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Hydrating serum made of potassium azeloyl diglycinate with CMC-Na variations as a gelling agent on physical-chemical and safety tests

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ABSTRACT: Potassium azeloyl diglycinate is an ingredient for moisturizing the skin. In this research, potassium-azeloyl-diglycinate was formulated into a gel-based hydrating serum. A good serum gel can be identified by its physicochemical properties and stability, which are determined by the composition of the ingredients used, one of which is CMC-Na, which is used as a gelling agent. The aim of this research was to test the effect of CMC-Na concentrations (1%, 1.25%, and 1.5%) on the physicochemical stability of serum and to find a formulation with the best stability by testing the water content and safety. The evaluation results show that increasing the concentration of CMC-Na has an impact on increasing viscosity, pH, and adhesion, thereby providing a different gel consistency in each formula and reducing its spreadability. The best formulation is Formula-2, which is then tested for water content on respondents. The research results show that this formula has the ability to hydrate the skin, as seen from an increase in oil content of 8.39% and an increase in water content of 8.86%. Initial acute dermal toxicity test results show that the serum is safe to use on the skin and does not cause side effects or toxicity reactions.

KEYWORDS: CMC-Na; gel; potassium azeloyl diglycinate; serum.

INTRODUCTION

Skin is the outermost tissue that functions to cover and protect the body and organs. Face is one part of the body that people really pay attention to, especially for the health and beauty of the skin. Facial skin is very sensitive to irritants, such as air pollution and extreme weather change. This can cause facial skin to become dry. Dry facial skin, rough skin texture, and skin that gets dull and peeled are caused by a lack of moisture [1]. Dry skin or xerosis describes skin condition with low moisture in the stratum corneum. Dry skin can be caused by water loss through the epidermal or trans epidermal water loss (TEWL). In general, there is a correlation between skin hydration and TEWL values, where a low TEWL value indicates more normal skin hydration [2].

Today, many topical skin care products contain compounds that moisturize facial skin. Moisturizers have occlusive properties and keep the skin hydrated. Occlusives ingredients work to reduce TEWL, while moisturizers reduce skin keratinization and increase water transport to cells, maintaining skin hydration [3]. One of the topical formulations for skin care is a facial serum formulation. Serum preparation is superior to other topical preparations due to its high concentration of active ingredients, which neither inhibit the physiological functions of the skin nor clog skin pores [4]. Serums, or concentrates as they are commonly called, contain ten times more bioactive substances than other topical preparations, making them work faster and more effectively. Serum has fast-absorbing properties and can penetrate deep into the skin.

A gel-based serum was chosen for this formula because it is a formulation that contains a high concentration of active ingredients that can enhance the effect of potassium azeloyl diglycinate on the skin. Moreover, the gel base ingredient has good spreading power, is not sticky, and is suitable for cosmetic ingredients. CMC Na was chosen as the gelling agent because this ingredient is a natural polymer and is stable at pH 5-9. Furthermore, it only takes a shorter time for CMC-Na to expand into a good gel structure [5].

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Chemical modification of azelaic acid and glycine to potassium azeloyl diglycinate results in similar activity, but much better from a technical point of view. Potassium azeloyl diglycinate is a water-insoluble derivative of azelaic acid and water-soluble glycine. Azelaic acid is inherently difficult to solubilize, so it is not recommended to use as a cosmetic ingredient since it is thick and difficult to apply. Condensation between one mole of azelaic acid and two moles of glycine can enhance each other's properties. The resulting potassium azeloyl diglycinate is water soluble, making it suitable for cosmetic formulation. Glycine is able to moisturize the skin, while azelaic acid is known for its antibacterial properties, which can regulate skin proliferation, dryness, and irritation. A study shows that glycine is an amino acid.

Amino acids and other chemicals form a natural moisturizing factor in the stratum corneum, which plays a role in moisturizing the stratum corneum [6]. This study aims to examine the effect of CMC-Na concentrations (1%, 1.25% and 1.5%) on the physicochemical stability of the formulation, which includes organoleptic, homogeneity, adhesion, spreadability, pH, and viscosity. Physicochemically, optimal formulations were tested for hydration on the skin to meet the criteria for a good gel formulation.

▪ MATERIALS AND METHODS

Material

Potassium azeloyl diglycinate (Nadev, Indonesia), CMC-Na (Agung Jaya, Indonesia), glycerin (Agung Jaya, Indonesia), propylene glycol (Agung Jaya, Indonesia), vanilla essence (Agung Jaya, Indonesia), methyl paraben (Techno Pharmchem, India), and aquadest (Agung Jaya, Indonesia).

Equipment

pH meter (LUTRON PH-208), water bath (MASPION S-302), viscometer (RION VT-04), oven (Memmert), refrigerator (SHARP), microscope (Nikon E100), and skin analyzer test (SK-8).

Hydrating serum formula made of potassium azeloyl diglycinate

The hydrating serum formula of potassium azeloyl diglycinate was made into 3 formulas by comparing the concentration variations of CMC Na, namely 1%, 1.25%, and 1.5%. The formula used can be seen in Table 1.

Table 1. Potassium azeloyl diglycinate hydrating serum formulation design.

No	Name of Ingredients	Formula and Composition (% b/v)		
		F1	F2	F3
1	Potassium azeloyl diglycinate	10	10	10
2	CMC Na	1	1.25	1.5
3	Glycerin	10	10	10
4	Propylene glycol	2	2	2
5	Methyl Paraben	0.005	0.005	0.005
6	Aquadest	Ad 100	Ad 100	Ad 100

F1 : CMC-Na content is 1%
F2 : CMC-Na content is 1.25%
F3 : CMC-Na content is 1.5%

Hydrating serum preparation of potassium azeloyl diglycinate

CMC Na was prepared with hot distilled water, then allowed to stand for 30 minutes until it swelled, stirred to form a gel mass, then added with propylene glycol, glycerin, methylparaben, and the remaining distilled water until evenly distributed. The final step was adding potassium azeloyl diglycinate with vanilla essence and stirring for 30 minutes until homogeneous.

Stability test on hydrating serum of potassium azeloyl diglycinate

Stability testing was carried out on day 0 (before the cycling test) and day 12 (after the cycling test) [7]. Serum gel was stored in the refrigerator at 4±2°C for 24 hours, then stored in the oven at 4±2°C for 24 hours (1 cycle). The test was carried out for 6 cycles, and physical changes were observed at the beginning and end of the test, including organoleptic, homogeneity, viscosity, adhesion, spreadability, and pH tests.

Organoleptic test

Organoleptic testing was carried out by observing the shape, smell, consistency, and color of the test preparations (F1, F2, and F3).

Homogeneity test

The homogeneity test was carried out using a glass plate smeared with serum gel to form a homogeneous composition, which was indicated by the presence of no solid material on the glass. Observation of homogeneity was carried out using a microscope [8].

pH test

The pH measurement was carried out by dipping the digital pH meter electrode into the sample calibrated in a buffer solution. The pH meter was turned on, and waited until the pH meter display showed a stable number [8].

Adhesion test

A total of 0.5 g of the preparation was smeared on a slide with an area of 2 x 2 cm, while another slide was placed on top of this preparation and pressed with a load of 1 kg for 5 minutes. The slide is inserted into the adhesion tester, the weights weighing up to 80 g were removed while recording the time until both glasses were removed [8].

Spreadability test

A total of 0.5 grams of the preparation was placed in a round glass, and another glass was placed on top of it, then left to stand for 1 minute. After that, a load weighing 150 grams was added, then let it stand for 1 minute, and the diameter measurement was repeated until a constant diameter distribution was obtained [8].

Viscosity test

The tested preparation was placed in a cylindrical housing, and the rotor was installed while ensuring that the rotor was immersed in the tested preparation. The viscometer was turned on while ensuring the rotor could rotate. It can be observed that the needle of the viscometer pointed to the number on the available spindle viscosity scale when the needle pointed in a steady direction [8].

Skin hydration ability testing

This test used a skin analyzer (SK-8) test tool. The tool will automatically detect skin conditions such as the oil and water content of the skin. This test was conducted on 5 female respondents aged 19-25 years. In this test, gel serum was applied to the back of the left hand once a day for 1 week. The preliminary test was carried out by measuring the water content and oil content of the skin before applying the gel serum, then it was measured again on day 7 [9]. The hydration test on the respondents' skin has received permission from the Research Ethics Commission Section of the Dr. Moewardi Regional General Hospital, Surakarta, No. 192/II/ HREC /2022.

Preliminary dermal toxicity test

This test was carried out on 3 groups of rats acclimatized for a week. The first group is Formula 1 (F1), the second group is Formula 2 (F2), and the third group is Formula 3 (F3). The rats' back hair was shaved the day before the test in an area of +/- 6 x 6 cm. The shaved areas were marked with a box for applying the test serum. Enough serum was applied to cover the entire shaved area of each formula in the rat group.

The area was covered with sterile gauze and covered with an elastic bandage. The test was carried out 1 time for 2 weeks (14 days). During preliminary testing of acute dermal toxicity observed, some rats died during testing. Re-confirmation test is needed by adding 1 rat. After 14 days, the rats were disposed of according to animal extermination procedures. Experimental animals were destroyed by euthanasia by administering high doses of ketamine (75-100 mg/Kg Body Weight) [10].

Data analysis

The data obtained were the results of pH, spreadability, adhesion, and viscosity tests, which were then analyzed by the Shapiro Wilk normality test and Levene's homogeneity test to determine whether the data were normally distributed and homogeneous. If the data were normally distributed and homogeneous, the Oneway ANOVA test was needed, with a 95% confidence level. Meanwhile, the results of hydration ability in the form of oil and water content were carried out using the Paired Samples t-test method.

▪ RESULTS AND DISCUSSION

Organoleptic test

Organoleptic tests were carried out using the five senses to describe the preparations. Organoleptic tests include color, odor, shape, preparation consistency, and homogeneity [11]. The results of organoleptic observations of serum preparations on day 0 (before cycling) and day 12 (after cycling) of storage can be seen in Table 2.

Table 2. Organoleptic test results for hydrating serum potassium azeloyl diglycinate.

Organoleptic	Formula 1		Formula 2		Formula 3	
	Day-0	**Day-12**	**Day-0**	**Day-12**	**Day-0**	**Day-12**
Color	Light brown	Light brown	Light brown	Light brown	Light brown	Light brown
Smell	Vanilla	Vanilla	Vanilla	Vanilla	Vanilla	Vanilla
Form	Gel	Gel	Gel	Gel	Gel	Gel
Consistensy	Liquid	Liquid	Rather thick	Rather thick	Thick	Thick
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous

The results of organoleptic tests, which included color, odor, shape, and consistency during 12 days of storage, showed that the three formulas were stable and had no significant changes. The test results showed differences in consistency between the three formulas. The F1 serum gel formulation has a slightly thicker liquid consistency than the F2, while the F3 had the highest consistency. CMC-Na is widely used in oral and topical pharmaceutical formulations, mainly due to its high viscosity.

The higher the concentration of Na CMC used, the higher the consistency of the resulting formulation. F1 used CMC Na with a concentration of 1%, F2 used CMC Na with a concentration of 1.25%, and F3 used CMC Na with a concentration of 1.5%. The results of the gel uniformity test showed no lumps or coarse particles, and it can be said that all formulations produced a homogeneous gel. Homogeneity testing showed that the active ingredients and excipients produce a good mix, so the active ingredients are evenly distributed in the preparation.

The results of these observations indicated that the concentration of CMC Na affects the physical properties of gel-based potassium azeloyl diglycinate derivative serum based on organoleptic parameters. CMC Na was chosen as a gelling agent because it has been widely used in various pharmaceutical, food, chemical, oil and textile industries, apart from that it is also stable over a wide pH range, namely pH 2-10; has the character of being easily dispersed in hot or cold water and is transparent in color after dispersing. Glycerin was chosen as a humectant because it dissolves in air to produce a stable mixture, is a hygroscopic liquid so it can maintain moisture in the preparation, does not oxidize easily if stored at room temperature, and has transparent physical characteristics [12].

Homogeneity test

The results of macroscopic visualization are a completely visible formula with a storage process of up to 12 days and,under stress conditions, show relatively homogeneous results. Based on test results carried out, a preparation that meets the homogeneity requirements is considered good, meaning no grains or missing ingredients are mixed into the preparation.

Viscosity test

Viscosity test was carried out to examine the viscosity of the three serum gel formulations at different concentrations of CMC Na and viscosity stability during storage. Observation results of the viscosity of the gel serum on day 0 (before cycle) and 12 (after cycle) of storage are shown in Table 3. Several factors affect viscosity, one of which is temperature. Viscosity is inversely proportional to temperature. If the temperature increases, the viscosity will decrease, and if the temperature is lowered, the viscosity will increase. This is due to the faster movement of material particles in line with the increase in temperature and decrease in viscosity [13].

Furthermore, the viscosity of the formula is also influenced by various factors, such as the mixing or stirring stages during the preparation process. The stronger the stirring power during the mixing process, the more freely the droplet particles move and collide with each other, resulting in easier blending. The binding of the droplet particles results in a weakening of the contact area between the droplet particles. The system consistency will decrease, causing the system viscosity to decrease during storage [14].

The difference in CMC Na concentration in each formulation can affect the viscosity of the formulation. This happens because of an increase in the concentration of Na-CMC in the water. The release of Na^+ ions to be replaced by H^+ ions becomes higher, increasing the formation of HCMC and viscosity [11]. Based on the One-way ANOVA test, the Sig value is <0.05 (p-value = 0.000), meaning that there is a significant difference between the viscosity of the formulas. This showed that variations in the concentration of CMC-Na affected the viscosity value of hydrating serum of Potassium azeloyl diglycinate preparations. There was no significant difference between the viscosity on storage using the influence of temperature (cycling test). In other words, the cycling test for 6 cycles did not affect the stability of the three viscosity preparations.

Table 3. The results of the stability test of hydrating serum potassium azeloyl diglycinate.

Test	Observation	Formula 1	Formula 2	Formula 3
Viscosity (cPs) ± SD	Day-0	523.50 ± 1.32	597.46 ± 2.49	655.33 ± 1.26
	Day -12	520.83 ±1.76	593.00 ± 2.00	653.00 ± 1.00
pH	Day-0	6.47 ± 0.06	6.70 ± 0.10	6.87 ± 0.08
	Day -12	6.30 ± 0.10	6.63 ± 0.06	6.72 ± 0.03
Spread power (cm) ± SD	Day -0	6.58 ± 0.08	6.28 ± 0.03	5.55 ± 0.05
	Day -12	6.82 ± 0.04	6.38 ± 0.02	5.99 ± 0.20
Adhesion (seconds)± SD	Day -0	0.78 ± 0.02	1.21± 0.04	1.55 ± 0.02
	Day -12	0.73 ± 0.02	1.13 ± 0.03	1.43 ± 0.04

pH Test

The pH test aims to examine the stability of the pH of the gel serum preparation during the storage period. This test aims to ascertain whether there is an effect of changes in pH due to differences in the concentration of CMC-Na and the cycling tests [11]. The results of observing the pH of serum gel preparations on day 0 (before the cycling test) and day 12 (after the cycling test) of storage can be seen in Table 3. It is known that there was an increase in the pH value of the three formulas as the CMC-Na concentration level increased. Based on the data, it can be seen that the addition of CMC-Na concentration tended to increase the pH value of gel serum preparation of potassium azeloyl diglycinate. This is because the pH value of CMC-Na, which is between 6.5-8.5 [15], is higher than the pH of potassium azeloyl diglycinate which has a pH range of 6.5-7.5 [16]. As a result, the higher the concentration of CMC-Na in the formula, the higher the pH of the serum gel preparation [15]. Topical preparations to be used on the skin must be in the skin pH range, namely 4.5-7. This is because too-acidic preparation causes skin irritation, while too-alkaline preparation can result in scaly skin [17]. Based on the One-way ANOVA statistical test, the Sig value was <0.05 (p = 0.000), meaning that there was a significant difference between the pH of each formula. This shows that variations in the concentration of CMC-Na affected the pH value of the hydrating serum preparation of potassium azeloyl diglycinate. In the pH stability test, which was affected by storage temperature for 6 cycles, there was no significant difference, meaning that the cycling test for 6 cycles did not affect the stability of the three pH preparations.

Spreadability test

Spreadability test was carried out to examine the ability of gel-based Potassium azeloyl diglycinate serum preparations when spreading on the skin. The greater the spreadability of preparation, the more practical the preparation is to be applied to the skin. The results of the spreadability test of serum preparations on day 0 (before the cycling test) and day 12 (after the cycling test) of storage can be seen in Table 3, which indicated that the scattering value decreased with increasing concentration of CMC-Na used. This is because CMC Na functions as a viscosity increaser and gelling agent [5]. The spreading power value was also higher after the 12-day cycling test. This is because, during the cycling test, there was a decrease in the viscosity of the hydrating serum preparation of potassium azeloyl diglycinate. The increase in spreading power is greatly influenced by the consistency of the preparation. The consistency of the preparation can be measured by the viscosity test. The greater the concentration of CMC-Na used, the higher the viscosity. Spreadability is inversely proportional to viscosity, where the higher the viscosity of preparation, the smaller the spreading power [14]. Based on the One-way ANOVA statistical test, the value of Sig was <0.05 (p-value = 0.000), meaning there was a significant difference between the dispersive power and the formula. This showed that variations in the concentration of CMC-Na affect the spreadability of the hydrating serum preparation of potassium azeloyl diglycinate. There was no significant difference in the stability test for the spreadability of the preparation in the cycling test. In other words, the cycling test for 6 cycles does not affect the spreadability of the preparation.

Adhesion test

The adhesion test aims to examine the ability of the preparation to adhere to the skin. The greater the adhesion value, the longer the contact with the skin will be. This will result in maximum absorption of the preparation by the skin. The adhesion test showed that formula 3 had the greatest adhesion, while formula 1 had the smallest adhesion. This is because CMC Na can increase the viscosity of the preparation in each formula. The concentration of CMC Na in formula 3 was higher than that found in formulas 1 and 2, so that the viscosity of formula 3 was higher than that found in formulas 1 and 2. The adhesion of preparation is closely related to its viscosity value. The higher the viscosity value, the thicker the preparation, resulting in greater adhesion to the skin. On the contrary, the lower the viscosity value, the thinner the preparation, causing less adhesion when used on the skin [11]. Based on the One-way ANOVA statistical test, the value of Sig was <0.05 (p value = 0.000), meaning that there was a significant difference in the stickiness of each formula. This shows that variations in the concentration of CMC Na affected the adhesion value of hydrating serum preparation of potassium azeloyl diglycinate. There was no significant difference in the adhesion test of each formula, meaning that the cycling test for 6 cycles did not affect the adhesion of the preparations.

Hydration test

The hydration test aims to examine the ability of the preparation to provide a hydrating effect on the skin. Skin moisture testing was carried out by measuring the hydration value of the stratum corneum of the test subject. The water content in the outer layer of the stratum corneum of normal skin was about 10%, and in the inner layer was about 30% [4],[18]. In the skin hydration test, the respondents used the best formula of the three hydrating serum formulas of potassium azeloyl diglycinate. Homogeneity, viscosity, spreadability, adhesion, and pH tests for the three formulas produced good test results and complied with the requirements for serum preparations.

There are differences in the results of the organoleptic test (consistency) for the three formulas. Formula 1 had a slightly runny gel preparation consistency, Formula 2 produced a slightly thick gel preparation consistency, and Formula 3 showed a thick gel preparation consistency. Formula 2, with CMC-Na concentration of 1.25%, was the best formula, based on the results of the organoleptic test (consistency), since the consistency of formula 2 is neither too thin nor too thick.

The observation results of the hydration test of serum preparations in respondents before (Pretest) and after using serum (Posttest) can be seen in table 4. Table 4 shows that the water content and oil content of the respondents increased, as shown in the posttest results. Based on these data, it can be concluded that hydrating serum of potassium azeloyl diglycinate is proven to increase skin moisture. In this study, there was an increase in the oil content by 8.39% and the water content by 8.86%. The composition of the hydrating serum Potassium azeloyl diglycinate consists of a glycine compound, which has the ability to hydrate the skin [16]. Previous research stated that glycine is an amino acid.

Amino acids added by other chemicals form a natural moisturizing factor in the stratum corneum, which functions to trap water to moisturize the stratum corneum [6]. In addition to the glycine content in the hydrating serum of potassium azeloyl diglycinate, there were also glycerin and propylene glycol, which function as humectants. Humectants are water-soluble ingredients with high water absorption abilities that help the skin retain moisture. Humectants are able to attract water from the atmosphere (when the atmospheric humidity is more than 80%) and the epidermis layer, increasing the water content in the stratum corneum.

This can happen because it contains a common hydroxyl group that allows it to form hydrogen bonds and absorb water. Humectants will penetrate into the stratum corneum and connect with stratum corneum lipids or proteins to increase moisture and hydration in the skin [3]. Based on the Paired-Samples T Test, it is known that there is an average difference between the results of the moisture content values between the pretest and the posttest, meaning that there is an effect of using potassium azeloyl diglycinate serum in increasing the moisture content in the skin of the five respondents.

Table 4. Hydrating serum Potassium azeloyl diglycinate hydration test results.

Respondent	Oil Content (%)		Water Content (%)	
	Pre test	Post test	Pre test	Post test
1	22.30	29.00	49.00	64.50
2	21.00	23.40	46.80	52.00
3	17.50	18.90	39.00	42.20
4	13.40	14.70	29.90	35.70
5	30.80	60.95	68.50	83.10
Average	21.00	29.39	46.64	55.50

Preliminary dermal acute toxicity test

Observations were made from day 1 after the application of the preparation until day 14 to find out whether any rats died. The results of observations in the eight test groups showed that there were no rats that died for 14 days. This indicates that potassium azeloyl diglycinate serum is safe for use on the skin and does not cause side effects or toxicity reactions.

Tabel 5. Results of the percentage of animal deaths.

Group	Number of dead
F1	0
F2	0
F3	0

▪ CONCLUSION

The stability of the physical and chemical properties of hydrating serum preparations can be tested by varying the three concentrations of CMC-Na. Increasing the concentration of CMC-Na will result in an increase in adhesion, pH, and viscosity. This reduces the spreadability of the preparation and increases the variation in gel consistency throughout the formula. Formula 2, which measures the respondent's water content, is the most effective formulation. The posttest findings on the water content of the respondents' skin, where the oil content increased by 8.39% and the water content by 8.86%, showed that this formula had the ability to hydrate the skin. This serum is safe to use based on preliminary acute dermal toxicity test results.

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