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## **Inorganic ions in the skin: allies or enemies?**

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### **Abstract**

Skin constitutes a barrier protecting the organism against physical and chemical factors. Therefore, it is constantly exposed to the xenobiotics, including inorganic ions that are ubiquitous in the environment. Some of them play important roles in homeostasis and regulatory functions of the body, also in the skin, while others can be considered dangerous. Many authors have shown that inorganic ions could penetrate inside the skin and possibly induce local effects. In this review, we give an account of the current knowledge on the effects of skin exposure to inorganic ions. Beneficial effects on skin conditions related to the use of thermal spring waters are discussed together with the application of aluminium in underarm hygiene products and silver salts in treatment of difficult wounds. Finally, the potential consequences of dermal exposure to topical sensitizers and harmful heavy ions including radionuclides are discussed.

## 1. Introduction

Ions take part in various life processes. Some of them are called essential as they are necessary for growth, reproduction, and maintaining overall good health. The presence of all types of ions reflect the dietary intake, environmental exposure or even geochemical origins of humans (Goldhaber, 2003; Nielsen, 1991). Even those present at very low concentrations, called trace elements, may be beneficial for health owing their biological activity. The main body elements (99 %) are light with atomic numbers lower than 36, except iodine (Fraústo da Silva and Williams, 2001; Frieden, 1985; Nielsen, 1976; Zoroddu et al., 2019). The most abundant ones are bulk structural elements: oxygen (25.4 % wet wt), carbon (9.4 % wet wt), hydrogen (62.8 % wet wt), and nitrogen (1.4% wet wt). Besides, macrominerals such as sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), chlorine (Cl), phosphorus (P) and sulphur (S) (mainly as phosphates ( $\text{PO}_4^{3-}$ ) and sulfates ( $\text{SO}_4^{2-}$ ) greatly contribute to the elementary composition of the body. All of the non-metals, excluding the inert gases, with atomic numbers smaller than that of bromine, are considered essential. Essential trace elements are present as amounts in the range of  $\mu\text{g}$  per g or less. Most of them are classified as transition metals or in a subdivision of this group (Table 1) (Frieden, 1985). Depending on the author, they may comprise: iron (Fe); zinc (Zn); copper (Cu), fluorine (F); iodine (I); selenium (Se); manganese (Mn); cobalt (Co); molybdenum (Mo) and chromium (Cr). Finally, ultra-trace elements have been detected in animal models: boron (B), vanadium (V), silicon (Si), nickel (Ni) and arsenic (As) (Table I) (Fraústo da Silva and Williams, 2001; Frieden, 1985; Nielsen, 1976, 1991; Zoroddu et al., 2019). All groups of elements of periodic table are present, except from the IIIA and IVA and inert gases (Table I). Consequently, many types of chemical interactions with biological tissues can be expected (Fraústo da Silva and Williams, 2001). The deprivation or over consumption of essential ions may lead to severe diseases (Edelman and Leibman, 1959; Elinder and Århem, 2003). Apart from C, N, H and O, which are essential for synthesis of biological structure of the body, some ions have essential functions for the transmission of information ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ), catalysis of biological reactions ( $\text{Fe}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Mo}^{2+}$ ), or electron transfer processes ( $\text{Fe}^{2+}$ ) (Fraústo da Silva and Williams, 2001). The characteristic electrolyte patterns of both intracellular and

extracellular fluids are crucial for cell survival and homeostasis (Lewis, 2012; Zoroddu et al., 2019). Some of the ions are necessary to ensure the electrical activity in the tissues; they are supporting muscle contractions and help at maintaining the function of nerve cells. At the cellular level, electrolytes are charged molecules that contribute to osmotic pressure and water exchanges between body compartments. They ensure several other functions such keeping electrical neutrality or maintaining acid-base balance and plasma isotonicity. They can also act as carriers, messengers, cofactors of enzymes and stabilizers of proteins and lipids. Sodium is the most abundant cation in extracellular fluids ( $140 \text{ mmol}\cdot\text{L}^{-1}$  against  $12 \text{ mmol}\cdot\text{L}^{-1}$  in the intracellular space) while potassium is its counterpart intracellular cation with  $140 \text{ mmol}\cdot\text{L}^{-1}$  against  $3.5$  to  $5 \text{ mmol}\cdot\text{L}^{-1}$  in the extracellular space (Berend et al., 2012). Chloride is the main anion in the body, mostly extracellular, and represents 70 % of the total content of anions (Reynolds et al., 2006). Calcium is the most abundant cation (1.5 % of total body weight) the main part (99 %) being found in bones and teeth in association with phosphate as hydroxyapatite. Calcium ions are also involved in blood clotting, normal muscle contraction and nerve activity. Magnesium ions,  $\text{Mg}^{2+}$ , are associated with the functioning of muscle and nerve tissue, bone formation and are cofactors of many enzymes. The detection of any deviation off the normal concentration ranges of these species is useful for the diagnosis of metabolic disorders such as diabetes, liver and renal dysfunction, or heart problems (Lansdown, 1995; Nielsen, 1991). Exposure to all of the abovementioned elements may cause either positive or detrimental effects which depend on their concentration and administration route. Finally, other ions of the periodic table of elements that are present in the environment may be ingested, inhaled or exposed topically and interfere with physiological processes involving ions. As example, heavier metals such as Cd, Hg, Pb or lanthanides are present in the environment (Earth's crust for example) and are toxic to most organisms.

The skin is the largest organ of the body and plays the role of a frontier between the external environment and the interior of the organism. It is an attractive administration route of drugs for therapies (either topical or systemic). But it may also be an entry for hazardous agents. As stated by Lansdown, "it must preserve a state of dynamic equilibrium with its surrounding environment and is

dependent on a correct intracellular and intercellular environment in achieving an optimal proliferation and differentiation” (Lansdown, 1995; Eisele and Eichelberger, 1945). Moreover, the skin may be regarded as one of the most important water-storage organs of the body. Its water content (623–717 g per kg of fat free skin) is considerably larger than in any other tissue of the body (Edelman and Leibman, 1959; Jackson, 1990; Eisele and Eichelberger, 1945). The first studies aiming at determining the ionic content of the skin were carried out in the eighties. Such late interest is quite amazing given that one of the most important functions of skin is the control of the fluxes of electrolytes and water across this tissue. Indeed, “taking the waters” is the most ancient water treatment promising relief to sick people (Forslind et al., 1999; Warner et al., 1988). The combination of hot or cold baths from volcanic or sea water is a beneficial treatment of numerous diseases including gout, paralysis, chronic pain, or convalescence after surgery. The distribution of ions in the skin serves as a reference for pathological changes occurring in the tissue as in the case of psoriasis or atopic dermatitis (Forslind et al., 1999). Conversely, some oral treatments or skin exposure may have adverse effects on the skin. As example, oral administration of lithium salts to psychiatric patients has been recognized to aggravate or provoke psoriasis (Hanumanthappa et al., 2010). Mercury salts, which are popular due to their skin-lightening effect, causes nephrotic syndrome (Chan, 2011). Skin sensitization has been reported after exposure to many ions including chromium(VI), cobalt, and nickel (Lansdown, 1995; Marinovich et al., 2014).

This review reports and analyses current knowledge regarding the effects of ions applied on the skin. It is focused on the passive absorption of ions into the skin with a particular emphasis on their effects on human health. Since ionic species are better dissolved in the tissue water, the distribution of both water and ions in the skin are intimately related. In the third section, the speciation of ions in biology is recalled. Next, the pathways for ions can cross biological membranes are described. The fifth part focuses on the effects of ions on human health. It mainly concerns the effects of cations on the skin as only few anions are recognized to disturb the skin when applied topically. Iodide is an exception that

is described in the last section dedicated to the transport of anions related to their physicochemical properties.

**Table 1.** Periodic table of elements: ions essential for life.

IA	IIA											IIIA	IVA	VA	VIA	VIIA	VIIIA
H												B	C	N	O	F	He
Li	Be											Al	Si	P	S	Cl	Ne
Na	Mg	IIIB	IVB	VB	VIB	VIIIB	VIII		IB	IIB		Ga	Ge	As	Se	Br	Kr
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	In	Sn	Sb	Te	I	Xe
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	Tl	Pb	Bi	Po	At	Rn
Cs	Ba	La-	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Pb	Bi	Po	At	Rn	
Fr	Ra	Ac-	Rf	Db	Sg	Bh	Hs	Mt	Ds	Tg	Cn	Nh	Fl	Mc	Lv	Ts	Og
		La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	
		Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr	

	Bulk elements
	Trace elements
	Possible trace elements

## 2. Water distribution in the skin

The distribution and fluxes of water in the skin are important for maintaining the physical properties of the tissue such as permeability and flexibility. The presence of water regulates also the activity of enzymes and cells because it is the solvent for many inorganic ions necessary for skin homeostasis.

Ions distribution is widely used as a convenient marker for the determination of the boundary between adjacent skin layers. It allows the measurement of the thickness of the *stratum corneum* by Confocal Raman Microspectroscopy.

The water distribution in the skin has been extensively studied by many authors with the help of various methods both *in vitro* and *in vivo* (Table 2) (Moretto et al., 1999; Rein, 1924; Scheuplein, 1976; Scheuplein and Blank, 1971; Wahlberg, 1965a; Warner et al., 1988). The semi quantitative measurement of the concentration profile of water must be carefully considered as it is based on two prerequisites: the frozen-hydrated cryo-sections should be uniformly thick, and uniform lateral shrinkage occurs throughout the section during the freeze-drying process (Imokawa et al., 2004; von

Zglinicki et al., 1993; Warner et al., 1988). These assumptions are also applicable to the distribution of ions in the skin.

Table 2 summarises available data on water distribution in the skin layers. It can be noticed that the *stratum corneum* has the lowest water content (10 to 35 % expressed as percent of total weight under the ambient conditions of relative humidity ranging from 0 to 75 %). Conversely, the viable epidermis and dermis contain 65–70 % of water (Imokawa et al., 2004; von Zglinicki et al., 1993; Warner et al., 1988). A discontinuity was also noticed between the *stratum corneum* and the *stratum granulosum*, the latter containing water-rich viable cells. Some authors have noticed that the free water (water that is not hydrogen-bonded to biomolecules but hydrogen-bonded to other water molecules) is located in the viable epidermis and dermis comparatively to *stratum corneum* (30–33 % water bound to biomolecules like proteins) or the reticular dermis (Imokawa et al., 2004). This bound water is swelling the multi-lamellar arrangement of the *stratum corneum* lipids and exerts a water-holding function (Foreman, 1976; Gniadecka et al., 1998; Imokawa et al., 2004; Kasting and Barai, 2003). Finally, the water content in the fatty tissues of hypodermis is low.

There are contradictory statements regarding water contents, probably because of the large variability of skin types and environmental conditions. Von Zglinicki et al.(1993) found an average water content of the *stratum corneum* quite higher (54 %) as compared to 15–40 % from Warner (1988) and a hydration of the dermis similar to that of the viable epidermis. This result is in accordance with Magnetic Resonance Imaging experiments by Richard et al. (1993) based on the variations of the relaxation times  $T_1$  and  $T_2$ . The  $T_1$  relaxation time related to the total water content is in the same range in both layers. The decrease of water proton mobility causes shorter  $T_2$  relaxation time. The very short  $T_2$  value obtained for dermis has therefore been related to a motional restriction of protons probably due to immobilisation of water in a highly ordered structure around collagen fibrils. Very short  $T_2$  values are also reported in the *stratum corneum*, which is not surprising considering that it is a dry layer composed of dead cells with water bound to keratins (Mirrashed and Sharp, 2004).

**Table 2.** Estimates of the water content in the skin.

	<b>Von Zglinicki et al. (1993) % of dry weight</b>	<b>Warner et al. (1988) % of dry weight</b>	<b>Caspers et al. (2001) % of wet tissue</b>
<b>Method</b>	Hydrated and freeze-dried samples	–	<i>In vivo</i> (6 volunteers)
<b>Apparatus</b>	X-ray microanalysis	–	Confocal Raman Microspectroscopy
<b>Location</b>	–	–	forearm
<b>Stratum corneum</b>	54	15–40	35
<b>Viable Epidermis</b>	75	40–65	65
<b>Dermis</b>	75	70–75	65
<b>Hypodermis</b>	75	–	NA

### 3. Distribution of endogenous ions in the skin

Just as water, ions are compartmentalized in the skin. Their distribution is an useful indicator of each skin layer (Moretto et al., 1999).

Several authors have studied the elemental distribution of ions generally expressed as mmoles per kg generally per dry weight. Among the first investigations on the ionic content in the body, Eisele and Eichelberger (1945) reported chemical analyses of fat-free skin after cryo-freezing, followed by a desiccation in an oven. The values in mmol·kg<sup>-1</sup> in skin samples from different parts of the body were: Cl<sup>-</sup>: 79 ± 5, Na<sup>+</sup>: 93 ± 8, K<sup>+</sup>: 16.5 ± 3, Ca<sup>2+</sup>: 2.7 ± 0.5, Mg<sup>2+</sup>: 2.1 ± 0.3; the total nitrogen was 45.5 ± 4 g and the total water was 718 ± 20 g. Since then, more sophisticated biophysical techniques have been used, such as ion capture cytochemistry with the oxalate-pyroantimonate-osmium technique (Menon et al., 1992, 1985), X-ray microanalysis (XRMA) (von Zglinicki et al., 1993), particle probe microanalysis (Forslind et al., 1995), electron probe analysis (Warner et al., 1988), and more recently, three different ion beam techniques methods: Scanning Transmission Ion Microscopy (STIM), Rutherford Backscattering Spectrometry (RBS), and Particle Induced X-ray Emission (PIXE) (Forslind et al., 1984; Gontier et al., 2005; Pallon et al., 1996; Veríssimo et al., 2007). XRMA is useful to study Na, P, S, Cl and K but is rather limited by its sensitivity threshold for elements such as Mg, Ca, Fe, Cu and Zn. PIXE is a better method for these latter elements (Forslind et al., 1997).



Within the above-mentioned studies, there was no gender-related variation. A wide range of values of elemental content has been found which is explained by the differences in sampling methods and by the fact that the entire skin was considered (Forslind et al., 1997). Moreover, a definite account of ions mapping is difficult because the units used to report results differ among papers and authors generally favoured figures rather than concentration tables to express their experimental data.

Ions distribution is often given layer by layer but does not always take into account the inter- and extracellular ion distribution together. Movements of mobile components (e.g. physiologically important ions such as  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ , and  $\text{Ca}^{2+}$ ) are possible; but they are minimized when fresh skin explants are quench-frozen at very low temperature immediately after their collection. Otherwise the results are given as an average of contents in intra- and extracellular compartments. Table 3 reports some ranges from papers in which the data report made it possible to get the values in  $\text{mmol}\cdot\text{kg}^{-1}$ .

The most prominent results found for cryopreserved skin may be summarised as follows:

- There are several ionic concentration gradients in the skin.  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations increase from the *stratum corneum* to the dermis (Forslind et al., 1997; Veríssimo et al., 2007; von Zglinicki et al., 1993). Von Zglinicki et al. (1993) noticed a concentration gradient for  $\text{Na}^+$  ( $50 \text{ mmol}\cdot\text{kg}^{-1}$  dry wt (SC) –  $200 \text{ mmol}\cdot\text{kg}^{-1}$  dry wt (D)) and  $\text{Cl}^-$  ( $200 \text{ mmol}\cdot\text{kg}^{-1}$ mol/kg dry wt (SC) –  $500 \text{ mmol}\cdot\text{kg}^{-1}$  dry wt (D)) from the *stratum corneum* to the dermis roughly in accordance with the results of Forslind et al. (1997). Both authors found that  $\text{Cl}^-$  is always the most abundant anion ( $175 \text{ mmol}\cdot\text{kg}^{-1}$ ), which concentration peaks in the dermis because of its extracellular nature. Warner et al. (1988) found the opposite result in the *stratum corneum* with a steep increase of both ions from the *stratum basale* to the surface of the *stratum corneum*. Veríssimo et al. (2007) reported an inverted gradient with a steep increase of  $\text{Cl}^-$  from the dermis to the *stratum granulosum* and a sharp decrease in the *stratum corneum*.
- The contents of  $\text{K}^+$  and P are low in the *stratum corneum*, high in the living cells of the epidermis ( $\sim 300 \text{ mmol}\cdot\text{kg}^{-1}$  mainly in the basal cell layer, and the lower *stratum spinosum*), and low again in the dermis, as expected for ions present in the extracellular compartment (Mitsuhiro Denda et al., 2000; Forslind et al., 1997; Veríssimo et al., 2007; von Zglinicki et al., 1993). Warner et al. (1988) found a high

concentration of  $K^+$  in the outer, and a very low one in the inner *stratum corneum*. The dermo-epidermal junction appears as a frontier beneath which a drastic decrease of phosphorus content is reported (Moretto et al., 1999). These results can be explained by the abundance of phospholipids and nucleic acids present in the living epidermis (Forslind et al., 1984; Malmqvist et al., 1987; Veríssimo et al., 2007). The authors explain the low contents of  $K^+$  and P in the *stratum corneum* by a recycling of these intracellular solutes within the viable tissue of epidermis.

**Table 3.** Average distribution of elements in  $\text{mmol}\cdot\text{kg}^{-1}$  adapted from von Zglinicki et al. (1993) (vZ) by XRMA and Forslind et al. (1997) (Fo) by XRMA and PIXE. The gradient observed in a layer is given from the bottom up in the same layer. In the study of von Zglinicki et al., the samples were shock-frozen and then freeze-dried. The samples reported in the paper of Forslind et al. were just quench-frozen.

#### A: Macrominerals

Ions ( $\text{mmol}\cdot\text{kg}^{-1}$ )	$\text{Na}^+$		$\text{Cl}^-$		$K^+$		$\text{Mg}^{2+}$	$\text{Ca}^{2+}$		P		S	
reference	vZ	Fo	vZ	Fo	vZ	Fo	vZ	vZ	Fo	vZ	Fo	vZ	Fo
<i>Stratum corneum</i>	75	85	200	60	75	10–25	70	5	20–30	100	32	100	125
<i>Stratum granulosum</i>	–	300–350	–	170–230	–	100–205	–	–	10–15	–	130	–	90–125
<i>Stratum spinosum</i>	100	695	300	230	200	330	40	5	3–5.5	275	40	125	62–90
<i>Stratum basale</i>	150	565	425	285	150	360	40	5	4.5	200	515	125	62
Upper dermis	150	1000	450	315	100	250	25	5	6	100	160	100	93
Reticular dermis	200		475		80		20	5		100		100	

#### B: Trace elements

Ions in ( $\text{mmol}\cdot\text{kg}^{-1}$ )	Fo
$\text{Fe}^{2+}$	0.14–0.27
$\text{Cu}^{2+}$	0.041–0.073
$\text{Zn}^{2+}$	0.44–0.91

- A  $K^+/Na^+$  ratio compatible with the ion content for viability of cells is only found in the *stratum basale* (Forslind et al., 1997). In the upper layers, the  $Na^+$  cell content increases while that of  $K^+$  decreases: the authors tentatively considered inward diffusion of salts ( $Na^+$  et  $Cl^-$ ) from sweat glands to the outer *stratum corneum* as the origin of this effect. Therefore, lower  $K^+/Na^+$  ratio was found in the *stratum spinosum* compared to the *stratum basale* (Forslind et al., 1997). This result disagrees with that of Warner et al. (1988) who found a rather uniform concentration of  $Na^+$  throughout the viable skin, so that  $K^+/Na^+$  ratios in the *stratum spinosum* were higher than those found in the *stratum basale*. No definite conclusion could be drawn by the authors.
- Sulfur has the same concentration in the dermis and in the *stratum corneum* but a little bit lower in the living cells of the epidermis (Forslind et al., 1997; Warner et al., 1988). Veríssimo found higher concentration of sulfur in the *stratum corneum* compared to the *stratum spinosum* (Veríssimo et al., 2007). This result is expected considering that keratinocytes differentiate into corneocytes, which leads to an increase in the content of cysteine-rich keratin (Gontier et al., 2005). Therefore, the sulfur content is an excellent marker of this layer (Moretto et al., 1999).
- The amount of calcium has been found in quite similar low range in all skin layers ( $5 \mu\text{mol}\cdot\text{kg}^{-1}$ ) (von Zglinicki et al., 1993). This value differs significantly from the results obtained by other teams who reported a concentration gradient increasing from the dermis up to the *stratum corneum*. The authors found around  $50 \text{ mmol}\cdot\text{kg}^{-1}$  in the upper skin layers (*stratum corneum* and *granulosum*). Calcium is particularly highly concentrated in the *stratum corneum*, the epidermal region with the lowest metabolism. There is a consensus to claim that human epidermis displays the highest  $Ca^{2+}$  concentration at the *stratum corneum/stratum granulosum* interface. In this latter layer, the  $Ca^{2+}$  ions are located in the extra- and intercellular compartments. In the *stratum spinosum* and *stratum basale*, very low amount of intracellular  $Ca^{2+}$  was found. Some authors claimed that there is no calcium in the *stratum corneum* except in case of skin disruption (Menon et al., 1992, 1985); but other teams found a high amount in this layer (Forslind et al., 1997; Moretto et al., 1999).

- Concerning the trace elements, the contents of  $\text{Zn}^{2+}$  and  $\text{Fe}^{2+}$  were two-fold higher at the epidermal/dermal interface as compared to the values observed in the *stratum corneum* and dermis (Forslind et al., 1997; Veríssimo et al., 2007). Neither  $\text{Fe}^{2+}$  nor  $\text{Zn}^{2+}$  were detectable in the *stratum corneum*. Forslind (1997) indicated that the ratio of  $\text{Zn}^{2+}$  content in epidermis/dermis was 3/1 in human normal skin. This result was in accordance with Lansdown et al. (2007) who reported a zinc concentration in epidermis higher than in dermis ( $50\text{--}70\ \mu\text{g}\cdot\text{g}^{-1}$  dry wt versus around  $10\ \mu\text{g}\cdot\text{g}^{-1}$  dry wt), probably due to the activity of zinc-dependent RNA and DNA polymerase in the *stratum basale* keratinocytes. An inverse gradient is observed between zinc and calcium across the epidermis. Finally, chromium, iron nickel and silicon appear only in the corneocyte layer (Moretto et al., 1999)

#### **4. Speciation of ions in the skin**

In all the skin layers, ions are dissolved in water ( $\text{pH} = 7$ ). *Stratum corneum* having a  $\text{pH}$  of 5.5 is the exception. Fraústo da Silva and Williams (2001) have analysed the speciation of ions with respect to biological systems. Some interesting statements can be made from the oxidation diagrams for metals and non-metals at  $\text{pH} = 7$ . The importance of the acidity and redox potential of specific cell compartments must be considered. Most of complexes formed by divalent transition metals and proteins are not stable at  $\text{pH}$  below 6, as in lysosomes for example. In such an acidic medium, the metal dissociates from the complex, the carrier protein is expelled back outside the cell, while the metal ion stays in the cytoplasm.

Monovalent ions such as  $\text{Na}^+$ ,  $\text{K}^+$  or  $\text{Cl}^-$  are very mobile. The sole oxidation state for both  $\text{Na}^+$  and  $\text{K}^+$  is +1, and these metals are rarely bound to enzyme reaction centres but can transfer their ionic charges.  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  are considered as semi-mobile ions compared to  $\text{Na}^+$  and  $\text{K}^+$ . Oxygen is the preferred donor atom for  $\text{Na}^+$  and  $\text{K}^+$  while carboxylate and phosphate are the preferred ligands for  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$ ;  $\text{Zn}^{2+}$  and  $\text{Ni}^{2+}$  that easily form complex species with molecules containing S and N. More than one oxidation state is possible for transition metals Fe, Cu Co, Mo and Mn (Figure 1). They bind very easily with N and S except for  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  for which carboxylate and phosphate are preferred ligands. These metals are generally considered static with the exception of  $\text{Mn}^{2+}$  and  $\text{Fe}^{2+}$  that are semi-mobile.

In aqueous media, the possible range of redox potentials is restricted to that of the systems  $\text{H}^+/\text{H}_2$  and  $\text{O}_2/\text{OH}^-$  ranging between  $-0.4$  and  $+0.8$  V. Water is reduced to  $\text{H}_2$  or oxidised to  $\text{O}_2$  above and below these values. Hence, metal ions have only a small range of stable oxidation states (iron: +2, +3; copper: +1, +2) while non-metals may take a wider range of values (i.e. S:  $-2$  to  $+6$ ; carbon:  $-4$  to  $+4$ ) (Figure 1).

Some of the ions, independently of their classification as metal or non-metals, can only occur in one very stable oxidation state. This is the case of alkali and alkaline-earth metals restricted to the oxidation state +1 or +2 and zinc and cadmium restricted to the +2 oxidation state.

Finally, phosphorus, boron and silicon are only present in the highest oxidation states, +3, +4 and +5 respectively as borate, silica and phosphate. Chlorine only exists as chloride and is never covalently bound (Fraústo da Silva and Williams, 2001).

## 5. Transport of ions in the skin

As seen above, the distribution of water and endogenous ions in the skin is not homogeneous. The local administration of ions from aqueous solution to cure skin diseases is compromised by the outermost skin layer. The *stratum corneum* is often considered the main, if not unique, barrier for permeation of xenobiotics in general. It is built of dead, nuclei-deprived keratinised cells (corneocytes) immersed in a lipid matrix making it highly lipophilic. This barrier strongly limits the transport of these low molar mass hydrophilic molecules, especially under their ionised state (Rein, 1924; Scheuplein and Blank, 1971; Wahlberg, 1965a; Warner et al., 1988). The topic on the pathway for ion transport is also controversial (Anigbogu and Maibach, 2008). Two mechanisms of passive diffusion through *stratum corneum* are possible: via trans- and intracellular route (Bolzinger et al., 2012).

### 5.1. Transport by passive diffusion

The intercellular pathway (Fig. 2B) is the continuous route for absorption of molecules and it has been thoroughly studied over the past decades (Boddé et al., 1991; Harada et al., 1992). It is the

predominant route for transport within the *stratum corneum*. The organisation of lipid bilayers within the intercellular space enables the transport through the lipid core via polar headgroups pathway (Cooper and Kasting, 1987; Scheuplein, 2011).

The intracellular route (Fig. 2A) is sometimes considered as a polar pathway within the *stratum corneum* (Cooper and Kasting, 1987). The Natural Moisturizing Factor (NMF) increases hydration in the cytoplasm and provides the intracellular matrix a polar environment. However, transcellular absorption requires multiple partitioning of the molecule between this polar environment and lipid external matrix.

The last permeation pathway within the horny skin layer is through the hair follicles (Fig. 2C). Scheuplein (2001) suggested that the contribution of this route is negligible compared to the intercellular one. More recently, hair follicles have been considered as “weak spots” in the skin barrier enabling faster penetration into the skin. Their contribution to the dermal absorption depends on the skin site and may not be neglected, for example on the forehead (Otberg et al., 2008). Transfollicular route is now considered as attractive for nanoparticle-based delivery systems and the role of hair follicles as long-term reservoirs for drug delivery has been extensively studied (Konráðsdóttir et al., 2009; Lademann et al., 2006, 2007). Recently, Chandrasekaran et al. (2016) reported significant contribution of follicular pathway for the skin absorption of magnesium cations.

## **5.2. Facilitated transport**

Although the penetration mechanisms of the majority of studied xenobiotics are restricted to the above-mentioned pathways, skin absorption of inorganic ions involves complementary routes of transport present in the deeper skin layers (viable epidermis and dermis) (Fig. 2). Some membrane proteins are channels or receptors allowing the specific passage of ions. Their activity may involve energy expenditure. Evidence of the presence of such proteins was given either by gene expression experiments or immunostaining of skin tissues (Proteinatlas.org).

### **5.2.1. Cations**

The first group of ion exchangers gathers the transport proteins that help to maintain the cationic gradients in the mammalian cells in general. Epithelial sodium channel (ENaC) actively transports hydrogen ions outside the cells by their exchange with sodium. In the skin, it allows resorption of  $\text{Na}^+$  excreted with the sweat. It is strongly expressed in viable epidermal layers as well as in sebaceous glands, eccrine glands and arrector pili smooth muscle cells (Hanukoglu et al., 2017). Lithium and ammonium cations are non-physiological ions that can be transported through this channel (Jakobsson et al., 2017; Nakhoul et al., 2017).  $\text{Na}^+/\text{K}^+$  ATPase is essential for maintaining the homeostasis of all cells, including skin cells. It allows active resorption of  $2\text{K}^+$  inside the cell by exchange with  $3\text{Na}^+$ , and is therefore involved in regulation of cationic gradients and osmotic pressure, resting and action potentials, water-electric balance, and transport of sugars and amino acids (Senol et al., 2007). Additionally, it is a pump that binds magnesium cations that are cofactors of enzymes at the active site (Jorgensen et al., 2003). A similar role is attributed to  $\text{Na}^+/\text{Ca}^{2+} - \text{K}^+$  exchanger (NCKX) that transports both  $\text{K}^+$  and  $\text{Ca}^{2+}$  inwards while evacuating  $\text{Na}^+$  to the extracellular medium (Lytton, 2007). Lamason et al. (2005) found that the levels of expression of *SLC24A5* gene encoding this exchanger are correlated with skin pigmentation processes.

Calcium plays a crucial role in intracellular signalling (secondary messenger) so that its concentrations both inside the cells and in the extracellular fluid are precisely controlled. For this purpose,  $\text{Ca}^{2+}$  ATPase helps cells to remove excess  $\text{Ca}^{2+}$  into the extracellular matrix. On the other hand, the inward flux of calcium is controlled by the second messenger-operated channels (SMOC), receptor-operated channels (ROC) and voltage operated channels (VOC) (Penner et al., 1993). Magnesium is another divalent cation of importance. It is mainly present in the intracellular medium and plays important roles in cell signalling, metabolism, growth and proliferation. Magnesium transporters (MgtE) help to maintain homeostasis related to their concentrations. But they are not very selective; they can also facilitate the transfer of other divalent cations ( $\text{Ba}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Mn}^{2+}$ ) (Sahni et al., 2013).

Other types of transport proteins of low specificity allow the transport of various divalent cations.

The initial role of the divalent metal transporter (DMT) is the transport of  $\text{Fe}^{2+}$  and  $\text{H}^+$ . Although it is primarily expressed in the intestine where it regulates  $\text{Fe}^{2+}$  absorption from the digestive tract, its overexpression in skin of patients suffering from leprosy is related to its impotence in pathogen-defence mechanisms (Pereira-Suárez et al., 2015). Depending on the pH, it may also transport other ions such as  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  and possibly  $\text{Zn}^{2+}$  (Jeong and Eide, 2013).

Zinc is an essential microelement involved in enzymatic and structural functions of various proteins. Two transporters regulate its cellular concentrations: the ZnT pumps the zinc outside the cell to decrease cytoplasmic zinc concentrations and the ZIP drives the inwards flux of  $\text{Zn}^{2+}$  ions (Bafaro et al., 2017). In the skin cells, the ZIP family is predominant, which may lead to facilitated absorption of  $\text{Zn}^{2+}$  and other divalent cations such as  $\text{Cd}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Fe}^{2+}$  (Hara et al., 2017).

### **5.2.2. Anions**

Chloride channels and transporters are the main group of proteins that are controlling anion flux in the skin cells. Lin and Gruenstein (1988) identified three main pathways of chloride transport in human fibroblasts. Calcium-activated chloride channels (CaCC) accounting for 20 % of total flux is a DIDS (4,4'-diisothiocyano-2,2'-stilbenedisulfonic acid) insensitive route which can be partially inhibited by anthracene-9-carboxylic acid. The second described group of transporters ( $\text{Na}^+/\text{Cl}^-$  co-transporter, NCC;  $\text{Na}^+/\text{Cl}^-$  co-transporter NKCC;  $\text{K}^+/\text{Cl}^-$  co-transporter, KCC) causing 25 % of the total efflux ensures a cation-coupled transport relying on the simultaneous presence of  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$ . Finally, up to 50 % of the total transport occurs via anion exchangers that could be inhibited by DIDS (chloride channels, CLC). Mastrocola et al. (1991) identified the chloride transport pathways in human keratinocytes. In that case, authors determined two predominant mechanisms: an anion exchange and an electrically conductive pathway whereas the cation-coupled transport was found to be negligible in case of this cell line. The transport via exchangers accounted for 50 % of the total efflux that was in line with the results found for the fibroblasts. The CCL were found yield approximately 40 % of the  $\text{Cl}^-$  flux.

The NCC, NKCC and KCC channels are not tissue specific and thus are expressed in all types of cells. Their role is to maintain the osmotic pressure and ionic concentration gradients. The anion exchanger



(AE) helps, in particular, at regulating the intracellular pH as its main substrates are bicarbonate (secretion) and chloride (uptake) (Nilius and Droogmans, 2003). Additionally to those chloride-transporters, the gene encoding  $\text{Na}^+/\text{I}^-$  symporter (NIS), whose primary role is to pump iodide ions inside the thyroid cells, has been detected in skin samples (Perron et al., 2001). Apart from  $\text{I}^-$ , NIS transports other monovalent anions in the following affinity order:  $\text{ClO}_4^- > \text{ReO}_4^- > \text{I}^- \geq \text{SCN}^- > \text{ClO}_3^- >> \text{Br}^-$  (van Sande et al., 2003).

Members of Chloride Channel family (CLC) are voltage-dependent channels and react on the changes of transmembrane electric field. The other substrates include  $\text{NO}_3^- > \text{Cl}^- > \text{Br}^- > \text{I}^-$  (Jentsch, 1993). Braun et al. (2010) suggested that the murine expression of CLCA5 member of this family is involved in maturation and keratinization of squamous epithelial cells. A similar conclusion was drawn by Plog et al. (2012) in the case of porcine CLCA2.

Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) is a cAMP-activated chloride channel of great importance in the skin. It regulates electrolyte and fluid secretion as well as absorption in the sweat glands (Saint-Criq and Gray, 2017). Chloride is not its only substrate and the difference between CFTR and CLC is the affinity to other monovalent anions:  $\text{I}^- > \text{NO}_3^- > \text{Br}^- > \text{Cl}^- > \text{F}^-$  (Linsdell et al., 1997).

Osmotic swelling resulting in a decrease of the intracellular ionic strength can also trigger the ionic flux through volume regulated anion channels (VRAC). Just as CFTR, they are able to transport various monovalent anions, with the following affinities:  $\text{SCN}^- > \text{I}^- > \text{NO}_3^- > \text{Br}^- > \text{Cl}^- > \text{F}^- > \text{gluconate}$  (Nilius and Droogmans, 2003). Raoux et al. studied the behaviour of normal human keratinocytes in the presence of heptylamine. They proposed that VRAC participate in the mechanisms underlying contact dermatitis caused by exposure to tested molecule, which actually is an inhibitor of the channel (Raoux et al., 2007).

$\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channels (CaCC/CLCA) react on an increase in intracellular  $\text{Ca}^{2+}$ . The expression of CLCA2 in human and pig skin was thoroughly studied. In human skin, the protein was detected in the basal epithelial cells (Connon et al., 2004). The porcine homologue was detected exclusively in the

mature keratinocytes of the epidermis and in the inner root sheath of hair follicles (Plog et al., 2012). Both authors linked the occurrence site with its structural role in epithelial stratification. Glycine receptor (GlyR) and  $\gamma$ -aminobutyric acid receptor (GABAR) channels are ligand-gated channels activated by binding glycine or GABA respectively. The affinity of both receptors to the monovalent anions follows the same order as in the case of CFTR and VRAC ( $\text{SCN}^- > \text{I}^- > \text{Br}^- > \text{Cl}^- > \text{F}^-$ ) (Bormann et al., 1987).

## **6. Effects of skin exposure to inorganic cations**

Available literature addressing dermal absorption of cations and their impact on the skin homeostasis is considerably richer than that of anions (Shani et al., 1985; Denda et al., 2000; Mudry et al., 2005, 2007; Larese et al., 2007; Merial-Kieny, 2011; Polefka et al., 2012). The following part is focused on the beneficial and detrimental effects related to dermal exposure to cations.

A large number of ions may enter the skin either by systemic transport: gastrointestinal absorption or inhalation of dusts and vapours or directly after dermal exposure. In this review, the effects of direct application or absorption of ions through topical exposure are reported.

### **6.1. Curative effects**

This section gathers the beneficial health effects of ions after topical application with a focus on ions found in thermal spring waters. Aluminium salts used in underarm hygiene products and silver salts applied in the wound treatment are also considered. Titanium dioxide and zinc oxide, although widely used in sunscreen formulations and make-up products, are excluded from the considerations because they are not soluble in water and thus cannot be absorbed through healthy skin to reach the blood circulation.

### **6.2. Composition of Thermal Spring Waters**

Thermal Spring Waters (TSW) are naturally mineralised waters that share three main characteristics: spring origin, bacteriological purity, and therapeutic potentials. Their common feature is the abundance in calcium and magnesium which is due to reactions and exchanges with the rocks surrounding the spring. Enrichment in calcium and magnesium occurs in exchange to sodium and potassium (that are usually present in high concentrations) (Mazor and Mero, 1969). The main anions present in the TSW are chlorides, sulfates and bicarbonates. TSW's differ in the proportions among those bulk minerals as well as in the content of trace elements, such as zinc, copper, strontium, selenium, iron, manganese, silica, fluoride, bromide, etc. (de Raimbouvill, 1898; Joly et al., 1998; Seite, 2013). Since the Antiquity, bathing was regarded as more than a simple cleansing procedure. Waters rich in sulphur have been popular to cure psoriasis and to help wound healing (Jackson, 1990). Recently, the clinical trials reported in the Cochrane library focused on the effects of balneotherapy on chronic painful diseases, as rheumatoid arthritis for example (Verhagen et al., 2015, 2007). Extensive research related to curative treatments of chronic dermatological diseases like ichthyosis, psoriasis and atopic dermatitis by hydrotherapy has been investigated since it has significant benefits on the quality of patients' lives (Bodemer et al., 2011; Gambichler et al., 2001, 2000; Péter et al., 2017). Huang et al. (2018) have recently published a review on the clinical applications of TSW on the skin, especially their immunomodulatory effect on inflammatory and autoimmune dermatologic diseases. According to the authors, the balneologic benefits of the Dead Sea Water, a very salty medium, are attributed to four variables: sunny climate (330 days/year) with moderate to high temperatures, low humidity, high barometric pressure with oxygenation of the air increased by 10 % and low pollen count (Huang et al., 2018).

The evaporation of the water creates an aerosol that could be inhaled. Mud-packs and bathing, sometimes in association with phototherapy, are used to relieve and soothe inflammatory lesions (Huang et al., 2018; Schiener et al., 2007). Several studies proved such a combined treatment more efficient in psoriatic patients than phototherapy alone (Brockow et al., 2007; Gambichler et al., 2000; Schiener et al., 2007). Proksch et al. (2005) gave evidence that bathing in Dead sea waters led to

enhancement of *stratum corneum* hydration, improvement of skin barrier function and reduction of skin roughness and inflammation in patients suffering from atopic dermatitis. Authors attributed these effects to the high content of magnesium.

These clinical data are supplemented by fundamental research on the skin absorption of ions associated to their effects at the cellular level (Joly et al., 2000; Levi-Schaffer et al., 1996; Merial-Kieny et al., 2011; Tacheau et al., 2018).

An *in vivo* experiment, in which shaved guinea pigs were “bathed” for 30 minutes in a solution of radiolabelled salts mimicking the composition of Dead Sea Water, was carried by Shani et al. (1985) as an extension of a clinical study. The results revealed high concentrations of ions retained in the skin as well as their significantly higher levels in various organs (ex. spleen, bones, heart). More recently, a report on the use of Epsom salt baths revealed significant increase of blood levels of  $Mg^{2+}$  and  $SO_4^{2-}$  in patients taking regular baths (Waring, 2014). Along with these clinical assays, several *in vitro* studies carried out using diffusion cells were reported. Tregear (1966) evaluated skin absorption of radiolabelled cations ( $^{24}Na^+$  and  $^{42}K^+$ ) from aqueous solutions using 3 skin models *in vitro* (rabbit, pig and human) and *in vivo* (human). His findings proved that both cations penetrated through the skin. However, the *in vitro* absorption of  $Na^+$  was slower than *in vivo*, and  $K^+$  penetrated faster through the rabbit skin as compared to porcine model. Skin absorption of copper and zinc after finite dosing of various topical formulations was also disclosed on a human skin model. Both cations were retained in the skin rather than penetrated through the tissue and the concentration in the epidermis was higher for  $Cu^{2+}$  than  $Zn^{2+}$  (Pirot et al., 1996a). After developing the novel method of blocking the follicular absorption route, Chandrasekaran et al. (2016) disclosed that these appendages played an important role in skin absorption of magnesium ions *in vitro*. Finally, our last study carried on viable porcine skin explants demonstrated measurable absorption of  $Ca^{2+}$  and  $Mg^{2+}$  from a marketed TSW. Both cations diffused through the skin samples after direct application of TSW and when the TSW was incorporated into model cosmetic formulations (Tarnowska et al., 2020).

Not surprisingly, these results triggered further research aiming at understanding the mechanisms underlying observed biological activities. Table IV summarises the effects on skin observed after application of various ions. The vast majority of beneficial effects of TSW on skin is due to its cationic composition ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Se}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Sr}^{2+}$ ,  $\text{Mn}^{2+}$ ...see [Table 4](#)).

Potassium is an abundant intracellular cation present in all living cells whose particular role in the skin is regulating the epidermal barrier homeostasis (along with  $\text{Ca}^{2+}$ ). Skin barrier disruption was found to decrease the endogenous concentrations of  $\text{K}^+$  in epidermis (Mauro et al., 1998), which is consistent with its role in barrier repair. Later on, Denda et al. (2007) indicated the importance of  $\text{K}^+$  flux in the skin barrier recovery. They observed that alterations of activity of  $\text{K}^+$  channels significantly affected skin barrier homeostasis: application of blockers of the  $\text{K}^+$  channels inhibited skin recovery while treatment with molecules opening the same channels or classified as  $\text{K}^+$  ionophore resulted in acceleration in skin recovery. Proposed mechanism of this regulation is based on the variations in intracellular  $\text{K}^+$  that either stimulate (low  $\text{K}^+$ ) or inhibit (high  $\text{K}^+$ ) secretion of lamellar bodies.

Calcium is an important ion in cell signalling (secondary messenger). In the skin, it is distributed according to a concentration gradient in the epidermis in such a way that the concentrations increase from the *stratum basale* to the *stratum granulosum*. In case of an external damage, this gradient is perturbed and activates skin repair mechanisms (Denda et al., 2000; Mauro et al., 1998). Thus, its topical application may lead to improvement of the skin barrier (Denda et al., 1999).  $\text{Ca}^{2+}$  was shown to play an important role in regulation of keratinocyte differentiation (Resing et al., 1993; Tu and Bikle, 2013). Denda et al. (2003) showed that its influx into keratinocytes induces the exocytosis of lamellar bodies and improves skin barrier function.

The concentration gradient of magnesium present within the epidermal layers is similar to that of calcium. Its role in the induction of skin barrier repair mechanisms is also analogical to the one described for  $\text{Ca}^{2+}$  (Denda et al., 2000). Topical application of some magnesium salts improved skin barrier recovery in hairless mice after tape stripping. However, observed effects depended on the counter-anion and association of magnesium with calcium salts (Denda et al., 1999). Moreover,

exogenous magnesium cations were shown to specifically inhibit Langerhans cells function and, by consequence, account to the anti-inflammatory properties of magnesium salt solutions observed after topical applications of Dead sea waters (Schempp et al., 2000).

Selenium is a trace element that is found in some TSW's. The research carried on skin fibroblasts cultured in selenite-supplemented medium disclosed its protective properties against UV-A radiation, even at low concentrations ( $0.1 \text{ mg} \cdot \text{L}^{-1}$ ). The survival rate of Se-cultured cells was significantly higher than in the control group. Authors attributed this action to enzymatic induction of glutathione peroxidase and the protection of cell membranes (Leccia et al., 1993). Moysan et al. (1995) also performed their study on human fibroblasts cultured in foetal calf serum depleted medium (2 %) that was enriched either by selenite alone or by the addition of Se-rich TSW (La Roche Posay). The observations from this study agreed with those of Leccia et al. (1993). Authors confirmed a protective effect of selenium after exposure to UV-A radiation, that was linked to the protection of dividing or quiescent fibroblasts against lipid peroxidation, and increased glutathione peroxidase activity in fibroblasts (Moysan et al., 1995). Similar results were obtained when TSW was used instead of a simple aqueous solution of selenite, which led to the conclusion that the capacity of La Roche Posay TSW to protect against UV-A radiation is due to its selenium content. No protective effect was observed in case of UV-B exposure.

Celerier et al. (1995) observed that the addition of selenium into a culture medium of reconstituted skin resulted in decreased production of IL-1 and IL-6 inflammatory cytokines, both at the intra- and extracellular levels. Authors linked their observations with the results of Leccia et al. (1993) and Moysan et al. (1995), and suggested that the mechanism of protection against *in situ* inflammatory reactions is due to the ability of selenium to catalyse the decomposition of reactive forms of oxygen. In the same study, the anti-inflammatory properties of TSW containing both selenium and strontium as well as strontium alone were shown. The effects observed for an aqueous solution of strontium were weaker and limited to the inhibition of IL-6. La Roche Posay TSW exhibited intermediate cytokine inhibition as it contained both minerals (Celerier et al., 1995).

In their investigation of the impact of trace elements on the expression of integrins crucial for skin wound healing processes, Tenaud et al. (1999) noted that manganese gluconate could modify the expression of integrins that are transmembrane receptors responsible for communication between cell and environment. They play a critical role in the regulation of adhesion, migration, proliferation, and differentiation of cells. They are necessary for the formation and maintenance of tissue structure integrity. Thus, modification of their expression may modulate wound healing mechanisms (Rippa et al., 2013). Manganese salt induced the expression of integrins  $\beta 1$  and  $\alpha 6$  in suprabasal keratinocytes. Observed effects were concentration-dependent and occurred both in the cell culture (keratinocytes) and in reconstructed skin samples. Authors did not notice increased expression of integrin  $\alpha 3$  which, considering the noted activation of integrin  $\alpha 6$ , led to the conclusions that the effect of manganese was more pronounced in keratinocytes during their differentiation phase than during their proliferation phase, and that it enhanced their migration and dermo-epidermal adhesion in the final healing phase (Tenaud et al., 1999).

Both copper and zinc are trace elements of TSW certainly contributing to their beneficial properties on health and beauty. Their effects observed after topical application were known since many centuries and resulted in the development of the widely used Dalibour cream ( $\text{CuSO}_4$ ,  $\text{ZnSO}_4$ ,  $\text{ZnO}$ , camphor, lanolin, water and vaseline), a medicine available in a number of countries worldwide, and zinc or Lasaar paste. Dalibour cream as well as other (dermo)cosmetics based on the association of  $\text{Zn}^{2+}$  and  $\text{Cu}^{2+}$  present soothing properties and are used to calm irritated and damaged skin. As mentioned above, absorptions of copper and zinc soluble salts are considered poor in human skin *in vitro*, but their application remains interesting in case of wound healing (Pirot et al., 1996a, 1996b).

The main biological activity of copper is improvement of wound healing processes that have been described by many authors. Tenaud et al. (1999) disclosed that incubation of keratinocytes or reconstituted skin in a medium supplemented with copper gluconate showed less activity on the integrins involved in the keratinocyte proliferation phase. On the other hand, the inductive action of copper gluconate was more specific towards differentiated keratinocytes, leading to enhanced

expression of  $\alpha 6$ ,  $\beta 1$  and  $\alpha 2$  integrins. Similarly to manganese,  $\text{Cu}^{2+}$  was found to play an important role in the final wound healing phase. These findings agreed with the results obtained *in vivo* on wounded rats. Siméon et al. (1999) investigated the efficacy of a tripeptide- $\text{Cu}^{2+}$  complex (Glycyl-L-Histidyl-L-Lysine- $\text{Cu}^{2+}$ , GHK-Cu) on activation of matrix metalloproteinases and their impact on wound healing *in vivo*. This study carried out on wounded rat model demonstrated that this complex did modulate the expression of various metalloproteinases at different stages of healing and altered wound remodelling.

Later on, the impact of the same complex has been evaluated on cultured fibroblasts and keratinocytes. GHK-Cu treated irradiated fibroblasts produced more growth factors ( $\beta$ -FGF and VEGF) as compared to the controls. This can be beneficial in the early stages of wound healing processes (Pollard et al., 2005). The tripeptide increased the proliferative potential of cultured keratinocytes by regulating extracellular matrix proteins such as  $\alpha 6$  and  $\beta 1$  integrins (Kang et al., 2009).

Apart from its undeniable benefits in wound healing processes, copper also enhances the production of collagen and elastin fibres in the skin. Clinical tests of cosmetic products containing copper-zinc malonate reported significant reduction of wrinkles after 8 -weeks of treatment. This result was supported by molecular and histological data showing regeneration of elastic fibre network in treated sites, which was due to enhanced synthesis of tropoelastin (Mahoney et al., 2009).

Zinc is a versatile cation regarding its biological activity within the skin. Zinc is located both intra- and extracellularly in epidermis while in dermal tissue it is bound to proteins. Its primary role is to stabilize cell membrane. It is also a cofactor with an important role in mitosis migration and maturation of cells (Lansdown et al., 2007). Many zinc salts are used in medicine and cosmetics: (i) zinc undecylenate as an antifungal agent, (ii) zinc pyrithione as an active agent for the control of dandruff, seborrheic dermatitis and scalp itch and, (iii) zinc acetate, zinc sulfate, zinc gluconate soothe the skin together with antimicrobial and seoregulatory effect, (iv) finally, nanoparticles of zinc oxide are applied for skin protection against UVA and UVB radiations (Polefka et al., 2012). Microparticles of ZnO are used to protect the skin of wetness, and as soothing and antimicrobial agent in case of diaper rash. At pH lower



than 6, ZnO can release  $\text{Zn}^{2+}$  ions that exhibit antimicrobial and soothing properties. As an example, in contact with acidic sweat ZnO hydrolyses into  $\text{Zn}^{2+}$  cations having protective effects against inflammation mediated by the UV radiations. Indeed, Leccia et al. (1993) demonstrated in their *in vitro* study that addition of  $\text{ZnCl}_2$  to fibroblasts culture protected the cells against UV-A irradiation and increased their survival rate. The role of zinc at the cellular level in wound healing processes recently reviewed by Lin et al. (2018) consists in an important modulation of all the stages of the cicatrization process. Just after wounding, zinc is involved in haemostasis. It enhances platelet activity and aggregation during blood clotting. Moreover, platelets and zinc are suggested to play an important role in initiating the inflammatory phase of wound healing. In the latter,  $\text{Zn}^{2+}$  reduces oxidative stress, ensures migration of neutrophils and leukocytes to infected/damaged sites, enhances neutrophilic phagocytosis and has an effect on cytokine production. In the next proliferation phase, zinc stimulates T-lymphocytes that regulate inflammation. Zinc regulates the expression of metalloproteinases involved in remodelling of extracellular matrix during the last stage of wound healing. They modulate growth factor activation, cleavage, degradation and composition of extracellular matrix, processing of cell-cell junctional adhesion molecules, cytokines and cell surface receptors and cell-matrix signalling during different stages of wound healing.

Zinc-based products are commonly used in the treatment of acne. Firstly,  $\text{Zn}^{2+}$  has significant anti-inflammatory potential. The presence of  $\text{Zn}^{2+}$  in keratinocyte culture suppresses cytokine-induced iNOS expression and IFN- $\gamma$  or TNF- $\alpha$  induced NO production (Yamaoka et al., 2000). This effect combines with its sebostatic efficacy based on inhibition of 5- $\alpha$ -reductase activity (Piérard-Franchimont et al., 1995; Stamatiadis et al., 1988; Sugimoto et al., 1995). In addition, its anti-microbial activity against *S. aureus*, *P. aeruginosa*, *C. albicans* and *A. brasiliensis* is maintained in topical formulations (Pasquet et al., 2015).

Finally, zinc in association with copper, enhances regeneration of elastic fibres within dermis, which results in the reduction of wrinkles (Mahoney et al., 2009).

**Table 4.** Biologically active ions found in TSW and their mechanisms of action.

	Ion	Compound and Dose	Details	Reference
Skin barrier enhancement	<b>Ca<sup>2+</sup></b>	10 mM	Hairless mice, mixture of magnesium and calcium salts accelerate skin barrier recovery. Calcium chloride delays skin barrier recovery	Denda et al., 1999
	<b>K<sup>+</sup></b>	Application of K <sup>+</sup> channel openers or blockers	Hairless mice, changes in K <sup>+</sup> levels modulate excretion of lamellar bodies	Denda et al., 2007
	<b>Mg<sup>2+</sup></b>	10 mM	Hairless mice, mixture of magnesium and calcium salts accelerate skin barrier recovery. Magnesium chloride accelerate the skin barrier recovery	Denda et al., 1999
	<b>Cl<sup>-</sup></b>		Hairless mice, acceleration of exocytosis of lipid- containing lamellar bodies after application of chloride ionophore	Denda et al., 2003
Anti-inflammation	<b>Mg<sup>2+</sup></b>	MgCl <sub>2</sub> 5 %	Specific inhibition of the antigen-presenting capacity of Langerhans cells and TNF-α production by epidermal cells	Schempp et al., 2000
	<b>Zn<sup>2+</sup></b>		Suppression of cytokine-induced iNOS expression and IFN-γ or TNF-α-induced NO production	Yamaoka et al., 2000
	<b>Se</b>	SeCl <sub>2</sub> , SeNaO <sub>3</sub> , SeNaO <sub>4</sub> (60 µg·L <sup>-1</sup> )	Reconstituted skin from biopsies (healthy and inflamed); moderate inhibitory effect on inflammatory cytokine production, particularly IL-6 and IL-1α	Celerier et al., 1995
	<b>Sr<sup>2+</sup></b>	SrNO <sub>3</sub> or SrCl <sub>2</sub> (260 µg·L <sup>-1</sup> )	Reconstituted skin from biopsies (healthy and inflamed), moderate (smaller than Se) inhibitory effect on inflammatory cytokine production, particularly on IL-6	Celerier et al., 1995
Wound healing	<b>Mn<sup>2+</sup></b>	Manganese gluconate 1 to 2 µg·mL <sup>-1</sup>	Keratinocyte culture and reconstituted skin; enhancement of expression integrins specific for the late phase of wound healing	Tenaud et al., 1999
	<b>Cu<sup>2+</sup></b>	Copper gluconate 2 and 4 µg·mL <sup>-1</sup>	Keratinocyte culture and reconstituted skin; enhancement of expression integrins specific for the late phase of wound healing	Tenaud et al., 1999
		Glycyl-L-histidyl-L-lysine-Cu(II) 2 mg per injection twice a week for 3 weeks	Wounded rats, induction of metalloproteinases (MMP-9 and MMP-2) involved in wound remodelling by GHL-Cu injections	Siméon et al., 1999
		10 <sup>9</sup> mol·L <sup>-1</sup>	Irradiated fibroblasts, increased production of βFGF and VEGF in cells treated with GHK-Cu (glycyl-L-histidyl-L-lysine-Cu <sup>2+</sup> )	Pollard et al., 2005
	<b>Zn<sup>2+</sup></b>	At physiological levels 1 to 3 % (topical) Zinc gluconate 1.8 µg·mL <sup>-1</sup>	- Regulation of all stages of wound healing processes  - Inductive effect of Zn Gluconate on integrins in the proliferative phase of wound healing	Lin et al., 2018 Tenaud et al., 1999
	<b>Zn<sup>2+</sup></b>			Mahoney et al., 2009

Elastin/collagen production enhancement	<b>Cu<sup>2+</sup></b>	0.1 % copper-zinc malonate	Clinical study, enhanced synthesis of tropoelastin leading to regeneration of elastic fibres within dermis	
UV protection	<b>Se</b>	0.1 mg·L <sup>-1</sup>	Fibroblasts, UV-A radiation cultured in Se or TSW-supplemented medium presented higher survival rate	Leccia et al., 1993
		320 nmol·L <sup>-1</sup>	Fibroblasts exposed to UV-A radiation cultured in Se-supplemented medium presented higher survival rate (inhibition of lipid peroxidation)	Moysan et al., 1995
	<b>Zn<sup>2+</sup></b>	6.5 mg·L <sup>-1</sup> as ZnCl <sub>2</sub>	Fibroblasts: UV-A radiation cultured in ZnCl <sub>2</sub> or TSW-supplemented medium presented higher survival rate	Leccia et al., 1993
Anti-acne	<b>Zn<sup>2+</sup></b>	1.2 % Zinc acetate 3 or 9 mmol·L <sup>-1</sup> 10 <sup>-6</sup> mol·L <sup>-1</sup>	Sebosuppression (inhibition of 5 α-reductase activity <i>in vitro</i> in cell cultures)	Piérard-Franchimont et al., 1995 Stamatiadi et al., 1988 Sugimoto et al., 1995
	<b>ZnO</b>	From 1 to 8 %	Antimicrobial	Pasquet et al., 2015

### 6.3. Aluminium

Aluminium compounds are used as inorganic very soluble salts (chloride, nitrate, sulfate, chlorate), partially soluble (acetate, benzoate and lactate) and as organic salts with a high molar mass counter-ions and poor solubility. Aluminium salts have been successfully used in antiperspirant products owing to the capacity of Al<sup>3+</sup> to bind –SH groups within epidermal keratin resulting in an obstruction of appendages (sweat ducts, hair follicles and sebaceous glands) (Lansdown, 1973; Lyon and Klotz, 1958). Some of them are used in toothpastes (aluminium fluoride), as colorants, lakes and pigments (aluminium silicate, aluminium hydroxide) or as viscosity agents (magnesium aluminium silicate). Medical applications of aluminium include its use as adjuvant in vaccines (aluminium phosphate and aluminium hydroxide) and as a symptomatic treatment against pathological hyperhidrosis (Klotz et al., 2017). The risk assessment of aluminium salts, especially those used in deodorants and antiperspirants, is continuously evaluated (European regulation on cosmetic products EC/1223, 2009). Aluminium chloride (also called aluminium trichloride) is unstable in water and forms the hexahydrate (Al(H<sub>2</sub>O)<sub>6</sub>Cl<sup>3+</sup>) which gives rise to Al(OH)<sub>3</sub> with release of HCl and heat. Therefore, AlCl<sub>3</sub> is very corrosive. To minimize this effect, hydrated aluminium chlorides which are partially hydrolysed are used, as aluminium chlorohydrate, for example. Double salts of zirconium and aluminium (aluminium zirconium tetra- or pentachlorohydrate) and the well-known “Alum stone” are also used. The use of some of

them is restricted in the Regulation on cosmetic products 1223/EC (Annex III). Aluminium neurotoxicity has first been reported a consequence of inhalation of aluminium dusts and in patients suffering from a kidney failure who were under dialysis. There is no report of such affects after the topical exposure. The cumulative dose over long times causes encephalopathies. A lot of controversy arose about its use in cosmetic products after the hypothesis given by Darbre (2001) claiming that “Underarm cosmetics are a cause of breast cancer”. This publication triggered the research on the link between the use of antiperspirants and deodorants and the risk of cancer. As a result, numerous case reports and clinical trials were published. Some of them claimed there was no correlation between hygiene habits of examined subjects and the occurrence of disease (Mirick, 2002; Namer et al., 2008). Simultaneously, other teams disclosed the mechanisms of  $\text{Al}^{3+}$  action in breast cancer (Bakir and Darbre, 2015; Exley et al., 2007) and published clinical studies proving the initial hypothesis. The morbidity of breast cancer was linked to the use of aluminium-based underarm cosmetics either in the population that started such hygiene patterns at early age (pre-puberty) or removed the auxiliary hair while using  $\text{Al}^{3+}$ -based underarm products (Darbre, 2003; Linhart et al., 2017; McGrath, 2003). This scientific discussion was then supported by the research focusing on the skin absorption of aluminium. Chronologically, the first study aiming at assessing skin absorption of Al was carried on by Anane et al. dates back to 1995 when aluminium was not yet suspected to increase the incidence of breast cancer but was possibly related to the development of neurodegenerative diseases. The *in vitro* study was performed on healthy shaved mice that underwent daily applications of low concentrations of aqueous solutions of aluminium chloride for 130 days. Authors demonstrated a significant retention of aluminium in brain (hippocampus) combined with an increase in concentrations of  $\text{Al}^{3+}$  detected in urine and serum as compared to non-treated animals (Anane et al., 1995). Given the anatomical differences between murine and human skin (namely the thickness and number of hair follicles per  $\text{cm}^2$ ), the following tests were performed as either clinical studies or with human skin explants. Within their *in vitro* (Franz cell) investigation of skin absorption of aluminium from commercial products (aerosol, stick and roll-on forms), Pineau et al. (2012) verified the impact of the physio-pathological state of the skin using both

intact and damaged (tape-stripped to mimic “after-shaving” conditions) skin models. Authors found significantly higher absorption of aluminium within the skin layers when the product was applied on damaged skin. Regardless the skin condition, the permeated dose (recovered in acceptor fluid) after 24 h was negligible. The majority of applied doses was recovered in the *stratum corneum* and viable epidermis. These results agreed with an earlier clinical study, in which a radiolabelled ( $^{26}\text{Al}$ ) aluminium chlorohydrate was applied in the axillary zone in male and female volunteers. The application of the product was unique within the study and was followed by hair removal (shaving with an electric razor). The attention was paid so as to avoid application to damaged skin. The treated axilla was kept under an occlusive bandage during 6 consecutive days and the application area was stripped twice. Absorption levels of 3.6  $\mu\text{g}$  (estimated by the renal clearance) of Al over 2 weeks were found, corresponded to 2.5 % of the dietary intake. Higher absorption rates in women were attributed to the damages of the skin occurring during the experiment and led to infer that the use of antiperspirants on damaged skin should be avoided (Flarend et al., 2001). This preliminary study examined the dermal absorption of aluminium after a single application without taking account daily use of underarm products in real life. Additionally, the authors confirmed the loss of 50–70 % of applied dose within the manipulations. The most recent clinical pharmacokinetic study on dermal absorption of Al used the “state-of-the-art” techniques to investigate the effects of daily exposure to  $^{26}\text{Al}$ -based underarm cosmetics. Tested conditions involved various regimens: application of  $^{26}\text{Al}$ -radiolabelled aluminium chlorohydrate. The results confirmed that antiperspirants play a minor role in the total systemic exposure to the aluminium salts. Moreover, shaving or daily use did not influence the absorbed quantities of  $^{26}\text{Al}$  (de Ligt et al., 2018).

#### **6.4. Silver**

Silver is not a trace metal and has no physiological role in the human body. It may be under three oxidation states: Ag(I), Ag(II) and Ag(III) but the only stable and biologically active ion is  $\text{Ag}^+$ . Different chemical forms of silver, for example metallic silver particles (nano or not), inorganic salts with different solubility ( $\text{AgNO}_3$ ,  $\text{AgCl}$ ) and organic complexes (silver proteins for example), are used in many

industries (Edwards-Jones, 2009; Lansdown, 2010, 2006). Because  $\text{Ag}^+$  is only active in solution, the role of its carrier is crucial. Silver derivatives are widely used as antimicrobial agents, with an activity proportional to the concentration of  $\text{Ag(I)}$ , in dentistry (dental amalgams), in medical devices, pharmaceutical products and cosmetics. Exposure to silver exhibits low toxicity in the human body except chronic ingestion or inhalation. The systemic absorption of silver-based drug may be toxic and cause local argyria of the skin. This is the most common manifestation of toxicity. Numerous cases of argyria have been described in literature and were related to the ingestion of metallic silver or silver compounds over periods of months or even years. A well-known case is that of an American woman who has been using nose drops for viral colds or allergy for years. Her skin had a characteristic irreversibly grey or blue-grey discoloration. Silver granules concentrate in basement membranes and elastic fibres surrounding sweat glands (ATSDR, 1990; Silver, 2003). *In vivo* evidence of argyria associated with modern silver wound dressings applied topically has never been observed (Cutting et al., 2007). Aqueous solution of silver nitrate has been used since many years in treatment of difficult wounds. Silver ions form insoluble complexes with proteins in skin wounds and with free anions that are abundant in the skin: chloride, phosphate or sulfate. This limits the availability of silver ions in the wound bed. Actually, silver nitrate has a very high ionizing capacity as compared to other silver compounds, and binds strongly to electron donor groups of biological molecules containing thiol groups, oxygen and nitrogen. The nature of the medium (salt and protein content) can have a great influence on the concentration of silver ions (Lansdown, 2006; Wilkinson et al., 2011). Silver nitrate is caustic and has astringent properties. Its main side-effect is related to reduction of colourless  $\text{Ag(I)}$  to black  $\text{Ag(0)}$ . To avoid this reaction, pharmacists precipitated silver in the form of silver proteinate or colloidal solution. The addition of sulfadiazine to stabilize  $\text{Ag(I)}$  was very useful and “marked the renaissance of the use of silver in wound care” (Lansdown, 2006). Sulfadiazine maintains  $\text{Ag(I)}$  in a stable form avoiding its reduction to  $\text{Ag(0)}$ . A cream containing 1 % silver sulfadiazine and 0.2 % chlorhexidine used for the treatment of serious burns which is a well-known silver preparation. This combination is also used in bandages, for treatments of burn wounds and traumatic injuries of humans

(Silver, 2003). Absorption of silver ion through the skin is very limited even in the case of skin injury. Lansdown et al. (1997) have studied the absorption of silver nitrate in wound therapy. Silver accumulated in sweat gland ducts and hair follicles but the new epithelialized tissue did not exhibit any staining suggesting that the silver bound to epidermal cells was eliminated with wound exudate, and epidermal cell debris. Absorption through intact skin has been estimated as low as 1 %/5 h by Skog and Wahlberg (1964) for concentrations of  $^{110m}\text{Ag}$  ranging between 0.00048 M and 4.87 M. This low absorption was confirmed by other teams (Coombs et al., 1992; Lansdown, 2010, 2006). Silver salts or colloidal silver are used in personal skin care products as preservatives or colorants in make-up, face masks or face care products, cleansers, antiperspirants or chemical exfoliating products, for example. Silver nitrate is reported in the Annex III of the European Regulation of cosmetic products only for colouring eyelashes and eyebrows with the following wording of “conditions of use and warning” clearly indicated on the packaging: “Contains silver nitrate. Rinse eyes immediately if product comes into contact with them”. Silver particles are also authorized as colorant in cosmetic products (Annex IV, CI 77820). Silver chloride deposited on titanium dioxide can be used as preservative (Annex V). In this latter case, the maximum concentration in ready to use preparation is 0.004 % as AgCl or 20 % AgCl (w/w) on  $\text{TiO}_2$ . It must not be used in products for children under 3 years of age in oral products and in eye or lip products (European regulation on cosmetic products EC/1223, 2009). Ag nanoparticles have been produced by different means with various shapes and sizes. The improvement of antibacterial activity depends on the size and shape with the triangular-shaped nanoparticles displaying better activity compared to rod-shaped particles (Edwards-Jones, 2009; Pal et al., 2007). Concerning this type of colloidal silver nanoparticles, the conclusions of the opinion of the Scientific Committee on Consumer Safety on colloidal silver (nano) were inconclusive due to insufficient data, especially concerning dermal absorption of such nanomaterials (*Opinion on colloidal silver (nano)*, 2018).

## **6.5. Ions as toxic agents**

The main toxic effects reported after skin exposure to cations are irritancy, immunological changes leading to delayed hypersensitivity, carcinogenicity and systemic effects being the results of percutaneous absorption. Within this category, we can distinguish sensitizers (Ni, Co and Cr) and metal ions which are highly toxic and cause long-term damages (As, Cd, Hg, Pb, Sb and lanthanides) (Lansdown, 1995; Marinovich et al., 2014; Thyssen and Menné, 2010).

Endogenous ions are essential for many processes that involve extensive cell proliferation in childhood. In the adulthood however, only some tissues are still proliferative; the epidermis that renews within 28 days is an example. It is therefore vulnerable to toxicity or deficiency of substances in its close environment. Today, dermatologists and toxicologists are able to carefully study hazards associated with vast arrays of ions because metals exposure has changed from an occupational to a consumer problem. For example, chromium allergy is related to occupational exposure to cement, metals and to leather consumer goods. Thyssen and Menné (2010) reported that leather exposure among chromium-allergic patients increased from 24.1 % between 1989 and 1994 to 45.5 % between 1995 and 2007. Cobalt allergy is associated to exposure to jewellery, alloys metals, prostheses, dental materials and paints. In the same way, higher prevalence of cobalt allergy has been observed in pierced people in comparison to non-pierced ones. The use of all these metals, especially Cr(VI), leads to serious health concerns in tattooed individuals because most pigments used in tattoo ink formulations are originally produced for other applications (such as textiles, dyes, car paints, and plastic pigments). Additionally, tattoos are persistent whereas consumers affected by other goods (jewellery, creams or textiles) can easily remove them to avoid the contact with allergen. Ink products contain numerous impurities such as Cr(VI), Cr oxides, and also nickel, copper and cobalt, iron oxides, aromatic amines in azo-colorants, and polycyclic aromatic hydrocarbons in carbon black (Bocca et al., 2018).

#### **6.5.1. Sensitizers: nickel, chromium III and VI and cobalt**

Nickel, cobalt, iron, palladium and platinum belong to the group VIII B and chromium to VIB of periodic table of Mendeleev. As transition elements, these metals generally exhibit high density, high melting point, magnetic properties, variable valence, and the formation of stable coordination complexes.



Thanks to these properties, they are applied in the modern industry in the form of alloys which are widely used to produce jewellery and medical prostheses (Lansdown, 1995).

The most stable valence states are trivalent Cr(III) and hexavalent Cr(VI). Cr(III) is an essential nutrient while Cr(VI) is highly toxic for human health. It is classified as human carcinogen (category 1) and induces allergies at lower exposure levels than most Cr(III). Cr(VI) is produced by industrial processes and is used in the manufacture of dyes and inks, in leather tanning, textile industries and as cement and wood preservative (Bocca et al., 2014).

In biological systems, +2 oxidation state is the most prevalent form of nickel and cobalt. These metals dissolve easily in acidic pH of sweat upon contact with the skin (Hostýnek et al., 1993). Nickel turns to a greenish hexahydrated  $[\text{Ni}(\text{H}_2\text{O})_6]^{2+}$  ionic species in aqueous medium at pH = 7 (Denkhaus and Salnikow, 2002). Although nickel, chromium and cobalt are considered as trace nutrients, they are the most frequent and strong sensitizers compared to other ions like iron, silver or gold (Schmidt and Goebeler, 2015). They cause Allergic Contact Dermatitis, and Allergic Contact Urticaria. Cross-allergy to nickel and cobalt is very common. It is estimated that up to 17 % of women and 3 % of men are nickel-allergic, whereas only 1–3 % are allergic to cobalt and chromium (Thyssen and Menné, 2010). Nickel, cobalt and even palladium interact directly with the specific histidine residues in the human Toll-Like Receptor 4 (TLR4), which normally acts as an innate immune receptor for bacterial lipopolysaccharide. Therefore, it activates the intracellular pro-inflammatory signalling pathways by mimicking pathogen-associated molecular patterns such as  $\text{TNF}\alpha$ , IL-8 and IL-6 (Schmidt and Goebeler, 2015). Nickel and chromium(VI) may bind to the major histocompatibility molecules (MHC) and, as a consequence, activate the cytotoxic responses of T-cells. This induces T-lymphocyte mediated delayed hypersensitivity reaction occurs after previous exposure and sensitisation to the metal (Schmidt and Goebeler, 2015). To exert these damages, nickel, chromium and cobalt must penetrate the skin. Skin absorption of metals is closely related to the capacity of the sweat to oxidise them. This penetration has been modelled as being skin-thickness and concentration-dependent (Ward et al., 2019).

Because nickel is ubiquitous (as the 24<sup>th</sup> element in the Earth's crust) and people are constantly exposed to it, its skin absorption has been thoroughly studied. Nickel chloride ( $\text{NiCl}_2$ ) has been found to penetrate the epidermis and accumulate there due to its affinity to the tissue. Nickel sulfate and nickel chloride hexahydrate permeate human skin easily: much better under occlusion than in non-occluded conditions. For nickel chloride, 4% of applied dose permeated under occlusion against 0.23 % in non-occluded conditions and after a very long lag-time (50 h). Fullerton et al. (1986) noticed a better permeation of  $\text{NiCl}_2$  compared to  $\text{NiSO}_4$  (50 times faster). Additionally, a considerable amount is bound to proteins (between 13 % and 43 % for nickel chloride and 4 to 7 % for nickel sulfate). Nickel ions bound to the dermis may act as a reservoir, and probably the threshold concentration producing an allergic reaction is related to the actual amount retained in epidermal tissue (Allenby and Basketter, 1993). Given that sweating enhances solubilisation of nickel, Menné et al. (1987) evaluated the patch test reactivity to nickel alloys corroded for a period of 6 weeks at 30 °C with a synthetic sweat composed of demineralized water, 0.5 % sodium chloride, 0.1 % lactic acid and 0.1 % urea and a pH adjusted to 6.5 with ammonium hydroxide. Discs of metals were applied *in vivo* to 267 patients with known or suspected allergy to nickel sulfate. Allergic reactions were linearly correlated with the amount of nickel released from discs. Roughly, alloys with a nickel release exceeding  $1 \mu\text{g}\cdot\text{cm}^{-2}/\text{week}$  gave a strong patch test reaction while those with a release less than  $0.5 \mu\text{g}\cdot\text{cm}^{-2}/\text{week}$  were poor sensitizers.

Larese et al. extended the experiment by measuring the dermal absorption of nickel but also that of cobalt and chromium using the Franz cell method. For that purpose, they dissolved nickel, cobalt and chromium powder and potassium dichromate in a synthetic sweat at pH 6.5 ( $50 \text{ g}\cdot\text{L}^{-1}$ ). After 30 min the authors found  $27 \text{ mg}\cdot\text{L}^{-1}$  of nickel,  $33 \text{ mg}\cdot\text{L}^{-1}$  of cobalt and no chromium in the donor phase. The transdermal fluxes were  $0.017 \mu\text{g}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$  for nickel,  $7.3 \mu\text{g}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$  for potassium dichromate and  $0.12 \mu\text{g cm}^{-2}\cdot\text{h}^{-1}$  for cobalt. The lag times were shorter than those reported by Fullerton (14 h for  $\text{Ni}^{2+}$  and  $\text{K}_2\text{Cr}_2\text{O}_7$  and only 1 h for  $\text{Co}^{2+}$ ). The donor media were not the same: water in the paper of Fullerton and a synthetic sweat in the papers of Larese et al. which can explain the observed differences (Larese

Filon et al., 2004; Larese et al., 2007). No Cr was detectable from the powder, maybe because Cr powder cannot be oxidised by synthetic sweat as opposed to Cr salts that are quickly absorbed into the skin (Hostynek, 2003; Larese et al., 2007). Gammelgaard et al. (1992) found that chromium(VI) permeates better than chromium(III) in experiments by the Franz cell method. However, it does not permeate well through the skin but accumulates in skin strata (Kimbrough et al., 1999; Wahlberg, 1965b; 1965c). Chromium applied onto the skin as chromate is reduced to chromic ions ( $\text{Cr}^{3+}$ ) by tissue proteins containing sulfhydryl groups and accumulates in the epidermis and dermis (Bolzinger et al., 2010). Cr(VI) as chromate or dichromate is seen to cross the skin with no chemical modification, showing that the skin has a limited capacity to reduce the chromate ion. Indeed, Samitz and Katz (Samitz and Katz, 1969) estimated that one gram of skin can reduce 1 mg of dichromate to  $\text{Cr}^{3+}$ . Finally, contact sensitization to platinum is increasingly reported because it is used extensively in jewellery and, like silver and gold, is reported to be an occasional sensitizer.

The decrease of the prevalence of metal allergy decreases with regulatory interventions. For instance, in Europe the prevalence of nickel allergy has decreased since the adoption of the “nickel directive EN 1811:2011+A1:2015”, and the European REACH regulations (Bogen and Garry, 2018; Thyssen and Menné, 2010). In the same way the E.U. REACH regulation 301/2014 restricted Cr(VI) content in leather products (< 3 ppm) (European regulation on cosmetic products EC/1223, 2009).

#### **6.5.2. Harmful effects of heavy metal ions**

Mercury, arsenic and lead are the most toxic elements present in the atmosphere, food and water, in our ecosystem. Actually, mercury (as well as arsenic oxide) in all forms alter the cellular functions by denaturation of the structure of proteins and by binding sulfhydryl and selenohydryl groups (Bernhoft, 2012; Rice et al., 2014). Inorganic mercury exists under several forms: (i) metallic mercury present in dental amalgam, thermometers, incandescent lights, batteries and the incineration of medical waste; (ii) mercury vapour ( $\text{Hg}_0$ ); (iii) mercurous salt ( $\text{Hg}_2^{2+}$ ), the famous Calomel, historically used as slight laxative; (iv) and mercuric salts ( $\text{Hg}^{2+}$ ). Organic mercurials include mercury bound to a chemical group, ex. methyl, ethyl or phenyl groups like in thiomersal, for example. Methyl mercury is the most toxic

mercury compound because it absorbs easily through biological membranes, and is excreted very slowly (> 70 days). *In vivo*, methyl mercury is demethylated to elemental mercury in the brain and its preferential form is  $\text{Hg}^{2+}$  (Bernhoft, 2012). This latter product has been widely used as an active agent for its antiseptic properties in biocides or as preservative in many drugs and cosmetics (thiomersal, Annex V of the cosmetic regulation N°1223/2009). Unfortunately, mercuric compounds (very often as  $\text{HgCl}_2$ , a certain oil-soluble salt, ammoniated mercury and mercurous oxide) are commonly misused as skin-lightening agents, especially in dark-skinned women from sub-Saharan Africa (Lerner, 1952; Mahé et al., 2003; Olumide et al., 2008). Mercury bleaches the skin probably by an effect on antityrosinase but paradoxically, chronic exposure darkens the skin by dispersion of metal granules (Olumide et al., 2008), which give skin a recognizable slate-grey pigmentation (Hostýnek et al., 1993; Lerner, 1952). Mercuric salts can penetrate the skin; their absorption is proportional to the applied concentration. Once in bloodstream, mercury can easily cross other biological membranes such as the blood-brain barrier which can lead to deposition of Hg in its metallic form (Skog and Wahlberg, 1964; Wahlberg, 1965c). Chronic topical exposure to Hg salts can result in neurologic, renal (nephrotic syndrome), and dermal toxicity (Chan et al., 2020; Mahé et al., 2003). Other possible cutaneous damages include burning of the face, contact dermatitis, flushing, erythroderma, purpura, and gingivostomatitis (Bocca et al., 2014).

Some heavy ions have also been studied for their radioactive properties. Radioactive sources are used in various fields of nuclear industry but also for medical purposes to prepare radiopharmaceuticals. A radiopharmaceutical is defined as a drug containing a radionuclide. Some of them, labelled with gamma-emitting isotopes, are used for diagnostic purposes, for example  $^{99\text{m}}\text{Tc}$ ,  $^{67}\text{Ga}$ ,  $^{111}\text{In}$ ,  $^{131}\text{I}$  and  $^{51}\text{Cr}$ . Therapeutic radiopharmaceuticals are meant to deliver a dose of cytotoxic radiation to the tumour cells while avoiding radiation damage in healthy tissues. They are generally low  $\beta$ -emitters and their radiation penetrates quite deeply in the tissue (2–12 mm), which is particularly important for the treatment of solid tumours. The choice of radiopharmaceuticals depends on their half-life, radiation type, other particulate radiation emissions, energy and biodistribution characteristics.

In the nuclear industry, thermal fission occurs when fissile elements are fabricated as nuclear fuel or reprocessed. This process results in formation of  $\alpha$ -emitting radionuclides, such as: uranium ( $^{235}\text{U}$ ), plutonium ( $^{239}\text{Pu}$ ,  $^{241}\text{Pu}$ ) or americium ( $^{241}\text{Am}$ ) whose nuclei contain odd numbers of neutrons. During the processing of nuclear fuels for recycling purposes, workers manipulate different actinide forms from extraction processes, or discharges of nuclear plants that may be a potential source of contamination (Tazart et al., 2018). Alpha particles are characterised by high radiation energy and a very short range (40  $\mu\text{m}$  in tissue) (Tazart et al., 2017; Tymen et al., 2000).  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$  nuclides are respectively  $\beta/\gamma$  emitter and pure  $\beta$  emitter and therefore belong to the most dangerous radionuclides. They are components of the long-lived fraction of fission products and cause severe health concerns after nuclear accidents. Indeed,  $\text{Sr}^{2+}$  is homologous to calcium in terms of chemical properties and thus it can compete with  $\text{Ca}^{2+}$  in the bone mineralisation processes (Bauerová et al., 2001). The limitation of the dose is of great importance for the workers manipulating high doses of radioactive products in industrial and hospital activities.  $\gamma$ - and  $\beta$ -rays are highly penetrating radiations.  $\alpha$ -emitters may locally emit very high dose in the case of repeated occupational exposure (Bolzinger et al., 2010; Liu, 2004; Tazart et al., 2017). The critical site of radiation resulting from topical contamination is the basal layer of epidermal cells. The radiation dose reaching the basal layer involves predominantly  $\beta$  and  $\gamma$  radiations (Bauerová et al., 2001). Grappin et al. reported a survey of 548 exposure cases which occurred in France between 1970 and 2003 and showed that approximately 54 % of them included skin exposure to actinides (uranium, plutonium and americium and fission products as  $^{137}\text{Cs}$  or  $^{90}\text{Sr}$ ), 17 % of which concerned contamination of healthy skin (Liu, 2004). More recently, Berard et al. (2010) reported that between 2003 and 2007, 100 to 150 cases including skin injuries occurred each year. Given that most contamination happens on the hands, the skin penetration of such heavy ions has been studied to develop effective countermeasures and decontamination guidelines.

$^{241}\text{Am}$ ,  $^{239}\text{Pu}$  and  $^{233}\text{U}$  have been studied *in vitro* using the Franz cell method or *in vivo* on rats by many researchers. These actinides are soluble in acidic medium. Americium is solely trivalent in water while plutonium is tetravalent  $\text{Pu(IV)}$ . Various uranyl isotopes are available but the most stable is  $^{235}\text{U}$ .

Uranium, plutonium and americium, in contact with the skin, form polymerized hydroxide particles  $(\text{UO}_2)_x(\text{OH})_y^{(2x-y)+}$ ,  $\text{Pu}(\text{OH})_z^{(4-z)+}$ ,  $\text{Am}(\text{OH})_x^{(3-x)+}$ , which are similar to the insoluble particles met in most human contamination cases (Tymen et al., 2000; Beall and Allard, 1981). In consequence, more than 90 % of the applied activity is concentrated at the surface and in the epidermis (Tymen et al., 2000). In Franz cell experiments on intact pig skin, Petitot et al. (2004) found a lag time of 4 h and a steady state flux of  $18 \pm 4 \text{ ng} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$  after application of 100  $\mu\text{L}$  of 60  $\mu\text{mol} \cdot \text{L}^{-1}$  uranyl nitrate solution (0.1N  $\text{HNO}_3$ ) containing 500 Bq of  $^{233}\text{U}$ . Americium contamination has been rarely studied and the most recent results were obtained by Tazart et al. on fresh and frozen pig skin explants using the *in vitro* Franz diffusion cell method (Tazart et al., 2017, 2018). In these studies, 4.1 kBq of  $^{241}\text{Am}$  were deposited in the donor chamber (270  $\mu\text{L}$  on 0.9  $\text{cm}^2$ ). The acceptor fluid of the Franz cell was collected after 4 h, and the distribution in the skin layers after 24 h was evaluated. The results showed that 90 % of the initial activity was recovered at the skin surface and 0.016 % and 0.003 % in the acceptor medium for the fresh and frozen skin respectively. Interestingly, even after washing the *stratum corneum* with water or a solution of a chelating agent, 8 to 10 % of the activity remained “stuck” within the *stratum corneum* layer. The same behaviour has also been observed in other studies for plutonium and uranium (Phan et al., 2013; Tymen et al., 2000).

The skin penetration of  $^{137}\text{Cs}^+$  and  $^{60}\text{Co}^{2+}$  through abdominal rat skin studied by Koprda et al. (2000) was shown proportional to the time for 5–7 hours of exposure. These heavy metal cations form covalent bonds with side chain polar groups of peptides ( $-\text{COOH}$ ,  $-\text{OH}$ , and  $-\text{NH}_2$ ) and phospholipids. They can also form multinuclear chelates (Bolzinger et al., 2010; Koprda et al., 2000; Reichert et al., 1999).

Concerning radionuclides used for diagnostic purposes, Bolzinger et al. (2010) have shown a low permeation of indium and gallium. These metal ions are stable only in the +3 oxidation state, due to the formation of insoluble hydroxides at  $\text{pH} = 5.5$  on the skin surface (Reichert et al., 1999). On the other hand, Technetium ( $^{99\text{m}}\text{Tc}$ ) permeated faster through the skin from its pertechnetate anion

( $\text{TcO}_4^-$ ). It was suggested that there was no effective chemical binding of  $^{99\text{m}}\text{Tc}$  to biomolecules in the skin under its oxidation state (VII) (Bolzinger et al., 2010).

## 7. Skin absorption of Hofmeister ions and their impact on absorption of other molecules

Three columns of the periodic table of the elements: IA, IIA and, VIIA must be considered specifically as they enter the classical Hofmeister series (Fig. 3). These ions display common physicochemical properties with two main classes: strongly hydrated (small size, high surface charge density) and weakly hydrated (large size, low surface charge density) species relative to the strength of water–water interactions, as written by Collins et al. (2004). Chaotropic ions are poorly hydrated in aqueous solutions and thus called “water-structure breakers”. They exhibit weaker interactions with water than water itself so that they interact strongly with non-polar substances, resulting in their increased solubility (salting-in effect). On the other hand, kosmotropes interact strongly with water molecules and reduce the solubility of non-polar substances (salting-out effect). These ions are also called “water structure makers”.

Considering these specific features, it has been attempted to investigate the properties of the ions classified in Hofmeister series in terms of their own skin absorption as well as their effects on skin penetration of other substances.

Tregear (1966) investigated *in vitro* and *in vivo* skin penetration of radiolabelled  $\text{Br}^-$  ( $^{82}\text{Br}$ ),  $\text{Na}^+$  ( $^{24}\text{Na}$ ) and  $\text{PO}_4^{3-}$  ( $^{32}\text{P}$ ) using different skin models (human and rabbit *in vitro* and *in vivo*, pig *in vitro*). Despite the differences in the flux depending on the model, all skin types used for *in vitro* and *in vivo* protocols were permeable to ions. The steady penetration rates of  $\text{Na}^+$  and  $\text{Br}^-$  through the skin were proportional to the concentration difference of each ion across the skin in accordance with a passive diffusion process following the Fick’s law. There were no significant differences between the penetration of different ions through the same skin model. Our team investigated percutaneous penetration of radioactive iodide ( $^{125}\text{I}$ ) in the context of skin contamination. Iodide penetrated full thickness pig skin over 24 h and had a 5 h lag time (Bolzinger et al., 2010). Iodide is an anion of a great

biological and pharmaceutical interest. It plays a central role in thyroid physiology and synthesis of thyroid hormones: triiodothyronine ( $T_3$ ) and thyroxine ( $T_4$ ). Thus, its systemic supplementation may be required in the case of hypothyroidism (Vitti and Hegedüs, 2018). On the other hand, radioactive iodine is a useful tool in the treatment of thyroid cancer and hyperthyroidism (Mettler and Guiberteau, 2018). Furthermore, iodine-based antiseptic formulations are commonly used topically in the treatment wounds difficult to heal and in pre-surgical preparations (Lou et al., 2013). Because of these applications, skin absorption of iodide has been thoroughly studied using Franz cell method. Lou et al. focused on transdermal delivery for iodide ions and enhancement of their penetration from various w/o microemulsions that could be an suitable formulation for administration. They demonstrated measurable skin penetration of iodide from KI aqueous solution that was significantly improved when the solution was incorporated into microemulsions. However, authors provided no information concerning the retention of the molecule within skin layers which could account to even greater absorption rates. The experiments used human excised skin and was carried over 24 h (Lou et al., 2013). In that study, the lag time observed for iodide was also 5 h, which was due to its high retention in *stratum corneum* at the beginning of absorption.

Our recent investigation showed that the flux of anion through the skin is proportional to applied concentration of  $I^-$  in the low concentration range; but in the case of highly concentrated solutions, the flux is better correlated to the activity of the salt solution (Tarnowska et al., unpublished). This did not agree with Nygren et al. (2017 reported a linear relationship between the transdermal flux and the tested concentration of iodide ( $^{131}I$ ). This disagreement is due to difference in the preparation and storage of skin samples (epidermal sheets prepared from frozen-stored explants vs full thickness fresh skin). Thus, the observations in the last study were only related to passive transport of the ion. Nygren et al. (2017) also noticed differences in absorption of iodide through human epidermal sheets depending on the acceptor medium used. Higher flux was obtained when a mixture of water and ethanol (1:1) was used as compared to Phosphate Buffer Saline. The very short observed lag time (5 min) was also due to the use of split frozen-stored skin model. Additionally, the preparation of epidermal discs



included heating up to 60 °C that could affect the structure of *stratum corneum* lipids (Nygren et al., 2017). The distribution of anion in the skin layers after 6 h exposure time revealed that there was no retention in the skin (Bolzinger et al., 2010).

These results were then extended in the work by Paweloszek et al. (2016a) on skin absorption of non-radioactive halide ions ( $F^-$ ,  $Cl^-$ ,  $Br^-$  and  $I^-$ ) as an important part of the Hofmeister series. The ions deposited as aqueous solutions of sodium salts permeated pig and human skin in a concentration-dependent manner. Moreover, tested anions had significantly higher penetration rates when fresh skin samples were used as compared to frozen-stored explants. These results, collected for both human and porcine skin, indicated that facilitated transport contributes to the skin penetration of anions. This was confirmed by applying a chloride channel blocker on fresh skin prior to its exposure to the salt solutions. Penetration rates in this experiment were similar to the ones obtained on frozen-stored skin (Paweloszek et al., 2016b). Finally, Tarnowska et al. (unpublished) tested skin permeation of the most chaotropic anions thiocyanate and perchlorate (also applied as aqueous solutions of sodium salts). These anions are known as endocrine disruptors as they can inhibit iodine uptake in the thyroid leading to insufficient synthesis of thyroid hormones (Oliveira et al., 2019). They were found to penetrate through viable skin *in vitro* over 24 h. The total absorbed amount of perchlorate was comparable to that of iodide while thiocyanate penetrated less.

Halogen anions studied by Paweloszek et al. (2016a,b) have some known biological effects. Especially chloride plays an important role in the skin biology. Denda et al. (2003) showed that GABA and glycine (that activates chloride channels) accelerated skin barrier repair in hairless mice having skin damaged by tape stripping and acetone treatment. The application of chloride ionophore accelerated the exocytosis of lipid-containing lamellar bodies, and thus the process of skin barrier repair. Mechanism suggested by the authors involved blocking the outward movement of the cell membrane and accelerated fusion of the lamellar body and cell membrane due to the repolarisation of cell membrane by the  $Cl^-$  efflux (Denda et al., 2003).

Skin exposure to high doses of fluoride, especially when related to skin absorption of the ion, may lead to reduced collagen content, as shown in rats and rabbits; but these effects were not observed for lower doses. Indeed, the mechanism of toxicity of sodium fluoride is known to cause deficient collagen fibres due to inadequate cross-links. Additionally, high doses of NaF were found to disturb terminal differentiation of cultured keratinocytes manifested by decreased keratin expression and failing in stratification, which finally impair skin barrier function (Drózd et al., 1980; Prado et al., 2011).

As chaotropic anions are “sticky ions” that bind to non-polar lipids or proteins and swell the extracellular lipid matrix of *stratum corneum* by a salting-in effect, they can contribute to hydration of lipid bilayers and then facilitate the skin absorption of drugs into the *stratum corneum*. The first study disclosing a significant improvement in skin absorption of neostigmine bromide, a positively charged organic model molecule, in the presence of NaCl and LiCl was reported by Michael-Baruch et al. (1994). Indeed, the drug did not permeate split skin from a simple aqueous solution (values below detection limit). The addition of NaCl and LiCl increased drug penetration rates in a concentration-dependent manner. The dependence of the permeability coefficient on inorganic ion concentration displayed two stages. A first stage was attributed to a decrease in the viscosity of the lamellar phase of *stratum corneum* lipids caused by the influx of salting-in cations which participated in the hydration of the lamellar phase. The second stage was ascribed to a Donnan-like effect where the poorly diffusible cations ( $\text{Na}^+$  and  $\text{Li}^+$ ) enhance the drug partitioning in the favour of the most diffusible ion, neostigmine (Michael-Baruch et al., 1994). In another study, Ko et al. tested the impact of different sodium salts of anions classified in the Hofmeister series ( $\text{NaF}$ ,  $\text{NaCl}$ ,  $\text{NaBr}$ ,  $\text{NaNO}_3$ ,  $\text{NaI}$ ,  $\text{NaSCN}$ ,  $\text{NaClO}_4$ ) on skin penetration of salicylic acid. The experiments were carried out using excised mouse skin mounted in Franz cells. The permeation-enhancing activities of tested salts only partially followed the order of the Hofmeister series. More chaotropic anions ( $\text{Br}^-$ ,  $\text{I}^-$  and  $\text{SCN}^-$ ) increased the flux of the tested molecule while the kosmotropic ones either did not have an effect ( $\text{Cl}^-$  and  $\text{ClO}_4^-$ ) or decreased ( $\text{F}^-$ ,  $\text{SO}_4^{2-}$ ) permeation rates. For the ions classified as permeation enhancers, concentration dependence of the effect was reported up to 2 M. Further increasing of the salt concentration did not cause additional

improvement of salicylic acid skin permeation. Authors confirmed these results by further investigation *in vivo*. Indeed, addition of NaI or NaBr to the vaseline ointment significantly increased the transdermal permeation of drug through rabbit skin. Authors suggested that these effects were due to alteration of the thermodynamic activity of salicylic acid in the donor solution in the presence of bromide and iodide (Ko et al., 1995). Finally, our recent studies investigated how mixing Hofmeister anions in bi- and ternary systems could affect their skin absorption *in vitro*. Two series of anions were tested using the Franz cell method while maintaining pork skin explants viable allowing facilitated transport. The first series included halide ions and their mixtures obtained in such a way that the total concentration of salts in deposited solution was constant. The results of skin absorption were expressed as an absorption coefficient which is the ratio of the total absorbed amount after 24 h exposure to the activity in the donor solution ( $k_{abs}=Q_{abs}/\gamma C$ ). Such conversion allowed direct comparison among  $k_{abs}$  values regardless different anion concentrations deposited in the donor compartment of Franz cell system. These studies revealed strong synergistic effect of mixing iodide and bromide. Addition of fluoride either did not contribute or slightly reduced overall penetration of other anions. In the case of more chaotropic anions:  $I^-$ ,  $SCN^-$  and  $ClO_4^-$  the total absorbed quantity of thiocyanate was the highest when applied in a ternary mixture. On the other hand, perchlorate had the highest permeation rates when applied alone. (Tarnowska et al., unpublished).

## 8. Conclusions

The effects of inorganic ions on human health depend on the type of ion and its dose. It is evident that bulk and macroelements are essential for healthy grow, development and functioning of the organism. Micro- and trace elements are necessary for proper functioning but their excessive administration can lead to serious adverse effects. Xenobiotic ions could also have either beneficial (ex.  $Ag^+$ ) or harmful effects on the organism (ex. lanthanides). In various studies, skin has been shown to be a potential route of entry of these inorganic ions. Depending on the physicochemical properties of ions, some of them permeate into the tissue while others can penetrate into the bloodstream. Generally, the

penetration is easier for anions and monovalent cations, while polyvalent ones tend to be retained within skin layers. Cations exert more biological functions in the skin than anions, so that the beneficial of thermal spring waters depend on their cationic composition. Also, harmful effects are often due to excessive exposure to cations of heavier metals.

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