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Research paper

Extraction of amino acids by reverse iontophoresis: Simulation of therapeutic monitoring *in vitro*

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ABSTRACT

Reverse iontophoresis across the skin has been investigated as alternative, non-invasive method for clinical and therapeutic drug monitoring. This research investigated the reverse iontophoretic extraction of 19 amino acids present at clinically relevant levels in the subdermal compartment of an *in vitro* diffusion cell. Over a simulated, systemic concentration range of 0–500 μM, the extraction of amino acids was linear. Charged amino acids were extracted towards the electrode of opposite polarity, while zwitterionic species were extracted to both anode and cathode with the latter predominating. The reverse iontophoretic extraction flux was a linear function of amino acid isoelectric point, reflecting the different contributions of electromigration and electroosmosis to electrotransport. Overall, the results confirm the feasibility of monitoring amino acids at clinically relevant levels and provide an incentive for *in vivo* research to further explore the clinical potential of reverse iontophoresis for the non-invasive monitoring of amino acids.

1. Introduction

Over the past two decades, reverse iontophoresis across the skin has been investigated as alternative, non-invasive method for clinical and therapeutic drug monitoring [1]. Its first application, the transdermal extraction of glucose [2], led to the development of the GlucoWatch® Biographer (Johnson & Johnson, New Brunswick, NJ). This FDA-approved device uses reverse iontophoresis to monitor glycaemia transdermally for up to 12 h [3]. More recent work confirmed the potential of the approach to track (both *in vitro* and *in vivo*) subdermal levels of lithium [4], phenytoin [5], valporate [6], lactate [7], and urea [8].

Amino acids are biological markers for the plasma levels of which may be used to detect inherited metabolic diseases [9]. Because of their small molecular weight and polar nature, these compounds are excellent candidates for non-invasive monitoring via reverse iontophoresis. Indeed, previous work has shown how the transport of amino acids across the skin can be significantly enhanced by iontophoresis [10,11], and pilot investigations (again, both *in vitro* and in vivo) on the reverse iontophoretic extraction of phenylalanine for the diagnosis of phenylketonuria have been reported [12,13].

Reverse iontophoresis uses a small electric current to drive charged and highly polar compounds across the skin at rates very much greater than their passive permeabilities [1]. Two major transport mechanisms are involved: electromigration and electroosmosis. Electromigration is the movement of small ions across the skin under the direct influence of the electric field. Electron fluxes are transformed into ionic fluxes via the electrode reactions, and ionic transport proceeds through the skin to maintain electroneutrality.

The total charge transported depends on the strength of the electric field and the duration of application. Iontophoresis sets in motion a number of ions across the skin, and all of them compete to carry a fraction of the current. According to Faraday's law, the flux of each ion in the iontophoretic circuit is given by

$$J_i = (t_i * I)/(F * z_i) \quad (1)$$

where J_i is the flux of the ith ion, t_i is its transport number, and z_i is the valence; F is Faraday's constant and I is the total current flowing. Iontophoretic transport numbers depend on the relative mobility and concentration of all mobile ions in the system.

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Electroosmosis is the principal transport mechanism of uncharged molecules and of high molecular weight cations. The skin is negatively charged at physiological pH and acts, therefore, as a permselective membrane to cations. This preferential passage of counterions induces an electroosmotic solvent flow that carries neutral molecules in the anode-to-cathode direction. The volume flow J_V (volume per unit time per unit area) is predicted [14] to be proportional to the potential gradient established by the electric field: The molar flux of a solute "j" present at concentration c_j is then

$$J_j = J_V * c_j \quad (2)$$

Electroosmosis depends on the charge on the membrane and may be modified by formulations (e.g., the pH) which "couple" the electrodes to the skin. Typically, for analytes such as amino acids, both transport mechanisms are involved in iontophoretic extraction, the relative contributions of electromigration and electroosmosis depending on the physicochemical properties of the analyte.

Many, diverse metabolic diseases are characterized by pathological, systemic levels of at least one amino acid [9]. As mentioned before, phenylketonuria is the most prominent example, in which elevated blood concentrations of phenylalanine (above 100 μM) can lead to impaired intellectual function. Other metabolic diseases include maple syrup urine disease caused by a deficiency of the enzyme branched-chain a-keto acid dehydrogenase. This leads to a build-up of the branched-chain amino acids leucine, isoleucine and valine (blood levels above 200, 100, and 350 μM, respectively) and their toxic by-products in blood and urine. Further, tyrosinemia is caused by the deficiency of different enzymes in the tyrosine metabolic pathway. The disease is characterized by elevated blood levels of tyrosine (above 100 μM) and its by-products and can either lead to liver and kidney failure, severe skin lesions or mental retardation.

Amino acid plasma concentrations can therefore be used to detect these diseases and to monitor the success of therapeutic measures, such as a strict diet. The advantage of reverse iontophoresis as a monitoring tool is the non-invasive nature of the technique, and the possibility to follow simultaneously a variety of analytes – for example, several amino acids – in the same skin extract. However, due to the skin's impressive barrier function, a transdermal extract contains a much lower concentration of the analyte than that present in the blood. A significant challenge, therefore, is to detect and quantify reproducibly the analyte of interest over the concentration range that is clinically relevant.

The objective of the research presented in this paper was to examine whether reverse iontophoresis, together with sample analysis using liquid chromatography coupled with mass spectrometry (LC-MS), would have sufficient sensitivity to permit the simultaneous detection of 19 naturally occurring amino acids in an *in vitro* simulation of a clinical monitoring protocol. The analytes were measured, subsequent to current passage, in both the anode and cathode chambers, permitting preliminary evaluation of the controlling structure-transport relationships in operation.

2. Materials and methods

2.1. Materials

Porcine ears were obtained from an abattoir (Société d'Exploitation d'Abbatage, Annecy, France) and cleaned under cold running water. Skin was removed with a dermatome set to 750 μm (Zimmer™ Air Dermatome, Dover, Ohio) and cut into 9 cm^2 pieces, which were individually wrapped in Parafilm™ and kept at −20 °C for up to 1 month before use.

Sodium chloride was purchased from Fluka (St. Quentin Fallavier, France). Phosphate buffered saline (reference P4417) and the amino acids Asn, Ser, Gly, Asp, Gln, Glu, Thr, Ala, Pro, Val, Tyr, Met, Ile, Leu, His, Lys, Phe, Arg, and Trp were purchased from Sigma–Aldrich (St. Quentin Fallavier, France). All chemicals were of analytical grade. All solutions were prepared with de-ionized water (resistivity >18 MΩ/cm^2).

2.2. In vitro iontophoretic experiments

The skin was mounted between the two halves of vertical iontophoresis cells [15]; the area available for transport was 0.8 cm^2. The anodal and cathodal compartments were filled with unbuffered 30 mM NaCl. The subdermal donor compartment was charged with physiological phosphate-buffered saline at pH 7.4, containing all 19 amino acids at levels of 100, 250, and 500 μM, respectively. All experiments, in which the 19 amino acids were extracted simultaneously, were performed 6 times. Direct current (0.4 mA) was applied for 6 h via Ag/AgCl electrodes from a power supply (KEPCO 1000M, Flushing, NY). The entire contents of the anodal and cathodal compartments were removed every hour and analysed for all amino acids using LC-MS as described below. In a control experiment, which investigated iontophoretic extraction of the endogenous amino acid reservoir ($n = 6$), the subdermal solution did not contain any exogenously added amino acids.

2.3. Analytical chemistry – liquid chromatography coupled to mass spectrometry (LC-MS)

The identification and simultaneous quantification of the 19 amino acids was based on a previously published LC-MS method [16,17]. LC–MS experiments were performed on a HP Series 1100 LC system (Agilent Technologies, Waldbronn, Germany) equipped with an autosampler and a binary pump. The Chemstation suite software (Agilent Technologies) was used for instrument control, data acquisition and data handling. Detection was carried out with a HP Series 1100 MSD (Agilent Technologies) equipped with an orthogonal electrospray interface. Nitrogen was used both as nebulizing (35 psi) and as drying gas at a temperature of 350 °C at a flow rate of 12 L min^{-1}. Electrospray and skimmer voltages were set at 1000 and 90 V, respectively. The quadrupole was operated in the selected ion monitoring (SIM) mode and was set on 20 ions. Amino acids are separated in 4 groups depending on retention time to reach a minimum dwell time of 50 ms for each ion. The protonated molecule $[M+H]^+$ was used as the selected ion. m/z are for group 1 from 5.1 to 15.9 min (Asn 133, Gly 76, Ser 106, Asp 134, Gln 147, Glu 148, Thr 120, Ala 90), group 2 from 16 to 19.7 min (Pro 116), group 3 from 19.8 to 23.2 min (Val 118, Tyr-D4 186, Tyr 182, Met 150) and group 4 from 23.4 to 29.5 min (Ile 132, Leu 132, His 156, Lys 147, Phe-D5 171, Phe 166, Arg 175, Trp 205).

The chromatographic separation was performed on a Symmetry C18, 3.5 μm, 2 mm i.d. × 150 mm (Waters, Switzerland) fitted with a Symmetry C18, 3.5 μm, 2 mm i.d. × 5 mm, (Waters) pre-column. The separation was carried out at a flow rate of 200 μL min^{-1} with the following solvent system: (A) a 20 mM nonafluoropentanoic acid solution, and (B) acetonitrile. 10 μL of internal standard solution (10 μM of Phe-D5 and Tyr-D4 in 30 mM NaCl) was added to 90 μL of sample. The samples were vortexed, centrifuged and transferred in HPLC-vials. The injection volume was 20 μL.

The gradient elution program started with 99% (A) for 5 min, changed linearly to 82% (A) in 7 min, then linearly to 74% (A) in 6 min, and linearly to 50% (A) in 6 min, followed by an isocratic elution at 50% (A) for 5 min. The analytical column was washed with 20% (A) for 3 min with at a flow rate of 300 μL min^{-1}. The system was equilibrated for 20 min in the initial conditions. Analyses were carried out at 10 °C.

This analytical method was fully validated to detect the amino acids in the iontophoretic matrix [18] according to regular guidelines (ICH) and results will be presented elsewhere. Briefly, trueness, repeatability (within-day variability) and intermediate fidelity (between-day variability) were evaluated for 3 series (independent analysis days) with two calibration curves (3 concentration levels, 0.1, 2.5 and 10 μM). Each day, 4 concentrations (0.1, 0.25, 1, 2.5 and 4 μM) were evaluated in triplicate with three batches of internal controls (validation standards) and with two batches of external control (0.25 and 2.5 μM). For calibration and control standards, a solution of 30 mM NaCl was used to mimic the iontophoretic cells composition. For all tested levels, trueness, repeatability and intermediate fidelity were between 5% and 15%. The working range for Glu, Thr, Ala, Pro, Val, Tyr, Met, Ile, Leu, His, Phe, Arg, Asp, Gln was 0.1–10 μM; for Gly and Lys, 1–10 μM; and for Ser, 2.5–10 μM. The lower limits of these ranges correspond to the limit of quantification (LOQ). The detection of Asp, Ser, Gly, Gln, and Asn was affected by signal suppression. Asn was the most compromised and could not be quantified at the concentration levels required for this series of experiments. It has to be noted that the high concentration of salts in the iontophoretic samples (30 mM NaCl and the other extracted salts) produced signal suppression in the first part of the chromatogram. Results for amino acid detection in this analysis time window (Asn, Ser, Gly, Asp and Gln) were slightly higher with a CV inferior to 25%. This value was considered acceptable given the important variation between extractions due to differences between different porcine ear skins.

2.4. Data analysis and statistics

Iontophoretic fluxes of all amino acids were directly calculated from the amount (mol) extracted in each collection interval normalized for the duration of the interval (h) and extraction surface (cm^2). For the analysis of transport direction, and efficiency of extraction, the 6-h steady-state fluxes, were considered. Linear regression of the amino acid fluxes as a function of their subdermal concentration was performed.

To identify a relationship between flux and degree of ionization (pI), the amino acid fluxes were normalized by the corresponding subdermal concentration.

Data are expressed as mean ± standard deviation (SD). Statistical tests and linear regression analysis were performed with GraphPad Prism 4.00 software (San Diego, CA). The significance of the linear regressions was tested by the corresponding ANOVAs. The level of statistical significance was fixed at $\alpha \leqslant 0.05$.

3. Results and discussion

3.1. Skin reservoir

Detectable amounts of all amino acids were extracted iontophoretically to both electrodes. Fluxes were highest in the initial phase of current passage (i.e., in the first collection interval), before decreasing to relatively constant values after ~3 h of iontophoresis (see Fig. 1). Even when the subdermal compartment contained no exogenously added amino acids, there were measurable quantities found in the electrode chambers suggesting the presence of a naturally occurring reservoir in the skin (as has been reported for a number of other substances, including glucose, urea and lactate, for example [3,7,8]). From the practical standpoint of monitoring systemic amino acid levels, this means that a pre-iontophoresis period would be necessary to deplete the analyte in the skin before the extraction flux could mirror accurately the subdermal concentration (as is the case for glucose, of course).

3.2. Amino acid electrotransport

Iontophoretic extraction of all amino acids increased with increasing subdermal concentration. Fig. 1 illustrates this behaviour for arginine, glutamic acid and proline, representing, respectively, examples of cationic, anionic, and zwitterionic amino acids at physiological pH. Table 1 summarises the steady-state iontophoretic fluxes of all the amino acids from subdermal solutions in which they were present in the concentration range of 0–500 μM.

For the positively charged amino acids Arg, Lys, and His, extraction towards the cathode was dominant and highly correlated with subdermal concentration (r^2 ranging from 0.90 to 0.96, Table 1). At the anode, extraction levels were close to or below the LOQ, and no correlation existed between subdermal concentration and extraction flux. Similarly, the negatively charged amino acids, Asp and Glu, were extracted efficiently towards the anode, with the extraction flux being highly correlated with subdermal concentration (r^2 of 0.88 and 0.95, respectively). At the cathode, in contrast, little or no amounts of these amino acids were detected. Zwitterionic amino acids were extracted towards both the anode and cathode, with the latter showing the higher fluxes. In both cases, extraction rates increased with subdermal concentration, the correlation being better towards the cathode (r^2 from 0.76 to 0.90) than the anode (r^2 from 0.41 to 0.82, or not significant). Because extraction levels were lower for the zwitterions, and much closer to the LOQ, analytical error has a greater impact on these data, as compared to those for the charged amino acids.

The charged amino acids are moved across the skin in the presence of an electric field by electromigration, while the principal mechanism operative for the zwitterionic species is the less efficient electroosmosis. Hence, Arg and Lys were extracted to the cathode at up to 8-fold, the rate observed for the zwitterions; similarly, the electrotransport of Asp and Glu to the anode was ~20 times that of the net neutral amino acids in this direction. It is worth noting, that for all amino acids, the absolute concentrations in the collected, hourly samples were in the nanomolar (and often low nanomolar) range.

The results for phenylalanine can be compared with previously published data on the iontophoretic extraction of this amino acid [12]. In that earlier work, Phe fluxes of 3.9, 19.5, and 33.6 nmol cm^{-2} h^{-1} were measured from subdermal concentration of 1, 5 and 10 mM, respectively. Fig. 2 plots these results, together with those from this study, on the same graph and demonstrates a highly linear correlation over a 100-fold range of concentration (and despite differences in the background electrolyte/buffer used). As plasma levels up to 1.5 mM have been observed in patients with phenylketonuria, it follows that the results in Fig. 2 are relevant to a clinical application of reverse iontophoresis, as has been very recently demonstrated by the work of Longo et al. [13].

As mentioned above, the principal direction of amino acid electrotransport depends on the degree and polarity of ionisation. Table 2 provides this information for 15 of the amino acids at physiological pH (Asn, Gln, Ser, and Gly are not considered here because their quantification was affected by signal suppression). Apart from the completely negatively charged Glu and Asp, therefore, extraction of all other amino acids would be expected to be primarily towards the cathode. Table 2 confirms that this was indeed the case. Parenthetically, the behaviour observed parallels earlier findings in which amino acids were delivered from the electrode compartments across the skin [19]. It should also be mentioned that the percentage extraction of the zwitterionic amino acids towards the cathode increased (from 63% to 80%) with increasing subdermal concentration. This can be explained by the fact that, when the subdermal concentration is higher, there is less 'contamination' of the amount extracted by amino acid originating

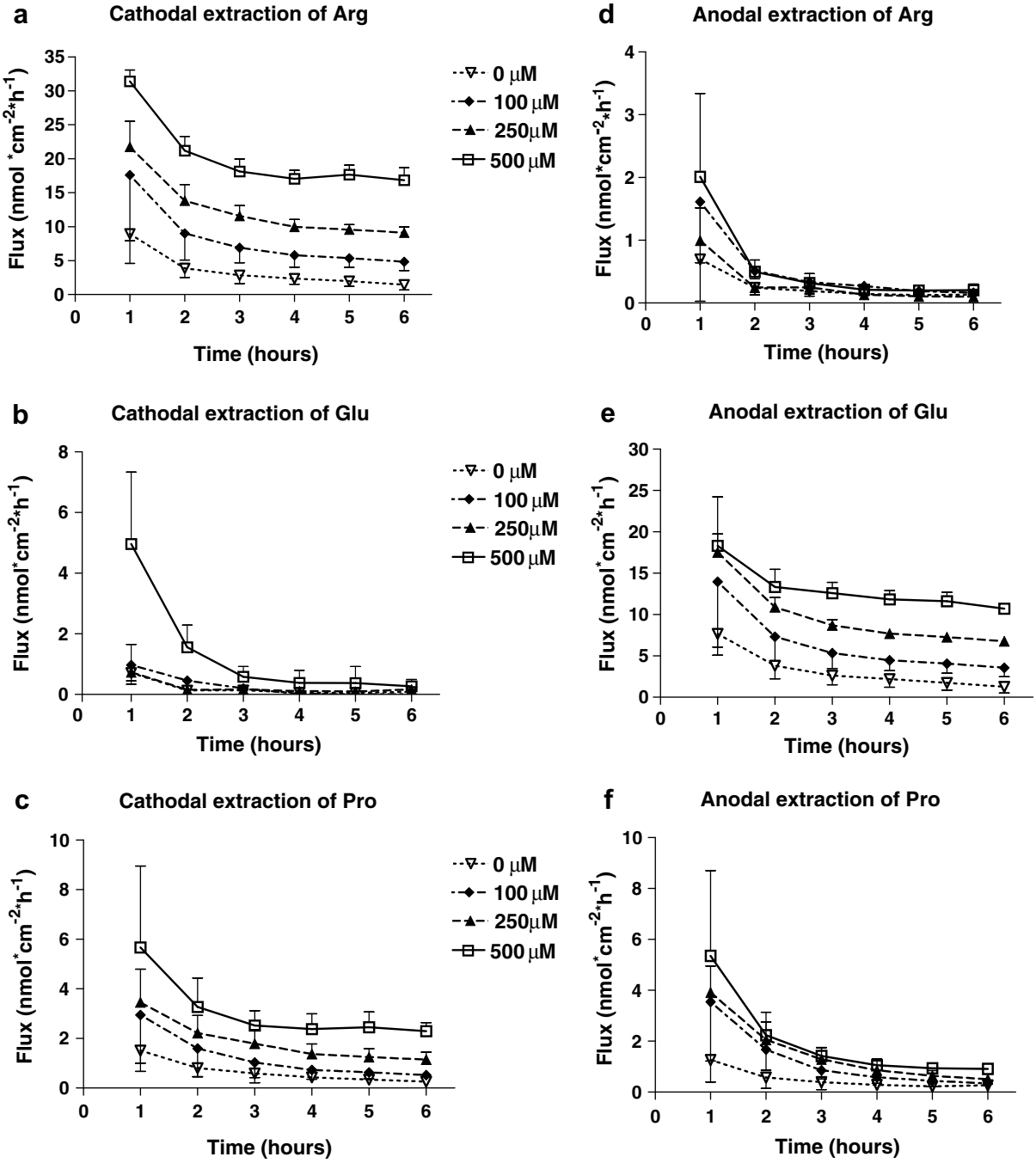

Fig. 1. Electrotransport of selected amino acids as a function of time and of their subdermal concentration: arginine (a and d), a cation; glutamic acid (b and e), an anion; and proline (c and f), a zwitterion. The panels on the left show extraction towards the cathode, those on the right towards the anode.

from the skin reservoir; furthermore, the analytical error is less because the concentrations detected are greater and further from the LOQ.

3.3. Structure-transport relationship

The typical parameters used to assess a relationship between iontophoretic transport and molecular structure include lipophilicity, charge-to-mass ratio, and molecular size (mobility) [19–22]. The amino acids chosen for investigation here, however, are rather limited in terms of the ranges of these properties which they cover. Nevertheless, it is clear that the degree and the polarity of amino acid ionisation is an important determinant, and this can be demonstrated by a consideration of their electrotransport as a function of the corresponding values of isoelectric point (pI).

To make maximum use of all the data obtained, the measured steady-state fluxes after 6 h of iontophoresis ($J_{ion,6}$) were first normalised by the relevant subdermal concentration (C_{subd}), yielding three iontophoretic permeability coefficients ($k_{ion,P} = J_{ion,6}/C_{subd}$) per amino acid. For cationic and zwitterionic amino acids, fluxes towards the cathode were used and were attributed a positive sign; for the negatively charged Asp and Glu, their fluxes towards the anode were employed and were assigned a negative value.

Fig. 3 is a plot of $k_{ion,P}$ as a function of pI, and demonstrates a strong correlation between these parameters. The small, but positive, electrotransport in the anode-to-cathode direction of the

Table 1
Iontophoretic extraction fluxes of amino acids after 6 h of current passage (mean ± SD; $n = 6$)

	Flux towards cathode (nmol cm^{-2} h^{-1})					Flux towards anode (nmol cm^{-2} h^{-1})				
	0 μM	100 μM	250 μM	500 μM	r^2	0 μM	100 μM	250 μM	500 μM	r^2
Arg	1.4 ± 0.7	4.8 ± 1.3	9.1 ± 0.8	16.1 ± 1.5	0.96	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	n/a
Lys	2.1 ± 0.8	5.3 ± 1.6	8.9 ± 0.9	16.5 ± 1.6	0.96	< LOQ	< LOQ	< LOQ	< LOQ	n/a
His	0.5 ± 0.3	1.6 ± 0.6	3.7 ± 1.1	5.4 ± 0.5	0.90	0.3 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.5 ± 0.2	ns[c]
Asp[a]	<LOQ	<LOQ	<LOQ	<LOQ	n/a[b]	0.3 ± 0.2	2.4 ± 0.7	4.6 ± 0.6	7.7 ± 1.7	0.88
Glu	<LOQ	<LOQ	0.2 ± 0.1	0.2 ± 0.2	n/a[b]	1.3 ± 0.7	3.6 ± 1.1	6.8 ± 0.5	10.4 ± 0.8	0.95
Asn[a]	<LOQ	<LOQ	<LOQ	<LOQ	n/a[b]	<LOQ	<LOQ	<LOQ	<LOQ	n/a[b]
Ser[a]	<LOQ	<LOQ	<LOQ	<LOQ	n/a[b]	<LOQ	2.9 ± 1.5	4.1 ± 1.8	3.2 ± 1.9	n/a[b]
Gly[a]	<LOQ	<LOQ	<LOQ	<LOQ	n/a[b]	1.0 ± 0.9	2.6 ± 1.2	3.3 ± 1.2	3.5 ± 1.1	ns[c]
Gln[a]	0.1 ± 0.1	0.2 ± 0.2	0.5 ± 0.2	0.5 ± 0.3	ns[c]	0.2 ± 0.2	0.2 ± 0.2	0.4 ± 0.1	0.4 ± 0.3	ns[c]
Thr	0.4 ± 0.3	0.8 ± 0.3	1.7 ± 0.5	2.6 ± 0.4	0.88	0.3 ± 0.2	0.6 ± 0.2	0.7 ± 0.2	0.8 ± 0.3	ns[c]
Ala	0.9 ± 0.5	1.5 ± 1.0	2.7 ± 0.5	3.3 ± 0.5	0.76	0.6 ± 0.4	0.7 ± 0.3	1.2 ± 0.4	1.4 ± 0.6	ns[c]
Pro	0.3 ± 0.2	0.5 ± 0.2	1.1 ± 0.3	2.3 ± 0.4	0.90	0.3 ± 0.3	0.3 ± 0.2	0.5 ± 0.2	0.7 ± 0.2	0.45
Val	0.3 ± 0.2	0.7 ± 0.2	1.0 ± 0.1	2.5 ± 0.5	0.87	0.1 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.6 ± 0.1	0.58
Tyr	0.2 ± 0.1	0.5 ± 0.2	0.6 ± 0.1	2.2 ± 0.5	0.81	0.2 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.5 ± 0.1	ns[c]
Met	0.1 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	1.9 ± 0.5	0.79	< LOQ	0.2 ± 0.3	< LOQ	0.3 ± 0.1	n/a[b]
Ile	0.1 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	2.1 ± 0.5	0.83	0.1 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	0.4 ± 0.1	0.82
Leu	0.3 ± 0.2	0.7 ± 0.3	1.1 ± 0.2	2.5 ± 0.5	0.88	0.2 ± 0.2	0.4 ± 0.2	0.4 ± 0.1	0.6 ± 0.2	0.41
Phe	0.2 ± 0.1	0.5 ± 0.2	0.7 ± 0.1	2.2 ± 0.6	0.80	0.1 ± 0.1	0.3 ± 0.2	0.3 ± 0.1	0.5 ± 0.2	0.42
Trp	0.1 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	2.3 ± 0.6	0.81	< LOQ	0.2 ± 0.2	< LOQ	0.6 ± 0.4	n/a[b]

[a] Result affected by signal suppression during LC-MS analysis.
[b] Not applicable. r^2 values were not calculated because the levels extracted, in at least one case, were below the LOQ.
[c] Slope not significant.

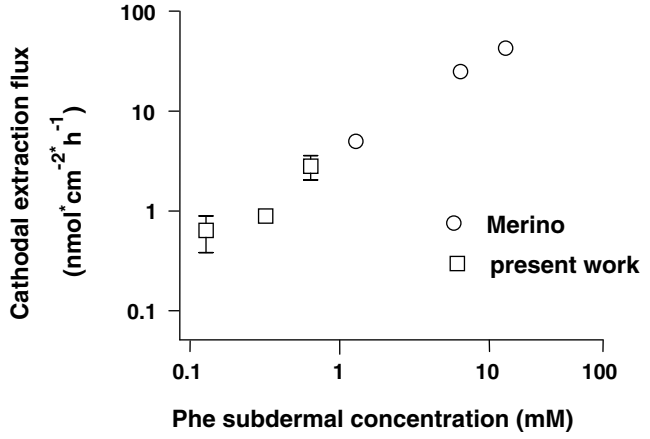

Fig. 2. Reverse iontophoretic extraction of phenylalanine towards the cathode as a function of its subdermal concentration. Results from this work, and from the earlier publication of Merino et al. [12], are shown.

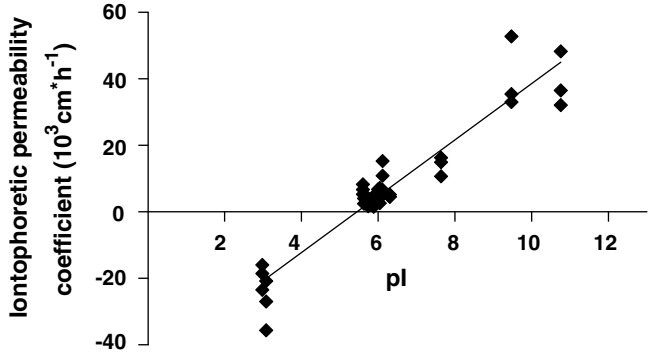

Fig. 3. Iontophoretic permeability coefficient ($k_{ion,P}$) plotted as a function of isoelectric point (pI) for a series of amino acids. The linear regression through the data is: $k_{ion,P} = 8.5\ pI - 46$; $r^2 = 0.90$.

Table 2
Ionisation state at pH 7.4 and transport direction of amino acids when present at subdermal concentrations of 100, 250 and 500 μM, respectively

	Cation	Anion[a]	Zwitterion	100 (μM)		250 (μM)		500 (μM)	
				% Anode	% Cathode	% Anode	% Cathode	% Anode	% Cathode
Arg	97.8	0	2.2	3	97	1	99	1	99
Lys	97.3	0.1	2.7	0	100	0	100	0	100
His	3.8	1.7	94.5	18	82	8	92	8	92
Asp	0	100	0	100	0	99	1	100	0
Glu	0	99.9	0.1	100	0	98	2	98	2
Thr	0	2.0	98.0	41	59	30	70	23	77
Ala	0	0.5	99.5	37	63	31	69	29	71
Pro	0	0.1	99.9	39	61	31	69	24	76
Val	0	0.6	99.4	36	64	25	75	19	81
Tyr	0	1.9	98.1	45	55	32	68	20	80
Met	0	1.5	98.5	33	67	16	84	14	86
Ile	0	0.6	99.4	34	66	23	77	16	84
Leu	0	0.6	99.4	34	66	26	74	19	81
Phe	0	1.8	98.2	40	60	28	72	19	81
Trp	0	1.0	99.0	33	67	19	81	20	80
			Average zwitterions	37 ± 4	63 ± 4	26 ± 5	74 ± 5	20 ± 4	80 ± 4

Note: Asn, Ser, Gly, and Gln are not included since data may be biased due to signal suppression.
[a] Since the fraction of di-anions is only minor, di-anions and mono-anions are grouped together. No di-cations are present at this pH.

zwitterions is the result of the skin's net negative charge at physiological pH and the predominant direction of electroosmotic flow. The strong contribution of electromigration to the transport of the charged amino acids is clear. Interestingly, the *x*-axis intercept of the graph occurs at pI = 5.4, a value not too different than that which has been reported in the literature for the isoelectric point of porcine skin [23].

In summary, it has been shown, in an *in vitro* simulation of a clinical situation, that the reverse iontophoretic extraction of naturally occurring amino acids is feasible and is sensitive to their subdermal concentrations. The efficiency of extraction was, not surprisingly, greater for charged amino acids than for zwitterionic species. There was a linear relationship between iontophoretic permeability and amino acid pI, with the electrotransport increasing as the difference between this parameter and the isoelectric point of the skin became greater. The results provide a foundation for further *in vivo* investigation of the non-invasive monitoring of amino acids by reverse iontophoresis.

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