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Research Article

Optimization of Propylene Glycol and Na CMC in Gel Serum Preparations of Chicken Bone Collagen Antioxidant *(Gallus gallus domesticus)*

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Abstract: One important component found in the dermis layer of the skin is collagen. A decrease in collagen levels can result in reduced skin thickness and strength, loss of elasticity, and decreased skin hydration. Chicken bones serve as a valuable alternative source for the commercial production of collagen, which can be used in easily applicable pharmaceutical preparations, such as gel serum. Antioxidant gel serum preparations are developed by optimizing chicken bone collagen gel serum combined with Na CMC (0-2%) and propylene glycol (8-10%) using the Simplex Lattice Design method, so it is obtained 8 consecutive runs, the comparison is RI and RIII (1%: 9%), RII and RVIII (2%: 8%), RIV (0.5%: 9.5%), RV (1.5%: 8.5%), RVI and RVII (0%: 10%). The results indicate that the optimal formula consists of 1.984% Na CMC and 8.016% propylene glycol, yielding a pH of 4.50, a viscosity of 1700 cPs, adhesion of 1.72 seconds, spreadability of 6.15 cm, and IC_{50} of 57.36 ppm. Validation tests using the T-test demonstrated no significant difference between the observation and prediction results, confirming that the obtained formula was valid.

Keywords: antioxidant, chicken bone collagen, Na CMC, optimization, propylene glycol

1. INTRODUCTION

One of the causes of skin damage is free radicals in the form of ultraviolet rays [1]. Decreased collagen levels lead to a decrease in thickness and strength, loss of elasticity, and reduced skin hydration [2], therefore collagen is needed form the outside to prevent this form happening. Chicken bone are a great alternative source for producing collagen commercially. Chicken bones contain about 15.8% to 32.8% collagen [3]. Collagen sourced from animals has many benefits such as antioxidant [4] and anti-aging [5]. Based on the the activity of chicken bone collagen, it needs to be developed into pharmaceutical preparations to facilitate its application such as gel serum.

Important components in gel serum include gelling agents and humectants, which influence the preparation's physical properties. Na CMC has several advantages as a gelling agent it is neutral, forms strong bonds with active compounds, and stabilizes emulsions. Additionally, Na CMC is non-toxic and not irritation [6]. Propylene glycol can enhance the formulation's stability, improve preservative solubility, and help retain moisture by binding water from humid air. The main purpose of adding propylene glycol is to maintain the water content in the preparation, which helps preserve the stability of the product's physical properties [7]. Optimizing gelling agents and humectants is essential to achieve the best physical characteristics for the preparation. Although research on chicken bone collagen extract in the form of cream has been carried out, its antioxidant activity is limited (medium category) [8], therefore the optimization of propylene glycol and Na CMC in gel serum preparations of chicken bone collagen extract must be done using the simplex lattice design method.

2. MATERIALS AND METHODS

The materials used in extraction of CH_3COOH (Merck-Pro analysis), NaOH (Hangzhou Lizu Co.,Ltd-Technical grade), chicken bone powder, standard bovine collagen(Hainan Huayan Collagen Technology Co,. Ltd-Pharmaceutical grade). The materials for the formulations process are Na CMC Da-chi Kagyo-Pharmaceutical grade), propylene glycol (Pharmaceutical grade), methyl paraben (Pharmaceutical grade), citrus essence, aquadest. The materials used in antioxidant activity test of methanol (Smart Lab-Pro analysis) and DPPH powder (Sigma Aldrich-Pro analysis).

2.1. Preparation of Chicken Bone Collagen Extract

Freeze Drying chicken bone collagen has characteristics such as a distinctive smell with a brownish-yellow color, and in the form of a coarse powder. The first step in this process involves sample preparation and the extraction of collagen from chicken bones. The chicken bone waste was carefully cleaned to remove any meat and dirt, then dried in an oven at 70°C for 6 hours. The dried chicken bones were soaked in a 0.1M NaOH solution at a ratio of 1:10 for 48 hours. Afterward, the bones were soaked in a 1M acetic acid (CH_3COOH) solution for 24 hours. The resulting filtrate, which contained collagen, was filtered using Whatman No. 1 filter paper and a Buchner funnel. Finally, the filtrate was concentrated using a rotary evaporator (Heidolph Hei-Vap) and dried with a freeze dryer (Alpha 1-2 LDplus) to obtain the dried collagen [5].

2.2. Analysis of Collagen Function Groups with FTIR Method

Two milligrams of freeze-dried chicken bone collagen are ground until smooth. The characteristics of collagen extracted from chicken bone were determined using the FTIR Spectrum-One, with a wavelength range of 750 to 4000 Cm^{-1} [9].

2.3. Formulation of Chicken Bone Collagen Gel Serum

Table 1. Formulation of Chicken Bone Collagen Gel Serum used Design Expert 13

Ingredients	RUN							
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**
Chicken Bone Collagen (%)	5	5	5	5	5	5	5	5
Na CMC (%)	1	2	1	0.5	1.5	0	0	2
Propylene glycol (%)	9	8	9	9.5	8.5	10	10	8
Methyl Paraben (%)	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Essence Citrus (%)	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Aquadest ad (%)	100	100	100	100	100	100	100	100

All ingredients in each formula (Table 1) are made with gel serum was prepared by swelling Na CMC in hot distilled water to form serum mass. Methyl paraben was dissolved in propylene glycol, mixed into the serum mass and stirred until homogeneous. The active substance in the form of chicken bone collagen is dissolved using distilled water, essence is added and then stirred until evenly distributed. The preparation was put into a serum container and sonicated for 1.5 hours to accelerate the dissolution of the substance [10].

2.4. Physical Charactericiation of Chicken Bone Collagen Gel Serum

The organoleptic test involves smell, texture, and color by observing visual appearance and skin sensation. Serum homogeneity is applied to transparent glass. The homogeneity is indicated by the absence of rough grains. Testing the pH of the preparation is measured using a pH meter, standard pH requirements for serum gel preparations are 4.5-5.5 [11]. Viscosity test using viscometer Brookfield spindle No. 63 and 64 at 10, 20, 50, and 100 rpm. The viscosity value of serum gel is in the range of 230-3000 cPs. Adhesion test as much 0.25 g of gel serum was placed between 2 glass object, then pressed a 1 kg load for 5 minutes, the load was lifted and given a load of 80 g on the tool and noted the gel serum release time [12]. The spreadability test of gel serum weighed as much as 0.5 g placed on glas and waited for 1 minute to be measured, then added every 50 g of load and allowed to stand for 1 minute and then measured the constant diameter. The requirement for spreadability is to have a diameter in the range of 5-7 cm [13].

2.5. Antioxidant Activity Test on Chicken Bone Collagen Gel Serum

In testing antioxidant activity with IC_{50} parameters using the DPPH method. The stock solution of gel serum preparation was pipetted 3.0 mL; 4.0 mL; 5.0 mL; 6.0 mL; and 7.0 mL so that 5 concentrations of gel serum were obtained. Each concentration was added with 4 mL of DPPH solution. The solution that has been mixed is then homogenized and allowed to stand for operating time and measured absorbance with a UV-Vis spectrophotometer at a maximum wavelength [14].

3. RESULTS AND DISCUSSION

The yield of chicken bone collagen from freeze drying was obtained as much as 6,59%.

3.1. Analysis of Collagen Function Groups with FTIR Method

The collagen produced through the freeze-drying process is analyzed for its functional groups using the FTIR method. This analysis helps determine the characteristics of the extracted collagen. The FTIR measurements were conducted in the spectral range of 750 to 4000 cm^{-1}. The results of the FTIR analysis are presented in the form of a graph, with the wave number on the x-axis and percentage transmittance on the y-axis.

The FTIR spectrum of collagen derived from chicken bones shown absorption peaks in the amide region, specifically including amide A, amide B, amide I, amide II, and amide III. These peaks correspond to the characteristic functional groups found in collagen [5]. Based on the infrared characteristics of the molecular function group, there is an N-H bond bending vibrations in the hydrogen bond of amide group A in the absorption region of 1620-1590 Cm^{-1} with strong intensity. Amida B showed the presence of a C-H bending group in the absorption region ~1340 Cm^{-1} with weak intensity. In the amide region I shows the presence of a C=C group which is a secondary structure of proteins in the absorption region ~1653 Cm^{-1} with moderate intensity. Amida II points to the N-H group in the absorption region of 1650-1550 Cm^{-1} with moderate intensity [15], in group III of the amide category, there is an N–H bond within the collagen helical structure, which appears in the absorption region of 1250-1350 cm^{-1} [16].

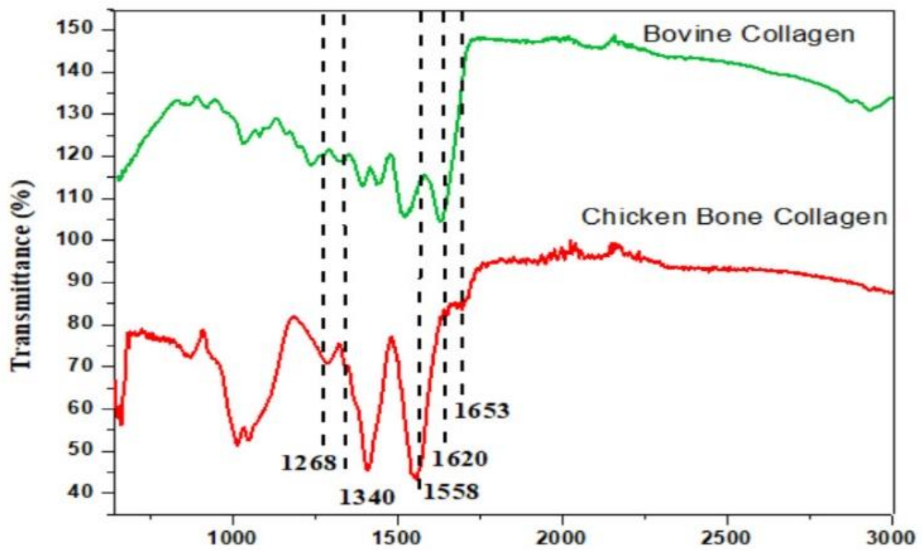

Figure 1. FTIR Spectrum Results of Broiler Bone Collagen and Standard Bovine Collagen.

Table 2. Functional Group Analysis Result of Chicken Bone Collagen By FTIR Method

Functional Groups		**Wave Numbers**		
Comparison Commercial Collagen "Bovine Collagen" (Cm^{-1})	**Chicken Bone Collagen (Cm^{-1})**	**Comparison Commercial Collagen "Bovine Collagen" (Cm^{-1})**	**Chicken Bone Collagen (Cm^{-1})**	**Functional Groups**
N-H	N-H	1620	1620	Amida A
C-H	C-H	1340	1340	Amida B
C=C	C=C	1653	1653	Amida I
N-H	N-H	1558	1558	Amida II
N-H	N-H	1268	1268	Amida III

3.2. Test of Physical Characteristics and Antioxidant Activity of Chicken Bone Collagen Gel Serum

In testing the physical characteristics and antioxidant activity of chicken bone collagen gel serum, the results can be seen in Table 3.

Table 3. Test of Physical Characteristics and Antioxidant Activity of Chicken Bone Collagen Gel Serum

Run	**Organoleptic**			**Homogeneity**	**pH**	**Viscosity (cPs)**	**Adhesion (s)**	**Spreada-bility (cm)**	**IC_{50} (ppm)**
	Color	**Odor**	**Texture**						
I	Bone-white	Citrus	Liquid	Homogeneous	4.33	164.4	0.99	6.6	61.49
II	Bone-white	Citrus	Slightly thick	Homogeneous	450	1732	1.66	6.2	53.35
III	Bone-white	Citrus	Liquid	Homogeneous	4.19	171.6	1.04	6.0	51.14
IV	Bone-white	Citrus	Liquid	Homogeneous	4.32	103.2	0.81	8.0	59.49
V	Bone-white	Citrus	Slightly thick	Homogeneous	4.42	248.3	1.45	5.7	64.40
VI	Bone-white	Citrus	Liquid	Homogeneous	4.35	224.4	0.54	9.3	47.87
VII	Bone-white	Citrus	Liquid	Homogeneous	4.40	148.8	0.53	8.9	47.22
VIII	Bone-white	Citrus	Slightly thick	Homogeneous	4.49	1932	1.77	6.3	59.97

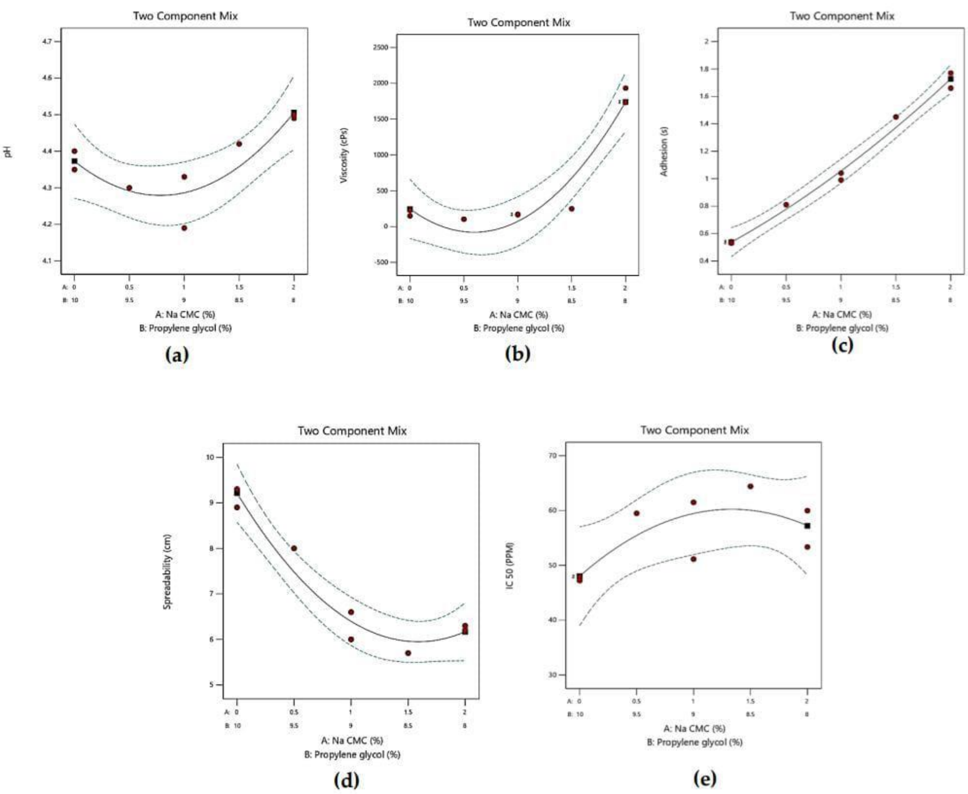

Figure 2. Graph of Effect Different Concentrations of Na CMC and Propylene glycol on Characteristic Test and Antioxidant Activity of Chicken Bone Collagen Antioxidant Gel Serum Based on Simplex Lattice Design : (a) pH (b) Viscosity (c) Adhesion (d) Spreadability (e) IC_{50}

Based on the results of pH testing, the Simplex Lattice Design equation is obtained Y= 1.72747 (A) + 0.437275 (B) – 0.152941 (A)(B). The coefficient value illustrates that Na CMC has more influence in increasing the pH of gel serum compared to propylene glycol. This is because of Na CMC has a pH of 6.0-8.5 while propylene glycol has a pH of 3-6 [17]. The addition of Na CMC causes an increase in pH due to the chemical properties of Na CMC as a salt of strong alkaline and weak acid, in Na CMC solution tends to produce hydroxide ions (OH-) so that the solution is more alkaline, besides Na CMC there is a hydrocolloid content which causes the pH to increase because it contains many carboxyl groups that will be hydrolyzed so that it affects the pH increase of Na CMC higher than propylene glycol [18]. The graph effect of different concentrations of Na CMC and Propylene glycol on pH can be seen in Figure 2 point (a).

Based on the results of viscosity testing, the Simplex Lattice Design equation is obtained Y= 8137.94059 (A) + 24.60007 (B) – 920.74118 (A)(B). The Na CMC increased the viscosity compared to propylene glycol. This is due to the increase in the number of Na CMC molecules responsible for the formation of the gel matrix, but the addition of excipients such as propylene glycol which has a liquid consistency can reduce the viscosity of the preparation [19]. Another reason is due to the nature of Na CMC as a cellulose derivative, it is often used in the pharmaceutical industry as a thickener, stabilizer, and has good binding power. The Na CMC increased the viscosity of the solution, the higher the concentration of Na CMC added in a formula, the higher the viscosity produced. The viscosity value of Na CMC is affected by its ability to bind water. The graph effect of different concentrations of Na CMC and Propylene glycol on viscosity can be seen in Figure 2 point (b).

Adeshion test equation Y = 1.25468 (A) + 0.053631 (B) – 0.075686 (A)(B). In the adhesion test, the coefficient value of Na CMC was higher than that of propylene glycol. This value indicates that the addition of Na CMC can increase the adhesion of gel serum compared to propylene glycol. This is caused by Na CMC can improve the adhesion of a preparation because it is related to the increase in viscosity, the higher the concentration of Na CMC, the higher the viscosity produced. Higher viscosity makes the preparation thicker so that it can adhere or stick longer to the skin surface. Na CMC also has a large cohesion force that allows its molecules to bind to each other well, this causes the gel to tend to collect and be difficult to spread, thereby increasing its ability to adhere to the skin [20]. The graph effect of different concentrations of Na CMC and Propylene glycol on adhesion can be seen in Figure 2 point (c).

Spreadability test equation Y = 9.75206 (A) + 0.921340 (B) – 1.29412 (A)(B). The coefficient of Na CMC is higher than coefficient of propylenglycol. The Na CMC increased the spreadability compared to propylene glycol. This is caused by the higher the concentration of Na CMC, the lower spreadability is resulted. In the test of spreadability, there is an inverse proportional correlation with viscosity, where the low viscosity of gel serum will produce high spreadability. This is because Na CMC functions as a gelling agent which tends to increase the viscosity of the preparation, while propylene glycol which functions as a humectant has the ability to reduce the viscosity of gel serum. The graph effect of different concentrations of Na CMC and Propylene glycol on spreadability can be seen in Figure 2 point (d).

The antioxidant activity of chicken bone collagen gel serum from Design Expert 13 formula was determined in vitro using UV-Vis double beam spectrophotometer with wavelength of 514,6 nm and operating time of 47 minutes. Based on the antioxidant activity test results, the equation Y = - 45.26700 (A) + 4.80300 (B) + 6.83333 (A)(B) was obtained. In this case, it means Na CMC and propylene glycol bases are appropriately used in the preparation of chicken bone collagen antioxidant gel serum, as evidenced by the increasing or decreasing concentration of Na CMC and propylene glycol does not have a significant effect on antioxidant activity because chicken bone collagen extract which is considered to have strong antioxidant activity after being formulated into preparations produces IC_{50} values that fall into the strong category. The graph effect of different concentrations of Na CMC and Propylene glycol on antioxidant activity can be seen in Figure 2 point (e).

The optimum formula was obtained from calculations using Design Expert 13 with parameters of pH, viscosity, adhesion, spreadability, and IC50 value of antioxidant activity of chicken bone collagen gel serum (Table 4). The optimum formula obtained is the ratio of Na CMC and propylenglycol with concentrations of 1.984% and 8.016%. The optimum formula produced was then made as many as 5 replicates and tested again including physical characteristics test and antioxidant activity test of chicken bone collagen with IC_{50} parameters.

The equation was validated using a One Sample T-test to confirm its accuracy, as derived from the Simplex Lattice Design method in the Design Expert 13 application. This analysis was conducted at a confidence level of 95%. The results of the T-test analysis can be found in Table 4.

Table 4. One Sample T-Test and Observation Result

Parameters Test	Observation Result	Prediction Result	Signification	Conclusion
pH	4.52 ± 0.02	4.50	0.070	Not significant difference
Viscosity (cPs)	1780 ± 69.31	1700.003	0.061	Not significant difference
Adhesion (s)	1.73 ± 0.02	1.716	0.239	Not significant difference
Spreadability (cm)	6.29 ± 0.24	6.152	0.265	Not significant difference
IC_{50} (ppm)	60.03 ± 8.16	57.365	0.504	Not significant difference

The results of the observation for each test parameter compared to the prediction results for validation of the Simplex Lattice Design equation using One Sample T-test on the formula showed that the test parameters of pH, viscosity, adhesion, spreadability, and IC50 were not significantly different because the significance value of the predicted results with the experimental results was >0.05. This shows that the T-test is valid and in accordance with the expectations of the researcher.

4. CONCLUSION

There is a significant effect of the ratio of Na CMC and propylene glycol on the physical characteristics of gel serum including pH test, viscosity, adhesion, spreadability, and antioxidant activity of chicken bone collagen gel serum. The concentration of Na CMC 1.984% and propylene glycol 8.016% it can produce chicken bone collagen gel serum formulation with optimal physical characteristics and antioxidant activity.

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