


Cite this: *RSC Adv.*, 2020, 10, 41727

The effect of alcohols as vehicles on the percutaneous absorption and skin retention of ibuprofen modified with L-valine alkyl esters†

Paula Ossowicz,^{ID}*^a Joanna Klebko,^{ID}^a Ewa Janus,^{ID}^a Anna Nowak,^{ID}^b Wiktoria Duchnik,^{ID}^b Łukasz Kucharski^{ID}^b and Adam Klimowicz^{ID}^b

The effect of various alcohols as vehicles on skin permeability was compared for unmodified ibuprofen (IBU) and ion pairs of ibuprofen with L-valine alkyl esters [ValOR][IBU], in which the alkyl chain R was changed from C1 to C8. *In vitro* permeation experiments were conducted in a Franz cell with porcine skin. Methanol, ethanol, and isopropanol solutions of 70% (v/v) were chosen as vehicles for penetrants and a buffer solution of pH 5.4 or 7.4 as the acceptor phase. The comparisons of permeation profiles for various [ValOR][IBU] from different alcohols were determined. The cumulative mass, skin accumulation, steady-state flux, diffusion coefficient, and lag time were investigated and compared. It was observed that *i*-propanol was the best enhancer of skin permeation of both unmodified ibuprofen and its salts with L-valine alkyl esters for both acceptor phases. The permeability of the various carriers increases with increasing chain-length of the alcohol. In most cases, significantly higher cumulative mass was found in the acceptor buffer of pH 7.4. The conjugate of ibuprofen with L-valine propyl ester [ValOPr][IBU] permeated the skin to the highest degree in comparison to unmodified ibuprofen. The accumulation of ibuprofen was higher for all salts in relation to the parent acid applied onto the skin. The greatest amounts of ibuprofen were accumulated in the skin when ibuprofen was used as the ionic pair with L-valine butyl ester, [ValOBu][IBU] in the *i*-propanol solution and pH 7.4 buffer as the acceptor phase.

Received 29th July 2020
Accepted 7th November 2020

DOI: 10.1039/d0ra06567f

rsc.li/rsc-advances

Introduction

Transdermal drug delivery stands as a convenient route for the administration of active substances since it allows minimization of the first-pass metabolism, avoiding the gastrointestinal degradation, and providing controlled and prolonged drug release into the systemic circulation.¹ The skin though, in particular, the stratum

corneum (SC) is the major limiting factor for percutaneous absorption of therapeutic agents.² The SC protects against external toxins and water loss but also acts as a barrier for drug penetration into the skin, which is highly dependent on lipophilicity, molecular size, and solubility of the active substance.³

Solvents, such as short-chain alcohols (ethanol, propanol, isopropanol) indicate high enhancing activity but also good

^aWest Pomeranian University of Technology, Szczecin, Faculty of Chemical Technology and Engineering, Department of Chemical Organic Technology and Polymeric Materials, Piastów Ave. 42, 71-065 Szczecin, Poland. E-mail: possowicz@zut.edu.pl

^bPomeranian Medical University in Szczecin, Department of Cosmetic and Pharmaceutical Chemistry, Powstańców Wielkopolskich Ave. 72, 70-111 Szczecin, Poland

† Electronic supplementary information (ESI) available: Amounts of substrates and yields of synthesis of L-valine alkyl ester hydrochlorides (Table S1); amounts of substrates and yields of synthesis of L-valine alkyl esters (Table S2); yields of synthesis of L-valine alkyl esters ibuprofenate (Table S3); statistical differences regarding the cumulative mass of active substance in relation to ibuprofen, taking into account all factors used (a type of alcohol and pH) by the Mann–Whitney test (Table S4); statistical differences between individual alcohols and between acceptor liquids with different pH using the Mann–Whitney test (Table S5); skin permeation expressed as % applied dose of IBU, after 24 h permeation of free acid and its salts with L-valine esters from methanolic, ethanolic and isopropanolic solution into acceptor phase at pH 7.4 and 5.4 (Table S6); skin accumulation expressed as % applied dose of

IBU, after 24 h skin permeation of ibuprofen free acid and its salts with L-valine esters from methanolic, ethanolic and isopropanolic solution into acceptor fluid at pH 7.4 and 5.4 (Table S7); NMR spectra of obtained compounds (Fig. S1, S2, S5, S6, S9, S10, S13, S14, S17, S18, S21, S22, S25, S26, S30, S31, S35 and S36) FT IR spectra of obtained compounds (Fig. S3, S7, S11, S15, S19, S23, S27, S32 and S37), the TG, DTG and c-DTA curves of obtained compounds (Fig. S4, S8, S12, S16, S20, S24, S28, S33 and S38); the DSC curves of L-valine obtained alkyl ester ibuprofenates (Fig. S29, S34 and S39). The cumulative mass of compound in skin after 24 hours of permeation ($n = 3$) – acceptor phase with pH 5.4 (Fig. S40). The cumulative mass of compound in skin after 24 hours of permeation ($n = 3$) – acceptor phase with pH 7.4 (Fig. S41). Hierarchical dendrogram of a mean cumulated mass of IBU (Fig. S42). Box and whisker plot of data from a mean cumulative mass of IBU depending on the type of ibuprofen derivative used (Fig. S43). Box and whisker plot of data from a mean cumulative mass of IBU depending on the type vehicles used (Fig. S44). Box and whisker plot of data from a mean cumulative mass of IBU depending on the pH used (Fig. S45). Diffusing of analyzed compounds through pig skin from alcoholic solutions to acceptor phase with different pH (Fig. S46–S56). See DOI: 10.1039/d0ra06567f



solvating power. Therefore, they are frequently used as enhancers and co-solvents for increasing the solubility of lipophilic compounds in aqueous vehicles, improving drug partitioning into a membrane and its thermodynamic activity.^{4–6} It has been reported that short-chain aliphatic alcohols can relatively enhance skin permeation of active substances when are applied to the drug.^{7,8} Moreover, it was demonstrated that the rate of adsorption of the drug on the skin and permeability coefficients increased with increasing alcohol chain length. This relationship applies to short-chain alcohols from C1 to C4.^{9–11}

Chandra *et al.* examined the influence of alcohols as chemical penetration enhancers on the *in vitro* permeation of ketorolac, the non-steroidal anti-inflammatory drug (NSAID) from hydrogel gel formulation across rat abdominal skin. The hydrogel of a nonionic polymer, methocel K15M (hydroxyl propyl methylcellulose, HPMC) with the addition of various alcohols (ethanol, *n*-propanol, isopropyl alcohol, *n*-butanol, *n*-pentanol, and propylene glycol) was used. The highest permeation coefficient was observed for isopropanol. Moreover, an increase in isopropanol concentration enhanced the permeation of ketorolac.¹²

Wenkers *et al.* investigated the skin penetration of series NSAIDs drugs, such as ibuprofen, ketoprofen, naproxen, diclofenac, *etc.* after application in the light mineral oil as a lipophilic vehicle. They showed that the skin permeability of NSAIDs is a function of hydrophilicity of the drugs, *i.e.*, of their partition coefficients between phosphate buffer saline (pH 7.4) and the lipophilic vehicle, which was $\log P_{\text{PBS/MO}} = -0.07$ for ibuprofen respectively. The experimentally determined skin permeabilities generally increase with increasing hydrophilicity of the NSAIDs. In this case, the viable epidermis instead of the stratum SC became the rate-limiting barrier for the transport of NSAIDs out of a lipophilic vehicle. Authors also revealed that the maximum flux of NSAIDs is primarily dependent on their vehicle solubility.¹³

Ibuprofen (IBU), the commonly used drug from the NSAID group has a high potential for application in transdermal systems. However, it is characterized by low solubility (21 mg dm⁻³ at 25 °C in water) and relative high lipophilicity ($\log P$ is in the range of 2.41–4.00 the value depends on the measurement method) which result in its poor permeation through the skin.^{14–21} Due to the acidic nature ($\text{p}K_{\text{a}} = 4.4$), its solubility is dependent on the pH of the environment – it increases with increasing alkalinity: it can vary from 0.024 mg cm⁻³ (pH = 2.2) to 14.8 mg dm⁻³ (pH = 9.2). This is closely related to the increase in the ionization degree, which in turn determines the ability of ibuprofen to penetrate the skin. It has been shown that the increased solubility of the ionized molecule reflected in the increased permeability at high pH.^{22–24}

The currently available on the market dermal dosage form of IBU is often prepared from the proper combination of water and alcohol.^{24,25} However, the composition of the vehicle has a significant influence on the percutaneous absorption of topical IBU preparations.²⁶ IBU moiety is a carboxylic acid, therefore its partition behavior also depends on $\text{p}K_{\text{a}}$ and ionization state.²³ Watkinson *et al.* investigated the influence of the

increasing amount of ethanol as a cosolvent on the solubility, ionization, and permeability of IBU in the human skin. The greater content of ethanol increased the $\text{p}K_{\text{a}}$ value and the proportion of unionized IBU and diffusion across the skin increased with the content of ethanol in the formulation. The optimal flux was indicated for 50 : 50 and 75 : 25 ethanol–water vehicles.²⁷ Additionally, various modifications of the ibuprofen structure are tested to increase ibuprofen solubility and skin permeability. One of the simplest ways is the formation of ibuprofen inorganic salts and ionic pairs with organic bases. In the literature, inorganic salts, such as sodium,²⁸ potassium, calcium, magnesium, aluminum,²⁹ copper,³⁰ zincum,³¹ and organic – *e.g.* lizynium,³² ranitidinium, diphenhydraminium,³³ benzalkonium, didecyldimethylammonium³⁴ salts. The ibuprofen alkylamine salts were obtained by Sarveiya *et al.* Those salts were characterized by the higher diffusion through the PDMS (polydimethylsiloxane) membrane compared to the sodium salt.³⁵ Wu *et al.* reported increased solubility in water of various aromatic, tetra-alkylammonium, and tetra-alkylphosphonium ibuprofen salts, compared to the parent acid and improved skin permeability compared to the ibuprofen sodium. In addition, they showed that tetrahexylammonium and didecyldimethylammonium salts provided more and faster ibuprofen skin permeation.³⁶ Furukawa *et al.* presented a combination of ibuprofen with proline ethyl ester as the base to form an ionic liquid with increased pig skin permeability.³⁷ Wang *et al.* presented a new combination of ibuprofen with lidocaine and ethanol in the form of liquid co-crystals in a deep eutectic form.³⁸ The authors proved that both lidocaine and ibuprofen are transported through the model membrane at much higher rates than the corresponding commercially available crystalline salts, *i.e.* lidocaine chloride and ibuprofen sodium.

In the recent study, we presented the ibuprofen derivatives made by its pairing with some L-valine alkyl esters counterion, which can improve the transport of ibuprofen through porcine skin from ethanol.¹⁷ The presented compounds combine the activity of ibuprofen and an amino acid, and thanks to the form of an alkyl ester, they provide increased water solubility and skin permeability. L-Valine was selected for the research due to the fact that it belongs to the essential exogenous amino acids and is involved in many processes in the body for example inhibits musclebuilding protein degradation process, triggers gluconeogenesis, intensifies the secretion of anabolic hormones. Furthermore L-valine is necessary for the synthesis of pantothenic acid.^{39,40} Therefore, the proposed compounds, apart from the therapeutic effect specific for ibuprofen, can provide a prophylactic and protective effect appropriate for L-valine.

In this study, we compared the effect of three alcohols – methanol, ethanol, and *i*-propanol, as vehicles, on the skin permeation of ibuprofen paired with L-valine alkyl esters, where alkyl chain was from the methyl (C1) to the octyl (C8). The reason of methanol application in this study was to evaluate the effect of the chain length on the permeability of the compounds obtained. These studies had a scientific, not application, purpose. Obviously, methanol is a toxic compound and will

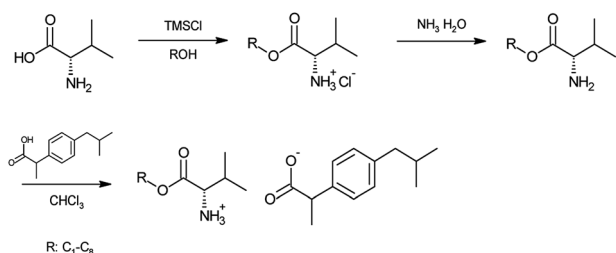


therefore not be used in commercial formulations for skin application. The use of methanol for this type of research is therefore justified, which is confirmed by research conducted by other scientists.⁴¹ Moreover, the pH value of the acceptor phase in permeation *in vitro* tests was set at 5.4 and 7.4 for simulation of the skin surface (stratum corneum) and underlying epidermis and dermis.

Results and discussion

Synthesis, identification, and characterization of the [ValOR][IBU]

Using previously described a three-step method,¹⁷ we synthesized salts of ibuprofen with L-valine alkyl esters (Scheme 1). The alkyl chain in the L-valine ester group was extended to methyl, heptyl, and octyl group (R) in comparison to the prior described C₂–C₆ esters.¹⁷ The compounds were obtained in high yields (92–98%) and were identified by ¹H and ¹³C-NMR, FTIR, and elemental analysis (see ESI†). In the first step, the hydrochlorides of L-valine alkyl esters (ValOR·HCl) were synthesized⁴² by the reaction of the proper alcohol with L-valine and chlorotrimethylsilane (TMSCl) as a chlorinating agent. Then the neutralization of the hydrochloride (ValOR·HCl), using the aqueous ammonia, gave the respective L-valine ester (L-ValOR), which was paired with an equimolar amount of ibuprofen to obtain [ValOR][IBU], without forming any by-products. The high purity of ibuprofen salts was confirmed by the content of individual elements (C, H, N, O) and NMR analysis. Additionally, ¹H NMR, ¹³C-NMR and FTIR spectra confirmed that synthesized compounds were organic salts consisting of L-valine alkyl ester cation and ibuprofenate anion – in the equimolar ratio each ion (see ESI†). The NMR spectra clearly show the ionic structure of the synthesized compounds. Signals for the protons of the protonated amino group (NH₃⁺) of the amino acid ester moiety appeared in CDCl₃ at the chemical shift (δ) in the range of 5.08 ppm for [ValOMe][IBU] to 6.03 ppm for [ValOOct][IBU]. The integration of these signals corresponds to the three protons. Moreover, on the ¹³C-NMR spectra of the obtained compounds, the signal of the carbonyl carbon of ibuprofen is observed at 179 ppm and is shifted about 2 ppm in comparison to the value for this carbon in the parent acid (181.16 ppm).^{43–45} Also, the FTIR analysis demonstrated the presence of strong bands at *ca.* 1600 and 1390 cm^{–1}, assigned to the symmetric and asymmetric stretching vibrations of carboxylate anion accordingly.



Scheme 1 The three-step synthesis of salts of ibuprofen and L-valine alkyl esters.

The difference between the frequency values assigned to $\nu(\text{COO}^-)_{\text{sym}}$ and $\nu(\text{COO}^-)_{\text{as}}$ vibrations is above 200 cm^{–1}.⁴⁶ Moreover, the absence of the specific, for unprotonated NH₂ group, vibrational N–H band at about 3390 and 3320 cm^{–1} in the FTIR spectra confirmed the formation of ionic structure of the ibuprofen derivatives. Also, the sharp absorption band is observed in the range of 1733–1740 cm^{–1}, which is characteristic of the C=O in the carboxylic group.

The obtained ibuprofen salts were white solids with melting points below 100 °C, therefore according to the accepted definition, can be qualified as ionic liquids. The melting point decreased with can be qualified as ionic liquids.

The melting point decreased with the increase in alkyl chain-length in the ester group of the L-valine ester moiety and was the highest for [ValOMe][IBU] (*T*_m = 89.4 °C), while the lowest for [ValOOct][IBU] (*T*_m = 62.0 °C) (Table 1). This dependence was also observed in the previously reported study.¹⁷ The crystallization temperatures of [ValOR][IBU], defined as the lowest point of the dip on DSC curves, were summarized in Table 1. The crystallization temperature depends on the length of the alkyl chain in the valine alkyl ester cation. The same relationship was observed for the melting point. The longer alkyl chain resulted in a lower crystallization temperature. The highest crystallization temperature was registered for [ValOMe][IBU] (*T*_c = 69.98 °C) while the lowest for [ValOOct][IBU] (*T*_c = 34.95 °C). The conjunction of ibuprofen with the *i*-propyl ester of L-valine was characterized by about 15 °C higher crystallization temperature than the conjunction with the *n*-propyl ester (*T*_c = 48.67 °C).

The thermal stability of the obtained ibuprofen derivatives was determined by TG analysis. The thermal degradation was estimated based on the *T*_{onset} value for the mass loss. All the obtained salts of ibuprofen, [ValOR][IBU] showed lower thermal stability compared to the parent acid, for which *T*_{onset} was 189.9 °C (Fig. 1). Moreover, the stability increased with an increase in the length of the alkyl chain in the ester group of the cation. The lowest onset point was demonstrated for ibuprofen salt paired with L-valine methyl ester [ValOMe][IBU] (*T*_{onset} = 82.9 °C), while the highest thermal stability was established for the salt with L-valine octyl ester [ValOct][IBU] (*T*_{onset} = 151.7 °C).

Table 1 The properties of ibuprofen and its L-valine ester derivatives^a

No.	Compound	Colour	<i>T</i> _m /°C	<i>T</i> _c /°C	<i>T</i> _{onset} /°C	[α] _D ^T
1	IBU ^b	White	77.51	—	189.8	—
2	[ValOMe][IBU]	White	89.40	69.98	82.9	+14.933
3	[ValOEt][IBU] ^b	White	77.99	55.20	89.5	+8.867
4	[ValOPr][IBU] ^b	White	79.81	48.67	109.5	+9.760
5	[ValOiPr][IBU] ^b	White	78.01	64.02	90.2	+11.852
6	[ValOBu][IBU] ^b	White	76.80	46.22	119.9	+11.094
7	[ValOAm][IBU] ^b	White	73.81	41.99	129.8	+10.076
8	[ValOHx][IBU] ^b	White	67.35	36.51	128.9	+8.987
9	[ValOHept][IBU]	White	63.30	37.51	135.7	+7.678
10	[ValOOct][IBU]	White	62.00	34.95	151.7	+8.300

^a *T*_c – cold crystallization temperature, *T*_m – melting point, *T*_{onset} – the onset of the thermal degradation. ^b Data for these compounds were earlier reported in ref. 17.



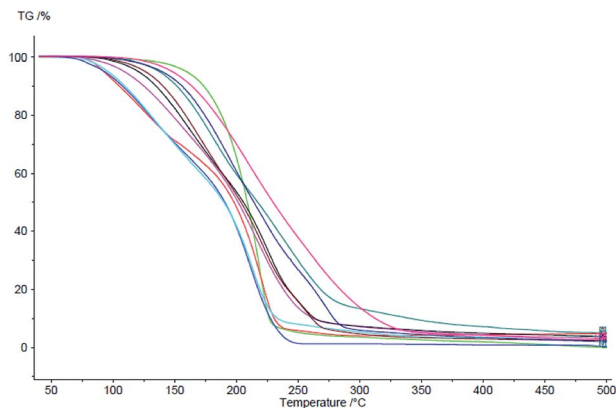


Fig. 1 The TG curves of ibuprofen and its L-valine ester derivatives red [ValOMe][IBU]; dark blue [ValOEt][IBU]; light blue [ValOiPr][IBU]; light purple [ValOPr][IBU]; black [ValOBu][IBU]; brown [ValOAm][IBU]; gray blue [ValOHex][IBU]; navy blue [ValOHept][IBU]; dark purple [ValOOct][IBU] and green, IBU.

All of the synthesized ibuprofen salts [ValOR][IBU] have two chiral centers, one in the amino acid cation and one in the ibuprofenate anion. Because they were synthesized from the racemic ibuprofen and pure L-valine enantiomer they reveal optical activity (Table 1). The value of specific rotation was the highest for [ValOMe][IBU], $[\alpha]_D^{20} = +14.933$, while for the rest of the synthesized salts this value was in the range of +7.678 for [ValOHept][IBU] and +11.852 (molar specific rotation: +43.325) for [ValOiPr][IBU].

The solubility of IBU salts was investigated in the selected polar and nonpolar solvents, following the modified Vogel's method at the temperature of 25 °C.⁴⁷ The obtained results were summarized in Table 2. The solvents were ranked with decreasing value of empirical polarity parameters ($E_T(30)$).⁴⁸ If the amount of substance dissolved in 1 cm³ was lower than 33 mg, the compound was marked as practically insoluble. The compound was described as partly soluble when amount of 33–100 mg dissolved in 1 cm³ and as soluble, when more than 100 mg of the compound was soluble in 1 cm³.

This study extended data from the previous work.¹⁷ There are an increasing ability of [ValOR][IBU] to dissolve in nonpolar *n*-hexane with elongation of the alkyl chain in the L-valine ester

group. The IBU and [ValOMe][IBU] were insoluble in *n*-hexane, while salt with [ValOAm] moiety was soluble partly. The novel salts were soluble in ethanol DMSO, chloroform, ethyl acetate, diethyl ether, and toluene. The obtained compounds were used in the skin permeation experiments as alcoholic solutions in concentration of 0.01 g cm⁻³, which was much lower than their solubility (over 0.100 g per 1 cm³ of 70% alcohol).

The solubility in deionized water and phosphate buffers at 25 °C at pH 5.4 and 7.4 was summarized in Table 3. The obtained results include data obtained in the previous work.¹⁷ The saturated concentration of the ibuprofen and its salts was expressed also as the concentration of the active substance. The presence of the amino acid alkyl moiety alters the solubility of the ibuprofen following the before described dependency. The solubility in water and both buffers decrease among with elongation of the carbon chain in the alkyl group and is the lowest for [ValOOct][IBU]. It results from weaker solvation power and stronger intermolecular interaction between the molecules, which requires more energy input.³ However, the [ValOMe][IBU] was characterized by a lower solubility than [ValOEt][IBU] in deionized water and pH 5.4 buffer but comparable at pH 7.4.

The solubility of an active substance in the buffer of pH 7.4 was higher than in the pH 5.4 buffer. The saturation concentration was in the range between 3.055 g IBU dm⁻³ for [ValOEt][IBU] and 0.259 g IBU dm⁻³ for [ValOOct][IBU]. In the pH 5.4 buffer, the solubility was 3.969 g IBU dm⁻³ for [ValOEt][IBU] and was more than 20-fold lower for salts with [ValOHex][IBU] and [ValOOct][IBU]. The effect of pH on solubility of sodium salt of ibuprofen was presented by Sarveiya *et al.* (2004),³⁵ showing increase solubility with an increase in the pH value.

Skin permeation and accumulation

In our research *in vitro* skin penetration studies were conducted using abdomen porcine skin. The porcine skin is frequently used for preliminary evaluation of percutaneous permeation of topically applied drugs due to its similar properties and similar permeability to human skin.⁴⁹ Porcine skin is considered appropriate as it is very close to human skin from physiological and histological viewpoints.^{50,51} *In vitro* penetration of ibuprofen through porcine skin has previously been evaluated in many studies.^{17,52–55}

Table 2 Solubility in water and organic solvents of ibuprofen and its L-valine derivatives at 25 °C

No.	Compound	Ethanol (51.9)	DMSO (45.1)	Chloroform (39.1)	Ethyl acetate (38.1)	Diethyl ether (34.5)	Toluene (33.9)	<i>n</i> -Hexane (31.0)
1	IBU (rac)	+ ¹⁷	+ ¹⁷	+ ¹⁷	+ ¹⁷	+ ¹⁷	+ ¹⁷	— ¹⁷
2	[ValOMe][IBU]	+	+	+	+	+	+	—
3	[ValOEt][IBU]	+ ¹⁷	+ ¹⁷	+ ¹⁷	+ ¹⁷	+ ¹⁷	+ ¹⁷	— ¹⁷
4	[ValOiPr][IBU]	+ ¹⁷	+ ¹⁷	+ ¹⁷	± ¹⁷	± ¹⁷	+ ¹⁷	— ¹⁷
5	[ValOPr][IBU]	+ ¹⁷	+ ¹⁷	+ ¹⁷	± ¹⁷	± ¹⁷	+ ¹⁷	— ¹⁷
6	[ValOBu][IBU]	+ ¹⁷	+ ¹⁷	+ ¹⁷	± ¹⁷	± ¹⁷	+ ¹⁷	— ¹⁷
7	[ValOAm][IBU]	+ ¹⁷	+ ¹⁷	+ ¹⁷	+ ¹⁷	+ ¹⁷	+ ¹⁷	± ¹⁷
8	[ValOHex][IBU]	+ ¹⁷	+ ¹⁷	+ ¹⁷	+ ¹⁷	+ ¹⁷	+ ¹⁷	+ ¹⁷
9	[ValOHept][IBU]	+	+	+	+	+	+	+
10	[ValOOct][IBU]	+	+	+	+	+	+	+



Table 3 The solubility of ibuprofen and its L-valine derivatives in water and phosphate buffers at 25 °C

Compound	Solubility in phosphate buffer					
	Solubility in water			pH = 5.4		
	g dm ⁻³	g IBU dm ⁻³	g IBU dm ⁻³	g dm ⁻³	g IBU dm ⁻³	g dm ⁻³
IBU (rac)	0.076 ± 0.001 (ref. 17)	0.076 ± 0.001 (ref. 17)	0.082 ± 0.001 (ref. 17)	0.082 ± 0.001 (ref. 17)	0.082 ± 0.001 (ref. 17)	0.432 ± 0.001 (ref. 17)
[ValOMe][IBU]	3.833 ± 0.005	2.343 ± 0.005	3.252 ± 0.007 (ref. 17)	3.929 ± 0.001	2.404 ± 0.001	4.937 ± 0.022
[ValOEt][IBU]	5.542 ± 0.007 (ref. 17)	3.252 ± 0.007 (ref. 17)	3.252 ± 0.007 (ref. 17)	3.969 ± 0.056 (ref. 17)	2.329 ± 0.056 (ref. 17)	5.206 ± 0.044 (ref. 17)
[ValOPr][IBU]	4.221 ± 0.033 (ref. 17)	2.382 ± 0.033 (ref. 17)	3.057 ± 0.045 (ref. 17)	3.057 ± 0.045 (ref. 17)	1.725 ± 0.045 (ref. 17)	3.055 ± 0.044 (ref. 17)
[ValOiPr][IBU]	3.468 ± 0.007 (ref. 17)	1.957 ± 0.007 (ref. 17)	2.371 ± 0.016 (ref. 17)	2.350 ± 0.027 (ref. 17)	1.326 ± 0.027 (ref. 17)	3.029 ± 0.019 (ref. 17)
[ValOBu][IBU]	3.125 ± 0.007 (ref. 17)	1.699 ± 0.007 (ref. 17)	2.371 ± 0.016 (ref. 17)	2.371 ± 0.016 (ref. 17)	1.289 ± 0.016 (ref. 17)	2.821 ± 0.018 (ref. 17)
[ValOAm][IBU]	1.798 ± 0.019 (ref. 17)	0.942 ± 0.019 (ref. 17)	1.380 ± 0.007 (ref. 17)	1.380 ± 0.007 (ref. 17)	0.723 ± 0.007 (ref. 17)	1.679 ± 0.015 (ref. 17)
[ValOHx][IBU]	1.058 ± 0.002 (ref. 17)	0.538 ± 0.002 (ref. 17)	0.759 ± 0.006 (ref. 17)	0.759 ± 0.006 (ref. 17)	0.386 ± 0.006 (ref. 17)	1.012 ± 0.008 (ref. 17)
[ValOHept][IBU]	0.533 ± 0.001	0.261 ± 0.001	0.401 ± 0.004	0.401 ± 0.004	0.192 ± 0.004	0.627 ± 0.001 (ref. 17)
[ValOOct][IBU]	0.300 ± 0.004	0.142 ± 0.004	0.390 ± 0.001	0.390 ± 0.001	0.190 ± 0.001	0.356 ± 0.002
						0.259 ± 0.001

Higher penetration of IBU derivatives through pig skin is confirmed by Furukawa *et al.*, where ibuprofen-ProOEt permeated ten-times higher than for free ibuprofen and variation in skin permeation depends on strong ion-pairing between a cation and an anionic drug.³⁷

In our *in vitro* study, the penetration of new ibuprofen derivatives was compared with the penetration of parent ibuprofen. The donor phase was a 1% (m/v) solution of the tested compound, which was dissolved in an aqueous solution of methanol, ethanol, or isopropanol (concentration of alcohol was 70% v/v). The acceptor phase was a buffer solution of pH 5.4 or pH 7.4.

The penetration of active compounds, which are topically applied, is restricted by stratum corneum. Lipophilic substances in the skin barrier inhibit the penetration of exogenous therapeutic and cosmetic compounds. Lipophilicity is an indicator that determines the penetration of substances through the skin. The increase in the lipophilicity of the compound could make faster penetration and contributes to achieving the desired therapeutic concentration of the drug.^{56,57}

Modifying the lipophilicity of the compound and selecting the appropriate vehicle could affect, to a large extent, on the penetration of active substances into the skin. Methanol, ethanol, and isopropanol are good solvents, wherein ethanol and isopropanol are often used as a solvent of the topical lipophilic drugs^{8,58–60}. In the present study, the effect of the solvent on the penetration of ibuprofen derivatives and unmodified IBU was also estimated. Into the donor chamber was used 70% of methanol, ethanol, and isopropanol. Krishnaian *et al.* demonstrated that this concentration of ethanol in ratio ethanol–water 70 : 30 (v/v) is an optimal vehicle of trans-epidermal application of the drug.⁶¹ Watkinson *et al.* demonstrated that used ethanol–water 75 : 25 (v/v) concentration caused the rate of the ibuprofen penetration was the most effective. The author suggests that the decrease of penetration of the substances from concentrated alcohol is caused by dehydration of the skin.²⁷ The permeation of glibenclamide and glipizide increased with the concentration of ethanol, reaching a maximum at 70% (v/v) and then decreasing with further increase concentration to 80% (v/v).⁶² Ethanol and isopropanol are promoters of transepidermal transport, which has an effect on the effectiveness of penetration of active substances into the skin. They are able to reversibly transform the structure of the lamellar system of the lipid matrix of the epidermis, thanks to which they can facilitate accelerate the diffusion of particles by stratum corneum. In addition, ethanol can disrupt the function of the skin barrier by affecting the cells between the cellular cement. This results in loosening the lipid layer and increasing its fluidity and consequently increases the degree of diffusion of active compounds.^{59,63}

Almost for each compound, there is the same tendency of an increase in the cumulative mass of ibuprofen that permeated through the skin, expressed as $\mu\text{g IBU cm}^{-2}$, with the change of the alcohol as a vehicle, in the sequence from methanol through ethanol to *i*-propanol, for both acceptors phases pH 5.4 and 7.4 (Table 4). It means, that *i*-propanol is the best enhancer of skin permeation of both unmodified ibuprofen and its salts with L-



Table 4 The cumulative mass of ibuprofen and its salts, expressed in $\mu\text{g IBU cm}^{-2}$, after 24 h permeation test^a

Compound	Cumulative mass of IBU, $\mu\text{g IBU cm}^{-2}$					
	pH 7.4			pH 5.4		
	MeOH	EtOH	iPrOH	MeOH	EtOH	iPrOH
IBU	229.27 \pm 5.53	302.84 \pm 3.63 ^b	380.11 \pm 3.44	219.18 \pm 0.64	240.48 \pm 4.85	336.10 \pm 5.58
[ValOMe][IBU]	226.49 \pm 3.82	251.69 \pm 16.29	290.27 \pm 21.81	197.25 \pm 6.75	235.37 \pm 12.57	265.64 \pm 8.35
[ValOEt][IBU]	200.82 \pm 1.62	215.19 \pm 8.14 ^b	281.29 \pm 19.98	203.77 \pm 4.16	190.01 \pm 1.14	216.60 \pm 1.11
[ValOiPr][IBU]	296.71 \pm 6.39**	341.20 \pm 4.87** ^b	410.76 \pm 26.72*	214.86 \pm 1.77	296.71 \pm 5.85**	352.54 \pm 3.97*
[ValOPr][IBU]	373.36 \pm 4.79**	382.35 \pm 1.05** ^b	411.76 \pm 1.78*	342.41 \pm 0.94**	313.16 \pm 3.07**	307.38 \pm 3.08
[ValOBu][IBU]	260.24 \pm 2.18**	289.74 \pm 1.05 ^b	371.75 \pm 14.92	248.78 \pm 3.89**	280.22 \pm 2.95**	285.75 \pm 5.08
[ValOAm][IBU]	245.97 \pm 6.13*	308.96 \pm 3.77** ^b	302.24 \pm 5.17	207.47 \pm 3.08	211.20 \pm 4.05	234.46 \pm 7.83
[ValOHex][IBU]	218.90 \pm 11.08	249.46 \pm 26.06 ^b	318.66 \pm 1.16	205.76 \pm 5.12	216.20 \pm 8.68	245.55 \pm 5.12
[ValOHept][IBU]	155.60 \pm 9.03	209.53 \pm 15.62	197.79 \pm 9.58	143.81 \pm 4.84	174.65 \pm 6.74	181.69 \pm 9.08
[ValOOct][IBU]	150.49 \pm 6.17	162.28 \pm 10.95	154.07 \pm 6.39	149.34 \pm 9.92	152.08 \pm 9.09	148.47 \pm 6.92

^a *Value is higher significantly from control (ibuprofen) ($P < 0.05$), **value is higher significantly from control (ibuprofen) ($P < 0.001$). ^b Data reported in ref. 17.

valine esters. The exceptions are three salts with the longest chain in ester group ([ValOAm][IBU], [ValOHept][IBU] and [ValOOct][IBU]) that permeated the skin in slightly larger amounts from ethanol than an *i*-propanol solution to a buffer of pH 7.4 as the acceptor phase and one salt ([ValOPr][IBU]), that the best penetrated the skin from methanol solution to the buffer at pH 5.4.

Among all the tested salts, the highest IBU cumulative masses, 411.76 $\mu\text{g IBU cm}^{-2}$ and 410.76 $\mu\text{g IBU cm}^{-2}$ were reached for the combination of ibuprofen with *L*-valine propyl ester – [ValOPr][IBU] and *L*-valine *i*-propyl ester – [ValOiPr][IBU] respectively, when the buffer pH 7.4 was acceptor phase and the *i*-propanol solution was the donor phase. For the buffer pH 5.4 as the acceptor phase, the highest IBU cumulative masses were also achieved for the same salts, for [ValOiPr][IBU] from *i*-propanol (352.54 $\mu\text{g IBU cm}^{-2}$) and [ValOPr][IBU] from methanol (342.41 $\mu\text{g IBU cm}^{-2}$). The cumulative masses in these cases were also higher than those obtained for the parent acid used under the respective conditions (Table 2).

Analyzing all used factors as acceptor chamber pH and solvents – the most preferred derivative is for [ValOPr][IBU]. This derivative penetrated the highest degree in comparison with unmodified ibuprofen, as confirmed by the Mann–Whitney test ($p = 0.000$) (see ESI, Table S4†) and by cluster analysis test (see ESI, Fig. S42†), and a box and whisker plot (see ESI, Fig. S43†).

In our study, significant differences in permeation efficiency were found, depending on the vehicle used (see ESI, Table S5†). Considering the average cumulative mass of the compounds, the permeation from the vehicles used was ranked in the following order: isopropanol > ethanol > methanol. All tested compounds best-penetrated from isopropanol with compared methanol, as confirmed by the Mann–Whitney test ($p = 0.000$) (see ESI, Table S5 and Fig. S44†).

The selection of an appropriate buffer to acceptor chamber in *in vitro* studies is very important. It is also very significant to create conditions very similar to those prevailing during *in vivo* studies. In our study analysis of the release of the drug was done

in physiology conditions using the acceptor solution of pH 5.4 and pH 7.4. The first of them is a value similar to pH on the skin surface, the other is a close parameter to conditions in the deeper layers of the skin.⁶⁴ In our research, in most cases, the significantly higher cumulative mass was in acceptor solution of pH 7.4. Considering all test substances, penetration into the acceptor fluid at pH 7.4 was significantly higher, as confirmed by the Mann–Whitney test ($p = 0.001$) (see ESI, Table S5 and Fig. S45†).

Moreover, the greatest amounts of ibuprofen, expressed in $\mu\text{g IBU per g of skin}$, were accumulated in the skin when ibuprofen was used as the ionic pair with *L*-valine butyl ester, [ValOBu][IBU] and in the *i*-propanol solution (Table 5). These values were 844.06 $\mu\text{g IBU g}^{-1}$ and 863.53 $\mu\text{g IBU g}^{-1}$ for respectively pH 5.4 buffer and pH 7.4 as the acceptor phase. The conjunction [ValOBu][IBU] was also the most accumulated in the skin from methanol and ethanol solution used as a vehicle in the permeation test. Only for the ethanol solution as donor phase and pH 5.4 as the acceptor phase, the highest accumulation was observed for [ValOiPr][IBU] (712.60 $\mu\text{g IBU g}^{-1}$) (Table 5).

Due to the decreasing dose of ibuprofen applied to the skin along with the longer alkyl chain in the *L*-valine ester cation, both the cumulative mass of the permeated ibuprofen and its accumulation in the skin were expressed in % of applied IBU dose. These values for individual salts were compared in the relation to that obtained for the parent acid applied and were expressed as the relative differences in skin permeation (RDSP) and skin accumulation (RDSA) of ibuprofen.

The relative percentage differences in IBU skin permeation (RDSP) in each vehicle (MeOH, EtOH, *i*PrOH) and pH value of the acceptor phase were calculated as follows:

$$\text{RDSP (\%)} =$$

$$\frac{\% \text{ permeated dose [IBU]}_{\text{salts}} - \% \text{ permeated dose [IBU]}_{\text{acid}}}{\% \text{ permeated dose [IBU]}_{\text{acid}}} \times 100\%$$



Table 5 Skin accumulation expressed as μg of IBU per g of skin, after 24 h skin permeation of ibuprofen free acid and its salts with L-valine esters from methanolic, ethanolic and isopropanolic solution into acceptor phase pH 7.4 and 5.4 (mean values \pm SD with $n = 3$)^a

Compound	Skin accumulation, $\mu\text{g IBU g}^{-1}$					
	pH 7.4			pH 5.4		
	MeOH	EtOH	iPrOH	MeOH	EtOH	iPrOH
IBU	554.82 \pm 59.51	745.69 \pm 61.87 ^b	710.36 \pm 91.49	506.47 \pm 56.04	580.70 \pm 63.80	600.97 \pm 64.54
[ValOMe][IBU]	484.87 \pm 40.50	447.17 \pm 90.02	512.80 \pm 42.16	451.50 \pm 68.28	416.91 \pm 39.528	469.08 \pm 27.88
[ValOEt][IBU]	472.97 \pm 54.40	412.19 \pm 22.57 ^{**b}	599.74 \pm 74.75	492.19 \pm 28.02	481.95 \pm 100.76	602.98 \pm 34.06
[ValOiPr][IBU]	702.31 \pm 38.82 [*]	726.42 \pm 63.99 ^{**b}	746.13 \pm 44.20	622.23 \pm 122.76 [*]	712.60 \pm 19.84 [*]	699.28 \pm 113.98
[ValOPr][IBU]	743.30 \pm 40.03 [*]	630.75 \pm 34.92 ^{**b}	823.06 \pm 30.98 [*]	574.53 \pm 56.38	702.22 \pm 36.01 [*]	788.16 \pm 49.91 [*]
[ValOBu][IBU]	778.50 \pm 8.60 [*]	815.14 \pm 32.03 ^{**b}	863.53 \pm 83.17 [*]	751.46 \pm 79.40 [*]	617.34 \pm 75.34	844.06 \pm 35.46 [*]
[ValOAm][IBU]	505.50 \pm 56.85	565.60 \pm 37.27 ^b	591.41 \pm 95.30	585.21 \pm 68.27	627.08 \pm 67.86	546.15 \pm 105.15
[ValOHex][IBU]	512.78 \pm 51.99	612.86 \pm 65.80 ^b	568.10 \pm 16.45	482.40 \pm 78.66	544.44 \pm 80.74	429.64 \pm 21.01
[ValOHept][IBU]	500.88 \pm 37.42	556.40 \pm 30.70	541.89 \pm 41.57	456.97 \pm 21.81	526.92 \pm 42.39	483.07 \pm 17.69
[ValOOct][IBU]	454.95 \pm 47.15	465.52 \pm 23.10	478.61 \pm 30.98	504.84 \pm 47.56	482.22 \pm 41.59	447.65 \pm 50.47

^a *Value is higher significantly from control (ibuprofen) ($P < 0.05$), **value is higher significantly from control (ibuprofen) ($P < 0.001$). ^b Data reported in ref. 17.

where % permeated dose $[\text{IBU}]_{\text{salts}}$ is the amount of IBU, used in the form of salt, expressed in % applied IBU dose, that has permeated the skin, % permeated dose $[\text{IBU}]_{\text{acid}}$ is the amount of IBU, used in the form of unmodified acid, expressed in % applied IBU dose, that has permeated the skin. The results were presented in Fig. 2 and 3, respectively for buffers of pH 5.4 and 7.4 as the acceptor phase. The % permeated dose of IBU after 24 h permeation of free acid and its salts with L-valine esters from different vehicles into different acceptor phase were presented in ESI (see ESI, Table S6†).

The relative percentage differences in IBU skin accumulation (RDSA) in each vehicle (MeOH, EtOH, iPrOH) and pH value of the acceptor phase were calculated as follows:

$$\text{RDSA (\%)} =$$

$$\frac{\% \text{ accumulated dose } [\text{IBU}]_{\text{salts}} - \% \text{ accumulated dose } [\text{IBU}]_{\text{acid}}}{\% \text{ accumulated dose } [\text{IBU}]_{\text{acid}}} \times 100\%$$

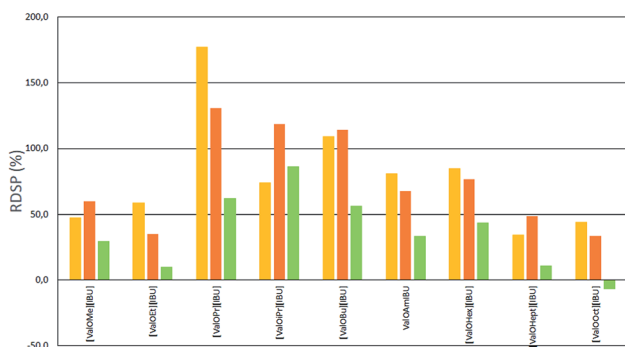


Fig. 2 The relative percentage differences in skin permeation of ibuprofen in the form of salt versus the parent acid form from different alcohols (yellow – methanol; orange – ethanol; green – isopropanol) (buffer pH 5.4 as the acceptor phase).

where % accumulated dose $[\text{IBU}]_{\text{salts}}$ is the amount of IBU, used in the form of salt and expressed in % applied IBU dose, that has accumulated in the skin, % accumulated dose $[\text{IBU}]_{\text{acid}}$ is the amount of IBU, used in the form of unmodified acid and expressed in % applied IBU dose, that has accumulated in the skin. The results were presented in Fig. 4 and 5, respectively for buffers of pH 5.4 and 7.4 as the acceptor phase. The % accumulation dose of IBU after 24 h permeation of free acid and its salts with L-valine esters from different vehicles into different acceptor phase were presented in ESI (see ESI, Table S7†).

As shown in Fig. 2 and 3, the application of L-valine ester ibuprofen salts in each of the alcohol solutions as vehicles led to a significant increase in the skin permeation of ibuprofen in comparison to ibuprofen applied in the acid form in the same vehicle. However, the highest RDSPs were found in methanol and ethanol solutions. When buffer pH 5.4 was used as the acceptor phase, high RDSP values, above 100% were found in permeation test from ethanol solution as a vehicle for [ValOiPr][IBU] and both methanol and ethanol solution for [ValOPr][IBU] and [ValOBu][IBU]. However, when buffer pH 7.4 was used as

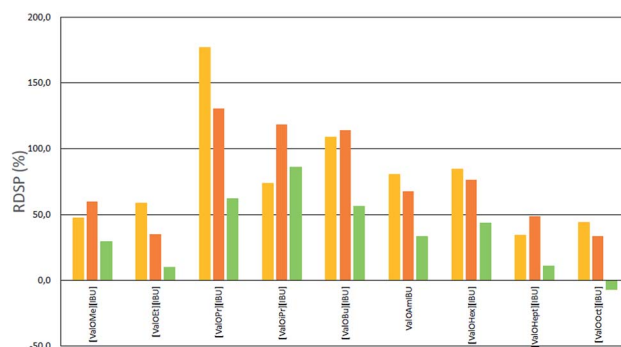


Fig. 3 The relative percentage differences in skin permeation of ibuprofen in the form of salt versus the parent acid form from different alcohols (yellow – methanol; orange – ethanol; green – isopropanol) (buffer pH 7.4 as the acceptor phase).



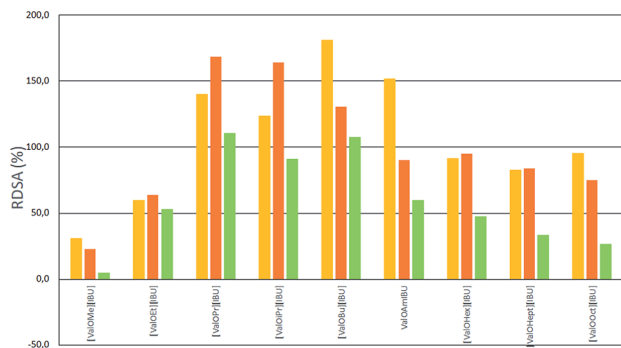


Fig. 4 The relative percentage differences in skin accumulation of ibuprofen (expressed as % IBU applied dose) in the form of salt *versus* the parent acid form from different alcohols (yellow – methanol; orange – ethanol; green – isopropanol) (buffer pH 5.4 as the acceptor phase).

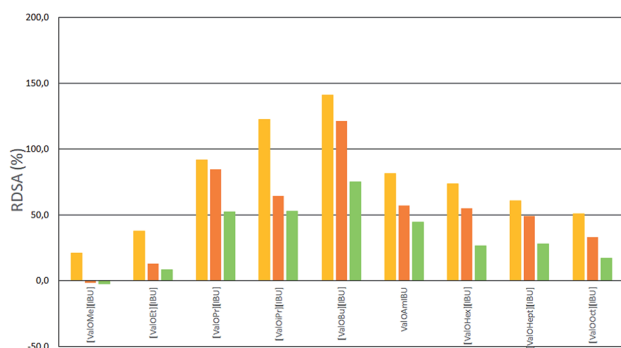


Fig. 5 The relative percentage differences in skin accumulation of ibuprofen (expressed as % IBU applied dose) in the form of salt *versus* the parent acid form from different alcohols (yellow – methanol; orange – ethanol; green – isopropanol) (buffer pH 7.4 as the acceptor phase).

acceptor phase, RDSP above 100% were observed from methanol solution as vehicle for [ValOPr][IBU], [ValOiPr][IBU], [ValOBu][IBU] and [ValOAm][IBU] and from ethanol for [ValOPr][IBU] and [ValOiPr][IBU]. The highest relative percentage difference in skin permeation was observed for [ValOPr][IBU] (RDSP 170%) and [ValOiPr][IBU] (RDSP 180%) in the system of methanol/buffer pH 5.4 and methanol/buffer pH 7.4 respectively. The lowest RDSP values, below 50%, were found in permeation test, from different alcohols as vehicles, for a combination of ibuprofen with the shortest (methyl and ethyl) and longest (heptyl and octyl) alkyl esters of L-valine. Particularly low differences in the increase of permeation of salts in relation to parent acid (RDSP values 5–25%), were determined when *i*-PrOH was used in the donor phase. RDSP values below zero for [ValOOct][IBU] mean that ibuprofen has permeated with a lower dose when used as [ValOOct][IBU] than as unmodified acid.

As shown in Fig. 4 and 5 the accumulation of ibuprofen was higher for all salts in relation to parent acid applied onto the skin. But the highest relative percentage differences in IBU skin accumulation (RDSA) were found for methanol and ethanol as

vehicles. Comparing data presented in Fig. 4 with data for the same salt and vehicle in Fig. 6, the higher RDSA values were observed for buffer pH 5.4 than pH 7.4 as the acceptor phase. This means that at a higher pH of the acceptor phase, the differences in the accumulation of ibuprofen as salt and unmodified acid are smaller than at a lower pH. This may be due to the better solubility of ibuprofen and the smaller differences between the solubility of ibuprofen and its salts at higher pH.¹⁷ When buffer pH 5.4 was used as the acceptor phase, the highest RDSA values (above 150%) were found in skin permeation of [ValOPr][IBU], [ValOiPr][IBU] from ethanol and [ValOBu][IBU] and [ValOAm][IBU] from methanol.

The profiles of ibuprofen permeation expressed in % of applied IBU dose are presented in Fig. 6 and compared for ibuprofen and its salts from different alcohols. All solutions permeated 5–15% of their drug content after 24 h. As can be seen from Fig. 6, the permeation of ibuprofen from each vehicle was biphasic, with an initial faster permeation followed by a period of slow-permeation. All solutions showed a slowdown permeation of ibuprofen after 4–5 h. The application of salts gives higher amounts of the permeated ibuprofen than the use of unmodified ibuprofen. For the acceptor phase of pH 7.4 after 24 h, between 6.51% and 14.59% (isopropanol solution); 6.85% and 13.55% (ethanolic solution) and 6.36% and 13.23% (methanolic solution) of ibuprofen was permeated to the acceptor phase, while for unmodified ibuprofen for only 7.60; 6.60 and 4.59%, respectively. Similarly, in the case of the acceptor phase with pH 5.4 after 24 h, it was between 6.27% and 12.49% (isopropanol solution); 6.24% and 11.10% (ethanolic solution) and 6.31% and 12.13% (methanolic solution) of ibuprofen were permeated to the acceptor phase, while for unmodified ibuprofen for only 6.72; 4.81 and 4.38%, respectively (see ESI, Table S6†). The profiles of ibuprofen permeation are very useful to obtain the permeation parameters such as the steady-state permeation flux, the diffusion coefficient, and the time required to reach steady-state permeation (lag time).

Permeation parameters

The maximum flux through the skin may occur at a pH where ibuprofen ionization is high, therefore the optimum topical formulations may not be for the free acid moiety. The total flux will result from diffusion of both the ionized and unionized species.^{23,24} The maximum flux is the function of the permeability coefficient and solubility of the penetrant. The solubility often increases more than the permeability decreases with the increasing ionization of drug.

The fluxes of the ten penetrants across pig epidermis from the three vehicles into acceptor fluid with two different pH were established. A summary of the permeability parameters (steady-state fluxes, permeability coefficients, and lag time for the permeation of ibuprofen and its salts from methanolic, ethanolic and isopropanolic solution into acceptor phase at pH 7.4) for ibuprofen and its derivatives from different vehicles is shown in Table 6 (results from acceptor fluid at pH 7.4) and Table 7 (pH 5.4). Lag time (L_T) was determined by extrapolating



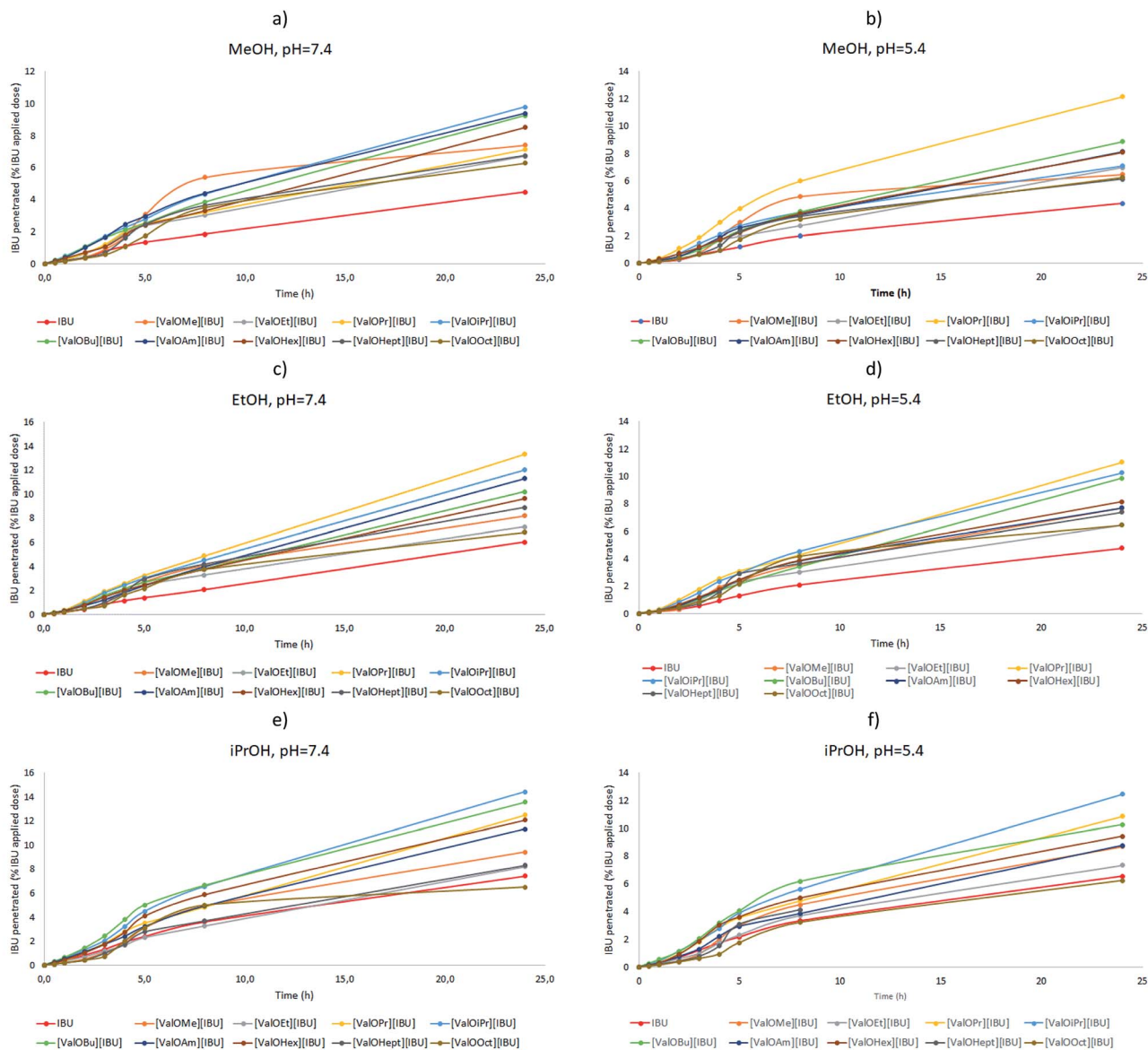


Fig. 6 The comparison of *in vitro* permeation profiles for different [ValOR][IBU] from methanol (a and b), ethanol (c and d), and i-propanol (e and f) solution to buffer solution of pH 5.4 (b, d and f) or 7.4 (a, c and e) as the acceptor phase.

the equation. The steady-state fluxes (J_{ss}) of ibuprofen and its derivatives through the skin were calculated from the slope of the plot of cumulative mass in the acceptor phase over time and were expressed as the amount active ibuprofen per skin area and time ($\mu\text{g IBU cm}^{-2} \text{ h}^{-1}$). In all solvent used, the highest rate of permeation of ibuprofen by connecting it with [ValOMe][IBU] and was 26.42, 27.82, and 33.10 $\mu\text{g IBU cm}^{-2} \text{ h}^{-1}$ in the system of methanol, ethanol, and isopropanol/buffer pH 7.4 respectively, and was 33.24, 25.12 and 31.70 $\mu\text{g IBU cm}^{-2} \text{ h}^{-1}$ in the system of methanol, ethanol, and isopropanol/buffer pH 5.4 respectively.

This derivative showed the fastest permeation of the skin. Moreover, the permeability coefficient (K_p), equal to the diffusion coefficient divided by the width of the membrane expressed in units of cm s^{-1} was also determined. This

coefficient is a quantitative measure of the rate at which a molecule can cross a skin. Comparing these values, the best medium is generally isopropanol. The pH of the acceptor fluid also affects the K_p value. Usually, these substances penetrate better into the acceptor fluid with a higher pH. Best permeation parameters including the highest permeation coefficient and the highest steady-state flux have [ValOMe][IBU] which penetrated from MeOH to acceptor at pH 5.4, and the lowest [ValOEt][IBU] which penetrated from iPrOH to acceptor at pH 7.4. The K_p values were 3.6 and 1.2 times higher, and J_{ss} values were 2.2 times higher and 1.5 times lower, respectively. The lag time is especially higher than ibuprofen for derivatives containing a long (C6–C8) or short (C1) alkyl-chain in the ester group. No relationship was found between the structure of the analyzed compound and permeation parameters.



Table 6 Measured parameters characterizing ibuprofen and its salts transport across porcine SC *in vitro*, after application of methanolic, ethanolic or isopropanolic solution into acceptor fluid pH 7.4

Compound	MeOH, pH 7.4			EtOH, pH 7.4			iPrOH, pH 7.4		
	J_{ss} , $\mu\text{g IBU cm}^{-2} \text{ h}^{-1}$	$K_p \times 10^3$, cm h^{-1}	L_T , h	J_{ss} , $\mu\text{g IBU cm}^{-2} \text{ h}^{-1}$	$K_p \times 10^3$, cm h^{-1}	L_T , h	J_{ss} , $\mu\text{g IBU cm}^{-2} \text{ h}^{-1}$	$K_p \times 10^3$, cm h^{-1}	L_T , h
IBU	16.45	1.61	0.69	15.83	1.58	0.49	26.28	2.57	0.34
[ValOMe][IBU]	26.42	5.40	2.25	27.82	4.52	1.90	33.10	4.29	1.62
[ValOEt][IBU]	17.91	3.00	0.97	17.63	2.99	0.87	18.25	2.66	0.77
[ValOiPr][IBU]	18.24	3.37	1.07	20.41	3.56	0.42	27.58	4.18	0.77
[ValOPr][IBU]	17.14	2.83	0.05	19.36	3.41	0.50	30.66	5.39	0.94
[ValOBu][IBU]	14.59	2.60	0.02	17.18	3.03	0.54	33.15	6.05	0.88
[ValOAm][IBU]	17.29	3.30	0.45	14.88	2.72	0.70	18.59	3.48	0.47
[ValOHx][IBU]	17.20	3.35	1.37	14.32	2.77	0.48	30.94	5.86	1.56
[ValOHept][IBU]	24.12	4.38	2.19	20.95	4.92	2.08	19.52	3.96	1.63
[ValOOct][IBU]	14.11	2.95	2.09	17.34	3.65	1.95	28.13	5.93	2.36

Experimental section

Materials

All reagents were commercially available materials and were used without further purification. (*RS*)-Ibuprofen (99%) was obtained from Acros Organics (Geel, Belgium). L-Valine ($\geq 99\%$) was purchased from Carl Roth (Karlsruhe, Germany). Trimethylsilyl chloride ($\geq 99\%$) (TMSCl) was provided by Sigma-Aldrich (Steinheim am Albuch, Germany). Methanol (MeOH), ethanol (EtOH), propan-2-ol (iPrOH), propan-1-ol (PrOH), butan-1-ol (BuOH), pentan-1-ol (AmOH), hexan-1-ol (HexOH), heptan-1-ol (HeptOH), acetic acid, potassium chloride, sodium chloride, orthophosphoric acid (98%), diethyl ether was high purity obtained from Chempur (Gliwice, Poland). Ammonium hydroxide solution 25% ($\text{NH}_3 \cdot \text{H}_2\text{O}$) was of analytical grade purchased from StanLab (Lublin, Poland). Acetonitrile ($\geq 99.9\%$) for HPLC gradient grade and *n*-octanol ($\geq 99\%$) were provided by Sigma-Aldrich (Steinheim am Albuch, Germany). Disodium hydrogen phosphate dihydrate ($\geq 99\%$) ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$), disodium hydrogen phosphate dodecahydrate (99%) ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) were provided by Fisher Bioreagents

(Pittsburgh, Pennsylvania, USA) and sodium dihydrogen phosphate anhydrous (98%) (NaH_2PO_4) was obtained from Acros Organics (Geel, Belgium). Potassium dihydrogen phosphate anhydrous (99%) was obtained from Merck (Darmstadt, Germany). Deuterated chloroform (CDCl_3) (99.8%) (+0.03% TMSCl) was purchased from Eurisotop (Cheshire, England).

Synthesis of the ibuprofen derivatives

General procedure for preparation of L-valine alkyl ester ibuprofenate ([ValOR][IBU]). The conjunctions of L-valine alkyl esters and ibuprofen were obtained according to the previously described method based on three-steps synthesis (Scheme 1).¹⁷ General procedure for obtaining the L-valine alkyl esters hydrochlorides (ValOR·HCl) include esterification and hydrohalogenation reactions of L-valine using alkyl alcohol such as methanol, ethanol, propan-2-ol, propan-1-ol, butan-1-ol, pentan-1-ol, hexan-1-ol, heptan-1-ol, and octan-1-ol and as hydrochloride agent – trimethylsilyl chloride (TMSCl). Two molar excess of TMSCl was used. The reaction was carried out at 60 °C for 24 h. The product was purified by distillation followed

Table 7 Measured parameters characterizing ibuprofen and its salts transport across porcine SC *in vitro*, after application of methanolic, ethanolic or isopropanolic solution into acceptor fluid pH 5.4

Compound	MeOH, pH 5.4			EtOH, pH 5.4			iPrOH, pH 5.4		
	J_{ss} , $\mu\text{g IBU cm}^{-2} \text{ h}^{-1}$	$K_p \times 10^3$, cm h^{-1}	L_T , h	J_{ss} , $\mu\text{g IBU cm}^{-2} \text{ h}^{-1}$	$K_p \times 10^3$, cm h^{-1}	L_T , h	J_{ss} , $\mu\text{g IBU cm}^{-2} \text{ h}^{-1}$	$K_p \times 10^3$, cm h^{-1}	L_T , h
IBU	15.43	1.53	1.00	16.92	1.69	1.22	25.34	2.47	0.56
[ValOMe][IBU]	33.24	5.46	2.29	25.12	4.10	1.68	31.70	5.15	2.09
[ValOEt][IBU]	16.27	2.78	0.94	17.88	3.03	1.24	17.23	2.92	1.10
[ValOiPr][IBU]	27.66	4.91	0.96	19.98	3.52	0.50	25.02	4.41	0.91
[ValOPr][IBU]	19.90	3.55	0.81	20.36	3.51	0.79	25.92	4.57	0.84
[ValOBu][IBU]	17.55	3.27	1.26	15.45	2.72	1.01	27.66	4.97	0.89
[ValOAm][IBU]	17.61	3.45	1.26	17.22	3.14	1.16	19.92	3.72	1.08
[ValOHx][IBU]	13.06	2.57	0.62	15.99	3.01	0.97	24.11	4.64	0.89
[ValOHept][IBU]	19.61	4.02	2.20	26.83	5.45	2.41	28.45	5.80	2.46
[ValOOct][IBU]	19.60	4.14	2.86	15.82	3.35	1.84	23.97	5.04	2.15



by washing with diethyl ether and drying at 60 °C under vacuum. In the next step obtained L-valine alkyl esters hydrochlorides were neutralized by the addition of one to three molar equivalents of 25% ammonium hydroxide aqueous solution. The received product was separated from the reaction mixture by extraction with diethyl ether. The organic layer was dried using anhydrous Na₂SO₄ and then concentrated under vacuum to receive L-valine alkyl ester (ValOR). Next, the equimolar reaction of ValOR with ibuprofen was carried out in chloroform at room temperature for 20 minutes. The solvent has been evaporated then under vacuum at 30 °C. The crude product was purified by recrystallization from ethanol. The obtained precipitate was filtered and dried in a vacuum oven at 60 °C for 24 h.

General analytical methods

Nuclear magnetic resonance spectroscopy (NMR). The NMR spectra were recorded with Bruker DPX-400 spectrometer (Billerica, MA, USA) at 400 MHz (¹H) and 100 MHz (¹³C) in CDCl₃ as a solvent. The chemical shifts (δ, ppm) are given relative to TMS used as an internal standard.

Total reflectance – Fourier transform infrared spectroscopy (ATR-FTIR). The FTIR data were collected on Thermo Scientific Nicolet 380 spectrometer (Waltham, MA, USA) equipped with an ATR diamond plate. The spectra were recorded in transmission mode in a range of 4000 to 400 cm⁻¹ at the resolution of 4 cm⁻¹.

UV-vis spectroscopy. UV-vis spectra were recorded on Spectroquant® Pharo 300 Spectrophotometer from Merck (Darmstadt, Germany). The solutions were prepared in absolute ethanol of concentration range 10⁻⁴ to 10⁻⁵ mol dm³. The measurements were performed in a 10 mm quartz cell in the wavelength range of 190–400 nm with an accuracy of ±1 nm.

Elemental analysis. The determination of elemental composition was carried out on Thermo Scientific™ FLASH 2000 CHNS/O Analyzer (Waltham, MA, USA). 2,5-Bis(5-(*tert*-butyl)-2-benzo-oxazol-2-yl)thiophene, L-cysteine, L-methionine, and sulphanilamide were used as standards in CHNS-mode and acetanilide and benzoic acid were used for calibration in O-mode respectively. The samples were prepared in a tin (CHNS analysis) or silver (O analysis) crucibles and were weighed with an accuracy of ±0.000001 g.

Thermogravimetric analysis. Thermogravimetric (TG) analysis of compounds was conducted with thermomicrobalance TG 209 F1 Libra® from NETZSCH (Selb, Germany). Samples were loaded in Al₂O₃ crucible and heated from 25 °C to 1000 °C at 10 °C min⁻¹ in the air (25 cm³ min⁻¹) with nitrogen flow (10 cm³ min⁻¹) as the purge gas.

Differential scanning calorimetry (DSC). The crystallization and melting behavior of the compounds were characterized by Differential Scanning Calorimetry (DSC) by the use of TA Instruments, model Q-100 DSC (New Castle, DE, USA). The measurements were carried out under a nitrogen atmosphere using approximately 10 mg of sample loaded in aluminum pans with a pierced lid. Before heating scans, the sample was cooled from 20 °C to 0 °C. The sample was then heated from 0 °C to set temperature, cooled to 0 °C, and again heated to the specified

temperature. The specified temperature was deemed as individual value for each compound and set at least 10 °C lower than the onset decomposition temperature (based on TG analysis). The rate of heating/cooling/heating was 10 °C min⁻¹. Indium and mercury were used as standards to calibrate the temperature. Heat calibration used indium.

Specific rotation. The measurement of specific rotation [α]_D²⁰ were performed on AUTOPOL IV Polarimeter from Rudolph Research Analytical (Hackettstown, NJ, USA). The concentrations of compounds were 0.01 g cm⁻³ in ethanol as a solvent. The angular rotation was determined at the accuracy of 0.001° at 20 ± 0.1 °C.

Solubility. The solubility of novel ibuprofen salts in conventional organic solvents (polar and nonpolar) have been evaluated following the modified Vogel method at the temperature of 25 °C.⁴⁷ For this purpose, dimethyl sulfoxide, ethanol, chloroform, ethyl acetate, diethyl ether, toluene, and *n*-hexane were used as solvents. The compounds were classified as soluble, partially soluble, and insoluble, based on the amount of compound dissolved in 1 cm⁻³ of proper solvent.

The solubility of ibuprofen and L-valine alkyl ester salts was evaluated in deionized water and phosphate buffers (pH 5.4 and 7.4) at 25 °C and phosphate buffers at 32 °C. The saturated solutions were prepared by adding the excess of the substance to 2 cm³ proper solvent in the screwed vial. The mixture was stirred vigorously at 25.00 ± 0.05 °C or 32.00 ± 0.05 °C, for 24 hours and subsequently centrifuged at the respective temperature. The liquid above was separated and diluted. The concentration of the substance was determined by HPLC method.

Permeation and skin accumulation studies

Chromatographic conditions. Liquid chromatography system (Knauer, Berlin, German) in skin permeation experiments for determination of compounds concentration in acceptor fluid and accumulation in the skin is a complex, modular system consisting of the following units: WellChrom model K1001 pump, model EuroChrom 2000 integrator, a Rheodyne model 7125 injector, model K2600 UV detector. Ibuprofen and its salts were analyzed on the Hypersil ODS (C18) column, 125 × 4.0 mm i.d., particle size 5 μm (Thermo Scientific™ Waltham, MA, USA). The mixture of acetonitrile – 1% (w/w) aqueous solution of acetic acid – methanol (45/45/10, v/v/v) was used as a mobile phase with 1.0 cm³ min⁻¹ flow rate. The column temperature was set at 25 °C, and the injection volume was 20 mm³. The signals were monitored at 264 nm. Quantitation was achieved by measurement of the peak area using a calibration curve method. Injections were repeated at least three times for each sample and the results were averaged.

Preparation of pig skin. In the *in vitro* penetration experiments, an abdomen, porcine skin was used due to its similar permeability to human skin.^{49,50} Numerous histopathological studies confirmed its similarity to human skin.^{65,66} The fresh skin was washed with PBS buffer (pH 7.4) several times. After drying, dermatome section skin 0.5 mm thick was prepared (Humby Dermatome, Surtex Instruments, New Malden, England). The samples of skin were wrapped in aluminum foil



and stored in a deep freezer at $-20\text{ }^{\circ}\text{C}$ until use not longer than 3 months. This time of frozen was safe to keep skin barrier properties.^{67,68} On the day of the experiment, the skin samples were slowly thawed at room temperature to 30 min and cut into appropriate pieces $2\text{ cm} \times 2\text{ cm}$ and were hydration by PBS buffer pH 7.4.^{60,69,70} The undamaged skin pieces (checked by measuring skin impedance) with an even thickness were chosen for the experiment.

In vitro skin permeation studies. The skin permeability for ibuprofen and its derivatives was studied *in vitro* by using Franz diffusion cell (SES GmbH Analyse Systeme, Germany) with a diffusion area of 1 cm^2 . The acceptor chamber was filled with the 0.1 mol dm^{-3} buffer solution of two different pH values 5.4 and 7.4. The volume of the acceptor chambers was 8 cm^3 and the volume of the donor chamber was approximately 2 cm^3 . The temperature of the acceptor chamber of each cell was maintained of $32 \pm 0.5\text{ }^{\circ}\text{C}$ ⁷¹ via thermostat (VEB MLW Prüfgeräte-Werk type of 3280). The content of the acceptor chamber was mixed with a magnetic stirrer. The diffusion cells were allowed to equilibrate at $32\text{ }^{\circ}\text{C}$ for 15 minutes. Once reached equilibrium, the donor chamber was filled with 0.500 cm^3 of the tested compound solution in 70% (v/v) either ethanol, methanol, or isopropanol. The concentration of the compound in this solution was 0.01 g cm^{-3} . The undamaged skin pieces were placed between the donor and acceptor chamber of Franz diffusion cells, then integrity has been checked. The skin samples were mounted in the diffusion cells in such a way that the stratum corneum side faced the donor chamber.⁷²

The experiment was carried for 24 hours. At each time point (0.5 h, 1 h, 2 h, 3 h, 4 h, 5 h, 8 h, and 24 h) 0.300 cm^3 samples of receptor solution were withdrawn and the chamber refilled with fresh buffer at the same pH. The drug concentration in the acceptor phase was measured by the HPLC method. The cumulative mass ($\mu\text{g cm}^{-2}$) was calculated based on this concentration. The steady-state flux $-J_{ss}$ (in $\mu\text{g cm}^{-2}\text{ h}^{-1}$) of the active drug – ibuprofen and its derivatives through the skin into acceptor fluid was determined as the slope of the plot of cumulative mass in the acceptor fluid *versus* time. Within the steady-state period, the flux is constant and can be determined as the slope of the linear regression of the permeated amount in the function of time. The diffusion coefficients (K_p) were determined by the following equation using Fick's law of diffusion:

$$K_p = \frac{J_{ss}}{C_d}$$

C_d was the initial drug concentration in the donor chamber.

Determination of ibuprofen and its derivatives concentration in the skin. After 24 hours experiment, each skin sample was removed from the Franz diffusion cell and carefully rinsed in PBS pH 7.4.⁷¹ The skin was then cut around the diffusional area (1 cm^2) and dried at room temperature. Next samples of skin were cut into very small pieces,⁶⁹ placed in 2 cm^3 of methanol, and incubated for 24 h at $4\text{ }^{\circ}\text{C}$. After this time skin samples were homogenized for 3 minutes using a homogenizer (IKA®T18 digital ULTRA TURRAX, Germany). The homogenate was centrifuged at 3500 rpm for 5 min. The supernatant was

collected and analyzed using HPLC to determine a concentration of ibuprofen.

Accumulation of the ibuprofen in the skin was calculated by dividing the amount of the drug remaining in the skin by a mass of skin sample and was expressed in mass of ibuprofen per mass of the skin ($\mu\text{g g}^{-1}$).

Skin electrical impedance. The measurements of skin electrical impedance were performed with an LCR meter 4080 (Votcraft LCR 4080, Conrad Electronic, Germany), which was operated in parallel mode at an alternating frequency of 120 Hz (error at $k\Omega$ values $< 0.5\%$). The tips of measuring probes were immersed in donor and acceptor chamber, filled with PBS buffer (pH 7.4).⁷¹ Skin samples with skin impedance of above $3\text{ k}\Omega$, which is a value like to the electrical resistance for human skin⁶⁸ were used to the experiment.

Conclusions

The ionic pairs of ibuprofen with the biocompatible counterions of L-valine alkyl ester, where the alkyl chain was between methyl and octyl, have better penetration through porcine skin compared to the starting acid. It was noticed that different vehicles and pH of the acceptor phase have influences on ibuprofen transport through porcine skin. Permeability tests through porcine skin have shown that [ValOipr][IBU] shows the best ibuprofen permeability, regardless of the vehicle used. This study showed that newly developed ibuprofen modifications could be promising active ingredients into formulations applied to the skin and employed as an ideal alternative to commercial ibuprofen. No relationship was found between the solubility of ibuprofen and its L-valine derivatives in water and phosphate buffers and skin accumulation.

Conflicts of interest

There are no conflicts to declare.

References

- 1 A. Alkilani, M. T. McCrudden and R. Donnelly, *Pharmaceutics*, 2015, 7, 438–470.
- 2 H. A. E. Benson, in *Topical and Transdermal Drug Delivery*, ed. H. A. E. Benson and A. C. Watkinson, John Wiley & Sons, Inc., Hoboken, NJ, USA, 2012, pp. 1–22.
- 3 M. E. Lane, P. Santos, A. C. Watkinson and J. Hadgraft, in *Topical and Transdermal Drug Delivery*, ed. H. A. E. Benson and A. C. Watkinson, John Wiley & Sons, Inc., Hoboken, NJ, USA, 2012, pp. 23–42.
- 4 N. Dragicevic, J. P. Atkinson and H. I. Maibach, in *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*, ed. N. Dragicevic and H. I. Maibach, Springer Berlin Heidelberg, Berlin, Heidelberg, 2015, pp. 11–27.
- 5 C. M. Heard, in *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement*, ed. N. Dragicevic and H. I. Maibach, Springer Berlin Heidelberg, Berlin, Heidelberg, 2015, pp. 151–172.



- 6 A. Schroeter, A. Eichner, J. Mueller and R. H. H. Neubert, in *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*, ed. N. Dragicevic and H. I. Maibach, Springer Berlin Heidelberg, Berlin, Heidelberg, 2015, pp. 29–37.
- 7 D. Horita, I. Hatta, M. Yoshimoto, Y. Kitao, H. Todo and K. Sugibayashi, *Biochim. Biophys. Acta, Biomembr.*, 2015, **1848**, 1196–1202.
- 8 A. Zhang, E.-C. Jung, H. Zhu, Y. Zou, X. Hui and H. Maibach, *Toxicol. Ind. Health*, 2017, **33**, 416–425.
- 9 C. Y. Goates and K. Knutson, *Biochim. Biophys. Acta, Biomembr.*, 1994, **1195**, 169–179.
- 10 H. V. Ly and M. L. Longo, *Biophys. J.*, 2004, **87**, 1013–1033.
- 11 S. C. McKarns, C. Hansch, W. S. Caldwell, W. T. Morgan, S. K. Moore and D. J. Doolittle, *J. Appl. Toxicol.*, 1997, **36**, 62–70.
- 12 A. Chandra, P. Sharma and R. Irchhiaya, *Asian J. Pharm.*, 2009, **3**, 37.
- 13 B. P. Wenkers and B. C. Lippold, *J. Pharm. Sci.*, 1999, **88**, 1326–1331.
- 14 A. Avdeef, K. J. Box, J. E. A. Comer, C. Hibbert and K. Y. Tam, *Pharm. Res.*, 1998, **15**, 209–215.
- 15 G. Bouchard, A. Galland, P.-A. Carrupt, R. Gulaboski, V. Mirčeski, F. Scholz and H. H. Girault, *Phys. Chem. Chem. Phys.*, 2003, **5**, 3748–3751.
- 16 A. Czyrski, *J. Chem.*, 2019, **2019**, 1–6.
- 17 E. Janus, P. Ossowicz, J. Klebko, A. Nowak, W. Duchnik, Ł. Kucharski and A. Klimowicz, *RSC Adv.*, 2020, **10**, 7570–7584.
- 18 *Clarke's analysis of drugs and poisons: in pharmaceuticals, body fluids and postmortem material*, ed. A. C. Moffat, M. D. Osselton, B. Widdop and J. Watts, Pharmaceutical Press, London, Chicago, 4th edn, 2011.
- 19 A. Pyka, *J. Liq. Chromatogr. Relat. Technol.*, 2009, **32**, 723–731.
- 20 T. Scheytt, P. Mersmann, R. Lindstädt and T. Heberer, *Water, Air, Soil Pollut.*, 2005, **165**, 3–11.
- 21 F. Stuer-Lauridsen, M. Birkved, L. P. Hansen, H.-C. Holten Lützhøft and B. Halling-Sørensen, *Chemosphere*, 2000, **40**, 783–793.
- 22 J. Hadgraft, J. du Plessis and C. Goosen, *Int. J. Pharm.*, 2000, **207**, 31–37.
- 23 J. Hadgraft and C. Valenta, *Int. J. Pharm.*, 2000, **200**, 243–247.
- 24 A. Patel, M. Bell, C. O'Connor, A. Inchley, J. Wibawa and M. E. Lane, *Int. J. Pharm.*, 2013, **457**, 9–13.
- 25 J. Irvine, A. Afrose and N. Islam, *Drug Dev. Ind. Pharm.*, 2018, **44**, 173–183.
- 26 J. Hadgraft, M. Whitefield and P. H. Rosher, *Skin Pharmacol. Physiol.*, 2003, **16**, 137–142.
- 27 R. M. Watkinson, C. Herkenne, R. H. Guy, J. Hadgraft, G. Oliveira and M. E. Lane, *Skin Pharmacol. Physiol.*, 2009, **22**, 15–21.
- 28 Strides Research and Specialty Chemicals Limited, US007084.299B2, 2006.
- 29 *Supported ionic liquids: fundamentals and applications*, ed. R. Fehrmann, Wiley-VCH, Weinheim, 2014.
- 30 A. L. Abuhijleh, *J. Inorg. Biochem.*, 1994, **55**, 255–262.
- 31 H. Abu Ali, S. N. Omar, M. D. Darawsheh and H. Fares, *J. Coord. Chem.*, 2016, **69**, 1110–1122.
- 32 Merck & Co., Inc., US005200558A, Rahway, N.J., 1993.
- 33 C. P. Frizzo, K. Wust, A. Z. Tier, T. S. Beck, L. V. Rodrigues, R. A. Vaucher, L. P. Bolzan, S. Terra, F. Soares and M. A. P. Martins, *RSC Adv.*, 2016, **6**, 100476–100486.
- 34 W. L. Hough and R. D. Rogers, *Bull. Chem. Soc. Jpn.*, 2007, **80**, 2262–2269.
- 35 V. Sarveiya, J. F. Templeton and H. A. E. Benson, *J. Pharm. Pharmacol.*, 2004, **56**, 717–724.
- 36 H. Wu, Z. Deng, B. Zhou, M. Qi, M. Hong and G. Ren, *J. Mol. Liq.*, 2019, **283**, 399–409.
- 37 S. Furukawa, G. Hattori, S. Sakai and N. Kamiya, *RSC Adv.*, 2016, **6**, 87753–87755.
- 38 H. Wang, G. Gurau, J. Shamshina, O. A. Cojocar, J. Janikowski, D. R. MacFarlane, J. H. Davis and R. D. Rogers, *Chem. Sci.*, 2014, **5**, 3449.
- 39 M. Kohlmeier, *Nutrient Metabolism*, Academic Press, San Diego, CA, USA, 2003.
- 40 V. Buddolla, *Recent Developments in Applied Microbiology and Biochemistry*, Academic Press, 2019.
- 41 M. S. Roberts and W. Kenneth, *Dermal Absorption and Toxicity Assessment*, CRP Press Taylor & Francis Group, LLC, 2nd edn, 2007.
- 42 J. Li and Y. Sha, *Molecules*, 2008, **13**, 1111–1119.
- 43 P. Ossowicz, E. Janus, G. Schroeder and Z. Rozwadowski, *Molecules*, 2013, **18**, 4986–5004.
- 44 R. Radeaglia, *J. Prakt. Chem.*, 1981, **323**, 1016.
- 45 Z. Rozwadowski, *J. Mol. Struct.*, 2005, **753**, 127–131.
- 46 S. Vairam, T. Premkumar and S. Govindarajan, *J. Therm. Anal. Calorim.*, 2010, **100**, 955–960.
- 47 *Vogel's textbook of practical organic chemistry*, ed. B. S. Furniss and A. I. Vogel, Pearson/Prentice Hall, Harlow, New, 5th edn, 2009.
- 48 C. Reichardt and T. Welton, *Solvents and solvent effects in organic chemistry*, Wiley-VCH, Weinheim, Germany, 4th edn, 2011.
- 49 B. A. Čuríková, K. Procházková, B. Filková, P. Diblíková, J. Svoboda, A. Kováčik, K. Vávrová and J. Zbytovská, *Int. J. Pharm.*, 2017, **534**, 287–296.
- 50 C. Génies, E. L. Jamin, L. Debrauwer, D. Zalko, E. N. Person, J. Eilstein, S. Grégoire, A. Schepky, D. Lange, C. Ellison, A. Roe, S. Salhi, R. Cubberley, N. J. Hewitt, H. Rothe, M. Klaric, H. Duplan and C. Jacques-Jamin, *J. Appl. Toxicol.*, 2018, **38**, 3730.
- 51 H. Bando, S. Mohri, F. Yamashita, Y. Takakura and M. Hashida, *J. Pharm. Sci.*, 1997, **86**, 759–761.
- 52 D. Celebi, R. H. Guy, K. J. Edler and J. L. Scott, *Int. J. Pharm.*, 2016, **514**, 238–243.
- 53 B. M. Jameel, A. Huynh, A. Chadha, S. Pandey, J. Duncan, M. Chandler and G. Baki, *Int. J. Pharm.*, 2019, **569**, 118549.
- 54 L. Luo, A. Patel, B. Sinko, M. Bell, J. Wibawa, J. Hadgraft and M. E. Lane, *Int. J. Pharm.*, 2016, **505**, 14–19.
- 55 O. Taofiq, F. Rodrigues, L. Barros, M. F. Barreiro, I. C. F. R. Ferreira and M. B. P. P. Oliveira, *Food Chem. Toxicol.*, 2019, **127**, 228–236.



- 56 M. Malinowska, E. Sikora and J. Ogonowski, *Wiad. Chem.*, 2013, **67**(3–4), 321–344.
- 57 H. Chen, X. Chang, D. Du, J. Li, H. Xu and X. Yang, *Int. J. Pharm.*, 2006, **315**, 52–58.
- 58 R. Intarakumhaeng and S. K. Li, *Int. J. Pharm.*, 2014, **476**, 266–276.
- 59 M. Jaworska, E. Sikora and J. Ogonowski, *Wiad. Chem.*, 2011, **65**(3–4), 301–320.
- 60 A. Haq and B. Michniak-Kohn, *Drug Delivery*, 2018, **25**, 1943–1949.
- 61 Y. S. R. Krishnaiah, V. Satyanarayana and R. S. Karthikeyan, *J. Pharm. Pharm. Sci.*, 2002, **5**, 123–130.
- 62 S. Mutalik and N. Udupa, *Pharm*, 2003, **58**, 891–894.
- 63 V. K. Llewelyn, L. Berger and B. D. Glass, *Heliyon*, 2019, **5**, e02127.
- 64 G. Marti-Mestres, J. Mestres, J. Bres, S. Martin, J. Ramos and L. Vian, *Int. J. Pharm.*, 2007, **331**, 139–144.
- 65 U. Jacobi, M. Kaiser, R. Toll, S. Mangelsdorf, H. Audring, N. Otberg, W. Sterry and J. Lademann, *Skin Res. Technol.*, 2007, **13**, 19–24.
- 66 M. Khiao, K. C. Richardson, A. Loewa, S. Hedtrich, S. Kaessmeyer and J. Plendl, *Anat., Histol., Embryol.*, 2019, **48**, 207–217.
- 67 M. M. Badran, J. Kuntsche and A. Fahr, *Eur. J. Pharm. Sci.*, 2009, **36**, 511–523.
- 68 D. J. Davies, R. J. Ward and J. R. Heylings, *Toxicol. In Vitro*, 2004, **18**, 351–358.
- 69 J. Kuntsche, H. Bunjes, A. Fahr, S. Pappinen, S. Rönkkö, M. Suhonen and A. Urtti, *Int. J. Pharm.*, 2008, **354**, 180–195.
- 70 A. Simon, M. I. Amaro, A. M. Healy, L. M. Cabral and V. P. de Sousa, *Int. J. Pharm.*, 2016, **512**, 234–241.
- 71 M. Kopečná, M. Macháček, A. Nováčková, G. Paraskevopoulos, J. Roh and K. Vávrová, *Sci. Rep.*, 2019, **9**, 14617.
- 72 A. Ahad, M. Aqil and A. Ali, *Pharm. Biol.*, 2016, **54**, 1042–1051.

