Poster Session 2 – Material Science

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Trimethylation of chitosans, its effect on toxicity and their application as a non-viral gene delivery system

T. Kean, S. Roth and M. Thanou

Centre for Polymer Therapeutics, Welsh School of Pharmacy, Cardiff University, Cardiff, UK, CF10 3XF, UK. E-mail: sphtjk@cf.ac.uk

Chitosans show great potential as non-viral gene delivery vectors due to their reported low toxicity (Thanou & Junginger 2004). However, their insolubility at physiological/alkaline pH presents a problem in the formulation of polyplexes with pDNA. Chitosan quaternisation, through trimethylation, provides a solution to this problem by increasing the pH range over which the derivative is soluble. It has the added benefit of presenting permanent positive charges which enable pDNA to be more effectively complexed (Thanou et al 2002). The effect of the degree of quaternisation on cytotoxicity has not been tested. Chitosan oligomer (3–6 kDa, 70% deacetylated) and low molecular weight chitosan (100 kDa, 97% deacetylated) were quaternised according to a previously described method (Thanou et al 2002). Investigation into the cytotoxicity of the derivatives was performed on COS-7 (monkey kidney fibroblast cell line) and MCF-7 (human breast cancer epithelial cell line) cell lines using the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) cytotoxicity assay (Sgouras & Duncan 1990). Derivatives were applied in the concentration range of 20–10 000 $\mu g\,mL^{-1}$. Complexes were formed between the chitosan derivatives and pGL3 luc, these were analysed by ethidium bromide agarose gel electrophoresis and used in transfection experiments. Quaternisation of both chitosans was achieved giving chitosan oligomer at 20%, 44%, 55% and 94% quaternisation (Trimethyl chitosan oligomers, (TMO)) and low molecular weight chitosan at 36%, 57%, 76% and 93% quaternisation (Trimethyl low molecular weight chitosans, (TMC)). IC50 values of oligomeric chitosan derivatives at 6 h on COS-7 cells were $> 2000\,\mu g\,mL^{-1}$ for all degrees of trimethylation (20 fold higher than transfection concentration). IC50 values for low molecular weight chitosan derivatives at 6 h on COS-7 cells were $> 10\,000$, 207.3 ± 62.7, 36.2 ± 2.85, $78.75 \pm 16.7\,\mu g\,mL^{-1}$ for 36%, 57%, 76% and 93% quaternisation, respectively. Increased incubation time augmented the toxicity of the derivatives. All chitosans retarded pDNA electrophoretic mobility on agarose gel. Transfection of MCF-7 cells with all derivatives at a 10:1 (polymer: DNA w/w ratio) resulted in TMO 44% and TMC 93% giving the greatest transfection efficiencies. Controllable quaternisation of chitosan is achievable in a time dependant reaction. Toxicity increases with increasing degree of quaternisation for both oligomers and polymers. All chitosans complexed DNA efficiently. It appears that transfection efficiency on breast cancer cells is influenced by both molecular weight and percentage of quaternisation. The effective concentration used in transfection is markedly lower than the IC50 for TMOs, and lower to approximately equivalent in TMCs.

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Curing properties and streptomycin release from bone cement materials

D. A. Raval and D. N. Parikh

Industrial Chemistry Department, ISTAR, Sophisticated Instrumentation Center For Applied Research & Testing, V. V. Nagar-388120, Anand, Gujarat, India. E-mail: deardipeshparikh@rediffmail.com

Reactive functionalized copolymers find widespread application as active materials based on their specific functional groups. Much interest has been generated in biomedical field in the utilization of such copolymers in development of polymeric bone cements and consequently improving compatibility as they promote the sustained release of antimicrobial agents from the bone cements via formation of drug–polymer conjugate system (Islas et al 2001). This study reports the effect of change in monomer ratio on curing properties and streptomycin release from bone cements prepared from functionalized methacrylates. A novel formulation procedure involving methamethacrylate (MMA), maleic anhydride (MA), benzoyl peroxide and N,N-dimethylaniline was used, which induced rapid polymerization and resulted into uniform dispersion of streptomycin throughout the polymeric strip. Streptomycin sulfate was incorporated in the strip at a fixed concentration of 2% w/w. The curing parameters of the resulting bone cements were evaluated with respect to change in monomer ratio and surrounding temperature during polymerization. The results of this study demonstrated that concentration of MA and surrounding temperature has a controlling effect on peak exotherm and curing time during polymerization. The curing time decreases and peak exotherm increases with increase in surrounding temperature and decrease in MA content. The hardness of the strip increases linearly with increase in MA content up to a certain extent. At a concentration higher than, and including, 40 mol % of MA, significant reduction in hardness was observed indicating limiting concentration for functionalized methacrylates based on MA. Streptomycin release was studied by placing 1 square inch of the strip in 100 mL of simulated gastric fluid (pH 1.2) in a stoppered flask in a shaking water bath (37°C, 100 oscillations min^{-1}) for 1 week. The amount of streptomycin released as a function of time was determined using spectrophotometer at 540 nm (Raval et al 1997). A sustained release profile for streptomycin was observed at low MA concentration in bone cements. Increasing concentration of MA in bone cement results in higher water absorbing capability leading to lower harness and rapid elution of streptomycin.

Table 1 Curing properties of bone cements prepared from functionalized mathacrylates

Sr	Feed ratio (mol)		Surrounding temp.							
			25°C		30°C		35°C		40°C	
	MMA	MA	Tm	tc	Tm	tc	Tm	tc	Tm	tc
1	0.09	0.01	65	20.2	70	14.6	72	11.4	79	08.2
2	0.08	0.02	70	12.4	74	11.8	80	07.6	82	05.2
3	0.07	0.03	72	10.7	78	07.3	82	05.3	83	04.4
4	0.06	0.04	73	07.5	82	05.5	86	04.1	86	03.6

Tm, Peak exotherm (°C); tc, curing time (min).

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Characterization of theophylline and nitrofurantoin adducts

A. Ademoye, S. R. Begum and P. J. Cox

School of Pharmacy, The Robert Gordon University, Schoolhill, Aberdeen, AB10 1FR, UK. E-mail: p.j.cox@rgu.ac.uk

High throughput crystallization techniques have been applied to the pharmaceutical discovery process (Morissette et al 2004) and medicinal substances can exist in many solid forms. This results in large numbers of polymorphs, solvates, hydrates, salts, co-crystals and amorphous solids that have to be examined. The optimum characterization technique used to analyse the crystalline solid state is X-ray crystallography. Hopefully, when suitable crystals are available the technique can be applied with great success but problems can occur. We have recently examined some adducts of theophylline and nitrofurantoin and present the preliminary results here. Prior to X-ray investigations, identification methods used on the samples included simple melting points, microscopic examination, FTIR, DSC and density determinations. Single crystal X-ray data, collected on a Bruker-Nonius KappaCCD diffractometer, were used to determine previously unknown crystal structures of four adducts. Co-crystals formed by theophylline and salicylic acid (1:1) have a very thin needle morphology and were reported by previous workers (Madarasz et al 2002) to be unsuitable for single crystal diffraction. After slow recrystallization from water we managed to grow a suitable crystal — albeit small — for data collection. The weakly diffracting crystal required a 7-fold increase in normal beam exposure time to obtain suitable diffraction intensity. Structure solution and refinement of the co-crystal was without further problems. Extensive hydrogen bonding, which stabilises the crystal structure, was characterized. Crystals of the theophylline:DMSO solvate (1:1) were twinned. Following the application of suitable software an X-ray data set was extracted for successful structure solution and refinement. Hydrogen bonding between drug and solvent involves N-H..O and C-H…O bonds. Crystals of the nitrofurantoin:DMSO (2:1) solvate contain solvent molecules disordered across a twofold axis corresponding to two confacial pyramidal orientations having common oxygen and carbon sites on a shared face. The DMSO molecule is linked to two nitrofurantoin molecules by the oxygen of the solvent acting as the acceptor in the formation of two N-H…O bonds. X-ray data for the nitrofurantoin:dimethylacetamide (1:2) co-crystal was originally collected in the space group $P2_1/a$ and then transformed to $P2_1/c$. One of the two DMA molecules in the asymmetric unit is disordered. This disordered molecule is linked to nitrofurantoin by an N-H…O hydrogen bond whereas the normal DMA molecule is only held through weak C-H…O bonding.

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An investigation into caking

D. W. Barrett and G. S. Webber

World Wide Physical Properties, Pharmaceutical Development, GlaxoSmithKline, New Frontiers Science Park, Harlow, Essex, CM19 5AW, UK.
E-mail: dave_w_barrett@gsk.com or greg_s_webber@gsk.com

The caking of pharmaceutical powders is not an uncommon phenomenon but is one that can have a significant impact, and can lead to an extra processing step to de-lump the material before it can be used. In this case study, a powder produced in Europe is transported to America for processing. By the time the material arrives it has caked. An investigation was undertaken to determine the cause of the caking and identify possible solutions to the problem. Two possible causes of the problem were considered: firstly, consolidation of the powder, which was investigated by tapping, using bulk and tapped density analysis; secondly, the effect of temperature and humidity, which was investigated in two parts — storage in a controlled environment followed by an assessment of cake hardness and also gravimetric vapour sorption at a range of different temperatures and humidities. Analysis showed that consolidation was not the main cause of the caking in this instance. Storage at elevated humidity conditions (75% relative humidity) for up to 5 weeks showed the formation of a cake with increasing hardness with time. This timescale was similar to the time between production and the use of the material. Gravimetric vapour sorption studies were performed using a Dynamic Vapour Sorption instrument. These showed low moisture uptake at ambient temperatures (25 and 30°C), but a significant increase in the uptake at elevated temperatures (35–50°C) and elevated humidities (greater than 60% RH). When the manufacturing process for the material was reviewed it was found that the final step was drying and collection. The material was discharged while still warm and in an uncontrolled humidity environment. This raised concerns about possible condensation as the material cools to ambient temperature in the final container. Standard psychometric charts were interrogated to assess the impact of the atmospheric conditions. It was found that if the material was discharged at 40°C and 60% relative humidity and sealed in a container, by the time the material had cooled to 30°C, the ambient humidity in the container would be close to 100%. This indicates that condensation was indeed possible, even likely. This investigation showed that the most probable cause of the caking was moisture related and was a result of the drying and collection step of the manufacturing process. Thus it was recommended that the material from the final manufacturing step should be collected at less than 30°C and less than 60% relative humidity.

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The phase behaviour of xanthan gum and sodium alginate solutions and the rheological effects of transition across the phase boundary

M. J. Boyd, J. R. Mitchell*, F. C. Hampson†, I. G. Jolliffe‡, P. W. Dettmar† and C. D. Melia

School of Pharmacy, University of Nottingham, University Park, Nottingham, NG7 2RD, *Division of Food Sciences, University of Nottingham, Sutton Bonington Campus, Sutton Bonington, Loughborough, Leics, LE12 5RD, †Technostics Ltd, The Deep Business Centre, Kingston-upon-Hull, East Yorkshire, HU1 4BG and ‡Reckitt Benckiser Healthcare (UK) Ltd, Dansom Lane, Hull, HU8 7DS, UK.
E-mail: colin.melia@nottingham.ac.uk

Xanthan gum and sodium alginate are widely used as suspending agents and anti-reflux agents. This study investigates mixtures of these polysaccharides, their aqueous phase behaviour and the viscosity changes that occur on dilution across a phase boundary. Mixtures were prepared from solutions of xanthan gum (Rhodigel 80; Caldic UK Ltd, Chesterfield, UK) and low viscosity sodium alginate (Protanal LFR 5/60; FMC Biopolymer Drammen, Norway) by high speed dispersion in deionised water. Viscosity and dynamic oscillatory rheology was undertaken using a Bohlin CVOR rheometer (25 ± 0.1°C, viscosity 0.01–1000 s^{-1}, delay = 30 s, integration = 30 s, oscillation strain value within the linear viscoelastic region, F = 0.1–3 Hz) and the viscosity change on dilution using a Newport Scientific Rapid Visco-analyzer (RVA) at 25 ± 0.5°C, using 30-g samples (undiluted) or 15-g samples diluted with 15 g of water or Simulated Gastric Fluid (No Enzymes, SGF) USP. 1% Xanthan solution showed pseudoplastic behaviour with high viscosity at low shear rate ($\eta = 448$ Pa s, 0.01 s^{-1}). Sodium alginate 5% solution showed low viscosity (0.08 Pa s, 1 s^{-1}) and near Newtonian behaviour. A mixed system containing 1% xanthan with 5% alginate showed intermediate viscosity ($\eta = 12$ Pa s, 0.01 s^{-1}) and pseudoplastic behaviour. However, when studied using dynamic oscillatory rheology, 1% xanthan exhibited weak gel-like properties ($G' > G''$) whereas the properties of the mixture were indicative of a low viscosity liquid. Microscopy revealed that this phenomenon was a result of phase separation. A phase diagram was constructed over a range of polymer concentrations and the consequences of dilution over the phase boundary investigated. A typical example is the phase separated mixture 1% xanthan 2% alginate, chosen close to the phase boundary (Table 1). Dilution 50% with water or SGF resulted in a viscosity increase and dynamic rheology showed the return of the weak gel-like properties ($G' > G''$) with SGF having a greater influence. A similar effect was also demonstrated using sodium carboxymethylcellulose, with similar effects on dilution being observed (Table 2). In conclusion, over appropriate concentration ranges, mixtures of these anionic biopolymers visibly phase separate, allowing the highly viscous properties of xanthan to be effectively hidden in a low viscosity continuous phase. Dilution across the phase boundary results in a rapid increase in viscosity, with the return of the weak gel-like properties previously hidden.

Table 1 The RVA viscosity (η at 200 rev min^{-1}) and dynamic moduli (G', G'' at 1.02 Hz) of a separated xanthan/alginate mixture undiluted, and the viscosity 10 min after 50% dilution with either water or SGF

Xanthan 1% and alginate 2%	η (mPa s)	G' (Pa)	G'' (Pa)
Undiluted	88 ± 10	0.87 ± 0.07	0.67 ± 0.03
Diluted to 50% with water	184 ± 6	9.35 ± 0.10	2.94 ± 0.02
Diluted to 50%with SGF	655 ± 19	109.6 ± 5.6	23.4 ± 0.8

Data are means ± s.d, n = 3.

Table 2 The RVA viscosity (η at 200 rev min^{-1}) of a separated xanthan/carboxymethylcellulose mixture undiluted, and the viscosity 10 min after 50% dilution with either water or SGF

Xanthan 1% and sodium carboxymethylcellulose 2%	η (mPa s)
Undiluted	127 ± 8
Diluted to 50% with water	180 ± 5
Diluted to 50% with SGF	199 ± 11

Data are means ± s.d., n = 3.

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Development of a test to measure the impact properties of coated tablets

S. Blackmore, K. Salmon, J. Vogwell, I. G. Turner and S. R. Brown*

Department of Engineering and Applied Science, University of Bath, Bath BA2 7AY and *Pfizer Global Research and Development, Pharmaceutical R&D, Sandwich, Kent CT13 9NJ, UK. E-mail: S.J.Blackmore@bath.ac.uk

Tablet coatings should not fail during processing or subsequent handling as the financial loss of a batch of tablets is significant. It is important that the performance of the coating be assessed and quantified at the development phase to reduce the risk of commercial failures. To achieve this, reliable test methods must be developed to study and measure coating performance. The current methods used to test uncoated tablets are of limited use for assessing the quality and robustness of coated tablets. The implications of 'scale-up' (i.e. from producing development batches to full-scale manufacturing capacities) can be problematic and unpredictable potentially resulting in inferior quality coatings. The development of new testing methods for coated tablets to better predict the effects of scale-up would lead to increased robustness and quality of the coatings finally achieved. The study that has been completed has involved developing a novel impact test to investigate the impact severity on the integrity of a range of tablet coatings. This was achieved using a modified pendulum impact testing technique based on Izod and Charpy methods, where the impacting pendulum head accommodated indenters of different sharpness to vary impact severity. The tablets used were based on a standard placebo formulation, coated with an HPMC-based film coating. Tablets were coated to four specific percentage weight gain (w.g.) values based on the mass of the tablet core. Tablets were impacted at three different locations: on the face centre; the edge of the face; and on the circumference. A ranking system was devised to grade the level of damage caused to tablets ranging from 1 (negligible damage) to 6 (severe damage). Also, micro-hardness values were obtained for each of the four coatings tested, and inspection of coating failures was carried out using scanning electron microscopy (SEM). Typical results are shown in Tables 1 and 2.

The results have identified differences in behaviour at each of the locations tested; the greatest damage occurring at the edge and the circumference. Generally, a sharper indenter was found to cause more localised damage whereas the thicker coatings often showed a greater tendency to chip. Results from the micro-hardness tests showed that tablet hardness increased with coating thickness, confirming the protective nature of the coating. SEM revealed correlations between surface roughness and coating thickness. In addition, the different modes of coating failure observed were found to be dependent of impact severity. In conclusion, the tests indicated that the coating performance differed across the tablet core on impact. It was also apparent that an impact test of the type developed could be used to compare different tablet types to assess their suitability for manufacturing scale-up.

Table 1 Typical impact testing results

Coating Quantity (w.g.)	Indenter sharpness (mm)	Mean damage ranking		
		Face (1)	Edge (2)	Circ. (3)
2.37%	<0.1	2.60 ± 0.55	3.00 ± 1.00	6.00 ± 0.00
	1	2.00 ± 0.00	2.60 ± 0.89	5.40 ± 0.55
	2	1.00 ± 0.00	1.60 ± 0.89	1.60 ± 0.89
	3	0.20 ± 0.45	1.20 ± 0.45	1.80 ± 1.10

Table 2 Vickers microhardness values

Coating quantity (w.g.)	Mean hardness (HV)
1.30%	11.08 ± 1.10
2.37%	12.68 ± 0.60
3.90%	15.00 ± 1.18
6.27%	16.70 ± 1.49

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A comparative evaluation of fish, bovine and porcine gelatins in the formulation of soft gelatin capsules

R. Burke, M. Melligan, A. M. Dyas and J. L. Ford

School of Pharmacy & Chemistry, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK. E-mail: m.a.dyas@livjm.ac.uk

Gelatin is an abundant, cheap and naturally occurring protein obtained by hydrolysis of collagen from animal skin and bones. It is the principal component in mass-produced capsule shells where, in the case of softgels, its properties are modulated by the inclusion of water and of plasticisers such as glycerol. Such mixtures are firstly formed into ribbons that are mechanically robust enough for use in high-speed softgel capsule filling equipment, suitably elastic for filling and have softening characteristics that allow the capsule to rapidly seal after filling. Traditionally, bovine and porcine gelatins have been used but recent concerns over the spread of BSE and vCJD, along with a growing vegetarian market, have led to a search for alternatives to mammalian gelatin. However, the manufacture of softgel capsules is a complex operation that is heavily dependent on thermo-mechanically important properties of the ribbon, which are themselves strongly influenced by the unique physicochemical properties of gelatin, making the search for a suitable substitute quite difficult. Fish gelatin offers a potential solution as it is has the typical properties of gelatin without the safety concerns of mammalian gelatin. Thus, the thermal and mechanical properties of a range of softgel ribbons, based on bovine, porcine or fish gelatin were evaluated using modulated differential scanning calorimetry (DSC), thermomechanical analysis (TMA) and tensiometry to determine the glass transition point (Tg), softening point (Tm) and elasticity (Young's modulus), respectively. The standard mixture used in the preparation of commercial softgel capsules comprises gelatin, water and glycerol in an approximate weight ratio of 40:40:20. Variants of this ratio were prepared and tested, although mixtures containing gelatin outside the range 20–50% w/w were either too rigid to be poured or yielded ribbons that were too fragile to be handled. Data were obtained indicating that increasing the gelatin concentration (at fixed water:glycerol ratio) increased the softening and glass-transition temperatures and decreased elasticity. Conversely, increasing either the glycerol content or the water content decreased both the softening and glass-transition temperatures and increased the elasticity. There was no discernible distinction seen between the effects of increasing the water or glycerol content (i.e. they appeared to act equally well as plasticizers). Comparatively, bovine and porcine gelatin behaved similarly but fish gelatin yielded a more elastic ribbon with a higher Tg/lower Tm. Fish gelatin also gave rise to a Tg > Tm while for mammalian gelatin the opposite situation was seen (i.e. Tg < Tm). These differences may relate to differences in the structure and composition of the gelatin, such as the significantly lower levels of proline/hydroxyproline seen in fish gelatin. However, the data indicate fish gelatin may prove a useful replacement for mammalian gelatin in the manufacture of pharmaceutical softgel capsules.

Table 1 The thermomechanical properties of standard[a] softgel ribbons

Gelatin source	Glass transition Tg (°C)	Softening point Tm (°C)	Young's modulus ($N\,mm^{-2}$)
Bovine	34.8	39.3	0.16
Fish	41.1	33.5	0.10
Porcine	32.0	38.8	0.18

[a]Gelatin:water:glycerol 40:40:20.

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Enzymatic synthesis of novel functionalised polyesters and their use as a biodegradable nanoparticle drug delivery system

M. C. Garnett, S. Higgins*, G. A. Hutcheon* and P. Kallinteri

School of Pharmacy, University of Nottingham, University Park, Nottingham, NG7 2RD and *School of Pharmacy and Chemistry, Liverpool John Moores University, James Parsons Building, Byrom Street, Liverpool, L3 3AF, UK. E-mail: G.A.Hutcheon@livjm.ac.uk

We have designed and developed a novel polymeric system for use as a biodegradable nanoparticle drug delivery system (Garnett et al 2003). The system has been developed to contain hydrophobic and hydrophilic sites, which allow drug incorporation and delivery to the target area. Furthermore the system is adjustable, during the synthesis, to allow for incorporation of various drug molecules. We have thus utilised the regio-selectivity of a hydrolytic enzyme to affect the polycondensation of an activated aliphatic diacid and an aliphatic diol (Kline et al 1998). By using glycerol, as the diol monomer, we are able to prepare a backbone linear polyester that contains pendant hydroxyl groups, which are available for further chemical functionalisation. Also by limiting the time in contact with the enzyme, we have been able to produce the required polyester with a molecular weight range of 1–20 kDa. The next stage, the functionalisation of the polymer backbone, was simply achieved by reaction of some of these pendant hydroxyl groups. To obtain the hydrophobic sites, for drug encapsulation, we reacted a percentage of the hydroxyl groups with various linear aliphatic acid chlorides. The nanoparticles dispersions were prepared using the interfacial deposition method (Fessi et al 1989). Using our library of polymers we have been able to produce nanoparticles of the correct size, 130–277 nm, and having a zeta potential ranging from -14 to -48 mV. Transmission electron microscope (TEM) images of these particles confirm the spherical nature of the particles and their nano-scale size. By using a fluorescent-labelled drug, we have confirmed the cellular uptake of our novel drug loaded nanoparticles. More importantly, we also get a steady release rate over a prolonged period. Our initial investigations have also proved that the polymer system showed no significant cytotoxicity towards the leukaemic suspension cell line (HL-60) or the hepatoma adherent cell line (HepG2) using the MTT assay. To conclude, we have prepared a library of polymers containing various degrees of hydrophobicity and hydrophilicity, specifically designed to enhance drug incorporation. The results for the best examples of our polymer system, far surpass those achieved with other biodegradable polymer systems currently under investigation (e.g. poly(lactic acid) (PLA) & poly(lactide-co-glycolide) (PLGA)), and indicate that our novel functionalised biodegradable polymer nanoparticle drug delivery system could have significant therapeutic use.

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Influence of sample pan design on the thermal characterisation of some cellulose derivatives

T. Watanabe and J. L. Ford

School of Pharmacy & Chemistry, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK. E-mail: J.L.Ford@livjm.ac.uk

Cellulose derivatives are used extensively in solid oral dosage forms. A knowledge of their properties, including water desorption and glass transition is

important since their properties influence the mechanical strength, water sorption manufacture and performance of granules or tablets containing the derivatives. Modulated temperature differential scanning calorimetry (MTDSC) is a technique that can detect dehydration and glass transitions as non-reversible and reversible responses, respectively. However, dehydration and glass transitions examined using MTDSC are affected by vapor pressure (Bravo-Osuna et al 2002). This study examines the influence of sample encapsulation on dehydration, glass transition temperature (Tg), melting and degradation of cellulose derivatives using MTDSC, thermogravimetric analysis (TGA) and thermomicroscopy. Ethylcellulose (Ethocel 10CP), hydroxypropylcellulose (HPC, Klucel-LF) and hydroxypropylmethylcellulose (HPMC, HPMC-K4M) were used. For MTDSC and TGA samples were placed into either hermetic aluminium pans with lids paced with or without crimping, into aluminium hermetic pans with pinhole-lids or into standard aluminium pans crimped with lids (all TA, Instruments, New Castle, USA). A Q1000 was used for MTDSC experiments. Samples were scanned from −20°C to 240°C, at 5°C min^{-1}, using a modulation amplitude of ±0.8°C every 60 s. Nitrogen was used as purge gas. TGA (TA Instruments 2050) and thermomicroscopy (Mettler FP82 Hot Stage) were performed at 5°C min^{-1} from room temperature to 240°C. For MTDSC and TGA samples were ∼2 mg and ∼6 mg, respectively. The order of the peak MTDSC dehydration temperature and the corresponding TGA temperature for weight loss was hermetic pans & lids without crimping < hermetic pans & pinhole-lids < standard pans & lids < hermetic pans & crimped lids. The actual TGA weight loss was in the reverse order. These results indicate that loosely adsorbed water was removed easily during TGA when the samples were placed in the atmosphere of the heating chamber. Glass transitions of Ethocel and HPMC were observed in the reversible MTDSC curves and during the thermomicroscopy as baseline change and fast contraction of the powder particles, respectively. A Tg was not found for HPC; The Tg of HPMC using different pans was in the reverse order of the dehydration temperature (range193–182°C). These changes in the glass transition temperature must result from the differing loss of water during heating. The moisture content of the hydrophobic Ethocel was small (1% w/w) and its Tg (∼128°C) was independent of sample pan setup. The onset melting temperatures of HPC (∼174°C) and Ethocel (∼163°C) were not affected by the difference in the pans. Following melting, further weight loss for HPC and Ethocel were detected indicating an instability of these materials.

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Development of an extracellular components based biomaterial for the study of novel delivery to solid tumours

S. Lélu, N. Tirelli and A. Pluen

School of Pharmacy and Phamaceutical Sciences, University of Manchester, Oxford Road, Manchester M13 9PL, UK. E-mail: sylvie.lelu@stud.man.ac.uk, alain.pluen@man.ac.uk

The success of some gene therapy treatments may ultimately depend on the ability of a drug to reach the targeted cell. The role of barriers such as the extracellular space (ECS) and the extracellular matrix (ECM) in the delivery of novel therapeutic agents has received little attention (Jain 1998). However, models have been proposed to describe macromolecular transport through the ECM. Whereas many transport models are able to describe the penetration mechanisms of macromolecules through simple gels such as agarose, the complexity of the interactions between the ECM components and the cells prevents the application of these models to the study of macromolecular transport through the interstitium. Historically hyaluronic acid was thought to be responsible for the transport hindrance presented by the ECM (Ogston & Sherman 1961) but recent investigations pointed out the role of the collagen fibres (Pluen et al 2001). In addition a recent model by Clague & Phillips (1997) suggests that both hyaluronic acid and collagen influence transport. In this study, a biomaterial based on main components of the extracellular space (collagen fibers, hyaluronic acid and a proteoglycan cross-linker) has been developed, firstly to understand the influence of collagen and glycosaminoglycans content and organisation inside the tumour ECM, secondly to use appropriate parameters in the transport models and thirdly to create an in-vitro system to evaluate the penetration of novel medicines in the ECS and to optimise these drugs. Furthermore, an ECM components-based gel also presents attractive properties in the tissue engineering field like biocompatibility and the ability of ECM to interact with the cells. In the first part of the study the experimental conditions were determined by rheology to evaluate the gelation point of our collagen and the mesh size of hyaluronic acid at different concentrations. Complex gels, based on the evidence from the rheological studies, were prepared. Visualisation by confocal microscopy allowed the determination of structural parameters (e.g. mesh size and the presence of micro-domains). The diffusion coefficient measurements of different macromolecules through the complex gels by confocal fluorescence spectroscopy will permit comparison of the diffusion of macromolecules in the gels and in the tumour ECM, and thus to get a better understanding of the structure and the organisation of the tumour interstitium.

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Development of anti-infective polymeric biomaterial surfaces

J. L. Gray, S. P. Gorman, D. S.Jones and C. P. McCoy

School of Pharmacy, Queen's University Belfast, Belfast, BT9 7BL, UK. E-mail: l.gray@qub.ac.uk

Endotracheal (ET) tubes are employed for the mechanical ventilation (MV) of patients within the intensive care unit (Chastre & Fagan 2002). Adherence of microorganisms to the surface of endotracheal tubes used for MV is of considerable importance as the biofilm formed is associated with increased bacterial resistance to antimicrobial agents and, furthermore, acts as a microbial reservoir for the development of Ventilator Associated Pneumonia (VAP) (Jones et al 1997). A rational strategy to reduce the incidence of VAP is to develop anti-infective polymeric biomaterial surfaces for the polymers used in ET tubes. This study focuses on the incorporation of Quaternary Ammonium Compounds (QACs) onto Polyvinyl Chloride (PVC), a polymer used in the manufacture of ET tubes. Three QACs were used: Cetylpyridinium Chloride (CPC), Hexadecyltrimethylammonium bromide (HTAB) and Benzalkonium Chloride (BAC). These are all currently used within the pharmaceutical industry and beyond (Russell et al 1999). This study investigates whether incorporation of QACs has any effect on the mechanical and physical properties of the polymer. The mechanical properties were investigated using tensile strength analysis and the results are summarised in Table 1. The physical properties were investigated using Dynamic Contact Angle studies and the results are summarised in Table 2. Statistical analysis of the mechanical properties showed that there was no significant difference in UTS between the 0.1% and 0.01% (w/w) loaded QAC polymers. There was however a significant difference between both concentrations of QAC with a blank polymer. Statistical analysis of the physical properties showed that there was no significant difference in the advancing or receding contact angle of 0.1% and 0.01% QAC loaded polymers compared with a blank polymer. It is apparent that the behaviour of the polymer is dependent on the type and concentration of the QAC incorporated. Further investigation is required to determine whether these changes are of clinical relevance. These findings offer the potential for an anti-infective polymer to be produced.

Table 1 Tensile strength results

QAC concn (% w/w)	Ultimate tensile strength (UTS) (MPa)
0	2.92 ± 0.57
0.01% CPC	1.72 ± 0.25
0.10% CPC	1.60 ± 0.20
1.00% CPC	0.43 ± 0.15
0.10% HAB	1.21 ± 0.21
1.00% HAB	1.02 ± 0.19
0.1% BAC	2.26 ± 0.36
1.00% BAC	2.00 ± 0.43

Table 2 Contact angle results

QAC concn (% w/w)	Advancing contact angle (°)	Receding contact angle (°)
0	94.28 ± 2.17	73.99 ± 3.36
0.01% CPC	87.92 ± 4.52	77.89 ± 8.01
0.1% CPC	91.08 ± 1.72	70.74 ± 2.18
0.01% HTAB	96.81 ± 2.73	72.70 ± 4.56
0.1% HTAB	90.19 ± 2.13	69.08 ± 1.91
0.01% BAC	94.51 ± 1.46	73.86 ± 1.69
0.1% BAC	89.53 ± 2.00	72.41 ± 1.86

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Influence of fatty acids on the release of chlorhexidine from poly ε-caprolactone films

F. Garvin, D. S. Jones and S. P. Gorman

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

A problem associated with urinary catheters is microbial biofilm formation, which frequently results in morbidity and mortality. One method by which medical device related infection may be reduced involves the controlled release of antimicrobial agents from the medical device directly to the attached biofilm. However, frequently the diffusion of antimicrobial agents from currently available medical device biomaterials is inappropriate and therefore alternative strategies are required to ensure the optimum drug release rate/duration. One method by which drug diffusion may be controlled is by the use of ion-pairing agents as this offers a means to modify the physicochemical properties of the diffusant. Therefore, in this study, the release of chlorhexidine (CHX) base and CHX that had been ion-paired with selected fatty acids from poly ε-caprolactone, a model biodegradable polyester biomaterial, has been examined. Films were produced containing, CHX 5% in PCL, CHX 5% with 1:1 and 1:2 molar ratios of oleic acid (OA) and CHX 5% with 1:1 and 1:2 molar ratios with myristic acid (MA). The mixtures were dissolved in 100 mL dichloromethane. The total added to the solvent amounted to 10 g in each case. When dissolved, 10-mL volumes were poured onto 10-cm diameter glass Petri dishes. Glass funnels were placed over the dishes to slow evaporation. The samples were left until the dichloromethane had evaporated. The resultant film discs were stored at room temperature for at least two weeks to ensure complete removal of dichloromethane. Three 2.4 mm $\times$ 2.4 mm samples were cut from each film and affixed to glass slides using a silicone adhesive. The slides were placed in beakers and immersed in pH 7.2 Tris buffer. The beakers were incubated in a water bath at 37°C and agitated at 30 rev min^{-1}. The buffer was sampled and replaced at 24 h and every 48 h thereafter up to seven days. The samples were assayed for chlorhexidine using HPLC at 257 nm. Mobile phase was acetonitrile 60% (v/v) methanol 33.2% (v/v), water 6.8% (v/v) glacial acetic acid 0.4% (v/v) and sodium lauryl sulphate 0.11%. The flow rate was 1 mL min^{-1} and a 250 $\times$ 4 mm GL WAKOSIL C18RS 5 μm column was used. Differences in release between the films were statistically analysed using a Kruskal-Wallis test ($P < 0.05$ denoting significance). The control films containing CHX alone showed a significantly greater initial release than ion-paired films (Table 1). The ratio of CHX to the ion-pairing agent but not the chemical nature of the ion pair significantly affected drug release. The release of CHX from films containing a 1:1 CHX:ion pair ratio were greater than from those containing a 1:2 ratio. The results of this release study demonstrate that the addition of fatty acids affects the release of CHX from PCL films. In particular greater control of CHX release was observed whenever the ion-pairing agents were included, highlighting the possibility of these systems for the controlled release of basic antimicrobial agents from biomaterials.

Table 1 Cumulative % CHX release from candidate films

	1 Day	3 Days	5 Days	7 Days
CHX 5%	24.8 ± 0.84	30.42 ± 0.96	31.41 ± 1.09	32.07 ± 1.09
OA 1:1	15.31 ± 1.22	20.86 ± 2.01	24.49 ± 3.32	28.04 ± 5.66
MA 1:1	15.15 ± 0.58	21.13 ± 1.34	27.98 ± 4.89	34.49 ± 4.03
OA 1:2	3.23 ± 0.55	6.25 ± 0.70	7.93 ± 0.65	9.35 ± 0.83
MA 1:2	3.91 ± 0.58	6.54 ± 0.53	8.23 ± 0.61	10.00 ± 0.40