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ORAL SESSIONS

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Preparation and pH-responsive release of organic nanoparticles

N. Grant, S. Rannard, A. Cooper and H. Zhang

Department of Chemistry, University of Liverpool, Liverpool, UK
E-mail: zhanghf@liv.ac.uk

Introduction and Objectives

The aim of this study was to prepare organic nanoparticles in porous polymers and tune their release into water by pH. A very wide range of useful organic compounds such as drugs are insoluble or poorly soluble in water, which has greatly limited their applications. The preparation of organic nanoparticles provides a potential strategy for their deployment in water-based formulations. Organic nanoparticles are formed *in situ* in porous polymer by the technique of freeze-drying emulsions. The nanoparticles are released from the polymer scaffold to form stable aqueous nanoparticle dispersions.

Methods

Oil-in-water emulsions containing chitosan in the continuous aqueous phase were firstly prepared. To the acetic acid solution, Triton X-405 was added as surfactant and stirred. Oil red (OR) solution in cyclohexane was added to form a high internal-phase emulsion (an emulsion in which the dispersed phase occupies more than 74% of the volume). Chitosan was then added to the formed emulsions and stirring was continued for approximately 5 min. The emulsions were frozen and then freeze-dried in a freeze dryer. Approximately 0.1 g of the freeze-dried sample was taken and placed in water at different pH values. The release of organic nanoparticles was monitored by ultraviolet.

Results and Discussion

OR is a poorly water-soluble organic dye and was used as a model organic compound. The rapid freezing in liquid nitrogen locked the emulsion–template porous structure. During the process of freeze-drying, both water and cyclohexane in the frozen emulsions were removed. The removal of organic solvent led to the assembly of organic molecules to form nanoparticles. Highly interconnected porous chitosan was produced with the in-situ formation of organic nanoparticles in the pores after freeze-drying. Because these particles were supported and separated by the polymeric structure, there was no issue of nanoparticle aggregation.[1] This porous nanocomposite was not soluble in water. The release of OR nanoparticles from porous chitosan scaffold into water under different pH values was investigated. OR organic nanoparticles were slowly released in basic and neutral water at room temperature. When the pH of the aqueous phase was reduced to 2, there was a much faster release of organic nanoparticles. The release of OR organic nanoparticles into water produced stable aqueous organic nanodispersion.

Conclusions

The utilisation of water-insoluble active species in aqueous systems is of great importance in many areas. The size of organic particles could be reduced to nanoscale and then dispersed in water to potentially solve this problem. We demonstrated the preparation of organic nanoparticles in the porous polymer by combining freeze-drying and emulsion templating. The pH-sensitive release of organic nanoparticles was also investigated to produce stable aqueous nanodispersions.

Reference

1. Zhang H *et al.* Formation and enhanced biocidal activity of water-dispersable organic nanoparticles. *Nat Nanotechnol* 2008; 3: 506–511.

2
The gel-layer and extended release properties of a hydroxypropyl methylcellulose matrix in fat-containing dissolution environments

H.D. Williams[a], K.P. Nott[b], R. Ward[c], I.J. Hardy[d] and C.D. Melia[a]

[a]School of Pharmacy, University of Nottingham, Nottingham, [b]Oxford Instruments Molecular Biotools Ltd, Oxfordshire, [c]Merck Sharp and Dohme, Hoddesdon, UK and [d]Merck and Co, West Point, Pennsylvania, US
E-mail: paxhdw@nottingham.ac.uk

Introduction and Objectives

Fed bioequivalence studies have, on occasion, reported increased bioavailability from hydroxypropyl methylcellulose (HPMC) matrices when taken with a high-fat breakfast.[1,2] In this work, we examine the gel-layer development and extended the release function of HPMC matrices in environments containing up to 30% emulsified fat.

Method

Tablets (8 mm, 250 mg) containing 10% wt/wt caffeine anhydrous, 30% HPMC (Methocel K4M-CR; Colorcon Ltd,

Dartford, UK) and lactose:microcrystalline cellulose in a 2 : 1 mixture were compressed at 230 MPa using an instrumented tablet press. Gel-layer development in milk (0.1 or 3.5% fat) and Intralipid (Fresenius Kabi, Sweden) (30% fat) plus water-soluble fluorescein (0.0001%) was imaged by confocal microscopy (Bio-Rad MRC-600; Bio-Rad, Hemel Hempstead, UK) described previously.[3] Combined magnetic resonance imaging (MRI)–drug release studies were undertaken using a bench-top 0.5Tesla-MRI with integrated USP-apparatus 4 (PharmaSense; Molecular Biotools, Oxford, UK) flushed continuously with dissolution media (16 ml/min, 37 ± 0.1°C). Images of hydrating matrices were captured periodically for 8 h and caffeine concentrations in media samples determined by high performance liquid chromatography.

Results and Discussion

Confocal imaging studies showed that matrices formed coherent gel-layers in all media containing up to 30% fat. This process was rapid and comparable to gel-layer development in water. Combined MRI-drug release studies showed that the gels formed in these fat-containing media maintained barrier properties for up to 8 h. Compared with water, however, drug release rates were slower in milk and even slower in Intralipid. Confocal and MRI work also provided evidence of fat depositions at the gel-layer surface, which accumulated with time. This feature was most prominent in Intralipid and manifested as a dark, nonfluorescent region in the confocal images or a bright layer around the hydrating tablet in MRI studies. The high signal intensity of this layer in MRI is the result of high ^{1}H density and/or short relaxation times (T_1), which is typical for fat-associated protons. The reduced surface area as a result of fat layer formation would explain the reduced drug-release rates observed in the fat-containing media.

Conclusion

HPMC matrices rapidly formed gel-layer barriers and improved their extended release in milk and other emulsions containing up to 30% fat. Drug release was progressively reduced with increasing media fat content, which may be a result of fat deposition at the gel-layer periphery. This work suggests that an environment containing emulsified fats is unlikely to promote accelerated drug release from HPMC matrices. A slowing of drug release is more likely, through an enhanced barrier at the gel-layer surface following fat deposition. This would give rise to a negative rather than a positive food effect in the fed state.

References

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3
Tissue viability and Langerhans cell behaviour in an ex-vivo human skin model for cutaneous DNA vaccination

K.W. Ng[a], M. Pearton[a], S. Coulman[a], A. Anstey[b], C. Gateley[b], A. Morrissey[c], C. O'Mahony[c], C. Allender[a] and J. Birchall[a]

[a]Welsh School of Pharmacy, Cardiff University, Cardiff, [b]Royal Gwent Hospital, Gwent Healthcare NHS Trust, Newport, UK and [c]Tyndall National Institute, Cork, Ireland
E-mail: ngkw2@cardiff.ac.uk

Introduction and Objectives

Epidermal Langerhans cells (LCs) present an attractive and accessible target for deoxyribonucleic acid (DNA) vaccination. Excised human skin is perhaps the most representative preclinical model attainable for immunological studies involving human LCs. In terms of DNA vaccination, explant viability and artefactual LC activation in response to culture conditions are important issues to be addressed in any potential ex-vivo human skin model. This study aims to optimise such a model for ex-vivo DNA vaccination studies by ascertaining the structural and functional viabilities of human skin explants, and minimising artefactual LC activation.

Method

Human breast skin was obtained by surgical procedures under ethical approval. Skin with just subcutaneous fat removed (full thickness) and the majority of dermis removed (split thickness) was cultured for up to 72 h in a Trowell-type organ culture system[1] using various culture media. LC activation was characterised by spatial density and cell size every 24 h. Skin integrity was determined by histological examination of tissue sections. β-Galactosidase reporter plasmid was delivered to the epidermis using a microneedle device at 24, 48 or 72 h. Tissue viability was defined as positive gene expression (determined by X-gal staining) 24 h later.

Results and Discussion

In the first 48 h, LCs exhibited a significant ($P < 0.01$) delay in activation profile in full-thickness skin compared with split-thickness skin (25 vs 54% decline in spatial density associated with a corresponding 122 vs 157% increase in cell size), possibly due to reduced physical damage of full-thickness skin during sample preparation. Preliminary results indicated that exclusion of potentially immunogenic fetal bovine serum (FBS) from culture medium based on Dulbecco's modified Eagle medium (DMEM) also attenuated LC activation in the first 48 h in full-thickness skin, elevating LC density by 8–29% ($P < 0.01$) from the levels observed in samples cultured using FBS-supplemented DMEM. Skin explants retained their structural integrity in DMEM after 72 h, but epidermal delamination was observed from 48 h in samples cultured in phosphate-buffered saline (PBS). Reporter gene expression was

detected in skin samples precultured for up to 72 h using either DMEM or PBS. Hence, the cellular machineries required for the expression of a DNA vaccine were functional for at least 72 h in this ex-vivo model system.

Conclusion

The study has demonstrated the temporal viability of the ex-vivo human skin model in line with the requirements of DNA vaccination. DMEM is more suitable than PBS for use with the ex-vivo model system, as it better preserves the structural integrity of excised skin for a longer time. Artefactual LC activation in skin explants can be minimised by utilising full-thickness skin instead of split-thickness skin, and excluding immunogenic additives from the culture medium. The ex-vivo model system offers a minimum window of 48 h within which the effects of DNA vaccination can be investigated.

Reference

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4
Comparative evaluation of different techniques of taste masking and their release profile for a water-soluble bitter drug

A. Rauf, F.J. Ahmad and R.S.R. Murthy

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

Introduction and Objectives

This study evaluates the different techniques[1,2] of taste masking (microencapsulation, ionic crosslinking, precipitation, drug-resin complex and ion-pair complex) of a water-soluble bitter drug. Dicyclomine hydrochloride was taken as a model drug, which is very bitter in taste and frequently used. The product was optimised by assessing drug to polymer, resin and pamoic acid ratio, drug loading, stirring speed and time and cost effectiveness. The products obtained were evaluated for taste masking and drug release in three different mediums (buffer pH 6.8 (pH of saliva), 0.1N HCl and buffer pH 7.4).

Method

Microencapsulation was performed by adding chitosan solution to liquid paraffin with constant stirring. Span 80 and Glutaraldehyde were used as emulsifying and cross-linking agent, respectively. Ionic crosslinking was carried out by adding TPP solution to chitosan solution with stirring until an opalescent suspension was obtained. Precipitation occurs when the pH of chitosan solution is increased from 3 to 6. On further increasing the pH to 8, Eudragit-E if present precipitates and coats the colloids. Drug-resin complex is formed by adding the drug solution to resin slurry with stirring to get tasteless product. Ion-pair complex is formed by adding the drug solution slowly to sodium pamoate solution.

Results and Discussion

Different parameters were used to optimise the products. All the optimised products were tasteless and showed no drug release in buffer pH 6.8 (pH of saliva). In microencapsulation, optimised microspheres (at drug to polymer 1 : 4, stirring speed 1500 rpm, mean particle size 67.5 μm and entrapment efficiency 59.5%) showed sustained release profile (~12% in 2 h in 0.1N HCl and 75% in 12 h in buffer pH 7.4). Similarly, optimised nanosuspension (at drug to polymer ratio 1 : 4 and mean particle size 158 nm) from ionic crosslinking gave sustained release product (~85% in 12 h in buffer pH 7.4). On the contrary, optimised colloidal suspension (at drug to polymer ratio 3 : 1, pH 7.5–8.0) from precipitation exhibited an immediate release product (~90% in 5 min in 0.1N HCl). Further coating of colloidal suspension by Eudragit-E (drug to polymer ratio 3 : 1 : 1.5) again showed immediate release (~90% in 10 min in 0.1N HCl). Optimised product developed (at drug to resin ratio 1 : 2, stirring time 6 h, stirring speed 750 rpm, rate of addition 1.5ml/min) from drug-resin complex showed moderate release profile (~93% in 2 h in 0.1N HCl), whereas the optimised product (at drug to pamoic acid ratio 1 : 0.6) obtained from ion-pair complex released ~85% of the drug in 2 h in 0.1N HCl.

Conclusions

Different techniques used for bitterness masking exhibited tasteless product and graded release profiles. For sustained effect (~75% in 12 h) of drug, microsphere-based formulations were the best. When the tasteless nanoparticles with sustained release are required, it is suggested to go with chitosan nanosuspension. Tasteless ion-pair complex and drug-resin complex may be used for the formulations when moderate release (~85–93% in 2 h) is required. Colloidal suspension is suggested for immediate release profile (~90% in 10 min). Drug-resin complex and ion-pair complex techniques were easy going, cost effective and reproducible at pilot and large scales.

References

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5
Measurement of amorphous lactose stability by terahertz spectroscopy

G. Smith[a], I. Ermolina[a], A. Pandya[a] and J. Alton[b]

De Montfort University, Leicester and [b]TeraView Ltd, Cambridge, UK
E-mail: gsmith02@dmu.ac.uk

Introduction and Objectives

The aim of this study was to analyse the stability of amorphous (freeze-dried) lactose on exposure to low and

high humidity using terahertz spectroscopy. Terahertz spectroscopy is a relatively new technique in pharmaceutical materials characterization but is particularly sensitive to structural changes in crystalline materials (e.g. polymorphism) owing to the various lattice vibrations that manifest as resonance peaks in the terahertz spectrum.

Method

Alpha-Lactose monohydrate was freeze-dried from a 10% aqueous solution to a residual moisture content of 3%. Both freeze-dried lactose and lactose monohydrate were mixed with polythene powder and then compacted (separately) to form pellets at a concentration of 10% lactose. The glass transition (T_g) of the unpelleted material was measured at 100°C/min using differential scanning calorimetry (DSC), and its moisture content was determined using thermogravimetric analysis (TGA). The transmission spectra of the lactose–polythene pellets were then measured using a TeraView Spectra3000 (TeraView Ltd, Cambridge, UK), following dry nitrogen purge of the sample compartment. Repeated measurements on the sample *in situ* showed that no moisture was lost from the sample.

Results and Discussion

α-Lactose monohydrate showed the various distinct peaks in the terahertz spectrum that were reported previously.[1] In contrast, the spectrum of the freeze-dried lactose was devoid of any discernable peaks, suggesting the material was totally amorphous (i.e. below the limits of the detection). The baseline of the amorphous lactose, however, was sensitive to moisture content, e.g. the absorbance decreases from 1.04 to 0.57 (at 2 THz) on decreasing the moisture from 3 to 1.3% w/w. Furthermore, when the freeze-dried material was exposed to 80% RH for 80 min, a rapid conversion to the hydrated forms of lactose occurred (as evident from the observation that peaks in the terahertz spectrum of this material were characteristic of α-lactose monohydrate and, to a much less extent, α-lactose anhydrate). However, the high baseline, broadness of the peak and the peak height suggested only a partial conversion. The DSC measurements on the partially converted material gave T_g = 65.5°C, which was similar to T_g = 64.5°C for the pure amorphous form (at low moisture content). This suggests that the moisture content of the amorphous regions was equivalent to that of the pure amorphous material (i.e. 1.3%). The total moisture content then determined by TGA and the subtraction of the moisture associated with the amorphous domains yielded a water content that was presumed to reside within α-lactose monohydrate. This assumption permitted an approximate calculation of the amount of lactose monohydrate present in the sample (48% w/w), which was then compared to an estimate from the terahertz spectrum. The main peak at 1.35 THz provided estimates of 49% w/w for lactose monohydrate, which is consistent with the amount estimated from the moisture content and T_g approach.

Conclusion

This study showed the sensitivity of terahertz spectroscopy to the moisture content of amorphous lactose and its transition to crystalline lactose on exposure to high humidity. However, the extent to which the technique might be used to quantify the extent of such transitions requires further work.

Reference

1. Zeitler JA *et al*. Terahertz pulsed spectroscopy and imaging in the pharmaceutical setting – a review. *J Pharm Pharmacol* 2008; 59: 209–223.

6
Synthesis of poly(amidoamine)s by microwave-assisted polymerization

N. Lavignac and J. Dubois

University of Kent, Chatham, Kent, UK
E-mail: n.lavignac@kent.ac.uk

Introduction and Objectives

Linear poly(amidoamine)s are water-soluble polymers. They display pH-dependent membrane activity and they have been used to deliver DNA and proteins into cells. They are also relatively nontoxic compared with other polycations. Poly (amidoamine)s are synthesized by Michael addition polymerization. The synthesis is carried out using conventional heating at 30°C for up to 5 days. In recent years, microwave irradiation has become an important tool for the synthesis of polymers due to increased kinetics rate. Here, the first synthesis of linear poly(amidoamine)s using microwave-assisted polymerization has been reported.

Method

Several poly(amidoamine)s were synthesized using microwave-assisted polymerization. The general procedure for the polymerization was as follows: equimolar amount of amine and bis-acrylamide was used, the reaction mixtures were heated at 60, 80 or 100°C and the microwave power was set up at 150 or 300 W. Structure of the polymers was identified by ^{1}H, ^{13}C nuclear magnetic resonance and Fourier transform infrared spectroscopy. Molecular weights and polydispersities were determined by gel permeation chromatography using poly(ethyleneglycol) as standards. Thermal analysis was carried out using differential scanning calorimetry.

Results and Discussion

ISA1 and ISA23 were synthesized by microwave-assisted polymerization. The results were consistent with those of polymers obtained by conventional heating. However, in most cases the microwave-assisted polymerization proceeded at a much faster rate and polymers with satisfactory molecular weight were obtained in 30 min (Figure 1).

Novel polymers were synthesized using a similar method. Their toxicity and delivery efficiency are currently investigated by cytotoxicity assay.

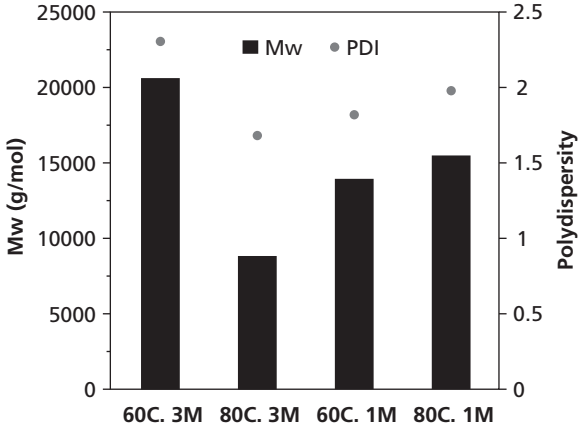

Figure 1 Synthesis of ISA23 by microwave-assisted polymerization. Molecular weight (Mw) and polydispersity (PD) of the reaction mixtures were determined at 30 min.

Conclusion

Gene delivery has been the preferred method to deliver proteins into cells. Although some successes have been achieved, no treatment has been approved by the Federal Drug Administration (FDA) yet. In past years, novel strategies to deliver proteins into cells have emerged and poly(amidoamine)s have been successfully used. Synthesis of a platform of novel poly(amidoamine)s using microwave-assisted polymerization will expand the tools to deliver functionally active proteins into cells. This could lead to the development of novel therapeutics with intracellular target and could also help scientists to investigate protein function.

New Scientists Session

7
Assessing drug release and dissolution in the stomach by means of Dynamic Gastric Model: a biorelevant approach

A. Mercuri[a,b], R. Faulks[b], D. Craig[a], S. Barker[a] and M. Wickham[b]

[a]University of East Anglia, Norwich and [b]Institute of Food Research, Norwich, UK
E-mail: a.mercuri@uea.ac.uk

Introduction and Objectives

Drug dissolution within the gastrointestinal tract is a critical step in achieving optimum drug bioavailability. For Biopharmaceutics Classification System class II and IV drugs, great variability in solubility/bioavailability can be observed in fasted/fed states. Solubility within the gastrointestinal fluids can be measured in human gastric aspirates in the fasted and fed states, using physiological media such as fasted-state simulating gastric fluid or recreating digestion 'snapshots'.[1] In this study, we used a new in-vitro system, the Dynamic Gastric Model (DGM) (Institute of Food Research, Norwich, UK), to assess the release of nifedipine from two different controlled release tablets.

Method

DGM was primed with 20 ml acid and salt solution to simulate the residual gastric juice in fasted conditions; one 60 mg nifedipine tablet was coadministered with 150 ml of water ($n = 4$). Physiological acid and enzymatic gastric secretions were added following variations in pH and calorific content as *in vivo*. Dissolved nifedipine and added internal standard (nimodipine) *in digesta* leaving the DGM was extracted into n-pentane : ethyl acetate (70 : 30 v/v) and quantified using reverse-phase high-performance liquid chromatography (Kromasil C-18 (4.6×250 mm) column, mobile-phase methanol : water 80 : 20 v/v and detection at 350 nm).

Results

The drug release from two commercially available tablets (a push-and-pull osmotic pump, Adalat LA from Bayer, and an erodible matrix, Coral from So.Se.PHARM), was measured using the DGM, which is able to reproduce the hydrodynamics and gastric secretions of the human stomach. During fasted digestion, the release of nifedipine from the two formulations was different. The drug started to appear within the gastric fluids after 11 min from Adalat LA, 0.003 μg/ml (SE $\pm$ 0.003), while the release was immediate (at 3 min) from Coral, 0.259 μg/ml (SE $\pm$ 0.156). At the end of digestion (13 min), the nifedipine release was 0.056 μg/ml (SE $\pm$ 0.044) and 3.77 μg/ml (SE $\pm$ 1.83) from Adalat LA and Coral, respectively. These results are consistent with in-vivo data[2] from which a lag time of 1 h was found in the fasted state for Adalat LA but not for Coral tablets.

Conclusion

The release of poorly water-soluble drug nifedipine in the fasted stomach mimicked by the DGM was found to be dependent on the type of formulation tested, and the results were consistent with in-vivo data. The study, therefore, supports the hypothesis that the DGM may provide a realistic temporal and dynamic model for stomach activity.

References

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