World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 4.223

FORMULATION OF CHONDROITIN SULFATE NANOPARTICLE WITH CHITOSAN AND KAPPA CARRAGEENAN USING THE IONIC GELATION METHOD

Danni Ramdhani*[1], Anis Yohana Chaerunisaa[2] and Aneu Nur Utami[2]

[1]Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy.
[2]Departement of Pharmaceutics, Universitas Padjadjaran, Jatinangor, Indonesia.

***Corresponding Author: Danni Ramdhani**
Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy.

Article Received on 03/12/2017 **Article Revised on 24/12/2017** **Article Accepted on 14/01/2018**

ABSTRACT

Objective. Chondroitin sulfate is a widely used dietary supplement formulations, effective for the treatment of osteoarthritis. In oral preparations, chondroitin sulfate has low bioavailability. The alternative to improve bioavailability of a drug is by using nanoparticle technology. This study was aimed to formulate and characterize chondroitin sulfate nanoparticles with chitosan as polymer and kappa carrageenan as crosslinker. **Methods.** Nanoparticles were prepared by using ionic gelation method that was based on the electrostatic interaction between opposite charges. **Results.** The results showed that F_1 containing 0.1% chitosan, 0.1% chondroitin sulfate, and 0.05% kappa carrageenan at volume ratio of 10: 1: 1 was the best formula with an average particle size 582.9 nm , polydispersity index value of 0.324, and zeta potential value as much as -0.47 mV.

KEYWORDS: Chondroitin sulfate, Chitosan, Kappa carrageenan, Nanoparticles, Ionic gelation.

INTRODUCTION

Osteoarthritis (OA) is a joint disorder that often occurs in the world.[1] Therapies commonly used are the nonsteroidal antiinflammatory drugs and preparations containing chondroitin sulfate. Several clinical trials showed that chondroitin sulfate has the ability to slow the progression of osteoarthritis.[2]

In recent years, much research that utilizes nanoparticle technology for drug delivery systems.[3] Various studies have also been developed, come to a focus in which pharmaceutical researchers strive to improve the effectiveness of the drug in the right amount. This is because the nanoparticles has many advantages such can penetrate the space between cells that small impenetrable by particles to large size, increasing the bioavailability of drugs.[4] In addition, the encapsulation process can maintain the stability of the active substance from enzymatic reactions during the first pass effect.[5]

Natural polymers often used in the manufacture of nanoparticles is chitosan and carrageenan. Chitosan is used very well because it has properties include safe, biodegradable, biocompatible, mucoadhesive, and hydrophilic.[6] There are studies showing that the combination of chitosan-carrageenan has a promising potential as a carrier in drug delivery systems. Carrageenan types used in these studies is kappa carrageenan.[7]

The method of making nanoparticles of the most interesting is the ionic gelation method. The principle of this method is the formation of particles based on ionic gelation method.

MATERIAL AND METHOD

Materials

Materials used in this study include Chondroitin sulfate (Bioiberica), Chitosan (Biotech Surindo), Kappa carrageenan (PT. Quadrant), Acetic acid and potassium bromide (merck), and aqua destilled (Brataco).

Methods

The method used in this study consists of several stages as follows:

1. Solubility test

Solubility testing chondroitin sulfate, chitosan, and kappa carrageenan based on the characteristics of each substance. A sample of 500 mg dissolved in the solvent and solubility observed.

2. Raw materials characterization by FTIR

Infrared spectrum of a sample powder of chondroitin sulfate, chitosan, kappa carrageenan, and the combination of chondroitin sulfate / chitosan / kappa carrageenan is measured. Each sample was weighed and crushed together with KBr in the ratio 1: 100. Then placed in a mold and compressed to form pellets. Pellets are placed in the sample container on the FTIR

instrument for analysis. Measurements were made at wave number 4000 cm^{-1} to 400 cm^{-1} using FTIR spectroscopy Jasco-4200 (8). The measurement results of the spectrum compared to the standard spectrum.

3. Determination of particle size of the chondroitin sulfate powder

Chondoitin sulfate was determined by Scanning Electron Microscope (SEM).

Optimization of chondroitin sulfate nanoparticles

Chitosan was dissolved in 1% acetic acid (v/v) and made various concentration (Table 1). Kappa carrageenan dispersed in distilled water at 60^0C and the concentration was fixed 0.05%. Chondroitin sulfate dissolved in distilled water to obtain a concentration of 0.1% chondroitin sulfate. Chondroitin sulfate solution, chitosan solution, and kappa carrageenan solution was homogenized using a vortex for 1 minute and a sonicator for 20 minutes.

Table 1: Composition formula of chondroitin sulfate nanoparticles.

F	Concentration in % (w/v)		
	Chitosan	Chondroitin Sulfate	Kappa Carrageenan
1	0,1	0.1	0.05
2	0.15	0.1	0.05
3	0.2	0.1	0.05
4	0.25	0.1	0.05
5	0.3	0.1	0.05

Each formula has been created then measured percent transmitannya using UV-Vis spectrophotometer at a wavelength of 650 nm. Three formulas have the highest percent transmittance will have to be formulated more.

Formulation of Chondroitin Sulfate Nanoparticles

Solution of chondroitin sulfate, chitosan, and kappa carrageenan was made with a concentration in accordance with formula chosen. Solution was homogenized using a vortex for 1 minute and then a sonicator for 20 minutes, respectively. Chitosan Chitosan solution was placed in a glass beaker on a magnetic stirrer that is rotated at a speed of 1500 rpm. Chondroitin sulfate solution was added dropwise to the chitosan solution using a syringe, and then left to stand for 60 minutes. Kappa carrageenan solution is added to the mixture and left for 30 minutes. The third formula that was created then characterized.

RESULTS AND DISCUSSIONS

Preformulation

1. Solubility tests

The test results showed that the solubility of chitosan chitosan 500 mg dissolved in 15 mL of 1% acetic acid, which means that 1 part chitosan dissolved in 30 parts of acetic acid. This is in accordance with the solubility of chitosan dissolved in an organic acid at a pH of less than 6.5 one acetic acid.[9] Amine group in chitosan will be groups protonated ($-NH_3^+$) in acid solution, so that will positively charged chitosan and soluble.[10] Results of testing the solubility of kappa karegenan showed that 500 mg of kappa carrageenan dissolved in 14 mL of distilled water temperature 60^0C, which means that the first part of kappa carrageenan dissolved in 28 parts distilled water, this is in accordance with the solubility of kappa carrageenan are soluble in hot distilled water.[11]

2. Raw materials characterization by FTIR

Characterization of chondroitin sulfate, chitosan, kappa carrageenan, and the combination of chondroitin sulfate / chitosan / kappa carrageenan has been done using FTIR. This characterization includes qualitative analysis aims to determine the purity of the material based on the spectrum of the sample compared to the reference spectra.

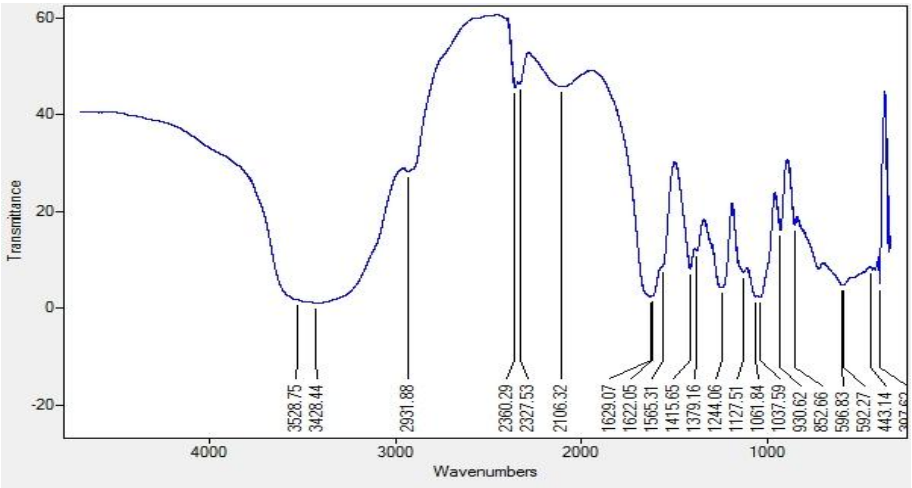

Figure 1: Spectrum of chondroitin sulfate sample.

Characterization of the peak of the spectrum of chondroitin sulfate sample that is at 3428.44 cm^{-1} indicate the presence of hydroxyl group (OH-free) and the symmetric NH_2 stretching, at wave number 2931.88 cm^{-1} indicate the presence of a chain aliphatic C-H, at 1629.07 cm^{-1} showed the group C=O, at 1565.31 cm^{-1} (deformation N-H), 1244.06 cm^{-1} indicated the group O-SO_3, and the wave number 1061.84 cm^{-1} shows the carboxyl group (C-O). The infra-red spectrum indicated that the chondroitin sulfate sample accordance with the reference.[12]

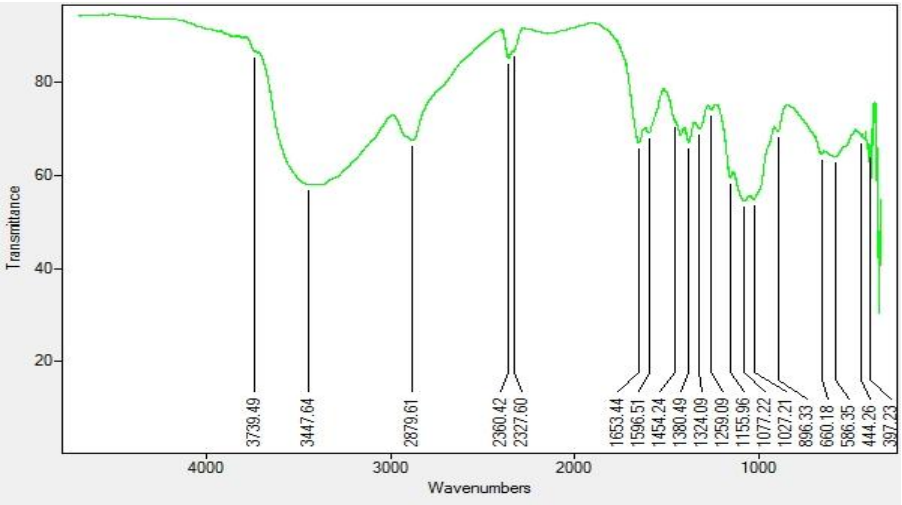

Figure 2: Spectrum of chitosan sample.

Based on wave numbers of samples, chitosan showed peaks characteristic of the group C=O stretching (amide I) at 1653.44 cm^{-1} and NH stretching (amide II) at 1596.51 cm-1 (Lawrie et al., 2007; Grenha et al., 2009). Characterization of others can be seen at the peak of 3447.64 cm^{-1} that showed stretching hydroxyl group (OH) and NH_2. In addition, the chain glycosidic (-C-O-C-) which looks at wave number 1077.22 cm^{-1}.[13]

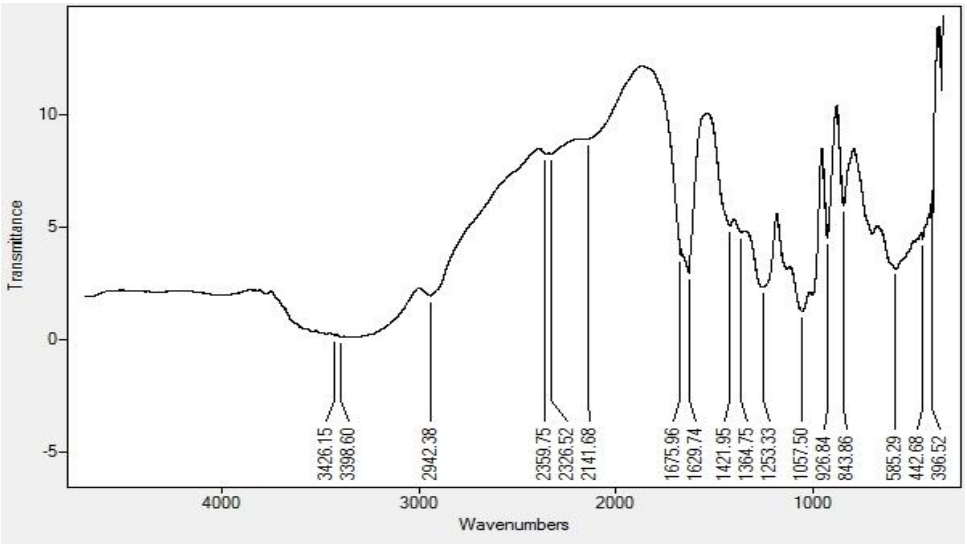

Figure 3: Spectrum of kappa carrageenan sample.

The pattern of kappa carrageenan sample in the 4000 - 400 cm^{-1} had similarities with the reference. Characterization of wave numbers of kappa carrageenan showed several peaks, one peak whose credibility is at 1253.33 cm^{-1} indicates that the sulfate group (O=S=O), the peak of 1057.50 cm^{-1} showed the chain glycosidic, peaks at 926, 84 cm^{-1} corresponding to 3,6-anhidrogalaktosa and peak 843.86 cm^{-1} in accordance with the galactose-4-sulfate.

3. Determination of Particle Size of The Chondroitin Sulfate Powder

Chondroitin sulfate powder was determinated by Scanning Electron Microscope (SEM) aims to determine the initial size.

Figure 4: Determination of particle size of the chondroitin sulfate powder.

Based on Figure 4. was known that the size of chondroitin sulfate before it is formulated into nanoparticles was 1.39 µm to 2.64 µm. The results of this determination can be compared with the size of chondroitin sulfate that had been formulated with the polymer and crosslinker into nanoparticles.

Optimization of chondroitin sulfate nanoparticles

The greater the percent transmittance indicated that the size of the particles in the dispersion system is getting smaller. Percent transmittance measurements performed using UV-Vis spectrophotometry at a wavelength of 650 nm and used distilled water as a blank. Formula was selected based on the percent transmittance was close to 100%, which means that the resulting dispersion system had a transparency similar to distilled water. This is because at a wavelength of 650 nm, the transmittance percent distilled water had a maximum of 100%.[15]

The ratio of the volume of chitosan (K), chondroitin sulfate (Ks), and kappa carrageenan (Kk) used in the fifth consecutive formula is 10: 1: 1 in units of mL.

Table 2: Percent transmittance of chondroitin sulfate nanoparticles.

Formula	Percent transmittance (%)			Mean
	I	II	III	
1	99,28	99,26	99,26	99,26
2	98,87	99,04	99,20	99,03
3	99,06	99,04	99,05	99,05
4	98,75	98,78	98,80	98,77
5	98,37	98,36	98,35	98,36

The results of measurements of percent transmittance by Table 2 it can be seen that from the fifth formula is made visible that Formula 1, Formula 2 and Formula 3 has a transmittance value percent higher than the formula 4 and formula 5. Based on these data the formula that will be used in the manufacture nanoparticles and characterized further was formula 1, formula 2 and formula 3 with a volume ratio chitosan: chondroitin sulfate: kappa carrageenan is 10 mL: 1 mL: 1 mL.

Formulation of chondroitin sulfate nanoparticles

Nanoparticles chondroitin sulfate had been made with the composition formula selected based optimizations performed.

Table 3: Formula of chondroitin sulfate Nanoparticles.

F	Consentration (%)		
	Chitosan	Chondroitin sulfate	Kappa Carrageenan
1	0,1	0,1	0,05
2	0,15	0,1	0,05
3	0,2	0,1	0,05

In the process of its formation, chitosan as polymer dissolved beforehand in a solution having a pH of acid with the aim of changing the amine group (NH_2) to ($-NH_3^+$). The group will interact positively ionized ionic negatively charged chondroitin sulfate. Kappa carrageenan is polianion added in order to stabilize the positive charge of the ammonium groups that did not interact.

CONCLUSION AND SUGGESTION

Conclusion

The composition formula chondroitin sulfate nanoparticles can be made with various concentrations of chitosan (K) 0.1%, 0.15% and 0.2%, while the concentration of chondroitin sulfate (Ks) and kappa carrageenan (Kk) was fixed at 0.1% and 0.05% with a volume ratio (K: Ks: Kk) is 10: 1: 1.

ACKNOWLEDGMENTS

The author would like to thank Richie A.I. Chandra, M.S.c., Apt for his cooperation in this study.

REFERENCES

1. Sinusas, K. Osteoarthritis: Diagnosis and Treatment. American Family Physician, 2012; 85(1): 50.
2. Uebelhart, D., Michel, M., Roberto, M., *et al*. Intermittent treatment of knee osteoarthritis with oral chondroitin sulfate: a one-year, randomized, double-blind, multicenter study versus placebo. *Osteoarthritis and Cartilage*, 2004; 12(4): 269–276.
3. Yeh, M.K., Kuang-ming, C., Chieh-shen, H., Yu-chuan, H., and Jenn-jong, Y. Novel protein-loaded chondroitin sulfate–chitosan nanoparticles: Preparation and characterization. *Acta Biomaterialia*, 2011; 7: 3804–3812.
4. Wu, Y., Yang, W., Wang, C., Hu, J., dan Fu, S. Chitosan nanoparticle as a novel delivery system for ammonium glycyrrhizinate. *International Journal of Pharmaceutics*, 2005; 295: 235-245.
5. Mozafari, M.R., J. Flanagan, L. Matia-Merino, A. Awati, A. Omri, Z.E. Suntres and H. Singh. Review: Recent trends in the lipid-based nanoencapsulation of antioxidants and their role in foods. *J Sci Food Agric*, 2006; 1-8.
6. Zhao, L., Burguera, E.F., Xu, H.H.K., Amin, N., Ryou, H., Arola, D.D. Fatigue and human umbilical cord stem cell seeding characteristics of calcium phosphate–chitosan–biodegradable fiber scaffolds. *Biomaterials*, 2010; 31: 840–847.
7. Grenha, A., Manuela, E.G., Màrcia, R., Vìtor, E.S., João, F.M., Nuno, M.N., Rui, L.R. Development of new chitosan/carrageenan nanoparticles for drug delivery applications. Journal of Biomedical Materials Research, 2009; 1265-1272.
8. Kumirska, J., Cherwicka, M., Kaczynski, Z., Bychowska, A., Brzozowski, K., Thoming, J., and Stepnowski, P. Application of spectroscopic methods for structural analysis of chitin and chitosan. *Mar Drugs*, 2010; 8(5): 1576-1636.
9. Tiyaboonchai, W. Chitosan nanoparticles: A promising system for drug delivery. *Naresuan University Journal*, 2003; 11(3): 51-66.
10. Kurita, K. Chitin and chitosan: Functional biopolymers from marine crustaceans. *Marine Biotechnol*, 2006; 8: 203–226.
11. Rowe, R.C., Sheskey, P.J., and Quinn, M.E. *Handbook of pharmaceutical excipients (6th ed)*. London: The Pharmaceutical Press, 2009; 45-58.
12. Ramasamy, T., Umadevi, S.K., Suresh, S., and Himabindhu, R. Formulation and Evaluation of Chondroitin Sulphate Tablets of Aceclofenac for Colon Targeted Drug Delivery. *IJPR*, 2012; 11(2): 465-479.
13. Ramya, R., P. N. Sudha, Dr. J. Mahalakshmi. Preparation and Characterization of Chitosan Binary Blend. *IJSRP*, 2012; 2(10): 1-9.
14. Dunn, L.N. and Pressman, A.E. *Integrated Remote Sensing System*. Las Vegas: Environmental Protection Agency, 1973.