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Selection and evaluation of anticholinergics for transdermal drug delivery

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Abstract

The receptor affinities of 18 anticholinergics, expressed as dissociation constants, were determined in order to select the most active compounds. Six drugs showed high affinities towards the muscarinic receptor ($K_d < 30$ nM), and these compounds were selected as possible candidates for transdermal drug delivery because they can theoretically provide therapeutic plasma concentrations by dermal application on a limited area. From the six selected anticholinergics (atropine sulphate monohydrate, benztropine mesylate, dexetimide hydrochloride, oxyphencyclimine hydrochloride, scopolamine hydrobromide trihydrate, tropicamide), the physicochemical characteristics are described, together with atropine base. These characteristics may be useful to explain possible differences in permeation experiments. The chemical structures, molecular weights, and pK_a values were available from the literature. The partition coefficients, and the solubility, pH and stability in the dosing and receptor solutions were experimentally determined.

Keywords: Anticholinergics; Receptor affinities; In vitro permeation; Transdermal delivery; Physicochemical characteristics

1. Introduction

Anticholinergics can be used as bronchodilators to prevent or diminish airway obstruction in patients with obstructive airway diseases. The only regularly used bronchodilator therapy with anticholinergics is the inhalation of ipratropium bromide (Pakes et al., 1980). However, because of the relatively short duration of action, it must be given four times a day. The transdermal route of administration may offer the advantage of producing a sustained, constant and controlled level of drug in the blood, resulting in a prolonged duration of action (Guy and Hadgraft, 1986).

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The transport of drugs through the skin depends on a number of factors such as the characteristics of the permeant, condition and type of skin, other chemicals present in the dosage form (e.g. enhancers) and external conditions (e.g. temperature). The factor with perhaps the greatest influence is the physicochemical character of the permeant (Wiechers, 1989; Smith, 1990). According to Fick's first law, the permeation of a drug through the stratum corneum, the main barrier in skin permeation, depends on the permeability coefficient and the concentration gradient of the permeant across the stratum corneum (Barry, 1983; Franz, 1983). The permeability coefficient is the product of the partition coefficient and the diffusion coefficient, divided by the length of the pathway through the stratum corneum. These factors, in turn, depend on variables such as molecular weight, size and structure, and degree of ionization of the permeant.

In this paper, we first investigated the suitability of a series of drugs with purported anticholinergic properties (anticholinergics) for transdermal drug delivery. Therefore, the affinities of 18 tertiary amines towards the muscarinic receptor were determined by radioreceptor assay (RRA). Only anticholinergics with high affinity for the muscarinic receptor can be considered as possible candidates for transdermal drug delivery because only very potent molecules can be delivered through a small skin surface.

From the selected anticholinergics, the physicochemical characteristics were determined using literature data, experimental measurements, and theoretical calculations. The chemical structures, molecular weights, and pK_a values were available from the literature (Moffat et al., 1986; Budavari et al., 1989; Reynolds et al., 1989). The partition coefficients, and the solubility and stability in the dosing and receptor solutions were experimentally determined. The pK_a values and the partition coefficients were also calculated with the PALLAS system which makes predictions based on the structural formulae of compounds. These physicochemical characteristics may be useful to explain possible differences in permeation between these anticholinergics (Bosman et al., 1998).

2. Materials and methods

2.1. Chemicals

[*N-methyl*-^{3}H]Scopolamine methyl chloride ([^{3}H]NMS, 81.5 Ci/mmol) was obtained from Du Pont NEN (Du Pont, Wilmington, DE). The anticholinergics, adiphenine hydrochloride, atropine, atropine sulphate monohydrate, benztropine mesylate, biperiden hydrochloride, dexetimide hydrochloride, dicyclomine hydrochloride, homatropine hydrochloride, methixene hydrochloride, orphenadrine hydrochloride, oxyphencyclimine hydrochloride, piperidolate hydrochloride, pirenzepine dihydrochloride monohydrate, procyclidine hydrochloride, profenamine hydrochloride, scopolamine hydrobromide trihydrate, terodiline hydrochloride, trihexyphenidyl hydrochloride, tropicamide, were all of pharmaceutical quality and obtained from local wholesalers. 1-Dodecylazacycloheptan-2-one (Azone®) was kindly supplied by Nelson Research (Irvine, CA). All other chemicals and solvents were of analytical grade and obtained from Merck (Darmstadt, Germany). Polyethylene tubes (12 ml) were obtained from Greiner (Alphen a/d Rijn, The Netherlands). The GF/B glassfibre filters were from Whatman (Maidstone, UK). Rialuma was used as scintillation liquid, obtained from Lumac (Olen, Belgium), in combination with mini-scintillation counting vials from Packard (Groningen, The Netherlands).

2.2. Preparation of solutions

The 50 mM sodium phosphate buffer, pH 7.4 (assay buffer), was prepared by dissolving 1.38 g $NaH_2PO_4 \cdot H_2O$ and 7.12 g $Na_2HPO_4 \cdot 2H_2O$ in 1 l distilled water.

Isotonic phosphate-buffered saline, pH 7.4 (PBS buffer), was prepared by dissolving 8.00 g NaCl, 0.20 g KCl, 0.20 g KH_2PO_4 and 1.44 g $Na_2HPO_4 \cdot 2H_2O$ in 1 l distilled water. PBS buffer was used as receptor solution in in vitro permeation experiments.

Ethanol/propylene glycol/PBS buffer/Azone 60:20:15:5 (v/v) was used as donor solution (vehicle) in in vitro permeation experiments (Swart et

al., 1992). The concentrations of anticholinergics in the vehicle were always 15 mg/ml.

Anticholinergic stock solutions of 1×10^{-3} M were prepared in ethanol and stored at -20°C.

2.3. Determination of receptor affinities

The receptor affinities of 18 tertiary anticholinergics towards the muscarinic receptor (Ensing et al., 1994), expressed as dissociation constants, K_d, were determined using radioreceptor assays with pre-incubation at 0°C, and [^{3}H]NMS was used as radiolabelled ligand.

From the anticholinergic stock solutions, appropriate dilutions were made in assay buffer, concentrations ranging from 1×10^{-9} to 1×10^{-5} M (calibration curve), and 50-μl aliquots of the solutions of anticholinergics were added to polyethylene tubes in duplicate, giving final assay concentrations ranging from 1×10^{-10} to 1×10^{-6} M. Then 400 μl receptor preparation, containing 2 mg lyophilized receptors in assay buffer (Ensing et al., 1994), were added. The tubes were vortexed and incubated during 60 min at 0°C before 50 μl of [^{3}H]NMS (4×10^{-9} M) were added. The tubes were vortexed again and incubated for another hour at 0°C. After the addition of 4 ml icecold assay buffer, the samples were immediately filtered through Whatman GF/B glassfibre filters under vacuum using a filtration apparatus (48S, University Centre for Pharmacy, Groningen, The Netherlands). The tubes were rinsed twice with 4 ml ice-cold assay buffer, which was also filtered. The total filtration and rinsing process, taking place in approximately 15 s, was carried out on each tube in turn. The filters were transferred into mini-scintillation vials and dispersed in 3.5 ml scintillation cocktail by shaking for 120 min. The vials were counted for 40000 counts or 5 min in a liquid scintillation counter (Minaxi, Packard, Groningen, The Netherlands), whatever came first; 50 μl of the used [^{3}H]NMS (4×10^{-9} M) solution were added to two mini-scintillation vials and counted as well. The curves were fitted with the ligand curve-fitting program to calculate the dissociation constants of the anticholinergics (Munson and Rodbard, 1980).

2.4. Partition coefficient

The *n*-octanol/water and *n*-octanol/PBS buffer systems were used to determine the partition coefficients of the selected anticholinergics, expressed as log *K*.

Then 5 ml of a solution of each anticholinergic (0.5 mM) in water or PBS buffer and 5 ml of *n*-octanol were added to glass tubes in triplicate (Amlacher et al., 1991). The tubes were shaken for 24 h and the concentration of the anticholinergic in the aqueous phase (water or PBS buffer) was determined by means of radioreceptor assay. Tropicamide and benztropine mesylate were analysed using RRA with pre-incubation at 0°C (Section 2.3). Atropine, atropine sulphate, dexetimide hydrochloride, oxyphencyclimine hydrochloride and scopolamine hydrobromide trihydrate were analysed using the RRA under equilibrium conditions (Section 2.7). Calibration curves were prepared and fitted with the ligand curve-fitting program (Munson and Rodbard, 1980). The unknown concentration of the anticholinergic in the aqueous phase was calculated by introducing the obtained binding values (Bq) in the fitted curves. When the final concentration of the anticholinergic in the aqueous phase exceeded the upper limit of quantitation, the samples were diluted (100–10000-fold) and reanalysed.

2.5. Solubility

The solubility of the anticholinergics in the vehicle and the receptor solution was measured in duplicate by allowing an excess of anticholinergic to equilibrate in 0.5 ml of the vehicle or 0.5 ml of PBS buffer while stirring at 20°C ($\pm$ 1°C). After 24 h the samples were centrifuged, diluted with the appropriate solvent and the drug concentration was analysed by ultraviolet spectrophotometry.

2.6. Stability

The stability of the anticholinergics at 37°C was tested in the donor solution and receptor solution using RRA. We chose a temperature of 37°C and

an incubation time of 24 h because these are the conditions used for the in vitro permeation experiments (Bosman et al., 1998).

To check the stability of anticholinergics in the receptor solution, appropriate dilutions from the anticholinergic stock solutions were made in PBS buffer, concentrations ranging from 1×10^{-9} to 1×10^{-5} M. Then 50-μl aliquots of the solutions of anticholinergics were added to polyethylene tubes in duplicate and analysed using RRA with pre-incubation at 0°C (Section 2.3) The remainder of the solutions was incubated at 37°C and, after 24 h, 50-μl aliquots of the incubated solutions were added to polyethylene tubes in duplicate and analysed as well.

To check the stability of anticholinergics in the vehicle, the dosing solutions of the anticholinergics were also incubated for 24 h at 37°C and diluted 10^5–10^6-fold with assay buffer before analysis.

The curves obtained with and without incubation at 37°C were fitted with the ligand curve-fitting program (Munson and Rodbard, 1980) to calculate the IC_{50} values (concentration which displaces 50% of radiolabelled drug); differences in IC_{50} values indicate that the anticholinergic is not stable during the incubation at 37°C for 24 h. The concentration of anticholinergic in the vehicle after incubation at 37°C for 24 h was calculated by introducing the obtained binding values (Bq) in the fitted curves.

2.7. RRA under equilibrium conditions

From the anticholinergic stock solutions, appropriate dilutions were made in assay buffer, concentrations ranging from 1×10^{-9} to 1×10^{-5} M (calibration curve). Then 50-μl aliquots of the solutions of anticholinergics were added in duplicate to polyethylene tubes, giving final assay concentrations ranging from 1×10^{-10} to 1×10^{-6} M. Then 50 μl of [^{3}H]NMS (4×10^{-9} M) and 400 μl receptor preparation, containing 2 mg lyophilized receptors in assay buffer (Ensing et al., 1994), were added to the polyethylene tubes. The tubes were mixed and incubated for 30 min at 37°C. The filtration, washing and counting procedures were performed as described in Section 2.3.

2.8. Prediction of pK_a and partition coefficient

PALLAS (CompuDrug Chemistry Ltd, Chemical Software Series, Version 1.2, 1994, Hungary) is a collection of tools for making predictions based on the structural formulae of the compounds. We used this system to predict the pK_a and partition coefficient of the selected anticholinergics, and plot the partition coefficient against pH.

The partition coefficient was predicted as the logarithm of the apparent partition coefficient ($\log P_{app}$) of compounds in octanol/water. The concentration of pairing ions (as Na^+ or Cl^-) in the aqueous phase can be changed to take into consideration the salt concentration of the buffer ($\log P_{app}$, octanol/buffer). We did not use the predicted logarithm of the partition coefficient ($\log P$), because this parameter is based only on neutral and non-ionic species.

3. Results and discussion

3.1. Selection of anticholinergics

The receptor affinities of 18 anticholinergics, expressed as dissociation constants, were determined in order to select the most active compounds (Table 1). Seven drugs had very low

Table 1
Affinities of 18 anticholinergics, expressed as dissociation constants (K_d)

Very low affinity ($K_d > 1$ μM)	Low affinity ($K_d > 30$ nM)	High affinity ($K_d < 30$ nM)
Adiphenine HCl	Biperiden HCl	Atropine sulphate·H_2O
Dicyclomine HCl	Pirenzepine 2HCl·$2H_2O$	Benztropine mesylate
Homatropine HCl	Procyclidine HCl	Dexetimide HCl
Methixene HCl	Terodiline HCl	Oxyphencyclimine HCl
Orphenadrine HCl	Trihexyphenidyl HCl	Scopolamine HBr·$3H_2O$
Piperidolate HCl		Tropicamide
Profenamine HCl		

Table 2
Solubility of the anticholinergics in the donor and receptor solutions, and apparent pH of the donor solutions

Anticholinergic	Solubility in donor solution (mg/ml)	Solubility in receptor solution (mg/ml)	Apparent pH in donor solution[a]
Atropine	290	12	9.8
Atropine sulphate·H_2O	>500	>1000	6.5
Benztropine mesylate	>500	>1000	6.5
Dexetimide HCl	55	56	5.7
Oxyphencyclimine HCl	45	5	6.6
Scopolamine HBr·$3H_2O$	150	270	5.3
Tropicamide	>500	4	6.8

[a] Concentration of the anticholinergic is 15 mg/ml.

affinities ($K_d > 1$ μM) to the muscarinic receptor, five drugs had low affinities ($K_d > 30$ nM) and six drugs showed high affinities with $K_d < 30$ nM. These six compounds were selected for in vitro permeation experiments because they may theoretically provide therapeutic plasma concentrations by dermal application on a limited area. Since we also wanted to examine possible differences between salts and bases, we also included atropine base in our further experiments.

Table 3
Partition coefficients of the anticholinergics, experimentally determined

Anticholinergic	Log K	
	Octanol/water	Octanol/buffer
Atropine	0.52 (0.05)	−0.45 (0.07)
Atropine sulphate·H_2O	−1.06 (0.06)	−0.63 (0.25)
Benztropine mesylate	−0.45 (0.12)	1.55 (0.03)
Dexetimide HCl	−0.03 (0.32)	1.93 (0.09)
Oxyphencyclimine HCl	0.03 (0.07)	1.91 (0.10)
Scopolamine HBr·$3H_2O$	−0.82 (0.20)	0.21 (0.08)
Tropicamide	1.28 (0.02)	1.40 (0.12)

Data represent mean values ± standard deviation ($n = 3$).

3.2. Characteristics of the selected anticholinergics

3.2.1. Chemical structure and molecular weight

The chemical structure may affect the partition coefficient, the interaction with the stratum corneum and the molecular weight and volume.

Table 4
The pH of 0.5 mM solutions of the anticholinergics in water, and their pK_a values

Anticholinergic	pH in water	pK_a	
		Literature[a]	Predicted[b]
Atropine	9.9	9.9	8.9
Atropine sulphate·H_2O	6.2	9.9	8.9
Benztropine mesylate	6.1	10.0	9.5
Dexetimide HCl	6.0	—	8.7/7.9
Oxyphencyclimine HCl	6.3	—	12.4
Scopolamine HBr·$3H_2O$	5.9	7.6	6.9
Tropicamide	6.8	5.2	5.4

[a] From Moffat et al. (1986) and Reynolds et al. (1989).
[b] With the PALLAS system.

Table 5
Partition coefficients of the anticholinergics, predicted with the PALLAS system

Anticholinergic	Log P^{a}_{app} (octanol/water)	Difference[b] log P_{app} − log K	Log P^{c}_{app} (octanol/buffer)	Difference[b] log P_{app} − log K
Atropine	1.85	1.33	0.42	0.87
Atropine sulphate·H_2O	−0.67	0.39	0.42	1.05
Benztropine mesylate	0.87	1.32	2.23	0.68
Dexetimide HCl	0.38	0.41	1.66	−0.27
Oxyphencyclimine HCl	3.26	3.23	3.26	1.35
Scopolamine HBr·$3H_2O$	−0.53	0.29	0.31	0.10
Tropicamide	1.19	−0.09	1.22	−0.18

[a] Partition coefficient, predicted at the pH of the 0.5 mM anticholinergic solution in water.
[b] Difference between predicted and experimentally determined partition coefficient.
[c] Partition coefficient, predicted at pH 7.4 and 0.150 mM salt concentration.

Small modifications in the structure may have a substantial effect on the permeability (Wiechers, 1989), as is the case for the selected anticholinergics.

The molecular weight and volume of a drug may influence skin permeation by affecting the diffusion coefficient which is a measure of the ease with which the molecule can move within the stratum corneum (Smith, 1990). However, up to a molecular weight of at least 500, or perhaps even 5000 Da, the molecular size plays no crucial role (Wiechers, 1989) which is the case for these anticholinergics.

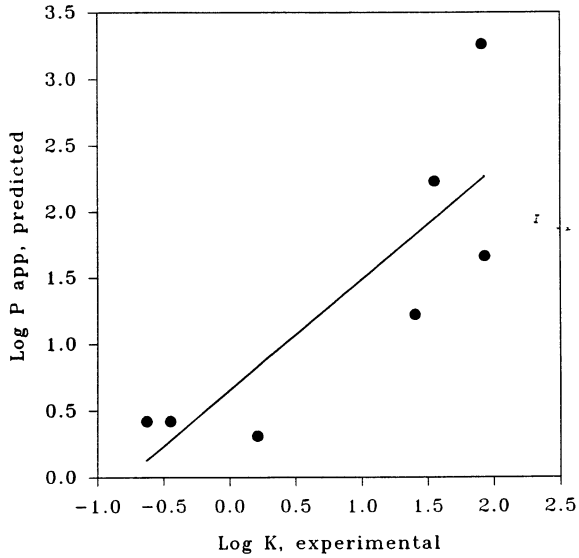

Fig. 1. The predicted partition coefficient (log P_{app}) versus the experimentally determined partition coefficient in octanol/buffer (log K).

Table 6
Stability of the anticholinergics in PBS buffer

Anticholinergic	Log IC^{a}_{50}	
	Without incubation	With incubation[b]
Atropine	−8.41	−8.35
Atropine sulphate·H_2O	−8.82	−8.84
Benztropine mesylate	−8.02	−8.00
Dexetimide HCl	−8.70	−8.64
Oxyphencyclimine HCl	−8.52	−8.30
Scopolamine HBr·$3H_2O$	−8.84	−8.82
Tropicamide	−7.28	−7.27

[a] Logarithm of the anticholinergic concentration which displaces 50% of [^{3}H]NMS.
[b] Incubation for 24 h at 37°C.

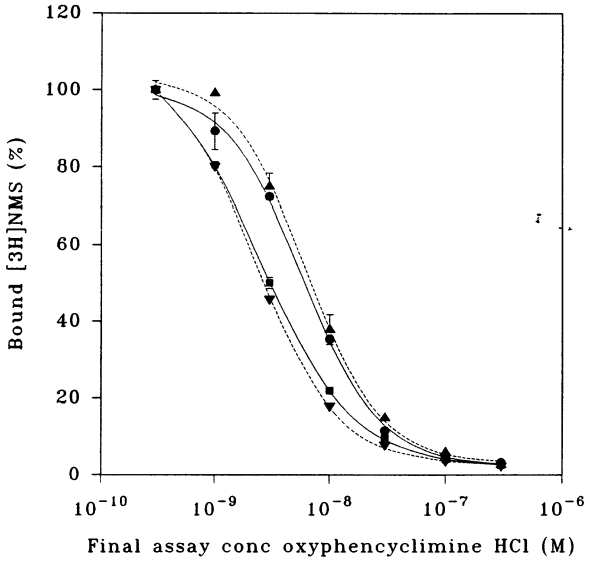

Fig. 2. Calibration curves of oxyphencyclimine hydrochloride with and without incubation. (■) Without incubation, first experiment; (●) incubation, first experiment; (▼) without incubation, second experiment; (▲) with incubation, second experiment.

3.2.2. Solubility

The solubility of a drug in the various phases during skin permeation can play a large part in determining the rate of permeation. A high lipid solubility in the stratum corneum relative to the vehicle is essential for a maximal input of the permeant into the stratum corneum, whereas a high aqueous solubility is essential for a maximal output into the viable epidermis, dermis and circulatory system (Barry, 1983; Wiechers, 1989; Smith, 1990).

We determined the solubility of the anticholinergics in ethanol/propylene glycol/PBS buffer/Azone 60:20:15:5 (v/v) and PBS buffer because these solvents were used in the in vitro permeation experiments as donor solution and receptor solution, respectively (Table 2). In the donor solution, the anticholinergics were all soluble to freely soluble (Moffat et al., 1986). However, in the receptor solution, the solubilities varied from very soluble for atropine sulphate and benztropine mesylate to slightly soluble for oxyphencyclimine hydrochloride and tropicamide (Moffat et al., 1986).

For all anticholinergics the pH (apparent) in the donor solutions was approximately 6, except for atropine (Table 2). The pH of atropine base in the donor solution was 9.8 compared to 6.5 for atropine sulphate, indicating that the concentration of non-ionized species in the donor solution of atropine base is increased which may result in higher permeation.

3.2.3. Partition coefficient and pK_a

The octanol/water partition coefficient of drugs is often considered as an estimate of the lipophilicity. Table 3 shows the partition coefficients of the anticholinergics determined in octanol/water and octanol/buffer. In octanol/buffer, the partition coefficients were higher than in octanol/water for all anticholinergics, except atropine. The differences between the two systems and between atropine and the others, can be explained by differences in pH of the aqueous phase. This pH, in combination with the permeant's pK_a, will determine the actual concentrations of ionized and non-ionized species and thus influence the partition coefficients (Tenjarla et al., 1994).

As shown in Table 4, the pH of a 0.5 mM solution of atropine in water is about 10. At this pH, the concentration of non-ionized species will be increased in comparison with pH 7.4 of the PBS buffer and will result in a higher partition coefficient in the octanol/water system.

Looking at the octanol/buffer system, relatively large partition coefficients were measured for dexetimide hydrochloride and oxyphencyclimine hydrochloride which may result in higher concentrations of these drugs in the stratum corneum.

We also calculated the partition coefficients with the PALLAS system, as shown in Table 5. These predicted values were compared with the experimentally determined values, showing no correlation ($r = 0.25$) for the octanol/water system and some correlation ($r = 0.84$) for the octanol/buffer system (Fig. 1). The predictions of log P_{app} depend on the predicted values of pK_a and predicted log P (partition coefficient for neutral species). If these predictions are incorrect (e.g. multiple pK_a values on small molecules, covalent hydration, conformational effects, hydrogen bonds), this will result in erroneous calculations of log P_{app}. As shown in Table 5, large differences in pK_a between literature and predictions with PALLAS were found. There-

fore, we may conclude that prediction with the PALLAS method failed for these compounds.

3.2.4. Stability

Table 6 presents the IC_{50} values of the calibration curves with and without incubation at 37°C for 24 h. If degradation of anticholinergics occurs during incubation, the calibration curve will shift to higher concentrations, resulting in a higher value of the IC_{50}. This shift was only seen for oxyphencyclimine hydrochloride and, after incubation, only 60% of the added concentration was left (Fig. 2). This means that during in vitro permeation experiments which will be performed at 37°C for 24 h, oxyphencyclimine present in the receptor solution will degrade. All other anticholinergics are stable in the receptor solution.

We also measured the anticholinergic concentration in the donor solutions after incubation and found concentrations varying from 85 to 120%, which means that all anticholinergics will be stable in the donor solution during the in vitro experiment.

4. Conclusions

Six anticholinergics were selected for in vitro permeation experiments, based on their high receptor affinities ($K_d < 30$ nM). Atropine base was included to examine possible differences in skin permeation between salts and bases.

The chemical structure of the anticholinergic is an important parameter in permeation experiments. However, the molecular weight may be irrelevant because of the small differences between the seven compounds. All anticholinergics were soluble to freely soluble in the donor solution, and the pH values were approximately 6, except for atropine base (pH 9.8). In the receptor solution, the solubilities varied from very soluble to slightly soluble; however, no solubility problems will be encountered because of the relatively large receptor compartment of the diffusion cells. The measured partition coefficients in octanol/buffer were higher than in octanol/water for all anticholinergics, except atropine. The predictions of the partition coefficients with the PALLAS system were incorrect. All anticholinergics are stable at 37°C for 24 h in the donor and receptor solution, except oxyphencyclimine hydrochloride.

References

Amlacher, R., Härtl, A., Neubert, R., Stöckel, U., Wenzel, K., 1991. Influence of ion-pair formation on the pharmacokinetic properties of drugs. Pharmacokinetic interactions of bretylium and hexylsalicylic acid in rabbits. J. Pharm. Pharmacol. 43, 794–797.

Barry, B.W., 1993. Basic principles of diffusion through membranes. In: Dermatological Formulations: Percutaneous Absorption. Marcel Dekker, New York, pp. 49–94.

Bosman, I.J., Ensing, K., de Zeeuw, R.A., 1998. Standardization procedure for the in vitro skin permeation of anticholinergics. Int. J. Pharm. 1, 65–73.

Budavari, S., O'Neil, M.J., Smith, A., Heckelman, P.E. (Eds.), 1989. The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals, 11th ed. Merck, Rahway, NJ.

Ensing, K., Bosman, I.J., Egberts, A.C.G., Franke, J.P., de Zeeuw, R.A., 1994. Application of radioreceptor assays for systematic toxicological analysis. 1. Procedures for radioreceptor assays for antihistaminics, anticholinergics and benzodiazepines. J. Pharm. Biomed. Anal. 12, 53–58.

Franz, T.J., 1983. Kinetics of cutaneous drug penetration. Int. J. Dermatol. 22, 499–505.

Guy, R.H., Hadgraft, J., 1986. Transdermal drug delivery: the ground rules are emerging. Pharm. Int. May, 112–116.

Moffat, A.C., Jackson, J.V., Moss, M.S., Widdop, B., Greenfield, E.S. (Eds.), 1986. Clarke's Isolation and Identification of Drugs in Pharmaceuticals, Body Fluids, and Post-mortem Material, 2nd ed. The Pharmaceutical Press, London.

Munson, P.J., Rodbard, D., 1980. Ligand: a versatile computerized approach for characterization of ligand-binding systems. Anal. Biochem. 107, 220–239.

Pakes, G.E., Brodgen, R.N., Heel, R.C., Speight, T.M., Avery, G.S., 1980. Ipratropium bromide: a review of its pharmacological properties and therapeutic efficacy in asthma and chronic bronchitis. Drugs 20, 237–266.

Reynolds, J.E.F., Parfitt, K., Parsons, A.V., Sweetman S.C. (Eds), 1989. Martindale. The Extra Pharmacopoeia, 29th ed. The Pharmaceutical Press, London.

Smith, K.L., 1990. Penetrant characteristics influencing skin absorption. In: Kemppainen, B.W., Reifenrath, W.G. (Eds.), Methods for Skin Absorption. CRC Press, Florida, pp. 23–34.

Swart, P.J., Weide, W.L., de Zeeuw, R.A., 1992. In vitro penetration of the dopamine D2 agonist N-0923 with and without Azone. Int. J. Pharm. 87, 67–72.

Tenjarla, S.N., Allen, R., Borazani, A., 1994. Evaluation of verapamil hydrochloride permeation through human cadaver skin. Drug Dev. Ind. Pharm. 20, 49–63.

Wiechers, J.W., 1989. The barrier function of the skin in relation to percutaneous absorption of drugs. Pharm. Weekbl. Sci. Ed. 11, 185–198.