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Stability study of blue phycocyanin from spirulina and compatibility evaluation in eye remover solution using Plackett-Burman design

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ABSTRACT

Phycocyanin is a blue pigment in spirulina that has been used as a colorant in the food, cosmetic, and pharmaceutical industries. However, it has a stability problem that is affected by numerous factors, such as light, temperature, and pH. The objectives of this study were to investigate the effects of temperature (40°C, 50°C, 60°C, 70°C, and 80°C) and pH (5.0, 6.0, 7.0, and 9.0) on phycocyanin stability. The results showed that the degradation of phycocyanin followed a first-order reaction. The maximum stability of phycocyanin was at pH 6.0 (the lowest degradation rate constant, 0.0480 h^{-1}). An increase in temperature significantly increased the degradation rate ($p < 0.05$) and correlated well with an Arrhenius plot of the degradation rate (ln k) against the reciprocal of kelvin (1/T), which showed a linear regression line with an R^2 value of 0.9656. The compatibility study of phycocyanin with various excipients in eye remover solution, as an example of cosmetic formulation, followed the Plackett-Burman design. The results showed that 47.0% w/w glycerin had a significant destabilizing effect on phycocyanin stability. These results indicate that a pH of 6.0 and lower temperatures increase the stability of phycocyanin.

Keywords: phycocyanin; stability; Arrhenius equation; Plackett-Burman design; pH-rate profile

1. INTRODUCTION

One of the key sensory factors that influence consumer acceptability of foods and cosmetics is their appearance. Therefore, natural and synthetic colorants are frequently added to products to improve their visual appeal. Due to safety and health issues, consumer concern about the use of synthetic colorants has increased (Yuan et al., 2022; Zahra et al., 2017). Because the US Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) limit the addition of certain synthetic colorants in foods and beverages due to their potential toxicity for humans, there is interest in developing natural colors to replace synthetic ones. Blue pigments extracted

from the *Spirulina platensis* have been shown to have an appealing color profile and health advantages. *Spirulina platensis* is a cyanobacterium with significant economic and nutritional value (Yuan et al., 2022). The phycobiliprotein, a complex of three light-harvesting proteins called phycocyanin, phycoerythrin, and allophycocyanin, is the primary component of the colors in *Spirulina platensis*. These pigments are located structurally on the periphery of the photosystem complex (phycoerythrin and phycocyanin) and surround the phycobiliprotein's core (allophycocyanin). Algal extracts are distinguishable by their bright blue color because of the presence of phycocyanin. The natural blue pigment phycocyanin is widely used in the pharmaceutical sector, used as biochemical tracers in immunoassays; the cosmetic sector, in lipstick and eyeliners; and the food sector, in chewing gum, dairy goods and jellies (Adjali et al., 2022; Gorgich et al., 2020).

Temperature, pH, and light are the main environmental stresses to which phycocyanin is particularly sensitive. Significant stability problems of precipitation, partial discoloration with changes from blue to green, or total discoloration, restrict the use of phycocyanin in product development in the food, cosmetic, and pharmaceutical industries (Adjali et al., 2022; Chaiklahan et al., 2012). Numerous studies on the stability of phycocyanin have been performed to identify the ideal conditions for preventing its degradation and extending its shelf life. Some studies have concentrated on factors influencing the chemical degradation of phycocyanin and the approaches developed to increase its stability. Stabilizing agents, such as proteins and polysaccharides, can be added to phycocyanin formulations to prevent them from aggregating and to improve their stability. Phycocyanin stability can also be preserved or improved by encapsulating it inside nano- or micro-structured products, such as microparticles, nanofibers, or nanoparticles (Adjali et al., 2022; Yuan et al., 2022). However, few studies have investigated the stability of blue phycocyanin and its compatibility with the excipients in cosmetic formulations.

The current study's goal was to examine the stability of phycocyanin and the factors affecting its stability, i.e., pH and temperature. The stability of phycocyanin was predicted using the Arrhenius equation. The compatibility of phycocyanin with different excipients in an example cosmetic formulation (eye remover solution) was examined using a Plackett-Burman experimental design. This study not only offers advice on how to maintain the stability of phycocyanin, but also provides helpful details on how to produce and utilize phycocyanin in products such as cosmetics.

2. MATERIALS AND METHODS

2.1 Materials

Spirulina platensis extract, phycocyanin, (Lot no. 5540172), marketed under the name Natpure xfine spirulina SL615, was purchased from Sensient Technologies Co. Ltd. (Guangzhou, China). Monobasic potassium phosphate (Lot no. A585525 450, VWR International Ltd., Poole, England) and sodium hydroxide (Batch no. 1823, Chemipan Co. Ltd., Bangkok, Thailand) were used to prepare a phosphate buffer. Excipients for the preparation of eye remover solution were obtained from the following sources and used as received: lauryl glucoside, surfactant (Lot no. K99A42J262, BASF Care Chemicals Co. Ltd., Shanghai, China), glycerin, humectant (Lot no. 0411-2020, Thai Glycerine Co. Ltd., Samut Sakhon, Thailand), propylene glycol, humectant and solvent for paraben (Lot no. C815K6FTC1, The Real three Co. Ltd., Nonthaburi, Thailand), PEG-7 glyceryl cocoate, surfactant (Lot no. 0021604444, The Real three Co. Ltd., Dusseldorf, Germany), methyl paraben, preservative (Batch no. 250146, Chemipan Co. Ltd., Bangkok, Thailand), propyl paraben, preservative (Batch no. S1120548, Chemipan Co. Ltd., Bangkok, Thailand). The remaining chemicals were all of analytical type.

2.2 Kinetic study on phycocyanin stability

2.2.1 Analysis of phycocyanin

A UV-visible spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) was used to measure the amount of phycocyanin in each sample. The phycocyanin content (PCs) was calculated from Equation 1 using the total amounts of C-phycocyanin (CPC) and allophycocyanin (APC). Measurements of absorption at 620 nm were used to determine concentrations of CPC and 652 nm for APC concentrations using Equations 2 and 3 (Böcker et al., 2019; Chaiklahan et al., 2012; Wu et al., 2016).

$$PCs = CPC + APC \quad (1)$$

$$CPC\ (mg/mL) = \frac{A_{620} - 0.474\ (A_{652})}{5.34} \quad (2)$$

$$APC\ (mg/mL) = \frac{A_{652} - 0.208\ (A_{620})}{5.09} \quad (3)$$

Equation 4 was used to calculate the percentage of remaining phycocyanin (PCs, %) at time t, as a proportion of the initial amount of phycocyanin at time zero (C_0), and the remaining amount of phycocyanin at time t (C).

$$PCs\ (\%) = \frac{C}{C_0} \times 100 \quad (4)$$

2.2.2 Determination of phycocyanin degradation kinetic order

The order of a reaction was determined using a graphical method. Equations 5, 6, and 7 illustrate how zero-order, first-order, and second-order kinetic models could be fitted to experimental data to establish the degradation kinetics of phycocyanin content. When the concentration is plotted against time, a straight line indicates a zero-order reaction. If a linear line is produced by plotting the concentration as a logarithm against time, this indicates a first-order reaction. When the initial concentrations are equal, it is a second-order reaction if the reciprocal of the concentration (1/C) vs. time produces a straight line (Yazar and Demiray, 2023; Hadiyanto et al., 2018).

$$C = C_0 - kt \quad (5)$$

$$\ln C = \ln C_0 - kt \quad (6)$$

$$\frac{1}{C} = \frac{1}{C_0} + kt \quad (7)$$

where C is the amount of phycocyanin (PCs) at time t, C_0 is the amount of phycocyanin at the beginning of time t, and k is the rate of kinetic degradation constant. The coefficient of determination (R^2) was utilized as a criterion for adequate fit after all the data were fitted to models.

2.2.3 Determination of the factors affecting phycocyanin stability

The variables determined to be affecting phycocyanin stability were pH (5.0, 6.0, 7.0, and 9.0) and temperature

(40°C, 50°C, 60°C, 70°C, and 80°C). Phycocyanin extracts were dissolved in a variety of phosphate buffer solutions to prepare the samples for testing. A phycocyanin concentration of 0.8 mg/mL was used to examine the effects of pH and temperature. The prepared samples were then kept in ovens with thermostatic controls (Model 500, Memmert GmbH, Germany) at 40°C, 50°C, 60°C, 70°C, and 80°C. The samples were tested at predetermined intervals of time. As described in Section 2.2.1, the concentrations of the remaining phycocyanin were measured using a UV-visible spectrophotometer. Each evaluation was performed in triplicate. Calculations were made using the values for k obtained at each temperature and pH level, and the relationships between temperature, pH and degradation rate constants were investigated. The Arrhenius equation was generated by Equations 8 and 9 and used to correlate temperature to the degradation rate constant. By using the Arrhenius equation, the accelerated data could be used to determine the stability at any lower temperature, including room temperature (Garrett, 1962).

$$k = Ae^{-E_a/RT} \quad (8)$$

$$\ln k = \ln A - \frac{E_a}{RT} \quad (9)$$

where k is the degradation rate constant, A is a constant that is also referred to as the frequency factor or the Arrhenius factor, E_a is the activation energy, R is the gas constant, 1.987 cal/deg mole, and T is the absolute temperature, kelvin (K, degree Celsius+273). The values of the constants A and E_a can be calculated by identifying k at various temperatures and graphing 1/T against ln k (Sinko, 2011).

2.2.4 Identification of the stabilizing and destabilizing effects of excipients on phycocyanin in cosmetic formulation (eye remover solution) using Plackett-Burman design

In this study, phycocyanin extract was used as a colorant (0.8 mg/mL) in eye remover solution. The compatibility of phycocyanin with different excipients in the formulation was examined using a Plackett-Burman experimental design.

At two levels of magnitude, five excipients (variables) of the eye remover solution were investigated. A (+) sign denotes the existence of a variable, and a (-) sign denotes its absence, with phycocyanin (0.8 mg/mL) as the constant concentration (Table 1). The compositions of the 12 solutions prepared for this investigation are presented in Table 2.

The solutions were placed in amber-glass bottles and incubated at 60°C in a thermostat-controlled oven. The amount of phycocyanin that remained in each bottle after 2 h of storage was determined using a UV spectrophotometer.

Table 1. (+) and (-) levels for the assigned variables used in the twelve-run Plackett-Burman design to study the effect of excipients on the stability of phycocyanin in eye remover solution

Variable	(+)	(-)
(A) Lauryl glucoside	5.0 % w/w	0
(B) Propylene glycol	45.3 % w/w	0
(C) Glycerin	47.0 % w/w	0
(D) PEG-7 glyceryl cocoate	2.0 % w/w	0
(E) Paraben concentrate	0.7 % w/w	0

Table 2. Twelve-run Plackett-Burman design to study the effect of excipients on the stability of phycocyanin in eye remover solution

Trial	Phycocyanin (mg/mL)	Assigned variables (A–E)				Unassigned variables (F–K)							% Remaining
		A	B	C	D	E	F	G	H	I	J	K	
1	0.8	+	+	-	+	+	+	-	-	-	+	-	
2	0.8	+	-	+	+	+	-	-	-	+	-	+	
3	0.8	-	+	+	+	-	-	-	+	-	+	+	
4	0.8	+	+	+	-	-	-	+	-	+	+	-	
5	0.8	+	+	-	-	-	+	-	+	+	-	+	
6	0.8	+	-	-	-	+	-	+	+	-	+	+	
7	0.8	-	-	-	+	-	+	+	-	+	+	+	
8	0.8	-	-	+	-	+	+	-	+	+	+	-	
9	0.8	-	+	-	+	+	-	+	+	+	-	-	
10	0.8	+	-	+	+	-	+	+	+	-	-	-	
11	0.8	-	+	+	-	+	+	+	-	-	-	+	
12	0.8	-	-	-	-	-	-	-	-	-	-	-	

2.3 Statistical analysis

The least squares method was used to determine the best-fit straight line. The determination coefficient of the curves was calculated. To investigate differences in the data averages, one-way analysis of variance (one-way ANOVA) or independent sample t-tests were performed. A 95% confidence level ($\alpha = 0.05$) was used to determine the significance of the differences.

3. RESULTS AND DISCUSSION

3.1 Kinetic analysis of the degradation of phycocyanin

UV-Vis spectroscopy is a typical analytical technique used for determining phycocyanin and was used in this research to determine the degradation kinetic order of phycocyanin at various pHs in phosphate buffer. A decrease in the phycocyanin concentration of the aqueous solution is shown by a decline in the relative intensities of the peaks at 620 and 652 nm (Wu et al., 2016). The determination coefficient (R^2) of all samples was determined after fitting a zero-order, first-order, or second-order kinetic model (Table 3).

The results showed that the degradation kinetics of phycocyanin in various pHs of phosphate buffer that were fitted by first-order kinetics had the highest R^2 value (0.7963-0.9550) when compared to those fitted by zero-order (0.6746-0.9309) or second-order kinetics (0.6933-0.9282). Therefore, the order of phycocyanin degradation kinetics followed a first-order reaction. These findings are in agreement with previous reports. Phycocyanin added to glucose, sucrose, or fructose, as well as preservatives, showed an identical trend in first-order kinetics (Hadiyanto et al., 2018; Antelo et al., 2008; Chaiklaha et al., 2012).

Table 3. The determination coefficient on phycocyanin degradation at various pHs (0.8 mg/mL, 60°C)

pH	Determination coefficient (R^2)		
	Zero-order	First-order	Second-order
5.0	0.7363	0.8549	0.7421
6.0	0.9309	0.9550	0.9282
7.0	0.8888	0.9324	0.8839
9.0	0.6746	0.7963	0.6933

3.2 Effect of pH

The pH of the formulation and the solvent are the most significant variables influencing phycocyanin stability (Wu et al., 2016). In the current research, the stability of phycocyanin in phosphate buffer at varied pH values was evaluated to identify the appropriate pH for phycocyanin stability. The first-order degradation rate constant (k) of phycocyanin was calculated using the percentage of residual phycocyanin as a graph of the natural logarithm over time, as shown in Table 4 and Figure 1. The highest k values were for the phosphate buffer at pH 9.0, followed by those at pH 5.0, then pH 7.0. Phycocyanin appeared to be most stable in the phosphate buffer at a pH of around 6.0 at 60°C, as indicated by the lowest degradation rate constant (0.0480 h^{-1}). These findings demonstrated that phycocyanin degradation was pH-dependent, with pH 6.0 being the optimum. The findings are in agreement with those of other investigations (Adjali et al., 2022; Chaiklahan et al., 2012; Martelli et al., 2014, Wu et al., 2016). At pH values between 4.0 and 4.8, which are relatively close to its isoelectric point (pH 3.4), phycocyanin became unstable. Because of the hydrophobicity of the protein's surface, the neutrally charged protein molecules are prone to aggregation (Li et al., 2021; Yuan et al., 2022). The pH of the medium alters the protein's spectral characteristics and color. At neutral pH, phycocyanin solution appears blue, while at acidic pH, it appears green (Adjali et al., 2022). At the same concentration, in comparison to pH 5.0, 6.0, and 7.0 at temperatures of 55°C and 65°C, room temperature phycocyanin solutions with an acidic pH of 3.0 and 4.0 displayed lower relative peak intensities at 620 and 652 nm and increased absorbance at 280 nm. This is explained by protein precipitation and possibly by the change in protein conformation (Adjali et al., 2022; Wu et al., 2016). The chromophore maintains its extended geometry for pH values greater than 4.0 and up to 6.0. However, as pH decreases, the chromophore folds into a cyclic conformation, altering its spectrum characteristics. Increasing the pH from 6.0 to 9.0 increased the degradation rate, indicating protein degradation, as was also evidenced by the appearance of some turbidity (Chaiklahan et al., 2012; Jespersen et al., 2005). These results agree with those of other studies. According to Martelli et al. (2014), pH 9.0 significantly reduces the stability of CPC at 49.9°C. Wu et al. (2016) determined the relative concentration of phycocyanin solution at five different pH levels (5.0, 5.5, 6.0, 6.5, and 7.0), incubated at 55°C and 65°C and found that the phycocyanin at pH 7.0 was the least stable. Chaiklahan et al. (2012) and Jespersen et al. (2005) also showed that phycocyanin solution at pH 7.0 precipitated after 30 min incubation at 64°C. The findings of numerous trials between pH 5.0 and 8.0 performed by researchers revealed that the optimal pH range for maintaining phycocyanin stability is between 5.5 and 6.0 (Adjali et al., 2022). Since phycocyanin contains an acyl group in its structure and has antioxidant activity, it could be suggested that it also degrades by hydrolysis and oxidation.

Table 4. Effect of pH on degradation rate constants (k) of phycocyanin in phosphate buffer (0.8 mg/mL) at 60°C

pH	k + SD (h^{-1})
5.0	0.9061 ± 0.0064
6.0	0.0480 ± 0.0026
7.0	0.6324 ± 0.0061
9.0	1.2763 ± 0.1042

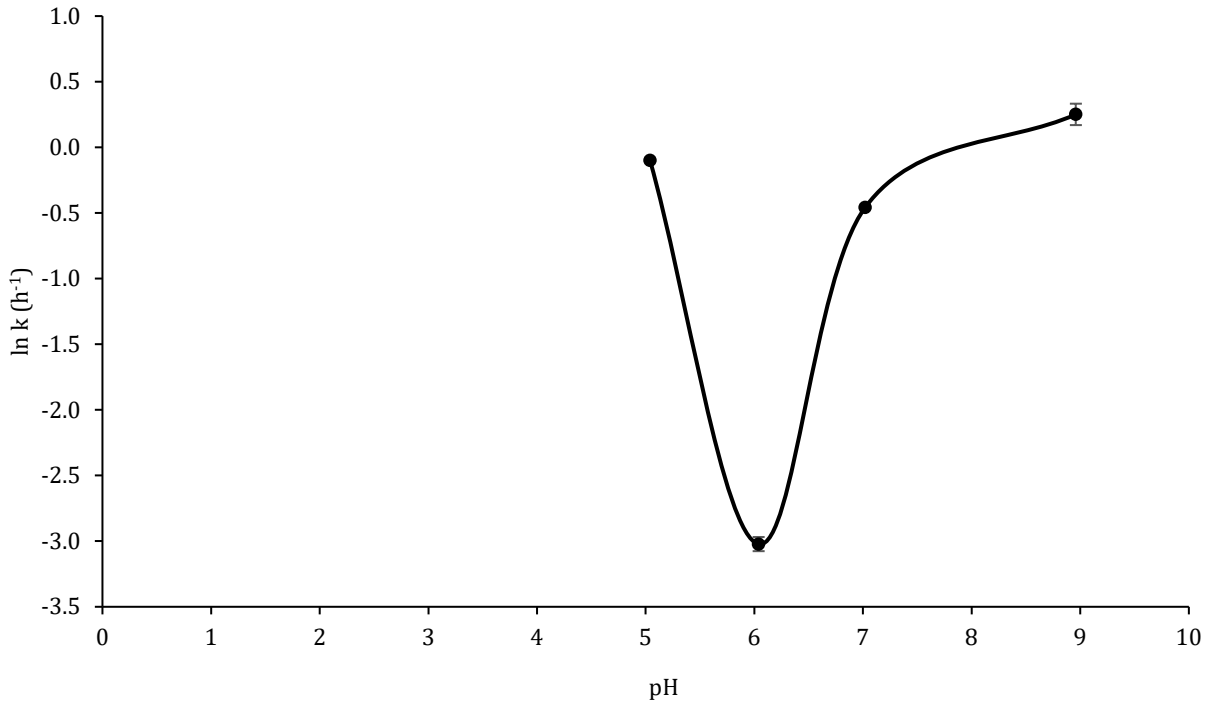

Figure 1. The pH-rate profile for phycocyanin degradation (0.8 mg/mL) at 60°C

3.3 Effect of temperature

The study of kinetics analysis in Section 3.1 indicates that phycocyanin degradation follows first-order degradation kinetics. The stability of phycocyanin at pH 6.0, which was the most stable pH in this research, was determined using the accelerated stability testing method. The samples of phycocyanin were prepared and then kept in ovens set to 40°C, 50°C, 60°C, and 80°C. Figure 2 displays the natural logarithm of the remaining percentage of phycocyanin as a function of time. Since all plots were determined to be linear, the phycocyanin concentration-dependent reaction was confirmed to be first-order. The slopes of the curves obtained from least squares fitting were used to calculate the rate constants (k) of phycocyanin degradation. The shelf life (t_{90}) of the formulations were calculated from Equation 10 using the predicted rate of degradation (k) (Treesinchai et al., 2020).

$$t_{90} = 0.105/k \quad (10)$$

where k is the first-order rate constant.

The degradation rate values and shelf life for each storage temperature are shown in Table 5. As can be seen, higher temperatures accelerated phycocyanin degradation. The shelf life of the phycocyanin solution decreased with increasing temperatures. This result corresponds to the research done by Antelo et al. (2008). They noted that the half-life values of aqueous phycocyanin crude extracts decrease as heat treatment increases. The impact of temperature on phycocyanin degradation has been examined in several studies. Chaiklahan et al. (2012) demonstrated that phycocyanin stability has a critical temperature of 47°C. In solutions at temperatures higher than that, a significant decrease in the phycocyanin half-life was observed, indicating that temperature may have a significant effect on the conformation of phycocyanin. Additionally, Zhang et al. (2021) found that after being heated at 90°C for 2.5 min, phycocyanin at pH 5.0 lost 60% of its color. This phenomenon might be caused by the apoprotein's conformational alterations interfering with the coupling and energy transfer pathways between the embedded chromophores (Li et al., 2021). Heating caused the phycocyanin to partially unfold, exposing hydrophobic groups that are normally located inside the molecule. Additionally, phycocyanin subunits may aggregate when treated to heat, which might cause phycocyanin to degrade and reduce its blue color (Yuan et al., 2022). Phycocyanin solution was reported to be more stable at low temperatures (4°C) (Chaiklahan et al., 2012). At temperatures near room temperature, phycocyanin solution also degrades very slowly, according to Adjali et al. (2022). This suggests that keeping the product at a lower temperature would increase its shelf life.

In this research, the Arrhenius equation was used to plot the temperature reciprocal against the natural logarithm of observed rate constants for the range of 40°C to 80°C (Carstensen, 2000). As shown in Figure 3, the plot of phycocyanin degradation in phosphate buffer at pH 6.0 exhibits linearity (R^2 = 0.9656). Equation 11, derived from Equation 9, shows the equation of the curves.

$$\ln k = 41.74 - 14.70 \times 1000/T \quad (11)$$

The slope of the straight line, as determined by least squares, was used to determine the heat of activation (E_a). The E_a and A values for phycocyanin degradation in pH 6.0 phosphate buffer were 29.21 kcal/mol and 1.34 x 10^{18} h^{-1}, respectively. The Arrhenius plot's linear nature is an excellent indication of the method's applicability in predicting rates of degradation at various temperatures (Garrett, 1962).

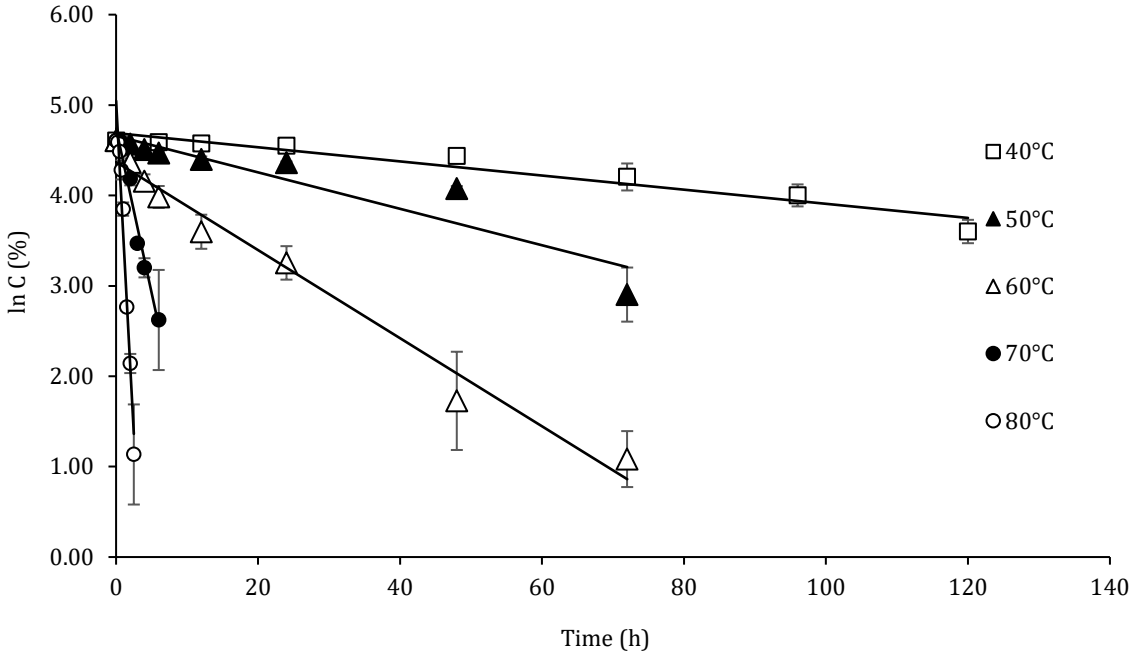

Figure 2. First-order plot for phycocyanin degradation in pH 6.0 phosphate buffer (0.8 mg/mL) over the temperature range of 40°C – 80°C

Table 5. Effect of temperature on degradation rate constants (k) of phycocyanin in pH 6.0 phosphate buffer (0.8 mg/mL)

Temperature (°C)	k (h^{-1}) ± SD	t_{90} (h) ± SD
40	0.0078 ± 0.0005	13.50 ± 0.95
50	0.0202 ± 0.0032	5.30 ± 0.92
60	0.0480 ± 0.0026	2.16 ± 0.12
70	0.3614 ± 0.0133	0.29 ± 0.01
80	1.4461 ± 0.1680	0.07 ± 0.01

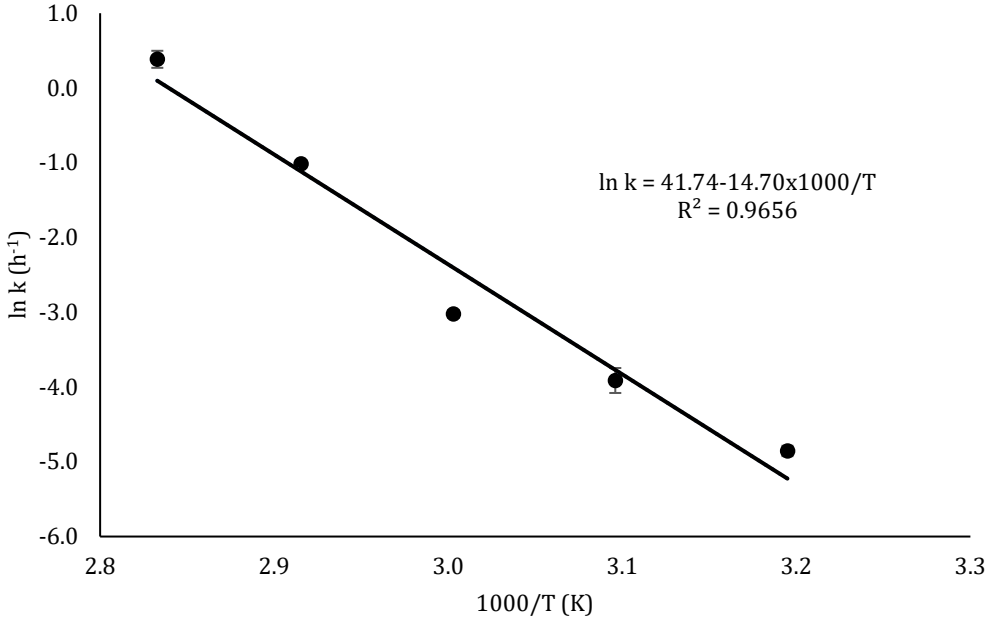

Figure 3. The Arrhenius plot for the first-order rate constant of phycocyanin degradation (0.8 mg/mL) in pH 6.0 phosphate buffer over the temperature range of 40°C – 80°C

3.4 Identification of the stabilizing and destabilizing effects of excipients on phycocyanin in cosmetic formulation (eye remover solution) using Plackett-Burman design

By using the Plackett-Burman design, it was possible to establish how the excipients in the example cosmetic formulation (eye remover solution) affected the stability of phycocyanin as a colorant. Table 6 displays the percentage of phycocyanin remaining from each excipient combination. The average effect for each assigned and unassigned variable was calculated from the percentage of phycocyanin remaining (Motola and Agharkar, 1992). The average effects of the unassigned variables were used to calculate the standard deviation of the effect of the factor (S_{FE}). The S_{FE} was then used to compute the minimum significant factor effect, E_{ms} (E_{ms} = t x S_{FE}), with a 90% level of confidence. The t-value in this study was 1.943 at a 90% confidence level. At this confidence level, the t-value is used where the number of degrees of freedom of t is equal to the number of unassigned variables (6 in this research). If the value of the average effect of each excipient is positive (+), it indicates that that excipient had a stabilizing effect on phycocyanin, whereas, if the value of the average effect of each excipient is negative (-), it indicates that the excipient had a destabilizing effect on phycocyanin. Importantly, any average effect with an absolute value larger than the E_{ms} was considered statistically significant.

Table 7 lists the average effects of the assigned variables compared to the E_{ms} at a 90% level of confidence. Glycerin appeared to be the dominant destabilizing substance because its average effect was negative (-) and higher than E_{ms}. Glycerin is used in a variety of pharmaceutical products, including ocular, parenteral, otic, and oral preparations. Glycerin is primarily used for its humectant and emollient properties in topical pharmaceutical formulations and cosmetic products. Glycerin also serves as a co-solvent or solvent in creams and emulsions (Rowe et al., 2009). It was unexpected that glycerin renders phycocyanin unstable. However, according to Braham et al. (2021), glycerin does not always have a stabilizing effect and it can occasionally cause some destabilization of the immobilized enzyme (for example, in the case of ficin extract, a protease). In the Braham et al. (2021) study, the destabilizing impact in the case of glyoxyl-ficin was obvious and very significant, confirming the impact of glycerin concentration on the stability of the enzyme. In addition, in our study, the pH of glycerin was determined to be about 7.0. It was observed that the high level of glycerin in the eye remover solution (47% w/w) could increase the pH of the formulation and destabilize phycocyanin. The pH of the mixtures containing glycerin in the twelve-run Plackett-Burman design was around 9.1–11.0, which is the pH range of high degradation in phycocyanin. For this reason, it is crucial to ensure that the impact is positive for a particular preparation under the usage conditions before using glycerin as a stabilizer agent. The other ingredients of the formulation, including lauryl glucoside, propylene glycol, PEG-7 glyceryl cocoate, and paraben concentrate, seemed to have a destabilizing effect on phycocyanin because their average effect values were negative (-), but were not significant since their average effects were less than E_{ms}.

4. CONCLUSION

This research demonstrated that phycocyanin degradation followed a first-order reaction. Phycocyanin was the most stable at pH 6.0. The degradation rate was considerably increased by an increase in temperature which correlates well with an Arrhenius plot. Plackett-Burman design demonstrated the ability to identify the compatibility of phycocyanin with different excipients in eye remover solution and found that only glycerin (47.0% w/w) had a significant destabilizing impact on phycocyanin stability. To summarize, a pH of 6.0 and lower temperatures increase the stability of phycocyanin. This information could be used to improve the color stability of phycocyanin as well as its biological activities. However, to stabilize phycocyanin in food, cosmetics, and pharmaceutical products, new strategies still need to be developed, which will require more research.

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Table 6. Plackett-Burman design shows the percentage remaining of phycocyanin for each trial (excipient combination)

Trial	Phycocyanin (mg/mL)	Assigned variables (A–E)					Unassigned variables (F–K)						% Remaining
		A	B	C	D	E	F	G	H	I	J	K	
1	0.8	+	+	-	+	+	+	-	-	-	+	-	8.46
2	0.8	+	-	+	+	+	-	-	-	+	-	+	17.52
3	0.8	-	+	+	+	-	-	-	+	-	+	+	26.10
4	0.8	+	+	+	-	-	-	+	-	+	+	-	18.17
5	0.8	+	+	-	-	-	+	-	+	+	-	+	58.29
6	0.8	+	-	-	-	+	-	+	+	-	+	+	76.61
7	0.8	-	-	-	+	-	+	+	-	+	+	+	50.15
8	0.8	-	-	+	-	+	+	-	+	+	+	-	1.28
9	0.8	-	+	-	+	+	-	+	+	+	-	-	61.31
10	0.8	+	-	+	+	-	+	+	+	-	-	-	9.97
11	0.8	-	+	+	-	+	+	+	-	-	-	+	18.42
12	0.8	-	-	-	-	-	-	-	-	-	-	-	78.18
Sum+		189.02	190.75	91.46	173.51	183.60	146.57	234.63	233.56	206.72	180.77	247.09	
Sum-		235.44	233.71	333.00	250.95	240.86	277.89	189.83	190.90	217.74	243.69	177.37	
Average effect		-7.74	-7.16	-40.26	-12.91	-9.54	-21.89	7.47	7.11	-1.84	-10.49	11.62	

Note: A - lauryl glucoside; B - propylene glycol; C - glycerin; D - PEG-7 glyceryl cocoate; E - paraben conc.; F to K - unassigned variables

Table 7. Summary of the compatibility test using a Plackett-Burman factorial design

Variable	Average effect	Significance* (E_{ms} =22.90)
Lauryl glucoside	-7.74	no
Propylene glycol	-7.16	no
Glycerin	-40.26	yes
PEG-7 glyceryl cocoate	-12.91	no
Paraben conc.	-9.54	no

Note: *90 % level of confidence

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