agents *etc.* The immobilized enzymes are also widely used in food industry, pharmaceutical industry, bioremediation, detergent industry, textile industry, *etc.* Enzyme immobilization improves operational stability and it is also due to increased enzyme loading which causes controlled diffusion (Nisha *et al.*, 2012).

MATERIALS AND METHODS

Extraction of Chitin and Chitosan

The exoskeletons of the prawn waste (shell) were removed and were rinsed thrice with tap water and twice with distilled water. Then, they were dried in a hot air oven for 24 h at 55 °C.

Deproteinization

The dried sample was soaked in 4% boiling NaOH for 1 h followed by cooling at room temperature for 30 min. The treated sample was ground into small pieces by using a grinder.

Demineralization

The crushed sample (32.8 g) was demineralized using 700 mL of 1% HCl and soaked for 24 h to remove minerals. The above samples were treated with 800 mL of 2% NaOH for 1 h, washed with deionized water and drained off.

Deacetylation

This process was carried out by adding 50% NaOH to the obtained sample and boiling it for 2 h at 100 °C followed by cooling it to room temperature. After allowing it to stand for 30 min at room temperature, it was washed repeatedly with 50% NaOH. It was left uncovered and oven-dried for 6 h at 110 °C (Puvvada *et al.*, 2012).

Magnetic Nanoparticles Synthesis (MNPs)

MNPs were synthesized as follows. In method one, a combination of $FeCl_3.6H_2O$ and $FeCl_2.4H_2O$ were used to synthesize nanoparticles. Then, to synthesize chitosan coated nanoparticles, the solutions were mixed with a solution of chitosan before dosing with ammonia. In method two, a combination of $FeCl_3.6H_2O$ and $FeSO_4.7H_2O$ were used directly to synthesize chitosan coated nanoparticles.

MNPs Synthesis using $FeCl_3.6H_2O$ and $FeCl_2.4H_2O$

The procedure started with mixing 50 mL of $FeCl_3.6H_2O$ (0.32 M) and 50 mL of $FeCl_2.4H_2O$ (0.2 M). Once Fe^{+2}, Fe^{+3}, solution reached the desired temperature of 50 °C, the dosing of 20 mL of the aqueous ammonia ($NH_4OH_{(aq)}$) was done at the rate of 0.5 mL/1-2 min. After dosing was over, the completion reaction was allowed to proceed for 20 min. At the end of the reaction, the particles were recovered by using a permanent magnet and washed 2 times with de-ionized water and the particle was analyzed by using a particle analyzer. The protocol was optimized for temperature (60 °C, 70 °C and 80 °C), and ammonia adding rate (0.5 mL/2-3 min, 0.5 mL/3-4 min).

Chitosan Coated Magnetic Nanoparticle

The procedure started with mixing 50 mL of $FeCl_3 \cdot 6H_2O$ (0.32 M) and 50 mL of $FeCl_2 \cdot 4H_2O$ (0.2 M). Then, 10 mL of chitosan solution was added to the reaction mixture and the temperature was raised to 50 °C. The dosing of 20 mL of the aqueous ammonia at 0.5 mL/1-2 min was started. After dosing was over, the completion reaction was allowed to proceed for 20 min. At the end of the reaction, the particles were recovered by using a permanent magnet, washed 2 times with de-ionized water and analyzed by using a particle analyzer. The protocol was optimized for temperature (60 °C, 70 °C and 80 °C) and ammonia adding rate (0.5 mL/2-3 min, 0.5 mL/3-4 min).

Chitosan Coated MNP Synthesis using $FeCl_3.6H_2O$ and $FeSO_4.7H_2O$

The procedure started with mixing 50 mL of $FeCl_3 \cdot 6H_2O$ (0.32 M) and 50 mL of $FeSO_4.7H_2O$ (0.2 M). Then, 1% chitosan solution (10 mL) was added to the reaction mixture and dosed with 20 mL of aqueous ammonia at 0.5 mL/1-2 min. After dosing was over, the completion reaction was allowed to proceed for 20 min. At the end of the reaction, the particles were recovered by using a permanent magnet, washed 2 times with deionized water and analyzed by using a particle analyzer. The conditions were optimized for pH (5, 6.5, 8 and 9.5), temperature (60 °C, 70 °C and 80 °C) and ammonia solution adding rate (0.5 mL/2-3 min, 0.5 mL/3-4 min).

RESULTS AND DISCUSSION

The result of FTIR spectra of shrimp shell chitin and chitosan are shown in Figure 3 and Figure 4, respectively.

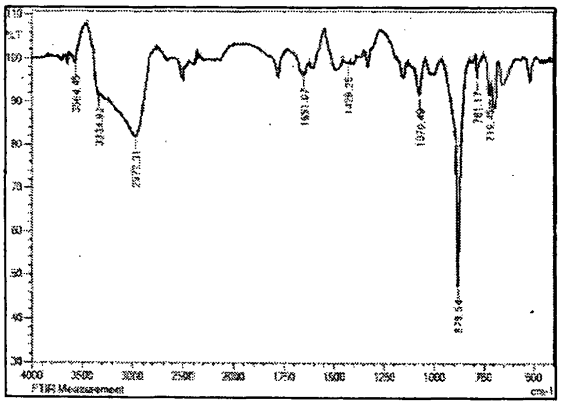

Figure 3. The fourier transform infra-red spectroscopy (FTIR) spectrum of chitosan

Table 1 compares the FTIR spectra of the chitin and chitosan synthesized in the present study to the FTIR spectra of commercial standards of chitin and chitosan. The FTIR spectra of chitosan exhibited a characteristic band at 3564.45 cm^{-1} that represents -OH group stretching vibration and the 3334.92 cm^{-1} due to the vibration of -NH. The stretching vibrations of methylene -C-H are indicated at 2972.31 cm^{-1}, while the absorption peak at 1651.07 cm^{-1} corresponds to $-NH_2$. The stretching vibration of $-CH_3$ is at 1429.25 cm^{-1} ; likewise, the stretching vibration of -C-O-C, $-NH_2$, and -N-H are at 1068.56 cm^{-1}, 781.17 cm^{-1}, and 719 cm^{-1} respectively. In the present study, similar absorption bands with slight shifts were observed at 3564.45, 3334.92, 2972.31, 1651.07, 1429.25, 1070.49, 781.17 and 719.45 cm^{-1}. This confirms the structure of chitosan; however, slight variations of the observed bands indicate that further purification is needed depending on the application (Figure 3).

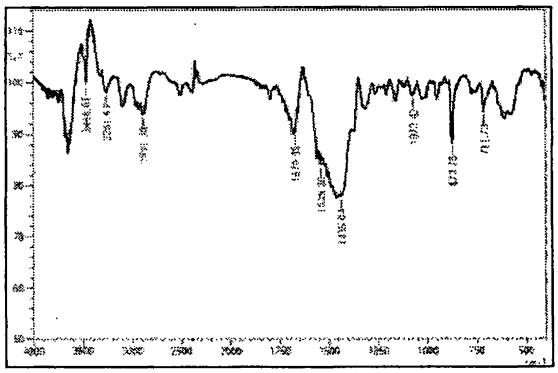

Figure 4. The fourier transform infra-red spectroscopy (FTIR) spectrum of chitin

Characteristic absorption bands of chitin are a broad absorption band at 3468.01 cm^{-1} attributed to -OH group stretching vibrations and the band 2891.3 cm^{-1} which is the aliphatic -C-H stretching band that converges to -OH stretching with -N-H. The characteristic carbonyl -C=O stretching of chitin at 1670.35 cm^{-1} is attributed to the vibration of the amide 1 group. The sharp band at 1435.04 cm^{-1} corresponds to a symmetrical deformation of $-CH_3$ group and at 1539.2 cm^{-1} corresponds to the -N-H deformation of amide 2.The vibration of -C-O-C and -N-H are at 1072.4 cm^{-1} and 711.73 cm^{-1} respectively (Rumengan *et al.*, 2014; Table 1 and Figure 4).

Table 1. Functional groups of shrimps chitosan and chitin compared to commercial standards of chitosan and chitin

Groups	Wave length (cm^{-1})			
	Standard chitosan	Shrimps Chitosan	Standard Chitin	Shrimps Chitin
OH	3450.0	3564.45	3448.0	3468.01
N-H stretching	3335.0	3334.92	3300-3250	3261.63
C-H stretching	2891.1	2972.31	2891.1	2891.30
C=O stretching	-	-	1680-1660	1670.35
NH2 cutting, N-H bending	1655.0	1651.07	-	-
N-H bending	-	-	1560-1530	1539.20
CH3	1419.5	1429.25	1419.5	1435.04
C-O-C	1072.3	1070.49	1072.3	1072.40
NH2	850-750	781.17	-	-
N-H	715.0	719.45	750-650	711.73

MNPs and CsMNPs Synthesis by using $FeCl_3.6H_2O$ and $FeCl_2.4H_2O$

An initial study was conducted to synthesize MNPs using $FeCl_2$ and $FeCl_3$ by changing temperature while keeping the other variables constant. According to the results, a 90 nm NP appeared at 70 °C. The same study was performed at same temperature alternatives with sonication. According to the results, 90 nm NP was observed again at 70 °C (Table 2 and Figure 5- A). Next, the study was conducted at 70 °C temperature by changing the ammonium solution adding rate while providing a uniform condition of the other variables. According to the result, the 90 nm NP appeared at 0.5 mL/1-2 min. The same study was repeated with sonication and observed the 90 nm NP at the 0.5 mL/1-2 min adding rate (Table 3 and Figure 5-B). Next, studies were conducted to synthesize CsMNPs with and without sonication with $FeCl_2/FeCl_3$ and $FeCl_2/FeSO_4$ combinations; however, we observed CsMNP of 90 nm at 70 °C only for $FeCl_2/FeCl_3$ without sonication (Table 4 and Figure 5-C).

Table 2. Synthesis of MNPs with and without sonication for different temperatures

Change the temperature				Without sonication			With sonication		
$FeCl_2$	$FeCl_3$	A.A.R (mL/min)	T °C	Max size (μm)	Min size (μm)	Mean size (μm)	Max size(μm)	Min size (μm)	Mean size(μm)
50 mL	50 mL	0.5/1-2	50	1.67	0.87	1.27	1.25	0.79	0.98
50 mL	50 mL	0.5/1-2	60	2.1	1.67	1.89	1.84	0.99	1.26
50 mL	50 mL	0.5/1-2	70	1.73	0.09	0.64	1.38	0.09	0.98
50 mL	50 mL	0.5/1-2	80	1.67	1.13	1.29	1.47	0.82	1.09

A.A.R- Ammonia adding rate, T-Temperature, MNP- Magnetic Nanoparticles

Table 3. Synthesis of MNPs with and without sonication for different NH_4OH_{aq} adding rate

Change the NH4OH Adding rate				Without sonication			With sonication		
$FeCl_2$	$FeCl_3$	A.A.R (mL/min)	T °C	Max size (μm)	Min size (μm)	Mean size (μm)	Max size(μm)	Min size (μm)	Mean size(μm)
50 mL	50 mL	0.5 /1-2	70	1.73	0.09	0.64	1.38	0.09	0.98
50 mL	50 mL	0.5/2-3	70	1.10	0.77	0.93	1.90	0.90	1.23
50 mL	50 mL	0.5/3-4	70	1.67	0.63	1.27	1.73	1.21	1.39

A.A.R- Ammonia adding rate, T-Temperature, MNP- Magnetic Nanoparticles

Table 4. Synthesis of CsMNPs with and without sonication for temperature alternatives

Change the temperature with chitosan solution				Without sonication			With sonication		
$FeCl_2$	$FeCl_3$	A.A.R (mL/min)	T °C	Max size (μm)	Min size (μm)	Mean size (μm)	Max size (μm)	Min size (μm)	Mean size (μm)
50 mL	50 mL	0.5/1-2	50	1.97	1.10	1.40	2.81	0.90	1.60
50 mL	50 mL	0.5/1-2	60	2.72	1.42	1.75	3.00	0.85	1.52
50 mL	50 mL	0.5/1-2	70	2.03	0.09	1.23	2.24	1.03	1.34
50 mL	50 mL	0.5/1-2	80	1.78	0.99	1.27	3.10	0.65	1.31

A.A.R- Ammonia adding rate, T-Temperature, CsMNPs – Chitosan coated magnetic nanoparticles

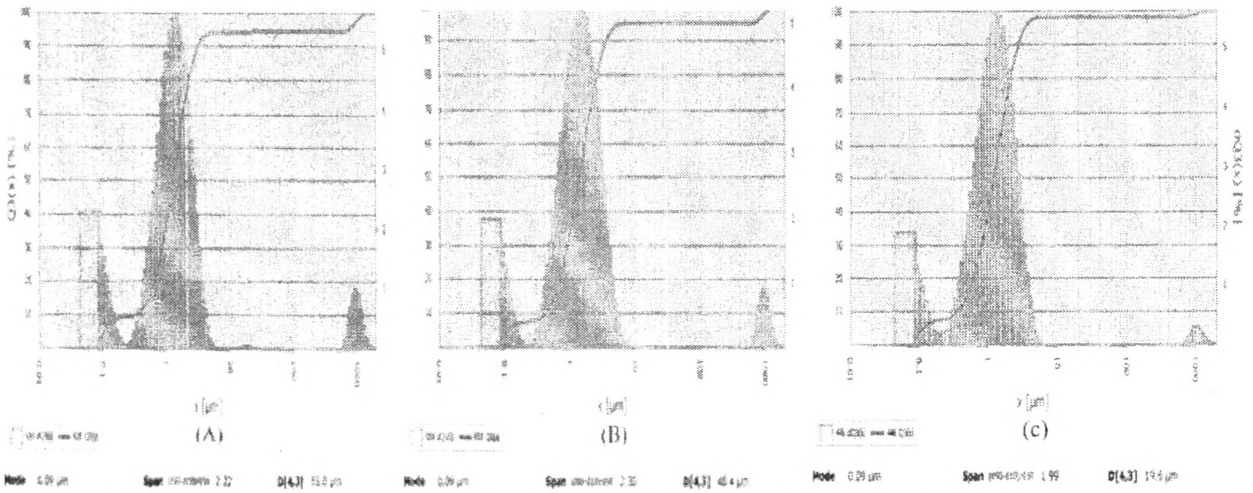

Figure 5. The size distribution graphs for the MNPs with $FeCl_2/FeCl_3$ combinations. *(A) MNP- the 90 nm NP at 70 °C and 0.5 mL/1 min NH_4OH_{aq} adding rate without sonication,(B) MNP -the 90 nm NP at 70 °C and 0.5 mL/1 min NH_4OH_{aq} adding rate with sonication,(C) CsMNPs -the 90 nm at 70 °C and 0.5 mL/1 min NH_4OH_{aq} adding rate without sonication*

Synthesis of CsMNPs by using $FeCl_3.6H_2O$ and $FeSO_4.7H_2O$

In the process of the synthesis of CsMNPs with $FeCl_2/FeSO_4$, the concentration of $FeCl_3.6H_2O$ and $FeSO_4.7H_2O$, and NH_4OH_{aq} remained constant. Then, the parameters such as temperature, pH and adding rate of NH_4OH_{aq} were changed one at a time to synthesize CsMNPs. When one parameter, was changed the other conditions were kept constant. According to the results, when the temperature was changed, the smallest size chitosan coated magnetic particle (1.73 µm) was observed at 80 °C. Chitosan coated magnetic particle size of 1.52 µm appeared at pH 9.5 and NH_4OH_{aq} adding rate of 0.5 mL/1-2 min (Table 5).

Table 5. Synthesis of CsMNPs by using $FeCl_3.6H_2O$ and $FeSO_4.7H_2O$

Condition change	Minimum size (µm)	Maximum size (µm)	Mean size (µm)
T °C			
50	2.03	2.81	2.34
60	2.10	2.10	2.10
70	1.78	2.72	2.08
80	1.73	2.24	1.92
pH			
5.0	1.73	2.24	1.92
6.5	1.73	2.03	1.84
8.0	1.78	2.72	2.08
9.5	1.52	2.17	1.83
A.A.R (mL/ min)			
0.5 /1-2	1.52	2.17	1.83
0.5 /2-3	1.57	1.78	1.64
0.5 /3-4	1.90	2.03	1.97

A.A.R- Ammonia adding rate, T-Temperature, CsMNPs – Chitosan coated magnetic nanoparticles

CONCLUSIONS

CsMNPs received much attention due to its wide range of potential applications. The -N-H stretching of chitosan and -C-O-C- stretching and -C-H- stretching of chitin have exact stretching vibration pattern similar to the standards and other characteristic band stretching vibrations showed slight deviation, from the standards. During the present study, several procedures were adopted to synthesize chitosan coated MNPs. Ninety nanometer MNPs and CsMNPs were observed in $FeCl_3.6H_2O$ and $FeCl_2.4H_2O$ combination at 70 °C and with dosing NH_4OH_{aq} at 0.5 mL/1-2 min. All the other combinations and conditions failed to give NPs below the range of 100 nm.

ACKNOWLEDGEMENTS

The authors would like to express their sincere thanks to all the academic and nonacademic staff members of the Department of Biotechnology, and all the members of Nanotech Laboratory specially, Ms. P.G.D.C.K. Karunarathna and S.P.A.U.K. Samarakoon and G.G. Chathuranga of the Faculty of Applied Science, Wayamba University of Sri Lanka, Kuliyapitiya.

REFERENCES

Dung, D.T.K., Hai, T.H., Phuc, L.H., Long, B.D., Vinh, L.K., Truc, P.N. (2009). Preparation and characterization of magnetic nanoparticles with chitosan coating. Journal of Physics Conference Series, 187 (2), 012036.

Hong, S., Chang, Y., and Rhee, I. (2010). Chitosan-coated ferrite (Fe_3O_4) nanoparticles as a T2 contrast agent for magnetic resonance imaging. Journal of the Korean Physical Society, 56 (3), 868-873.

Nisha, S., Karthick, S.A. and Gobi, N. (2012). A review on methods, application and properties of immobilized enzyme. Chemical Science Reiew and Letters, 1 (3), 148–155.

Osuna, Y., Gregorio-Jauregui, K.M., Gaona-Lozano, J.G., De La Garza-Rodrguez, I.M., Ilyna, A., Barriga-Castro, E.D. and L pez, R.G. (2012). Chitosan-coated magnetic nanoparticles with low chitosan content prepared in one-step. Journal of Nanomaterials, 327562.

Puvvada, Y.S., Vankayalapati, S., and Sukhavasi, S. (2012). Extraction of chitin from chitosan from exoskeleton of shrimp for application in the pharmaceutical industry. International Current Pharmaceutical Journal, 1 (9), 258–263.

Rumengan, I.F.M., Suryanto, E., Modaso, R., Wullur, S., Tallei, T.E. and Limbong, D. (2014). Structural characteristics of chitin and chitosan isolated from the biomass of cultivated rotifer, brachionus rotundiformis, 3 (1), 12–18.

Safari, J., and Javadian, L. (2014). Chitosan decorated Fe_3O_4 nanoparticles as a magnetic catalyst in the synthesis of phenytoin derivatives. *Royal Society of Chemistry Advances*, **4** (90), 48973–48979.

Zhao, L., Shi, L., Zhang, Z., Chen, J., Shi, D., Yang, J., and Tang, Z. (2011). Preparation and application of chitosan nanoparticles and nanofibers. Brazilian Journal of Chemical Engineering, 28 (3), 353–362.