# The effects of esterified solvents on the diffusion of a model compound across human skin; an ATR-FTIR spectroscopic study

W.J. McAuley[1,3], S. Chavda-Sitaram[1], K.T. Mader[2], J.Tetteh[2], M.E. Lane[1] and J. Hadgraft[1]

[1]School of Pharmacy, University College London

[2]Medway Sciences, University of Greenwich

[3]Current address School of Life and Medical Sciences, University of Hertfordshire

**Corresponding author:** William J. McAuley

School of Life and Medical Sciences, University of Hertfordshire

Hatfield

HERTS, AL10 9AB

UK

Email: w.j.mcauley@herts.ac.uk

Tel: +44 (0) 1707 281052

**Abstract**

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy has been used to investigate the effects of three fatty acid esters on skin permeation. Propylene glycol diperlargonate (DPPG), isopropyl myristate (IPM) and isostearyl isostearate (ISIS) were selected as pharmaceutically relevant solvents with a range of lipophilicities and cyanophenol (CNP) was used as a model drug. The resultant data were compared with that obtained when water was used as the solvent. The diffusion of CNP, DPPG and IPM across epidermis were successfully described by a Fickian model. When ISIS was used as a solvent Fickian behaviour was only obtained across isolated stratum corneum suggesting that the hydrophilic layers of the epidermis interfere with the permeation of the hydrophobic ISIS. The diffusion coefficients of CNP across epidermis in the different solvents were not significantly different. Using chemometric data analysis diffusion profiles for the solvents were deconvoluted from that of the skin and modelled. Each of these solvents was found to diffuse at a faster rate across the skin than CNP. DPPG considerably increased the concentration of CNP in the stratum corneum in comparison with the other solvents indicating strong penetration enhancer potential. In contrast IPM produced a similar CNP concentration in the stratum corneum to water with ISIS resulting in a lower CNP concentration suggesting negligible enhancement and penetration retardation effects for these two solvents respectively.

**Keywords**

Penetration enhancement, Human skin, Diffusion, ATR-FTIR spectroscopy

## Introduction

The stratum corneum, the outermost layer of skin is the main barrier to the absorption of drug molecules across and into human skin. This barrier has important implications for the topical treatment of skin conditions as it may render a particular treatment ineffective. Modification of the barrier properties of the stratum corneum is often sought primarily to increase drug penetration and the use of chemical penetration enhancers in a formulation is perhaps the most common strategy used to achieve this. A variety of different types of molecules have been shown to have the potential to affect drug permeation including alcohols, esters and fatty acids (Gorukanti et al., 1999; Liu et al., 2009; Ogiso et al., 1995). These molecules modify the properties of the stratum corneum altering drug flux across the skin. However it is difficult to elucidate the mechanisms through which they exert their actions. Improved knowledge of how individual enhancers modify skin penetration would facilitate formulation design and perhaps also give insight into how combinations of enhancers can have synergistic effects greatly increasing skin penetration (Goldbergcettina et al., 1995), allowing this effect to be utilised in formulations.

Fatty acid esters have been commonly used as penetration enhancers, with IPM being the most commonly used (Goldbergcettina et al., 1995; Gorukanti et al., 1999; Kikwai et al., 2002; Leichtnam et al., 2006; Liu et al., 2006; Yamato et al., 2009). Other examples of such molecules which have been investigated for this effect include ethyl oleate, decyl oleate, propylene glycol laurate, propylene glycol monolaurate, propylene glycol monocaprylate/caprate, glyceryl monocaprylate/caprate and ISIS (Cornwell et al., 1998; Gwak and Chun, 2002; Kikwai et al., 2002; Liu et al., 2006; Ozawa et al., 1988; Takahashi et al., 1996).

These molecules exhibit considerable variation in their physiochemical properties, differing in hydrocarbon chain length, number of hydrocarbon chains and polarity. The effect of these molecules as chemical penetration modifiers of drug transport is however variable. For example IPM as a sole agent may increase drug permeation significantly (Gorukanti et al., 1999) but the effect may be modest in other cases and some of the other esters such as propylene glycol laurate and glyceryl monocaprylate/caprate have been demonstrated to be superior to IPM for particular drug molecules (Cornwell et al., 1998; Gwak and Chun, 2002). Also in combination with other enhancers these esterified solvents have shown a synergistic penetration enhancement effect, though this is not always the case (Alberti et al., 2001; Gorukanti et al., 1999; Gwak and Chun, 2002; Liu et al., 2009). Better insight into the mechanism of action of these types of enhancers would facilitate their selection and help rationalise the topical formulation development process.

Diffusion across a membrane may be followed using ATR-FTIR spectroscopy which potentially offers improved mechanistic insight into the role of penetration enhancers on drug diffusion than can be gained with conventional *in vitro* diffusion experiments such as those performed using Franz cells. The technique makes it easier to separate the effect of the enhancer on the concentration and diffusion coefficient of the drug in the stratum corneum and can also give molecular insight into the mechanism of action. For example Harrison et al have correlated the increase in the diffusion coefficient of a model compound, cyanophenol across the stratum corneum with increased fluidity of the intercellular lipids (Harrison et al., 1996). The technique also allows the diffusion profile of the enhancer to be monitored and can give insight into drug transport mechanisms. Tantishaiyakul et al used ATR-FTIR spectroscopy

to follow the diffusion of ion pairs and McAuley et al have reported the diffusion of hydrogen bonded species across model membranes (McAuley et al., 2009; Tantishaiyakul et al., 2004).

In this study the effect of three fatty acid ester solvents on the transport of a model drug CNP across human epidermis have been investigated using ATR-FTIR spectroscopy. The esters examined, IPM, ISIS and DPPG are all used in topical formulations and have a range of polarities and hence should provide insight into the effects of these types of enhancers on drug transport across human skin.

## Materials

CNP was obtained from Fisher Scientific (Loughborough, UK). IPM was obtained from Sigma-Aldrich (Poole, UK). ISIS was received as a gift from Uniqema (Gouda, The Netherlands) and DPPG was received as a gift from Gattefosse (Saint-Priest, France).

## Methods

### Tissue preparation

Dermatomed human thigh skin from a single female patient was obtained from the International Institute for the Advancement of Medicine. Separated epidermis was prepared by blunt dissection following heat separation. The dermatomed skin was defrosted for two hours at room temperature before being immersed in a water bath at 60°C for 1 minute. The tissue was then pinned to a cork board and the epidermis was peeled away from the dermis using tweezers. Isolated stratum corneum was

prepared by soaking the epidermis in phosphate buffered saline containing 0.0001% trypsin for 24 hours at 37°C (Pellett et al., 1997b). The stratum corneum was then rinsed thoroughly with deionised water. All skin tissue was stored frozen at -20°C and allowed to thaw at room temperature prior to use.

ATR-FTIR spectroscopy studies

Diffusion experiments were conducted at ambient temperature (21 ± 2 ºC) using a Nicolet Avatar 360 FTIR spectrometer fitted with a multibounce ATR accessory with a Zinc Selenide (ZnSe) crystal. The incident angle of the IR radiation was 45°. Ten scans were taken every 60 seconds with resolution of 2 $cm^{-1}$ and an average spectrum was produced at each time point. All experiments were repeated in triplicate. Spectral analysis was performed using Opus® 5.5 software.

Epidermis was placed on the ZnSe crystal so that the stratum corneum was in direct contact with the crystal. Intimate contact between the epidermis and crystal was assessed visually. This type of experimental set up using epidermis for ATR-FTIR spectroscopic studies of diffusion has been described previously (Tetteh et al., 2009). An aluminium trough constructed specifically for the diffusion experiments was placed on top of the epidermis and was sealed with silicone grease. A saturated CNP solution was applied to the membrane and an aluminium lid was placed on top, again sealed with silicone grease.

When isolated stratum corneum was used it was placed on the ZnSe crystal so that the outer surface was in direct contact with the crystal, thus the stratum corneum

was placed on the ATR crystal in the same orientation as in the epidermis experiment.

Saturated solutions of CNP in the three esters were prepared by constant agitation of excess of CNP in the solvent in the presence of an immiscible water layer for 48 hours. The aqueous layer was then removed prior to the solution being used. The CNP solutions were equilibrated with water to prevent them from dehydrating the skin tissue and causing it to curl off the ATR crystal. The saturated solution of CNP in water was prepared by constant agitation of an excess of CNP in water for 48 hours.

Data modelling

Assuming that the Beer-Lambert law applies, the increase in IR absorbance associated with either the drug or solvent molecule with time is directly related to the concentration of the species in the membrane. The increase in absorbance can therefore be modelled by fitting appropriate boundary conditions to Fick's second law,

$$\frac{\partial C}{\partial t} = -D\frac{\partial^2 C}{\partial x^2} \qquad \text{Equation 1}$$

where C is the concentration of the diffusing species in the membrane, D is the diffusion coefficient of the diffusing species, x is the diffusional pathlength, and t is time. In this study the method of Laplace transformation was used to solve Equation 1 to obtain diffusion coefficients for the permeating molecules across skin. This

approach has been reported previously (McAuley et al., 2010). The diffusion coefficients were obtained by fitting the normalised data with Micromath Scientist® 3.0 for Windows, using the following Laplace transformation;

$$\frac{A}{A_\infty} = \frac{\bar{C}}{\bar{C}_\infty} = \frac{\cosh\left(h \cdot \sqrt{s/D}\right)}{s} \qquad \text{Equation 2}$$

where $A$ is the absorbance, $A_\infty$ is the absorbance at infinite time, $\bar{C}$ is the concentration in the Laplace domain, $\bar{C}_\infty$ is the concentration in the Laplace domain at infinite time, s is the Laplace variable and h is the diffusional pathlength. Statistical analyses were made using Graphpad Prism 5 software. Comparisons of the calculated diffusion coefficients were made using the Kruskal Wallis test with post hoc comparison made using the Mann-Whitney U test. Significance was accepted at the $p \leq 0.05$ level.

Solubility studies

The solubilities of CNP at 21 ± 2°C in water, IPM, DPPG and ISIS were measured by UV absorbance at 249nm. Saturated solutions of the model drugs were prepared using constant agitation for 48 hours. An aliquot of this was then centrifuged to separate any solid material and the supernatant was sampled, diluted as necessary and analysed.

Chemometric data analysis

Chemometric data analysis was used to obtain the diffusion profiles of the solvents. The multivariate target factor analysis approach was used to deconvolute the solvent profile from that of the skin and CNP. The analysis was conducted using InSight (InSight 4.0a, 2009) software coded in Matlab® (Matlab, R2009a). The algorithms and methods used by this programme are described in more detail elsewhere (Dias et al., 2004).

**Results**

Figure 1 shows IR spectra taken at different time points following application of CNP in DPPG to human epidermis. The increase in the CN stretch of CNP at 2227 $cm^{-1}$ can clearly be seen as can that associated with the carbonyl band of DPPG at 1742 $cm^{-1}$. The Amide I and II bands of the stratum corneum are also identifiable confirming good contact between the epidermis and crystal throughout the experiment.

The CN stretching absorption band of CNP occurs in a spectrally silent skin region that also does not overlap with absorption bands associated with the solvents used in this study. Assuming the Beer-Lambert law applies the absorbance of this band can be directly related to the concentration of CNP in the stratum corneum and through monitoring the change in absorbance with time a diffusion profile of CNP across the membrane can be obtained. Figure 2 shows diffusion profiles of CNP in the different solvents used in this study across human epidermis.

Typical Fickian plots were observed for the diffusion of CNP in DPPG, water and IPM across epidermis with the expected lag, exponential and plateau phases (Pellett

et al., 1997a). The diffusion traces of CNP in IPM and water are similar whereas the plateau absorbance of CNP in DPPG is much greater, indicating that a higher concentration of CNP is obtained in the stratum corneum when DPPG is the vehicle. The CNP in ISIS diffusion experiment again shows the typical lag and exponential diffusion phases, however the plateau absorbance appears to slowly decrease rather than maintain an equilibrium value. This slowly decreasing plateau was observed to correspond to the permeation of ISIS into the membrane. Figure 3 shows two spectra taken from the CNP in ISIS diffusion experiment and shows a decrease in the magnitude in the CN stretch, indicating a lower CNP concentration in the stratum corneum with increasing ISIS permeation into the membrane as observed through a stronger carbonyl absorption which is associated with the solvent.

From the ATR-FTIR spectra the diffusion profiles of the different solvents used in the study can be obtained. The solvents, IPM, ISIS and DPPG all have an ester carbonyl group which absorbs in a region where the absorption of skin components is relatively weak (see Figure 1). However this functional group is often involved in hydrogen bonding, which alters the frequency of absorption and may affect the absorption coefficient limiting the usefulness of this band for diffusion profile analysis. Indeed CNP is known to hydrogen bond to these types of solvents (McAuley et al., 2009). Instead a chemometric approach has been used to deconvolute the spectral signal of the solvent from that of the other components in the 1478-1350cm$^{-1}$ wavenumber window which is not believed to change with interactions between the molecules. Figure 4 shows the separation of the spectral profiles associated with DPPG and the stratum corneum extracted by the InSight

software and the corresponding evolution of each signal with time. Typical Fickian profiles were observed for the three solvents but ISIS had a significantly longer lag time than either DPPG or IPM. These diffusion profiles for CNP, IPM and DPPG could be modelled using Scientist to obtain a diffusion coefficient for the permeating species. Figure 5 shows example normalised diffusion profiles and model fittings for CNP and IPM across epidermis following application of a saturated solution of CNP in IPM. The profiles are normalised by setting the plateau value in each case ($C_\infty$) to 1 enabling presentation on the same scale. The calculated diffusion coefficients are given in Table 1. These are reported as pathlength normalised values ($D/h^2$) as the diffusional pathlength across the stratum corneum is unknown. Table 1 also provides the equilibrium solubility results for CNP in each of the solvents and the plateau absorbance values for each of the diffusion experiments. Model fitting of the ISIS diffusion profile obtained using epidermal tissue was less successful however as the lag time was observed to be overly long in comparison with the exponential phase. To investigate whether the more hydrophilic layers of epidermis were affecting the permeation of ISIS into the stratum corneum the CNP in ISIS experiment was repeated using stratum corneum prepared from the same skin tissue. Using isolated stratum corneum typical Fickian diffusion plots were observed for both CNP and ISIS allowing diffusion coefficients to be calculated, again shown in Table 1. For comparative purposes the diffusion of CNP across epidermis and stratum corneum from an ISIS vehicle are shown in Figure 6. The experiment across stratum corneum has a lower plateau absorbance than that obtained across epidermis but remains constant as is expected if the diffusion follows Fick's law rather than the slowly decreasing value obtained in the CNP across epidermis experiment.

The ingress of ISIS into the stratum corneum appears to lower the concentration of CNP in the stratum corneum to less than that produced when water is the vehicle. In this case ISIS would be expected to act as a penetration retarder. This data suggests that the hydrophilic layers in the epidermis affect the ingress of the hydrophobic ISIS into the stratum corneum in the epidermal tissue experimental design and that for excipients such as ISIS, use of stratum corneum is preferable.

Table 1. The solubility of CNP in each solvent, the plateau absorbance of CNP in the stratum corneum and the calculated diffusion coefficient for CNP (mean values ± the range, n=3). ND not determined.

| **Solvent** | **CNP solubility ($mol/dm^3$)** | **Plateau absorbance** | **CNP $D/h^2$ ($x\ 10^{-5}\ s^{-1}$)** | **Solvent $D/h^2$ ($x\ 10^{-5}\ s^{-1}$)** |
|---|---|---|---|---|
| Dipropylene glycol perlargonate | 1.185 | 6.31 (6.09-6.55) | 9.4 (9.0-9.9) | 17.7 (11.5-27.2) |
| Isopropyl myristate | 0.681 | 3.51 (2.86 4.00) | 7.3 (6.9-7.8) | 21.1 (9.0-27.2) |
| Isostearyl isostearate | 0.387 | 1.93 (1.55 – 2.53) | 13.8 (11.7-15.8) | 28.8 (27.2-30.4) |
| Water | 0.123 | 3.26 (2.85 - 3.82) | 10.3 ( 9.4 – 11.2) | ND |

## Discussion

Since the initial publications using ATR-FTIR spectroscopy to investigate drug diffusion across skin in the mid 1990s, only a few studies have been published using skin tissue. This is likely to be a result of difficulties in performing the experiment using stratum corneum. Mounting the stratum corneum onto the ATR crystal is

difficult as the tissue is fragile and is readily damaged or torn. In this study we have reported using human epidermis instead of isolated stratum corneum for ATR-FTIR spectroscopic studies across skin. The skin is positioned such that the stratum corneum is in direct contact with the ATR crystal. This ensures that the data obtained from the experiment relates to the concentration of the permeants in the stratum corneum. Using epidermal tissue allows reliable, reproducible contact to be made between the membrane and the ATR crystal. Such an experiment design studying diffusion across human epidermis has been reported using an ATR-FTIR spectroscopic imaging setup (Tetteh et al., 2009). Pellet et al have previously investigated the effect of stratum corneum orientation on the ATR crystal with permeability and did find some differences depending on whether the inner or outer surface of the stratum corneum was placed on the ATR crystal (Pellett et al., 1997b). However it is thought that this is unlikely to affect interpretation of data in a consistent experimental design where relative differences between formulations are being used to gauge the effects of excipients to promote transport across skin.

Typical Fickian diffusion profiles were obtained for the permeation of CNP across the epidermis which allowed it to be modelled, enabling calculation of the diffusion coefficients of CNP in the different vehicles and assessment of the effects of the vehicle on the concentration of CNP in the stratum corneum. The plateau absorbance value obtained during the permeation experiment relates to the concentration of CNP in the membrane. Solvents capable of increasing the concentration of the CNP in the stratum corneum are likely to act as penetration enhancers (Moser et al., 2001). DPPG was found to produce much higher concentrations of CNP in the membrane than the other solvents. This is likely to be a

feature of both the good solubility of CNP in DPPG and the significant uptake of the solvent into the stratum corneum, which was observed through the increase in the absorbance of the solvent's carbonyl band during the experiment. The data indicates that DPPG is likely to act as a strong penetration enhancer for CNP( and presumably other structurally similar molecules) across skin. In contrast CNP in IPM showed a similar plateau absorbance to that of CNP in water, suggesting limited enhancing potential through altering the concentration of CNP in stratum corneum. This result agrees with previous findings which reported the ability of IPM to act as an enhancer as a single solvent for particular drugs to be modest (Kikwai et al., 2002). In the CNP in ISIS experiment using epidermal sheet, ISIS appeared to reduce the concentration of CNP in the slowly, such that the diffusion profile did not come to an equilibrium plateau absorbance over the timescale of the experiment. The slowly decreasing plateau absorbance of the CN stretch was observed to correspond to the permeation of ISIS into the membrane and analysis of the solvent diffusion profile suggested that it was non Fickian indicating that ISIS was behaving differently to the other solvents. To investigate this further the CNP in ISIS experiment was repeated using stratum corneum prepared from the same piece of skin tissue and placed on the ATR crystal in the same orientation as it is in the epidermis experiment. This allowed investigation of whether the inner, more hydrophilic epidermal layers may have impeded the permeation of ISIS into the stratum corneum, preventing the effects of the solvent on CNP diffusion into the skin from being readily apparent. The resultant data produced typical Fickian diffusion plots for the CNP diffusion across stratum corneum, with an equilibrium plateau absorbance lower than that obtained when water is the solvent, indicating that ISIS lowers the concentration of CNP in the stratum corneum. The data indicate that whilst use of human epidermis appears to

be valid for the use of DPPG and IPM with the more hydrophobic solvents such as ISIS the presence of the more hydrophilic epidermal layers will affect the observed diffusion profiles of permeating species and that in these circumstances, stratum corneum should be used. That ISIS lowers the concentration of CNP in the stratum corneum suggests that it acts as a penetration retarder. This is despite the solubility of CNP in ISIS being approximately three times larger than that of CNP in water and significant absorption of ISIS into the stratum corneum. This may be a result of the hydrophobic ISIS providing a less favourable environment than the endogenous lipids of the stratum corneum. There are cases where retardation of skin permeation is beneficial, typically when a drug or active is being targeted to the skin surface. Examples can include sunscreens and topical anti-microbials where a penetration retarder may prevent systemic exposure to the drug and thereby reduce toxicity (Hadgraft et al., 1996). ISIS may be a useful excipient for such formulations.

The diffusion coefficients of CNP in DPPG, IPM and water across epidermis were similar. CNP in ISIS across stratum corneum did diffuse faster than those systems which were tested across epidermis as would be expected given that the lower epidermal layers will provide some diffusional resistance. However this difference is small which is consistent with the stratum corneum being the main barrier to the ingress of substances across skin (Hadgraft and Lane, 2011). The calculated diffusion coefficients are of a similar magnitude to those calculated by Pellett et al for the diffusion of CNP in water across stratum corneum (Pellett et al., 1997b). Thus the data suggest that the solvents tested do not enhance skin permeation by decreasing the membranes diffusional resistance, though it may be possible that pre-treatment of the skin with a solvent is necessary to fully elucidate the effects on

the diffusion coefficient in this type of experimental set up. Chemometric analysis software (InSight) was used to extract diffusion profiles of the ester solvents across the skin tissue. A spectral region was chosen where issues such as hydrogen bonding between the CNP and the solvent would not interfere with the observed diffusion profile. The diffusion profiles of DPPG and IPM across epidermis and ISIS across stratum corneum were obtained and could be modelled to obtain diffusion coefficients. Each of the fatty acid ester solvents was found to diffuse at a faster rate than the CNP. Previous ATR-FTIR spectroscopic diffusion studies across silicone membrane found that CNP diffused more quickly across that membrane than ISIS and that this seemed to correlate well with the increased molecular size of the ISIS molecule (McAuley et al., 2009). This was not observed in this study for diffusion across human tissue but potentially be explained through consideration of the functional groups of the molecules. Drug diffusion across skin has been inversely correlated to the number of hydrogen bonding groups on a molecule, with strong hydrogen bonding groups such as hydroxyl groups slowing diffusion to a greater extent that for example a carbonyl group(Pugh et al., 1996; Roberts et al., 1996). This effect is particularly strong when the number of hydrogen bonding groups on the molecule is small. Thus the presence of both hydroxyl and the hydrogen bond accepting nitrile groups on CNP may explain its slower diffusion across skin in comparison to the esterified solvents.

## Conclusions

ATR-FTIR spectroscopy has been used to study the diffusion of CNP across human epidermis in three separate fatty acid ester solvents and water. The diffusion of CNP in water, DPPG and IPM across human epidermis were modelled using Fick's second law. In the case of CNP in ISIS, Fickian diffusion profiles were only obtained when stratum corneum was used. No significant differences were observed between the diffusion coefficients of CNP in the different vehicles across epidermis. CNP in ISIS diffused more quickly across stratum corneum though this difference was small and is probably linked to the removal of the lower hydrophilic epidermal layers. Using chemometric software, diffusion profiles of the fatty acid ester solvents were extracted and each of the esterified solvents was found to diffuse at a faster rate across skin tissue than the model drug. DPPG was found to be able to increase the concentration of CNP in the stratum corneum suggesting that it possesses strong penetration enhancing potential. IPM appeared to have a limited or negligible ability to enhance CNP permeation whereas, in contrast ISIS is able to lower the CNP concentration obtained in the stratum corneum suggesting that it has a penetration retarding effect.

## References

Alberti, I., Kalia, Y.N., Naik, A., Bonny, J.D., Guy, R.H., 2001. Effect of ethanol and isopropyl myristate on the availability of topical terbinafine in human stratum corneum, in vivo. International Journal of Pharmaceutics 219, 11-19.

Cornwell, P.A., Tubek, J., van Gompel, H., Little, C.J., Wiechers, J.W., 1998. Glyceryl monocaprylate/caprate as a moderate skin penetration enhancer. International Journal of Pharmaceutics 171, 243-255.

Dias, M., Hadgraft, J., Raghavan, S., Tetteh, J., 2004. The effect of solvent on permeant diffusion through membranes studied using ATR-FTIR and chemometric data analysis. Journal of Pharmaceutical Sciences 93, 186-196.

Goldbergcettina, M., Liu, P.C., Nightingale, J., Kuriharabergstrom, T., 1995. Enhanced Transdermal Delivery of Estradiol in-Vitro Using Binary Vehicles of Isopropyl Myristate and Short-Chain Alkanols. International Journal of Pharmaceutics 114, 237-245.

Gorukanti, S.R., Li, L.L., Kim, K.H., 1999. Transdermal delivery of antiparkinsonian agent, benztropine. I. Effect of vehicles on skin permeation. International Journal of Pharmaceutics 192, 159-172.

Gwak, H.S., Chun, I.K., 2002. Effect of vehicles and penetration enhancers on the in vitro percutaneous absorption of tenoxicam through hairless mouse skin. International Journal of Pharmaceutics 236, 57-64.

Hadgraft, J., Lane, M.E., 2011. Skin: the ultimate interface. Physical Chemistry Chemical Physics 13, 5215-5222.

Hadgraft, J., Peck, J., Williams, D.G., Pugh, W.J., Allan, G., 1996. Mechanisms of action of skin penetration enhancers retarders: Azone and analogues. International Journal of Pharmaceutics 141, 17-25.

Harrison, J.E., Watkinson, A.C., Green, D.M., Hadgraft, J., Brain, K., 1996. The relative effect of Azone(R) and Transcutol(R) on permeant diffusivity and solubility in human stratum corneum. Pharmaceutical Research 13, 542-546.

InSight 4.0a User manual 2009, DiKnow Ltd. UK.

445 Kikwai, L., Kanikkannan, N., Babu, R.J., Singh, M., 2002. Effect of vehicles on the
446 transdermal delivery of melatonin across porcine skin in vitro. Journal of Controlled
447 Release 83, 307-311.
448 Leichtnam, M.L., Rolland, H., Wuthrich, P., Guy, R.H., 2006. Identification of
449 penetration enhancers for testosterone transdermal delivery from spray formulations.
450 Journal of Controlled Release 113, 57-62.
451 Liu, H.Z., Li, S.M., Wang, Y.J., Yao, H.M., Zhang, Y., 2006. Effect of vehicles and
452 enhancers on the topical delivery of cyclosporin A. International Journal of
453 Pharmaceutics 311, 182-186.
454 Liu, P., Cettina, M., Wong, J., 2009. Effects of Isopropanol-Isopropyl Myristate Binary
455 Enhancers on In Vitro Transport of Estradiol in Human Epidermis: A Mechanistic
456 Evaluation. Journal of Pharmaceutical Sciences 98, 565-572.
457 McAuley, W.J., Lad, M.D., Mader, K.T., Santos, P., Tetteh, J., Kazarian, S.G.,
458 Hadgraft, J., Lane, M.E., 2010. ATR-FTIR spectroscopy and spectroscopic imaging
459 of solvent and permeant diffusion across model membranes. European Journal of
460 Pharmaceutics and Biopharmaceutics 74, 413-419.
461 Matlab R2009a, The Mathworks, USA.
462 McAuley, W.J., Mader, K.T., Tetteh, J., Lane, M.E., Hadgraft, J., 2009. Simultaneous
463 monitoring of drug and solvent diffusion across a model membrane using ATR-FTIR
464 spectroscopy. European Journal of Pharmaceutical Sciences 38, 378-383.
465 Moser, K., Kriwet, K., Naik, A., Kalia, Y.N., Guy, R.H., 2001. Passive skin penetration
466 enhancement and its quantification in vitro. European Journal of Pharmaceutics and
467 Biopharmaceutics 52, 103-112.
468 Ogiso, T., Iwaki, M., Paku, T., 1995. Effect of Various Enhancers on Transdermal
469 Penetration of Indomethacin and Urea, and Relationship between Penetration

Parameters and Enhancement Factors. Journal of Pharmaceutical Sciences 84, 482-488.

Ozawa, Y., Yamahira, T., Sunada, H., Nadai, T., 1988. Influence of Fatty-Acid Alcohol Esters on Percutaneous-Absorption of Hydrocortisone Butyrate Propionate. Chemical & Pharmaceutical Bulletin 36, 2145-2151.

Pellett, M.A., Watkinson, A.C., Hadgraft, J., Brain, K.R., 1997a. Comparison of permeability data from traditional diffusion cells and ATR-FTIR spectroscopy .1. Synthetic membranes. International Journal of Pharmaceutics 154, 205-215.

Pellett, M.A., Watkinson, A.C., Hadgraft, J., Brain, K.R., 1997b. Comparison of permeability data from traditional diffusion cells and ATR-FTIR spectroscopy .2. Determination of diffusional pathlengths in synthetic membranes and human stratum corneum. International Journal of Pharmaceutics 154, 217-227.

Pugh, W.J., Roberts, M.S., Hadgraft, J., 1996. Epidermal permeability - Penetrant structure relationships .3. The effect of hydrogen bonding interactions and molecular size on diffusion across the stratum corneum. International Journal of Pharmaceutics 138, 149-165.

Roberts, M.S., Pugh, W.J., Hadgraft, J., 1996. Epidermal permeability: Penetrant structure relationships .2. The effect of H-bonding groups in penetrants on their diffusion through the stratum corneum. International Journal of Pharmaceutics 132, 23-32.

Takahashi, K., Matsumoto, T., Kimura, T., Sakano, H., Mizuno, N., Yata, N., 1996. Effect of polyol fatty acid esters on diclofenac permeation through rat skin. Biological & Pharmaceutical Bulletin 19, 893-896.

Tantishaiyakul, V., Phadoongsombut, N., Wongpuwarak, W., Thungtiwachgul, J., Faroongsarng, D., Wiwattanawongsa, K., Rojanasakul, Y., 2004. ATR-FTIR

495 characterization of transport properties of benzoic acid ion-pairs in silicone
496 membranes. International Journal of Pharmaceutics 283, 111-116.
497 Tetteh, J., Mader, K.T., Andanson, J.M., McAuley, W.J., Lane, M.E., Hadgraft, J.,
498 Kazarian, S.G., Mitchell, J.C., 2009. Local examination of skin diffusion using FTIR
499 spectroscopic imaging and multivariate target factor analysis. Analytica Chimica Acta
500 642, 246-256.
501 Yamato, K., Takahashi, Y., Akiyama, H., Tsuji, K., Onishi, H., Machida, Y., 2009.
502 Effect of Penetration Enhancers on Transdermal Delivery of Propofol. Biological &
503 Pharmaceutical Bulletin 32, 677-683.
504
505

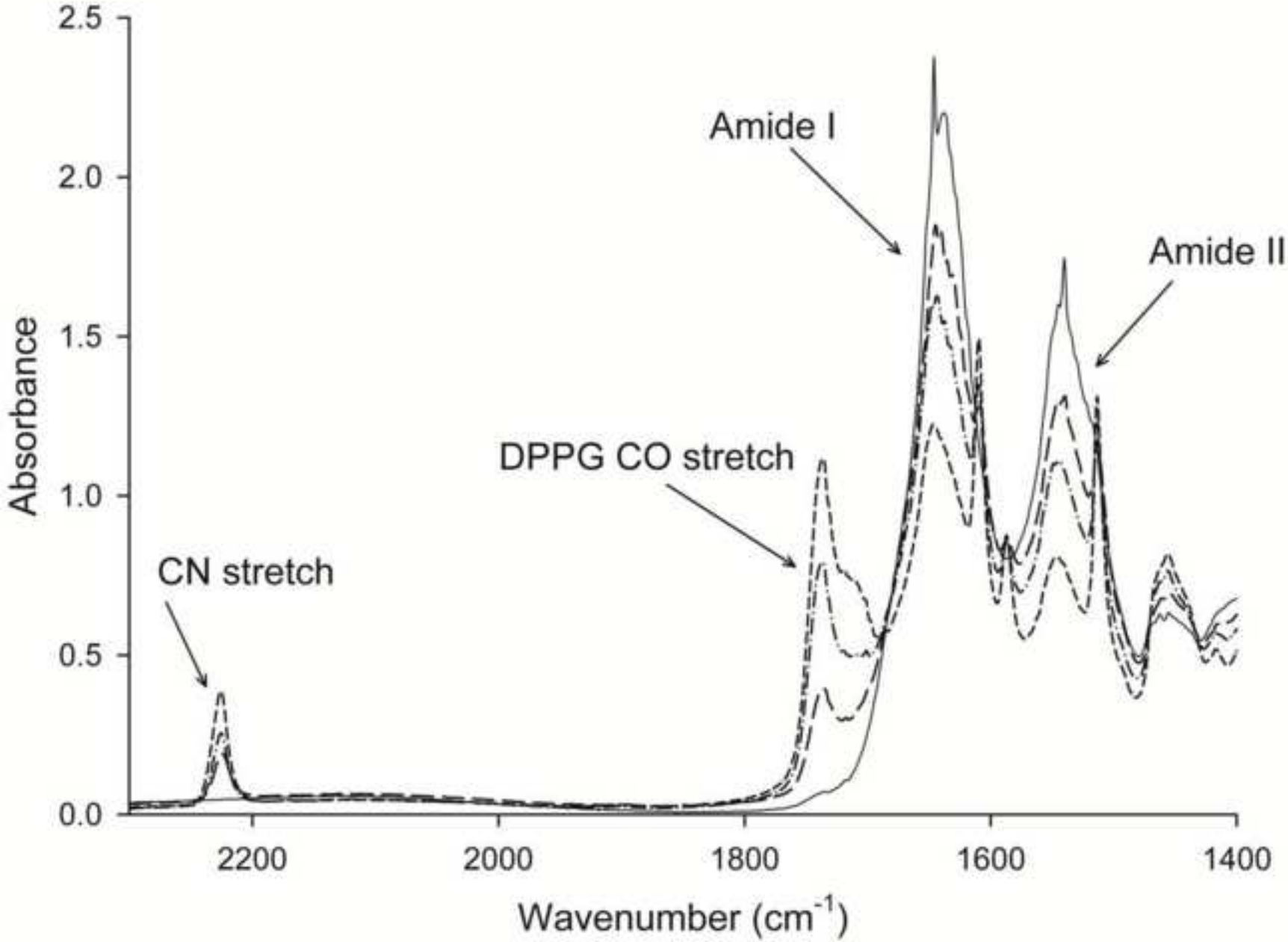
**Table(s)**

Table 1. The solubility of CNP in each solvent, the plateau absorbance of CNP in the stratum corneum and the calculated diffusion coefficient for CNP (mean values ± the range, n=3). ND not determined.

| **Solvent** | **CNP solubility ($mol/dm^3$)** | **Plateau absorbance** | **CNP $D/h^2$ ($x\ 10^{-5}\ s^{-1}$)** | **Solvent $D/h^2$ ($x\ 10^{-5}\ s^{-1}$)** |
|---|---|---|---|---|
| Dipropylene glycol perlargonate | 1.185 | 6.31 (6.09-6.55) | 9.4 (9.0-9.9) | 17.7 (11.5-27.2) |
| Isopropyl myristate | 0.681 | 3.51 (2.86 4.00) | 7.3 (6.9-7.8) | 21.1 (9.0-27.2) |
| Isostearyl isostearate | 0.387 | 1.93 (1.55 – 2.53) | 13.8 (11.7-15.8) | 28.8 (27.2-30.4) |
| Water | 0.123 | 3.26 (2.85 - 3.82) | 10.3 ( 9.4 – 11.2) | ND |

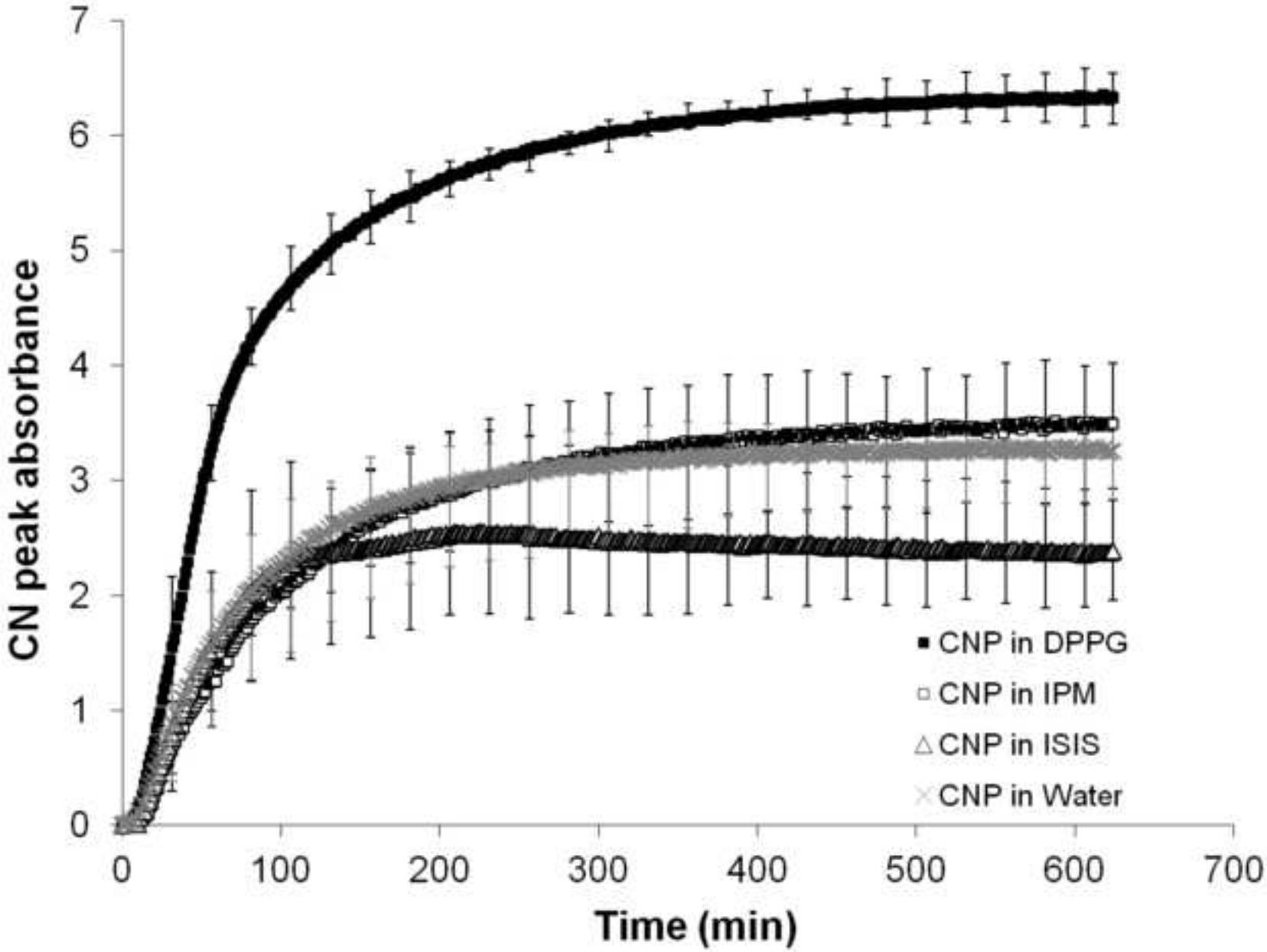
Figure(s)

Click here to download high resolution image



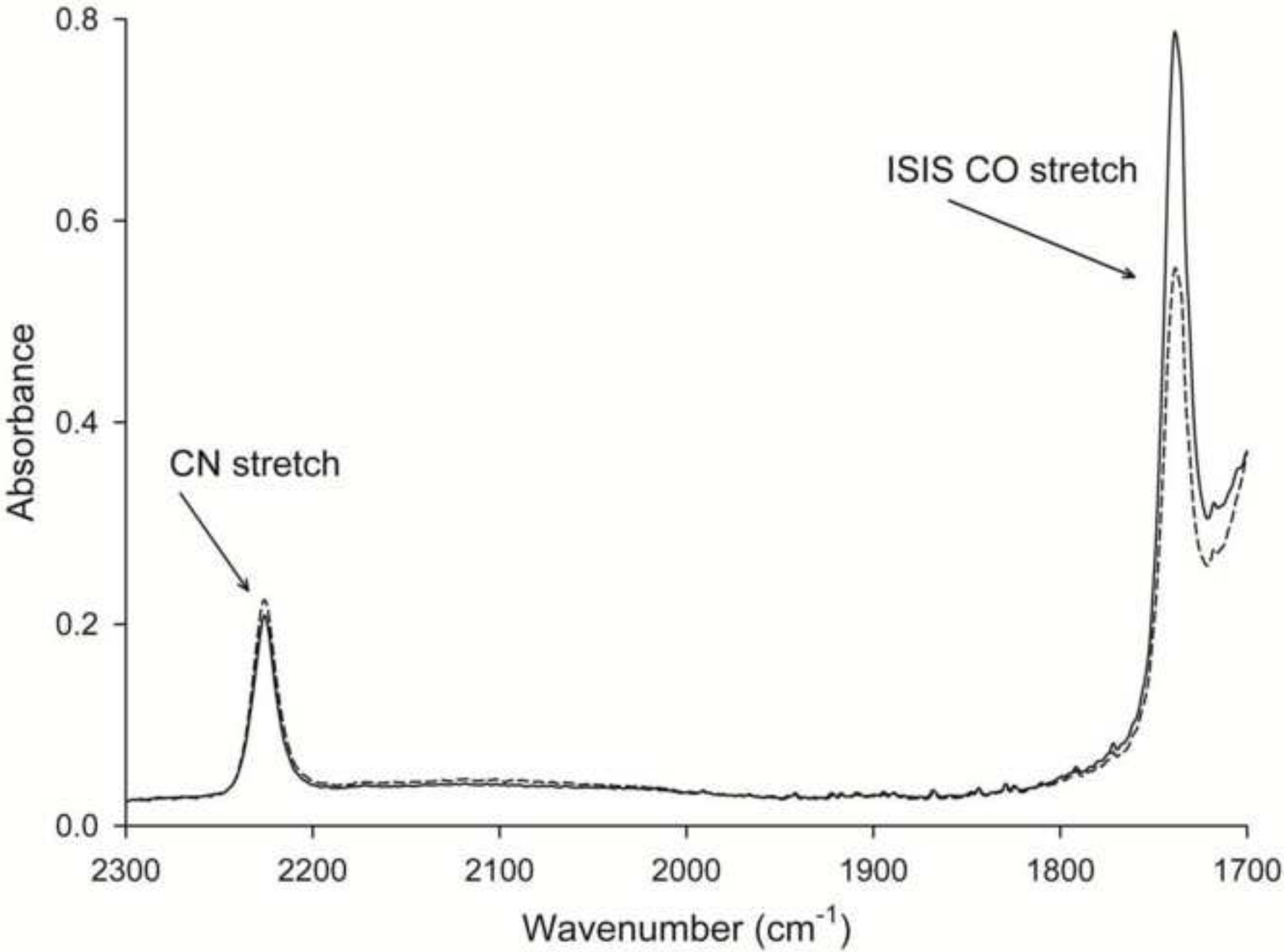
Figure(s)

Click here to download high resolution image



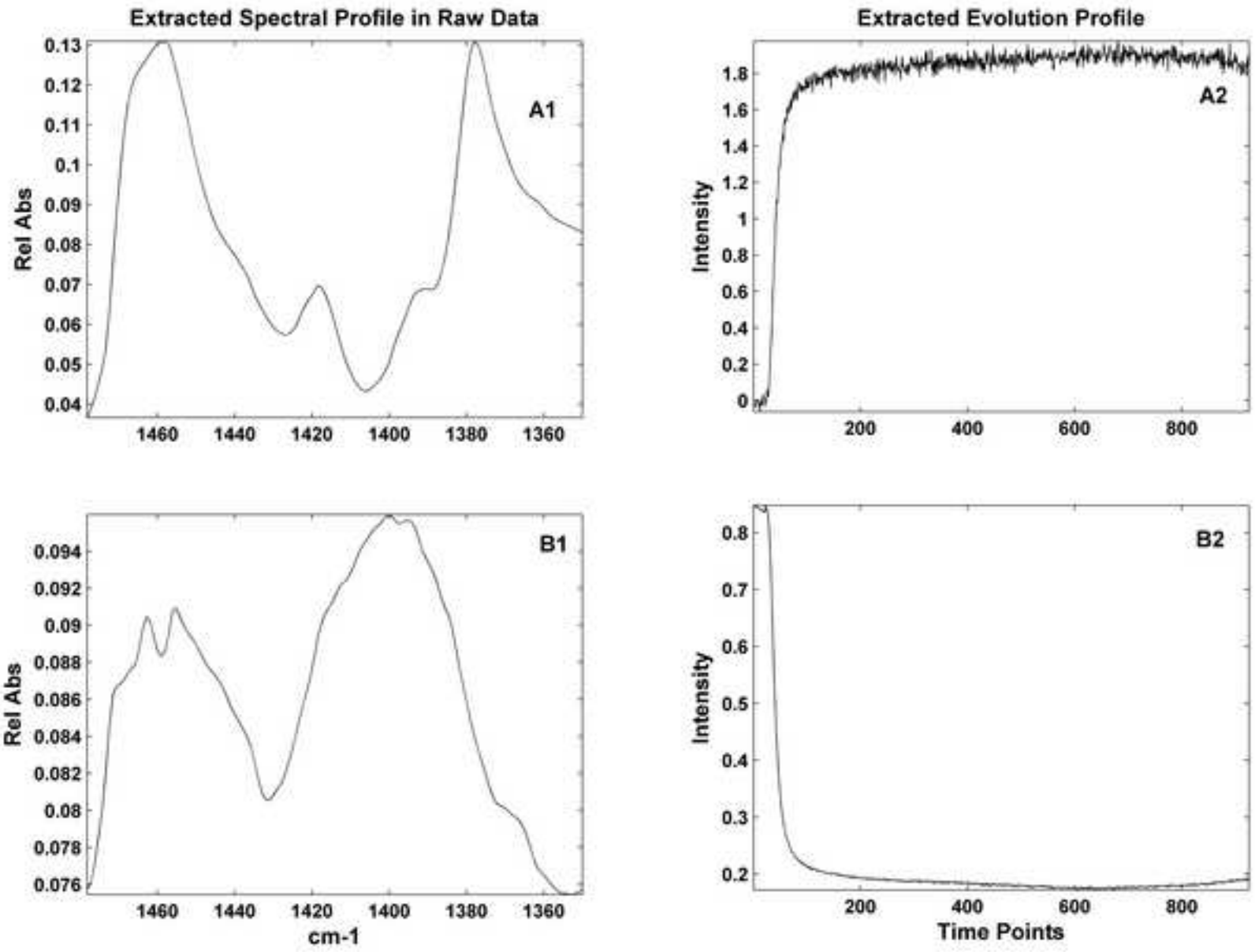
Figure(s)

Click here to download high resolution image



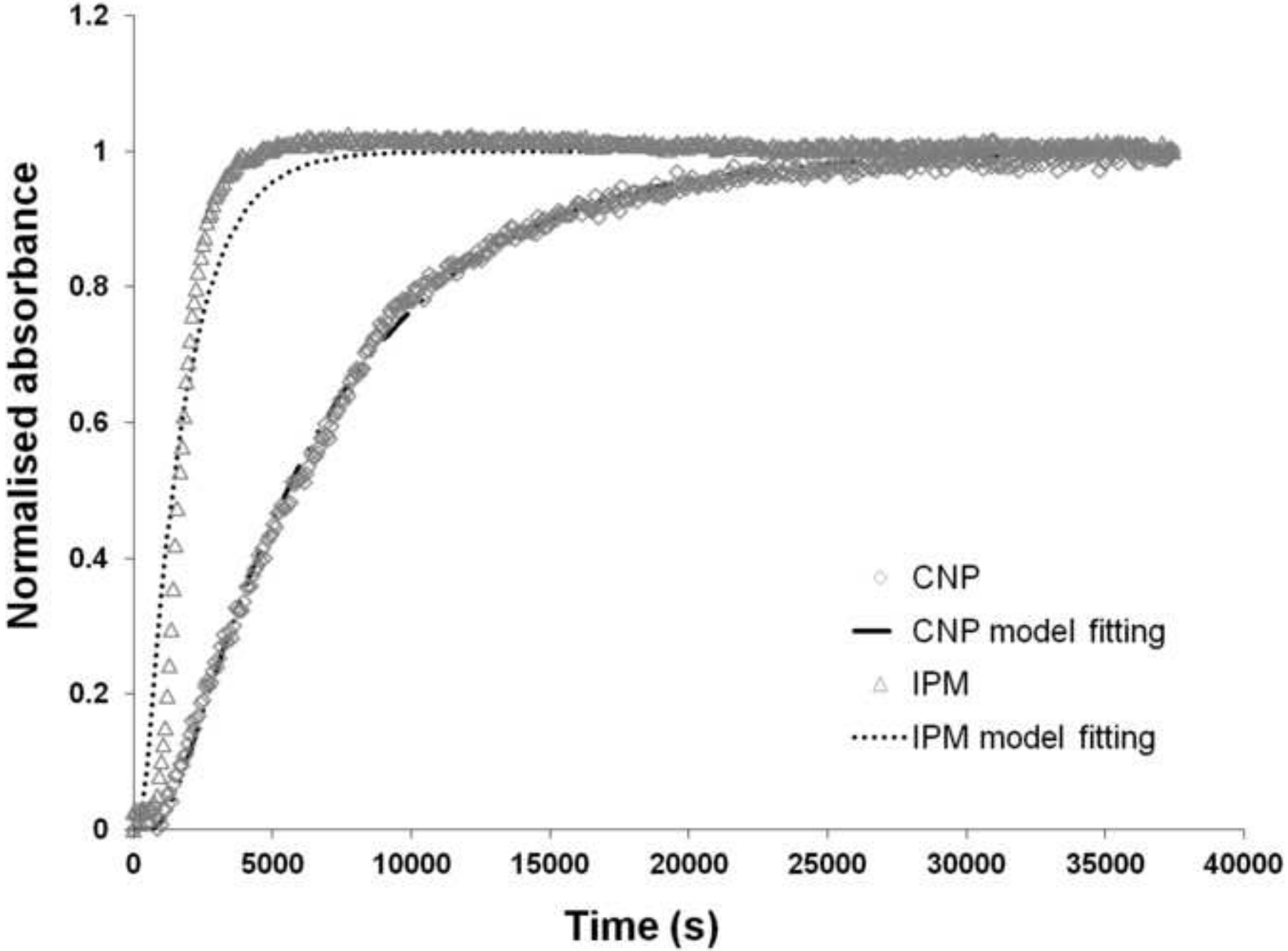
Figure(s)

Click here to download high resolution image



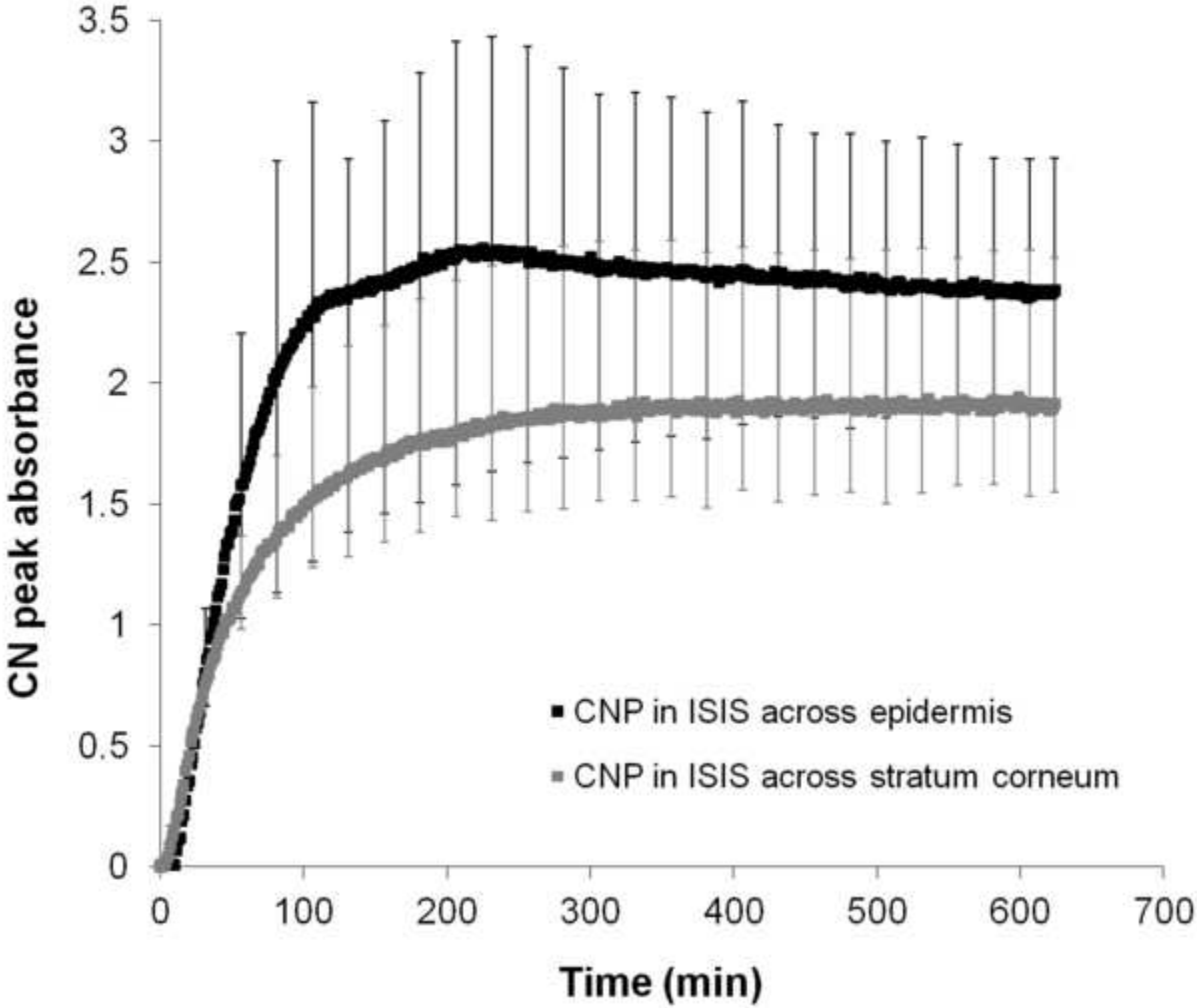
Figure(s)

Click here to download high resolution image



Figure(s)

Click here to download high resolution image



**Figure(s)**

Figure 1. ATR-FTIR spectra, taken as a function of time of a CNP in DPPG across epidermis experiment.

Figure 2. Increase in CN peak absorbance with time of CNP in DPPG, IPM, ISIS and water across human epidermis. Error bars show the range (n=3).

Figure 3. Decrease in CN stretch absorbance with increasing ISIS penetration into skin. Dashed line spectrum taken at 280 minutes, solid line spectrum taken at 620 minutes

Figure 4. Deconvoluted spectrum of the DPPG signal (A1) from that of the stratum corneum (B1) in the 1478-1350 $cm^{-1}$ spectral window. Plots on the right hand side (A2 and B2) show the corresponding evolution profiles with time.

Figure 5. Typical normalised absorbance plots showing the diffusion of CNP and IPM across human epidermis with associated model fittings.

Figure 6. Increase in CN peak absorbance with time of CNP in ISIS across human epidermis and human stratum corneum. Error bars show the range (n=3).