



Skin absorption of mixed halide anions from concentrated aqueous solutions

Malgorzata Tarnowska, Yves Chevalier, Stéphanie Briançon, Claire Bordes,
Jacqueline Resende de Azevedo, Delphine Arquier, Thierry Pourcher,
Marie-Alexandrine Bolzinger

► To cite this version:

Malgorzata Tarnowska, Yves Chevalier, Stéphanie Briançon, Claire Bordes, Jacqueline Resende de Azevedo, et al.. Skin absorption of mixed halide anions from concentrated aqueous solutions. *European Journal of Pharmaceutical Sciences*, 2021, pp.105985. <10.1016/j.ejps.2021.105985>. <hal-03330438>

HAL Id: hal-03330438

<https://hal.science/hal-03330438v1>

Submitted on 31 Aug 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Skin absorption of mixed halide anions from concentrated aqueous solutions

Małgorzata Tarnowska^a, Yves Chevalier^a, Stéphanie Briançon^a, Claire Bordes^a, Jacqueline Resende de Azevedo^a, Delphine Arquier^a, Thierry Pourcher^b, Marie-Alexandrine Bolzinger^a

^a Univ Lyon, Université Claude Bernard Lyon 1, CNRS UMR 5007, Laboratoire d'Automatique, de Génie des Procédés et de Génie Pharmaceutique (LAGEPP), 43 bd du 11 Novembre 1918, 69622, Villeurbanne, France.

^b Laboratory Transporter in Imaging and Radiotherapy in Oncology (TIRO), University Côte d'Azur, Institut de biosciences et biotechnologies d'Aix-Marseille (BIAM), Commissariat à l'Énergie Atomique, Faculté de Médecine, 28 av de Valombrose, Nice, France.

Abstract

Non-ideal behaviour of mixed ions is disclosed in skin absorption experiments of mixed halide anions in excised pig skin. Comparison of skin absorption of pure and mixed ions shows enhanced penetration of chaotropic ions from mixed solutions. An experimental design and statistical analysis using a Scheffé {3,2} simplex-lattice allows investigating the full ternary diagram of anion mixtures of fluoride, bromide and iodide. Synergism in mixed absorption is observed for chaotropic bromide and iodide anions. A refined analysis highlighting specific interactions is made by considering the ratio of the absorbed amount to the ion activity instead of the directly measured absorbed amount. Statistical analysis discards non-significant effects and discloses specific interactions. Such interactions between bromide and iodide cause an absorption enhancement of their partner by a factor of 2–3 with respect to the case of ideal mixing. It is proposed that enhanced absorption from mixed solution involves the formation of neutral complex species of mixed bromide and iodide with endogenous magnesium or calcium inside *stratum corneum*.

Keywords: Skin absorption; Anions absorption; Ionic interactions; Hofmeister series; Halide ions; Experimental design

1. Introduction

Skin plays a key role in separation of human body from external environment. This is mainly due to *stratum corneum*, the outermost layer made of dead cells (corneocytes) surrounded by a lipid intercellular medium. *Stratum corneum* is organised into a brick-and-mortar-like structure where the bricks are corneocytes and the mortar is made of the intercellular lipids. Such highly hydrophobic structure protects body from excessive water loss on one hand and from pathogen and xenobiotic absorption on the other. Because of this hydrophobic barrier, skin absorption of simple inorganic ions has long been believed limited, or even impossible [1]. However, several recent studies have proved this concept wrong [2,3]. Recent work by Paweloszek *et al.* [4] shed new light on the possible mechanisms of halide ion transport across the skin including facilitated transport in viable skin by ionic channels and ion transporters. Skin absorption of ionic species is much more complex than just passive diffusion as described for various hydrophobic molecules because of the complex skin structure organized in layers. Outermost poorly hydrated *stratum corneum* with high content of lipids could indeed act as a simple passive barrier. On the other hand, viable epidermis is characterised by higher water content (up to 70 %) and living cells producing variety of proteins [5,6]. Similar water content is found in dermis that includes collagen and elastin in the intercellular matrix. Therefore, various types of skin-ion interactions are possible.

Anions interacting with biological systems have been classified in Hofmeister series (F^- , Cl^- , Br^- , NO_3^- , I^- , SCN^- , ClO_4^-) based on their interactions with macromolecules in aqueous solutions [7]. Ions binding to organic materials is related to their “salting in” or “salting out” properties [8]. The Hofmeister series classifies ions that are strongly hydrated in aqueous solution as “kosmotropic” or “water-structure makers”. The solubility of neutral organic molecules is reduced in the presence of kosmotropes (salting-out effect). On the other hand, “chaotropic” ions are poorly hydrated in aqueous solution (“water-structure breakers”). They interact strongly with non-polar substances, which results in their increased solubility (salting-in effect) [7,9–12]. The properties described by Hofmeister series determine various physicochemical phenomena occurring in biological systems. For instance, binding of anions to model phospholipid bilayers was found to increase following the Hofmeister series of anions and binding of chaotropic ions was reported to affect the lipid packing within bilayers [13,14]. Similar effects can be expected within *stratum corneum* as it has been disclosed by Paweloszek *et al.* [15]. Moreover, a significant contribution of facilitated transport of tested ions in viable skin was observed [4]. Beyond these observations, the involved physicochemical phenomena still remain poorly understood issues. It is worth noticing that such different phenomena as anion adsorption/absorption to hydrophobic *stratum corneum* and facilitated anion transport by proteins depend on the nature of anions in the same order: The Hofmeister series.

The present work addresses mixed solutions of anions where non-ideal mixing (interactions with ions of different chemical nature) influence skin absorption. Some clues regarding salts effects have been given in studies of skin absorption of organic molecules from ionic solutions. Thus, Michael-Baruch *et al.* [16] reported that skin permeation of neostigmine was enhanced in a concentration-dependent manner in the presence of LiCl and NaCl. Soon after, Ko *et al.* [17] studied transdermal penetration of salicylic acid from mixed water-ethanol medium at pH 2 containing sodium salts of inorganic anions classified in lyotropic Hofmeister. They reported increased skin permeation in the presence of NaBr, NaI and NaSCN while other salts had no or a reverse effect. As a whole, the Hofmeister series was not followed; the authors ascribed the effects to interactions in solution [16].

Absorption of halide ions from concentrated aqueous solutions of single or mixed sodium halides (NaF, NaBr, NaI) into freshly excised skin was investigated. It has been done on excised porcine skin while keeping its metabolism in a suitable survival medium [18]. Young swine skin is the most accurate

animal model for human skin in terms of permeation properties [19]. It is recommended for *in vitro* experiments in diffusion Franz cells. Chloride was excluded from the study because its high endogenous concentration in skin [4,20] causes analysis issues. Indeed, chloride is the most abundant anion in the body, especially in extracellular medium [21]. Cross-effects of halides on one another's on skin absorption were investigated in binary and ternary mixtures. Experimental results were exploited in a {3,2} simplex-lattice design model allowing quantitative statistical analysis.

2. Materials and Methods

2.1. Chemicals

NaF, NaBr and NaI were purchased from Fisher Scientific (Illkirch, France). Aqueous solutions of salts were prepared in ultrapure water (resistivity > 18 MΩ·cm at 25 °C) by weighing, providing concentrations expressed as molality (mol·kg⁻¹).

2.2. Water activity and density measurements

Freezing point depression was measured with a Gonotec Osmomat 3000 freezing point osmometer in order to assess water activity a_w . The osmometer was calibrated with pure water. Measurements were performed in triplicate with 50 µL of ionic solutions. The reading expressed in Osmol·kg⁻¹ units was converted into water activity as

$$\text{Osmolality} = -\frac{\ln(a_w)}{M_{\text{mol},w}} \quad \text{Eq. 1}$$

where $M_{\text{mol},w}$ is the molar mass of water (0.018 kg·mol⁻¹).

Densities of ionic solutions needed for the conversion of molality (m , mol·kg⁻¹) into concentration (C , mol·L⁻¹) were measured in triplicate at 32 °C with a 50 cm³ pycnometer calibrated with pure water at 32 °C (0.995 kg·dm⁻³).

Experimental values of densities and osmolalities are given in [Table S1](#) of [Supporting Information](#).

2.3. Skin samples preparation

Full-thickness porcine flank skin explants were obtained from five young female pigs (30 ± 1 kg), sacrificed at the École de Chirurgie, University Claude Bernard Lyon 1 (Lyon, France). Freshly excised tissue samples were immediately (< 1 h) used to evaluate skin absorption of anions. The bristles were cut with an electrical clipper, and the explants were rinsed in water. The subcutaneous adipose tissue was carefully removed using a scalpel. Skin samples prepared to the final thickness of 1.35 ± 0.02 mm (Micrometer Mitutoyo) and cut into round sections of 3 cm². Skin integrity was assessed by measuring the Trans Epidermal Water Loss (TEWL) (Tewameter TM210, Monaderm, Monaco). Samples presenting TEWL values larger than 15 g·h⁻¹·m⁻² after 1 min measurements were discarded. The average TEWL values of skin samples used for experiments was 9.85 ± 0.50 g·h⁻¹·m⁻².

2.4. Permeation and ion distribution in skin layers after 24 h exposure

Skin absorption was carried out for 24 h in static Franz diffusion cells. Skin explants were mounted into two-chamber glass cells [22] (exposure area = 2.54 cm²) with *stratum corneum* facing up. Acceptor compartment was filled with 10 mL of salt-free survival medium ensuring skin viability over the duration of the experiment. The isotonic survival medium called betaine-glucose (BG) [18] contained glucose 1 g·L⁻¹, betaine 2.99 g·L⁻¹, and halide-free phosphate buffer at pH 7.4 (NaHCO₃ 0.35 g·L⁻¹, Na₂HPO₄ 0.048 g·L⁻¹, KH₂PO₄ 0.060 g·L⁻¹). Mounted Franz cells were placed in a water bath at 37 °C

under magnetic stirring, providing a temperature of 32 °C at the skin surface due to heat loss. After 30 min of stabilization, the acceptor fluid was renewed and 1 mL of aqueous solution of sodium halides was placed on the skin surface. Aqueous solutions of NaF, NaBr and NaI alone, in binary and ternary mixtures at the total concentration of 500 mmol·kg⁻¹ were studied.

At the end of the experiment, Franz cells were dismantled and skin layers were separated. *Stratum corneum* (SC) was removed using cyanoacrylate glue (Loctite SuperGlue-3, Henkel) spread on a glass plate, according to the method for cyanoacrylate surface biopsies [23]. Then, the viable epidermis (VE) was separated from the dermis (D) by immersing the sample in water at 60 °C for 45 s. The hot water used for this separation was pooled with the extraction medium of the dermis. The underlying assumption is that extracted ions by hot water mainly came from the dermis, a skin layer of high water content where ions mobility is high. The extraction of ions from each skin layer was performed by sonication for 30 min at 60 Hz followed by extraction with a water/dichloromethane (1:1 v/v) mixture for 17 h. Recovered quantities of ions were measured in the donor medium (DM), the acceptor medium (AM) and the different skin layers. The background amounts coming from endogenous ions was measured in a control experiment without donor fluid under the same conditions as the samples. They were subtracted from the absorbed amounts of the samples. Each measurement was performed on 9 replicates with skin pieces excised from 5 different pigs according to the OECD guidelines [24].

2.5. Analysis of ions concentrations

Collected samples were analysed using ion chromatography (930 Compact IC Flex, Metrohm, Switzerland) equipped with a chemical suppressor and conductivity detection. Elution solvent was 8 mmol·L⁻¹ sodium carbonate (Fisher Scientific, Illkirch, France) in ultrapure water. Metrosep A Supp 5 250/4.0 column with an adequate pre-column at a temperature of 35 °C was used for all analyses. Calibration curve was linear from 0.06 to 2000 µmol·kg⁻¹ ($R^2 = 0.999$).

2.6. Data reduction

Results were expressed as recovered quantities in µmol·cm⁻². The mean value and standard error of the mean (*sem*) were calculated from the $n = 9$ repetitions of each sample. Q_{abs} corresponds to the cumulated absorbed amount of ions recovered in SC + VE + D + AM. Comparison of formulations for their significant differences was performed with a Student *t*-test ($p < 0.05$).

3. Statistical model for ion mixtures

A mixture design approach was used to investigate the influence of the ionic composition on the skin permeation of each ion. Such approach allows regular screening of the entire experimental domain while limiting the number of experiments.

The experimental domain corresponds to the full ternary diagram drawn in mole fractions of the three ions ranging from zero to unity. This simplex experimental domain allows accommodating a polynomial equation that describes the studied phenomenon over all compositions of mixed ions [25,26]. The used {3,2} simplex-lattice consisted of the three vertices and the three binary blends located at the midpoints of the edges. According to the Scheffé's design, six aqueous solutions were tested: three single-anion solutions NaF, NaBr and NaI at the concentration of 500 mmol·kg⁻¹ each, $(x_F, x_{Br}, x_I) = (1,0,0)$, $(0,1,0)$ and $(0,0,1)$, and three binary mixtures made of equal molar amounts of both ions, $(x_F, x_{Br}, x_I) = (1/2, 1/2, 0)$, $(1/2, 0, 1/2)$ and $(0, 1/2, 1/2)$.

The {3,2} simplex-lattice allows estimating the six coefficients of a {3,2} polynomial equation; i.e. a second-degree model for a three-component system is written as follows:

$$\hat{y} = b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 \quad \text{Eq. 2}$$

where \hat{y} is the predicted response for each observed anion, x_i are the mole fractions of components i in the mixture ($x_F + x_{Br} + x_I = 1$) and the parameters $b_i x_i$ and $b_{ij} x_i x_j$ are respectively the linear and nonlinear mixing terms. In a first analysis, responses \hat{y} are measured absorbed amounts per unit skin area (Q_{abs} for F^- , Br^- and I^-). Thereafter, a second analysis was performed where the ratio of Q_{abs} to ion activity was introduced as a new response.

An additional experiment corresponding to the centroid of the simplex (ternary mixture of NaF, NaBr and NaI at $167 \text{ mmol} \cdot \text{kg}^{-1}$ each, $(x_F, x_{Br}, x_I) = (1/3, 1/3, 1/3)$), has been added as a check point to test the adequacy of the model.

Multiple linear regression calculations, analysis of variance (ANOVA) and statistical analyses were performed with Umetrics MODDE 12.0 software (Umetrics, Umeå, Sweden).

4. Results and discussion

4.1. Experimental results

Skin penetration of ions has long been believed limited and most of available research on the subject have considered cations far more often than anions. Previous study by Paweloszek *et al.* [15] initiated research on cutaneous absorption of halide anions by investigating skin absorption of NaF, NaCl, NaBr and NaI from aqueous solutions of equal mass concentrations. In the present study, molal concentrations were set equal because they are more closely related to thermodynamic parameters such as activity and chemical potential. Chloride was excluded from experiments for the sake of feasibility and reproducibility. Indeed, chloride anions are abundant endogenous ions in the skin tissue; this causes analysis problems for distinguishing endo- and exogenous ions [4,20].

4.2. Effect of ionic concentration

The effect of ionic concentration was firstly addressed. Indeed, a higher concentration in the donor side creates a larger concentration gradient through the skin towards the acceptor medium. The amount of ions penetrating skin was very low compared to the amount in the donor solution, so that ion absorption did not significantly deplete the donor fluid; experiments were performed under “infinite dose” conditions. The concentration in the acceptor medium also increased very little compared to that of the donor. As a consequence, the concentration gradient driving passive diffusion was kept constant over the whole experiment duration. Changing the concentration in the donor fluid changes the concentration gradient to the same extent, so that the flux of passive diffusion might be proportional to the concentration in the donor fluid. The sample concentration scale was molality (number of moles of solute per unit mass of solvent, $\text{mol} \cdot \text{kg}^{-1}$) throughout the whole paper. As shown in Fig. 1, the total absorbed amount of iodide ions penetrating skin actually did not vary linearly with respect to the concentration. It curved downwards at the highest concentrations. Since the ionic concentrations were fairly high, the activity should be better considered than the concentration. Following Stokes and Robinson [27], the activity coefficient of anions, γ , includes an electrostatic contribution given by the Debye–Hückel relationship and a contribution coming from ion hydration expressed with the help of the water activity, a_w , in compliance with Gibbs–Duhem. For a 1:1 electrolyte at 32°C and concentrations expressed on the molality scale, it reads [28]

$$\ln(\gamma) = -\frac{z^2 e^3 N_A v^{1/2}}{4\pi (\epsilon_0 \epsilon kT)^{3/2}} \frac{\sqrt{I}}{1+b\sqrt{I}} - \frac{n}{2} \ln(a_w) - \ln(1 - M_{\text{mol},w} (n-2) m) \quad \text{Eq. 3}$$

where symbols have their usual meaning. $I = \frac{1000}{2} \sum_{ions} z_i^2 C_i$ is the ionic strength; b is a constant taken as $1.2 \text{ L}^{1/2} \cdot \text{mol}^{-1/2}$ [28]; $M_{\text{mol,w}} = 0.018 \text{ kg} \cdot \text{mol}^{-1}$ is the molar mass of water; and m is the molality of solutions. There is a large variability of experimental values of hydration numbers. The hydration number of sodium halides, n , was taken from mean values compiled by Israelachvili [29]: $n = 7$ for NaF, $n = 6$ for NaBr and $n = 5$ for NaI. Hydration numbers are close to each other because the main contribution comes from the hydration of the sodium cation. The mean value of $n = 6$ was taken for the mixtures of all sodium halides. It is noticed that the Debye–Hückel part of Eq. 3 is expressed as a function of molarity (C_i , $\text{mol} \cdot \text{dm}^{-3}$) whereas the two other terms are functions of the molality (m , $\text{mol} \cdot \text{kg}^{-1}$). Conversion from one into another is made using measured densities.

The calculation of the activity of mixed ions is a difficult task because too many parameters have to be taken into account. A clear review of the topic is given by Blandamer et al. [30]. The case is especially dramatic when mixed ions have different valences. In the present case, only monovalent anions having related properties (halides) are mixed; the cationic counter-ion is sodium in every case. The activity coefficients were calculated considering mixed ions as single species.

The relationship between absorbed amount and ion activity in the donor medium was linear (Fig. 1). Since the activity correction only considers interactions taking place in bulk aqueous solution, any supplementary non-ideal behaviour of ions absorption from mixed ionic solutions should come from specific interactions operating inside skin.

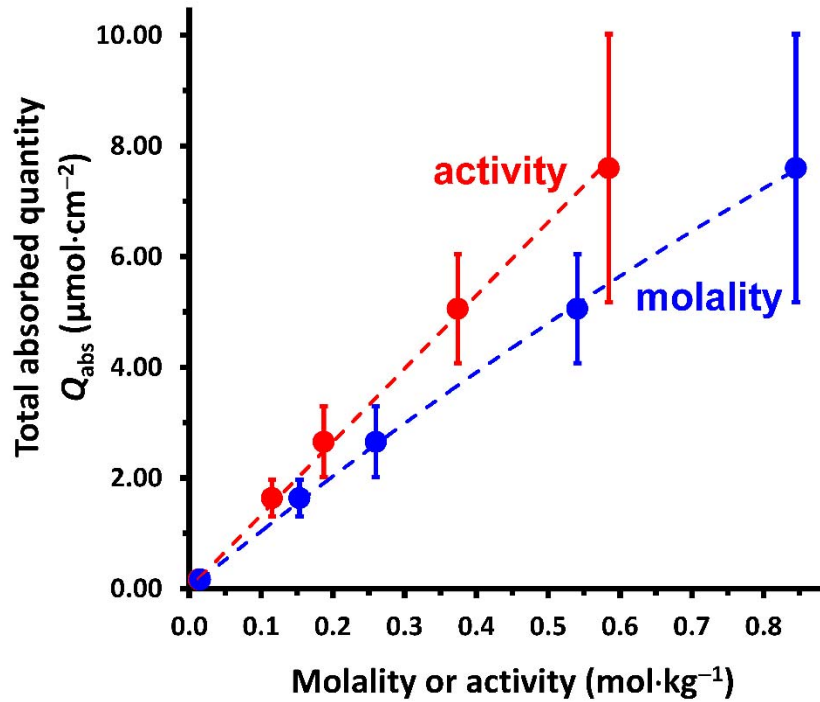


Fig. 1. Total absorbed amount of iodide ions ($\mu\text{mol} \cdot \text{cm}^{-2}$) after 24 h exposure to sodium iodide solutions as a function of iodide concentration (blue) and iodide activity (red).

As a consequence of the linear behaviour with respect to ions activity, it makes sense to consider the ratio of the absorbed amount to ion activity when experiments at different ionic concentrations are compared. Similar ratio has been introduced a long time ago as the permeation coefficient k_p defined as the ratio of the permeation flux in steady state regime to the concentration in the donor medium [31]. We define here the “absorption coefficient”, k_{abs} , as the ratio of the total absorbed amount after 24 h exposure to the activity in the donor solution as:

$$k_{\text{abs}} = \frac{Q_{\text{abs}}}{\gamma m}$$

Eq. 4

4.3. Absorption of single and mixed anions

The total absorbed quantity of halide anions tested alone at 500 mmol·kg⁻¹ did not differ significantly (Fig. 2). The same was observed for concentrations of 250 mmol·kg⁻¹ (data not shown). The present results differ from the ones reported previously because the studied solutions were containing the same mass concentration (ppm) for each anion [15] instead of the present molality concentration scale. Given the large differences in molar masses of F (19 g·mol⁻¹), Br (80 g·mol⁻¹) and I (127 g·mol⁻¹), it is easy to notice that conversion into molar concentrations makes the observed variations weaker. Moreover, the survival medium used as the acceptor medium was also different: ultrapure water supplemented with glucose only created a flux of ions towards the hypotonic acceptor solution [15]. In the present study, the acceptor medium was an isotonic composition ensuring equilibrium of osmotic pressure with skin cells and longer viability of skin samples [18].

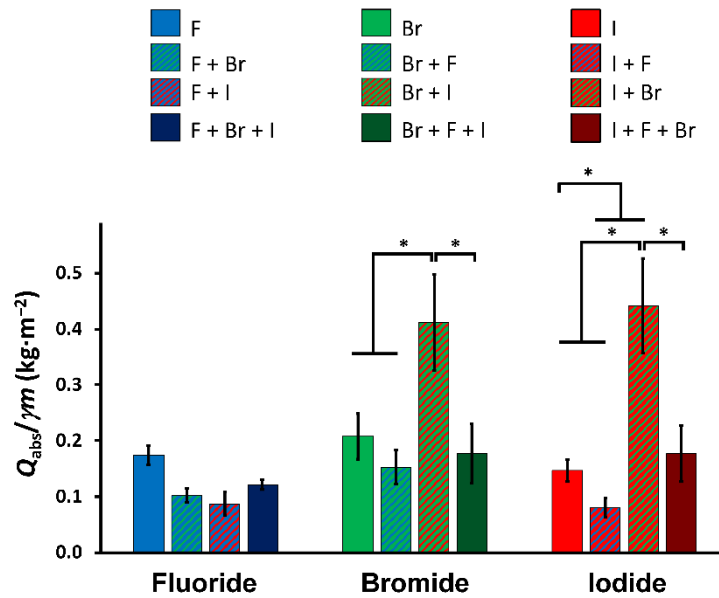


Fig. 2. Absorption coefficient $k_{\text{abs}} = Q_{\text{abs}}/\gamma m$ (kg·m⁻²) after 24 h exposure time for skin exposed to binary and ternary mixtures of halide salts compared to single halide salt as control. Data represent mean \pm sem carried out on $n = 9$ individual skin explants taken from 5 different pigs. Asterisks (*) indicate $p < 0.05$ in t -test analysis.

Investigation of skin permeability for anions in binary mixtures revealed significant differences in their absorption depending on the composition. Synergistic effects were observed for mixtures of NaBr and NaI. The values of k_{abs} of both anions were significantly higher in the case of paired salts than alone. These observations agree with Ko *et al.* [17] who studied the effects of addition of NaF, NaCl, NaBr, NaI on skin penetration of salicylic acid. They found that addition of NaBr or NaI to the vaseline-based formulation significantly increased absorption of an organic compound through murine skin *in vitro* and rabbit skin *in vivo*. On the contrary, addition of NaF and NaCl decreased it. Similarly, the present k_{abs} values for NaI were significantly reduced when mixed with NaF. Skin penetration of fluoride was also impaired in the presence of NaBr and NaI.

Ko *et al.* [17] also observed that salts increased leaching of sterol off excised skin, thereby impairing the initial hydrophobic barrier. This led to infer that enhanced penetration of salicylic acid in presence of NaBr or NaI might be due to the reduction of the barrier function of *stratum corneum* by

disorganization of the crystalline structure of its lipids, thereby allowing better swelling. According to Elden [32], lyotropic swelling of collagen also occurs in the order: $F^- < Cl^- < Br^- < SCN^-$. The main presumption from these studies is that penetrating chaotropic ions disorganize the skin barrier and make it more permeable to penetrating ionic or nonionic molecules. There is no experiential evidence of such phenomenon, however. The present study goes deeper into the mechanisms by considering the distribution of anions in skin layers: *stratum corneum*, viable epidermis, dermis, and acceptor medium (SC, VE, D and AM). Indeed, anions distribution in skin layers after 24 h exposure (Fig. 3) was also altered depending on the composition of tested solution. Simultaneous exposure to Br^- and I^- led to increased recovery in AM as compared to Br^- and I^- alone. This was associated with reduced values in D, VE and especially SC, indicating lower tendency for accumulation within the tissues. These observations contradict the postulated idea of a disorganization of skin structure by the presence of absorbed anions. Indeed, structural modification of skin layers would require accumulation of ions inside them. On the other hand, the trends are reversed for iodide distribution in the presence of NaF: recovery in AM slightly decreased for mixed ions and absorbed amount in SC increased. This effect was less pronounced in the case of NaF + NaBr.

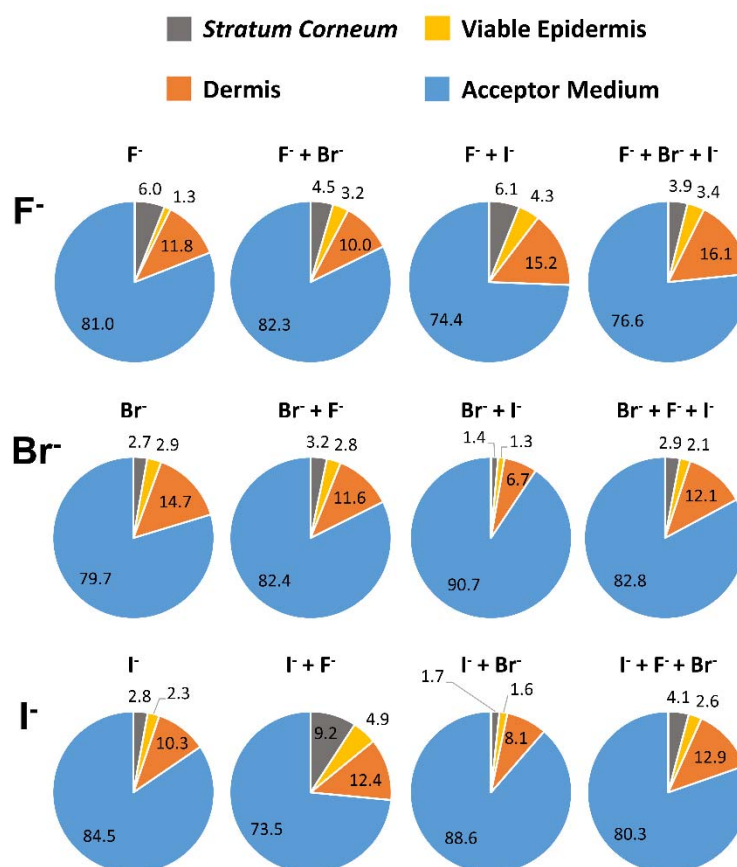


Fig. 3. Distribution of halide anions in skin layers after 24 h exposure. Data represent mean value of absorbed amount expressed as a percentage of the total absorbed amount Q_{abs} . Results are means of $n = 9$ independent measurements.

The observed effects are quite complex because they result from several contributions: interactions between ions in the bulk donor solution, adsorption of ions to the skin surface, transfer into *stratum corneum* and passive diffusion inside it, facilitated transport in the viable epidermis with the help of membrane proteins in keratinocytes (ion channels, ion transporters).

Interactions in bulk solution are considered non-specific and are accounted for by the activity coefficient involving electrostatic and ion hydration contributions. Facilitated transport in viable skin is predominant; indeed, Paweloszek *et al.* [4] disclosed a large difference between viable and non-viable skin. Passive diffusion through *stratum corneum* is necessary for ions to reach viable epidermis where facilitated transport operates. Most elementary steps for ion absorption are interdependent. Transport phenomena depend on ion type in the same way according to Hofmeister series. The best strategy for addressing such a complexity is a global approach undertaken here using an experimental design.

4.4. Statistical Modelling

An alternative way to exploit experimental results consists in fitting them to statistical models. The beneficial input of such data analysis is a rigorous statistical framework. The outcome is a quantitative parametric assessment of non-ideal behaviour through the coefficients of the Scheffé {3,2} polynomial equation (Eq. 2). The \hat{y} -responses are either absorbed amounts Q_{abs} or absorption coefficients $k_{\text{abs}} = Q_{\text{abs}}/\gamma m$. Experimental values of Q_{abs} and k_{abs} are given in Table S2 of Supporting Information. They must fully fill a matrix containing absorption data of ions though some of them are not present in the solution of mixed ions. As example, a binary mixture of fluoride and bromide does not contain iodide, but the matrix for iodide absorption data does contain a figure pertaining to iodide absorption from a mixed solution of fluoride and bromide. This is mandatory to fully fill the \hat{y} -response matrix in the mathematical framework of Scheffé simplex-lattice design. This is easy to manage when Q_{abs} is considered as a response: Q_{abs} is zero when the anion is not present. When responses are absorption coefficients, the correct values are $\lim_{m \rightarrow 0} \left(\frac{Q_{\text{abs}}}{\gamma m} \right)$. They were obtained by extrapolation from experiments at several concentrations. Although those concentrations were fairly high (> 100 mM) for ensuring enough experimental accuracy, extrapolation to infinite dilution was quite robust, owing to the almost linear dependence of Q_{abs} with respect to γm in the low concentration range. A first approach was to determine the coefficients of the polynomial using the six experiments of the {3,2} simplex-lattice design for the skin absorption of each anion. Since there are mixtures where some ions are absent, their coefficient in the polynomial is undetermined, and it has been removed from the polynomial model. Keeping these coefficients led to statistically non-significant values ($p > 0.75$). As example, because there is no I^- in a binary mixture of F^- and Br^- , the term containing the coefficient b_{FBr} was removed from the polynomial pertaining to Q_{abs} or k_{abs} of iodide. The modified Eq. 2 became the set of Eqs 5 to 7 for Q_{abs} and Eqs 8 to 10 for k_{abs} :

$$Q_{\text{abs}}(\text{F}^-) = a_{\text{F}}x_{\text{F}} + a_{\text{Br}}x_{\text{Br}} + a_{\text{I}}x_{\text{I}} + a_{\text{FBr}}x_{\text{F}}x_{\text{Br}} + a_{\text{FI}}x_{\text{F}}x_{\text{I}} \quad \text{Eq. 5}$$

$$Q_{\text{abs}}(\text{Br}^-) = a_{\text{F}}x_{\text{F}} + a_{\text{Br}}x_{\text{Br}} + a_{\text{I}}x_{\text{I}} + a_{\text{FBr}}x_{\text{F}}x_{\text{Br}} + a_{\text{BrI}}x_{\text{Br}}x_{\text{I}} \quad \text{Eq. 6}$$

$$Q_{\text{abs}}(\text{I}^-) = a_{\text{F}}x_{\text{F}} + a_{\text{Br}}x_{\text{Br}} + a_{\text{I}}x_{\text{I}} + a_{\text{FI}}x_{\text{F}}x_{\text{I}} + a_{\text{BrI}}x_{\text{Br}}x_{\text{I}} \quad \text{Eq. 7}$$

$$k_{\text{abs}}(\text{F}^-) = b_{\text{F}}x_{\text{F}} + b_{\text{Br}}x_{\text{Br}} + b_{\text{I}}x_{\text{I}} + b_{\text{FBr}}x_{\text{F}}x_{\text{Br}} + b_{\text{FI}}x_{\text{F}}x_{\text{I}} \quad \text{Eq. 8}$$

$$k_{\text{abs}}(\text{Br}^-) = b_{\text{F}}x_{\text{F}} + b_{\text{Br}}x_{\text{Br}} + b_{\text{I}}x_{\text{I}} + b_{\text{FBr}}x_{\text{F}}x_{\text{Br}} + b_{\text{BrI}}x_{\text{Br}}x_{\text{I}} \quad \text{Eq. 9}$$

$$k_{\text{abs}}(\text{I}^-) = b_{\text{F}}x_{\text{F}} + b_{\text{Br}}x_{\text{Br}} + b_{\text{I}}x_{\text{I}} + b_{\text{FI}}x_{\text{F}}x_{\text{I}} + b_{\text{BrI}}x_{\text{Br}}x_{\text{I}} \quad \text{Eq. 10}$$

Q_{abs} was first analysed because this was the actually measured parameter. The models for Q_{abs} (Eqs 5, 6 and 7) were first used to predict the amounts of absorbed ions for the centroid experiment (ternary mixture) as a validation tool of the model: residuals of 0.5, -1.6 and -1.3 $\mu\text{mol} \cdot \text{cm}^{-2}$ were respectively obtained for $Q_{\text{abs}}(\text{F}^-)$, $Q_{\text{abs}}(\text{Br}^-)$ and $Q_{\text{abs}}(\text{I}^-)$. They were considered satisfactory compared to the experimental standard deviation. The centroid experiment was then used in combination with the 6 experiments of the simplex-lattice design to fit once again the models for Q_{abs} and k_{abs} to the full experimental data. The best statistical models (characterized by highest values of R^2) are presented in

Table 1. For each model, ANOVA analysis indicated the high significance of the fits ($p < 0.05$); and the corresponding determination coefficient R^2 greater than 0.97 (**Table 1**) also showed their satisfactory adequacy.

Once validated, the models were used to plot the estimated surface contours of the studied responses in the investigated experimental domain, i.e. in a classical ternary diagram in mole fractions of ions (**Fig. 4**) where the colour scale stands for Q_{abs} .

The variation of Q_{abs} of F^- in **Fig. 4** shows a continuous increase of Q_{abs} as the mole fraction of fluoride increases, which is the obvious consequence of the increase of fluoride concentration in the donor solution. The iso- Q_{abs} lines are almost horizontal in the triangular presentation where pure fluoride is at the apex, showing that the effects of bromide and iodide ions are identical and negligible. As a whole, there is no specific effect of mixed anions. Conversely, absorptions of bromide and iodide anions show a maximum in the presence of the other. There is no apparent effect of fluoride. The statistical analysis disclosed a specific interaction between bromide and iodide that caused enhanced skin absorption of these anions. The maxima of absorption occur when the solution is rich in the observed anion because of the trivial effect of the higher concentration of the observed anion as the corner of pure anion is approached. This analysis considering Q_{abs} includes contributions of the concentration of observed anions and specific interactions with their partners. A quantitative approach is to consider the a_{ij} coefficients (**Table 1**) that have statistical significance. The a_{F} , a_{Br} and a_{I} coefficients of the same anions (e.g. a_{F} for Q_{abs} of F^-) are highly significant ($p \leq 0.01$) and positive. They reflect the trivial effects of increased absorption as a function of concentration. The a_{F} , a_{Br} and a_{I} coefficients of different anions (e.g. a_{F} for Q_{abs} of Br^-) are statistically not significant (SD is larger than the coefficient value and $p = 0.93$). a_{FBr} , and a_{FI} negative coefficients are also not significant owing the large SD and p -value. The striking feature is the large and significant positive values of a_{BrI} , showing a specific interaction between bromide and iodide anions.

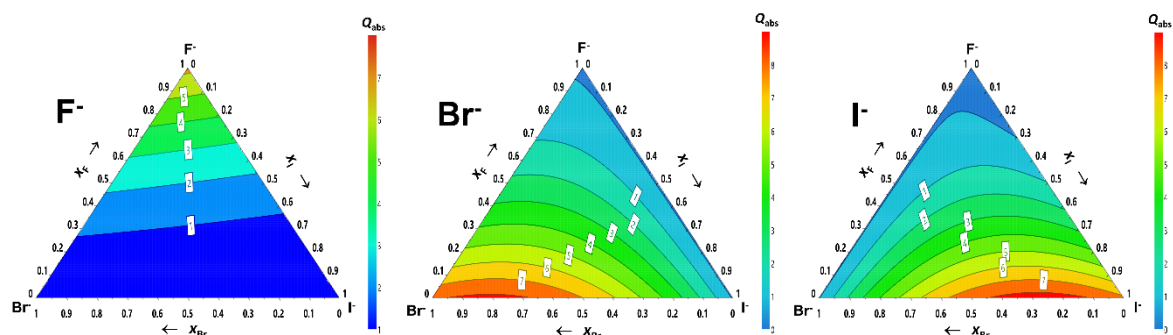


Fig. 4. Contour plots of the predicted absorbed amounts (Q_{abs} , $\mu\text{mol}\cdot\text{cm}^{-2}$) of F^- , Br^- and I^- ions after 24 h exposure in the composition space of ionic mixtures expressed in mole fractions.

Table 1

Coefficients a_{ij} ($\mu\text{mol}\cdot\text{cm}^{-2}$) of the {3,2} polynomial models of absorbed amounts Q_{abs} (Eqs 5, 6 and 7) together with the corresponding determination coefficient R^2 , standard deviation (SD) and p -value of the ANOVA test between brackets. All values are given with two digits with no consideration to their statistical significance.

Ions (i,j)	F^-	Br^-	I^-
	$a_{ij} (SD, p)$	$a_{ij} (SD, p)$	$a_{ij} (SD, p)$
F^-	6.26 (0.3, 0.001)	−0.08 (0.9, 0.93)	−0.07 (0.7, 0.93)
Br^-	0.03 (0.3, 0.93)	7.80 (0.9, 0.01)	−0.07 (0.7, 0.93)
I^-	0.03 (0.3, 0.93)	−0.08 (0.9, 0.93)	7.20 (0.78, 0.01)
F/Br^-	−3.53 (1.4, 0.13)	−6.50 (4.3, 0.27)	—
F/I^-	−5.65 (1.4, 0.06)	—	−10.1 (3.6, 0.11)
Br/I^-	—	12.7 (4.3, 0.10)	−10.1 (3.6, 0.11)

R^2	0.994	0.975	0.983
-------	-------	-------	-------

The global statistical analysis of skin absorption of mixed anions disclosed a specific interaction between them, but neither had it allowed inferring the nature of the interactions, nor the location of them in the complex layered structure of skin (donor solution, *stratum corneum*, viable epidermis and dermis). A refined analysis was performed considering the absorption coefficient $k_{\text{abs}} = Q_{\text{abs}}/\gamma C$ in place of Q_{abs} . This suppresses the effects of the concentration of observed anion and interactions taking place in the bulk donor solution through the activity coefficient. The same statistical analysis as for Q_{abs} was made. Results are given in Table 2 and Fig. 5.

Substitution of k_{abs} for Q_{abs} eliminates the effects of ion concentrations in a similar way as applying a background flattening image processing to the triangular composition maps of Fig. 4. The sole remaining effects are specific cross-interactions. The contour plot for fluoride is almost flat with a constant value of $k_{\text{abs}} = 0.1 \text{ kg}\cdot\text{m}^{-2}$ (Fig. 5). This feature shows the overall absence of specific interactions of bromide and iodide with fluoride. Conversely again, the k_{abs} maps of bromide and iodide anions show a maximum at the centre of the bottom side. Substitution of k_{abs} for Q_{abs} shifted the location of the absorption maximum at the bottom side of the triangle from close to the vertex of adsorbed ion to the mid-point of the Br–I axis, $(x_{\text{F}}, x_{\text{Br}}, x_{\text{I}}) = (0, 1/2, 1/2)$. Such symmetrical position suggests a 1:1 stoichiometry of specific interaction between bromide and iodide anions that could not be detected in the raw Q_{abs} map. Many of the b_{ij} coefficients (Table 2) have poor statistical significance. So, the significance of outcoming b_{ij} coefficients is first discussed. The distribution of p -values was definitely bimodal with either very large or moderate values. $p < 0.1$ was taken for claiming significance in the present case instead of the classical criterion $p < 0.05$ for the full population of low/moderate p -values is taken as significant. Under this condition, the b_{F} , b_{Br} and b_{I} coefficients are significant ($p < 0.07$). The b_{BrI} and b_{IBr} are also of medium significance; they assume a high positive value. The negative b_{FBr} and b_{FI} coefficients involving the F^- anion are not significant. They nevertheless give a contribution to other coefficients in the statistical model. Thus, it is clear from the flat F^- map (Fig. 5) that skin absorption of fluoride was not influenced by the presence of other ions, so that the positive values of b_{F} coefficients contradicted the overall features of the F^- map of k_{abs} . It is suspected that the effects of the positive values of b_{F} and the negative values of b_{FBr} and b_{FI} compensated such that the balance is null. Because of the lack of statistical significance of b_{FBr} and b_{FI} coefficients for absorption of all anions, it is considered that the positive values of b_{F} are biases coming from a too large variability of skin absorption measurements. Such high variability is intrinsic of experiments performed on skin and living matter in general.

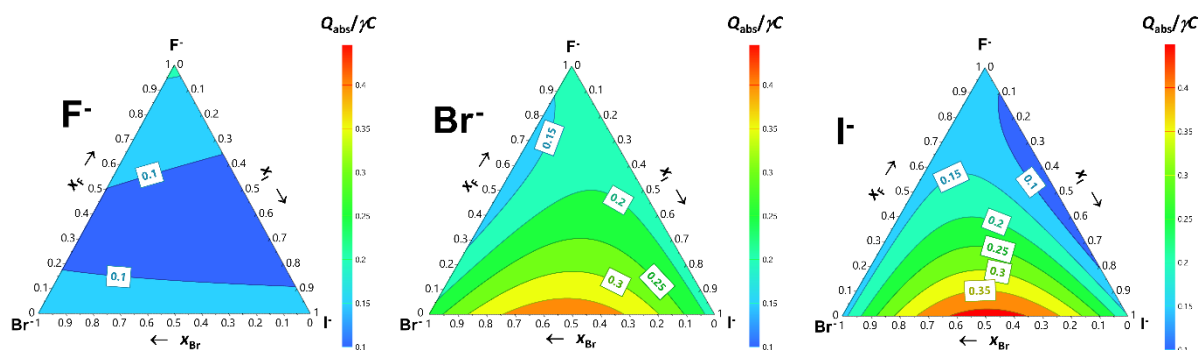


Fig. 5. Contour plots of the predicted absorption coefficients ($k_{\text{abs}} = Q_{\text{abs}}/\gamma C$, $\text{kg}\cdot\text{m}^{-2}$) of F^- , Br^- and I^- ions after 24 h exposure in the composition space of ionic mixtures expressed in mole fractions.

Table 2

Coefficients b_{ij} ($\text{kg}\cdot\text{m}^{-2}$) of the {3,2} polynomial models of absorption coefficients k_{abs} (Eqs 8, 9 and 10) together with the corresponding determination coefficient R^2 , standard deviation (SD) and p -value of the ANOVA test between brackets. All values are given with two digits with no consideration to their statistical significance.

Ions (i,j)	F ⁻ b_{ij} (SD, p)	Br ⁻ b_{ij} (SD, p)	I ⁻ b_{ij} (SD, p)
F ⁻	0.17 (0.017, 0.010)	0.17 (0.048, 0.071)	0.17 (0.041, 0.051)
Br ⁻	0.12 (0.016, 0.016)	0.22 (0.053, 0.056)	0.17 (0.041, 0.051)
I ⁻	0.12 (0.016, 0.016)	0.17 (0.048, 0.071)	0.18 (0.044, 0.055)
F ⁻ /Br ⁻	-0.14 (0.080, 0.21)	-0.28 (0.24, 0.37)	–
F ⁻ /I ⁻	-0.20 (0.080, 0.13)	–	-0.48 (0.20, 0.14)
Br ⁻ /I ⁻	–	0.76 (0.24, 0.089)	0.96 (0.20, 0.042)
R^2	0.86	0.88	0.95

Refinement of the analysis by considering k_{abs} may allow discrimination between the ion effects coming from alteration of the medium (ionic strength, dielectric properties...) through the b_{F} , b_{Br} and b_{I} coefficients, and from specific interactions through the b_{FBr} , b_{FI} and b_{BrI} coefficients. This was successful and led to supplementary conclusions:

- Owing to the linear dependence of Q_{abs} with respect to activity for F⁻, Br⁻ and I⁻ (Fig. 1), the values of b_{F} , b_{Br} and b_{I} should be null. The positive values of b_{F} , b_{Br} and b_{I} ($\approx 0.2 \text{ kg}\cdot\text{m}^{-2}$ for all anions) cannot be considered as a non-specific effect of the presence of other ions inside skin. Their contribution is compensated by negative b_{ij} coefficients that are not significant as it has been shown from the flat k_{abs} map for F⁻ absorption.
- The high positive values of b_{BrI} indicates a strong specific interaction between bromide and iodide. This is the main outcome of the statistical analysis.

A specific interaction operating between bromide and iodide was already inferred from the raw experimental data. The statistical analysis showed that this was the sole significant specific interaction. The absorption coefficient k_{abs} of Br⁻ in the presence of an equal amount of I⁻ was $0.41 \text{ kg}\cdot\text{m}^{-2}$ against $0.21 \text{ kg}\cdot\text{m}^{-2}$ for Br⁻ alone (Table S2 and Fig 2), which was twice an absorption enhancement caused by non-ideal mixing. In the same symmetrical case, k_{abs} of I⁻ increased by a factor of three in the presence of an equal amount of Br⁻ ($k_{\text{abs}} = 0.44 \text{ kg}\cdot\text{m}^{-2}$ against $0.146 \text{ kg}\cdot\text{m}^{-2}$ for I⁻ alone). Such interactions between bromide and iodide caused an absorption enhancement of their partner by a factor of 2–3 with respect to the case of ideal mixing.

4.5. Discussion

The location of disclosed interactions is difficult to discuss in details despite the distribution of ions stored in the different skin layers has been measured (Fig. 3). As main the feature, very few figures show significant differences. The main contributions to differences of total absorbed amount Q_{abs} come from the amounts that reached the acceptor medium. The very small differences of amounts in SC suggest that SC is just a passive diffusion barrier where permeation is only driven by the concentration gradient. There is no accumulation of ions inside SC, showing that there is no specific binding of anions there. There are also small differences of ion amounts in viable epidermis and dermis. It is known that these skin layers are not passive diffusion barriers because they contain ion transporters contributing a lot to the overall absorption [15]. The absorbed amounts per unit skin area can be converted into concentrations in $\text{mmol}\cdot\text{L}^{-1}$ taking the thickness of SC, VE and D as 20 μm , 50 μm and 1.25 mm. The result is a smooth concentration gradient along skin depth in all cases (Fig. 6). One consequence is that concentrations in different layers are interdependent. There is no indication of accumulation coming either from specific interactions with skin materials or any ion-specific barrier in SC or VE.

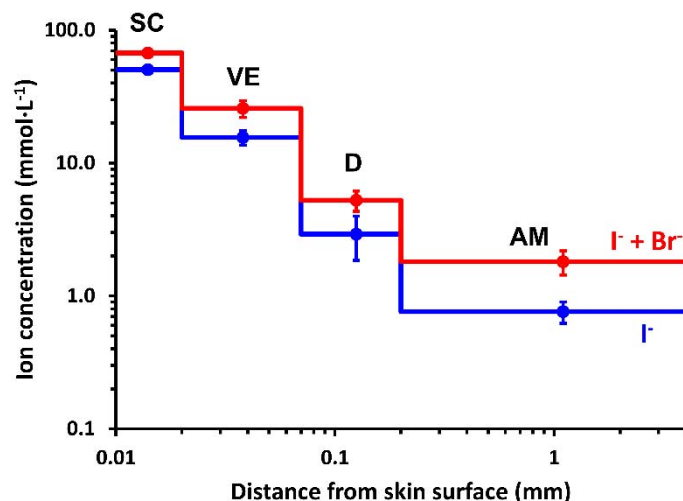


Fig. 6. Concentration profile of iodide ions inside skin layers ($\text{mmol}\cdot\text{L}^{-1}$) as a function of depth showing the concentration gradient from the surface to the acceptor medium. Concentrations are taken homogeneous in each skin layer, *Stratum corneum* (SC), Viable epidermis (VE), Dermis (D) and Acceptor medium (AM).

The nature of interactions operating in skin absorption experiments cannot be inferred from the present data analysis that remains a global approach. Non-specific electrostatic interactions do operate in the concentrated donor solution; they have been accounted for by considering the activity coefficients. Ions absorption cannot contribute to electrostatic effects inside skin because the concentrations of penetrating ions inside skin layers were low with respect to the concentrations of endogenous ions [4]. As example taking the thickness of viable epidermis as 50 μm and that of dermis as 1.3 mm, the mean concentration of endogenous chloride in viable epidermis and dermis are respectively 200 $\text{mmol}\cdot\text{L}^{-1}$ and 15 $\text{mmol}\cdot\text{L}^{-1}$; the concentrations of sodium are three times larger and those of potassium are also larger than those of chloride [4]. All presently measured anions concentrations were less than 50 $\text{mmol}\cdot\text{L}^{-1}$ in viable epidermis and less than 10 $\text{mmol}\cdot\text{L}^{-1}$ in dermis.

A specific interaction between bromide and iodide ions should be considered. These two anions are chaotropic in the Hofmeister series of halides because of their large size and high polarizability allowing strong attractive dispersion interactions and their poor hydration. Hydration of ions [33,34] and dispersion interactions [35] are the two main rationalizing factors for the Hofmeister series. As outlined early by Fajans [36], both phenomena are interrelated as they originate from the size and polarizability of ions [37–39]. Both Hofmeister and anti-Hofmeister behaviours have been reported [40,41]; their concomitance may lead to compensation of effects and even reversal of the global balance. Owing to the poor current knowledge on skin ultrastructure and interactions operating inside skin layers, going farther would require a full account of many interactions before a simplified view retaining only the predominant interactions could be reached. A recent study of water sorption by wood is a successful example of such an approach [42]. Electrostatic repulsions act against association of Br^- and I^- anions, so that a cation is necessarily involved in the association. Possible complex species may result from the association of Br^- , I^- and Na^+ ions as the NaBrI^- monovalent anion. Another option is the association with endogenous magnesium or calcium cations by formation of the neutral MgBrI and CaBrI species. Indeed, magnesium and calcium are abundant endogenous ions inside skin, with the presence of a Ca^{2+} gradient from the *stratum basale* to the *stratum granulosum* where it peaks before declining in *stratum corneum* [43]. On the basis of size and polarizability of ionic partners, Mg^{2+} , Ca^{2+} , Br^- and I^- [37–39], such complex association with magnesium or calcium looks quite a likely mechanism for anions to pass through the *stratum corneum*.

5. Conclusion

Skin absorption of mixed halide ions shows synergistic effects between chaotropic bromide and iodide anions. This has been clearly disclosed by a statistical analysis of absorption experiments on excised pig skin mounted in diffusion Franz cells. Statistical analysis allowed identification of interacting anions pairs from skin absorption experiments that show a large variability. Considering the ratio of the absorbed amounts to the ion activity discards the trivial contributions of ion concentration and non-specific effects of electrostatic interactions in the aqueous solution; such data reduction process highlights the effects of specific interactions.

It has been inferred that polarizable hydrophobic anions associate in the *stratum corneum* as complex species together with cations, in particular with magnesium and calcium, in order to counterbalance their electrostatic repulsions.

The present results together with our previous work [15], definitely show that ionic species do penetrate skin. They are of relevance to practical ionic solutions such as spring waters used in cosmetic products.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

MT acknowledges a PhD grant from the University of Lyon.

Appendix A. Supplementary data

Supplementary material related to this article can be found in the on line version of [Supporting Information](#) file

References

- [1] J. Hadgraft and C. Valenta, *Int. J. Pharm.*, 200 (2000) 243–247.
- [2] T. D. La Count and G. B. Kasting, *J. Pharm. Sci.*, 102 (2013) 2241–2253.
- [3] M.-A. Bolzinger, C. Bolot, G. Galy, A. Chabanel, J. Pelletier and S. Briançon, *Int. J. Pharm.*, 402 (2010) 44–49.
- [4] R. Paweloszek, S. Briançon, Y. Chevalier, N. Gilon-Delepine, J. Pelletier and M.-A. Bolzinger, *Pharm. Res.*, 33 (2016) 1564–1575.
- [5] T. von Zglinicki, M. Lindberg, G. M. Roomans and B. Forslind, *Acta Derm. Venerol.*, 116 (1993) 434–442.
- [6] R. R. Warner, M. C. Myers and D. A. Taylor, *J. Invest. Dermatol.*, 90 (1988) 218–224.
- [7] M. G. Cacace, E. M. Landau and J. J. Ramsden, *Q. Rev. Biophys.*, 3 (1997) 241–277.
- [8] W. Kunz, P. Lo Nostro and B. W. Ninham, *Curr. Opin. Colloid Interface Sci.*, 9 (2004) 1–18.
- [9] K. D. Collins and M. W. Washabaugh, *Q. Rev. Biophys.*, 18 (1985) 323–422.
- [10] M. W. Washabaugh and K. D. Collins, *J. Biol. Chem.*, 261 (1986) 2477–2485.
- [11] K. D. Collins, *Proc. Natl Acad. Sci. USA*, 92 (1995) 5553–5557.
- [12] K. D. Collins, *Methods*, 34 (2004) 300–311.
- [13] R. Bartucci and L. Sportelli, *Biochim. Biophys. Acta*, 1195 (1994) 229–236.
- [14] R. Bartucci, S. Belsito and L. Sportelli, *Chem. Phys. Lipids*, 79 (1996) 171–180.

- [15] R. Paweloszek, S. Briançon, Y. Chevalier, N. Gilon-Delepine, J. Pelletier and M.-A. Bolzinger, *Pharm. Res.*, 33 (2016) 1576–1586.
- [16] E. Michael-Baruch, Y. Shiri and S. Cohen, *J. Pharm. Sci.*, 83 (1994) 1071–1076.
- [17] Y. I. I. Ko, S. S. Kim and S. K. Han, *Arch. Pharm. Res.*, 18 (1995) 231–236.
- [18] M. Tarnowska, S. Briançon, J. Resende de Azevedo, Y. Chevalier, C. Barratier, T. Pourcher and M.-A. Bolzinger, *Int. J. Cosmet. Sci.*, 41 (2019) 617–623.
- [19] M. J. Bartek, J. A. LaBudde and H. I. Maibach, *J. Invest. Dermatol.* 58 (1972) 114–123.
- [20] H. Brown, *J. Biol. Chem.*, LXVIII (1926) 260.
- [21] K. Berend, L. H. van Hulsteijn and R. O. B. Gans, *Eur. J. Int. Med.*, 23 (2012) 203–211.
- [22] T. J. Franz, *J. Invest. Dermatol.*, 64 (1975) 190–195.
- [23] M. Förster, M.-A. Bolzinger, M.-R. Rovère, O. Damour, G. Montagnac and S. Briançon, *Skin Pharmacol. Physiol.*, 24 (2011) 103–112.
- [24] OECD, *Guidance Document for the Conduct of Skin Absorption Studies*, 2004, vol. 2.
- [25] H. Scheffé, *J. R. Stat. Soc. Ser. B*, 25 (1963) 235–251.
- [26] J. Cornell, *Experiments with Mixtures: Designs, Models, and the Analysis of Mixture Data*, New York, 3rd edn, 2002.
- [27] R. H. Stokes and R. A. Robinson, *J. Am. Chem. Soc.*, 70 (1948) 1870–1878.
- [28] K. S. Pitzer, *J. Phys. Chem.* 77, (1973) 268–277.
- [29] J. N. Israelachvili, *Intermolecular & Surface Forces*, Academic Press, London, 2nd edn, 1991, p. 55.
- [30] M. J. Blandamer, J. B. F. N. Engberts, P. T. Gleeson and J. C. R. Reis, *Chem. Soc. Rev.*, 34 (2005) 440–458.
- [31] M. S. Roberts, Y. G. Anissimov and R. A. Gonsalvez, in R. L. Bronaugh and H. I. Maibach (Eds), *Percutaneous Absorption Drugs–Cosmetics–Mechanisms–Methodology*, Marcel Dekker, New York, 3rd edn, 1999, pp. 7–8.
- [32] H. R. Elden, in H. R. Elden (Ed.), *Biophysical Properties of the Skin*, Wiley-Interscience, New York, 1971, pp. 115–123.
- [33] E. Ruckenstein and M. Manciu, *Adv. Colloid Interface Sci.*, 105 (2003) 63–101.
- [34] A. Kalra, N. Tugcu, S. M. Cramer and S. Garde, *J. Phys. Chem. B*, 105 (2001) 6380–6386.
- [35] B. W. Ninham and V. Yaminsky, *Langmuir*, 13 (2002) 2097–2108.
- [36] K. Fajans, *Naturwiss.*, 9 (1921) 729–738.
- [37] D. F. C. Morris, *Struct. Bond.*, 4 (1968) 63–82.
- [38] K. D. Collins, *Biophys. J.*, 72 (1997) 65–76.
- [39] K. D. Collins, G. W. Neilson and J. E. Enderby, *Biophys. Chem.*, 128 (2007) 95–104.
- [40] D. F. Parsons, M. Boström, T. J. Maceina, A. Salis and B. W. Ninham, *Langmuir*, 26 (2010) 3323–3328.
- [41] N. Schwierz, D. Horinek and R. R. Netz, *Langmuir*, 26 (2010) 7370–7379.
- [42] L. Bertineti, P. Fratzl and T. Zemb, *New J. Phys.*, 18 (2016) 083048.
- [43] T. Mauro, G. Bench, E. Sidderas-Haddad, K. Feingold, P. Elias and C. Cullander, *J. Invest. Dermatol.*, 111 (1998) 1198–1201.