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Liquisolid technique for sustaining the drug release from compacts

A. Nokhodchi[1], Y. Javadzadeh[2,3] and L. Mosaalrezaei[1]

[1]University of Kent, Medway School of Pharmacy, Central Ave, Chatham Maritime, ME4 4TB, Kent, UK, [2]Tabriz University of Medical Sciences, Daneshghah St., Tabriz, 51664, USA and [3]Drug Applied Reserach Centre, Daneshghah St, Tabriz, USA.
E-mail: a.nokhodchi@kent.ac.uk

Objectives There are several techniques for preparation of sustained release formulations, among which control of drug dissolution is one of the best and most successful methods due to its simplicity and low cost (Banker & Rhodes 1996). Liquisolid technique has been used to enhance the dissolution rate of poorly water soluble drugs (Nokhodchi et al 2005; Spirease & Sadu 1998). It is suggested here that the method has the potential to be optimized for the reduction of drug dissolution rate and thereby production of sustained release systems. Propranolol hydrochloride has been used as the model water soluble drug.

Methods In order to prepare liquisolid compacts propranolol hydrochloride was dispersed in polysorbate 80 as the liquid vehicle. Then a binary mixture of carrier-coating materials (Eudragit RL or RS as the carrier and silica as the coating material) was added to the liquid medication under continuous mixing in a mortar. The final mixture was compressed using the manual tableting machine. The effect of drug concentration and loading factor on release profile of propranolol hydrochloride from liquisolid compacts were investigated at two pH values (1.2 and 6.8). The release rate of propranolol from liquisolid compacts was compared with the release of propranolol from conventional tablets. X-ray crystallography and DSC were used to investigate the formation of any complex between drug and excipients or any crystallinity changes during the manufacturing process.

Results Propranolol tablets prepared by liquisolid technique showed greater retardation properties in comparison with conventional matrix tablets. For example liquisolid compact containing 30% liquid medication with a loading factor of 0.225 released only 80% of drug within 8 h but this amount of release in its counterpart, the conventional matrix tablet, was obtained within 3 h. This indicates that liquisolid systems showed better retardation in comparison with conventional matrix system. The results also showed that wet granulation had remarkable impact on release rate of propranolol from liquisolid compacts, reducing the release rate of drug from liquisolid compacts. The kinetics studies revealed that most of the liquisolid formulations followed the zero-order release pattern. X-ray crystallography and DSC ruled out any changes in crystallinity or complex formation during the manufacturing process of liquisolid formulations.

Conclusion This work showed that liquisolid technique can be optimized for the production of sustained release matrices of water-soluble drugs. Polysorbate 80

was used as the liquid medication. The release of drug from these formulations followed zero-order release kinetics. No crystallinity changes or interaction was observed during the process.

Banker, G. S., Rhodes, C. T. (1996) *Modern pharmaceutics*. 3rd Edn, Marcel Dekker, New York, pp 575–593
Nokhodchi, A., et al (2005) *J. Pharm. Pharm. Sci*. **8**: 18–25
Spirease, S., Sadu, S. (1998) *Int. J. Pharm*. **166**: 177–188

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Modelling and control of batch fluidized bed dryers

M. Li[1], G. Smith[1] and S. Duncan[2]

[1]De Montfort University, School of Pharmacy, Leicester, LE1 9BH and [2]University of Oxford, Department of Engineering, Parks Road, Oxford, OX1 3PJ, UK. E-mail: mli@dmu.ac.uk

Objectives Batch fluidized bed dryers are widely used for drying moist powders and granular solids in the food, biotechnology and pharmaceutical industries because they provide good solid mixing and intensive heat and mass transfer between the solid and hot gas phases. Generally, large energy inputs are needed during a drying process because of the high latent heat of water evaporation. As a result, modelling and control of the fluidized bed dryer is important in order to minimize energy usage.

Methods A simple two-phase model is used to describe the dynamics of a fluidized bed dryer, which includes a bubble phase and an emulsion phase consisting of an interstitial gas phase and a solid phase. The model describes the mass and heat transfer between the phases and the effect of the drying-wall is also taken into account. Two distinct issues are addressed in a batch fluidized bed dryer control: one is to determine the optimal operation so that energy consumption is reduced and the other is on-line control of the material drying time to achieve the desired drying rate. In terms of the optimal operation conditions for batch fluidized bed dryers, the control strategy should be a compromise between the energy consumption and the drying time required to achieve the desired end moisture content, which is a typical nonlinear programming problem. It has been shown that the performance of a fluidized bed dryer is dominated by the inlet gas superficial velocity rather than the inlet gas temperature based on the model parameter sensitivity analysis so that the inlet gas superficial velocity is chosen as the only manipulated variable for control while the inlet gas temperature is kept constant. The optimal inlet gas superficial velocity profile can be obtained by solving the energy consumption index with the constraints on the manipulated variable and the end point of particle moisture content. In order to achieve a desired drying rate of powders in a fluidized bed dryer, a feedback controller called generic model control (GMC) is designed. The significant advantages of using the GMC for control of a batch fluidized bed dryer include: (a) the developed fluidized bed dryer model can be used directly in the control algorithm; (b) The GMC can handle a large degree of process model mismatch. In order to implement optimization and on-line feedback control for a batch fluidized bed dryer, a nonlinear observer using an Extended Kalman filter (EKF) has been developed to estimate the particle moisture content in cases where the on-line particle moisture content measurement is not available

Results The experimental validation shows that the developed dynamic model can be used to predict the particle moisture content and temperature profiles during the drying process in a fluidized bed dryer. The sensitivity analysis of model parameters has been carried out using different simulations, indicating that the performance of a batch fluidized bed dryer is affected significantly by the inlet gas superficial velocity and temperature. It is found that the optimal operation condition of a batch fluidized bed dryer can be obtained by setting the inlet gas superficial velocity to its maximum allowable value and the proposed GMC strategy can be successfully used to control a batch fluidized bed dryer.

Conclusions Modelling and control of a batch fluidized bed dryer is required in many industries. The dynamic model and control strategy developed in this study have proved that they can provide satisfactory estimation and control of the particle moisture content.

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In-Vitro and In-vivo evaluation of controlled release erythromycin formulations

A. Elkordy[1] and M. Yusif[2]

[1]University of Sunderland, Wharncliffe street, Sunderland, SR1 3SD, UK and [2]University of Tanta, Tanta, Egypt. E-mail: amal.elkordy@sunderland.ac.uk

Objectives Erythromycin is a broad spectrum antibiotic that is effective against a wide variety of micro-organisms. Erythromycin base is unstable in acidic media. This represents a problem in stability of the drug in stomach juice. The aim of this study was to protect erythromycin base from hydrolysis by gastric acid via controlled release (enteric-coated) formulations by microencapsulation and solid dispersions using Eudragit-L100 and stearic acid as coating materials.

Methods Enteric-coated erythromycin was prepared as follows: (i) microencapsulation using: fluidised-bed coating in which 5%w/v Eudragit-L100 was used as a coating material, and emulsion technique in which different ratios of the drug and stearic acid were used in presence of Tween 80 (1 mg%—this percentage was used as it produced the highest yield) as emulsion stabiliser. The formed microcapsules were separated by filtration, washed, dried and sized. (ii) Solid dispersions using melting and solvent methods. Erythromycin microcapsules and solid dispersions were evaluated via yield percentage, drug incorporation efficiency, particle size distribution analysis, X-ray diffractometry, Fourier transform Infra-red spectroscopy (FT-IR), differential thermal analysis (DTA), microscopic examination, percentage of protecting efficiency against acidic degradation and *in-vitro* and *in-vivo* (for selected erythromycin formulations in tablet and capsule dosage forms) drug release.

Results The yield values, using emulsion method, were inversely proportional to the concentration of the drug incorporated in the microcapsules (e.g. yield was 93.8 ± 2.9 and 73 ± 1.7% for 10% and 40% erythromycin, respectively). The drug incorporation efficiencies (DIE) for microcapsules prepared by fluidised-bed coating and emulsion method were 91.2 ± 8.3 and 96.7 ± 5.7%, respectively. For solid dispersions, the DIE was 96.5 ± 2.8%. The sphericity and individuality, as indicated by microscopic examination, of microcapsules increased by increasing the concentration of stearic acid from 60 to 90% in the emulsion preparation. The narrowest particle size distribution was found when the microcapsules were prepared with 10% erythromycin and 90% stearic acid. FT-IR spectra revealed appearance of a new peak at 1660–1500 cm^{-1} in case of solid dispersion preparations, indicating drug-stearic acid interaction (salt formation). For erythromycin microcapsules, the FT-IR spectra were similar to that of erythromycin, suggesting neither decomposition nor interaction between the drug and stearic acid or Eudragit-L100. X-ray diffractometry and DTA data confirmed the FT-IR data. For release of erythromycin from microcapsules (emulsion method) of different sizes, the dissolution data revealed that with decrease in particle size, there was an increase in the dissolution rate of the drug, due to large surface area. The release was characterised by zero-order kinetic for all types of microcapsules and approximately 60% of the drug was released after 30 min. However, microcapsules prepared by fluidised-bed coating showed rapid initial drug release (51.5 ± 0.4% after 5 min). The extent of erythromycin release from solid dispersions was less than that from microcapsules. Proposed formulations were subjected to *in-vivo* test in human subjects (after ethical approval). Microcapsules prepared by fluidised-bed coating showed the highest serum drug concentration after 3 h compared with other preparations. There was a correlation between the *in-vitro* and *in-vivo* data.

Conclusions Microencapsulations and solid dispersions using Eudragit-L100 and stearic acid for protection of erythromycin base against gastric acid hydrolysis are feasible.

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Analysis of tablet film coating quality using terahertz pulsed imaging

L. Ho[1,2], R. Müller[4], M. Römer[5], K. C. Gordon[6], J. Heinämäki[5], P. Kleinebudde[4], M. Pepper[2,3], T. Rades[1], Y. C. Shen[1], C. J. Strachan[7], P. F. Taday[3] and J. A. Zeitler[1,2]

[1]School of Pharmacy, University of Otago, P.O. Box 56, Dunedin, New Zealand, [2]Cavendish Laboratory, University of Cambridge, CB3 0HE, Cambridge, UK, [3]TeraView Ltd, St John's Innovation Park, CB4 0WS, Cambridge, UK, [4]Institute of Pharmaceutics and Biopharmacetuics, Heinrich-Heine-University, Universitätsstr.1, Düsseldorf, D-40225, Germany, [5]Division of Pharmaceutical Technology, FI 00014 University of Helsinki, Finland, [6]Department of Chemistry, University of Otago, P.O. Box 56, Dunedin, New Zealand and [7]Drug Discovery and Development Technology Centre, University of Helsinki, Finland. E-mail: lchh2@cam.ac.uk

Objectives Tablet coating quality analysis has long been a subject of great interest to the pharmaceutical community, as the consequences of poor coating quality have serious legal and commercial implications. To date there are various techniques capable of investigating aspects of the coating quality available. We report the first application of terahertz pulsed imaging (TPI) to coating quality analysis. TPI operates and obtains information from around 2–120 cm^{-1}. In this range, most pharmaceutical excipients are semi-transparent, thus allowing the non-destructive investigation and accurate determination of coating layer thickness. This study investigates various coating qualities, including coating layer thickness variability and coating distribution. Validation of the TPI measurements with optical microscopy imaging and investigations of the TPI measurement repeatability are also included in the study.

Methods Ten sustained-release (polyvinyl acetate) coated tablets were randomly selected from the same lab-scale batch. Nine were subjected to TPI analysis before the subsequent destructive microscopy validation. One tablet was kept for long-term measurement repeatability analysis. For short-term measurement repeatability determination, triplicate measurements on tablet surfaces side

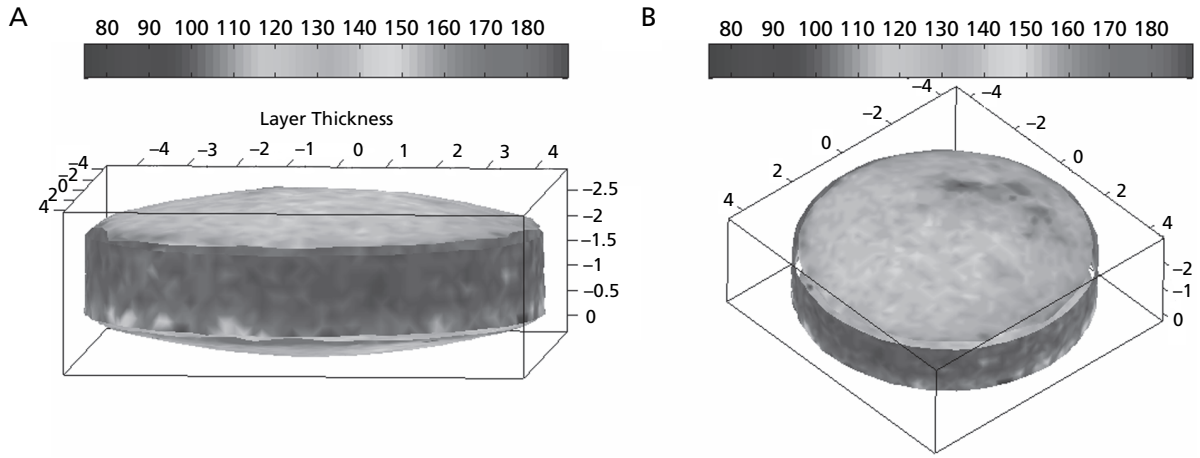

Figure 1 Terahertz 3D models of a tablet. The colour bar is in micron to indicate coating layer thickness. It is visible that the tablet coating thickness on the central band is thinner than that of the tablet surfaces. A defect is also visible in image B.

a, side b and the central band were analysed separately to avoid variations introduced by coating defects. The long-term measurement repeatability was determined by measuring the same tablet once, on nine separate days spanning over a total period of 17 days.

Results Coating layer thickness varied within the same tablet. Some tablets exhibited one side of the tablet surface being at least 10 μm thicker than the other. Bimodal distribution on some tablet surfaces was also observed. For all ten tablets investigated, areas of coating weakness were found around the central band, which was at least 30% thinner than that on the tablet surfaces (Figure 1). Inter-tablet coating examination showed a 7% variation within the batch. Validations with microscopy showed excellent agreement. The short-term measurement repeatability yielded a precision of 0.2% while the long-term measurement repeatability was 0.5%.

Conclusions Terahertz pulsed imaging affords non-destructive, detailed coating analysis on various aspects of coating quality. The validation with microscopy images found excellent agreement with high measurement repeatability. Further research is underway to better understand how the terahertz images reflect the pharmaceutical-performance of the product.

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Properties of inter-granular binder bridges: making the nano to bulk formulation link

B. Crean[1], M. Perkins[2], X. Chen[1], S. R. Banks[3], W. G. Cook[3], C. D. Melia[1] and C. J. Roberts[1]

[1]University of Nottingham, University Park, Nottingham, NG7 2RD, [2]Molecular Profiles, 8 Orchard Place, Nottingham Business Park, Nottingham, NG8 6PX and [3]AstraZeneca R&D Charnwood, Bakewell Road, Loughborough, LE11 5RH, UK. E-mail: paxbc@nottingham.ac.uk

Objectives One of the challenges in the pharmaceutical industry is control of wet granulation processes so that manufacturing conditions and formulation properties may be predicted and optimised. The purpose of this project is to develop novel methods to determine the micro-mechanical and rheological properties of inter-particulate bridges to facilitate their characterisation at high shear rates.

Methods Granules were prepared from α-lactose monohydrate (Pharmatose-450M, DMV International, Holland) with deionised water as a granulating fluid and polyvinylpyrrolidone (PVP) (Plasdone, ISP, Germany) as a polymeric binder. Normal force/distance curves between an Atomic Force Microscope (AFM) cantilever of known spring constant and the surface of a granule were investigated as a function of relative humidity (R_H). Applying the *Voigt* spring and dashpot model, AFM may be used as a nano-rheometer to measure storage (G') and loss moduli (G'') of polymers. AFM has been used to investigate G' and G'' of inter-particulate bridges at constant oscillation amplitude (~3 nm) and fixed frequencies (50, 100, 500 & 1000 Hz) as a function of R_H and cantilever/granule separation distance by measuring amplitude/phase/deflection/distance curves. X-ray micro computed tomography (μCT) was performed on individual granules, made from two different grades of PVP (K29/32 and K90). Confocal Raman microscopy was employed to investigate the distribution of the components within the granule. Cross-sections of the granule were prepared using an ultramicrotome.

Results A marked increase in the energy of adhesion was observed at 80% R_H due to the formation of a viscous bridge in contrast to elastic adhesion at lower R_H. G' and G'' values for inter-particulate bridges are dependent on both frequency and R_H; the bridges are more compliant at higher R_H values and are more elastic in character. As the material bridges, formed at 80% R_H, are stretched G' decreases sharply and G'' initially increases to a maximum before decreasing as the bridge breaks. At the point of rupture at 80% R_H G'' always exceeds G' whereas at lower R_H G' is dominant. Bridge length increases with oscillation frequency. μCT analysis illustrates the morphology and binder distribution of the granules. PVP K90 does not spread over lactose as efficiently as K29/32 due to its higher surface energy and viscosity. Therefore PVP K90 yields granules of low porosity with concentrated dense regions and a few very large pores, while granules made from PVP K29/32 are more porous and have a more uniform pore distribution. Confocal Raman data are consistent with the view of granule micro-structure determined by μCT, where small domains of PVP are surrounded by a lactose matrix.

Conclusions AFM may be employed as a nano-rheometer to investigate the rheology of inter-granular material bridges. This novel method has been used to ascertain how binders of different viscosities and surface energies, granulated with different diluent fillers, behave at high shear rates. Micro X-ray computed tomography (μCT) can be applied to investigate granular micro-structure and resolve porosity as well as excipient and binder volumes. Combining this technique with chemical imaging provides further structural information and confirms the interpretations of the μCT images.

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Swellable elementary osmotic pump (SEOP) as an effective device for delivery of a poorly water-soluble drug (indometacin)

A. Nokhodchi[1], J. Shokri[2], M. Shahsavari[2], P. Ahmadi[2] and P. Rashidi[2]

[1]University of Kent, Medway School of Pharmacy, Central Av., Chatham Maritime, ME4 4TB, Kent, UK and [2]Tabriz University of Medical Sciences, Daneshghah St., Tabriz, 51664, USA. E-mail: a.nokhodchi@kent.ac.uk

Objectives Osmotic drug delivery systems have been considerably developed in recent years and a large number of articles and patents have been devoted. Among different types of per oral osmotic devices, the elementary osmotic pump (EOP) is one of the technically simplest and efficient systems that is suitable for the delivery of highly/moderately water-soluble drugs. The aim of this study is to design a new type of EOP for efficient delivery of poorly water-soluble/practically insoluble drugs. From this system, called swellable elementary osmotic pump (SEOP), drug is released through the delivery orifice in the form of a very fine dispersion in gel ready for dissolution and absorption.

Methods SEOPs were prepared by compressing a mixture of micronized drug and excipients into convex tablets using a single punch tableting machine with 9 mm concave punches and factors affecting the release of drug from the SEOP containing a poorly water-soluble drug, indomethacin, were explored extensively. To this end, the effect of various swelling and wetting agents, orifice size, concentration of osmotic agent and hydrophobic plasticizer were investigated. The tablets were coated with cellulose acetate by dip coating technique. Then a small orifice was drilled through the one side of each coated tablet by a standard mechanical micro-drill. The release behaviour of indometacin from these dosage forms was studied at pH 6.8 for a period of 24 h. The formulations were compared based on four comparative parameters, namely, $D_{24\,h}$ (total release after 24 h), t_L (lag time), RSQ_{zero} (R square of zero order equation) and $D\%_{zero}$ (deviation percent from zero order kinetics).

Results The drug release profile from osmotic devices showed that the type of polymer in the core formulation can markedly affect the drug release. For example, $D_{24\,h}$ was 85.07, 74.29, 99.59, 18.35 and 86.19% for the core formulations containing HPMC K100M, HPMC E50LV, HPMC E15LV, HPMC E5LV and PVP K30, respectively. The results showed that concentration of suspending agent (SLS) in the core formulation was very important parameter in $D_{24\,h}$ and release pattern of indometacin from SEOP system. Incorporation of 15 mg SLS to the core formulation increased D_{24h} from 17.23 to 80.58%. Increasing the amount of suspending agent to an optimum level (60 mg) significantly increased $D_{24\,h}$ and improved zero-order release pattern of indometacin. Increasing hydrophobicity of semi-permeable membrane of the devices by changing percent of hydrophobic (caster oil) or hydrophilic (glycerin) plasticizers in coating formulation markedly increased t_L and decreased $D_{24\,h}$. For instance, the absence of glycerin from semi-permeable membrane prolonged the lag time from 2.47 h to 4.44 h and decreased $D_{24\,h}$ from 85.07 to 68.46%. The results also demonstrated that aperture size is a critical parameter and should be optimized for each SEOP system. Optimum amount of aperture diameter was determined to be 650 μm for zero-order release pattern. t_L and $D\%_{zero}$ were dramatically decreased whereas $D_{24\,h}$ and RSQ_{zero} increased with increasing the aperture size to optimum level. This study also revealed that optimization of semi-permeable membrane thickness is very important for approaching zero-order kinetics.

Conclusion The SEOP was simple to prepare, because there was no need for a push compartment. The results showed that the SEOP can be a very effective device for the delivery of poorly water-soluble drug with zero-order pattern. These devices can release their drug contents in a form of soluble or solid suspended particles out of the system by constant release rate. The main system characteristics including $D_{24\,h}$, t_L, RSQ_{zero} and $D\%_{zero}$ can be improved by optimizing the formulation parameters. The optimized system in this study was able to release indometacin at a zero-order kinetics for 24 h when tested in pH 6.8 medium.

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Simulation and experimental studies of packing characteristics of pellets and effects on the consistency of capsule filling

A. M. Abdel Haleem, M. de Matas, P. York and R. C. Rowe

Institute of Pharmaceutical Innovation, University of Bradford, Richmond Road, Bradford, BD7 1DP, UK. E-mail: a.m.a.ali@bradford.ac.uk

Objectives Pellets are usually large in size (800–1400 μm) compared with powders and granules and have different shapes and surface properties. Therefore, filling of pellets into hard gelatin capsules requires different techniques from those used for powders and granules (Jones 2004). Whilst there is a lack of information on how variations in the shape of pellets influence their packing into capsules (Chopra et al 2001), computer simulations can be used to study these effects. This research uses computer simulation to model the packing of pellets into hard gelatin capsules and to compare weight variations calculated using the simulation tool with experimental measurements.

Methods Lactose monohydrate (Lactochem, UK) and Avicel PH101 (FMC Biopolymer, Ireland), in 1:1 ratio were mixed in a Hobart planetary mixer with water as the binder. Pellets were prepared using an Alexanderwerk extruder (AGM/R Remscheid, Germany) and a 15 inch spheronizer (Caleva Ltd., UK). Pellets obtained after 10 min spheronization were dried in a vacuum oven and then sieved using woven-wire test sieves (BS: 410–1969). Size and shape distributions were evaluated using image analysis (Scion Corp., USA). One size fraction of the pellets (1.0–1.2 mm) was filled into hard gelatin capsules of sizes 0, 1, 2 and 3 using a manual capsule filling machine (Series 60, Copley Scientific Ltd., UK). Macropac software (Intelligensys Ltd., UK) was used to simulate the packing of pellets of similar size and shape to those manufactured. For computational packing studies, pellets with different aspect ratios (AR) were designed using ShapeBuilder (Intelligensys Ltd., UK).

Results Specifically, the simulated packing of pellets of slightly different size and aspect ratio (1.00:1.15:1.20) in similar proportions (35:40:25% by number) to those found experimentally was shown to correlate to the experimental packing of the (1.0–1.2 mm) fraction of pellets more effectively (Table 1). Fill weight variations observed in simulation studies and those of practical experiments indicated similar trends of increasing coefficient of variation (% CV) with decreasing capsule size. However, using uniform pellet size, the simulated % CVs were smaller than experimental values (see Table 1). By including measures of pellet size and shape distribution in the simulations, the % CVs approached those of practical experiments.

Table 1 % CV calculated after simulation and experimental filling of pellets fraction (1.0–1.2 mm) into hard gelatin capsules

Capsule size	Pellets	Simulated spheres		Simulated shapes	
	1.0–1.2 mm	1.0 mm	1.0–1.2 mm	1.15 AR (1.0–1.2 mm)	1.00:1.15:1.20 35:40:25% (by No.)
0	1.93	0.51	0.69	0.64	0.71
3	2.20	1.03	1.21	1.26	2.00

Conclusions Computer simulation has proved to be a valuable tool in modelling the packing of pellets into hard gelatine capsules, especially when appropriate choices of pellet size and shape are made. In particular, calculated weight variations compare well with those observed by experiment.

Chopra, et al, (2001) *Pharm. Dev. Tech*. **6**: 495–503

Jones, B. E., Podczeck, F. (eds) (2004) *Pharmaceutical capsules*. London: Pharmaceutical Press

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Structural changes in sodium lauryl sulphate/cetostearyl alcohol/water ternary systems and the corresponding liquid paraffin in water nanoemulsions induced by ultrasonication

J. H. Kim[1], J. J. Rouse[1], M. J. Lawrence[2] and G. M. Eccleston[1]

[1]University of Strathclyde, Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS), Glasgow, G4 ONR and [2]Kings College, Department of Pharmaceutical Sciences, London SE1 9NH, UK. E-mail: g.m.eccleston@strath.ac.uk

Objectives Nanoemulsions are kinetically stable systems, which have potential for dermatological drug delivery because the nano-sized droplets provide a large interfacial area in contact with skin. In previous work, nanoemulsions formed by the ultrasonication of liquid paraffin-in-water macroemulsions with mixed emulsifier sodium lauryl sulphate (SLS) and cetostearyl alcohol (CSA) were examined. It was shown that the lamellar gel networks, formed in the continuous phase when the mixed emulsifier interacts with water (ternary system) were destroyed by ultrasonication, but that structure rebuilt with time (Kim et al 2006). The aim of this work is to use differential scanning calorimetry (DSC) and small angle neutron scattering (SANS) on selected systems to investigate whether the structural changes after ultrasonication are due to the re-building of lamellar phase.

Methods Ternary (SLS/CSA/water) systems and liquid paraffin-in-water macroemulsions (3 g) containing either 4% w/w or 10% w/w mixed emulsifier (9:1 CSA/SLS ratio) were sonicated at 20 KHz, 30% amplitude, by ultrasound for 30 min (Sonics Vibra cell VCX 500) and examined at 25 °C before and after ultrasonication. Techniques used include microscopy (Polyvar, UK), rheology (CSL 100 Rheometer, TA instruments, UK), differential scanning calorimetry, DSC (DSC 822^e, Mettler, UK) and small angle neutron scattering (4% w/w systems only) using LOQ at ISIS (Oxford, UK).

Results Before ultrasonication, all the systems were semisolid with apparent viscosities ranging from 0.24 to 1.16 Pas. Anisotropic lamellar structures observed microscopically gave a broad endotherm in DSC peaking at ~66 °C, with a shoulder at 62 °C due to the presence of fatty alcohol crystals. The *d*-spacing and bilayer thickness obtained from SANS experiments for the 4% w/w ternary system and (in parenthesis) nanoemulsion were 309Å, 46.3Å (233 Å, 47.9 Å) respectively. All systems were fluid immediately after ultrasonication (apparent viscosities in the range 0.004–0.09 Pas). Lamellar structures were not visible microscopically and the main endotherm (~66°C) in DSC was reduced in the ternary systems and absent in the emulsions. On storage over 4 weeks, all systems became semisolid except the 4% w/w nanoemulsion, which remained fluid. In the aged semisolid systems, the main endotherm due to lamellar phase had re-formed on storage. The lamellar structures (4% w/w ternary system) were shown by SANS to have a similar d-spacing (314 Å) and bilayer thickness (44.5 Å) to those obtained before ultrasonication. In contrast, the main lamellar endotherm did not re-form in the fluid 4% w/w nanoemulsion, and the 'layer' thickness of 28 Å measured implies that although a layer of SLS/CSA is present at the o/w interface, there was insufficient excess mixed emulsifier to form lamellar structures in the continuous phase.

Conclusions DSC and SANS data support the hypothesis that lamellar phase is destroyed by ultrasonication, but rebuilds with time. In nanoemulsions, there is a large increase in interfacial area and extra emulsifier is required to form both an oil droplet monolayer and a lamellar continuous phase.

Kim, J. H., et al (2006) *J. Pharm. Pharmacol*. **58**: A12–A13

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Proton nuclear magnetic resonance (NMR) spectroscopy: a novel application for investigating interactions between a model drug and self-emulsifying drug delivery systems (SEDDS)

A. Mercuri, P. S. Belton, P. G. Royall and S. A. Barker

School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich NR4 7TJ, UK. E-mail: Annalisa.Mercuri@uea.ac.uk

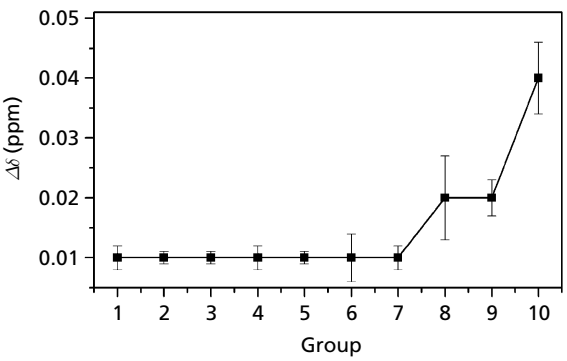
Objectives NMR has found wide applications other than the straightforward identification of chemical compounds; in fact, it can be used to determine interactions

between molecules, protein structures, emulsion droplet size, etc. The proton chemical shift is influenced by the solvent used, neighbouring protons and the chemical nature of the molecule itself. This effect has been used here to investigate drug-excipient interactions and therefore determine the relative position of a probe molecule (Reichardt's dye) within a SEDDS droplet (composed of soybean oil, Tween 80 and Span 80).

Methods The optimal amount of SEDDS placebo was added to a 10^{-3} mol L^{-1} NaOD/D_2O solution (pH 12) containing a dye concentration of 150 μM. Samples were mixed until an even distribution was reached. All spectra (n = 6) were recorded using a NMR Varian 400 MHz; chemical shifts were transformed into the TMS scale by using the partially deuterated water HDO signal ($\delta_{water} = 4.63$ ppm) as an internal reference (Derome 1987). Differences ($\delta\delta$; in ppm) between the chemical shift of the sample containing the dye and a blank derive from the equation $\triangle\delta = \delta_{(sample)} - \delta_{(sample + dye)}$.

Results Figure 1 illustrates the $\triangle\delta$ values observed. Internal protons from the aliphatic and olefinic chains of the surfactants and oil showed minimal difference in the presence and absence of the dye. However, protons arising from the polyoxyethylene (POE) chains show significant changes, indicating that the dye is solubilised in this region of the system. Moreover, the more internal POE units are shifted upfield, indicating that the dye is solubilised deeply inside the palisade layer formed by the surfactant's hydrophilic head.

Conclusions NMR spectroscopy has proved to be a powerful tool for the characterization of interactions between a model drug and the SEDDS. Our data indicates that strong interactions between formulation components are possible and should be investigated. These results may explain why a decrease in formulation performance is often observed as a consequence of drug loading.



N	Proton	Group	N	Proton	Group
1	CH_3	Methyl group	6	CH_2CH_2COOR	β-carboxyl
2	CH_2	Methylene groups	7	CH_2CH_2COOR	α-carboxyl
3	CH = CH-CH_2-CH = CH	Diacyl	8	CH_2OCOR	Glycerol
4	CH_2-CH = CH	α-olefinic	9	CH_2OCOR	Glycerol
5	$CH = CH$	Olefinic	10	OCH_2CH_2O	Methylene

Figure 1 Observed $\triangle\delta$ values for SEDDS protons.

Derome, A. E. (1987) *Modern NMR techniques for chemistry research*. Pergamon

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Drug–excipient interactions in a self-emulsifying drug delivery system (SEDDS) as studied by proton nuclear magnetic resonance (NMR) spectroscopy

A. Mercuri, P. S. Belton, S. A. Barker and P. G. Royall

School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich NR4 7TJ, UK. E-mail: Annalisa.Mercuri@uea.ac.uk

Objectives As a consequence of drug loading, a change in the self-emulsification performance may be observed in SEDDS. This problem may be due to drug interactions with different formulation components. In this study we selected three molecules from the BCS class II as model drugs (naproxen, ibuprofen, flurbiprofen) with similar chemical structures, but different values of aqueous solubility, partition coefficient and pKa.

Methods The optimal amount of SEDDS placebo was added to a D_2O solution containing different drug concentrations (300, 200 and 100 μM). For the blank samples, the same SEDDS amount was added to a DCl/D_2O solution (pD = pH drug-loaded formulation). Samples were mixed until equilibrium was reached. All spectra (n = 3) were recorded using a NMR Varian 400 MHz; chemical shifts were transformed into the TMS scale using the partially deutereted water HDO signal (4.63 ppm) as internal reference.

Results From the spectral analysis (Figure 1), it is possible to highlight that, for all three drugs tested, the polyoxyethylene (POE) units are the preferred solubilisation loci. The POE chains show, in the absence of the drug, a broad signal between 3.4 and 3.5 ppm, as a result of the overlapping signals from different oxyethlene protons. In the presence of a model drug, the POE signal shows a change in peak shape (upfield broadening); this effect is caused by the different environment experienced by those protons that are spatially close to the drug molecules. Furthermore, these changes are dependent on the chemical structure of the drug, as indicated in Figure 1, and its concentration (data not shown).

Conclusions Solubilisation of drugs at the SEDDS droplet surface may explain the observed change in self-emulsification performance. If the drug behaves as a surface-active agent (Fini et al 1995), the optimal SEDDS surfactants ratio changes, and therefore the SEDDS capability to be successfully formed.

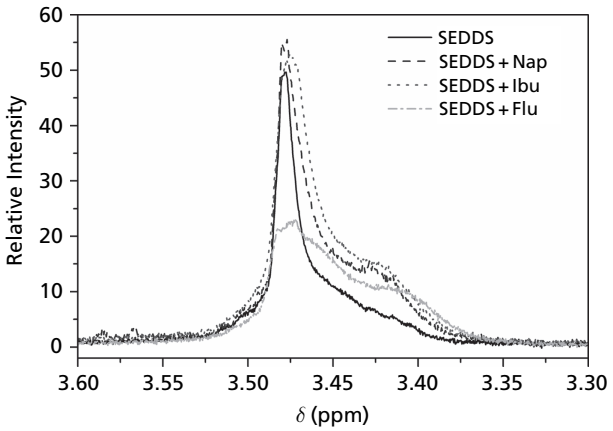

Figure 1 ^{1}H NMR spectra of POE units for SEDDS placebo and in mixtures with naproxen (Nap), ibuprofen (Ibu) and flurbiprofen (Flu) (300 μM).

Fini, A., et al (1995) *Int. J. Pharm.* **126**: 95–102

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Pharmaceutical tablet manufacture: effect of powder processing parameters on granule properties

Z. Langham[1,2], B. Gururajan[2], D. Brooks[2] and J. Kraunsoe[2]

[1]University of Nottingham, School of Pharmacy, University Park, Nottingham NG7 2RD and [2]AstraZeneca R&D, Charnwood, Bakewell Road, Loughborough – LE11 5RH, UK. E-mail: Bindhu.Gururajan@astrazeneca.com

Objectives Wet Granulation is an important step in the manufacture of pharmaceutical tablets, and there are numerous variables to be considered in this process. These include the amount and type of binder, the amount of water and water addition method and also the time for the granulation to occur. The aim of this study was to assess which of these factors has the greatest influence on granule properties for a placebo formulation.

Methods Fifteen granulations were performed using a High Shear Fielder GP1 granulator (Aeromatic Fielder, UK) with a 10-litre bowl, using the same placebo formulation, but changing one of the above-mentioned variables for each. The granulations were monitored *in-situ* using impellor power in order to detect any significant differences that occurred between batches during processing. A series of standard laboratory analyses were also performed on the resultant granules to determine whether the different granulation conditions had an influence on physical properties of the granules produced. Measurement of the Carr's Indices and Critical Orifice Diameter were used as indication of the powder flow properties, backed up by the use of a RST-XS Ring Shear Tester (Dr Dietmar Schulze, Wolfenbüttel, Germany). Sieve analysis and Laser Light Scattering (Malvern Mastersizer) were used to determine the particle size distributions for the materials produced.

Results The analyses showed that the most influential factor on the granulation process for the formulation used in this study was the water addition method. This is likely to be due to larger droplet sizes from pouring the water resulting in larger granule size, compared with using a spray nozzle or dual fluid water addition system. This effect was also seen for the HPC grades that were prepared in solution before use and added wet to the granulation via pouring. Amount of binder, amount of water and time for granulation also had effects on the particle size distributions of the resultant granules.

Table 1 Analyses of variables during wet granulation process

Water Addition method	Addition method of HPC	Amount of binder (%)	Max impellor power (W)	Mean particle size D_{50}	Flow function ffc
Pouring	Dry	3	655	308.986	53
Spray Nozzle	Dry	3	444	153.974	33
Dual Fluid	Dry	3	513	163.595	19
Spray Nozzle	Dry	2	517	137.362	28
Spray Nozzle	Dry	6	810	758.105	4.2
Pouring	Wet	2	730	392.527	27
Pouring	Wet	3	1503	225.823	25

Conclusions All of the granulate produced was shown to have good flow properties as shown in Table 1, therefore demonstrating that despite differences in the particle size distributions, this formulation is robust. This study has also shown that particle size distribution does not necessarily have a direct correlation with poor flow properties as is sometimes assumed. Further work is required to determine how the addition of an API to the formulation will affect this robustness.

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Micro-reactor for forming protein drug particles via supercritical anti-solvent process

T. Z. Du, S. M. Jang and G. S. Huang

Industrial Technology Research Institute, T100, MCL/ITRI 321 Kuang Fu Road, Sec.2, Hsinchu, Taiwan 300, Taiwan. E-mail: DuTzBang@itri.org.tw

Objectives Supercritical carbon dioxide anti-solvent (SAS) process is one of the effective routes to form micro particles. The advantage of low process temperature is suitability for handling heat sensitive materials such as drugs. However, due to the large dead volume existing in such supercritical apparatus (e.g., fittings, valves and cartridge), the recovery of drug particles is low. Also, a large amount of raw material is needed to adjust the process conditions. The drawbacks limit the application of SAS method in drug particle formation process, especially for the expansive protein drugs such as bone growth factor protein drugs. As a result, we have developed a micro-reactor for SAS process with high recovery of protein particles.

Methods A porous metal filter cap for collecting drug particles is combined with a 1-mL mini-vial for loading solution. The combination kit is used as micro-reactor and locked into a high-pressure cartridge. To begin SAS in the micro-reactor, drug solution is firstly added into the mini-vial. The vial is transfer to the bottom of the cartridge and located by the cartridge hole. The mini-vial is then capped with the porous metal filter cap. The mini-vial and the porous metal filter cap are fixed and press each other as the cartridge screws tight. The cartridge with micro-reactor is vertically place into a thermostat water bath. Supercritical carbon dioxide ($SCCO_2$) is filled into the micro-reactor from the bottom of the cartridge to reach the experimental pressure. After a period of batch anti-solvating, the cartridge is reversed for 180° up and down to enable the product precipitated in the mini-vial to drop into the porous metal filter cap. $SCCO_2$ is applied to eliminate the solvent in the micro-reactor from the top of the cartridge. Finally, we release the pressure of the cartridge and dry drug particles are obtained in the micro-reactor.

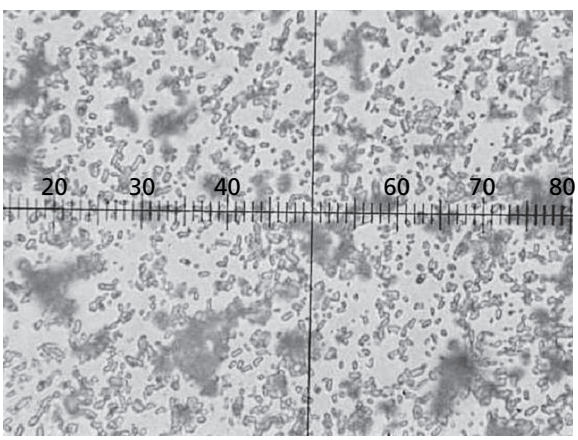

Figure 1 Optical microscope (200×) photograph for BSA particles obtained by SAS process in the micro-reactor.

Results Bovine serum albumin (BSA) is used to verify the micro-reactor applying to SAS process. When operating under 3000 PSI and 35 °C $SCCO_2$ with 0.05 g 10 wt% BSA aqueous solution loaded 0.0046 g of BSA particles are collected. The particles size are about 2~10 μm as shown in Figure 1 and the particle recovery is 92%.

Conclusions The micro-reactor for obtaining high recovery protein particle is verified by forming bovine serum albumin particles via supercritical carbon dioxide anti-solvent process in this study. The particle size is maintain within 2~10 μm and recovery of the forming particles in supercritical process can reach 92%. As a result, the micro-reactor can promisingly reduce the cost of applying supercritical anti-solvent process to form drug particles, especially for those drugs with high unit price.

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Development and evaluation of time dependent pulsatile drug delivery system for the treatment of rheumatoid arthritis

J. Qureshi, J. Ali, A. Ahuja, S. Baboota and S. Hasan

Jamia Hamdard, Hamdard University, Department of Pharmaceutics, Faculty of Pharmacy, Hamdrad Nagar, New Delhi-110062, India. E-mail: dr.sohailhasan@gmail.com

Objective To develop and evaluate a pulsatile drug delivery system having predetermined lag time of 6 h that can meet the circadian requirement associated with morning arthritic pain. The system is based on a drug containing inner core, which was coated with a swelling layer and an outer enteric layer.

Methods Preparation of inner core tablet: core tablets (200 mg) were prepared by dry granulation. Each core tablet consisted of aceclofenac (100 mg), Ac-di-sol (10 mg), Prosolv 90 (silicified microcrystalline cellulose) and magnesium stearate (2 mg). Ac-di-sol was added to obtain fast disintegration of core tablet after a predetermined lag time. Slugs were prepared by single flat punch. Granules obtained were further compressed in to a tablet at an applied force of 4000 kg using 8 m round convex punch. Preparation of press coated tablet: core tablets were press coated with a swelling comprising of 170 mg hydroxy propyl cellulose (HPC) M and 30 mg of ethyl cellulose. Half of the coating material was placed in the cavity, the core tablet was carefully positioned in the centre of the die, and cavity was filled with the other half of the coating material. Coating materials was compressed around the core tablet at a force of 5000 kg using 12 mm diameter round convex punch. Application of enteric coating: compressed coated tablets were further coated with enteric polymer Eudragit L 30 D by conventional coating pan, rotating at 35 rpm with inlet temperature around 35–40 °C and at spraying rate of 2 mL/min. coating suspension consisted of eudragit L 30 D and PEG 400 (20% of total polymer content). Suspension was further diluted with purified water to make the total solid content up to 15%. Tablets of different coating levels (4, 6 and 8% w/w) were with drawn and evaluated.

Results Dissolution studies were carried out in pH 1.2, 6.5, 6.8 and 7.4 using USP apparatus II. Samples were withdrawn at regular intervals and analysed by UV spectrophotometry at 276 nm for the presence of drug. The best results were obtained with 6% w/w enteric coating. For coated tablets there was no release of drug for 6 h (± 0.5 h). After the lag time a rapid burst and complete release was observed.

Conclusion The obtained system can be used to effectively adjust the drug release of aceclofenac in rheumatoid arthritis, which is dependent on circadian rhythm.

Fukui, E., et al (2000) *Int. J. Pharm.* **204**: 7–15
Zhang, Y., et al (2003) *J. Controlled Release* **89**: 47–55

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Gellan-chitosan: a novel polymer matrix for lipase immobilization (part B)

S. A. Pawar, V. B. Pokharkar, N. R. Solanki and A. V. Bhosale

Seth Govind Raghunath Sable College of Pharmacy, saswad, Ta-Puandhar, Pune 412301, India. E-mail: nikunj_nikee@rediffmail.com

Objective The aim of this study was to investigate the application of chitosan crosslinked gellan gum as a novel matrix for lipase immobilization for the formulation of a controlled delivery system.

Method The gellan-chitosan beads were prepared and optimized using a 3^2 factorial design by ionotropic gelation. The 2 variables, gellan and chitosan concentrations, were 1, 1.5, 2% w/v and 0.3, 0,6, 1% w/v, respectively. The lipase-loaded beads were evaluated for the percent yield, swelling index, loading efficiency, release profile, reusability and storage stability. The interactions between gellan and chitosan were confirmed by solid state IR studies. Activity of both immobilised and free lipase was checked in aqueous and organic medium using the substrate p-nitrophenylpalmitate. In aqueous medium, the reaction mixture consisted of lipase (free/immobilized beads) + p-NPP solution in 2-propanol + Tris HCl buffer + deionized water. The reaction was carried out at 37 °C and was incubated for 30 min in an incubator with shaking. The activity was calculated by measuring the absorbance with UV-Visible Spectrophotometer. The effect of added water on the lipase activity in organic medium was studied by adding water in the reaction mixture containing

free lipase/its immobilized counterpart. Kinetics constants were estimated by nonlinear regression (Graph Pad Prism). The surface and cross-sectional morphology of the freeze-dried beads was studied using SEM (JEOL JSM 840 A).

Results It was found that the activity in the aqueous phase is significantly greater compared with any of the organic solvents used. Similarly, amongst organic solvents, hydrophobic solvents like cyclohexane and heptane gave a relatively higher activity than polar solvents like acetone and carbon tetrachloride. It was observed that with no addition of water, the rate of hydrolysis was significant. Thus, the amount of water in the system was enough for high enzyme activity. The increment in the amount of water from 10 to 500 μL added led to an increase in the activity of both free and the immobilized lipase. Also, it was seen that the hydrolysis rate increased with the substrate concentration. Similarly, the kinetics in both aqueous and organic media showed a linear increase in the concentration of para-nitrophenol with time for the first reaction samples and then a plateau corresponding to almost 100% conversion of substrate. Operational stability indicated that the beads were used successfully for 7–9 cycles and retained 50% of the activity after 4 reuses.

Conclusions Gellan gum, a novel microbial exopolysaccharide, and chitosan, a heteropolysaccharide from crustacean shells, along with lipase from porcine pancreas were evaluated for their application in the area of immobilization, which resulted in matrix compositions exhibiting controlled-release therapeutic as well as its industrial applicability owing to the good mechanical strength of the beads.

Desai, P. D., et al (2004) *J. Mol. Catal. B: Enz.* **31**: 143–145
Fadnavis, N., et al (2003) *Biotechnol. Prog*. **19**: 557–564

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Improved Tat-mediated gene delivery in monolayer cell culture and multicellular tumour spheroids

A. F. Alhaj Saleh, H. S. Aojula and A. Pluen

University of Manchester, School of Pharmacy and Pharmaceutical Sciences, The University of Manchester, Stopford Building, Oxford Road, M13 9PT, UK.
E-mail: amer.saleh@postgrad.manchester.ac.uk

Objectives Cell penetrating peptides (CPPs) have been shown to enhance the internalization of different bioactive molecules across the plasma membrane. Tat-derived peptide, one of the best characterized CPPs, has been demonstrated to enhance the cellular uptake of plasmid DNA (pDNA) (Ignatovich et al 2003). However, the use of Tat-derived peptide as a gene carrier may be restricted due to possible entrapment of the Tat/pDNA complexes within endolysosomal compartments. In addition, most biological studies utilizing CPPs were based on cells grown as two-dimensional (2D) monolayer culture, which does not accurately represent the tumour microenvironment. Three-dimensional (3D) multicellular tumour spheroids (MCTS) provide a useful model that mimics the complexity of solid tumours in vivo. In this study, we designed a peptide conjugate composed of Tat motif for cell permeation and a membrane active peptide sequence (LK15) for endosomal escape. The ability of peptides to transfect MCTS and 2D monolayer cell culture was evaluated.

Methods The pDNA condensation property of Tat and the composite peptide Tat-LK15 was evaluated by fluorescence quenching assay. Gene transfer into HT29 cells grown as 2D monolayer culture and 3D MCTS was optimized by a luciferase reporter gene. Using a fluorescent pDNA, we quantified the cellular uptake of peptide/pDNA complexes in monolayer culture by flow cytometry. The localization of EGFP expression and the uptake of peptide/DNA complexes in MCTS were investigated by confocal fluorescence microscopy.

Results Both peptides (Tat and Tat-LK15) were able to condense DNA at a (+/−) charge ratio of 2. Optimal (+/−) charge ratio that provided the maximum level of transfection was 10:1 and 3:1 for Tat and Tat-LK15 peptides, respectively. A significant improvement in transfection efficiency was observed with Tat-LK15 peptide compared with Tat in HT29 cells grown as 2D monolayer culture. Similarly, Tat-LK15/pDNA resulted in higher gene expression in MTCS compared to Tat; however, gene expression using Tat-LK15 was 9% of the expression in monolayers. Treatment of HT29 cells (in 2D cultures) with chloroquine resulted in a 2-fold increase in level of gene transfer mediated by Tat-LK15. The cellular uptake of Tat-LK15/pDNA complexes was up to 3-fold higher than Tat/pDNA complexes in 2D cell culture as analyzed by flow cytometry. Using confocal microscopy, EGFP expression mediated by Tat-LK15 or Tat peptides was localized to the outer cell layers of the MCTS. Likewise, we found that fluorescently labelled complexes could only be detected at the outer cell regions of the MTCS.

Conclusions Attachment of membrane active sequence to Tat-derived peptide can be used to improve the transfection efficiency in both 2D and 3D cell cultures. This may be attributed in part to higher cellular uptake of the peptide/pDNA complexes as measured by flow cytometry. Gene expression was remarkably reduced in MCTS compared to monolayers. Accordingly, limited penetration of the peptide/DNA complexes into deeper central regions of MCTS may represent a major barrier for efficient gene delivery.

Ignatovich, I. A., et al (2003) *J. Biol. Chem.* **278**: 42625–42636