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Research paper

Electroosmotic flow as a result of buccal iontophoresis – Buccal mucosa properties

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ABSTRACT

The objective of this study was to investigate and to better understand the properties of buccal mucosa as a semipermeable membrane and a portal for drug administration by iontophoretic and electroosmotic means. In vitro experiments showed that buccal mucosa at the pH of about 7.4 behaved as a cation-exchange membrane and non-linear resistor. It had lower resistance and was more permeable for water than a skin. The electroosmotic volume flow through mucosa depended on current density, mucosa resistance and electrolyte concentration. Sodium dodecyl sulfate (in concentration range 0.001–0.005 mol L^{-1}) and urea (in concentration range 0.42–1.67 mol L^{-1}) did not promote a water transfer through buccal mucosa, however, both substances enhanced flow through the skin.

1. Introduction

The most popular oral route of drug administration is very simple and convenient but has some disadvantages like degradation of pH-sensitive substances or peptide-drugs digestion in stomach and sometimes low bioavailability caused by first pass hepatic effect. To overcome these problems scientists are working on new delivery systems targeting new drug absorption areas, like skin or buccal mucosa.

The external layer of skin, stratum corneum, is the main barrier to transdermal diffusion. It consists of keratinized cells surrounded by lipid lamellae containing cholesterol, free fatty acid and ceramides [1–3]. Transfer of hydrophilic drug through this structure is hindered; however, hydrophilic regions of the lipid lamellae enable migration of small ions by intracellular pathway [4]. Because of the pH gradient across the stratum corneum (outer side pH 5.0, inner side pH 7.4) the ionization of lipid head groups ranges from 10% (outer side) to 90% at the inner side [5]. This phenomenon is responsible for a negative net charge of the skin [6–8]. Human buccal mucosa is devoid of keratinized cell layer, so this tissue is more permeable than skin and much more suitable for hydrophilic drug transfer [9]. However, buccal mucosa acts also as a barrier to xenobiotics absorption and has some protection region as well. This defence line is positioned in the depth of tissue and consists of low molecular weight polar lipids, which do not aggregate to form filaments and are in extracellular spaces [10,11]. This lipid barrier and a small area of absorption via buccal mucosa make administration of drugs in therapeutic quantities difficult, which forces pharmacists to use enhancements.

Looking for more efficient drug transfer, application of iontophoresis (electrophoresis) was proposed. In this process moderate electric field propels drug ions to move towards the direction of counter-charged electrode [12]. The phenomenon of iontophoresis is known since 1900 [13] and now typical area of its application in medicine is transdermal drug delivery, but it may also be used for enhancement of drug transfer through buccal mucosa [14–17].

Iontophoresis enhances drug transfer by mechanisms of electromigration and electroosmosis [18,19]. In transdermal delivery, taking into consideration the negative net charge of skin, both these mechanisms play synergetic roles in the transport of cationic drugs. For anionic molecules only electromigration is efficient and electroosmosis reduces anionic drug flux. Nonionic substances may be transferred by electroosmosis. Electroosmotical flux of cationic drug depends on its concentration and electroosmotic solvent flux (called in short electroosmosis). This solvent flux is induced by charge transfer through skin under electric field and was reported in 1924 by Rein [20], whereas electroosmotically enhanced transport of neutral species was presented in 1980 by Gangarosa et al. [21]. Electroosmosis may be observed only if electromigration of cations and anions in a system is not symmetrical – like in ion-exchange membranes. Results for transdermal iontophoresis [21–26] show increased mobility of cations and the cation-exchange nature of skin. There is no similar knowledge about transport through mucosa.

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The aim of this work was to investigate the properties of buccal mucosa as a portal for drug delivery by iontophoretic and particularly electroosmotic means and to define intensity of electroosmosis. The purpose of experiments carried out in our laboratory was to show cation-exchange nature of buccal mucosa and better permeability for water than skin. Additionally, the investigations showed electrical properties of mucosa.

2. Materials and methods

2.1. Materials

All chemicals were of analytical grade. Phosphate-buffered saline (PBS), pH 7.4, was prepared by dissolving KH_2PO_4 (0.2 g), anhydrous Na_2HPO_4 (0.92 g), NaCl (8.0 g) and KCl (0.2 g) in 1 L of distilled water. Specific conductivity of distilled water at 22 °C was 2.9×10^{-4} S m^{-1}. Salts that were used came from Chempur (Piekary Slaskie, Poland).

As promoters of water transfer sodium dodecyl sulfate (SDS) from POCH (Gliwice, Poland) and urea from Chempur (Piekary Slaskie, Poland) were applied. Urea is known for its hydrating properties of stratum corneum [27] and for keratolytic properties [28]. SDS reduces barrier properties of skin and buccal mucosa by extracting lipids above its critical micelle concentration [29,30].

Silver wire 1 mm in diameter for electrodes came from Mennica Metali Szlachetnych Ltd. (Warsaw, Poland). In the studies silver/silver chloride electrodes were used which were prepared according to the typical procedure [14].

In experiments pig buccal mucosa and skin were used. They are known as good models of human tissues [31,32]. Domestic pig cheeks had been received from the local slaughterhouse (Sochaczew, Poland). Hairs were gently removed from skin using scissors. The buccal mucosa or skin slices 0.2 cm thick were mechanically cut out using a scalpel and kept deep-frozen. A piece of tissue for experiment was defrosted and washed in PBS for 45 min before inserting into measuring apparatus.

2.2. In vitro electroosmosis studies

Experiments were performed at 22 °C. The in vitro electroosmosis studies were conducted in the specially designed horizontal two-chamber permeation cell (Fig. 1) with power supply of direct current.

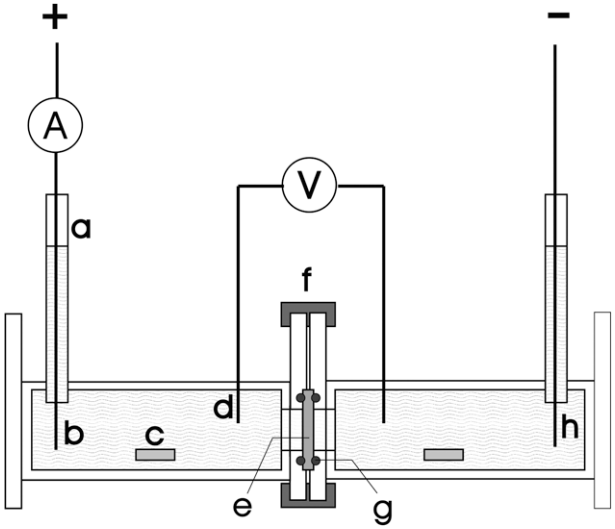

Fig. 1. Schematic picture of two-chamber permeation cell for electroosmosis studies. Where: a, tube for measuring volume flow; b, anode (silver); c, stirring bar; d, electrode (silver) for measuring voltage; e, tissue slice positioned natural external side in anodal direction; f, clamp; g, 'o' ring; h, cathode (silver chloride).

The anodal chamber was filled with PBS buffer or with solution of urea (concentration range 0.42–1.67 mol L^{-1}) or SDS (concentration range 0.001–0.005 mol L^{-1}) in the buffer or sodium chloride solution (concentration range 0.02–0.76 mol L^{-1}) in water. The cathodal chamber was filled with PBS buffer. The volume of chambers was 6.7 ml each. A slice of tissue was inserted between chambers with natural-external surface turned to anode. The anodal chamber was always filled with PBS to simulate internal organism conditions. The electroosmosis was investigated by measuring the solvent volume flux from anodal chamber to cathodal chamber. The voltage in electric circuit: silver electrode, electrolyte solution, tissue, electrolyte solution, silver electrode was also recorded. This measurement was made usually every 10 min. To determine the voltage drop on the tissue only a voltage in the same circuit without tissue and with chambers filled with electrolyte was measured. The voltage drop on tissue was calculated by subtracting the electrolyte voltage for circuit without tissue from voltage for circuit with tissue. The area of tissue surface which was in contact with electrolyte (0.5 cm^2) was used to calculate the current density.

All experiments were carried simultaneously with and without electric field to investigate the influence of osmotic pressure on osmotic volume flow. The current was turned on from the beginning of the experiment. The current density range which was applied in experiment was 0.25–1.0 mA cm^{-2}. This range is typical for iontophoretic drug delivery and the higher current may cause tissue damage.

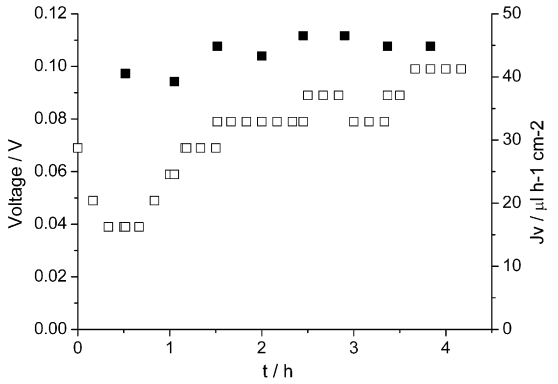

Fig. 2. The example of experiment course. Anodal and cathodal chambers filled with PBS, buccal mucosa, current density 1.0 mA cm^{-2}. The voltage drop on mucosa – empty squares; J_v – filled squares.

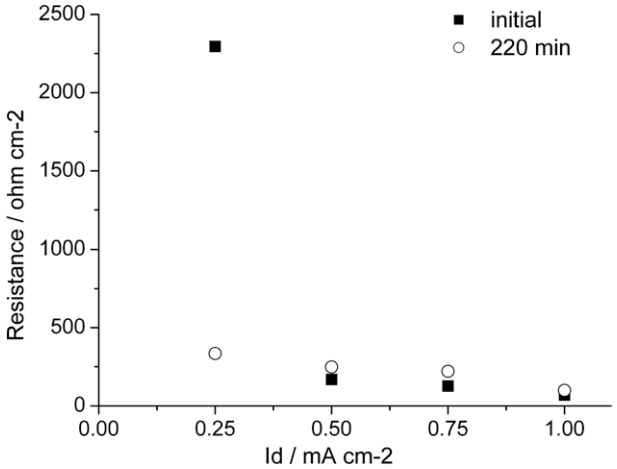

Fig. 3. The influence of current density on mucosal resistance at the beginning of the experiment and after 220 min. Anodal and cathodal chambers filled with PBS. Initially mucosa is a non-linear resistor.

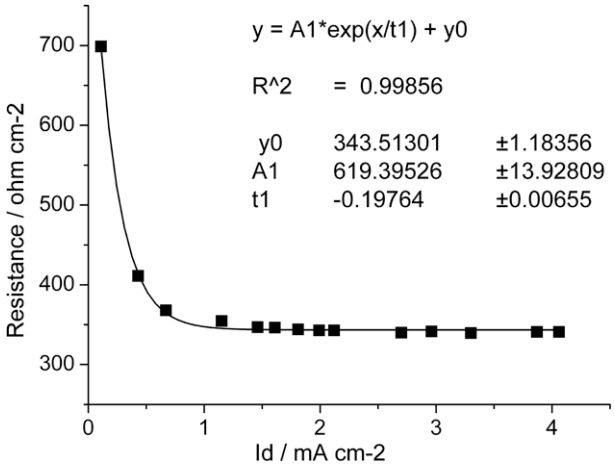

Fig. 4. The PBS resistance calculated from Ohm's law from voltage measurements as a function of current density. PBS was a linear resistor above 1 mA cm^{-2}. Measurements were conducted in permeation cell filled with PBS.

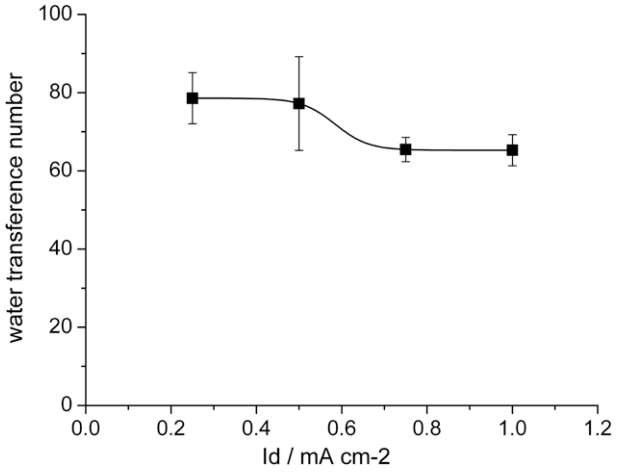

Fig. 6. The current density influence on water transference number in electroosmosis process. Anodal and cathodal chambers filled with PBS, buccal mucosa.

Measurements were conducted until the process stabilized and at least a minimum of three successive readings of solvent volume changes were taken. The scale of tube for volume flow measurements was 0.01 ml, so it was the minimal volume change which could be observed.

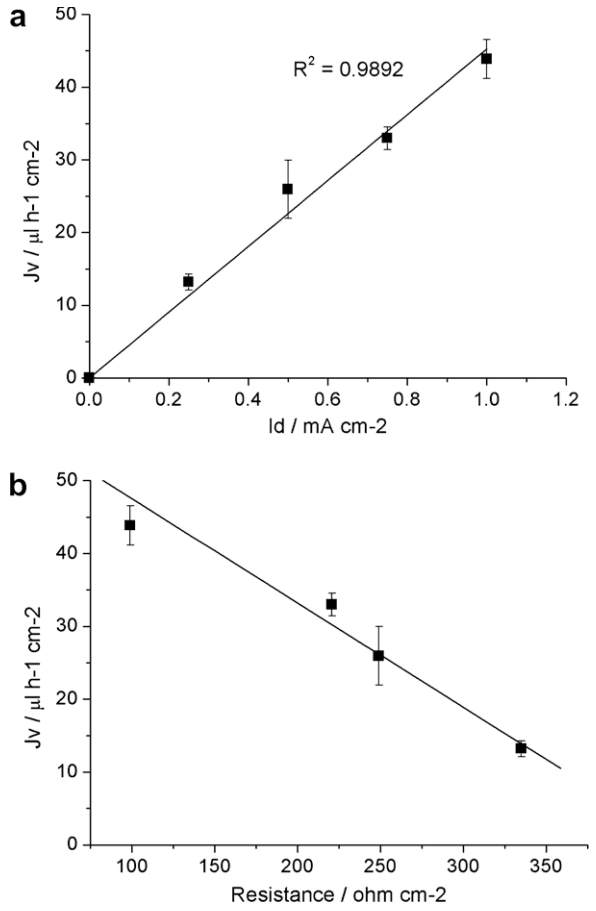

Fig. 5. (a) The current density influence on J_v; (b) the correlation of mucosal resistance and J_v (resistance for 220 min of experiments). Anodal and cathodal chambers filled with PBS, buccal mucosa.

Data presented in Figs. 3–6 are arithmetical averages from measurements with standard deviations, number of measurements: $3 \leqslant n \leqslant 9$.

3. Result and discussion

The purely osmotic volumetric flow of the solvent in the experiment conditions was smaller than measurement accuracy, so results presented in this paper takes into consideration the electroosmotic flow (J_v) only. The water flow from positive to negative electrode was observed during experiments in electric field.

The J_v stabilized just after the beginning of the process. It was found that during all experiments the voltage drop, caused by the change in the tissue sample resistance, was falling at the beginning and after 20–40 min started to increase – like in Fig. 2. We did not notice the stop of this growth during all the experiments (the longest experiment took 320 min). An average percentage growth of voltage drop was calculated by comparison of measurements at 220 min of experiment to beginning values for four experiments (PBS in both chambers, current density: 0.25, 0.5, 0.75, 1.0 mA cm^{-2}). It was 40 ± 11%.

3.1. Resistance

Observed voltage drop during the measurements was proportional to the electrical resistance of the tissue sample, so after the electric current had been applied at the beginning of experiments the resistance decreased, and afterwards it started to increase. The result suggests that at the beginning mucosa hydration enables formation of new pathways for ion transport, but next the amount of water transferred into the tissue causes dilution of electrolytes which hinders the charge transport. This mechanism may be confirmed by a significant swelling of mucosa which was observed in all experiments. The resistance decrease during the current treatment was also reported for skin [22,33,34]. For this tissue after the first hour of experiment the resistance is stabilized [22] or it still decreases, but at slower rate [34].

Experiments proved that initial mucosa resistance was 2300–70 Ω cm^{-2} and depended on current density (see Fig. 3). After 220 min of current treatment the resistance was in the range of 335–100 Ω cm^{-2}, so the current dependence became weaker. The resistance dependence on current was noted earlier for skin [22]. Moreover skin is also a nonlinear system in which reduction in

resistance often correlates with increased permeability [34]. Results presented in Figs. 3 and 5b confirmed the same properties for mucosa. Comparison of mucosal resistance with measurements for skin at similar current density [22] revealed that mucosa had a few times lower resistance (2 times before and 5 times during the current treatment).

The function of resistance (Fig. 3), especially for initial resistance, has a non-linear, but decreasing trend. Result presented by Pikal and Shah [22] for skin resistance after electroosmosis also exhibited decreasing dependence, but the tendency was linear in the range of 0.3–3.0 mA cm^{-2}. Extrapolation of their results to zero of current density and comparing to presented data for 220 min once more revealed that the resistance of mucosa was lower than that for skin, however, the order of this value was the same.

The resistance of PBS, used in this work to simulate the internal conditions of body, was also calculated from measurements of voltage drop (Fig. 4). The buffer proved to be a non-linear resistor like a mucosa and the resistance, although a little higher, was in the same order of magnitude as for tissue.

3.2. Volume flow

Observed J_v through buccal mucosa was approximately a linear function of current density (Fig. 5a); however, this regression was not well fitted to measurements at low current density. To explain this result the water transference number was calculated.

The electroosmotic flow is also defined as a water transference number which is the number of water moles transported per equivalent of electricity passed through membrane [35]. Pikal [23] stated that water transference number is independent of current density. However, for diluted solutions the voltage needed to sustain constant current is too high to retain the linear Ohm's law. Data given in Fig. 6 were calculated from equation presented by Pikal [23] and showed the water transference number dependence on current density for system PBS–buccal mucosa–PBS.

This dependence in spite of wide error bars was confirmed by dependence of PBS and tissue resistance on current density. Comparison of dependence of water transference number on current density with results for ion-exchange artificial membranes [36] showed similar behaviour.

3.3. Electrolyte concentration

Results presented in Fig. 7 showed that J_v was a function of electrolyte concentration and this dependence was stronger for lower concentration of electrolyte. Obtained data had a similar trend as the data for skin [22], however, for skin the J_v is one order of magnitude smaller.

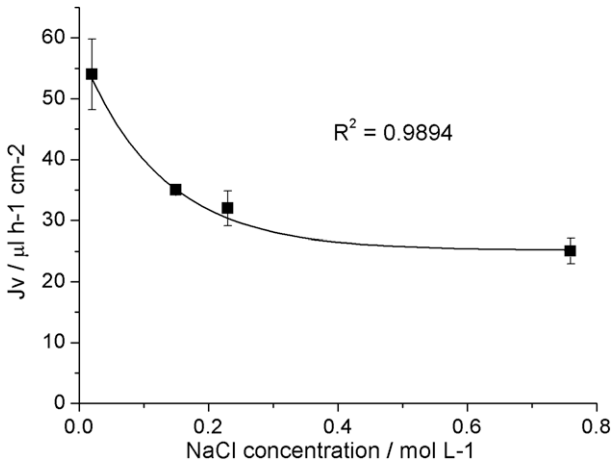

Fig. 7. The influence of electrolyte concentration in anodal (donor) chamber on J_v. Cathodal chamber filled with PBS, buccal mucosa, current density 0.75 mA cm^{-2}.

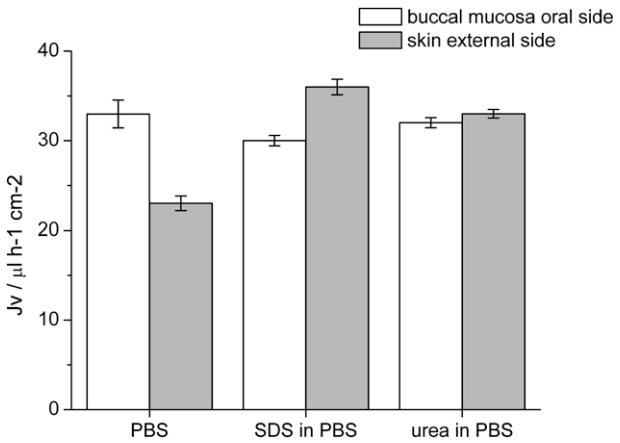

Fig. 8. Comparison of electroosmosis in buccal mucosa and skin. Current density 0.75 mA cm^{-2}, cathodal chamber filled with pure PBS, anodal: pure PBS, 0.0019 mol L^{-1} SDS solution in PBS and 0.98 mol L^{-1} urea solution in PBS.

3.4. Comparison to skin

Comparison of results for skin and mucosa shows that stratum corneum of skin is a strong barrier for water transfer – the J_v through mucosa was 1.4 times bigger than that for skin (Fig. 8). The addition of SDS and urea had different influence on water flow via these two types of tissues (Fig. 8). SDS did not promote a water transfer through buccal mucosa in concentration range 0.001–0.005 mol L^{-1}, however, in experiment with skin, SDS enhanced the J_v above the level of non-keratinized tissue. The addition of urea had no significant influence on water transport via mucosa in concentration range 0.42–1.67 mol L^{-1}, but promoted electroosmosis in skin. These results suggest that SDS and urea – typical permeation enhancers for transdermal iontophoresis, had an influence only on transport through lipid layer of stratum corneum and did not change the properties of lipid barrier in buccal mucosa.

In conclusion buccal mucosa at pH 7.4 showed bigger mobility of cations than anions and in field of direct current the J_v from cathode to anode was observed. Mucosa had better water permeability than skin which suggests that this could be a better area for iontophoretical delivery of hydrophilic and particularly cationic drug. An internal side of cheek may also be a more convenient portal for electroosmotic administration of the neutral substances than skin area. The electrically enhanced drug delivery via buccal mucosa may be as effective as simultaneously electrically and chemically enhanced transfer through skin. The authors expect that if for SDS there is no improvement in electroosmotic water flow through mucosa, also application of the other surfactants (e.g. nonionic which have a weaker action) will not bring enhancement of electroosmosis in mucosa. During buccal iontophoresis new hydrophilic pathways are created mainly by electrically enhanced solvent flux which accompanies the flux of cations and loosens the tissue structure. It seems that chemical enhancers have not as strong influence on mucosa hydration as in case of keratinized tissue, so during buccal iontophoresis there is no necessity to use them.

Taking into account better permeability of mucosa than skin all substances which are administrated by transdermal iontophoresis probably may be delivered by buccal iontophoresis. However, it is necessary to emphasize that for buccal absorption, drugs which are safe to be swallowed and which have an acceptable taste for pa-

tients can only be used. This type of electric enhancement in buccal delivery may be useful for local and also systemic drug delivery. The current density limitation may be more restrictive than for skin, because of the bigger sensitivity to pain; however, we expect that buccal administration via iontophoresis is more efficient and will require lower current density than transdermal application. The buccal mucosa, as an area of absorption, enables design of electronic intraoral implants for long-term-controlled delivery [37] and invent of devices for needleless drug 'injection'. The localization of the mucosa allows location of the electrodes set on the same surface or only the donor electrode may be positioned in a mouth and the acceptor one may be on the external side of cheek. This opposite location may increase the current efficiency of drug transfer.

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