### 085
### Temperature controlled Raman microscopy for the imaging of crystallisation and polymorphic transitions in frozen systems

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Mannitol is extensively used as an excipient in freeze-dried pharmaceutical formulations since it promotes the formation of an elegant product. However, there are also problems associated with its use, including crystallisation upon reheating leading to vial cracking (Williams et al 1986) and reduction in its ability to act as a cryoprotectant (Izutsu et al 1993). Previous work (Kett et al 2003) using DSC, cold stage microscopy (CSM) and temperature controlled X-ray diffractometry (TXRD) has indicated that mannitol in frozen 3% w/w solutions crystallises into the $\beta$ polymorph. In this investigation, this process has been probed using temperature controlled Raman microscopy (TRM), which enables the location of specific chemical functionalities within a 3D structure. The objectives were to locate the crystallising mannitol within the frozen solution and if possible to determine into which form(s) crystallisation is preferred. Mannitol (Roquette) was used as 3% w/w solutions in distilled water throughout the study. Samples were mounted in a Linkam TMS 600 biological cryostage with $N_{2(g)}$ purge and temperature controller and analysed using a Raman microscope (Horiba Jobin-Yvon LabRam HR800) equipped with an x-y motorised stage and an Olympus BX41 microscope ($\times$ 50 magnification was used, with an excitation wavelength of 514 nm). Raman data of single crystals of the $\alpha$, $\beta$ & $\gamma$ polymorphs were acquired in each of the three possible orientations (x, y and z) with respect to the path and polarisation of the incident beam. The mannitol solution was cooled at 10°C min$^{-1}$ to −30°C before annealing and data acquisition. Raman maps at the edges of the frozen annealed samples were recorded at 10-min intervals in the 2600–3600 cm$^{-1}$ spectral region over an area of $12 + 28\,\mu m^2$ using a spacing of $3\,\mu m$. The spectral region for mapping was chosen to enable simultaneous monitoring of CH and OH vibrational modes of mannitol. The proportion of the spectrum arising from the different polymorphs and orientations was determined using linear combinations to fit the model spectra to the spectra at each point in the map. Significantly different spectra were obtained for each of the polymorphs and each of their orientations, allowing assignment of the polymorphic type and the orientation with respect to the incident laser pathway. The Raman maps obtained confirmed the presence of a boundary region that was rich in mannitol and also revealed that the mannitol concentration in this region increased with time. It was also demonstrated that, over time, this mannitol-rich region expanded inwards with the main icy body of the drop receding. Temperature controlled Raman microscopy has confirmed that the events observed using DSC correspond to crystallisation of the mannitol. TXRD had indicated that this was exclusively into the $\beta$ form. However, Raman microscopy has shown that both the $\beta$- and $\alpha$-forms are present. The technique has located, for the first time, the crystallising material within this sample. It clearly has potential for further use in following crystallisation processes in frozen pharmaceutical formulations.

Izutsu, K.-I. et al (1993) *Pharm. Res.* **10**: 1232–1237
Kett, V. L. et al (2003) *J. Pharm. Sci.* **92**: 1919–1929
Williams, N. A. et al (1986) *J. Parent. Sci. Technol.* **40**: 135–141

## Poster Session 2 – Drug Delivery

### 086
### Delivery of temozolomide hexyl ester prodrug through skin from VE TPGS microemulsion systems

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In an attempt to develop a skin-deliverable congener for temozolomide (TMZ) to treat skin cancers, temozolomide hexyl ester (TMZ-HE) was identified as a potential prodrug because it is readily converted into parental temozolomide acid (TMZA) and has a promising permeability coefficient ($K_p$) and flux value (J) through rat and human skin (Wang et al 2002). TMZ-HE also demonstrated an equal cytotoxicity against the cancer cell lines as TMZ and TMZA, and significantly inhibited tumour growth in mice inoculated with melanoma via topical administration (Wang et al 2004). A formulation to deliver TMZ-HE through skin must demonstrate a high drug loading and stability of the system and the prodrug. Microemulsions (ME) were proposed as they are promising vehicles for skin delivery of drugs, demonstrating high drug loading capacity and a penetration enhancer effect (Kreilgaard 2002). As a starting point, we chose Vitamin E-TPGS (VE-TGPS) as a surfactant, oleic acid (OA) or isopropyl myristate (IPM) as an oil phase and isopropyl alcohol (IPA) as co-surfactant where appropriate. A gel VE-TPGS ME system was developed and a number of formulations were prepared (Table 1). The ME system and formulations were characterized using polarization microscopy and freeze fracture electron microscopy (FFEM). In vitro permeation of TMZ-HE from the ME formulations was studied using silicone membrane for 8 h and full-thickness hairless mice skin for 24 h with Franz diffusion cells. In the mouse skin permeation studies, TMZ-HE was extensively hydrolyzed by skin esterases to give TMZA (Wang et al 2002). The cumulative permeated TMZ-HE and TMZA were assayed using HPLC. The flux value (J) and permeability coefficient ($K_p$) were calculated. Skin stripping was used to determine TMZ-HE retention in the skin following skin permeation experiments. Drug retained in the skin were extracted and determined by HPLC. Compared with an aqueous control, VE-TPGS MS formulations increased the prodrug loading 35- to 75-fold and enhanced the permeation rate up to 7-fold through silicone membrane compared (Table 1). The fluxes and permeability coefficients of the prodrug from the OA ME and the IPM ME were significantly higher than from neat OA and IPM preparations. The OA ME showed more than 2-fold drug retention in stratum corneum (SC) than the IPM ME. In conclusion, VE-TPGS ME systems were showed to be a stable and effective vehicle resulting in high TMZ-HE loading and a permeation enhancing effect. The inclusion of OA is promising for a topical formulation, while IPM may promote transdermal absorption.

**Table 1** Composition of the ME formulations, drug loading and drug flux through silicone membrane[a]

| Formulation | Drug load (mg mL$^{-1}$) | Flux (J) (nmol cm$^{-2}$ h$^{-1}$) | Ingredient (% w/w)[b] | | | | |
|---|---|---|---|---|---|---|---|
| | | | W | OA | IPM | V | IPA |
| ME 1 | 15.3 | 67.64 ± 2.23 | 20 | 50 | — | 30 | — |
| ME 2 | 20.8 | 79.01 ± 5.04 | 10 | 50 | — | 40 | — |
| ME 3 | 31.8 | 101.00 ± 1.83 | 11 | 33 | — | 56 | — |
| ME 4 | 22.4 | 162.86 ± 19.26 | 8 | — | 35 | 57 | — |
| ME 5 | 21.6 | 263.58 ± 4.90 | 10 | — | 40 | 40 | 10 |
| Control[c] | 0.4 | 35.14 ± 5.95 | — | — | — | — | — |

[a]n = 3; mean ± s.d, [b]W = Water, V = VE-TPGS, [c]10% w/w propylene glycol in water.

Kreilgaard, M. (2002) *Adv. Drug Deliv. Rev.* **1**: S77–S98
Wang et al (2002) CN 02131346.6
Wang et al (2004) CN2004100686.80.7

### 087
### Skin permeation studies of transdermal formulations of fluorescein through porcine ear stratum corneum

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Pressure sensitive drug-in-glue transdermal formulations offer a simple means of preparing a formulation for application to the skin. This study compared the rate of drug release from three commercially available acrylic based glues and noted the effect of in vitro permeation with the addition of permeation enhancers. Disodium fluorescein was used as a model drug in all cases. The rate of drug release was measured from the patches into phosphate buffer solution (PBS) pH 7.4 over 72 h. The drug flux was calculated as the gradient of the mass released per cm$^2$ versus the time, this data is shown in Table 1. The results show that Durotak 87900A had the lowest rate of drug release and this glue was used in subsequent studies to note the effect of permeation enhancers on skin permeability. The effect of penetration enhancers, oleic acid and polyethylene glycol 400(PEG), singly or synergistically, on fluorescein permeation across full thickness dorsal porcine ear skin in static Franz diffusion cells was monitored. Porcine skin was excised from fresh carcasses and full thickness dorsal skin was dissected carefully with scalpel and scissors. PBS pH 7.4 was used as the receiver medium

and this was stirred continuously using Teflon coated magnetic stirrer. The skin was mounted between the donor and receiver compartments with the stratum corneum facing the donor compartment. A 2-$cm^2$ patch was used in each experiment; the patches matched the diameter of the Franz cell. A 1-$cm^3$ sample was drawn and replaced with freshly prepared PBS at set time points; 0, 4, 24, 48 and 72 hours. The skin was then cut into pieces, homogenized in 10 mL PBS, stored at 37°C overnight and the homogenate was filtered using a 0.2 $\mu$m filter before analysis. All analysis was carried out using fluorescence-spectrometry at excitation wavelength 490 nm and emission 515 nm. The flux, J, and skin concentration of the drug were calculated for these new formulations and the data is shown in Table 2. These results suggest that oleic acid enhances the permeation of fluorescein through porcine skin; this may be explained by earlier studies suggesting the induction of phase separation of stratum corneum lipids (Moser et al 2001), which led to increased diffusivity. PEG did not enhance the permeation of the model drug alone, yet in combination with oleic acid an enhancement in skin concentration was observed. The solubility of the drug in the matrix mixed with and without enhancers needs to be assessed to examine the effect of PEG and oleic acid on fluorescein solublity.

**Table 1** Comparison of steady state flux of fluorescein from 2 cm diameter patches containing 2.5 mg $mL^{-1}$ of the model drug

| Matrix (Durotak) | Flux ($\mu g\,cm^{-2}\,h^{-1}$) |
|---|---|
| 87-201A | 21.242 |
| 87-900A | 1.631 |
| 87-2677 | 5.377 |

**Table 2** Effect of 5% oleic acid, 5% PEG and 3.3% oleic acid + 5% PEG on the flux and skin concentration of fluorescein through porcine ear skin delivered by transdermal patches formulated using Durotak 87-900A

| Formulation | Flux ($\mu g\,cm^{-2}\,h^{-1}$) | Skin concn ($\mu g\,g^{-1}$) |
|---|---|---|
| 87-900A | 1.866 | 22.687 |
| 87-900A + Oleic acid | 2.904 | 26.871 |
| 87-900A + Oleic acid + PEG | 1.347 | 28.227 |
| 87-900A + PEG | 0.147 | 11.077 |

Moser, K. et al (2001) *Eur. J. Pharm. Biopharm.* **52**: 103–112

### 088
### Hydrophobic ion-pairing as a strategy to increase drug release from polymer devices

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The release of drug from non-degradable, non-swellable polymer matrices is affected by two important variables (Malcolm et al 2002) – the inherent solubility of the drug to be released in the polymer and its diffusivity within the polymer. For ionizable molecules that might otherwise have poor permeation characteristics, the formation of a hydrophobic ion-pair complex might be a useful strategy for enhancing release, whereby the hydrophobic ion-pairing will increase the solubility of the drug within the polymer. However, the ion-pair will be larger than the parent drug molecule and therefore diffusivity is likely to be reduced. For this enhancement strategy to work the increase in hydrophobicity of the ion-pair must greatly outweigh the decrease in diffusivity. Studies have shown that fluoxetine HCl releases poorly from silicone elastomer due to its hydrophillicity. In this study, sodium 1-hexanesulfonate ion-pair complexes were formed to evaluate potential for enhanced release. Fluoxetine HCl and sodium 1-hexanesulfonate were dissolved in HPLC grade water, and the solutions mixed to form the ion-pair. The ion-pair was extracted into HPLC grade dichloromethane, which was then removed using a rotary evaporator. Reservoir silicone devices of 10% drug-loading were formulated containing fluoxetine HCl, a mix of fluoxetine HCl and sodium 1-hexanesulfonate, and the isolated ion-pair. The reservoir device consisted of a drug-loaded core completely encased by a layer of silicone. In vitro release studies were performed employing a release medium of 30% ethanol and 70% HPLC grade water. Release data was determined by HPLC. The release of the ion-pair was approximately 2000% of fluoxetine HCl. Fluoxetine HCl and the simple mix of the two parent compounds had virtually no difference in the amount of drug released. The enhanced release is attributed to the greater hydrophobicity of the ion-pair complex. Consequently the obtained results demonstrate proof of concept for the ion-pair theory as an enhancement strategy to augment the release of drug from reservoir polymer devices. The next phase of this work will be to investigate the effect of the number of carbons and molecular size of the ion-pairing agent on the release dynamics of fluoxetine from the polymer devices.

Malcolm, R. K. et al (2002) *Biomaterials* **23**: 3589–3594

### 089
### Investigations into the use of gelatin and polyacrylamide gels as skin simulants for jet injections

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Jet injection (needle free) technology works by forcing an ultra-fine stream of liquid medication at high velocity (> 100 $ms^{-1}$) through a tiny orifice (400–100 $\mu$m) to penetrate the skin. A recent study (Schramm-Baxter et al 2004) showed that a mechanistic understanding of jet injection into skin might be developed using cross-linked polyacrylamide gels as mechanical simulants for skin. However, such gels are expensive and are susceptible to variation in preparation due to the large number of components. The aims of this work are, firstly, to investigate the potential use of gels prepared from gelatin compared with polyacrylamide gels and, secondly, to assess the suitability of Young's modulus (in unconfined compression) as a qualitative/quantitative strength measure with which to correlate gel properties to jet injection parameters in relation to skin in vivo. Polyacrylamide gels (5–30% w/w) were prepared by in situ co-polymerisation (Schramm-Baxter et al 2004). Gelatin gels (16–52% w/w) were prepared by dissolving gelatin in hot water (65°C) and cooling (4°C) to set. Both gels were prepared as cuboids at a range of dimensions (cross-sectional area, $A_0$, and length, $l_0$) in moulds. Gel strength parameters in compression (e.g. apparent Young's modulus, $E$, and work of fracture $W$) were determined (Texture Analyser TAXT21, Stable Microsystems, UK) for a range of gel dimensions, probe diameters and probe velocities ($v$). For visualization experiments, gel samples were injected with dye solution (sulforhodamine B) using a commercial jet injector (MHI 500; Medical House Industries Ltd), and penetration characteristics correlated with gel strength parameters. Table 1 shows representative data for the series of gelatin gels. Similar trends (i.e. increase in E and W and decrease in normalized penetration depth with increasing gel concentration) were shown with the polyacrylamide gels and with different gel dimensions. Both gel types displayed similar visual injection characteristics. Changing probe speed indicated that E did not display rate dependency within the range studied. It was concluded that both gels are equivalent as visualization aides. Young's modulus, as determined by unconfined compression may be a suitable qualitative parameter for jet penetration studies, but may not be suitable quantitatively and thus applicable to skin in vivo.

**Table 1** Selected strength measures for gelatin at room temperature (~18°C)

| Concn gelatin (% w/w) | $E$ (±s.d.) (MPa) | $W$ (±s.d.) (mJ) | Normalized penetration depth (±s.d.) |
|---|---|---|---|
| 15.7 | 1.24 ± 0.04 | 1.49 ± 0.94 | 0.74 ± 0.17 |
| 20.1 | 1.65 ± 0.10 | 1.68 ± 0.88 | 0.8 ± 0.05 |
| 34.9 | 4.02 ± 0.47 | 3.90 ± 1.00 | 0.5 ± 0.08 |
| 52.3 | 9.22 ± 0.99 | 18.71 ± 5.29 | 0.41 ± 0.09 |

Penetration depth = 2 mm, $l_0$ = 30 mm, $A_0$ = 500 $mm^2$, $v = 10^{-4}$ $ms^{-1}$.

Schramm-Baxter, J. et al (2004) *J. Biomech.* **37**: 1181–1188

### 090
### Effect of a model lipophilic compound on the phase behaviour of hydrophilic self-micro emulsifying lipid formulations

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There has been growing interest in the self-emulsifying lipid technology in recent years as an approach to improve the oral bioavailability of poorly

water-soluble compounds. Nonetheless, for the design of successful lipid formulations with a potential to maximize the bioavailability of lipophilic drugs, key elements in the lipid composite in relation to the physicochemical state of drug after dispersion need to be optimized. This process includes determination of drug solubility in the lipid matrix, controlling factors that influence the hydrophilicity of the lipid vehicle, such as type of oil, oil–cosurfactant ratio, type of surfactant and the inclusion of hydrophilic co-solvent. Also, it is important to investigate the effect of drug on the emulsification performance of these systems in suitable media simulating the physiological conditions of the gastrointestinal tract. In this study, various lipid formulations were optimized for oil-in-water micro-emulsion drug delivery systems. The effect of ibuprofen (a model lipophilic drug) on the emulsification behaviour of these systems was also investigated by conducting phase behaviour studies and particle size measurements. Representative ternary mixtures of Miglyol 812, Imwitor 308 and various non-ionic hydrophilic surfactants; Cremophor RH40, Tagat S2 or Tagat S were blended at 10% intervals. One gram of each mixture was introduced into 100 mL of Milli-Q water or media simulating gastrointestinal fluids and emulsified at 37°C for 15 min by gentle agitation. Pseudo-ternary diagrams were constructed by blending oil mixtures of {Miglyol 812/Imwitor 308} 5:5 and increasing concentrations of surfactant (10–90% w/w), demineralized water was then sequentially added at 2.5% w/w interval into the oil mixture under agitation. Systems were then allowed to equilibrate at 37°C for a few minutes. The addition of water was continued until phase change was observed. Phases were identified as isotropic $L_2$ (oil-based liquids) or $L_1$ (aqueous-based liquids), liquid crystalline phases (LC) and multiphasic turbid mixtures ($L_1 + L_2$). The phase behaviour was also carried out in lipid mixtures containing Ibuprofen at 100 mg g$^{-1}$. Optimum self-micro-emulsifying systems were obtained by using oil blends of {Miglyol 812/Imwitor 308} at ratio of 5:5 in the case of Cremophor RH40 or Tagat S2 and 4:6 for Tagat S. Ternary phase equilibrium diagrams reveal extended regions of $L_2$ phase when no drug was added to the lipid mixtures. However, in the case of lipid formulations containing ibuprofen at 100 mg g$^{-1}$, small limited areas of $L_2$ phase was observed, which suggests that the drug can interfere in the mechanistic processes of emulsification. In the case of lipid mixtures containing Cremophor RH40, the emulsification performance has shown high resistance to the effect of temperature or electrolytes present in the emulsification media. On the contrary, for formulations containing Tagat S2, ionic strength of the media has dramatically influenced emulsification performance. This effect, however, was retarded in the case of using Tagat S as a nonionic surfactant, which has a relatively high HLB value. To design successful self-micro-emulsifying lipid systems, pre-formulation studies that affect the performance of resultant dispersions of these systems with and without drug should be carried out using suitable emulsification media.

## 091
## Gamma-irradiation of lyophilised wafers

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Lyophilised wafers are being developed as stable drug delivery systems that can be applied directly to the surface of suppurating wounds. The rate at which these freeze-dried polymers absorb wound exudate and form viscous gel systems can be tailored to suit the varying degrees of suppuration encountered with a wide variety of wound types. The risk of contamination of the wound-site by exogenous bacteria can be minimised by sterilisation of the applied treatment (Walker et al 2003). To test the performance of lyophilised wafers following sterilising doses of gamma-irradiation (25 and 40 kGray), rheological studies on two separate formulation types were undertaken. Type I wafers were formed from solutions of low molecular weight sodium alginate (SA), viscosity modified with high molecular weight methylcellulose (MC) whereas Type II wafers consisted of combinations of xanthan gel (XG) and MC. Type I wafers were produced by the initial addition of powdered MC to hot (70°C) stirred solutions of SA (5% w/v) followed by casting to circular moulds (6-well polystyrene culture plates). After cooling to below the 'incipient gelation temperature' (IGT) of MC (Sarkar 1979) the samples were freeze-dried. For Type II wafers, this process was repeated using XG (2% w/v) in place of SA. The resultant shaped wafers were easily removed from their moulds and stored in a desiccator before gamma-irradiation within a Cobalt-60 source. A third of the wafers were kept as non-irradiated reference samples. Following irradiation, individual wafers were reconstituted as their pre-lyophilised solutions or gels by the addition of precisely 5 mL of distilled water. Rheological testing using a cone-and-plate rheometer under isothermal conditions of continuous shear ('shear-rate sweep cycle') produced definitive viscosity profiles (shear-stress as a function of shear-rate) for individual samples. The flow curves produced were quantified using the 'Power Law' equation, modified to account for a yield stress evident for Type II wafers containing xanthan:

$$\sigma = \eta' \gamma^c + \sigma_\circ,$$

where $\sigma$ = shear stress (Pa); $\eta'$ = viscosity coefficient; $\gamma$ = shear rate (s$^{-1}$); c = 'rate index' of pseudoplasticity and $\sigma_\circ$ = yield stress (Pa). Reference to the computed values of $\eta'$ (Table 1), indicates that degradation of Type I wafers had occurred at the lower sterilising dose of 25 kGy. In surprising contrast, the effects of gamma-rays on Type II wafers was less noticeable, particularly for those wafers unmodified with MC. At the higher dose of 40 kGy, no discernable decrease in $\eta'$ was evident. Indeed, unmodified XG appeared to show an overall increase in consistency as well as a measurable increase in yield stress. These observations were in agreement with earlier work (Hanna et al 1997) suggesting that xanthan was able to tolerate the effects of sterilising doses of radiation. Production of a sterile, wound-healing wafer appears feasible.

**Table 1** Rheological data for irradiated and non-irradiated wafers

| Wafer | Composition | Dose (kGy) | $\eta'$ | c | $\sigma_\circ$ |
|---|---|---|---|---|---|
| Type I | 100:0 | 0 | 0.20 | 0.82 | — |
| (SA:MC) | | 25 | 0.02 | 0.80 | — |
| | 90:10 | 0 | 0.61 | 0.74 | — |
| | | 25 | 0.04 | 0.86 | — |
| Type II | 100:0 | 0 | 0.24 | 0.54 | 1.12 |
| (XG:MC) | | 25 | 0.21 | 0.55 | 1.27 |
| | | 40 | 0.30 | 0.52 | 1.76 |
| | 90:10 | 0 | 0.39 | 0.51 | 1.28 |
| | | 25 | 0.16 | 0.61 | 2.32 |
| | | 40 | 0.12 | 0.64 | 2.02 |

Hanna et al (1997) *J. Food Sci.* **62**: 816–820
Sarkar, N. (1979) *J. Appl. Polym. Sci.* **24**: 1073–1087
Walker, M. et al (2003) *Biomaterials* **24**: 883–890

## 092
## Optimisation of low-frequency ultrasound parameters for transcutaneous protein delivery

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Transdermal delivery using low-frequency ultrasound has been largely focused on small drug molecules and knowledge on macromolecular delivery is scant. As we are interested in the application of ultrasound waves for vaccine delivery to the skin, the aim of this study was to establish optimum ultrasound setting for parameters such as type of wave (continuous or pulse), application time, duty cycle, pulse length and possible synergistic action with chemical enhancer (1% SLS aqueous solution) for the transcutaneous delivery of a model antigen (BSA). Permeation studies using Franz cells were conducted with full-thickness rat skin and PBS as the receptor medium. The donor compartment was filled with 2 mL of coupling medium (1% SLS aqueous solution or water) and ultrasound (19% amplitude for SLS and 50% for water; either continuous or pulsatile – 10% or 50% duty cycle) was applied with the transducer being 10 mm from the skin surface. Following ultrasound application, the coupling medium was removed from the donor compartment, the skin was rinsed and blotted dry, and 50 $\mu$L of Iodine-125 labelled BSA was applied onto the skin. Twenty-four hours later, the levels of radioactivity in the receptor compartment and in the skin were measured using a gamma counter. Gel electrophoresis on the receptor phase confirmed the presence of BSA. It was found that application of ultrasound, inclusion of SLS, a pulse wave, 50% duty cycle (0.5 s on 0.5 s off) and longer application time (3 min) compared with absence of ultrasound and of SLS, a continuous wave, 10% duty cycle (0.1 s on, 0.9 s off) and shorter application time (1 min), respectively (Table 1), resulted in greater permeation and deposition of BSA in the skin as expected and as reported previously for small molecular weight drugs (Mitragotri et al 1996). However, the use of longer pulse length (5 s on, 5 s off) resulted in lower protein permeation through the skin compared with a shorter pulse length (0.5 s on, 0.5 s off) even though the total time was the same. This is due to depletion of gas in the coupling medium during the initial long pulses. A shortage of gaseous molecule inhibits effective formation and collapse of bubbles (or microjets), which is believed to cause skin disruption and permeation of drugs. From these

preliminary studies we can conclude that ultrasound does influence permeation of the large molecule BSA into and through the skin, total pre-treatment time matters and duty cycle and pulse length are important parameters. Finally pulse mode is also better than continuous mode even though less sound waves generated.

**Table 1** Amount of labelled BSA that permeated skin at 24 h

| Ultrasound potocol | Amount of BSA, cpm (n = 4) |
|---|---|
| Continuous mode + water | 54 ± 2.4 |
| 0.5 s on pulse wave + 1% SLS (3 min) | 220 ± 46.5 |
| 0.1 s on pulse wave + 1% SLS (3 min) | 112.5 ± 2.1 |
| 0.5 s on pulse wave + water (1 min) | 55 ± 3.6 |
| 5.0 s on pulse wave + water (3 min) | 107 ± 2 |
| 0.5 s on pulse wave + water (3 min) | 154 ± 19.5 |
| Control | 30 ± 7.2 |

Mitragotri, S. et al (1996) *Pharm. Res.* **13**: 411–420

## 093
## Effect of probe distance on sonophoresis of proteins through rat skin

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Low frequency ultrasound (US) is an effective physical enhancer in delivering various molecules through the skin. Among the mechanisms that have been proposed are heating, cavitation (formation and collapse of bubbles in a medium) and acoustic microstreaming (unidirectional flow currents of fluid caused by sound waves). Unfortunately, many important experimental parameters for optimal drug permeation have yet to be established. One of them is the probe distance from the skin surface. So far, most experiments have been conducted at 10 mm. The aim of this study was to establish the relationship between the probe distance and protein permeation through skin. Permeation studies using Franz cells were conducted using full-thickness rat skin as the membrane. The receptor medium was phosphate buffered saline (PBS). The donor compartment was filled with 20 mL of coupling medium (either water or 0.04% SLS aqueous solution) and ultrasound was applied, using a probe placed at varying distances (5, 7.5, 10, 12.5, 15 mm) from the skin. The ultrasound protocol was fixed at 30% amplitude and 0.5 s on, 0.5 s off pulse wave for a total sonication time of 2 min. Following US application, the coupling medium was removed, the skin was rinsed, blotted dry and 50 $\mu$L of iodine-125 labelled bovine serum albumin (BSA) was applied onto the skin. After 24 h, 400 $\mu$L samples were taken from the receptor compartment and the radioactivity level was counted using a gamma counter. Gel electrophoresis on the receptor phase confirmed the presence of BSA. Increasing the probe distance was found to result in decreased protein permeation when water was used as coupling medium. The decreased protein permeation is thought to be due to decreased damage caused by mechanical effects of US to the skin and was reflected in a reduced number of pits formed with increasing probe distance when aluminium foil was used as the membrane and US was applied. The pits on the aluminium foil give an indication of indentations that may be formed in skin by US via microjet formation on skin and through which protein can permeate into the skin and then into the receptor phase. Interestingly when 0.04% SLS aqueous solution was used as the coupling medium, a different trend was observed. Increasing distance from 5 to 12.5 mm resulted in increased permeation; further increase in probe distance (to 17.5 mm) resulted in decreased in permeation. This increased and decreased permeation with increasing probe distance reflects the increase and decrease in pitting on aluminium foil when the latter was used as the membrane. Although it is not clear why the presence of 0.04% SLS causes such as difference in permeation profile, this study has highlighted the importance of probe distance on permeation and the existence of different optimal distances for different coupling media.

## 094
## Effect of coupling medium volume on sonophoresis of proteins through rat skin

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Low-frequency ultrasound has been found to increase transdermal drug delivery; and proposed mechanisms include thermal effect, acoustic cavitation (formation and collapse of bubbles in a medium) and acoustic streaming (Lavon & Kost 2004). During the cavitation process, 2 types of cavitation can occur: stable cavitation (regular bubble formation and collapse) and inertial cavitation (violent bubble formation and collapse) (Mitragotri & Kost 2004). However, inertial cavitation is thought to increase skin permeability by the formation of microjet bubbles. In most studies, ultrasound has been applied via small volumes (e.g., 1 mL) of coupling media. The disadvantages of small volumes are loss of coupling media due to splashing and excessive temperature rise. This could be remedied by increasing the volume of the coupling medium. However, ultrasound (US) wave generation and propagation in large volumes and the effects on transdermal delivery are unknown. The aim of this study was therefore to explore the relationship between coupling medium volume and transdermal protein delivery. Permeation studies using Franz cells were conducted with full-thickness rat skin as the membrane and PBS as the receptor medium. The donor compartment was filled with different volumes (10, 20, 30, 40, 50 mL) of coupling medium (water) and US was applied at 30% amplitude, probe distance of 5 mm and pulse wave of 0.5 s on, 0.5 s off for a total sonication time of 2 min. Following US application, the coupling medium was removed, the skin was rinsed and blotted dry and 50 $\mu$L of iodine-125 labelled bovine serum albumin (BSA) was applied onto the skin. After 24 h, the levels of radioactivity in the receptor compartment and in the skin were measured using a gamma counter. Gel electrophoresis on the receptor phase confirmed BSA presence. It was found that that increasing the coupling medium volume resulted in increased protein permeation into the receptor phase. There could be two possible explanations for the higher protein permeation when large volumes of coupling medium were used. Firstly, the effect of atmospheric pressure caused by the mass of water found on top of the skin may enhance the impact of microjets hitting the skin surface and result in deeper and greater protein penetration. Secondly, the abundance of gaseous molecules in larger volumes may result in increased inertial cavitation, air bubbles being essential for cavitation to occur. Further work must be conducted to assess the damage caused to skin by larger volumes of coupling medium to skin.

Lavon, I., Kost, J. (2004) *Drug Disc. Today* **9**: 670–676
Mitragotri, S., Kost, J. (2004) *Adv. Drug Deliv. Rev.* **56**: 589–601

## 095
## Effect of surfactant concentration on sonophoresis of proteins through rat skin

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The synergistic effect of sodium lauryl sulphate (SLS) normally at 1% and low-frequency ultrasound (US) in transdermal drug delivery has been established (Mitragotri et al 2000). However, due to the potential skin irritancy of SLS, the use of 1% surfactant concurrently with US could be pharmaceutically unacceptable. The aim of this study was to explore the relationship between surfactant concentration and enhancement of transdermal protein delivery by low-frequency ultrasound. Permeation studies using Franz cells were conducted using full-thickness rat skin as the membrane, and the receptor phase (4 mL) was phosphate buffered saline (PBS). The donor compartment was filled with 20 mL of coupling medium (water or SLS aqueous solution at different concentrations: 0.001, 0.004, 0.01, 0.04, 0.1 and 1%) and ultrasound (30% amplitude, 0.5 s on, 0.5 s off pulse wave for a total sonication time of 2 min) was applied with the transducer being 5 mm from the skin surface. Following ultrasound application, the coupling medium was removed from the donor compartment and the skin was rinsed and blotted dry, and 50 $\mu$L of iodine-125 labelled bovine serum albumin (BSA) was applied onto the skin. After 24 h, the levels of radioactivity in the receptor compartment and in the skin were measured using a gamma counter. Gel electrophoresis on the receptor phase confirmed BSA presence. It was found that when 0.001% and 0.004% SLS solutions were used as the coupling medium, protein permeation into the receptor phase was similar to those obtained when water was used as coupling medium. Surprisingly, an increase in SLS concentration to 0.01% and 0.04% resulted in a marked decrease in protein permeation. A further increase in SLS concentration to 0.1% to 1% resulted in increased protein. To understand the protein permeation profile at the different surfactant concentrations, the surface tension of the different SLS solutions were measured and aluminium foil pitting experiments were conducted. The latter are conducted by applying ultrasound waves to an aluminium foil using different SLS solutions as coupling medium and counting the number of pits formed on the aluminium foil. The number of pits gives an indication of the damage caused to skin by ultrasound. There seems to be some correlation between surface tension of SLS solutions and number of pits formed and protein permeation at low SLS concentrations. Increasing surfactant concentration from 0 to 0.04% led to decreased surface tension, decreased number of pits and decreased protein through the skin. The reduced damage caused to skin (equivalent to fewer pits on aluminium foil) may be responsible for the decreased protein permeation. At higher SLS concentrations

(e.g., 0.1%), protein permeation into skin was increased despite the lack of pits formed on aluminium foil. This indicates that protein was permeating through the skin via pathways that could not be related to pits formed on aluminium foil. This study has shown that very low concentrations of SLS (e.g., 0.001%) resulted in similar protein permeation to those achieved by 1% SLS. The lower SLS concentration has advantages, such as lower toxicity.

Mitragotri, S. et al (2000) *J. Pharm.Sci.* **89**: 892–900

### 096
### $N^4$,$N^9$-Dimyristoyl spermine is a non-viral cationic lipid vector for plasmid DNA formulation

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The design and formulation of novel non-viral gene transfer (nano) particles have to overcome different barriers from the site of administration until they actively transport the DNA payload to the nucleus of target cells. Cationic lipids, as a major class of non-viral gene carriers, can be further classified as liposomal and non-liposomal non-viral DNA-delivery vectors. Lipopolyamines, especially those based upon the tetra-amine spermine, are promising non-viral gene delivery vectors, with significantly less toxicity and more controlled production than cationic polymers (Blagbrough et al 2003). These polyamine conjugates efficiently condense DNA into nano-particles for cellular uptake by endocytosis, with ultimate DNA delivery to the nucleus in a variety of cell lines. In this study, we synthesized and formulated a novel non-liposomal lipospermine in which the tetra-amine spermine (the cationic moiety) and two C14 dimyristoyl fatty-acid chains (the lipophilic moiety) are linked by amide bonds at the secondary amino groups of spermine to form $N^4$,$N^9$-dimyristoyl spermine. These amide linkers have the advantages of being both biodegradable and less toxic than the ether bonds in DOTMA (Tranchant et al 2004). Furthermore, the characteristics of this specific lipospermine with its cationic headgroup and two saturated long fatty-acid derived chains, render this conjugate a promising carrier for DNA by lipoplex formation. The binding affinity of this novel non-viral vector for calf thymus DNA (Sigma-Aldrich) was determined using an ethidium bromide (EthBr) fluorescence-quenching assay ($\lambda_{ex} = 260$ nm, $\lambda_{em} = 600$ nm; Geall & Blagbrough 2000). To quantify the ability of this polyamine conjugate in DNA condensation, the binding constant with DNA was calculated. Also, particles of condensed DNA were detected using a UV light scattering assay at $\lambda = 320$ nm. The results indicate the ability of $N^4$,$N^9$-dimyristoyl spermine efficiently to condense DNA by the significant decrease in (intercalated) EthBr fluorescence intensity, from 100% normalized intercalated EthBr without lipopolyamine, reduced to 9% at an ammonium/phosphate (N/P) charge ratio of 2.8. There was a matching increase (from 0%, no particles) to 100% in relative apparent absorption in the light scattering assay, displaying a maximum absorption at N/P = 3.0. The transfection results revealed high transfection efficiency in an immortalized cancer cell line (HeLa derived HtTA cells) and in primary skin cell-lines (FEK4 and FCP cells) using plasmid DNA encoding for enhanced green fluorescent protein (pEGFP, Clontech) as the reporter macromolecule with its fluorescent imidazolidinone moiety analysed by Fluorescent Activated Cell Sorter (FACS). The viability of the cells was studied using an MTT assay (e.g. 50% FEK4 cell survival at N/P = 15). All the studies were investigated in comparison with the commercially available non-liposomal cationic lipid Transfectam (Promega). These results obtained with novel $N^4$,$N^9$-dimyristoyl spermine show it to be an efficient non-viral gene delivery vector in both lipoplex formation and lipofection.

We acknowledge the financial support of the Egyptian Government (studentship to O.A.A.A.). We are grateful to Prof. R. M. Tyrrell (University of Bath) for the HtTA and FEK4 cell lines.

Blagbrough, I. S. et al (2003) *Biochem. Soc. Trans.* **31**: 397–406
Geall, A. J., Blagbrough, I. S. (2000) *J. Pharm. Biomed. Anal.* **22**: 849–859
Tranchant, I. et al (2004) *J. Gene Med.* **6**: S24–S35

### 097
### Varying the unsaturation in C18-lipid moieties of spermine-based cationic lipids for more efficient non-viral gene delivery

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The use of an efficient vector for nucleic acid delivery is one of the determining factors for the successful application of gene therapy to difficult-to-treat diseases. Among non-viral delivery systems, non-liposomal cationic lipids are promising, non-toxic gene carriers. The synthesis by Behr et al (1989) of the lipopolyamine dioctadecylamidoglycylspermine (DOGS, Transfectam), as an efficient non-viral transfection agent, encouraged several laboratories to focus on the synthesis of novel cationic lipids based on the naturally occurring polyamine spermine. In an effort to improve DNA delivery, we investigated novel spermine-based cationic lipid formulations. Following on from our promising results obtained with $N^4$,$N^9$-dioleoyl spermine (Ahmed et al 2005), we have investigated the effect of the degree of unsaturation in the lipophilic moiety of our novel lipopolyamines on the overall transfection efficiency. The synthesis of saturated C18 ($N^4$,$N^9$-distearoyl spermine), and the doubly-unsaturated C18 (cis 9, 12) $N^4$,$N^9$-dilinoleoyl spermine followed that of the mono-unsaturated C18 (cis 9) $N^4$,$N^9$-dioleoyl spermine (Ahmed et al 2005). The binding affinities of these compounds for calf thymus DNA were determined using an ethidium bromide (EthBr) fluorescence-quenching assay ($\lambda_{ex} = 260$ nm, $\lambda_{em} = 600$ nm; Geall & Blagbrough 2000) utilising the ability of EthBr as a cationic dye that displays a marked increase in fluorescence on binding with DNA through the intercalation of the EthBr phenanthridinium moiety between adjacent base-pairs along DNA sequences. The increase in the apparent absorbance due to light scattering as a result of the formation of condensed DNA nanoparticles was also detected at $\lambda = 320$ nm. $N^4$,$N^9$-Dilinoleoyl spermine induced a marked decrease in intercalated EthBr fluorescence intensity from 100% (EthBr-DNA complex without cationic lipid) reduced to 10% at an ammonium/phosphate (N/P) charge ratio of 1.5 and a maximum (100%) relative apparent absorption at this same charge ratio. The transfection efficiencies of the synthesized lipopolyamines were studied in primary skin cells (FEK4 and FCP cells) and in an immortalized (HeLa derived HtTA) cancer cell line using plasmid DNA encoding for enhanced green fluorescent protein (pEGFP, Clontech) as the reporter macromolecule with its fluorescent imidazolidinone moiety analysed by Fluorescent Activated Cell Sorter (FACS). The results revealed improved transfection with $N^4$,$N$-dilinoleoyl spermine, typically 90% efficiency in primary cell lines, higher values than were obtained with the saturated $N^4$,$N^9$-distearoyl spermine, about 25–40% transfection efficiency in primary cell lines. The cytotoxicity of these compounds was studied in both primary skin and immortalised cancer cell lines using an MTT assay (about 70% viability at N/P 5.5). All these studies were compared with commercially available non-liposomal cationic lipid DOGS (Transfectam, Promega, typically 75% transfection and 75% cell viability at N/P 7.8). The results show that an increase in the number of double bonds in the long C18 fatty chain of the cationic lipid improves the ability of the non-viral vector to deliver the DNA payload to the cultured cells.

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Ahmed, O. A. A. et al (2005) *Pharm. Res.* **22**: 972–980
Behr, J.-P. et al (1989) *Proc. Natl Acad. Sci. USA* **86**: 6982–6986
Geall, A. J., Blagbrough, I. S. (2000) *J. Pharm. Biomed. Anal.* **22**: 849–859

### 098
### Predicting drug–polymer interactions

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This study aims to establish the validity of mesoscale computational models in predicting drug-polymer interactions. The encapsulation and release profile of two model drugs (prednisolone and isoniazid) from poly (lactide) (PLA) and poly (lactide-co-glycolide) (PLGA) matrices was studied using both experimental and computational methods. A high throughput approach, involving the simultaneous preparation of 24 different formulations, was used to prepare PLA and PLGA microspheres loaded with the various levels of the model drugs. The influence of polymeric composition, polymer type, polymer molecular weight and drug loading on drug retention by these formulations was studied. Drug content was determined by a reverse phase HPLC method. Mesoscale modelling (Dissipative Particle Dynamics; DPD) was used to simulate drug retention in these formulations. DPD is a mesoscale simulation technique that is used for the description of dynamic behaviour of matter. Such a technique may allow us to gain important information on drug availability from a polymer formulation without the need for experimentation. DPD simulations of drug–polymer interactions were performed using MS modelling 3.0 software (Accelrys Inc.). Experimental studies for the encapsulation of prednisolone showed that, within the molecular weight class 40–75 kD, PLGA microspheres had higher encapsulation efficiencies (69.31% —

PLGAI) than PLA microspheres (47.62% — PLAI). These trends were also observed in the in silico data (Table 1). Experimental data on percentage encapsulation of isoniazid showed very low levels of encapsulation (mostly in the range of 1–10%, Table 2). Simulations were unable to show these low encapsulation values. Simulations of in vitro release study were performed by forcing the model drugs into the polymer matrix and then restarting the simulations with real interaction parameters. The interaction parameters used for forcing the drug into the polymer were based on the hypothesis that an interaction parameter value of zero or less corresponds to a favourable interaction (Groot & Warren 1997). In vitro release data for the hydrophilic model drug isoniazid showed that the microspheres released the drug at a very rapid rate. While simulations predicted the rapid release of isoniazid, simulations predicted that no prednisolone would be released. The latter finding was contrary to experimental observations. Microsphere drug retention variables and in vitro release were studied using experimental and mesoscale modelling methods. It is concluded that mesoscale simulations may be used to predict trends in polymer–drug interactions.

**Table 1** The maximum drug encapsulation efficiency (% for PLA I and PLGA I polymers – a comparison of the in-silico and experimental data

| Polymer type | In-silico data | Experimental data ± s.d. |
|---|---|---|
| PLA I | 12.5 | 47.62 ± 4.7 |
| PLGA I | 44.44 | 69.31 ± 7.5 |

**Table 2** Encapsulation efficiency (%) of isoniazid in PLA and PLGA matrices: experimental data

| Drug amount (mg) | % Encapsulation ± s.d. | | | | | |
|---|---|---|---|---|---|---|
| | PLGA1 | PLGA2 | PLGA3 | PLGA 4 | PLA 1 | PLA 2 |
| 1 | 7.50 ± 3.1 | 3.05 ± 1.9 | 3.99 ± 3.4 | 9.98 ± 2.9 | 3.72 ± 2.2 | 3.27 ± 1.5 |
| 5 | 1.10 ± 1.5 | 2.30 ± 0.0 | 2.51 ± 0.5 | 2.16 ± 0.8 | 4.56 ± 1.1 | 7.00 ± 1.4 |
| 9 | 2.10 ± 0.7 | 0.85 ± 1.3 | 3.43 ± 2.5 | 1.59 ± 0.1 | 5.02 ± 3.1 | 5.59 ± 4.0 |
| 12 | 2.84 ± 0.4 | 2.01 ± 2.4 | 2.77 ± 1.8 | 2.30 ± 1.6 | 8.14 ± 0.8 | 17.79 ± 1.0 |

Groot, R. D., Warren, P. B. (1997) *J. Chem. Phys.* **107**: 4423–4435

### 099
### Validation of Franz Cell diffusion experiments and evaluation of drug release through various cellulose membranes

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Franz cells are commonly used with synthetic membranes for quality control studies of topical formulations because of their simplicity, ease of monitoring, and supposed good reproducibility. A recent study using a synthetic membrane under quasi-standardised condition showed up to 35% variation in drug flux within 18 laboratories (Chilcott et al 2005). Although large result variability can occur with natural skin due to biological variations, such variation should not occur with carefully manufactured synthetic membranes. Un-validated equipment and methodology may cause such variability. The aims of this study were, firstly, to validate our Franz cell equipment and methodology to minimise result variation and, secondly, to use the validated equipment to investigate the influence of cellulose membrane structure on drug flux. Ibuprofen drug release from a gel through a Visking membrane was conducted for 7 h using the Franz cell equipment before and after validation of the variables. After validation, the influence of three types of cellulose membrane (Visking, Cuprophan and benzoylated) on the flux of ibuprofen from saturated solutions was investigated. The dimensions of each individual receptor and donor Franz cell were found to vary (donor heights 4.5–5.8 cm, angle of the receptor arms 124–132°, receptor volumes 11.55–11.76 mL, effective diffusion area 72.26–81.95 $cm^2$) and the shape of base of the receptors ranged from flat to convex. Each stirring block gave different stirring efficiencies with specific receptor cells. Overall at ~200 rev $min^{-1}$, a dye ($KMnO_4$) was effectively distributed throughout the receptor cell within 60 s using a stirring bar without a pivot ring as it did not create a vortex. The membrane also showed an appreciable weight gain (127.39–171.65%) after 24 h. It was concluded that the cellulose membranes must be allowed to hydrate for at least 24 h, and that the donor and receptor cells must be matched and placed in allocated stirrer blocks. A speed of 200 rev $min^{-1}$ with a non-pivot stirring bar was required to give effective distribution of the drug. Post-validation testing of equipment and techniques supported these conclusions as the coefficient of variation for ibuprofen diffusion from the gel reduced from 25.7% to 0.4% (n = 6). The membranes influenced the flux values obtained for the drug. The flux for the relatively hydrophilic Cuprophan membrane (9.52 mg $cm^{-2}$ $h^{-1/2}$) was significantly lower ($P < 0.05$) than the flux values for the other membranes 12.84 mg $cm^{-2}$ $h^{-1/2}$ (Visking) and 14.14 mg $cm^{-2}$ $h^{-1/2}$ (benzoylated). The data suggest that drug interaction with membrane surface groups influence the diffusion. Cuprophan may produce lower flux for the relatively hydrophobic ibuprofen due to the high density of hydroxyl groups on the membrane surface while the less hydrophilic benzoylated membrane gave higher flux. It was concluded that after validation of methodology and equipment, the variations in our drug release results were solely due to the properties of the membrane and the drug.

Chilcott R. P. et al (2005) *J. Pharm. Sci.* **94**: 632–638

### 100
### The choice of non-ionic surfactant in self-micro-emulsifying lipid formulations for the oral delivery of poorly water-soluble compounds

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Self-micro-emulsifying drug delivery systems (SMEDDS) are isotropic mixtures of oils and non-ionic surfactants that spontaneously emulsify in water upon gentle agitation producing oil-in-water fine dispersions of almost optical clarity. These systems can enhance the bioavailability of lipophilic drugs. They provide a reservoir of drug dissolved in the lipid matrix, which upon administration and making contact with gastrointestinal fluids spontaneously emulsify producing dispersions of small particle size with large surface area available for drug diffusion. The reformulation of Cyclosporine A as a pre-concentrate microemulsion system as in Neoral (Novartis) has given new impetus to this evolving technology. In this study, blends of Miglyol 812 (medium-chain triglyceride), Imwitor 988 ($C_8/C_{10}$ mono, di-glycerides) and various non-ionic surfactants with HLB in the range of 11–16.5 were optimised for self-micro-emulsification. Phase behaviour studies were also conducted to correlate with the size profiles of resultant dispersions and to establish the mechanism of the emulsification process. Various oil blends of {Miglyol 812/Imwitor 988} at ratios of 10/0, 9/1 to 0/10 were mixed with increasing concentrations of various non-ionic surfactants. One gram of each mixture was introduced into 100 mL of Milli-Q water and emulsified at 37°C for 15 min by gentle agitation. Dispersions were analysed for particle size using laser diffraction and photon correlation spectroscopy. Phase behaviour studies of lipid–water systems were conducted by constructing pseudo-ternary diagrams. Self micro-emulsifying systems of particle size 30–40 nm were obtained by using optimum oil blends of {Miglyol 812/Imwitor 988} at ratios of 5:5 in the case Cremophor RH40 or Cremophor EL, at ratios of 4:6 in the case of Crillet 4 or Tagat O2 and at 7:3 for Tagat TO. As Table 1 illustrates, to obtain fine dispersions of oil in water, the oil mixture has to be either slightly lipophilic or hydrophilic depending on the HLB value of the surfactant. The higher the HLB of the surfactant, the less Miglyol 812 is used in the oil blend. In this case, Imwitor 988, which is a polar oil, acts as a co-surfactant. Phase behaviour studies have shown that 'Diffusion and Stranding' is the proposed mechanism of emulsification whereby, on dispersion, the hydrophilic components in the oil mixture tend to diffuse out into the aqueous phase and hence oil nucleation starts to occur as the solvent capacity is lost. Self-micro-emulsifying lipid systems with the potential to enhance the bioavailability of lipophilic compounds were developed by blending various ratios of {Miglyol 812/Imwitor 988} with a wide range of non-ionic surfactants of varying degrees of hydrophilicity. There is a correlation between surfactant HLB and the percentage ratio of the non-polar oil in the formulation mixture required to produce microemulsion systems.

**Table 1** Effect of surfactant HLB on the % of Miglyol in the oil blend {Miglyol/Imwitor} needed to obtain SMEDD formulations

| Surfactant | Surfactant HLB | Miglyol 812 Wt% |
|---|---|---|
| Tagat TO | 11 | 70 |
| Cremophor EL | 12–14 | 30–60 |
| Cremophor RH40 | 14–16 | 30–60 |
| Crillet 4 | 15 | 40 |
| Tagat O2 | 15 | 40 |

## 101
## Nanocarriers for siRNA based on cationic polymers: chitosan and TAT peptide

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Gene silencing by RNA interfering (RNAi) has been receiving increased attention over the past few years and this powerful sequence-specific regulation of gene expression is mediated through small interfering RNA (siRNA). As a result, double stranded siRNA has become a preferred tool for target validation as well as potential agents in the treatment of a wide range of diseases (Urban-Klein et al 2005). The wide use of siRNA, however, is hampered by their rapid degradation and poor cellular uptake into the cells in vitro or in vivo. Consequently, the delivery of unassisted or unmodified siRNA molecules into the cells is challenging. One approach that has been explored and widely used to deliver siRNA in vitro is lipofectamine, which also has limited application due to its related toxicity and specific to only certain types of cells (Chiu et al 2004). Therefore, new and safer strategies to deliver siRNA successfully are needed. We have explored siRNA delivery system based on a cationic polymer chitosan due to its low toxicity, biodegradability and biocompatibility, as well as a cationic peptide, TAT, as it is rapidly taken up by a broad spectrum of cell types. In this study, we explored the use of both chitosan and TAT peptide to produce nanoparticles by simple complexation through electrostatic interactions between the opposite charges of siRNA and chitosan or TAT peptide. The effects of certain parameters (e.g. type and molecular weight of chitosan) had also been investigated on physical characteristics of the complex (e.g. size, surface charge and morphology), as well as the condensation capacity of siRNA by gel retardation assays. In addition, siRNA was adsorbed onto the surface of preformed chitosan nanoparticles and its loading efficiency was determined by gel electrophoresis and spectrophotometry. Both siRNA-chitosan and TAT complexes were shown to be approximately 200 nm in size, depending on the concentration ratio of chitosan or TAT peptide to the siRNA. In the case of chitosan, the size of complex also depended on the type and molecular weight of the chitosan used. In addition, the TAT peptide appears to be forming stronger complexes than the chitosan for all types and molecular weights of chitosan tested in this study. Interestingly, preformed chitosan nanoparticles showed very high siRNA loading efficiency, with almost 100% of siRNA adsorbed onto the surface of the particles. It was also found that the loading efficiency of siRNA for these carriers was highly dependent on the concentration ratio of the carrier to the siRNA. This was expected due to the higher density of positive charge as the surface charge of the complex increased as the chitosan or TAT concentration ratio increased. The surface charge of the TAT-siRNA complexes at different concentrations is shown in Table 1. This study has shown that by using simple complexation method, we are still able to obtain high siRNA loading with TAT without the need of chemical conjugation (Chiu et al 2004). We believe that chitosan and TAT peptide have promising prospects to deliver siRNA and the studies of the application of these systems in mammalian cells in vitro and in vivo is on-going.

**Table 1** Surface charge of TAT-siRNA complex

| TAT concn ($\mu g\,mL^{-1}$) | Surface charge (mV) |
|---|---|
| 80 | $-6.3 \pm 0.3$ |
| 100 | $+24.0 \pm 0.5$ |
| 150 | $+30.6 \pm 0.4$ |
| 200 | $+33.9 \pm 0.3$ |

Chiu, Y.-L. et al (2004) *Chem. Biol.* **11**: 1165–1175
Urban-Klein, B. et al (2005) *Gene Ther.* **12**: 461–466

## 102
## Synthesis and biological testing of lipopolyamine $N^4$,$N^9$-Dioleoylspermine: self-assembly of a nanopharmaceutical for plasmid delivery

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We are designing novel, small molecule DNA-condensing agents, modified synthetic polyamine-conjugates (lipopolyamines) (Blagbrough et al 2003) using spermine as the polyammonium ion moieties conjugated with lipophilic groups, such as steroids (e.g. cholesterol), bile acids, long alkyl or alkenyl chains (e.g. $C_{18}$ fatty acids), to develop more efficient non-viral gene delivery systems to improve DNA uptake by target cells for safe non-viral gene therapy (NVGT). Non-viral vector mediated cell transfection remains a poorly understood phenomenon, but it is inherently safer (less immunogenic) and can transport a significantly larger DNA payload than using a viral vector. $N^4$,$N^9$-Dioleoylspermine (LipoGen) (Ahmed et al 2005) is a lipospermine with two oleoyl chains acylating both secondary amines. This small molecule combines the characteristics of both a cationic lipid and a fusogenic (lipid bilayer disrupting) lipid in its structure. Trifluoroacetylation with ethyl trifluoroacetate (2.0 eq.) was successfully used to protect only the primary amino functional groups in spermine (1.0 eq.). $N^1$,$N^{12}$-Di-(trifluoroacetyl)spermine was reacted with oleic acid (2.2 eq.) to form $N^4$,$N^9$-dioleoyl-$N^1$,$N^{12}$-di-(trifluoroacetyl)spermine using 1,3-dicyclohexylcarbodiimide (DCC) (2.4 eq.) and catalysed by 1-hydroxybenzotriazole (HOBt) (1.0 eq.). The removal of the protecting groups was easily carried out at alkaline pH (11.0) in methanolic ammonia. Given $N^4$,$N^9$-dioleoylspermine carries two positive charges at neutral pH, the removal of unreacted starting materials and by-products (e.g. oleic acid, dicyclohexylurea, etc.) was carried out by column chromatographic elution over flash silica gel with DCM/MeOH (5/2 v/v) as mobile phase. Then, $N^4$,$N^9$-dioleoylspermine was collected by elution with DCM/MeOH/$NH_4OH$ (25/10/1 v/v/v) and characterised by NMR and MS. The ammonium ions interact with and then condense DNA, so we are studying the formulation and analytical chemistry of these polyamine conjugates acting as histone mimics. DNA condensation, the first step in gene delivery achieved by DNA phosphate charge neutralisation with cationic lipopolyamines, was studied by the fluorescence quenching of ethidium bromide (EthBr) (Geall & Blagbrough 2000) to monitor the formation of nanoparticles and to determine the efficiency of the condensation process. The fluorescence yield of EthBr ($\lambda_{ex} = 260$ nm, $\lambda_{em} = 600$ nm) increased on intercalation between adjacent base-pairs, and then gradually decreased as the DNA phosphate anions were neutralised by titration with increasing ammonium/phosphate (N/P) charge ratio. We showed that lipophilic modification of spermine resulted in a more efficient Enhanced Green Fluorescent Protein (pEGFP) cDNA condensation (15% residual fluorescence, in the EthBr assay, at N/P charge ratio 2.5) compared with tetracationic spermine (50% at N/P charge ratio 3.0). Particle formation was confirmed by measuring UV light scattering (LS), recorded as increased absorption at $\lambda = 320$ nm. This can be compared with the efficiency of primary FEK4 cell line transfection with plasmid DNA encoding EGFP condensed by the same lipopolyamine; transfection efficiency (62% at optimal charge ratio 2.5) is significantly higher than in the control cells transfected with cationic lipid (DOTMA/DOPE) (10–40%). Thus, this is an efficient novel lipopolyamine for NVGT in vitro.

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## 103
## Enhanced fine particle dose of nebulised salbutamol sulphate using a proliposome approach

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Proliposomes are alcoholic solutions of phospholipid, which generate liposomes on adding aqueous phase (Perrett et al 1991). Liposomes may entrap drugs and, when nebulised to the lungs, localise and prolong drug action within airways (Taylor et al 1989). In this work, we developed a cholesterol-containing proliposome formulation for generating liposomes by adding aqueous phase containing salbutamol sulphate. The effect of formulation on drug delivery to a "twin impinger" (TI) was studied using an Aeroneb Pro vibrating-mesh nebuliser. Soya phosphatidylcholine and cholesterol (50 mg; 1:1 mole ratio) were dissolved in ethanol (100:120 w/w) by heating at 70°C for 1 min. Salbutamol sulphate (5 mg) was dissolved in 5 mL isotonic NaCl (0.9%) for nebulisation or for hydration of proliposomes, with hand shaking for 1 min, before nebulisation. The TI was set up at 60 L $min^{-1}$ and NaCl (0.9%) was used as the collection medium. The nebuliser was filled with formulation (5 mL) and directed to the 'throat' of the TI, and nebulisation commenced to 'dryness'. Aerosol mass output was calculated by weight difference before and after nebulisation, and phospholipid output and distribution in the TI were

estimated analytically (Stewart 1980). Drug output and distribution in the TI were analysed using HPLC after releasing liposome-entrapped drug using Triton X (0.5%). The surface tension of each formulation was measured using a Kibron Delta-8 multichannel microtensiometer. The morphology of liposomes retained in the nebuliser and those delivered to the impinger were investigated using transmission electron microscopy. Liposomes generated from proliposomes were multilamellar and oligolamellar. Liposomal salbutamol sulphate provided slightly but significantly ($P < 0.05$) greater mass and drug outputs compared with salbutamol sulphate solution (Table 1). Compared with the drug solution, liposomes significantly ($P < 0.05$) decreased drug deposition in the upper stage and increased deposition in the lower stage (cut-off diameter 6.4 $\mu$m) of the TI (Table 2), suggesting increased likelihood of deposition in the peripheral airways. These differences might be attributed to the lower surface tension of the liposomal formulation ($31.38 \pm 1.97\,mN\,m^{-1}$) compared with the solution ($72.10 \pm 0.57\,mN\,m^{-1}$). This study shows that a proliposome approach has provided a liposomal salbutamol sulphate formulation that when delivered using a vibrating-mesh nebuliser gives greater aerosol mass and drug outputs, and a larger "fine particle dose" compared with the conventional drug solution. This was attributed to the lowered surface tension provided by phospholipids inclusion. Overall, this study suggests that liposomes may provide enhanced drug delivery using this novel nebuliser.

**Table 1** Effect of formulation on mass and drug output (%) of salbutamol sulphate (SS) preparations

| Formulation | Mass output (%) | Drug output (%) |
|---|---|---|
| Liposomal SS | $89.94 \pm 2.24$ | $85.90 \pm 2.31$ |
| SS solution | $84.30 \pm 0.89$ | $80.03 \pm 2.55$ |

SS, salbutamol sulphate. Data are means ± s.d., n = 3.

**Table 2** Effect of formulation on the drug distribution in the nebuliser and TI

| Formulation | Nebuliser (%) | Upper stage (%) | Lower stage (%) |
|---|---|---|---|
| Liposomal SS | $14.10 \pm 2.31$ | $19.53 \pm 0.24$ | $66.37 \pm 2.11$ |
| SS solution | $19.97 \pm 2.55$ | $22.52 \pm 0.81$ | $57.50 \pm 1.80$ |

SS, salbutamol sulphate. Data are means ± s.d., n = 3.

Perrett, S. et al (1991) *J. Pharm. Pharmacol.* **43**: 154–161
Stewart, J. C. M. (1980) *Anal. Biochem.* **104**: 10–14
Taylor, K. M. G. et al (1989) *Pharm. Res.* **6**: 633–636

## 104
## Influence of proliposome hydration medium on the delivery of liposomes using a vibrating-mesh nebuliser

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Vibrating-mesh nebulisers generate aerosol by means of perforated plates vibrating at high frequency (Dhand 2002). Proliposomes are alcoholic solutions of phospholipid, which produce liposomes on adding aqueous phase (Perrett et al 1991). In this study, liposomes were generated from a novel cholesterol-containing proliposome formulation. Using a two-compartment vibrating-mesh nebuliser (Aeroneb Go), the delivery of vesicles dispersed in isotonic solutions of NaCl (0.9%) or sucrose (9.25%) was investigated. Proliposomes were formed by dissolving soya phosphatidylcholine and cholesterol (50 mg; 1:1 mole ratio) in ethanol (100:120 w/w) at 70°C for 1 min. NaCl (0.9%) or sucrose (9.25%) solution (5 mL) was added, with vigorous hand shaking for 1 min, to generate liposomes. The dispersion was transferred to the nebuliser and nebulisation commenced to "dryness". Aerosol mass output was calculated by weight difference before and after nebulisation and phospholipid output was estimated analytically (Stewart 1980). Aerosol droplet size was measured using laser diffraction, and viscosity of each hydration medium measured using a suspended level viscometer at 25°C. Proliposomes hydrated with NaCl solution generated aerosols with significantly ($P < 0.05$) smaller volume median diameter (VMD) and 90% undersize than those hydrated with sucrose solution (Table 1), indicating that aerosols generated from the two hydration media are likely to have different deposition profiles in the airways. However, for both formulations, mass output was greater than phospholipid output (Table 2), indicating concentration of phospholipids within nebulisers during nebulisation. Sucrose solution reduced the mass and phospholipid outputs and increased phospholipid accumulation within the nebuliser compared with the NaCl solution (Table 2). The lower reservoir (trap) of the nebuliser retained phospholipids, with greatest retention for the sucrose-hydrated formulation (Table 2), probably as a result of the larger droplet size generated from the sucrose-hydrated formulation (Table 1). Sucrose (9.25%) solution ($1.0904 \pm 0.0125$ mPas s) has higher viscosity than NaCl (0.9%) solution ($0.8983 \pm 0.0021$ mPas s), and this may be responsible for the poor delivery and the relatively large droplet size of the sucrose-hydrated formulation, as fluid viscosity is often key the physicochemical property affecting nebuliser performance. In conclusion, the performance of a vibrating-mesh nebuliser for delivering liposomes generated from proliposomes, was greatly influenced by the hydration medium. Hydration with NaCl (0.9%) solution produced aerosols with smaller droplet VMD and 90% undersize, and higher mass and phospholipid outputs than sucrose, suggesting NaCl solution is the appropriate medium for dispersing this proliposome formulation using this nebuliser.

**Table 1** Effect of proliposome hydration medium on the aerosol droplets size distribution

| Hydration medium | VMD ($\mu$m) | 0% undersize[a] ($\mu$m) |
|---|---|---|
| Sucrose (9.25%) | $5.58 \pm 0.40$ | $11.14 \pm 0.57$ |
| NaCl (0.9%) | $3.89 \pm 0.25$ | $8.67 \pm 0.45$ |

Data are means ± s.d., n = 3. [a]Size below which 90% of droplets, by volume, fall.

**Table 2** Phospholipid (PL) output and distribution between the reservoir upper (mesh unit) and lower (trap) compartmentS of the Aeroneb Go nebuliser

| Hydration medium | PL in upper reservoir (%) | PL in lower reservoir (%) | Output (%) | |
|---|---|---|---|---|
| | | | PL | Mass |
| Sucrose | $7.72 \pm 1.37$ | $73.24 \pm 1.79$ | $19.03 \pm 1.90$ | $32.47 \pm 4.82$ |
| NaCl | $17.64 \pm 6.15$ | $17.64 \pm 1.37$ | $64.71 \pm 5.36$ | $81.92 \pm 0.91$ |

Data are means ± s.d., n = 3.

Dhand, R. (2002) *Respir. Care* **47**: 1406–1418
Perrett, S. et al. (1991) *J. Pharm. Pharmacol.* **43**: 154–161
Stewart, J. C. M. (1980) *Anal. Biochem.* **104**: 10–14

## 105
## Emulsions as locally acting drug delivery formulations targeting the oesophagus: in vitro adhesion and drug release

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Adhesive liquids permit targeted drug delivery to the oesophagus for treating a range of diseases. Emulsions that adhere to the oesophagus for extended periods of time have been reported (Cuca et al 1999). This study investigated oesophageal retention of emulsions in vitro, and the rate of nifedipine (NIF) release from these emulsions. NIF is a calcium channel blocker used in treating motility disorders of the oesophagus, hence was chosen as a model drug. Emulsions were prepared using heavy (HMO) or light (LMO) mineral oil in water, with Tween 85 as the surfactant. NIF was incorporated into the lipid phase of the emulsions at either $2.5\,mg\,mL^{-1}$ or $1.0\,mg\,mL^{-1}$ final concentration. All chemicals were supplied by Sigma, UK. Bioadhesion was assessed by testing the retention of a 1-mL dose of radiolabelled emulsion on ex-vivo porcine oesophagus, using a model described by Batchelor et al (2002) incorporating simulation of saliva flow. All formulations were retained on porcine oesophageal tissue better than a viscosity-matched glycerol control (Table 1). The two emulsions without drug incorporated demonstrated comparable

bioadhesion to the tissue. For both emulsions, an increasing drug concentration led to an increased impairment of retention. Drug release rate was studied from the NIF-loaded emulsions. Drug release over 4 h was determined using Franz cells with dialysis membrane (MW cut-off 12–14 kDa) between compartments. Formulation (1 mL) was placed in the donor compartment, the receiver fluid (acetonitrile:water 60:40) was sampled at regular intervals and NIF content determined by HPLC. Drug release over time was calculated as $\mu$g diffused per unit area. NIF was released linearly from emulsions over a 4-h period. Drug release was more rapid at higher concentrations. At 2.5 mg mL$^{-1}$, there was no significant difference between release rates from LMO or HMO (0.14 vs 0.20 $\mu$g cm$^{-2}$, respectively at 15 min). At 1.0 mg mL$^{-1}$, after 60 min, drug release rate was faster from LMO than HMO (0.36 vs 0.33 $\mu$g cm$^{-2}$), although this difference was not significant. Emulsions show adhesion to oesophageal tissue in vitro, although incorporation of drugs may affect the adhesive properties. NIF was released within a short time and continued to be released linearly for an extended period. Mineral oils could be used as adhesive drug delivery systems in the oesophagus. A drug could be released during prolonged epithelial contact, to deliver drug locally at the oesophagus. This strategy could be used for treatment of oesophageal motility disorders without the use of systemic drugs.

**Table 1** % retention of formulations at varying time points

| Formulation | 5 min | 10 min | 30 min |
|---|---|---|---|
| HMO | 2.16 (0.83) | 1.26 (0.38) | 0.64 (0.31) |
| HMO + NIF 1.0 | 0.97 (0.36) | 0.52 (0.23) | 0.24 (0.07) |
| HMO + NIF 2.5 | 0.91 (0.38) | 0.45 (0.15) | 0.17 (0.02) |
| LMO | 4.65 (5.51) | 3.38 (4.17) | 2.11 (2.76) |
| LMO + NIF 1.0 | 3.35 (1.40) | 1.29 (0.25) | 0.72 (0.32) |
| LMO + NIF 2.5 | 0.99 (0.42) | 0.62 (0.27) | 0.34 (0.19) |
| Glycerol | 0.81 (0.34) | 0.63 (0.40) | 0.46 (0.43) |

s.d. given in parentheses.

Batchelor, H. K. et al (2002) *Int. J. Pharm.* **238**: 123–132
Cuca, R. C. et al (1999) US Patent 5,858,391

### 106
### Particle engineering from emulsions by controlled spray drying

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Particulate fabrication by spray drying allows engineering of particles with varying size, density and morphology (Bain et al 1999). In this study, the Niro SD Micro Spray Drier System was used to produce particles in an inert atmosphere of nitrogen, allowing the safe use of organic solvents. Additionally, it is possible to precisely control critical processing parameters, including nozzle position, drying gas flow and pressure, atomisation pressure, inlet and outlet temperatures and evaporation rate as a function of liquid pump capacity. We now describe how we have utilised this system to examine the formation of particles by spray drying emulsions (Kusonwiriyawong et al 2004). This abstract describes how particle surface charge can be varied while maintaining particle size and morphology. Double emulsions comprised of PLGA (50:50 PLGA 2A) and PVA (weight average approximately 18 000 g mol$^{-1}$) in a weight ratio of 1:1 were prepared by homogenisation in ethyl acetate and water. The PLGA (1.1 g) was dissolved in ethyl acetate (10.0 mL) and a 5% (w/v) aqueous PVA (2.0 mL) solution was added and homogenised for 2 min with an Ultraturrax. This water in oil emulsion was immediately transferred to a conical flask (100 mL) that contained 2.5% (w/v) aqueous PVA solution (40 mL), which was further homogenised for 7 min to produce the double emulsion, which was immediately spray dried to give yields in the range of 30–0%. It was important to keep the outlet temperature of the spray drier below the Tg of PLGA. When the outlet temperature was above 30°C, the particle yield was below 5% due to excess polymer sticking to the surfaces within the spray drier. Composition was varied in two ways during the formation of the primary emulsion. First, stearylamine (SA) was added to the ethyl acetate as a means to vary surface charge (PLGA:PVA:SA, 1:1:0.1). Secondly, either pluronics F68 or a novel PEG derived polyacetal (0.05 to PLGA) (Tomlinson et al 2002) was added to the aqueous PVA solution. The spray dried particles were then characterised by SEM, Mastersizer, Zetasizer and DSC. All the particles displayed a wrinkled, raisin-like, appearance by SEM. The surface charge became positive with the addition SA (Table 1). The positive charge then decreased significantly by the small addition of either of the water soluble PEG derived polymers. The reason for this is that both of these polymers mainly consist of PEG, which has a tendency, due to its surfactant character, to migrate to the particle surface, to displace the SA and decrease the charge at the surface. Thus, by adding a small amount of minor polymeric excipients, it was possible to alter the surface charge while maintaining the morphology and particle size. The formulations with the PEG derived polymers displayed only slightly reduced Tg. In conclusion, the addition of the two PEG derived polymers offers the possibility to alter the charge while maintaining particle size and overall surface morphology.

**Table 1** Physical chemical characterisation of different formulations

| Formulation | Size ($\mu$m) | Charge (mV) | $T_g$ (°C) |
|---|---|---|---|
| PLGA + PVA | 3.8 (± 1.2) | −2.50 (± 0.1) | 34.05 |
| PLGA + PVA + Stearylamine | 4.1 (± 0.9) | 32.00 (± 0.9) | 33.60 |
| PLGA + PVA Stearylamine + Pluronic F68 | 3.9 (± 0.7) | 15.40 (± 0.2) | 28.65 |
| PLGA + PVA Stearylamine + Polyacetals | 4.0 (± 1.0) | 9.20 (± 1.0) | 28.10 |

Data are means ± s.d., n = 3.

Bain, D. F. et al (1999) *J. Microencapsul.* **16**: 453–474
Kusonwiriyawong, C. et al (2004) *J. Microencapsul.* **21**: 25–36
Tomlinson, R. et al (2002) *Macromolecules* **35**: 473–480

### 107
### Evaluating the interactions and release parameters of various drugs from hydrogels

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Due to the excellent water solubility exhibited by poly-ethylene glycol (PEG), it is thought that the inclusion of PEG into hydrogels may increase the hydrophilicity and provide a preferable environment for drug to alter the release properties (Kim & Peppas 2003). In the past there has been considerable work performed in the development of crosslinked polymer networks that possess a PEG backbone or are grafted with a PEG pendent group and therefore would be a desirable system for controlled release. This work aims to synthesize a series of hydrogels with different properties and investigate the macroscopic swelling properties. This will help us to better understand the fine structure of the hydrogels and to investigate the loading and release and interactions between drugs and hydrogels. Poly(methacrylic acid) P(MAA) and poly (methacrylic acid grafted PEG1000) P(MAAEG1000) hydrogels were prepared by free-radical photopolymerization. The swelling and release studies were performed in pH 2.2 and pH 7.4 phosphate-citrate buffer solutions. Disks of hydrogels were soaked in drug solution for loading and after four days put drug-loaded hydrogels in buffer for release. Temperature of the solution was controlled by the water bath at 25°C with 150 strokes. The PEG400 (1:0.1) and PEG400 (1:0.5) hydrogels were obtained by chemical crosslinking of poly (ethyleneglycol) through urethane groups by incorporating diisocyanate and triol. Two model drugs, metronidazole and diclofenac, were used for the loading and release. The results obtained indicate that at pH7.4, methacrylic acids in PMAA were charged and repelled each other. This resulted in a greater degree of swelling compared with those at pH2.2, which were not charged. In the case of PMAA, It was considered that the positively charged metronidazole in pH 7.4 solutions interacted electrostatically with negatively charged carboxyl groups of PMAA and the negatively charged diclofenac. Because PMAAEG1000 (EG:MAA, 1:1) has fewer methacrylic acid groups than PMAA, the gels did not show significant swelling differences between pH 2.2 and pH 7.4 buffers. The swelling of PMAA and PMAAEG after equilibrium provided further evidence that the charge of methacrylic acid in the corresponding media played an important role in the swelling effect of the gel. According to the results of macroscopic swelling, the PMAAEG1000 network exhibited the highest equilibrium swelling ratio. This indicated that PEG 1000 groups absorb more water than MAA and PEG400 groups. According to the results from this work, metronidazole-loaded PEG400 (1:0.1) released more than PEG400 (1:0.5). This indicated that the density of crosslinkers in the

hydrogels had an influence on the swelling properties and release mechanisms of the hydrogels. Table 1 showed that diclofenac-loaded PMAAEG1000 released more than PMAA and the PMAAEG400 swelled to a lesser extent but released more than PMAA. This indicated that PEG grafted in the PMAA groups changed the internal environment of the hydrogels and may possibly interact with the drugs to change the properties of release.

**Table 1** Shows release data of drugs from hydrogels

| Gel samples | Amount of drugs released ($\mu$g/mg dry gel) average ± s.d | |
|---|---|---|
| | Metronidazole | Diclofenac |
| PMAA | 3.603 ± 0.121 | 2.464 ± 0.093 |
| PMAAEG400 (4:1) | 4.415 ± 0.212 | 3.991 ± 0.234 |
| PMAAEG1000 (1:1) | 12.707 ± 0.081 | 23.966 ± 0.132 |
| PEG400 (1:1) | 4.224 ± 0.091 | 12.900 ± 0.140 |
| PEG400 (1:0.5) | 3.797 ± 0.106 | 5.651 ± 0.157 |

The loading in the metronidazole and diclofenac solutions: 1 mg ml$^{-1}$.Values are means ± s.d., n = 3.

Kim, B., Peppas, N. A. (2003) *Int. J. Pharm.* **266**: 29–37

## Poster Session 2 – Materials Science

### 108
### Relationship between the mechanical and molecular properties of HPMC solutions during the thermally modified sol:gel transition

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Hydroxypropyl methylcellulose (HPMC), a non-ionic cellulose ether, is frequently used as a rate control polymer in extended release hydrophilic matrices. An unusual property of HPMC is its ability to undergo a reversible sol:gel transition at elevated temperatures in aqueous solutions. Modifications in the dynamic viscoelastic functions of HPMC solutions during the phase transition have been reported (Haque et al 1993), although detailed information relating to changes occurring at a molecular level are lacking. As a result we use Attenuated Total Reflectance–Fourier Transformed Infrared Spectroscopy (ATR-FTIR) to probe molecular changes during the phase transition. This study was undertaken to investigate the relationship between the molecular and mechanical changes occurring in HPMC solutions during the thermally modified sol:gel transition, employing ATR-FTIR and oscillatory rheology experiments, respectively. All spectra of 2% w/w HPMC solutions (Methocel E4M; Colorcon Ltd, Dartford, UK) were collected using a single reflection heated diamond ATR cell (Graseby Specac, UK) coupled to a ThermoNicolet Magna FTIR spectrometer. The dynamic viscoelastic functions (storage modulus G′ and loss modulus G″) were determined using a Bohlin C-VOR (Bohlin Instruments Ltd, UK) fitted with acrylic parallel plates (40 mm). A continuous temperature sweep (10–85°C) at a rate of 1°C/min was undertaken. Measurements were carried out at an angular frequency of 0.5 Hz and at 5% strain to ensure the linearity of viscoelasticity. From the ATR-FTIR data, the $\upsilon$(CO) band that is associated with the C-O stretches of methoxyl, hydroxyl and hydroxypropyl functional groups of HPMC, as well as the glycoside link, showed a marked increase in intensity at approximately 56°C; this marked the onset of gelation. From the rheology data, the crossover of G' and G'' (i.e. tan $\delta = 1$), which has been traditionally used as an indication of the sol:gel transition point, also occurred at 56°C. Before the crossover ($G' < G''$), the system demonstrated common viscoelastic behaviour of a liquid, whereas following the crossover ($G' > G''$) a weak but elastic structure was displayed. A large drop in G'' was also observed at approximately 56°C. Upon cooling, both the $\upsilon$(CO) band intensity and dynamic viscoelastic functions illustrated a deviation from the heating curve; a hysteresis loop was observed during the thermal cycle. This has been attributed to the lifetime of certain polymer interactions such as hydrophobic bonding. A change in the shape of the $\upsilon$(CO) band was identified as a function of temperature. The methoxyl band (1197 cm$^{-1}$) component displayed a change in relative intensity during the sol:gel transition with respect to other components of the $\upsilon$(CO) band, therefore illustrating the prominent role of the hydrophobic group during the phase transition. The ATR-FTIR data was in good agreement with rheological measurements conducted on the same system, molecular and mechanical changes were detected during the sol: gel transition. Changes in the shape of the $\upsilon$(CO) band indicated hydrophobic polymer chain interactions were involved during the phase transition.

Haque, A. et al (1993) *Carbohydrate polymers* **22**: 175–186

### 109
### Development of tests to measure the impact and abrasion properties of film-coated tablets

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Failure of tablet coatings during processing and subsequent handling can result in significant financial losses. There is therefore a need to develop tests to assess the potential behaviour of coatings to ensure that they are sufficiently robust to withstand all the forces they will experience in situ. The aim of this project was to develop reliable laboratory scale test methods to measure coating performance with a strong emphasis on assessing mechanical damage. The implications of scale-up, from development batches to full-scale manufacturing capacity, can be unpredictable due to large differences in processing conditions, potentially resulting in inferior quality coatings. The development of new testing methods for coated tablets to better predict the effects of scale-up would lead to increased coating robustness and quality minimising commercial losses. This study has involved the development of novel impact and abrasion testing methods that can be applied to tablet coatings. The impact technique determines the energy required to cause given levels of damage at the face centre and face edge of the tablets. The abrasion technique determines the relative wear rates of the coatings. Tests were carried out on tablet batches with a variety of placebo core formulations (standard, as well as those exhibiting brittle and plastic like properties) and HPMC based film coatings (a conventional coating, A, applied to all cores; and a spray dried coating, B, applied to the standard formulation only) to encompass a range of tablet properties in terms of core hardness and coating quality. Scanning electron microscopy (SEM), compression testing and surface profilometry were used to further characterise the range of tablets. Tables 1 and 2 show typical results from impact and abrasion testing, respectively. The results identify differences between tablet types. The coated brittle cores proved to be the weakest in terms of both impact and abrasion testing, and based on results from Tables 1 and 2, were up to approximately 77% weaker during impact, and wore through after up to 11% less distance (per unit coating thickness). The coated plastic core formulation exhibited significantly better resistance on impact testing, but was slightly inferior during abrasion. The standard core formulation with coating A showed higher impact resistance than coating B, however differences during abrasion were of little significance. Results from compression testing showed that while coating tablets improved their strength, the properties of the core predominate. SEM analysis did not reveal any major differences between the tablet types and in some cases the theoretically better quality coating appeared more porous than the spray dried one. In conclusion, the results indicate that, in coated tablets, the resistance to damage is affected by the core formulation and to some extent the presence of a coating (mainly due to its thickness). The techniques will enable coated tablet performance to be evaluated more thoroughly at an early stage of the development process. This will increase the understanding of the coatings' performance under impact and abrasion conditions and will potentially optimise their performance. The methods developed show potential for future research and development, to generate useful data modelled on real life situations that can be used to predict coated tablet behaviour.

**Table 1** Typical impact testing results with 1 mm diameter indenter at tablet face edge – damage threshold energies

| Tablet type | | Minimum energy required (mJ/$\mu$m) | |
|---|---|---|---|
| Placebo core | Coating quality and mean thickness | Coating fracture | Total tablet fracture |
| Brittle | A, 24.9 $\mu$m | 0.31 | 2.39 |
| Plastic | A, 28.1 $\mu$m | 0.56 | 6.22 |
| Standard | A, 33.7 $\mu$m | 1.32 | 3.71 |
| Standard | B, 26.3 $\mu$m | 1.25 | 4.60 |