Original Article

Development of Nanoemulsion-based Hydrogel Containing Andrographolide: Physical Properties and Stability Evaluation

Oktavia Indrati1,2, Ronny Martien2, Abdul Rohman3, Akhmad Kharis Nugroho2

1Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Islam Indonesia, Sleman, Yogyakarta, Indonesia, 2Department of Pharmaceutics, Faculty of Pharmacy, Universitas Gadjah Mada, Sekip Utara, Yogyakarta, Indonesia, 3Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada, Sekip Utara, Yogyakarta, Indonesia

Introduction: Andrographolide is a compound that shows various pharmacological activities, which can be applied topically or orally. Nanoemulsion can improve drug solubility and stability, but has limitations for topical application. Incorporation of nanoemulsion into hydrogel can increase the viscosity of the system which can prolong the drug residence time. The aim of this study was to develop andrographolide nanoemulsion-based hydrogel for topical application.

Method: Andrographolide nanoemulsion was prepared using Capryol 90 as the oil, Kolliphor RH 40 as the surfactant, and propylene glycol as the cosurfactant. Droplet size and polydispersity index of the nanoemulsions were evaluated using particle size analyzer. D-optimal mixture design was employed to generate the total number of runs (formulation), and obtain the optimum formulation. Fourteen formulations of nanoemulsion-based hydrogel were prepared by incorporating nanoemulsion into the hydrogel base (1:1). Carbopol was employed as the gelling agent, whereas other excipients including propylene glycol, oleic acid, triethanolamine, methylparaben, and propylparaben were also added to produce hydrogel base. Nanoemulsion-based hydrogel was evaluated for its pH, viscosity, and physical appearance (after 8 weeks of storage).

Results: The result revealed that nanoemulsion-based hydrogel containing 34.65% of carbopol, 1.35% of triethanolamine, and 9% of propylene glycol was selected as an optimum formulation which shows acceptable pH, viscosity, and physical appearance. This optimum nanoemulsion-based hydrogel has pH of 6.50 ± 0.02, and 2492.33 ± 36.91 cP of viscosity with milky white color, and smooth homogeneous texture.

Conclusion: This study suggested that andrographolide can be successfully formulated into an acceptable nanoemulsion-based hydrogel.

Keywords: Andrographolide, D-optimal mixture design, hydrogel, nanoemulsion

Submitted : 13-Feb-2020
Revised : 10-May-2020
Accepted : 11-Jun-2020
Published : 05-Nov-2020

INTRODUCTION

In the past decades, nanotechnology has become a trend in the development of a drug delivery system. Nanoemulsion is an isotropic system based on nanotechnology that comprises of oil, surfactant, cosurfactant, and water. This system offers various advantages such as ease of preparation, good stability, and enhancement of drug solubility and bioavailability. However, nanoemulsion has a low viscosity that becomes its limitation for topical application. This drawback can be overcome by incorporating nanoemulsion into a hydrogel resulting in the increasing of viscosity and prolonging of drug residence time. This unique system is well known as nanoemulsion-based hydrogel or nanoemulgel.

Address for correspondence: Dr. Akhmad Kharis Nugroho, M.Si, Apt, Faculty of Pharmacy, Universitas Gadjah Mada, Sekip Utara, Yogyakarta 55281, Indonesia.
E-mail: a.k.nugroho@ugm.ac.id
Andrographis paniculata is a medicinal plant widely distributed in South East Asian countries, China, and India and has been used empirically to cure diarrhea, cough, ulcer, fever, and inflammation.[6,7] The active substance, andrographolide, was reported to have vast pharmacological activities including anti-inflammatory, and antioxidative activity.[6,8] Andrographolide has a bitter taste and poor aqueous solubility which is a challenge for drug formulations.[9,10] To increase the acceptability of the active substance for topical use, the nanoemulsion-based hydrogel was selected as a drug delivery system for andrographolide. Several publications on andrographolide formulation are readily available including andrographolide in self-nanoemulsifying drug delivery,[11] and solid self-nanodispersion,[12] but so far there is no report about nanoemulsion-based hydrogel containing andrographolide. D-optimal design was successfully employed in the formulation of nanosuspension,[13] and emulsion.[14] Hence, this study aimed to develop andrographolide nanoemulsion-based hydrogel using D-optimal mixture design.

**Materials and Methods**

Andrographolide was purchased from Sinobright Pharmaceutical (Guangdong, China). Capryol 90 was bought from Gattefosse (Saint-Priest, France), Kolliphor RH 40 was purchased from BASF Indonesia (Jakarta, Indonesia), whereas carbopol, triethanolamine, propylene glycol (PG), oleic acid, propylparaben, and methylparaben were obtained from Brataco (Yogyakarta, Indonesia).

**Nanoemulsion preparation**

Andrographolide (5 mg) was dissolved in Capryol 90 as the oil component using an ultrasonic probe. Kolliphor RH 40 as the surfactant and PG as the cosurfactant were then added to form preconcentrate nanoemulsion. This preparation will be used in further evaluation.

**Particle size and polydispersity index determination**

Preconcentrate nanoemulsion was diluted to 100 times, droplet size and polydispersity index were then determined using the particle size analyzer (Horiba SZ100, Japan). The measurement was done in triplicate.

**Nanoemulsion-based hydrogel preparation**

D-optimal mixture experimental design containing 14 runs (formulation) was generated by Design Expert software version 10 (Stat-Ease Inc., Minneapolis, USA). D-optimal mixture design is a flexible design that enables the researcher to optimize based on customs model and constrained or unequal components range. In this study, the concentrations of carbopol, triethanolamine (TEA), and PG were selected as the independent variables, whereas viscosity (Y1), pH (Y2), and stability (Y3) were chosen as the dependent variables. The lower and upper limits of each independent variables were selected as follows: carbopol as X1 (57%–79%), TEA as X2 (1%–3%), and PG as X3 (20%–40%). The software generates 14 runs that consist of 6 required model points (quadratic model), 5 lack of fit points, and 3 replicate points. In the studied experimental design, the total concentration of carbopol, TEA, and PG are 100% but in the actual formulation of nanoemulsion-based hydrogel, the total concentration of the three of them is 45% (the other 65% are water and other excipients).

Carbopol solution (1%) was prepared by dissolving 1g carbopol into 100mL of distilled water under magnetic stirring and it was then kept overnight to swell. Andrographolide nanoemulsion was mixed with carbopol solution and other excipients (oleic acid, methylparaben, and propylparaben). Lastly, TEA was added to the mixture, whereas stirred using magnetic stirrer. Oleic acid was added as the penetration enhancer, whereas methyl and propylparaben as the preservative.

**pH determination**

Determination of pH values was performed using pH meter (Horiba, Japan) which were done in triplicate.

**Viscosity determination**

The viscosity of nanoemulsion-based hydrogels (0.5 g) was measured using cone and plate viscometer, spindle 52 at 50 rpm and room temperature (Brookfield, Middleborough, USA).

**Stability determination**

The developed formulation was subjected to 8-week storage at room temperature. Any changes in physical appearance including homogeneity and phase separation were observed. The results were recorded and assessed carefully. A score of 1 was given to the formulation that becomes inhomogenous and undergoes phase separation after storage. Meanwhile, a score of 2 is given for hydrogel that is still homogenous and does not undergo phase separation.

**Results**

Droplet size is an important parameter for assessing nanoemulsion. It is found that the droplet size of the nanoemulsion was 56.5 ± 1.92 nm; meanwhile, the polydispersity index was 0.455 ± 0.01. This nanoemulsion was incorporated with hydrogel and other excipients and then evaluated. The organoleptic evaluation showed that the nanoemulsion-based hydrogels have milky white color, distinctive aroma,
and smooth homogeneous texture. The composition of nanoemulsion-based hydrogels and the result of the evaluation are presented in Table 1. On the stability evaluation, “1” indicates that the formulation was unstable; meanwhile, “2” implies that the formulation was stable during storage.

It is found that the model F-value of pH, viscosity, and stability were 80.23, 72.70, and 16.58, respectively, which implied that the model is significant for each variable. For pH variable, the coefficient values were +5.63 (X1), +14.98 (X2), and +5.92 (X3). Meanwhile for viscosity variable, the regression coefficient values of components were +1504.11 (X1), +2976.60 (X2), +757.74 (X3), +11071.35 (X1 × 2), −43.84 (X1 × 3), and −365.25 (X2 × 3). For stability variable, the coefficient value were +2.24 (X1), +38395.48 (X2), and −0.076 (X3).

The desirability function of numerical optimization was employed to select the optimum formulation of the nanoemulsion-based hydrogel. The selection of optimum formulation was based on the criteria of attaining acceptable pH and viscosity for topical application and good stability during 8-week storage. In the topical drug development, it is important to obtain formulation that has pH value as close as the pH of the skin to prevent skin irritation. As for viscosity and stability, they play an important role in the consumer acceptability. The goal for independent variables including carbopol, TEA, and PG was setted as “in range.” Meanwhile, the goal for responses was settled as “in range” for pH, and “maximize” for viscosity. It is found that formulation consists of 77% of carbopol (34.65% of total formulation), 3% of TEA (1.35% of total formulation), and 20% of PG (9% of total formulation) has desirability value of 0.982 [Figure 1]. This formulation was then prepared in triplicate and characterized. The predicted values of the responses generated from the software were 6.48 for pH and 2522.96 cP for viscosity. It is found that the observed values from experiments were 6.50 ± 0.02 for pH, and 2492.33 ± 36.91 cP for viscosity. The p-value from one sample t-test was 0.2872 and 0.3001 for viscosity and pH, respectively, which indicated that there were no significant differences between predicted and observed values.

**DISCUSSIONS**

On the development of nanoemulsion, selection of the oil, surfactant, and cosurfactant is an important step. Capryol-90 was selected as the oil, whereas Kolliphor RH 40 and PG were chosen as the surfactant and cosurfactant, respectively. Capryol 90 is a water-insoluble substance containing mainly monoesters and a small fraction of diesters of C8 fatty acid (caprylic acid) which has surfactant properties. Previous studies revealed that oils with medium carbon chain length and higher hydrophilic-lipophilic balance (HLB) value, such as Capryol 90, are preferable in the production of nanoemulsion compared to longer chain length and lower HLB value oils. Kolliphor RH 40 is a nonionic surfactant with hydrophilic-lipophilic balance (HLB) value in the range of 14–16. Nonionic surfactants are widely used in the nanoemulsion formulation as it is less toxic and less affected by pH, and more biocompatible compare to ionic surfactants. The combination of selected oil, surfactant, and cosurfactant could produce an emulsion with nanometer-scale droplet size.

Regarding the result of the statistical analysis in D-optimal design, a positive value of the regression coefficient implies a direct proportionality between

| Carbopol | TEA | PG | Viscosity | pH | Stability |
|----------|-----|----|-----------|----|-----------|
| 1        | 0.77| 0.03| 0.2       | 2586| 6.45      | 2         |
| 2        | 0.69| 0.03| 0.28      | 1759| 6.63      | 2         |
| 3        | 0.62| 0.01| 0.37      | 976 | 5.72      | 2         |
| 4        | 0.59| 0.01| 0.4       | 748.8| 5.81    | 1         |
| 5        | 0.76| 0.01| 0.23      | 1275.3| 5.61    | 2         |
| 6        | 0.57| 0.03| 0.4       | 980.5| 6.72     | 1         |
| 7        | 0.63| 0.03| 0.34      | 1480| 6.39     | 1         |
| 8        | 0.66| 0.02| 0.31      | 1346| 6.34     | 1         |
| 9        | 0.59| 0.01| 0.4       | 830.2| 6.01     | 1         |
| 10       | 0.78| 0.01| 0.2       | 1894.1| 5.74    | 2         |
| 11       | 0.78| 0.01| 0.2       | 1726.6| 5.98    | 2         |
| 12       | 0.72| 0.01| 0.27      | 1366| 5.83     | 2         |
| 13       | 0.57| 0.03| 0.4       | 872.6| 6.86     | 1         |
| 14       | 0.73| 0.03| 0.24      | 2214| 6.49     | 2         |
the independent and dependent variables (responses); meanwhile, a negative value indicates an inverse relationship between them. The concentration of carbopol and TEA has a positive effect on the viscosity of the formulation. The effect of carbopol was lower compared to those of TEA. The interaction effect of carbopol-TEA also has a positive effect on viscosity which much higher compared to those of TEA. When TEA was added into the mixture, the acid residue on carbopol molecule will become neutral resulting in the gelatination process and increasing of viscosity. TEA has a positive effect on pH as TEA is an alkaline substance that could increase the pH value of the formulation. The effect on these characteristics was greater compared to those of carbopol and PG. After 8 weeks of storage, some of the formulations showed phase separation. This phenomenon could occur because the surfactant and cosurfactant could no longer capable to solubilize the oil phase hence the oils (Capryol 90 and oleic acid) separated from the aqueous phase.

The resulted desirability index is considerably high (over 0.7), implying that the model was able to optimize all factors (Xn and Yn) concurrently until reaching the optimum level. In addition, the predicted values of viscosity and pH calculated by equations were close to those obtained from the experiments, indicating that the used experimental design can predict the results accurately.

**Conclusion**

The studied nanoemulsion-based hydrogel showed acceptable characteristics including viscosity, pH, and physical appearance. The nanoemulsion-based hydrogel was optimized using D-optimal design and the resulted optimum formulation comprised 34.65% of carbopol, 1.35% of triethanolamine, and 9% of PG. It can be concluded that andrographolide can be successfully formulated into a nanoemulsion-based hydrogel although more evaluations are still needed for its development.

**Acknowledgement**

The authors greatly acknowledge the Indonesia Endowment Fund for Education (BUDI DN-LPDP) for providing financial support for this study.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.
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