manufacturing site so reducing the quarantine time. A key paper from Blanco & Romero (2001) proposed the use of cascading libraries for NIR spectroscopy. In this work, an NIR spectral library of excipients and active pharmaceutical ingredients (APIs) was constructed and used to compare five identification methods. Reflectance NIR spectra were collected over the range 1100–2500 nm on a FOSS 6500 Spectrophotometer fitted with a Rapid Content Analyser for 210 pharmaceutical compounds. Five pattern recognition methods were compared, based on second derivative spectra: Wavelength Correlation (WC); WC with wavelength selection (WCWS); Maximum Distance in Wavelength Space (MDWS); Peak Positioning (PP); and Soft Independent Modelling of Class Analogies (SIMCA). A reduced library of 55 compounds (containing at least eight batches of each substance) was used to optimise each method. The number of compounds correctly identified by each individual method is given in Table 1. SIMCA was the best method, but it required extensive data analysis for little advantage compared with the other methods. The next best method was PP; but it was discarded because robustness was an issue over time. MDWS came next, but multiple sample spectra are required for this procedure. While WC was the worst performing method, it was easy to update (i.e. add new compounds) and it also gave a good indication of the 'problem' compounds highlighted by the other methods. Identification was best performed by using a cascading approach, initially using WC to divide the spectral library into groups. Each group was then sub-divided sequentially using WCWS, MDWS and SIMCA. Where the group contained both chemically and physically different compounds, then WCWS was next applied. For groups containing only physically different compounds (e.g. particle size), or mixtures with different ratios of components, then MDWS was optimal. SIMCA was finally used for any groups that still remained unresolved. The procedure was initially applied to the library of 55 compounds; 36 compounds were correctly identified with the remainder grouped into starch and starch derivatives or compounds that differed only by the grade of material (Table 1). Applying the procedure to the 210 compounds, for which there were 1–91 batches for each compound, 167 compounds were correctly identified, leaving 43 that fell into 14 groups. Of these 14 groups, only one involved different chemical compounds (ceratonia, guar galactomannan and tara gum). In this study, there were insufficient batches for these compounds to allow further discrimination. In conclusion, while SIMCA is the best individual method, the cascading approach advocated is much better.

Table 1 Number of compounds identified using different pattern recognition methods

Procedure	Method				
	WC	WCWS	MDWS	SIMCA	PP
Single method (n = 55)	24	—	29	33	30
Cumulative procedure (n = 55)	24	29	33	36	—
Cumulative procedure (n = 210)	124	137	151	167	—

Blanco, M., Romero, M. A. (2001) *Analyst* **126**: 2212–2217

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Identification of counterfeit Cialis, Levitra and Viagra tablets by near-infrared spectroscopy

M. Shin[1,2], R. D. Jee[1], K. Chae[2] and A. C. Moffat[1]

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The Busan Regional Korea Food and Drugs Administration (BRKFDA) analysed suspected Cialis, Levitra and Viagra tablets by high-performance liquid chromatography (HPLC), but the analyses took a long time and could not differentiate between excipients. Near-infrared (NIR) spectroscopic analysis was therefore examined as it had previously been used successfully for the identification of counterfeit Viagra tablets (Wilson & Moffat 2004) and to compare parallel imports (Yoon et al 2004). Different authentic batches of Cialis (20 mg tadalafil), Levitra (20 mg vardenafil) and Viagra (100 mg sildenafil) tablets were purchased from a local London pharmacy and authentic Viagra tablets were also obtained from seven different countries. Counterfeit tablets were supplied by the BRKFDA. They were scanned using a FOSS NIRSystems 6500 spectrometer fitted with a Rapid Content Analyser over the range 110–2500 nm taking only 40 s per tablet. Data processing was carried out using Vision software (FOSS) and The Unscrambler (CAMO). Reference analysis (HPLC) was made using a CAPCELL PAK C18 column. All the counterfeit Cialis tablets could be identified from their NIR spectra by using a correlation in wavelength space threshold of 0.90 (using second derivative and standard normal variate (SNV) pre-treatments). A principal components analysis (PCA) model using three principal components easily distinguished counterfeit from authentic Cialis tablets. HPLC analysis revealed that one batch of the counterfeit tablets contained 103 mg sildenafil instead of 20 mg tadalafil. Examination of the NIR spectra of the authentic and counterfeit Levitra tablets showed only small differences. Also, the NIR spectra of the counterfeit Levitra tablets were very similar to authentic Viagra tablets. This was explained by HPLC analysis, which showed that the counterfeit Levitra tablets each contained 1.3 mg sildenafil (the active ingredient of Viagra tablets) and 17 mg of tadalafil (the active ingredient of Cialis tablets) instead of 20 mg of vardenafil. Adequate discrimination was obtained when SNV and second derivative transformations were used together with correlation in wavelength space and maximum wavelength distance algorithms. PCA was the most effective tool for identification. Authentic and counterfeit Levitra tablets could easily be differentiated from the plots by using only the first two principal components. This was true also for centre of gravity plots (Yoon et al 2000). The NIR spectra and second derivative spectra for the authentic and counterfeit Viagra tablets were very similar with correlation in wavelength space not discriminating them. However, maximum wavelength distance (using SNV and second derivative pre-treatments) could discriminate authentic from counterfeit preparations using a threshold of six. Much better was the use of PCA, which could barely differentiate batches of authentic tablets from seven different countries using soft independent modelling class analogy and six principal components, but could easily show the difference between authentic and counterfeit tablets using this model. In conclusion, NIR spectroscopy using PCA is an effective, fast and non-destructive method for identifying counterfeit Cialis, Levitra and Viagra tablets.

Yoon, W. L. et al (2000) In: Davies, A. M. C., Giangiacomo, R., (eds) *Near infrared spectroscopy: proceedings of the 9th International Conference*. Chichester: NIR Publications, pp 547–550
Yoon, W. L. et al (2004) *J. Pharm. Biomed. Anal.* **34**: 933–944
Wilson, N. D., Moffat, A. C. (2004) *J. Pharm. Pharmacol.* **56** (Suppl.): S-3

Short Talks on Pharmaceutics and Drug Delivery

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Enhanced release of acyclovir from intravaginal rings using common vaginal excipients

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The present scale of the HIV pandemic and the failure to develop an effective vaccine have forced scientists to evaluate alternative preventative strategies for reducing the rate of HIV transmission. Since over 90% of new infections result from heterosexual intercourse, the most viable, short-term strategy being pursued is the development of vaginal HIV microbicides. In particular, vaginal rings providing long-term, controlled-release of antiretrovirals over days/weeks/months are actively being investigated for this purpose. Several studies have demonstrated that prior infection with herpes simplex virus type 2 (HSV-2) is an important risk factor for HIV acquisition (Holmberg et al 1988), attributed to the presence of vaginal lesions and subsequent infiltration of CD4 cells to the lesion sites. Therefore, preventing and treating HSV-2 vaginal infections may be a useful strategy in reducing the incidence of female HIV infection. In this study, we evaluate the potential for controlled release of the anti-HSV-2 drug acyclovir from silicone vaginal rings. Silicone, matrix-type vaginal rings containing 10% w/w acyclovir and optionally 15% w/w of various pharmaceutically-acceptable, hydrophilic, vaginal excipients (crosscarmellose, polyacrylic acid, ascorbic acid, octyl dodocanol, povidone iodine, undecylenic acid) were manufactured according to standard methodologies (Malcolm et al 2003). In vitro release studies (37°C, 100 mL aqueous release medium, orbital incubation) were performed to assess the potential of each excipient for providing enhanced release of acyclovir from the silicone devices. Briefly, samples of the release medium were taken daily over a 14-day period, the release medium replaced to ensure sink conditions, and the samples subsequently analysed by UV-HPLC for determination of drug concentrations. For

manufacturing site so reducing the quarantine time. A key paper from Blanco & Romero (2001) proposed the use of cascading libraries for NIR spectroscopy. In this work, an NIR spectral library of excipients and active pharmaceutical ingredients (APIs) was constructed and used to compare five identification methods. Reflectance NIR spectra were collected over the range 1100–2500 nm on a FOSS 6500 Spectrophotometer fitted with a Rapid Content Analyser for 210 pharmaceutical compounds. Five pattern recognition methods were compared, based on second derivative spectra: Wavelength Correlation (WC); WC with wavelength selection (WCWS); Maximum Distance in Wavelength Space (MDWS); Peak Positioning (PP); and Soft Independent Modelling of Class Analogies (SIMCA). A reduced library of 55 compounds (containing at least eight batches of each substance) was used to optimise each method. The number of compounds correctly identified by each individual method is given in Table 1. SIMCA was the best method, but it required extensive data analysis for little advantage compared with the other methods. The next best method was PP; but it was discarded because robustness was an issue over time. MDWS came next, but multiple sample spectra are required for this procedure. While WC was the worst performing method, it was easy to update (i.e. add new compounds) and it also gave a good indication of the 'problem' compounds highlighted by the other methods. Identification was best performed by using a cascading approach, initially using WC to divide the spectral library into groups. Each group was then sub-divided sequentially using WCWS, MDWS and SIMCA. Where the group contained both chemically and physically different compounds, then WCWS was next applied. For groups containing only physically different compounds (e.g. particle size), or mixtures with different ratios of components, then MDWS was optimal. SIMCA was finally used for any groups that still remained unresolved. The procedure was initially applied to the library of 55 compounds; 36 compounds were correctly identified with the remainder grouped into starch and starch derivatives or compounds that differed only by the grade of material (Table 1). Applying the procedure to the 210 compounds, for which there were 1–91 batches for each compound, 167 compounds were correctly identified, leaving 43 that fell into 14 groups. Of these 14 groups, only one involved different chemical compounds (ceratonia, guar galactomannan and tara gum). In this study, there were insufficient batches for these compounds to allow further discrimination. In conclusion, while SIMCA is the best individual method, the cascading approach advocated is much better.

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Short Talks on Pharmaceutics and Drug Delivery

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Enhanced release of acyclovir from intravaginal rings using common vaginal excipients

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The present scale of the HIV pandemic and the failure to develop an effective vaccine have forced scientists to evaluate alternative preventative strategies for reducing the rate of HIV transmission. Since over 90% of new infections result from heterosexual intercourse, the most viable, short-term strategy being pursued is the development of vaginal HIV microbicides. In particular, vaginal rings providing long-term, controlled-release of antiretrovirals over days/weeks/months are actively being investigated for this purpose. Several studies have demonstrated that prior infection with herpes simplex virus type 2 (HSV-2) is an important risk factor for HIV acquisition (Holmberg et al 1988), attributed to the presence of vaginal lesions and subsequent infiltration of CD4 cells to the lesion sites. Therefore, preventing and treating HSV-2 vaginal infections may be a useful strategy in reducing the incidence of female HIV infection. In this study, we evaluate the potential for controlled release of the anti-HSV-2 drug acyclovir from silicone vaginal rings. Silicone, matrix-type vaginal rings containing 10% w/w acyclovir and optionally 15% w/w of various pharmaceutically-acceptable, hydrophilic, vaginal excipients (crosscarmellose, polyacrylic acid, ascorbic acid, octyl dodocanol, povidone iodine, undecylenic acid) were manufactured according to standard methodologies (Malcolm et al 2003). In vitro release studies (37°C, 100 mL aqueous release medium, orbital incubation) were performed to assess the potential of each excipient for providing enhanced release of acyclovir from the silicone devices. Briefly, samples of the release medium were taken daily over a 14-day period, the release medium replaced to ensure sink conditions, and the samples subsequently analysed by UV-HPLC for determination of drug concentrations. For

six of the seven excipients, acyclovir release was enhanced over the 14 days compared with the ring formulation containing no excipient (crosscarmellose 152%, povidone iodine 23%, ascorbic acid 18.2%, polyacrylic acid (MW 2100) 11.2%, polyacrylic acid (MW 5100) 10.8% and octyl dodecanol 3.4%). The results demonstrate that hydrophilic excipients provide greatest release enhancement and have the potential to extend the utility of the rings to the release of substances that might otherwise be difficult to release from the hydrophobic silicone elastomer. The enhanced release may be attributed to a number of factors, including water ingress and subsequent dissolution of the acyclovir. Further studies are required to determine the vaginal concentration of acyclovir required to reduce HIV transmission and to optimise the release profile from these modified ring formulations.

Holmberg, S. D. et al (1988) *JAMA* **259**: 1048–1050
Malcolm, K. et al (2003) *J. Control. Release* **90**: 217–225

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An investigation into protein stability following low-frequency ultrasound application

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Low-frequency ultrasound (US) has been shown to be effective in enhancing protein and DNA permeation (Boucaud et al 2002; Tezel et al 2004). US can be applied to the skin as a pre-treatment (i.e. before drug application) or concurrently with the drug. The possible advantage of the latter is potentially higher protein permeation into the skin while a possible disadvantage is protein degradation due to heat and other processes generated by US (e.g. cavitation). This study was conducted to determine the stability of a model protein when exposed to US. A solution of bovine serum albumin (MW 66 kDa) in a thermally insulated container was exposed to US (20 kHz) 70% amplitude for a total sonication time of 5 min using a calibrated sonicator (VCX500) in 3 different modes of US application: continuous mode, 5 s on 5 s off pulse mode (50% duty cycle) and 0.1 s on 0.9 s off pulse mode (10% duty cycle). Temperature changes in the coupling medium were measured throughout the experiments. At the end of the experiments, gel electrophoresis was performed and band intensities were analysed using Scion Image software (Maryland, USA) to assess changes in protein structure. A control experiment where a temperature change of 50% duty cycle US was mimicked was conducted to determine the contribution of heat changes in protein stability, if any. It was found that continuous mode produced the greatest rate of increase in temperature (~50°C) in 5 min. 50% Duty cycle (5 s on, 5 s off) pulse mode caused a similar temperature rise, but over a duration of 10 min. In contrast for the 10% duty cycle (0.1 s on, 0.9 s off) the maximum temperature rise was ~15°C. Analysis of band intensities following SDS-PAGE revealed that continuous mode caused the most severe BSA degradation, followed by 50% duty cycle, then by 10% duty cycle (Table 1). Interestingly the control experiment, which mimicked the temperature rise of 50% duty cycle, showed only a small percentage of degradation. This shows that BSA degradation upon sonication was not wholly due to the heat generated during US application but was related to other effects of US such as cavitation. We conclude that US treatment causes degradation of protein and damage is more likely when low frequency US is applied continuously compared with application in pulses. Thermal effects contribute to protein degradation but are not solely responsible for the degradation. Severe protein degradation during simultaneous sonophoresis may result in loss of activity. Therefore, ultrasound pre-treatment will be considered for our future experiments.

Table 1 Percentage of BSA left after sonication

Ultrasound protocol	Percentage of BSA (n = 4)
Continuous mode	48.4 ± 8.6
5 s on 5 s off pulse mode (50% duty cycle)	71.9 ± 10.5
0.1 s on 0.9 s off pulse mode (10% duty cycle)	87.2 ± 15.5
Control	91.5 ± 7.2

Boucaud, A. et al (2002) *J. Control. Release* **81**: 113–119
Tezel, A. et al (2004) *Pharm. Res.* **21**: 2219–2225

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Polymer-based gene delivery system with a novel polymer-multilayer encapsulating structure

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Polymer-based gene delivery systems have been studied as promising formulations for gene therapy. The studies in this area mainly focus on the systems formed by DNA and merely one polycation, which can be denoted as DNA/polycation delivery system. A variety of natural or synthetic polycations, such as polyethyleneimine, polylysine, chitosan, etc., have been used. Although considerable progress has been made in this area, the design of "artificial virus" system that would contain different functional components has not yet been achieved. The objective of this study is to apply a layer-by-layer self-assembly approach (Sukhorukov et al 1998) to build a novel polymer-based gene delivery system, which has an "onion-like" multilayer structure. We apply this approach on gene delivery system in the way of forming a core by condensing DNA with a polycation, and then depositing a polyanion on the formed cores to build an outer layer. The alternative deposition of oppositely charged polymers can be repeated until the desired number of layers is achieved. It is hoped that by formulating such a multilayer structure, the drawbacks of DNA/polycation system can be overcome; moreover, multifunctionality of the delivery system can be further developed based on such a structure. Our study has been carried out using two species of DNA (calf thymus DNA and salmon sperm DNA), the polycations of polyethyleneimine, chitosan and poly (2-(diethylamino)ethyl methacrylate), and the polyanions of poly(styrene sulfonate), poly(aspartic acid) and hyaluronan (both undegraded and degraded). The medium is 10 mM Tris-HCl buffer, at pH 7.4 and pH 4.0, respectively. Ethidium bromide displacement assay, size measurement by PCS, zeta-potential measurement and gel electrophoresis were used to study formation and characterize the system. The results show that the polycations used condense DNA into particles to different degrees, whereby polyethyleneimine appears the most efficient. After the polyanion addition to the DNA/polycation particles, "free" DNA was detected in the case of poly(styrene sulfonate) and poly(aspartic acid), indicating polyelectrolyte competition and DNA displacement from the complexes (Danielsen et al 2005); hence the polymers were not appropriate for our aim. However, when hyaluronan was used, DNA seemed not be released from the system: no "free" DNA was observed in the medium. Moreover, in optimized conditions, particles of smaller size and improved colloidal stability could be achieved. Therefore, we conclude that combination of polyethyleneimine and hyaluronan may be a potential polyelectrolyte system to take part in the building of multilayers. Moreover, as a natural polymer of non-toxic, non-immunogenic and with potentially targeting ability, hyaluronan is advantageous to be used in gene delivery systems.

Danielsen, S. et al (2005) *Biopolymers* **77**: 86–97
Sukhorukov, G. B. et al (1998) *Polym. Adv. Technol.* **9**: 1–9

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Hydrogels based on PLGA-PEG-PLGA triblock co-polymers as sustained release reservoirs for the delivery of pDNA to microneedle treated human skin

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The ability to deliver to and express plasmid DNA (pDNA) within the viable epidermis of skin holds significant therapeutic potential, most notably in genetic vaccination. In this respect the skin is an attractive target due to its considerable immune surveillance capabilities, a consequence of its proximity to the physical environment. However, there are a number of formidable barriers that restrict cutaneous gene delivery; these include the stratum corneum (SC) and the rapid elimination of the vector by the host cells (Hengge et al 1995). To address these issues, microfabricated microneedles were utilized to compromise the SC barrier, creating channels primarily in the epidermis, permitting direct access to the viable cells in a precise and potentially pain free manner. Hydrogel formulations, based on Carbopol-940 and PLGA-PEG-PLGA, were used to provide a sustained release environment for pDNA, replacing that previously eliminated by the host cells. Microfabricated micro-

needle arrays, first described by Prausnitz in 1998 (Henry et al 1998), were manufactured using both wet- and dry-etch methodologies and subsequently characterized via scanning electron microscopy (SEM). SC disruption was determined using heat separated human epidermal membranes and human split thickness skin treated with the microneedle device. Conformation of channel formation was made both by SEM and transmission electron microscopy (TEM) and determining the trans-epithelial water loss (TEWL) of skin. Carbopol-940 gels were prepared at varying strengths, each loaded with 100 μg of the pCMVß reporter plasmid, and neutralized with TEA. Polymers of PLGA-PEG-PLGA were synthesized by ring-opening-polymerization (ROP) using stannous octoate as the catalyst (Chen et al (2004) and structurally characterized by ^{1}H NMR, GPC and HPLC. Hydrogels were formed from the polymer at strengths (20–30% w/v) and loaded with 100 μg of the pCMVß reporter plasmid. Transfection studies were performed on fresh, split thickness human skin obtained immediately after surgery. Following microneedle disruption and hydrogel application the tissue was maintained in organ culture for 24 h followed by X-gal staining to give a visual indication of positive transgene expression. Other samples were subject to RNA isolation followed by entry into RT-PCR reactions containing primers specific for the reporter gene messenger RNA (mRNA) transcript. Microneedle treatment resulted in comprehensive disruption of the SC and the formation of channels of 150–200 μm length (i.e. to the basement of the viable epidermis). Additionally, a significant increase in TEWL, through skin treated with both wet- and dry-etched microneedles, was also observed, with the highest levels observed by the dry-etched microneedle array. Analysis of the PLGA-PEG-PLGA polymers showed that its chemical identity conformed to that predicted before synthesis. Hydrogels based on both Carbopol-940 and PLGA-PEG-PLGA could harbour and release functional pDNA, as determined by electrophoresis, which was subsequently shown, by RT-PCR and X-gal staining, to successfully transfect microneedle treated human skin in organ culture. Initial studies suggest that transfection is enhanced when pDNA is released from PLGA-PEG-PLGA based hydrogels compared with those based on Carbopol-940, though more studies are required to verify this and elucidate the reason why. Using an innovative combination of microfabricated microneedles and pDNA-loaded hydrogels it is possible to successfully deliver and express transgenes in human skin maintained in organ culture. These delivery platforms warrant further investigations for their potential in the cutaneous administration of genetic vaccines.

Chen et al (2004) *Int. J. Pharm.* **288**: 207–218
Hengge et al (1995) *Nat. Genetics* **10**: 161–166
Henry et al (1998) *J. Pharm. Sci.* **87**: 922–925

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The influence of alkyl chain symmetry on liposomal encapsulation of poorly water soluble midazolam

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The biphasic nature of the liposomal systems enables efficient solubilisation of both water soluble and insoluble drug moieties. However, careful bilayer characteristics need to be studied to enhance the entrapment of poorly water soluble drugs. This work investigates the role of structural geometry of phosphatidylcholine (PC) analogues on the encapsulation of midazolam, a poorly water soluble model drug candidate. Multi-lamellar vesicles (MLV) were formulated via the hand shaking method, with the addition of 1.0 mg of poorly water soluble midazolam (experimental logP = 3.88; Monzon & Yudi 2001). The lipids investigated include symmetrical (1,2-distearoyl-sn-glycero-3-phosphocholine, DSPC; 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, DPPC; 1,2-dimyristoyl-sn-glycero-3-phosphocholine, DMPC) and asymmetrical (1-myristoyl-2-stearoyl-sn-glycero-3-phosphocoline, MSPC; 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, POPC) types, along with the addition of 11% cholesterol (Chol) in all cases. Liposome encapsulation was measured by spectrophotometric analysis of the supernatant following MLV separation by centrifugation, and mean volume diameter noted using a Malvern Mastersizer X. Initial optimisation studies showed that of those tested, 11% cholesterol content encapsulated the highest quantity of midazolam and yielded the largest liposomal diameter size (6.24 μm), therefore suggesting this to be the most efficient and to be investigated further with the various PC analogues and cholesterol. Incorporation studies using PC and its derivates suggest that symmetrical PC derivatives (i.e. DMPC, DPPC and DSPC) provide more efficient encapsulation of midazolam when compared with that of their asymmetrical counterparts and a trend of increasing midazolam incorporation with increasing lipid alkyl chain length in the order of DSPC = DPPC > DMPC > PC (Table 1). This trend correlates with previous work investigating the solubilisation of ibuprofen in liposomes (Mohammed et al 2004) and micellar solubilisation of barbiturates (Arnarson et al 1980) and may be attributed to the increased lipophilic area within these longer lipid bilayers. The two asymmetric lipid (MPSC, POPC) formulations yielded the lowest encapsulation (~4% mol/mol) and a lower MLV size than DSPC, DPPC, and DMPC liposomes, therefore, suggesting geometry to play a key role in drug bilayer loading. Statistical variance analysis showed that the encapsulation values were significantly different for all the formulations ($P < 0.05$) except when comparing DMPC:Chol with MSPC:Chol. The decrease in encapsulation could be as a result of bilayer formation via a mismatch interaction between asymmetric lipids, therefore reducing the overall hydrophobic region when compared with that of symmetric lipid bilayers. In conclusion, these results suggest that alkyl chain symmetry plays a significant role in the incorporation of the poorly water soluble drug midazolam. Investigations continue into combinations of both symmetrical and asymmetrical lipids.

Table 1 The influence of long-chain symmetric and asymmetric lipids on midazolam encapsulation and mean volume diameter

Lipid category	Formulation	Encapsulation (% mol/mol)	Size (μm)
Symmetric	DSPC:Chol	7.09 ± 0.33	8.49 ± 0.19
	DPPC:Chol	6.46 ± 0.12	7.42 ± 0.10
	DMPC:Chol	5.13 ± 0.36	8.32 ± 0.67
	PC:Chol	4.79 ± 0.30	6.24 ± 0.35
Asymmetric	POPC:Chol	4.06 ± 0.48	7.23 ± 0.20
	MSPC:Chol	4.01 ± 0.91	7.14 ± 0.18

Result represent mean ± s.e., n = 3.

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Amphiphilic lower generation polypropylenimine dendrimer as a gene delivery agent

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An ideal gene medicine would be orally active, transforming only the cells within the target tissue. Polypropylenimine or DAB (diaminobutamine) dendrimers contain 100% protonable nitrogens and have been used for gene delivery. DAB-16-Am (generation 3, 1687 Da, 16 terminal amine groups) is able to completely condense DNA (Zinselmeyer et al 2002). Administration of DAB-16-Am-DNA complexes via the tail vein to mice was found to produce preferential liver expression of the reporter gene, in contrast with a commercial linear polyethylenimine formulation that resulted in high levels of gene expression in the lung tissue (Schätzlein et al 2005). Intravenous injection of DAB-16-Am-DNA complexes to CD1 nude mice bearing A431 xenografts has also been shown to result in a localisation of gene expression in the tumours (Dufes et al 2005). Lung avoidance is therefore advantageous and possibly occurs because the dendrimer can resist aggregation in the lung capillary bed, an effect which may be influenced by amphiphilicity, molecular size and overall charge of complexes. To test this hypothesis, a cetylated derivative of DAB-16-Am has been synthesised. DAB-16-Am and cetyl DAB-16 dendrimers were complexed with DNA at a 5:1 weight ratio (nitrogen:phosphate molar ratio 30:1) for comparison (Table 1). Visualisation in liquid using tapping mode AFM reveals flower-like strands of DNA protruding from DAB-16-Am-DNA complexes whereas DNA complexed with cetyl DAB-16 appears to be completely 'coated' by the dendrimer. The mean IC_{50} of the DAB-16-Am-DNA formulation determined by MTT assay of treated A431 cells (92.7 $\mu g\,mL^{-1}$) is reduced by one order of magnitude after modification. Cetyl DAB-16 self assembles into bilayer vesicles (of approximately 50 nm diameter) in aqueous media in the presence of cholesterol. This vesicle structure does not alter the mean size and charge of the cetyl DAB-16-

DNA complex but there is a modest improvement in the biocompatibility of the formulation. At the ratio tested, cetyl DAB-16 complexed with DNA transfects A431 cells with the β-galactosidase reporter gene at least as efficiently as the parent dendrimer and is comparable with DOTAP (a commercial in vitro lipid transfection reagent). This amphiphilic dendrimer has potential as an in vivo carrier of therapeutic genes and as a tool to identify key determinants of the biodistribution profile of complexes and factors influencing complex uptake by solid tumours.

Table 1 Key features of the dendrimers and their complexes

Dendrimer (complex with DNA N:P = 30)	Molar % cetylation (chains per molecule)	Mean % DNA binding (n = 3)	Mean hydro-dynamic diameter of complex (nm) (n = 3)	Mean zeta potential (mV) (n = 3)
DAB-16-Am	0 (0)	80.5 ± 1.0	310.2 ± 0.4	+11.4 ± 0.8
Cetyl DAB-16	2.4 (0.72)	97.2 ± 1.0	244.7 ± 0.9	+32.8 ± 0.9

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pH-temperature sensitive microgel particles as (trans)dermal drug delivery systems

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Microgels are dispersions of particles in the size range of 1 nm to 1 μm. They have a high degree of sensitivity to changes in environmental conditions (i.e. a change in temperature, pH, ionic strength and solvency). As a function of these changing conditions, microgels undergo rapid changes in their physical properties, including changes in particle size (behaving like "micro-sponges") and surface charge density; taking these properties into account, the term "smart materials" is often applied to microgel particles. In this study, microgels will be used as novel drug carriers (i.e. a novel controlled drug delivery system) for either dermal or transdermal delivery. The steps followed in this project were, firstly, to investigate the uptake and release of model compounds with different physico-chemical properties (i.e. solubility and $\log K_{oct/w}$) to and from a colloidal gel system. Secondly, the study of the permeation of the model compounds across human epidermis. The first part of the project was to co-synthesise pH- and temperature-sensitive colloidal microgels particles based on a co-polymer system of poly(N-isopropylacrylamide) (85%) -co-butyl acrylate (10%) -co-methacrylic acid (5%) (NIPAM/BA/MAA) (85/10/5) (% w/w), in the presence and in the absence of methyl paraben (MP), propyl paraben (PP) and salicylamide (SA), by a surfactant-free emulsion polymerisation (SFEP) in water. Physico-chemical properties of the microgels were determined using different techniques, including Photon Correlation Spectroscopy (PCS) and Nuclear Magnetic Resonance (^{1}H and ^{13}C spectroscopy). The uptake and release of the model compounds to and from colloidal microgel particles was investigated by partitioning (i.e. centrifugation) and in vitro drug release experiments (i.e. dialysis). Results showed that there is a relationship between the amount of compound entrapped and the solubility and $\log K_{oct/w}$ of the compounds. Diffusion studies, across human skin, were performed at 305 K in the range of pH 3–7 for saturated solutions of SA, MP and PP, and for microgel particles incorporating the three compounds. The transport rate for the microgels incorporating MP was reduced by 2–3 fold compared with the saturated solution, by one order of magnitude for PP, meanwhile the transport rate for the microgels incorporating SA is the same order of magnitude as that for the corresponding saturated solutions. (Trans)dermal release studies of the saturated colloidal dispersions indicated that pH control of the drug release was marginal. The incorporation of compounds into the pH/temperature sensitive co-polymer (NIPAM/BA/MAA) (85/10/5) and the subsequent release depends on the $\log K_{oct/w}$ and solubility of the respective compound.

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Drug delivery system induced gene expression changes in cells in vitro and in vivo

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Gene therapy confers enormous potential for the successful treatment of genome-based diseases. To date, a number of gene therapy strategies have reached the stage of clinical trials. The success of gene therapy mainly depends upon the development of suitable delivery systems for in vivo gene transfer. Many clinical trials have been performed with viral vectors, but the potential risks of undesired immune and toxic side reactions have raised concern (Somia & Verma 2000). Furthermore, the potential of viral recombination to replicate competent wild-type virus and insertional mutagenesis needs to be more fully evaluated (Whitehouse 2003). Non-viral vectors are a promising alternative to viral vectors since they offer a higher degree of safety, as well as ease of manufacture. Polycations of lipid and polymers provide great potential for non-viral gene delivery, in which DNA or RNA form lipoplexes and polymers polyplexes with lipid and polymer formulations, respectively. However, little is known about the impact of non-viral vectors at the subcellular level. Here we investigated the toxicogenomics of the cationic polymers, linear and branched poly(ethyleneimine) (PEI), using microarray analysis of both cultured cells and in vivo tumour samples. The carcinoma cell line, A431 was the culture model utilised, both in vitro and in vivo as the basis of the tumour model. Microarray was carried out using 10 k and 20 k gene human arrays (MWG-Biotech), following the protocol described by Omidi et al (2003). The quality of mRNA isolates was confirmed before Cy-dye labelling and microarray hybridisation. Images were then analysed using Imagene 5 and Genesight packages (BioDiscovery, Inc.); further annotation and gene ontology analysis was undertaken using DAVID EASE software. Verification of microarray data was performed using semi-quantitative rt-PCR. Western blot analysis of specific protein expression was used to further verify the microarray findings. Cells in culture were treated with PEI and harvested for analysis 24 or 48 h later. An increased number of gene expression changes was observed with increasing concentration of branched PEI whereby 2.5 μg of PEI effected 875 gene expression changes by greater than 2-fold while at 10 μg PEI, 3468 genes were altered in their expression after 24 h. In a study of the effect of PEI exposure time on toxicogenomics, gene expression changes at 48 h post-transfection were reduced by 45% compared with 4 h post-transfection for branched PEI. In vivo administration of PEI by intra-tumoural injection into A431 xenografts showed that greater than 4 times more gene expression changes in a wide range of gene ontologies were induced by branched PEI compared to linear PEI, suggesting that toxicogenomics are influenced by polymer architecture. These studies suggest that drug delivery systems can markedly alter target cell genomics. They also show that polymer architecture may be an important determinant of the final number and type of genes that are affected in target cells. Thus, a detailed understanding of delivery system genomics will help guide their appropriate use in vivo.

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Application of real time characterisation techniques during Zydis product development

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The Zydis dosage form is a fast dispersing tablet designed to disintegrate in less than 10 s and thus dissolves instantly to release the drug when placed on the tongue. The Zydis process involves dispersing the active pharmaceutical ingredient (API) in an aqueous solution of gelatin and mannitol, which is then dispensed into preformed blister pockets. The dispensed aqueous "dispersion" is then frozen and freeze-dried to form a light porous freeze-dried tablet. The API may be dispersed as a suspension or solution and held within the aqueous phase for several hours. Therefore, the ability to monitor API uniformity and potential for morphological changes on line is a significant advantage. Real time particle characterisation is one of several PAT tools being evaluated by

Cardinal Health for Zydis product development. This abstract summarised a study on the use of real time analysis to monitor morphological changes, crystal growth, suspension homogeneity and impact of processing conditions, potential physical interactions of excipients and API, for a Zydis "dispersion". For this work, three model Zydis "dispersions" were evaluated using two PAT tools, namely Focus Beam Reflectance Measurement (FBRM) and the Particle Vision and Measurement (PVM) systems. In Example 1, the FBRM tracked the change in the crystal habit of a model drug "A" known to undergo pseudo-polymorphic transformation on prolonged suspension hold and or with an increase in suspension temperature. The data indicated that the particles of the original polymorph dissolved and then transformed into the new pseudo-polymorphic form with new habits. These observations were confirmed by off line microscopy. In Example 2, FBRM and PVM were used to monitor the uniformity of the dispersion and the physical interactions of the API and excipients. The FBRM tracked the particle size and number during the preparation of Zydis suspensions for a model compound "B" with a range of excipients typically used in Zydis formulations. The FBRM was able to quantify the changes to the particle size and particle number as each ingredient was added. The effects of addition of excipients, even in relatively small quantities (e.g. oily flavour, colour pigments, surfactant) were detected. The dispersion and de-aggregation of API particles to reach a stable suspension were also successful detected. The PVM captured images of the dispersion on line, showing clearly the physical interactions of the excipients with each other and with the API. In Example 3, the FBRM tracked the changes that took place as a result of different solid loading of model drug "C", the influence of process conditions on particle dispersion, and stability of the homogenised model suspension while held in an Intermediate Storage Vessel (ISV). In summary, the above examples demonstrated the usefulness of FBRM and PVM in product development by providing real time data for Zydis "dispersion" on events such as changes in crystal growth and habits, physical interactions between the formulation ingredients and the influence on processing conditions on the stability and uniformity of the dispersion.

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Gastro-retentive dosage forms: the characterisation of floating calcium alginate beads

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Gastro-retentive dosage forms have the potential to improve drug bioavailability compared with that from many commercially available immediate release and modified release products. A dosage form, based on freeze-dried calcium alginate beads has been developed and shown to have prolonged gastro-retention in the fed state. The aims of this work were to obtain information regarding the structure, floating ability and changes that occur when the dosage form is placed in aqueous media. Calcium alginate beads were prepared by extruding sodium alginate solution 2% w/w into calcium chloride 0.02 M solution. The precipitated gelled calcium alginate beads were freeze-dried. The formula was modified so that calcium alginate beads containing a model drug, riboflavin, were produced. The characterisation of the calcium alginate beads was divided into five main categories: physical parameters, floating ability, imaging, and release rate of riboflavin from the calcium alginate beads. The weight and diameter of the calcium alginate beads were assessed. The floating ability of the calcium alginate beads was assessed using the resultant weight technique (Timmermans & Moës 1990). The technique considers vertical and gravitational forces exerted by the dosage form, hence providing a quantitative measure of floating ability. The internal and external morphology of the calcium alginate beads was viewed using SEM. Digital photography studied the effect of the aqueous environment on dried calcium alginate beads. Confocal laser scanning microscopy measured the diffusion rate and movement of riboflavin from the calcium alginate beads when the beads were placed in aqueous media. X-ray microanalysis was used to determine the presence of phosphorous and the distribution of riboflavin-5′-phosphate within dried calcium alginate beads. The weight and diameter of the calcium alginate beads varied according to formulation. Calcium alginate beads containing riboflavin were 16.3% larger in diameter, and had a mass 3.2% greater, than placebo calcium alginate beads. The resultant weight technique demonstrated that the calcium alginate beads floated for a time in excess of 12 h, regardless of formulation. SEM showed all calcium alginate beads to be spherical and consist of air filled cavities that enabled floatation. X-ray microanalysis showed the presence of calcium and chlorine, which was expected. The X-ray microanalysis data plot of calcium alginate beads containing riboflavin-5′-phosphate did not show the presence of phosphorus. Only a small part of the riboflavin-5′-phosphate molecule is phosphorous. The absence of phosphorous in the X-ray microanalysis results may be due to insufficient amounts of phosphorous in the calcium alginate bead. Therefore, distribution of riboflavin-5′-phosphate within the calcium alginate beads was not observed. Confocal laser scanning microscopy showed that the movement of riboflavin throughout the bead and rate of riboflavin release from the calcium alginate beads occurred rapidly, (diffusion coefficient $24.70 \times 10^8\,cm^2\,s^{-1}$). Therefore, the drug would not remain in the dosage form long enough to demonstrate prolonged gastro-retention without further formula modifications. Digital photography showed that when calcium alginate beads were placed in aqueous media, air filled cavities remained. The development of a gel barrier, that slows the ingress of the dissolution medium, was also observed. Drug release may therefore occur by erosion and diffusion. The characterisation of calcium alginate beads of different formulations has resulted in obtaining an understanding of the properties of the floating dosage form. The characteristics of the calcium alginate beads make them suitable for further investigation as modified release gastro-retentive dosage form.

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An investigation into the physical properties of a wet granulated formulation at different stages of production

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The prediction of the properties of granules made by wet granulation is one objective the pharmaceutical industry currently works towards, to optimise products and manufacturing processes and ultimately minimise development times. The aim of this research was to study the physical properties of granules, here by using macro-granules made by a beam formation process from the wet mass, and the final product (i.e. tablets) made from conventional granules of the same wet mass. Wet granulation masses containing microcrystalline cellulose and lactose monohydrate with varying concentrations of a liquid binder, polyvinylpyrrolidone (PVP), dissolved in water (0–6%), were produced using a Glatt high shear mixer granulator. A percentage of the wet mass was used to form model granules in the form of beams as described in a previous study (Pettersson et al 1997). The beams were dried in two ways, in an oven for 1 h at 60°C and at room temperature for 1 week. A notch of defined dimensions was introduced centrally to the beams, which were then subjected to bending stress using a CT5 strength tester with a 3-point bending rig attached. The remaining wet mass was screened and dried and granule size analysis was carried out, retaining the relevant size fractions for tablet production and for further assessment. The results obtained from granules dried at 60°C showed no clear trends between the tablet and beam tensile strengths. Beams containing 3% PVP, which were oven dried, were found to be brittle to an extent where a satisfactory notch could not be introduced into the beams. This study was repeated and the beams produced were left to dry for a week at room temperature. This method of drying resulted in specimens that were easier to handle, and not as brittle. The results of the repeated study showed that a correlation between beam and tablet strength did exist and the tensile strength of the beams and tablets were similar (Table 1). One observation, which occurred in both studies, was that between 3 and 4% PVP the tablet and beam tensile strength increased and then decreased significantly between 4–6%. This was more

Table 1 Tablet and beam tensile strength at varying PVP concentrations

PVP concn (%)	Granule size fraction (μm)	Tablet tensile strength (MPa)	Beam tensile strength (MPa)
0	355	3.44 ± 0.23	3.43 ± 0.66
	500	3.14 ± 0.22	
1	355	2.89 ± 0.24	3.71 ± 0.73
	500	2.74 ± 0.39	
2	355	2.65 ± 0.31	2.50 ± 1.58
	500	2.65 ± 0.13	
3	355	2.76 ± 0.13	2.31 ± 0.99
	500	2.78 ± 0.24	
4	355	2.98 ± 0.28	2.75 ± 1.14
	500	3.19 ± 0.34	
5	355	2.25 ± 0.18	0.83 ± 0.43
	500	2.40 ± 0.27	
6	355	2.36 ± 0.08	1.48 ± 0.71
	500	2.43 ± 0.33	

pronounced in the beam data and was clearly visible when the results were illustrated graphically. This trend was also observed in a recent study into the strength and morphology of solid bridges in pharmaceutical powders (Bika et al 2005), where PVP was used as a binder with mannitol. The study has demonstrated that the properties of the model granules and the properties of the tablets are comparable and estimations of the physical properties of the tablets produced from the same formulation could be made from such model specimens.

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Small Angle Neutron Scattering (SANS) from polymer-stabilised drug nanoparticles

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An increasing number of new drugs exhibit extremely poor water-solubility, and hence, low and erratic oral bioavailability. One solution to this problem is to mill the drug in the presence of an aqueous solution of polymeric stabiliser, to produce crystalline drug nanoparticles of less than 400 nm. While this novel technology has been successful in producing stable nanoparticles of a wide range of hydrophobic drugs, the development process has thus far been largely empirical, with very little understanding gained into the fundamental interface science involved in the stabilisation. The aim of this study was to rectify this deficiency and in particular, to determine the amount and conformation of stabilising polymer adsorbed onto the drug nanoparticles with a view to understanding why some stabilisers are more effective than others. Nanoparticles of two poorly water soluble drugs, nabumetone and halofantrine were prepared by milling 4 g of nabumetone or 6 g of halofantrine in the presence of a 1.5% w/v solution of either hydroxypropyl cellulose (HPC) or hydroxypropylmethyl celluloses (HPMC) for nabumetone and HPMC or polyvinylpyrrolidone (PVP) for halofantrine. After removal of the excess polymer, the nanoparticles were re-suspended in either a 31.3 or 33.8 vol% D_2O/H_2O mixture (according to the experimentally determined 'contrast-match' point of nabumetone and halofantrine, respectively). Under these conditions only the scattering from the polymer stabiliser was detected. SANS experiments were performed at the Institute Laue Langevin, France, over the momentum transfer (Q) range 0.007–0.035 Å^{-1}. The neutron data were analysed using a "volume fraction profile independent surface Guinier model" (King et al 2000). This allowed , the mass of polymer adsorbed per unit area, also known as the adsorbed amount (in mg m^{-2}) to be determined, as well as s, the 'second moment' of the layer; the distance of the centre-of-mass of the adsorbed polymer layer from the interface (Table 1). There was little change in either the second moment of the polymer layer or the amount of polymer adsorbed onto the nabumetone nanoparticles with molecular weight of HPC and HPMC. Under the present experimental conditions, nabumetone nanoparticles could not be prepared using HPMC of molecular weight greater than 7 kg mol^{-1}. Compared with nabumetone, HPMC adsorbed to the halofantrine nanoparticles to a lesser extent, forming a thinner adsorbed layer and indicating that a different conformation is adopted at the drug nanoparticle surface. Similarly PVP only formed a relatively thin absorbed layer on the halofantrine nanoparticles. To our knowledge these SANS studies are the first that have been performed on polymer-coated drug nanoparticles.

Table 1 Characterisation (using SANS) of polymer layer on nabumetone and halofantrine nanoparticles as a function of polymer molecular weight

Nanoparticle composition	Polymer molecular weight (M_η kg mol^{-1})	σ (Å)	Γ (mg m^2)
Nab-HPC	110	80.4	11.4
	95	76.0	11.6
	80	78.9	11.6
	65	76.5	11.0
	55	78.3	11.0
	45	80.3	11.3
Nab-HPMC	7	76.8	10.5
	5	80.8	10.8
Halo-HPMC	7	42.3	6.9
	5	33.4	7.7
Halo-PVP	46	52.7	1.8
	3	51.6	1.9

Nab, nabumetone; Halo, halofantrine; σ second moment of the adsorbed polymer layer; Γ adsorbed amount of polymer.

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Poster Session 1 – Analytical Chemistry

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A transdermal delivery system for aspirin as an antithrombotic drug

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More than 100 years after its introduction into therapy, aspirin may be the most widely used medication in the world. Aspirin has become the gold standard with which newer antiplatelet drugs are compared for reducing risks of cardiovascular diseases, while keeping low cost. Aspirin has been, and will be, the drug of choice for the long-term treatment of platelet hyperactivity. It imparts its primary antithrombotic effect through the irreversible inhibition of PGH-synthase/COX. The resultant decreased production of prostaglandins and TXA_2 accounts for the therapeutic effects, as well as the toxicity, of aspirin. Unfortunately, aspirin has a well-recognized repertoire of unwanted gastrointestinal side effects that can affect both the upper and lower gut and may occur even at very low oral doses. These can offset its clinical benefit by predisposing patients to gastrointestinal haemorrhage. Also, orally administered aspirin requires high frequent dosing because it undergoes extensive presystemic hydrolysis in the gut and the liver into salicylic acid, which is devoid of anti-platelet activity. Because of its extremely widespread, continuous and growing use, there is a need to minimize adverse effects of aspirin while maintaining its benefits. Transdermal delivery offers an alternative route for administering aspirin that bypasses the gut and may be a more convenient, safer and non-invasive means for aspirin delivery, especially for long-term use. This study comprised formulation of aspirin in different bases for topical application (Hydrophilic Ointment USP, Polyethylene Glycol Ointment NF, carboxymethyl cellulose gel, hydrocarbon gel (Plastibase) and vaseline bases). Release studies carried out according to the paddle method using the USP dissolution tester revealed that hydrocarbon gel base allowed the highest drug release. Ex-vivo permeation studies through full-thickness rat abdominal skin using Franz diffusion cell revealed the highest permeation of the drug from hydrocarbon gel. Several chemical penetration enhancers were surveyed for augmenting the permeation of aspirin from this base; these comprised oleic acid, methyl myristate, a combination of propylene glycol and alcohol, limonene, dimethyl sulfoxide, urea, β-cyclodextrin, hydroxypropyl-β-cyclodextrin and dimethyl-β-cyclodextrin. The combination of propylene glycol and alcohol showed maximum enhancing effect and, hence, was selected for biological investigation. The biological performance of the selected formulation was assessed turbidometrically by measuring the inhibition of platelet aggregation in male Wistar rats relevant to different dosage regimens aiming to minimize, as far as possible, both the dose of the drug and its frequency of application. The results demonstrated the feasibility of successfully influencing platelet function by the selected formulation containing a low dose of aspirin and revealed that the therapeutic efficacy of the drug in a transdermal delivery system (TDS) is dose-independent, consistent with saturability of platelet COX-1 inhibition by aspirin at very low doses, which support the use of the lowest effective dose. Aiming to justify the goal of this study (i.e., designing a safe, stable and effective transdermal delivery system for aspirin), the biological performance of the selected formulation was re-assessed after storage at 37°C for 7 months. The results showed that the biological performance was not affected by storage, ensuring stability and persistent therapeutic efficacy.