ABSTRACT

Objective: The objectives of this study were to formulate and characterize nanoparticles gel of timolol maleate (TM) by ionic gelation method using chitosan (CS) and sodium alginate (SA).

Methods: Optimization was carried out by factorial design using Design Expert®10.0.1 software to obtain the concentration of CS, SA, and calcium chloride (CaCl₂) to produce the optimum formula of TM nanoparticles. The optimum formula was characterized for particle size, polydispersity index, entrapment efficiency, Zeta potential, and molecular structure. Hydroxy Propyl Methyl Cellulose (HPMC) K15 was incorporated into optimum formula to form nanoparticles gel of TM and carried out in vivo release study using the Franz Diffusion Cell.

Results: TM nanoparticles was successfully prepared with concentration of CS, SA, and CaCl₂ of 0.01 % (w/v), 0.1 % (w/v), and 0.25 % (w/v), respectively. The particle size, polydispersity index, entrapment efficiency, and Zeta potential were found to be 200.47±4.20 nm, 0.27±0.0154, respectively. The result of FTIR spectra indicated TM-loaded in the nanoparticles system. In vitro release profile of TM-loaded nanoparticles gel showed controlled release and the Korsmeyer-Peppas model was found to be the best fit for drug release kinetics.

Conclusion: TM-loaded CS/SA nanoparticles gel was successfully prepared and could be considered as a promising candidate for controlled TM delivery of infantile hemangioma treatment.

Keywords: Timolol maleate, Nanoparticles, Infantile hemangioma

INTRODUCTION

Infantile hemangioma (IH) is the most prevalent vascular benign tumor in children with an incidence of about 4 to 10 % in the first year of life [1]. Although it will improve spontaneously, about 10 to 20 % of infants who have IH require interventions to prevent and reduce complications associated with the proliferation phase [2]. In addition, IH has implications for parental psychology and visual disfigurement of children [3]. IH can occur in any part of the body, but the most often found in the head and neck area so that it can trigger deformity [4]. Topical TM is a promising alternative treatment for IH. The use of topical TM for IH treatment was first reported in 2010. Since then, many case reports and case series have demonstrated the efficacy of TM for the treatment of IH. TM can inhibit growth and promote the regression of IH [5]. However, TM has systemic absorption due to it is administered topically and then result in unwanted effects [6].

The therapeutic effectiveness of topical dermatology formulation can be improved by nanotechnology-based delivery systems. Nanoparticles are of great interest in drug delivery due to their comparable size of the component in the human cell. Size matching is important in carrying out any activities in the biological system [7]. The successful implementation of nanoparticles for drug delivery depends on their ability to penetrate through several anatomical barriers, sustained release of their contents, and their stability in the nanometer size [8]. Polymeric nanoparticles are solid colloidal particles with a diameter ranging from 1 nm to 1000 nm. It is developed by using biodegradable and biocompatible polymers offer interesting options for controlled drug delivery and drug targeting [9].

Biopolymers are polymer molecules that have been used extensively as biomaterials in drug delivery systems. The use of biopolymers has several advantages due to their properties, such as biocompatible, non-toxic, non-irritant, and can form the network of particles matrix. The preparation of biopolymers system uses double polymer which has opposite charges to form a matrix, so it can trap drug molecules. CS and SA are great combinations of biopolymers that can be used in drug delivery system [10]. Biopolymeric nanoparticles of CS and SA can deliver active ingredients with controlled drug release profile. Therefore, it can increase the efficacy of therapy, reduce systemic drug absorption, and reduce the frequency of administration [11].

CS/SA nanoparticles can be prepared by ionic gelation methods using ions of calcium. Mechanism of interaction is based on the negative charge of the uronic acid carboxylic groups of SA with the positive surface charge of the protonated amino groups of CS. The formation of CS/SA nanoparticles by the ionic gelation method produces a pre-gel that consists of very small aggregates of gel particles, which are then followed by the addition of a polycationic solution to form the polyelectrolyte complex. CS/SA nanoparticles can protect drug encapsulation from enzymatic degradation, drug delivery to target organs, prolong contact time of active ingredients with target epithelial cells, and control drug release [12].

MATERIALS AND METHODS

Materials

TM was purchased from Octagon Chemical Limited (China). CS (molecular weight: 200 kDa to 500 kDa with 94.88 % of deacetylation degree) was purchased from local company Chimultiguna (Cirebon, Indonesia), SA was purchased from Sigma Aldrich (Darmstadt, Germany), CaCl₂ was purchased from Merck (Germany), glacial acetic acid, hydrochloric acid, and sodium hydroxide were purchased from Merck (New Jersey, USA).

Optimization of TM nanoparticles

Optimization of TM nanoparticles was prepared based on the 2ⁿ factorial design to select independent variables that were needed to
produce the optimum formula of TM nanoparticles. The selected three independent variables were the concentration percentage of CS, SA, and CaCl₂.

Characterization of TM nanoparticles

Particle size, polydispersity index, and Zeta potential

Particle size, polydispersity index, and Zeta potential were analysed by the method of dynamic light scattering (DLS). The TM nanoparticles were examined using Zetasizer Nano ZS (Malvern, UK) to determine the average of particle size and polydispersity index (distribution of particles). A certain amount of TM nanoparticles sample was dispersed in 5 ml of aquadest and then placed in disposable cuvette. Zeta potential of TM nanoparticles was also examined using Zetasizer Nano ZS (Malvern, UK) to determine surface charge of particles. A certain amount of TM nanoparticles was placed in dip cell carefully. Replications were done for each measurement of the same samples for 3 times. This instrument was controlled with Malvern software [13].

Entrapment efficiency

Entrapment efficiency (EE) of TM nanoparticles was measured indirectly. Samples of TM nanoparticles were centrifuged using Refrigerated Centrifuge (Velocity 1R8) at 15,000 rpm for 30 min at 4 °C to separate unloaded nanoparticles. The supernatant (unloaded nanoparticles) was analyzed using UV-Vis spectrophotometer (Thermo Scientific Genesys 10S UV) at 296 nm. The following equation was used to calculate entrapment efficiency (EE) [12].

\[
EE (\%) = \frac{\text{total amount of TM—unloaded nanoparticle of TM in supernatant}}{\text{total amount of TM}} \times 100
\]

RESULT AND DISCUSSION

Particle size, polydispersity index, and entrapment efficiency

Table 1: The result of an average of particle size (nm), polydispersity index, and entrapment efficiency (%) as the response of full factorial design experimental (n=8)

| Formulation codes | Independent variables | Responses | Polydispersity index | Entrapment efficiency (%) |
|-------------------|-----------------------|-----------|----------------------|---------------------------|
|                   | CS | SA | CaCl₂ | Particle diameter (nm) |                        |                        |
| F1                | -1 | -1 | -1    | 473.10±75.86         | 0.37±0.0392          | 35.12±4.73           |
| F2                | +1 | -1 | -1    | 676.33±107.76        | 0.51±0.0489          | 33.20±7.16           |
| F3                | -1 | +1 | -1    | 489.30±15.14         | 0.51±0.0934          | 39.01±2.86           |
| F4                | +1 | +1 | -1    | 350.00±64.88         | 0.66±0.0345          | 37.59±3.86           |
| F5                | -1 | -1 | +1    | 200.47±4.20          | 0.27±0.0154          | 35.23±4.55           |
| F6                | +1 | -1 | +1    | 589.23±9.93          | 0.45±0.0326          | 33.71±3.99           |
| F7                | -1 | +1 | +1    | 447.83±100.54        | 0.49±0.0965          | 35.63±4.77           |
| F8                | +1 | +1 | +1    | 791.10±15.59         | 0.53±0.0349          | 39.77±3.01           |

*Data from each response is presented in mean±SD (n=3). CS (-1 = 0.01 % w/v; +1 = 0.1 % w/v), SA (-1 = 0.1 % w/v; +1 = 0.5 % w/v), CaCl₂ (-1 = 0.05 % w/v; +1 = 0.25 % w/v)

The particle size is a critical factor in the preparation of nanoparticles. The particle mean diameter obtained for all formulations were in range 200.47±4.20 nm to 791.10±15.59 nm (Table 1). At the low concentration of CS (0.01 % w/v), SA (0.1 % w/v), and CaCl₂ (0.05 % w/v), particles diameter were obtained in range 200.47±4.20 nm to 489.30±15.14, 200.47±4.20 nm to 676.33±107.76 nm, and 350±64.88 nm to 676.33±107.76 nm, respectively. Meanwhile, at the high concentration of CS (0.1 % w/v), SA (0.5 % w/v), and CaCl₂ (0.25 % w/v) particles diameter were obtained in range 350±64.88 nm to 791.10±15.59 nm, 350±64.88 nm to 791.10±15.59 nm, and 200.47±4.20 nm to 791.10±15.59 nm, respectively. Based on the polynomial term of full factorial design experimental and contour plot that was showed in fig. 1 predicted effect of each independent variables to particle size. The greater concentration of CS, SA, and CaCl₂ was used in the formula; the greater particle size of nanoparticles was obtained. The main factor in nanoparticle formation is the tendency of functional groups of SA chains, especially carboxylic groups could form complex structures with calcium ions. These findings are in accordance with the previously study [15]. Daemi and co-worker reported that the high concentration of SA caused more functional groups to gather around calcium ions, then the more SA chains binded to form complexes with calcium cations. Thus, the more concentration of SA used in the formula the larger size of particle produced. Meanwhile, increasing the concentration of calcium ions caused less amount of polymer chains involved with a greater content of calcium cations. So that, increasing of CaCl₂ led the smaller size of particle produced. Manish and co-workers were also reported that increasing the concentration of SA and Ca increased particle size and ultimately decreased stability of nanoparticles due to aggregation and precipitation [16]. Polydispersity index describes the uniformity of particles size distribution produced. The smaller the polydispersity index obtained
The more uniform the particle size would be. Uniformity of the particle size distribution could affect the properties of particles produced due to it would not be easier to settle [17]. The polydispersity index value<0.5 indicates a narrow size distribution of particles. Meanwhile, polydispersity index value>0.5 indicates a broad particle size distribution of particles. Increase of polydispersity index could lead aggregation due to instable system [18].

The polydispersity index obtained for all formulations was in range 0.27±0.0154 to 0.66±0.0345 (table 1). At the low concentration of CS (0.01 % w/v), SA (0.1 % w/v), and CaCl2 (0.05 % w/v) polydispersity index of nanoparticles were obtained in range 0.27±0.0154 to 0.51±0.0934, 0.27±0.0154 to 0.51±0.0489, and 0.37±0.0582 to 0.66±0.0345, respectively. Meanwhile, at the high concentration of CS (0.1 % w/v), SA (0.5 % w/v), and CaCl2 (0.25 % w/v) polydispersity index of nanoparticles were obtained in range 0.45±0.0526 to 0.66±0.0345, 0.49±0.0965 to 0.66±0.0345, and 0.2±0.0154 to 0.53±0.0349, respectively. Based on polynomial term of full factorial design experimental and contour plot that was showed in fig. 2 predicted the effect of each independent variables to polydispersity index. The greater concentration of CS and SA were used in the formula the greater polydispersity index of nanoparticles was obtained. Meanwhile, the greater concentration of CaCl2 the smaller polydispersity index was obtained.

Entrapment efficiency is an important parameter in the overview of the effectiveness of active binding compounds by polymer complexes in the nanoparticles system [19]. The percentage of entrapment efficiency obtained for all formulations was in range 33.20±7.16 % to 39.77±3.01 % (table 1). At the low concentration of CS (0.01 % w/v), SA (0.1 % w/v), and CaCl2 (0.25 % w/v) percentage of entrapment efficiency of nanoparticles were obtained in range 33.71±4.73 % to 39.01±2.86 %, 33.20±7.16 % to 35.23±4.55 %, and 33.20±7.16 % to 39.01±2.86 %, respectively. Meanwhile, at the high concentration of CS (0.1 % w/v), SA (0.5 % w/v), and CaCl2 (0.25 % w/v) percentage of entrapment efficiency of nanoparticles were obtained in range 33.20±7.16 % to 39.77±3.01 %, 35.63±4.77 % to 39.77±3.01 %, and 33.71±3.99 % to 39.77±3.01 %, respectively. Based on polynomial term of full factorial design experimental and contour plot that was showed in fig. 3 that predicted effect of each independent variables to percentage of entrapment efficiency. The greater concentration of CS, SA, and CaCl2 was used in the formula the greater entrapment efficiency of nanoparticles was obtained. It is clear that increase in polymer concentration lead increases the drug entrapment efficiency [20].

The particle size, polydispersity index (PDI), and entrapment efficiency obtained through optimization of 8 formulas were used to generate the optimum formula of nanoparticles. Based on prediction of factorial design as a statistical tool, Formula 5 was selected as the optimum formula with concentration of CS, SA, and CaCl2: 0.01 % (w/v), 0.1 % (w/v), and 0.25 % (w/v), respectively.
percentages of SA revealed a negatively charged Zeta potential. Morsi and co-workers reported that the nanoparticles with small value of Zeta potential (-3.7 mV) despite having higher SA content and this probably because of the amounts of CS and CaCl₂ were still insufficient to interact with all carboxylic groups of SA. However, increasing the concentration of CaCl₂ or increasing the concentration of CS shifted the Zeta potential to positive values. These findings were also in accordance with the previously reported data [23].

Huang and co-workers reported that the negative value for Zeta potential of TM liquid crystalline nanoparticle.

**FTIR analysis**

The FTIR spectra could be used to get information about molecular structure from the vibration of functional groups at the certain wavenumber [24]. The FTIR spectra of TM nanoparticles were showed in fig. 5.

The FTIR spectra of TM nanoparticles in fig 5 indicate the interaction among nanoparticles components. CS indicates a broad band appearing at 3354 cm⁻¹ due to C-H stretching vibrations. The band for C≡C stretching vibrations appears at 2113.39 cm⁻¹. The peak at 1645 cm⁻¹ is due to C=N stretching vibrations. The peak at 1419.57 cm⁻¹ is due to O-H bending vibrations. The C-O stretching vibrations appears at wavenumber 1149.91 cm⁻¹, 1061.63 cm⁻¹, and 1025.46 cm⁻¹, respectively. The peak at 894.14 cm⁻¹ is due to C-H bending vibrations. Meanwhile, SA indicates a broad band appearing at 3357 cm⁻¹ due to O-H stretching vibrations. The band for C≡C stretching vibrations appears at 2099.38 cm⁻¹. The peak at 1597.67 cm⁻¹ is due to C=C stretching vibrations. The peak at 1409.45 cm⁻¹ appears due to O-H bending vibrations. The peaks indicate the C-O stretching vibrations at wavenumber 1292.88 cm⁻¹, 1262.02 cm⁻¹, and 1197.37 cm⁻¹, respectively. The C=C bending vibrations appears at wavenumber 898.17 cm⁻¹, 890.02 cm⁻¹, and 806.50 cm⁻¹, respectively.

After loading TM in the nanoparticles, there is broadband at wavenumber 3354.92 cm⁻¹ due to O-H stretching vibrations. The band for C≡C stretching vibrations appears at wavenumber 2106.60 cm⁻¹. Both of these peaks are similar to the peak of CS and SA. There is a sharp peak at wavenumber 1603.44 cm⁻¹ due to C=N stretching vibrations. The peaks at 1416.91 cm⁻¹ due to O-H bending vibrations. The peaks indicate the C-O stretching vibrations at wavenumber 1076.31 cm⁻¹ and 1026.10 cm⁻¹, respectively. These peaks are also similar to the peak of CS and SA. There are peaks at wavenumber 888.67 cm⁻¹ and 817.51 cm⁻¹ due to C=C bending vibrations. These peaks are similar to peak of TM. These results confirmed with the characterization of nanoparticles component, so that indicated TM loaded in the nanoparticles system.
Ionic interaction involved an ionic cross-linking between cations on the backbone of CS and anion of SA [25]. CaCl$_2$ was used as stabilizer, which contributed to the formation of the particle core. Although SA can be complex with CS alone and can form nanoparticles through formation of a simple polyelectrolyte complex, the formation of a pre-gel phase between Ca$^{2+}$ions and SA allows the formation of nanoparticles with increased compact structure [22]. Ionic interaction of TM, CS, SA, and CaCl$_2$ was showed in fig. 6.

In vitro release study

The release profile of TM from nanoparticles gel in PBS was showed in fig. 7. The release profile exhibited a controlled release phase lasting for long period. The controlled release phase may be attributed to the swelling or degradation of the polymer, which leads to the formation of pores within the polymeric matrix [26]. This result was also in accordance with the previously study that reported drug release was sustained for a long period which may be due to the hydration capability of CS which on coming in contact with dissolution medium result to the formation of gelatinous mass that act as a retardant material for the drug to get diffused out [27]. The data obtained from in vitro release study of TM from CS/SA nanoparticles gel was further analysed with different kinetic models (zero orde, first orde, Higuchi, Korsmeyer-Peppas, and Hixson-Crowell). Hence, the Korsmeyer-Peppas model was found to be the best fit for drug release kinetics. Korsmeyer-Peppas is a mathematical model that describes drug release from polymeric system [28]. The finding was also in accordance with the previously study [29, 30]. These studies reported that Korsmeyer-Peppas is the best fit of drug release from nanoparticle polymeric system. Mathematical Model in TM Release Kinetics was showed in table 2.
CONCLUSION

We have prepared successfully TM-loaded nanoparticles gel by ionic gelation using biopolymer CS and SA. The best formula was prepared with concentration of CS, SA, and CaCl2 of 0.01 % w/v, 0.1 % w/v, and 0.25 % w/v, respectively. The particle size, polydispersity index, entrapment efficiency, and Zeta potential were found to be 200.47±4.20 nm, 0.27±0.0154, 35.23±4.55 %, and-5.68 ±1.80 mV, respectively. The result of FTIR spectra indicated TM loaded in the nanoparticles system. In vitro release profile of TM-loaded nanoparticles gel showed controlled release and Korsmeyer-Peppas model was found to be the best fit for drug release kinetics. Finally, we concluded that TM-loaded CS/SA nanoparticles gel can be considered as a promising candidate for controlled TM delivery of IH treatment.

ACKNOWLEDGMENT

Author thanks to The Ministry of Research, Technology, and Higher Education of the Republic of Indonesia for its grant so that this research can be done.

AUTHORS CONTRIBUTIONS

RA compiled data dan prepared manuscript, LN, RM and RD designed research, analysed data, and made critical thinking on the manuscript.

CONFLICT OF INTERESTS

The author declares that there is no conflict of interest.

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