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Biocompatibility and Skin Performance of Fe/Mn-Doped Hydroxyapatite Derived from *Meretrix lyrata* Shell in Emulsion-Based Cosmeceutical Formulations

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Skincare represents a comprehensive methodology for preserving dermal health, given that the integument functions as a critical shield against various environmental hazards. Skincare prevents issues like cancer and promotes youthful skin through hydration and wrinkle reduction. Sunscreens contain various components designed to manage UV radiation. This research investigates alternative compounds and physical barriers that suitable for formulating a sunscreen formulation. The synthesised hydroxyapatite (*Meretrix lyrata*, bivalve shells originated) combined with iron (II) oxide and manganese oxide in formulations were characterised to determine their toxicity, pH value and skin assessments. All samples show optimal pH value, non-toxic and great skin assessment studies, meanwhile, the Fe-doped HAp emulsion exhibiting the most favourable hydration, minor reduction in the transepidermal water loss (TEWL) values, superior elasticity and higher rate of melanin degradation showed characteristics compare to other samples. These results support the commercial feasibility of these formulations in the cosmetic sector that should be further confirmed in specific future research such as in vivo UV protection testing.

Keywords: ultraviolet (UV) filters; hydroxyapatite (HAp); skin assessment; *Meretrix lyrata*

I. INTRODUCTION

Skincare encompasses far more than a superficial routine; it represents a comprehensive approach to nurturing and sustaining the vitality of the integumentary system. The skin serves as an essential protective barrier against numerous external environmental hazards, including ultraviolet radiation, pollutants, and extreme weather conditions. In these past years, people have been encouraged to choose cosmetic products that infused with ultraviolet filter (UVF) qualities in accordance with the ongoing El Niño phenomenon. Unprotected exposure to the sun's heat can cause sunburn and lead to skin issues over time such as skin cancer, discolouration, and wrinkles, even on cloudy days (Merin *et al.*, 2022; Ismailov *et al.*, 2024). Sunscreens are photoprotective lotion required to prevent UV radiation–induced skin damage and skin malignancies. Both organic (chemical) and inorganic (physical) filters are extensively utilised to produce broad-spectrum protection.

The most prevalent organic compounds in commercial sunscreens are titanium dioxide (TiO_2) and zinc oxide (ZnO). These are often included in formulations because of their increased effectiveness when combined as nanoparticles, providing protection against ultraviolet (UV) radiation across the entire UV spectrum (Babarus *et al.*, 2023). However, there alarming insights about the environmental impact and photostability of TiO_2 and ZnO as conventional filters. These metal filters in nanoparticulate forms can display photocatalytic activity, generating reactive oxygen species (ROS) that potentially irritate the skin or accelerate degradation of formulation ingredients (Majumder *et al.*, 2020). Besides, the growing use of sunscreen over the years has demonstrated the cumulative negative impacts of some active components, especially ZnO and TiO_2, on the environment, as evidenced by the detection of significant

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concentrations in coastal waters that represent a direct threat to the ecosystem of marine habitats, particularly coral (Vasyukova *et al.*, 2021). These filters are detrimental and hazardous to marine organisms, requiring regulatory action in tourism-sensitive areas (Lozano *et al.*, 2020).

In order to fill these gaps, there is an upmost need to produce alternative, eco-friendly UV filters to address these problems. In recent years, the biocompatible calcium phosphate hydroxyapatite (HAp) has emerged as a promising alternative HAp can be prepared from calcium (Ca) from shell debris like clam with a phosphate (PO_4) precursor (Fitriyana *et al.*, 2019; Murugiah *et al.*, 2021). The HAp was demonstrated to be a good UV ray protector and a circular-economy pathway that promoted marine biomass and reduced the dependence on manufactured metal oxides (Pal *et al.*, 2020; Ghazali *et al.*, 2021). It was suggested that to extend its UV absorption and improve photoprotective performance, HAp can b doped with transition metal ions such as iron (Fe) and manganese (Mn), which suggested (Hadagalli *et al.*, 2021; Kera & Ray, 2024; Khan *et al.*, 2024). This study investigates the formation of photoprotective emulsions employing a hybrid, metal compound doped clam shell, particularly *Meretix lyrata,* then, were synthesised incorporated with iron (II) oxide and manganese oxide, to assessing their skin compatibility and toxicity provides a way to eco-safe photoprotective formulations.

II. MATERIALS AND METHOD

A. Emulsification of Sunscreen

The formulation is delineated into the oil phase and the aqueous phase throughout the emulsification procedure. The petroleum jelly, serving as a thickening agent, along with the Igepal CO-520 and Tween 80 surfactants (ratio of 50:50) and virgin coconut oil (VCO) was integrated as oil phase following a heating process. Upon achieving the requisite temperature of 70 °C, the oil phase is introduced into the aqueous phase (containing only distilled water) while being subjected to vigorous agitation. Once the amalgamation attains a state of homogeneity, it is systematically stirred using a mechanical stirrer until the temperature diminishes to 35 °C. The samples were subsequently preserved in a cool and dark environment. The samples were evaluated daily to detect any potential modifications.

The most stable emulsions were selected and infused with undoped HAp, Fe-doped HAp, and Mn-doped HAp for subsequent analysis. Both iron-doped HAp sample was prepared by mixing 90 g of raw HAp with diluted 0.5 M iron chloride ($FeCl_2$) and 0.5 M manganese chloride ($MnCl_2$) separately. The samples were then swirled continuously at 60-80 °C for 3 hours. Each solution is acidic, thus 30 mL of 1.0 M NH_4OH was added until the pH level increased from 6.0 to 8.0 (Rozaini *et al.*, 2023). pH of 8.0 was chosen to promote nucleation of HAp, suitable incorporation of Fe^{2+} in the HAp lattice (substituting Ca^{2+}) and to prevent the formation of secondary phosphate phases. The doped samples were subjected to a similar ageing process and then filtered, dried and calcined. Control sample of undoped HAp was used for comparison with metal doped ones.

B. pH

The ideal pH is influenced by the specific ingredients and their applications, as well as the skin's natural pH range. The skin's pH is generally slightly acidic, between 4.5 and 5.5; thus, formulating skin creams with a similar pH facilitates deeper penetration of active components into the dermis (Piccirillo *et al.*, 2017; van Hoogevest & Fahr, 2019; Lukić *et al.*, 2021). Deviations from this pH range in emulsions can compromise the skin's barrier function, leading to inflammation and other adverse effects (Lee & Lee, 2014). All formulated samples were characterised using a pH meter calibrated with standard buffer solutions.

C. Cytotoxicity

The cytotoxicity analysis is crucial for developing new formulations of cosmeceutical products to guarantee the safety of end-users. The cytotoxicity property of emulsion samples was utilised to determine their cell viability in this investigation through the implementation of the MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay against RAW264.7 cell lines (cells derived from mouse ascites leukaemia-induced) as conducted in previous studies (Álvarez-Gómez *et al.*, 2019; Chittasupho *et al.*, 2022; Niveditha *et al.*, 2022). The RAW264.7 cell line was selected

for its established role in assessing inflammatory and toxicological responses, as well as its effectiveness for transfection (Wang *et al.*, 2020; Jannus *et al.*, 2021). In sunscreen cytotoxicity test, the most common cell lines used are human keratinocytes and mouse fibroblasts (Hayden *et al.,* 2005; Maciel *et al.,* 2019; Svobodová *et al.,* 2019).

The RAW264.7 cells were maintained at 37°C in Dulbecco's Modified Eagle Medium (DMEM) with 10% Fetal Bovine Serum (FBS), 1% antibiotics, in 5% CO_2 environment. Phosphate Buffered Saline (PBS) was formulated following the manufacturer's guidelines. Each PBS tablet was dissolved in 100 mL deionised water and autoclaved for refrigeration. The cells were allowed to achieve 80-90% confluence for experimentation. The frozen cells were thawed rapidly and transferred to a cryovial, which was subsequently cleaned with ethanol. The cells were then introduced into a centrifuge tube incrementally. The cell suspension was enhanced with pre-warmed 1% penicillin-streptomycin and DMEM growth medium containing 10% PBS. These additives were incorporated into the tube. The cells underwent centrifugation for one minute at 500 rpm and 4 °C. After centrifugation, additional 1% penicillin-streptomycin, 10% PBS, and 10 mL of pre-warmed DMEM were added to the supernatant.

The cell suspension was gently reconstituted to avoid bubble formation. A cell culture flask was prepared, and the cell suspension was transferred into it. A 75 cm^3 culture flask was utilised to hold 15 mL of growth medium, incubated at 37 °C with 5% CO_2. The cells were undisturbed for at least twenty-four hours for observation of contamination or deterioration. The previous culture medium was discarded, and 5 mL of PBS was added to rinse out the cells. The cells were incubated at 37 °C with 5% CO_2 in a Sigma-Aldrich humidified incubator.

The haemocytometer was prepared under a microscope while the surface was disinfected, dried, and cleaned. Ten microliters of trypan blue dye were pipetted onto parafilm, followed by the addition of 10 µL of RAW264.7 cell suspension. This mixture was thoroughly blended using a pipette. The suspension was then positioned at the periphery of the haemocytometer chamber. An inverted microscope was employed to examine the slide. Viable cells, identifiable by their brightness, were counted and recorded in the corners of the haemocytometer. The concentration of cells was calculated using equation provided below:

$$\text{Cells concentration (cells/ml)} = [(A+B+C+D)/4] \times 2.104$$

whereas,

A, B, C, D is the number of cells counted in each of the four corner (or designated) squares on the haemocytometer.

2.104 is a specific dilution/calibration factor.

D. Skin Assessments

An investigation was conducted employing skin analysis to assess the efficacy of experimental formulations on the skin. This study was a small, exploratory human trial designed to assess the skin compatibility of hydroxyapatite (HAp)-based emulsions containing undoped, Fe-doped, and Mn-doped HAp in a stable formulation emulsion. Skin analysis was performed on a cohort of ten volunteers, evenly divided by sex and aged 18 to 60 years on average. Ten healthy adult volunteers were recruited, each serving as their own control in a randomised, within-subject design that also includes a vehicle-only formulation. Ethical approval was obtained from an Ethics Committee, and written informed consent were collected from all participants. Eligible individuals were selected based on adults with healthy skin and no known sensitivities to formulation components.

Each formulation was applied to separate, randomized forearm using an occlusive patch test for four hours. Skin responses were evaluated focusing on four biophysical skin parameters—transepidermal water loss (TEWL), hydration, skin elasticity, skin colour (melanin), high-resolution ultrasound imaging were evaluated on participants.

Data were be analysed using repeated measures statistical methods ANOVA, with post hoc comparisons where needed and a significance level of 0.05. Due to the small sample size, the study is considered a pilot, aimed at generating preliminary safety and tolerability data to support future research.

III. RESULT AND DISCUSSION

A. Emulsification of Sunscreen

Figure 3 illustrates the stable physical appearance of four formulations after centrifugation and four weeks of storage. The undoped HAp, doped HAp-Fe, and doped HAp-Mn, were incorporated into formulations MEM-B, MEM-C, and MEM-D at 10%, whereas MEM-A served as a blank emulsion devoid of active ingredients as shown in Table 1. Samples were centrifuged after four weeks to evaluate formulation shelf-life (Badolato, 2008; Akhtar *et al.*, 2010). Observations indicated no phase separation in freshly prepared samples during the experiment. This stability may result from appropriate homogenisation that prevented formulation breakage (Sainorudin *et al.*, 2015).

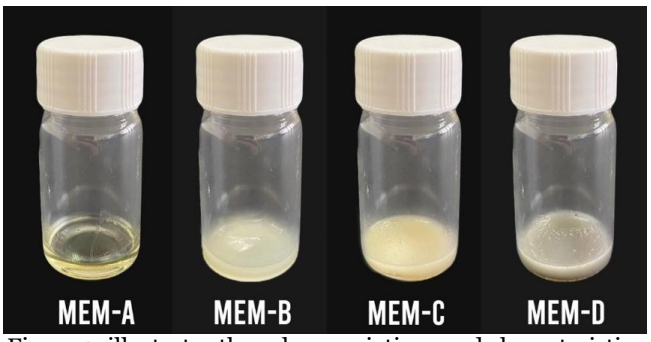

Figure 3. illustrates the colour variations and characteristics of four emulsion formulations. MEM-A served as the control emulsion, while MEM-B represented the undoped Hap sample. MEM-C comprised the doped HAp-Fe sample, and MEM-D corresponded to the doped HAp-Mn sample.

Table 1. Formulation of emulsion samples infused with different UV filters agent (active ingredient)

Sample	Ingredients Added					Type of Active Ingredient (AI)
	Water (%)	Igepal CO-520 (%)	Tween 80 (%)	Virgin Coconut Oil (%)	AI amount added (%)	
MEM-A	15	34	34	17	0	Not available
MEM-B	15	34	34	17	10	Undoped HAp
MEM-C	15	34	34	17	10	Doped HAp-Fe
MEM-D	15	34	34	17	10	Doped HAp-Mn

Table 2. pH value of formulation samples

Sample	Duration				
	Day 1	Day 7	Day 14	Day 21	Day 28
MEM-A	5.34 ± 0.10^{a}	5.25 ± 0.05^{a}	5.24 ± 0.21^{b}	5.20 ± 0.10^{ab}	5.19 ± 0.11^{b}
MEM-B	5.45 ± 0.06^{a}	5.43 ± 0.12^{ab}	5.38 ± 0.18^{b}	5.32 ± 0.08^{a}	5.31 ± 0.21^{a}
MEM-C	5.42 ± 0.21^{b}	5.40 ± 0.16^{b}	5.38 ± 0.15^{ab}	5.35 ± 0.05^{a}	5.32 ± 0.20^{a}
MEM-D	5.48 ± 0.16^{b}	5.47 ± 0.12^{b}	5.45 ± 0.06^{a}	5.42 ± 0.12^{ab}	5.39 ± 0.16^{ab}

Values are the mean ± standard deviation of n=3 with $P<0.05$ using the ANOVA Test.

B. pH Value Analysis

Based on Table 2, all samples exhibited consistently acidic pH levels with negligible decreases during the experiment. All formulations demonstrated non-irritating pH values, rendering them suitable for cosmetic product manufacturing. Furthermore, the variance in pH values correlates with the diverse active ingredients utilised in the formulations. The emulsions produced in this study exhibited pH values ranging from 5.41–5.48 on day one, decreasing slightly to 5.19–5.39 after 28 days.

The pH of topical products and specific ingredients are crucial for skin health and acidification mechanisms. Alpha,

beta, and polyhydroxy acids have been widely used in dermatology and cosmetology for thirty years (Lukić *et al.*, 2021).

Their efficacy depends on the type, pH, and concentration of the acids used. Although not fully elucidated, their action is linked to H^+ ion concentration and the dissociation of free acids, ideally occurring in the epidermis (Jiang & Qureshi, 1998; Draelos, 2000).

The general pH range of the skin is 5.4 to 5.9, crucial for stratum corneum integrity, with surfactants affecting this layer. Surfactants may disrupt skin pH, impacting enzyme synthesis essential for lipid production and NMF components in the stratum corneum.

Regulation of this process is affected by skin microorganisms, endocrine gland substances, and natural moisturising factor (NMF) constituents. The literature highlights the interactions between surfactants and skin that may cause irritation. Research on surfactants centres on their effects on stratum corneum surface proteins. This interaction can lead to protein denaturation, modification of intercellular cement structure, and lipid removal, compromising the epidermal barrier and affecting deeper skin layers (Seweryn *et al.*, 2018).

B. Cytotoxicity

Based on the findings in Figure 4, none of the samples displayed toxicity to the RAW264.7 cell line. The lead formulation, MEM-B (undoped HAp emulsion), showed cell viability exceeding 100%. This was succeeded by MEM-C (Fe-doped HAp emulsion) at 100% viability. The MEM-A (blank emulsion) exhibited a viability percentage of 95%, while MEM-D (Mn-doped HAp emulsion) recorded 85%.

Moreover, viable cells were identified even at the highest concentration. A thorough assessment of the cytotoxicity was performed by evaluating their inhibition values. Subsequently, none of the microemulsion samples showed toxicity toward RAW264.7 cells, as their IC_{50} (IC_{50} = Half Maximal Inhibitory Concentration) values all exceeded 30 µg/mL. Therefore, it was concluded that the four formulation samples are safe for future human skin analysis due to the results indicated that the IC_{50} value for the samples is at least 30 µg/ml.

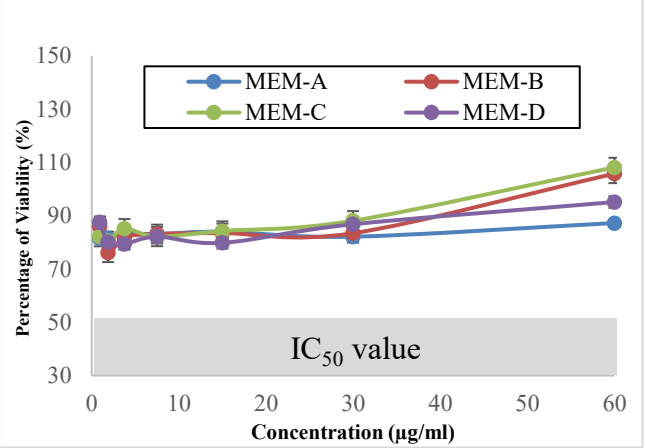

Figure 4. Cytotoxicity property of microhedral formulations against RAW264.7 cell lines. The IC_{50} values are categorised as follows: >30.0 µg/mL is non-toxic, <1.0 µg/mL denotes high toxicity, 1.0-10.0 µg/mL indicates toxicity, and 10.0-30.0 µg/mL reflects moderate toxicity (Syed Abdul Rahman *et al.*, 2013; Niebles *et al.*, 2017; Bakar *et al.*, 2019).

B. Skin Assessment: Hydration, Transepidermal Water Loss (TEWL), Skin elasticity and Melanin

Based on Table 3, for the control emulsion (MEM-A), the increase difference was 30.33 ± 0.08^{a} in males and 36.44 ± 0.12^{a} µS in females. Undoped HAp emulsion (MEM-B) displayed a rise of 30.95 ± 0.15^{b} in males and 39.19 ± 0.15^{b} in females. For sample of doped HAp-Fe emulsion (MEM-C), males experienced a rise of 38.92 ± 0.05^{a} in while females increase of 45.35 ± 0.20^{ab}. The doped HAp-Mn emulsion (MEM-D) recorded increases of 31.56 ± 0.12^{ab} in males and 39.82 ± 0.08^{a} in females. This showed that the hydration values for MEM-A, MEM-B, and MEM-D indicated an upward trend relative to prior measurements. Nonetheless, these values did not surpass those of the MEM-C emulsion's hydration rate.

Table 4 indicated a reduction in transepidermal water loss (TEWL) values for MEM-A of 10.43 g/m2/h ± 0.05^{a} in males and 7.34 g/m2/h ± 0.13^{a} in females. For MEM-B, males exhibited a decrease of 8.26 g/m2/h ± 0.015^{ab}, whereas females showed a reduction of 8.98 g/m2/h ± 0.12^{a}. In the case of MEM-C, males experienced a decrease of 4.88 g/m2/h ± 0.22^{ab}, while females had a reduction of 5.12 g/m2/h ± 0.24^{ab}.

Table 3. Hydration value of samples

Sample	Males			Females		
	Before	After	Hydration Rate	Before	After	Hydration Rate
MEM-A	157.07 ± 0.30^{a}	187.40 ± 0.10^{a}	30.33 ± 0.08^{a}	158.68 ± 0.12^{a}	195.12 ± 0.10^{a}	36.44 ± 0.12^{a}
MEM-B	165.89 ± 0.15^{a}	196.83 ± 0.12^{b}	30.95 ± 0.15^{b}	159.52 ± 0.10^{a}	198.71 ± 0.13^{a}	39.19 ± 0.15^{b}
MEM-C	174.08 ± 0.08^{ab}	212.99 ± 0.15^{ab}	38.92 ± 0.05^{a}	165.02 ± 0.05^{b}	210.38 ± 0.15^{a}	45.35 ± 0.20^{ab}
MEM-D	174.05 ± 0.10^{b}	205.61 ± 0.10^{a}	31.56 ± 0.12^{ab}	164.87 ± 0.13^{ab}	204.70 ± 0.06^{a}	39.82 ± 0.08^{a}

Values are the mean ± standard deviation of n=3 with P<0.05 using the ANOVA Test.

Table 4. TEWL value of samples

Sample	Males			Females		
	Before	After	TWEL Rate	Before	After	TWEL Rate
MEM-A	17.85 ± 0.15^{a}	7.42 ± 0.10^{a}	10.43 ± 0.05^{a}	16.12 ± 0.10^{b}	8.75 ± 0.05^{a}	7.34 ± 0.13^{a}
MEM-B	16.65 ± 0.13^{b}	8.39 ± 0.08^{a}	8.26 ± 0.15^{ab}	17.53 ± 0.12^{ab}	8.55 ± 0.17^{a}	8.98 ± 0.12^{a}
MEM-C	14.65 ± 0.22^{b}	10.02 ± 0.05^{a}	4.88 ± 0.22^{ab}	16.52 ± 0.08^{a}	11.40 ± 0.12^{ab}	5.12 ± 0.24^{ab}
MEM-D	14.45 ± 0.08^{ab}	8.82 ± 0.13^{ab}	5.63 ± 0.18^{a}	16.84 ± 0.05^{a}	8.59 ± 0.21^{a}	8.25 ± 0.27^{ab}

Values are the mean ± standard deviation of n=3 with P<0.05 using the ANOVA Test.

Table 5. Elasticity value of samples

Sample	Males			Females		
	Before	After	Elasticity Rate	Before	After	Elasticity Rate
MEM-A	8.99 ± 0.15^{a}	6.26 ± 0.23b	2.74 ± 0.10^{a}	11.60 ± 0.12^{a}	7.95 ± 0.10^{a}	3.65 ± 0.06^{a}
MEM-B	11.39 ± 0.05^{a}	7.06 ± 0.17^{a}	4.34 ± 0.13^{b}	11.20 ± 0.16^{b}	6.95 ± 0.12^{a}	4.61 ± 0.10^{a}
MEM-C	11.59 ± 0.17^{ab}	7.66 ± 0.12^{a}	3.94 ± 0.04^{ab}	10.80 ± 0.22^{ab}	6.75 ± 0.15^{b}	4.05 ± 0.12^{b}
MEM-D	12.19 ± 0.23^{b}	7.06 ± 0.07^{ab}	5.14 ± 0.10^{a}	13.00 ± 0.07^{a}	8.35 ± 0.22^{ab}	4.65 ± 0.23^{ab}

Values are the mean ± standard deviation of n=3 with P<0.05 using the ANOVA Test.

Table 6. Melanin value of samples

Sample	Males			Females		
	Before	After	Melanin Rate	Before	After	Melanin Rate
MEM-A	40.59 ± 0.10^{a}	38.66 ± 0.08^{a}	1.95 ± 0.05^{a}	38.81 ± 0.03^{a}	36.55 ± 0.10^{b}	2.50 ± 0.09^{ab}
MEM-B	41.19 ± 0.15^{b}	38.13 ± 0.22^{b}	3.06 ± 0.12^{b}	38.84 ± 0.06^{a}	36.34 ± 0.13^{a}	2.50 ± 0.17^{b}
MEM-C	40.81 ± 0.22^{ab}	35.44 ± 0.14^{b}	5.38 ± 0.18^{b}	38.80 ± 0.12^{b}	34.14 ± 0.16^{a}	4.65 ± 0.25^{ab}
MEM-D	39.99 ± 0.12^{a}	35.44 ± 0.18^{b}	4.58 ± 0.20^{b}	37.58 ± 0.11^{b}	34.54 ± 0.21^{ab}	3.04 ± 0.31^{ab}

Values are the mean ± standard deviation of n=3 with P<0.05 using the ANOVA Test.

The MEM-D exhibited a decrease of 5.63 g/m2/h ± 0.18^{a} in males, and females showed a decrease of 8.25 g/m2/h ± 0.27^{ab}. Notably, emulsion MEM-C demonstrated the lowest TEWL values for both genders compared to the other emulsions. This suggests that the skin retains optimal hydration levels following the application of the emulsion sample.

This finding corroborates Salvo *et al.* (2014), which states healthy skin typically has TEWL values between 4 and 8 g/m2/h. This indicates the formulation effectively retained moisture and reduced water loss. Moreover, outcomes revealed a slight water depletion in healthy skin's stratum corneum. The lower TEWL indicate higher skin water retention and better skin protective coat and minor loss of water and are persistent with reduced damage of the stratum corneum barrier function (Buraczewska *et al.*, 2007;

Ferguson *et al.*, 2013). TEWL is influenced by numerous factors, including humidity, temperature, seasonal changes, hydration, and moisturiser usage (Green *et al.*, 2022). Additionally, TEWL values can reflect chemical and physical impacts on the skin, such as abrasion-induced irritation.

Based on Table 5, the elasticity difference for MEM-A was 3.65 kPa ± 0.06^{a} in females and 2.74 kPa ± 0.10^{a} in males. The MEM-B showed a reduction of 4.34 kPa ± 0.13^{b} in males and 4.61 kPa ± 0.10^{a} in females. Comparatively, MEM-C exhibited values of 3.94 kPa ± 0.04^{ab} in males and 4.05 kPa± 0.12^{b} in females. Males recorded a MEM-D value of 5.14 kPa± 0.10^{a}, while females had 4.65 kPa± 0.23^{ab}. It indicates that the elasticity of the blank emulsion surpassed that of the active ingredient (AI) sample. The inclusion of active ingredients may potentially reduce the elasticity value of the sample. When contrasting AI-containing samples with the emulsion MEM-C, the latter demonstrated enhanced elasticity, as evidenced by significant differences in pre- and post-experimental data.

While on Table 6, males exhibited a skin colour disparity (MEM-A) of 2.50± 0.09^{ab}, while females showed 1.94± 0.05^{a}. The MEM-B score decreased by 3.06 ± 0.12^{b} for males and 2.50± 0.17^{b} for females. Males had a MEM-C value of 5.38± 0.18^{b} in contrast to females with value of 4.65± 0.25^{ab}. The MEM-D value was 4.58± 0.20^{b} for males and 3.04± 0.31^{ab} for females. The samples did not significantly affect skin melanin levels. Melanin plays an essential biological role in protecting against solar damage. It protects the epidermis from UV light. The MEM-C sample shows greater melanin degradation.

Moreover, considering four biophysical properties of skin, the hydration values for all emulsion samples were enhanced, with the Fe-doped HAp emulsion demonstrating superior hydration properties in comparison to the other formulations. TEWL values displayed a slight reduction in participants following application, suggesting an improvement in skin condition consistent with effective moisture retention and diminished transepidermal water loss. Additionally, a slight decrease in stratum corneum hydration was recorded in healthy skin, influenced by environmental factors such as humidity, temperature, and the application of moisturisers. Although the elasticity of active ingredient may compromise elasticity, the Fe-doped HAp emulsion exhibited superior elasticity based on substantial pre- and post-experimental data differentials. The emulsions did not significantly alter melanin levels in the skin, despite the protective role of melanin against UV-induced damage. Conversely, other emulsions can lower melanin levels compared to the Fe-doped HAp emulsion.

C. Skin Assesment: High Resolution Ultrasound Skin Imaging

In Figures 6, dermascan images of male and female subjects are compared. Initial findings revealed fragmented and irregular lines at the subcutis-dermis interface. Post-treatment images indicated enhanced compactness, rigidity, and elasticity of skin tissues, reflecting strong connective tissue. The black cavities in dermascan images were linked to water/lymphatic fluid and adipose cells. This also indicated a significant reduction in the echo regions of free interspaces. Furthermore, the cavities exhibited solidified structures post-treatment due to an emulsion component that enhances skin appearance. High resolution ultrasound imaging confirmed that collagen and elastic fibres in the dermis and subcutis became denser post-treatment, attributed to physiological gender differences. Male skin is generally thicker and oilier due to a higher vascular density compared to female skin. Consequently, as female skin exhibits accelerated aging, it necessitates increased hydration and more anti-aging interventions. Additionally, TEWL, hydration levels, and melanin were significantly influenced by photoprotective emulsions, physical activity types, and hydration practices. Reflecting on this, it is apparent that human skin composition and subcutaneous tissue vary among individuals due to numerous factors, including disease, sex, and genetic predispositions. A significant contrast in skin characteristics was observed between male and female participants. Due to androgen (testosterone) effects, male skin is approximately 22 percent thicker and possesses a more resilient texture compared to female skin (Surakiatpinyo & Moungkhem, 2010). Consequently, melanocyte production increases with sun exposure, thereby enhancing protection against harmful UV radiation (Brenner & Hearing, 2008).

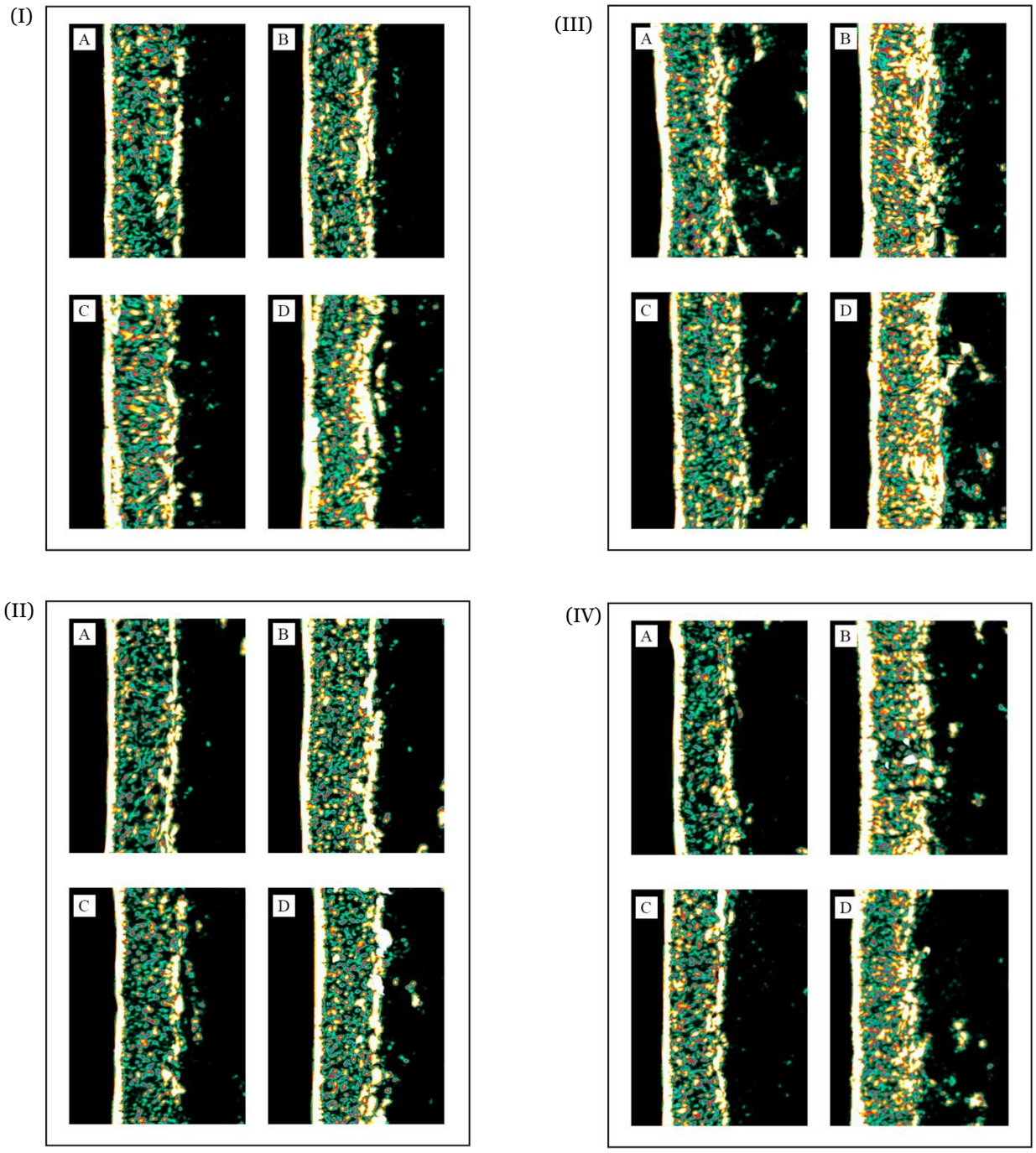

Figure 6. DermaScan images of skin volunteer for (I) MEM-A (blank emulsion), (II) MEM-B (undoped HAp), (III) MEM-C (Fe-doped HAp) and (IV) MEM-D (Mn-doped HAp).
(A) Before treatment of male volunteer. (B) After treatment of male volunteer. (C) Before treatment of female volunteer. (D) After treatment of female volunteer

IV. CONCLUSION

In conclusion, hydroxyapatite synthesised in conjunction with iron (II) oxide and manganese oxide, as the alternative compounds and physical barriers incorporated in emulsion formulations yielding a non-toxic, appropriately pH-balanced, non-irritating properties. It was subsequently observed that none of the emulsion samples exhibited toxicity. Moreover, considering four biophysical properties of skin, all emulsion samples were enhanced, with the Fe-doped HAp emulsion demonstrating superior hydration, good reduction in TEWL level, good elasticity and higher melanin rate compared. These outcomes substantiate the commercial viability of these formulations within the cosmetic industry which must further in specific future studies, such as in vivo UV protection testing.

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