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A quantitative minimally invasive assay for the detection of metals in the stratum corneum

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Abstract

A quantitative, minimally invasive tape-stripping assay for the detection of metals on and in skin that also has application to the detection of metallic elements on dry surfaces (where human contact could occur) has been developed. This development included construction, using commercial products, of an approximately 25 μm thick, low-metal content tape suitable both for tape-stripping and elemental analysis. Individual tapes were sequentially applied to the skin surface and then removed, taking with them a sample of the dead outer layer of the skin (stratum corneum). Analysis of such tape strip samples by particle induced X-ray emission (PIXE) — a well-characterized, sensitive, analytical technique based on X-ray spectrometry — identified and accurately quantified the metals in the sample. The assay had elemental sensitivities of approximately 1 ng/cm^2 for many metals and analysis of elemental contents could be performed in as little as 5 min. The feasibility of the assay for measuring metals in the stratum corneum was demonstrated on the forearms of healthy human volunteers. Samples from approximately half the subjects were found to contain zirconium, possibly arising from the use of roll-on antiperspirants. The assay has potential as a tool: (1) for risk assessment, (2) to identify exposure levels following possible contact with a hazardous metal, and (3) to determine the effectiveness of cleanup or removal measures.

Keywords: Tape-stripping; Skin; Particle induced X-ray emission; Stratum corneum

1. Introduction

Many metals and metal-based compounds, which are inherently toxic to biological systems, are present in occupational and environmental settings [1]. Growing occupational and environmental exposure to metals and metal-based compounds raises concerns about their potential effects on human health.

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Three major routes of chemical uptake are ingestion, inhalation and dermal absorption. The skin is the largest organ of the body (surface area approximately 18 000 cm^2), giving it the potential to absorb significant amounts of contaminants [2]. Although skin is an effective barrier to most xenobiotics, skin contact remains an important route of exposure for some hazardous metals or metal compounds since (a) metals are absorbed through the skin; (b) skin can act as a reservoir for metals; (c) skin surface deposition can be an important source of secondary contamination; and (d) impairment/loss of skin barrier function can occur. Upon skin entry, metals can have many deleterious health effects that include hypersensitive/allergic response or skin cancer [3]. Although skin is an important route of exposure for some hazardous metals or metal compounds there are no well developed, minimally-invasive assays that can assess trace metal contents in the outer dead layers of the skin (stratum corneum).

A reliable minimally-invasive assay for the detection of metals on and in the skin would be a valuable tool: (1) for risk assessment; (2) to identify exposure levels following possible contact with a hazardous metal, particularly if some indication of the sites and routes of deposition were obtainable; and (3) to determine the effectiveness of cleanup or removal measures. Such an assay might also aid in the diagnosis of individuals with allergic or contact hypersensitivity reactions and could also provide insight into the extent and time course of the percutaneous absorption of metals and metal compounds for which there are inadequate animal models and little epidemiological data. Furthermore, if the assay has general application to the detection of metals on a variety of surfaces it could be used to establish the presence of potentially toxic metals at a particular site (or under certain conditions), and thus the potential for exposure.

This paper details the development of a quantitative, minimally invasive tape-stripping assay for the detection of metals on and in skin that also has general application to detection of elements on a variety of surfaces including surfaces where human contact could occur. Assay development involved the manufacture of a low-metal content adhesive tape using commercially available components suitable both for tape-stripping applications and elemental analysis. The individual tapes were applied to the skin surface and then removed, taking with them a sample of stratum corneum. Analysis of these tape strip samples by particle induced X- ray emission (PIXE) — a well-characterized, analytical technique based on X-ray spectrometry [4] — identified and accurately quantified the metals in the sample. The feasibility of the assay for measuring metals on and in the stratum corneum was demonstrated on the forearms of healthy human volunteers.

2. Materials and methods

2.1. Tape-stripping of the skin surface as an analytical technique

The outermost layer of the human epidermis, the stratum corneum, consists of dead cells (corneocytes) in a lipid matrix. A sample of the stratum corneum (essentially a sheet of corneocytes) can be obtained by evenly applying and then carefully removing a strip of adhesive tape. This technique has been widely used to study the localization and distribution of substances within the superficial layers of the skin [5–7]. Approximately 30 such tape strips will remove most of the stratum corneum on the inner surface of the forearm [8]. While the amount of stratum corneum removed with each such tape strip varies as a function of several factors [8–11], tape-stripping is an inexpensive, minimally invasive means of sampling skin, and is of sufficient utility to have recently been proposed by the FDA as part of a standard method to evaluate bioequivalence of topical dermatological dosage forms [11].

2.2. Particle induced X-ray emission (PIXE)

PIXE utilizes a high energy (MeV) proton beam that interacts primarily with atomic electrons in a specimen to create vacancies within inner electron shell orbitals. When a vacancy is filled by an outer shell electron, the excess energy resulting from the transition can be released as an X-ray photon

whose energy is characteristic of the emitting atom. The technique is a routine analytical tool for determining the concentration of elements with atomic number greater than 12 in a variety of specimen types [4]. PIXE can be utilized to provide a bulk analysis of a sample's elemental contents and spatial microanalyses of element distributions within a sample [4,12,13].

PIXE analysis is similar in concept to standard elemental analysis with electron microscopy. However, the lack of primary particle bremsstrahlung enables PIXE to be carried out with little X-ray background in comparison to the electron analogue [4]. This reduction in X-ray background enables high analytical sensitivity to be obtained and elemental sensitivities can approach the 0.1 mg/kg level [4]. PIXE is analytically quantitative and is much less affected by problems of quantitative accuracy than mass spectrometry techniques. With appropriately chosen beam currents and beam current densities thermal damage to the specimen is minimal thereby ensuring the quantitative accuracy of PIXE analyses [4]. Furthermore, PIXE possesses simultaneous multi-element detection resulting in time efficient elemental analyses. Owing to it's sensitivity, simultaneous multi-element detection, and ease of quantitation PIXE is well suited to the quantitative analysis of major, minor and trace metals in materials sampled by tape-stripping.

2.3. Assay requirements

In order to reliably quantitate the elemental contents of a sample obtained by tape-stripping the tape should ideally contain no elements of interest or, more practically, contain concentrations of these elements that are below the analysis technique's limit of detection. Since samples were to be analyzed by PIXE endogenous metal contents in the tapes needed to be below the mg/kg level.

As MeV energy protons can penetrate many microns beneath the surface of the specimen, PIXE yields a volume analysis [4]. If the material to be analyzed on a tape is thin ($\leq$ a few μm) use of too thick a tape can result in reduced analytical sensitivity. Additionally, if the proton beam does not penetrate through the tape, highly localized energy deposition can occur at the beam's end of range. With large current densities the energy deposition can be sufficient to result in sample distortion and, in some instances, destruction as the irradiated region of the tape melts. To obtain good sensitivity and to minimize the thermal effects the tape should be as thin as practical yet strong enough to resist the strain of application to and removal from the sampled surface.

Nine commercial 'sticky tapes' were initially analyzed with PIXE and all were found to contain unacceptable levels (>20 mg/kg) of one or more transition metal elements. Furthermore, two thirds of the tapes were sufficiently thick to stop the 3 MeV proton beam. Consequently, a thin low metal content custom tape was developed using commercially available components suitable both for tape-stripping applications and PIXE analysis.

2.4. Manufacture of custom tapes

Tapes usually consist of two parts, a backing (usually some type of plastic) and an adhesive. In addition, an easy release removable plastic covering or release liner protects the tack (stickiness) of the adhesive prior to use and protects the virgin tape from potential contamination.

Several commercially available plastics were analyzed for suitability as the tape backing. The majority of these plastics contained significant amounts (>50 mg/kg) of transition metals. However, Dartek 0-401 machine direction oriented nylon type 6,6 film (DuPont Canada, Whitby, Ont., Canada) was found to contain no discernible quantities of elements with an atomic number greater than 12 when analyzed via PIXE. Furthermore, this nylon could be readily obtained from Dupont in a film of thickness of 15.2 μm and a density of 1.135 g/cm^3. The film had a tensile strength of 2450 kg/cm^2, a tensile modulus of 23 000 kg/cm^2 and a tear strength of 32 g/μm.

Duro-Tak (R) 87-2510 liquid resin adhesive (National Starch and Chemical, Bridgewater, NJ, product number 87-2150) was chosen as the adhesive (However, other metal free adhesives may work equally well and could provide a range of tack). PIXE analyses revealed that this adhesive

contained no detectable quantities of elements with an atomic number greater than 12. The adhesive also had sufficient tack to remove samples of stratum corneum in skin tape-stripping applications. A 76 μm thick clear polyester plastic, Rexam Grade 15787 S 3MIL CL PET 92A/100 (Rexam Release, Bedford IL) was selected for the release liner. One side of this plastic was coated with an ultraviolet cured silicone formulation. PIXE analyses revealed that the coated side contained no detectable elements with an atomic number greater than 12 apart from silicon at a concentration of approximately 2 $\mu g/cm^2$, while the uncoated side contained no detectable quantities of elements with an atomic number greater than 12.

The tapes were constructed from Dartek 0-401 film, Duro-Tak (R) 87-2510 liquid resin adhesive and Rexam release liner using the National Starch and Chemical Company, 'Standard Coating, Drying and Transfer Procedure for Solution Pressure Sensitive Adhesives' (National Starch and Chemical, Bridgewater, NJ, Standard operating procedure Number 250, revision date 25 July 1995).

With this procedure adhesive was initially applied to the silicone coated side of an approximately 1 m length of the release liner (the Rexam release liner was supplied as a roll of width 30 cm and a length of several meters) using a drawdown coater (ChemInstruments, Fairfield, OH). The coated release liner was then cut into individual sheets of size approximately 20×30 cm^2 using a paper cutter and a pre-cut piece of release liner to act as a barrier between the cutter and the adhesive. The adhesive on each sheet was allowed to dry at room temperature for 20 min before the sheets were placed in a LAC series bench oven (Despatch Industries, Minneapolis, MN) with forced convection airflow at 250°F for 3 min. The sheets were subsequently cooled to room temperature.

Test samples (2.5×12.5 cm^2) were cut from the first and the last individual sheets. The adhesive was manually removed from these test samples by rolling it between the fingers. Complete removal of the adhesive from the release liner was readily achieved owing to the silicone formulation coating on the liner. The adhesive from each test sample was subsequently weighed using a self-calibrating microbalance (with 10 μg precision and accuracy). Knowing the weight of the adhesive, the adhesive density and the area of the release liner from which the weighed adhesive was obtained an estimate of the adhesive thickness could be made. Use of this method yielded an average thickness for the adhesive of approximately 10 μm.

Dartek backing was subsequently placed over the uncovered portions of adhesive and smoothed down with light pressure applied by a squeegee. This layered structure was then cut into 7.5×2.5 cm^2 pieces with a paper cutter to produce tapes protected by a release liner. The protected tapes were stored in sealed, ultra-clean, dry plastic containers.

As the Dartek backing had a thickness of approximately 15 μm, and the adhesive had a thickness of approximately 10 μm the manufactured tapes had a nominal thickness of approximately 25 μm. This thickness was verified by measuring the thickness of nine tapes (with the release liner removed) using a micrometer. All nine tapes were found to have a thickness between 24 and 28 μm.

2.5. Tape-stripping protocol

An approximately 7.5×2.5 cm^2 area on the volar forearm of each human subject was selected as the skin site to be tape stripped. Subjects participating in the tape-stripping studies were asked to use no lotions (or similar topical creams with the exception of soap) on or near the site to be tested for 3 days prior to testing. All jewelry, watches and clothing were removed from the sampled site on each subject prior to tape-stripping. Using a cotton swab, the site was swiped with distilled water five times to clean the skin and compressed air was used to dry the site for a duration of 30 s. An area outlining the area to be tape-stripped was then marked with a permanent marker. To reduce the risk of sample contamination the ink markings were made on the boundaries of an approximately 8.5×3 cm^2 area. The 7.5×2.5 cm^2 tapes were placed within this area and had no contact with the ink. No ink markings were made in the area from where the samples were taken.

Prior to the preparation of each skin site the release liners were removed from the tapes to be used in the sampling. The silicone coating on the release liner enabled easy release of the liner from the adhesive. In no instance was adhesive visually detected on the release liners that had been removed from the tapes. The uncovered tapes were weighed using a self-calibrating microbalance (with 10 μg precision and accuracy) and then placed in a sealed ultra-clean plastic container prior to skin sampling to reduce the possibility of contamination by air. The average mass of the virgin tapes (expressed as a mean $\pm$ S.D.) was 53 ± 1 mg and all tapes had a mass between 50 and 57 mg. Whenever possible the tapes were handled with plastic tweezers during the sampling of the stratum corneum to reduce the possibility of elemental contamination.

For skin sampling the tapes were placed within the selected skin site. To ensure that the tape adhered to the stratum corneum surface, pressure was applied in a back and forth motion for 7–10 s with the rounded (closed) end of a pair of plastic tweezers. The tape was then gently peeled away from the skin taking with it a sample of stratum corneum. If required this procedure was repeated several times on the selected site to obtain a depth profile of a substantial fraction of the stratum corneum. All tape strips were again weighed individually after tape-stripping to obtain an estimate of the mass of stratum corneum removed from the sample area.

After sampling the stratum corneum each tape was cut in half to produce two samples. Each sample was stretched over a 15 mm diameter hole in a 4×2 cm^2 plastic PIXE analysis target frame. The remaining tack of the adhesive on the tape enabled the samples to adhere to the target holders. Excess tape protruding over the edges of the target holder was trimmed off with a sharp knife. The samples were then placed in separate sealed, dry ultra-clean plastic containers to reduce the possibility of contamination. For each tape PIXE analysis was performed on one sample while the second sample was archived.

2.6. PIXE analysis of tapes

The samples were examined at the PIXE/nuclear microprobe facility at the Lawrence Livermore National Laboratory [12]. With this system, approximately 25 keV H^- ions were produced by an off-axis duoplasmatron ion source and accelerated and stripped in a tandem electrostatic accelerator to produce MeV energy protons. The proton beam energy utilized in these studies was 3 MeV. These energetic ions subsequently passed through the field of an energy analyzing magnet and a slit which controlled the energy stability of the accelerator. The PIXE beamline/nuclear microprobe lay 0.5 m downstream of the energy stabilization slit. At the entrance to the proton microscope the central portion of the beam core was first selected and then further collimated by sets of slits before interacting with the specimen in the target chamber. The target chamber was maintained at a pressure of approximately 10^{-6} torr during sample analysis.

Samples were analyzed using a collimated 3 MeV proton beam of dimensions 1×1 mm^2. Only regions of tape located over the 15 mm diameter hole in each target frame were analyzed. The 3 MeV proton beam could readily penetrate the approximately 25 μm thick tape with a residual energy of approximately 2.5 MeV enabling reliable charge collection to be made by a Faraday cup located 10 cm behind the sample (reliable charge collection is a requirement for accurate and precise elemental quantitation). Analyzed areas on each tape sample were irradiated with an exposure of 4.0 μC.

Proton induced X-rays were detected with an energy dispersive Iglet-X X-ray detector (EG&G Ortec, Oakridge, TN) with a 100 μm thick beryllium window. The window was sufficiently thick to prevent scattered protons from entering and potentially damaging the detector crystal. However, the window heavily attenuated low energy (approximately 1 keV or less) X-rays including those from elements with atomic number up to 12 making analysis of elements such as sodium and magnesium impractical. The detector was located at an angle of 135° with respect to the incident beam, and subtended a solid angle of approxi-

mately 100 msr to the specimen. For the stratum corneum data shown in the results no additional X-ray filters were placed between the sample and the X-ray detector. As a result beam currents of several nA produced X-ray pulse pileup peaks from the relatively intense characteristic X-rays arising from S, Cl, K and Ca in the stratum corneum samples. These pulse pileup peaks reduced sensitivity for several transition metal elements. To achieve improved sensitivity for transition metals beam currents were limited to 2 nA resulting in X-ray count rates of approximately 2000/s and irradiation times of approximately 0.5 h.

Alternatively, additional X-ray filters could have been utilized to reduce the intensity of characteristic S, Cl, K and Ca X-rays. After collection of the stratum corneum data reported in the results a 0.2 mm thick annular mylar filter (inside diameter 1 mm, outside diameter 16 mm) was utilized to determine if the tapes could be analysed more time efficiently. The annular filter significantly attenuated the relatively intense X-ray signals from S, Cl, K and Ca whilst minimally affecting the count rates from X-rays with energies greater than 5 keV. For tapes containing samples of stratum corneum it was found that with this filter beam currents of 10 nA could be used for sample irradiation with minimal pulse pileup peaks present in the X-ray spectrum. Furthermore, analysis times could be reduced to approximately 5 min while maintaining the same analytical sensitivity, and precision as analyses conducted at lower beam currents without the use of a mylar X-ray filter.

Various beam current densities were examined to determine if the irradiation conditions employed for the PIXE analyses adversely affected the tape/stratum corneum sample integrity and element quantitation from stratum corneum samples. For current densities of up to 20 nA/mm^2 X-ray yields of elements per unit dose remained constant throughout the irradiation. Furthermore, the stratum corneum samples suffered no discernible morphological changes on the micron scale when viewed after irradiation apart from a discoloration of the irradiated region to a golden-brown color. However, elemental losses were observed for current densities of greater than 20 nA/mm^2 and the irradiated region of the tape melted for current densities of greater than 50 nA/mm^2.

X-ray spectra were stored on computer and analyzed off-line [14]. To obtain quantitative elemental concentration data all X-ray spectra were analyzed using the computational iterative PIXE spectrum fitting code PIXEF [15] to extract characteristic X-ray peak areas (or yields) for elements of interest. PIXEF has been tested on a range of certified standards and has a quantitative accuracy of better than 5% for a wide range of samples [15]. As the stratum corneum on each tape was less than 1 μm thick (determined by optical microscopy) and the tape strips were orientated with the stratum corneum facing both the incident ion beam and the X-ray detector elemental contents were calculated from the X-ray yields using the thin film approximation [4]. The measured elemental contents had units of g/cm^2 and a quantitative accuracy of approximately 95%.

PIXEF also determined the minimum number of X-rays needed for positive identification of a particular element in an X-ray spectrum [15]. This was accomplished by integrating the energy window corresponding to the background signal underneath the characteristic X-ray emission line used for the analysis of a given element. The extremes of this energy window were taken to be $\pm 3\sigma$ from the peak centroid where σ is the detector energy resolution at the peak centroid. The minimum number of X-rays or minimum detection limit (MDL) needed for positive identification of a particular element was taken to be $3.29 \times \sqrt{\text{background}}$ [15]. When appropriate, minimum detection limits were subsequently converted to minimum detectable concentrations using the thin film approximation.

To ensure the quantitative accuracy of the PIXE data the efficiency of the X-ray detection system was verified using a series of thin film elemental standards, each of thickness approximately 50 μg/cm^2 and having an uncertainty of 5% in certified thickness. These standards were analysed under similar irradiation conditions used

for the analysis of the tape strip samples. PIXE analysis yielded a measured elemental thickness for each standard that was within 5% of the certified thickness.

2.7. Tape-stripping studies

PIXE analysis was performed on nine blank tapes to determine whether any elements with atomic number greater than 12 were present in the blank tapes and to determine the minimum detectable concentrations for elements in material sampled by tape-stripping.

To test the feasibility of the tape-stripping protocol, tape-strip samples of stratum corneum were taken from 13 healthy human volunteers consisting of seven males and six females. For all the males and for five females in the study group, only one tape strip from the surface of the stratum corneum was taken. The other female had one sample of stratum corneum removed via tape-stripping per day from the same region on her forearm for 8 consecutive days. On a separate day the same female had her forearm consecutively sampled with ten tapes to obtain a depth profile of the outermost regions of the stratum corneum.

The degree of variation in X-ray yields arising from inhomogeneities in the stratum corneum coverage on tapes was examined by analyzing each of three tapes that had sampled stratum corneum in six separate 1×1 mm^2 regions. The three tapes came from different individuals: one female and two males. The six regions analyzed on each tape were from the four corners, the center and a region 2 mm directly above the center of an 0.8×0.8 cm^2 area located in the middle of the 15 mm diameter aperture in the target frame.

2.8. Analysis of zirconium and titanium contents in toiletries

Several of the tape strips from the human subjects contained detectable amounts of zirconium and titanium. Consequently, the female subject who participated in both the depth profile and time course studies supplied samples of her soap, shower gel, shampoo and roll-on antiperspirant for analysis of zirconium and titanium content to determine if the toiletries could be a possible source of these metals. Samples of soap, shower gel, shampoo and antiperspirant were mounted on blank tapes and allowed to dry. The sample masses on each tape were determined by weighing the mass of the tape before the application of the sample and after the sample had dried. The samples were subsequently analyzed by PIXE using the same protocol employed for analyses of the tape strips. The soap, shower gel and shampoo samples and one antiperspirant sample each had a dry mass of approximately 5 mg/cm^2. In addition a second antiperspirant sample was made using a thin layer of the antiperspirant smeared onto a blank tape. The dry mass of this thin antiperspirant sample was approximately 0.1 mg or approximately 5 μg/cm^2. Information on toiletry use by the other human subjects was not obtained. Furthermore, tape-strip samples were obtained in the middle of winter. Although some sunscreens do contain titanium, due to the season in which the tape strip samples were collected it was felt unlikely that the subjects would have used sunscreens the preceding month before sampling. Consequently, no inquiry was made as to sunscreen use.

2.9. Data presentation

When relevant, mean element concentrations and S.E.s in element concentrations were calculated from associated PIXE analyses of tape strip samples. Means and S.E.s in the mass of the stratum corneum sampled by the tapes were also calculated when relevant. Differences in the elemental contents between the male and female samples of stratum corneum were assessed by unpaired two tailed Student's t-tests as the t-tests were performed on independent groups. Differences in the mass of the stratum corneum sampled via tape-stripping for male and female subjects were also assessed by unpaired two tailed Student's t-tests. A significance level of < 0.05 was considered meaningful for the t-tests.

3. Results

PIXE analysis of the nine blank tapes revealed the tapes contained no detectable elements with atomic number greater than 10 apart from silicon. The silicon content in the blank tapes ranged from approximately 10 to 50 mg/kg (or 30–150 ng/cm^2 assuming silicon was on the tape surface). Silicon most likely arose from the silicone coating on the release liner adhering to the tape when the release liner was removed from the adhesive. Owing to the varying silicon content in the blank tapes the silicon contents were not determined in tapes that had sampled the stratum corneum. The minimum detectable concentrations of elements with atomic numbers between 13 and 40, apart from silicon, were calculated (Table 1). The minimum detectable concentrations ranged from approximately 30 ng/cm^2 for aluminum down to approximately 1 ng/cm^2 for many elements with atomic number greater than 25.

Table 1
Minimum detectable concentrations (MDL) of elements with atomic numbers between 13 and 40 apart from silicon in tapes derived from the analysis of nine blank tapes

Element	MDL (ng/cm^2)
Al	34
P	16
S	12
Cl	10
Ar	10
K	9
Ca	8
Sc	6
Ti	5
V	4
Cr	3
Mn	3
Fe	2
Co	2
Ni	1
Cu	1
Zn	1
Ga	1
Ge	1
As	0.5
Se	0.5
Br	0.5
Kr	0.5
Rb	0.5
Sr	0.5
Y	0.5
Zr	0.5

The study of all the tapes that had sampled the stratum corneum with a low power optical microscope revealed the distribution of stratum corneum coverage on each tape to be homogeneous at the millimeter level. Consequently, the areal mass of skin measured on each tape was assumed to be uniformly distributed across the tape at the millimeter level.

Table 2 shows a summary of all detectable elements, except silicon, from the PIXE analyses of six separate 1×1 mm^2 regions on the tapes that had sampled the stratum corneum from three different individuals. In no instance was an element detected in some regions of a tape and not detected in other regions of the same tape. For each tape sample the S.E.s in the element concentrations when expressed as a percentage of the mean element concentration obtained from the six measurements were typically less than 10%. Furthermore, the percentage S.E.s for a particular element, were similar in each of the three tapes. Areal skin masses are also listed in Table 2 and were consistent to within 6% for the three tapes.

Table 3 shows a summary of all detectable elements, except Si, from PIXE analyses of the tapes used to sample the same region of one individual's forearm once per day for 8 consecutive days. These elements were present in detectable quantities in all eight tapes. The S.E.s in the element concentrations when expressed as a percentage of the mean element concentration obtained from the eight measurements ranged from approximately 10 to 30%. Fig. 1 shows a plot of the element concentration versus day of sampling for the elements S, Cl, K, Ca, Ti, Fe, Cu, Zn and Zr. The average mass of the stratum corneum sampled on the eight tapes (expressed as a mean $\pm$ S.E.) was 0.52 ± 0.01 μg/cm^2 while the minimum and maximum masses were 0.49 and 0.54 μg/cm^2 respectively. Consequently, assuming uniform mass distribution of the stratum corneum across each tape at the millimeter level, the element concentration data from different tapes could be compared without normalization to the

Table 2
Mean element concentrations (ng/cm^2), and S.E.s expressed as a percentage of the mean from six separate 1×1 mm^2 regions on tapes used to sample the stratum corneum from three different individuals[a]

Element	Individual 1 (female) (mass = 52 μg/cm^2)		Individual 2 (male) (mass = 53 μg/cm^2)		Individual 3 (male) (mass = 50 μg/cm^2)	
	Mean	S.E.	Mean	S.E.	Mean	S.E.
S	703	2	938	3	539	3
Cl	189	6	277	8	298	8
K	82	11	141	9	204	10
Ca	187	3	128	7	163	5
Ti	10	9	7	7	4	8
Fe	25	8	20	10	35	7
Cu	3	5	1	5	2	5
Zn	5	7	3	7	6	8
Br	2	8	14	6	4	6
Zr	29	3	ND	NA	25	4

[a] Also included is the average mass of skin sampled by each tape. ND means not detected and NA means not applicable.

sampled skin areal density with an inaccuracy of approximately 10%.

Fig. 2 shows bar graphs of S, Cl, K, Ca, Ti, Fe, Cu, Zn and Zr concentration from the analysis of the tape strips sampling the stratum corneum from 13 human volunteers. The elements shown in Fig. 2 were present in detectable quantities in all 13 tapes. S.E.s in the element concentrations displayed in Fig. 2 when expressed as a percentage of the mean ranged from approximately 10 to 50%.

Si and Br were also present in all of the tape strip samples. The average Br concentration was approximately 4 ng/cm^2 in each of the total, male and female populations. However, the S.E.s associated with these Br element concentrations when expressed as a percentage of the mean were greater than 100%. The only other element detected in some of the samples was zirconium. Although, tape strips from five females and one male contained detectable amounts of zirconium, the other seven individuals did not contain zirconium in their stratum corneum down to the minimum detectable limits of approximately 0.5 ng/cm^2. Table 4 shows the measured concentration of zirconium in the tape strips from each of the 13 human volunteers and the mass of the sampled skin per square centimeter in the tape strips.

The average mass of the stratum corneum sampled on the 13 (expressed as a mean $\pm$ S.E.) was 0.51 ± 0.01 μg/cm^2. The average mass of the stratum corneum sampled on the six tapes from the female subjects (expressed as a mean $\pm$ S.E.) was 0.51 ± 0.01 μg/cm^2. The average mass of stratum corneum sampled on the seven tapes from the male subjects (expressed as a mean $\pm$ S.E.) was 0.51 ± 0.01 μg/cm^2. Student's t-tests performed on the skin mass distributions obtained from the male and female populations revealed no significant differences between the two populations at

Table 3
Mean element concentrations (ng/cm^2), and S.E.s expressed as a percentage of the mean obtained from analysis of the eight tapes used to sample the same region of one individuals forearm once per day for 8 consecutive days

Element	Mean	S.E.
S	843	15
Cl	326	15
K	204	25
Ca	215	18
Ti	18	29
Fe	32	12
Cu	2	7
Zn	5	11
Br	7	14
Zr	30	8

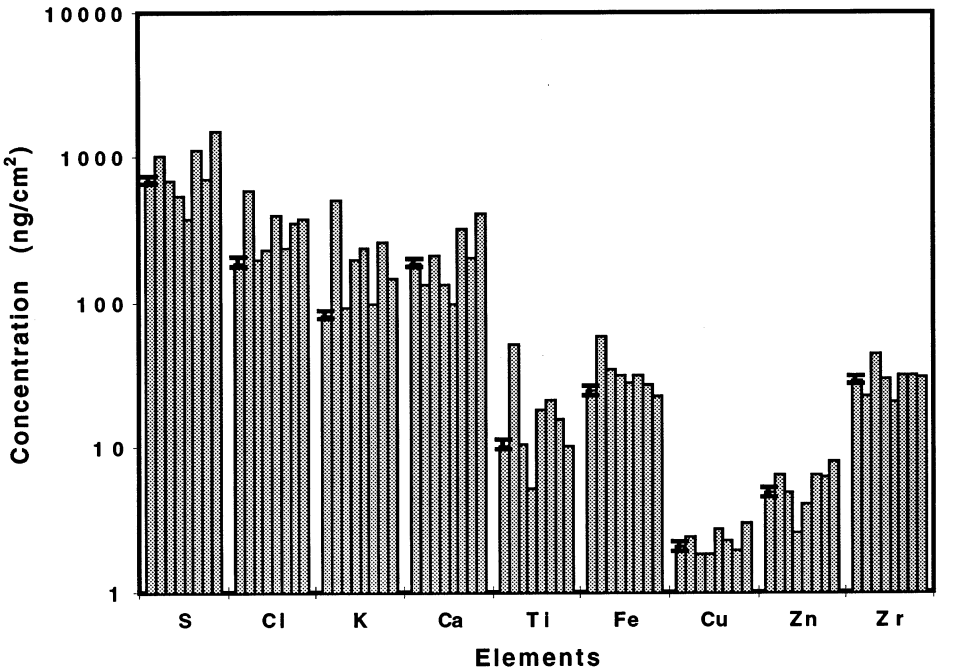

Fig. 1. Bar graphs of element concentration from analysis of the eight tapes used to sample the same region of one individual's forearm once per day for 8 consecutive days. For each element the data points from left to right correspond respectively with days 1–8. For each element the error bar on the day 1 data point corresponds to the uncertainty associated with the PIXE measurement of the element concentration.

significance levels of at least 0.05. The mass of stratum corneum on the 13 tapes varied between 0.48 and 0.54 μg/cm^2. Thus assuming uniform mass distribution of stratum corneum across each tape at the millimeter level the element concentration data from different individuals or groups of individuals could be compared without normalization to the sampled skin areal density with an inaccuracy of approximately 10%.

Student's *t*-tests were performed on each of the S, Cl, K, Ca, Ti, Fe, Cu, Zn and Br element concentration distributions (shown in Fig. 2) obtained from the male and female populations. For each element the *t*-test revealed no significant differences in element concentration between the two populations at significance levels of at least 0.05.

Table 5 shows the mass of skin on ten tapes used to obtain a depth profile of the outermost regions of the stratum corneum on one female's skin areal masses revealed a reduction in the amount of skin removed by sequential tape strips probing the stratum corneum. Microscopic analysis of the ten tape strip samples also revealed that the surface tapes sampled a greater amount of skin than the tape strips probing deeper into the stratum corneum. From Table 5 the mass of skin on the final tape is only two thirds of the mass of skin on the initial tape. Consequently, to reliably compare data from the ten tapes, element concentrations were normalized to the sampled skin areal density. This was achieved by dividing the elemental concentrations (ng/cm^2) from each tape by the associated areal mass of skin on the tape from Table 5 (μg/cm^2) to achieve normalized element concentrations in units of mg/kg.

Fig. 3A shows the plots of element concentration versus cumulative areal mass sampled by the tape strips for the elements S, Cl, K, and Ca, while Fig. 3B is a similar plot for the elements Ti, Fe, Cu, Zn, Br and Zr. Owing to the use of cumulative areal mass on the horizontal axis Fig.

3A and B are element depth profiles in the outer layers of the stratum corneum.

PIXE analysis of the tapes containing approximately 5 mg/cm^2 samples of toiletries revealed that the soap contained Ti at a concentration of approximately 4000 mg/kg and several other elements with atomic number between 14 and 30. However, the soap contained no zirconium at levels above 0.5 mg/kg. The shampoo and shower gel contained no detectable quantities of metals with an atomic number between 21 and 30, while the antiperspirant (Arm & Hammer deodorant anti-perspirant) was found to contain approximately 10 000 mg/kg Zr and approximately 3000 mg/kg Al. PIXE analysis of the tape containing a thin layer of the roll-on antiperspirant (approximately 5 μg/cm^2) revealed the presence of zirconium at a concentration of approximately 50 ng/cm^2, but not aluminum whose expected concentration, based on the analysis of the thicker antiperspirant sample, was approximately 15 ng/cm^2. The minimum detection limit for Al in the tape containing the thin sample of antiperspirant was calculated to be 33 ng/cm^2.

4. Discussion

Quantitative elemental contents of sodium, phosphorus, sulfur, chlorine, potassium, calcium, iron, copper and zinc in the different layers of the human epidermis have been previously reported and summarized [16]. Conversely, reports of titanium, and zirconium contents in human stratum corneum are not readily found in the scientific literature. Furthermore, previous methods have lacked quantitation and/or have not possessed sufficient spatial resolution to quantitatively study element depth profiles in the stratum corneum. The minimally invasive tape-stripping assay allows quantitative study of elemental profiles in the stratum corneum.

In this study phosphorus was not observed in any stratum corneum samples obtained by tape-

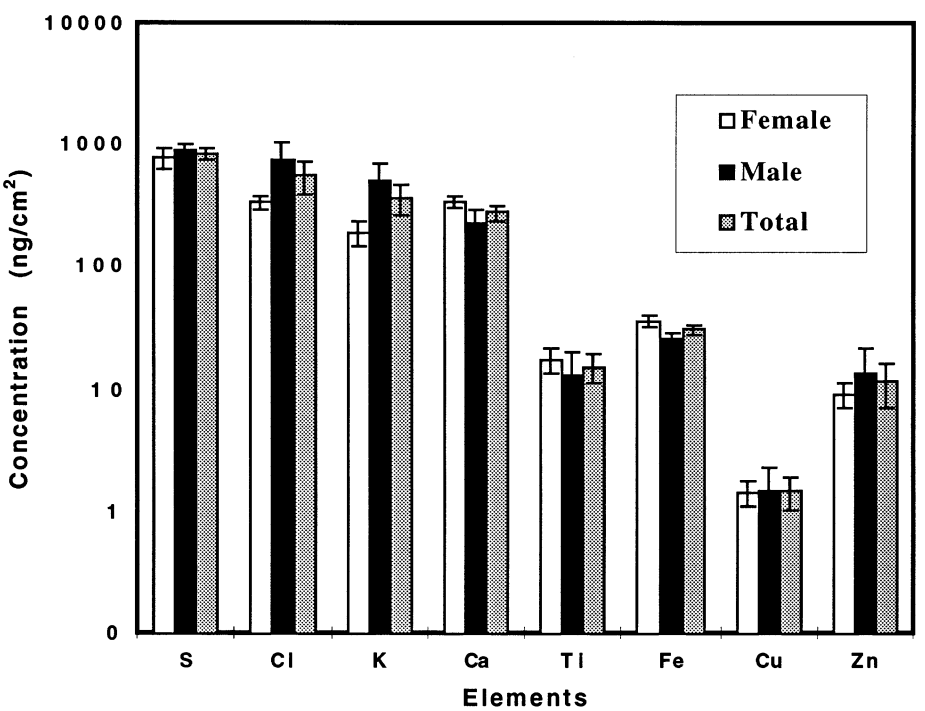

Fig. 2. Bar graphs of element concentration from the analysis of the tape strips sampling stratum corneum from human volunteers. Data points are expressed as the mean ± S.E. of measurements from six females, seven males and the total pool of 13 individuals.

Table 4
Measured zirconium concentrations in the stratum corneum specimens sampled by tape-stripping together with the mass of skin sampled by tape-stripping from 13 individuals[a]

Individual	Mass of skin ($\mu g/cm^2$)	Zirconium concentration (ng/cm^2)
Female 1	48 ± 2	13 ± 2
Female 2	52 ± 2	46 ± 6
Female 3	53 ± 2	58 ± 7
Female 4	49 ± 2	ND
Female 5	51 ± 2	9 ± 2
Female 6	50 ± 2	18 ± 3
Male 1	54 ± 2	25 ± 3
Male 2	52 ± 2	ND
Male 3	49 ± 2	ND
Male 4	51 ± 2	ND
Male 5	51 ± 2	ND
Male 6	50 ± 2	ND
Male 7	50 ± 2	ND

[a] The error associated with each mass measurement corresponds to the uncertainty in determining the mass of stratum corneum. The error associated with each zirconium measurement corresponds to the uncertainty associated with the PIXE measurement of the zirconium concentration.

Table 5
Measured mass of skin on ten tapes used to obtain a depth profile from the outermost regions of the stratum corneum on the forearm of one female subject[a]

Sample number	Areal mass of skin ($\mu g/cm^2$)	Cumulative mass ($\mu g/cm^2$)
1	51 ± 2	51 ± 2
2	43 ± 2	94 ± 4
3	44 ± 2	138 ± 6
4	40 ± 2	178 ± 8
5	41 ± 2	219 ± 10
6	38 ± 2	257 ± 12
7	42 ± 2	299 ± 14
8	36 ± 2	335 ± 16
9	30 ± 2	365 ± 18
10	35 ± 2	400 ± 20

[a] Sample 1 corresponds to the first tape strip (stratum corneum surface) while sample 10 corresponds to the final tape strip. The third column shows the cumulative areal mass sampled by consecutive tape strips.

stripping. From Table 1 the minimum detection limit of the tape-stripping assay for phosphorus was 16 ng/cm^2. The mass of stratum corneum sampled by the tapes was approximately 50 $\mu g/cm^2$, thus the approximate weight part per million minimum detection limit for phosphorus in the stratum corneum was approximately 300 mg/kg. Null detection of phosphorus is not surprising as, owing to the low levels of phospholipids and nucleic acids, the phosphorus content is less than 100 mg/kg in the stratum corneum [16]. Sodium and magnesium were also not observed in any stratum corneum samples owing to the low detection efficiency and the relatively poor sensitivity of PIXE for X-rays produced by these elements.

The sulfur, chlorine, potassium, copper and zinc areal masses shown in Tables 2 and 3 and

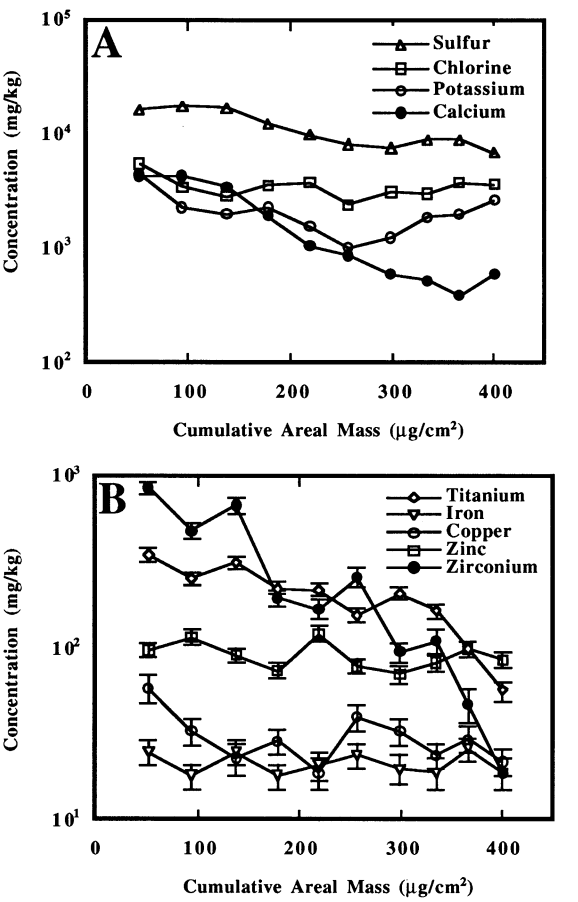

Fig. 3. Element concentration: (A) S, Cl, K and Ca; and (B) Ti, Fe, Cu, Zn and Zr vs. cumulative areal density from ten tapes used to obtain a depth profile from the outermost approximately 4 μm of the stratum corneum on the forearm of one female subject. In (A) the error bars are not shown as they were are smaller than the data points.

Figs. 1 and 2 when normalized to the mass of stratum corneum sampled by tape-stripping (approximately 50 μg/cm^2) were two to six times higher than the respective average elemental contents in human stratum corneum obtained from the PIXE analysis of skin punch biopsies [16,17]. The most likely reason for these differences is that the two techniques sampled different depths of the stratum corneum. For the PIXE analysis of the punch biopsies the derived ion concentration values were averages over the whole depth of the stratum corneum (approximately 10–20 μm depth). Conversely, the tape strip data shown in Tables 2 and 3 and Figs. 1 and 2 were obtained from tape strips that sampled only the surface layer of the material associated with the stratum corneum (approximately 0.5 μm depth). On the other hand, the calcium data were consistent with the average calcium contents in human stratum corneum [16] and the calcium gradient observed in Fig. 3B had a slope that was consistent with observations of the calcium gradient in other studies [16,17].

All stratum corneum samples obtained by tape-stripping contained Br. The Br contents of the stratum corneum had significant variability between individuals (greater than 100% S.E.) that was at least twice the percentage S.E. for elements with an atomic number less than 31. The variability of multiple measurements on individuals had a percentage S.E. that only ranged from 6 to 8% for Br (Table 2) and the variability of Br over time (14% S.E.) in one individual (Table 3) was also significantly less than the variability between individuals. A possible explanation for this variability between individuals could be caused by a variance in exposure to Br by either diet (sea food), environment (trihalomethanes from water treatment), or occupation. Br is commonly found in human and animal tissues. Since it is commonly found in mammalian tissues and the scope of this paper is to demonstrate the quantitative nature of this tape-stripping technique for the detection of metals we have not chosen to test any of these hypotheses for the possible variation in Br contents between individuals.

All the stratum corneum samples obtained by tape-stripping contained Ti and several contained Zr. Ti and Zr contents in samples of human stratum corneum have not been widely reported. The depth profiles in Fig. 3B showed that both Ti and Zr exhibited strong gradients with the element concentration decreasing with increasing stratum corneum depth. These profiles suggested that the presence of Zr and Ti in the stratum corneum could have resulted from the use of soap, shampoos or other personal hygiene products. Many soaps contain Ti and Zr is commonly found in roll on or stick antiperspirants as the active ingredient aluminum zirconium tetrachlorohydrex. Although antiperspirants are not usually applied to the forearm, it is plausible that antiperspirants previously applied to other body regions could be dispersed during cleansing activities (such as washing or showering) resulting in the presence of Zr on the volar forearm.

PIXE analysis of toiletries used by the female subject who participated in both the depth profile and time course studies suggest that toiletries could be a possible source of Zr and Ti. PIXE analyses revealed that this individual's soap contained Ti at a concentration of approximately 4000 mg/kg, but no zirconium at levels above 0.5 mg/kg; while the antiperspirant contained approximately 10 000 mg/kg Zr and approximately 3000 mg/kg Al.

The absence of Al in all the tapes containing samples of stratum corneum might appear to contradict the hypothesis that zirconium found in the stratum corneum samples could derive from the use of antiperspirants. However, the tape-stripping assay had much better sensitivity for detection of zirconium than for aluminum (see Table 1). As the zirconium content of the antiperspirant was approximately three times that of the aluminum content it is quite feasible to only detect zirconium in a tape strip sampling μg/cm^2 or smaller quantities of antiperspirant. PIXE analyses of the 5 mg/cm^2 and 5 μg/cm^2 samples of dried antiperspirant on a blank tape illustrated this conjecture. The 5 mg/cm^2 sample was found to contain approximately 10 000 mg/kg Zr and approximately 3000 mg/kg Al while the 5 μg/cm^2 sample contained zirconium at a concentration of approximately 50 ng/cm^2 but not aluminum whose expected concentration was approximately

15 ng/cm^2. As the minimum detection limit for Al in a tape strip is 34 ng/cm^2 (see Table 1), the amount of Al present in a 5 μg/cm^2 sample of the antiperspirant is below the minimum detectable limits of the assay.

5. Future Directions

Although a collimated 1×1 mm^2 beam was used to determine the metal contents in samples of stratum corneum for this study, the proton beam can also be focused down to micron sized spots and repeatedly scanned over the specimen enabling the spatial microanalysis of elemental distributions [4,12,13]. Following data acquisition, maps of the element concentration can be generated from the data and X-ray spectra from beam locations corresponding to any region of interest can be extracted for quantitative analysis [14,15].

The feasibility of mapping element distributions via PIXE on tapes that had sampled stratum corneum was examined using the first tape obtained from the depth profiling study. A 1 nA proton beam was focused down to a spot size of a few microns and rapidly scanned over an approximately 1×1 mm^2 area of the tape for a total dose of 10 μC. Spatial maps of S, Cl, K, Ca, transition metals, Br and Zr were subsequently generated. Analysis of the data revealed that X-ray yields and the spatial distribution of elements per unit dose remained constant throughout irradiation. Furthermore, the irradiated region on the sample suffered no discernible morphological changes on the micron scale when viewed after irradiation apart from a discoloration of the specimen to a golden-brown color. These observations indicated that rapid and repeated scanning of a focused 1 nA beam over the sample did not appear to adversely affect element distributions in the sample or element quantitation. Consequently, the ability to focus the proton beam down to micron sized spots and scan the focused beam over a tape strip may make it possible to study the spatial elemental distributions associated with specific morphological features (e.g. hair follicles, moles, sweat glands) in stratum corneum samples and may also make it possible to study the spatial elemental distributions in areas of the skin that are in contact with or exposed to jewelry, watches or chemical agents, or arising from tattoos, lotions, etc.

6. Conclusions

A quantitative, minimally invasive tape-stripping assay for the detection of metals on and in the skin that also has application to detection of elements on a variety of surfaces including surfaces where human contact could occur has been developed. The development included manufacture of a low-metal content tape suitable both for tape-stripping and elemental analysis. Individual tapes were applied to the skin and subsequently removed, taking with them a sample of the dead outer layer of the skin (stratum corneum). Analysis of the tapes by PIXE identified and accurately quantified the metals. The assay had elemental sensitivities of approximately 1 ng/cm^2 for many metals and analysis of elemental contents could be performed in as little as 5 min. The assay has potential as a tool: (1) for risk assessment, (2) to identify exposure levels following possible contact with a hazardous metal, and (3) to determine the effectiveness of cleanup or removal measures.

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