Synthesis of nanomagnetite coated with carboxymethyl kappa carrageenan (CMKC) as a binder of sodium diclofenac

B Zuhroti¹, I K Kusumaningrum¹*, A R Wijaya¹ and N N Purnama¹

¹ Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Jl. Semarang 5 Malang 65145, Indonesia

*E-mail: irma.kartika.fmipa@um.ac.id

Abstract. To increase the effectiveness of therapy and prevent such damage, nanocarriers can be used as drug carriers through intraarticular injection. The active ingredient of the drug is bound to the nanoparticle-wrapping membrane which is controlled by a magnetic field from the outside. The nanoparticles used in this study were nanomagnetites which were synthesized by the coprecipitation method. Then nanomagnetite is coated with a coating agent that can bind the active ingredients of the drug while preventing agglomeration and oxidation. The coating used carboxymethyl kappa-carrageenan (CMKC). CMKC synthesis was carried out by modifying kappa-carrageenan through two steps, alkalization of k-carrageenan using NaOH, to form alkoxy-k-carrageenan, then etherification of alkoxy-k-carrageenan with monochloroacetic acid (MCA) as reactant to produce carboxymethyl k-carrageenan (CMKC) in isopropanol and ethanol solvent media. The absorption of diclofenac sodium into nanomagnetite coatings was carried out on a phosphate buffer media of pH 7.4 with an absorption time of 30 minutes at room temperature. From the results of the research note that sodium diclofenac successfully adsorbed onto nanomagnetite coatings.

1. Introduction
Nonsteroidal anti-inflammatory drugs are drugs to treat pain, fever, and anti-inflammatory which are often prescribed in Indonesia. One of them is sodium diclofenac or better known as Non-Steroidal Anti-Inflammatory Drugs (NSAID). NSAIDs are drugs to treat acute or chronic pain, oral administration that is too often avoided because it can cause stomach damage [1]. The dosage and formulation for its use must be planned because sodium diclofenac has a low half-life, 1 to 2 hours [2]. One of the slow off drug administration can be done by injection into the joint. In order to slow the delivery of drugs can increase the effectiveness of therapy and deliver drugs according to the target, then used nanocarrier.

In the nanocarrier, the active ingredient is bound to the magnetic nanoparticle membrane, then the movement of the drug is controlled by a magnetic field from the outside. One type of magnetic nanoparticle that is often used is nanomagnetite, because it has superior characteristics such as superparamagnetic, high surface and volume ratio, and biocompatible [3]. Nanomagnetite is very biocompatible in the body because of the absorption of iron, transport and storage in the liver which is regulated by proteins such as ferritin and transferrin [4].

Nanomagnetite synthesis can be carried out by the coprecipitation method, which is the simplest method and can be carried out under a temperature of 100 °C [5]. Although it has several advantages,
nanomagnetic has disadvantages when applied, including having a high level of oxidation, so that its stability is low and tends to be agglomerated [6]. An alternative solution to prevent agglomeration of magnetite particles is by coating. Commonly used coatings are polysaccharides and their derivatives with carboxyl groups or hydroxyl groups are hydrophilic and non-toxic, such as chitosan, alginate, and carrageenan [7].

Carrageenan is a linear sulfate polysaccharide, there are several types of carrageenan. Taetapi that is often used is kappa-carrageenan which is a natural marine sulfate polysaccharide which has prominent properties, namely as an anticoagulant, antithrombotic, and strong anti-inflammatory [8].

The functional group in kappa-carrageenan can be modified to improve the nature of carrageenan through CMKC synthesis. CMKC synthesis can be carried out through two stages, namely alkalinization and etherification [9] using isopropanol and ethanol as solvent media. Carboxymethylation can be done with monochloro acetic acid (MCA) reagents.

The type of interaction that occurs between nanomagnetites and the compounds to be bound is influenced by the type of functional group, charge, morphology, and coating pores. CMKC and diclofenac sodium are possible to interact because of the morphology and pore coatings. Research on nanomagnetite coating using CMKC is an effort to find alternative methods of nanocarrier coating of drugs that are biocompatible and are able to bind drug compounds optimally.

2. Methods

2.1. Synthesis of CMKC in Isopropanol-Etanol-Acetone as Reaction Medium
1 gram of k-carrageenan powder was suspended into 25 mL of isopropanol and stirred for 15 minutes at room temperature, then ethanol 96% was added. Furthermore, 2.4 g of NaOH powder was added to the suspension 3 times every 15 minutes and heated for 1 hour at 70°C. Then solvent media was added by adding total volume of 5 mL (ethanol: acetone 2: 3) and stirred for 10 minutes. Next, the mixture was added with 2.5 grams of MCA while heated at 70°C for 4 hours. The precipitate obtained was filtered, dissolved in 96% ethanol, then neutralized with glacial acetic acid. The neutral precipitate was washed 3 times with 30 mL of ethanol each then in the oven at 50°C for 24 hours (modified from) [8 10, 11]

2.2. Synthesis of nanomagnetites
Synthesis of nanomagnetite was carried out by adding 10 ml of 0.125 M FeSO₄ solution and 10 mL of 0.25 M FeCl₃ solution into 40 mL of 1 M NaOH solution dropwise and stirred with magnetic stirrer for 30 minutes. The black precipitate obtained was filtered and neutralized with distilled water. Then dried in the oven at 50°C for 5 hours, then mashed.

2.3. Functional Group Analysis
Analysis of functional groups from CMKC and nanomagnetite used Fourier transform infrared (FTIR Prestige 21 Shimadzu). The range of wave numbers was analyzed at 4000 cm to 400 cm⁻¹.

2.4. Nanomagnetic Coating with CMKC
0.5 g CMKC was mixed into 500 mL of aquademin and stirred with a magnetic stirrer for 3 hours. From this solution 100 mL is taken to continue stirring and then added with 0.5 g of nanomagnetic and stirred for 2 hours. The precipitate obtained was filtered and roasted at 50°C for 5 h [12].

2.5. Analysis of Magnetization Strength
Analysis of the magnetization strength of nanoparticles that have not been coated or that have been coated are performed with a vibrating magnometer (VSM). VSM analysis was carried out to determine the value of CMKC coated nanomagnetite and nanomagnetite nanomagnetites.

2.6. Surface Shape and Size Analysis
Surface shape analysis of nano and nano coated is done using a scanning electron microscopy (SEM), while the surface size of the particle is done by calculating the average size per hundred particles through Ms. Word.

2.7. Adsorption of Sodium Diclofenac
0.01 g of coated nanomagnetic was added to the diclofenac sodium solution (solvent = phosphate buffer pH 7.4) at concentrations of 9 and 12 ppm. The mixture is stirred with a shaker for 30 minutes (150 ppm). The remaining diclofenac concentration can be known from the UV test results.

3. Results and Discussion
Nanomagnetite (Fe₃O₄) is a nano-molecular particle that has superparamagnetic properties. Magnetite synthesis was carried out by the coprecipitation method (Hong), namely by mixing FeSO₄·7H₂O and FeCl₃·6H₂O with NaOH solution. The resulting precipitate is solid black. The results of nanomagnetite synthesis were then characterized by XRD (X-Ray Diffraction), FTIR, SEM, and VSM. This characterization was carried out to confirm the formation of Fe₃O₄ and its crystallinity in Figure 1.

![Figure 1. XRD Characterization of Nanomagnetite](image)

In accordance with the results obtained, the magnetite characterization with XRD is indicated by the results of the intensity and angle (2θ). Standard pattern of pure magnetite X-ray diffraction (Fe₃O₄) according to the Joint Committee for Powder Diffraction Standard) at 2θ are 18.2°, 30.1°, 35.4°, 37.1°, 53.4°, 56.9°, 62.5°, 65.7°, 70.9°, and 73.9° (JCPDS No. 19-0629). The standard data is used for comparison with characterization of synthesis results. Based on nanomagnetite synthesis results obtained angles that appear at the top of XRD diffratogram, namely 35°, 43°, 56.5°, 57°, 63°. The peak produced is still in the range of 30.5° and 63.1° which indicates that the magnetite oil in the sample is a mineral crystal [13].

Nanomagnetic has disadvantages when applied, including having a high level of oxidation, so that its stability is low and tends to be agglomerated [6]. The agglomeration is caused by the presence of a large enough hydrophobic surface on the magnetite particles and the repulsion between the particles, so that their interaction with water is low. An alternative solution to prevent agglomeration of magnetite particles is by coating. Coatings can overcome the problem of agglomeration as well as provide a group of active ingredient binding agents. Commonly used coatings are polysaccharides and their derivatives with carboxyl groups or hydroxyl groups are hydrophilic and non-toxic, such as chitosan, alginate, and carrageenan [7].
The functional group in kappa-carrageenan can be modified to improve the nature of carrageenan. Carboxymethylation of carrageenan is an attempt to substitute the hydroxyl group with a carboxyl group to increase the interaction of polysaccharides with the polar environment, this also affects the polysaccharide characteristics such as solubility, water binding ability, and viscosity [8]. Carboxymethylation of carrageenan is carried out in two stages, namely alkalization (Figure 2.a) and etherification (Figure 2.b). The alkalization step is carried out by reacting NaOH with k-carrageenan in the isopropanol and ethanol reaction media to produce alkoxy-k-carrageenan, while the etherification step is carried out by reacting alkoxy-k-carrageenan with the etherification agent monocloroacetic acid (MCA) on the ethanol and ethanol-acetone reaction media.

K-carrageenan is a polymer that has many hydroxyl groups, which indicates that k-carrageenan has properties as a weak acid. The presence of NaOH in the reaction will attract H\(^+\) from the k-carrageenan hydroxyl group and bind with OH\(^-\) from NaOH, whereas Na\(^+\) from NaOH will refer to O\(^-\) in k-carrageenan to produce alkali-k-carrageenan. The addition of alkali-k-carrageenan MCA aims to substitute the carboxyl group to replace Na\(^+\) in Alkoxy-kappa-carrageenan.

\[ \text{Alkoxy-kappa-carrageenan} \]

![Figure 2](image)

**Figure 2.** (a) Kappa-Carrageenan Reaction with NaOH and (b) Reaction of Alkoxy k-Carrageenan with Monochloroacetic Acid (MCA)

The success of CMKC synthesis and nanomagnetite coating with CMKC can be confirmed through the FTIR spectrum.

![Figure 3](image)

**Figure 3.** FTIR Spectrum: a. Kappa-Carrageenan, b. Carboxymethyl Kappa-Carrageenan

Based on Figure 3 it can be seen that some of the hydroxyl groups have been replaced with carboxyl groups. This is indicated by the appearance of absorption peaks at wave numbers 1774.51 cm\(^{-1}\) and 1417 cm\(^{-1}\) which indicate the formation of C=O groups, where the absorption peaks do not
appear in the k-carrageenan FTIR spectra. O-H group in k-carrageenan appears absorption peak with wavelength of 3205.69 cm\(^{-1}\) with high intensity, whereas in CMKC spectra O-H group appears at wavelength of 3197.98 cm\(^{-1}\) with lower intensity. This shows that in the k-carrageenan carboxymethylation process, not all O-H groups were successfully converted to C=O groups. Particle morphology can be observed through SEM as shown in Figure 4.

![Figure 4. SEM Characterization: a. Nanomagnetite, b. Nanomagnetite Coated with Carboxymethyl Kappa-Carrageenan](image)

Before being coated, nanomagnetite has a smaller diameter of 58 nm (Figure 4.a) than those that have been coated with carboxymethyl kappa-carrageenan i.e 99 nm (Figure 4.b). Nanomagnetite which has been coated by CMKC has a larger and separate particle size than that which has not been coated.

![Figure 5. VSM Characterization: a. Magnetite, b. CMKC coated magnetite](image)

Coating is one factor that can reduce nanomagnetite magnetism (Kalkan, et al., 2011). The magnetic properties of nanomagnetites before and after coated by CMKC were tested with VSM as indicated by the difference in the hysteresis curve produced. The magnetic properties of CMKC coated magnetite nanoparticles showed a lower magnetization saturation value of 18 emu/ g (Figure 5.b) compared to nanomagnetite with a magnetization saturation value of 40 emu / g (Figure 5.a). This happens because the coating by CMKC can cover the surface of the nanomagnetite so that its magnetic properties are reduced.4 Adsorpsi natrium diklofenak oleh adsorben nano terlapis

Diclofenac ions contained in phosphate buffer pH 7.4 can enter the pore in nanomagnetite coatings, in the form of CMKC in the adsorption process. Adsorption of diclofenac by CMKC coated nanomagnetite adsorbents can be tested by UV Spectrophotometer at a wavelength of 268 nm. Data obtained from the UV test in the form of a concentration of the solution in mg / L units.
Table 1. Adsorption of Sodium Diclofenac into CMKC Coated Nanomagnetites

| Sodium diclofenac (ppm) | Remain concentration (ppm) | Absorbance |
|-------------------------|-----------------------------|------------|
| 12                      | 7.63                        | 0.57       |
| 9                       | 8.61                        | 0.63       |

From Table 1 it was found that sodium diclofenac was successfully adsorbed onto carboxymethyl-coated kappa carrageenan nanomagnetite.

4. Conclusion

Kappa-carrageenan (CMKC) carboxymethyl can be synthesized with monochloro acetic acid etherification agent (MCA). The FTIR spectrum which marks the formation of CMKC is characterized by the appearance of absorption peaks at wave numbers 1774.51 and 1417 cm⁻¹. Diclofenac sodium can be absorbed by CMKC coated nanomagnetite which is proven by UV testing.

References

[1] Temporelli P L, Zito G B, Pedretti R F, Belisarii F I, Putorti G, and Faggiano P 2015 *Monaldi Arch. Chest Dis.* **82** 1-10
[2] Anggraeni Y, Hendradi E, and Purwanti T 2012 *PharmaScientia* **1** 15-21
[3] Jianga W, Yanga H C, Yang S Y, Horng H E, Hung J C, Chene Y C, Hong C-Y 2004 *J. Magn. Magn. Mater.* **283** 210–14
[4] Corchero J L and Villaverde A 2009 *Trends Biotechnol.* **27** 468–76
[5] Hong R, Li J, Wang J and Li H 2007 *China Particuology* **5** 186–191
[6] Si S and Singhal M 2015 *Transl. Biomed.* **6** 1-10
[7] Jayakumar R, Menon D, Manzoor K, Nair S V and Tamura H 2010 *Carbohydr. Polym.* **82** 227–232
[8] Fan L, Wang L, Gao S, Wu P, Li M, Xie W, Liu S, Wang W 2016 *Carbohydr. Polym.* **86** 1167–1174
[9] Ambjörnsson H A, Schenzel K, and Germgård U 2013 *BioResources* **8** 1918–1932
[10] Kusumaningrum I K, Wijaya A R, Marfuah S and Fadilah M N 2019 *IOP Conf. Ser. Earth Environ. Sci.* (Munich) Vol 299 (Amsterdam: North-Holland/American Elsevier) p 517
[11] Dorniani D, Kura A U, Ahmad Z, Shaari A H, Hussein M Z and Fukurazi S 2012 *IJN* **11** 5745-51
[12] Mahdavinia G and Etemadi H 2015 *Arab. J. Chem.* **12** 1-9
[13] Fajaroh F, Setyawan W, Widiyastuti W and Winardi S 2012 *Adv. Powder Technol.* **23** 328–333