Vibrational Spectroscopy 97 (2018) 119–128

Contents lists available at ScienceDirect

Vibrational Spectroscopy

journal homepage: www.elsevier.com/locate/vibspec

Study of interactions between Gallic Acid and Skin Surface using Infrared Spectroscopy

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ARTICLE INFO

Keywords:
Skin
Gallic acid
Effect of solvents
Solubility and temperature
Infrared spectroscopy
Multivariate statistical analysis

ABSTRACT

The transdermal transmission of model substance on the pigskin samples was investigated using the attenuated total reflection (ATR) technique of infrared (IR) spectroscopy. The collected vibrational spectroscopic data were evaluated by multidimensional statistical methods as principal component analysis (PCA), linear discriminant analysis (LDA) and partial least squares (PLS) regression which enable detection of individual substances in the skin, their identification and mutual differentiation. Gallic acid (GA), a natural phenolic anti-oxidant with many potential healing properties suitable e.g. for atopic dermatitis treatment, was used as an analyte. Effect of GA on the skin surface was examined for four different solvents namely ethanol (EtOH), methanol (MeOH), dimethyl sulfoxide (DMSO) and ultrahigh purity water (H_2O). Moreover, the effects of temperature related to GA solubility in H_2O were investigated. During the series of experiments, nonsystematic changes of untreated skin samples were observed; while systematic changes are evident after the skin treatment. The systematic effects correspond to structural changes of the skin constituents during substance penetration.

1. Introduction

The skin forms an important natural barrier between the body and external environment and plays a key role in the field of transdermal (drug) delivery. The uppermost layer of the skin is *stratum corneum*. This crucial barrier consists of dead keratin cells with membranes formed by ceramides and it creates the main obstacle for permeation of substances through the skin primarily due to the structural arrangement and extending of the lipid layer [1–4]. Penetration of substances through the whole skin, it means to the bloodstream, depends on the possibility of molecules to overcome mechanically and chemically resistant skin barrier [5]. Interactions of delivered molecules with proteins in *stratum corneum*, especially with keratins, could have either reversible or irreversible character. Binding of some substances to the specific sites on the skin surface may lead to the physiological reactions [6,7]. Penetration of substances into the skin can be based on the passive physicochemical kinetic process known as diffusion. One of the possibilities of penetration kinetic monitoring is the use of Fourier transform infrared (FTIR) spectroscopy with attenuated total reflection (ATR) technique. This method is noninvasive and nondestructive. However, measurements are spatially limited by experimental sampling depth (related to the refractive index of the crystal used together with general and optical properties of a sample) in the range ca. 0.7–2.1 μm [7,8]. The advantage of ATR technique is quite simple experimental setup and short individual data acquisition, which allow us to perform kinetic studies.

In many cases, pigskin is used for study of different drugs penetration in dermatology, cosmetic or medical field due to the permeation properties analogous to the human skin [1,5,9]. Comparative studies of these two types of skin were performed on the *stratum corneum* by confocal Raman microspectroscopy [5]. Any significant structural or concentration differences of measured components were not observable in normalized Raman spectra. Nevertheless, human skin contents higher amount of hyaluronic acid and carotenoids than pigskin, which has been proven by application of multivariate statistical methods [5]. Multivariate statistical methods such as principal component analysis (PCA), partial least squares (PLS) and linear discriminant analysis (LDA) are widely used in many fields of chemistry [10–13], physics [14,15], biology [16–19], pharmacology [20–23], clinical chemistry [24–27] or industrial process control [28–30].

IR spectrum of pigskin measured by ATR IR exhibits characteristic bands of CH_3- and $-CH_2$- functional groups of the saturated aliphatic skeletons due to the contribution of aliphatic chains in keratins and ceramides as constituents of *stratum corneum*. The high content of keratins in *stratum corneum* is reflected by strong bands of amide I and amide II vibrational modes. A band at 1745 cm^{-1} represents C=O vibrations of unsaturated fatty esters somewhat overlapped by vibrations of melanin, the epidermal cutaneous polymeric pigments. Amount of

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https://doi.org/10.1016/j.vibspec.2018.06.009
Received 15 February 2018; Received in revised form 9 May 2018; Accepted 15 June 2018
Available online 18 June 2018
0924-2031/

water in the cuticle and the presence of the melanin molecules rich in hydroxy-groups are revealed further by strong bands of OH- stretching vibrational modes [4].

In this research study, pigskin (in the following text only "skin") was used instead of human skin because of biological similarity. Gallic acid (GA, 3,4,5-trihydroxybenzoic acid) was selected as a representative model analyte. It represents a polyhydroxy phenolic derivative, included in many natural plants (grapes, strawberries, bananas, lemons, etc.), exhibiting various biological functions. The most important roles of natural phenolic derivatives are anti-oxidant, anti-viral, anti-bacterial, anti-inflammatory and furthermore anti-tumor effects [31–37]. GA was also tested for treatment of atopic dermatitis. In the case of this skin disease, the pruritogenic cytokine IL-31 activates eosinophils interacting with skin cells and IL-33 is passively released during tissue damage. GA could suppress the release of pro-inflammatory cytokine in IL-31- and IL-33-treated eosinophils-dermal fibroblasts co-culture [36]. Topical application of GA is an effective de-pigmenting or skin lightening cosmetics approach because of inhibition of melanin production caused by UVB-induced hyperpigmentation in mice skin [38]. Different types of microcapsules containing GA are tested for topical treatment [35,37] e.g. against oxidative damage of skin [35].

This study is focused on the use of simple solutions of GA in different solvents instead of complicatedly prepared microcapsules just to elucidate the role GA and various solvents on the skin properties. For this research, we choose ultrahigh purity water (H_2O), ethanol (EtOH), dimethyl sulfoxide (DMSO) and methanol (MeOH) as solvents for the preparation of GA solutions. H_2O was selected due to the non-toxicity, involvement in physiologic processes and general use of this liquid as a suitable polar solvent. Organic solvents such as EtOH, MeOH or DMSO are typically used in pharmacy as co-solvents to facilitate solubilization of lipophilic compounds [39,40]. Non-aqueous solvents may positively influence transfer (absorption) of active pharmaceutical ingredient (API) into the biosystem/skin but water as a solvent could sometimes strongly affect the stability of drugs. Besides, organic solvents are used during film-coating processes to obtain a high-quality tablet coating because they evaporate quicker than aqueous systems [39]. MeOH is used as a denaturant agent and (co-)solvent in cosmetic formulations and pharmaceutical industries [41], and it is used as an extraction agent in the case of extraction of GA from natural materials [41–43]. DMSO is highly used in dermatologic and pharmaceutical research as a hydrophilic penetration enhancer [44,45]. Systematic toxicity of DMSO is considered nowadays as quite low. DMSO´s therapeutic effects include enhanced penetration of added substances throw the biologic membranes or release of histamine by mast cells or free radical scavenging [46]. Zhang´s group demonstrated high effectivity of IR spectroscopy for evaluation of penetration of substances through *stratum corneum* and for clarification of intermolecular interactions of exogenous agents with internal components of this skin uppermost layer where DMSO, used as a keratin-denaturing agent, explains transcellular penetration processes [47]. It is known that the use of EtOH leads to disruption of the lipid structure of *stratum corneum* and thus it reduces the protective effect of a natural barrier. EtOH also increases apparently a permeation capability of transdermal transfer especially for various drugs [48]. The effect of EtOH on the skin permeation is considered as reversible. Penetration of EtOH to the skin can be used to design a transdermal system with increased flux. For example, the linear dependence between permeation of nitroglycerin into the skin barrier and the transdermal flux of EtOH was described [49] and the skin permeation of nitroglycerin can be controlled by the delivery of EtOH [49]. However, organic solvents may alter the activities of some enzymes by modifying their integrity or their native environment [40]. Hence, the solvent dosage has to be regulated carefully.

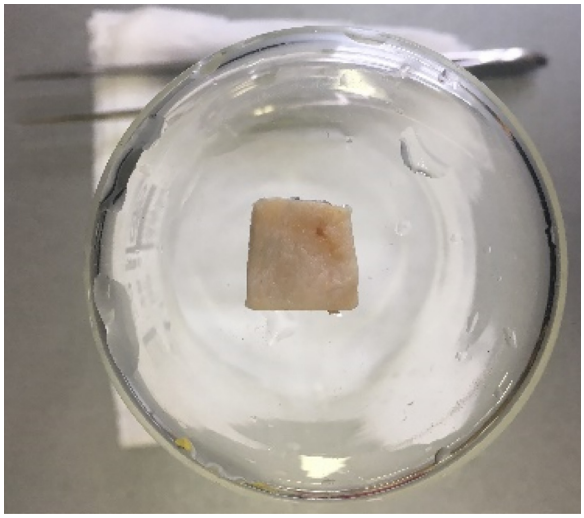

Fig. 1. Sample of skin immediately before application of the treating solution.

2. Experimental

2.1. Materials and chemicals

2.1.1. Samples of skin

Skin samples from pig ears were provided by the Department of Organic Technology University of Chemistry and Technology Prague, CZ. The skin samples originate from the dorsal side of pig auricle containing epidermis, dermis, and subcutis without subcutaneous fat. They were thick ca. 1 mm. Skin pieces with the size ca. 1 × 1 cm (Fig. 1) stored in the freezer at less than −18 °C were used immediately after defrosting for the treatment by the analyte solutions (volume of the individually used solution was 10 µl).

2.1.2. Chemicals

Gallic acid (GA, $C_7H_6O_5$, Sigma Aldrich, ≥ 99.0%, USA) was dissolved in four different solvents: ethanol (EtOH, ≥ 96.0%, PENTA, CZ), methanol (MeOH, ≥ 99.0%, PENTA, CZ), dimethyl sulfoxide (DMSO, ≥ 99.0%, PENTA, CZ) and ultrahigh purity water purified using a Milli-Q® system (H_2O).

2.2. Methods

ATR IR reflectance spectra were collected by Nicolet iS50 FT-IR (Thermo Scientific, USA) spectrometer equipped with single-bounce ATR adapter with non-toxic diamond crystal. The individual IR 4-cm^{-1} resolved spectra were results of 128 co-added scans (zero filling 0) acquired under control of the software Omnic 8.1 (Thermo Scientific, USA). The spectrometer is equipped with high-efficient pyroelectric detector DLaTGS (L-alanine-doped deuterated triglycine sulfate).

2.3. Preparation of samples

In the first study, GA was dissolved in H_2O, EtOH, MeOH, and DMSO at 298.75 K. The concentration limit of GA solubility in H_2O is 13.00 ± 0.65 $g{\cdot}L^{-1}$ [50], the solubility values in DMSO, EtOH and MeOH are much higher than in water. Considering the comparison of results, concentration of GA in each solvent was just 13.00 ± 0.65 $g{\cdot}L^{-1}$.

In the second study, GA was dissolved in H_2O at three different temperatures (293.15 K, 298.75 K, and 314.65 K). The highest temperature is the temperature at which the physiological state of the skin surface is not disturbed yet. Saturation limit concentration values at the individual used temperatures are presented in Table 1.