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Amperometric monitoring of quercetin permeation through skin membranes

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ABSTRACT

Transdermal delivery of quercetin (QR, 3,3′,4′,5,7-pentahydroxyflavone), a natural flavonoid with a considerable antioxidant capacity, is important for medical treatment of, e.g., skin disorders. QR permeability through skin is low, which, at the same time, makes the monitoring of percutaneous QR penetration difficult. The objective of this study was to assess an electrochemical method for monitoring QR penetration through skin membranes. An electrode was covered with the membrane, exposed to QR solution, and electrode current was measured. The registered current was due to electro-oxidation of QR penetrating the membrane. Exploiting strict current-QR flux relationships diffusion coefficient, *D*, of QR in skin and dialysis membranes was calculated. The *D* values were strongly dependent on the theoretical model and parameters assumed in the processing of the amperometric data. The highest values of *D* were in the range of $1.6–6.1 \times 10^{-7}$ cm^2/s. This was reached only for skin membranes pretreated with buffer-ethanol mixture for more than 24 h. QR solutions containing penetration enhancers, ethanol and L-menthol, definitely increased *D* values. The results demonstrate that electrochemical setup gives a possibility to assess penetration characteristics as well as enables monitoring of penetration dynamics, which is more difficult by traditional methods using Franz cells.

1. Introduction

Skin is the largest organ of human body and it provides a protective function against external factors such as mechanical injuries, chemical toxins, ozone or UV radiation. To fulfill this role, skin is composed of two main parts: dermis and epidermis. Dermis is the inner part of the skin which contains blood capillaries, sweat glands, sebaceous glands, hair follicles and nerves. The outer part of the skin is called epidermis. It is built up of skin cells (keratinocytes) at different stage of differentiation: from active cells with the ability to divide, to the most outer layer of dead cells (corneocytes), which together with the extracellular lipids form the most impermeable part of the skin-the stratum corneum. Although stratum corneum is a tough barrier, it is challenged by topical application of drugs. Topically applied drugs may diffuse through or be trapped in epidermis, or penetrate to dermis and reach blood circulation. Transdermal drug transport is reported to occur across the skin cells (transcellular route), lipids present in stratum corneum (intracellular route) or the hair follicles and sweat ducts (Moser et al., 2001; Barry, 2002).

The skin, as an outer tissue of a body, is exposed to high risk of oxidative stress and increased amount of reactive oxygen species (ROS), which are one of the reasons of skin damage, photo-ageing and cancerous lesions. The generation of ROS in skin is related to external factors, especially to the UV irradiation, which penetrates through stratum corneum to deeper skin layers: UVB reaches mostly epidermis and UVA reaches both epidermis and dermis where the pathological changes starts (Farkas et al., 2002). Skin has its own protection mechanisms from ROS, such as p53 tumor suppressor gene (Yamaguchi et al., 2008) or creatine kinase system (Lenz et al., 2005), but in the conditions of excessive exposure to harmful agents it needs additional protection, e.g., topical application of antioxidants.

Flavonoids, natural compounds found in plants, have strong antioxidant properties and, thus, are great agents to prevent the photooxidative stress in skin. QR is a flavonoid and is considered as one of the most powerful natural antioxidants (Bonina et al., 1996). It is widely used in medical treatment due to its anti-inflammatory (Lin et al., 2012), antibacterial (Rigano et al., 2007), antiviral (Ganesan et al., 2012), anticancer (Caltagirone et al., 2000) and

Abbreviations: CNP, carbon nanoparticles; EtOH, ethanol; PBS, phosphate buffer saline; QR, quercetin; MT, L-menthol.

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antioxidative properties. It has been found that the amount of QR in apple skin increases with intensified exposure to sunlight as a possible protective reaction against UV-B radiation in plants (Solovchenko and Schmitz-Eiberger, 2003). The increased resistance to oxidative stress after QR application was observed in microorganisms (Belinha et al., 2007) and worms (Kampkötter et al., 2007). Casagrande et al. (2006) confirmed anti-inflammatory and anti-oxidative effect of QR on UVB-exposed skin of mice. The same was later confirmed on rats (Liu et al., 2013).

From the discussion of QR effects on skin, it is obvious that topical application should be designed to optimize QR accumulation in epidermis or delivery to dermis and ultimately uptake in the blood. As concerns QR delivery to the blood, the main and most common route is oral administration. However, the bioavailability of QR from the gastrointestinal tract is limited. It was estimated that absorption of QR after oral application was less than 17% in rats (Khaled et al., 2003) and varied between 17 and 52% in human (Hollman et al., 1997). Topical QR delivery to the blood might, thus, be considered as an interesting alternative in the future.

Despite the great anti-oxidative properties of QR, the drug is characterized by very low skin permeation and, thus, restricted percutaneous delivery (Saija et al., 1998; Liu et al., 2013; Lin et al., 2012). This was confirmed by a number of in-vitro penetration assays using Franz cells (Casagrande et al., 2007; Vicentini et al., 2008; Dal Belo et al., 2009; Bose et al., 2013). It is known that some compounds as alcohols, glycerides, fatty acids, terpenes or phospholipids are able to influence the transdermal permeation of drugs (for a review, see Sinha and Kaur, 2000). The use of penetration enhancers have been tested to improve QR permeation through skin. Some of them, inter alia D-limonene and lecithine had no effect (Saija et al., 1998), but others as QR-loaded lecithin–chitosan nanoparticles significantly increased the amount of QR accumulation in skin layers reaching 9 $\mu g/cm^2$ and 3.3 $\mu g/cm^2$ in epidermis and dermis, respectively (Tan et al., 2011).

In all above mentioned investigations rather tedious methods to study QR penetration through skin membranes are used. The current study describes optimization and application of an electrochemical technique to monitor QR permeation through dialysis and skin membranes in the absence and the presence of penetration enhancers. To the best of our knowledge this is the first report on application of skin membrane covered electrodes for amperometric in-vitro registration of QR penetration. General aspects of the methodology has been briefly introduced in our recent publication (Gari et al., 2015). This relatively simple, quick and highly convenient amperometric method allows the monitoring of QR permeation through membranes in real-time which is registered as an electric current. By knowing the mechanism of drug electro-oxidation at the electrode, the amperometric response, i.e., the electrode current, enables assessing the values of the flux and the diffusion coefficient of QR in skin membranes.

2. Material and methods

2.1. Materials

Phosphate buffer saline (PBS) tablets, quercetin (QR), carbon nanopowder (CNP, with carbon particle size less than 50 nm), methanol, ethanol-(EtOH) and L-menthol (MT) were purchased from Sigma–Aldrich (St. Louise, USA). The solutions were prepared using water purified by Milli-Q system (Merck Millipore, Billerica, USA) with a resistivity of 18.2 MΩ cm. pH of solutions was adjusted by using 2.5 M HCl and 1.0 M NaOH. The stock solution of QR at concentration 0.01 M was prepared by diluting 15 mg of QR in 5 ml of methanol. The fresh stock solution was prepared weekly and was stored in the fridge at +4 °C, in dark (wrapped in aluminum foil).

For monitoring the penetration of QR through membranes, skin membranes were prepared from pig's ears provided by a local abattoir. As a model membrane a dialysis tubing cellulose membrane with cut-off 12 kDa (Sigma–Aldrich, St. Louise, USA) was used.

2.2. Electrode preparation and experimental conditions

Electrochemical measurements were performed using CompactStat potentiostat from IVIUM Technologies (Eindhoven, Netherlands). Three electrode electrochemical setup (Fig. 1(1)) was used. A platinum wire and an Ag/AgCl/(KCl saturated) electrode were used as a counter and reference electrodes, respectively. To enhance electro-oxidation of QR at the working electrode, a platinum disk electrode was modified with CNP

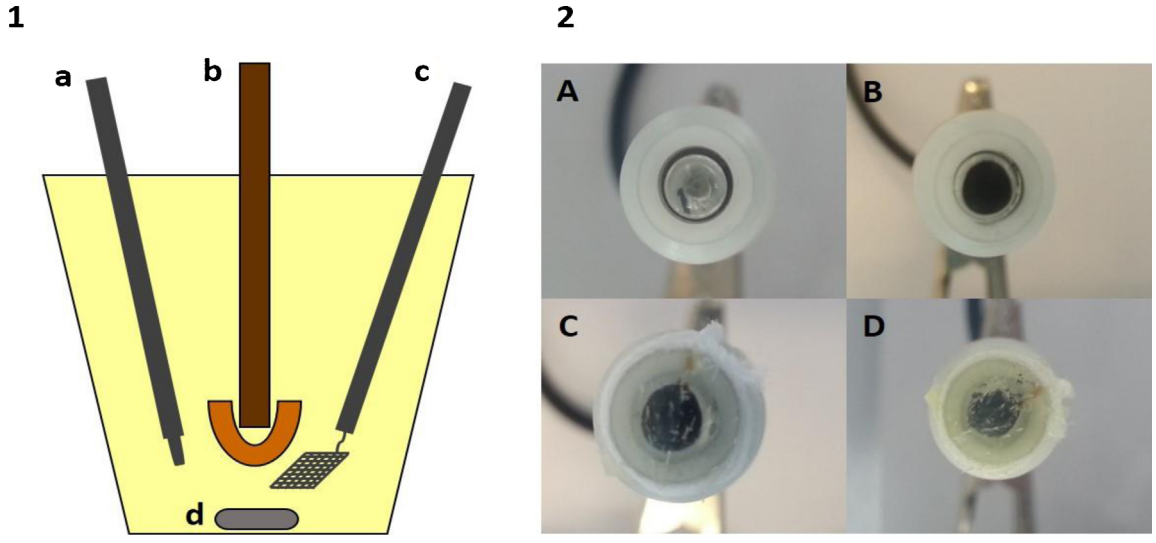

Fig. 1. (1) A schematic representation of the electrochemical cell for amperometric measurements (a) Ag/AgCl reference electrode, (b) working electrode covered with a membrane, (c) platinum mesh counter electrode, and (d) magnetic stirring. (2) A photo of the tip of the working electrode after different steps of the preparation (A) surface of platinum working electrode, dia. 0.2 mm, after polishing with emery paper, (B) surface of the working electrode after modification by CNP dispersion, dia. 2 mm, (C) working electrode covered with skin membrane, and (D) skin membrane covered working electrode after QR monitoring (yellow color is due to accumulation of QR in the membrane). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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