

## Effect of formulations on *in vitro* skin permeation of some metals from mineral clays

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### Abstract

Mineral clay from the evaporites of the Cretaceous to early Tertiary Maha Sarakham Formation in some regions of the Northeast of Thailand containing  $\text{Cu}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Zn}^{2+}$ , in various mineral forms, was used for external applications. Samples of mineral clay were collected in Udonthani and pulverized by heat to obtain plain clay (particle sizes of  $< 75 \mu\text{m}$ ) and then maturated to obtain mature clay. Two gel formulas, a polymer gel (formula 1) and a mineral gel (formula 2), were prepared to incorporate plain clay. *In vitro* skin permeation of  $\text{Cu}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Zn}^{2+}$  from plain clay, mature clay and plain clay in formula 1 or formula 2 through full-thickness skin of newborn pigs ( $n=6$ ) were compared by using Franz diffusion cells and analyzed by atomic absorption spectroscopy. Trace amounts of these metals were detected in the receptor fluid. Maturation of clay with smaller size significantly enhanced skin permeation of  $\text{Cu}^{2+}$  ( $p = 0.04$ ),  $\text{Mn}^{2+}$  ( $p = 0.04$ ) and  $\text{Zn}^{2+}$  ( $p = 0.01$ ). Formula 1 and size reduction affect permeation of  $\text{Cu}^{2+}$  ( $p = 0.04$ ) and  $\text{Zn}^{2+}$  ( $p = 0.01$ ), while formula 2 and size reduction affect permeation of  $\text{Mn}^{2+}$  ( $p = 0.02$ ) and  $\text{Zn}^{2+}$  ( $p = 0.01$ ). Initial content and skin permeation of plain clay are comparatively in the same order, as follows:  $\text{Mg}^{2+} > \text{Mn}^{2+} > \text{Cu}^{2+} > \text{Zn}^{2+}$ . Therefore, some trace elements,  $\text{Cu}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Zn}^{2+}$  from mineral clay can permeate the skin by diffusion with additional effects from formulation and particle size reduction.

**Keyword:** mineral clay, *in vitro* skin permeation,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$

### 1. INTRODUCTION

Minerals are topical used for cosmetic as well as therapeutic purposes.<sup>1</sup> Releasable mineral salts in skin care products containing salts of magnesium ( $\text{Mg}^{2+}$ ), manganese ( $\text{Mn}^{2+}$ ), copper ( $\text{Cu}^{2+}$ ), zinc ( $\text{Zn}^{2+}$ ) and several others play vital roles in maintaining skin conditions by regulating water retention, osmotic pressure, pH and bio-electrical potentials.<sup>2</sup> Topical mud pack improves local microcirculation and vasomotion stimulation.<sup>3</sup> High mineral contents of the Dead Sea mud and mineral water are shown to have beneficial effects for treatment of psoriasis.<sup>4,5</sup> Certain factors, such as climate,

intense sunlight and mineral-rich mud influence the effect of mud therapy on rheumatic disorders. The evaporites of the Cretaceous to early Tertiary Maha Sarakham Formation (MSF) are typically found in the Khorat Plateau in the Northeast of Thailand.<sup>6</sup> Salt lies close to the surface, so-called rock salt, of the MSF is rich in manganese, magnesium, copper and etc.<sup>7</sup> Residues or wastes of the salt farms in the region are the underneath clays which have been soaked with the evaporating salt water and exposed to intense sunlight for months, forming fine mineral-rich clays. To prove that the salt-farming wastes, the mineral clay, can provide mineral permeation through

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the skin, *in vitro* skin permeation is the model for screening tests.

Copper, one of the micronutrients, maintains the performances of the immune and antioxidant systems as well as affect health aging and longevity.<sup>8</sup> Its local distribution in biological tissues in the forms of enzymatic metalloproteins supports the utilization of oxygen/energy and synthesis of essential compounds.

Manganese is classified as 'probably essential' trace element to human health. Manganese superoxide dismutase, for instance, dismutates the superoxide anion ( $O_2^{\cdot -}$ ) derived from the reduction of molecular oxygen to hydrogen peroxide ( $H_2O_2$ ), which is detoxified by glutathione peroxidase to water and molecular oxygen.<sup>9</sup>

Magnesium is the second most abundant intracellular divalent cation which involves major metabolic reactions in the body involving disease prevention and overall health.<sup>10</sup> Dead Sea salt solution with high magnesium content was shown to improve skin barrier function, enhance stratum corneum hydration, and reduce skin roughness and inflammation.<sup>11</sup> Magnesium salts bind water, influence epidermal proliferation and differentiation, and enhance permeability barrier repair. Zinc, the second most abundant trace metal found in eukaryotic organisms and one of the trace elements vital to human health, is involved in activities of numerous metalloenzymes or as a stabilizer of the molecular structure of subcellular constituents and membranes. It is essential for the skin protein, DNA and collagen syntheses and its deficiency retards wound healing. It modulates the production of inflammatory mediators and also possesses antioxidant and antiandrogenic properties. Mineral clays are topically applied for pharmaceutical and cosmetic purposes<sup>12</sup> with several therapeutic approaches.<sup>1,13</sup> High adsorptive and cation exchange capacities of clays are useful for the skin, i.e. removal of excess secretions, coat and protection against external physical and chemical substances, as well as antiseptic and refreshing actions.

This enables soluble trace elements adsorption onto the clay surface and could deliver them to exert activities as co-enzymes and bio-catalysts, regulation of pH osmotic pressure and bio-electrical potentials.<sup>2</sup> Maturation of clays with salt or mineral water to alter the physical characteristics, e.g. plasticity, absorption capacity, cooling indexes and particle size, is one of the modified treatments to the clays.

*In vitro* permeation studies are particularly useful for screening dermal absorption of chemicals in industrial settings, e.g. cosmetic, detergent, agrochemical. Limitations of the studies could be due to the availability and variability of the human skin, the most acceptable barrier membrane. Despite the differences in anatomical and chemical compositions, several animal skin models have been used as the alternative including shed snake skin<sup>14</sup>, rat skin, porcine skin and good correlated results to the human skin were suggested under certain experiment control. The fact that the barrier function of the skin lies on non-living tissue attributed to the stratum corneum.<sup>15</sup>

The healing use of mud pack therapy was attributed to ion transport across the skin, while water insoluble elements were available when abundant perspiration triggers cation exchange with clay materials. Thus, an *in vitro* study shows an exchangeable radiolabeled  $Ca^{2+}$  from Ca-bentonite across the guinea pig skin and delivers  $Ca^{2+}$  to deposit into the hydroxylapatite matrix.

In this study, experiments are performed using porcine skin with the purpose of comparing the permeation of some ions found in the salt-rich mineral clays of the region. For comparison of the permeation profiles, the effects of maturation and formulations were also investigated.

## 2. MATERIALS AND METHODS

The standards used,  $Mg^{2+}$  (magnesium ions),  $Mn^{2+}$  (manganese ions),  $Cu^{2+}$  (copper ions) and  $Zn^{2+}$  (zinc ions) were all ICP standard 1,000 mg/L (Merck, Germany). Deionized water was used throughout the study.

### **2.1. Collection, preparation and weight distribution of mineral clay**

Samples of raw clay were randomly collected from salt farms after harvesting during dry season in Udonthani, one of the districts situated on the salt beds of the MSF deposits in the northeastern Thailand. The raw clay was dried to constant weight and heated at the temperature over 200°C for about 1 h, followed by a series of sieving to further separate the clay samples into 2 ranges based on the particle sizes.

The weight distribution of the clay samples were investigated and classified, as 'fine clays' being those passed through the sieve no. 200 but retained on the sieve no. 325 or particle size range 45 – 75 µm and 'extra fine clays' being those passed through the sieve no. 325 or particle size of less than 45 µm. Sterilization of the clay or its products was conducted using an autoclave at 121°C for 20 min.

### **2.2. Formulations of mineral clay**

#### **2.2.1. Formula 1**

50% of the clay samples, 30% polyethylene glycol 1000, cetomacrogol 1%, and deionized water being used to adjust to the final weight. The clay sample was thoroughly mixed with melted PEG and cetomacrogol using a homogenizer, followed by adjusting the final weight with deionized water.

#### **2.2.2. Formula 2**

20% of the clay samples, 15% bentonite, 0.2% sodium benzoate and water for adjust to the final weight. Bentonite, fine powder, was dispersed in water (at about 50°C) and allowed to fully hydrated (at about 25°C) for 24 h to form gel. The gel was mixed with the fine clay, followed by sodium benzoate solution and water.

### **2.3. In vitro permeation**

#### **2.3.1. Membrane preparation**

Fresh porcine ears (n = 6) were

obtained from local abattoir and treated within 2 h after sacrifice. After cleaning with water, the skin was excised with a scalpel. Specimen of the porcine skin was obtained by heat separation: Pieces of fresh full-thickness skin were immersed in water at about 60°C for 45 sec following which the epidermis was carefully separated from the dermis with tweezers and stored at –20°C until use. The frozen specimen was washed and soaked with water, at 35°C for about 1 h, and cut to size before use. In average, the thickness of the PS was 1.1 mm (n = 20).

#### **2.3.2. Preparation of Franz diffusion cells**

Firstly, receptor compartments of glass Franz diffusion cells were filled with 5 mL of water and a magnetic follower controlled at 37°C with water jacket. A piece of the pre-treated porcine skin used the barrier membrane was placed between the donor and receptor compartments by facing the outer towards to the donor compartment. Great care was taken to prevent air bubble formation between the membrane and the solution. Donor chamber was then place above the barriers and clamped the three compartments of donor, barrier and receptor together with cell clamp. Subsequently, the donor compartment was filled with 1 mL of test materials. The receptor compartment was constantly stirred by a magnetic follower (inside the receptor compartment) to mix the permeated compound with the receptor medium. Samples of receptor solutions were collected via sampling port and receptor medium (37°C) of the same volume was replaced the sample taken at predetermined time interval. The sample was analyzed by atomic absorption spectrometry.

To enable passive diffusion particularly from solid or semi-solid samples, the testing materials, i.e. the clay samples, matured clay, formula 1 or formula 2, were diluted with an equal amount of water before filling the donor chamber of the pre-packed Franz diffusion cells.

#### 2.4. Quantitative analysis of $\text{Cu}^{2+}$ , $\text{Mg}^{2+}$ , $\text{Mn}^{2+}$ and $\text{Zn}^{2+}$

The samples were digested with concentrate nitric acid, using a respective volume ratio of 4:1, at about 95°C for 2 h, and then diluted to the range of the relevant standard curves. The dilutions were subjected to analysis by inductively coupled plasma – optical emission spectrometer (Optima 4300 DV, Perkin Elmer, U.S.A.) and inductively coupled plasma mass spectrometer (Agilent 7500, Japan) using  $\text{Ar}_2$  as the carrier gas at 0.9 L/min. The results were obtained by calculating from the relevant standards; i.e 0 – 25 ng/mL for  $\text{Mn}^{2+}$ , 0-100 ng/mL for  $\text{Mg}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$ .

#### 2.5. Data analysis

Cumulative amounts of each metal ions permeated were plotted with each time interval. The slopes obtained from each plot was used to estimate the rates of permeation (flux) which were averaged and permeabilities were estimated based on donor concentrations of the relevant metal ions.

Statistical analysis was carried out to study the significance of the results by comparing the data at 95% confidence. Results of the same permeation durations of samples were compared by paired t-test (2 products) or ANOVA (> 2 products). Permeation rates of the products were compared by non-parametric Chi-square test.

**Table 1.** Effect of dry heat treatment on weight distribution of the clay samples

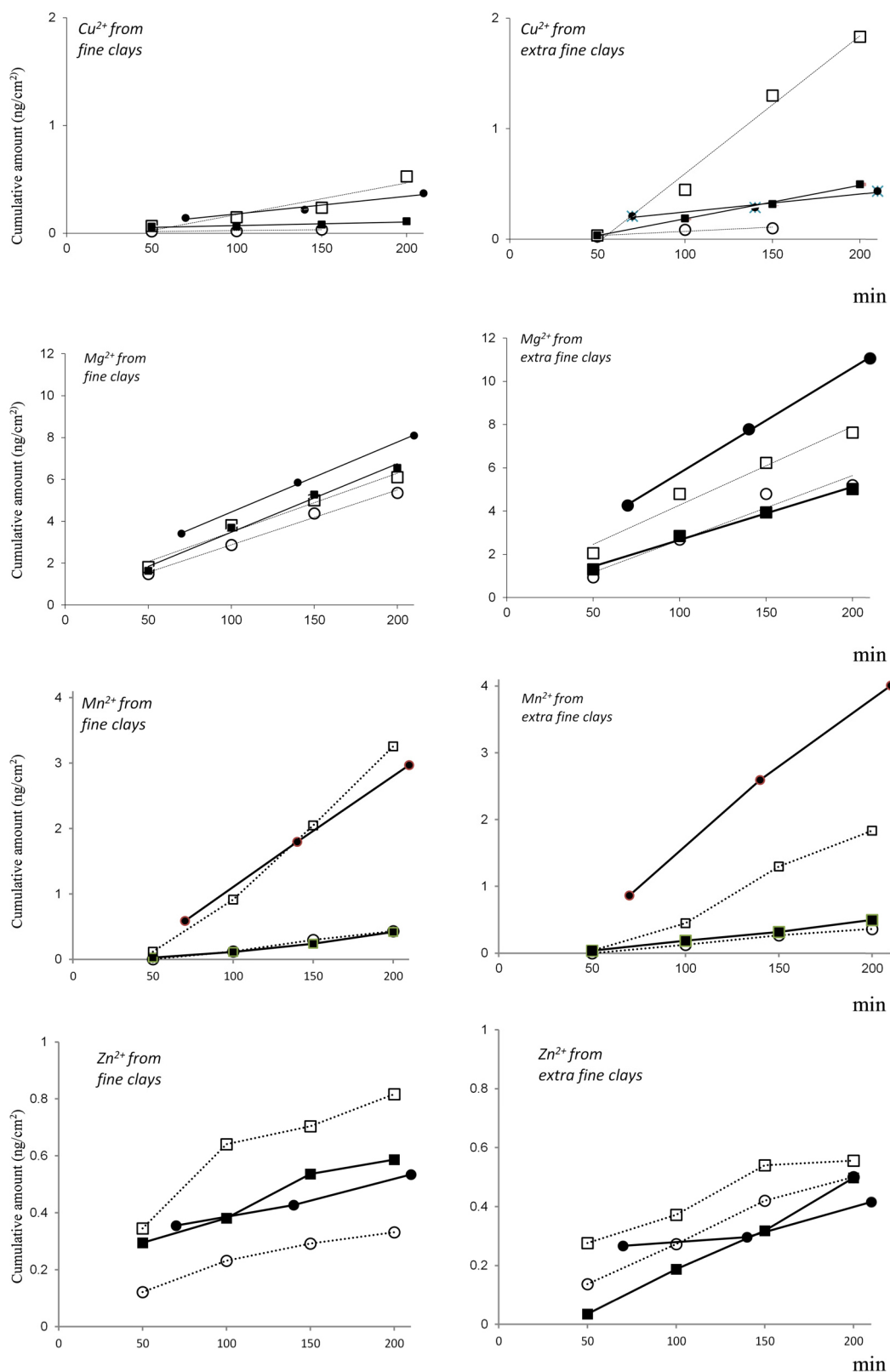
Particle size ( $\mu\text{m}$ )		Weight distribution upon dry heating (%)	
		Before	After
> 106	-	86.5	66.5
75 – 106	-	11.1	19.9
45 - < 75	Fine clay	2.1	10.8
< 45	Extra fine clay	0.3	2.8

### 3. RESULTS AND DISCUSSION

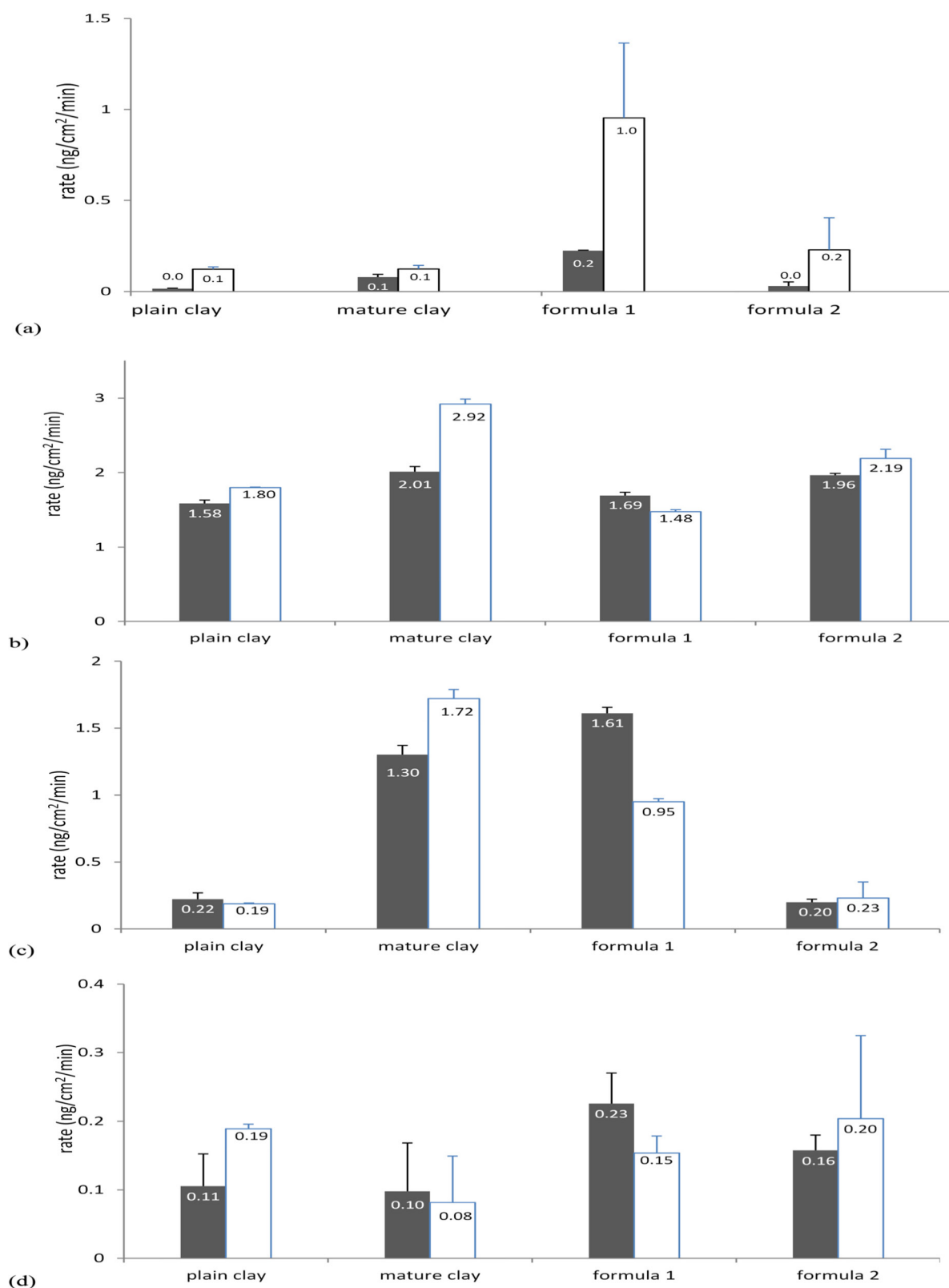
Table 1 compared the weight distribution of the clay samples before and after the dry heat treatment for pulverization of the mineral clay. After dry heating the raw clay at about 200°C for 30-45 min, some moisture in the clay evaporates. Vapor and heat segregated the particles, resulting in size reduction of the clay which could be observed as clay powder. An additional heating duration of 15-30 min was applied to treat the clay powder in order to allow heat transfer into the tortuous pores for further segregation of the solid powder. The resultant from the dry heat process was a reduction of particle sizes of the raw clay. Pulverization by heat increases the particle sizes of < 75  $\mu\text{m}$  from about 2% of the raw clay (before) to

about 13% of the plain clay (after), as shown in Table 1.

The particle size ranges of particular interest those less than 45 mm (extra fine clay) and between 45 to 75 mm (fine clay). Thus, fine and extra fine clays were separated from the raw clay for use in this study. The permeation profiles of  $\text{Cu}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Zn}^{2+}$  through the porcine skin (barrier membrane) are compared between fine and extra fine clays in formulas 1 and 2 and blank plain clay to matured clay, as shown in Figure 1. Despite the presence of  $\text{Cu}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Zn}^{2+}$  in trace amounts in the plain clay, the permeated quantities were detectable from the receptor fluids. Overall, the permeated quantities were ranked, as follows:  $\text{Mg}^{2+} > \text{Mn}^{2+} > \text{Cu}^{2+} > \text{Zn}^{2+}$ .



**Figure 1.** Cumulative amount (ng/cm<sup>2</sup>) permeated of Cu<sup>2+</sup>, Mg<sup>2+</sup>, Mn<sup>2+</sup> and Zn<sup>2+</sup> from plain clay (—○—), maturated clay (—●—), plain clay in formula 1 (—□—) and plain clay in formula 2 (—■—) detected in the receptor phase at time (min), compared between fine clay (45-75 mm) and extra fine clay (<45 mm) at 37°C. (n = 3 each)



**Figure 2.** Comparative effects of formulations on average (with standard deviations as shown in error bars) *in vitro* permeation rate (ng/cm<sup>2</sup>/h) of (a)  $\text{Cu}^{2+}$ , (b)  $\text{Mg}^{2+}$ , (c)  $\text{Mn}^{2+}$  and (d)  $\text{Zn}^{2+}$  through porcine skin (n = 6); grey columns – fine clays and clear columns – extra fine clay.



**Table 2.** Some elements found in the clay samples, in comparison of before and after the heat treatment.

	Before	After
Cu <sup>2+</sup> (mg/g)	0.02	0.02
Mg <sup>2+</sup> (mg/g)	8.25	5.65
Mn <sup>2+</sup> (mg/g)	4.10	3.50
Zn <sup>2+</sup> (mg/g)	0.03	0.01

The processes used to treat the mineral clay deviate skin permeation of some trace elements. Maturation with the mineral salt solution of the same area, profoundly affect the permeation of Mn<sup>2+</sup>, Cu<sup>2+</sup> and Zn<sup>2+</sup>. Extra fine clay with maturation significantly increased permeation of Cu<sup>2+</sup> ( $p = 0.04$ ), Mn<sup>2+</sup> ( $p = 0.04$ ) and Zn<sup>2+</sup> ( $p = 0.01$ ), but insignificantly affected that of Mg<sup>2+</sup> ( $p = 0.23$ ). Saturation of the minerals on the clay surface should occur during maturation, and thus, the matured clay provides higher content of minerals which increases the gradients between the skin used as the barriers. This effect is not significant with Mg<sup>2+</sup> which initially contains the highest level and may have been limited by the barrier function of the skin. The polymer gel of formula 1, but not mineral gel of formula 2, significantly enhanced the permeation of Cu<sup>2+</sup> ( $p = 0.04$ ) and Zn<sup>2+</sup> ( $p = 0.01$ ) from extra fine clay, in comparison to the clay with smaller size. Similarly, formula 2 affects skin permeation of Mn<sup>2+</sup> from extra fine clay ( $p = 0.02$ ). Polymers used to form formula 1 could facilitate even distribution and readiness to partition through the skin of Cu<sup>2+</sup> and Zn<sup>2+</sup>. It is surprised to learn that the permeation extent of Mn<sup>2+</sup> and Zn<sup>2+</sup> from formula 2 was enhanced with size reduction ( $p = 0.02$  and  $0.01$ , respectively).

Fine clays and extra fine clays exhibited a similar pattern of permeation of Cu<sup>2+</sup>, Mg<sup>2+</sup>, Mn<sup>2+</sup> and Zn<sup>2+</sup>, for example, lowest permeation from blank clays and higher from matured clays. Maturation of the blank clays was expected to increase mineral contents adsorbed onto the clay particles and thus should enhance permeation. The results obtained from the blank clays

and matured clays were intended to verify this *in vitro* permeation studies.

Formulas 1 affects the skin permeation of Zn<sup>2+</sup> and Mn<sup>2+</sup> in both fine and extra fine clays, and Cu<sup>2+</sup> and Mg<sup>2+</sup> in extra fine clays, when compared to those of the plain clays. Formula 2 slightly enhances permeation of Zn<sup>2+</sup> in fine clays but not in extra fine clays which may be a result of thoroughly mixing of the mineral clay and the inorganic clay gel. The polymers in formula 1 may promote even dispersion of the clay particles and enhance dissolution of the inorganic cations. Clays in the formula 2 and the mineral clays are inorganic clays which are similar materials and may have interacted such that permeation of the mineral contents was not promoted.

It is worth notified that the temperature control in this study was 1-2°C higher than the skin temperature (about 35°C) since it was well defined that topical applications of mud for 15 – 30 mm elevated skin temperature.<sup>3</sup> Initial contents of the metal ions in the clay samples used directly affect each individual permeation. Table 2 shows that the 4 minerals in the clay samples were, as follows: Mg<sup>2+</sup> > Mn<sup>2+</sup> > Zn<sup>2+</sup> > Cu<sup>2+</sup> which is also the case with permeation. Mg<sup>2+</sup> which is also high compositions in Dead Sea salts is shown to be high in our mineral clay but at a lower extent than the Dead Sea products. However, Mg<sup>2+</sup> in our mineral clay is also permeated at the highest extent and rate when compared to Cu<sup>2+</sup>, Mn<sup>2+</sup> and Zn<sup>2+</sup>. Thus, results from this study indicate that skin permeation of mixtures of metal ions occurs by diffusion. However, further studies to investigate the chemical forms of these metal ions which may have some functional effects *in vivo*.

## 4. CONCLUSION

Mineral clay sampled from one site of the MSF was able to be pulverized by heat with an increase in extra fine clay ( $< 75 \mu\text{m}$ ) and found to contain  $\text{Mg}^{2+} > \text{Mn}^{2+} > \text{Zn}^{2+} > \text{Cu}^{2+}$ . Maturation of the clay greatly promotes the skin permeation rates of  $\text{Cu}^{2+}$ . Formula 1, incorporated with extra fine clay, enhances the rates of skin permeation of  $\text{Cu}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Zn}^{2+}$ . Formula 2 does not as greatly promote the skin permeation of these cations as formula 1, possibly due to interactions of the gel base and the incorporated mineral clay. The *in vitro* permeation of  $\text{Cu}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Zn}^{2+}$  through the skin occurs by diffusion, in the same order as the contents.

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