In Vitro Evaluation of a Hydroxypropyl Cellulose Gel System for Transdermal Delivery of Timolol

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Abstract: In this work, the development of a gel reservoir for a timolol (TM) transdermal iontophoretic delivery system is investigated. TM gel is prepared using hydroxypropyl cellulose (HPC) and the permeability of TM from the gel through an artificial membrane (Polyflux®) and pig stratum corneum (SC) is studied.

For a constant TM donor concentration, the TM transport across the Polyflux® membrane alone decreases when the concentration of the gel increases due to increase of the gel viscosity. For constant gel concentration, however, the TM permeation across the membrane increases when the TM donor concentration increases. In addition, no effect of the electrical current (iontophoresis, current density 0.5 mA cm^{-2}) on the TM permeation is found.

For the combination of the Polyflux® membrane with pig SC, the TM transport is much lower than for the membrane alone and the SC fully controls the TM delivery. In this case, the application of electrical current enhances the TM delivery 13-15 times in comparison to passive (no current) transport. According to our estimation, the daily TM dose (10-60 mg) can be delivered by an iontophoretic patch with Polyflux® membrane area of 6 - 36 cm^2 containing 20 % (w/w) HPC gel and 15 mg cm^{-3} of TM.

Keywords: Transdermal, Timolol, Hydroxypropyl cellulose, Gel reservoir, Iontophoresis.

1. INTRODUCTION

Timolol (TM) is a nonselective beta-adrenergic blocking agent, which is used in the management of hypertension, angina pectoris, myocardial infraction and glaucoma. The systemic absorption of TM may cause respiratory and cardiovascular side effects. It is therefore important to minimize the systemic absorption and enhance the local TM bio-availability [1]. Previous studies concerning the TM delivery include ocular [2-4], intravenous [5] as well as transdermal systems [6-14].

Transdermal administration of TM can overcome the above-mentioned side effects and provide controlled delivery for an extended period of time. The transdermal TM delivery may be:

- *Passive*, where the driving force for the TM transport is only the concentration gradient between the delivery device and the skin [6, 7, 10, 11]),
- *Active,* where the TM transport is enhanced by the application of either constant electrical current (maximum 0.5 $mAcm^{-2}$, a method called iontophoresis [8, 9, 12, 13]) or pulsed electrical current (a method called electroporation [14]).

In earlier work performed in our group, we investigated the TM delivery from liquid buffer solution through artificial membranes alone [12], pig stratum corneum (SC) alone and in combination with artificial membranes [13]. We found that the application of porous membranes to a TM iontophoretic transdermal delivery system is feasible. In commercial transdermal systems, however, the drug is often incorporated in a gel reservoir. In the present work, we aim to evaluate the application of a hydroxypropyl cellulose (HPC) gelling agent, which is commonly used in transdermal drug delivery [15-16], as TM reservoir. Our objectives are to develop a topical gel TM formulation and to study its *in vitro* permeation characteristics through an artificial membrane (Polyflux®) alone and in combination with pig SC. The latter proved to be a good model for the TM delivery through human skin [13]. To optimize the TM formulation, the effects of HPC gel concentration, TM concentration and current application on the TM permeation rate are evaluated for the Polyflux® membrane alone. As it will be shown, the membrane imbibes the gel and the TM transport across the membrane probably takes place through the gel – filled membrane pores. However, when the artificial membrane is combined with pig SC the control of the TM transport is determined by the transport through the pig SC.

2. EXPERIMENTAL

2.1. Materials

Timolol maleate salt and hydroxypropyl cellulose (MW 80 kDa) were obtained from Sigma – The Netherlands. Gambro (Hechingen - Germany) kindly provided the Polyflux® artificial flat sheet membrane (MWCO: 10 kDa, geometrical thickness of the dry membrane: 61±3 μm, material: polyarylethersulfone). All the other reagents were of analytical grade and were used without any pre-treatment.

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TM was dissolved in citrate buffer saline solution 0.148 M at pH 4.7 (Citrate buffer: NaCl (8 g l^{-1}), citric acid 1.07 g l^{-1}, sodium citrate 2.11 g l^{-1} in ultra pure MilliQ = deionised water). In some experiments, lower NaCl concentrations were applied too (see results later). Under this pH condition (pH = 4.7), TM is positively charged (pK_a of TM is 9.21). The Institute of Animal Science and Health (ID-Lelystad, The Netherlands) kindly provided the pig skin and the SC was obtained following the procedure described elsewhere [17].

2.2. Preparation of TM gel

The HPC is derived from cellulose by substitution of the hydroxyl group. Its backbone is therefore that of cellulose with a repeat anhydroglucose unit. It is easily dissolved in water and forms gels at high concentrations. HPC solutions of concentrations up to 20 % (w/w) in TM – citrate buffer solution (pH 4.7) were prepared at room temperature. In our experiments, a TM base concentration in the range of 5-20 mg cm^{-3} was used.

2.3. Gel imbibition by the Membrane

The amount of the gel uptake by the membrane was determined by weight measurements. Between 3 and 5 mg of Polyflux® membrane were accurately weight and soaked in buffer solution and in HPC gel at various gel concentrations. Imbibition occurred rather fast and a constant weight was reached within hours, as was ascertained by monitoring the weight for at least 3 days. The amount of imbibed buffer or gel into the membrane (% (w/w)) was determined from the weight difference between the membrane soaked in buffer or gel and the dry membrane, over the weight of the dry membrane.

2.4. Membrane Characterization

In order to get a sufficient insight on the structure of the Polyflux® membrane, the clean water permeability in the pressure range of 1-3.5 bar at 20 ± 2 °C, was measured. The permeation experiments were performed in a dead-end filtration set-up [18a]. Each cell contains an effective membrane area of 3.14 cm^2. A porous disc supported the membrane. For the permeation experiments, clean ultra pure MilliQ = deionised water was placed in a feed tank and pressurised with N_2 gas. The water flux through the Polyflux® membrane was calculated by the following equation:

$$J_w = V_w / (A\ t) \quad (1)$$

where J_w is the water flux (in l m^{-2} h^{-1}), V_w is the permeate volume of water (in l), A is the membrane area (in m^2), t is the permeation time (in h). The flux of water increases with the increase of the applied pressure following the equation:

$$J_w = P_w \Delta p \quad (2)$$

where Δp is the transmembrane pressure difference (in bar) and P_w is the clean water membrane permeability (in l m^{-2} h^{-1} bar^{-1}).

The morphology of the Polyflux® membrane was visualised by Scanning Electron Microscopy (SEM, Microscope Jeol JSM-5600LV). The samples were fractioned in liquid N_2 and sputtered with gold under vacuum for 300 s, at a current of 15 mA.

2.5. Diffusion Cell – Experimental Procedure

All TM transport experiments (passive diffusion or iontophoresis) were performed in a three chamber continuous flow through diffusion cell designed at Leiden University (see detailed description in [12, 19]). The available area for transport was 0.64 cm^2. Circulating water at 37°C controlled the temperature of the acceptor chamber (containing phosphate buffer saline (PBS) 0.153M solution at pH 7.4 [PBS: NaCl (8 g l^{-1}), KCl (0.19 g l^{-1}), KH_2PO_4 (0.2 g l^{-1}), $Na_2HPO_4.12H_2O$ (2.86 g l^{-1}) in ultra pure MilliQ = deionised water]) and the temperature in the donor chamber reached 32°C. A silver plate electrode and a silver/silver chloride electrode were used as driving electrodes in the anodal and cathodal compartment, respectively. Silver/silver chloride electrodes were also used as reference electrodes for the measurement of the electrical resistance. The pig SC was hydrated for two hours in buffer solution prior to use. The SC was very fragile; therefore, it was supported by a dialysis membrane (Dialysis-5, neutral cellulose, MWCO 5 kDa, Diachema - Germany). In a previous study [13], it was found that the contribution of this membrane to the overall TM permeability (through the SC and dialysis membrane) could be ignored.

For the transport experiments, the membrane pieces only or in combination with SC were introduced between the cell chambers and the cell acceptor chamber was equilibrated with PBS at a flow rate of 6.5 ml h^{-1} for 30 min, using a peristaltic pump. The TM gel was introduced in the anodal chamber and citrate buffer without TM in the cathodal chamber. No stirring was applied. In the iontophoretic experiments, current density of 0.5 mA cm^{-2} was used. Digital multi-meters connected to the reference electrodes monitored the resistance of the cell. All transport experiments were performed for 6 - 8 hours and for 8-10 samples for each case. The average and standard deviation for these samples were calculated and statistical analysis was made using the t-test. The probability value of less than 0.05 was considered to be significant. The "flow through" acceptor solution was collected every hour and the concentration of TM was determined by UV for the Polyflux® membrane alone and by HPLC for experiments when pig SC was used [13]. The apparent flux of TM through the membrane, J_{app}, is obtained using the equation:

$$J_{app} = F\ C_{acceptor} / A \quad (3)$$

where $C_{acceptor}$ is the TM acceptor concentration (in mg cm^{-3}), A is the available membrane area for diffusion (in cm^2) and F is the acceptor flow rate (in cm^3 h^{-1}). The flux reaches steady state after a certain time. This steady state flux (J_{ss}, in mg cm^{-2} h^{-1}) is obtained from the linear part of the cumulative flux and expressed as:

$$J_{ss} = Q / t = (K_P)_{memb}\ C_{donor} \quad (4)$$

where Q is the amount of TM permeated through the membrane (in mg cm^{-2}) in experimental time t (in h), C_{donor} is the concentration of TM in the donor chamber (in mg cm^{-3}) and $(K_P)_{memb}$ (in cm h^{-1} or cm s^{-1}) is the permeability of TM through the membrane.

RESULTS AND DISCUSSION

3.1. Characterization of the Polyflux® membrane

The average value of the clean water membrane permeability measured for at least 5 membrane samples is $P_w = 28.8 \pm 4.9$ l m^{-2} h^{-1} bar^{-1}. In the literature [18b], the value of the clean water permeability is often used to calculate the membrane porosity and tortuosity. However, this calculation requires the application of models, which assume that the pores have a well-defined shape (Hagen – Poiseuille equation for capillary pores and Kozeny – Carman equation for pores that are interstices between closed packed spheres [18b]). For the Polyflux® membrane, the SEM pictures do not show a well-defined pore structure (Fig. **1**). The membrane has an asymmetric structure and its pore size differs between the membrane sides. The Polyflux® membrane, in the form of hollow fibre, is used in haemodialysis [20]. The denser layer, which is in contact with the blood, contains fine nano-pores and is supported by a thicker solid structure with interlinked voids, which provide mechanical support for the dense layer. The asymmetry in the membrane structure is clear when looking at the two membrane surfaces. One surface has much smaller pores than the other (Fig. **1b, 1c**). Based on these observations, we avoid applying any of the proposed models to calculate further membrane characteristics (porosity, tortuosity etc).

3.2. Gel Imbibition

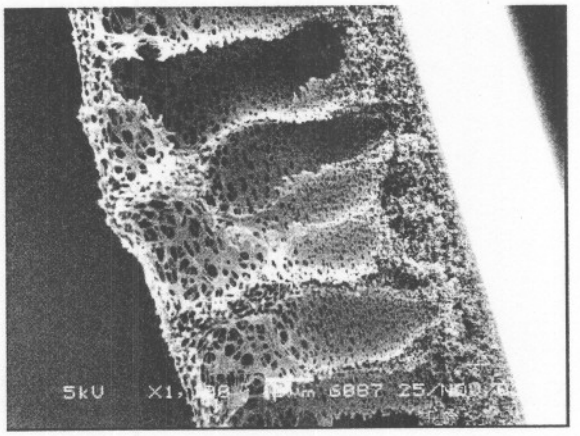
The liquid buffer and gel are imbibed by the membrane replacing the air from the pores. Table **1** shows these results for liquid buffer and for the HPC gel at various gel concentrations. The imbibition of liquid does not differ significantly in comparison to that of the HPC gel and the thickness of the wet membranes does not differ from that of the dry membrane. We assume that the gel fills up the pores of the membrane without causing further changes to the membrane structure.

3.3. Permeation across the Polyflux® membrane

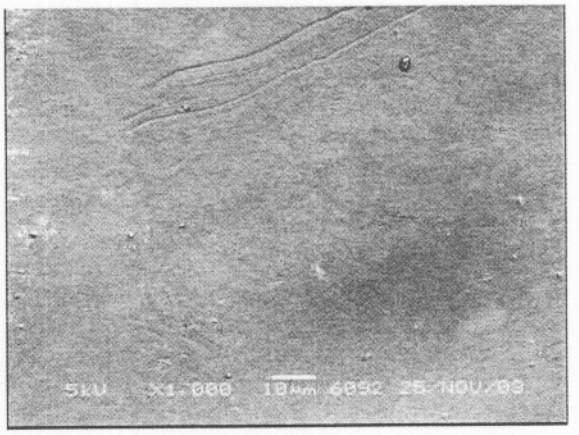
3.3.1. Effect of Gel Concentration on the Permeation

Figure **2** shows a typical result of the effect of HPC gel concentration on the TM permeation across the Polyflux® membrane, at 37°C (for these experiments, the TM donor concentration was 14-16 mg cm^{-3}). The amount of TM permeating across the membrane is linear with the experimental time. The higher the gel concentration, the slower is the TM transport. Using eqn. 4, the TM permeability at various gel concentrations is calculated and presented in Table **2**. The apparent reduction of TM transport at higher gel concentrations is attributed to the increase of gel viscosity. It is important to note that even though the TM transport decreases with the increase of gel concentration, the gel concentration should be high enough for a stable patch reservoir. From our experience in this work with this gel, a concentration of 20% (w/w) seems more suitable for the TM patch. Therefore, all the *in vitro* studies of the membrane in combination with the pig skin were performed using this 20% (w/w) HPC gel concentration (see results later).

(a) Polyflux® membrane: cross-section



(b) Polyflux® membrane: top surface



(c) Polyflux® membrane: bottom surface

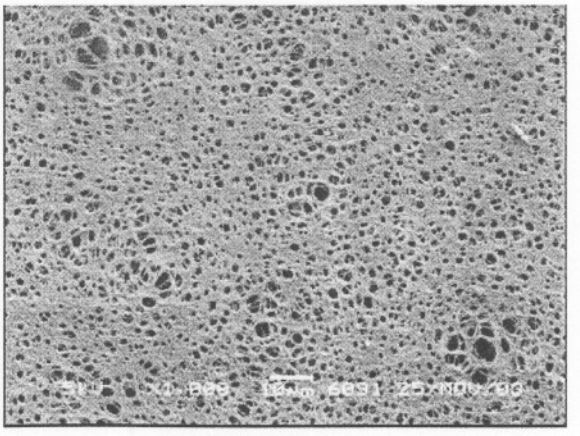


Fig. (1). SEM pictures of the Polyflux® membrane. (a) cross section, (b) top surface (c) bottom surface.

Table 1. Gel Imbibition by the Membrane as a Function of HPC Concentration

HPC (%, w/w)	Polyflux® Imbibition (%, w/w)	Polyflux® thickness, h (μm)
0	311 ± 4	61 ± 3
10	325 ± 13	63 ± 2
20	323 ± 7	63 ± 2

Table 2. Passive and Iontophoretic TM Permeability from the HPC Gels through the Polyflux® Membrane Alone and in Combination with Pig SC

TM - reservoir	HPC (%, w/w)	Kp x 10^6 (cm s^{-1}) Passive diffusion	Kp x 10^6 (cm s^{-1}) Iontophoresis	EF
Polyflux ®				
8 g/l NaCl	0	108.3 ± 18.6	101.9 ± 7.5	0.9
8 g/l NaCl	10	58.8 ± 4.9	59.5 ± 4.8	1.0
8 g/l NaCl	20	37.5 ± 2.6	40.1 ± 1.6	1.1
4 g/l NaCl	20	35.6 ± 5.0	37.0 ± 5.3	1.0
Polyflux® +pig SC				
8 g/l NaCl	20	0.1 ± 0.1	1.3 ± 0.4	13.0
4 g/l NaCl	20	0.1 ± 0.1	1.5 ± 0.3	15.0

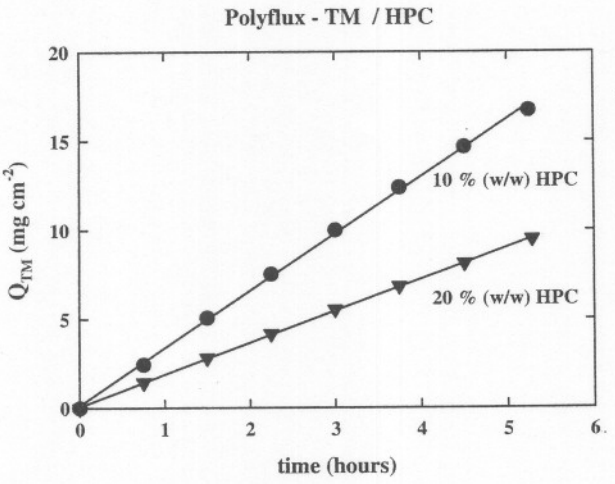


Fig. (2). Amount of TM permeated from the HPC gel across the Polyflux® membrane at various gel concentrations. Temperature= 37°C. Gel contains 14-16 mg cm^{-3} TM.

3.3.2. Effect of TM Concentration on the Permeation

Figure **3a** shows a typical plot of the amount of TM delivered from the 20% (w/w) HPC gel at various TM donor concentrations. Figure **3b** shows the TM steady state flux ($J_{ss,TM}$) through the Polyflux® membrane versus the TM donor concentration for various HPC gel concentrations. The TM flux increases with the increase of TM concentration (driving force) as expected from eqn. 4. However, the TM permeability, K_P, does not depend upon the TM concentration (Fig. **4**) as expected from the same equation. The permeability is a characteristic parameter of the specific system and should be independent of the TM concentration if there is no interaction between the TM and the membrane - gel. In order to investigate possible interaction between the TM and the Polyflux® membrane, we measured the static adsorption of TM to the Polyflux® membrane. The membrane samples were dipped in a TM solution of concentrations in the range 5-25 mg cm^{-3}, at 37°C for at least 3 days. The membranes were then removed from the solution and the concentration of the TM adsorbed to the membrane was calculated from the difference between the concentration of the TM solution before and after membrane dipping. The average equilibrium amount of TM adsorbed to the membranes was very low, ~10 μg cm^{-2}, proving the low interaction between TM and the Polyflux® membrane.

3.3.3. Effect of Current Application on the Permeation

Figure **4** also shows the results of the TM permeability from the 20% (w/w) HPC gel across the Polyflux® membrane, under passive and iontophoretic conditions (current density 0.5 mA cm^{-2}). It shows no significant effect of the current to the TM permeation. The electrical resistance of the membranes during iontophoresis is low and in the range of 0.12-0.50 kΩ cm^2. Table **2** also shows that the enhancement factor (EF, ratio of TM permeability across the membrane during iontophoresis over passive diffusion) at

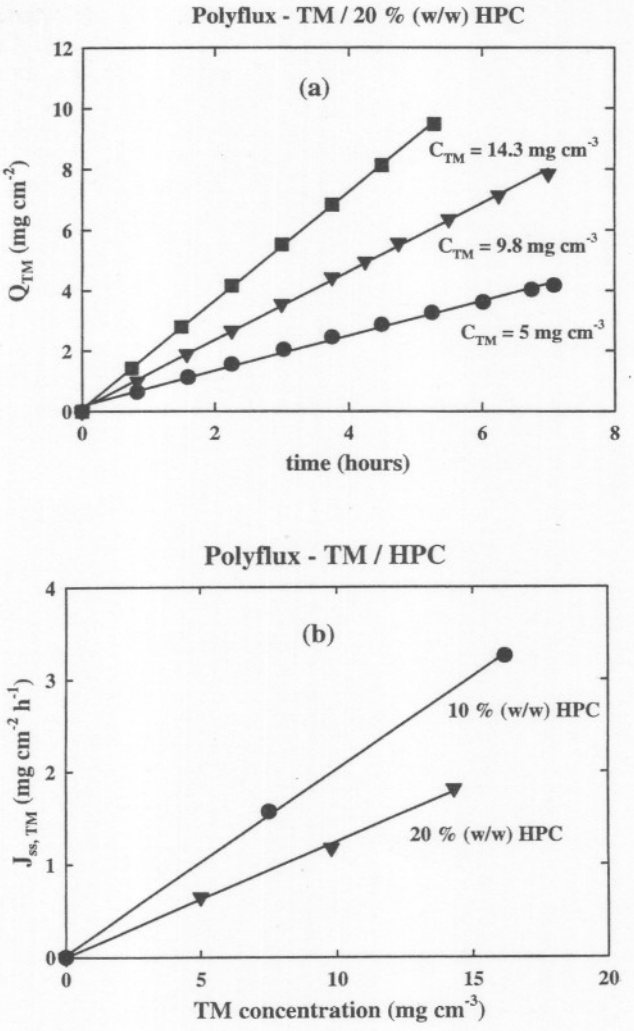


Fig. (3). (a) Amount of TM permeated from the 20% (w/w) HPC gel across the Polyflux® membrane at various TM donor concentrations. Temperature = 37°C. (b) The steady state flux of TM ($J_{ss,TM}$) through the Polyflux® membrane versus the TM donor concentration at various HPC gel concentrations.

various HPC gel compositions is ~1. Apparently, the contribution of passive diffusion greatly outweighs the contribution of electrical current, thereby making the TM transport with and without applied current indistinguishable. Namely, the passive diffusion is very high due to the high membrane pore size and the small extra iontophoretic driving force (0.5 mAcm^{-2}) do not cause significant increase of the TM transport. Then as expected, the decrease of competition from the Na^+ and Cl^- ions (by lowering the NaCl concentration) does not cause significant changes on the iontophoretic TM transport, too. (Table **2**, the NaCl is used for the reaction at the Ag / AgCl electrodes [1]).

3.4. Permeation Across Combination of Polyflux® Membrane and Pig SC

As mentioned earlier, a stable viscous gel is required for the TM patch reservoir and therefore, a HPC gel

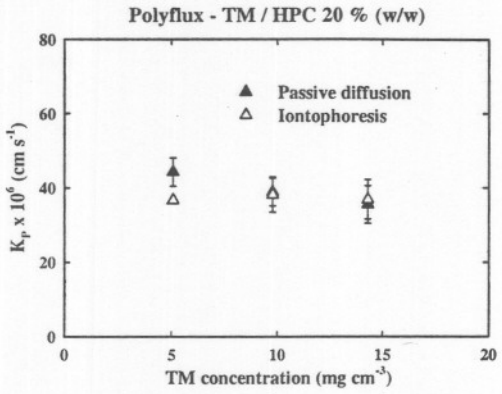


Fig. (4). Effect of the TM concentration on the TM permeability from a 20% (w/w) HPC gel across the Polyflux® membrane. Full symbols: passive diffusion, open symbols: iontophoresis (current density of 0.5 mA cm^{-2}). Temperature = 37°C.

concentration of 20 % (w/w) was selected for the experiments with the pig SC. For these experiments, the Polyflux® membrane was placed in front of the pig SC as it happens during the application of a patch. Then, the overall permeability through the membrane and pig SC was measured. The HPC-TM gel was applied only to the anode compartment [12].

Figure **5a** shows typical results of the permeated TM from the 20% (w/w) HPC gel through the combination of the Polyflux® membrane and pig SC under iontophoretic conditions (current density: 0.5 mAcm^{-2}). The TM permeation through the combination is much lower than through the membrane alone indicating the significant role of the SC to the TM delivery. It is worth to note that in our laboratory we also measured the transport of TM from Carbopol gel (1 %, (w/w), the Carbopol 980NF gelling agent was kindly provided by Noveon Inc. – Belgium) through a polyethersulfone artificial membrane (PES 30, MWCO 30 kDa kindly provided by Sartorius - Germany) alone and in combination with pig SC. We found that the permeability through the latter controls the overall TM delivery. The TM permeability through the combination of PES 30 and pig SC during passive diffusion and iontophoresis was 0.1 ± 0.1 x10^{-6} cm/s and 1.2 ± 0.3 x 10^{-6} cm/s, respectively, in excellent agreement with the results of the current work. These results prove once more the significant role of the SC to the TM delivery.

In contrast to the Polyflux® membrane alone, for the combination of membrane and pig SC, the EF for the TM iontophoretic transport is considerably higher than the passive transport (see Table **2** and Fig. **5b**). During the first few minutes of the current application, the electrical resistance of the combination of membrane and pig SC drops sharply from ~16 kΩ cm^2 to ~2.4 kΩ cm^2 and stays at the lower levels for several hours of iontophoresis. Figure **5b** also shows that for both passive diffusion and iontophoresis, a small lag time (0.5 - 1 h) is needed to achieve the steady state TM delivery. This finding is a little surprising. We

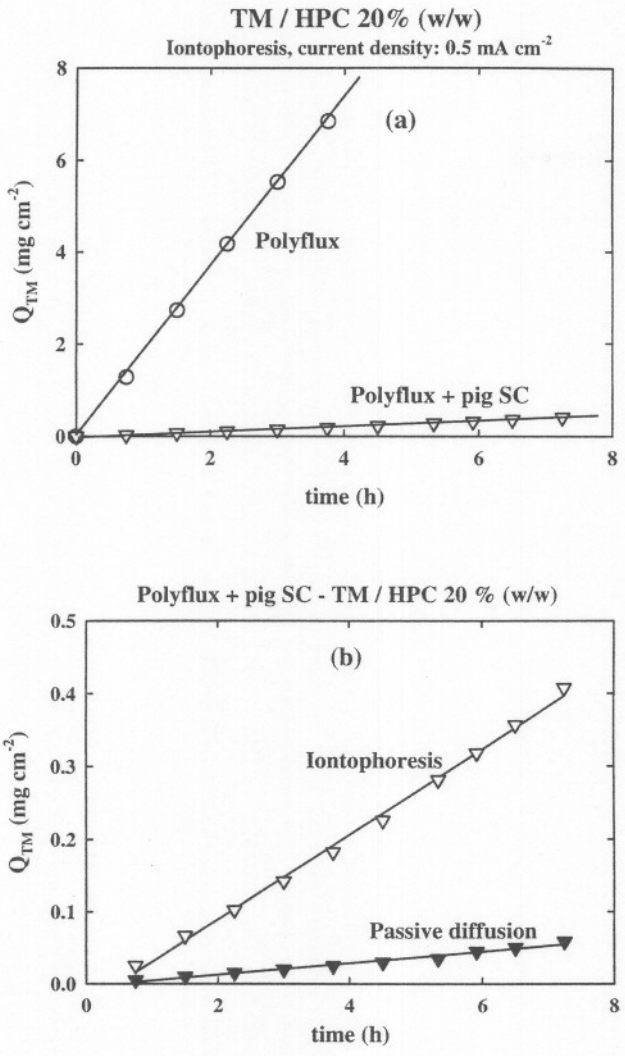


Fig. (5). a) Iontophoretic TM permeability (current density: 0.5 mA cm^{-2}) from a 20% (w/w) HPC gel across a Polyflux® membrane alone (circles) and in combination with pig SC (triangles). (b) TM permeability from HPC 20 % (w/w) gel across the combination of Polyflux® membrane and pig SC under passive (full symbols) and iontophoretic conditions (open symbols). Temperature = 37°C. Gel contains 14-16 mg cm^{-3} TM.

would generally expect that the lag time for the TM iontophoretic delivery would be lower than for the passive delivery. In our experiments, however, such difference is not observed.

As for the Polyflux® membrane alone, the iontophoretic TM permeability through the combination of membrane and pig SC does not change significantly when the concentration of NaCl electrolyte in the gel decreases (see Table **2**). However, in this case the reason is different than that for the membrane alone. The TM transport is fully controlled by the SC, which contains much smaller pores than the artificial membrane. When the constant current is applied, there is competition for the current between the bulky TM and the smaller charged ions Na^+ and Cl^- present in the solution. It seems that only a small amount of the current is transported by TM but mostly it is transported by other ions. The fact that the decrease of the NaCl concentration in the donor solution does not cause a significant increase on the TM transport indicates that probably the current is transported by the Cl- ions coming from the backside of the membrane. Recently, Marro *et al.* [21] have also reported that for the iontophoretic delivery of propranolol (a beta blocking agent, like TM), the major charge carrier was the Cl^- moving from beneath the skin into the anodal chamber. This behaviour has been reported in other studies as well [22, 23].

For the combination of the Polyflux® membrane and pig SC, we can estimate the TM patch specifications, using eq. 4. The daily TM dose (10-60 mg, depending on the disease and treatment) can be delivered by an iontophoretic patch (current density: 0.5 mA cm^{-2}) containing:

- **Gel reservoir:** HPC 20% (w/w), 15 mg cm^{-3} of TM
- **Membrane:** Polyflux® membrane area in the range of 6 - 36 cm^2.

The required Polyflux® membrane area is acceptable, having in mind that the maximum acceptable patch size is generally ~ 50 cm^2 [1].

4. CONCLUSIONS

In this work, the transport of TM from a HPC gel system through the Polyflux® artificial membrane and pig SC was investigated. Permeation experiments performed using the membrane alone showed that for constant TM donor concentration, the TM transport across the membrane decreased when the concentration of the HPC gel increased, due to increased gel viscosity. However, for constant gel concentration, the TM permeation increased when the TM donor concentration increased. In addition, no effect of the electrical current on the TM permeation was found.

For the combination of the Polyflux® membrane with pig SC, the TM transport was much lower than for the membrane alone, which indicated that the SC fully controlled the TM delivery. In this case, the application of electrical current enhanced the TM transport significantly in comparison to the passive diffusion (EF =13-15). Our estimation showed that the daily TM dose (10-60 mg) could be delivered by an iontophoretic patch containing 20% (w/w) HPC gel, 15 mg cm^{-3} of TM and a Polyflux® membrane area in the range of 6 - 36 cm^2.

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LIST OF SYMBOLS

A	=	Membrane surface area (in m^2 or cm^2)
$C_{acceptor}$	=	Acceptor concentration (in mg cm^{-3})
C_{donor}	=	Donor concentration (in mg cm^{-3})
Δp	=	Transmembrane pressure difference (in bar)
EF	=	Enhancement factor
F	=	Acceptor flow rate (in $cm^3\ h^{-1}$)
J_{ss}	=	Steady state flux (in mg $cm^{-2}\ h^{-1}$)
J_w	=	Water flux (in l $m^{-2}\ h^{-1}$)
h	=	Membrane thickness (in μm)
I	=	Electrical current (in mA)
K_P	=	Permeability (in cm h^{-1} or cm s^{-1})
P_w	=	Water permeability (in l $m^{-2}\ h^{-1}\ bar^{-1}$)
Q	=	Amount of drug permeating through the membrane (in mg cm^{-2})
t	=	Time (in h)
V_w	=	Volume of water (in l)

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