Synthesis and transdermal properties of acetylsalicylic acid and selected esters

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Abstract

The primary aim of this study was to determine the transdermal penetration of acetylsalicylic acid and some of its derivatives, to establish a correlation, if any, with selected physicochemical properties and to determine if transdermal application of acetylsalicylic acid and its derivatives will give therapeutic drug concentrations with respect to transdermal flux. Ten derivatives of acetylsalicylic acid were prepared by esterification of acetylsalicyloyl chloride with ten different alcohols. The experimental aqueous solubility, log $D$ and transdermal flux values were determined for acetylsalicylic acid and its derivatives at pH 4.5. In vitro penetration was measured through excised female human abdominal skin in diffusion cells. The experimental aqueous solubility of acetylsalicylic acid (6.56 mg/ml) was higher than that of the synthesised acetylsalicylate derivatives (ranging from $1.76 \times 10^{-3}$ to 3.32 mg/ml), and the log $D$ of acetylsalicylic acid ($-0.85$) was lower than that of its derivatives (ranging from $-0.25$ to 1.95). There was thus an inverse correlation between the aqueous solubility data and the log $D$ values. The experimental transdermal flux of acetylsalicylic acid (263.83 nmol/cm$^2$ h) was much higher than that of its derivatives (ranging from 0.12 to 136.02 nmol/cm$^2$ h).

Keywords: Acetylsalicylic acid; Skin penetration; Transdermal delivery
1. Introduction

The skin is the most extensive and readily accessible organ in the body. In an average adult it covers an area of about 1.73 m² and receives one third of circulating blood through the body at any given time. The potential of using intact skin as the site of administration for dermatological preparations to elicit pharmacological action in the skin tissue has been well recognised (Barr, 1962). The permeation of chemicals, toxicants and drugs are much slower across the skin when compared to other biological membranes in the body, due to the excellent barrier provided by the outermost layer of the skin, the stratum corneum (Harrison et al., 1996). The percutaneous delivery of drugs is an effective way of achieving controlled drug delivery. Unfortunately it is only suitable for a limited number of drugs that possesses the appropriate physicochemical characteristics to allow them to cross the stratum corneum (Harrison et al., 1996). The lipophilic stratum corneum is responsible for the primary barrier function of the skin and provides an extensive challenge to scientists in their pursuit to develop drugs for transdermal delivery (Pefile and Smith, 1997).

Acetylsalicylic acid (1) possesses anti-inflammatory, analgesic and antipyretic activity. (1) is also used in the prevention of thromboembolic disorders, reducing the incidence of colon cancer and it delays the onset of Alzheimer’s disease (Insel, 2001; Giovannucci et al., 1995; Rang and Dale, 1999). The most common adverse effect of (1) occurring with therapeutic doses is gastro-intestinal disturbances (Reynolds, 1984). Winek et al. (2001) gave the therapeutic blood level of (1) for analgesic use as 20–100 µg/ml (0.11–0.56 mM) and the level for prevention of thromboembolic disorders is lower since the dose for these conditions is lower.

Transdermal drug delivery offers a few advantages over oral and parenteral delivery. They include avoiding hepatic first pass metabolism, maintaining constant blood levels for longer periods of time, improving bioavailability, decreasing the administered dose, adverse effects and gastrointestinal side effects, easy discontinuation in case of toxic effects and improved patient compliance (Mitragotri, 2000).
The aim of this study was to determine the transdermal penetration of acetylsalicylic acid and some of its derivatives. Nielsen and Bundgaard (1989) evaluated various esters of (1) as potential prodrugs and found that (2) was not a prodrug for (1).
2. Materials and methods

2.1 Materials
Acetylsalicyloyl chloride was purchased from Sigma-Aldrich South Africa Ltd, and HPLC grade acetonitrile was obtained from LabChem South Africa Ltd. All the other reagents and chemicals were of analytical grade.

2.2 General procedures
The $^1$H and $^{13}$C NMR spectra were recorded on a Varian Gemini 300 spectrometer in CDCl$_3$, at a frequency of 300.075 MHz and 75.462 MHz, respectively with tetramethylsilane as internal standard. The MS spectra were recorded on an analytical VG 7070E mass spectrometer using electron impact (EI) at 70 eV as ionisation technique. Melting points were determined by differential scanning calorimetry (DSC) with a Shimadzu DSC-50 instrument.

2.3 High pressure liquid chromatography (HPLC)
The HPLC system consisted of a HP (Hewlet Packard) Agilent 1100 series auto sampler, HP Agilent 1100 series variable wave detector (VWD) and HP Agilent 1100 series isocratic pump. A Phenomenex (Luna C-18, 5 µm, 250 mm x 4.60 mm) column was used together with a Securityguard pre-column (C-18, 4 mm x 3 mm) insert (Phenomenex) in order to prolong column life and the Agilent Chemstation for LC Systems software package was used for data analysis. The flow rate was 1.2 ml/min with detection at 225 nm while the mobile phase compositions and retention times for each of the acetylsalicylic acid derivatives are presented in Table 1. Orthophosphoric acid (OPA) was used to adjust the pH of the mobile phase to 2.25. A volume of 100 µl was injected for each of the samples. Recycling of the mobile phase did not adversely affect the HPLC analysis. Calibration curves were constructed ranging from concentrations of 0.125–5.0 µg/ml.

Table 1:
HPLC conditions

2.3.1 Esterification
To a well-stirred mixture of acetylsalicyloyl chloride (5.96 g, 30.0 mmol) in pyridine (1.2 ml, 14.9 mmol) was added one of 10 different alcohols (excess) to form a clear solution. Stirring was continued for 24 h whereafter aqueous HCl (20 ml, 2M) was added followed by an excess of dichloromethane. The organic phase was collected and washed with 5% NaHCO₃ (20 ml) and water (3 x 20 ml). The organic phase was then dried over anhydrous MgSO₄, the dichloromethane removed under vacuum and the resulting oil collected. The prepared compounds were purified using column chromatography on silica gel.

### 2.3.1.1 Methyl acetylsalicylate (2)

A yield of 1.3 g (21.7%) of white crystalline compound was obtained. M.p. 46.80 °C (47–49 °C; Nielsen and Bundgaard, 1989), C₁₀H₁₀O₄, ¹H NMR δ (ppm) 2.32 (s, 3H, H-3’’), 3.85 (s, 3H, H-3’), 7.08 (dd, 1H, J = 8.1, 1.1 Hz, H-6), 7.28 (ddd, 1H, J = 7.6, 7.6, 1.2 Hz, H-4), 7.53 (ddd, 1H, J = 7.4, 7.4, 1.7 Hz, H-5), 8.00 (dd, 1H, J = 7.9, 1.7 Hz, H-3). ¹³C NMR δ (ppm) 20.90 (C-3’’), 52.08 (C-3’), 123.16 (C-2), 123.76 (C-6), 125.92 (C-4), 131.70 (C-3), 133.78 (C-5), 150.68 (C-1), 164.84 (C-1’), 169.57 (C-2’’). MS m/z 194 (14.2), 163 (83.0), 153 (92.5), 120 (52.8), 93 (67.0), 63 (100.0), 43 (49.8).

### 2.3.1.2 Ethyl acetylsalicylate (3)

A yield of 0.3 g (20.9%) of clear, light orange-yellow oil was obtained, C₁₁H₁₂O₄, ¹H NMR δ (ppm) 1.34 (t, 3H, J = 7.1 Hz, H-4’), 2.32 (s, 3H, H-3’’), 4.31 (q, 2H, J = 7.1 Hz, H-3’), 7.07 (dd, 1H, J = 8.1, 1.1 Hz, H-6), 7.28 (ddd, 1H, J = 7.6, 7.6, 1.2 Hz, H-4), 7.52 (ddd, 1H, J = 7.4, 7.4, 1.7 Hz, H-5), 8.00 (dd, 1H, J = 7.8, 1.8 Hz, H-3). ¹³C NMR δ (ppm) 14.17 (C-4’’), 20.93 (C-3’’), 61.02 (C-3’), 123.57 (C-2), 123.69 (C-6), 125.88 (C-4), 131.68 (C-3), 133.60 (C-5), 150.55 (C-1), 164.47 (C-1’), 169.46 (C-2’’). MS m/z 208 (10.3), 163 (67.7), 121 (95.7), 92 (84.4), 65 (63.8), 43 (90.4), 28 (100.0).

### 2.3.1.3 n-Propyl acetylsalicylate (4)

A yield of 0.6 g (8.4%) of clear, yellow oil was obtained, C₁₂H₁₄O₄, ¹H NMR δ (ppm) 0.99 (t, 3H, J = 7.4 Hz, H-5’), 1.74 (m, 2H, H-4’’), 2.32 (s, 3H, H-3’’), 4.21 (t, 2H, J = 6.7 Hz, H-3’), 7.07 (dd, 1H, J = 8.1, 1.1 Hz, H-6), 7.28 (ddd, 1H, J = 7.6, 7.6, 1.2 Hz, H-4), 7.53 (ddd, 1H, J = 7.4, 7.4, 1.7 Hz, H-5), 8.00 (dd, 1H, J = 7.8, 1.7 Hz, H-3). ¹³C NMR δ (ppm) 10.38 (C-5’), 20.95 (C-3’’), 22.00 (C-4’’), 66.66 (C-3’),
123.56 (C-2), 123.74 (C-6), 125.90 (C-4), 131.66 (C-3), 133.62 (C-5), 150.63 (C-1), 164.50 (C-1’), 169.52 (C-2’’). MS m/z 222 (12.8), 181 (80.1), 163 (100.0), 65 (98.2), 41 (80.7), 39 (87.3), 27 (87.7).

2.3.1.4 Isopropyl acetylsalicylate (5)

A yield of 0.4 g (6.6%) of clear, dark yellow oil was obtained, C_{12}H_{14}O_4, \(^1\)H NMR δ (ppm) 1.32 (d, 6H, J = 3.7 Hz, H-4’, H-5’), 2.32 (s, 3H, H-3’’), 5.20 (m, 1H, J = 6.3 Hz, H-3’), 7.06 (dd, 1H, J = 8.0, 1.1 Hz, H-6), 7.27 (ddd, 1H, J = 7.6, 7.6, 1.2 Hz, H-4), 7.51 (ddd, 1H, J = 7.4, 7.4, 1.7 Hz, H-5), 7.98 (dd, 1H, J = 7.8, 1.7 Hz, H-3). \(^13\)C NMR δ (ppm) 20.99 (C-3’’), 21.82 (C-4’, C-5’), 68.50 (C-3’), 123.63 (C-2), 124.05 (C-6), 125.86 (C-4), 131.64 (C-3), 133.44 (C-5), 150.44 (C-1), 164.06 (C-1’), 169.41 (C-2’’). MS m/z 222 (12.5), 180 (99.0), 163 (79.9), 121 (100.0), 92 (88.0), 65 (68.5), 39 (60.2).

2.3.1.5 n-Butyl acetylsalicylate (6)

A yield of 0.5 g (7.3%) of clear, light orange-yellow oil was obtained, C_{13}H_{16}O_4, \(^1\)H NMR δ (ppm) 0.95 (t, 3H, J = 7.4 Hz, H-6’), 1.43 (m, 2H, H-5’), 1.70 (m, 2H, H-4’), 2.32 (s, 3H, H-3’’), 4.26 (t, 2H, J = 6.7 Hz, H-3’), 7.07 (dd, 1H, J = 8.1, 1.1 Hz, H-6), 7.28 (ddd, 1H, J = 7.6, 7.6, 1.2 Hz, H-4), 7.53 (ddd, 1H, J = 7.4, 7.4, 1.7 Hz, H-5), 7.99 (dd, 1H, J = 7.8, 1.7 Hz, H-3). \(^13\)C NMR δ (ppm) 13.66 (C-6’), 19.15 (C-5’), 20.95 (C-3’’), 30.67 (C-4’), 64.94 (C-3’), 123.55 (C-2), 123.74 (C-6), 125.89 (C-4), 131.66 (C-3), 133.61 (C-5), 150.64 (C-1), 164.49 (C-1’), 169.51 (C-2’’). MS m/z 194 (100.0), 138 (96.3), 121 (92.3), 92 (74.2), 43 (86.1), 29 (61.3); FAB 237 ((M+H’) 30.0%), 195 (78.0%), 163 (54.0%), 149 (31.5%), 138 (53.0%), 121 (100.0%).

2.3.1.6 1-Methylpropyl acetylsalicylate (7)

A yield of 0.6 g (8.3%) of clear, dark yellow oil was obtained, C_{13}H_{16}O_4, \(^1\)H NMR δ (ppm) 0.94 (t, 3H, J = 7.4 Hz, H-5’), 1.29 (d, 3H, J = 6.3 Hz, H-6’), 1.66 (m, 2H, H-4’), 2.32 (s, 3H, H-3’’), 5.04 (m, 1H, H-3’), 7.07 (dd, 1H, J = 8.0, 1.0 Hz, H-6), 7.28 (ddd, 1H, J = 7.6, 7.6, 1.2 Hz, H-4), 7.51 (ddd, 1H, J = 7.4, 7.4, 1.7 Hz, H-5), 7.99 (dd, 1H, J = 7.9, 1.7 Hz, H-3). \(^13\)C NMR δ (ppm) 9.66 (C-5’), 19.42 (C-6’), 21.01 (C-3’’), 28.87 (C-4’), 73.06 (C-3’), 123.70 (C-2), 124.05 (C-6), 125.88 (C-4), 131.62 (C-3), 133.45 (C-5), 150.53 (C-1), 164.16 (C-1’), 169.48 (C-2’’). MS m/z 236 (9.3), 194 (89.5), 163 (84.7), 121 (100.0), 92 (84.0), 43 (94.9), 29 (71.3).
2.3.1.7  Tert-butyl acetylsalicylate (8)

A yield of 0.5 g (7.1%) of clear, light orange-yellow oil was obtained, C₁₃H₁₆O₄. ¹H NMR δ (ppm) 1.55 (s, 9H, H-4’, H-5’, H-6’), 2.32 (s, 3H, H-3’’), 7.04 (dd, 1H, J = 8.1, 1.2 Hz, H-6), 7.26 (ddd, 1H, J = 7.6, 7.6, 1.2 Hz, H-4), 7.46 (ddd, 1H, J = 7.4, 7.4, 1.8 Hz, H-5), 7.89 (dd, 1H, J = 7.8, 1.8 Hz, H-3). ¹³C NMR δ (ppm) 21.08 (C-3’’), 28.13 (C-4’, C-5’, C-6’), 81.54 (C-3’), 123.46 (C-2), 125.41 (C-6), 125.77 (C-4), 131.47 (C-3), 132.96 (C-5), 150.12 (C-1), 163.89 (C-1’), 169.39 (C-2’’). MS m/z 181 (60.6), 163 (100.0), 138 (56.5), 121 (71.2), 43 (57.3); FAB 237 ((M+H⁺) 35.0%), 195 (87.5%), 163 (61.0%), 149 (32.0%), 138 (55.0%), 121 (100.0%).

2.3.1.8  n-Pentyl acetylsalicylate (9)

A yield of 0.6 g (8.0%) of clear, light yellow oil was obtained, C₁₄H₁₈O₄. ¹H NMR δ (ppm) 0.90 (t, 3H, J = 7.1 Hz, H-7’), 1.37 (m, 4H, H-5’, H-6’), 1.72 (m, 2H, H-4’), 2.32 (s, 3H, H-3’’), 4.25 (t, 2H, J = 6.8 Hz, H-3’), 7.07 (dd, 1H, J = 8.1, 1.1 Hz, H-6), 7.28 (dd, 1H, J = 7.6, 7.6, 1.2 Hz, H-4), 7.52 (ddd, 1H, J = 7.4, 7.4, 1.8 Hz, H-5), 7.99 (dd, 1H, J = 7.8, 1.7 Hz, H-3). ¹³C NMR δ (ppm) 13.89 (C-7’), 20.97 (C-3’’), 22.30 (C-6’), 28.07 (C-5’), 28.34 (C-4’), 65.25 (C-3’), 123.58 (C-2), 123.75 (C-6), 125.91 (C-4), 131.67 (C-3), 133.61 (C-5), 150.65 (C-1), 164.51 (C-1’), 169.53 (C-2’’). MS m/z 209 (75.1), 208 (94.9), 163 (77.7), 138 (90.2), 121 (86.4), 120 (100.0); FAB 251 ((M+H⁺) 30.5%), 209 (82.0%), 163 (55.0%), 138 (63.0%), 121 (100.0%).

2.3.1.9  1-Methylbutyl acetylsalicylate (10)

A yield of 0.2 g (15.5%) of clear, colourless oil was obtained, C₁₄H₁₈O₄. ¹H NMR δ (ppm) 0.92 (t, 3H, J = 7.3 Hz, H-6’), 1.30 (d, 3H, J = 6.3 Hz, H-7’), 1.38 (m, 2H, H-5’), 1.60 (m, 2H, H-4’), 2.32 (s, 3H, H-3’’), 5.12 (m, 1H, H-3’), 7.07 (dd, 1H, J = 8.1, 1.1 Hz, H-6), 7.28 (ddd, 1H, J = 7.6, 7.6, 1.2 Hz, H-4), 7.51 (ddd, 1H, J = 7.4, 7.4, 1.8 Hz, H-5), 7.98 (dd, 1H, J = 7.8, 1.7 Hz, H-3). ¹³C NMR δ (ppm) 13.88 (C-6’), 18.62 (C-7’), 19.95 (C-5’), 21.01 (C-3’’), 38.15 (C-4’), 71.65 (C-3’), 123.70 (C-2), 124.06 (C-6), 125.88 (C-4), 131.62 (C-3), 133.44 (C-5), 150.53 (C-1), 164.14 (C-1’), 169.46 (C-2’’). MS m/z 250 (7.9), 208 (88.4), 163 (83.1), 139 (58.1), 121 (100.0), 92 (80.9), 65 (58.2).

2.3.1.10  1-Ethylpropyl acetylsalicylate (11)
A yield of 0.2 g (10.9%) of clear, colourless oil was obtained, C₁₄H₁₈O₄, ¹H NMR δ (ppm) 0.92 (t, 6H, J = 7.5 Hz, H-5’, H-7’), 1.65 (m, 4H, H-4’, H-6’), 2.32 (s, 3H, H-3’’), 4.96 (m, 1H, H-3’’), 7.07 (dd, 1H, J = 8.1, 1.1 Hz, H-6), 7.28 (ddd, 1H, J = 7.6, 7.6, 1.2 Hz, H-4), 7.52 (ddd, 1H, J = 7.4, 7.4, 1.8 Hz, H-5), 8.00 (dd, 1H, J = 7.8, 1.7 Hz, H-3). ¹³C NMR δ (ppm) 9.56 (C-5’, C-7’), 20.99 (C-3’’), 26.47 (C-4’, C-6’), 77.54 (C-3’), 123.75 (C-2), 124.02 (C-6), 125.88 (C-4), 131.59 (C-3), 133.44 (C-5), 150.60 (C-1), 164.33 (C-1’), 169.47 (C-2’’). MS m/z 208 (80.7), 163 (94.2), 139 (21.0%), 121 (100.0%), 92 (74.3), 43 (63.4); FAB251 ((M+H+) 23.0%), 209 (10.0%), 181 (16.5%), 163 (35.0%), 139 (21.0%), 121 (100.0%).

2.4 Physicochemical properties

2.4.1 Solubility determination

The aqueous solubility of acetylsalicylic acid and its derivatives was obtained by preparing saturated solutions in a tris-(hydroxymethyl)aminomethane buffer (TRIS) at pH 4.5. The slurries were stirred with magnetic bars in a water bath at 37 °C for 24 h. An excess of solute (oil or crystals) was present at all times to provide saturated solutions. After 24 h, the solutions were filtered and analysed directly by HPLC to determine the concentration of solute dissolved in the solvent. The experiment was done in triplicate.

2.4.2 Experimental log D

Equal volumes of n-octanol and TRIS buffer (pH 4.5) were saturated with each other under vigorous stirring for at least 24 h. Each of the acetylsalicylic acid derivatives (10 mg/ml) was dissolved in 3ml pre-saturated n-octanol, stoppered and agitated for 10 min. Subsequently 3ml pre-saturated TRIS buffer was transferred to assay tubes containing before-mentioned solutions. The tubes were stoppered and agitated for 45 min. Thereafter, they were centrifuged at 4000 rpm for 30 min. The n-octanol and aqueous phases were separately analysed by HPLC. The aqueous phase was analysed directly by HPLC and the n-octanol solutions were diluted 1:1000 with acetonitrile (solvent) prior to being analysed by HPLC. The log D values (log (octanol: pH 4.5 buffer partition coefficient)) were calculated as logarithmic ratios of the acetylsalicylic acid derivative concentrations in the n-octanol phase to the concentrations in the TRIS buffer. The experiment was done in triplicate.
2.5 Permeation experiments

2.5.1 Preparation of donor solutions
Donor solutions of the acetylsalicylate derivatives were obtained by the equilibration of excess amounts of solute in TRIS buffer (pH 4.5). TRIS buffer was used because over the period of the experiment the derivatives were stable in it, while in phosphate buffer solution (PBS) the derivatives were absent after 24 h. The slurries were prepared in stoppered flasks with stirring in a water bath at 37 °C over a period of 24 h solvent saturation to occur. An excess amount of solute was present at all times.

2.5.2 Skin preparation
Female human abdominal skin, obtained after cosmetic procedures, was used for the permeation studies. A scalpel was used to separate the skin from the fat layer; subsequently the epidermis was removed by means of immersion in 60 °C HPLC water for 60 s (Kligman and Christophers, 1963). The epidermis was gently teased away from the skin with forceps. Special care was taken that the integrity of the epidermis was not ruptured, as this would compromise the validity of the results. The epidermis was placed in a bath filled with HPLC water and carefully set on Whatman® filter paper, left to air dry and was wrapped in foil. The foil containing the epidermis was stored in a freezer at −20 °C and was used within 6 months after being prepared. Prior to use, the epidermis was thawed and visually examined, before it was mounted on the Franz diffusion cells.

2.5.3 Method for skin permeation
Vertical Franz diffusion cells with 2.0 ml receptor compartments and 1.0751 cm² effective diffusion area was used for the permeation studies. The epidermal skin layer was carefully mounted on the lower half of the Franz cell with the stratum corneum facing upwards. A clamp was used to fasten the upper and lower parts of the Franz cell together, with the epidermis separating the donor and receptor compartments. The receptor compartments were filled with isotonic TRIS buffer (pH 7.4). Special care was taken that no air bubbles came between the buffer solution and the epidermis. The Franz cells, containing buffer solution, were equilibrated for 1 h in the water bath at 37 °C, prior to the addition of the saturated solutions to the donor compartments. Only the receptor compartments were submerged in the water and were equipped
with stirring magnets. After a period of 1 h, 1.0 ml of freshly prepared saturated solution with an excess oil or crystals were added to each donor compartment, which was immediately covered with Parafilm® to prevent the evaporation of any constituents from the saturated solution for the duration of the experiment. An excess amount of solute was present in the donor compartments at all times during the experimental procedure.

The entire receptor volumes were withdrawn and replaced with 37 °C fresh buffer solution (pH 7.4) after 2, 4, 6, 8, 10, 12 and 24 h. The entire receptor volumes were withdrawn to mimic sink conditions as they occur in the human body. The experiments were conducted over 24-h periods.

The withdrawn samples were assayed immediately by HPLC to in each case determine the drug concentration of (1) or the derivative (2)–(11) that had permeated the epidermis. In no case any salicylic acid or (1) was observed in the chromatograms of the receptor phase of the derivatives in the TRIS buffer, although (2)–(11) showed an extra peak at 1.790–2.170 min in the chromatogram. The experiment was done until six values within 30–40% of one another were obtained. Flux was calculated from the gradient of the cumulative concentration versus time graph. At least six data points on the steady-state part of the curve were used.
3. Results

Table 2:
Aqueous solubility, partition coefficient and flux of acetylsalicylic acid and its derivatives
4. Discussion

4.1 Structures of the products

Fig. 1. Structures of acetylsalicylic acid and its esters.

4.1.1 1-Methylbutyl acetylsalicylate (10)

The MS data (FAB) confirmed the presence of the molecular ion of (10) at \( m/z \) 250, corresponding to a molecular formula of \( \text{C}_{14}\text{H}_{18}\text{O}_{4} \). The molecular ion was not observed in the electron impact mass spectrum. The \( ^{13}\text{C} \) NMR data of (10) were similar to that of (1), except that the signal of the carbonyl carbon atom has shifted to a slightly lower magnetic field at \( \delta \) 164.14 in the \( ^{13}\text{C} \) NMR spectrum, while the presence of the methyl groups were indicated by signals at \( \delta \) 13.88 and 18.62, with the methine group represented by the peak at \( \delta \) 71.65. The methylene groups were indicated by signals at \( \delta \) 19.95 and 38.15. In the \( ^{1}\text{H} \) NMR spectrum the triplet at \( \delta \) 0.92 and the doublet at \( \delta \) 1.30 represent two methyl groups. The multiplets at \( \delta \) 1.38 and 1.60 represent two methylene groups, while the signal at \( \delta \) 5.12 represents the methine group. The structural properties of the remaining derivatives were consistent with that of (10) and the structures of all the derivatives (Fig. 1) were determined in the same fashion.

4.2 Physicochemical properties

A higher degree of ionisation leads to lower concentrations of unionised drug available for transdermal penetration. Hence, unionised species permeate the stratum corneum better than the ionised form (Abdou, 1989; Jack et al., 1991). (1) is a relatively strong acidic drug with a pKa of 3.50 (Florey, 1979), thus at a pH of 3.5 (1) is 50% unionised. This provides a problem for transdermal application, for if the pH is lower than 4.5 it will irritate the skin. This was the motive for choosing a pH of 4.5, where (1) was 9.09% unionised. To compare the synthesised derivatives with (1), it was necessary to determine the physicochemical properties at the same pH.

The aqueous solubility of (1) is higher and the log \( D \) of (1) is lower than that of the synthesised acetylsalicylate compounds. Table 2 shows that in the straight chain series (2), (3), (4), (6) and (9) the aqueous solubility on a molar basis decreases and the log \( D \) increases with an increase in alkyl chain...
length, which is in accordance with the findings of Abdou (1989). The effect of branching in the
cromoioety leads to an increase in aqueous solubility and a decrease in log $D$ values in the series (6)–(8)
and (9)–(11), but not for (4) and (5). These results are in agreement with predictions in the literature
(Sloan et al., 2003).
The results indicate that esterification of (1) leads to lower aqueous solubilities and higher log $D$ values.
These results validate the aqueous solubility data, whereby compounds with higher log $D$ values present
with an increased lipophilicity and lower aqueous solubility.

4.3 Transdermal properties

The experimental transdermal flux of (1) (47.53 µg/cm$^2$ h) was much higher than that of its derivatives,
with the (3) (28.32 µg/cm$^2$ h), (2) (10.06 µg/cm$^2$ h) and (8) derivatives (7.30 µg/cm$^2$ h) being the only
compounds with appreciable flux. (1) and (2) were both crystalline compounds, the rest of the
acetylsalicylate derivatives were oils. Therefore, in the supersaturated buffer solutions (1) and (2) had
crystals at the bottom of the donor phase, on top of the skin, keeping the resultant solution homogenous
which may explain the higher flux observed for these compounds. The other derivatives were found
floating on top of the saturated solution and as there was no stirring in the donor phase to keep the
resultant solution homogenous, the solution closest to the skin became unsaturated and this may describe
the decreased flux observed for these compounds, except for (3). If solution of the compound from the oil
phase is slow, it is possible that the concentration immediately adjacent to the skin surface will not be
replenish faster than it is absorbed into the skin. In this case there will be a depletion of the concentration
that drives the flux.

As a general rule, a drug substance with an aqueous solubility of less than 1 mg/ml may represent a
potential bioavailability problem. A drug should have optimal permeation and highest flux values if it has
reasonable solubility in both water and oils and has a log $D$ in the range of 1–2 (Sloan, 1989; Hadgraft,
1996; Roberts and Sloan, 2000). (1), (2), and (3) had higher flux values; probably due to the aqueous
solubilities (ranging from 3.32 to 6.56 mg/ml) being higher than 1 mg/ml. (8) had an aqueous solubility
close to 1 mg/ml (0.76 mg/ml) and a log $D$ between 1 and 2 (1.09), which could explain the higher flux
obtained. (6), (7), (9), (10) and (11) had aqueous solubilities far less than 1 mg/ml (ranging from $1.76 \times 10^{-3}$ to 0.20 mg/ml) and log $D$ values between 1 and 2 (ranging from 1.09 to 1.95), leading to lower flux values.

Yano et al. (1986) investigated the skin permeability of eight salicylates, which showed a decrease in absorption with an increase in chain length. A series of investigations have been done on the transdermal delivery of (1). The following flux values for (1) were obtained: 21.81 ± 3.11 µg/cm$^2$ h (Feldmann and Maibach, 1970), and in vivo 24.8 ± 4.4 µg/cm$^2$ h and in vitro 29.0 ± 3.1 µg/cm$^2$ h (Bronaugh et al., 1982). The previous studies were all performed at neutral pH. In this study the flux for (1) was 47.53 ± 5.8 µg/cm$^2$ h at pH 4.5, which is a higher flux value than the values obtained in previous studies. Since (1) is 99.99% ionised at pH 7.4 and 91.91% ionised at pH 4.5, it makes sense that the flux values obtained in this study was higher, due to the fact that (1) is more unionised.
5. Conclusion

The acetylsalicylic acid derivatives were successfully synthesised and the structures were verified by \(^1\)H and \(^{13}\)C NMR and MS spectroscopy. The higher flux values of (1), (2) and (3) can be due to the aqueous solubilities being higher than 1 mg/ml. (8) had an aqueous solubility of 0.76 mg/ml and a log \(D\) of 1.09, which could explain the higher flux. The aqueous solubility values of the other acetylsalicylate derivatives were far less than 1 mg/ml, but they had log \(D\) values between 1–2, which lead to lower flux values, due to the fact that the lipophilic compounds had difficulty leaving the stratum corneum or for being oils that were found floating on top of the saturated solution. The more lipophilic derivatives may exhibit lower flux values than (1), since they are less water soluble. In this study the flux for (1) was 47.53 ± 5.8 µg/cm\(^2\) h at pH 4.5, which is a higher flux value than those obtained in previous studies, due to the fact that (1) is more unionised. The flux of (1) is also higher than that of any of the derivatives prepared in this study. Their transdermal application thus has no practical advantage.
Acknowledgements

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References


### Table 1: HPLC conditions

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<tr>
<th>Compound</th>
<th>Mobile phase</th>
<th>Retention time (min)</th>
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<tbody>
<tr>
<td>Acetylsalicylic acid (1)</td>
<td>50:50</td>
<td>3.47</td>
</tr>
<tr>
<td>Methyl acetylsalicylate (2)</td>
<td>50:50</td>
<td>6.25</td>
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<tr>
<td>Ethyl acetylsalicylate (3)</td>
<td>50:50</td>
<td>8.65</td>
</tr>
<tr>
<td>Propyl acetylsalicylate (4)</td>
<td>33:67</td>
<td>5.66</td>
</tr>
<tr>
<td>Isopropyl acetylsalicylate (5)</td>
<td>33:67</td>
<td>5.38</td>
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<td>n-Butyl acetylsalicylate (6)</td>
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<td>7.39</td>
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<td>1-Ethylpropyl acetylsalicylate (11)</td>
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<td>9.15</td>
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<tr>
<td>Compound</td>
<td>Aqueous solubility (mg/ml)</td>
<td>Aqueous solubility (mM)</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>----------------------------</td>
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<tr>
<td>Acetylsalicylic acid (1)</td>
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<td>36.41</td>
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<td>Methyl acetylsalicylate (2)</td>
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<td>0.20</td>
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<tr>
<td>1-Ethylpropyl acetylsalicylate (11)</td>
<td>0.11</td>
<td>0.44</td>
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</table>
Figures:

1: R = H
2: R = CH₃
3: R = C₂H₅
4, 5: R = n-C₃H₇, i-C₃H₇
6, 7, 8: R = n-C₄H₉, 2°-C₄H₉, t-C₄H₉
9, 10: R = n-C₅H₁₁, 2°-C₅H₁₁
11: R = CH(C₂H₅)₂

Fig. 1. Structures of acetylsalicylic acid and its esters.