Poster Session 3 – Material Science

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DNA binding with aza-cyclic derivatives

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DNA binding is a pre-requisite for all DNA delivery agents and while the aza-cyclic molecule hexacyclen (1,4,7,10,13,16-hexaazacyclooctadecane) does not bind DNA, cross linked nanoparticles and amphiphilic derivatives have been found to successfully bind DNA. The wide spread use of gene therapeutics has been stalled by delivery obstacles, as therapeutic genes cannot be delivered successfully into the human body, owing to the generation of immune responses and the presence of intracellular barriers (Brown et al 2001). In this work amphiphilic and cross linked derivatives of hexacyclen were synthesised by reaction with 1-bromohexadecane to yield cetyl hexacyclen (cHex), 1,16-dibromohexadecane to yield a cetyl hexacyclen bolaamphiphile (cBo) and cross linked with 1,8-dibromooctane to yield cross linked nanoparticles (CL). Structures were confirmed by ^{1}H, ^{13}C, 2D-COSY NMR and mass spectrometry. Self-assembly was assessed in the presence and absence of cholesterol by monitoring methyl orange hypsochromic shifts and electron microscopy studies; while DNA binding was assessed by measuring the exclusion of ethidium bromide from the collapsed DNA molecule. Biological characterisation involved a study of the haemocompatibility as assessed using a red blood cell lysis assay, as well as a study of the compound's cytotoxicity against the A431 cell line using the MTT assay. The structure of the compounds was confirmed by NMR and the cross linked compound had a hexacyclen, octane ratio of 13:10. Table 1 reveals that in aqueous media, the hexacyclen derivatives were found to self-aggregate into 100–400 nm structures. Electron micrographs showed that in the presence of cholesterol, bilayer vesicles were produced from cHex and dense nanoparticles were produced from cHex alone, cBo alone and CL alone. The conversion from the conventional amphiphile to the bolaamphiphile reduced surface activity, due to an increase in the proportion of hydrophilic areas in the molecule (Table 1). An examination of the minimum N:P ratio needed for DNA condensation revealed that converting hexacyclen to an amphiphile (cHex or cBo) or to a macromolecule (CL) was a key factor in promoting DNA binding. It is clear that cooperativity between the different amine units is required for DNA binding and that the amine units have to make simultaneous multiple contacts with the DNA molecule for DNA to condense into colloidal units that may be delivered to cells. This cooperativity is either obtained by the hydrophobic associations between individual amphiphiles within the vesicles/nanoparticles (cHex-cholesterol, cHex or cBo) or from the covalent attachment of one amine molecule to another (CL). The vesicular assembly produced a more efficient DNA binding unit than the nanoparticle (Table 1), due to the presentation of a greater number of hexacyclen head groups on the particle surface as the particle moved from a dense structure (cHex alone) to a vesicle (shell-like) structure (cHex, cholesterol). Unfortunately, all compounds proved to be more cytotoxic and haemotoxic than hexacyclen alone and were unable to transfer genes to A431 cells.

Table 1 New Hexacyclen derivatives

Sample	Critical aggregation concn ($mg\,mL^{-1}$)	Aggregate particle size (nm)	Hexacyclen, DNA ratio for DNA condensation (N:P)	% Haemolysis ($0.1\,mg\,mL^{-1}$)	IC50 ($\mu g\,mL^{-1}$)
Hexacyclen	N/A	139	No DNA binding	1	255.8
cHex	0.05	99	83	50	5.4
cHex/chol (2:1)	0.05	241	10	40	5.7
cBo	0.50	133	20	85	7.2
CL	0.05	422	22	76	8.4

Brown, M. D., Schatzlein, A. G., Uchegbu, I. F. (2001) *Int. J. Pharm.* **229**: 1–21

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Investigation of protein adherence to various surfaces via the quartz crystal microbalance

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Upon implantation in the body, many biomaterials in contact with blood or living tissue may trigger adverse responses such as thrombosis, inflammation and device-associated infections (Kinshott et al 1999). For this reason, poor protein adhesion is vital for devices such as catheters and contact lenses. Some situations also arise, as is the case with bone implants, whereby complete integration of the implant with the body is essential and hence strong protein adhesion is necessary to achieve a compact implant–bone interface (Marxer et al 2003). As a result of this, various techniques have been involved in the surface modification of polymeric implants to regulate cellular and protein adhesion (Schierholz et al 2002). Recently, the use of the quartz crystal microbalance (QCM) as an analytical technique has gained attention. The variations in the oscillation frequency of a thin quartz disk, associated with changes in adsorbed mass upon the exposed surface, can be used to investigate many important physical and chemical processes including the adsorption of proteins. In this study, the QCM was used to investigate the adsorption of bovine serum albumin (BSA) onto both gold and polyurethane-coated 5 MHz sensor crystal surfaces in addition to investigating the effects of pre-exposing these surfaces to a range of varying molecular weight poly (vinyl pyrollidone) (PVP) molecules before exposure to the BSA solution. BSA solutions (0.025%) were prepared in phosphate-buffered saline (PBS) while 0.1% solutions of PVP (10 000 Da, 24 000 Da , 40 000 Da and 360 000 Da) were also prepared in PBS. A 5% solution of polyurethane in dioxane was prepared for coating of the sensor crystal, which was achieved via spin coating. After finding the resonant frequency of the sensor crystal in PBS, each solution under examination was allowed to flow into the measurement chamber where measurements where then made under static conditions. Table 1 shows the maximum frequency shifts and dissipation values achieved after an equilibrium state was reached and loosely bound molecules had been removed via washing through the measurement chamber with PBS. Although a range of molecular weight PVP's were initially investigated only the results of albumin adhesion to the 360 000 Da PVP-modified surfaces are shown here as the lower molecular weight molecules were shown to be largely removed from the crystal during the washing procedure. The adsorption of BSA onto gold resulted in the lowest shift in resonant frequency indicating the higher affinity of BSA for the coated surfaces. Almost a 4-fold increase in BSA adsorption was observed for the polyurethane-coated surface with the dissipation factor also being substantially increased, indicating the formation of a more viscoelastic film on the surface of the crystal, while PVP adsorption onto this surface before BSA exposure served to minimise BSA adsorption as indicated by a less substantial decrease in the resonant frequency.

Table 1 Maximum frequency shifts and dissipation values obtained via QCM on various surfaces

Exposed surface	Max. frequency shift (Hz)	Max. dissipation ($\times 10^{-6}$)
Gold	-3.467 ± 0.153	0.215 ± 0.062
PVP 360 000 Da on gold	-8.017 ± 0.051	0.362 ± 0.034
PVP 360 000 Da on polyurethane	-10.663 ± 0.035	0.412 ± 0.009
Polyurethane	-13.140 ± 0.271	0.705 ± 0.125

Kinshott, P., et al (1999) *Curr. Opin. Solid State Materials Sci.* **4**: 403–412
Marxer, C. G., et al (2003) *J. Colloid Interface Sci.* **261**: 291–298
Schierholz, J. M., et al (2002) *Int. J. Microb. Agents* **19**: 511–516

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The use of macroporous hydrogels containing gentamicin, as a potential coating for the endotracheal (ET) tube, to prevent ventilator-associated pneumonia (VAP)

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Microbial biofilm on the endotracheal (ET) tube surface provides a reservoir of opportunistic microorganisms and has been implicated in the development of ventilator-associated pneumonia (VAP) (Adair et al 1999). The difficulty in

eradicating established microbial biofilm using parenteral antibiotics has led to the assessment of nebulised gentamicin as a preventative strategy for VAP with effective results (Adair et al 2002). However in an effort to enhance the attachment of gentamicin to the surface of the ET tube, this study develops a macroporous hydrogel system loaded with gentamicin, to potentially coat the ET tube to reduce bacterial colonisation and prevent VAP. A series of macroporous poly(2-hydroxyethylmethacrylate) (p(HEMA) hydrogels were prepared using aqueous sodium chloride solutions of various concentrations (0.1, 0.3, 0.5 and 0.7 M) as diluents in the polymerisation medium. The hydrogels were loaded with drug by either soaking in a buffered gentamicin solution (Tris pH 7.4, 50 mg mL^{-1} gentamicin sulphate) after polymerisation or gentamicin was incorporated into the diluent before polymerisation. To quantify the mass of gentamicin that had been incorporated into each of the hydrogels, the drug was extracted five times into a buffered solution (Tris pH 7.4). The mass of gentamicin in the various extracts was quantified using HPLC. Tensile properties were analysed using an SMS TA-XT^2 Texture Analyser, a technique that allows ultimate tensile strength (UTS), Young's modulus and strain at failure to be calculated (Jones et al 2002). The results demonstrated that greater gentamicin loading was obtained with drug incorporated before polymerisation as opposed to soaking the hydrogels in a buffered gentamicin solution after polymerisation (Table 1). The ultimate tensile strength (UTS) and Young's modulus decreased with increasing NaCl concentration in the polymerisation medium, which was attributed to the onset of pore interconnectivity, as confirmed by SEM micrographs. The study has illustrated the ability of macroporous hydrogel matrices to facilitate the absorption and release of gentamicin. The use of such coating may further allow entrapment of gentamicin following nebulisation in-situ. This strategy shows initial promise for the prevention of VAP.

Table 1 Gentamicin loading and physicochemical properties of various macroporous p(HEMA) polymers

Diluent NaCl concn	Drug loading Incorp. before curing	(μg) Soaking post curing	UTS (MPa)	Young's modulus (MPa)
0.1 M	397 ± 14	106 ± 13	0.430 ± 0.083	0.643 ± 0.031
0.3 M	547 ± 28	96 ± 28	0.332 ± 0.040	0.602 ± 0.029
0.5 M	474 ± 31	265 ± 24	0.071 ± 0.006	0.230 ± 0.020
0.6 M	736 ± 52	271 ± 53	0.049 ± 0.007	0.214 ± 0.052
0.7 M	602 ± 57	163 ± 57	0.036 ± 0.005	0.250 ± 0.044

Adair, C. G., Gorman, S. P., Feron, F. B., et al (1999) *Intensive Care Med.* **25**: 1072–1076
Adair, C. G., Gorman, S. P., Byers, L. M., et al (2002) *Intensive Care Med.* **28**: 426–431
Jones, D. S., et al (2002) *Biomaterials* **23**: 4449–4458

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Characterisation of protein-containing binary polymeric gel systems designed for the treatment of periodontal disease

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Periodontal diseases comprise a variety of conditions affecting the health of the periodontium including gingivitis and periodontitis (Eber et al 2002). Periodontal disease is an inflammatory condition that results in the generation of a periodontal pocket and in severe cases alveolar bone destruction and tooth loss. It is well documented that localised mucoadhesive gel drug delivery systems designed for insertion into the periodontal pocket are advantageous in many ways over traditional therapies, most notably because of the localised release of antibiotics in a controlled fashion (Jones et al 2000). While most gel systems are designed to deliver agents that will alter the local microenvironment to decrease bacterial load and stabilise the disease process, there is also significant potential for alternative treatment strategies to deliver recombinant growth factors for periodontal regeneration (Cochran et al 2000). Although there are many antibiotic-containing gel systems possessing ideal mechanical and rheological properties to aid easy insertion/retention within the periodontal pocket there are few reports describing the use of protein containing gel systems and the effect such large molecules may have on the rheological and mechanical properties. This study therefore examined the effect of a model protein, bovine serum albumin (BSA) on the rheological and mechanical gel properties. Gel formulations were manufactured from PVME/MA (15% w/w) and PVP (5, 10 & 15% w/w) with BSA (0.1, 1, 2 & 5% w/w) dispersed in the system. Flow and oscillatory rheology was performed using a TA AR2000 rotational rheometer with 2 and 4 cm diameter parallel plate geometry and a 1 mm plate gap. Texture analysis of all formulations was performed as previously described, Jones et al (1997). The results are shown in Table 1. Increasing the concentration of PVP and BSA resulted in an increase in the rheological (zero-rate viscosity, consistency, G', G'', & η') and textural (hardness, adhesiveness and compressibility) properties of the formulations. As the concentration of both PVP and BSA was increased, the number of large polymer chains in solution increased and hence the network structure of the system was enhanced. This increased network structuring increased resistance to viscous flow whereas increased chain connectivity increased system elasticity. This study illustrated that through the use of simple formulation changes a diverse range of rheological and textural properties were achieved by varying the concentration of PVP and BSA. In addition we have highlighted the potential of gel systems both rheologically and mechanically for the local delivery of large protein molecules for use in periodontal regeneration. The delivery of recombinant growth factors for the treatment of periodontal regeneration is becoming another viable method in the treatment of periodontal diseases. This investigation provides fundamental information concerning the use of bioadhesive gel systems to deliver proteins to the periodontal pocket and the effect such large molecules may have on product performance.

Table 1 Consistency, hardness & storage modulus for six gel systems

PVP (% w/w)	BSA (% w/w)	Hardness (N)[a]	Compressibility (N s)[a]	G' at 10 Hz[a] (Pa)
5	0.1	0.66 ± 0.03	0.55 ± 0.02	1583.08 ± 25.67
5	5	1.07 ± 0.03	0.82 ± 0.02	1722.28 ± 41.95
10	0.1	1.58 ± 0.02	1.28 ± 0.14	2695.01 ± 129.71
10	5	2.22 ± 0.15	1.48 ± 0.24	3992.00 ± 80.98
15	0.1	2.72 ± 0.04	2.16 ± 0.03	5209.45 ± 155.63
15	5	4.77 ± 0.16	3.74 ± 0.11	6930.00 ± 216.33

[a]Mean ± s.d. of five replicate measurements.

Cochran, D. L., et al (2000) *J. Periodontol.* **71**: 1241–1257
Eber, R. O., et al (2002) *J. Clin Periodontol.* **29**: 400–410
Jones, D. S., et al. (1997) *Int. J. Pharm* **151**: 223–233
Jones, D. S., et al (2000) *J. Controlled Release* **67**: 357–368

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Characterisation of binary, polymeric gel systems containing metronidazole for the treatment of periodontitis

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Periodontitis is clinically characterised by both the loss of attachment and inflammation of the gum, resulting in the formation of a periodontal pocket. As the disease progresses, there is subsequent destruction of the periodontal tissues, increasing the mobility of the tooth and reducing the functionality. If left untreated, the support structures degenerate to a point were repair is not possible and tooth loss results (Jeffcoat 1994). Consequently, the purpose of any treatment is to arrest the progression of the disease by means of reducing the inflammation of surrounding tissue, decrease the number of pathogenic bacteria and eliminate the depth of the periodontal pocket. Injectable gel systems are highly desirable as treatment strategies due to their ease of application, reduced therapy costs and ultimately the ability of these systems to fill the periodontal pocket and reach a large proportion of pathogens (Esposito et al 1996). In this study, gel systems loaded with metronidazole have been rheologically and mechanically characterised for their potential use in the treatment of periodontitis. The neutralised gel formulations were composed of PVME/MA (10 & 15% w/w) and PVP (10 & 15% w/w) with metronidazole (10% w/w) dispersed in the system. Flow rheology was performed using a Carri-Med CSL2 – 100 rheometer with 2 cm diameter parallel plate geometry and a 1 mm plate gap. Texture analysis of all formulations was performed as previously described, Jones et al (1997); results are shown in Table 1. The effect of PVME/MA and PVP concentration on the textural and rheological properties were statistically evaluated using a two way analysis of variance ($P < 0.05$ denoting significance). Increasing the concentration of PVME/MA and PVP resulted in an increase in the rheological (consistency) and textural (hardness, adhesiveness and compressibility) properties of the formulations. As the concentration of each polymer was increased, the number of polymer chains in solution increased and hence the network structure of the system is increased,

which offers an increased resistance to flow, and deformation during TPA. Formulations with wide ranging rheological and textural properties were achieved by varying the concentration of each polymer. As a result optimum gel formulations can be determined to fulfil the rheological and textural requirements for insertion in the periodontal pocket and retention at site. This study has increased the fundamental understanding of the properties required to optimise the clinical performance of these systems for the management of periodontitis.

Table 1 Consistency, hardness & compressibility for 10% w/w metronidazole

PVME/MA (% w/w)	PVP (% w/w)	Hardness (N)[a]	Compressibility (N mm)[a]	Consist (KPa s)[a]
10	10	2.65 ± 0.12	2.39 ± 0.26	1.55 ± 0.08
10	15	4.71 ± 0.18	4.17 ± 0.24	3.17 ± 0.15
15	10	5.98 ± 0.56	5.29 ± 0.50	7.43 ± 0.35
15	15	9.28 ± 0.08	8.24 ± 0.29	13.37 ± 1.05

[a]Mean ± s.d. of five replicate measurements.

Esposito, C. E., et al. (1996) *Int. J. Pharm.* **142**: 9–23
Jeffcoat, M. K. (1994) *Prev. Med.* **23**: 704–708
Jones, D. S., et al (1997) *Int. J. Pharm.* **151**: 223–233

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Rheological characterisation of novel bioadhesive, polymeric gel networks for the treatment of dental hypersentivity

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Dentine sensitivity is a common condition wherein exposure to non-noxious stimulus gives rise to a range of symptoms (Nagata et al 1994). This condition may be a result of exposure of the tooth root surface following periodontal surgery or gingival recession. Formulations designed for the delivery of desensitising agents are most commonly dentifrices and mouthwashes (Uchida et al 1980). Although there have been reports describing the clinical efficacy of mouthwashes and dentifrices, there are few reports describing the use of bioadhesive gels as the desensitising delivery platform. Bioadhesive gel systems offer significant potential, since they would be easy to apply and remove and would increase the residence time on the tooth surface thereby increasing the bioavailability of a desensitising agent. Therefore, the aim of this study is to design novel bioadhesive gel systems for the delivery of strontium chloride, a common desensitising agent. The neutralised gel formulations were composed of PVME/MA (15% w/w), PVP (5 & 10% w/w) and glycerol (5% w/w) with strontium chloride (0 & 5% w/w) dispersed in the system. Continuous shear and oscillatory rheometry was performed using a Carri-Med CSL^2 – 100 rheometer with 4 or 6 cm diameter parallel plate geometry and a 1 mm plate gap. Oscillatory rheometry was performed at a strain of 6.5×10^{-3} and a frequency range of 1–20 Hz. The results are shown in Table 1. The effect of PVP and strontium chloride concentration on the rheological properties were statistically evaluated using a two-way analysis of variance ($P < 0.05$ denoting significance). All formulations were found to exhibit pseudoplastic flow with minimal thixotropy. An increase in the concentration of PVP was found to increase all rheological (consistency, storage modulus, loss modulus and dynamic viscosity) characteristics with the loss tangent decreasing. The addition of strontium chloride significantly decreased the rheological structure for all formulations. This effect was attributed to electrostatic interaction occurring between the PVME/MA and strontium. With an increase in frequency there was an increase in the storage and loss moduli but a decrease in dynamic viscosity and loss tangent. This response is consistent with the Maxwell model for viscoelastic materials to oscillatory stresses. The increase in rheological characteristics with increase in PVP concentration was ascribed to an increase in polymer chains hence reduction in polymer chain mobility. We can conclude that these formulations offer distinct rheological advantages as treatments for dental hypersensitivity.

Table 1 Consistency, storage (G′) and loss (G″) modulus (frequency 1 Hz) for 15% PVME/MA

PVP (% w/w)	Strontium chloride (% w/w)	Consist (Pa s)[a]	G′ (Pa)[a]	G″ (Pa)[a]
5	0	177.02 ± 9.95	262.80 ± 14.81	348.22 ± 13.88
5	5	42.77 ± 1.95	57.75 ± 2.38	129.92 ± 8.50
10	0	891.91 ± 62.30	1207.32 ± 48.13	1074.56 ± 86.09
10	5	270.38 ± 13.48	414.83 ± 22.64	502.21 ± 28.24

[a]Mean ± s.d. of five replicate measurements.

Nagata, T., et al (1994) *J. Clin. Periodontol.* **21**: 217–221
Uchida, A., et al (1980) *J. Periodontol.* **51**: 578

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Examination of polymer–polymer entanglements at the air–water interface

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Recently an interest has developed in the use of binary polymer networks as platforms for topical formulations due to the unique rheological and mechanical properties of such systems (Jones et al 2002). In the design of polymer networks identification and characterisation of the polymer interactions is required. One method by which the interaction between two polymer components may be characterised is interfacial rheometry although there have been few reports of the use of this technique for this application. Previously we have described the interaction between polymethylvinylether–comaleic anhydride (PVME/MA) and polyvinylpyrrolidone (PVP) in aqueous solution using bulk rheological techniques. It is proposed that this interactive polymer blend may be employed in the formulation of disperse systems in which the polymer–polymer interactions at an interface are important. Therefore, this study describes interfacial interactions between PVME/MA and PVP at a model air/aqueous solution interface. The neutralised dilute polymer solutions were composed of PVME/MA (0.5% w/w) and PVP (0.5, 1 & 3% w/w). Interfacial rheometry was performed using a Camtel CIR–100 Interfacial Rheometer. Samples were placed in a standard glass sample vessel (46 mm i.d.) and a 13 mm Pt/Ir Du Noüy ring used as the measuring geometry. The ring was automatically placed at the liquid/air interface and the surface of the sample was subjected to a sinusoidal change of shape for a period of 30 min at a frequency of 3 Hz, strain amplitude 5000 mrads and at 20°C. The effect of time, concentration of PVME/MA and PVP on the surface elasticity and surface viscosity were statistically examined using a three-way analysis of variance ($P < 0.05$ denoting significance, Table 1). In the presence of PVME/MA or PVP the surface viscosity and elasticity of the interface significantly increased, with PVME/MA having a more pronounced effect on these parameters than PVP. Both the surface viscosity and elasticity for all formulations were found to be independent of time. Interestingly a rheological interaction between the two polymers was observed at the interface, as denoted by rheological synergy. This infers that an interaction between the polymers present at the interface occurred, thereby resulting in the formation of an elastic interface. Furthermore, by manipulation of the concentrations of the two polymers, the rheological properties of the interface may be successfully engineered. This study has therefore highlighted the substantial interaction between two hydrophilic polymers at an air/water interface. It is suggested that the surface elasticity and viscosity of the polymeric interfaces offer particular promise for the formulation of stable, bioadhesive disperse systems as drug delivery platforms.

Table 1 Surface elasticity and surface viscosity of polymeric systems (15 min)

PMVE/MA (% w/w)	PVP (% w/w)	Surface elasticity (uN m^{-1})[a]	Surface viscosity (uN s m^{-1})[a]
0.5	0.80	44 ± 5.84	110.85 ± 7.30
0	0.5	3.09 ± 0.18	2.34 ± 0.22
0.5	0.5	244.11 ± 20.88	244.85 ± 18.26
0	1	31.98 ± 2.73	15.43 ± 1.32
0.5	1	374.95 ± 31.91	282.73 ± 22.44
0	3	113.68 ± 10.25	41.53 ± 3.63
0.5	3	1258.09 ± 82.90	427.94 ± 29.75

[a]Mean ± s.d. of five replicate measurements.

Jones, D. S. et al (2002) *J. Pharm. Sci.* **97**: 2090–2101