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Physically cross-linked chitosan hydrogels as topical vehicles for hydrophilic drugs

T. Cerchiara, B. Luppi, F. Bigucci, I. Orienti and V. Zecchi

Abstract

Physically cross-linked chitosan hydrogels with lauric, myristic, palmitic or stearic acid were prepared by freeze-drying and have been studied for topical use. This study selected propranolol hydrochloride as a hydrophilic model drug to design a transdermal delivery system. We evaluated the effect of the nature of the cross-linker on drug permeation through porcine skin and the main permeation parameters (diffusion coefficient, flux and lag time) were calculated. All the chitosan hydrogels analysed provided more transcutaneous permeation of propranolol hydrochloride than the corresponding solution of the commercial drug. Among the different chitosan vehicles, chitosan–laurate and chitosan–myristate hydrogels enhanced lyophilised drug diffusion through the skin with respect to chitosan–palmitate and chitosan–stearate hydrogels. This can been explained by the interaction of the hydrogels with the stratum corneum, increasing the solubility of the drug in the skin.

Introduction

In the field of controlled drug delivery systems, transdermal drug delivery systems (TDSs) have biopharmaceutical benefits such as increasing bioavailability and therapeutic efficacy, bypassing hepatic first-pass metabolism (Jona et al 1995; Thacharodi & Rao 1995; Fuhrman et al 1997; Modamio et al 2000).

Propranolol hydrochloride is a non-selective β-adrenergic blocking agent widely used in the treatment of hypertension and other cardiovascular disorders. It suffers from a high degree of first-pass metabolism resulting in very low bioavailability ($< 10\%$) following administration in conventional oral formulations (Krishna & Pandit 1996) and is characterized by a short elimination half-life of about 3 h (Zhao & Singh 1999). To circumvent this significant therapeutic hurdle, a hydrophilic vehicle was devised to attain propranolol hydrochloride concentrations suitable for efficient transdermal delivery. This work describes the use of physically cross-linked chitosan obtained from the reaction with lauric, myristic, palmitic or stearic acid as supporting material for the preparation of hydrogels able to release propranolol hydrochloride and, more generally, hydrophilic drugs.

Chitosan, a cationic natural biopolymer produced from deacetylation of chitin, has been widely used for drug carrying devices in controlled drug delivery systems (Paul & Sharma 2000; Ravi Kumar 2000). This natural polysaccharide possesses useful properties such as non-toxicity, high biocompatibility (Sawayanagi et al 1983; Hirano et al 1990) and non-antigenicity that offer advantages for possible clinical use (Suheyla Kas 1997). Chitosan was selected as a starting material that on attachment of a strategic number of hydrophobic groups yields a gel on freeze-drying. The use of an amphiphilic polymer to prepare hydrogels has the added advantage of potentially improving the permeation of drug substances through the biological membranes (Noble et al 1999).

In particular, we evaluated the correlations between the physico-chemical characteristics of physically cross-linked chitosan hydrogels and the functional properties of the gels with the aim of determining the conditions favouring drug permeation through the skin.

Department of Pharmaceutical Sciences, Bologna University, Via S. Donato 19/2, 40127 Bologna, Italy

T. Cerchiara, B. Luppi, F. Bigucci, I. Orienti, V. Zecchi

Correspondence: B. Luppi, Department of Pharmaceutical Sciences, Bologna University, Via S. Donato 19/2, 40127 Bologna, Italy. E-mail: bluppi@biocfarm.unibo.it

Materials and Methods

Materials

High-molecular-weight chitosan (MW 600 000, viscosity 400 mPas (1% solution in 1% acetic acid), degree of deacetylation 85%) was purchased from Fluka (Buchs, Switzerland). Propranolol hydrochloride was purchased from Sigma-Aldrich; lauric, myristic, palmitic and stearic acids were from Fluka. Acetonitrile (HPLC grade), triethylamine and orthophosphoric acid were from Romil Pure Chemistry. Phenomenex Luna C18(2) column was obtained from Chemtek Analitica. Other organic and inorganic chemicals were commercially available and used without further purification.

Synthesis of physically cross-linked chitosan hydrogels

Chitosan (0.25 g) was dissolved in 0.1 M HCl (20 mL) to which was added absolute ethanol (25 mL). To this solution was added, drop-wise, an ethanolic solution of lauric acid *N*-hydroxysuccinimide (33.9 mg mL^{-1}, 30 mL); myristic acid *N*-hydroxysuccinimide (38.3 mg mL^{-1}, 30 mL); palmitic acid *N*-hydroxysuccinimide (43.3 mg mL^{-1}, 30 mL) or stearic acid *N*-hydroxysuccinimide (47 mg mL^{-1}, 30 mL). These mixtures were left stirring for 24 h protected from light. At the end of this period, acetone was added to the reaction mixture. The product was isolated by evaporating most of the ethanol and extraction of the remaining aqueous dispersion of the polymers with diethyl ether (×3). Exhaustive dialysis of these dispersions was carried out in Visking tubing (molecular weight cut-off = 12–14 kDa) against water with 6 changes over 24 h. The dialysed dispersions were then freeze-dryed.

^{1}H NMR studies

^{1}H NMR (with integration; Gemini 200 MHz) was performed on a solution of the physically cross-linked chitosan hydrogels in $(CD_3)_2SO$ to assign non-exchangeable coupled protons.

Fourier transform infrared spectroscopy (FTIR)

Infrared (IR) spectra were recorded with a Jasco FT-IR-410 spectrophotometer. KBr discs were prepared by mixing the polymer and dry KBr in a weight ratio of 1:9 and subsequently compressing this physical mixture by a punch press working at 7 ton cm^{-2}.

Preparation of the hydrogels

The gels of the physically cross-linked chitosan (1%, w/w) were prepared by dispersing 1 g of polymer in 99 g of a water–glycerol mixture (5:1). Each gel (2 g) was supplemented with lyophilised propranolol hydrochloride, 50 mg (2.5%) for unsaturated gels or 200 mg (10%) for supersaturated gels. As a comparison, a liquid solution or suspension of lyophilised propranolol hydrochloride and commercial propranolol hydrochloride (Sigma-Aldrich) in the same water–glycerol mixture was prepared, 50 mg (2.5%) for the unsaturated solution or 200 mg (10%) for the supersaturated suspension. The pH of each gel, and the pH of the solution and the suspension were measured with a pH meter (Crison micropH 2000) and adjusted to 5.5.

Viscosity tests

The viscosity of all the chitosan hydrogels were tested at 37°C, before and after the addition of propranolol hydrochloride, using a Visco Star-R (Fungilab-Spain) viscosimeter.

Water uptake

The water-absorbing capacity of each gel was determined by a gravimetric method. Polymer (15 mg) was immersed in pH 5.5 or pH 7.4 aqueous buffer (25 mL) at 37°C and the decreasing weight of the non-absorbed aqueous buffer was measured for 6 h (the time predetermined for permeation studies). The water uptake of each polymer was determined as follows:

$$\text{Water uptake} = (Wa - Wb)/Wc \quad (1)$$

where Wa is the weight of the initial water, Wb is the weight of the non-absorbed water at time t and Wc is the weight of the polymer. The reproducibility of the method was 2.3%.

In-vitro percutaneous studies through porcine skin

In-vitro percutaneous studies were performed as previously described by Orienti et al (2000). Briefly, porcine ears were obtained from a local slaughterhouse. Porcine skin was separated into circular segments and hydrated in phosphate saline buffer at 4±1°C for 24 h. Only the segments of thickness 1.50±0.05 mm were selected for the study. The permeation study was conducted in a Franz-type permeation cell with a diffusional area of 10.7 cm^2. At time zero, 2 g of gel sample or liquid solution or suspension sample were placed on the skin in the donor compartment. The receiver phase (100 mL of an isotonic phosphate buffer solution, pH 7.4, maintained at 37°C by means of a surrounding jacket) was stirred constantly and at predetermined time intervals was withdrawn and replaced with blank buffer in order to maintain sink conditions. The amount of propranolol hydrochloride in the receiving phase was analysed by HPLC. Three parallel experiments were conducted with each gel and with the liquid solution and suspension. The studies were carried on for 6 h.

Measurements of drug solubility in the skin

Direct measurements of the drug solubility in the skin in the presence of the different vehicles examined were made (Orienti et al 2000). Briefly, the oversaturated gels or the

liquid suspension were placed in contact with the skin treated in the same way as for the in-vitro percutaneous studies. The skin was mounted in the Franz-type permeation cell previously described. The receiver phase was not replaced over time and when a concentration equilibrium had been achieved between vehicle, skin and receiver phase, the skin segment was rinsed with water and gently dried with a cotton swab. Following the addition of 5 mL acetone–ethanol (1:1, v:v), the skin sample was subjected to ultraturrax at 10000 rev min^{-1} for 5 min (Ultra-Turrax T8, IKA-WERKE, Staufen, Germany) and an ultrasonic (Vibracell VCX, West Kenosia Ave., Danbury, CT) treatment at an elevated temperature ($50 \pm 5^{\circ}C$, 5 min). Subsequently, the suspension was centrifuged at 15000 rev min^{-1} (ALC 4239R, Milano, Italy). Then 2.5 mL from the supernatant was desiccated by vacuum rotation and the remainder was resolved in 0.5 mL ethanol. The drug in the solvent was finally determined by HPLC.

Apparatus

Chromatographic separations were performed using a Shimadzu (model LC-10AT_{VP}) liquid chromatograph equipped with a UV-VIS detector (model SPD-10A_{VP}) and with a CromatoPlus computerised integration system (Shimadzu Italia, Milan, Italy). Manual injections were made using a Rheodyne 7125 injector with a 20-μL sample loop.

Chromatographic conditions

Chromatographic separations were performed on a C18 Phenomenex Luna (3 μm, 150 × 4.60 mm i.d.) (Chemtek Analitica, Bologna, Italy) column at room temperature using a mixture of acetonitrile–pH 3.0 solution of triethylamine (50 mM) 70:30 (v/v) at a flow rate of 0.8 mL min^{-1}. UV detection was at 290 nm. The limit of detection (signal to noise ratio 3:1) was 30.1 ng mL^{-1} propranolol hydrochloride. Reproducibility was 2.8%.

Statistical analysis

All the data are the means of results from three experiments ± s.d. Statistical data analysis was performed using the one-way analysis of variance, with $P < 0.05$ as minimum level of significance.

Results and Discussion

^{1}H NMR

Proton assignments for chitosan hydrogels in $(CD_3)_2SO$ (relative to dimethyl sulfoxide δ 2.50): δ 2.01 ppm = CH_3 (residual acetyl of chitosan), δ 3.20–4.00 ppm = non-exchangeable sugar protons, δ 0.93 ppm = CH_3 (lauroyl), δ 1.10 ppm = CH_2 (lauroyl), δ 1.80 ppm = CH_2 (lauroyl deshielded by carbonyl), δ 0.96 ppm = CH_3 (miristoyl), δ 1.10 ppm = CH_2 (miristoyl), δ 1.82 ppm = CH_2 (miristoyl deshielded by carbonyl), δ 0.92 ppm = CH_3 (palmitoyl), δ 1.20 ppm = CH_2 (palmitoyl), δ 1.45 ppm = CH_2 (palmitoyl deshielded by carbonyl), δ 0.82 ppm = CH_3 (stearoyl), δ 1.10 ppm = CH_2 (stearoyl, δ 1.70 ppm = CH_2 (stearoyl deshielded by carbonyl). The level of substitution, calculated from the ^{1}H NMR spectrum, was determined as 14.5%. This data was obtained by comparing the signal of lauroyl (δ 0.93 ppm), miristoyl (δ 0.96 ppm), palmitoyl (δ 0.92 ppm) and stearoyl (δ 0.82 ppm) protons with that of acetyl protons (δ 2.01 ppm) present at 15% in the chitosan.

FT-IR

Figure 1 shows the FT-IR spectra of the samples under study. The FT-IR spectra of chitosan reveal characteristic absorption bands at 3436, 2916 and 2850 cm^{-1}, that represent the presence of OH group, CH_2 and CH_3 groups (aliphatic groups), respectively. The amino group has a characteristic absorption band in the region of 3400–3500 cm^{-1}, which must have been masked by the absorption band due to the OH group (Shanmugasundaram et al 2001). The presence of a significant peak at 1732 cm^{-1} (due to CH_3–C=O group denoting the presence of acetyl group) confirms that chitosan is a partially deacetylated product, unlike chitin which is a completely acetylated moiety. Chitosan showed a sharpening of the amine group peak at 1669 cm^{-1}.

The FT-IR spectra of lyophilised chitosan–laurate, –myristate, –palmitate and –stearate, respectively, revealed a relatively smaller amide peak at 1660, 1636, 1647 and 1637 cm^{-1}, thus confirming the reaction of the amine group with lauric, myristic, palmitic and stearic acid.

Viscosity tests

The viscosity of all the chitosan hydrogels before the addition of propranolol hydrochloride (Table 1) was near 4000 cPs and, in particular, it increased from the chitosans substituted with lower acyl chain length to the chitosans substituted with higher acyl chain length. This behaviour can be due to the stronger hydrophobic interaction resulting from the compenetration of longer acyl chains (palmitic and stearic acids) with respect to the interaction resulting from the compenetration of shorter acyl chains (lauric and myristic acids). The presence of propranolol hydrochloride in the chitosan hydrogels did not modify the viscosity (data not reported).

Water uptake

The water-absorbing capacity of polymers depends upon the nature of the polymer, temperature and polymer–solvent interactions (Khare et al 1992). The importance of quantifying swelling properties is obvious, not only with regard to design and development of controlled release forms, but also for topical formulations (Valenta et al

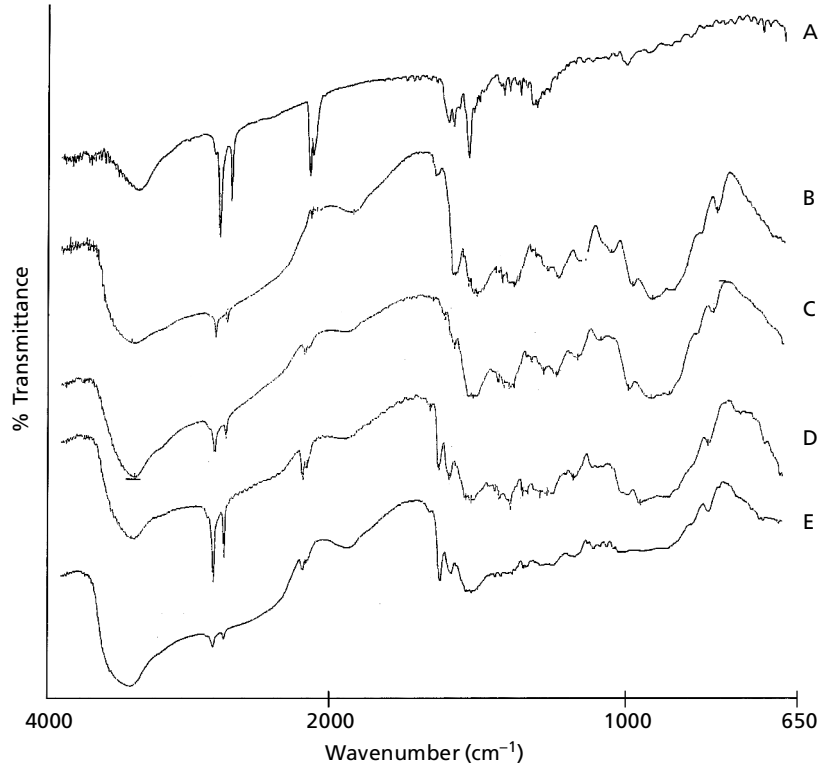

Figure 1 FT-IR spectra of chitosan (A) and the physically cross-linked chitosans: chitosan–laurate (B); chitosan–myristate (C); chitosan–palmitate (D); chitosan–stearate (E).

Table 1 Viscosity of the different chitosan hydrogels.

Vehicle	Chitosan–laurate	Chitosan–myristate	Chitosan–palmitate	Chitosan–stearate
Viscosity (cPs)	3500±100	3720±50	4050±70	4250±100

Data are means±s.d.; n = 3.

1998). The reduced swelling properties of each polymer in pH 5.5 (Figure 2A) is in contrast to most chitosan-based materials which show increasing swelling properties in acid media (Deyao et al 1993; Guan et al 1996; Chen et al 1997) as a result of their protonated amine groups (Deyao et al 1994) which are subsequently hydrated. This may be attributed to the acylation of chitosan (15% acetyl group of the chitosan purchased from Fluka and 14.5% acyl group of the substituted polymers), which reduces the number of available amine groups and hence the pH sensitivity of the gel.

Hydrogels exhibited lower swelling properties at pH 5.5 than at pH 7.4 (Figures 2A, B). This can be explained by the presence of hydrophobic and hydrophilic groups in the polymer that profoundly affected the swelling properties. At acidic pH the polymers did not solubilize or swell; at alkaline pH chitosan–laurate dissolved readily, while all other polymers swelled slowly. The swelling properties of hydrogels were in the order: chitosan–laurate >chitosan–myristate >chitosan–palmitate >chitosan–stearate.

Skin permeation of propranolol hydrochloride from the chitosan hydrogels

The in-vitro permeation of propranolol hydrochloride from the oversaturated chitosan hydrogels is shown in Figure 3A. The permeation profile of commercial propranolol hydrochloride was lower than the corresponding lyophilised propranolol hydrochloride due, probably, to the reduction in crystalline structure produced by the freeze-drying method (Patel & Amiji 1996), enhancing drug solubility in the skin. The permeation profiles of all the chitosan hydrogels analysed were higher than that of the commercial propranolol hydrochloride. Instead, only the permeation profiles of the lower acyl chain physically cross-linked chitosan hydrogels (chitosan–laurate and

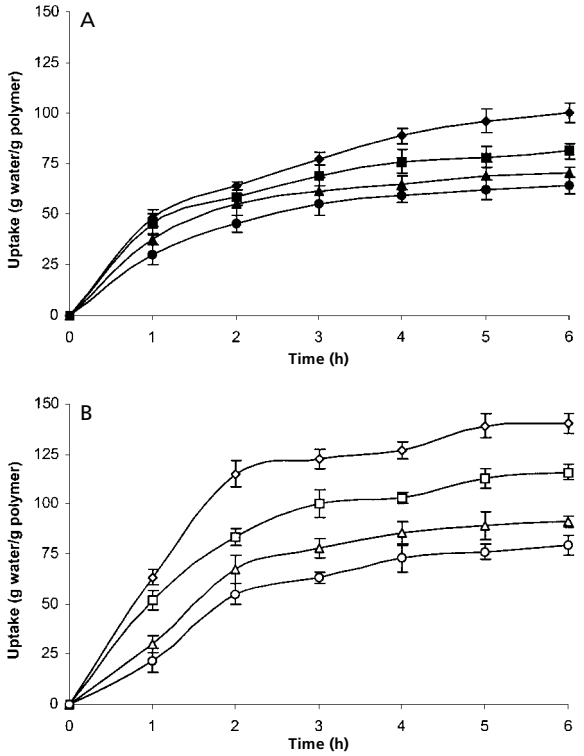

Figure 2 Water uptake (±s.d.; n = 3) of the physically cross-linked chitosans at pH 5.5 (A) and pH 7.4 (B). Diamonds, chitosan–laurate; squares, chitosan–myristate; triangles, chitosan–palmitate; circles, chitosan–stearate.

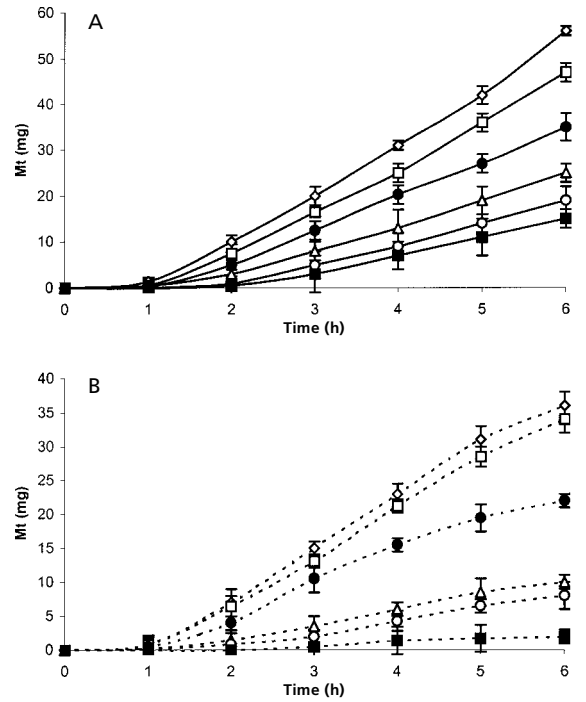

Figure 3 In-vitro permeation profiles of propranolol hydrochloride through porcine skin from oversaturated hydrogels (A) and unsaturated hydrogels (B) and liquid suspension (A) or solution (B). Diamonds, chitosan–laurate; open squares, chitosan–myristate; triangles, chitosan–palmitate; open circles, chitosan–stearate; closed circles, lyophilised propranolol hydrochloride; closed squares, commercial propranolol hydrochloride.

–myristate) were higher than that of the lyophilised propranolol hydrochloride. Decreasing physical cross-linker chain length in the chitosan hydrogels enhanced the permeation of propranolol hydrochloride through the skin. In particular, the permeation of lyophilised propranolol hydrochloride from the supersaturated chitosan–laurate and chitosan–myristate hydrogels was higher than the corresponding supersaturated liquid suspension and the permeation of lyophilized propranolol hydrochloride from the supersatured chitosan–palmitate and chitosan–stearate hydrogels was lower than the corresponding supersaturated liquid suspension.

The solubility and diffusion coefficient of the drug in the skin were determined by equations 2 and 3 (Higuchi 1960; Buri et al 1985):

$$(dM/dt)_{ss} = ADC_s/h \quad (2)$$

$$t_L = h^2/6D \quad (3)$$

where $(dM/dt)_{ss}$ is the permeation rate at the steady state, A is the skin surface area, D is the diffusion coefficient of the drug in the skin, C_s is the solubility of the drug in the skin, t_L is the lag time before the steady-state diffusion is achieved and h is the skin thickness. The slopes of the linear portion of the permeation profiles obtained by the oversaturated vehicles and from the liquid suspension represent the permeation rates at the steady state; the intersections of these linear portions with the abscissa axis represent the lag times. The data of C_s and D obtained from equations 2 and 3, and the solubility of the drug in the skin obtained by direct measurements are reported in Table 2. The C_s values obtained from equations 2 and 3 are similar to those determined by direct measurements. In particular, chitosan–laurate and chitosan–myristate gels were higher than those obtained from the liquid suspension of lyophilized propranolol hydrochloride and commercial propranolol hydrochloride. This can be explained by the interaction of the gels with the stratum corneum, increasing the solubility of the drug in the skin (Table 2) and hence the permeation rate. This interaction can be attributed to the amphiphilic properties of the polymeric network due to the presence of hydrophylic (chitosan molecule) and lipophilic (acyl chains) portions (Cooper 1985; Chien & Lee 1987) and to its ability to partially solubilize in the upper parts of the stratum corneum. The nature of the different acyl chains influences the interaction with the stratum corneum. The C_s values obtained from the chitosan–palmitate and chitosan–stearate hydrogels were lower than that from the liquid suspension of lyophilised propranolol hydrochloride, but higher than that obtained from the liquid suspension of commercial propranolol hydrochloride. This was due to the reduced solubility of propranolol hydrochloride in these hydrogels, which may

Table 2 Permeation parameters of propranolol hydrochloride from physically cross-linked chitosans oversaturated (10% propranolol hydrochloride) and unsaturated (2.5%) hydrogels and from liquid suspension (2.5%) or liquid solution (10%) through porcine skin.

Oversaturated (10% propranolol hydrochloride)

Vehicle	Flux dM/dt S ($mg\ h^{-1}\ cm^2$)	Lag time t_L (h)	Diffusion coefficient D ($cm^2\ h^{-1}$)$\times 10^{-3}$	Solubility in skin (indirect)[a] C_S ($mg\ g^{-1}$)	Solubility in skin (direct)[b] C'_S ($mg\ g^{-1}$)
Chitosan–laurate	1.00±0.02	1.05±0.04	9.90±0.01	25.30±0.05	29.30±0.03
Chitosan–myristate	0.87±0.05	1.11±0.06	9.31±0.02	24.10±0.03	27.80±0.05
Chitosan–palmitate	0.47±0.03	1.22±0.03	8.34±0.05	13.90±0.08	17.32±0.03
Chitosan–stearate	0.37±0.01	1.47±0.04	7.12±0.02	12.90±0.04	15.06±0.04
Lyophilised propranolol hydrochloride suspension	0.68±0.04	1.24±0.02	8.43±0.03	20.24±0.02	23.85±0.03
Commercial propranolol hydrochloride suspension	0.29±0.09	1.57±0.06	6.59±0.01	11.18±0.05	13.73±0.01

Unsaturated (PHCL 2.5%)

Vehicle	Flux dM/dt S ($mg\ h^{-1}\ cm^2$)	Concn in skin (indirect)[c] C ($mg\ g^{-1}$)
Chitosan–laurate	0.71±0.05	16.80±0.03
Chitosan–myristate	0.67±0.01	16.32±0.07
Chitosan–palmitate	0.22±0.06	7.52±0.04
Chitosan–stearate	0.18±0.03	7.32±0.01
Lyophilised propranolol hydrochloride suspension	0.48±0.02	12.61±0.03
Commercial propranolol hydrochloride suspension	0.08±0.01	4.90±0.04

[a]Solubility of the drug in the skin determined by indirect measurements; [b]solubility of the drug in the skin determined by direct measurements; [c]concentration of the drug in the skin determined by indirect measurements. Data are means±s.d.

be sterically hindered and more hydrophobic than the chitosan–laurate and chitosan–myristate gels. The D values were lower from the chitosan–palmitate and chitosan–stearate hydrogels than the liquid suspension of lyophilised propranolol hydrochloride (Table 2), indicating that the interaction of the polymer with the stratum corneum could decrease the drug diffusibility through the skin. The hydrogels produced increased flux at steady state through the skin and also decreased lag times with respect to the liquid suspension of commercial propranolol hydrochloride. Only chitosan–laurate and chitosan–myristate presented a higher flux than the liquid suspension of lyophilised propranolol hydrochloride. A comparison between the different physically cross-linked hydrogels disclosed that the flux at steady state increased, and the lag time decreased, by decreasing the cross-linker acyl chain length. This is a consequence of the C_s and D variations: C_s decreases and D increases by decreasing the cross-linker chain length. The increased solubility of propranolol hydrochloride and its increased diffusion coefficient in the skin observed in the presence of the physically cross-linked chitosan hydrogels (with respect to the liquid suspension) produced enhanced fluxes for oversaturated vehicles, where the drug activity is maximal, and also for the unsaturated vehicles. To obtain information on the unsaturated vehicles, permeability studies were also conducted on the gels containing 2.5% drug. The flux was evaluated from the equation 4 (Higuchi 1960; Buri et al 1985):

$$dM/dt = ADC/h \quad (4)$$

where dM/dt is the flux of the drug through the skin from an unsaturated vehicle, thus in non steady-state conditions, A is the skin surface, D is the drug diffusion coefficient through the skin, C is the concentration of the drug in the skin in equilibrium with the concentration of the drug in the vehicle and h is the skin thickness.

The flux was determined by the slopes of the linear portion of the permeation profile obtained for no longer than 1 h after the lag time, as in this time period no deviation from linearity was observed (Figure 3B). The fluxes from the unsaturated gels were lower than the oversaturated ones, but the fluxes from the chitosan–laurate and chitosan–myristate hydrogels were higher than that of the corresponding liquid solution of lyophilized propranolol hydrochloride and the fluxes from the chitosan–palmitate and chitosan–stearate hydrogels were lower than the corresponding liquid solution of lyophilized

propranolol hydrochloride (Table 2). This behaviour was in accordance with the favourable effect of the vehicle (chitosan–laurate and –myristate) in enhancing the solubility of the drug in the skin. In fact, at the examined formulative concentration (2.5%, under saturation), the vehicle also raised the flux with respect to the aqueous solution by increasing the concentration of the drug in the skin (Table 2).

Conclusions

Physically cross-linked chitosans with lauric and myristic acids made hydrogels able to increase the skin permeation of propranolol hydrochloride with respect to the liquid solution or suspension. This effect seems to be linked to an increased drug solubility in the skin, probably produced by the interaction of the polymer with the stratum corneum. The maximum enhancement of drug permeation was observed in the presence of the lowest length of the cross-linker acyl chain. Moreover, the physically cross-linked chitosan hydrogels with hydrophobic palmitic and stearic groups reduced the skin permeation of lyophilized propranolol hydrochloride with respect to the liquid solution or suspension. This is the result of the reduced solubility of propranolol hydrochloride in the skin and consequently the permeation rate.

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