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In vitro comparative studies of transdermal nicotine delivery systems

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Abstract

In vitro release rates of nicotine from three trandsermal systems available in the Portuguese and Spanish markets have been compared in vitro by methods based on recently proposed USP release tests and assay, and using Franz diffusion cells with membranes of 'full-thickness' porcine ear or human breast skin. No significant differences were found between the release profiles obtained by the different release test methods for each device and it may be possible and desirable to further standardise testing recommendations. Total nicotine content could not be determined for one device at modest agitation speeds by methanol extraction. Some simple approaches to comparing animal models and release tests with human skin experiments in vitro are discussed in relation to potential applications to quality control of TDS and traditional topical products. © 1997 Elsevier Science B.V.

Keywords: Transdermal drug delivery; Nicotine; In vitro release test; Franz diffusion cells

1. Introduction

Quality control of transdermal systems and comparative studies of products with varying designs from different manufacturers present particular challenges for developers of in vitro release tests in the pharmaceutical industry and related bodies. Recent developments and design criteria of suitable tests have recently been discussed (Shah et al., 1995) with the ideal aim, often stated as for the dissolution testing of other dosage forms, being the definition of conditions which predict meaningful pharmaceutical differences affecting the in vivo bioavailability of drug. In many cases similar considerations to those for TDS, as well as additional difficulties apply to the development of release tests for traditional topical formulations e.g. creams and ointments (Shah and Elkins, 1995). The recommendation of a release test implies that this type of assay can report on

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the overall quality of a formulation lot, to monitor drug stability, quality of the manufacturing process and batch to batch uniformity (Shah et al., 1995), conveying information not contained in the results of other quality control testing procedures which may be employed, e.g. total content of principal ingredients, rheological measurements, particle size analysis etc.

Traditional dissolution/release testing usually exposes the sample to an aqueous medium which may interact with polymeric constituents of a TDS and modify its release characteristics. Components of other topical formulations may dissolve in addition to the drug of interest and the bulk of the sample may absorb the dissolution medium to an extent depending on its physicochemical nature. The conditions prevailing in such tests clearly differ markedly from the clinical situation when the TDS remains attached to the skin surface and traditional formulations undergo changes due to exposure to the atmosphere and skin secretions and specific excipients may enter the stratum corneum facilitating the penetration of active ingredients.

An additional assessment of penetration rates across excised skin samples may be carried out to determine the significance of differences noted by the simple release method and to indicate the relative resistances within the device and the skin (Hadgraft et al., 1991; Guy and Hadgraft, 1992). It was demonstrated that marketed nitroglycerine (GTN) TDS having widely varying dissolution behaviour produce similar delivery rates across intact human skin and good agreement was observed between the stated in vivo dose and that measured in simple in vitro diffusion cells when portions of the device corresponding closely to the cell aperture can be excised and mounted on human skin membranes. When human samples can be obtained these systems offer the closest approximation to the living patient that can be achieved in vitro to model passive drug transport through the epidermis. They are, therefore, convenient systems with which to compare alternative, in vitro, quality control tests.

As human skin is difficult to obtain routinely the availability of animal models or reliable synthetic membranes which mimic transport rates of particular compounds through human skin would offer convenient alternatives. However, suitable models have proven difficult to validate in practice and are required to demonstrate consistently the ability to discriminate between batches of the same product having different delivery characteristics in vivo.

In this work, comparative release studies of nicotine from cut down patches across porcine ear skin and human breast skin mounted in Franz-type diffusion cells were performed. In addition recent proposals for dissolution methods (Drug Release Tests 2, 3 and 4) applicable to TDS delivering nicotine and the assay for total nicotine content published by the United States Pharmacopeia (Pharmacopeial Forum, 1995) have been applied to three products available in Portugal and Spain.

The histology and permeability characteristics of pig skin, for hydrophilic and hydrophobic penetrants, have been reported to make it a suitable model (Dick and Scott, 1992) closer in permeability to human than several species including rat. However, considerable inter-species differences are observed for some substances and conflicting reports for variation of permeability with lipophilicity exist in the literature which may be partly explained by differences in the experimental systems used.

In a recent study of nicotine containing devices, Ho and Chien (1993) investigated the permeation kinetics of nicotine from solution and four designs of TDS mounted whole in diffusion cells using hairless rat and human cadaver skin. Good agreement was observed between the permeation profiles across both skin types. However, the receptor area exposed to the skin sample in these experiments was much less than the release area of the devices and therefore the observed amounts crossing the specimen are exaggerated due to lateral diffusion within the system which could also modify the observed release kinetics.

Table 1
Transdermal delivery systems examined

TDS	Nicotine content (mg)	Area (cm^2)	Claimed in vivo release (mg)	Lot. no.	Valid until
(A) Nicotinell	35	20	14/24 h	156200	12/96
(B) Nicotinell	35	20	14/24 h	154600	12/96
(C) Nicotinell	35	20	14/24 h	153800	11/96
(D1) Nicotrans	24.9	30	15/16 h	I-05	09/95
(D2) Nicotrans	24.9	30	15/16 h	J-01	11/97
(E) Nicodisc	30	—	22/24 h	DA6304	05/96

2. Materials and methods

2.1. Materials

The six lots of three transdermal systems examined in this work are described in Table 1. Nicotinell (A and B) were supplied by Ciba-Geigy Portuguesa Lda; (C) by Ciba-Geigy, Sociedad Anonima, Barcelona; Nicotrans (D1 and D2) Laboratorios PENSA, Barcelona (licensed by Kabi Pharmacia AB, Sweden) and Nicodisc (E) Lácer S.A., Barcelona (licensed by Elan Pharma, Ireland). D2 was received after tests on the other devices had been substantially completed in order to compare a newer batch of Nicotrans.

Nicotinell consists of a methacrylic acid copolymer solution of nicotine dispersed in a pad of nonwoven viscose and cotton, on which a layer of adhesive is laminated (Ho and Chien, 1993). Nicotrans has a polyisobutene/polybutene adhesive layer containing nicotine on a silvery-metallic backing film carrying an outer plastic layer with product details. A polyester release liner protects the adhesive reservoir. Nicodisc is a round, flat, beige coloured, foam pad carrying a pressure-sensitive acrylate adhesive with a round well in the centre containing nicotine in a carageenan, hydrogel matrix with a heavy foil and release liner protective cover which is discarded on application. The claimed in vivo deliveries (Table 1) are the systemic absorptions which differ slightly from the determined nicotine release from the devices, reportedly 98% of nicotine released for Nicotinell and Nicodisc and 95% for Nicotrans (Benowitz, 1995).

2.2. Preparation of porcine ear and human skin specimens

Full thickness, human breast skin was obtained by blunt dissection of tissue taken during biopsy at a local hospital and stored frozen before use.

Pig ears were obtained from a local abattoir and also stored frozen until use. After cleaning under cold running water hair was roughly trimmed from the specimen with scissors. Whole skin membranes carefully removed from both sides of the ear using forceps and scalpel.

Each porcine specimen could be mounted in 10–12 Franz-type diffusion cells (aperture 1.0 cm^2). Cells were filled with isotonic phosphate buffered saline pH 7.4 (4.0 ml) and equilibrated in a water bath at 37°C for ca. 1 h before application of the TDS. Generally duplicate cells carrying a portion of a patch of each lot available were prepared from each specimen. Only one lot of Nicotinell was tested using human skin due to the limited availability of tissue.

2.3. Preparation of transdermal systems and application to diffusion cells

In the case of Nicotinell and Nicotrans TDS circular portions (1.77 cm^2) were removed using a steel punch which overlapped slightly the aperture of the receptor chamber. Clamping of the upper chamber seals the edge of the patch between ground glass surfaces of the cell. The possibility of loss of nicotine from this region was considered, particularly in the case of Nicotinell where the thickness of the reservoir material is slightly greater. A preliminary experiment compared permeation from (A) across pig skin, from simple cut

patches, samples where the edge was lightly sealed with a silicone grease or where the skin and patch were covered in a tight layer of Parafilm or of aluminium foil. No significant differences were observed between the treatments.

The hydrogel reservoir of Nicodisc frequently separates from the adhesive backing material when opened, remaining in the well of the metallic release layer and has no well defined area. To obtain a suitable sample for application, the following procedure was adopted.

(1) Remove the gel entirely to a tared watchglass and cover with a second to minimise evaporation. Weigh the gel.

(2) Punch out circular samples (diam. 1.3 cm) and apply to skin cells, surrounding each with one or two cardboard washers. These are covered with aluminium foil or patch backing material held by the cell top. Later experiments comparing only Nicodisc patches utilised single steel washers, either placed around the gel and below a piece of aluminium foil or on top of a piece of foil slightly formed to fit directly on top of the gel and achieve a light pressure forcing the gel against the membrane. Improved contact between the gel and skin appeared to increase nicotine flux.

(3) Weigh the remaining gel and use the mean difference weight as the amount applied to each cell.

2.4. Release testing of transdermal systems

Details of the testing procedures suggested by the USP are given in Table 2 (Pharmacopeial Forum, 1995). Assays (5 ml with replacement) were taken at various time-points in order to generate dissolution profiles. These tests are based on the methodologies of individual manufacturers and the table indicates the appropriate manufacturer/distributor and brandname in the US with the corresponding product name used in Europe. The following modifications were adopted when comparing the behaviour of each system in each testing procedure and because of difficulty in obtaining some materials:

Scotch pressure sensitive tape (ref. 4945, 3M) was used to fix devices to the cylinder. However, Nicotrans became detached between the polymer backing and the underlying layer and the Nicodisc gel also requires to be held in place. Nicotinell was attached to the cylinder using tape alone or tape and a covering of netting (tied with similar material) as were systems D and E. Adapting methods published by the FDA (Shah et al., 1988) using aluminium screen to secure TDS to watch glasses, locally available high density polystyrene netting of similar mesh was utilised in this work to secure all systems to watch glasses and to the cylinder. The United States Pharmacopoeia (Vol. 23) (Pharmacopeial Forum, 1995) describes Cuprophan dialysis membrane and Dow Corning 355 medical adhesive to attach TDS to the cylinder or adhesive alone to a disk. However, this adhesive was not available during this work and Scotch 468MP Hi Performance adhesive was tested. Neither adhesive nor tape prevented detachment of the dialysis membrane but adhesive was successful in securing Nicotinell patches to watch glasses in some experiments.

Tests 3 and 4: 8-cm watch glasses were used for all systems. Nicotinell was attached by adhesive or under netting as were D and E. Plastic curtain supports were found suitable to hold the screen onto watchglasses.

2.5. HPLC analysis of nicotine

Analysis of skin cell samples was carried out using a similar system to Ho and Chien, 1993. Analytical column; Waters Nova-Pak C18: guard column; Waters C18: mobile phase; USP pH 7.4 phosphate buffer/methanol/acetonitrile (40:30:30, v/v): flow rate 0.5 ml/min: detection; Waters 440 UV Detector at 254 nm. This method was also applied to some dissolution test samples. The retention time of the nicotine peak varied ca. 4.5 min to 5.3 min. Calibration curves of 7 or 11 standards were determined with each batch of samples and the unweighted linear regression line used for quantification. Although Test 2 specifies HPLC rather than UV determination of dissolution samples the profiles presented were wholly determined by UV absorbance. Some early profiles of all three tests and selected samples from later runs were confirmed by HPLC analysis with no evidence of interference.

Table 2
USP drug release tests for transdermal devices containing nicotine

USP drug release test	Apparatus and conditions	Analysis	Tolerances (% labelled dose)
Test 2 (Ciba-Geigy, Habitrol/Nicotinell)	Apparatus 6 Cylinder 50 rpm. TDS attached using double-sided tape. 10-ml aliquot. Medium 40 g NaCl, 1.0 g KCl, 8.66 g Na_2HPO_4, 1.0 g KH_2PO_4 in 5.0 l of water. 500 ml.	HPLC packing L1 12.5 cm × 4.6 cm Detection 260 nm. Inject filtered samples of ca. 100 μl. Compare with a standard solution of similar concentration	6 h 71% to 157%; 24 h 156% to 224%
Test 3 (Lederle, Prostep/Nicodisc)	Apparatus 5 Paddle over disk 50 rpm. Use an 8-cm watchglass for a 22-mg TDS. Apply the TDS using a suitable adhesive. Medium: water 900 ml.	UV absorbances at 259 nm compared with a standard solution in water of similar concentration using a water blank.	1 h 35% to 75%; 2 h 55% to 95%; 4 h not less than 73%
Test 4 (Pharmacia, Nicotrol/Nicotrans)	As Test 3. Medium 0.025 N hydrochloric acid 600 ml	As Test 3.	4 h 36% to 66%; 16 h 72% to 112%

2.6. Total nicotine content of TDS

USP Assay for Uniformity of Dosage Units (Pharmacopeial Forum, 1995) was modified to reduce the number of devices used. One or two of each available lot were placed in 200 ml of methanol in a 500 ml stoppered flask, shaken for 3 h (120 or 100 min^{-1} on a flat bed laboratory shaker) and then analyzed by HPLC. In some cases this procedure was repeated to confirm results. Hydrogel discs were assayed from Nicodisc patches and the remaining patch materials were also determined separately to assess loss of available nicotine during handling.

3. Results and discussion

3.1. Skin cells-nicotinell

Fig. 1 shows release of nicotine from Nicotinell patches expressed as the quantity of nicotine released from whole patches across porcine ear and human skin. Error bars are included only for sample A of the porcine data. No significant differences are seen between the lots using pig skin. Permeated amounts at 24 h as a % ± cv of the stated dose and in addition at 28 h in one case were as follows:

Pig: (A) 174.5 ± 14.5%; (B) 179.0 ± 18.4%; (C) 170.9 ± 21.8%

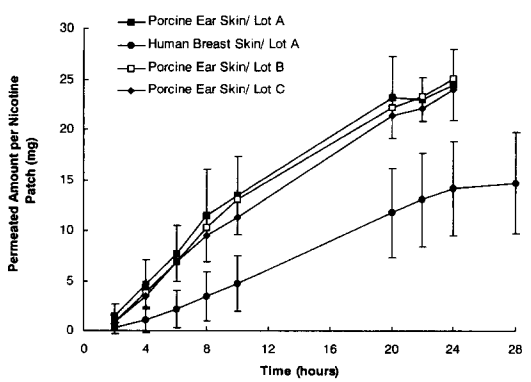

Fig. 1. Comparison between the amounts of nicotine penetrated through human (4 cells, 3 specimens) and porcine (8 cells, 4 specimens) skin from three lots (A, B, C) of Nicotinell transdermal systems.

Human: (A) 24 h, 101.3 ± 32.9% (A) 28h, 104.9% mean of 2 results

Two cells were allowed to run for 28 h after removal of the patches and the amount of nicotine in the receptor continued to rise. Increases over the 24 h levels were 12.2% and 24.4%. In vivo it has been reported that cutaneous vasoconstriction may limit transdermal nicotine absorption indicating the significance of the barrier properties of both the viable epidermis and dermis for this compound (Benowitz et al., 1992; Benowitz, 1995). More consistent results might be expected using dermatomed or heat separated human membranes.

Interpretation of in vitro skin cell data is complicated by the occurrence of an appreciable reservoir effect in vivo which leads to delayed absorption of nicotine after patch removal (Benowitz, 1995; Benowitz et al., 1991) and the possibility of lateral spreading of drug within the skin below a TDS can produce a larger effective area for absorption into the systemic circulation than the simple geometric area defined in a Franz cell experiment. Measurement of drug permeation across excised skin over the application period is therefore likely to underestimate the true in vivo delivery. It was reported (Benowitz, 1995; Benowitz et al., 1991) that for Habitrol (Nicotinell) TDS, 21 mg/24 h, nicotine continued to be absorbed at a low rate for at least 8 h after the application period accounting for about 10% of the total dose absorbed. The mean terminal half-life of nicotine after removal of the patch (4.3 h) was consequently considerably longer than that after i.v. infusion (2.8 h). However, the average absolute bioavailability of nicotine in the study was estimated to be only 82% of the nicotine released from the patches (mean 110.5% of the claimed in vivo dose, 21 mg), bioavailability range 12.5–25 mg, raising the possibility of metabolism of nicotine by the skin or loss of nicotine from the surface after patch removal. Differences in experimental procedure, sample handling etc., which contribute to the high observed inter-subject variability are likely to make agreement between the claimed systemic delivery and limited independent study results, particularly in vitro, difficult to

achieve, however the skin cell data obtained here does appear to both quantitatively and qualitatively reflect the claimed in vivo behaviour of the Nicotinell TDS.

Continued application of patches during therapy will eventually achieve steady-state plasma levels which may be estimated from the pharmacokinetic equation describing steady state during a constant rate of infusion (Guy and Hadgraft, 1992), $A_{24h}/T_{app} = C_L * C_{ss}$, where A_{24h} is the amount delivered by the patch during $T_{app} = 24$ h, C_{ss} is the plasma concentration attained and C_L denotes the clearance of the drug.

For Nicotinell patches delivering 14 mg, C_{ss} based on a C_L of 69.7 l/h (Benowitz et al., 1991) has a value of 8.37 ng/ml, below the lower end of the range of plasma levels associated with active smokers 15–45 ng/ml (Shulgin et al., 1987). This size of patch is normally utilised during the second stage of treatment (Benowitz, 1995) after 4–8 weeks of receiving 21 mg/24 h.

Pig skin may be considered analogous to human skin having a reduced barrier function and using the mean 24 h permeated amount for B (ca. 25.1 mg), a corresponding delivery in a human subject might be expected to produce a C_{ss} of 15.0 ng/ml while a proportionate increase in the delivery of the 21 mg device would be expected to produce levels of about 22.5 ng/ml. These values are clearly not dangerous although peak concentrations are not estimated but may sustain typical smoking levels rendering the treatment less effective in some individuals. The value of 25 mg/24 h reflects the highest bioavailability observed in the study by Benowitz, 1995.

For quality control purposes pig skin has a much lower barrier property than human skin but generating permeation profiles over a reduced time period corresponding to a total delivery equivalent to the human case followed by scaling of the time axis to represent delivery across the human membrane could be one approach to using this animal membrane as a routine test system. Taking the 24 h human skin mean delivery of this study and obtaining a corresponding time for permeation across pig skin by interpolation, the resulting scaling factor has been used to transform the porcine ear data time axis. The data sets (Fig. 2) can be seen to agree very closely as a result of the almost zero order delivery observed in the original data. For other devices alternative mapping procedures might be utilised.

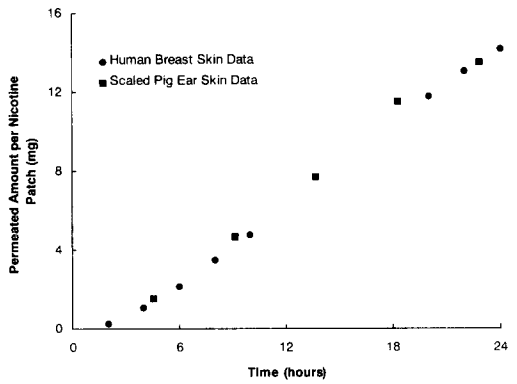

Fig. 2. Permeation data for Nicotinell lot A across human skin compared with porcine skin data scaled according to the time obtained by interpolation at which the same mean permeated amount is measured as for the human data at 24 h.

3.2. Skin cells-nicotrans

In the case of Nicotrans, Fig. 3, the permeated amounts at 16 and 24 h as a % ± CV of the stated in vivo dose were:

Pig: (D1) 16 h, 49.4 ± 20.8%; 24 h, 68.2 ± 22.8%

Human: (D1) 16 h, 38.5 ± 14.0%; 24 h, 52.5 ± 14.5%

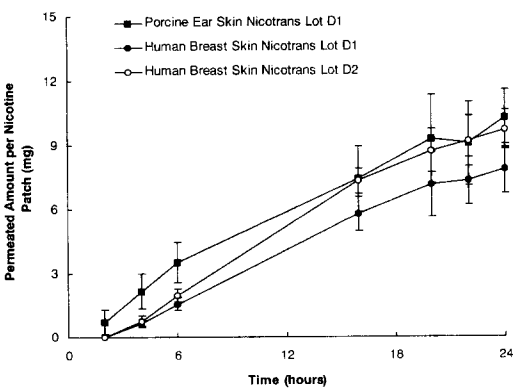

Fig. 3. Comparison between the amounts of nicotine penetrated through human (lots D1, 5 cells, 3 specimens and D2, 4 cells, 2 specimens) and porcine skin (D1 only, 8 cells, 4 specimens) from Nicotrans transdermal systems.

(D2) 16 h, 48.8 ± 8.3%; 24 h, 64.7 ± 16.3%

The profiles are very low with less than 50% the predicted delivery at 16 h and although lot D1 was nearing the end of its validity period this should not affect the measured amount in such a dramatic manner. The results for D1 and D2 are the means of five cells, three specimens and four cells, two specimens, respectively, the latter two specimens having been used for both lots. Excluding the specimen used for D1 only raises the mean values slightly (16 h, 40.2%; 24 h, 54.4%) and hardly alters the observed differences. Removal of patches after 16 h again revealed a slow release of nicotine from the skin and rising receptor levels with no significant differences between specimens carrying patches and those without. The values at 24 h may therefore give a better guide to total in vivo delivery, still below 65% of dose and with a considerable inter-lot difference.

Loss of nicotine on storage of Nicotrans systems or age related alterations in the adhesive, reservoir layer could be responsible for the reduced delivery noted here. Losses during handling and from the cut patch material during the experiment may be to blame although no improvement was observed for permeation across pig skin with different methods of sealing the patch edge and the construction of the device would appear at least as favourable to these manipulations as Nicotinell. The only slightly higher permeation from D1 across pig skin indicates the greater control exerted by this device compared to Nicotinell although this interpretation is complicated by the possibility of losses etc. affecting delivery.

The dosing regime of Nicotrans allows a washout period of ca. 8 h each night before applying a fresh device and the steady-state approximation is not strictly appropriate to estimate the effect of this apparent reduction in delivery on plasma levels but performing the calculation as before assuming a dosing schedule of 24 h, a claimed in vivo delivery of 15 mg yields a C_{ss} of 9.0 ng/ml which reduces to 4.7 ng/ml if delivery is 52.2% of this target. These are low values, in the latter case as low as non-smokers who are exposed to high levels of passive smoking (Shulgin et al., 1987) although the daytime levels achieved during application should be higher.

Other workers have reported changes in the intrinsic amount of oestradiol released from a TDS after storage of 11 months and between batches of different ages (Tymes et al., 1990; Brain et al., 1993) which may be related to loss of ethanol from that device with time. These gave rise to significant differences of in vitro skin permeation of drug and a pharmacokinetic model was used to estimate steady state plasma concentrations from the observed permeation rates which varied two-fold. In this work the differences between mean in vitro human skin penetration of nicotine from lots D1 and D2 are 26.8% at 16 h and 23.2% at 24 h and such differences in vivo may be expected to give rise to clinically significant differences in effectiveness of treatment for some patients although the impact on efficacy is difficult to assess. As stated by Benowitz (1995) in a comparative study of blood plasma profiles of nicotine: 'the pharmacodynamic implications of pharmacokinetic differences among transdermal systems, and implications for therapeutic efficacy, have not been elucidated'.

3.3. Skin cells-nicodisc

Nicodisc (E) also produced results lower than the claimed in vivo delivery. Where two fibre washers topped by aluminium enclosed the hydrogel sample the gel appeared slightly shrunken at the end of the experimental period and frequently adhered to the foil. Other treatments gave apparently better skin contact and slightly higher permeation rates.

All human experiments were carried out using a steel washer pressing down on an aluminium foil covering over the gel sample.

Permeated amounts after 24 h as a % ± CV of dose were:

Pig: (E) 24 h, Single washer 81.1 ± 14.3%; Double washer 65.3 ± 14.9%

Human: (E) 24 h, Single washer 51.5 ± 37.8% (means of 4 cells, 3 specimens)

(E) 28 h, Single washer 65.4% and 37.8% (115.5% and 140.6% of 24 h values, 2 cells, 2 specimens patches removed at 24 h)

Combined results are shown in Fig. 4. One very low result was excluded as the cell became off-

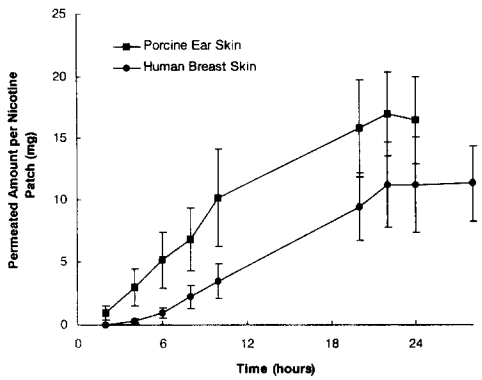

Fig. 4. Comparison between the amounts of nicotine penetrated through human (4 cells, 3 specimens) and porcine (15 cells, 5 specimens) skin from one lot (E) of Nicodisc transdermal systems.

centred due to an ill-fitting clip. Good contact between the gel and skin is clearly of importance to the delivery and in the clinical situation may be massaged into the skin during application. Some losses of available nicotine also occur during dismantling of the patches (see Section 3.4). Once again an appreciable reservoir effect is evident where the cells have been assayed at 28 h although this insufficient to account for the shortfall observed in the permeated amount.

Applying the steady-state calculation to the expected delivery over 24 h yields a value of $C_{ss} =$ 13.2 ng/ml. This patch size is the initial dose for treatment with this device (Benowitz, 1995) and a reduction of as much as 50% would be expected to result in low plasma levels ca. 6.1 ng/ml. However, the problems associated with testing this device in vitro probably render the results obtainable only useful in lot to lot comparisons albeit with difficulty due to the high variability observed and no direct quantitative extrapolation to in vivo delivery is possible. As the release area of the patch is quite small it may be possible to construct a cell which allows testing of whole devices on human or animal skin in order to approximate closely application in vivo.

The relatively higher expected depletion from Nicotrans (60.2%) and Nicodisc (73.3%) compared to Nicotinell (40.0%) as well as the higher resistance of Nicotrans means that there is less capacity for these devices to supply an excess dose. Guy and Hadgraft (1992) point out that, 'it is important for safety reasons to attempt to make the drug loading as close as possible to the amount absorbed'. The widest safety margins appear to be achieved in the present case by the devices having the greatest and least control respectively.

3.4. Total nicotine content of TDS

Available devices (A, B, C, D1, E) were initially assayed during December 1995 with a shaking period of 3 h and an agitation speed of 120 min^{-1}. Very good agreement was observed with the labelled amount for Nicotinell but Nicotrans (D1) gave very low and variable values even below those observed in the skin permeation experiments (Table 3). Subsequently lot D2 was received and further results obtained on Nicotrans and Nicodisc after 3 h and 6 h at 100 min^{-1}. Assays of D1 gave an even lower result after 3 h while the newer lot (D2) produced higher values similar to the first assay of D1. After 6 h increased concentrations of nicotine were measured for both lots highlighting the unreliability of methanol extraction over the recommended period at modest agitation speeds. The adhesive reservoir layer was only partially eroded by the end of the experiment and some indication of minimum agitation conditions and/or possible alternative solvent systems and procedures may be advisable for some TDS. The values obtained were consistently below 50% of stated content and therefore inconsistent with the deliveries measured across skin making it uncertain whether a low content in addition to altered release characteristics and/or experimental losses contribute to the low permeated amounts seen in the skin cell experiments.

Nicodisc gel yields a value consistent with the claimed content after 3 h (Table 3) but some nicotine still appears to be associated with the other patch materials which were discarded in the skin experiments. The hydrogel is insoluble in methanol and becomes hard and shrunken during immersion but assays at 3 and 6 h indicated no significant retardation of release rate during the test period.

Table 3
Summary of nicotine content assay results

Device (time)	Date	Total Nicotine % of Label Content	Device (time)	Date	Total Nicotine % of Label Content
A (3 h)	Dec. 95	98.1	D2 (3 h)	Feb. 96	27.6, 27.2
B (3 h)	Dec. 95	103.1	D2 (6 h)	Feb. 96	40.8, 39.2
C (3 h)	Dec. 95	103.1, 101.2	Ea (3 h)	Dec. 95	98.2 (9.3)
D1 (3 h)	Dec. 95	27.8, 32.0, 26.1	Eb (3 h)	Feb. 96	101.0
D1 (3 h)	Feb. 96	17.7	Eb (6 h)	Feb. 96	97.3
D1 (6 h)	Feb. 96	23.3			

Ea, assay of hydrogel only; (), assay of release liner and outer patch materials; Eb, total patch less release liner.

3.5. USP drug release tests

Release profiles for test 3 applied to each of the devices are shown in Fig. 5.

For each device release was apparently independent of the medium and apparatus used and almost superimposable profiles were generated under all three sets of test conditions. No interference with the UV assay was revealed by media from vessels without TDS and confirmation of certain profiles (all three test methods and devices) by HPLC analysis of samples produced excellent agreement with the UV absorbance data.

Table 4 presents % release data at each time-point specified by the USP for each test, and for each device confirming the similarity of values obtained. No significant differences were observed for different methods of attachment to cylinder or watch glass.

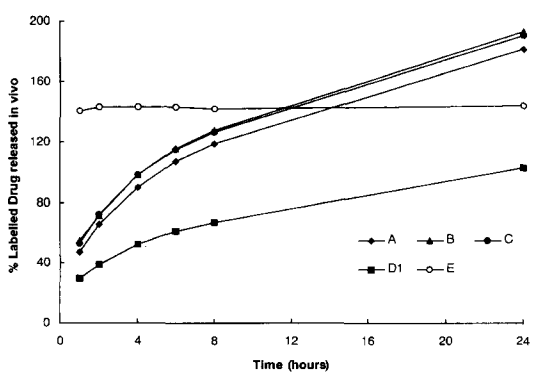

Fig. 5. Original in vitro release profiles, as determined by USP release test 3 (paddle over watchglass), for the three nicotine-containing transdermal systems.

In the case of Nicotinell, coefficients of variation less than 4.4% were obtained at all time-points with release after 24 h almost twice the stated dose. All lots were found to comply with the required specifications.

Nicotrans (D1) produced a gradual release profile which only after 24 h reached the stated in vivo dose at 16 h.

Nicodisc has the highest expected depletion of any of the devices, 73.3% of content. The carageenan hydrogel dissolves and nicotine release is rapid and complete under the conditions of each of the release tests. Consequently complete profiles were only generated for test 3. Assuming the stated content and dose we would expect complete dissolution to give 136% of the in vivo release but we measured 141% (CV 2.0%, $n = 3$) after only 1 h. Correcting the patch content for our assay results in Section 3.4 (107.6% dose) would predict 146.7% after total dissolution. As a result of the rapid dissolution of the gel the device does not comply with the test specifications at the first two time-points. Use of dialysis membrane as described in the United States Pharmacopoeia (Vol. 23) (Pharmacopeial Forum, 1995), does not appear mandatory in the test description but may slow dissolution of the reservoir sufficiently to bring the release characteristics within the specified ranges. However, the relevance of basing quality control testing of the product on this combined property of the release test apparatus membrane and drug reservoir must be questioned.

A simple model characterising the skin and device as diffusional resistances in series was carried out to explain some aspects of the behaviour

Table 4
Summary of mean % release from each device at time-points specified in USP proposals for tests 2, 3 and 4

Test	Time (h)	A	B	C	D1	D2	E
2, 3, 4	6	111, 108, 108	123, 116, 122	121, 115, 115	—	—	—
2, 3, 4	24	183, 181, 190	200, 193, 198	200, 191, 191	—	—	—
2, **3**, 4	1	—	—	—	—	—	(138), 141, (133)
2, **3**, 4	2	—	—	—	—	—	(142), (143), (142)
2, **3**, 4	4	—	—	—	—	—	nd, 144, nd
2, 3, **4**	4	—	—	—	46.8, 50.4, 46.5	nd, nd, nd	—
		—	—	—	nd, nd, 45.7	nd, nd, 52.3	—
2, 3, **4**	16	—	—	—	nd, nd, 83.5	nd, nd, nd,	—
		—	—	—	nd, nd, 87.4	nd, nd, 94.6	—

$n = 2$, 3, or 4 except for () = single profile.
nd, not done.
Highlighted (bold) test number is the specified test for the particular device whose results are tabulated in the corresponding row.

of GTN TDS (Hadgraft et al., 1991) and to interrelate in vitro data for release into an aqueous sink and in vitro delivery across human skin. The assumptions and limitations of this approach were further discussed by Guy and Hadgraft (1992). In particular the assumption of constant resistances for the skin and device were shown to depend on the relatively small depletion of the devices during the experiment. Nicotinell (rated to release 40% of its content in vivo) seems a reasonable candidate for this analysis. In the case of Nicodisc, since the device is clearly 'interacting' with the drug reservoir it is inappropriate to attempt to relate the observed flux to variations in vivo by the simple series resistances model.

The relationship between the total resistance (R_T) to drug delivery through the skin and the resistances to drug release from the device and to drug transport across the skin (R_d and R_s) may be written in terms of the reciprocal fluxes, assuming steady-state conditions and estimating the values from the average permeation rates over the application time, as

$$\frac{1}{J_T} = \frac{1}{J_d} + \frac{1}{J_s}$$

Considering the middle (190% dose) and high and low extremes (156 and 224%) of the release test 2 tolerance range to define values of J_d and assuming J_s to be a constant determined by the stated dose we can use the above equation to estimate the range of J_T observed for devices showing extreme but allowable release test behaviour. The low limit produces a value of 12.6 mg (89.7% of stated dose) and the high limit a value of 15.2 mg (108.6% of stated dose). Applying the steady-state pharmacokinetic equation as before clearly implies a proportional variation in steady state plasma levels due to variation in device behaviour which are in addition to the inter-subject variation which has been shown clinically (Benowitz, 1995).

If the intrinsic resistance of the device in contact with an aqueous receptor medium and in contact with the skin are similar then the release rate in the former case cannot be less than the permeation rate across the additional skin barrier and our measurements of less than 100% of stated dose released in vitro by Nicotrans imply that over the period of application (16 h) less drug is necessarily delivered from these lots. The midpoint of the tolerance range given for test 4 is only 92% of the claimed in vivo dose. In vivo depletion of the device after the application period is higher than for Nicotinell at 60.2%. It appears that in practice, $1/J_T \approx 1/J_d$ the resistance of the skin having a negligible contribution overall and the tolerance range of the release test might be expected to translate directly into a similar range of deliveries across skin, i.e. ca. 72–112%. However the simplifications inherent in the model ignore the fact that device/skin contributions to the overall flux vary during the delivery period and that initially the skin is unlikely to

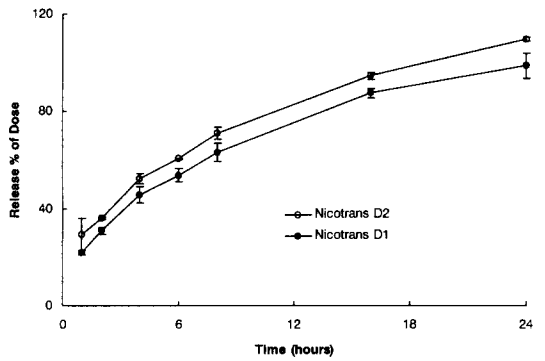

Fig. 6. In vitro release profiles, as determined by USP test 4 (paddle over watchglass), for lots D1 and D2 of Nicotrans transdermal systems.

contribute zero resistance to the delivery of drug. A low drug content in a device of normal resistance will result in lower than calculated drug delivery during the initial phase of application, i.e. due to additional skin resistance which exaggerates the expected reduction in delivery. The effect of the lower resistance of the porcine membrane leads to just the reverse effect, an increase in the early flux observed in Fig. 3 compared to the human with the profiles almost parallel thereafter.

Transfer of control to the device during the course of application results in a reduction in the expected delivery due to the lower reservoir capacity of the device producing lower effective concentrations during the latter stages of drug delivery. Rate-limiting devices are therefore expected to be highly sensitive to the accuracy of the drug levels they contain especially where an appreciable depletion of the drug reservoir is expected over the course of application.

It was shown for GTN containing devices (Hadgraft et al., 1991) that effective rate control by the patch led to reduced variability between skin samples and the CVs observed for the three devices in this work also broadly reflect the order of increasing control by the device, Nicodisc < Nicotinell ≈ Nicotrans, although the range of release behaviour and drug contents are less than for the GTN systems.

All three sets of data support a low release and/or content for D1. However, it still meets the suggested specifications of release test 4. Repeating test 4 with both lots of Nicotrans (3 × D2, 2 × D1) and a Nicotinell system (C) produced virtually identical profiles to those obtained previously but lot D2 which appeared to release nicotine more readily into methanol in the content assay gave a slightly higher release compared to D1 at all timepoints (see Table 4 and Fig. 6). This difference is quite small in comparison with those observed between these lots for permeation across human skin (Fig. 3).

Extraction into methanol appeared to be a more sensitive test of differences between these Nicotrans lots than release into an aqueous medium.

Release test specifications for Nicotrans are tighter than the other devices but it may be expected that these should reflect more directly on the in vivo delivery as a result of the rate determining nature of the device than for those systems which present more highly available reservoirs to the skin. The skin penetration data (Section 3.2) indicates that in particular a low drug content and/or reduced release rate albeit within the existing specifications may result in considerable reduction in drug delivery although this should be confirmed by in vivo studies. TDS design may be viewed as a compromise between the requirements of delivering a consistent and sufficient dose across all patients while minimising the possibility of excessive delivery and toxic side-effects. The three devices tested are not designed to be bioequivalent having different dosages, delivery rates and application periods but illustrate three different approaches to TDS design and the resulting problems that arise in devising and interpreting in vitro test data.

References

Benowitz, N.L., Clinical pharmacology of transdermal nicotine. *Eur. J. Pharm. Biopharm.*, 41 (1995) 168–174.

Benowitz, N.L., Chan, K., Denaro, C.P. and Jacob, P., Stable isotope method for studying transdermal drug absorption: the nicotine patch. *Clin. Pharmacol. Ther.*, 50 (1991) 286–293.

Benowitz, N.L., Jacob, P., Olsson, P. and Johansson, C.J., Intravenous nicotine retards transdermal absorption of nicotine: evidence of blood flow-limited percutaneous absorption. *Clin. Pharmacol. Ther.*, 52 (1992) 223–230.

Brain, K.R., Hadgraft, J., James, V.J., Shah, V.P., Walters, K.A and Watkinson, A.C., In vitro assessment of skin permeation from a transdermal system for the delivery of oestradiol. *Int. J. Pharm.*, 89 (1993) R13–R16.

Dick, I.P. and Scott, R.C., Pig ear skin as an in vitro model for human skin permeability. *J. Pharm. Pharmacol.*, 44 (1992) 640–645.

Guy, R.H. and Hadgraft, J., Rate control in transdermal drug delivery? *Int. J. Pharm.*, 82 (1992) R1–R6.

Hadgraft, J., Lewis, D., Beutner and Wolff, H.M. In vitro assessments of transdermal devices containing nitroglycerin. *Int. J. Pharm.*, 73 (1991) 125–130.

Ho, H. and Chien, Y.W., Kinetic evaluation of transdermal nicotine systems. *Drug Dev. Ind. Pharm.*, 19 (1993) 295–313.

Pharmacopeial Forum, *United States Pharmacopoeia*, 1995, Vol. 21, pp. 585–589 and 23, pp. 1796–1799.

Shah, V.P. and Elkins, J.S., In vitro release from corticosteroids ointments. *J. Pharm. Sci.*, 84 (1995) 1139–1140.

Shah, V.P., Tymes, N.W. and Skelly, J.P., Comparative in vitro release profiles of marketed nitroglycerin patches by different dissolution methods. *J. Control. Release*, 7 (1988) 79–86.

Shah, V.P., Lesko, L.J. and Williams, R.L., In vitro evaluation of transdermal drug delivery. *Eur. J. Pharm. Biopharm.*, 41 (1995) 163–167.

Shulgin, A.T., Jacob, P., Benowitz, N.L. and Lau, D., Identification and quantitative analysis of cotinine-*N*-oxide in human urine. *J. Chromatogr.*, 423 (1987) 365–372.

Tymes, N.W., Shah, V.P. and Skelly, J.P., In vitro release profile of estradiol transdermal therapeutic systems. *J. Pharm. Sci.*, 79 (1990) 601–602.