Poster Session 1 – Analytical Chemistry

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Analysis of multi-layer packaging systems for pharmaceuticals, using AFM, SThM, and confocal Raman microscopy

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Packaging of pharmaceuticals often requires the formation of complex multi-layer systems. The design of the packaging is governed by many factors, including mechanical strength, stability to sterilization and heating, barrier properties, chemical resistance and formation of a good seal. So as to satisfy the above requirements, packaging comprising many differing layers is employed, with each of these layers, imparting a specific property to the overall product. Typically the layers of a packaging material will comprise polymeric faces enabling formation of a peel-able or weld seal, an aluminium or 'glass' barrier layer and an outer polymer layer providing mechanical stability and potentially a printable surface. The integrity of the interfaces formed between these layers and at the sealed interface is of critical importance to the packaging properties.
Here we have characterised a multi-layer pharmaceutical packaging system using atomic force microscopy (AFM), scanning thermal microscopy (SThM) and confocal Raman microscopy. Raman spectroscopy and scanning thermal microscopy SThM have been used to identify the composition of the polymeric layers, to be polypropylene and polyethylene terephthalate. SThM provides localised measurement (1 μm) of the material properties over a temperature ramp applied to the probe. The melting points of both polymers are reflected by inflection in the cantilever position, and show good correlation to melting point values from bulk samples
The sealed interface and interfaces formed between the polymer and barrier layers have also been characterised at high resolution by both AFM phase imaging and force volume imaging. AFM phase imaging can identify between two polymers depending on factors such as stiffness, adhesion and elasticity, and here provides a useful tool in identification of the interfaces. Force volume imaging involves the acquisition of an array of force curves over a predetermined area of the sample. The image can then be generated for a given displacement on the force curves, so as to provide an image representative of adhesion, elasticity or long-range forces.
In conclusion, surface analytical techniques have been used to characterise a polymeric packaging material and investigate on a nano-scale the interfaces formed therein.

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Effect of toothpaste sodium tripolyphosphate (STP) upon inhibition and removal of tea stain from hydroxyapatite

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Many dietary compounds are known to cause staining and discoloration of the teeth by the formation of chromogens on the tooth surface. Examples of such compounds include tea, coffee and red wine. Extrinsic dental stain may be removed to some degree on a daily basis through brushing and the use of tooth pastes that incorporate abrasives or chemical, tooth-whitening agents. Long-term build up of dental stain often requires restorative dental techniques, therefore the demand for over the counter tooth whitening products which may be integrated into a daily oral hygiene regime is high.
Significant information on the potential stain removal and inhibition properties of sodium tripolyphosphate (STP) has been obtained in studies by Addy & Moran (1995) and Watts & Addy (2001) using either bovine or synthesised tooth substrates. Each of these techniques has limitations relating to dissimilarity to human tooth structure and composition.
In this study, commercially available, stoichiometric, hydroxyapatite (HA) powders were used to investigate and substantiate the stain removal and inhibition properties of STP, established using both real-time and static model systems in studies by Pavey et al (2001), Tantbirojin et al (1998) and Langford et al (2002). The stain deposition onto the HA surface was achieved using standardised samples of selected red wine and tea products under controlled conditions. The stain removal capacity of varied concentrations of STP, 2, 5 and 10% w/v, using first-derivative UV spectrometry showed that significant stain removal was achieved (74%) within minutes of contact with the HA substrate. Stain inhibition studies using a 2% w/v STP pre-treatment onto the HA substrate, subsequently subjected to standardised tea stain, showed inhibition of stain above 94% within 1–3 min. These results not only correlate with, but confirm the claims that STP is an effective tooth-whitening agent. The method offers a rapid, label-free method for screening the potential of stain removal/inhibition agents for incorporation into dental products.

Addy, M., Moran, J. (1995) *Adv. Dent. Res.* **9**: 450
Langford, J., et al (2002) *Analyst* **127**: 360–367
Pavey, K. D., et al (2001) *Analyst* **126**: 426
Tantbirojin, D., et al (1998) *Esthetic Dent.* 29: 28–36
Watts, A., Addy, M. (2001) *Br. Dent. J.* **190**: 309

019
Effects of stearyl alcohol on the molecular dynamics of Eudragit RSPO

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Eudragits are copolymers (based on polymethacrylates) that are used widely in the pharmaceutical industry for sustained-release and coating formulations. Eudragit RSPO (Rohm GmbH) is a thermoplastic, pharmaceutical grade, ionic copolymer, composed of three different monomer units and was chosen for the study of model controlled-release multiparticulates. This study uses low frequency dielectric relaxation spectroscopy (DRS) and differential scanning calorimetry (DSC) for the characterisation of the bulk molecular dynamics of Eudragit RSPO as a function of small concentrations (1–5%) of stearyl alcohol (plasticiser/release modifier) and temperature.
Dielectric relaxation measurements were carried out in the frequency range 10^{-1} to 10^6 Hz and temperature range 25–130°C. Samples were sandwiched between two polished brass parallel plate electrodes (25 mm in diameter, 1 mm separation, with an air capacitance of $C_o = 4.35$ pF) and characterised using a Solatron 1296 dielectric interface, connected to a Solatron 129610A temperature controller/cryostat system and a Solatron 1255 frequency response analyser. Differential scanning calorimetric (DSC) measurements were taken using pierced, standard aluminium, 50 μL crimped pans under a nitrogen purge. Samples, 10-12 mg in weight, were heated from 25°C to 150°C, at a rate of 10°C min^{-1}.
Polymers usually exhibit multiple dielectric relaxation processes: a low frequency α-response, a high frequency β-response, and a number of subsidiary processes at even higher frequencies (Blythe 1979). The glass transition (T_g) of polymers is analogous to the dielectric α-response and is attributed to major changes in segmental chain motion (Blythe 1979). The β-response is usually attributed to motions of parts of the main chain, or rotational movements of side groups attached to the main chain (Blythe 1979). Two temperature-dependent dielectric responses are observed in all samples, a fast (i.e., high frequency/low temperature) and broad β-response (with small Δε) and a slow (i.e., medium frequency/high temperature) and narrow, α-response (with large Δε). Both relaxation peaks shift to higher frequencies with elevated temperature. Data curve-fitting analysis for both responses, involved the combined use of a Havriliak-Negami (Fitzgerald & Runt 1997) (HN)/Davidson-Cole[2] (DC) element in parallel with a Constant Phase (CPE)

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element. Dielectric relaxation strength plots ($\Delta\varepsilon_*$ T vs T) illustrate the change in dipolar cooperativity for both processes as the temperature was elevated through T_g (in the case of the β-response) and the softening temperature (in the case of the α-response). Both DRS and DSC profiles show a reduction in T_g as the concentration of stearyl alcohol was increased. From Arrhenius plots (Inτ vs 1000/T), relaxation times (τ) for both responses clearly shorten with increased quantities of stearyl alcohol accompanied by significant decreases in enthalpy (ΔH).

The study shows that the dielectric properties (e.g., cooperativity, τ and T_g) of Eudragit RSPO are significantly affected by small concentrations of the stearyl alcohol, due to changes in the molecular dynamics of the amorphous copolymer network. These observations have implications for improving our understanding of how the molecular dynamics of these materials impact on their suitability and function as controlled release matrices.

Blythe, A. R. (1979) *Electrical properties of polymers*. Cambridge University Press, Cambridge, pp 38–67

Fitzgerald, J. J., Runt, J. P. (eds) (1997) *Dielectric spectroscopy of polymeric materials, fundamentals and applications*. American Chemical Society, Washington, pp 81–106

020

New methods for the isolation of minor cannabinoids from *Cannabis sativa*

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In addition to the psychoactive cannabinoid, Δ^9THC, (which is characteristic of the drug type chemovars) and CBD, (which is characteristic of the fibre type chemovars), there are a wide range of other minor cannabinoids that are also present in *Cannabis sativa*. Following recent promising findings regarding the pharmacology of both Δ^9THC and CBD, the study of the pharmacology of the minor cannabinoids is now also becoming of increasing interest and hence, there is a need to isolate quantities of the pure materials. In addition, as analytical techniques improve, there is a requirement for purified primary standards of these compounds.

These minor cannabinoids are found in herbal cannabis. These may be intermediates in the biosynthesis of the major cannabinoids and for this reason only exist at low concentrations. Such an example is cannabigerol (CBG). Other minor cannabinoids may represent the end point of an alternative biosynthetic pathway to that leading to the formation of the major cannabinoids Δ^9THC and CBD. These cannabinoids are frequently more abundant in the plant, an example being cannibichromene (CBC). Another example is the cannabinoid Δ^9tetrahydrocannabidivarin (Δ^9THCV), which is closely related to Δ^9THC, and is considered to be characteristic of the sub-species *Cannabis indica*.

The isolation of these minor cannabinoids is hampered by the relatively low abundance and their occurrence in combination with larger quantities of closely related principal cannabinoids. Extraction and purification methods to isolate milligram to gram quantities of the purified compounds (85–98+%) are described.

THCV is obtained by the column chromatography of non-polar extracts of certain cannabis chemovars as an off-white crystalline solid of high purity, which rapidly takes on a purple colour when exposed to air. Identity of the product was confirmed by comparison of GC and HPLC retention time indices to a sample previously authenticated by MS data. The yield from 4.0 g of crude extract (with 55% w/w THCV) was 1.3 g of crystalline THCV (32% yield on mass basis). Melting point, 41–45°C; chromatographic purity, >99%; THC content, 0.5% w/w.

CBC and CBG are obtained as pale yellow semi-solids, (with chromatographic purities in the region of 90% by area normalisation), by column chromatography of non-polar extracts of cannabis herb chemovars. The identity of the products were confirmed by comparison of GC and HPLC retention-time indices with those available in the published literature. The yield of CBG was 300 mg from 100 g of herbal material (0.3%) with a chromatographic purity of 92%. The yield of CBC was again approximately 300 mg from 100 g of material.

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Influence of concentration in diffusing wave spectroscopy measurements of turbid suspensions

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Although light scattering techniques such as Photon Correlation Spectroscopy (PCS) and Mie diffraction may be used to determine particle sizes in the sub-micron range, their application to turbid systems necessitates sample dilution, which may affect the particle size characteristics of the sample. Diffusing Wave Spectroscopy (DWS) extends the principles of PCS to the interpretation of scattering data from turbid, highly scattering, suspensions. In this work the ability of a DWS probe to discriminate concentrated samples according to their size is studied using four polymer latexes with a range of particle sizes and volume fractions (Vf) up to 0.18.

The DWS probe used in this work is that described by Rega et al (2001) and consists of two monomode optical fibres, one of which is used to illuminate the sample while the other collects the light backscattered by the sample. A laser is used as the light source, and a photomultiplier connected to a correlator is used to collect and process the data, which are analysed using custom software. The latexes were prepared using a method similar to that of O'Callaghan et al (1995), and the mean weight average diameters (D_w) of suspensions of the latexes in water were determined using Disc Centrifuge Photosedimentometry (DCP).

Table 1 Characteristic times of the decay of the ACF from DWS

Dw	Decay time ($s^{1/2}$)			
(nm)	Vf = 0.02	Vf = 0.04	Vf = 0.09	Vf = 0.18
90	2.41 ± 0.01	2.02 ± 0.01	2.01 ± 0.01	2.24 ± 0.02
430	7.03 ± 0.05	4.89 ± 0.02	3.22 ± 0.01	2.42 ± 0.01
740	8.56 ± 0.10	7.76 ± 0.04	5.13 ± 0.02	3.82 ± 0.03
1305	15.29 ± 0.00	14.85 ± 0.00	9.68 ± 0.00	7.04 ± 0.00

The DWS data presented in Table 1 may be used to indicate the sensitivity of the DWS probe to changes of particle size in the latex suspensions. It is seen that the characteristic decay time of the ACF of the scattered intensity is a good indicator of particle size in the range of particle sizes studied and for volume fractions of up to 0.09. At a volume fraction of 0.18, the resolution of the probe is limited for particles smaller than approximately 500 nm, but differences in size for larger particles can still be resolved.

The modular design of the DWS equipment, together with its ability to monitor particle size differences in concentrated suspensions suggest the potential of this equipment as a tool for in-line monitoring of particle size in pharmaceutical size reduction processes.

O'Callaghan KJ et al (1995) *J. Polym. Sci. Polym. Chem. Ed.* **33**: 1849–1857

Rega, C., et al (2001) *Appl. Opt.* **40**: 4204–4209

022

Sensitivity and specificity enhancement in fluorescent analysis

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In both microanalysis and drug delivery there is a continuous drive to reduce measurement duration and volume. Fluorescent analysis is widely used as a detection technique in diagnostics, drug screening and monitoring of drug delivery due to its minimal effect on the sample (Fritschy & Härtig 2000). The major

problem associated with the use of fluorescent techniques, however, lies in discriminating between non-specific background fluorescence and the specific signal (Billinton & Knight 2001).

A novel, generic method is described, which has been developed through the combination of fluorescent lifetime analysis, self-quenching and photobleaching. Photobleaching experiments show an enhanced signal, along with a reduction in the levels of background fluorescence. Fluorescent lifetime analysis shows improved specificity and sensitivity, using a single fluorescent dye. By exploiting the fluorescent properties of several widely used dyes (Calcein, pyrene and perylene), this technique can be applied to improve the sensitivity of many existing assays by decreasing detection limits.

Billinton, N., Knight, A. W. (2001) *Anal. Biochem.* **291**: 175–197

Fritschy, J.-M., Härtig, W. (2000) *Encyclopaedia of life sciences.* http://www.els.net

023

The association between the concentration of total plasma homocysteine detected by HPLC-ECD and the risk among ischaemic stroke patients in Taiwan

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Many case-control and cohort studies have identified a strong, independent and dose-related association between moderately elevated total plasma homocysteine (tHcy) and atherosclerotic vascular disease, including stroke, coronary heart disease and peripheral vascular disease (Hankey & Eikelboon 1999).

From November 1, 1999, to April 30, 2001, we conducted hospital cases with a first-ever stroke in Armed Forces Taoyuan General Hospital. Within 7–10 days of the acute stroke event, an overnight fasting venous blood sample with EDTA as anticoagulant was used for the measurement of tHcy, folate and cobalamin. For tHcy analysis, venous blood samples were centrifuged within 30 min at 2000 rev min^{-1} for 10 min. The plasma was then separated and stored at –70°C until analysis. Concentrations of tHcy were measured by high-performance liquid chromatography–electrochemical detector (HPLC-ECD) assay as we developed. Plasma folate and cobalamin were determined by automated chemiluminescence immunoassay (ACLIA).

An ion-paired reversed-phase HPLC-ECD method for the suitable assay of tHcy was developed in this study. The optimal separation of homocysteine was achieved at a mobile phase of pH 2.6, 0.01 M monosodium phosphate buffer (containing 26 mM octane sulfonate)–methanol, 81:19, and 700 mV at E2 (ECD) was employed. The extraction ratio of tHcy ranged from 96.7% to 106.2% at concentration of 0.5–5 $\mu g\,mL^{-1}$ in plasma. The limits of quantitation for intra-assay and inter-assay were 0.15 $\mu g\,mL^{-1}$ (1.12 μM) and 0.27 $\mu g\,mL^{-1}$ (1.97 μM), respectively. A case-control study of 231 subjects (117 cases with acute ischaemic stroke and 114 control subjects) was conducted. Our results revealed that mean fasting tHcy was significantly higher in ischaemic stroke patients than in control subjects (7.90 ± 3.93 vs 6.31 ± 2.27 μM, $P < 0.001$). These differences remained statistically significant after adjustment for age, sex, conventional risk factors, folate and cobalamin levels ($P = 0.002$). Plasma folate levels were significantly lower in ischaemic stroke than in controls (9.32 ± 6.04 vs 11.19 ± 6.82 $ng\,mL^{-1}$, $P = 0.028$). There was no difference in plasma cobalamin levels between ischaemic stroke and controls (576.50 ± 410.45 vs 616.73 ± 311.30 $pg\,mL^{-1}$, $P = 0.403$). Compared with the lowest quartile (< 4.86 μM), the highest quartile (> 8.50 μM) of tHcy was associated with an adjusted odds ratio (OR) of ischaemic stroke of 1.85. The adjusted OR for ischaemic stroke of a 5 μM tHcy increment was 2.31 ($P = 0.004$). It is suggested that increasing tHcy should be a strong and independent risk factor of ischaemic stroke in Taiwan.

Hankey, G. J., Eikelboon, J. W. (1999) *Lancet* **354**: 407–413

024

A voltammetric method for L-Ascorbic acid assay

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Voltammetric techniques based on the measurement of current resulting from an oxidation or reduction at an electrode surface following the application of a potential difference at an electrochemical cell, have assumed an important place in the arsenal of analytical techniques for the identification and determination of trace concentrations of many organic, organometallic and inorganic biological molecules. L-ascorbic acid (AA) is found throughout the plant and animal kingdoms. A major function of AA is as an antioxidant, protecting tissues from harmful free radicals and maintaining certain enzymes in their reduced form. The redox chemistry of AA in solution phase is extremely complicated, involving numerous radical, ionic and complexation species. Even the thermodynamics of this redox reaction are uncertain because of the instability of the AA oxidation product (dehydroascorbic acid) in aqueous environments. Thus, the formal electrode potential (E°) of AA with respect to the saturated calomel electrode can be estimated in a range from –190mv to 600mv, although adsorption of AA on the electrode might distort such estimates.

This study is based on an analysis of the I-E curves for AA at varying pH values. An analysis was also carried out on the influence of the concentration of the electroactive species, the velocity of the potential sweep on the I-E curves. Voltagrams of AA on a gold electrode show the existence of two oxidation waves but no reduction wave. The peak height of first oxidation wave is used for AA assay. It is shown that AA can be determined in aqueous media in the range 0–175 $\mu g\,mL^{-1}$ by linear sweep voltammetry on a gold electrode. The optimum pH and sweep rate for AA determination were 3.2 and 7500 $mv\,s^{-1}$, respectively. Under these conditions, the detection limit of the method was 0.2 $\mu g\,mL^{-1}$. Repeatability of the method expressed by relative standard deviation for AA was calculated 0.83, 3.7 and 13% at concentrations 50, 10 and 1 $\mu g\,mL^{-1}$, respectively. The calibration curve was linear over the range 0–175 $\mu g\,mL^{-1}$ ($r^2 = 0.9977$, $P < 0.001$).

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Differentiation between bovine and porcine gelatins by principal component analysis

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Gelatin is derived from collagen (a main component of connective tissue of animals). It has a very large number of applications in the pharmaceutical, food and adhesive industries and photography. Similarity in structure and properties makes it very difficult to differentiate between gelatins. Chemometric methods as principal component analysis can help classification and characterization of components when there is a large amount of similar data. Principal component analysis is a projection method that reduces the dimensionality in a data matrix while retaining most significant information (Ren et al 1997). In this study, 10 bovine and 5 porcine gelatins were examined. The analysis procedure involved complete hydrolysis of samples by classic hydrolysis to release their amino acids and separation and determination of amino acids by reversed-phase high-performance liquid chromatography (HPLC) followed by precolumn derivatization (Reichlet et al 1999). Orthophtaldialdehyde (OPA) and 4-chloro-7-nitrobenzofurazane (NBD-Cl) were derivatization reagents. 3-Mercaptopropionic acid was used as a thiol reagent to enhance fluorescence emission (Mengerlink et al 2002). Twenty peaks resulted from HPLC analysis. One of these peaks was very typical in bovine

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gelatin. Some factors of peak report (such as height, area, percent of area and width) were used to make matrixes. Principal component analysis by MATLAB program was used to differentiate between these gelatins.

Ren, Y., et al (1997) *Talanta* **44**: 1823–1831
Reichlet, M., et al (1999) *Chromatographia* **49**: 59–65
Mengerlink, Y., et al (2002) *J. Chromatogr. A* **949**: 9–124

026
Determination of colorant matter mixtures in foods by spectrophotometry

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Pigments are important food additives and are used commercially in dyeing foods, drinks, medicines and cosmetics. These colorants normally contain azo and aromatic ring structures, therefore they are somewhat harmful to health (Yang et al 1989). Generally, a number of official methods such as extractive, chromatographic, gravimetric, titrimetric and spectrophotometric procedures are reported for the determination of colour additives. Our intent is to design a method for the simultaneous determination of the most commonly used additives in any commercial food. For this purpose different chemometric algorithms, namely classical least squares (CLS), principal component regression (PCR) and partial least squares (PLS-1 and PLS-2), have been widely used in a number of spectrophotometric analyses and satisfactory results have been reported. In the calibration phase of such chemometric procedures, the mathematical model is developed that relates the multiple spectral intensities from many calibration samples to the known concentration of the analytes in this samples. Then this method is used for the prediction of the analyte content in the unknown sample. The efficiency of different multivariate calibration models was compared and discussed in many papers and it can be assumed that PLS algorithm is very flexible and for low precision data can give better results (Marten et al 1993). In this work a new, simple, sensitive and inexpensive method for simultaneous determination of carmoisine, ponceau4R and sunset yellow in mixtures is proposed. The data obtained from experiments were processed by PLS method, using the normal, first- and second- derivative absorbance spectra. Forty mixtures of colorants with three combinations were evaluated and the proposed method was applied satisfactorily to the determination of colorants in several commercial food products. The relative standard error (R.S.E.) for the carmoisine, ponceau4R and sunset yellow were calculated as 4.8, 5.6 and 3.5 %, respectively, and the result showed that calibration based on PLS chemometric method are suitable for determination of colorant matters in food.

Marten, et al (1993) *Multivariate calibration*. John Wiley
Yang, et al (1989) *New edition handbook of food additives*. Chinese Agriculture Publishing Press

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