The Optimum Conditions of Carboxymethyl Chitosan Synthesis on Drug Delivery Application and Its Release of Kinetics Study

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ABSTRACT

In this paper, carboxymethyl chitosan (CMC) was synthesized and studied as a carrier to encapsulate vitamin (as drug model) and controlled release. Chitosan (CS) is a polycationic derivated from chitin, which suitable for active substance carrier system on biomedical function. CS has good properties such as non-toxic, biodegradable, and biocompatible. However, CS insoluble in an aqueous solvent so CS was modified chemically into CMC. CMC was formed by reacting CS and monochloroacetic acid with sodium hydroxide (NaOH) as a catalyst. Optimation was performed by varying the NaOH concentration during alkalizing the CS and the temperature reaction. The functional group and crystallinity of CS and CMC were estimated by FTIR and XRD. The degree substitution of carboxymethylation has an average value of 0.60. The results show optimum temperature reaction and NaOH concentration were 60 °C and 40% (w/v). The nicotinamide (NA), a hydrophilic vitamin, was loaded within CMC matrix system through in vitro precipitation method. To confirm the encapsulation of NA in CMC and the release kinetics of NA from CMC in distilled water was studied through UV-Vis spectrophotometry. The release profile of NA from CMC matrix system carried out for 3 h and 12 h. The rate of NA release from CMC increases with increasing time and the follows a zero order, Higuchi, and Korsmeyer-Peppas kinetics rules.

Keywords: *carboxymethyl chitosan; temperature reaction; NaOH concentration; encapsulation; release kinetics*

ABSTRAK

Dalam penelitian ini, karboksimetil kitosan (CMC) telah disintesis dan dipelajari sebagai pembawa untuk mengenkapsulasi vitamin (sebagai model obat) dan pelepasan terkontrol. Kitosan (CS) merupakan polikationik hasil deasetilasi kitin, yang cocok digunakan sebagai matriks pembawa zat aktif dalam bidang biomedis. CS bersifat tidak beracun, biodegradabel, dan biokompatibel. Namun, CS tidak larut dalam pelarut air sehingga CS dimodifikasi secara kimia menjadi CMC. CMC dibentuk dengan mereaksikan CS dan asam monokloroasetat dengan natrium hidroksida (NaOH) sebagai katalis. Optimasi dilakukan dengan memvariasikan temperatur reaksi dan konsentrasi NaOH selama alkalisasi CS. Gugus fungsi dan kristalinitas dari CS dan CMC dianalisis dengan FTIR dan XRD. Derajat substitusi (DS) tingkat karboksimetilasi memiliki nilai rata-rata 0,60. Hasil penelitian menunjukkan temperatur optimum dan konsentrasi NaOH yaitu 60 °C dan 40% (w/v). Nikotinamida (NA) merupakan vitamin hidrofilik yang dienkap dalam sistem matriks CMC melalui metode presipitasi in vitro. Enkapsulasi NA di CMC dan kinetika pelepasan NA dari CMC dalam air destilated dipelajari melalui spektrofotometri UV-Vis. Profil pelepasan NA dari sistem matriks CMC dilakukan selama 3 dan 12 jam. Laju pelepasan NA dari CMC meningkat dengan bertambahnya waktu dan mengikuti aturan kinetika orde nol, Higuchi dan Korsmeyer-Peppas.

Kata Kunci: *karboksimetil kitosan; temperatur reaksi; konsentrasi NaOH; enkapsulasi; kinetika pelepasan*

INTRODUCTION

Over the past several decades, many scientists have focused their attention on the development of ideal drugs that specifically target the site of action. Synthesis of drug loaded polymer particles has attracted extensive attention due to the protection function and controlled release properties of the particles. The drug can be either incorporated by passive absorption or chemical conjugation into the carrier matrix. An example of a natural polymer that can be used as a carrier matrix of the active substance is chitosan. Chitosan is usually preferred as carrier matrix because of its biocompatibility, biodegradability, and non-toxicity [1-7].

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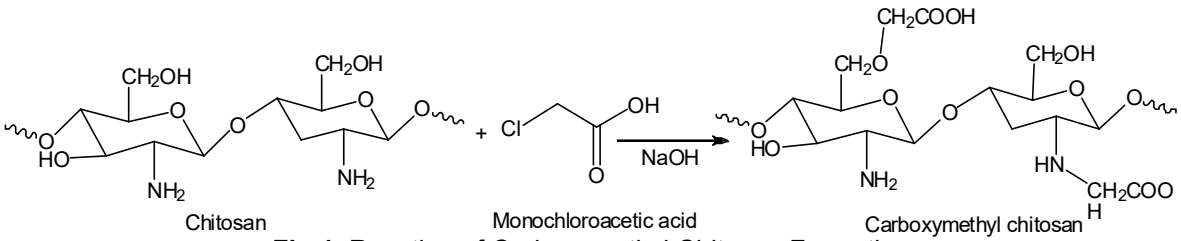

Fig 1. Reaction of Carboxymethyl Chitosan Formation

Chitosan (CS) or β-(1,4) -D-glucosamine is cationic polysaccharide main derivative of chitin which is from the shells of crustaceans, so it is available very abundant in nature [8-13]. Chitosan insoluble in neutral and alkaline pH because of the amino groups of chitosan are weak bases which predominantly protonated when pH<6.5 and it very stable crystalline structure arising from strong hydrogen bonds [14]. Therefore, the solubilization of chitosan only dilutes in acid solutions. So the poor solubility of chitosan when pH>6.5 is a serious drawback in many of its applications, especially as a drug carrier matrix. However, chitosan has reactive amino, primary hydroxyl, and secondary hydroxyl groups which can be used for chemical modification under mild reaction condition to overcome its limited solubility in aqueous media [15-16]. Many water soluble chitosan derivatives have been prepared by introducing hydrophilic groups, such as carboxymethyl groups. CMC has a higher solubility than chitosan in the neutral solvent due to the hydrophilic groups. This advantage makes CMC widely used in various fields: food industry, agriculture, cosmetic, biomedicine, and pharmaceutical [17-20].

Carboxymethyl chitosan (CMC) can be made through the alkylation process by using sodium hydroxide (NaOH) and reacted with the monochloroacetic acid ($ClCH_2COOH$) as depicted in Fig. 1, thus forming carboxymethyl groups on the chitosan polymer chain. Some factors that influence CMC forming are the ratio of water: isopropyl alcohol, the degree of deacetylation (DD), temperature reaction and alkaline concentration (NaOH) [14,21]. The increase of the ratio water: isopropyl alcohol decrease the fraction of carboxymethylation. The increase of the temperature reaction will increase the fraction of carboxymethylation [17]. The degree of substitution (DS) carboxymethyl groups strongly dependent on NaOH concentration used. NaOH aqueous solution as a catalyst. The employment of high NaOH concentration promoted side reaction between NaOH and chloroacetic acid and the available chloroacetic concentration for the reaction decreased accordingly [14].

In biomedical function, CMC used as carrier matrix in encapsulation system. Encapsulation is one of the quality preservation techniques of sensitive substances and a method for production of materials with new valuable properties [22]. The encapsulation technique of choice depends on the type and physical properties of the core and shell material. Different methods have been used to encapsulation. Selection of any of the methods depends on factors such as particle size requirement, the thermal and chemical stability of the active agent, reproducibility of the release kinetic profiles, the stability of the final product and residual toxicity associated with the final product [23]. Some methods of encapsulation are emulsion cross-linking, emulsion-droplets coalescence, ionotropic-gelation, precipitation, reverse micelles, a sieving method, spray drying [24]. Precipitation is quite methods. Particles are produced by blowing polymer solution into an alkali solution like sodium hydroxide [23].

Encapsulation is a phenomenon of intermolecular interaction. Chitosan has lower encapsulation efficiency than CMC due to its hydrophobicity. In this paper, chitosan is modified into CMC to improve the encapsulation efficiency and release kinetics. Encapsulation efficiency between chitosan and CMC was determined by nicotinamide (NA) as drug model [25-26]. Rate release of nicotinamide from matrix system will be determined by slow release kinetics mechanism [27-30].

EXPERIMENTAL SECTION

Materials

Materials used in this study were chitosan with a degree of deacetylation 71.90%, sodium hydroxide (Merck), monochloroacetic acid (Merck), and nicotinamide (Sigma Aldrich). All chemical and reagents used in this experiment were of analytical grade.

Instrumentation

The instruments in this experiment were glass tools Pyrex ® $Iwaki_{TE-32}$ made in Thailand, analytical balance KERN ALS 220-4N made in Germany, oven Fisher Scientific model 630F made in USA, filter paper Whatman® Schleicher and Schuell 110 mm made in England, thermometer, hotplate and magnetic stirrer, pH meter Handylab Schott, HANNA conductometer model H1991300 made in Romania, Hettich centrifuges

EBA 20 made in Germany, Shimadzu FTIR Spectrometer made in Japan, UV Vis 1601 Spectrophotometer model T60 U made in Germany and Shimadzu XRD-7000 made in Japan.

Procedure

Preparation of carboxymethyl chitosan

Chitosan (2 g) was dispersed in 20 mL of isopropanol and stirring for 20 min at room temperature. Next 5 mL of aqueous NaOH was added than 45 min stirring and 12 g of monochloroacetic acid was then added to the suspension. The reaction proceeded to the 3 h with temperature, 40 °C, 50 °C, 60 °C, 70 °C, 80 °C, 90 °C, and 100 °C for each mixture. Then the solid product was then filtered, suspended in 100 mL methanol. The product was dried at 60 °C in an oven for 12 h and finally in a vacuum desiccators at room temperature. FTIR used to structural characterization.

Different carboxymethyl chitosan samples was prepared by employing different temperature reaction (room temperature /CMC-A, 40 °C/CMC-B, 50 °C/CMC-C, 60 °C/CMC-D, 70 °C/CMC-E, 80 °C/CMC-F, 90 °C/CMC-G, and 100 °C/CMC-H for each mixture) and concentration of NaOH (20%/CMC-D1, 30%/CMC-D2, 40%/CMC-D3, 50%/CMC-D4, 60%/CMC-D5, 70%/CMC-D6, and 80%/CMC-D7 (w/v) for each mixture).

Solubility and conductivity test

A 100 mg CMC was inserted into a glass beaker and 20 mL double distilled water was added to it slowly to dissolve of the carboxymethyl chitosan completely. After 1 h, carboxymethyl chitosan remaining separated by vacuum drying. Then the residue has dried to determine the amount of mass loss in dissolved water. The conductivity was tested every 10 min during 1 h. The solubility and conductivity were testing at room temperature.

$$\text{Carboxymethyl chitosan solubility}(\%) = \frac{Mi - Mf}{Mi} x100 \quad (1)$$

M_i = initial mass of carboxymethyl chitosan (g)
M_f = final mass of carboxymethyl chitosan (g)

Degree of substitution

Worked by de Abreu et al. [31], the degree of substitution was determined by automatic titrator (Schott Titronic Universal) with an accuracy of 0.05 mL. In our lab we did not have these instruments, so we follow Rahmawati et al. [32] method, the degree of substitution (DS) of this carboxymethyl chitosan was calculated following the equation (2).

A 100 mg of CMC in 10 mL of 0.12 M NaOH was prepared. The mixture was stirred 30 min at room temperature. Methyl red as an indicator was added and the mixture was titrated with 0.13 M HCl until the mixture became reddish.

$$DS = \frac{MW\,x\,M\,x\,(B - S)}{1000\,x\,W} \quad (2)$$

MW = molecular weight of monomer chitosan (g/mol)
B = volume of HCl blank (mL)
S = volume of HCl sample (mL)
M = molarity of HCl (mol/L)
W = mass of sample (g)
DS = number of substituted hydroxyl group

Characterization of chitosan and carboxymethyl chitosan

FTIR and XRD were used to the characterization the functional groups and crystallinity of CS and CMC.

Encapsulation efficiency and nicotinamide (NA) release

Encapsulation was done on 500 mg of CS and CMC. The samples were dissolved in 1% acetic acid. Then the sample solution was added by 40 mL NA 2500 ppm and stirring for 5 min. Furthermore, the mixture was precipitated by 1 N NaOH. The separation of encapsulated NA using a centrifuge at 5000 rpm for 30 min. The filtrate was subjected to spectrometric measurement at 263 nm with UV Vis 1601 Spectrometer. The encapsulation efficiency was determined by:

$$\text{Encapsulation efficiency}(\%) = \left(\frac{\text{Ci of NA} - \text{Cf of NA}}{\text{Ci of NA}}\right) x10 \quad (3)$$

Fifty (50) mg of NA-CS and NA-CMC was added into 40 mL distilled water and the release was estimated at different time points. Three milliliters of the sample was taken at each time interval then analyzed in UV Vis 1601 Spectrometer to estimate the nicotinamide concentration at 263 nm.

Data obtained from *in vitro* release studies were fitted to various kinetics equations to discover the mechanism of drug release from prepared formulations. The kinetic models used were zero order, first order, Higuchi and Korsmeyer-Peppas models. The rate constants were also calculated for the respective models.

Zero order:

$$Q_t = Q_o + K_o t \quad (4)$$

where Q_t is the amount of drug dissolved at time t, Q_o is initial drug concentration, k_o the kinetics constant.

First order:

$$\ln Q_t = \ln Q_o + K_1 t \quad (5)$$

where k_1 the constant rate of the first order.

Korsmeyer-Peppas model (eq.7) to explain drug release from the polymeric system:

$$M_t / M_\infty = K.t^n \quad (6)$$

where M_t/M_∞ is cumulative drug released at time t, k the kinetics constant, and n the release exponent and determines the release mechanism.

Higuchi models (eq.8) used to study of drug release, either water soluble or slightly soluble in water, a polymeric matrix solid or semi-solid:

$$M_t / M_\infty = K_H . \sqrt{t} \quad (7)$$

where k_H the kinetics constant.

RESULT AND DISCUSSION

Synthesis of Carboxymethyl Chitosan

The aim of this study was to obtain the optimum condition for the synthesis of carboxymethyl chitosan with the highest solubility in water. Therefore, the various type carboxymethyl chitosan was made with various concentration of NaOH and temperature reaction. The works in the reference report that the carboxymethyl chitosan occurs selectively according to the conditions used in the reaction, a complex mixture of products is generally obtained when ordinary conditions are used. During carboxymethylation of chitosan with monochloro acetic acid in the mildly alkaline medium of pH ± 8, only the amine groups activated and so only N-substitution will take place. The products result from variation in temperature can be seen in Fig. 2.

From varying the temperature of reaction (Fig. 2), it can be seen that the products have different color due to increasing temperature. CMC-A until CMC-D have the same color, but start from CMC-E to CMC-H have a darker color. According to Wijaya et al. [33], the chitosan TGA results that at 57.54–153.76 °C occurs water vaporing and followed by decomposition of chitosan. It proves that CMC-E to CMC-H has a different color. This prediction supported by solubility and conductivity test results (as shown in Fig. 3).

Based on the results, the optimum temperature of carboxymethyl chitosan synthesis at 60 °C (CMC-D) with solubility in water 43.50%.

After the optimum temperature was found then the optimum concentration of NaOH on the synthesis of carboxymethyl chitosan was determined. The products result from the various concentration of NaOH have the same color (Fig. 4) indicating that variation concentration of NaOH did not have impact physically property.

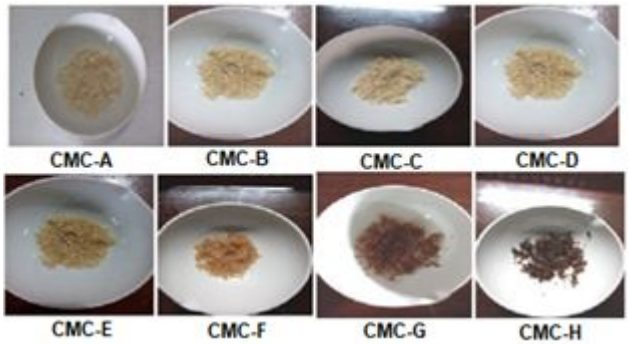

Fig 2. CMC varied by temperature

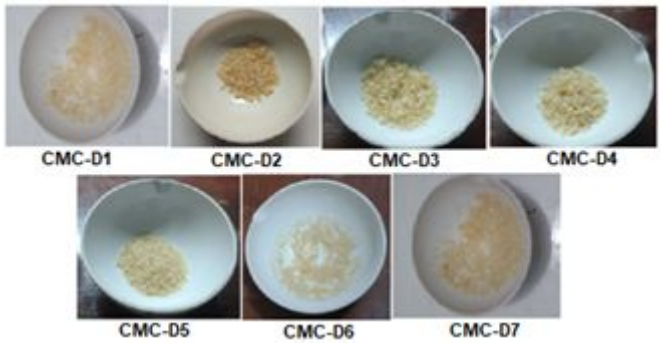

Fig 4. CMC varied by NaOH concentration

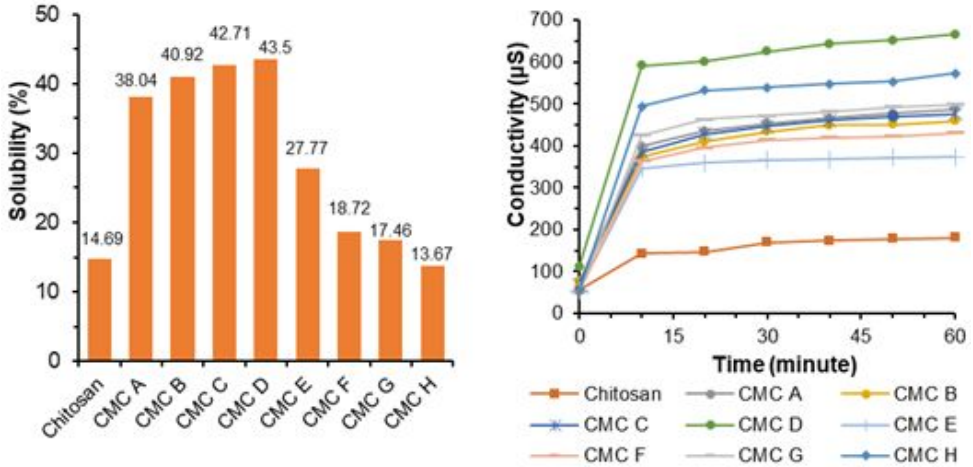

Fig 3. Solubility and conductivity test of chitosan and CMC varied by temperature

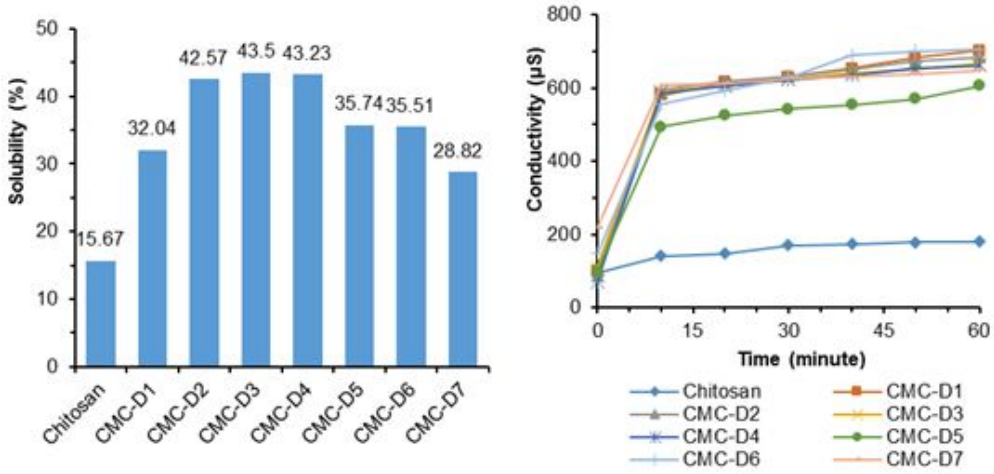

Fig 5. Solubility and conductivity test of chitosan and CMC varied by NaOH concentration

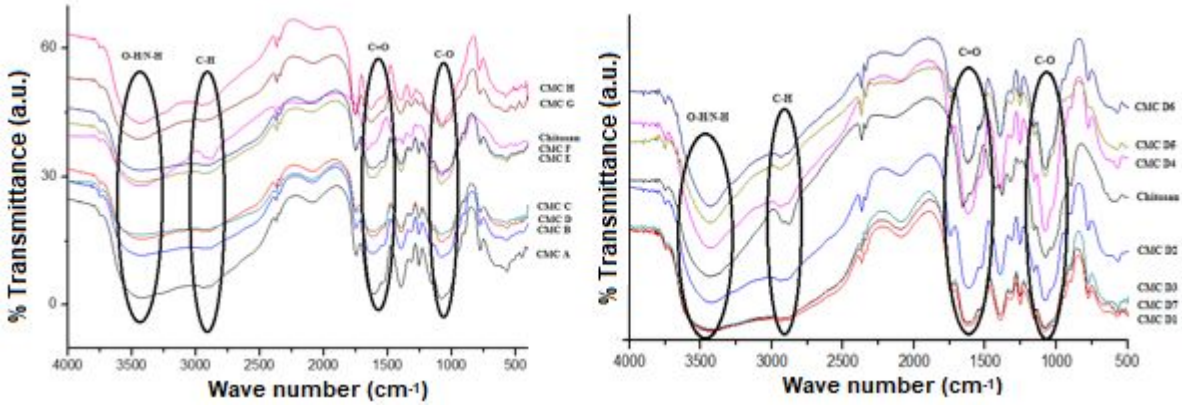

Fig 6. FTIR spectra of chitosan and CMC varied by temperature and varied by NaOH concentration

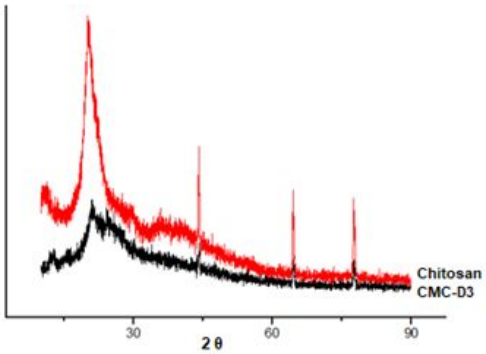

Fig 7. XRD diffractogram of chitosan and CMC-D3

Table 1. Value of DS carboxymethyl chitosan determined by titration

Sample	DS
CMC-D1	0.68
CMC-D2	0.69
CMC-D3	0.69
CMC-D4	0.67
CMC-D5	0.64
CMC-D6	0.62
CMC-D7	0.60

The degree of substitution (DS) strongly dependent on NaOH concentration used the value of DS as shown in Table 1. The rigid crystalline structure of chitosan was difficult to disrupt to ensure penetration of the monochloroacetic acid at lower concentration of NaOH into the interlocking polymer chains resulting in a lower degree of substitution. In contrast, employing a high concentration of NaOH promoted side reaction between NaOH and monochloroacetic acid and the available monochloroacetic concentration for reaction forming carboxymethyl chitosan reduced accordingly.

The DS value of the sample CMC-D3 and CMC-D2 (Table 1) are same, but the solubility of CMC-D3 higher than D2. It is indicated that the synthesis of carboxymethyl chitosan optimum with 40% (w/v) NaOH addition. The solubility and conductivity test results are shown in Fig. 5.

FTIR and XRD Study

The infrared absorption spectrum of chitosan and carboxymethyl chitosan are shown in Fig. 6. The specific

wavenumber 3441 cm^{-1} shows OH and NH stretching vibration, 2924-2854 cm^{-1} shows the CH stretching vibration, and 1635 cm^{-1} shows the C=O bond, and 1080 cm^{-1} for C-O bond [20]. The peaks that appear in the infrared spectrum of chitosan and carboxymethyl chitosan are identical. In the infrared spectra of chitosan, the peak observed at a wavenumber 3425.58 cm^{-1} is the stretching vibration of NH_2 and OH groups. The peak at wavenumber 1604.77 cm^{-1} in the bending vibration of NH_2 group. The C-O stretching vibration at 1080 cm^{-1}.

According to Mi et al. [19], the specific peaks of carboxymethyl chitosan at wavenumber 1744 cm^{-1} (C=O of carboxylic acid), 1608 cm^{-1} (stretching vibration of $–COO^-$ antisymmetric of salt carboxylic), and 1421 cm^{-1} (stretching vibration of $–COO^-$ symmetric). Based on spectra FTIR of chitosan (Fig. 6), all carboxymethyl chitosan have specific peaks at wavenumber 1743 cm^{-1}, 1620 and 1396 cm^{-1} both in temperature and concentration of NaOH variation. The based on Fig. 6 that carboxymethyl been formed.

Based on the obtained result of XRD diffractogram as depicted in Fig. 7, CS can be recognized with the emergence of the sharp characteristic peak at an angle of 20°, while CMC can be recognized by the appearance of the characteristic peak that is less sharp than CS at an angle of about 33° [34]. In this study, the characteristic peak of CS appears at an angle of 20.6393°, while the characteristic peak of CMC appears at an angle of 32.9299°. Based on XRD diffractogram, CS has crystalline structure while CMC has an amorphous structure. This difference of their structure proves that CMC more soluble than CS in water.

Encapsulation Efficiency and Nicotinamide Release

The encapsulation efficiency of NA-CS and NA-CMC was shown in the Fig. 8. Compared with the CS, the CMC yielded a higher EE. A possible explanation for this difference was that CMC possessed a hydrophilic group, which allowed for higher capacity of NA incorporation. This result shows that with more functional groups involved in the polymer-vitamin interaction [21].

The release of three samples at 3 h early in accordance with the 0-order kinetics with correlation coefficient >0.99. Kinetics of the 0-order on drug encapsulation system by using a controlled release polymer can be obtained. When the initial concentration of the drug beyond the solubility of the drug in the system and the concentration of the drug is constant. Drug release will be proceeded by the dissolution of the existing drug on the surface of the system and follow by swelling matrix polymer. The drug

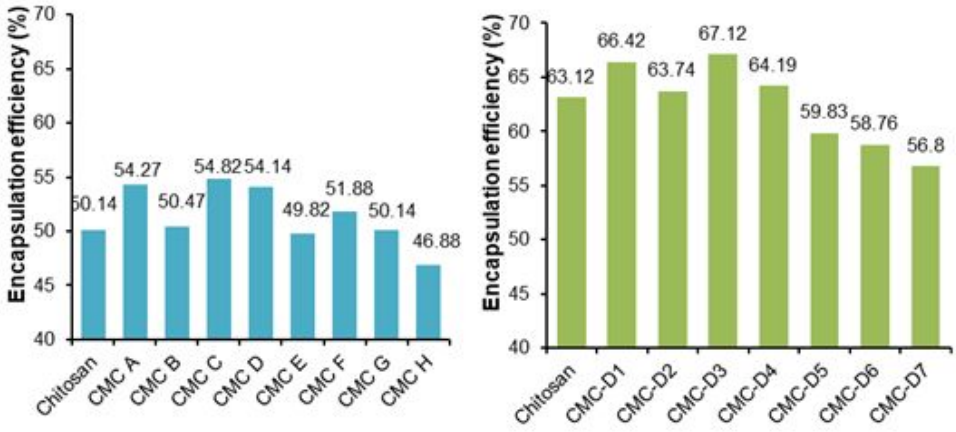

Fig 8. Encapsulation efficiency of chitosan and CMC

Table 2. Time release during 3 h

Kinetics Models	Parameters	Samples		
		Chitosan	CMC-D1	CMC-D3
0 Order	R^2	0.9917	0.1161	0.9926
	k_0	0.0336	0.9894	0.0689
1st Order	R^2	0.9791	0.9818	0.9925
	k_1	0.0025	0.0016	0.0017
Korsmeyer-Peppas	R^2	0.9857	0.9789	0.9455
	k_{KP}	1.6252	1.3759	1.3906
	n	0.2109	0.1386	0.1432
Higuchi	k_H	0.0408	0.1338	0.0075
	R^2	0.9969	0.9958	0.9739

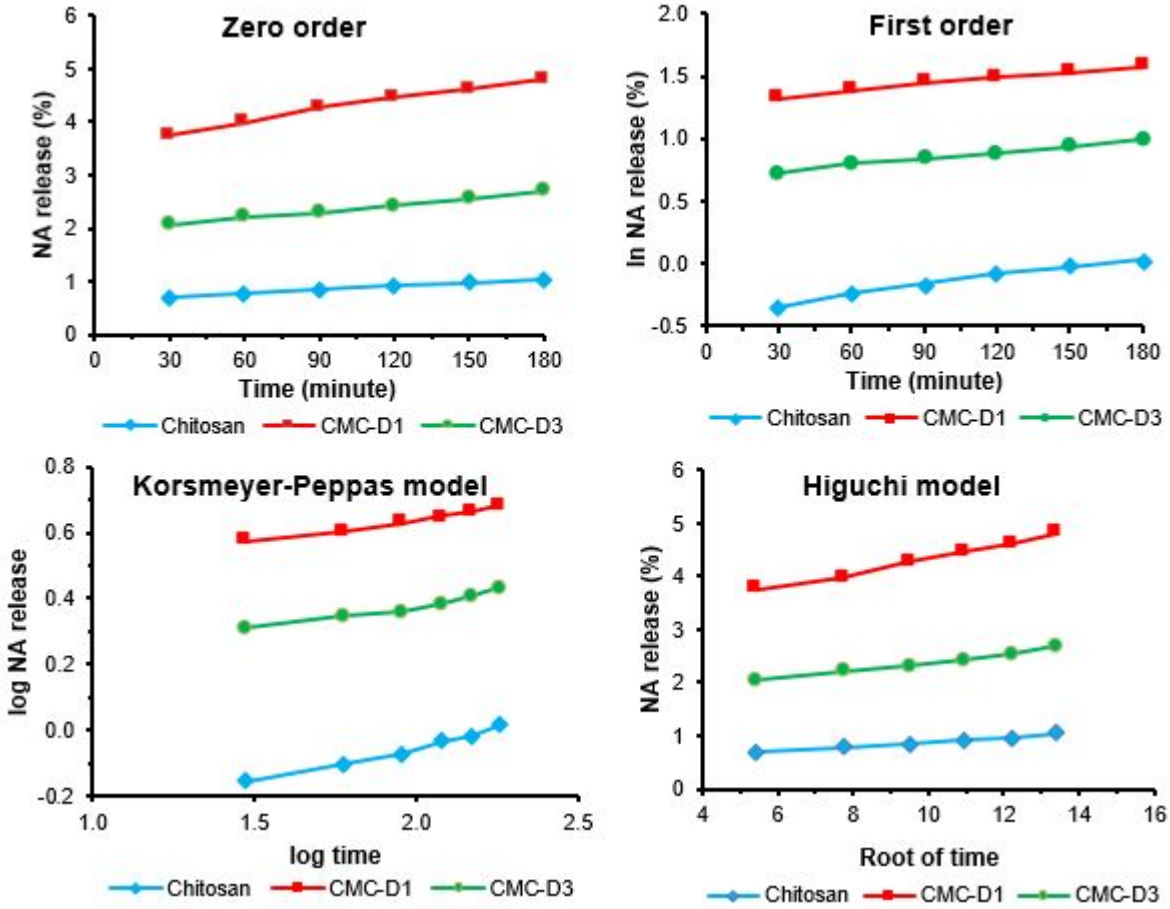

Fig 9. Time release during 3 h

Table 3. Time release during 12 h

Kinetics Models	Parameters	Samples		
		Chitosan	CMC-D1	CMC-D3
0 Order	R^2	0.9964	0.9970	0.9825
	k_0	0.5816	0.1390	0.3619
1st Order	R^2	0.9908	0.9970	0.9851
	k_1	0.0269	0.0032	0.0089
Korsmeyer-Peppas	R^2	0.9671	0.9406	0.9016
	k_{KP}	1.4632	1.0406	1.1160
	n	0.1653	0.0173	0.0477
Higuchi	k_H	0.1810	0.0105	0.1043
	R^2	0.9911	0.9839	0.9524

in the deeper parts will come out of the system when the drug on the surface had separated, so the concentration increases [35].

The release profile of NA from NA-CS, NA-CMC-D1, and NA-CMC-D3 with respect to time can be shown in Table 2 and Fig. 9.

All of the samples follow 0-order kinetics. It is indicated drug release occurring at any given time is relatively constant, so the drug can last longer on the system. The drug concentration release from CMC higher than CS. This will lead to determining the amount of the drug on the encapsulating system when designing long-term controlled release of the drug. Increasing concentration of drug release from CMC higher than CS because CMC more easily dissolve in water. So that the drug will more easily separate from CMC than CS matrix due to the interaction between water and hydrophilic groups of CMC.

A mathematical model that is also in accordance with the all of the sample drug release is Korsmeyer-Peppas and Higuchi with the correlation coefficient for each sample >0.97. Both of this model that has been

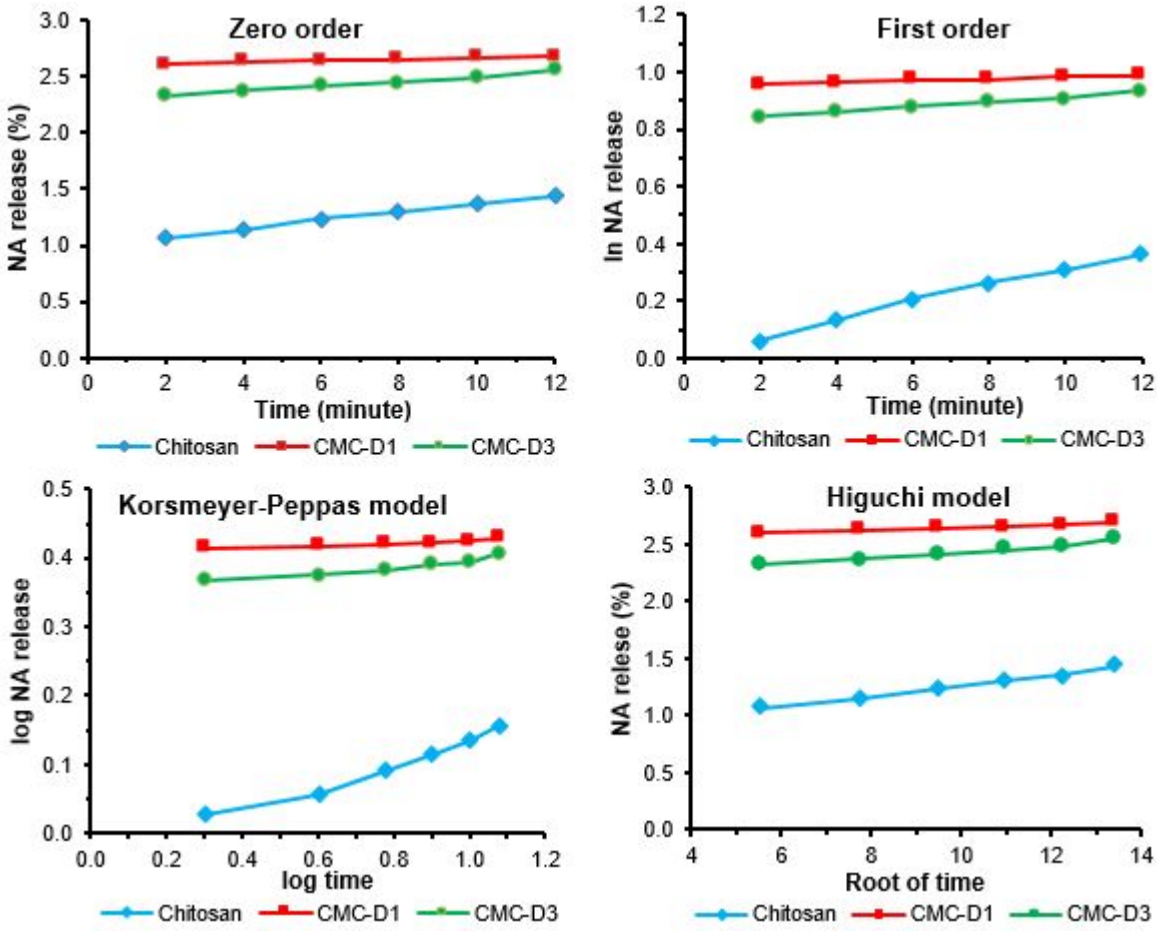

Fig 10. Time Release during 12 h

recognized and often used to determine the drug release mechanism. Drug release mechanism can be seen from the value of *n* resulted. The *n* value is between 0.13-0.21. It indicates that drug release mechanism is not only influenced by the drug diffusion process, but also degradation of the polymeric matrix. Based on these models can be estimated that the release process through 2 steps, (i) drug release fully caused by drug diffusion and (ii) the degradation of polymer matrix [35]. Determination of drug release of all samples was done in a longer time that is 12 hours to know the stability of the system as depicted in Table 3 and Fig. 10.

The based on Table 3 show that the accumulation of drug concentration release from CMC matrix higher than CS. During 12 h of drug release making the polymeric matrix unstable. According to the Korsmeyer-Peppas and Higuchi models, the *n* value and the correlation coefficient of 12 h drug release lower than 3 h.

The initial release of NA can be attributed to release of NA adsorbed onto the surface of CS and CMC matrix system. CMC-D3 had a slow release of nicotinamide thus making CMC-D3 a potential candidate as matrix system of encapsulation.

CONCLUSION

The optimum synthesis conditions of carboxymethyl chitosan obtained in this study are the use of temperature reaction at 60 °C and 40% (w/v) NaOH concentration for alkalizing chitosan respectively. The highest solubility in water of CMC-D3 is 43.50%. The encapsulation efficiency of NA-CMC-D3 is 67.12% and follows zero order kinetic model.

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