JURNAL FARMASI SAINS DAN KOMUNITAS
Journal of Pharmaceutical Sciences & Community

p-ISSN 1693-5683; e-ISSN 2527-7146

Vol. 19, No. 2, November 2022, pp. 62-70 Research Article

Optimization of *Nanosilver* Synthesis Process with Bioreductor of Binahong Leaf Extract (*Anredera cordifolia* (Ten.) Steenis)

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doi https://doi.org/10.24071/jpsc.004465 J. Pharm. Sci. Community, 2022, 19(2), 62-70

Article Info

Received: 10-03-2022
Revised: 20-07-2022
Accepted: 21-07-2022

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Keywords:
Binahong; bioreductant; flavonoid; nanosilver; sonication

ABSTRACT

Nanosilver is a type of metallic silver nanoparticles and has a particle size of 1-100 nm. The method used in the synthesis of nanosilver is a chemical reduction method with silver metal precursor $AgNO_3$ and a bioreductant of binahong leaf extract containing flavonoid compounds. This study aims to obtain the duration, temperature and optimum point in the synthesis of nanosilver with the bioreductant of binahong leaf extract using the sonication method. This type of research is purely experimental with a 2x2 factorial design. The nanosilver characterization was conducted by determining the optimum wavelength and the transmittance value of the nanosilver formed. The effect of temperature and duration of sonication on the wavelength and % transmittance of the synthesized nanosilver in this study were analyzed by ANOVA statistical test using the Minitab 19 application with a 95% confidence level. In this study, the temperature and duration of sonication as well as their interactions have an effect on the transmittance response. The statistical results of the model have a significant effect on the % transmittance response only with p-value <0.05. The optimum point was found in the synthesis of nanosilver with the bioreductant of binahong leaf extract (*Anredera cordifolia* (Ten.) Steenis) using a 2x2 factorial design, namely at a sonication temperature of 80°C for 10 minutes, a sonication temperature of 70°C for 15 minutes and a sonication temperature of 80°C for 15 minutes.

INTRODUCTION

Nanoparticle technology at this time has become an alternative in drug delivery systems. Nanoparticles is a technology that can be used to develop the particle size of a pharmaceutical preparation in the size range of 1000 nm (Martien *et al*., 2012). One type of nanoparticles that can be applied to pharmaceutical preparations is nanosilver. Nanosilver is a nanoparticle synthesized from silver metal which has a size of 1-100 nm and can be applied as antibacterial, antifungal, antiviral and anti-inflammatory (Ge *et al*., 2014). The nanosilver synthesis process can be done both top-down and bottom-up. On a top-down basis, macro-sized silver metal will be broken down to nano-sized by physical methods, such as ultrasound (Muhriz, Subagio, and Pardoyo, 2011; Kosimaningrum, Pitaloka, Hidayat, Aisyah, Ramadhan, and Rosyid, 2020). On a bottom-up basis, silver Ag^+ ions will be reduced in solution to form Ag0 silver atoms which then undergo nucleation and grow into nano-sized Ag particles, namely nanosilver (Kosimaningrum, *et al*., 2020). In general, when nanosilver is synthesized by chemical reduction methods, metal ions will be reduced using a reducing agent to produce the most optimal and stable nanosilver particle size (Saputra et.al., 2011; Oktaviani and Amrullah, 2015).

One of the metal ions that is often used is $AgNO_3$ (silver nitrate). $AgNO_3$ in the synthesis of nanosilver acts as a metallic silver precursor (Salasa, *et al*., 2016). The reducing agents commonly used are ascorbic acid, sodium borohydride and trisodium citrate (Apriandanu, Wahyuni, and Hadisaputro, 2013). However, the chemical reduction method with the reducing agent is not environmentally friendly because it can pollute the surface of the nanosilver that is formed, so a more environmentally friendly chemical reduction method is used, namely by using plant extracts as the reducing agent or commonly referred to as bioreductors (Ge *et al*., 2014; Rengga, Yufitasari and Adi, 2017; Fabiani, Sutanti, Silvia, and Putri, 2018). By using a chemical reduction method that is more environmentally friendly, the synthesized nanosilver is more stable and the nanosilver can remain stable for a long time (Ge *et al*., 2014). Synthesis of nanosilver using plant extracts as reducing agents is more environmentally friendly because it utilizes secondary metabolites contained in the extract (Tapa, Suryanto, and Momuat, 2016; Tjiang, Aritonang, and Koleangan, 2019). The secondary metabolites in question are phenolic compounds that are thought to function as reducing agents in the synthesis of nanosilver, namely to reduce silver metal ions (Ag^{+}) (Bere, Sibarani, and Manurung, 2019; Oktavia and Sutoyo, 2021). One of the plant extracts containing phenolic compounds is binahong leaf extract (*Anredera cordifolia* (Ten.) Steenis) (Selawa, Runtuwene, and Citraningtyas, 2013).

Binahong leaf extract (*Anredera cordifolia* (Ten.) Steenis) contains various secondary metabolites, such as polyphenolic compounds, saponins, steroids, alkaloids and triterpenoids (Garmana, Sukandar, and Fdrianny, 2014; Imadahidayah, 2015). One example of polyphenolic compounds found in binahong leaf extract is flavonoids which have the potential to reduce silver ions (Ag+) so that they can be used as bioreductants in nanosilver synthesis (Yudhaprawira, 2014; Lestari, Suprihatin and Sibarani, 2019). One method that can help in the synthesis of nanosilver is the sonication method. Sonication is one of the methods in the top-down nanosilver synthesis process (Candani, Ulfah, Noviana, and Zainul, 2018). The sonication method is a method that utilizes ultrasonic waves where an ultrasonic electric generator will generate an electrical signal which is then converted into physical vibrations or ultrasonic waves so that it has a very strong effect called the cavitation effect on the solution which causes the particles of the solution to break (Rusdiana, Hambali, and Rahayuningsih, 2018). The factors that influence the formation of nanoparticles using the sonication method are the sonication temperature and duration of sonication as well as the ultrasonication frequency used (Delmifiana and Astuti, 2013; Nuraeni, 2017; Rusdiana *et al*., 2018).

Optimization needs to be done to obtain the optimum sonication temperature and duration of sonication in the nanosilver synthesis process in order to obtain the appropriate nanosilver characterization. In this study, the optimization design used is a 2x2 factorial design. With a 2x2 factorial design, all possible combinations of the two desired factors with the two specified levels can be analyzed (Salomon, Kokasih, and Angkasa, 2015). The factorial design was chosen in the experimental research design because it has high flexibility to research or increase treatment variations and is efficient to test the main effects and interactions between factors or variables in research (Tisngati, Martini, Meifiani and Apriyani, 2019). Based on the description above, the research was conducted on the optimization of temperature and duration with the sonication method in the synthesis of nanosilver using a bioreductant of binahong leaf extract (*Anredera cordifolia* (Ten.) Steenis).

METHODS

Materials

The materials used in this study were fresh binahong leaves obtained from Manisrenggo, Klaten, silver nitrate pro-analysis ($AgNO_3$), aquabidest, and aquadest. The tools used in this study were glassware (Pyrex), pipette pump, hotplate (Thermo), UV-Vis spectrophotometer (Shimadzu UV-Vis 1800 double beam), thermometer, stopwatch, analytical balance (Mettler Toledo), filter paper, microtube, vortex (Thermo), centrifugator (Thermo), water purificator (Thermo), and sonicator bath (Elmason).

Binahong Leaf Collection

The binahong (*Anredera cordifolia* (Ten.) Steenis) leaves used were obtained from the Manisrenggo area, Klaten. Binahong leaves were collected fresh, dark green in color without yellow spots, black spots and holes, harvested at the age of 3-4 months after planting and picked in the morning (BPOM, 2016; Taek, 2018; Auw, 2020).

Binahong Plant Determination

The determination of the binahong plant (*Anredera cordifolia* (Ten.) Steenis) was done at the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta.

Extraction of Binahong Leaf

Binahong leaf extract was prepared using the infusion method with 5% w/v concentration of binahong leaf extract in the infusion. The binahong leaves were prepared fresh and 10 grams were weighed in 200 mL of aquabidest then heated on a hotplate to 90°C for 15-30 minutes and stirred occasionally (Ardianti, Guntarti, and Zainab., 2014; Auw, 2020).

Preparation of 1 mM $AgNO_3$ Solution

One mM $AgNO_3$ solution was prepared by diluting 10 mM $AgNO_3$ solution. Silver nitrate ($AgNO_3$) was weighed as much as 0.17 grams and dissolved in 100 ml of aquabidest then 20 ml was taken and put into a 200 ml volumetric flask then aquabidest was added to the mark.

Optimization Design of Nanosilver Synthesis

Optimization of temperature and duration of sonication refers to research conducted by Kurniawati (2018) and Dewi *et al.* (2019) which was modified. Variations in temperature and duration of sonication are shown in Table 1.

Table 1. Optimization Design of Sonication Temperature and Duration

Treatment	Sonication Temperature	Sonication Duration
F1	70°C	10 minutes
Fa	80°C	10 minutes
Fb	70°C	15 minutes
Fab	80°C	15 minutes

Nanosilver Synthesis and Purification

Synthesis of nanosilver was conducted by mixing binahong leaf extract and $AgNO_3$ solution in a ratio of 1:9.45 mL of 1 mM $AgNO_3$ solution was heated on a hotplate to adjust the temperature in the sonicator bath. After that, it was put into a container in the sonicator bath and 5 mL of binahong leaf extract were added. Then, the solution mixture was heated using a sonicator bath with variations in temperature and duration determined in the optimization design. Replication of nanosilver synthesis was done six times (Massake *et al.*, 2015; Dewi *et al.*, 2019). Next, nanosilver purification was done, in which the sonicated solution was centrifuged at 2000 rpm for 10 minutes to remove any impurities. Then, to obtain a nanosilver solution, the first purified supernatant was centrifuged again at 11,000 rpm for 15 minutes. The results of the second centrifugation were used for the characterization of the nanosilver (Dewi *et al.*, 2019).

Nanosilver Characterization (UV-Vis Spectrophotometer)

The characterization of the synthesized and purified nanosilver was analyzed using a double beam UV-Vis spectrophotometer. The formation of nanosilver is characterized by the presence of an absorption peak at a wavelength of 400-450 nm. The blank used was aquabidest (Dewi *et al.*, 2019).

Determination of %Transmittance value

Determination of the transmittance value was done by dissolving 100 µL of the synthesized nanosilver solution into 5 mL of aquabidest, then vortexed for 1 minute. Then, the absorbance was measured at the maximum wavelength obtained previously and the blank used was aquabidest (Huda and Wahyuningsih, 2010).

Result Analysis

The results of the study were analyzed using a two-way ANOVA statistical test using the Minitab 19 application with a 95% confidence level (CI) to see the effect value of the factors and their interaction with the response and p-value. Followed by looking for contour plots of each factor against the dependent variable to determine the optimal point of the combination of temperature and duration of sonication using DOE (experimental design).

RESULTS AND DISCUSSION

Results of Determination of Binahong Leaves (*Anredera cordifolia* (Ten.) Steenis)

The binahong plant was determined at the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta with No. 15.20.9/UN1/FFA/BF/PT/ 2021. The determination results obtained indicate that the binahong plant used in this study corresponds to the plant in question, namely binahong leaf (*Anredera cordifolia* (Ten.) Steenis).

Binahong Leaf Infusion Extract

Binahong leaf extract was prepared using the infusion method (Auw, 2020). The infusion method was chosen because it is more affordable and easier to use (Ainia, 2017). Binahong leaf extract contains flavonoid active compounds that are soluble in water, so the solvent used in the binahong leaf extraction process is aquabidest (Auw, 2020; Kemit, Widarta and Nocianitri, 2017). Aquabidest was chosen because it is polar so it can attract polar active flavonoid compounds (Harsanti and Yasi, 2019).

Nanosilver Synthesis and Purification Process

In this study, the synthesis of nanosilver was conducted bottom-up by the chemical reduction method and top-down using sonication method. The nanosilver synthesis process occurs due to a chemical reduction reaction, namely binahong leaf extract as a bioreductor containing flavonoid compounds that have a hydroxyl group so that they are able to donate electrons to silver ions (Ag^{+}) which causes Ag^{+} (oxide 1) to decrease to silver metal Ag^{0} (oxide 0) and produce nanosilver (Ag) Then, there is nucleation of Ag^{0} and growth into silver nanoparticles (nanosilver) (Fajaroh, 2018; Kurniawati, 2018).

The formation of nanosilver is indicated by a change in the color of the solution from light yellow to yellow-brown to reddish-brown (Dewi, *et al*., 2019; Irwan, Zakir and Budi, 2016). Color changes occur due to the influence of reducing agents or bioreductors used and due to the excitation of surface plasmon vibrations on nanosilver or the so-called surface plasmon resonance (SPR) phenomenon of nanosilver (Irwan *et al*., 2016; Rahmayani, Zulhadjri and Arief, 2019; Cahyani, 2020). Surface plasmon resonance is a collection of oscillations to the conduction of electrons that occur on the surface plasmon of silver nanoparticles (Irwan *et al*., 2016).

Synthesis of nanosilver using a bioreductant of binahong leaf extract produced a yellow-brown nanosilver solution as shown in Figure 1A. Then, the wavelength produced by the nanosilver solution was in the range of 400 - 450 nm. In this study, nanosilver purification was done by centrifugation twice, then the second centrifuged solution (Figure 1B) was used for the characteristics of nanosilver (Dewi *et al*., 2019).

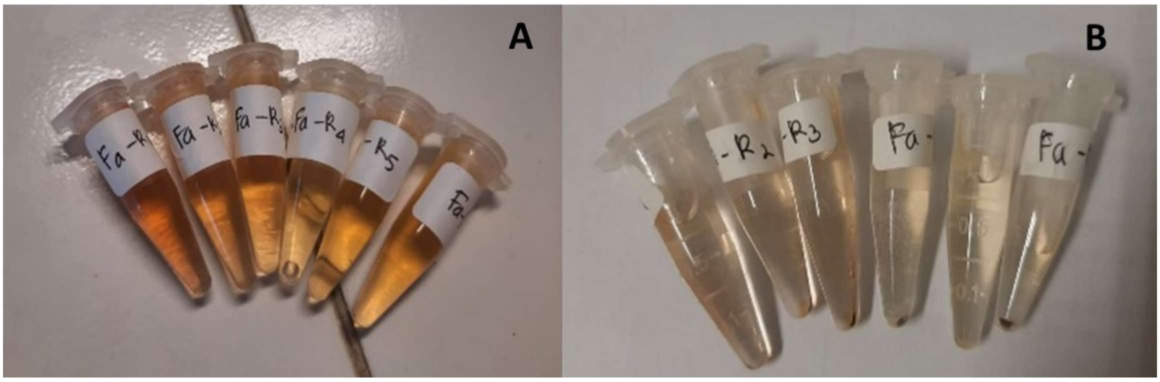

Figure 1. Nanosilver yield 400-450 nm (yellow brown) (A), and second time centrifuged nanosilver solution (B).

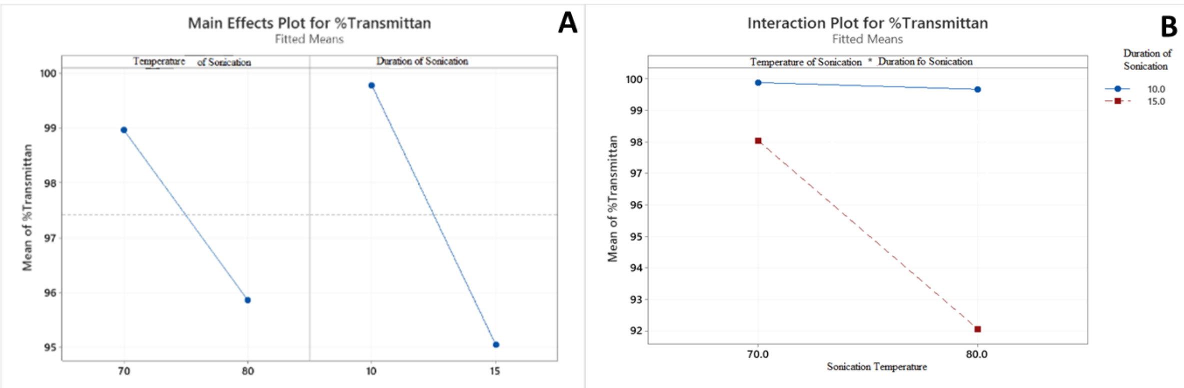

Figure 2. Main effect plot for % transmittance value (A) and interaction plot for % transmittance value (B).

Table 2. Wavelength results and % transmittance after purification

Experiment	Wavelength (nm)	CV	Transmittance (%)	CV
F1	457.67 ± 2.56	0.56%	99.89% ± 0.04	0.04%
Fa	413.33 ± 68.04	16.46%	99.67% ± 0.48	0.48%
Fb	438.67 ± 21.74	4.96%	98.03% ± 3.49	3.56%
Fab	437 ± 14.50	3.32%	92.05% ± 2.13	2.32%

Table 3. Effect value on nanosilver wavelength and % transmittance responses

Term	Wavelength				%Transmittance			
	Effect	Coef	T-value	*p*-value	Effect	Coef	T-value	*p*-value
Constant		436.67	53.56	0.000		97.412	210.68	0.000
Temperature	-23.00	-11.50	-1.41	0.174	-3.095	-1.548	-3.35	0.003
Duration	2.33	1.17	0.14	0.888	-4.728	-2.364	-5.11	0.000
Temp*Duration	21.33	10.67	1.31	0.206	-2.880	-1.440	-3.11	0.005

Results Characteristics of Nanosilver

The characteristics of nanosilver were examined using a UV-Vis spectrophotometer to determine the presence of nanosilver in solution at the optimum absorption and wavelength of nanosilver formation (Rahmayani *et al.*, 2019; Palupi and Suparno, 2020). If an absorption peak is produced at a wavelength of 400-450 using a UV-Vis spectrophotometer, it indicates that the nanosilver formed has a particle size of 1-100 nm. The characteristics of the nanosilver can then be determined by measuring the % transmittance value of the nanosilver formed. The % transmittance value close to 100% indicates clear and transparent dispersions with particle sizes reaching nanometers (Huda and Wahyuningsih, 2010).

In this study, each treatment of nanosilver synthesis was replicated six times. The following are the results of the average wavelength and % transmittance value from the optimization results of nanosilver synthesis after purification.

In this study, wavelength measurements were made for the negative control used, namely silver nitrate solution and binahong leaf extract which had been treated with sonication at 80°C for 15 minutes. The results of the wavelength of silver nitrate solution, which are at 260 nm and 289 nm and the wavelength of the binahong leaf extract, which is in the range of 217-282 nm which is not the optimum nanosilver wavelength because it is below 400 nm. Based on the Table 2, it can be seen that in the treatment of Fa, Fb, and Fab the wavelengths produced meet the optimum nanosilver wavelength range, which is 400-450 nm (Jannah and Amira, 2020). In the F1 treatment, the resulting wavelength did not meet the nanosilver wavelength range. This finding shows that the stability of nanosilver is still low because there has been an aggregation or agglomeration event (Masakke and Rasyid, 2015; Jalestri and Taufikrohmah, 2016).

Then it can be seen that in the F1, Fa, Fb, and Fab treatments, the nanosilver % transmittance results that meet the nanosilver % transmittance target are in the range of 91%-99% (Johannes, 2021). The % transmittance results indicate that the size of the nanosilver particles formed is getting smaller. If the CV value is < 10%, it can be said that the results of the data in the study have high precision for each replication (Johannes, 2021). The % transmittance values in the F1, Fa, Fb, and Fab treatments obtained CV values < 10%, so it can be said that the data obtained had high precision.

In this study, the response seen is the wavelength (nm) and the transmittance value of each treatment in the optimization design. Then the response was analyzed using a two-way ANOVA statistical test. Based on the results of the analysis in this study, the effect value was obtained to determine the factors and interactions that would have the most dominant influence on the wavelength response and the nanosilver % transmittance value (Tantri, Widiharih and Wuryandari, 2015). The effect values of each factor, namely the sonication temperature and duration of sonication and their interactions with each response are shown in Table 3.

The factors and interactions in the optimization design used are not significant to the nanosilver wavelength response, so the effect values obtained cannot be used to determine the factors and interactions that will have the most dominant influence on the wavelength response. In the % transmittance response, the optimization design factors and interactions used are significant on the nanosilver % transmittance response, so it can be said that the effect values obtained can be used to determine the factors and interactions that will have the most dominant influence on the % transmittance response. The sonication duration factor is a factor that has the most dominant influence on the response of the transmittance value because it has an effect value of -4.728, but the result is negative which indicates that the sonication duration factor can reduce the response of the nanosilver % transmittance value produced so that the transmittance value will shift down from the expected value of % nanosilver transmittance, which is in the range of 91%-99%.

In the wavelength response, plots of the main effects and interactions in this study could not be made, because the p-value of the effect of factors and their interactions on the wavelength response was not significant. The main effect plot for % transmittance (Figure 2A) shows that the temperature and sonication duration lines are tilted to the left so that they are negative, which means that the temperature and duration of sonication will have an effect on the decrease in the % transmittance value of nanosilver. Then the factor interaction plot for the % transmittance value (Figure 2B) shows that the lines of the two factors do not intersect each other in the range of factor values used so that the interaction of sonication temperature and sonication duration does not give a strong effect value to influence the response of the nanosilver % transmittance value.

The optimization design model has a real or significant effect if it has a p-value <0.05 (Quintero-Quiroz *et al*., 2019). The *p*-value of the model on the wavelength response obtained is more than 0.05, which is 0.321, indicating that the optimization design model used is not significant for the nanosilver wavelength response. Additionally, the *p*-value results of sonication temperature, sonication duration and interaction obtained *p*-value results that were not significant. This can happen because the wavelength response in all treatments is in the optimum nanosilver wavelength range and the difference is not significant, so it can be said that the optimization design model in this study cannot predict the nanosilver wavelength response (Parera, 2021). The regression equation for the wavelength model is as follows: Wavelength = 1403 – 12.97 Sonication Temperature – 63.5 Sonication Duration + 0.853 Sonication Temperature × Sonication Duration (1)

The results of the analysis of the % transmittance response obtained that the *p*-value of the model is less than 0.05, which is 0.000 which indicates that the optimization design model used is significant to the nanosilver % transmittance response, so it can be said that the optimization design model in this study can predict the % transmittance response. The regression equation for the % transmittance value model is as follows: %transmittance = 24.4 + 1,130 Sonication Temperature + 7.69 Sonication Duration – 0.1152 Sonication Temperature × Sonication Duration (2)

Based on the results of the study, three optimum treatments were selected in the synthesis of nanosilver by sonication using a bioreductant of binahong leaf extract, namely Fa treatment with a sonication temperature of 80°C for 10 minutes, Fb treatment with a sonication temperature of 70°C for 15 minutes and Fab treatment with a sonication temperature 80°C for 15 minutes. Based on the results of the optimization experiments done, the three treatments had wavelengths and transmittance values that matched the target range. The three treatments were chosen because they were the optimum sonication temperature and duration of sonication in this study and also entered the optimum point in the synthesis of nanosilver with bioreductant of binahong leaf extract using a 2x2 factorial design.

In this study, three optimum points were selected with the aim of providing several options for the formulator to be able to choose the best composition of the nanosilver formulation process according to the considerations that are made.

CONCLUSIONS

Based on the results of the study, it can be concluded that the sonication temperature and the duration of sonication as well as their interaction have an insignificant effect on the nanosilver wavelength response. However, they have a significant effect on the % transmittance response of nanosilver produced with the most dominant factor being the duration of sonication. Three combinations of sonication temperature and optimum sonication duration were found in the synthesis of nanosilver using a bioreductant of binahong leaf extract (*Anredera cordifolia* (Ten.) Steenis) using the sonication method. The optimum point was found in the synthesis of nanosilver with the bioreductant of binahong leaf extract (*Anredera cordifolia* (Ten.) Steenis) using a 2x2 factorial design, namely at a sonication temperature of 80^{o}C for 10 minutes, a sonication temperature of 70^{o}C for 15 minutes and a sonication temperature of 80^{o}C for 15 minutes.

ACKNOWLEDGEMENTS

This research was financially funded by Program Penelitian Pusat Studi Universitas Sanata Dharma 2022 (No.017/Penel./LPPM-

USD/IV/2022) awarded to Dr. apt. Rini Dwiastuti, M.Sc.

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