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Original Article

OPTIMIZATION OF ACYCLOVIR SUSPENSION: FORMULATING DEVELOPMENT USING NA-CMC AND QUALITY CONTROL OF FINISHED PRODUCTS

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ABSTRACT

Objective: Acyclovir is widely used as a potent antiviral drug to treat viral infections of herpes and varicella-zoster families. Unfortunately, the drug has a very poor oral bioavailability character (15-30%). The purpose of this study was to develop a formulation of acyclovir suspension with a simple suspension method using Na-CMC (carboxymethylcellulose sodium), and to carry out quality control of the finished product.

Methods: The formulation was developed by designing variations in Na-CMC concentration and quality control, including pH, viscosity, dispersibility, storage stability, microscopic measurement, sedimentation volume, and evaluation of acyclovir levels. Quality control is to evaluate the suspension in order to obtain good and stable physicochemical properties of the suspension.

Results: Design variations of Na-CMC concentrations of 1.4%, 1.5%, and 1.6% resulted in a homogeneous suspension and easily dispersed perfectly. The three formulas did not have a significant difference in the value of viscosity, permeability, and sedimentation volume, which were not significant. All formulas have pseudoplastic flow properties, with good particle size uniformity in the range of 0–13 µm. The stability of pH during storage time was shown by the formula with 1.5% Na-CMC.

Conclusion: The acyclovir suspension with 1.5% Na-CMC concentration was the best compared to the other formulas in terms of stability and physicochemical properties.

Keywords: Acyclovir, Suspension, Formulation, Quality control, Na-CMC, Spectrophotometer UV-Vis

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INTRODUCTION

Acyclovir is widely used for the treatment of infections with the herpes simplex virus, varicella-zoster virus (chickenpox) and herpes zoster (shingles) [1, 2]. Acyclovir, with the molecular formula $C_8H_{11}N_5O_3$, has a maximum solubility in the water at 37 °C is 2.5 mg/ml, and pKa is 2.27 and 9.25, causing poor bioavailability values. As a result, more than half of the dose taken orally is found in the stool [3, 4].

Acyclovir that has entered cells infected by the viral thymidine kinase will be converted into its monophosphate, and then by the cell, kinase itself is converted into triphosphate compounds. This triphosphate compound is an actual compound that inhibits specific DNA polymerase in viruses [5, 6].

The most widely used dosage form of acyclovir is suspension. This form is preferred by children compared to tablets or capsules. Suspension preparations have several advantages, such as ease of administration of relatively large doses, safe, easy to administer, and can be easily formulated to cover the unpleasant taste of the active pharmaceutical ingredients [7, 8].

Suspension formulations require a suspension agent, also called a protective colloid, whose role is to increase the viscosity and maintain the stability of the suspension [9]. Furthermore, it also produces a structure that helps the phase dispersion in the suspension. Na-CMC is anionic, water-soluble, which can act as a suspending agent that can improve the flow properties of the suspension, acting as a stabilizer [10-12]. This study reports the manufacture of a stable acyclovir suspension formula using Na-CMC (carboxymethylcellulose sodium), supported by a quality control test of the suspension formula.

MATERIALS AND METHODS

The materials used in this study were acyclovir, methylparaben, propylparaben, sodium carboxymethylcellulose, vanilla purchased from Sigma-Aldrich, and sorbitol, glycerin obtained from Merck. All ingredients were pharmaceutical grade. The tools used in this research were Sartorius Entries 224-1S Analytical Balances, ultra turrax (Heildolph®), viscometer (Brookfield LVT®), pH meter (Metrohm®), SM852B 3 Phase Rotation Tester Digital Phase, and ultraviolet-visible spectrometer (GENESYS™ 180).

Preformulations and preparation of acyclovir suspension

The initial stage is to develop a formula from ingredients that comply with guidelines such as the Indonesian Pharmacopeia, the United States Pharmacopeia or British Pharmacopeia [13, 14]. The formula was made with 3 variations of Na-CMC concentration 1.4%; 1.5%; and 1.6% (table 2) refers to existing textbooks [13, 14]. Acyclovir suspension was made by dissolving Na-CMC into 50 ml of heated distilled water, then stirring until a clear mass of mucus was formed. Acyclovir dissolved in sorbitol stirred until dissolved, then added to the mixture. Methyl paraben and propyl paraben were dissolved in glycerin, stirred until dissolved, then added to the mixture. Aquadest was added slowly to a volume of 100 ml and stirred until the mixture was homogeneous.

Quality control of acyclovir suspension

Quality control of Acyclovir suspension. Evaluation of the quality of acyclovir suspension includes parameters of pH, viscosity, microscopic observations, dispersion test, flow properties, and sedimentation volume [9, 15]. The content uniformity of each formula was analyzed by an ultraviolet-visible spectrometer at a wavelength of 266 nm [16]. Data analysis was performed using a completely randomized block design based on ANOVA tables with a 95% confidence level.

RESULTS

Preformulations and preparation of acyclovir suspension

All excipients and active pharmaceutical substances in the formulation of acyclovir suspension are listed in the Indonesian Pharmacopoeia IV edition [13]. However, the sorbitol used in this formulation is used in liquid form, not powder.

Table 1: Physicochemical properties of substances used in the formulation

Components	Specification	Inspection results	Conclusions
Acyclovir	Description	Crystal Powder, white.	Appropriate
	Solubility	Insoluble in water.	Appropriate
Methylparaben	Description	White crystalline powder, odorless.	Appropriate
	Solubility	Difficult to dissolve in water, soluble in glycerin.	Appropriate
Propylparaben	Description	White powder.	Appropriate
	Solubility	Difficult to dissolve in water, soluble in glycerin.	Appropriate
Na CMC	Description	Powder, beige.	Appropriate
	Solubility	Disperse in water, insoluble in ethanol.	Appropriate
Sorbitol	Description	A clear liquid, colorless, sweet taste.	Appropriate
	Solubility	Easily dissolves in water and ethanol.	Appropriate
Glycerin	Description	The clear, colorless, viscous, sweet taste.	Appropriate
	Solubility	Mixes easily with water.	Appropriate
Vanillin	Description	Needle smooth, white, distinctive taste and smell.	Appropriate
	Solubility	It dissolves in glycerin and hot water.	Appropriate
Aquadest	Description	Clear, colorless, odorless liquid.	Appropriate

The results of the suspension formulations used are summarized in table 2.

Table 2: Acyclovir Suspension formula

Components	F_0 (%)	F_1 (%)	F_2 (%)	F_3 (%)
Acyclovir	4	4	4	4
Methylparaben	0.1	0.1	0.1	0.1
Propyl paraben	0.02	0.02	0.02	0.02
Na CMC	0.0	1.4	1.5	1.6
Sorbitol	10.0	10.0	10.0	10.0
Glycerin	10.0	10.0	10.0	10.0
Vanilin	q. s	q. s	q. s	q. s
Aquadest	ad 100	ad 100	ad 100	ad 100

F0: Suspension formula without suspending agent, F1: Suspension formula with 1.4% Na-CMC, F2: Suspension formula with 1.5 % Na-CMC, F3: Suspension formula with 1.6% Na-CMC

The complexity of additives in a formula plays a role in their respective functions, such as surfactants, suspending agents, buffers, preservatives, sweeteners, flavorings, and flavors.

Quality control of suspension preparations

The suspension evaluation included organoleptic observation, sedimentation volume, viscosity value, suspension pH, dispersibility test, microscopic observation, suspension fluid properties during storage time, and measurement of acyclovir concentration in suspension.

Organoleptic observations

The results of the observations include color, taste, and smell. There were no changes in all suspension formulas. Changes occurred on the 35th day, where it can be concluded that the changes occurred because they were influenced by storage conditions such as container closure, temperature, and exposure to sunlight.

Viscosity measurement

Viscosity shows the viscosity of the suspension, where a high value means that the viscosity of the suspension is getting higher [19]. Viscosity was measured with a Brookfield LV viscometer with spindle No. 3 and a speed of 60 rpm, and repeated 3 times for each treatment. The test results of the three formulas are summarized in table 3. In the formula without Na-CMC, the viscosity value is 0 mPa s, indicating that the viscosity is highly dependent on the suspending agent.

Table 3: Viscosity results of the three formulas during storage time

Days	F_0 (mPa. s)	F_1 (mPa.s)±SD (n=3)	F_2 (mPa.s)±SD (n=3)	F_3(mPa.s)±SD (n=3)
0	0	550±0.58	765±0.10	820±0.28
7	0	545±0.20	620±0.10	800±0.28
14	0	530±0.20	605±0.10	790±0.15
21	0	530±0.20	600±0.20	785±0.20
28	0	530±0.20	570±0.10	780±0.20

F0: Suspension formula without suspending agent, F1: Suspension formula with 1.4% Na-CMC, F2: Suspension formula with 1.5% Na-CMC, F3: Suspension formula with 1.6% Na-CMC

Based on Sigma-Aldrich data, the viscosity of Na-CMC is 400-800 MPa. s, so the concentration range is 1.4%-1.6%. The result of the viscosity of formula 3 is 820, exceeding the viscosity value of Na-CMC 800 mPas. Statistical analysis with 95% confidence level and the Newman-Keuls test showed no significant difference in viscosity during storage time.

pH measurement

The results of measuring the pH of the three formulas during the storage process with 3 repetitions are shown in table 6. Statistical analysis with 95% confidence level and the Newman-Keuls test showed that there was a significant difference in pH changes that occurred during the storage process. This is because the suspension preparation is not added with a buffer solution, which can maintain the pH value.

Measurement of sedimentation volume

The sedimentation volume test is useful to find out how much sediment is formed in the suspension preparation during the storage process. Every day the sediment height of each formula formed was

measured. During the storage period, the sedimentation volume increased because more sediment was formed, which was different from the formula without Na-CMC [20]. The initial hypothesis showed that there is a change in sedimentation in each formula. Further statistical tests with Newman-Keuls showed that there was no significant difference in sedimentation volume during storage time.

Table 4: The pH values of the three formulas during storage time

Days	$F_{0\pm}$SD (n=3)	$F_{1\pm}$SD (n=3)	$F_{2\pm}$SD (n=3)	$F_{3\pm}$SD (n=3)
1	7.18±0.005	7.34±0.020	7.39±0.005	7.46±0.005
7	7.12±0.005	7.30±0.011	7.35±0.010	7.38±0.005
14	6.99±0.010	7.26±0.011	7.32±0.010	7.30±0.005
21	7.04±0.005	7.23±0.020	7.28±0.020	7.22±0.010
28	7.00±0.011	7.37±0.010	7.29±0.011	7.27±0.005

F0: Suspension formula without suspending agent, F1: Suspension formula with 1.4% Na-CMC, F2: Suspension formula with 1.5% Na-CMC, F3: Suspension formula with 1.6% Na-CMC

Dispersibility test

This test was carried out using a rotator tester with a speed of 20 rpm. This test provides information on the ability of the suspension to be redispersed without forming a precipitate [21]. Good dispersion results were found from the three formulas, and there was no significant difference in the dispersion results.

Determination of flow properties

Flow properties are measured to determine the flow properties of the suspension based on the type of suspension substance used. The flow characteristics of sodium carboxymethylcellulose are pseudoplastic; that is, the higher the shear velocity results in a decrease in the shear stress [22]. On day 0, the viscosity decreases with increasing shear velocity, which proves that the flow properties are pseudoplastic. On day 28, pseudoplastic flow properties were also found, that the higher the velocity, the lower the viscosity.

Microscopic measurement

Measurement of the particle size of acyclovir suspension with a microscope aims to determine the level of particle size, the uniformity of the substance in the suspension. The results of the observations provide information that the acyclovir suspension has a particle size of 0-13 µm, and the category of good suspension preparations (<50 µm) [23].

The content uniformity of acyclovir suspension–spectroscopy study

The content uniformity test was performed on freshly prepared samples of the three formulas. The calibration curve was made to determine the relationship between 2 variables, the concentration and absorbance value of the sample [24]. This curve was created by measuring the five-point concentration (5, 10, 15, 20, 25 ppm) of acyclovir in an acid solvent. Acyclovir is insoluble in water; it is an amphiphilic molecule; therefore, acid medium is used to produce ionized acyclovir species.

The concentration for each formula was estimated by application of the linear equation (y = 0.0362x-3E-05, linear regression coefficient R^2= 0.09996), which was derived from the calibration curve made using the standard acyclovir solution shown in fig. 1. Based on the proximity of the absorbance value to the standard solution, the formula with a Na-CMC concentration of 1.5% was the best formula.

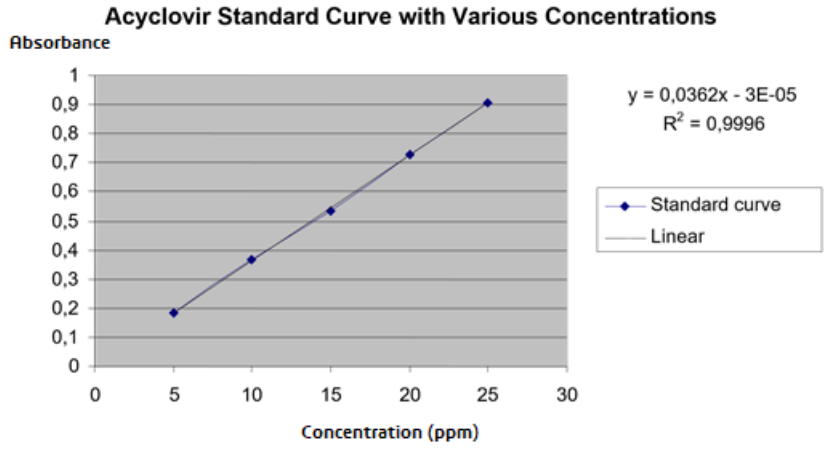

Fig. 1: Standard curves of acyclovir

DISCUSSION

According to Pharmacopoeia Indonesia Edition IV, it is easier and more practical to use in formulations because it did not need to be dissolved in aquadest before use. Formulation is an important step in the manufacture of pharmaceutical preparations [17]. The suspension preparation formula is added with additional substances that are useful for maintaining the uniformity of physical form, texture, stability, dosage, taste, and overall appearance so that it is stable during storage.

Formula differences were carried out to influence the significant role of Na-CMC in acyclovir suspension preparations [18]. Na-CMS has a role to regulate viscosity and is a good stabilizer for suspension preparations. Optimization of the viscosity of acyclovir suspension has been carried out with variations in the concentration of Na-CMC 1.0%; 1.25%; 1.5%; 1.75%, and 2.0%. The result that meets the viscosity requirements is 1.5%. Therefore, in this study, the formulation of acyclovir suspension was carried out with an approach at the Na-CMC concentrations of 1.4%, 1.5% and 1.6%.

Quality control of the final product of acyclovir suspension, starting from physical observations, sedimentation volume, suspension viscosity, pH value, dispersibility test, microscopic observation, is needed to determine the fluid properties of the resulting acyclovir suspension. This property will be closely related to the stability of the suspension preparation during the storage period so that the expected therapeutic effect is maintained.

The stability of the suspension can be improved by using Na-CMC while making suspensions. The preparation's stability can be attributed to the system's capacity to preserve Gibbs free energy, which makes the interfacial tension relatively thermodynamically stable [25]. Agglomeration is a function of activation energy, which is impacted by the addition of stabilizers to the system (such as, surfactants and polymers). Na-CMC stabilizes the mixture and serves as a wetting agent by lowering the interfacial tension between the particles and the dispersion medium. In order to prevent agglomeration caused by electrostatic attraction (ionic surfactants) or steric stabilization (non-ionic surfactants and polymers), the second necessity is to provide a barrier between the drug particles [26].

CONCLUSION

The formulation results showed that the formula with 1.5% Na-CMC was the best formula, taking into account the analysis of the acyclovir content in the suspension, viscosity, pH, dispersibility, sedimentation volume, and stable flow properties of the suspension during the storage process. Microscopic tests also showed that the preparation of acyclovir suspension was good, with relatively uniform particle size homogeneity.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

There is no conflict of interest between the authors.

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