



Research Article

PREPARATION AND *IN VITRO* DRUG RELEASE OF SODIUM DICLOFENAC NANOPARTICLES USING MEDIUM CHAIN CHITOSAN AND TRIPOLYPHOSPHATE

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ABSTRACT

Diclofenac sodium is a non-steroid anti-inflammatory drug for inflammation, analgesic and antipyretic. To obtain optimal therapeutic effects, diclofenac sodium has several drawbacks, e.g. high adverse effects, undergoes gastrointestinal degradation and first pass metabolism. The aims of this research were to investigate the characterization and the stability of chitosan-loaded sodium diclofenac in dissolution medium. Preparation of diclofenac sodium nanoparticle was to improve the delivery in the nanoparticles form in which medium chain chitosan as polymer and sodium tripolyphosphate as a cross-linker. The characterization of nanoparticle chitosan-loaded sodium diclofenac included morphology, distribution and particle size, zeta potential, entrapment efficiency and yield percentage respectively. The stability test was conducted in simulated gastric fluid (SGF) pH 1.2 ± 0.1 and artificial intestinal fluid (AIF) pH 7.0. The result of the research showed that preparation of chitosan-loaded sodium diclofenac nanoparticles can be made by an ionotropic gelation method using medium chain chitosan and sodium tripolyphosphate as a cross-linker. The obtained nanoparticles size were in the range of 200-500 nm with spherical shape, zeta potential was + 4.66 mV, entrapment efficiency ranged from 95.616 to 97.056 % and the yield percentage ranged from 55.61 to 184.26 %. *In vitro* release tests showed that nanoparticles stability depends on pH and ionic strength of the medium used. Nanoparticle chitosan-loaded diclofenac sodium was stable in simulated gastric fluid (SGF) pH 1.2 ± 0.1 and artificial intestinal fluid (AIF) pH 7.0 respectively.

Keywords: nanoparticles, diclofenac sodium nanoparticles, ionotropic gelation.

INTRODUCTION

Sodium diclofenac is non-steroid anti-inflammatory drugs (NSAIDs) derived from phenyl salicyl acid. It is used to relieve pain and inflammatory in some condition such as musculoskeletal problem and joint such as rheumatoid arthritis, osteoarthritis and ankylosing spondylitis, periarticular problems such as bursitis and tendinitis and other pain condition such as stomach ache, acute gout, dysmenorrhea, migraine and post-surgery procedure. Sodium diclofenac has a higher therapeutic index compared to the others NSAIDs¹. Despite its efficient activity, this NSAID suffers from several drawbacks, mainly a short biological half-life (due to a very rapid metabolism), a high percentage of protein binding and a very high pre-systemic metabolism. This generating the need of using high doses, simultaneously leading to severe dose-limiting side effects (including cardiac, gastrointestinal, hepatic and renal adverse events²). Sodium diclofenac also has some adverse effects such as abdominal distension, abdominal pain, constipation, diarrhea, dyspepsia, flatulence, gastrointestinal (GI) perforation, heartburn, nausea, peptic ulcer/GI bleed, vomiting³. In order to reduce the side effects and improve the therapeutic efficiency, it is formulated based on nanoparticles using polymer⁴. Nanoparticle is polymer particle in which particle size under 1 µm and promise for parenteral, ocular, oral delivery and as adjuvant of vaccines⁵. Nanoparticle can deliver chemicals better into the small units in body; overcome the resistance problem caused by body's physiological barriers related to the pore-size factor; and it is possible to be targeted; so it can reduce toxicity and improve drug distribution efficiency⁶. Chitosan, a natural polysaccharide, is an N-deacetylated derivative of chitin which can be obtained from crustaceans, insects, fungi etc. Several interesting properties of chitosan such as film forming ability,

gelation characteristics, bioadhesion properties and penetration enhancing effects which were explained by opening tight junctions of epithelial cells have been reported⁷⁻⁹. Chitosan is non-toxic, biocompatible and biodegradable cationic polymer, soluble in acid and has positive charge and able to improve trans-cellular and para-cellular as well transport across epithelium¹⁰. Because of its cationic properties, it is expected to interact with negatively charged molecules or polymer¹¹. Chitosan is widely used together with poly anion TPP in various nanoparticle formulation study by ionic gelation method. Ionic gelation method is engaged to the forming of complex by the two oppositely charged structures that then form nanoparticle gel. Ionic gelation is a very simple and easy preparation method¹². Commercially, chitosan is widely available in various type and quality with a different molecular weight, deacetylation degree and viscosity which exhibit a difference in physical-chemical properties¹³. Almost of the functional properties of chitosan depend on chain length, charge density and distribution¹⁴. Based on the explanation above, it would be conducted sodium diclofenac nanoparticle with medium chain chitosan as polymer and sodium tripolyphosphate as cross-linker into sodium diclofenac nanoparticles using ionic gelation to improve the bioavailability, stability to reduce the side effects of sodium diclofenac in gastrointestinal tract.

MATERIAL AND METHOD

Materials used are diclofenac sodium p.a (Sigma Aldrich, USA), medium chain chitosan p.a (Sigma Aldrich, USA), sodium tripolyphosphate (technical grade), hydrochloric acid (Merck, Germany), acetic acid, ethanol (95 %) p.a (Merck, Germany), magnesium chloride, calcium chloride, sodium bicarbonate,

potassium chloride, sodium chloride, sodium hydroxide were purchased from CV INTRACO. Makassar, Indonesia.

Co-polymer preparation of chitosan-loaded diclofenac sodium

This research was conducted experimentally and laboratory-scale. In order to formulate diclofenac sodium nanoparticles using ionotropic gelation method medium chain chitosan as polymer were used in various of concentrations (0.2 %, 0.4 %, 0.6 %, 0.8 % and 1.0 % w/v), and sodium tripolyphosphate (TPP) as cross-linker were (0.5 %, 1.0 %, 1.5 % and 2.0 % w/v) while diclofenac sodium in one concentration 10.0 mg. Chitosan was dissolved in 1 % of acetic acid, sodium diclofenac was dissolved in ethanol and sodium tripolyphosphate was dissolved in purified water. Chitosan and diclofenac sodium solution were stirred at 500 rpm for 10 minutes and then TPP solution added and continued stirring. The nanoparticle was obtained when the solution become opalescent. There were 20 formula produced each in 20.0 mL. Formula A1 to A20 (not shown in this paper).

Scale up and freeze drying

The optimum preparation of sodium diclofenac chitosan produced from co-polymer process were formula A11, A16 and A17 then scaled up into 500.0 mL in volume. Subsequently, the solution was evaporated to remove the ethanol content and was made into powder using freeze drying technique.

Nanoparticle Characterization

Morphology

Observation of nanoparticle morphology was conducted by using Transmission Electron Microscope (TEM) and Scanning Electron Microscope (SEM). Samples of nanoparticle were spilled over copper grid and then by means of auto carbon coated (JOEL JEM, Japan) for 5 seconds, dried out at room temperature for 24 hours. Once the nanoparticle samples were dried, they coated again with carbon as mentioned above then copper grid placed into holder and analyzed by the voltage acceleration on 120 kV and 60.000 magnification.

Distribution and size of nanoparticle

The size and distribution of nanoparticle measured by using Nicomp™ 380 ZLS Submicron Particle Sizer with 90° angle at 23°C. 2 drops of nanoparticle samples at pH 4.0 were added 5.0 mL purified water, mixed by shaking the conical tube contradictorily. Subsequently 3.0 mL of mixture was taken and entered into cuvette and analyzed the size and distribution of nanoparticles.

Entrapment efficiency

The calculation of sodium diclofenac in nanoparticle chitosan conducted by using extraction method. The lyophilized diclofenac sodium-chitosan nanoparticles were dissolved in purified water and Was treated using vortex until the dispersed nanoparticles were formed. The free sodium diclofenac in nanoparticle dispersion was separated from the supernatant and then measured for the absorbance using spectrophotometry at 276 nm wavelength.

$$EE (\%) = \frac{\text{Total diclofenac sodium amount} - \text{free diclofenac sodium}}{\text{total diclofenac sodium amount}} \times 100$$

Yield percentage determination

To determine whether the method for loading the drug into polymer is efficient or not. The raw material, active ingredients, and the other parameter process are a factor to produce the product when the manufacturing process of nanoparticle. The produced results were determined by weighing the nanoparticles and determined the percentage result of added material weight that were the weight of drug and polymer which were used.

$$P.Y (\%) = \frac{\text{Nanoparticle weight}}{\text{Total solids weight (chitosan + TPP + diclofenac sodium)}} \times 100$$

Stability tests of nanoparticles

Simulated gastric fluid (SGF) pH 1.2 ± 0.1 and artificial intestinal fluid (AIF) pH 7.0

As much as 100.0 mg of nanoparticles powders were weighed then conducted the release test of sodium diclofenac using basket method. The dissolution medium used was 900.0 mL of simulated gastric fluid (SGF) pH 1.2 ± 0.1 for 3 hours at 37.5 ± 0.5°C at 50 rpm. As much as 5.0 mL samples were taken at 0, 30, 60, 90, 120, 150 and 180 minutes¹². Samples of 5.0 mL then changed with the same volume of fresh simulated gastric fluid (SGF) pH 1.2 ± 0.1 to maintain the volume constant. After filtration, the released of sodium diclofenac in the samples were measured using spectrophotometer UV-Vis at a wavelength of 266 nm. Every withdrawal of samples conducted for triplication. The same treatment performed with artificial intestinal fluid (AIF) pH 7.0. The withdrawal of samples performed until minutes of 240 and the measurement of free sodium diclofenac conducted at a wavelength of 276 nm.

RESULT AND DISCUSSION

Formulation of diclofenac sodium nanoparticles

The optimum composition of diclofenac sodium-chitosan produced from co-polymer process that were formula A11, A16 and A17 which each ratio of chitosan and TPP concentrations were 0.09:0.075 (A11), 0.09:0.1 (A16) and 0.18:0.1 (A17), then scaled up into 500.0 mL in volume and the residue of ethanol was evaporated to remove ethanol content of solution. The next process was freeze drying to produce nanoparticle powders. Formulation of chitosan nanoparticle-loaded sodium diclofenac based on ionotropic gelation. Chitosan at lower pH possess positive charge due to the amine (NH₂) group of chitosan protonated into NH₃⁺. Subsequently, NH₃⁺ group will interact with negative charge of sodium diclofenac. TPP then added into formula as cross-linker to stabilize the formed binding between chitosan and sodium diclofenac. TPP possess negative charged (polyanion) will interact with chitosan (positively charged polymer) to form nanoparticles.

Characterization of nanoparticles

Morphology of nanoparticles

From the result (Figure 2b), it showed that nanoparticles were spherical form and the particle size ranged in nanosize. The morphology of nanoparticles (Figure 2a) showed the grinding of nanoparticle form larger aggregates. The formation of aggregates possibly caused by the zeta potential value close to neutral.

Determining size and size distribution of particles

The particle size influences the physical and chemical properties of nanoparticles to interact with environment and biological system. The particle size is an important factor affecting the uptake process of nanoparticle in mucosa and epithelium tissues. The measurement of nanoparticle size conducted using Transmission Electron Microscope (TEM) (TESCAN 1400, Germany). From the measurement, it showed that the particles size ranging from 200-500 nm.

The Measurement of nanoparticles surface properties

The zeta potential measurement of chitosan-sodium diclofenac nanoparticles performed by using Nicomp™ 380 ZLS Particle Sizing System (USA). Potential zeta is an electric potential in interface of double layer between medium and stationary layer adhering in dispersed particles¹⁵. The zeta potential produced in this

research was + 4.66 mV (Figure 3) (the showed potential zeta is the most stable formula in the SGF and AIF A16). The value of potential zeta caused by the counter ion effect between positive charge of chitosan and negative charge of sodium diclofenac and TPP. The higher concentration of chitosan would cause the higher density of positive charge so that zeta potential will increase. The higher of chitosan concentration will cause the more group of NH_3^+ interacting with TPP and sodium diclofenac so that it will increase the number of potential zeta of nanoparticles. Entrapment efficiency leads to the capability of polymer to entrap a drug which is stated as the total number of sodium diclofenac entrapped into the matrix. The ratio of chitosan and TPP in formula: A11 (0.09:0.0075); A16 (0.09:0.1) and A17 (0.18:0.1). The results showed that there were influences of chitosan in entrapment efficiency of sodium diclofenac. The lower concentration of chitosan, the higher the entrapment efficiency (Figure 4). This because of a lower concentration of chitosan will produce less viscous solution so that Drug will be easily to diffuse into the matrices of the polymer. Beside the chitosan concentration effect, the concentration of TPP also plays an important role to the entrapment efficiency percentage. It is possibly because the diclofenac sodium in its solution has O^- charge¹⁶. The higher number of TPP needed to stabilize the cross linking will precisely cause the repulsion forces between PO_4^- charge of TPP and O^- charge of diclofenac so that it will weaken interaction strength which has been formed before between chitosan and diclofenac so that it will enable the drug to come out to the continuous phase. It showed that the number of EE percentage, beside it affected by polymer concentration; it is also influenced by charge interaction among the groups of polymer and drug. It agrees with the research of Wu *et al* (2005) and Zhang *et al* (2010) which showed that the higher concentration of chitosan, the lower of the entrapment efficiency^{17,18}. The formation of nanoparticles with the best entrapment efficiency is with a lower concentration of chitosan and TPP.

Yield percentage

The determination of yield percentage aims to determine whether the method used to combine drug into polymer efficient or not. Yield percentage depends on concentration of added chitosan. The result of study showed that method which was used to load drug into polymer was efficient. It was proved by the increasing of yield percentage by the low concentration of polymer and cross-linker (Figure 5).

The binding stability test in simulated gastric fluid (SGF) pH 1.2 ± 0.1 and artificial intestinal fluid (AIF) pH 7.0

Drug release of the nanoparticles exhibit stability behaviour of the particles in the gastrointestinal tract (GIT). The drug release of particulate system based on chitosan depends on morphology, size and density of particulate system, the physicochemical properties of drug and also with the existence of adjuvant. It also depends on pH, polarity and the existence of enzyme in dissolution medium. The drug release of chitosan particulate system involves some different mechanisms that are the release in particle surface, diffusion across the swelling matrix and the release facilitated by polymer¹⁹. The binding stability test of chitosan-sodium diclofenac nanoparticle was conducted by using simulated gastric fluid (SGF) medium pH 1.2 ± 0.1. Physiological salts used were sodium chloride as one of the electrolyte composition in gastric fluid. The results showed that all formula in constant stability (Figure 6). It is possibly because electrostatic interaction undergone between sodium diclofenac and chitosan was strong enough so that there was not sodium diclofenac release significantly in dissolution medium. The drug release mechanism of chitosan-diclofenac sodium nanoparticles in SGF medium is possibly by swelling mechanism. It is because in acid condition, the amino group of chitosan would be protonated so that lead to electrostatic repulsion force between polymer chains, so that one to another would apart away which allow the water molecule easy to penetrate in entire chitosan network. It would lead much amount of water to enter in hydrogel network and facilitate erosion process²⁰. The stability test in artificial intestinal fluid (AIF) was conducted at pH 7. It used physiological salts such as sodium diclofenac, potassium chloride, magnesium chloride, calcium chloride and sodium bicarbonate. The use of physiological salts agrees with composition of electrolyte in intestinal fluid. The release of sodium diclofenac made by ionotropic gelation in artificial intestinal fluid was highly influenced by ionic strength of medium (Figure 7). The release of nanoparticle in artificial intestinal fluid (AIF) medium produced possibly by diffusion of sodium diclofenac across the wall of chitosan polymer. The release of sodium diclofenac from Nanoparticle was possibly caused by the weak electrostatic interaction formed between chitosan, sodium diclofenac and TPP so that the existence of physiological salts would compete with chitosan. The competition would lead chitosan dissociate and release sodium diclofenac. It is because at pH 7.0 NH_3^+ group of chitosan would release H atom to be NH_2 . As a result, sodium diclofenac on the particle surface released into the dissolution medium.

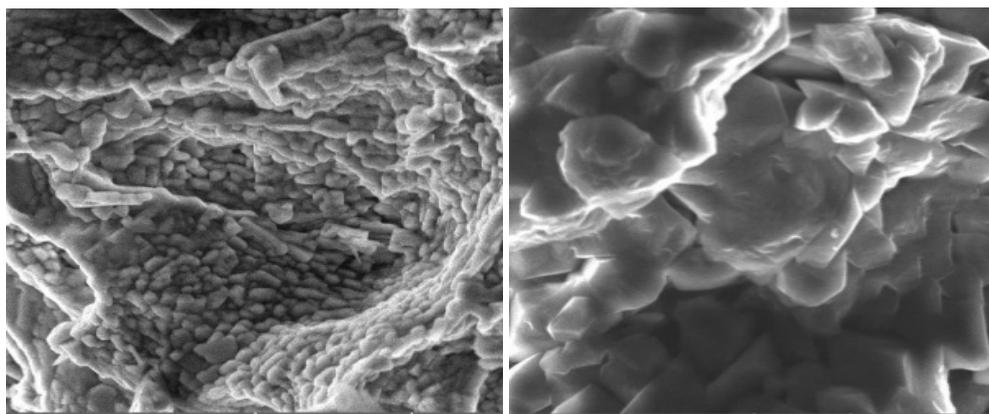


Figure 1: Scanning Electron Microscope (SEM) of chitosan- diclofenac sodium nanoparticles 15 kV voltage, magnification 500x (a) and 100x (b)

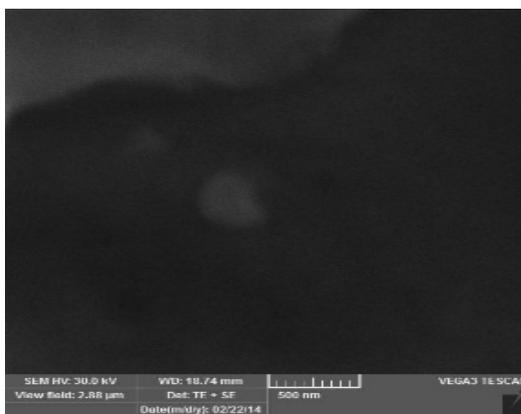


Figure 2: TEM of chitosan- diclofenac sodium nanoparticle, 30 kV voltage, 500000x

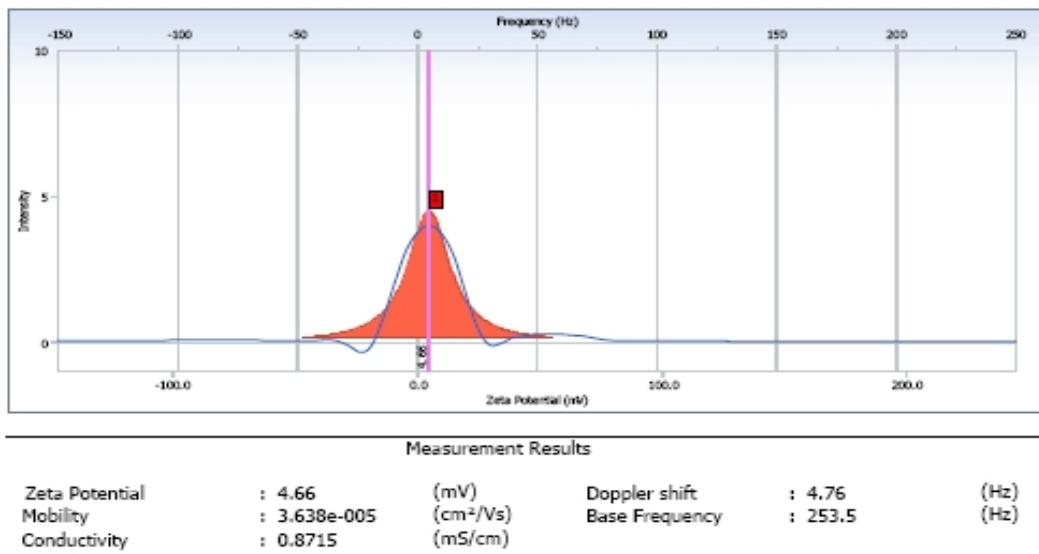


Figure 3: Zeta potential measurement

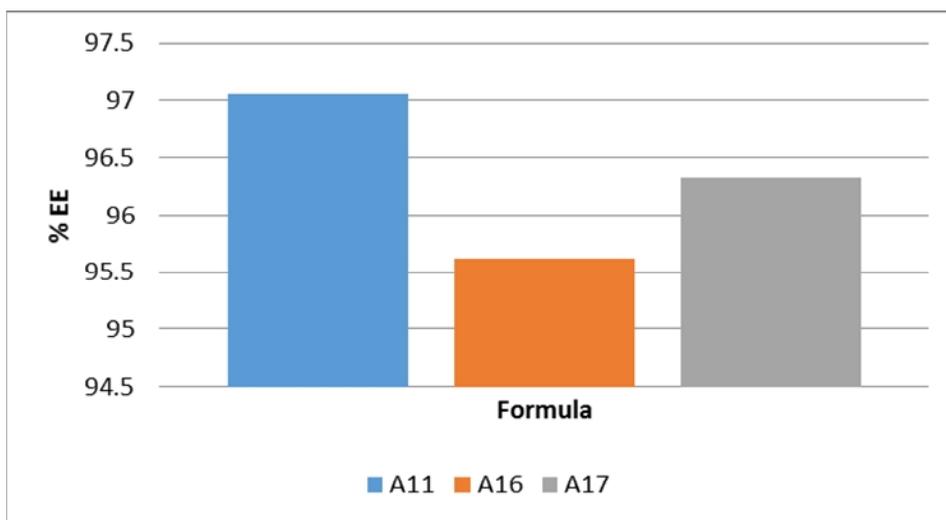


Figure 4: Entrapment efficiency percentage

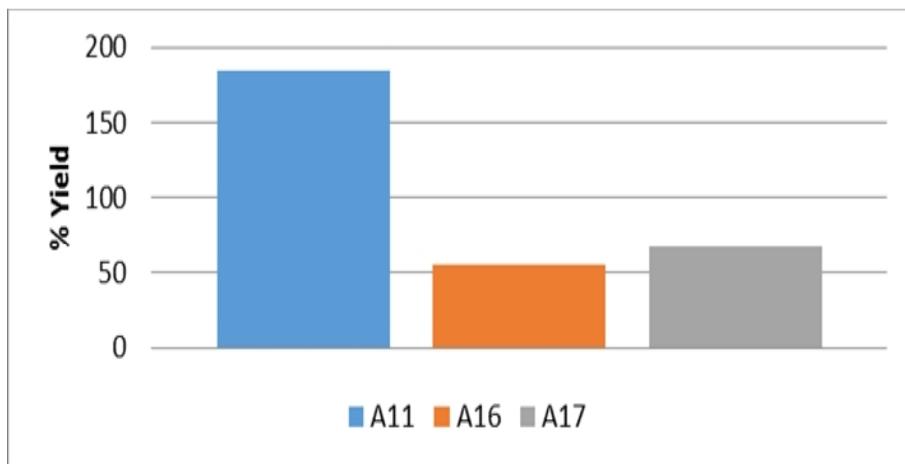


Figure 5: Yield percentage

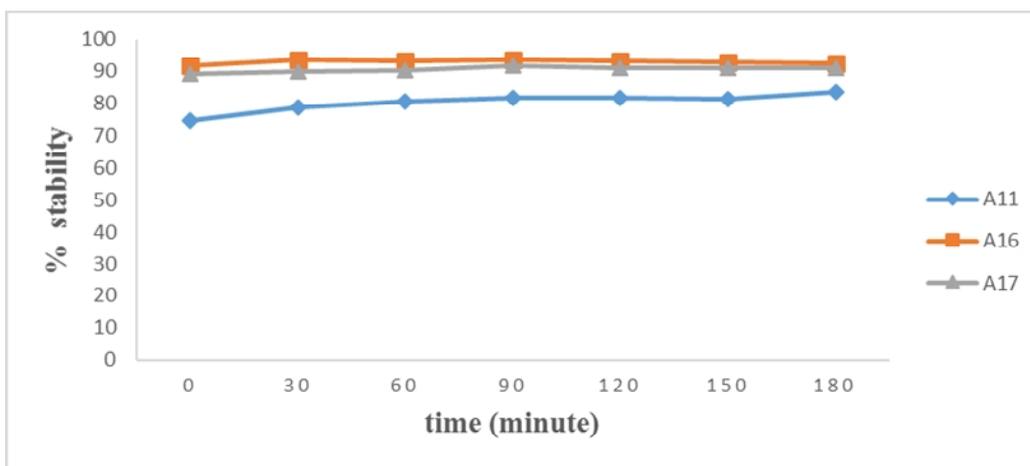


Figure 6: Stability curves of diclofenac sodium in simulated gastric fluid (SGF) pH 1.2 ± 0.1

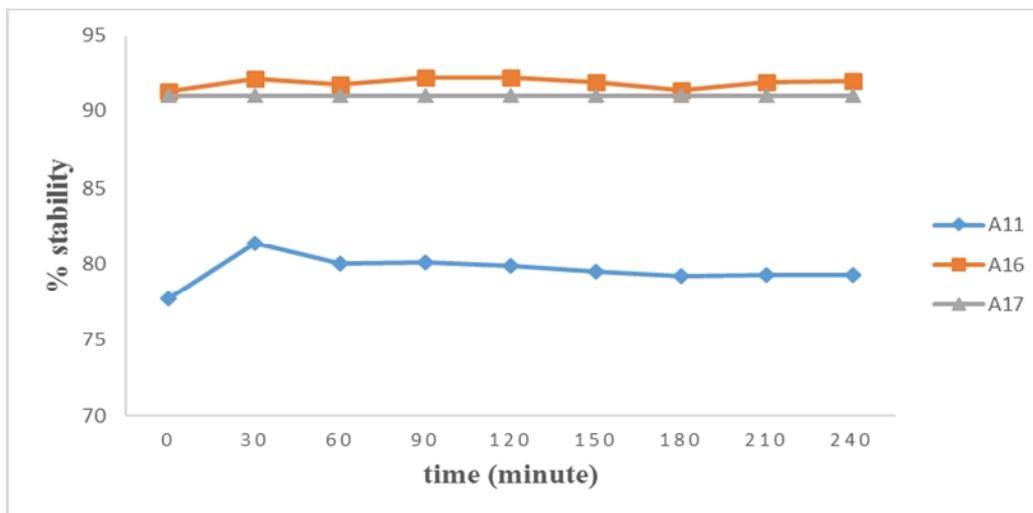


Figure 7: Stability curves of diclofenac sodium in artificial intestinal fluid (AIF) pH 7.0

CONCLUSION

Based on the result of study, it can be concluded that sodium diclofenac can be formulated into nanoparticle using medium chain chitosan and sodium tripolyphosphate as cross-linker by ionotropic gelation with yield percentage 55.61-184.26 %, potential zeta + 4.66 mV, entrapment efficiency ranging 95.616-97.056 %, particle size 200-500 nm and spherical in shape. The *in vitro* stability test showed that sodium diclofenac nanoparticles were stable in simulated gastric fluid (SGF) pH 1.2 ± 0.1 and artificial intestinal fluid (AIF) pH 7.0.

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