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Critical Quality Attributes (CQA) and Critical Process Parameters (CPP) roles in the pilot scale up process and stability of a cosmetic cream

Sharifah Shakirah Syed Omar[1], Hazrina Ab Hadi[1,*] and Abd Almonem Doolaanea[1]

ABSTRACT

Introduction: The objective of this study was to scale up a natural based cream containing Piper betle L. extract to pilot scale and asses it's stability over time.

Method: During the scale up process, the critical process parameters (CPP) were monitored closely to ensure the critical quality attributes (CQA) are maintained within a desired range. To ensure the formulation was stable, a 6-months stability study was done where the organoleptic properties, particle size, zeta potential, viscosity, microbial limit and hydroxchavicol content were assessed. The formulation was characterized repeatedly at 0-month, 1-month, 2-month, 3-month and 6-month time point after being stored in 30°C±2 and 40°C±2 at 75%±5% RH.

Results: CPPs in each process were maintained at certain values throughout to achieve CQAs. Time, speed and temperature during mixing and homogenization process were monitored and maintained. Stability study over 6 months proved the ability of the product to remain stable with pH < 5, particle size < 2µm, low span value, zeta potential < -50mV, apparent viscosity < 1.2Pa.s^{-1} and no microbial growth.

Conclusion: In conclusions, CPPs played a major role in enabling the formulation produced attain acceptable CQAs. Identifying CQA and CPP ensured the production of quality and stable product.

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***Corresponding author:**

Email address: hazrina@iium.edu.my

Authors' Affiliation:

[1] *Department of Pharmaceutical Technology, Kulliyyah of Pharmacy, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia*

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Introduction

According to the Federal Food, Drug and Cosmetic Act and Article 2 of the EU Cosmetics Regulation the definition of cosmetics are products that are meant to be applied on body parts either by being rubbed, poured, sprinkled, or sprayed on or introduced in to, for the purpose of changing the appearance, enhancing beauty, improving attractiveness cleaning, perfuming and preserve the skin's good condition (European Comission, 2009; U.S Congress, 1934). Moving forward the use of cosmetics have widen its horizon to maintaining healthy skin, protecting from harmful hazards as well as prevent or remedy for the many skin issues faced by all races, genders, and age globally (Arti et al., 2014).

Based on Euromonitor, the skincare segment recorded a turnover of US$107 billion worldwide in 2013, where 65% of it is contributed by facial treatments alone and 30% of the facial treatments are from skin lightening products (Euromonitor, 2016). In 2018, Asia pacific lead the world's skincare sales with China followed by Japan and Korea at the forefront. Sales of skincare in Malaysia, Indonesia and Thailand is also expected to grow exponentially (Lueng, 2019). Malaysia is one of the countries that is showing growth in the demand of skin lightening products. These products garner attention in the management of hyperpigmentation (Charles & McLean, 2017; Desai, 2014). Skin lightening cosmetic products offer solutions that are accessible and affordable with multiple choices.

Although with abundance of skin-lightening products already available in the market, many are opting for natural based skincare products due to the occurrence of adverse effects (Desai, 2014; Oudenhoven et al., 2015). Piper Betle L. was found to have skin lightening effect due to its ability to inhibit tyrosinase enzyme in the melanogenesis pathway (Majeed et al., 2012). Piper betle L. is an evergreen perennial plant native to peninsular Malaysia from the Piperaceae family (Gibson, 2016; Singtongratana et al., 2013). This skin lightening effect is believed to be contributed by a compound name hydroxychavicol or its chemical name 1-allyl-3, 4-dihydroxybenzene.

However, the stability of natural based formulation is concerning especially the effects of scale up on the formulation if commercialization is considered. Scale up is the process of increasing a batch size with the same procedures to produce a larger output. Scaling up to pilot scale means that the batch size is increased to intermediate batch size which is representative of the procedures to be applied in manufacturing scale (Gibson, 2016). Scale up is important as it provides information on suitability of the equipments, identifying critical parameters of the process (FDA, 2011) and plausibility to produce a stable product (CTFA, 2004). Identification of critical quality attributes (CQA) and critical process parameters (CPP) that should be monitored since laboratory scale formulation and method development is indispensable (EMA, 2016).

Methodology

The formulation consists of aqueous phase, oil phase and cooling phase. Aqueous phase compose of water, lecithin (Tianjin Hexiyuan lecithin Technology Co Ltd, Chin), glycerin (IOI Acidchem Sdn Bhd, Malaysia), chitosan acetate which the chitosan is from Sigma-Aldrich (Missouri, United states) while the acetic acid from Merck (New jersey, United states); disodium edetate (Xi'an lyphar Biotech Co Ltd Shaanxi,China) and Piper Betle L. (PBL) extract. The Piper betle leaves extract was extracted through subcritical water extraction using an accelerated solvent extractor (ASE) with temperature of 100°C for 60 minutes. Anti-tyrosinase assay of the extract has been quantitatively determined and found that crude Piper betle leaves extract possess activity to confer skin lightening effect (Omar et al., 2021).

The oil phase comprises of beeswax (Kahl beeswax, Germany), xanthan gum (Deosen biochemical ltd., Unites states), refined coconut oil and olive oil from Natures natural India (New Delhi, India), cocamidopropyl Betaine (Haihang Industry Co, Ltd., China) and squalane from Kono Chem Co. Ltd. (Xi'an, China). Finally, the cooling phase includes ascorbic acid from Xi'an lyphar Biotech Co Ltd (Shaanxi, China), tocopherol acetate from Natures natural India (New Delhi, India), pectin from Kono Chem Co. Ltd. (Xi'an, China), Euxyl-PE (IMCD Malaysia Sdn. Bhd, Malaysia) and chamomile essential oil (Hunan Nutramax Inc., China).

Raw material identification

Identification test was done using Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR). A total of 16 scans were run at a wave region of 400 – 4000 cm^{-1} at temperature of 25ºC using Spectrum RX Two Instrument (PerkinElmer Inc., USA) complete with Spectrum 3.5 software.

Scale up procedures

The aqueous phase was placed in the aqueous pot, while the oil phase was placed in the main pot of ZJR-30 Vacuum Emulsification Machine (Wuxi YK Automation Technology, China). Both phases were heated to 75±5ºC with continuous agitation. The aqueous and oil phase were mixed and homogenized for 30 minutes then left to cool to room temperature before addition of cooling phase and homogenized again.

The CPP were monitored closely to ensure that the CQA were maintained within a desired range. CQA are parameters physical, chemical, or biological that should be maintained within an acceptable limit to achieve products that are safe, effective and of high quality (FDA, 2011).

CPP on the other hand are factors whose inconsistency may affect CQA, hence should be monitored to produce product of good quality (ICH, 2009). Table 1 shows CQA and CPP involved.

Table 1: CQA and CPP

CQA	CPP	Risk assessment
Viscosity	Homogenization time, homogenization speed, temperature	Monitor and adjust the parameters accordingly.
Homogeneity	Homogenization time, homogenization speed, speed of pouring	
Particle size	Homogenization time	

Characterisation

Physical analysis

The organoleptic properties as well as the physical appearance of the cream were assessed for acceptability to potential users. The properties include appearance, colour, odour and texture of the formulation (Smaoui et al., 2012).

Forced centrifugation

10 g of the cream formulation was placed in a centrifugal tube and centrifuged at 5000 rpm for 10 minutes at 25°C (Smaoui et al., 2013). Centrifugation study was employed to study the resistance of emulsion towards being centrifuged (Ghosh et al., 2013; Rodrigues et al., 2016).

pH

pH of the formulation was measured using a calibrated pH meter (Metler Toledo, Switzerland). The pH of the formulation was determined by diluting 1 g of the formulation in 9 mL of distilled water to form a concentration of 10% (w/w) (Mahmood et al., 2015).

Particle size analysis

Particle size was analysed using a laser particle size analyser (BT-9300H, Dandong Baite Instrument Co. Ltd., China). Cream was diluted to approximately 1:1000 in the sample cell containing distilled water to prevent multiple scattering effect before running the machine (BahramParvar & Goff, 2013). A refractive index of 1.46 and 1.33 was used for the fat and dispersing medium respectively (Kirtil & Oztop, 2016).

Zeta potential determination

The zeta potential was measured using Zetasizer (ZEN 1600, Malvern, UK). Samples were diluted with deionized water to 1:100 to avoid multiple scattering effects (Khor et al., 2014). All measurements were carried out at temperature of 25 °C (Esteban et al., 2016).

Rheological analysis

The rheological parameter was measured using stress-controlled rheometer (HAAKE MARS, Thermo Scientific, Germany). Sample was loaded on to the lower plate of the rheometer and allowed to equilibrate to temperature of 25°C (Khor et al., 2014). A shear rate ramp with shear rates of 0.1 s^{-1} - 100 s^{-1} was applied to the sample (Kirtil & Oztop, 2016).

Microbial limit count

1 g of the sample was diluted to a 1 in 10 dilution using phosphate buffer solution pH 7.2. 10 mL of the sample was used for inoculation on the Tryptic soy broth which was then incubated at 30-35°C for 18-24 hours. Sample was sub-cultured on a Mannitol Salt Agar for Staphylococcus aureus and on Cetrimide Agar for Pseudomonas aeruginosa, then incubated for 18-72 hours at 30-35°C (USP, 2014b).

For the determination of Total Aerobic Microbial Count (TAMC) and Total Yeast and Mold Count (TYMC) a dilution of 1:10 of the cream in phosphate buffer solution pH 7.2 was prepared. Plate-count method was employed, specifically pour-plate method. 1 mL of the diluted sample was added in a petri dish and 15-20 mL of Tryptic soy agar for TAMC was added. Plate was then incubated at 30-35°C for 5 days . Similar steps were repeated on Sabouraud dextrose agar for TYMC. The plate for TYMC was then incubated at 20-25°C for 5 days (USP, 2014a).

Stability study

Stability study provides evidence on how different environmental factors affect the formulation thus provide information on the shelf life and recommended storage conditions. An accelerated stability study was employed at temperatures of 30°C±2 and 40°C±2 at 75%±5% RH for 6 months (ASEAN, 2013; ICH, 2003). Samples were assessed initially, then after 1 month, 2 months, 3 months, and 6 months, which is a total of 5 collection points (Singh et al., 2016). Data was analyzed using Statistical Package for the Social Science (SPSS) software version 20 applying repeated measures ANOVA.

Hydroxychavicol Content Analysis with High Performance Liquid Chromatography (HPLC)

A validated HPLC method was used to identify the presence of hydroxychavicol in the formulation (Omar et al., 2021). Analysis of the samples was done using an isocratic system with Agilent G1310A pump linked with diode array detector (Agilent, Stevens Creek Blvd Santa Clara, USA). The column used was a C-18 silica-based column (5 µm, 250 × 4.6 mm) (Phenomenex, Torrance, USA) which makes it a reversed phase chromatography. Acetonitrile and 2% acetic acid in water with ratio of 25:75 was used as the mobile phase with a flow rate of 1.0 mL/min.

Results

Raw material identification

All the raw materials were identified. Shown in Table 2 is the fingerprint for each raw material.

Critical Quality Attributes (CQA) and Critical Process Parameters (CPP)

The CPPs played a major role in enabling the formulation produced attain acceptable CQAs. As shown in table 3 the CPPs in each process were maintained at certain values.

Table 3: CPP during scale up.

Process		Process parameter	
Oil pot and Aqueous pot	Mixing	Time	30 minutes
		Speed	1400 rpm
		Temperature	75 ± 5 °C
Main pot	Homogenization	Time	30 minutes
		Homogeniser speed	3000 rpm
		Agitatator speed	2700 rpm
		Temperature	OFF
		Vacuum	ON

Characterisation

Physical analysis and forced centrifugation study

The colour of the formulation was light beige while texture-wise, it appears smooth with a slight shine. As the formulation had chamomile essential oil, the odour of the cream was pleasant and showed no phase separation. The results are as reported in Table 4.

Table 4: Physical Observations of Cream Containing Piper Betle L. (PBL) Extract Stored in 30°C/75%RH and 40°C/75%RH at 0 Month, 1 Month, 2 Months, 3 Months, and 6 Months

Characteristic	Storage	PBL cream				
		0M	1M	2M	3M	6M
Colour	30°C/75%RH	LB	LB	LB	LB	B
	40°C/75%RH	LB	LB	LB	LB	B
Texture	30°C/75%RH	S	S	S	S	S
	40°C/75%RH	S	S	S	S	S
Odour	30°C/75%RH	P	P	P	P	P
	40°C/75%RH	P	P	P	P	P
Phase separation	30°C/75%RH	X	X	X	X	X
	40°C/75%RH	X	X	X	X	X

Abbreviations: 0M is day 0 month; 1M is 1 month; 2M is 2 months; 3M is 3 months; 6M is 6 months; LB is light beige; B is beige; S is smooth; P is pleasant; X is absent

pH

As shown in Figure 1, pH of the formulation throughout the 6 months period ranged between 4.3-4.7. The change in pH was found to be significant over time (p=0.000).

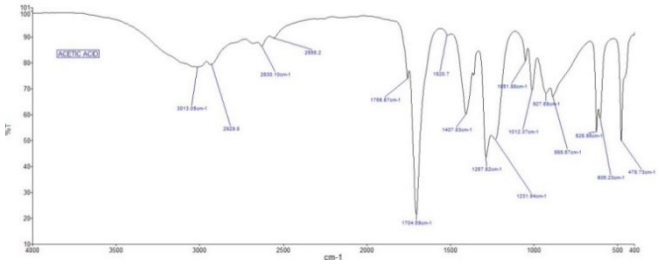
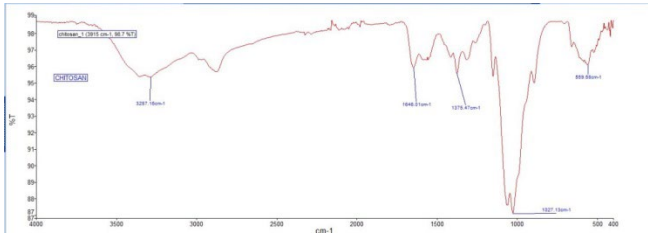
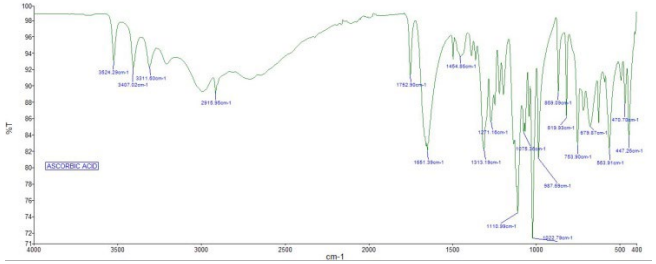
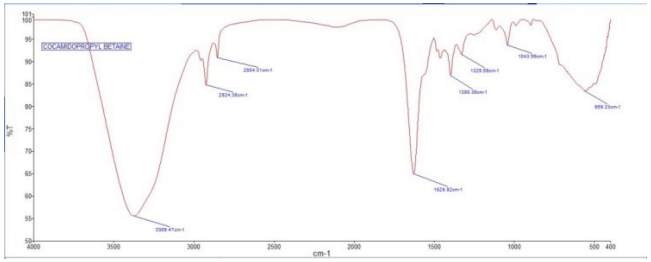
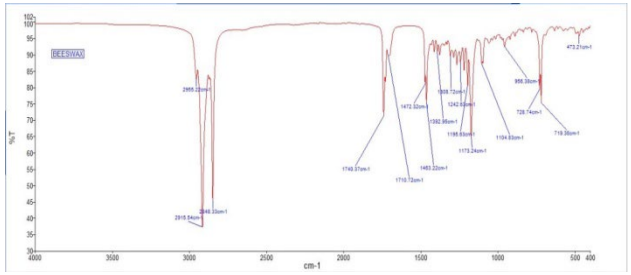
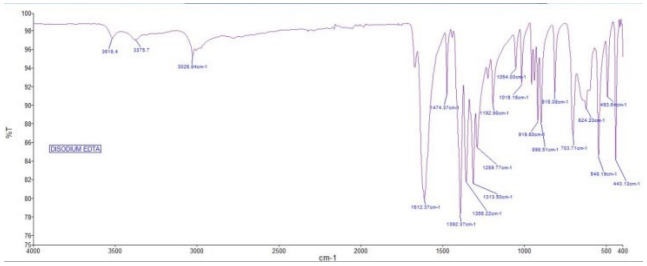
Particle size

Figure 2 shows both formulations have particle size ranging from 0.99 µm - 1.92 µm like most commercialized emulsions having sizes of 0.3-2 µm (Terjung et al., 2012). Although the changes of particle size over time is statistically significant (p=0.000), the size is still considerably small. The formulation maintained a span value below 2, except for at 3 months and 6 months as shown in Figure 3. Despite the significant changes (p=0.000), the cream did not show any observable changes or signs of instability.

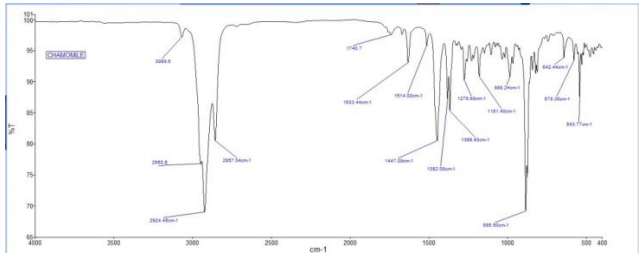
Zeta potential

Zeta potential also known as electro kinetic potential is the measure of repulsive or attractive charge between particles (Honary & Zahir, 2013). Results in Figure 4 show both formulations in both conditions having high zeta potentials mostly below -50. Over 6 months, zeta potential showed significant change (p=0.000) but remain well below the agglomeration threshold.

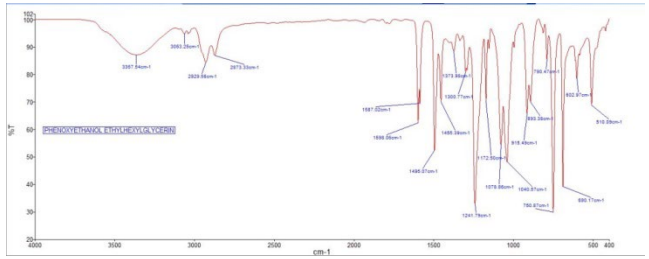
Table 2: ATR-FTIR results for each raw material.

Name	IR Fingerprint	Name	IR Fingerprint
Acetic acid		Chitosan	
Ascorbic acid		Cocamidopropyl betaine	
Beeswax		Disodium Ethylenediaminetetraacetic Acid (EDTA)	

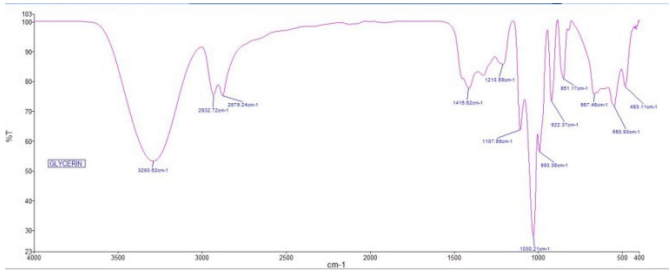
Chamomile essential oil



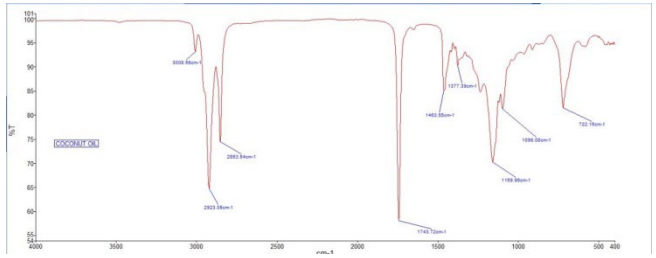
Euxyl-PE



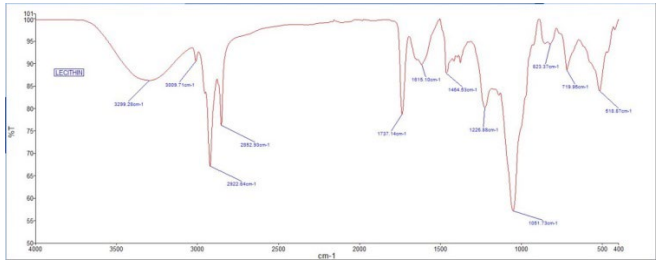
Glycerin



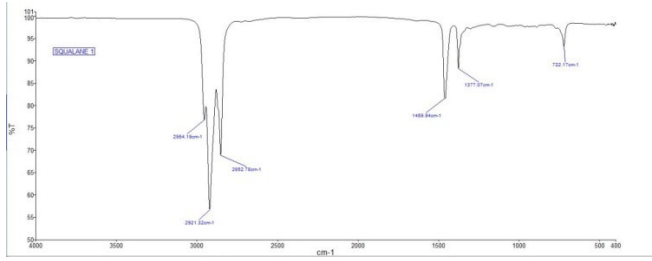
Refined coconut oil



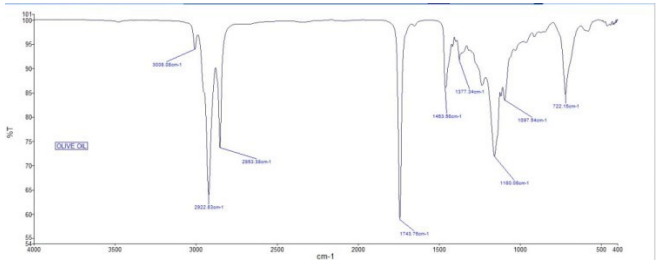
Lecithin



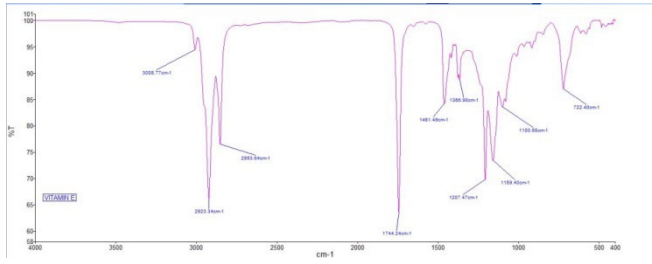
Squalane



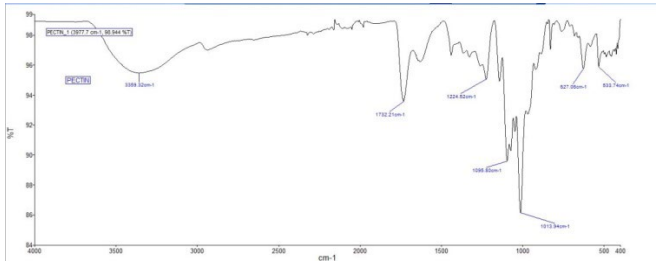
Olive oil



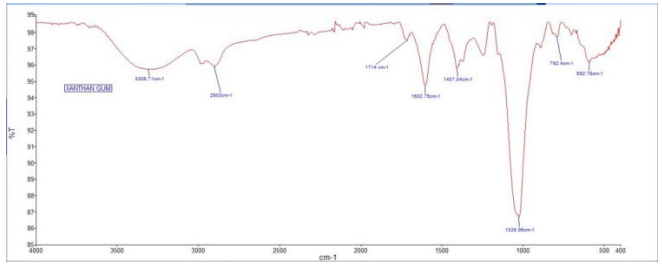
Tocopherol acetate



Pectin



Xanthan gum



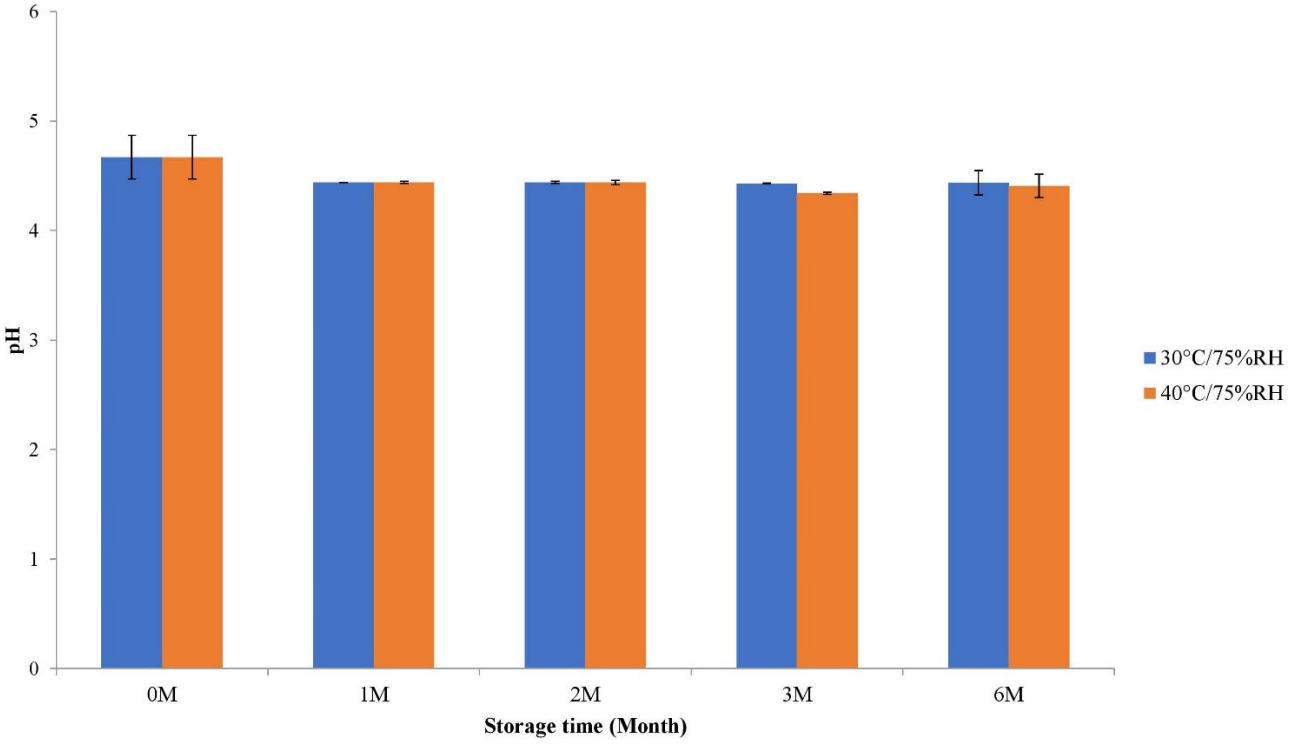


Figure 1: pH Values of Cream Containing *Piper Betle* L. (Mean ± SD, n = 3). Abbreviations: 0M is 0 month; 1M is 1 month; 2M is 2 months; 3M is 3 months; 6M is 6 months.

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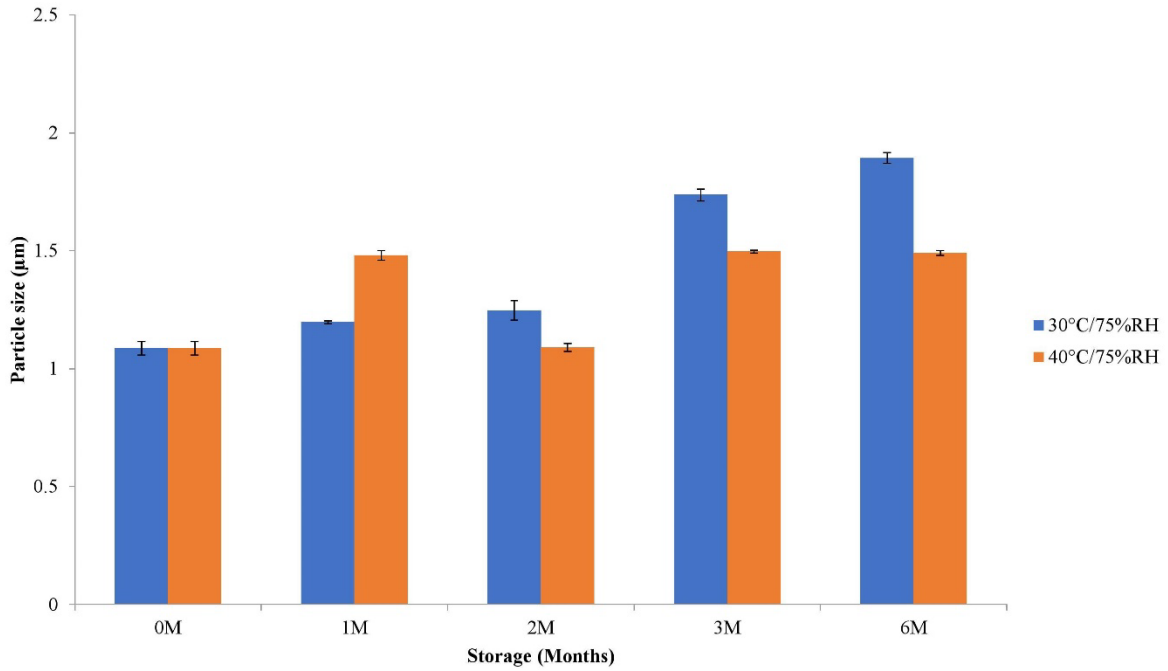

Figure 2: Particle size of Cream Containing Piper Betle L. (Mean ± SD, n = 3).

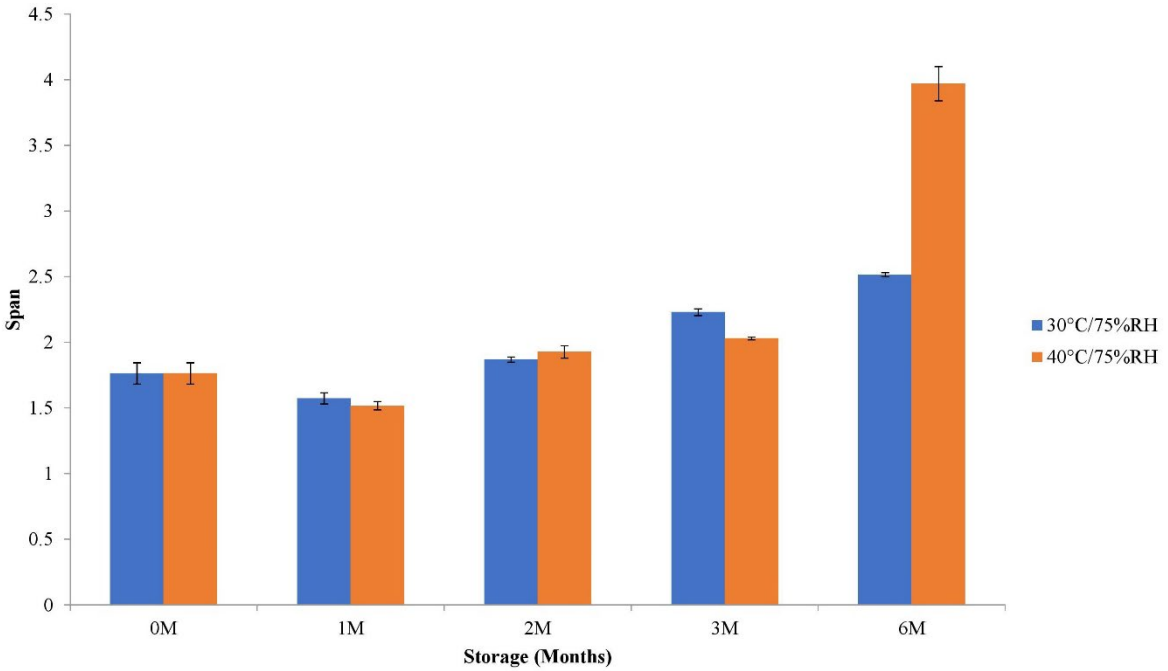

Figure 3: Span of Cream Containing Piper Betle L. (Mean ± SD, n = 3).

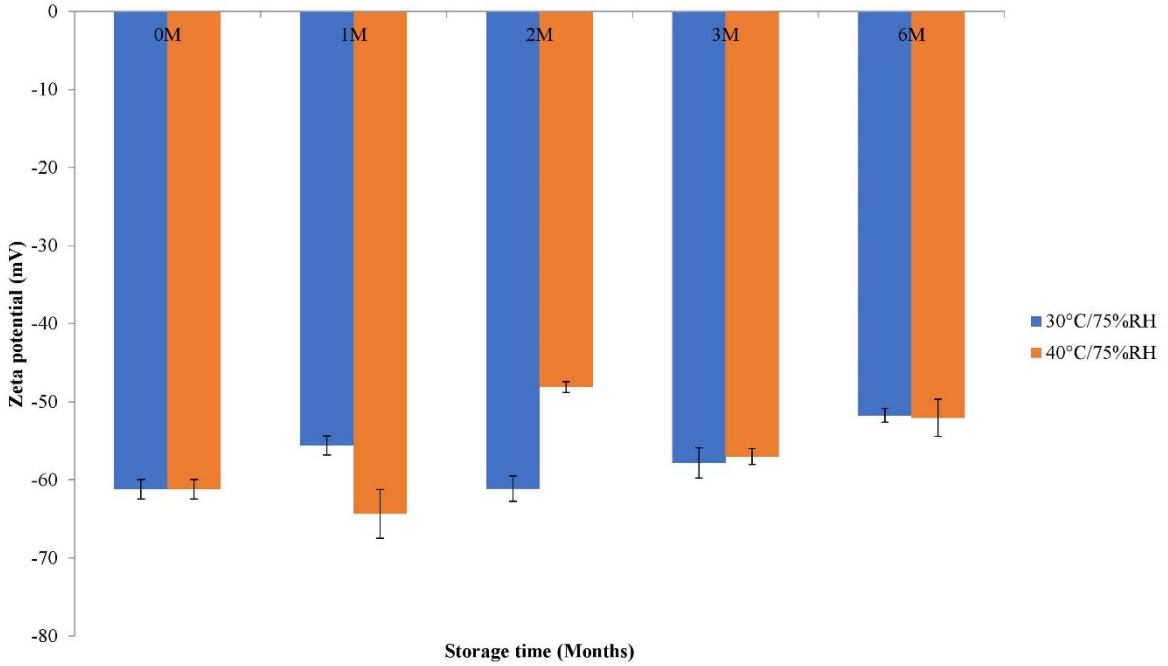

Figure 4 Zeta potential of Cream Containing Piper Betle L. (Mean ± SD, n = 3).

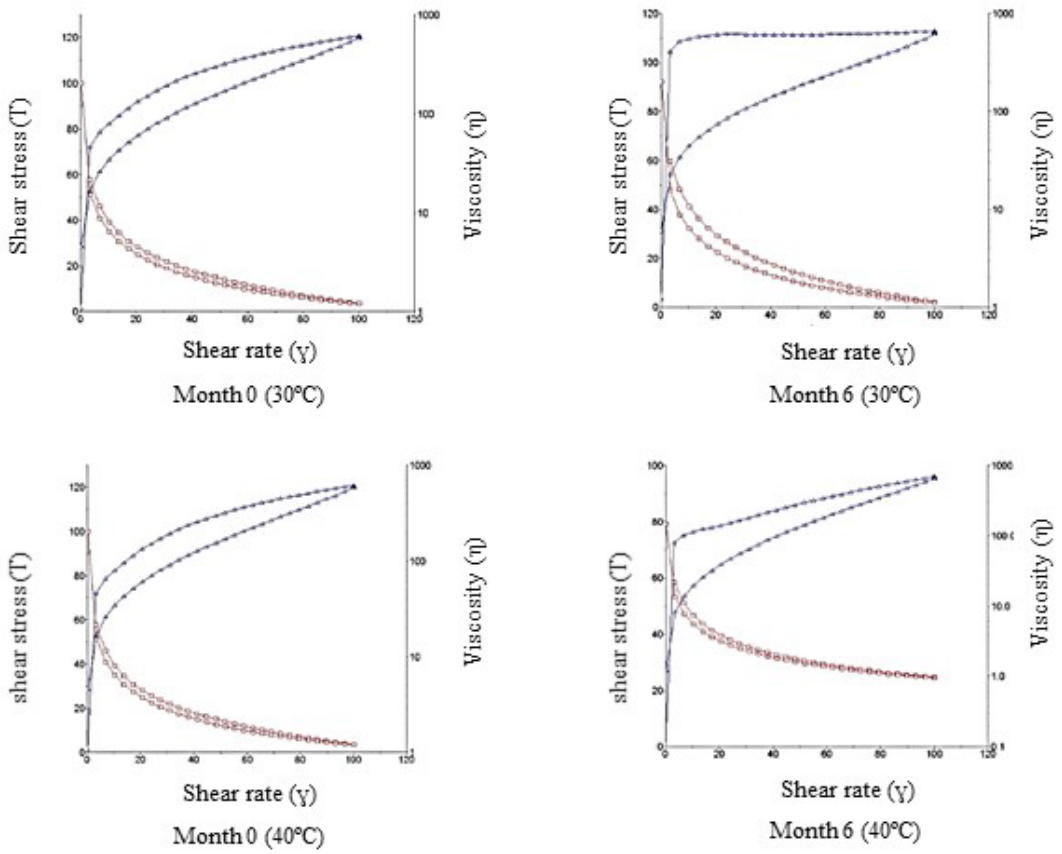

Figure 5: Rheological analysis graphs for PBL cream. Blue line shows relationship of shear stress (T) and shear rate (ɣ), while red line shows relationship of viscosity (ŋ) and shear rate (ɣ).

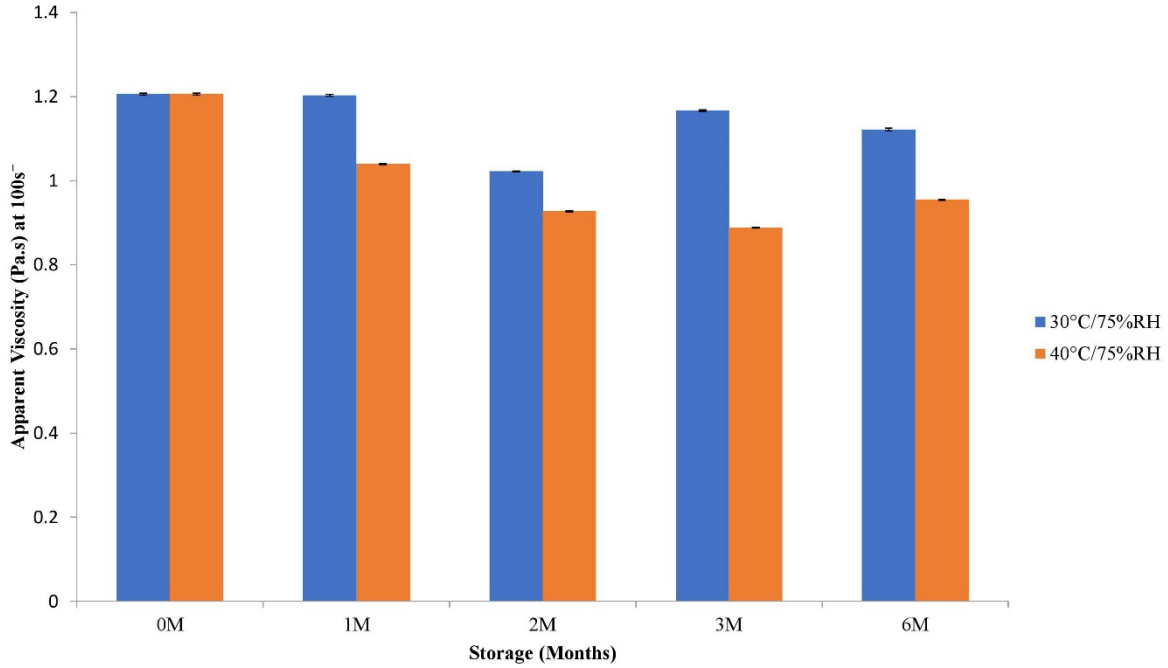

Figure 6: Apparent Viscosity (Pa.s) at $100s^{-1}$ (Mean ± SD, n = 3).

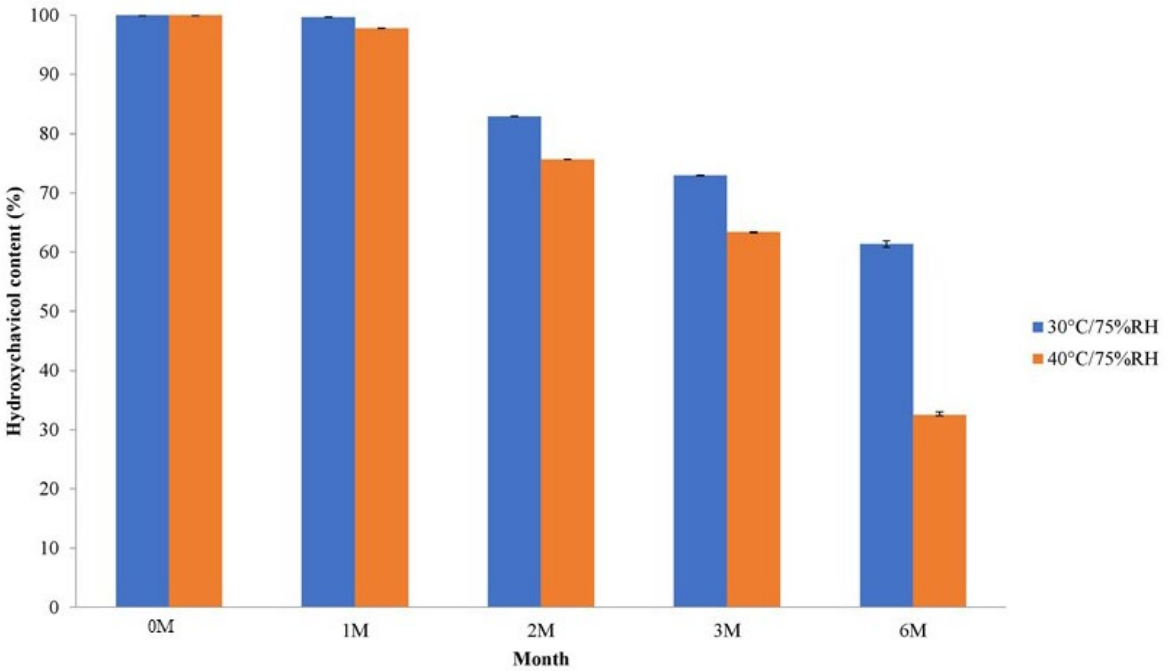

Figure 7: Hydroxychavicol Content in Cream Containing Piper Betle L. (Mean ± SD, n = 3).

Rheological properties

Rheology is the science related to the flow of fluids which is associated with processes such as mixing, pouring, filling containers and extrusion of product through container (Niu et al., 2016). Based on Figure 5, formulations follow a non-Newtonian flow, where the relation between shear stress (mPa) and shear rate (1/s) is not linear. This is shown by the red lines depict decreasing viscosity as a result of increasing shear rate. The blue line forms a hysteresis loop which is a characteristic sign of a thixotropic system. Thixotropy is a time-dependent shear thinning property where with stress; fluid will flow as viscosity reduces. The property of the formulation remained the same throughout the 6 months stability study. Apparent viscosity is the applied shear stress on a liquid divided by the shear rate. The apparent viscosity (Pa.s) at shear rate of $100s^{-1}$, as presented in Figure 6 shows low values maintained below 1.2 Pa.s, despite being significantly different over time (p=0.000).

Microbial limit study

Results of the study shows no growth was observed for all tests which included TAMC, TYMC and the specific tests for Staphylococcous aureus and Pseudomonas aeruginosa over 6 months.

Hydroxychavicol Content Analysis

Figure 7 shows the hydroxychavicol content throughout the 6 months stability study. Formulation stored in both conditions showed a declining trend but differ in the extent of degradation. The final content for formulations stored in 30°C/75%RH and 40°C/75%RH were 61.36 ± 0.53% and 32.46 ± 0.12% respectively. Storage condition caused significant (p=0.000) reduction in the hydroxychavicol content over 6 months' time. The effect of the different conditions towards the formulation was also found to be significant (p=0.000).

Discussion

The light beige colour of the Piper betle Leaves (PBL) cream was caused by the dark brown colour of the extract mixed with white base cream. After 6 months of storage, PBL cream became a tone darker. Raised temperature is known to cause physical changes to formulation including its colour (Deuschle et al., 2015). Creaming, foaming, and phase separation was not exhibited in the PBL cream during stability study. The presence of emulsifying agent lecithin conferred stability to the cream (Fernandes et al., 2012). In addition to that, emulsion stabilizers such as xanthan gum, beeswax and pectin helped prevent separation to its oil and water components (Krstonošić et al., 2015). The acidic pH is considered suitable for skin application with potentially no irritation as it lies within pH of 3-9 and the narrower range of 4-6 of ideal pH for skin formulation (S. Ali & Yosipovitch, 2013; Contri et al., 2014). An acidic pH is preferred because the human skin is covered with an acidic mantle that can be maintained at an average pH 5.5 with the help of an acidic moisturizer (Khan et al., 2013).

The particle size ranges from 0.99 µm - 1.92 µm is considered small and is favoured as it confers higher stability (Khor et al., 2014). The gradual increase in particle size which is especially evident at 6 months can be caused by Ostwald ripening, flocculation, or coalescence of the droplets (Ahmed et al., 2012; Long et al., 2012). The span value remained below 2 except at 3 months and 6 months. A small span value <2 indicates monodispersity of particles in the formulation and is favoured as polydispersity can lead to instability (Santos & Gaspar, 2018: Saberi et al., 2013). Despite increase in particle size and span value, the emulsion remained stable as no visible, creaming or phase separation occurred.

The further the value of the zeta potential from 0, negative or positive, the greater repulsive force between particles, which ensures emulsion stability. As the values obtain are below -50, the formulation remained stable although size and span increased over time. Zeta potential values of -41 to -50 have good stability, compared to values of -11 to -20 which are less stable as it is near the agglomeration threshold (Salkar & Tembhurkar, 2016). Although results show significant difference (p=0.000) in zeta potential values, no significant change in the formulation stability was observed as a difference of more than 10mV frombaseline to month 6 is required to exhibit a significant change (Lu & Gao, 2010).

Both formulations portray a pseudo plastic flow or also known as the shear-thinning system. This system shows that increase in shear stress will increase the shear rate hence reduce viscosity (Rodrigues et al., 2016). The longer time the fluid undergoes stress, the lower the viscosity. The hysteresis loop which represents thixotropy is formed because the breakdown of the formulation does not return to its original state immediately after stress is removed. The increase in shear rate surpasses the Brownian motion hence cause less resistance to flow (Niu et al., 2016).

Topical application has been associated with shear rates of up to 120 s^{-1} (Henderson et al., 1961). Lower viscosity at around these shear rates may represent the good spreadability of the cream for consumers as shown by PBL formulation (Adeyeye et al., 2002). The outcome which showed reduction over time most apparent at 40°C/75%RH however is different from past research which found apparent viscosity to increase over time (Goyal et al., 2015).The product is deemed stable as it can prevent microbial growth (CTFA, 2004). One of the contributing reasons is the natural antimicrobial activity conferred by Piper betle leaves itself on top of the presence of antioxidant and preservative (Pradhan et al., 2013).

Hydroxychavicol content in formulation stored in 30°C/75%RH shows degradation of hydroxychavicol content less than that of the ones stored in a higher temperature of 40°C/75%RH. A study on the stability of phenolic compounds in Piper betle at 5ºC and 25ºC showed that temperature influences the stability of the compounds whereby higher temperature will lead to degradation of the compounds (A. Ali et al., 2018). As hydroxychavicol is a part of the propenylphenol compound, it is also subjected to degradation with higher temperature (Singtongratana et al., 2013). Although the study found minimal effect on hydroxychavicol particularly compared to other compounds, that study was done at a temperature of 25ºC.

Conclusion

As a conclusion, a stable cosmetic cream containing *Piper Betle* L. extract was able to be produced in large scale. Identifying CQA and CPP were an integral step to ensure the production of quality product. Stability study over 6 months proved the ability of the product to remain stable physically and in pH, zeta potential, particle size, viscosity also the hydroxychavicol content.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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