inhibitor's side chain as opposed to any interaction between the inhibitor and the active site corresponding to the C-20 area.

Ahmed, S., Denison, S. (1998) *Bioorg. Med. Chem. Lett.* **8**: 2615–2620

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Novel inhibitors of oestrone sulphatase based on the derivatives of 4-aminophenol

A. Whaeed, T. Cartledge, C. P. Owen and S. Ahmed

Department of Pharmacy, Kingston University, Kingston-upon-Thames, UK.
E-mail: s.ahmed@kingston.ac.uk

Objectives We have previously reported the design and synthesis of a number of compounds against the enzyme oestrone sulphatase (ES) and have suggested a number of physico-chemical properties that we considered to be important in the design of potent irreversible sulphamate-based inhibitors of ES (Ahmed et al 2000, 2001). However, the compounds synthesized were found to be highly unstable and were prone to rapid degradation. In an effort to improve the stability, we have investigated a number of derivatives of benzamides, which, although they were found to be weak inhibitors, were more stable than the ester-based compounds previously reported by us. We report here the initial results of our study into the synthesis and biochemical evaluation of a series of sulphamated derivatives of 4-aminophenol as potential inhibitors of ES.

Methods In the synthesis of the target compounds, we followed literature procedure (Ahmed et al 2002). That is, following the derivatization of the amino functionality involving the reaction between 4-aminophenol and an anhydride, the resulting benzamide compound was aminosulphonated using aminosulphonyl chloride. In the biochemical evaluation, we used a modified literature procedure (Ahmed et al 2001), the modification being the use of rat liver microsomal enzyme.

Results The target compounds were obtained in relatively moderate yield (ranging from 40 to 60%) without any major problems. In general, the potential inhibitors proved to be weaker inhibitors than the standards used, namely EMATE (which was found to possess 99% inhibition at an inhibitor concentration of 50 μM) and 667-COUMATE (which was also found to possess 99% inhibition under similar conditions). The most potent compound was found to be the sulphamic acid 4-butyrylamino-phenyl ester (possessing 55% inhibition under similar conditions). With regards to the structure–activity relationship, from an initial consideration of the initial screening data, it would appear that, as previously shown by us, the logP of the inhibitor (and in particular, the carbon backbone) is an important physico-chemical factor in the inhibition of ES by the compounds under consideration. The compounds also proved to be somewhat more stable (chemically) than the previously reported benzoic acid esters which were highly potent inhibitors. In particular, the amide moiety was found to be resistant to degradation: it should be noted that the sulphamate moiety has always proved to be unstable and would appear to be an important characteristic for the compounds to possess inhibition.

Conclusions We have provided some novel ES inhibitors with limited stability so as to allow us to design further novel, and more potent, inhibitors of ES.

Ahmed, S. et al (2000) *Biochem. Biophys. Res. Commun.* **272**: 583–585
Ahmed, S. et al (2001) *Bioorg. Med. Chem. Lett.* **11**: 899–902
Ahmed, S. et al (2002) *Bioorg. Med. Chem. Lett*. **12**: 2391–2394

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Novel inhibitors of the enzyme complex 17α-hydroxylase (17α-OHase) and 17,20-lyase based on a phenyl acyl azole backbone

W.-Y. Lee, I. Shahid, C. P. Owen and S. Ahmed

Department of Pharmacy, Kingston University, Kingston-upon-Thames, UK.
E-mail: s.ahmed@kingston.ac.uk

Objectives Conversion of pregnanes to androgens is catalysed by the enzyme complex 17α-hydroxylase/17,20-lyase ($P450_{17\alpha}$). The overall process involves 17α-hydroxylation of the C-17 position of the steroid backbone (involving the 17α-hydroxylase (17α-OHase) component) followed by the cleavage of the C-17–C-20 bond (involving the 17,20-lyase (lyase) component). We have previously reported the substrate–haem complex (SHC) approach for $P450_{17\alpha}$, which allowed the production of an approximate model for the active site of $P450_{17\alpha}$ since the crystal structure is currently unavailable (Ahmed 1999). Using the SHC approach, we undertook the design of compounds based upon the phenyl acyl backbone. We hypothesized that the C=O moiety would utilize potential hydrogen-bonding interactions, thereby increasing inhibitory activity, a characteristic that was lacking in the previously synthesized compounds (Owen et al 2006, Patel et al 2006). Here, we report the synthesis and evaluation (against the two components of $P450_{17\alpha}$) of a range of phenyl acyl imidazole-based inhibitors.

Methods The target compounds were synthesized through the N-alkylation of the azole functionality involving the use of the appropriate derivatives of 2-bromo-1-phenyl-ethanone, a suitable base and anhydrous tetrahydrofuran as solvent. Biochemical evaluation of the compounds was undertaken using literature assay procedure (Owen et al 2006).

Results The reactions in the synthesis of the target compounds proceeded in good yield (ranging from 50 to 80%) and no major problems were encountered. The biochemical evaluation shows that the compounds were, in general, weaker inhibitors than the standard compound, namely ketoconazole (KTZ) (found to possess 62 and 79% inhibitory activity against 17α-OHase and lyase respectively), although a few were found to be equipotent to KTZ. The most potent was 4-bromophenyl acyl imidazole, which was found to possess 55 and 70% inhibitory activity against 17α-OHase and lyase respectively. The compounds were also found to possess slightly greater inhibitory activity against the lyase component than the 17α-OHase: this is a useful feature as it suggests that these compounds would lack major side effects associated with the inhibition of corticosteroid biosynthesis. The inhibitory activity observed has been rationalized using molecular modelling and suggests that interaction between the substituent on the phenyl moiety and the enzyme active site of $P450_{17\alpha}$ results in increased potency and not the C=O moiety: the carbonyl functionality was positioned such that any groups interacting with this group would undergo steric interaction with the haem, thereby lowering the inhibitory activity.

Conclusions The compounds synthesized in the present study are therefore good lead compounds in the design of further novel and specific inhibitors of $P450_{17\alpha}$.

Ahmed, S. (1999) *Bioorg. Med. Chem.* **7**: 1487–1496
Owen, C. P. et al (2006) *Bioorg. Med. Chem. Lett*. **16**: 4011–4015
Patel, C. H. et al (2006) *Bioorg. Med. Chem. Lett.* **16**: 4752–4756

Drug Delivery

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Development of oral sustained-release formulation of isoniazid/alginate/chitosan-blend microspheres for intestinal delivery

A. Samad, Y. Sultana, R. K. Khar, M. Aqil and A. K. Misha

Jamia Hamdard, New Delhi, India. E-mail: samadpharma@gmail.com

Objectives The objective of this work was the development of an oral sustained-release formulation of alginate/chitosan blend microspheres of isoniazid to increase the bioavailability of the drug by targeting the intestine. This would improve the absorption and reduce the toxicity of isoniazid and improve the compliance of the patient by decreasing the frequency of dosing.

Methods Microspheres were prepared by water in the oil-emulsification method. The concentration of polymer and crosslinker as well as crosslinking time were varied to note the effects on microsphere characteristics. The shape and surface characteristics were determined by scanning electron microscopy using a gold-sputter technique (Leo435P microscope). Particle sizes of both placebo- and drug-loaded formulations were measured using scanning electron microscopy and the particle-size distribution was determined using an optical microscope. The entrapment was measured using UV methods. (Drug-loaded microspheres were sonicated for 1 hour in simulated intestinal fluid, pH 7.4, to lyse the particles. The extent of drug loading was determined by measuring the absorbance.) The bioadhesive potential of the microspheres was measured in the rat intestine using 50 mg of the microspheres. The adhered microspheres were allowed to hydrate for 20 minutes and dried. After 20 minutes the adhered microspheres were separated and weighed. Percentage bioadhesion was then calculated. The physical state of the drug in the formulation was determined by differential scanning calorimetry (DSC). The release profiles of isoniazid from microspheres were examined in simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.4). γ-Scintigraphic studies were carried out to determine the location of ^{99m}Tc-labelled microspheres, following oral administration to New Zealand white rabbits, and the extent of transit through the gastrointestinal tract.

Results The microspheres had a smooth surface and were found to be discreet and spherical in shape. Heterogeneous size distribution was found with an average diameter of 3.849 μm. Results indicated that the mean particle size of the microspheres increased with an increase in polymer and crosslinker concentration as well as the crosslinking time. The entrapment efficiency was found to be in the range of 52–94% w/w. Concentration of the crosslinker up to 7.5% caused an increase in the entrapment efficiency and the extent of drug release. Optimized isoniazid/alginate/chitosan-blend microspheres were found to possess good

bioadhesion (75.53 ± 1.034%). The bioadhesive property of the particles resulted in prolonged retention in the small intestine. Microspheres could be observed in the intestinal lumen at 4 hours and were detectable in the intestine 24 hours post-oral administration, although the percentage of radioactivity had significantly decreased ($t_{1/2}$ of ^{99m}Tc = 4–5 hours).

Conclusions In the study, spherical microspheres able to prolong the release of isoniazid were produced by a modified emulsification method, using sodium alginate as the hydrophilic carrier. Variables such as the drug/polymer ratio, crosslinker concentration and crosslinking time were investigated. Increased drug loading (94%) was observed for the optimized formulation (smoother surface, average particle size of 3.849 μm, good bioadhesion, high entrapment efficiency and sustained release action), suggesting the efficiency of the method. Nearly 19.04% of isoniazid was released in SGF, pH 1.2, in 6 hours and 85.95% in SIF, pH 7.4, in 36 hours. No significant drug–polymer interactions were encountered. Dissolution and γ-scintigraphy studies have shown promising results, proving the utility of the formulation for enteric drug delivery.

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Development of a model membrane representing skin with different water contents for investigation of drug release from polymeric microneedles

K. E. Migalska, A. D. Woolfson, P. A. McCarron, D. Morrow, R. Thakur and R. F. Donnelly

School of Pharmacy, Queen's University, Belfast, UK. E-mail: kmigalska01@qub.ac.uk

Objectives Upon insertion into skin, drug release from water-soluble microneedles is governed by the dissolution rate in moisture from the viable skin layers. Inter-individual differences in the skin water content can possibly influence drug release. The object of this work was to develop a model membrane for investigation of the effect of different skin water contents on drug release from water-soluble microneedles. As free water content in the skin increases from 32–35% w/w in the normal state to about 50% w/w upon hydration, it was decided that model membranes would contain 30, 40 and 50% w/w water at equilibrium.

Methods Membranes made of poly(methylvinylether/maleic acid) (PMVE/MA) crosslinked by polyethyleneglycols (PEGs) of varying chain lengths (200, 400, 1000, 6000, 8000 and 10000 Da) in the PMVE/MA:PEG ratio of 2:1 were prepared. Poly (hydroxyethylmethacrylate) (pHEMA) membranes were prepared by thermal polymerization (90°C and 2 hours) using ethylene glycol dimethacrylate (EGDMA), in the concentration range 0–7% w/v, as a crosslinking agent. Benzoyl peroxide (2% w/v) was used as the polymerization initiator. Swelling studies were performed in phosphate-buffered saline (PBS), pH 7.4, using a gravimetric method. Permeation through membranes of the model drugs, methylene blue and fluorescein sodium, was investigated using side-by-side Franz diffusion cells.

Results PMVE/MA membranes prepared using PEGs with lower molecular weight (i.e. 200, 400 and 1000 Da) disintegrated in PBS, pH 7.4. Membranes crosslinked by PEGs of higher molecular weight (6000, 8000 and 10000 Da) imbibed 83, 93 and 89% w/w water at equilibrium, respectively. pHEMA membranes crosslinked by EGDMA at concentrations of 0, 1 and 5% w/v retained approximately 47, 40 and 31% w/w water, respectively. Methylene blue transport across pHEMA membranes containing 47 and 40% w/w water at equilibrium was undetectable during the first 6 hours: only 0.015 and 0.008% of the total amount of drug permeated through these membranes, respectively, after 24 hours. No drug permeation through membranes containing 31% w/w water was detectable. Permeation of fluorescein sodium across membranes containing 47 and 40% w/w water was first observed after 1 hour; 2.26 and 0.26% of the total amount of drug traversed these membranes, respectively, after 24 hours. No drug permeated membranes containing 31% w/w water, even after 24 hours.

Conclusions PMVE/MA membranes crosslinked by PEG 200, 400 and 1000 Da were unsuitable as model membranes due to disintegration in PBS, pH 7.4, caused possibly by the crosslinks being too short. PMVE/MA membranes crosslinked by PEG 6000, 8000 and 10000 Da retained 83, 93 and 89% of water, respectively, possibly owing to lower crosslinking density and longer crosslinks, which disqualified them as model membranes. Fluorescein sodium permeation through pHEMA membranes was higher compared with methylene blue, possibly because of weaker hydrophobic interactions between its structure and pHEMA membranes. Overall, the poor drug transport across pHEMA membranes may be due to the relatively large size of the model compounds along with the hindrance posed by the tortuous networks of the membranes. The rate of permeation proved to be dependent mostly upon properties of the membrane, which may obscure the effect of microneedle devices on drug release in future studies. However, if drugs were to be extracted from the membrane at the end of the experiment, then pHEMA membranes may still prove to be useful in evaluating drug release from water-soluble microneedles.

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IPSE, a glycoprotein released from *Schistosoma mansoni* eggs as a novel gene-delivery vector

I. Kaur[1], S. Stolnik[1], K. Kindle[1], D. Heery[1], G. Schamm[2], H. Haas[2] and F. Falcone[1]

[1]School of Pharmacy, University of Nottingham, Nottingham, UK and [2]Research Institute, Borstel, Germany. E-mail: paxik1@nottingham.ac.uk

Objectives Though long sought, gene delivery is still limited by safety, efficacy and several biological barriers. Viral vectors themselves can induce a toxic immunogenic response potentially leading to a fatal outcome. Non-viral vectors have very low efficiency and are limited by the cell-membrane barrier, endolysosomal degradation and nuclear membrane barrier. In an attempt to resolve the present limitations IPSE is being studied, owing to its unique and important properties, which may make it useful as a gene-delivery vehicle. IPSE is an interleukin-4-inducing glycoprotein released from *Schistosoma mansoni* eggs. IPSE binds to mammalian transferrin and has a predicted C-terminal nuclear localization signal (NLS) with the sequence PKRRRTY. Based upon these two properties, our working hypothesis is that IPSE uses the transferrin receptor to gain entry into host cells, translocates to the cytoplasm, and from there enters the nucleus via its NLS.

Methods The predicted NLS in IPSE has been studied for its functionality by transfecting the U2-OS cell line with an enhanced green fluorescent protein (EGFP)–IPSE reporter construct and assessing its nuclear localization by confocal laser microscopy. The function of the NLS was further analysed using an alanine scan approach in which every single amino acid in the NLS was mutagenized to Ala. IPSE–transferrin binding and IPSE–DNA binding have been studied by western blotting, surface plasmon resonance and electrophoretic mobility shift assays. IPSE's binding to the transferrin receptor (TfR1), its uptake via the TfR and recycling of TfR is further being studied by confocal microscopy. The effect of IPSE on the growth of Jurkat cells under conditions where transferrin-dependent iron uptake limits proliferation has also been studied to investigate the impact of IPSE on the transferrin pathway.

Results The presence and functionality of the NLS in IPSE has been confirmed by transfecting U2-OS and Huh7 cell lines with EGFP–IPSE with and without NLS. The functionality of the NLS has further been shown with the alanine-mutagenized NLS by transfecting the U2-OS cell line. The mutagenized NLS shows that lysine and all three arginines (1–3) are essentially required for nuclear localization. The percentage decreases in nuclear localization by mutation were 95% (lysine), 80% (arginine 1), 40% (arginine 2) and 50% (arginine 3). However, mutation all three arginines 1–3 results in complete disruption of nuclear localization. IPSE also shows binding to transferrin and DNA. A concentration-dependent inhibitory effect of IPSE on Jurkat cell proliferation was also observed, possibly indicating disruption of physiological iron-transport mechanisms via this pathway.

Conclusions In line with our hypothesis, IPSE binds to transferrin and has a functional NLS. Based upon these findings, IPSE can potentially enter into the nucleus after entry into the cell via the transferrin receptor route. If IPSE escapes endolysosomal degradation (which remains to be shown) it could be used as a potential gene-delivery tool, balancing between harmful effects of viral vectors and low efficiency of non-viral vectors.

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Correlation between *in vitro* and *in vivo* release rates of the anti-retroviral candidate, dapivirine, from silicone elastomer vaginal rings

C. F. McCoy[1], R. K. Malcolm[1], A. D. Woolfson[1], J. Romano[2], A. Nel[2], S. Smythe[2], K. Young[2] and Z. Rosenberg[2]

[1]Queen's University, Belfast, UK and [2]International Partnership for Microbicides, Silver Spring, MD, USA. E-mail: clare.mccoy@qub.ac.uk

Objectives Dapivirine, an anti-retroviral candidate, is currently in development as a vaginal microbicide for the prevention of HIV transmission. In this study, the sustained *in vitro* release characteristics from dapivirine-loaded vaginal rings (VRs) were assessed and compared with residual drug contents after 28 days' use in humans. Although most VR release studies are performed *in vitro* under sink conditions in a solvent/water dissolution medium, this method may not best represent conditions *in vivo*. In order to achieve an understanding of release rates *in vivo*, an extraction method enabling quantification of residual dapivirine content in rings post-release was developed.

Methods Dapivirine (25 mg) matrix and reservoir VRs were manufactured from MED-6382 silicone elastomer. Content uniformity studies were performed prior to dissolution studies to confirm accurate dapivirine loadings. *In vitro* dissolution studies were performed into 100 mL isopropanol/water (1:1 v/v) at

37°C at 60 rpm in an orbital shaking incubator. Daily sampling and complete replacement of the release medium were performed over a 28-day period. All samples were analysed using UV high-performance liquid chromatography (HPLC). Alongside the *in vitro* release studies, a 24-participant phase I 28-day clinical trial was conducted involving eight matrix, eight reservoir and eight placebo VRs. Following completion of the clinical trial residual dapivirine content was determined. The amount of drug released *in vivo* was then calculated by subtracting total drug extracted from initial VR loading.

Results During the 28-day *in vitro* dissolution studies reservoir VRs displayed a day-1 burst of between 95 and 125 μg followed by the development of a steady-state release rate of approximately 60 μg/day. Matrix VRs produced daily release rates of approximately 3000 μg on day 1, decreasing gradually to approximately 250–300 μg on day 28. *In vitro*, matrix rings provided significantly higher daily release rates than reservoir VRs with the same initial loading. Content uniformity analysis performed on a selection of VRs prior to the phase I trial determined mean content uniformity values for matrix and reservoir VRs to be 25.9 and 26.3 mg, respectively. Following extraction of the rings post-use, predicted dapivirine-release levels were calculated by subtracting the measured residual drug content of each VR from the mean content uniformity values to give total drug released. For reservoir, matrix and placebo VRs the mean residual dapivirine content after 28 days' use was determined to be 24.33 ± 0.72, 14.51 ± 0.41 and 0 mg respectively. The calculated mean totals released *in vivo* over 28 days were calculated to be 1.97 and 11.89 mg for reservoir and matrix VRs, respectively. The total amounts released after 28 days *in vivo* from both matrix and reservoir devices were similar to those obtained *in vitro*, thereby confirming that sink conditions were achieved *in vivo*.

Conclusions Determination of residual dapivirine content analysis and subsequent release rates confirm that sink conditions are achieved *in vivo*. The results demonstrate that matrix VRs provide significantly faster release rates *in vivo* than reservoir rings, just as they do *in vitro* under sink conditions. If matrix rings are shown to maintain the cervicovaginal concentration at levels capable of providing effective protection against HIV then they may have significant potential for the delivery of microbicides.

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Effect of environmental conditions on the crystallization patterns and *in vitro* release of ibuprofen from drug-in-adhesive acrylic layers

K. Dodou, N. Martin and Z. Ali

School of Pharmacy, University of Sunderland, Sunderland, UK.
E-mail: kalliopi.dodou@sunderland.ac.uk

Objectives To examine the effect of different temperature and relative humidity conditions on the crystallization patterns of ibuprofen in drug-in-adhesive acrylic layers and to examine the *in vitro* thermodynamic activity of the layers before and after storage. Such knowledge can allow prediction of the long-term stability and performance of the transdermal drug-delivery system (Variankaval et al 1999).

Methods Drug-in-adhesive layers of ibuprofen in an acrylic polymer adhesive Duro-Tak®-87-4287 (National Adhesive and Starch Corporation, Zutphen, Netherlands) were prepared in triplicate over a range of ibuprofen concentrations and examined for the presence of ibuprofen particles immediately after drying, using a Swift M4000-D microscope (Swift Instruments International) fitted with a Quodmaster® 100 polarizing (10 ×) objective. The lowest ibuprofen concentration at which ibuprofen particles were present in the layer after drying was regarded as the saturation solubility of ibuprofen in the adhesive. Unsaturated (10 and 20% w/w ibuprofen) drug-in-adhesive layers were then prepared in triplicate for each concentration and were exposed to three different sets of environmental conditions; condition A = 1.5 ± 1°C and 41 ± 3% relative humidity (RH); condition B = 20 ± 3°C and 41 ± 3% RH; condition C = 20 ± 3°C and 61 ± 2% RH. Temperature and relative humidity were monitored using a temperature/humidity meter. The layers were examined microscopically twice a week and the time of initial crystal formation was recorded. Crystal morphology was observed using an Olympus BH-2 microscope fitted with a camera (AxioCam MRc-ZEISS, UK) and AxioVision (version 4.4) software. Ibuprofen release from the 10% w/w layers after preparation and after 14 weeks' storage was studied using a dissolution test in phosphate buffer (pH 5.4) at 32°C for 6 hours.

Results Crystal nuclei of ibuprofen appeared in the following order: 1 week in the 20% w/w layers (conditions A and C); 2 weeks in the 20% w/w layers (B); 10 weeks in the 10% w/w layers (B); 12 weeks in the 10% w/w layers (A and C). Crystal length ranged between 0.5–2.5 μm and 50–200 μm for the 10% and 20% w/w layers, respectively. Plate crystals with smooth edges were formed at ambient conditions (B) and angular crystals with sharp edges were formed at high relative humidity (C) and low-temperature conditions (A). The rate of crystal growth was drug concentration-dependent; 10% w/w < 20% w/w. A dendrite crystal was also formed, attributed to crystallization of the acrylic polymer. Ibuprofen release from the 10% w/w layers was significantly lower (one-way analysis of variance, $P < 0.01$) after storage under low temperature (condition A) and high relative humidity (condition C) compared with the freshly prepared layers.

Conclusions The plate ibuprofen crystals formed after storage of the drug-in-adhesive layers at ambient conditions did not alter significantly the *in vitro* thermodynamic activity of the patches. The sharp-edged angular ibuprofen crystals formed at low temperature and high relative humidity conditions significantly decreased the *in vitro* thermodynamic activity of the patches compared with the freshly prepared layers.

Variankaval, N. E. et al (1999) *J. Biomed. Mater. Res.* **44**: 397–406

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Formulation and evaluation of novel dosage forms of cysteamine for the potential treatment of cystinosis

B. E. Buchan, G. Kay, K. H. Matthews and D. Cairns

School of Pharmacy and Life Sciences, The Robert Gordon University, Aberdeen, UK.
E-mail: 0201071@rgu.ac.uk

Objectives Cystinosis is a rare autosomal recessive disease characterized by raised intracellular levels of the amino acid cystine. Treatment for cystinosis involves the oral administration of cysteamine (Cystagon™), an aminothiol which possesses an offensive taste and smell and, along with its metabolites, is excreted in breath and sweat, causing halitosis and body odour as well as gastric irritation. As a result, patient compliance may be poor (Cairns et al 2002). The aim of the current work was to create and evaluate novel formulations of cysteamine and to investigate possible alternative routes of administration for the agent. Initially, dosage forms for rectal administration were evaluated.

Methods An experimental cysteamine conjugate, which possessed a chromophore, was synthesized to facilitate quantitative evaluation of release of the active agent. Various suppository bases were evaluated, including poly (ethylene glycol) (PEG), Witepsol and Gelucire. The method employed followed the standard British Pharmacopoeia test for suppositories: one suppository in a basket rotating at 100 rpm in a 1 L water bath at 37°C, sampling at 5 minute intervals for 1 hour. The concentrations, and hence the release profiles, were determined by recording the UV absorbance of experimental drug at 256 nm.

Results Results are shown in Table 1.

Table 1 Percentage release data from five suppositories containing the tagged drug

	Time to release active drug (minutes)				
	10%	25%	50%	75%	100%
PEG 1500	1.75	4	7.75	13	60
Blend A (PEG 8000/600)	1	2.5	5	8	50
Blend B (PEG 14000/600)	1.75	2.5	7	13	60
Gelucire 39/01	0.5	1	2	2.5	25
Witepsol W35	0.5	1	2	2.5	20

Conclusions Suppositories composed of PEG produced a sustained release of the active agent, whereas fatty bases such as Witepsol released nearly 100% of the drug at 20 minutes. Optimal appearance, hardness and ease of manufacture were obtained with PEG blending. Two blends composed of PEG 8000/600 (blend A) and PEG 14000/600 (blend B) produced suppositories with excellent physical characteristics and controlled-release profiles (50 and 60 minutes, respectively). Currently studies to evaluate further analogues of cysteamine are underway in our laboratories.

Cairns, D. et al (2002) *J. Pharm.* **269**: 615–616

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Exploring the nature of chitosan–mucin interactions

I. A. Sogias, A. C. Williams and V. V. Khutoryanskiy

School of Pharmacy, University of Reading, Reading, Berkshire, UK.
E-mail: i.sogias@reading.ac.uk

Objectives Chitosan is a well-established biodegradable and biocompatible cationic amino-polysaccharide which is soluble in aqueous solutions at pH values of less than 6.5. It has been used in biomedical and therapeutic applications due to its low toxicity, good biocompatibility and anti-microbial properties, and in drug-delivery systems because of its excellent mucoadhesive properties. Mucoadhesion

is the ability of some natural or synthetic molecules to adhere to mucosal membranes. Mucin is the main component of the mucus layer, produced by the mucosal membranes which line the walls of various body cavities such as the gastrointestinal, respiratory and reproductive tracts. Although chitosan mucoadhesion has been extensively studied, the importance of the amino groups and the nature of interactions between chitosan and mucin remain poorly defined. We have examined the role of chitosan primary amino groups in mucoadhesion by reducing their number through acetylation. The role of electrostatic attractions, hydrogen bonding and hydrophobic effects on aggregation of gastric mucin in the presence of chitosan was also explored to elucidate the mechanisms underlying chitosan mucoadhesion.

Methods Chitosan is approximately 12% acetylated on the primary amines. Our acetylated derivative of chitosan was produced by treatment with acetic anhydride to synthesize modified chitosan with an approximately 50 ± 2% degree of acetylation. Mucin dispersions and their aggregates in the presence of chitosans were studied by dynamic light scattering and transmission electron microscopy. Mucin dispersions were also titrated with increasing ratios of polymers in zeta-potential measurements and turbidimetric titrations to probe the aggregation profile of the mucin–polymer interactions.

Results When the number of free amino groups in chitosan is reduced through their half-acetylation, this results in an expansion of chitosan's pH solubility up to pH 7.4, but is also accompanied by a reduction in its ability to cause mucin aggregation. We showed that electrostatic interactions between chitosan and gastric mucin are suppressed in solutions with increased ionic strength (0.2 mol/L sodium chloride); however, this does not prevent the aggregation of mucin particles in the presence of chitosan, indicating that other forces play a role in mucoadhesion. The presence of 8 mol/L urea or 10% v/v ethanol in mucin dispersions also affects the aggregation in the presence of chitosan, demonstrating the roles of hydrogen bonding and hydrophobic effects, respectively, in mucoadhesion.

Conclusions Mucoadhesive interactions between chitosan and mucin are a complex phenomenon, where the free amino groups play a key role. Aggregation is driven by electrostatic attractions, hydrogen bonding and hydrophobic effects. Electrostatic attraction appears to be the major mechanism for chitosan mucoadhesion, but is also accompanied by contributions from hydrogen bonding and hydrophobic effects.

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Gas-filled microbubbles: development and optimization as ultrasound contrast agents

R. Kaur[1], R. Morris[2], G. Cave[2], M. Bencsik[2], M. P. Krafft[3], G. Waton[3], T. Rades[4] and Y. Perrie[1]

[1]School of Life and Health Sciences, Aston University, Birmingham, [2]Nottingham Trent University, Nottingham, UK, [3]Institut Charles Sadron, Strasbourg, France and [4]University of Otago, Dunedin, New Zealand. E-mail: kaurr3@aston.ac.uk

Objectives Gas-filled microbubbles (GFMs) have the potential to be used as ultrasound contrast agents. However, they must be able to withstand the static pressure and tension effects of the human vasculature and also have the required stability to resist dissolution and/or destruction during insonation. Therefore we have been looking at a range of formulations and methods to prepare GFMs for application as ultrasound contrast agents.

Methods Microbubbles were prepared using methods as described by Vangala et al (2007) and Gerber et al (2006). The first method involved GFMs being prepared by homogenizing 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) in water at temperatures below the phase-transition temperature (T_c, ≈60°C) of the lipid using a high-shear mixer. GFM formulations were prepared in the presence or absence of cholesterol and/or polyethylene glycol distearate (PEG-distearate). Integrity and structural stability were assessed in terms of vesicle size over 7 days stored at 25°C. Vesicle size distribution was measured on Sympatec Helos and sizes were validated using a graticule under an optical microscope. The second method involved microbubbles being stabilized by a perfluoroalkylated glycero-phosphatidylcholine (*F*-GPC) as a shell component and a fluorocarbon gas as part of the filling gas. Microbubbles were prepared by dissolving fluorinated surfactant F8H11PC and Pluronic F-68 at a molar ratio of 10:1 in ISOTON II at a final concentration of 20 mM. The dispersions were sonicated, with the volume above the dispersion being filled with perfluorohexane-saturated N_2. Vesicle sizes were measured by a Zeta-Plus Analyser.

Results Initial vesicle sizes of DSPC-based GFM were 30.9 ± 1.2 μm; however, on day 7 the sizes increased to 44.5 ± 8.3 μm. This increase could be due to aggregation and/or fusion of vesicles. Formulations containing DSPC supplemented with cholesterol were similar in vesicle size (31.6 ± 1.2 μm). However, over the 7 days, the vesicle size of formulations with cholesterol remained stable, suggesting that cholesterol has a stabilizing effect on these gas-filled vesicles in a similar nature to aqueous-filled liposomes. The addition of an emulsifier, PEG-distearate, to DSPC and cholesterol formulations at 5 and 10% resulted in an increase in vesicle size (40.9 ± 3.8 and 40.4 ± 1.0 μm, respectively) compared with preparations without PEG-distearate. The inclusion of the emulsifier resulted in vesicle sizes remaining unchanged after 7 days (40.9 ± 1.4 and 39.0 ± 2.9 μm, respectively). Initial studies on vesicles prepared using *F*-GPC found that vesicle sizes of less than 500 nm were produced when fluorinated lipid constituted the shell component and an internal fluorocarbon gas component was used. Further studies on the long-term stability of both these systems and the DSPC formulations will be reported in combination with nuclear magnetic resonance (NMR) studies.

Conclusions Three different liposome formulations encapsulating gas have been prepared and tested, and they show substantial differences in stability and physico-chemical characteristics. Further work is required and our results promise great hope in the design of novel, optimized ultrasound and NMR contrast agents.

Gerber, F. et al (2006) *New J. Chem.* **30**: 524–527
Vangala, A. et al (2007) *J. Liposome Res.* **17**: 263–272

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Investigation of fluorescent derivatives as potential probes for novel transglutaminase inhibitors

N. Daneshpour, R. Collighan, Y. Perrie, D. Telci, A. Mongeot, D. Rathbone and M. Griffin

School of Life and Health Sciences, Aston University, Birmingham, UK. E-mail: govaranooshin@yahoo.com

Objectives Transglutaminase inhibitors can be used in the treatment of transglutaminase-relevant human diseases including chonic neurodegeneration, neoplastic diseases, autoimmune diseases and fibrosis (Griffin et al 2002). This work investigates whether fluorescent derivatives of transglutaminase inhibitors can be used as appropriate probes to characterize toxicity and efficacy of these novel transglutaminase inhibitors.

Methods The *in vitro* efficacy of a novel transglutaminase inhibitor (Griffin et al 2004), R281 (0–500 μM), and its fluorescent derivative, FL-281 (0–500 μM), in the inhibition of tissue transglutaminase was investigated by studying the dose dependency of their effect on the activity of purified guinea-pig liver transglutaminase using an enzyme-linked sorbent assay (ELSA) based on biotinylated cadaverine (BTC) incorporation into *N*,*N*′-dimethyl casein (DMC) as previously described (Griffin et al 2004). The absorbance, which is directly proportional to transglutaminase activity, was measured at 450 nm. Consequently, the half-maximal inhibitory concentrations (IC50 values) of both inhibitors for tissue transglutaminase (tTG) were measured. For the cytotoxicity study, initially albino mouse embryo 3T3 fibroblasts were seeded at a concentration of 5000 cells/well in a 96-well plate and incubated at 37°C until the cells reached confluence. Subsequently, the medium was supplemented with different concentrations (0–500 μM) of both inhibitors. After 48 hours, the *in vitro* cytotoxicity of both transglutaminase inhibitors was assessed by an XTT-based colorimetric cellular cytotoxicity assay and compared with the control (Tominaga et al 1999). To each well of the 96-well plate containing treated fibroblast cells, 15 μl of XTT labelling mixture (XTT labelling reagent and electron coupling reagent) was added and incubated for 3 hours at 37°C. After the incubation period, orange formazan solution was formed, which was spectrophotometrically quantified at 490 and 750 nm.

Results As the concentration of inhibitors increases, measured absorbance and hence the activity of the transglutaminase enzyme decrease; however, FL-281 showed higher efficacy of inhibition against tTG, as FL-281 showed a lower IC50 value (7 μM) than R281 (10 μM). In terms of cell toxicity of the inhibitors, there was not a significant difference in the viability of the cells at concentrations approximately 40-fold higher (up to 400 μM for both inhibitors). However, at concentrations of 500 μM the viability decreases by 50% for FL-281 compared with R281.

Conclusions Both transglutaminase inhibitors (R281 and FL-281) can act as an enzyme inhibitor for tTG in a concentration-dependent manner. Furthermore, FL-281 showed higher inhibition activity than R281 for the tTG tested. However, modification of the physico-chemical properties of the inhibitor by fluorescent modification of the inhibitor may increase toxicity and this needs to be taken into account when using as a probe to investigate biodistribution.

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Griffin, M. et al (2002) *Biochem. J.* **368**: 377–396
Griffin, M. et al (2004) World patent WO2004113363
Tominaga, H. et al (1999) *Anal. Commun.* **36**: 47–50

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Effect of propylene glycol on *in vitro* membrane transport of ionized diclofenac diethylamine from saturated binary cosolvent mixtures

F. Benaouda[1], G. P. Martin[1], S. A. Jones[1] and M. B. Brown[2]

[1]Pharmaceutical Science Division, King's College London, London and
[2]School of Pharmacy, University of Hertfordshire, Hatfield, UK.
E-mail: faiza.benaouda@kcl.ac.uk

Objectives For saturated formulations, where thermodynamic activity (α) is equal to 1, the flux of the permeant through a membrane should theoretically be constant irrespective of the delivery vehicle (Higuchi 1960). Although some studies have supported this hypothesis for saturated binary mixtures of propylene glycol (PG)/water (e.g. Twist and Zatz 1986), variations from this ideal behaviour have been reported (e.g. Pellett et al 1994). Therefore, no definitive conclusions have been made regarding the influence of PG on the membrane transport rate of drugs from saturated vehicles. The aim of this study was to investigate systematically the impact of PG on the permeation of one drug, diclofenac diethylamine (DDEA), from a series of saturated binary mixtures, through model membranes.

Methods The permeation profile of DDEA (pK_a 4.87) through silicone membrane (120 μm thickness) and regenerated cellulose membrane (RCM; cut-off pore size, 12–14000 Da) following the application of saturated formulations containing 20–65% (v/v) PG at pH 7.6 was assessed at 25°C, using individually calibrated upright Franz diffusion cells. DDEA saturated solubility in the binary PG/phosphate-buffered saline (0.172 M) mixtures was determined by high-performance liquid chromatography (a method previously shown to be fit for purpose). The effects of the formulations on the membrane thickness and on the diffusivity were determined using a micrometer and lag times respectively.

Results There was an exponential increase in the saturated solubility of DDEA with increased PG content in the binary cosolvent mixture up to 80% (v/v) PG, when a plateau was reached. The steady-state flux of DDEA from drug-saturated 20% PG was significantly greater ($P < 0.05$, analysis of variance) than the equivalent flux obtained from drug-saturated 65% (v/v) PG when using both a silicone membrane (4.43 ± 0.99 compared with 0.84 ± 0.05 μg cm^{-2} hour^{-1}) and an RCM (652.32 ± 34.60 compared with 498.74 ± 31.43 μg cm^{-2} hour^{-1}). The vehicle composition was also found to influence the lag time, and hence the diffusivity; however, the solvents induced no significant effect in the diffusional pathlength of either membrane.

Conclusions DDEA permeation through model membranes was influenced by the ratio of PG in the vehicle, despite the drug being formulated to have equal thermodynamic activity. As the PG content of the vehicle was increased, the permeation rate of DDEA ionized species through the model membranes was decreased. This was, in part, a consequence of a reduced release rate from the vehicle. The results obtained using silicone membranes indicated that the vehicle might also have an effect on drug partitioning.

Higuchi, T. (1960) *J. Soc. Cosmet. Chem.* **11**: 85–97
Pellett, M. A. et al (1994) *Int. J. Pharm.* **111**: 1–6
Twist, J. N., Zatz, J. L. (1986) *J. Soc. Cosmet. Chem.* **37**: 429–444

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Interaction of tea tree oil and its constituents with over-the-counter topical medicines

C. Martin, J. O'Neill and K. Chan

University of Wolverhampton, Wolverhampton, UK. E-mail: claire.martin2@wlv.ac.uk

Objectives The focus of these investigations is the potential interaction between the essential oil tea tree oil (TTO) and its constituent terpene derivatives with three model topical medicaments. In addition to cineole, terpinen-4-ol is one of the major constituents of TTO, with defined penetration-enhancer activities (Williams and Barry 2004). Potential for enhanced dermal transport is of concern when co-administered with other medicaments; increased speed and extent of absorption may lead to inadvertent overdose from topical formulations containing pain-relieving or anaesthetic agents. These experiments investigated the interactions between TTO, TTO components and three commonly used, over-the-counter (OTC) creams and gels: (1) anti-fungal (Daktarin™), (2) anti-inflammatory (Ibuleve™) and (3) anti-septic (Savlon™).

Methods Partitioning (between octan-1-ol and phosphate buffered saline, pH 7.4) of the topical medicines in the presence of cineole, limonene, terpinen-4-ol or whole TTO was examined by the shake-flask method; quantification was undertaken with thin-layer chromatography (TLC) using toluene/ethyl acetate (85:15 v/v) as the mobile phase and *p*-anisaldehyde II stain (anisaldehyde/$HClO_4$/acetone/water 1:10:20:80 by vol.) for specific visualization of terpenoids (the presence of creams or gel was confirmed by UV detection). The effect of pH on precipitate formation and differential partitioning into octan-1-ol was also examined by incubating each topical medicine with a buffer of pH 4.6, 5.0, 6.0 or 7.0.

Results TLC analysis of TTO constituents indicated the following R_f value ranges: cineole, 0.72–0.77, limonene, 0.69–0.74 and terpinene-4-ol, 0.60–0.62 respectively. Whole TTO alone presents several component terpene bands, but when combined with the aqueous gel Ibuleve™ only a single spot results, with no differentiation between the various terpene components. Comparing these results with the lipophilic anti-fungal cream Daktarin™, there is clear separation between TTO constituents when the whole oil is incubated with the anti-fungal cream where each spot has a comparable R_f value to the pure component. The effect of acidity on R_f value was investigated for Daktarin™, Ibuleve™ and Savlon™, as different disease states are known to alter skin pH from its normal slightly acidic state (Schmid-Wendtner and Korting 2006). Incubation of Ibuleve™ gel over a range of pH values resulted in an average R_f of 0.50 ± 0.05 compared with a distinct spot of R_f = 0.77 ± 0.04 in the presence of whole TTO, possibly corresponding to the cineole fraction. In contrast, Savlon™ displayed a wide range of R_f (0.20–0.80) possibly indicating ionization and hence altered solubility of major components, such as chlorhexidine gluconate.

Conclusions Results indicate that the presence of TTO or its constituents on the skin prior to the application of a topical medicament may enhance or impede its absorption and/or therapeutic activity. The implications of the findings are that the use of cosmetics containing of TTO or TTO components may lead to over- or under-dosing of medicinal creams and gels applied to the skin. Further work is needed to clarify the extent of absorption, or barrier action, of essential oils and their components, as well as potential interactions with topically applied, freely available, OTC medicinal products.

Schmid-Wendtner, M.-H., Korting, H. C. (2006) *Skin Pharmacol. Physiol.* **19**: 296–302
Williams, A. C., Barry, B. W. (2004) *Adv. Drug Del. Rev.* **56**: 603–618

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Controlled-release formulation of tea tree oil and its constituents

C. Martin, U. Bhujbal and M. A. Kenward

University of Wolverhampton, Wolverhampton, UK. E-mail: claire.martin2@wlv.ac.uk

Objectives This study investigated the development of stable, anti-microbially active liposomal formulations of tea tree oil (TTO). TTO is derived from steam distillation of *Melaleuca alternifolia* leaves and twigs and is predominantly composed of terpenes and sesquiterpenes. Pharmaceutical-grade TTO contains less than 5% cineole and more than 35% terpinen-4-ol, in addition to more than 100 other constituent materials, and is available commercially as essential oil, cream, ointment and lotion formulations (see SCCP Opinion on TTO, http://ec.europa.eu/health/ph_risk/committees/04_ sccp/docs/sccp_o_00c.pdf). The aims of this study were to investigate incorporation of TTO and its constituents into liposomes by (1) developing stable, pharmaceutically acceptable TTO emulsion formulations and (2) designing an optimized protocol for the preparation of liposomal TTO emulsions for controlled, sustained anti-microbial natural product release.

Methods Thin-layer chromatography (TLC) was used to confirm the presence of major TTO constituents, including cineole and terpinen-4-ol, in emulsified and liposome-encapsulated formulations. An optimized TLC system of toluene/ethyl acetate (85:15, v/v) mobile phase and *p*-anisaldehyde stain II (anisaldehyde/perchloric acid/acetone/water (1:10:20:80, by vol.)) were selected for further experiments. Emulsions of TTO (10% v/v) and different molecular weights of poly(vinyl alcohol) (PVA; 13–23 kDa ($PVA_{13–23kDa}$), 30–70 kDa ($PVA_{30–70kDa}$) and 70–100 kDa ($PVA_{70–100kDa}$)) at two concentrations (0.1 and 1.0% w/v) were prepared by high-shear homogenization. Liposomally encapsulated TTO-PVA emulsions were prepared from phosphatidylcholine (a kind gift from Lipoid GMBH) and cholesterol (2:1 molar ratio) with TTO-PVA emulsion as the bulk aqueous phase. All formulations were assessed for PVA using the iodine assay (Takeuchi et al 1998), dispersed droplet size by laser diffraction (Malvern Instruments, UK) and TTO loading in liposomes visualized by TLC. All materials were from Sigma UK unless otherwise stated and each experiment was conducted in triplicate.

Results Particle-size analysis for PVA-emulsified TTO indicated that the lowest-molecular-weight polymer ($PVA_{13–23kDa}$) produced the smallest dispersed droplet sizes. TLC analysis of TTO-PVA emulsions was undertaken to (1) visually approximate the quantity of TTO in emulsion formulations and (2) examine differential partitioning of the constituents of TTO during the preparation process. No major differences were found in R_f values between whole oil and TTO-PVA

emulsions of various molecular weights. Liposomal formulations of TTO had large particle sizes (>10 μm), which is believed to result from the formation of a visible precipitate within the formulation. TLC analysis of the precipitate indicated that it was an amalgam of several TTO components, in addition to phosphatidylcholine and cholesterol. Whereas the trigger for precipitation is unclear, it may result from the differential solubility of individual TTO components within both the bulk aqueous medium and organic solvent (chloroform) used during the preparation of liposomes. Percentage encapsulation of PVA (EE%) for liposomal TTO-PVA emulsions indicated that 1.0% w/v $PVA_{30–70kDa}$-TTO emulsion showed the highest PVA EE% of 78.5%, compared with all other concentrations and molecular weights of liposome-encapsulated polymer.

Conclusions In conclusion, a stable, low-viscosity delivery vehicle of PVA-emulsified TTO which can be encapsulated into liposomes has been developed. These chimaeric delivery systems may be able to provide prolonged and sustained release of anti-microbial natural products for treatment of a variety of bacterial, fungal and viral infections.

Takeuchi, H. et al (1998) *Int. J. Pharm.* **164**: 103–111

Material Science

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An investigation into the thermal behaviour of different grades of hydroxypropyl methylcellulose for the preparation of amorphous dispersions by spray-drying

M. Zhao[1], C. McGregor[2], S. A. Barker[1] and D. Q. M. Craig[1]

[1]School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich and [2]Development Laboratories, Merck Sharp and Dohme, Hoddesdon, UK.
E-mail: min.zhao@uea.ac.uk

Objectives Paracetamol is known to form a low-stability amorphous phase (Sheng et al 2008) hence there is considerable potential benefit in developing means by which such drugs, with a low glass transition temperature (T_g), may be stabilized. The dispersion of drugs in hydrophilic polymers with a relatively high T_g is a practical approach to enhancing both the dissolution rate and stability of amorphous drugs (Bruno and George 1997). In this study, hydroxypropyl methylcellulose (HPMC) was chosen as the model polymer and five grades of such polymer – HPMC A, B, C, D and E – were explored for the variation in their thermal behaviour as a means of stabilizing amorphous paracetamol within a spray-dried solid disperse system.

Methods Each grade of HPMC raw material and their corresponding spray-dried products were characterized for T_g and moisture content, using modulated-temperature differential scanning calorimetry (MTDSC) and thermogravimetric analysis (TGA), respectively. Dispersions with a paracetamol/HPMC ratio of 1:1 (w/w) were prepared by spray-drying and characterized afterwards in comparison with equivalent physical mixtures via thermal techniques and X-ray powder diffraction (XRPD). All spray-drying processes in this study were performed with the same inlet temperature of 110°C and a pump rate of approximately 5 mL/minute.

Results It was found that water contents of HPMC A and B were dramatically increased after spray-drying while those of HPMC C, D and E were all decreased. Curves obtained by TGA demonstrated that all spray-dried HPMC products had a lower water-loss temperature than their corresponding raw materials, which is in good agreement with DSC findings. In terms of raw materials, HPMC B failed to show a marked T_g. However, a glass transition at 149.9, 159.9 and 158.8°C, each with an enthalpic relaxation in the non-reversing heat flow, was obtained for HPMC C, D and E. A comparatively higher T_g at 187.1°C without relaxation was shown for the HPMC A raw material. Further studies on the drug-loaded systems showed a melting response for paracetamol for all samples. However, a paracetamol recrystallization peak at 66.7°C was found exclusively for systems containing HPMC C. In addition, the XRPD patterns produced by this sample showed partially amorphous character.

Conclusions Different grades of HPMC have different thermal behaviours due to the changes in polymer molecular weight, substitution type, viscosity, T_g, moisture content, etc., resulting in the variation in stabilizing effects on drugs within solid dispersions. In particular, all findings in this study are indications that HPMC C is a promising polymer for inhibiting the crystallization of paracetamol within solid dispersions.

Bruno, C. H., George, Z. (1997) *J. Pharm. Sci.* **86**: 1–12
Sheng, Q. et al (2008) *Eur. J. Pharm. Biopharm.* in press

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The stability of biodegradable polyesters and polyester-co-lactones utilized for drug delivery

A. Iftikhar, E. Gaskell, G. A. Hutcheon and C. Rostron

Liverpool John Moores University, Liverpool, UK. E-mail: G.A.Hutcheon@ljmu.ac.uk

Objectives Although it is desirable for biodegradable biomaterials to degrade after administration, depending on how they are stored such materials are also prone to degrade prior to use. The aim of this study was to investigate the stability of a library of biodegradable polymers under various environmental conditions to establish optimal storage conditions and provide an estimation of shelf life. This information will contribute to existing studies on these polymers, which have recently shown promise for drug-delivery applications (Kallinteri et al 2005).

Methods Polymers were synthesized by a combination of enzyme-catalysed condensation and ring-opening polymerization of divinyl adipate with either glycerol or butanediol and with and without pentadecalactone. All materials were characterized by nuclear resonance spectroscopy, infrared spectroscopy and gel-permeation chromatography (GPC) (Gaskell et al 2008). Poly(glycerol adipate) (PGA), poly(glycerol adipate-co-pentadecalactone) (PGA-co-PDL), poly(butanediol adipate) (PBA) and poly(butanediol adipate-co-pentadecalactone) (PBA-co-PDL) were used to investigate the effect of temperature (4, 25 and 40°C) and humidity (0, 25 and 75% relative humidity) on the polymer structure over a 6 month period according to published guidelines (Mathews 1999). Samples of polymer were removed at 20 day intervals for 3 months and then after 6 months of storage, and compositional and physical changes of the polymers were monitored by GPC and infrared spectroscopy.

Results Polymer degradation was observed to increase with temperature. At 4°C there was less than a 5% decrease in molecular weight over 6 months compared with up to a 50% decrease at 40°C. Similarly, at all temperatures, polymer degradation was observed to accelerate with increasing humidity. However, this was much more noticeable at 25 and 40°C. Under the same environmental conditions, low-molecular-weight polymers degraded more quickly than those with a higher molecular weight and, for a comparable molecular weight, those in a solid form degraded less than liquid polymers. PBA and PBA-co-PDL demonstrated longer estimated shelf lives at both 4 and 25°C than PGA and PGA-co-PDL. This is probably due to the presence of hydroxyl groups on the polymer backbone, which increase the hydrophilic character of these polymers. This leads to an increase in degradation by hydrolysis compared with those without backbone hydroxyl groups.

Conclusions These results indicate that increasing temperature and/or humidity increases polymer degradation, suggesting that the degradation of these polymers occurs by random chain scissions induced by thermolysis and hydrolysis of labile ester bonds. Hence, to minimize degradation, storage at 4°C, preferably under a dry atmosphere, is advised. Minimization of degradation is crucial, both when evaluating these polymers for potential and in actual use in drug-delivery applications. Further studies will determine how the presence of chemical moieties conjugated to the polymer backbone via the free hydroxyl groups affects degradation of both the backbone and conjugate ester bonds.

Gaskell, E. E. et al (2008) *J. Microencapsul.* in press
Kallinteri, P. et al (2005) *Biomacromoles* **6**: 1885–1894
Mathews, B. R. (1999) *Drug Dev. Ind. Pharm.* **25**: 831–856

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The study of the effect of photodegradation products on the phase transitions in crystalline and amorphous nifedipine using differential scanning calorimetry

R. Patil and M. Antonijevic

School of Science, University of Greenwich, Chatham Maritime, UK.
E-mail: M.Antonijevic@gre.ac.uk

Objectives To study the use of differential scanning calorimetry (DSC) to analyse the influence of photodegradation products on the phase changes in nifedipine. In addition, to investigate the effect of the UV irradiation on solid–solid phase transitions. The study was conducted by comparing various exothermic and endothermic processes (glass transition, solid–solid transition, crystallization and melting) of crystalline and UV-irradiated nifedipine.

Methods DSC experiments were carried out using a Mettler Toledo DSC823^e with a sample size in the range of 6–8 mg. All experiments were analysed in the temperature range 10–210°C and performed using sealed pans and following thermal cycles: heating/cooling/reheating at 5, 10, 20 and 40° min^{-1}. An irradiated sample was prepared by exposing a thin bed of nifedipine to UV irradiation in a UV chamber Spectroline CC80 for 114 hours.