CORPUS PUBLISHERS

Advance Research in Dermatology & Cosmetics (ARDC)

Volume 1 Issue 1, 2022

Article Information

Received date : December 02, 2022

Published date: December 21, 2022

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DOI: 10.54026/ARDC/1004

Keywords

Mass spectrometry technology; Isotopic labeling; Cosmetology; Chromatographic analysis

Mini Review

Mini Review: Mass Spectrometry Technology for Molecule Distribution inside Skin

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Abstract

The functional ingredients for skin-related products have been highly recognized accompanied by the consumers' curiosity and instrumental improvement. The targeted location of a functional active ingredient in the skin organelle could be very critical information for its product development, safety evaluation, and benefit claims. Due to those factors, mass spectrometry technology exhibited rapid growth in those research areas because it can accurately and sensitively determine the ingredient molecular weights and locations. In this min review, a summarized figure was created for mass spectrometry technology applications in skin research in the last two decades or so. Compared to other related technologies, the uniqueness and advantage of chromatographic-mass spectrometry and mass spectrometry imaging were also emphasized. Several typical examples from separation, isotopic labeling, and combination with Franz cell were discussed.

Introduction

A molecular destination inside the skin since its deposition on the skin surface cannot be overvalued for skin care and topical drug product development. The human skin is the largest multilayered organelle of the body with a variety of advanced functions. It protects the body from xenobiotics and microbial attacks, helps regulate the body temperature and permits sensations of touch, heat, and cold. Skin functions as a barrier that regulates the adsorption and penetration of various compounds. The study of human skin represents an important area of research and development in dermatology, toxicology, pharmacology, and cosmetology [1]. The studies in this field underwent significant transformations throughout the centuries [2]. From the first descriptions of skin diseases in Egyptian papyri and Hippocratic writings to the first treatises on dermatology, important individuals and discoveries have marked the specialty. In the 18th and 19th centuries, the specialty consolidated itself as a field of medical study based on the first classifications of dermatoses, diagnostic methods, and drug treatments. In the 20th century, the scientific and technological revolution transformed dermatological practice, incorporating new therapeutic resources, as well as surgical and aesthetic procedures [3]. With the development of human civilization and beauty needs, the study of skin has been extended to cosmetics and skin care products, which have played many varied and important roles in human history. Skincare products have influenced everything from religious practices to the health of the general population. The Food and Drug Administration (FDA) defines cosmetics as products meant to be used by humans on their bodies for cleansing, beautifying, appealing enhancement, or adjusting appearance [4]. Its research fields include soap and cleansing, colored facial, eye, and lip cosmetics. Today, the market for transepidermal products is on the rise, as it is a comfortable, safe, and non-invasive route for healthy consumers and patients [5-7].

The history of drug treatment, cosmetics, and skincare products always parallels many important technological developments in chemistry, instrumentation, materials, and packaging innovations [8]. To improve active ingredient (AI) functionality in the formula, two efforts need to be given. One is a product development and the other is a selection of evaluation tools. Those two efforts are specifically important in the early stages of topical drug and cosmetic product development since an AI can be economically screened to optimize AI selection, concentration, and formula before significant investigation for clinical and marketing progresses [9]. The transdermal delivery systems have been intensively studied to overcome the skin barrier for more effective application of pharmaceutical and cosmetic products [10,11]. Although the cosmeceutical industry has made substantial progress in the development and incorporation of new and effective AI in their products, the barrier function of the skin remains a limiting factor in the penetration and absorption of these actives. This limiting factor brings us an important need and challenge for skin barrier function evaluation. Understanding the spatial and temporal distributions of AI and its delivery in target skin tissues is of paramount importance in both pharmaceutical and cosmetic product development [12,13]. To approach those understandings, many technologies and instrumentations have been applied in skin studies [12,14-16]. Each instrument has its unique insights for the product functions. In this mini-review, we will briefly describe those instrument and their challenges for AI distribution inside the skin and emphasize MS technology at the molecular level.

Challenges

Many technologies have been applied for AI distribution studies in skin organs. The radioisotopic method still is a gold standard [17,18] for skin distribution studies despite recent papers showing good agreement between radiolabeling and classical chromatographic analysis. The combination of vertical diffusion Franz cell with liquid chromatographic analysis of the fluid reservoir, tape stripping, and skin layer separation has become the main method to study skin penetration [19-23]. However, their capability could be limited without mass spectrometry (MS) detection coupling, which will be discussed in the MS technology section.

Confocal microscopy is an optical imaging technique designed to increase the optical contrast and resolution of a sample using a spatial pinhole to block out any out-of-focus light in the background of the image. It is also known as confocal laser scanning microscopy (CLSM) or laser confocal scanning microscopy (LCSM). Confocal fluorescence microscopy uses ex vivo human skin biopsies, which can be taken and analyzed either in 2D with histology or in 3D with confocal fluorescence via fresh or whole-mount methods [13]. It offers an improved image contrast over conventional fluorescence imaging due to the elimination of out-of-focus fluorescence and improved lateral (~200–400nm) and axial (~0,4–1μm) resolutions. The practical imaging resolutions depend on the fluorophore emission wavelength, the numerical aperture of the objective,

and the index of refraction of the sample. In vivo confocal fluorescence imaging can follow and quantify the penetration of a wide range of fluorescent compounds and nanoparticles within skin tissue [24]. It should be noted that confocal fluorescence methods can cause rather significant photobleaching of fluorescence compounds, as all fluorophores along the optical axis are excited. This can also lead to photodamage of the skin tissues, as the excited fluorophores can generate reactive radical species [25]. Skin autofluorescence is highly heterogeneous from person to person. In a recent study examining facial skin samples, a six-fold difference in total auto-fluorescence intensity was observed across more than forty subjects, showcasing a challenge in quantitatively measuring fluorescent drug uptake in the skin [13,26]. More problematically, the skin structures that give rise to autofluorescence are themselves heterogeneous: individual structures, such as sebaceous glands, have non-identical levels of autofluorescence and are spatially distributed heterogeneously within skin [27]. This degree of subject and spatial heterogeneity has motivated the need to study larger numbers of skin specimens with advanced forms of fluorescence microscopy. Multiphoton microscopy (MPM) opens up the ability to image fluorescent molecules deep within human skin in vivo and has many applications in applied cosmetic and pharmaceutical research [28].

Raman microspectroscopy provides a non-destructive, non-invasive, and chemically specific methodology for both in vitro and in vivo investigations. It can provide a powerful alternative to the current gold standard methods approved by regulatory bodies such as the European Union [1]. The use of excised human skin has been adopted by the "Guideline test" 428 of the organization for economic co-operation and development (OECD) [17,18]. The Raman spectrum can be considered as a spectral fingerprint of the molecule. The additional advantage of Raman spectroscopy compared to IR is its ability to provide confocal information presenting the possibility of in vivo analysis and, for example here, a real-time evaluation of AI permeation mechanisms. Specifically, confocal Raman spectroscopy has the advantage to study skin and skin hydration by assessing water concentrations and the effects of moisturizing factors [29-32]. However, Raman is hard for biological ingredient identification such as amino acids and lactic acid because of the same ingredients or similar chemical functional groups present in both formulated ingredients and skin matrix.

The morphology of the skin surface can be studied by using atomic force microscopy (AFM) and an optical microscope, specifically for the comparison of skin surface stiffness, roughness, hydrophilic properties, viscous friction, and the adhesive force between the virgin and cream-treated skins [33]. Co-localized AFM and Raman instruments have been studied [22,34]. The physical morphology and ingredient functional group have been characterized at the same spatial location, which built an accurate relationship between AI and skin structural changes.

Transmission electron microscopy (TEM) and X-ray microanalysis (XRMA) with cryopreserved human skin are good tools to study ingredient passive diffusion and the effect of iontophoresis on percutaneous ion transport [35]. TEM can offer an excellent resolution of ultrastructural details (0.1nm) for skin visualization. But it causes damage to the specimen and suffers from fixation and sectioning artifacts. Only small specimen areas can be directly visualized and quantification of a permeating AI molecule is impossible.

The correlations between Raman spectroscopy, tensile testing, AFM, scanning electron microscopy (SEM), and MPM for swine and human skin have been studied [36]. All the technologies described above have their strength based on the flexibility of *in vitro* and *in vivo*, the correlation between physical and chemical properties, and high spatial resolution. However, they have a common limitation on intact AI molecular information and detection sensitivity compared to MS technology.

Mass Spectrometry Technology

MS is an analytical tool capable of producing and separating ions according to their mass-to-charge ratio (m/z). This capability can detect most AI molecules and differentiate them from the sample matrix either skin extracts or whole skin biopsy. Since the "Potts & Guy equation" was built for AI transdermal prediction [37], more research works have been leveraged at the molecular level. Due to its sensitive detection of an intact molecular weight, MS technology has grown very rapidly in skin transdermal studies. This conclusion can be obtained from our survey shown in figure 1. For review purposes, a survey of mass spectrometry technology in skin deposition study from 1999 to July 2022 has been taken. The article selection was based on the ScienceDirect® database with the search term {mass spectrometry skin deposition}. A few publications were tracked by recent articles, which were not listed in ScienceDirect® but used in this survey. Several publications were listed in ScienceDirect®, but were not from peer-reviewed journals and thus not included in this survey. In total, 11,8886 articles were listed in this survey.

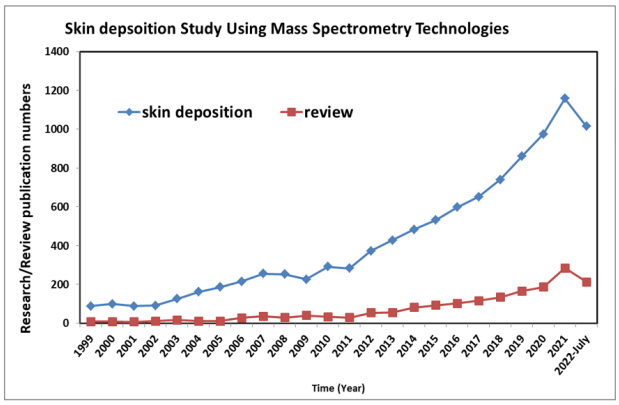

Figure 1: Research and review articles related to mass spectrometry technology and skin deposition found in the ScienceDirect® database from 1999 to July 2022.

If two phrases, "skin deposition" and "mass spectrometry" were given to search the literature, the information in figure 1 can be found. Using MS to study skin deposition has grown significantly since 2011. One reason for this growth is the improvement of mass spectrometry instrumentation. The other important reason is consumers like to know more details about AI location and amount after either topical medicine or cosmetic products were applied to the skin surface. An intact AI molecular weight, especially using a high resolution MS, provided more confidence to researchers and consumers.

If only skin surface deposition data is needed, the sampling procedure usually is conducted by solvent extraction and/or tape stripping after AI testing samples are applied on the skin surface. Sterile hollow glass or steel cylinders can be used for solvent extraction [38-40]. Solvent extraction is a very convenient method to evaluate the AI deposition on the skin surface but it cannot provide deposition depth information. The striped tapes contain not only information about the AI deposited amount but also the AI distribution in the different depths of the stratum corneum layers. The striped tape can get the stratum corneum layer off separately by continuous stripping up to 16 times on the same skin locations and first striped tape (number 1) is on top of the surface and the last striped tape (number 16) is the deepest stratum corneum layer based on the standard protocol [41-43]. To enhance the sample detectability, the same number of striped tapes can be pooled together for AI extraction.

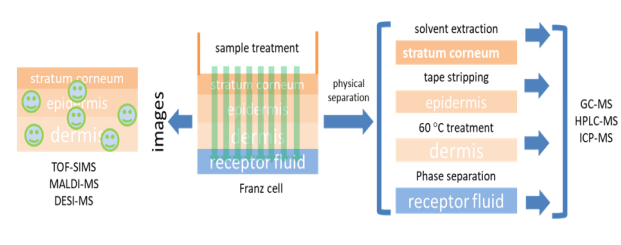

Figure 2: An Outline of active ingredients distribution inside the skin by using mass spectrometry technology.

If AI distribution and penetration information in whole skin layers is needed, MS technology should be coupled with Franz cell equipment, which is shown in figure 2. The sample treatment is applied on the skin surface in the Franz cell. After the incubation/treatment time, AI will be distributed in the skin and even penetrated over the skin tissue into receptor fluid, which is shown in figure 2 center. AI distribution in the stratum corneum layer can be obtained either by using solvent extraction or tape stripping, which is shown in figure 2 right top. The epidermal and dermis layers can be separated with the established methods at 60 °C treatment [42]. In addition, more separation systems have been designed and applied to split epidermal and dermis layers, including chemical reagents, enzymes, heat, and mechanical procedures. Their

advantages and disadvantages have been compared and reviewed [44]. The AI in those separated skin layers plus receptor fluid can be analyzed using MS technologies, which include GC-MS [45,46], LC-MS, and ICP-MS [47]. The solvent extraction, chromatography, and MS detection constitute a sequential filtering system like three-dimension separation. The solvent solubility executes the first (X-axis) dimension of AI separation from the sample matrix. The chromatography carries out the second (Y-axis) dimension of separation based on the different AI molecular characters like polarity. The MS detector achieves the third (Z-axis) dimension of separation based on their molecular weights. This entire system can effectively filter out most interference present in the skin matrixes and sample formula. The more accurate and reliable results from this system make MS technology more widely applied in cosmetic and topical drug research. Many new delivery systems such as microneedle [48], Colloidal nanodispersions [49], Polymeric micelle mediated follicular [50], Sulfhydryl modified polymers [51], and polyamidoamine dendrimers [52] have been evaluated with MS technologies. In addition, LCMS is a very quick and effective tool to determine AI for skin benefits and a great linkage between traditional raw materials from botanical and food resources and skin efficacy validation [53]. It can play a critical role when an artificial membrane was used to mimic the interactions between AI and the cell lipid membrane [54,55]. Sensitizing metals like nickel, cobalt, and chromium can activate the localized immune response in allergic individuals, ultimately resulting in contact dermatitis. The inductively coupled plasma mass spectrometer (ICP-MS) can be applied to study metal permeation in the skin [56,57], specifically for risk assessments. Due to the specificity of the mass spectrometer to all different metals, ICP-MS can effectively differentiate the metal permeation rate when combined with typical Franz cell experiments described above.

When an AI is the same or very similar to the ingredients naturally present in the skin, most detectors could face challenges due to the difficulty of differentiation. In those cases, LC-MS and/or GC-MS can exhibit their very unique differentiation capabilities for very subtle variation and even the same structures of molecules. A good example is hemp seed oil deposition on the skin surface [40]. Pure ethanol extraction can remove most dead cell residues and salts on the skin surface. A reversed phase column can effectively separate the skin ceramides and triacylglycerides (TAG) from the TAG in hemp seed oil. After a chemical marker (glycerol, tri-γ-linolenicate) with an exact molecular weight (890.7216, $M+NH_4^+$) and retention time of 30.47min in hemp seed oil was established, hemp seed oil deposition can be confidently quantified without significant interference of skin components. The results are shown in figure 3. The chromatographic peak at 30.47 min can be well separated from other lipid ingredients. LC-MS can not only differentiate the botanical TAG from skin TAG, but also determine the skin lipid profiles [58]. Another important example was using LC-MS technology to localize the AIs and their metabolites across human skin *in vitro* [59]. This is very important for the research of organic sunscreen products and many organic UV filters could be metabolized into toxic compounds in the aquatic environment [60].

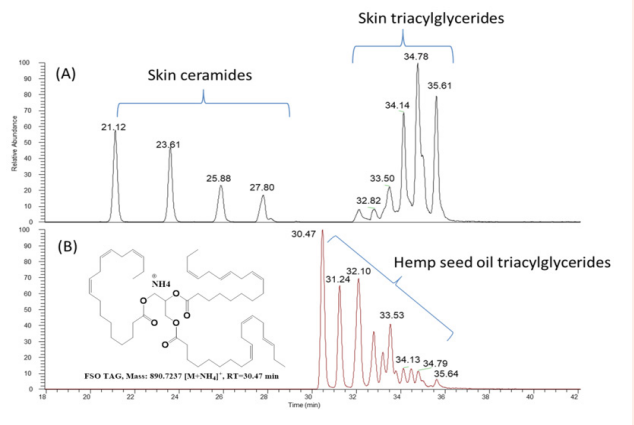

Figure 3: LCMS chromatogram of skin sample with hemp seed oil treatment. (A-top) ceramides and triacylglycerides from pig skin and (B-bottom) triacylglycerides from hemp seed oil. The chemical structure in (B) is the chemical marker in hemp seed oil.

Another superior advantage of MS technologies to other methods is the application of stable isotope labeling of AIs. Compared to the gold standard method using radioisotopic AI [17,18], the safety requirement of a stable isotope is low and it is much easy to handle in a routine analytical lab. The most common application is the isotope dilution method. For example, synthetic peptides can be used to track and quantify the disease-related protein level during proteomics studies [61]. The isotope dilution method has been widely used to determine sunscreen ingredient adsorption [62] and metabolite level in skin tissues [63,64]. The stable isotopic internal standards can be used in most MS technology to improve the method's accuracy and reliability. To differentiate the endogenous from exogenous ingredients like essential fatty acids, deuterated linoleic and alpha-linolenic acids have been combined with GC-MS for their quantification.

The combination between mass spectrometry technology and Franz cell provided an excellent analytical tool to quantify the distributed molecules in skin organelles. However, the artificial factors during the physicochemical separation limited the entire in situ information. Those limitations can be avoided by using mass spectrometry imaging (MSI) as a complementary method. It takes advantage of the selectivity and sensitivity of mass spectrometry, which is superior to spectroscopic techniques, in an experiment where the mass spectrometric analysis is performed directly from the sample rather than from sample extracts as done with the conventional LC-MS, GC-MS, and ICP-MS techniques. Currently, secondary ion mass spectrometry (SIMS), desorption electrospray ionization (DESI), and matrix-assisted laser desorption/ionization (MALDI) are the most commonly used ionization methods for MSI [65,66]. The comparison between MSI and extract analysis is demonstrated in figure 2. MSI is a label-free technique allowing the detection of both endogenous and exogenous compounds in parallel, especially when stable isotopic ingredients are involved. The high resolutions from both mass weight and spatial scale have made this technology gain rapid growth [65,67-71].

A good example was coming from chlorhexidine gluconate (CHG) characterization [72]. TOF-SIMS analysis provides excellent sensitivity to the ppm range and it demonstrates the ability to image samples with high chemical specificity without the need for radiolabels. Classical in vitro Franz cell permeation studies can be combined with the TOF-SIMS analysis of the skin. A comprehensive characterization of CHG skin permeability was reported [73]. The results indicated that the TOF-SIMS method has a lower limit of detection than the tape stripping methods. CHG distribution was mapped throughout the skin sections and confirmed that it was predominantly localized within the stratum corneum.

TOF-SIMS has been applied to analyze the skin by several researchers to explore the effects of photo-aging on human skin and to characterize the distribution of synthetic pseudo ceramides delivered from a cosmetic formulation [74]. Most recently, this method has been applied to the characterization of age-related changes to human stratum corneum lipids following in vivo sampling, which identified alterations to the spatial distribution of several stratum corneum lipids associated with membrane stability [75].

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