Enhanced Drug Release of Poly(lactic-co-glycolic Acid) Nanoparticles Modified with Hydrophilic Polymers: Chitosan and Carboxymethyl Chitosan

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Abstract: *The biodegradable polymer poly(lactic-co-glycolic acid) (PLGA) is a biomaterial with great potential as a drug delivery carrier and a tissue engineering scaffold. Using diclofenac sodium (DS) as a drug model, PLGA/DS nanoparticles were synthesized by modification with two hydrophilic polymers: chitosan and carboxymethyl chitosan (CMCh). The introduction of chitosan and CMCh enhances the efficiency encapsulation, capacity loading of the nanoparticles, and DS release at pH 6.8 and minimum release at pH 1.2. Synthesis of nanoparticles was carried out using a double emulsion (water/oil/water) solvent evaporation method. Characterization using an Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectrophotometer indicates that the interaction between DS and polymer on nanoparticles is non-covalent with a spherical shape based on a transmission electron microscope (TEM) and scanning electron microscope (SEM) characterization. From the various formulation studied, nanoparticles with the ratio chitosan-PLGA-DS and CMCh-PLGA-DS of 2:20:4 proved to be the optimum model carrier with the required release profile and could be the alternative for DS delivery systems.*

Keywords: *diclofenac sodium; PLGA; chitosan; carboxymethyl chitosan; controlled release*

■ INTRODUCTION

Diclofenac sodium (DS) is a non-steroidal anti-inflammatory drug (NSAID) widely used to relieve pain and anti-inflammation in various diseases like osteoarthritis and rheumatoid arthritis [1-2]. Due to its about two hours of biological half-life, frequent administration is necessary to maintain therapeutic drug-blood levels. DS is reported to cause gastrointestinal troubles, peptic ulceration, and renal damage if large dosing is taken orally [1,3-4]. A controlled drug is required to maintain drug efficacy and reduce side effects. This can be accomplished using a drug encapsulation technique to deliver the drug to a specific target [5]. Nanoparticles are one of the most extensively exploited for drug delivery systems due to their unique properties related to their size, capacity for drug protection, and controlled drug release [6]. Encapsulation with nanoparticles is one technique for enhancing drug stability by protecting it from enzymatic degradation, controlling drug release, increasing drug solubility, absorption by target cells or tissues, and drug safety [7-8]. The biodegradable polymer poly(lactic-co-glycolic acid) (PLGA) is a biocompatible and biodegradable copolymer commonly utilized as a nanoparticle matrix [2]. Several methods to prepare PLGA nanoparticles include the single or double emulsion method, salting-out, and nanoprecipitation [9]. PLGA has been modified with other biodegradable polymeric materials to increase its use as a drug carrier.

Chitosan and its derivatives are hydrophilic polymers that can be used as a surface modification of PLGA. Chitosan has been widely applied in tissue engineering, biomedical implants, and drug delivery devices as a natural biopolymer. However, it has limitations, such as being insoluble at physiological pH

and poor mechanical stability [10]. Carboxymethyl chitosan (CMCh) is a chitosan derivative that enhances its solubility at neutral pH. The presence of a carboxyl group at C-6 of chitosan produces carboxymethyl chitosan, which is soluble in both neutral and alkaline pH solutions. CMCh can be applied as a surface coating for polymer nanoparticles in the drug encapsulation process. The pH sensitivity of CMCh can be utilized for controlled drug release in delivery systems based on gastrointestinal pH changes [11]. CMCh improves the stability of polymer nanoparticles, hence diminishing drug burst release from polymer nanoparticles [12]. Furthermore, minimizing the size of particles of the drug can increase the solubility of the drug [13-14].

In this study, PLGA nanoparticles containing different amounts of chitosan or CMCh and DS have been fabricated via a double emulsion (w/o/w) solvent evaporation technique. The effect of chitosan and CMCh on PLGA nanoparticles was studied by an *in vitro* DS release study, determining the percentage of encapsulation efficiency and loading capacity. The effect of chitosan and CMCh on PLGA nanoparticles in *in vitro* DS release study, percentage of encapsulation efficiency, loading efficiency, and morphology were compared with unmodified PLGA nanoparticles.

■ EXPERIMENTAL SECTION

Materials

The materials used in this study were diclofenac sodium (National Agency of Drug and Food Control-Republic of Indonesia) as a drug model, poly(D,L-lactic-co-glycolic acid) lactic acid-glycolic acid ratio 50:50 with carboxyl end groups, Mw = 7–17 kDa (Nomisma Healthcare, India) as a nanoparticle material, poly(vinyl alcohol) (PVA) 87–90% hydrolyzed (Sigma Aldrich) as an emulsifier, low molecular weight chitosan with a degree of deacetylation > 75%, Mw = 50–190 kDa (Sigma Aldrich), O-carboxymethyl chitosan (CMCh) with a degree of substitution > 80% (Xi'an Herben Biotech), dichloromethane (Merck, Germany) was used as a PLGA solvent. Sodium chloride (Merck, Germany), hydrochloride acid (37%, Merck, Germany), and sodium hydroxide (Merck, Germany) were used to prepare the release medium. In addition, glacial acetic acid (Merck, Germany) was used to dissolve chitosan, methanol (LC grade, Merck, Germany), and deionized water (Milli-Q, Millipore) used for analysis using High-Performance Liquid Chromatography.

Instrumentation

A probe ultrasonicator (Hielscher UP 200st, PT Petra Karunia Persada) and freeze dryer (Thermo Scientific) were used in the preparation of nanoparticles. Nanoparticles were characterized using an ATR-FTIR spectrometer (Shimadzu IRSpirit). The size of nanoparticles was analyzed by particle size analyzer (Horiba SZ 100z, Integrated Laboratory and Research Center, Universitas Indonesia), and the morphology was analyzed by using a scanning electron microscope (SEM-Quanta) and transmission electron microscope (TEM-FEI Tecnai G2, Integrated Laboratory and Research Center, Universitas Indonesia). Determination of DS was performed by high-performance liquid chromatography (HPLC Shimadzu LC-20 AD) and UV-Vis Spectrophotometer (Shimadzu UV-1800).

Procedure

Preparation of DS nanoparticles

The nanoparticles were prepared using the double emulsion solvent evaporation technique as reported by Khanal et al. [11] with modification. As much as 200 mg of PLGA was dissolved in dichloromethane, and the DS solution was added and sonicated for 30 s using a probe ultrasonicator. The mixture was added dropwise to 5 mL of 0.3% polyvinyl alcohol (PVA) solution under a constant vortex. The solution was emulsified in an ice bath for 3 min using a probe ultrasonicator (water in oil emulsion). The emulsified solution was transferred into 45 mL of 0.3% PVA solution containing CMCh or chitosan solution, and the emulsion was stirred at 500 rpm for 5 h at room temperature (water in oil in water emulsion) and centrifuged the emulsion at 8000 rpm for 30 min at 4 °C. Finally, the nanoparticles were lyophilized for 24 h. The composition of ingredients is described in Table 1 and Table 2.

Table 1. Variation of composition of CMCh and chitosan on PLGA-DS

Formulation	Code	Weight ratio CMCh-PLGA-DS
PLGA-DS	F-0	0:20:2
CMCh-PLGA-DS	F-1	1:20:2
CMCh-PLGA-DS	F-2	2:20:2
CMCh-PLGA-DS	F-3	3:20:2
CMCh-PLGA-DS	F-4	4:20:2
Chitosan-PLGA-DS	H-1	1:20:2
Chitosan-PLGA-DS	H-2	2:20:2
Chitosan-PLGA-DS	H-3	3:20:2
Chitosan-PLGA-DS	H-4	4:20:2

Table 2. Variation of composition of DS on CMCh-PLGA and chitosan-PLGA

Formulation	Code	Weight ratio PLGA-DS
CMCh-PLGA-DS*	F-A	20:1
	F-B	20:2
	F-C	20:4
Chitosan-PLGA-DS*	H-A	20:1
	H-B	20:2
	H-C	20:4

*the composition of CMCh and chitosan used was the optimum composition obtained in the optimization of Table 1

Determination of loading capacity and encapsulation efficiency

The nanoparticles loading capacity (LC) and encapsulation efficiency (EE) were determined directly using a method described in Farmakope Indonesia 6th edition as follows: Accurately weighed 5 mg of nanoparticles was put into a 20 mL volumetric flask, added 0.5 mL of dichloromethane, then shaken using a vortex mixer for 3 min. The nanoparticles were added with methanol, sonicated for 20 min, and diluted with methanol to volume. The amount of DS in lyophilized nanoparticles was analyzed using an HPLC UV detector at 254 nm. An octyl silane column was used with methanol and 0.01 M phosphate buffer pH 2.5 (7:3) as the mobile phase with a 1 mL/min flow rate. The LC and EE were given according to Eq. (1) and (2).

$$LC = \frac{\text{weight of the drug}}{\text{total weight of the drug loaded nanoparticles}} \times 100\% \quad (1)$$

$$EE = \frac{\text{weight of drug in nanoparticles}}{\text{weight of drug added}} \times 100\% \quad (2)$$

In vitro *drug release study*

In vitro release studies were conducted to determine the effect of chitosan and CMCh on PLGA/DS nanoparticles. As much as 50 mg of each formulation were immersed in 50 mL pH 1.2 solution and stirred at 100 rpm for 2 h at a temperature of 37 ± 0.5 °C. About 2 mL of filtrate was taken and replaced with preheated fresh medium at various periods. After 2 h, the pH of the release medium was increased to 6.8 by adding 2.5 M sodium hydroxide solution [15], and the release was continued for a further 24 h. About 2 mL of filtrate was taken and replaced with a preheated fresh medium within 24 h. The concentration of drug release was measured by UV-Vis spectrophotometer at wavelength 277 nm using an absorbance-drug concentration calibration curve.

■ RESULTS AND DISCUSSION

FTIR Spectroscopy Analysis

Characterization of the nanoparticles was performed by ATR-FTIR, investigating interactions between the drug and polymer (Fig. 1). Pure PLGA exhibits peaks at 1713 and 1051 cm^{-1}, corresponding to -C=O stretching and C-O-C bending [16-17]. CMCh displayed the characteristic peaks at 1547 and 1024 cm^{-1} indicating COO stretching and C-O-C bending [16,18]. A suggested COO band at 1547 cm^{-1} overlaps the NH_2 band at 1600 cm^{-1}. The CMCh/PLGA/DS nanoparticles spectrum displayed band at 1774 cm^{-1} indicates PLGA. The relative weak band was at 1534 and 712 cm^{-1}, indicating a typical band absorption CMCh and DS, respectively. The same profile occurred in the Chitosan/PLGA/DS nanoparticles spectrum, where the characteristic peaks of PLGA, DS, and Chitosan appeared at 1718, 1606, 1064, and 713 cm^{-1}. IR analysis showed no change in the peaks of each material which indicates there was no new absorption formed in the nanoparticle spectrum. The spectrum of nanoparticles shows a characteristic absorption band of the drug, and its polymer indicates the absence of covalent interactions between the drug and the polymer [19], suggesting the

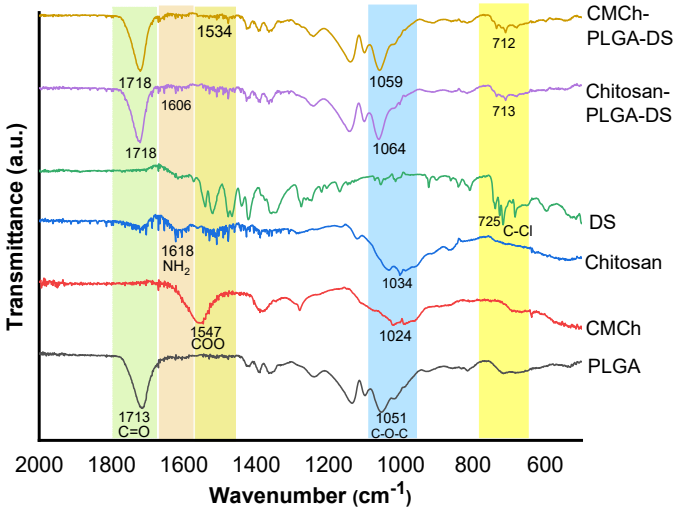

Fig 1. FTIR spectra of PLGA, CMCh, Chitosan, DS, Chitosan-PLGA-DS, and CMCh-PLGA-DS nanoparticles

interaction between PLGA and CMCh was a non-covalent bond. Non-covalent bonds on the surface of nanoparticles can occur through electrostatic interactions [20].

Particle Size, Surface Charge, and Morphology

PSA analysis

The addition of CMCh and chitosan increased the hydrodynamic diameter of the nanoparticles, according to the dynamic light scattering technique. The increase in size was due to the increased viscosity of the external phase containing CMCh or chitosan, which reduces shear stress during stirring so that larger emulsion droplets are formed [21]. The modifier composition enhanced the particle size [17,22]. Khanal et al. also reported the size increase of modified PLGA nanoparticles using chitosan [11]. Fig. 2 shows the size distribution taken from three samples that were F-0, F-2, and H-2. Table 3 shows the hydrodynamic size for all of the nanoparticles. The size difference between the unmodified and modified nanoparticles demonstrates that carboxymethyl chitosan or chitosan was adsorbed on the PLGA surface [23].

The presence of chitosan or CMCh layer on the nanoparticle surface was caused by the electrostatic interaction between the amine group and the negatively charged surface of the PLGA. This was the dominant interaction in the formation of the first adsorption layer. The adsorption of chitosan or CMCh can continue where hydrogen bonds can be involved in the adsorption. Therefore, the chitosan or CMCh layer can be adsorbed at high concentrations on the first layer. More layers allow the chitosan or CMCh chains to repel

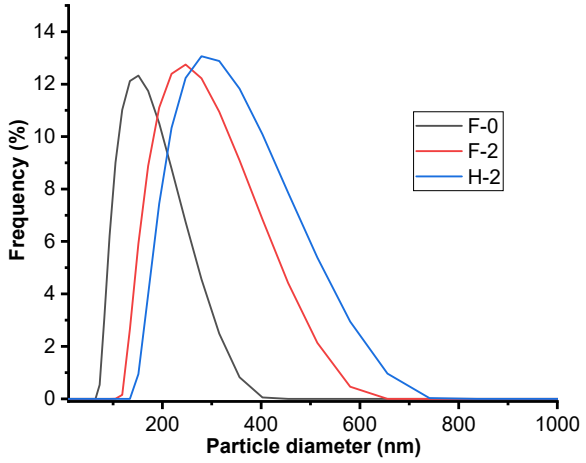

Fig 2. Size distribution of the F-0, F-2, and H-2 nanoparticles determined by dynamic light scattering measurement

Table 3. The hydrodynamic size of nanoparticles

Formulation code	Size ± SD (nm)	Polydispersity ± SD	Zeta Potential ± SD (mV)
F-0	140 ± 4	0.14 ± 0.09	-52 ± 0.7
F-1	173 ± 7	0.26 ± 0.02	
F-2	233 ± 2	0.14 ± 0.07	
F-3	238 ± 30	0.30 ± 0.17	
F-4	197 ± 2	0.23 ± 0.07	-43.4 ± 1.1
H-1	399 ± 38	0.41 ± 0.04	
H-2	267 ± 2	0.18 ± 0.07	
H-3	289 ± 5	0.35 ± 0.09	
H-4	425 ± 5	0.39 ± 0.08	24.3 ± 0.8

because they have the same charge but interact through van der Waals forces and hydrogen bonds [22]. However, in Table 3, the increase in the ratio of chitosan and CMCh to PLGA nanoparticles does not seem to give a significant difference in size increase. The small amount of chitosan or CMCh or the amount of chitosan or CMCh undergoing leaching may be due to non-optimal interactions. It is necessary to prove it by studying the addition of chitosan or CMCh with a more significant amount.

The polydispersity index (PDI) value shows the distribution of suspended particles, where the value is between 0–1. PDI values below 0.5 indicate that the nanoparticles have a homogeneous size distribution and good stability [24]. The PDI resulting from each nanoparticle formula shows good homogeneity. It can be seen from its value which is below 0.5. A large positive or negative value of zeta potential has been shown to prevent agglomeration due to electrostatic repulsion and increase colloidal stability [25-26]. We measured the zeta potential of three different formulas as representatives. It was found that the PLGA-DS nanoparticles have a zeta potential of -52 ± 0.7 mV due to carboxyl end groups on nanoparticle surfaces. The zeta-potential of the CMCh-PLGA-DS (F-4) was slightly less negative (-43.4 ± 1.1 mV) compared to the PLGA-DS nanoparticles because CMCh has an amine group and a carboxyl group, so the amine group may reduce the negative value of the nanoparticle surface, which indicates the CMCh effectively adsorbed onto the PLGA nanoparticle surfaces. In contrast to chitosan-PLGA-DS nanoparticles (H-4), a positive value for zeta potential resulted due to the presence of an amine group, which was 24.3 ± 0.8 mV.

SEM and TEM analysis

TEM measurements were conducted to investigate the morphology and size of the particle. In addition, the surface morphology of the PLGA-DS, CMCh-PLGA-DS, and Chitosan-PLGA-DS nanoparticles was observed through SEM images. Fig. 3 shows the morphology of the nanoparticles discovered to be spherical and have smooth surfaces. SEM images of the nanoparticles after the release of the drug show interparticle fusing. The morphology of

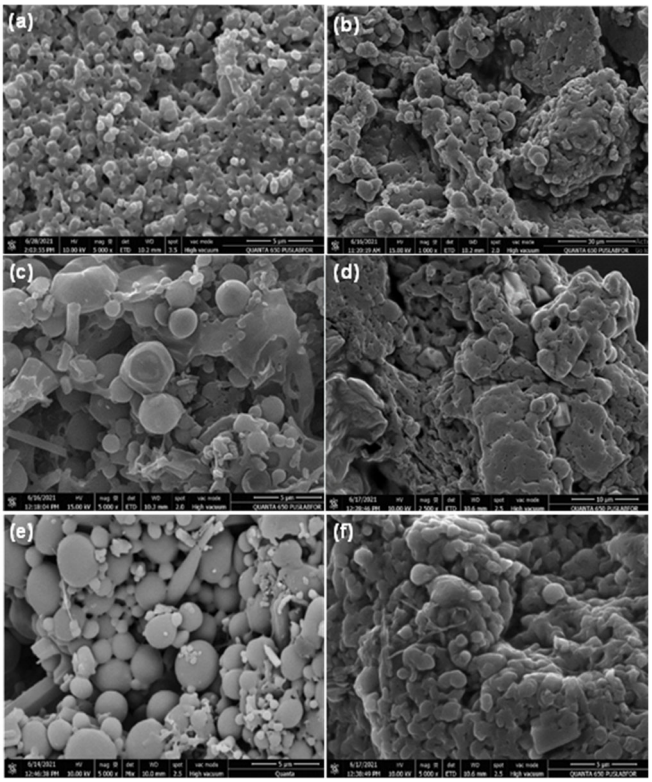

Fig 3. SEM images of nanoparticles: (a) F-0 before release; (b) F-0 after release; (c) F-2 before release; (d) F-2 after release; (e) H-2 before release (f) H-2 after release

the nanoparticles remain, suggesting that most of the release of the drug occurs through diffusion across the polymeric matrix [27].

Nanoparticles coded F-0, F-2, and H-2 were taken as samples to be measured using TEM, where the F-2 and H-2 are nanoparticles with an optimum release profile compared to other formulas. TEM images of the PLGA-DS, CMCh-PLGA-DS, and chitosan-PLGA-DS in Fig. 4 suggested that a layer of CMCh and chitosan was attached to the surface of the nanoparticles due to non-covalent interactions that may occur between CMCh with PLGA. The measurement results with TEM and PSA were only slightly different. The size of nanoparticles of F-2 based on TEM measurements was about 300 nm, and 280 nm for H-2. In general, there are differences in particle size with PSA measurements with TEM particle sizes, which can be caused by differences in the methods used. In contrast to measurements using TEM, which produces a 2D image projected from the

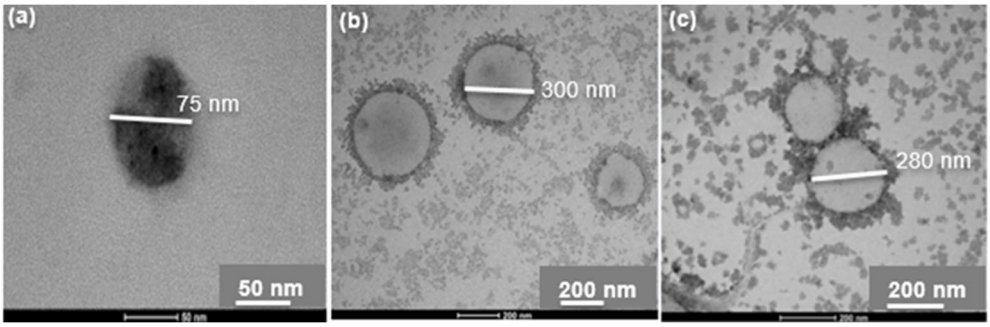

Fig 4. TEM images of nanoparticles: (a) F-0, (b) F-2, (c) H-2

nanoparticles, the dynamic light scattering (DLS) method used in PSA is based on hydrodynamic measurements of nanoparticles that produce a dispersed particle size distribution [28]. The presence of aggregation in the dispersion can encourage the distribution of particles to tend to be larger [29].

The Effect of CMCh and Chitosan Composition on EE and LC

The effect of CMCh and chitosan to PLGA weight ratio on EE of DS encapsulated in the PLGA-CMCh and PLGA-Chitosan matrix is presented in Fig. 5. This study evaluated four different weight ratios of CMCh and chitosan to PLGA. CMCh-modified PLGA (F-1 to F-4) and chitosan-modified PLGA (H-1 to H-4) nanoparticles have an encapsulation efficiency that tends to increase as the composition of CMCh and chitosan increases. As shown in Fig. 5, the EE of the PLGA nanoparticles without modification (F-0) was 59.2%. Meanwhile, the EE values of modified PLGA nanoparticles were higher. The result is higher than the optimal EE in the previous study by Khanal et al., 52% [11]. The interaction of nanoparticles with DS tends to increase with the increasing amount of chitosan and CMCh. Chitosan and CMCh have an amine group that can interact electrostatically with DS and PLGA, retaining DS in the matrix. Therefore, variations in the ratio of chitosan and CMCh to PLGA showed an increase in size, which allowed higher drug entrapment.

As shown in Fig. 6, these results have a similar trend, namely, the increasing composition of CMCh in the matrix enhances the EE and LC. These results, as observed in a previous study by Khanal et al., obtained an optimal LC of 6% [11].

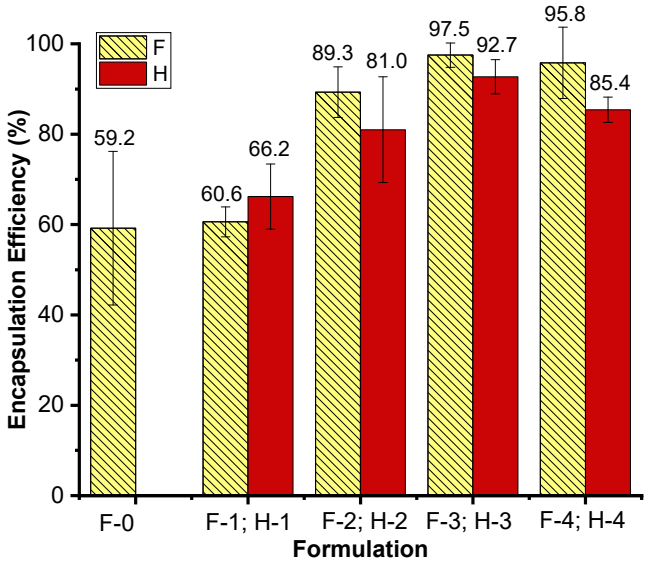

Fig 5. Effects of weight ratio of CMCh and chitosan to PLGA on encapsulation efficiency

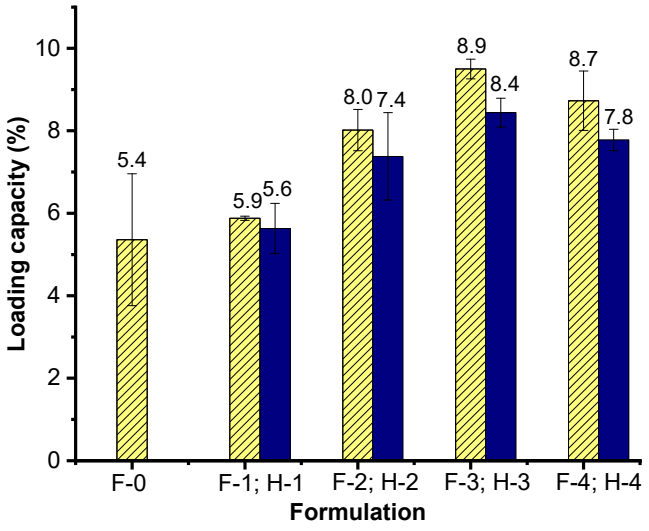

Fig 6. Effects of weight ratio of CMCh and chitosan to PLGA on loading capacity

The Effect of CMCh and Chitosan Composition on Release Drug

In vitro drug release study was performed to evaluate the release of DS from nanoparticles. Several factors influence drug release from nanoparticle-based formulations, including pH, temperature, drug solubility, desorption of surface-bound or adsorbed drugs, drug diffusion, nanoparticle matrix swelling, erosion, and the combination of erosion and diffusion processes [30]. The percentage of cumulative drug release is presented in Fig. 7(a). The results showed that DS was released from the PLGA matrix faster than from the CMCh-PLGA matrix. Approximately more than 70% of the cumulative drug release from CMCh-PLGA nanoparticles occurred in 24 h, whereas for the cumulative drug release from the PLGA matrix, only 49%. The results showed that all formulations had a biphasic release profile, characterized by an initial rapid release and continuous release [31]. As seen in Fig. 7(b), all formulas show resistance to pH 1.2. This is indicated by the low percentage of release at pH 1.2, and the highest release is only about 1.5%. The presence of CMCh was thought to play a role in protecting the surface of the PLGA matrix so that drug release could be reduced.

Compared with PLGA-DS nanoparticles, the cumulative drug release of CMCh-PLGA-DS nanoparticles was higher at pH 6.8. It was estimated that CMCh affects the release of DS in the medium. CMCh is more hydrophilic than PLGA, allowing pH 6.8 medium solution to penetrate the nanoparticle matrix more quickly and release more drug molecules [11]. The increase of CMCh concentration suggested increasing the medium penetration and hydration rate of the CMCh-PLGA matrix. The behavior of CMCh, which has a solubility at neutral pH, allows increasing the release rate at pH 6.8. At neutral pH, the carboxyl group in CMCh was deprotonated, leading to dissolution in the medium. This provides the possibility for hydration of the matrix. These factors may contribute to a higher cumulative drug release from CMCh-PLGA nanoparticles. From all formulas, the composition of CMCh to PLGA in formula F-2 seemed to have the best release profile, which resulted in low drug release at pH 1.2 was 0.2%, and sustained release up to 24 h, which reached 90.9%.

The release profile of chitosan-PLGA-DS had almost the same trend. The release study was low at pH 1.2 and relatively high at pH 6.8 for all formulas, as seen in Fig. 8. The maximum release was 1.3% at pH 1.2, while the lowest was 0.8%. Chitosan can dissolve in acidic media, so at pH 6.8, the contact of chitosan-PLGA-DS with a pH of 1.2 might cause protonation of chitosan, weakening the interaction of chitosan with PLGA to dissolve in the medium and facilitating the diffusion of DS from the matrix. The solubility of DS is known to increase at this pH. The addition of chitosan

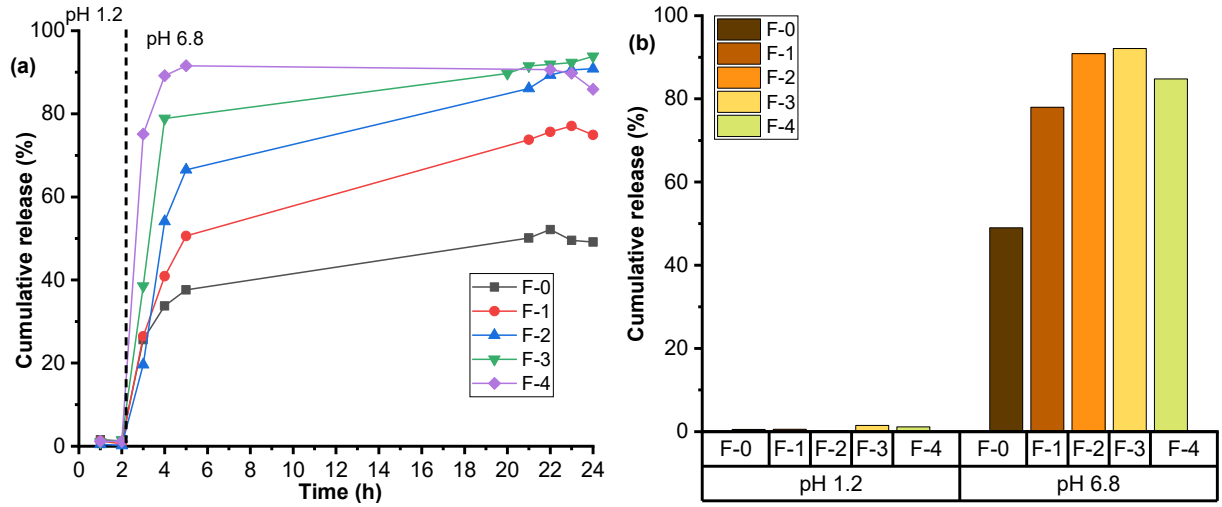

Fig 7. Effect of weight ratio of CMCh to PLGA (a) and different pH media (b) on profile release of diclofenac sodium

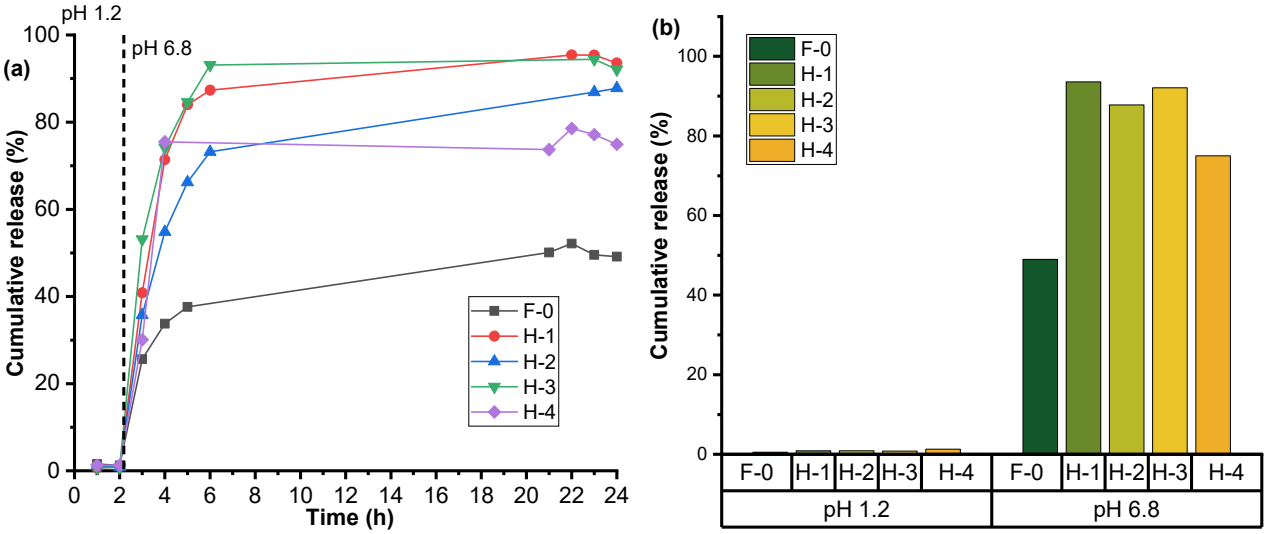

Fig 8. Effect of weight ratio of chitosan to PLGA (a) and different pH media (b) on profile release of diclofenac sodium

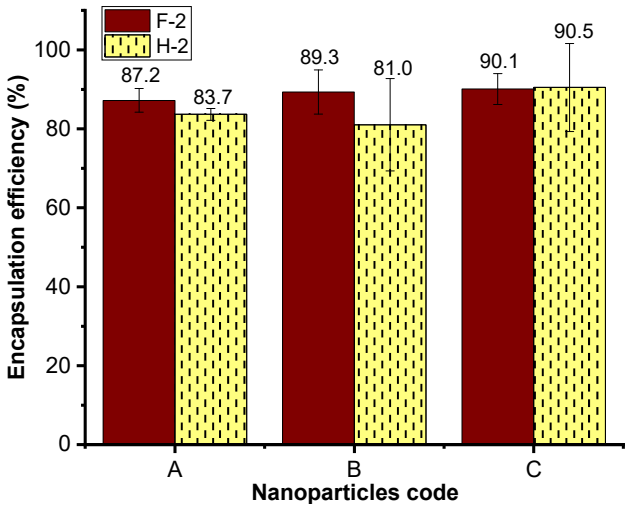

Fig 9. Effect of weight ratio of diclofenac sodium to PLGA on efficiency encapsulation

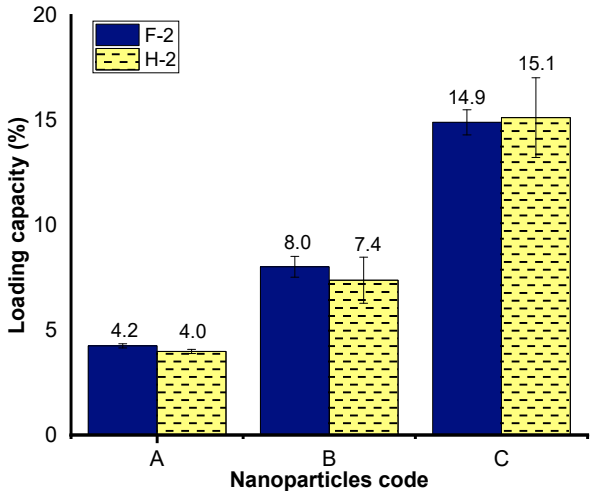

Fig 10. Effect of weight ratio of diclofenac sodium to PLGA on loading capacity

was adequate in this study to withstand the release of DS at low pH.

According to the release profile, the formula F-2 and H-2 were the optimum since they had the lowest release at pH 1.2, followed by a fairly significant release at pH 6.8. This formula is used to optimize the PLGA-DS composition.

The Effect of Diclofenac Sodium Composition on EE and LC

Fig. 9 shows the percentage of encapsulation efficiency as the mass ratio of DS to PLGA varies. All formulas, both CMCh and chitosan-modified, have excellent encapsulation efficiency. So, this suggests that adding DS composition does not seem to influence the percentage of EE.

Fig. 10 illustrates the percentage loading capacity of the optimized DS composition. The higher the DS composition in the matrix, the higher the percentage of LC obtained. The higher percentage of LC reveals that the polymer matrix might contain drugs in a 20:4 ratio for both F-2C and H-2C, enhancing the effectiveness of nanoparticles. Although various nanoparticle systems with various characteristics have been successfully synthesized, the loading capacity of most of the nanoparticle systems is relatively low, generally less than 10%. As an outcome, developing techniques to improve

drug loading is necessary [32].

■ CONCLUSION

A modified PLGA nanoparticle was prepared successfully using carboxymethyl chitosan and chitosan to encapsulate DS. It could improve the loading capacity, efficiency encapsulation, and drug release of nanoparticles with increased size. The formula obtained the best formulation of CMCh-PLGA-DS with the best LC and EE. The minimum drug release at pH 1.2 medium and the maximum drug release at pH 6.8 with a low initial burst release, namely F-2C and H-2C.

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