

Poster Session 1 – Drug Delivery

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Carrying effect of dichloromethane outflow on protein leaking during the encapsulation process in preparing protein-loaded PLGA microspheres

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To investigate the effects of salt in the external phase on the evaporation rate of dichloromethane (DCM) for exploring the formation mechanism indented poly (dl-glycolide-co-lactide, 50:50) (PLGA) microparticles (Chen et al 2002; Yeh et al 2002). Ovalbumin (OVA) was used as a model protein and encapsulated in PLGA microparticles using W/O/W solvent evaporation method. In the solvent evaporation process, DCM evaporation was carried out at 25°C with a serial stirring rate (400 ~1000 rev min^{-1}), and the evaporation rates in the external phase and total disperse system were determined by GC method (Ruchatz et al 1997). Bovine serum albumin conjugated with fluorescein isothiocyanate (BSA-FITC) was adsorbed on the surface of OVA-loaded microparticles and measured by spectrofluorophotometery. During the evaporation of DCM, OVA was carried by DCM to leak from the inner aqueous phase of the primary emulation into the external aqueous phase of the multiple emulsion. By increasing the stirring rate from 400 to 1000 rev min^{-1}, the OVA leaking rate is increased from 8.8 to 315.1 $\mu g\,mL^{-1}$ as increasing the DCM evaporation rate. The result suggested that the extracted DCM from the organic phase into the external aqueous phase is an important factor for the leaking of OVA. Combined 3~5% sodium chloride in the external phase improved the yield of microsphere and OVA content from 76 to 89% and 51 to 73 $\mu g\,mg^{-1}$, respectively. The entrapment efficiency was increased from 43 to 72%. The specific surface area of microparticle was also increased from 1.93 to 2.50 $m^2\,g^{-1}$ and the adsorption of BSA-FITC on the surface of microparticles increased from 10.02 to 15.46 $mg\,m^{-2}$. During microsphere solidification, the leaking of protein was significantly affected by the outflow of DCM. By combining sodium chloride in the external phase formed an indent surface structure of microparticle and significantly enhanced the entrapment of protein in microparticle.

Chen, J. L., Chiang, C. H., Yeh, M. K. (2002) *J. Microencapsulation* **19**: 333–346
Ruchatz, F., Kleinebudde, P., Muller, B. W. (1997) *J. Pharm. Sci.* **86**L: 101–105
Yeh, M. K., Chen, J. L., Chiang, C. H. (2002) *J. Microencapsulation* **19**: 203–212

037

A scintigraphic investigation of the precorneal residence time of TS polysaccharide formulations in mild to moderate KCS patients

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Repair and restoration of the tear film in conditions involving mucin disorders such as keratoconjunctivitis sicca (KCS) requires the administration of hydrophilic polymers which are able to resist dessication and provide lubrication for lid and eye movement. The most effective preparations developed to date have been those based on hyaluronic acid or oily bases, although the latter may cause blurring of vision. Formulations based on other biopolymers such as xanthan gums and alginates have also been introduced. Recently, Burglassi and colleagues have described the properties of formulations containing polysaccharides derived from the tamarind seed (TS polysaccharide) (Burglassi et al 1999). Such formulations have high viscosity and mucoadhesive properties. Gamma scintigraphy provides a quantitative measurement of the precorneal distribution and drainage (Wilson 1999).

This was a single-centre, randomised, analyst-blind, four-way crossover scintigraphic study in twelve diagnosed mild to moderate KCS patients aged 35 to 75 (4 females, 8 males). The formulations studied were 0.5%, 1.0%, 2.0% w/v TS polysaccharide and 0.4% w/v hyaluronic acid. These were aseptically labelled with a radioactive marker ^{99m}Tc-DTPA (10µl per gram). The addition of this label had no significant effect on the viscosity of the formulations.

Subjects were seated at an ophthalmic table positioned 75 mm from a low-energy high-resolution collimator with chin and forehead supported. Twenty-five microlitres of the appropriate formulation (approx. dose 1 MBq) was instilled onto the corneal surface of the specified eye using a positive displacement pipette. A dynamic view was recorded for 10 min post dosing. Subjects were instructed to remain still and maintain as normal a blink rate as possible. Subsequently static views were collected every 5 min for a period of 30 min, then every 15 min until 2 h post dose.

Dynamic corneal residence-time curves (first 10 min post instillation) show a greater retention of 2.0 and 1.0% w/v TS-polysaccharides formulations than the hyaluronic acid formulation. The relative proportions of the total label remaining on the corneal surface at the end of the dynamic acquisition (mean ± s.d.) were 66.9 ± 18.2% for 2.0% and 42.1 ± 22.9% for 1.0% compared with 22.2 ± 20.0% for the hyaluronic acid formulation. The 0.5% formulation had a comparable profile to the hyaluronic acid solution (relative proportions 20.2 ± 16.4% compared with 22.2 ± 20.0%).

All materials were retained on the cornea for a prolonged period (up to 2 h). The greatest retention was observed with the higher percentage concentrations of TS polysaccharide. This pattern of retention strongly suggests a tear-structuring effect of the TS polysaccharide. All compounds were well tolerated and no reflex tearing was noted.

Burglassi, S., et al (1999) *Ophthal. Res.* **31**: 229–235
Wilson, C. G. (1999) *Pharm. Sci. Tech. Today.* **2:** 321–326

038

The effects of gastric emptying and disintegration rate on the absorption of Panadol Actifast tablets

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Panadol Actifast is a new rapidly absorbed paracetamol tablet containing sodium bicarbonate. Pharmacokinetic studies in man have shown a significantly shorter t_{max} (both fed and fasted states) and a significantly higher C_{max} (fasted state) for Panadol Actifast than for conventional paracetamol tablets (Panadol), following oral dosing (Grattan et al 2000; Rostami-Hodgson et al 2002). To investigate the hypothesis that this is due to enhanced gastric emptying and dissolution/disintegration mechanisms, a combined scintigraphy and pharmacokinetic study was conducted.

A two-tablet dose of each formulation, radiolabelled with 2 MBq ^{99m}Tc-DTPA, was administered in both fasted and fed states. Eleven healthy subjects completed the study. Scintigraphic images and blood samples were acquired at pre-defined intervals over a 10-h period. Serum paracetamol levels were measured using a validated UV-HPLC assay.

In the fasted state, Panadol Actifast tablets emptied from the stomach faster than Panadol, but the difference was not significant. However, in two subjects, emptying of Panadol Actifast was dramatically retarded. This may be due to the fact that both subjects were menstruating at this stage of the study. The menstrual cycle has been linked to changes in gastric emptying patterns (Wald et al 1981). If data from these two subjects is excluded, onset of emptying, time to 50% gastric emptying (t_{50}) and time to 90% gastric emptying (t_{90}) were all significantly faster for Panadol Actifast than for Panadol ($P = 0.0448$, 0.0112 and 0.0042, respectively).

In the fed state, onset of emptying, t_{50} and t_{90} were all faster for Panadol Actifast than for Panadol, although the differences were not significant. Since the meal will dominate the emptying process under these conditions, this is to be expected and is consistent with the findings of the recent pharmacokinetic study (Rostami-Hodgson et al 2002). $AUC_{0\text{-}60\,min}$ correlated with t_{50} for all treatment arms, and with t_{90} for all treatment arms except Panadol Actifast fed (Table 1). This indicates that the extent of early absorption (and by extension the absorption rate) was dependent on the rate of gastric emptying. This is supported by the correlation of t_{max} with t_{50} and t_{90} for all treatment arms except Panadol fed (Table 1).

These results confirm faster gastric emptying and disintegration of Panadol Actifast tablets compared with conventional Panadol tablets. While these effects exist in both the fed and fasted states, the differences in gastric emptying are more pronounced in the fasted state and the differences in disintegration are more pronounced in the fed state.

Table 1 Correlation of pharmacokinetic parameters with gastric emptying times

	Panadol Actifast		Panadol	
	Fasted	Fed	Fasted	Fed
	P	*P*	*P*	*P*
t_{max} v t_{50}	0.0002	0.0049	0.0002	0.0631
t_{max} v t_{90}	0.0002	0.0006	0.0002	0.2723
$AUC_{0\text{-}60}$ v t_{50}	0.0011	0.0017	0.0013	0.0201
$AUC_{0\text{-}60}$ v t_{90}	0.0011	0.3195	0.0003	0.0276

Rostami-Hodgson, A., et al (2002) *Drug Dev. Ind. Pharm.* **28**: 535–545
Grattan, T. J., et al (2000) *Eur. J. Pharm. Biopharm.* **49**: 225–229
Wald, A., et al (1981) *Gastroenterology* **80**: 1497–1500

039
Investigation of factors controlling the erosion of press-coated pulsed-release tablets

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Previous studies by this group (Leaokittikul et al 2001a, b) have investigated the formulation factors involved in the performance of pulsatile press-coated tablets. Dissolution studies demonstrated that the drug release (both lag time and $T_{50\%}$) is controlled by varying the thickness of a hydrophobic erodible press coating around a core tablet or the proportional weight ratio between the two components of the coating granules (glyceryl behenate (GB; Gattefossé, France) and low-substituted hydroxypropyl cellulose (L-HPC; Shin-Etsu, Japan)). Since the swelling property and erosion of the coat controls the drug release, it is important to investigate the sensitivity of such formulations to water uptake and to agitation conditions as a prelude to the formulations being evaluated in a clinical study.

In this study, the exposure of coating granule formulations to moisture vapour was studied (Table 1) using dynamic vapour sorption (DVS, Surface Measurement Systems, UK) at 25°C. In addition, the effect on the release performance of variation of dissolution paddle speeds (0, 18, 25, 50, 100, 150 and 200 rev min^{-1}) as well as pH of the medium (2, 4, 7.4) have also been investigated.

Table 1 Granule formulations and moisture uptake at 90% RH

Formulation	Ratio of glyceryl behenate and L-HPC	% Change in mass (at 90% RH)
A	50:50	13.64
B	60:40	10.19
C	65:35	8.97
D	70:30	6.96
E	75:25	5.13

Table 2 Effect of dissolution paddle speed on drug release

Formulation	Paddle speed			
	18 rev min^{-1}		200 rev min^{-1}	
	Lag time (min)	$T_{50\%}$ (min)	Lag time (min)	$T_{50\%}$ (min)
A	83 (34)	200 (42)	35 (8)	39 (9)
B	83 (15)§	175 (28)	81 (11)§	85 (12)
C	96 (15)	170 (20)	64 (15)	70 (18)
D	140 (20)	182 (27)	70 (14)	75 (14)
E	196 (38)	219 (47)	135 (10)	139 (11)

Data are means (s.d.), n = 5 or 6, ($F > 4.96$ at $\alpha = 0.05$, except §)

The DVS results (Table 1) demonstrate that as the amount of GB increases, the water uptake decreases, reflecting the increasing hydrophobicity of the granules. These findings correlate well with the dissolution results in that the lag time and $T_{50\%}$ are also prolonged as the content of GB increases. Paddle speed (Table 2) has less of an effect on the lag time compared with a pulsed-release system where erosion alone is the controlling mechanism (Ross et al 2000). This indicates that the tablet performance is determined more by the active swelling property of the L-HPC than by the fluid dynamics of the dissolution medium itself. However, $T_{50\%}$ is prolonged considerably at the lower agitation rates. Notably, as the rotation speed decreases, the dissolution profile exhibits sustained release, rather than the pulsatile release profile prevalent at higher agitation rates.

Leaokittikul, D., et al (2001a) *BPC Science Proceedings*. p.54
Leaokittikul, D., et al (2001b) *BPC Science Proceedings*. p.77
Ross, A. C., et al (2000) *J. Pharm. Pharmacol.* **52**: 909–916

040
5-Aminolevulinic acid for photodynamic therapy of vulval intraepithelial neoplasia. Assay development and release from a proprietary formulation

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Vulval intraepithelial neoplasia (VIN) is a pre-cancerous dysplastic lesion of the vulva which can progress to invasive cancer. Current treatment methods for VIN include surgical excision, laser ablation and topical application of cytotoxic drugs. Photodynamic therapy (PDT) is a relatively new technique by which a combination of visible light and a sensitising drug causes the destruction of selected cells. A particularly interesting agent used in PDT is the naturally occurring haem precursor 5-aminolevulinic acid (ALA). ALA causes preferential accumulation of the photosensitiser protoporphyrin IX in neoplastic cells following topical application (Gannon et al 1999). Due to its rapid kinetics and topical applicability, ALA represents a promising alternative for the treatment of VIN.

The delivery of ALA to the vulva using topically applied creams for photodynamic therapy is problematic. Determination of an appropriate and exact dose of drug depends on the correct amount of cream being applied. This is highly variable, given that the cream is located under an occlusive barrier with no control over thickness. Furthermore, clinical response depends on maintaining the formulation in place sufficiently to allow drug to penetrate into the lesions. In practice, occlusive dressings are poor at staying in place around the lower-reproductive-tract area, where shear forces are high in mobile patients.

To date, there has been no published data on the release of ALA from topically applied vehicles. The objective of this work was to evaluate release of ALA from a proprietary cream formulation (Porphin, 20% w/w ALA in Unguentum Merck) with a view to re-evaluating the dosing strategy and method of administration of ALA to the vulva for the treatment of VIN.

The release of ALA from Porphin was investigated using Franz cells and a model Cuprophan membrane. Due to poor UV absorbance, ALA was quantified by a

modified HPLC method employing pre-column derivatisation with acetyl acetone and formaldehyde followed by fluorescence detection (Oishi et al 1996).

A typical biphasic release profile was observed with an initial rapid release followed by a plateau region. After 6 h, which is the typical application time in-vivo, only approximately 50% of the drug was released possibly due to the hydrophilic nature of the drug and the water in oil nature of the formulation. This low level of drug release will further add to the uncertainty of achieving consistency in dose during topical administration of ALA which arises due to uneven cream thickness under occlusion.

Gannon, M. J., Brown, S. B. (1999) *Br. J. Obstet. Gynaecol.* **106**: 1246–1254
Hillemanns, P., Untch, M., Dannecker, C., et al (2000) *Int. J. Cancer* **85**: 649–653
Oishi, H., Nomiyama, H., Nomiyama, K., et al (1996) *J. Anal. Toxicol.* **20**: 106–110

041

Relationship between HLB of waxes and Log P of drugs on dissolution from hydrophobic matrix pellets

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Hydrophobic matrix pellets formed by direct warm-spheronisation without prior extrusion have previously been shown by Lee et al (2001a) to retard drug release. Paracetamol release from the wax pellets was shown by Lee et al (2001b) to increase as the HLB value of the wax matrix increased and was also influenced by type and content of hydrophilic excipient incorporated in the formulation. The aim of this study is to investigate the relationship between drug log P and wax HLB on the resultant dissolution profile.

β-Antagonist drugs in free-base form were used as the model drug series. Free-base propranolol was obtained from HCl salt solution by precipitation with excess alkali and extraction with diethyl ether, with purity confirmed from melting point determination. Nadolol and atenolol were obtained in free-base form and used as received. Waxes of a range of known HLB values were prepared using either pure waxes or admixtures of waxes.

Wax pellets of size range 1001–1180 μm containing wax–dicalcium phosphate–drug (50:48:2) were prepared as previously described. The dissolution profile of each pellet formulation (equivalent to 20 mg drug) was determined using an automated dissolution apparatus (USP XIII Apparatus 2) containing 1000 mL deaerated distilled water, operated at 50 rev min^{-1} paddle speed and maintained at 37°C bath temperature.

All formulations in each drug series exhibited Higuchian release. The release profiles were faster as the HLB value of the wax matrix increased for each drug series (Table 1). As drug log P value in the formulation increased, drug dissolution rate decreased for a given HLB series.

Table 1 Dissolution of β-antagonist drugs from wax matrix pellets

Drug	Log P	Time to reach 25% drug release, $T_{25\%}$ (min) Wax pellet HLB				
		2.0	3.5	5.0	6.5	9.5
Atenolol	0.27	307	19	13	6	n.d.
Nadolol	0.85	112	33	14	6	n.d.
Propranolol	3.37	n.d.	71	29	14	8

As the wax matrix becomes more hydrophilic, drug release is promoted. However, as drug log P increases, drug release is retarded. The relationship between wax HLB and drug log P is therefore an important parameter in influencing drug partitioning from the wax matrix to bulk phase water.

Lee, J. J. N., et al (2001a) *Proc. Int. Symp. Control. Rel. Bioact. Mater.* **28**: 720–721
Lee, J. J. N., et al (2001b) *Proc. Int. Symp. Control. Rel. Bioact. Mater.* **28**: 742–743

042

Effect of storage on dissolution and DSC profile of a hydrophobic matrix pellet formulation

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Ageing properties of triglyceride waxes have been well documented by Sutananta et al (1994) and may affect the stability of formulations incorporating such waxes. The aim of this study is to investigate how ageing affects drug release from a hydrophobic matrix pellet formulation containing saturated monoglyceride.

Pellets containing glyceryl monostearate–dicalcium phosphate–paracetamol (50:40:10) were prepared using a direct warm spheronisation technique (without prior extrusion) described previously by Lee et al (2001). The pellets were stored at 25°C and 37°C and the dissolution profile of the fresh and aged pellets determined in 1000 mL deaerated distilled water using an automated dissolution apparatus (USP XIII Apparatus 2) operated at 50 rev min^{-1} paddle speed and maintained at 37°C bath temperature. Pellets equivalent to 12.5 mg drug were analysed (n = 6).

Thermal analysis of the formulations was investigated using DSC. Sample (6.5–8.0 mg) were sealed in aluminium crucibles (40 μL) with a pierced lid. A similar empty crucible was used as the reference. Each sample was heated from 10 to 80°C at a heating rate of 2°C min^{-1}.

Surface morphology of the fresh and aged pellets was analysed using SEM at 50×, 800×, 1600× and 3200× magnification at 6.0 kV. Samples were mounted for microscopy on aluminium stubs with carbon tape and then gold coated.

All formulations exhibited Higuchian release during the study period. As storage time increased, the pellet dissolution rate decreased and the DSC peak temperature increased (Table 1). The pellet surface morphology changed visibly during the storage period.

Table 1 Drug release rates and DSC peak values for pellet formulations stored at 25°C

Storage time (month)	Rate of drug release, k (min^{-1})	Peak temperature (°C)
0	6.873	55.79
1	6.297	55.92
2	5.711	56.06
3	4.835	56.07
4	3.474	56.23

When stored at 37°C, the DSC profile indicated the appearance of an additional shoulder at a higher melting point.

The reduction in dissolution rate and the alteration in surface morphology are indicative of polymorphic transformation of the glyceryl monostearate. The elevation of DSC peak temperature is consistent with the changes observed by Whittam & Rosano (1975) on trimyristin wax.

Lee, J. J. N., et al (2001) *Proc. Int Symp. Control. Rel. Bioact. Mater.* **28**: 720–721
Sutananta, W., et al (1994) *Int. J. Pharmaceutics* **111**: 51–62
Whittam, J. H., Rosano, H. L. (1975) *J. Am. Oil Chem. Soc.* **52**: 128–133

043

Viscoelastic properties of a novel glucose-sensitive gel for self-regulated insulin delivery

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A self-regulating insulin delivery system would improve the management of diabetes. This is because such a closed-loop delivery system would mimic physiological patterns of insulin secretion and thereby minimise the complications of diabetes which are a result of poor glycaemic control. Stimuli-sensitive gels that

undergo changes in state in response to changes in environmental conditions have potential applications as smart biomaterials for polymeric controlled drug delivery. Development of glucose-sensitive gels may therefore advance the construction of a self-regulating drug delivery system for the management of diabetes.

Formulations of the plant lectin, concanavalin A (con A), and dextran have been shown to produce glucose-sensitive gels (Taylor 1992). These gels have been used as part of an in-vitro self-regulating drug-delivery system based on a polysaccharide displacement mechanism. This design delivers insulin from a reservoir through a gel membrane, the viscosity of which varies reversibly according to glucose content. However, a problem with the design of these gels was the leaching of the mitogenic lectin. This has been addressed previously by the covalent coupling of the lectin to periodate oxidised polysaccharide and to a carbomer resin using carbodiimide chemistry (Tanna et al 1999, 2002). Both of these methods have resulted in stabilised gels that maintain their ability to respond to glucose and deliver insulin, but suffer minimal loss of components.

This study examined the viscoelastic properties of novel carbomer-stabilised glucose-sensitive gel formulations, reported previously for their ability to modulate insulin delivery in response to glucose (Tanna et al 2002). These oscillatory tests were conducted because they could provide useful information about the glucose-provoked gel-sol transformations for candidate gels because of the separation of parameters relating to solid and liquid behaviour. Oscillatory rheometry was performed using a Haake Rheostress RS75 rheometer at 37 $\pm$ 0.5°C in conjunction with cone and plate geometry (C35 with 2° angle). Samples were subjected to a constant stress (100 Pa) and defined viscoelastic parameters, namely storage modulus (G'), loss modulus (G"), loss tangent (tan δ) and complex dynamic viscosity (η*), measured over a defined frequency range (0.01–10 Hz). As the oscillatory frequency was increased, G' and G" of all formulations increased, whereas η* decreased. The values for tan δ were below one, indicating a predominantly elastic system. Increasing formulation glucose concentration decreased G' and G", observations that may be attributed to the physical state of the glucose-responsive formulations. For the design of a closed-loop insulin delivery device, a gel formulation exhibiting a greater elastic component could be useful for providing the low basal rate which might show little increase in insulin diffusion rate until the gel lattice was affected by critical glucose levels. This study has shown the applicability of oscillatory rheometry for both characterisation and selection of candidate glucose-sensitive gels for clinical evaluation. The final choice of formulation for clinical evaluation would involve a compromise between viscoelastic behaviour and acceptable drug delivery characteristics.

Tanna, S., et al (1999) *J. Pharm. Pharmacol.* **51**: 1093–1098
Tanna, S., et al (2002) *J. Drug Target*. In press
Taylor, M. J. (1992) Patent WO 93/13803

044

Tween 80 improves folding reversibility, integrity and biological activity of spray-dried and crystallised lysozyme

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Stabilization of the native folded protein conformation is critical for preparation of proteins as therapeutic products. A protein must be able to find the folding pathway in a short time from a denatured to folded, active state. Proteins undergo various structural changes if physiological conditions alter. Accordingly, they may denature and the denatured protein tends to adsorb to surfaces and aggregate with other protein molecules. Hence, the protein loses its stability and activity. Consequently, a non-ionic surfactant (Tween 80, 0.09% w/v) was added during preparation of crystallised and spray-dried lysozyme to decrease the interfacial tension and adsorption of protein to air/water interface and the results were monitored.

Our aims were to evaluate the influence of Tween 80 on folding reversibility and conformational integrity of lysozyme formulations in solution using high-sensitivity differential scanning calorimetry (HSDSC) and Fourier transform-Raman (FT-Raman) spectroscopy, respectively, and to determine if folding reversibility correlates with biological activity.

HSDSC determined folding reversibility of thermally denatured unprocessed and processed lysozyme samples in solution (5 and 20 mg protein/1 mL of 0.1 M sodium acetate buffer, pH 4.6) by employing two consecutive upscans from 20 to 90°C at 1°C min^{-1}. Enthalpy ratios of the second upscan (ΔH2)/first upscan (ΔH1) were taken as measures of folding reversibility. FT-Raman spectra of aqueous lysozyme solutions (1 and 15% w/v) were the average of 2000 scans. Enzymatic assay of thermally denatured samples was performed after cooling (in HSDSC).

HSDSC and biological activity analyses (Table 1) indicated that Tween 80 enhanced the folding reversibility of protein samples compared with samples without surfactant, accompanied by increased activity. There were no significant differences between folding reversibility of protein samples with surfactant at low and high protein concentration, indicating Tween 80 greatly decreased the formation of aggregates. FT-Raman demonstrated that samples (15% protein w/v) containing Tween 80 exhibited identical spectra to native lysozyme solution (1% w/v) (i.e. no aggregation). Spectra of high protein concentration without Tween 80 showed marked shifts especially in amide I (~6 cm^{-1}) and III (~7 cm^{-1}). In all experiments, crystals were least affected by aggregation.

Table 1 Folding reversibility (percentage of ΔH2/ΔH1) and enzymatic activity of thermally denatured lysozyme solutions upon cooling

Sample	Tween 80 absent		Tween 80 present	
	Folding reversibility	% Activity	Folding reversibility	% Activity
Control				
5 mg mL^{-1}	59.5 ± 2.2	61.4 ± 2.0	64.6 ± 1.3	69.5 ± 1.0
20 mg mL − 1	51.6 ± 2.1	49.9 ± 3.2	63.5 ± 2.0	68.6 ± 1.6
Crystals				
5 mg mL^{-1}	66.5 ± 1.4	65.7 ± 1.4	73.6 ± 0.9	71.3 ± 1.4
20 mg mL^{-1}	52.4 ± 1.6	52.7 ± 2.3	72.5 ± 1.2	70.5 ± 2.0
Spray dried				
5 mg mL^{-1}	50.6 ± 3.1	43.9 ± 3.1	55.3 ± 1.7	51.5 ± 1.6
20 mg mL^{-1}	42.5 ± 2.3	40.3 ± 2.3	53.9 ± 1.2	49.7 ± 2.3

n = 3, with s.d.

In conclusion, the surfactant appears to decrease the aggregation process that competes with lysozyme refolding. Notably, in the presence of Tween 80, lysozyme crystals showed highest activity and reversibility compared with unprocessed and spray-dried protein, supporting our earlier findings that protein crystals may have advantageous drug delivery properties.

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Clinical trial of a pressure sensitive anaesthetic patch system for use on neonates

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Aqueous gel compositions containing the local anaesthetic amethocaine (tetracaine) provide effective percutaneous local anaesthesia of intact, healthy skin (Woolfson et al 1990), thus enabling invasive procedures such as intravenous injection or cannulation to be performed without causing undue stress to the patient. Amethocaine has been chosen as an ideal candidate for local anaesthesia due its phase change in aqueous media at 32°C (i.e. skin temperature) (Woolfson & McCafferty 1993). This study aimed to assess the activity of a self-adhesive amethocaine patch through a placebo-controlled double-blind clinical trial. The device has been specifically designed for application to neonates before routine venepuncture and heel stabs.

The films investigated were cast from 5% hydroxypropylcellulose (HPC) gels of 0.4% w/w amethocaine loading. The gel was dried after casting using a cool air convection system producing a film containing 1 mg cm^{-2} of amethocaine. A non-

porous, adhesive backing film was formulated with the desired adhesive qualities required for adhesion to immature, and thus fragile epidermis, as seen in the premature neonate. The final device was of an island design. An identical patch was formulated using micro-crystalline cellulose (Avicel) in place of amethocaine to produce a placebo device. A randomised, double-blind, placebo-controlled trial was conducted within the Neonatal Intensive Care Unit and delivery wards of the Royal Maternity Hospital Belfast. A minimum of 30 newborn infants was recruited, incorporating both term and preterm infants from 32 to 42 weeks gestation (median 36) at 3–18 days of age (median 6). The disc at the centre of the patch system was moistened with sterile water and applied to the chosen area. The patch was left in place for 30 min before the venepuncture procedure. Pain was assessed in response to needle insertion in the infant. A validated adaptation of the neonatal facial coding score (NFCS) and the presence of crying was used to assess this pain (Jain & Rutter 2000). This method scores each of a number of facial characteristics as being present (scoring one point) or absent (no score is received) over the 5 s after the painful procedure. A cumulative score of 10 or less within the 5 s was defined as an indication clinically effective anaesthesia (Jain & Rutter 2000).

Table 1 Cumulative NFCS over the five seconds following painful procedure

Group	n	Gestation (weeks)	Median cumulative NFCS (range)
Amethocaine	15	35 (32-42)	0 (0–13)
Placebo	16	37 (34-41)	12.5 (0–23)

The median cumulative NFCS over the 5 s immediately following the heel stab was 0 in the active patch group compared with 12.5 in the placebo group. There was a significant difference in the cumulative NFCS between the two groups following the procedure ($P = 0.0002$, Mann Whitney test). Amethocaine-treated patients (14 of 15, 93%) showed little or no pain in response to the heel stab compared with 5 of 16 (31%) in the placebo group. The results show a definite anaesthetic response produced by the patch device following the 30 min application time. The practicality of the patch was proved in a clinical environment, and shown to be a simple and effective pre-venepuncture procedure in neonates.

Woolfson, A. D., et al (1990) *Br. J. Clin. Pharmacol.* **30**: 273–279
Woolfson, A. D., McCafferty, D. F. (1993) *Int. J. Pharmaceutics* **94**: 75–80
Jain, A., Rutter, N. (2000) *Arch. Dis. Child. Fetal Neonatal Ed.* **83**: F207–F210

046
Preliminary investigations into a novel controlled release mechanism: the influence of water uptake rates

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Low-substituted hydroxypropylcellulose (grade LH21) is widely used as a tablet binder and a disintegrant. Its ability to swell upon contact with water has been utilised in various controlled-release formulations such as the time-controlled explosion system patented by Ueda et al (1989).

We report our initial studies into the use of LH21 in a novel time-controlled release capsule that relies on the hydrostatic pressure generated by the swelling of LH21 upon water ingress to rupture a water-insoluble, brittle outer coating resulting in the release of drug. We evaluated the importance of water uptake rates on the swelling of LH21 as a mechanism for allowing programmed rupture of the capsule coat.

A modified version of the apparatus employed by Kawashima et al (1993) was used to measure LH21 water uptake. It comprised of a sintered glass filter funnel as a sample holder attached to a horizontally positioned graduated pipette via a PVC tube. Water was filled into the apparatus, noting the initial position of the meniscus in the pipette. The sample was then poured into the funnel and the volume of water absorbed over time recorded.

Studies were carried out on 5-mL dispersions of LH21 in corn oil (2–40% w/v) with and without addition of a surfactant. Increasing the concentration of LH21 in corn oil increased the rate of water uptake with all LH21 concentrations absorbing approximately 2–5 times their own dry weight in water after 30 min (Table 1).

Table 1 Influence of LH21 concentration in corn oil dispersion on the rate of water uptake

Concn of LH21 (% w/v)	Initial rate of water uptake (mL min^{-1})[a]	Weight of water absorbed:dry weight of LH21[b]
2	0.02	5.40
5	0.05	5.04
10	0.08	4.32
20	0.10	3.03
30	0.12	2.32
40	0.13	1.94

[a]Measured over initial 20 min; [b]at 30 min

Surfactants sodium dodecyl sulphate (SDS) and Span 80 decreased the rate of water uptake into the LH21–corn oil dispersions; the extent of reduction enhanced by the increasing amount of surfactant added (Table 2).

Table 2 Influence of surfactant type and concentration on the rate of water uptake into a 10% w/v LH21–corn oil dispersion

	Rate of water uptake (mL min^{-1})[a] Surfactant concn (% w/v)			
	0.00	0.25	0.50	1.00
SDS	0.0800	0.0333	0.0192	0.0046
Span 80	0.0800	0.0510	0.0421	0.0005

[a]Measured over initial 5 min

These results indicate that water uptake rates can be controlled by judicious selection of excipients in the proposed device and therefore offer a means of programming capsule rupture. Additional control can also be obtained by optimisation of the type and thickness of the outer coating.

Kawashima, Y., et al (1993) *Pharm. Res.* **10**: 351–355
Ueda, Y., et al (1989) US Patent 4,871,549

047
Characterisation of non-aqueous bioadhesive drug delivery systems by differential scanning calorimetry

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One strategy that has been proposed to prolong the retention of a topical formulation on a mucosal surface is to present the bioadhesive polymer in a non-aqueous vehicle (NAP) (Zaman et al 2001). It is proposed that such formulations will hydrate slowly and therefore prolong the time between application and adhesive failure. The interaction and distribution of water in hydrophilic polymer gel systems is critical to their properties and affects behaviour including, rheology, sol-gel transformation and adhesion (Carstensen & Li Wan Po 1992; Haque & Morris 1993), as well as their use in matrices employed for controlled drug release.

The aim of this study was to investigate, using differential scanning calorimetry (DSC), the effect of water on a series of NAP systems differing in polyethylene glycol 400 concentration, and to identify the different states of water (if any) within the polymer gel.

Four NAP formulations were prepared using combinations of either polyethylene glycol 400 (P), Carbopol 974 (C) or glycerin (G). These were designated G100 (100% (w/w) G), CG2 (98% G, 2% C), PCG2 (92.5% G, 2% C, 5.5% P) and PCG2,30 (68% G, 2% C, 30% P). Experiments were carried out in a DSC (TA Instruments Ltd, UK), using hermetically sealed aluminium pans. Water was mixed into samples of the NAP formulations, and allowed to equilibrate for 12 h, such that the final aqueous content was 40, 60 and 80% w/w, respectively. Samples (~15 mg) were then cooled from +20°C to −55°C at a rate of 10°C min^{-1} and, subsequently, heated to +55°C at 10°C min^{-1} (n = 6).

Unhydrated NAP samples or those containing up to 40% w/w water, did not exhibit any peaks in the thermograms, reflecting an absence of phase transitions under the experimental conditions employed. However, at 60% and 80% w/w water content, freezing and melting transitions were observed. At 80% w/w water, the endothermic peak was shifted towards 0°C and there was a marked increase in melting enthalpies (Table 1) compared with those observed in systems containing 60% w/w water. At 60% w/w water, formulations containing either C or P had a significantly higher enthalpy compared to the formulation containing only G; this difference was not apparent when the water content was increased to 80% w/w.

Table 1 Enthalpies (J g^{-1}, final hydrated sample) for NAP formulations containing 60 or 80% w/w water

NAP	Enthalpy (J g^{-1})	
	60%	80%
G100	87 ± 2	198 ± 3
CG2	100 ± 2	197 ± 4
PCG2	104 ± 3	199 ± 6
PCG2,30	105 ± 2	199 ± 4

Data are presented as means ± s.d., n = 6

The endothermic (melting) peak was broad, possibly due to the interaction of the water molecules with hydrophilic non-aqueous liquid components such as G and P. The results of this study demonstrate that DSC can be employed to differentiate between freezable and non-freezable water in hydrated, non-aqueous based formulations.

Carstensen, J. T., Li Wan Po, A. (1992) *Int. J. Pharmaceutics* **83**: 87–94
Haque, A., Morris, E. R. (1993) *Carb. Polym.* **22**: 161–173
Zaman, M. A., et al (2001) *British Pharmaceutical Conference Science Proceedings*. Pharmaceutical Press, London, p. 66

048
Self-regulated insulin delivery in-vivo

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In the absence of normal pancreatic control, blood glucose concentration is a function of calorie intake. Approximations to good control using current treatment are inadequate for prevention of the serious long term complications of diabetes mellitus and ideally an automated, self-regulated system is needed. Several such systems have been proposed, of which most contain flaws such as non-specificity for glucose, the need for chemically modified insulin and a failure to deliver insulin in a dose-related manner. The development of beta cell transplants, while making progress, is considered unlikely to address the worldwide extent of the problem in the foreseeable future.

The design presented here is for a reservoir device in which the rate control resides in a membrane comprising a glucose-sensitive gel formulation. The material at its simplest, is a mixture of the lectin, concanavalin A which contains glucose receptors and a polysaccharide bearing terminal glucose units. The combination forms a gelatinous complex in which the polysaccharide acts as a temporary cross linker for the lectin, with reversible structural collapse occurring on interaction with free glucose. Thus the viscosity of the gel depends on glucose content. To prevent loss of the components from the device, loose cross links have been introduced with permanent Schiff base bonds to form larger interactive structures. These retain the capability to respond to glucose with a viscosity change and can still therefore create glucose-dependent insulin transport.

In this study, a prototype device contained a thin layer of the bonded gel to govern output from a reservoir of insulin. The gel comprised either the variable viscosity agent or a constant viscosity carbomer counterpart. The carbomer viscosity was chosen to represent a value between the high and low extremes of the responsive gel (Taylor 2002).

Devices were implanted into the peritoneal cavity of rats and after recovery, diabetes was induced by administration of the pancreatic toxin streptozotocin. The rats were subjected to a large oral glucose challenge several days after this and resulting glucose levels monitored. In saline-treated controls this provoked extremely high blood glucose levels even following an injected substantial insulin bolus dose. With zero-order release insulin therapy the rise in glucose was more subdued, although still frequently reached abnormal levels. For example, early or late glucose breakthroughs developed using a 250 U mL^{-1} reservoir concentration while the higher insulin outputs produced hypoglycaemia. In contrast, those treated with the responsive version of the device remained normal throughout the challenge period. This design has therefore performed well in a glucose challenge in-vivo study. It is an automated self-regulated system without cellular, electronic or moving components and relies on a highly specific glucose response independent of any intermediate pH reaction. It also avoids the need to use chemically modified insulin.

Taylor, M. (2002) *Drug Deliv. Syst. Sci.* **1**: 101–105

049
The influence of particle size on transdermal permeation of nanoparticulate vaccine formulations

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Transdermal immunisation has been shown to stimulate both cell-mediated and humoral responses (Glenn et al 1998; Hammond et al 2001) and this has been has confirmed by our recent work (Somavarapu et al 2001). The prevalence of Langerhans cells in the viable epidermis provides the reasoning for this. Such antigen presenting cells have an affinity to particles, and the use of particulate vaccine formulations will serve to exploit the transdermal route to its full immunological potential.

In this study we investigated the relationship between nanoparticle size and the degree of permeation through skin. In addition, we aim to synthesize biodegradable polylactic acid (PLA) nanoparticles to be used for further in vitro studies.

Experiments were performed using florescent latex particle suspensions (Polysciences Inc., USA) of 50 m and 100 m, which were diluted to give a 1% v/v suspension.

Full thickness porcine skin was used as the model membrane for human skin and permeation experiments were performed in a diffusion cell. The cell was heated to 32°C using a water jacket and continuously stirred PBS was used as the receptor fluid. One millilitre of each particle sample was added as the donor phase. A 0.5-mL sample of the receptor phase was removed before starting the experiment and at 0, 1, 2, 3, 4 and 6 h intervals. PBS was added to replace receptor fluid after each sample was removed. Concentrations of particles permeated were determined by measuring fluorescence. Each sample was measured in triplicate. PLA nanoparticles were also prepared by a modified double-emulsion method

The particle permeation studies showed a greater concentration of particles in the receptor phase with 50-nm particles, compared to the 100-nm particles. After 6 h 0.19% of 50-nm particles permeated against 0.07% of 100-nm particles, showing a significant increase ($P < 0.005$) in permeation of 171%.

PLA particles were sized at 132.4 nm (polydispersity 0.23) by photon correlation spectroscopy (Zetasizer 2000, Malvern, UK). With further manipulation of process parameters to reduce size, the performance of these particles will be assessed to establish if they match the pattern achieved with the latex particles.

These data suggest that nanoparticles of a reduced size permeate the skin to a greater extent than those of a larger size. Further modifications of the particles will be aimed at enhancing percentage particle permeation. This will allow greater presentation of antigen-loaded nanoparticles to the Langerhans cells and hence increase the potential of eliciting an increased immune response.

Glenn, G., et al (1998) *Nature* **391**: 851

Hammond, S., et al (2001) *Vaccine* **19**: 2701–2707

Somavarapu, S., et al (2001) *Immunology* **104**: 4

050
The factors affecting liquid and semi-solid mucoadhesion to the oral cavity and oesophagus

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Liquid and semi-solid formulations adherent to the mouth, pharynx and oesophagus may provide mechanical protection, palliative relief and treat local disease. These mucosae also offer a substantial area of well-perfused tissue, perhaps suitable for systemic drug delivery. However, successful formulation design has been curtailed by the lack of a generally accepted method for evaluating mucoadhesive performance. Moreover, the mucoadhesive interaction is a complex phenomenon rather than a single-process bond. Therefore, an understanding of the relationship between mucoadhesive performance and the physicochemical properties must be elucidated to allow rational design of retentive dosage forms.

The porcine oesophageal mucoadhesion test system provides an accepted in-vitro technique for observing the mucoadhesive performance of liquid and semi-solid formulations (Young & Smart 1998; Riley et al 2002). To the planar epithelial surface, maintained in near-physiological condition in respect of temperature, humidity and (artificial) salivary flow, a sample of test formulation is applied and the elution of both the applied carrier formulation and a model water-soluble drug determined. The recovery of aqueous dispersions of synthetic and naturally occurring mucoadhesive polymers follows pseudo-first-order elimination from the mucosal surface to leave a baseline of mucoadherent formulation. The half-life of the elimination process and the mucoadherent polymer surface-concentration have been determined and employed as indices of mucoadhesive performance. These parameters are related to the surface nature and bulk rheology of polymer dispersions determined by contact angle goniometry and dynamic oscillatory shear rheometry respectively.

The calculated the work of adhesion of hydrated polymer to mucin in water is proportional to mucoadhesive polymer-surface concentration (Table 1), demonstrating the fundamental importance of surface interactions to mucoadhesion. As the extent of adsorption is proportional to the work of adhesion, these data are suggestive of a dynamic equilibrium of mucoadhesion and disassociation. This is further supported by the Freundlich adsorption isotherm demonstrated for a concentration series of hydroxypropyl-methylcellulose dispersions. An observed power relationship between formulation initial half-life and dynamic viscosity supports the hypothesis that the exponential elimination phase represents the sloughing of excess hydrogel matrix from the surface (Young & Smart 2000). The elimination rate is unrelated to mucoadhesive performance.

Table 1 Calculated work of adhesion vs mucahesive polymer surface concentration

Hydrogel delivery System	Caclulated work of adhesion of hydrated polymer to mucin in water ($mJ\ m^{-2}$)	Mucoadhesive polymer surface concn ($mg\ m^{-2}$)
Noveon AA1 0·5% (w/v)	5·72 ± 0·50	51·6 ± 10·9
Carbopol 934P 0·5% (w/v)	13·77 ± 0·29	515·7 ± 128·9
Sodium carboxy Methylcellulose 1·0% (w/v)	25·56 ± 0·38	2881·5 ± 58·7
Poly(ethylene oxide) 4·0% (w/v)	52·71 ± 1·58	8774·3 ± 522·0
Hydroxypropyl Methylcellulose 4·0% (w/v)	72·80 ± 11·60	12527·3 ± 626·3

Data are means ± s.d.

It is concluded that mucoadhesion is primarily a surface interaction resulting in a multi-layered adsorption of polymer matrix at the mucosal surface. Once a mucoadhesive interaction is established, excess formulation sloughs from the surface at a rate determined by the dynamic viscosity of the bulk.

Riley, R. G., et al (2002) *Int. J. Pharmaceutics* **236**: 87–96

Young, S. A., Smart, J. D. (1998) *J. Pharm. Pharmacol.* **50**: s167

Young, S. A., Smart, J. D. (2000) *J. Pharm. Pharmacol.* **52**: s167

051
Electrochemical stability of insulin under iontophoretic conditions

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Transdermal iontophoresis (TI) is a potential enhancement strategy for the delivery of charged molecules using a small electric current and it underwent a renaissance with the emergence of rDNA technology that has made it possible to produce peptide/protein drugs in large numbers. Commercial and therapeutic feasibility of TI is influenced by a number of technical and developmental issues (Panchagnula et al 2000). One of the key issues is ensuring the stability of peptides and proteins starting from formulation development till it reaches the site of action. In this respect, the electrochemical stability of insulin, a model peptide, was investigated under various iontophoretic conditions by SDS-PAGE, HPLC and in-vivo hypoglycaemic activity. Insulin solution ($1\ mg\ mL^{-1}$) at various pHs were applied to the donor compartment of Franz diffusion cells and separated from the receptor compartment by Parafilm. Currents of varying strength and duration were applied using a constant-power supply unit through platinum electrode and the donor solution was analysed for insulin content at the end of iontophoresis. Out of the five pH levels investigated in the study by both anodal and cathodal iontophoresis, maximum degradation was observed at pH 7.4 during anodal iontophoresis, while degradation was minimal at pH 3.6. The degradation is attributed to the pH shift due to electrolysis of water at the platinum electrode surface, which was high at pH 7.4, and minimum at pH 3.6. However, the intensity of insulin bands in SDS-PAGE matched with HPLC results only at pH 3.6. Despite 70% degradation at pH 7.4, the hypoglycaemic activity was comparable with the activity shown by freshly prepared insulin solution. Further, at pH 3.6, the degradation increased as a function of current strength and significant degradation was observed at $1\ mA\ cm^{-2}$. On the other hand, the degradation did not differ significantly with increasing duration of current application at $0.5\ mA\ cm^{-2}$. The results of HPLC and SDS-PAGE were comparable to each other; however further studies with other analytical methods to investigate the nature of degradation products would provide strategies to reduce the electrochemical degradation of insulin under the application of electric current.

Panchagnula, R., et al (2000) *Curr. Opin. Chem. Biol.* **4:** 468–473

052
Transdermal delivery of naloxone: in-vivo evaluation of gel formulation

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Naloxone (NLX) is a potent, pure, competitive opioid antagonist. It is the drug of choice in treatment of narcotic overdose and in the management of post-anaesthetic depression induced by opioids. It rapidly and completely antagonizes respiratory and central nervous system signs associated with opiate overdose, while having no intrinsic antagonistic or respiratory depressant actions. But, NLX is not effective when administered perorally because of its high first pass metabolism and short duration of action. The transdermal delivery can be a solution to these problems. This route can circumvent problems associated with first-pass metabolism, short elimination half life and repeated injections, can maintain constant blood levels that will result in better management of narcotic antagonism and needs no motivation in administration of dosage form. The feasibility studies on transdermal delivery of NLX were performed in our laboratory (Jaiswal et al 1999; Panchagnula et al 2001) and a gel formulation was developed with hydroxypropyl cellulose as gelling agent and, using ethanol and propylene glycol as vehicle incorporating oleic acid as penetration enhancer. Hence, the objective of present investigation is to evaluate the gel formulation in-vivo in Sprague-Dawley rats.

Pharmacokinetic parameters of NLX in rats were determined after intravenous administration of drug ($5\,mg\,kg^{-1}$). From the pharmacokinetic parameters obtained from intravenous administration, and ex-vivo permeation, in-vivo levels of drug after transdermal administration were predicted. To predict plasma concentrations, transdermal delivery was assumed to be modeled by constant infusion of drugs with a lagtime as described by Takayama & Nagai (1991).

The hair on dorsal rat skin was clipped with an animal hair clipper (Aesculap, Germany). A teflon ring was fixed to that area using a commercial adhesive, and into that about 20 g of gel ($20\,mg\,g^{-1}$ NLX) was applied. After application, ring was covered with the help of a teflon cylinder fitted to the dimensions of the ring. Samples were collected at predetermined time points and the radioactivity in them was counted with a liquid scintillation counter (Wallac 1409, Finland). At the end of study, skin surface was cleaned and observed for visual signs of inflammation, edema, or other damage. None of the rats showed any signs of skin damage after 48 h. The in-vivo levels obtained were in the range of $8–16\,\mu g\,mL^{-1}$ from 8–48 h. These levels were much higher than the values predicted from ex-vivo flux. After removal of formulation, NLX plasma concentrations were found to sustain for 5 h in the range of $12–16\,\mu g\,mL^{-1}$ suggesting the possibility of depot formation in skin. The blood levels achieved were much higher than those predicted with the help of ex-vivo data for up to 48 hs. The C_{ss} obtained from the transdermal gel is 4 times higher than that predicted from ex-vivo data. Hence, there can be flexibility in the size of the patch to design various dosage regimens dictated by clinical need or the concentration of drug can be decreased in the formulation.

Jaiswal, J., et al (1999) *Int. J. Pharmaceutics* **179**: 129–134
Panchagnula, R., et al (2001) *Int. J. Pharmaceutics* **219**: 95–105
Takayama, K., Nagai, T. (1991) *Int. J. Pharmaceutics* **74**: 115–126

053
Flow properties of lyophilised wafers on a model gelatine surface

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Lyophilised wafers are being developed for the delivery of drugs to suppurating wounds. It is proposed that a freeze-dried formulation containing an appropriate medication is applied directly to the target surface with immediate adhesion. Once in place the wafer continually absorbs fluid, changing from a solid to a highly viscous gel. In this state, the contained drug might be expected to migrate throughout the wound environment. Such a system may have advantages over conventional topical formulations.

To test the hydration and flow properties of these wafers, a simple model was developed with a moist surface to which formulated wafers could adhere and hydrate. To test the model, wafers were fabricated from low molecular weight sodium alginate modified with high-molecular-weight methylcellulose.

Sodium alginate (Hopkins & Williams) was dissolved in deionised water at a solids content of 5%w/v and the solution heated to 60–70°C. Sodium fluorescein (Sigma) was added to the stock solution as a visible model for a soluble drug. Methylcellulose (Methocel A4M, Dow) was then added in precise amounts to stirred 50-mL batches of the heated solution to produce a series of modified formulations. While still hot, equal amounts of the mixtures were poured to 6-well polystyrene microplates (Costar) and allowed to cool below the gelation temperature of methylcellulose (Sarkar 1979). Lyophilisation was undertaken in a laboratory scale freeze-drier (Virtis) using a controlled cycle (24 h).

After completion of the cycle, identically sized wafers were placed on the flat surface of a gelatine medium (4% w/v) contained in individual petri dishes. The transparent medium permitted the transmission of incident light and the edge of the wafers cast a sharp shadow onto the surface of an accurately ruled card placed below. It was possible to gauge the diameter of the discs to within $\pm$ 0.25 mm. The diameters were measured as a function of time and the 'creep ratio' obtained by division with the original unswollen diameter.

Table 1 Creep ratio of wafers as a function of time Time Methylcellulose content (%)

Time	Methylcellulose content (%)					
(h)	0	10	20	30	40	50
0.0	1.00	1.00	1.00	1.00	1.00	1.00
1.5	1.08	1.07	1.03	1.02	1.03	1.03
3.0	1.15	1.13	1.06	1.05	1.05	1.03
4.0	1.19	1.17	1.13	1.06	1.07	1.06
1.23	1.21	1.17	1.10	1.10	1.08	
1.25	1.23	1.19	1.11	1.11	1.10	
24.0	1.57	1.44	1.40	1.39	1.34	1.24
48.0	1.78	1.64	1.55	1.51	1.44	1.33
72.0	1.94	1.81	1.70	1.60	1.51	1.38

From the results in Table 1, the relative creep rates of wafers could be compared. Increased methylcellulose content decreased the swelling rate while producing higher gel viscosities. Consequently, the gels flowed outwards at decreasing rates. The dye front preceded the edge of the swollen wafer in all cases.

Sarkar, N. (1979) *J. Appl. Pol. Sci.* **24**: 1073–1087

054
The release of gentamicin sulfate from acrylic bone cement

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Acrylic bone cements (ABC) have been used successfully for the fixation of prosthetic components in orthopaedic surgery. Post-operative infection cannot be adequately controlled by parenterally administered antibiotics alone. Gentamicin sulfate (GSO_4), an aminoglycoside antibiotic, when added directly to ABCs, was shown to provide considerably reduced post-operative infection rates. Antibiotic

concentrations at the operative site were found to be higher than could be achieved by parenteral administration alone and with systemic levels well below those that might give rise to toxic side-affects associated with this class of antibiotic.

However, it is well recognised, that the amount of antibiotic released in-vitro tends to be both low and of limited duration, due to the low diffusivity of ABCs (Bayston & Milner 1982).

The aim of this study was to investigate the influence of the initial loading of antibiotic in ABCs and of a channelling agent upon the amount of antibiotic released in-vitro.

Table 1 Cement powder formulation

	CMW^{1RO} (g)	GSO_4 (g)	NaCl (g)
A	37.64	2.00	0
B	35.28	5.00	0
C	39.77	2.11	1.01
D	38.03	2.02	3.02

ABCs (CMW^{1RO}, DePuy) were prepared containing different amounts of GSO_4 either with or without added channelling agent (NaCl) (Table 1). Ten test specimens (75 mm × 15 mm), of different known thicknesses, were prepared from each cement formulation. The specimens were incubated at 37°C in PBS, under sink conditions, in a shaking water bath and GSO_4 release was monitored by HPLC for up to 49 days.

Table 2 Cumulative amount of GSO_4 released

	Amount released (mg (± s.d.))			
	1 h	12 h	96 h	49 days
A	0.81 (0.10)	0.87 (0.10)	0.75 (0.08)	–
B	2.97 (0.26)	3.48 (0.28)	3.12 (0.27)	–
C	0.95 (0.17)	1.21 (0.21)	1.52 (0.21)	2.37 (0.50)
D	0.69 (0.08)	1.08 (0.09)	1.72 (0.16)	3.43 (0.51)

The majority of the GSO_4 released from the cements containing only GSO_4 (A and B), was found in the eluate after one hour (Table 2). Thereafter, no measurable, additional release was observed. These findings suggest that the release of GSO_4 from ABCs can be said to be an essentially surface phenomenon. The addition of salt to the cement formulation dramatically altered the amount and duration of release of GSO_4. The reduced amount of GSO_4 released initially (first 12 h) from cement D (3 g NaCl) was less than that from cement C (1 g NaCl) which may be attributed to a proportionally lower surface concentration of GSO_4 in cement D relative to that of cement C. The addition of NaCl to the cement not only increased the amount of GSO_4 released but also extended the duration of discernable release.

Bayston, R., Milner, R. D. G. (1982) *JBJS* **64-B:** 460–464

055

Aqueous soluble dendrimers with shape-persistent hydrophobic cores for drug solubilisation

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Dendrimers are a class of polymers in which the monomer units branch out from a central core molecule in concentric tiers to form spherical monodisperse macromolecules (Tomalia & Naylor 1990). In recent years, there has been much interest in applying this type of compound to the field of drug delivery/solubilisation. Drug moieties can be conjugated to the exterior (Milhem & Mobedi 2001), or the internal cavities of the dendrimer can be used for the inclusion of drugs (Liu & Kono 1999).

Previous studies of dendrimers as inclusion compounds have focused on species with flexible linkages, which are susceptible to conformational collapse in solution. In this study, a novel series of first and second generation dendrimers based on highly rigid polyphenylenes have been prepared. The accessible hydrophobic cores enable a good affinity for many aromatic drugs. With the addition of solubilising groups to the exterior of the macromolecule, it is anticipated that they will resemble covalent micelles.

These shape-persistent dendrimers were assembled using the highly efficient Suzuki coupling reaction (Miller & Neenan 1992). Structural elucidation via NMR spectroscopy indicated the formation of desired dendrimers with terminal moieties suitable for the facile attachment of solubilising polymer chains. Mass spectrometry indicated the formation of dendrimers with molecular masses in the range of 942–2928.

In conclusion, the encapsulating and solubilising characteristics suggest these dendrimers could be employed as effective drug delivery/solubilising agents.

Liu, M., Kono, K. (1999) *J. Polym. Sci: Part A: Polym. Chem.* **37**: 3492–3503
Milhem, O. M., Mobedi, H. (2001) *Polym. Mater.: Sci. Eng.* **84**: 721–723
Miller, T. M., Neenan, T. X. (1992) *J. Am. Chem. Soc.* **114:** 1018–1025
Tomalia, D. A., Naylor, A. M. (1990) *Angew. Chem.* **102:** 119–157