the transfer standard measured on A and B or C respectively. These corrected spectra were then used to predict the paracetamol content using a model developed on A.

Results A three-factor partial least squares regression (PLSR) calibration model (1100–2498 nm) using SNV + first-derivative spectral pre-treatment on instrument A gave an RMSEP of 0.57% m/m. Direct transfer of spectra from instrument B gave an RMSEP of 0.81% m/m. Corrected spectra gave slightly better results: for example, avicel PH101 in vial (0.95% m/m), avicel PH101 in cell (0.60% m/m), paracetamol in vial (1.14% m/m), sucrose in vial (0.58% m/m), sucrose in cell (0.81% m/m) and mean sample spectrum (0.56% m/m). Transfers between different instrument types (A and C) were less successful: direct transfer (0.97% m/m), avicel PH101 in vial (0.75% m/m), avicel PH101 in cell (1.60% m/m), paracetamol in vial (0.54% m/m), sucrose in vial (1.07% m/m) and sucrose in cell (1.03% m/m). However, correction using the mean sample spectrum was best (0.52% m/m). Many factors influenced the success of transfer. Model selection was important; for example, increasing the level of smoothing during spectral pre-treatment generally improved transfer. Matching the transfer standard dimensions to that of the paracetamol tablets was also an important factor. In general the transfer standard needs to closely match the sample both chemically and physically.

Conclusions Transfer using simple pure compounds, while useful, was not to be preferred over mean sample spectrum correction.

Biologics

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Synthesis and evaluation of an affinity-based probe for fibroblast-activation protein α (seprase) activity

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Objectives Fibroblast-activation protein a (FAPα; also known as seprase) is a 170 kDa serine gelatinase and prolyl dipeptidyl peptidase that consists of two identical N-glycosylated 97 kDa subunits embedded in and integral to the cell membrane. FAPα is expressed during tumour cell invasion at the invasive fronts of melanoma cells and breast carcinoma cells, in gastric carcinoma and also in rheumatoid arthritis. FAPα belongs to a family of post-proline-cleaving proteases, many of which share a high degree of homology and/or substrate specificity. This is particularly true of dipeptidyl peptidase IV (DPP-IV), another therapeutically important protease. The purpose of this study was to design, synthesize and evaluate an affinity-based probe for the selective disclosure of FAPα activity in biological milieux where other closely related peptidase activities are present.

Methods The affinity-based probe, Bio-PEG-T-S-G-$P^P(OPh)_2$, was synthesized by a combination of solid- and solution-phase synthesis. The amino acid sequence was based on previous work by our group which indicated that Gly-$Pro^P(OPh)_2$ was a potent irreversible inhibitor of both DPP-IV and FAPα (Gilmore et al 2006), and a recent report indicating that FAP could cleave extended substrates (Edosada 2006), thus permitting the design of selective inhibitors which do not target closely related peptidases such as DPP-IV. The inhibitor was evaluated by continuous fluorimetric assay against FAP, DPP-IV, DPP2 and DPP7. Labelling of peptidases was conducted as described by Gilmore et al (2007) using standard electrophoresis and western-blotting methodologies. Labelled peptidases were detected by streptavidin-HP chemiluminescence on photographic film.

Results The inhibitor probe Bio-PEG-T-S-G-$P^P(OPh)_2$ was found to be a moderately potent but highly selective inhibitor of FAPα, having an overall second-order rate constant (k_i/K_i) of 3.8×10^3 M^{-1} min^{-1}. Importantly, Bio-PEG-T-S-G-$P^P(OPh)_2$ exhibited no inhibitory activity against DPP-IV, DPP2 or DPP7. The probe was subsequently used to label recombinant FAPα and shown to be directed by the active site, since incorporation of the probe was blocked by pre-incubation of the enzyme with pefabloc.

Conclusions In this report we detail the synthesis of a moderately potent, selective affinity-based probe for the disclosure of FAPα activity in complex biological milieux even where closely related peptidase activities are present. This probe may have utility in further elucidating the role of this peptidase in diseases such as cancer and rheumatoid arthritis.

Edosada, C. Y. et al (2006) *FEBS Lett*. **580**: 1581–1586
Gilmore, B. F. et al (2006) *Biochem. Biophys. Res. Commun*. **346**: 436–446
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Chemistry

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Photothermal microspectroscopy: a new technique for spatially differentiating between crystalline and amorphous materials

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Objectives This study demonstrates the ability of photothermal microspectroscopy (PTMS), a new technique (Hammiche et al 2004), to differentiate between both the physical and chemical nature of a model GlaxoSmithKline drug in its amorphous and crystalline forms.

Methods The model GlaxoSmithKline drug was used as received in its crystalline form. The amorphous form was prepared using a Büchi Mini-Spray Dryer B-290. Compacts of the samples were formed using a 13 mm infrared sample press with an applied pressure of 1 ton. PTMS was performed by interfacing a Caliber AFM equipped with a Wollaston wire thermal probe to a Bruker FTIR spectrometer. Local thermal analysis experiments were conducted with an underlying heating rate of 10°C s^{-1} from 60 to 200°C. Infrared spectra were collected using 200 scans and a resolution of 8 cm^{-1}, across the range 4000 to 500 cm^{-1}. Modulated-temperature differential scanning calorimetry (MTDSC) and attenuated total reflectance-Fourier transform infrared spectroscopy were used to validate the PTMS results.

Results PTMS demonstrated its ability to distinguish between the pure amorphous and crystalline surfaces. The topography of the crystalline and amorphous surfaces showed clear differences although it was not possible to state which surface represents which material from topography alone. Local thermal analysis, whereby the temperature of the tip is raised and the temperature of penetration noted (Royall et al 2001), was able to discriminate on the basis of the glassy or melting behaviour of the system. This agrees with the MTDSC response seen. For the first time we were also able to differentiate via the photothermal infrared spectra using PTMS, shown in Figure 1, with spectral differences noted in terms of peak broadening and disappearance in the 3500–2000 cm^{-1} region. This broadening is due to non-specific intermolecular bonding in the amorphous material.

Conclusions PTMS was shown to be a highly promising new technique for determining and differentiating between pure amorphous and crystalline materials at precise topographic locations. Subsequent work is focused on mixed systems and results to date are highly encouraging.

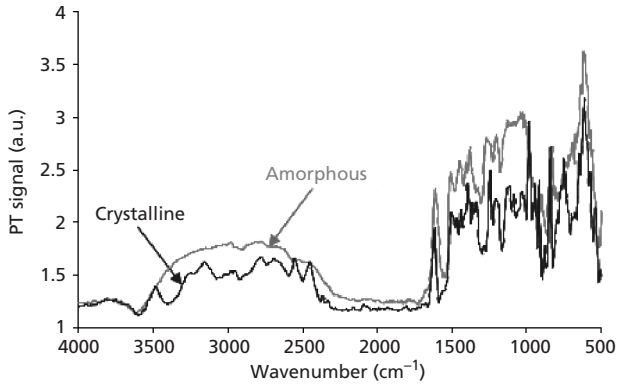

Figure 1 Photothermal IR spectra of the amorphous and crystalline samples.

Hammiche, A. et al (2004) *J. Microsc.* **213**: 129–134
Royall, P. G. et al (2001) *J. Phys. Chem. B* **105**: 7021–7026

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Synthesis, characterization, *in vitro* hydrolysis and biological evaluation of amino acid methyl ester conjugates of valproic acid: a prodrug approach

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Objectives Valproic acid (VPA) is an extensively used anti-epileptic drug for the treatment of various kinds of epilepsies and administered orally several times a day due to short half-life. It has been proven to possess the life-theatening side effects of hepatotoxicity, teratogenicity and gastric irritation (Sobol et al 2004, Bryant and