Poster Session 1 – Analytical Chemistry

017
Generation of derivative curves of absorption spectra using Glenn's method of orthogonal functions

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Glenn's method of orthogonal functions (Glenn 1963) is an original work for generating derivative spectra. Thus, an absorption curve f(λ) can be expanded as follows:

$$f(\lambda) = p_0P_0 + p_1P_1 + p_2P_2 + p_3P_3 + \ldots\ldots\ldots\ldots + p_nP_n \quad (1)$$

where p_j are the coefficients of the orthogonal polynomials P_j for n + 1 points. The linear, p_1, quadratic, p_2 , cubic, p_3, quartic, p_4, etc., coefficients are analogous to the first derivative, ^{1}D, second derivative ^{2}D, third derivative ^{3}D, and fourth derivative,^{4}D, etc., of the absorption curve, respectively. By convoluting an absorption curve (i.e. plotting p_j against λ_m the mean of the set of wavelengths over which a coefficient is calculated (Agwu & Glenn, 1967; Wahbi 1967, 1971) using the orthogonal polynomials from P_1 to P_n), the respective derivative curves are obtained. Convoluted curves of Gaussian bands were found to be similar to the respective derivative curves with regard to position of optima and zero crossings. The p_1 convoluted curve of the p_1 convoluted curve (double convolution) of any curve is the p_2 convoluted curve — a situation similar to recording the first derivative curve of the first derivative curve, giving the second derivative curve. Table 1 shows the p_1 and p_2 convoluted absorption curves of a phenol solution where the optima and zero crossings were found to be within ± 1 nm from the corresponding ^{1}D and ^{2}D instrumental curves, respectively. The p_2 convoluted curve of a toluene solution gave optima at ± 1 nm from the published ^{2}D data by the BP. The orthogonal functions method has the advantage that can be applied in a combined polynomial form (Wahbi & Ebel 1974) to avoid measurements at slopes in the derivative curves. Application to A versus wavenumber (non-linear scale) curves is also an important advantage (unpublished data). BASIC programs facilitate the computation.

In conclusion, orthogonal function-spectrophotometry (Glenn 1963) is equivalent to derivative spectrophotometry in all quantitative and qualitative aspects.

Table 1 Derivative and convoluted curves of absorption curves

	^{1}D curve	p_1 curve
	Optima (nm)	
Phenol 48 μg mL^{-1} in 0.05 M H_2SO_4	254 and 284	255 and 285
λ_{max} = 270 nm	Zero crossings (nm)	
	257 and 281	258 and 282
	^{2}D curve	p_2 curve
	Optima (nm)	
	270	269
	Zero crossings (nm)	
	257 and 281	258 and 282
	^{2}D curve	p_2 curve
	Optima (nm)	
Toluene 0.02%v/v in methanol (BP 2001)	261, 263	262, 264,
265, 268	265, 269	

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018
Novel thermal methods for the characterisation of polymorphic transitions

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The present work describes the application of a novel approach to the characterisation of polymorphic transitions. More specifically, we describe the use of modulated temperature differential scanning calorimetry (MTDSC) in quasi-isothermal mode, whereby a sample is held at a series of temperature steps and modulated for a specified time. This allows the heat capacity to be measured in a manner that allows the reduction of kinetic effects.

Anhydrous caffeine exists in two enantiotropic polymorphic forms, II and I, that are stable below and above a transition at ~150°C, respectively (Cesaro *et al* 1980). On heating Form II through this temperature it transforms to Form I, thereby providing a controlled model system. Form I and Form II caffeine were prepared (Bothe *et al* 1979). All thermal analyses were performed in hermetic aluminium pans using a TA 2920 MTDSC calibrated appropriately. DSC studies were performed at a range of heating rates (Table 1). For standard MTDSC studies, samples were heated (2°C min^{-1}, ± 0.75°C, over 80 s, nitrogen purge) to 280°C. In the quasi-isothermal studies, the same modulation was used, (increment 0.2°C, isothermal period 30 min) from 120°C to 160°C. Caffeine Form II was analysed by X-ray powder diffractometry on a Siemens D5000 powder diffractometer equipped with a variable divergence slit and a position sensitive detector. Diffractograms were collected using copper Kα radiation (λ = 1.5418 Å) from 2 to 90° 2θ in steps of 0.007° 2θ and a step time of 0.1 s. Diffractograms were collected at 30°C and then from 130 to 160°C in steps of 2°C.

Table 1 shows the DSC data corresponding to the effect of increasing heating rate causing the transition to occur at a higher temperature and over a broader range, implying that there is a kinetic component to the transition.

Table 1 Effect of heating rate on polymorphic transition of caffeine

Heating rate	Onset temperature	Transition width	Enthalpy (ΔHf) (KJ mol^{-1})
0.1°C min^{-1}	139.5°C (0.3)	2.9°C	2.62 (0.35)
1°C min^{-1}	141.5°C (0.3)	4.6°C	2.61 (0.04)
2°C min^{-1}	141.9°C (0.4)	7.7°C	2.84 (0.06)
5°C min^{-1}	145.2°C (0.5)	9.8°C	2.95 (0.04)
10°C min^{-1}	147.0°C (1.3)	11.6°C	2.47 (0.03)

The MTDSC response of Form II showed a transition as an endotherm at 141°C in the total heat flow (equivalent to standard DSC) with an associated small step change in the reversing heat flow indicating the change in heat capacity between the two forms. The quasi-isothermal studies enabled direct collection of the heat capacity data through the transition itself, thereby removing effects caused by the heating process and yielding a transition value of 137.6°C.

The study has indicated that quasi-isothermal MTDSC represents a novel and potentially highly important method of studying polymorphic transitions.

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019
Inhibitory effect of SDS on subunit dissociation of hCG

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The placental hormone, human chorionic gonadotropine (hCG), and the pituitary

glycoprotein hormones (luteinizing hormone, follicle-stimulating hormone and thyrotropin) contain two subunits, the alpha and beta chains (Andrew 1991). The alpha chains are very similar. Each, however, has a unique beta chain; giving them their individual functions (Catt 1973; Forastieri & Ingham 1980).

In this study, intensity of ANS (8-anilino-1-naphtalen sulfonate) fluorescence at 480 nm has been taken as a measure of the amount of hCG in the solution (Ingham & Bolotin 1978). The rate of subunit dissociation of hCG has turned out to be as a first order reaction in the range of pH = 2–10 at different temperatures (25–70°C) (Strickland & Puett 1982). The dissociation rate constant in acid medium is higher than other mediums. Gel filtration study has shown that the elution volume of hCG decreases as the pH decreases. Sodium dodecyl sulphate (SDS) slows down the subunit dissociation of hCG through the concentration range used (0.3, 0.5, 0.7, 1.0 and 1.5 mM). The best inhibitory concentration of SDS is found to be 0.7 mM.

The activation energies were calculated by Arrhenius equation (Higuchi *et al* 1950). This method is in order to calculation of rate constants (k) of dissociation reaction, and then activation energies from the slope of the plot of lnk against 1/T ($^{\circ}K^{-1}$). The results showed the activation energies increase at all pHs in the presence of SDS. The maximum value of activation energies correspond to the concentration of 0.7 mM of SDS. It has been evident that the gel filtration causes, at all pHs and the presence of SDS (0.7 mM), the increase of elution volume of hCG. The decrease in the rate of subunit dissociation in addition to the increase in activation energy in the presence of SDS could be accounted for the partial folding of hCG.

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020
Characterisation of solute dissolution in simulated intestinal fluids by solution calorimetry

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Solution calorimetry measures the enthalpy of solution of a solid dissolving into a solvent. The objective of this study was to investigate the potential of solution calorimetry to characterise dissolution in simulated intestinal fluids (SIFs), by measuring the enthalpy of solution of two solutes using SIFs as the solvent. SIFs mimic conditions found in the intestine, as they contain mixtures of bile salts and lipid, which form mixed micelles (Castro *et al* 2001). The concentrations of bile salts and lipid were varied to model the environment present in the fed and fasted intestinal lumen. The two solutes, mannitol and propranolol, were chosen as they have previously been used to evaluate the drug delivery potential of mixed surfactant:lipid micelles (Gould 1996). It was reported that micellar systems retarded the transepithelial transport of propranolol but not mannitol — a finding that was attributed to an interaction of propranolol, but not mannitol, with the micellar phase. In this study, $\Delta_{sol}H$ was measured using a Thermometric 2225 Precision Solution Calorimeter (Thermometric AB Sweden). The solvents used were fed state SIFs, fasted state SIFs (Patel *et al* 2001), and Hanks Balanced Salt Solution (HBSS).

Both solutes exhibited endothermic reactions in all solvents. The $\Delta_{sol}H$ for propranolol in the SIFs differed from those values determined in HBSS; however, for mannitol there were no significant differences (Table 1). The difference in enthalpy for propranolol between HBSS, fed and fasted state SIFs is consistent with the solute partitioning into the micellar phase, as proposed by Gould (1996). A constant value of $\Delta_{sol}H$ confirmed a minimal interaction between mannitol and the micellar phases present in both SIFs. The raw temperature offset data was converted to the dynamically corrected heat flow data using a thermistor time constant of two seconds. The resultant power time plots showed that for propranolol the dissolution process was faster in the fed state SIF compared with the fasted state SIF.

Table 1 Enthalpies of solution of solutes in solvents

Solvent	$\Delta_{sol}H$ Propranolol (kJ mol^{-1})	$\Delta_{sol}H$ Mannitol (kJ mol^{-1})
Fed state SIF	15.4 ± 0.12	21.5 ± 0.19
Fasted state SIF	23.6 ± 0.25	21.9 ± .40
HBSS	25.0 ± 0.21	21.9 ± .67

Data are means ± s.d., n = 3

This study indicates that solution calorimetry has the potential to discriminate both dynamic and thermal characteristics of drug dissolution in complex media.

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021
Characterisation of Sporanox® and three unlicensed itraconazole solid oral capsules employing complementary in-situ microanalytical methods

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Itraconazole, a broad-spectrum antifungal agent, is a highly lipophilic weak base with an aqueous solubility at neutral pH estimated at about 1 ng/ml. The development and commercialisation of a bioavailable solid oral dosage form of this poorly water-soluble antifungal has been possible through the coating of inert sugar spheres with a solid solution containing itraconazole and hydroxypropylmethylcellulose (HPMC). This product, Sporanox®, releases the molecularly dispersed drug to give a supersaturated solution in the stomach. Sporanox® and three unlicensed itraconazole solid oral capsules have been characterized utilizing state-of-the-art surface analytical tools. The goal of this study was to investigate physical, chemical and morphological differences between these 4 formulations by scanning electron microscopy (SEM), Raman confocal spectroscopy and scanning thermal microscopy.

For Sporanox®, the release of itraconazole is complete at 60 minutes whereas substantially lower release rates are encountered for the three unlicensed products. A structural investigation using scanning electron microscopy reveals a similar overall structure, namely an itraconazole/HPMC coating onto inert sugar spheres. However, significant differences in the homogeneity of the coating of the active component are observed. This was further investigated using confocal Raman microspectroscopy and scanning thermal analysis (SThM). These results show significant differences in the distribution of the drug within HPMC. Significantly, whilst the data strongly indicated the formation of a homogenous itraconazole/HPMC solid solution in the case of Sporanox®, the unlicensed products gave spectral and localised thermal analysis (LTA) data that was consistent with the presence of discreet crystalline drug particles within the HPMC polymer. The results correlate with the release and bioavailability data and provide a valuable insight into the microstructure of the formulation in relation to the release profile. Furthermore, the results highlight the significant advances in mapping physicochemical properties using state-of-the-art chemical mapping (Raman microspectroscopy) and imaging technologies (scanning thermal microscopy (SThM)) and the unique opportunities these techniques give to understand structure-property relationships.

022
An optimized HPLC-ECD detection method for the assay of homocysteine

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For more than 20 years, many studies had demonstrated a positive correlation between elevated total plasma homocysteine (tHcy) and vascular disease. A total of 10% of the risk of coronary arterial disease (CAD) appears to be attributable to tHcy (Boushey *et al* 1995). An ion-paired reversed-phase high-performance liquid chromatography with electrochemical detection (HPLC-ECD) was developed for the determination of tHcy. The method provides a simple, precise and reliable tHcy assay at low cost. To achieve optimal separation of homocysteine from other electrochemically active components, the key approach is to find the most appropriate mobile phase in the HPLC-ECD system. The major factors examined in this study were buffer pH, buffer concentration, buffer material, ion-pair concentration and methanol concentration in the mobile phase. Retention time or capacity factor (k') were used as the parameter to evaluate the influence of each factor After the optimal separation condition was obtained, the selectivity of the internal standard, specificity, recovery and precision of the assay method was investigated. The optimal assay of homocysteine was achieved with a mobile phase of pH 2.6, 0.01 M monosodium phosphate buffer (containing 26 mM octane sulfonate sodium)—methanol in a 81:19 (v/v) ratio) with detection at guard cell 800 mV, analytical cell E1 500 mV, and E2 700 mV. The recovery of tHcy ranged from 96.7% to 106.2% at the concentration range of 0.5–5 $\mu g\ mL^{-1}$ in plasma. The mean correlation coefficients of intra-assay (n = 6) and inter-assay (n = 6) calibration curves for tHcy were both 0.999 over a concentration range of 0.1–10 $\mu g\ mL^{-1}$. The limit of detection for intra-assay and inter-assay was 0.049 $\mu g\ mL^{-1}$ (0.37 μM) and 0.088 $\mu g\ mL^{-1}$ (0.65 μM), respectively, and the limit of quantitation was 0.151 $\mu g\ mL^{-1}$ (1.12 μM) and 0.266 $\mu g\ mL^{-1}$ (1.97 μM), respectively. An ion-paired reversed-phased HPLC assay with electrochemical detection for tHcy suitable for clinical laboratory use was developed in this study. The method was simple, precise and accurate for the determination of tHcy levels and enabled comparatively easy processing of large numbers of specimens. The assay is suitable for the determination of tHcy levels in clinical stroke patients.

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023
Reductants comparison for determination of total plasma homocysteine

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Homocysteine (Hcy), increasingly being recognized as a risk factor for vascular disease, is found primarily in plasma in the form of homocystine and mixed disulfides, both protein-bound and unbound; total plasma homocysteine (tHcy) is the sum of all Hcy species obtained after quantitative reduction. Measurement of tHcy is an independent and predictive biomarker in cardiovascular disease. The validation of assay in determination of tHcy is a key; besides, the efficiency and reaction condition of the reductant in the assay are important. Several methods are currently used to measure tHcy in plasma, including GC/MS, ion-exchange chromatography, HPLC-ECD, HPLC-FD, enzyme immunoassay and fluorescence polarization immunoassay (Pfeiffer *et al* 1999). In the most popular HPLC techniques, any homocystine and homocysteine-mixed disulfides present are reduced with 1,4-dithiothreitol (DTT) or tris 2-carboxyethyl phosphine (TCEP) to Hcy and detected with HPLC-ECD.

In this study, the tHcy was determined by HPLC-ECD and used to investigate the relationship of reductants DTT and TCEP in various reaction conditions, including reaction time, temperature and the concentration in the reduction reaction.

Seven reduction times of 1, 3, 5, 10, 15, 20 and 30 min at room temperature and in a 37°C water bath were tried to understand the tHcy level in the reduction reaction. The tHcy level in the reduction reaction was determined at seven different concentrations of 1, 3, 5, 7, 10, 15 and 20 $mg\ mL^{-1}$. The tHcy concentration of fifteen samples was measured by DTT and TCEP reductant separately.

Assay of fifteen patient samples with a normal range of homocysteine values showed excellent correlation ($R^2 = 0.9370$). Analysis of residuals by a run test and examination of the Bland-Altman plot (Bland *et al* 1986) revealed no significant nonlinear trend. The study found that the best efficiency of two reductants is at 37°C water bath, with a reaction time of 15 min and a concentration with 15 $mg\ mL^{-1}$. It also demonstrated the agreement between reductant DTT and TCEP in plasma samples by statistics.

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024
Production and analysis of thiolated polymeric excipients

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Polymers that have free pendent sulfhydral groups are known as thiomers (Bernkop-Schnurch *et al* 1999). This important class of polymers can display mucoadhesive properties by a combination of interactions: (1) covalent interactions due to disulphide exchange reactions with the cysteine rich subdomains of mucous glycoproteins and (2) non-covalent interactions of polar pendent groups along the polymer mainchain.

Typically thiomers are prepared using broad molecular weight distribution poly(methaacrylic) acids or polycarbophils (Bernkop-Schnurch & Steininger 2000). This process yields broad molecular weight thiolated acidic polymers. Our intent was to prepare and evaluate narrow molecular weight thiomers that possess neutral chemical functionality. This was accomplished utilising methodology recently developed by Godwin *et al* (2001) where narrow molecular weight active ester precursor polymers can be used to provide families of pharmaceutically relevant functionalised polymers for study.

The thiomers were prepared by first conjugating cystamine hydrochloride (10–50% loading) followed by addition of 1-amino-2-propanol to provide the desired thiomers. Conjugations of non-thiol aliphatic amines are typically monitored by FT-IR where loading matches the stoichiometry of the amine being used. For cystamine, there can be competitive conjugation of the thiol moiety to yield an intermediate, labile thiol ester that is then displaced by amine in the second conjugation step. Competitive thiol ester formation appears to be a function of pH, concentration and stoichiometry, however it was possible to conjugate cystamine where the IR loading was within about 5–15% of the expected value.

It was anticipated that during isolation and storage, the free thiol moieties could undergo disulfide exchange reactions. It was critical to assay for free thiol content and to use GPC to determine if increases in molecular weight occurred over time. Three different assays were examined to evaluate which was best suited for thiolated polymers. There was concern that intramolecular interactions along the polymer mainchain could influence assay results or that prolonged experiment times could cause polymeric disulfide formation. The assays were (1) iodometric titration with starch as an indicator, (2) ammonium thiocyanate back titration using ferric alum solution as an indicator following the addition of excess silver nitrate and (3) the use of 2,2-dithiodipyridine (DTDP), which undergoes rapid exchange reactions with free thiol groups providing a UV detectable chromophore.

Table 1 Determination of thiol loading of a model thiomer with 26% theoretical loading

Iodometric	$AgNO_3$	DTDP
3.17	26.13	36.45
2.02	7.06	16.78

Listed in Table 1 are the results for using the three assays for one batch of thiomer prepared with a 26% theoretical loading. Only two determinations are shown The IR loading had indicated that 30% cystamine was conjugated. After thiomer isolation, iodometric titration suggested that there was a deficiency of free thiol groups. This trend was observed for many batches of thiomer that was prepared. The ammonium thiocyanate/$AgNO_3$ back-titration and the DTDP assay suggested thiol content was near the theoretical amount or indicated a low thiol content suggesting crosslinking had occurred. This was confirmed when GPC results showed that a sample thiomer gained molecular weight over time (from 28 000 to over 700 000 g mol^{-1} over two weeks in storage at ambient temperature). Thiolated polymers are fast becoming an important class of new excipients, however care is required during preparation, evaluation, handling and storage to avoid formation of polymeric disulfides.

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