Journal of Materials and Electronic Devices 5 (2020) 6-11

The Use of Chitosan Nanoparticles Obtained by Ionic Gelation Method as a Drug Delivery System

Deniz Ismik[1,2*], Demet Sezgin Mansuroglu[2,3], Erdi Bulus[2,4], Yesim Muge Sahin[2,5]

[1]Yıldız Technical University, Institute of Science, Bioengineering Program, Istanbul, 34220, Turkey.

[2]Istanbul Arel University, Arel POTKAM (Polymer Technologies and Composite Application and Research Center), Istanbul, 34500, Turkey

[3]Istanbul Cerrahpasa University, Institute of Science, Chemistry Program, Istanbul, 34320, Turkey.

[4]Kocaeli University, Institute of Science, Polymer Science and Technology Program, Kocaeli, 41000, Turkey.

[5]Istanbul Arel University, Faculty of Engineering and Architecture, Biomedical Engineering Department, Istanbul, 34500, Turkey.

Chitosan, a biocompatible and biodegradable polymer, has attracted the interest of scientists in recent years due to its antibacterial, antimicrobial and antifungal effects. In this study, the usage of chitosan nanoparticles (CNP) as a drug delivery system is investigated. In this study, the effect of chitosan / cross-linker ratio and pH parameters on CNP produced by ionic gelation technique is observed. Tripolyphosphate (TPP) is used as a cross-linker. For the usage of CNP as a drug delivery system, salicylic acid, a hydrophilic drug-acting substance, is loaded as a model drug to the resulting nanoparticles and release kinetics are determined for 72 hours in phosphate buffer solution (PBS). For the structural characterization of CNP produced by ionic gelation technique, Fourier Transform Infrared Spectroscopy (FTIR) is used. Field Emission Gun Scanning Electron Microscopy (FEG-SEM) is used for surface characterization. In addition, UV-spectrophotometer is used to obtain drug release properties of the produced nanoparticles. CNP drug delivery systems produced in this study; is a candidate material which can be used in many areas due to the fact that degradation products of nanoparticles that are disintegrated in the physiological environment are non-toxic, have high active ingredient loading capacity and can be designable as desired according to the usage areas (cosmetic, pharmaceutical, biomedical and tissue engineering, etc.).

Keywords: *Chitosan, drug delivery system, ionic gelation, nanoparticle, salicylic acid.*

Submission Date: 21 September 2020
Acceptance Date: 10 November 2020

**Corresponding author: denizismik@arel.edu.tr (Deniz ISMIK) Tel: +905532786911*

1. Introduction

Today, major progress has been made in drug design and effectiveness by dint of developing technology and nano-dimensional production techniques. The conventional drugs taken orally or intravenously at frequent intervals and high doses have given their place to nanotechnological drug delivery systems that are effective only in the targeted site thanks to their targetability, require a low dose of active substance for being active in a niche site, reduce the dosing intervals owing to their intelligent release characteristics,

and are free from side and toxic effects. One of those drug delivery systems is the "nanoparticles".

Nanoparticles (also called "nanospheres" or "nanocapsules" based on the method of preparation) are the matrix systems prepared using natural or synthetic polymers and having a size ranging from 10 to 1000 nm, where the active substances are dissolved, detained in the particle and/or adsorbed or bound to the surface. The nanoparticles used while targeting the peptide and genes to the related tissue offer two major advantages. The first of these is their small particle sizes. This allows the active substance to accumulate in the targeted site. The second advantage thereof, however, is the use of biodegradable materials in the preparation of small particles. Biodegradable materials ensure a long-term controlled delivery of active substances. Nanoparticles also enhance the stability of drugs/proteins or peptides. Aside from the fact that they can be easily sterilized, they have a high capacity of active substance loading, which thereby increases the delivery and bioavailability of the drug administered orally in the form of nanoparticles.

Nanoparticles are the matrix systems with dimensions ranging from 10 to 1000 nm, prepared using natural or synthetic polymers and called as nanocapsules or nanospheres based on the preparation method, where the active substances are dissolved, detained in the particle and/or adsorbed or bound to the particle surface. There are two outstanding advantages of nanoparticles, which are used for the delivery of active substances to the desired area, tissue or organ. The first of those advantages is that nanoparticles have very small particle sizes. Owing to the small sizes of particles, they can penetrate into the cell and thus, the active substance they carry can accumulate in the desired tissue, organ or site. The second important advantage offered by the nanoparticles is the use of biodegradable materials in the preparation of these small particles. Biodegradable materials ensure a controlled delivery of the active substance for a desired period of time. Additionally, nanoparticles increase the stability of the active substances they carry, are easy to sterilize and have a high active substance carrying capacity. Those characteristics of nanoparticles enhance the delivery and bioavailability of the active substance they carry.

Chitosan (CTS) is a natural polymer obtained through deacetylation of chitin, which is abundant especially in the shells of marine crustaceans. They have many uses in the medical, agricultural and food industries thanks to their antibacterial and antifungal properties and for being both biocompatible and biodegradable [1].

This study aimed to research the use of chitosan nanoparticles (CNP) obtained using ionic gelation technique and loaded with salicylic acid as a controlled drug delivery system. Tripolyphosphate (TPP) is used as crosslinker and the effect of crosslinker amount and pH on nanoparticle formation is investigated. Also, the study used Fourier Transform Infrared Spectroscopy (FT-IR) to identify structural properties of empty and drug-loaded nanoparticles, and Scanning Electron Microscope (FEG-SEM) for their morphology, and UV Spectrophotometer treated for 72 hours in phosphate buffer for their drug loading and delivery behavior.

2. Experimental Studies

2.1. Materials

Chitosan (of medium molecular weight), degree of deacetylation 75-85% [Brookfield viscosity 200 to 800 cP, Sigma Aldrich], Sodium Tripolyphosphate (TPP, Sigma Aldrich), Acetic Acid (CH_3COOH, Sigma Aldrich, 100%), Sodium hydroxide [(NaOH), Sigma Aldrich], Disodium hydrogen phosphate dihydrate [($NaH_2PO_4.2H_2O$), Sigma Aldrich], Disodium hydrogen phosphate heptahydrate [($NaH_2PO_4.7H_2O$), Sigma Aldrich], Salicylic acid [($C_7H_6O_3$), Merck].

2.2. Preparation of drug-free and drug-loaded nanoparticles

While preparing the drug-free CNP, chitosan is dissolved in 1% (v/v) acetic acid at a concentration of 4 mg/ml. The TPP, which is used as cross-linker, is prepared at a concentration of 2 mg/ml. The resulting TPP solution is dripped into the chitosan solution using a peristaltic pump and dispersed in the ultrasonic bath for an hour. A total of 4 samples with a different chitosan/TPP ratio and pH different values are prepared to analyze the effects of cross-linker amount and pH level on the CNP formation [2]. The compositions of samples are presented in Table 2.1. The resulting 4 different CNP solutions were centrifuged at 10000 rpm and at 4 °C for 50 minutes and then, the supernatant was poured off and the pellet was washed 3 times with distilled water and freeze-dried at -80 °C.

Table 2.1: CNP prepared under diverse conditions

Sample	CTS:TPP	pH
CNP-1	2:1	3.5
CNP-2	2:1	5
CNP-3	5:1	3.5
CNP-4	5:1	5

The salicylic acid-loaded CNPs were also obtained employing a similar method. Among those 4 different CNPs, the one which has the most appropriate rates for drug loading was identified as a result of the characterizations performed, and the drug loaded CNPs were produced repeating the production process of this sample. To produce those salicylic acid (SA)-loaded particles, the CTS solution with a concentration of 4 mg/ml and the SA solution with a concentration of 1 mg/ml were put inside a magnetic stirrer and stirred for 30 minutes. The TPP solution with a concentration of 2 mg/ml was dripped into the chitosan solution using a peristaltic pump and dispersed in the ultrasonic bath for an hour. The CNP solution prepared were centrifuged at 10000 rpm and at 4 °C for 50 minutes and then, the supernatant was poured off and the pellet was washed 3 times with distilled water and freeze-dried at -80 °C.

2.3. Characterization of drug-free and drug-loaded nanoparticles

The formation of CNP cross-linked with TPP obtained by ionic gelation method as well as the bonds in the structures of related groups were analyzed using a Fourier Infrared Conversion Spectroscopy (FTIR) of Jasco FT-IR 6600 brand/model. The equipment operates based on the principle that chemical bonds absorb infrared energy at specific frequencies or wavelengths. The spectra of the samples were taken within the range of 600-4000 cm^{-1}. The functional groups of the samples were identified at the pre- and post-procedure period. The surface properties, shapes and dimensions of the resulting CNP were analyzed using a FEGSEM device of FEI brand and Quanta FEG 450 model and in WETSTEM-FEG mode.

2.4. Drug loading and release studies

In order for in-vitro analysis of the drug release behavior of drug-loaded nanoparticles, the salicylic acid-loaded CNP were incubated in the 75 ml phosphate buffer adjusted to pH 7.4, and their drug release behaviors were followed up for 2 hours, 4 hours, 6 hours and then 3 days on a daily basis and, thereby, the drug release percentages of the nanoparticles produced were identified. Those drug release studies were performed inside a shaking water bath at 37 °C. The drug loading and drug release studies were performed using a UV-Vis Spectrophotometer of Jasco V-750 brand and at a wavelength of 248 nm.

During the drug loading and release studies, the drug concentrations loaded/released were identified using an ultraviolet-visible spectroscopy. The absorbance-concentration correlation in UV-visible spectroscopy is represented with the "Lambert Beer Equation" (Equation 1) below.

$$A = \varepsilon.b.C \quad (1)$$

The slope of the calibration graph represents the molar absorption coefficient. The drug concentrations were calculated using the molar absorption coefficient, accompanied by absorbance measurements.

The amount of drug loaded to CNP obtained by ionic gelation method in drug loading studies was calculated by Equation 1 using the absorbance of the supernatant liquid drug solution obtained at the centrifugation stage at 248 nm after loading and the molar absorption coefficient obtained from the calibration graph.

The amount of drug loaded in the nanoparticles (mg) was calculated by Equation 2.

$$q_e = \frac{V_x(C_0 - C_t)}{m} \quad (2)$$

The drug loading capacity (%) was calculated by the Equation 3 given below.

$$\% \text{ (weight)} = (m_{\text{drug / nanoparticle}}) / m_{\text{nanoparticle}} \quad (3)$$

The drug loading efficiency (%) was calculated by the Equation 4 given below.

$$\frac{(C_0 - C_t)}{C_0} x100 \quad (4)$$

The amount of release was calculated using the equation for time-dependent mass calculation.

$$M_t = C_t \times V + \sum C_{t-1} \times V_s \quad (mg) \quad (5)$$

Mt, Amount of drug at time t (mg), V, Volume of release medium (L), Ct, Drug concentration at time t (mg / L), Vs, Volume of release medium taken at specific intervals for the measurement (L).

To calculate the release amount in %;

$$\%M = \frac{M_t}{M_0} \times 100 \quad (6)$$

3. Result and Discussion

3.1. FT-IR analysis results

3.1.1. FTIR analysis results of CNP prepared by ionic gelation method

Figure 3.1-3.4 shows the FTIR analyzes of CNP prepared by ionic gelation method under 4 different conditions within the study.

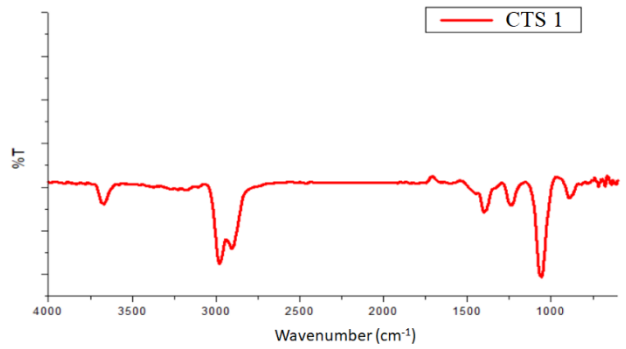

Figure 3.1 Nanoparticles obtained at pH:3.5 with a CTS: TPP ratio of 2:1 by volume

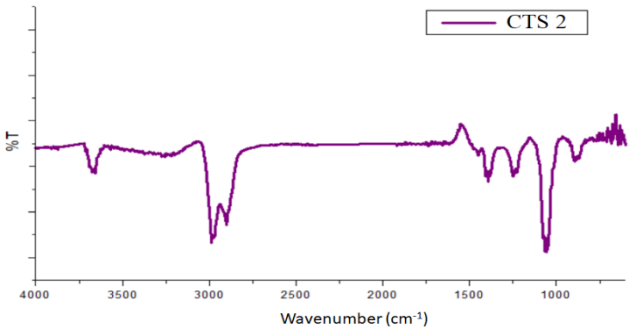

Figure 3.2 Nanoparticles obtained at pH:5 with a CTS: TPP ratio of 2:1 by volume

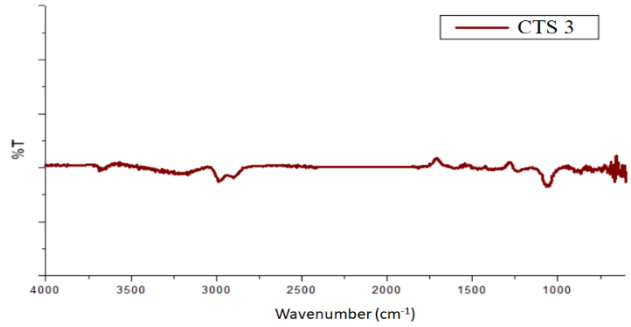

Figure 3.3 Nanoparticles obtained at pH:3.5 with a CTS: TPP ratio of 5:1 by volume

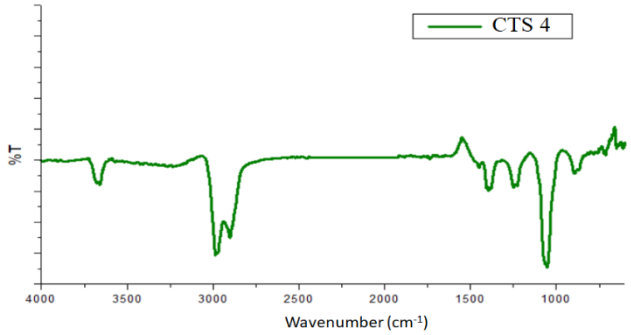

Figure 3.4 Nanoparticles obtained at pH:5 with a CTS: TPP ratio of 5:1 by volume

The nanoparticles containing different amounts of CTS:TPP and prepared at different pHs demonstrated specific signal peaks representing different functional groups (C-O-C, C-H, C-O, OH strain and P-O, PO_2, PO_3 and P-O-P) related to chitosan and TPP in FTIR spectra, which were almost similar in all samples. In a combination of stretching modes of OH and N-H bonds in the chitosan matrix, a wideband was observed at 3352.64 cm^{-1} and 3585.14 cm^{-1}. In CNP, this band became larger and shifted to the range of 3690,12 cm^{-1} to 3658.3 cm^{-1}. This proves that the presence of strong electrostatic interactions and the development of hydrogen bond interactions. The C-O bond at 1075,33 cm^{-1} and 1025,18 cm^{-1} in chitosan shifted to the range of 1074,16 cm^{-1} to 1053,91 cm^{-1} during the formation of nanoparticles. This, however, represents that C-O groups were also involved in the non-binding interaction. Plus, the NH_2 bending band of chitosan samples at 1578,45 cm^{-1} shifted to a wavenumber of about 1550 cm^{-1} in nanoparticles. Other peaks noted in nanoparticles include the peaks created by P = O strain at 1250.61 cm^{-1}, 1242.3 cm^{-1} and 1229.4 cm^{-1} wavelengths, P-O bend at the wavelength of 1027,87 cm^{-1} and P-O bond around a wavelength of 1053,91 cm^{-1} [3].

3.1.2. FTIR analysis result of salicylic acid-loaded CNP

In reference to the FTIR and FEG-SEM analyses, the sample most suited for use in drug loading and release studies was found to be the sample containing 5:1 CTS:TPP by volume and obtained at pH:5 (CNP-4). Therefore, this sample was the one used for drug loading procedure. Figure 3.5 shows the FTIR analysis result of the sample containing a CTS:TPP of 5:1 by volume, obtained at pH:5 and loaded with salicylic acid.

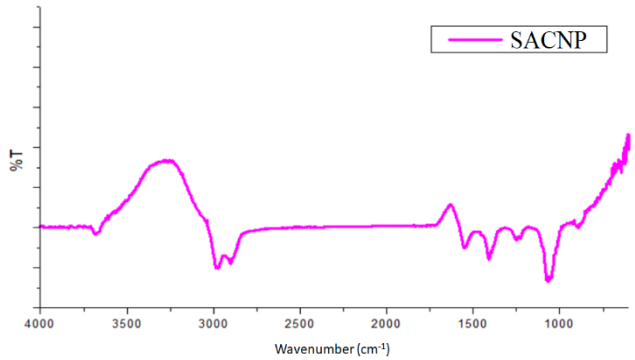

Figure 3.5 Salicylic acid-loaded CNP

In examining the FTIR spectra of salicylic acid-loaded CNP, the characteristic absorption peaks of salicylic acid at 1700 cm^{-1} and 1650 cm^{-1} reveal the acetoxy-group and carboxylic-

group bendings. The peak at 1300 cm^{-1} shows the C-N group bending for the interaction between the carboxylic (-COOH) group of salicylic acid and the primary amide of chitosan, which suggests the successful loading of salicylic acid into the CNP [4].

3.2. FEG-SEM analysis results

3.2.1. FEG-SEM analysis results of CNP prepared by ionic gelation method

The shapes and sizes of nanoparticles containing CTS:TPP in different proportions by volume and prepared at different pHs were found using SEM. In Figures 3.6 to 3.9, it is clearly shown that the resulting nanoparticles are spherical in shape, and the particle sizes vary depending on the CTS: TPP ratio and pH in the light of SEM images of nanoparticles prepared at different pHs and containing different amounts of CTS: TPP by volume.

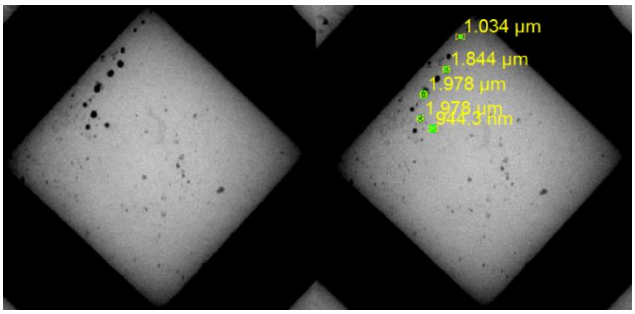

Figure 3.6 FEG-SEM images of nanoparticles obtained at pH:3.5 with a CTS:TPP ratio of 2:1 by volume

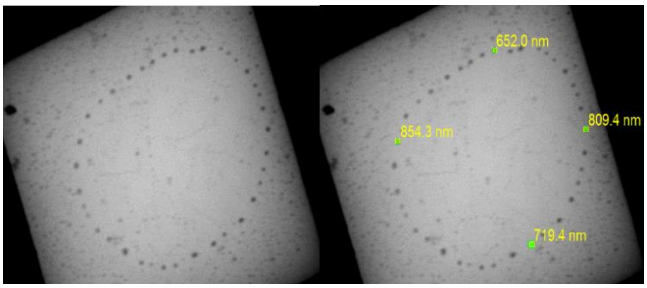

Figure 3.7 FEG-SEM images of nanoparticles obtained at pH:5 with a CTS: TPP ratio of 2:1 by volume

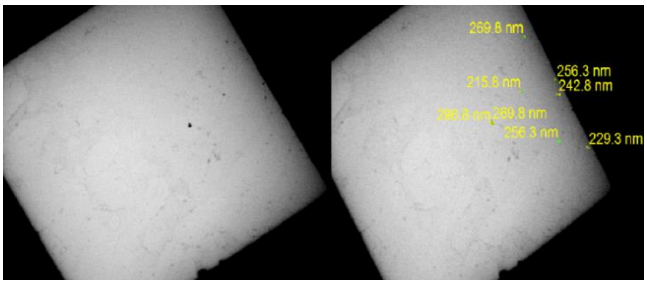

Figure 3.8 FEG-SEM images of nanoparticles obtained at pH:3.5 with a CTS: TPP ratio of 2:1 by volume

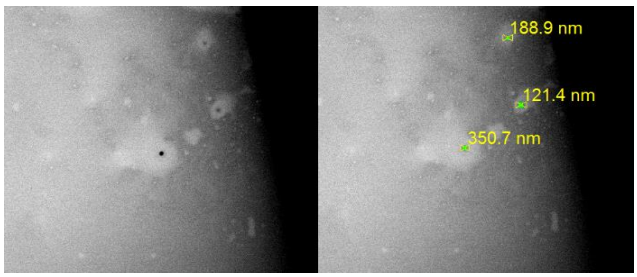

Figure 3.9 FEG-SEM images of nanoparticles obtained at pH:5 with a CTS: TPP ratio of 5:1 by volume

3.2.2. FEG-SEM analysis results of salicylic acid-loaded CNP

Figure 3.10 shows the results of FEG-SEM analysis of salicylic acid-loaded CNP.

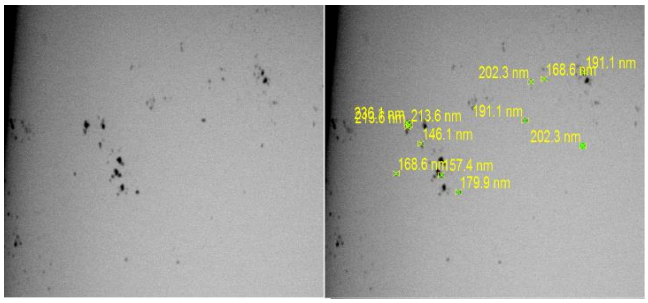

Figure 3.10 FEG-SEM images of salicylic acid-loaded nanoparticles obtained at pH:5 with a CTS: TPP ratio of 5:1 by volume

In examining the SEM images, the CNP prepared by ionic gelation method appear to form under the given 4 different conditions. As is seen in the images obtained, the size of the particles formed increases as the amount of cross-linker (TPP) used is increased, whereas their sizes become smaller as the particle formation pH is increased from 3.5 to 5, i.e. when the basicity of the medium is enhanced.

3.3. Drug loading and release studies

During the drug loading and release studies, the drug concentrations loaded/released were identified using an ultraviolet-visible spectroscopy. Towards that end, first the absorbance values of the prepared solution at different concentrations were read using an UV-Visible Spectrophotometer at 248 nm wavelength and then the salicylic acid/distilled water calibration curve was plotted (Figure 3.11).

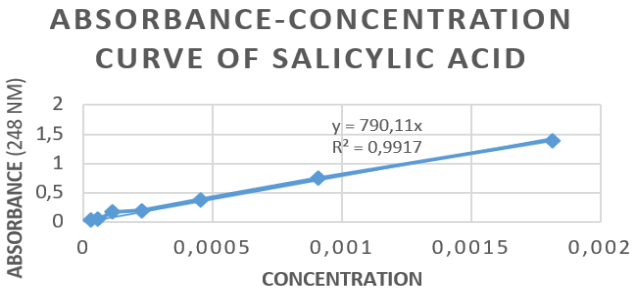

Figure 3.11 Absorbance-Concentration Curve of Salicylic Acid/Distilled Water

Table 3.1 shows the amount of drug loaded into the sample CNP-4.

Table 3.1: Amount of drug loaded into the sample CNP-4

Sample	pH	qE (mg/g)	Loading Efficiency (%)	Drug Loading Capacity (%)
CNP-4	5	857.327	85.73	78.00

Table 3.2 shows the drug release values of the sample CNP-4.

Table 3.2: Drug release values of the sample CNP-4

Time (hours)	Release (%)
2	25.78
4	19.42
6	18.42
24	17.55
48	16.88
72	64.00

Figure 3.12 presents the release-time graph in % of the salicylic acid loaded into the sample CNP-4 in phosphate buffer.

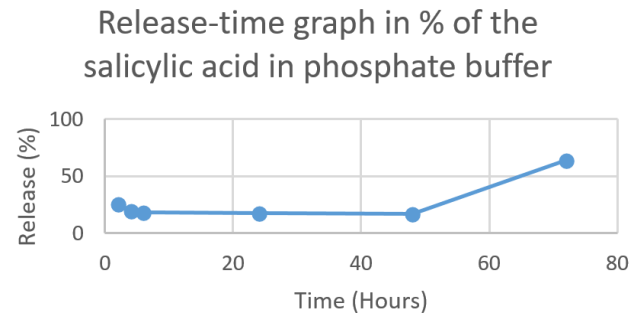

Figure 3.12 Release-time graph in % of the salicylic acid loaded into the sample CNP-4 in phosphate buffer

4. Conclusion

This study analyzed the effect of chitosan/cross-linker ratio and pH parameters on CNP produced by ionic gelation technique and explored the use of the resulting nanoparticles as a drug delivery system. Both the FTIR spectra and FEG-SEM images of the samples show that the CNP prepared by ionic gelation method can emanate under 4 different conditions having variable pHs and cross-linking ratios. In examining the images obtained by FEG-SEM, it is seen that the size of the particles become larger in parallel to an increase in the amount of cross-linker (TPP) used. However, their sizes are found to become smaller as the particle formation pH is increased from 3.5 to 5, i.e. when the basicity of the medium is enhanced. As a result of the FTIR and FEG-SEM analyses, the sample CNP-4 was found to be suited for use in the drug loading studies. To this end, the salicylic acid-loaded SACNP sample was synthesized at the ratios used in the preparation of the sample CNP-4. The supernatant extracted from the synthesized SACNP was analyzed using a UV-spectrophotometer to find out the amount of the drug loaded. Those processes showed us that the sample SACNP had a drug loading capacity of 78% and a loading efficiency of 85.73%. The studies on drug release of the salicylic acid-loaded CNP were performed at room temperature for 72 hours in a phosphate buffer of pH:7.4 and using a shaking water bath. As a result of the drug release studies, the drug release percentages of the sample SACNP was found to be 25.78% at the 2nd hour, 19.42% at the 4th hour, 18.42% at the 6th hour, 17.55% at the 24th hour, 16.88% at the 48th hour and 64% at the 72nd hour. Those values suggest that drug-loaded CNPs ensure the highest drug release between the 2nd and 3rd days.

Acknowledgements

The authors are thankful for the opportunities that ArelPOTKAM (Polymer Technologies and Composite Materials R&D Center) has offered for the production and characterization of the materials.

References

[1] S. Yasmeen, M. K. Kabiraz, B. Saha, M. R. Qadir, M. A. Gafur & S. M. Masum, International Research Journal of Pure and Applied Chemistry, 10(2016), 1
[2] F. Martins, D. M. de Oliveira, A. G. Pereira, A. F. Rubira & E. C. Muniz, International journal of biological macromolecules, 51(2012), 1127
[3] T. Gomathi, P. N. Sudha, J. A. K. Florence, J. Venkatesan, & S. Anil, International journal of biological macromolecules, 104 (2017) 1820
[4] J. Ji, S. Hao, D. Wu, R. Huang & Y. Xu, Carbohydrate polymers, 85(2011) 803