Optimization of Chlorphenesin Concentration and Solubilizer for Wet Wipes Through Challenge and Accelerated Stability Tests

M. Arif Lukman Hakim [a,1], Adi Permadi [b,2,*], Erna Astuti [a,3], Asdarina binti Yahya [b,4], Farahidah Mohamed [c,5]

[a] Department of Chemical Engineering, Faculty of Industrial Technology, Universitas Ahmad Dahlan, Yogyakarta, Indonesia
[b] School of Chemical Engineering, College of Engineering, Universiti Teknologi MARA (UiTM), Selangor, Malaysia
[c] Department of Pharmaceutical, Kulliyyah of Pharmacy, International Islamic University Malaysia, Pahang, Malaysia
[1] 2308054011@webmail.uad.ac.id; [2] adi.permadi@che.uad.ac.id*; [3] erna.astuti@che.uad.ac.id; [4] asda@uitm.edu.my; [5] farahidah@iium.edu.my
* corresponding author

ARTICLE INFO

Article history
Received June 11, 2025
Revised July 20, 2025
Accepted July 21, 2025

Keywords
Accelerated stability test
Challenge test
Chlorphenesin
Preservative
Wet wipes

ABSTRACT

The household health equipment industry, particularly wet wipes, is growing rapidly, with a projected CAGR of 2.60%. Key concerns include microbial contamination and high preservative concentrations that can cause skin irritation. This study aimed to optimize the concentration and type of chlorphenesin solvent used as a preservative to mitigate these issues. The study tested chlorphenesin at concentrations of 0.20%, 0.25%, and 0.30% with two solvents, propylene glycol and ethanol. Two experimental tests were conducted: the challenge test, which focused on the ability of the preservative system to inhibit the growth of pathogenic microorganisms, such as Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, and Aspergillus brasiliensis, over 28 days, on the other hand, the accelerated stability test, focused to evaluates the preservative's ability to maintain the physicochemical properties of the product, including visual appearance, aroma, sensory attributes, and pH, over 6 months under extreme conditions (40 °C; 75% RH). This study found that the formulation with 0.30% chlorphenesin, using either propylene glycol or ethanol as a solvent, showed the best reduction against pathogenic microorganisms (S. aureus, P. aeruginosa, C. albicans, and A. brasiliensis) on the second day of the challenge test. In the accelerated stability test, the formulation with 0.30% chlorphenesin and propylene glycol received the highest score (9.000) across all parameters (visual, homogeneity, odor, and sensory applications). However, at the same concentration, when ethanol was used as the solvent, the formulation exhibited lower scores in odor and sensory applications. Therefore, the recommended formulation for wet wipes is a formula containing 0.30% chlorphenesin with propylene glycol as the solvent.

1. Introduction

The utilization of wet wipes has exhibited a marked increase over the past decade, particularly in the context of personal cleanliness and hygiene. These wipes find application in diverse domains, including personal care, baby care, and household care, thus occupying a prominent position in the consumer market [1]. Wet wipes have been the subject of continuous market expansion annually. From 2011 to 2016, the annual growth rate of wet wipes worldwide increased by up to 5.10%. In the American market, it is predicted that the average yearly growth rate of wet wipes from 2020 to 2030 will reach 2.60% [2], [3]. The use of wet wipes has been steadily increasing, particularly in personal care, baby care, and household hygiene. One of the main challenges in the development of damp

wipe products is dealing with microbial contamination caused by the cellulose content in non-woven fabrics and the high water content in the wet wipe formulation [4], [5], [6]. Another issue with wet wipe products is the use of preservatives, which can increase the risk of irritation, allergic reactions, and sensitization in users. Therefore, there is a need for preservatives that are sufficiently safe for use in wet wipe products [7], [8], [9].

This study aims to test and optimize the concentration and type of solvent used for chlorphenesin in wet wipe formulations. A challenge test was conducted to assess chlorphenesin's ability to prevent the growth of pathogenic microorganisms, while an accelerated stability test was performed to evaluate the long-term stability of the product, including parameters such as pH, visual appearance, odor, homogeneity, and sensory perception [10], [11], [12]. The novelty of this research lies in testing chlorphenesin as an alternative preservative in wet wipes, which is expected to address microbial contamination issues without causing irritation or adverse skin effects, while also providing new insights into the stability of wet wipe formulations with this preservative. The results of this study are expected to make a significant contribution to the development of safer and more effective preservatives for wet wipe products, as well as open up opportunities for the broader application of chlorphenesin in the personal care industry.

2. Research Methodology

2.1. Materials

The materials utilised in this study are categorised into two distinct groups: materials employed in the formulation of products and materials used for testing purposes. The materials employed in the formulation of the raw materials are as follows: purified water (99% purity from PT. Mulia Artha Saudara), chamomile extract (99% purity from PT. Merpati Mahardika), propylene glycol (99% purity from Coswealth SDN. BHD), chlorphenesin (99% purity from PCI Innovative Chemicals SDN. BHD), PEG 40 hydrogenated castor oil (99% purity from Trimak Co. Ltd), ethanol (96% purity from PT Karsavicta), fragrance fresh leaves (99% purity from Givaudan Co.), disodium calcium EDTA (99% purity from PT. Lug Chemicals), and lactic acid (98% purity from Corbion Co.), while the test materials used were nutrient tryptic soy agar (TSA), polysorbate 80, sabouraud dextrose agar, peptone solution 0.1%, NaCl, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 10231, and *Aspergillus brasiliensis* ATCC 16404. It is noteworthy that all test materials are pro-analysis grade and provided by PT Saraswanti Indo Genetech.

2.2. Wet Wipes Formulation

The wet wipe formulation consists of two types. Formula ‘A’ contains 20% propylene glycol as a solvent with chlorphenesin at 0.20% to 0.30%. Formula ‘B’ uses 10% ethanol as a solvent with chlorphenesin at 0.20% to 0.30%. The composition is shown in Table 1.

Table 1. Wet wipes liquid formulation

Raw Material	Percentage (%)					
	A-1	***A-2***	***A-3***	***B-1***	***B-2***	***B-3***
Purified water	78.83	78.78	78.73	88.83	88.78	88.73
Chamomile extract	0.25	0.25	0.25	0.25	0.25	0.25
Ethanol	-	-	-	10.00	10.00	10.00
Propylene glycol	20.00	20.00	20.00	-	-	-
Chlorphenesin	0.20	0.25	0.30	0.20	0.25	0.30
PEG 40 hydrogenated castor oil	0.30	0.30	0.30	0.30	0.30	0.30
Fragrance	0.12	0.12	0.12	0.12	0.12	0.12
Disodium calcium EDTA	0.20	0.20	0.20	0.20	0.20	0.20
Lactic acid	0.10	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00

The liquid formulation of the wet wipes is prepared by combining chlorphenesin with propylene glycol or ethanol as a solvent, in premix tank -01. Fragrance and PEG 40 hydrogenated castor oil are then added to premix tank -02. In the mixer tank, water, chamomile extract, disodium calcium EDTA, and lactic acid are thoroughly mixed. The chlorphenesin-preservative mixture is then

transferred to the mixer tank and stirred until homogeneous. Finally, the premix from tank-02 is added to the mixer tank and stirred until homogeneous.

2.3. Challenge Test

The challenge test in this study follows the method outlined in "Regulation of the Head of the Food and Drug Supervisory Agency of the Republic of Indonesia No. HK. 03.1.23.08.1107331 of 2011" to assess the preservative effectiveness of the cosmetic product [13]. A total of 150 sheets of wet wipes, containing 483 g of liquid formula, were homogenized using an STO-4 paddle blender. Next, 100 g of liquid from the wipes was transferred into four sample bottles. Two bottles were inoculated with bacterial suspensions of *S. aureus* and *P. aeruginosa* at concentrations of 10^5–10^6 cfu/mL, while the other two were inoculated with *C. albicans* and *A. brasiliensis* fungi at concentrations of 10^4–10^6 cfu/mL. Then, 20 g of liquid from each bottle was transferred into new bottles, and the samples were incubated at 22°C in the dark for observation on days 2, 7, 14, and 28. The number of surviving microorganisms was determined by performing serial dilutions (10^-1 to 10^-4), adding 1 mL of each dilution to a Petri dish with the appropriate medium (TSA for bacteria, SDA for fungi). Bacterial plates were incubated at 35°C for 2 days, while fungal plates were incubated at 25°C for 3 days. The challenge test was performed with two replicate trials for each sample. Log reduction was calculated using the formula: $log_{reduction} = logN_0 - logN_t$, where N_0 is the bacterial count on day 0, and N_t is the count on days 2, 7, 14, and 28.

2.4. Procedures

Accelerated stability testing was performed on two aspects: the wet wipes formula liquid and the complete wet wipe product (non-woven fabric + formula liquid). Testing was conducted at 40°C and 75% RH, with the parameters listed in Table 2.

Table 2. Accelerated stability test parameters on wet wipes

Parameters	Method	Applications
Visual testing	Organoleptic test (SNI 01-2346-2006)	Liquid formula and wet wipes product
Odor testing	Organoleptic test (SNI 01-2346-2006)	Liquid formula and wet wipes product
Homogeneity testing	Organoleptic test (SNI 01-2346-2006)	Liquid formula
Wet wipes usage sensations testing	Organoleptic test (SNI 01-2346-2006)	Wet wipes product
pH	Potentiometry (SNI-8526-2018)	Liquid formula

2.5. Procedures

Six wet wipes were tested for organoleptic properties, including visual appearance, odor, homogeneity, and skin sensation. A rating scale was used: a 'perfect' sample showed no changes, a 'good' sample had slight modifications, and a 'not good' sample had significant changes. The test was conducted by eight trained panelists following SNI 01-2346-2006, with eight replications per sample. The results were analyzed statistically using the Kruskal–Wallis test. If no significant differences were found, no further testing was needed; otherwise, the Mann–Whitney U test was applied.

The pH of the wet wipes fluid is measured by calibrating the pH meter with buffer solutions at pH 4.01, 6.86, and 10.00. After calibration, the electrode is rinsed with distilled water, dried with tissue, and then immersed in the sample solution until the pH stabilizes before recording the result.

3. Results and Discussion

3.1. Evaluating the Efficacy of Preservatives in Wet Wipes Through Challenge Test

The challenge test assesses chlorphenesin's effectiveness as a preservative in inhibiting microorganism growth over 28 days. A high log reduction indicates the preservative's ability to reduce microorganisms in wet wipes significantly. A preservative is effective if bacterial and yeast populations decrease by at least 99% on day 2, 99.9% on day 7, and remain stable by day 28.

The challenge test on the gram-positive bacterium *S. aureus* (concentration range of 1.1 x 10^6 to 1.5 x 10^6 cfu/g) in Fig. 2a demonstrated that chlorphenesin at 0.20%, using either propylene glycol or ethanol as solvents, reduced the bacterium by 2.60 (SD: 0.02 cfu/g) log reduction and 2.55 (SD: 0.04 cfu/g) log reduction on day 2, respectively. Both solvents achieved complete reduction,

resulting in more than 6 log reduction from day 7 to day 28. At concentrations of 0.25% and 0.30%, chlorphenesin with either solvent eliminated *S. aureus* by more than 6 log reduction by day 2, with no bacterial growth observed until day 28. Gram-positive bacteria, such as *S. aureus*, have thinner cell walls compared to gram-negative bacteria. At a concentration of 0.20% chlorphenesin, the preservative system can reduce *S. aureus* by more than 6 log reduction on day 7 of the test. Increasing the chlorphenesin concentration accelerates bacterial reduction. This occurs because chlorphenesin releases chlorine ions in solution, which can destroy the cell wall of *S. aureus*. The higher the chlorphenesin concentration, the more chlorine ions are present in the product, leading to faster bacterial death [15], [16], [17].

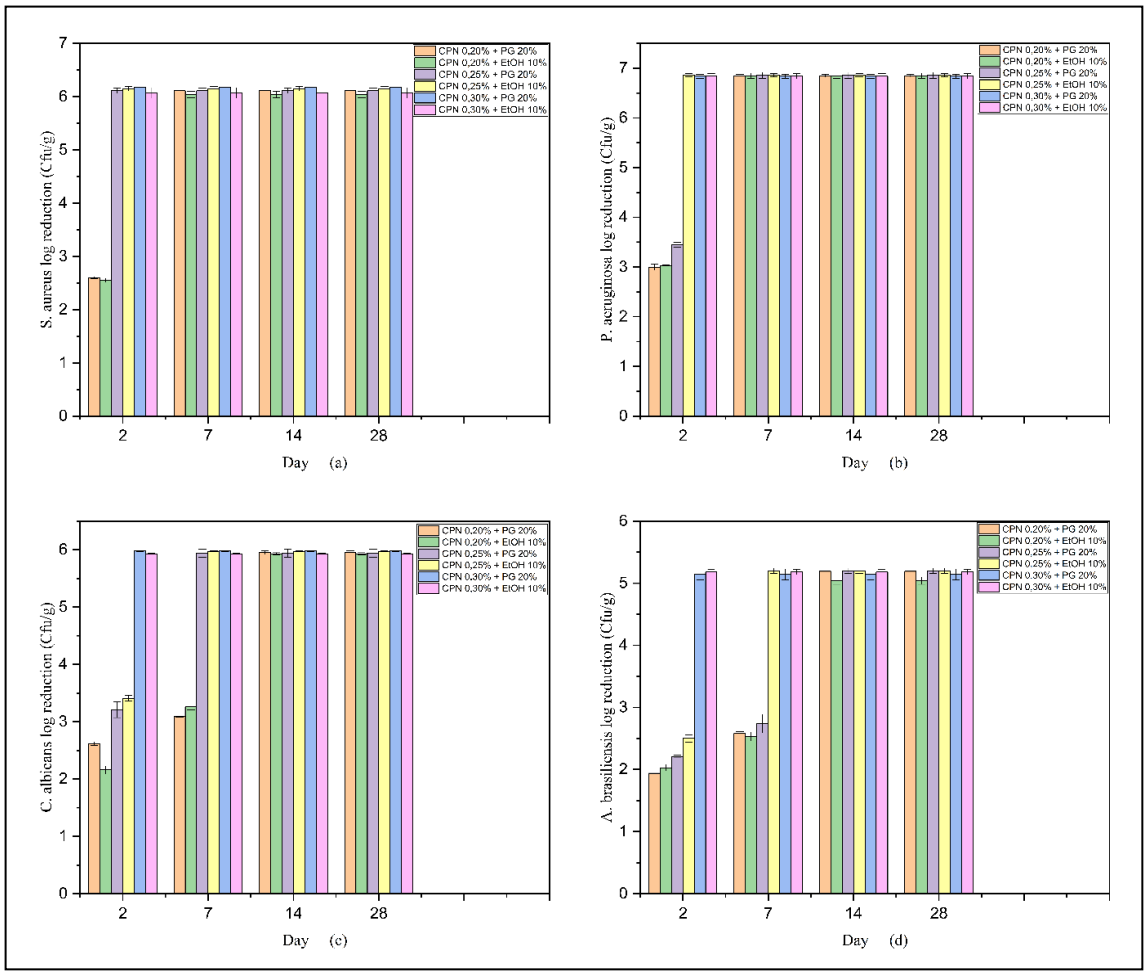

Fig. 1. Wet wipes liquid challenge test results. (a) reduction against *S. aureus*, (b) reduction against *P. aeruginosa*, (c) decrease against *C. albicans,* and (d) reduction against *A. brasiliensis*

As shown in Fig. 2b, the challenge test of wet wipe fluid against *P. aeruginosa* at a concentration of 7.1 x 10^6 cfu/g demonstrated that chlorphenesin at 0.20% concentration, with either propylene glycol or ethanol as solvents, reduced the bacterial count by 3.00 log reduction (SD: 0.06 cfu/g) and 3.03 (SD: 0.01 cfu/g) log reduction on day 2, respectively, with complete bacterial eradication by days 7, 14, and 28. At 0.25%, chlorphenesin reduced the bacterial count by 3.45 log reduction (SD: 0.05) and more than 6 log reduction (SD: 0.06 cfu/g) on day 2, and eliminated microbial growth by more than 6 log reduction on days 7, 14, and 28. At 0.30%, chlorphenesin, with either solvent, eliminated more than 6 log reduction (SD: 0.03 cfu/g) by day 2, with no *P. aeruginosa* growth detected until day 28. This research shows that chlorphenesin effectively inhibits microbial contamination in wet wipe formulations at a minimum concentration of 0.20%, with propylene glycol or ethanol as solvents. The ideal preservative concentration is 0.25% using ethanol as a solvent, as it eliminates more than 6 log reduction of *P. aeruginosa* by day 2. This case shows that the choice of solvent significantly impacts bacterial reduction in the short term, particularly on day 2 of the challenge test for samples with 0.25% chlorphenesin. This is due to ethanol's strong

synergistic effect with this preservation system, as it disrupts microbial lipid membranes through dehydration and protein denaturation [18].

Figure 2c illustrates the results of the challenge test on *Candida albicans* with a concentration range of 8.5 x 10^5 to 9.5 x 10^5 cfu/g. Chlorphenesin at a 0.20% concentration, using propylene glycol and ethanol as solvents, achieved a reduction of *C. albicans* by 2.62 log reduction (SD: 0.03 cfu/g) and 2.15 log reduction (SD: 0.07 cfu/g) on day 2, respectively. On day 7, chlorphenesin (0.20%) dissolved in propylene glycol reduced *C. albicans* by 3.09 log reduction (SD: 0.01 cfu/g), while chlorphenesin (0.20%) in ethanol resulted in a reduction of 3.26 log reduction (SD: 0.05 cfu/g). By days 14 and 28, chlorphenesin at 0.20%, with either propylene glycol or ethanol as a solvent, achieved near-complete elimination of *C. albicans* (> 5 log reduction). At a 0.25% concentration, chlorphenesin, using propylene glycol and ethanol as solvents, reduced *C. albicans* by 3.21 log reduction (SD: 0.14 cfu/g) and 3.41 log reduction (SD: 0.05 cfu/g), respectively. This concentration also resulted in a reduction of over 5 logs in the elimination of *C. albicans* on days 7, 14, and 28. Finally, chlorphenesin at a 0.30% concentration, regardless of solvent, eliminated *C. albicans* by day 2 (> 5 log reduction), with no fungal growth observed until day 28. All tested concentrations passed the challenge test, regardless of whether propylene glycol or ethanol was used as the solvent. The optimal chlorphenesin concentration was 0.25% in both solvents, as it demonstrated the same reduction rate as the 0.30% concentration. Therefore, the 0.25% formulation is more cost-effective. Additionally, the type of solvent had no significant impact on *C. albicans*, as no substantial difference in log reduction was observed between the two solvents. In a previous study, an article stated that the minimum inhibitory concentration (MIC) of chlorphenesin against *C. albicans* yeast is 0.20%. Therefore, when chlorphenesin concentrations exceed the MIC, it can rapidly reduce *C. albicans*. However, when the chlorphenesin concentration is at the MIC, it can still reduce the number of *C. albicans*, but the process takes a relatively longer time [19], [20].

The challenge test on *A. brasiliensis*, conducted with concentrations ranging from 1.1 x 10^5 to 1.6 x 10^5 (Fig. 2.d), showed that chlorphenesin at 0.20% reduced the microorganism by 2.09 log reduction (SD: 0.00 cfu/g) and 2.03 log reduction (SD: 0.05 cfu/g) on day 2 when using propylene glycol and ethanol, respectively. On day 7, chlorphenesin in propylene glycol achieved 2.56 log reduction (SD: 0.03 cfu/g), while ethanol resulted in 2.53 log reduction (SD: 0.07 cfu/g). Both systems achieved more than 5 log reduction by day 14 and maintained it until day 28. At 0.25%, chlorphenesin in propylene glycol reduced *A. brasiliensis* by 2.21 log reduction (SD: 0.02 cfu/g) on day 2, 2.74 log reduction (SD: 0.15 cfu/g) on day 7, and more than 5 log reduction by day 14. However, in ethanol, chlorphenesin showed 2.50 log reduction (SD: 0.06 cfu/g) on day 2, reaching more than 5 log reduction by day 7. The most effective reduction was seen with 0.30% chlorphenesin, which achieved more than 5 log reduction by day 2, regardless of the solvent used, with no microbial growth detected until day 28. In this study, all tested formulations passed the challenge test according to BPOM regulations. However, chlorphenesin at 0.20%, using either propylene glycol or ethanol as the solvent, showed the slowest reduction of *A. brasiliensis*, achieving more than 5 log reduction by day 14. The optimal concentration was 0.30%, which achieved more than 5 log reduction by day 2, regardless of the solvent used. A significant difference was observed at 0.25% chlorphenesin, where the formulation with propylene glycol reduced *A. brasiliensis* by 2.74 logs on day 14, while the formulation with ethanol reduced it by more than 5 logs by day 7. Ethanol is known to have a synergistic effect with preservatives, aiding in the breakdown of microbial cell walls and accelerating microorganism reduction [21].

This study found that all tested formulations effectively inhibited the growth of *S. aureus*, *P. aeruginosa*, *C. albicans*, and *A. brasiliensis*. Chlorphenesin at 0.20%, using either propylene glycol or ethanol as the solvent, showed a slow reduction profile. At this concentration, the wet tissue formula reduced bacteria by more than 6 log reductions by day 7, while molds and yeasts were decreased by over 5 logs by day 14. Chlorphenesin at 0.25% exhibited a moderate reduction profile. For some microorganisms, such as *S. aureus* and *P. aeruginosa*, the reduction at 0.25% was similar to that at 0.30%, achieving a >5 log reduction in bacteria by day 2. However, for molds and yeasts, the reduction at 0.25% was slower by 5 days compared to 0.30%. Notably, at 0.25%, a synergistic effect was observed between chlorphenesin and ethanol, particularly for *P. aeruginosa* and *A. brasiliensis*. The reduction in gram-negative bacteria and molds was more than 1 log higher when chlorphenesin was dissolved in ethanol compared to propylene glycol. The most optimal formula in the challenge test was the 0.30% chlorphenesin formulation, whether using propylene glycol or

ethanol. Both formulations showed the fastest reduction, achieving >5 log reductions for all microorganisms by day 2 of the challenge test.

Chlorphenesin at a concentration of 0.30% was found to be more effective than commonly used preservatives in wet wipe formulations, including a mixture of sodium benzoate (0.50%), potassium sorbate (0.60%), and cetyl pyridinium chloride (0.05%) (preservative system 1), a mixture of polyaminopropyl biguanide (0.50%) and caprylyl glycol (0.50%) (preservative system 2), and a mixture of sodium benzoate (0.50%), potassium sorbate (0.60%), and phenoxyethanol (0.80%) (preservative system 3). This was demonstrated by the reduction of *P. aeruginosa*, *C. albicans*, and *A. brasiliensis* by preservative systems 1, 2, and 3. Preservative systems 1 and 3 reduced *P. aeruginosa* by more than 6 log reductions by day 14, while preservative system 2 achieved over 6 log reductions by day 7. Systems 1 and 3 reduced *C. albicans* by more than 5 log reductions by day 28, whereas system 2 achieved a more than 5 log reduction by day 14. For molds, preservative system 1 reduced mold growth by more than 5 log reductions by day 14. In comparison, systems 2 and 3 were unable to achieve more than 5 log reductions by day 28, indicating that some mold remained viable until the 28th day of the challenge test. In contrast, chlorphenesin at 0.30% was able to reduce all tested microorganisms by day 2 of the challenge test [22].

3.2. Accelerated Stability Test in Wet Wipes

Accelerated stability testing is a method used to evaluate the shelf life of cosmetic products by simulating extreme storage conditions. It examines the effects of environmental factors, such as temperature, light, and humidity, on the product's formulation over time. This process involves maintaining the product at 40°C and 75% relative humidity [12]. The study analyzes six parameters in wet wipe fluid and products, including visual characteristics, homogeneity, odor, sensory application, and pH. The data were analyzed using two statistical methods: multi-element variance analysis with the Kruskal-Wallis test and two-group variance analysis with the Mann-Whitney U test. Data are considered significantly different when the P value is < 0.05, and not significantly different when the P value is > 0.05 in the Kruskal-Wallis test. If no significant differences are found between two or more groups, the analysis ends after the Kruskal-Wallis test. However, if substantial differences are observed, the Mann-Whitney U test is used to identify which groups differ. In the Mann-Whitney U test, two groups are considered significantly different if the P value is < 0.05 and not significantly different if the P value is > 0.05. Results are presented in Tables 3 and 4.

Table 3. Result of accelerated stability test on wet wipes fluids

Formula	Parameters			
	Visual	***Homogeneity***	***Odor***	***pH***
A1 (CPN 0.20% + PG 20%)	9.000 ± 0.000^{a}	9.000 ± 0.000^{a}	8.850 ± 0.160^{a}	4.758 ± 0.007^{a}
A2 (CPN 0.25% + PG 20%)	9.000 ± 0.000^{a}	9.000 ± 0.000^{a}	9.000 ± 0.000^{b}	4.736 ± 0.015^{b}
A3 (CPN 0.30% + PG 20%)	9.000 ± 0.000^{a}	9.000 ± 0.000^{a}	9.000 ± 0.000^{b}	4.416 ± 0.047^{c}
B1 (CPN 0.20% + EtOH 10%)	9.000 ± 0.000^{a}	9.000 ± 0.000^{a}	7.906 ± 0.386^{c}	4.675 ± 0.015^{d}
B2 (CPN 0.25% + EtOH 10%)	9.000 ± 0.000^{a}	9.000 ± 0.000^{a}	8.251 ± 0.549^{cd}	4.561 ± 0.017^{e}
B3 (CPN 0.30% + EtOH 10%)	9.000 ± 0.000^{a}	9.000 ± 0.000^{a}	8.655 ± 0.301^{ad}	4.503 ± 0.008^{f}

Table 3 highlights four key parameters: visual characteristics, homogeneity, odor, and pH. Visual changes, such as color shifts, can impact the quality of wet wipe products. Odor variations may result from factors like solvent evaporation and microbiological contamination. Homogeneity is vital for the efficacy of active ingredients, as it influences the effectiveness of individual components. pH is critical for preserving the physical and chemical integrity of cosmetic formulations. A pH range of 3.50 to 7.50 is recommended, as an excessively acidic pH can lead to skin irritation or dryness [23], [24].

According to Table 3, no signs of emulsion separation were observed in the wet wipe fluid, nor were there any color changes or black spots on the non-woven fabric. This evaluation was conducted by eight panelists over a 24-week (6-month) period, assessing six sample types stored at 40°C and 75% RH. The panelists' assessments reflected these findings, with all eight panelists awarding a perfect score of 9.000 out of 9.000 for all formulas. When analyzed using the Kruskal-Wallis method, no significant differences were found among the tested formulations, confirming that all were stable (P value = 1.00). This stability is attributed to the effect of solvents, such as propylene

glycol or ethanol, which enhance chlorphenesin's solubility in water. As a result, chlorphenesin, which has low water solubility, remains stable without recrystallization during the accelerated stability testing [25].

While the visual stability remained consistent over 24 weeks, several formulations were found to have poor aroma stability. Using the Kruskal-Wallis method, significant differences were observed (P value < 0.01), leading to further analysis with the Mann-Whitney U test. The results indicated no significant differences between formulas A2 and A3 (P value = 1.00), A1 and B3 (P value = 0.161), B1 and B2 (P value = 0.195), or B2 and B3 (P value = 0.083). Among these, formulas A2 and A3 demonstrated the best aroma stability, receiving perfect scores of 9 out of 9 from the panelists, making them the most stable formulations. Other formulas, particularly those using ethanol as a solvent, experienced the most significant aroma changes. This is because ethanol, being a polar compound with high volatility, strongly influences the volatility of aromatic compounds in fragrances. As a result, the fragrance does not last as long in products containing ethanol, as it evaporates along with the ethanol [26].

As shown in Table 3, the pH of the product decreases as the concentration of chlorphenesin increases. This is because a small portion of chlorphenesin converts into two compounds: protonated phenol (C-C6H4-OH) and enol. Protonated phenol is acidic, while enol has a neutral pH. Therefore, higher chlorphenesin concentrations lead to more protonated phenol in the wet wipe fluid, resulting in a lower pH. The solvent type also significantly impacts pH. Chlorphenesin dissolved in ethanol generally has a lower pH compared to when it is dissolved in propylene glycol. This is because ethanol has a lower pKa value (15.9) compared to propylene glycol (16.47), making ethanol slightly more acidic [27], [28]. Despite this, the pH range throughout the stability period remains within the requirements set by SNI 8526: 2018, which specifies a pH range between 3.5 and 7.5 [23].

Table 4. Result of accelerated stability test on wet wipe product

Formula	Parameters		
	Visual	***Odor***	***Sensory Application***
A1 (CPN 0.20% + PG 20%)	9.000 ± 0.000^{a}	8.942 ± 0.106^{a}	8.684 ± 0.211^{a}
A2 (CPN 0.25% + PG 20%)	9.000 ± 0.000^{a}	9.000 ± 0.000^{a}	8.885 ± 0.122^{b}
A3 (CPN 0.30% + PG 20%)	9.000 ± 0.000^{a}	9.000 ± 0.000^{a}	9.000 ± 0.000^{c}
B1 (CPN 0.20% + EtOH 10%)	9.000 ± 0.000^{a}	7.646 ± 0.231^{b}	8.166 ± 0.244^{d}
B2 (CPN 0.25% + EtOH 10%)	9.000 ± 0.000^{a}	8.106 ± 0.532^{bc}	8.310 ± 0.275^{d}
B3 (CPN 0.30% + EtOH 10%)	9.000 ± 0.000^{a}	8.540 ± 0.301^{c}	8.712 ± 0.295^{ab}

According to Table 4, the study examines the stability of wet wipes, focusing on three parameters: visual, odor, and user experience. The results show no significant differences between formulas A1 to B3, with a value of 9.000 (P value = 1.00) in visual parameters. Unstable wet wipes typically show color changes, such as turning yellow, and the presence of black spots on the non-woven fabric. However, the panelists did not observe any color changes or black spots over the 6-month (24-week) period, indicating that the visual parameter remained stable.

According to Table 4, the aroma parameter of the wet wipe products showed significant differences in the groups tested using the Kruskal-Wallis method, with a P value < 0.01. Formulas A1, A2, and A3 demonstrated the best aroma stability, with scores of 8.942 for formula A1 and 9.000 out of 9.000 for formulas A2 and A3. Further analysis using the Mann-Whitney U test showed no significant differences between the groups, with a P value of 1.00 for the comparison between A2 and A3, and a P value of 0.442 for comparisons between A1 and A2 or A1 and A3. This indicates that formulas A2 and A3 did not exhibit any detectable aroma changes, as identified by the panelists. Although formula A1 showed some aroma changes, they were subtle enough to be still considered stable. On the other hand, formulas using ethanol as a solvent showed noticeable aroma changes. B1 and B2 did not show significant differences (P value: 0.105), but they had the poorest aroma stability, with complaints about the fragrance fading. This is similar to the behavior observed in the wet wipe fluid, where the high volatility of ethanol accelerates the evaporation of the fragrance. As a result, the fragrance does not last long, particularly when stored under extreme conditions (40°C and 75% RH), which speeds up the evaporation of both ethanol and the fragrance [26].

Table 4 presents the results of the Kruskal-Wallis test, which revealed significant differences in the sensory application parameter, with a P value < 0.001. The best results were seen in the wet wipe product using formula A3 (chlorphenesin 0.30% with propylene glycol as the solvent), which earned a perfect score of 9.000 out of 9.000. This indicates that panelists did not report any change in user sensation over the 6 months. In contrast, formulas A2 and A1 were associated with a sticky feeling, as noted by the panelists. This effect is likely due to the use of propylene glycol, a humectant that draws moisture from the environment into the skin. When stored at 40°C, some water evaporates, increasing the concentration of propylene glycol in the wet wipe product. Upon application to the skin, propylene glycol binds moisture from the air, forming a thick layer that creates the sticky sensation [29]. At the 0.30% chlorphenesin concentration, however, this effect does not occur because the addition of chlorphenesin lowers the water vapor pressure, slowing the rate of evaporation, by the colligative properties of solutions [30]. The poorest stability in sensory application was observed in formulas A2 and A3, where no significant differences were found using the Mann-Whitney U test (P value: 0.279). Panelists noted that the wet wipe products felt drier and that the packaging appeared swollen, which was attributed to the evaporation of ethanol in the product [31].

Accelerated stability testing revealed that increasing the concentration of chlorphenesin enhanced the stability of the product (visual, aroma, homogeneity, and sensory application) when the same solvent was used. However, the impact of the solvent on stability differed from the challenge test results. In the challenge test, ethanol provided better results than propylene glycol at a chlorphenesin concentration of 0.25%. Yet, during accelerated stability testing, products with ethanol as the solvent demonstrated significantly poorer stability in aroma and sensory application compared to those with propylene glycol. These results align with previous studies that suggest ethanol can have a synergistic effect with certain preservatives, though only for a short period. On the other hand, propylene glycol ensures long-term stability of the product

4. Conclusion

This study found that the optimal formulation in the challenge test was the formula containing chlorphenesin at 0.30%, with either propylene glycol or ethanol as the solvent (A3 and B3). These formulations were able to reduce all tested microorganisms by more than 5 logs on day 2 of the challenge test. In contrast, the formulation with the best long-term stability was the one using chlorphenesin 0.30% with propylene glycol as the solvent. This formula earned a perfect score of 9.000 out of 9.000 (rated by eight trained panelists) in terms of visual characteristics, homogeneity, aroma, and sensory application. Additionally, the pH of this formulation remained within the acceptable range specified by SNI 8526:2018. Therefore, the most optimal and recommended formulation for widespread use in wet wipes is the one with chlorphenesin at 0.30%, using propylene glycol as the solvent. Ethanol, although better at reducing bacteria, is not recommended due to its inferior long-term stability compared to propylene glycol.

Acknowledgment

The author would like to thank Ms. Nurani Sofiana and Ms. Stradivary Maulida Firdaus for their contributions in providing suggestions and critiques for this review article. This research is funded using personal finances.

References

[1] T. Hadley, K. Hickey, K. Lix, S. Sharma, T. Berretta, and T. Navessin, “Flushed but not forgotten: The rising costs and opportunities of disposable wet wipes,” *BioResources*, vol. 18, no. 1, Dec. 2022, doi: 10.15376/biores.18.1.Hadley.

[2] Grand View research, “Wet Wipes Market Size And Share Analysis Report, 2030,” Grand View research, San Francisco, United States, Market Report GVR-4-68039-713-5, 2022. Accessed: Sep. 26, 2024. [Online]. Available: https://www.grandviewresearch.com/industry-analysis/wet-wipes-market-report/methodology

[3] S. Kaplan, S. Pulan, and S. Ulusoy, “Objective and subjective performance evaluations of wet wipes including herbal components,” *J. Ind. Text.*, vol. 47, no. 8, pp. 1959–1978, May 2018, doi: 10.1177/1528083717716165.

[4] W. Siegert, "Preservative Trends in Wet Wipes," *SOFW J.*, vol. 137, no. 5, pp. 43–51, May 2011.

[5] P. Salama, A. Gliksberg, M. Cohen, I. Tzafrir, and N. Ziklo, "Why Are Wet Wipes So Difficult to Preserve? Understanding the Intrinsic Causes," *Cosmetics*, vol. 8, no. 3, p. 73, Aug. 2021, doi: 10.3390/cosmetics8030073.

[6] T. Harter, H. Steiner, M. Bračič, R. Kargl, and U. Hirn, "Deteriorating dispersibility of flushable wet wipes during storage: Role of fibre swelling and ionic shielding," *J. Ind. Text.*, vol. 52, p. 15280837221139236, Jun. 2022, doi: 10.1177/15280837221139236.

[7] K. Uzdrowska and M. Górska-Ponikowska, "Preservatives in cosmetics technology," *Aesthetic Cosmetol. Med.*, vol. 12, no. 2, pp. 73–78, Apr. 2023, doi: 10.52336/acm.2023.008.

[8] M. Corazza, A. Virgili, A. M. Bertoldi, S. Benetti, and A. Borghi, "Allergic contact dermatitis caused by insect repellent wipes," *John Wiley Sons Ltd*, vol. 74, pp. 295–319, 2016, doi: https://doi.org/10.1111/cod.12509.

[9] M. A. Aranzabal, N. Arruti, A. Joral, E. M. Lasa, S. Martínez, and M. A. Echenagusia, "Contact urticaria caused by phenoxyethanol in ultrasound gel," *Contact Dermatitis*, vol. 81, no. 2, pp. 132–133, Aug. 2019, doi: 10.1111/cod.13255.

[10] F. A. M. Fiorentino, M. Chorilli, and H. R. N. Salgado, "The use of the challenge test to analyse preservative efficiency in non-sterile cosmetic and health products: applications and critical points," *Anal. Methods*, vol. 3, no. 4, p. 790, 2011, doi: 10.1039/c0ay00597e.

[11] A. D. Russell, "Challenge testing: principles and practice," *Int. J. Cosmet. Sci.*, vol. 25, no. 3, pp. 147–153, Jun. 2003, doi: 10.1046/j.1467-2494.2003.00179.x.

[12] S. Banerjee, P. Chattopadhyay, A. Ghosh, S. S. Bhattacharya, A. Kundu, and V. Veer, "Accelerated stability testing of a transdermal patch composed of eserine and pralidoxime chloride for prophylaxis against (±)-anatoxin A poisoning," *J. Food Drug Anal.*, vol. 22, no. 2, pp. 264–270, Jun. 2014, doi: 10.1016/j.jfda.2014.01.022.

[13] Badan Pengawas Obat dan Makanan Republik Indonesia, "Peraturan Kepala Badan Pengawas Obat dan Makanan Republik Indonesia Tentang Metode Analisis Kosmetika," Jakarta, HK.03.1.23.08.11.07331 Tahun 2011, Agustus 2011.

[14] L. I. Ceja-Medina, M. Jiménez-Fernández, I. Andrade-González, A. Navarrete-Guzmán, M. A. Chacón-López, M. L. García-Magaña, J. A. Bonilla-Cárdenas, and R. I. Ortiz-Basurto, "Microbiological stability and general sensory acceptance of microfiltered skim milk with agave fructans of a high degree of polymerization added," *J. Food Saf.*, vol. 40, no. 5, p. e12844, Oct. 2020, doi: 10.1111/jfs.12844.

[15] E. H. Hill, D. G. Whitten, and D. G. Evans, "Computational Study of Bacterial Membrane Disruption by Cationic Biocides: Structural Basis for Water Pore Formation," *J. Phys. Chem. B*, vol. 118, no. 32, pp. 9722–9732, Aug. 2014, doi: 10.1021/jp504297s.

[16] E. M. Houang, F. S. Bates, Y. Y. Sham, and J. M. Metzger, "All-Atom Molecular Dynamics-Based Analysis of Membrane-Stabilizing Copolymer Interactions with Lipid Bilayers Probed under Constant Surface Tensions," *J. Phys. Chem. B*, vol. 121, no. 47, pp. 10657–10664, Nov. 2017, doi: 10.1021/acs.jpcb.7b08938.

[17] H. Lygre, G. Moe, R. Skålevik, and H. Holmsen, "Interaction of triclosan with eukaryotic membrane lipids," *Eur. J. Oral Sci.*, vol. 111, no. 3, pp. 216–222, Jun. 2003, doi: 10.1034/j.1600-0722.2003.00034.x.

[18] S. Seleshe and S. N. Kang, "In Vitro Antimicrobial Activity of Different Solvent Extracts from Moringa stenopetala Leaves," *Prev. Nutr. Food Sci.*, vol. 24, no. 1, pp. 70–74, Mar. 2019, doi: 10.3746/pnf.2019.24.1.70.

[19] G. M. Josephe and C. J. Noel, "Utilisation De La Chlorphenesine Comme Agent Conservateur," FR 2748208A1, 1997

[20] H. Gomyo, M. Ookawa, K. Oshibuchi, Y. Sugamura, M. Hosokawa, N. Shionoiri, Y. Maeda, T. Matsunaga, and T. Tanaka, "Evaluation of a Microbial Sensor as a Tool for Antimicrobial Activity Test of Cosmetic Preservatives," *Biocontrol Sci.*, vol. 20, no. 4, pp. 247–253, 2015, doi: 10.4265/bio.20.247.

[21] C. S. Moreau, B. D. Wray, J. E. Czekanski-Moir, and B. E. R. Rubin, "DNA preservation: a test of commonly used preservatives for insects," *Invertebr. Syst.*, vol. 27, no. 1, p. 81, 2013, doi: 10.1071/is12067.

[22] M. A. L. Hakim, A. Permadi, E. Astuti, and F. Mohamed, "Comparative Efficacy of Wet Wipe Preservatives: Antimicrobial Performance to Inhibit Microbial Growth," *J. Biol. Trop.*, vol. 25, pp. 2379–2389, 2025.

[23] Badan Standardisasi Nasional, "Standar Nasional Indonesia (SNI) 8526 : 2018," Badan Standardisasi Nasional, Jakarta, ICS 11.08.20; 85.080.20, Mar. 2018.

[24] E. H. Choi and H. Kang, "Importance of Stratum Corneum Acidification to Restore Skin Barrier Function in Eczematous Diseases," *Ann. Dermatol.*, vol. 36, no. 1, p. 1, 2024, doi: 10.5021/ad.23.078.

[25] W. Hong, S. Jia, Z. Li, W. Li, and Z. Gao, "Solubility Determination and Thermodynamic Correlation of Chlorphenesin in 12 Pure Solvents from 288.15 to 328.15 K," *J. Chem. Eng. Data*, vol. 66, no. 1, pp. 822–831, Jan. 2021, doi: 10.1021/acs.jced.0c00900.

[26] E. Sikora, M. Małgorzata, K. Wolinska Kennard, and E. Lason, "Nanoemulsions as a Form of Perfumery Products," *Cosmetics*, vol. 5, no. 4, p. 63, Oct. 2018, doi: 10.3390/cosmetics5040063.

[27] M. Ben Ouaghrem, S. De Vaugelade, S. Bourcier, C. Genty, S. Pirnay, and S. Bouchonnet, "Characterization of photoproducts and global ecotoxicity of chlorphenesin: A preservative used in skin care products," *Int. J. Cosmet. Sci.*, vol. 44, no. 1, pp. 10–19, Feb. 2022, doi: 10.1111/ics.12760.

[28] H. Hartmann and X. Yu, "On the Protonation and Deuteration of Simple Phenols**," *ChemistrySelect*, vol. 7, no. 22, p. e202201083, Jun. 2022, doi: 10.1002/slct.202201083.

[29] B. Gesslein W. and M. Rosen R., *Harry's Cosmeticology 9th edition*, 9th ed., vol. 1, 3 vols. United States of America: Chemical Publishing Company, 2015.

[30] R. Chang, *Kimia Dasar Konsep-konsep Inti*, 3rd ed. in 2, no. 3. Jakarta: Erlangga, 2005.

[31] C. S. Manuel, D. J. Yeomans, J. A. Williams, C. Fricker, K. Kucera, D. Light, and J. W. Arbogast, "Presence of unsafe chemical impurities, accelerated evaporation of alcohol, and lack of key labeling requirements are risks and concerns for some alcohol-based hand sanitizers and dispenser practices during the COVID-19 pandemic," *PLOS ONE*, vol. 17, no. 3, p. e0265519, Mar. 2022, doi: 10.1371/journal.pone.0265519.