# Comparison of Fourier Transform Raman Spectra of Mammalian and Reptilian Skin*

**Adrian C. Williams and Brian W. Barry**

*Postgraduate Studies in Pharmaceutical Technology, The School of Pharmacy, University of Bradford, Bradford, UK BD7 1DP*

**Howell G. M. Edwards**

*Chemistry and Chemical Technology, University of Bradford, Bradford, UK BD7 1DP*



**Human skin offers potential advantages for the administration of therapeutic agents for both local and systemic use. However, in studies of transdermal drug delivery, problems with the supply, storage, and use of human tissue have encouraged workers to seek alternative animal materials to model drug diffusion across human skin. We obtained Fourier transform Raman spectra from mammalian (human and pig) and reptilian (snake) skins, and considered structural dissimilarities in the light of differences observed in the diffusion of drugs across the tissues.**

***Keywords:*** *Fourier transform Raman spectroscopy; human skin; pig skin; snake skin; transdermal drug delivery*

## Introduction

Human skin provides a self-repairing barrier against physical, microbial, and chemical assault. The stratum corneum is the outermost layer of this tissue and, although only 6–30 μm thick on most body sites (excepting the palms of the hands and the soles of the feet), it provides the main barrier to ingress of environmental contaminants, and thwarts researchers' and clinicians' attempts to administer many therapeutic agents *via* the transdermal route. This barrier function derives from the unique morphology of the stratum corneum, which comprises keratin-rich anucleate cells embedded in a matrix of multiple lipid bilayers.[1]

Many workers have studied the transdermal permeation of a wide variety of therapeutic agents, and the molecular basis for the barrier function of skin, as recently reviewed by Williams and Barry.[2] However, difficulties with supply, storage and use of biohazardous human tissue have encouraged some workers to seek alternative animal models for their investigations. Pig skin closely resembles that of humans both morphologically[3,4] and in diffusional characteristics for many drugs.[4,5] Snake skin, however, shows some considerable structural variations to human tissue,[6,7] being based on a hinge and scale construction; the hinges consist largely of flexible α-keratin whilst the scales contain β-keratin folds, beneath which are lipoidal domains. Although snake skin possesses different diffusional characteristics compared with human skin,[8] it is a popular model membrane,[9–13] because mature snakes regularly shed their skin, thus providing plentiful samples without sacrifice that do not present the biohazard of human tissue.

The molecular basis for the barrier nature of human stratum corneum has been probed by Fourier transform (FT) infrared spectroscopy,[14,15] a technique that has also been applied to the study of fatty acid interactions with porcine stratum corneum.[16] However, as a naturally hydrated biomembrane, the molecular nature of skin samples can be more clearly studied by FT Raman spectroscopy.[17–20] We now report FT Raman spectra and band assignments for human, pig, and snake skin to describe structural dissimilarities between the tissues, and relate these to reported variations in permeability characteristics to therapeutic agents.

## Experimental

### *Preparation of Stratum Corneum Membranes*

Caucasian abdominal midline skin, obtained post-mortem, and freshly excised porcine skin were stored frozen at −20 °C.[21] Epidermal membranes, incorporating the anucleate stratum corneum and nucleate epidermal tissue, were prepared by a heat-separation technique;[22] skin samples trimmed of fatty tissue were immersed in water at 60 °C for 45 s, after which the epidermal membranes were teased off the underlying dermis. Stratum corneum samples were then prepared from the epidermal membrane;[22] the epidermal tissue was floated overnight on an aqueous solution of trypsin (0.0001% m/v) and sodium hydrogencarbonate (0.5% m/v) at 37 °C. The enzyme digests the nucleate epidermal tissue allowing the remnants to be removed by swabbing. The pig and human stratum corneum samples were then washed with water before being stored dry. Prior to spectral analysis the stratum corneum samples and the snake skin (requiring no special preparation) were rinsed for 10 s with acetone to remove surface contamination (*e.g.*, fatty deposits) and were hydrated over saturated aqueous salt solutions to a water content of 20–40% m/m.

### *Fourier Transform Raman Spectra*

Fourier transform Raman spectra were obtained using a Bruker IFS 66 with FRA 106 FT Raman attachment. Sample excitation was by an Nd–YAG laser operating at 1.064 μm with a maximum power of 700 mW. Typically, the laser beam was focused to a spot of approximately 100 μm diameter at the skin samples, which were packed into stainless-steel sampling cups. The samples were not prone to thermal degradation at the incident laser power levels used in the experiments (usually 200–300 mW) with typically, 500 scans at 4 $cm^{-1}$ being collected for the human and pig tissue. The snake skin tended to fluoresce, but good quality spectra were obtained through using low laser powers, a de-focused laser beam and prolonged collection of many scans (*e.g.*, 4000). A liquid nitrogen cooled germanium detector with an extended spectral bandwidth was used over the wavenumber range 3500–100

* Presented at the XXVIII Colloquium Spectroscopicum Internationale (CSI), York, UK, June 29–July 4, 1993.

$cm^{-1}$. Spectral response was corrected for white light and the observed band wavenumbers were calibrated against internal laser frequencies; these are correct to better than $\pm 1$ $cm^{-1}$.

## Results and Discussion

Examples of FT Raman spectra from human and pig stratum corneum, and from snake skin are shown in Fig. 1. The spectra from a given species were found to be reproducible in terms of peak positions and relative intensities from a particular specimen. However, some minor alterations were found in relative peak intensities (but not positions) between different samples of the same species, this was ascribed to natural variability of skin component compositions.[23]

All three tissue samples show remarkably similar Raman spectra, with those from the human and porcine tissue appearing almost identical. Fig. 2(*a*) and (*b*) allows closer examination of the spectra in the wavenumber regions 2750–3250 $cm^{-1}$ (C–H stretching region) and 900–1800 $cm^{-1}$ (functionality region); the spectra show subtle differences, such as altered band shapes and wavenumbers.

The wavenumbers and approximate descriptions of the Raman vibrational modes for human, pig, and snake skins are compared in Table 1; with over 40 bands listed in Table 1, several of which are relatively weak spectral features, and with inevitable mode mixing from the complex tissues, some of the assignments are necessarily tentative.

Mammalian stratum corneum is naturally hydrated, a state that can be problematic in infrared investigations.[17] Figs. 1 and 2 illustrate the value of FT Raman spectroscopy in structural studies of biological tissues, with good quality spectra being obtained relatively free from interference by water bands.

The C–H stretching vibrations of lipids within the skin samples are shown in the spectra in Fig. 2(*a*). Porcine and human skin contain similar lipids but in slightly different amounts.[3] This variation is reflected in the spectra where band intensity differences are seen between the tissues, especially for the features at 2931 and 2883 $cm^{-1}$. However, considering bandwidths and wavenumber positions it is likely that whilst lipid contents vary, the state of order of the lipid component in human and pig skin is similar. Snake skin provides a similar Raman spectrum to those from human and pig tissue, a somewhat surprising result when considering environmental and physiological differences between the species. However, the lipid component of snake skin may be in a less ordered state, and a feature at 2975 $cm^{-1}$ evident in the snake skin spectrum is not seen in spectra from mammalian tissues. Such differences in the lipid content and order are of particular interest as it is this component of skin that provides the main barrier to the transdermal permeation of polar drugs.[2]

The three skin samples also provide similar Raman spectra in the 'fingerprint region', 900–1800 $cm^{-1}$ [Fig. 2(*b*)]. Again, the tissues appear to contain similar components in slightly differing amounts and states, with spectra from pig and human stratum corneum appearing nearly identical. One notable difference in the spectrum from reptilian skin compared with those from mammalian tissues is due to the keratin present in the samples. Shed snake skin consists of three distinct layers; the outermost β layer is rich in β-keratin, beneath which is the mesos layer containing α-keratin in a lipid matrix, followed by the lower α-keratin-rich α layer. In contrast, the keratin in mammalian stratum corneum is largely in the α conformation, although some unordered and/or β-keratin may be present. These conformational dissimilarities are clearly seen in the wavenumber positions of the amide I and amide III vibrational modes (Table 1).

Other dissimilarities in the spectra from the three animals are also evident in Fig. 1. Pig stratum corneum appears to have a greater sulfur content than the human tissue; stretching modes of C–S (around 644 and 620 $cm^{-1}$) and S–S (around 545 and 525 $cm^{-1}$) bonds are relatively more intense in the pig compared with the human tissue. This could possibly be explained by the hirsute nature of pig skin. However, by examining stratum corneum samples we have avoided this potential source of error, as the hair follicles with their hair shafts remain embedded in the dermis when heat-separating the skin as described earlier. It is more likely that these

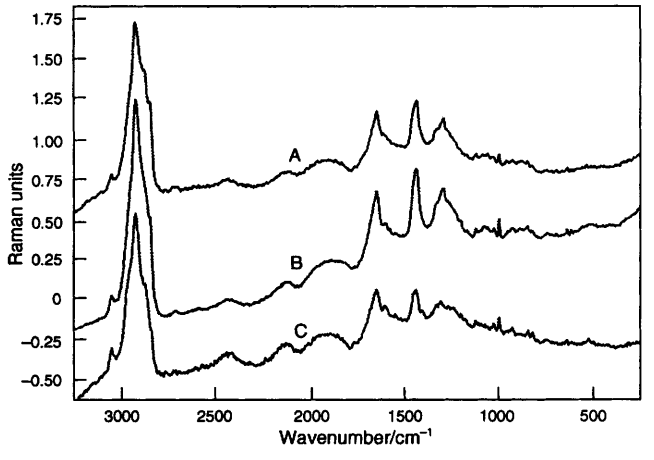


**Fig. 1** FT Raman spectra of A, human stratum corneum, B, pig stratum corneum, and C, snake skin over the wavenumber range 250–3250 $cm^{-1}$. Spectra not corrected for instrument response

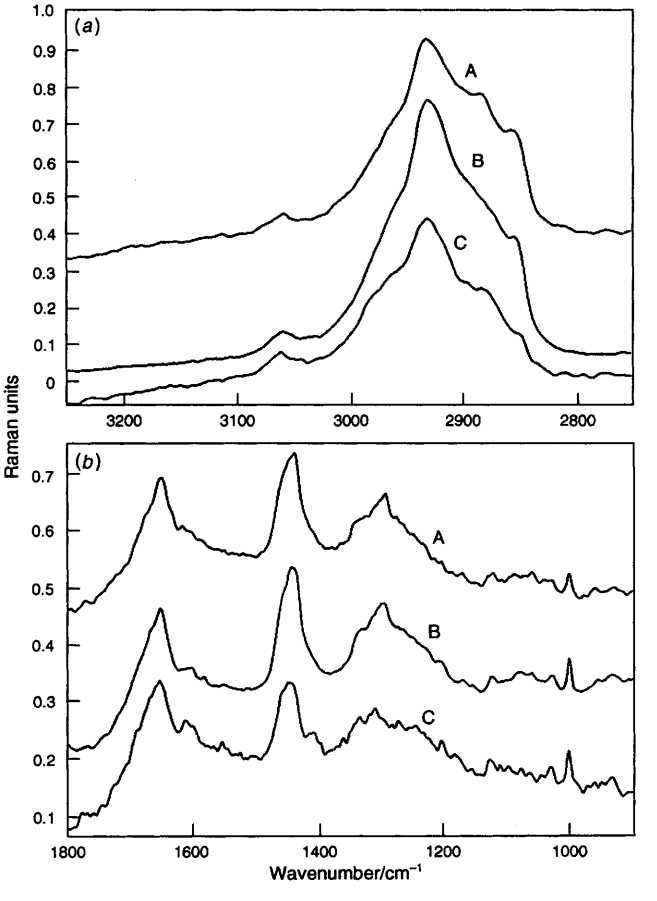


**Fig. 2** Expansion of Fig. 1: (*a*) wavenumber range, 2750–3250 $cm^{-1}$; and (*b*) wavenumber range, 900–1800 $cm^{-1}$. A, Human stratum corneum; B, pig stratum corneum; and C, snake skin

observed differences in the spectra from mammalian tissues arise from dissimilarities in amino acid contents. These sulfur-related stretching vibrations are largely absent in the spectrum of the snake skin, suggesting that these vibrations may result from proteins in the mammalian tissue.

The comparative diffusional resistances of the tissues to therapeutic agents are dependent, in part, on the nature of the permeant. However, the differences described for the structures of human and pig skins appear to be a minor consideration in terms of the tissues' diffusional characteristics; nicorandil [*N*-(2-hydroxyethyl)nicotinamide nitrate] has near identical permeability coefficients in human and pig skin.[4] Also, in an extensive study, Hawkins and Reifenrath[5] have shown that there is a good correlation ($r = 0.79$) between the percutaneous penetration of 11 test compounds in pig and human skin. In contrast, the structural differences seen between snake skin and the mammalian tissues may underlie some diffusional discrepancies. Permeation of a polar drug, 5-fluorouracil ($\log P_{\text{octanol/water}} = -0.89$) is nearly 10 times faster through snake skin than through human skin.[8] This may be partially due to the nature of the lipids in reptilian skin; these appear to be less ordered than the lipids in mammalian tissues. However, the literature on the use of snake skin as a model for human tissue is unclear; water permeation is similar through human and snake skin,[4] phenol and hydroxyprogesterone permeate less well in snake skin compared with that of humans, whereas corticosterone penetrates better in reptilian tissue compared with mammalian skin.[24] The permeation rate of salicylic acid (at pH 4) through snake skin was similar to that of human skin sites—breast, thigh, back, lower leg, foot (not sole)—although only single determinations were performed with sites other than thigh and breast.[25] However, in experiments where chemical treatment was intended to alter the barrier nature of skin samples, the β-keratin layer of snake skin provided a barrier that protected the tissue, making treatment less effective;[4] removal of the β layer with adhesive tape provided a more reliable model system for these studies.

## Conclusion

This study has provided good quality FT Raman spectra of pig, human, and snake stratum corneum that have allowed molecular comparison of the three tissues. Structural differences evident from the spectra may help partially to explain some of the observed differences in the percutaneous absorbance of therapeutic agents. Structurally and diffusionally, pig skin appears to be a good mimic for the human tissue, whereas snake skin may offer a reasonable alternative (for some compounds) that is readily available, but which, with its β-keratin layer, may possess an additional barrier to permeation of, in particular, lipophilic molecules. Clearly, further work is required before the value of pig or snake skin as models for human tissue in transdermal drug delivery studies can be fully assessed.

## References


1 Michaels, A. S., Chanderasekaran, S. K., and Shaw, J. E., *AIChEJ.*, 1975, **21**, 985.
2 Williams, A. C., and Barry, B. W., *Crit. Rev. Ther. Drug Carrier Syst.*, 1992, **9**, 305.
3 Squier, C. A., Cox, P., and Wertz, P. W., *J. Invest. Dermatol.*, 1991, **96**, 123.
4 Sato, K., Sugibayashi, K., and Morimoto, Y., *J. Pharm. Sci.*, 1991, **80**, 104.
5 Hawkins, G. S., and Reifenrath, W. G., *J. Pharm. Sci.*, 1986, **75**, 378.
6 Banerjee, T. K., and Mittal, A. K., *J. Zool. (London)*, 1978, **185**, 415.
7 Roth, S. I., and Jones, W. A., *J. Ultrastruct. Res.*, 1970, **32**, 69.
8 Rigg, P. C., and Barry, B. W., *J. Invest. Dermatol.*, 1990, **94**, 235.
9 Fleeker, C., Wong, O., and Rytting, J. H., *Pharm. Res.*, 1989, **6**, 443.
10 Wong, O., Huntington, J., Nishihata, T., and Rytting, J. H., *Pharm. Res.*, 1989, **6**, 286.
11 Ogiso, T., and Shintani, M., *J. Pharm. Sci.*, 1990, **79**, 1065.


**Table 1** Vibrational wavenumbers and assignments for human stratum corneum, pig stratum corneum, and shed skin of the American black rat snake*

| Wavenumber/cm$^{-1}$ | | | Approximate description of vibrational mode | Wavenumber/cm$^{-1}$ | | | Approximate description of vibrational mode |
|---|---|---|---|---|---|---|---|
| Human | Pig | Snake | | Human | Pig | Snake | |
| 3060w | 3059w | 3060w | ν (CH) olefinic | 1244w, sh | — | 1240mw | ν (CN) amide III β sheet/disordered; δ ($CH_2$) wagging |
| 3000vw, sh | — | 3002w | ν (CH) aromatic | | | | |
| — | — | 2975mw, sh | ν ($CH_3$) asymmetric | — | 1208mw | — | ν (CC) ring |
| 2958m, sh | 2957m, sh | 2956mw, sh | ν ($CH_3$) asymmetric | 1172w | 1172w | 1170w | ν (CC) |
| 2931s | 2931s | 2933s | ν ($CH_3$) asymmetric | 1155w | 1156w | 1151w | ν (CC); δ (COH) |
| 2883ms | 2883ms | 2882m | ν ($CH_2$) asymmetric | 1126mw | 1126mw | 1124mw | ν (CC) skeletal, *trans* conformation |
| 2852m | 2852m | 2852mw, sh | ν ($CH_2$) asymmetric | 1082mw | 1084mw | 1085w | ν (CC) skeletal, random conformation |
| 2723w | 2719w | 2720vw | ν (CH) aliphatic | | | | |
| 1768vw | 1769vw | — | ν (COO) | 1062mw | 1062mw | 1055vw | ν (CC) skeletal, *trans* conformation |
| 1743vw | 1742vw | — | ν (C=O) lipid | 1031mw | 1030mw | 1031w | ν (CC) skeletal, *cis* conformation |
| — | 1670vw, sh | 1672mw, sh | ν (C=O) amide I β sheet | 1002m | 1002m | 1004w | ν (CC) aromatic ring |
| | | | | 956w | 957w | — | ρ ($CH_3$); δ (CH) olefinic |
| 1652s | 1652s | 1656ms | ν (C=O) amide I α helix | 931w, br | 934w, br | 937mw, br | ρ ($CH_3$) terminal; ν (CC) α helix |
| | | | | 883mw | 891mw | 870w, br | ρ ($CH_2$) |
| — | — | 1614mw | ν (C=C) olefinic | 850w | 850w | — | δ (CCH) aromatic |
| 1585w | 1584w | — | ν (C=C) olefinic | 827w | 828w | — | δ (CCH) aliphatic |
| 1552w | 1554w | 1540mw | ν (CN) and δ (NH) amide II | 746w, br | 743w | 750w | ρ ($CH_2$) in-phase |
| — | — | 1473w, sh | δ ($CH_2$) | 644w | 643mw | — | ν (CS); amide IV |
| 1438s | 1443s, br | 1452m | δ ($CH_2$) scissoring | 623w | 620mw | — | ν (CS) |
| 1421w, sh | — | 1417w | δ ($CH_3$) | 600w, br | — | — | ρ (CH) wagging |
| 1385vw | 1388w | 1394w | δ ($CH_3$) symmetric | — | 545w | — | ν (SS) |
| — | 1336m | — | δ (CH) | 526mw, br | 524m | 491vw, vbr | ν (SS) |
| 1296ms | 1299ms | 1294mw | δ C($CH_2$) twisting | 424w, br | 417w | — | δ (CCC) skeletal backbone |
| 1274mw | 1276m, sh | — | ν (CN) and δ (NH) amide III α helix | | | | |
| — | 1267w, sh | 1267w | δ ($CH_2$) wagging | | | | |

* v = very; s = strong; m = medium; w = weak; sh = shoulder; br = broad, ν = stretching; δ = deformation; and ρ = rocking.

12 Itoh, T., Magavi, R., Casady, R. L., Nishihata, T., and Rytting, J. H., *Pharm. Res.*, 1990, **7**, 1302.
13 Bhattachar, S. N., Rytting, J. H., Itoh, T., and Nishihata, T., *Int. J. Pharm.*, 1992, **79**, 263.
14 Golden, G. M., Guzek, D. B., Harris, R. R., McKie, J. E., and Potts, R. O., *J. Invest. Dermatol.*, 1986, **86**, 255.
15 Potts, R. O., *J. Soc. Cosmet. Chem.*, 1986, **37**, 9.
16 Golden, G. M., McKie, J. E., and Potts, R. O., *J. Pharm. Sci.*, 1987, **76**, 25.
17 Williams, A. C., Edwards, H. G. M., and Barry, B. W., *Int. J. Pharm.*, 1992, **81**, R11.
18 Barry, B. W., Edwards, H. G. M., and Williams, A. C., *J. Raman Spectrosc.*, 1992, **23**, 641.
19 Barry, B. W., Williams, A. C., and Edwards, H. G. M., *Spectrochim. Acta, Part A*, 1993, **49**, 801.
20 Edwards, H. G. M., Farwell, D. W., Williams, A. C., and Barry, B. W., *Spectrochim. Acta, Part A*, 1993, **49**, 913.
21 Harrison, S. M., Barry, B. W., and Dugard, P. H., *J. Pharm. Pharmacol.*, 1984, **36**, 261.
22 Kligman, A. M., and Christophers, E., *Arch. Dermatol.*, 1963, **88**, 702.
23 Williams, A. C., Barry, B. W., Edwards, H. G. M., and Farwell, D. W., *Pharm. Res.*, 1993, **10**, 1642.
24 Itoh, T., Xia, J., Magavi, R., Nishihata, G., and Rytting, J. H., *Pharm. Res.*, 1990, **7**, 1042.
25 Harada, K., Murakami, T., Kawasaki, E., Higashi, Y., Yamamoto, S., and Yata, N., *J. Pharm. Pharmacol.*, 1993, **45**, 414.

*Paper 3/04103D*
*Received July 13, 1993*
*Accepted September 9, 1993*