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Tuesday Poster Sessions

Biopharmaceutics

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Investigation of high permeability standards for BCS classification in Caco-2 cell monolayers

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Introduction and Objectives

The biopharmaceutics classification system (BCS) provides a framework for the waiver of regulatory requirements for in-vivo bioequivalence studies for IR oral products. While the definition of high permeability (>90% in-vivo absorption) in the guidance documents is clear, translation to practice can be challenging through the rigid interpretation of in-vitro permeability limits imposed through the use of metoprolol, the most widely accepted high permeability standard. This study was conducted to understand the impact of pH on metoprolol permeability and the potential use of alternative high permeability compounds to decrease the in-vitro high permeability threshold.

Methods

Caco-2 cells (obtained from ATCC) were used between passages 21 and 41. Falcon 24-well plates (1-μm polyethylene terepthlate (PET) membrane) were seeded with 1.6×10^5 cells/ml. Permeability measurements (P_{app}, cm/s) were conducted between days 15 and 28 in both absorptive (apical to basolateral, A–B) and secretory (basolateral to apical, B–A) directions. Monolayer integrity was assessed using 10 μM nadolol in Hank's Balanced Salt Solution (HBSS) containing 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) or 10 mM 2-(N-morpholino) ethane sulphonic acid monohydrate (MES). Only monolayers with P_{app} values $<1.2 \times 10^{-6}$ were used in data generation. Drug transport was measured at concentrations of 25 μM. Samples were analysed using liquid chromatography-mass spectrometry (LC-MS/MS).

Results and Discussion

Table 1 shows that timolol and pindolol P_{app} values were lower than the metoprolol P_{app} at pH 7.4. Both compounds exhibit >90% absorption in man and could be considered as alternatives to metoprolol to define the high permeability threshold.[1,2] In addition, metoprolol flux at pH 6.5 was significantly reduced compared to that observed at pH 7.4. Depending on the pK_a of a test compound, the use of a lower apical pH could significantly alter the permeability classification obtained. Further work is ongoing with labetalol and minoxidil (both >90% absorbed) to investigate how far the boundary of in-vitro high permeability could be lowered.

Conclusion

This study shows that there are alternative high permeability standards to metoprolol with lower flux that could be used for BCS permeability classification. In addition, the pK_a of the high permeability standard and the apical–basal pH profile selected are important considerations in study design. Regulatory acceptability of alternative high permeability standards remains to be determined.

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Investigation of the influence of formulation on genomic signature during permeability studies

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Table 1 Caco-2 P_{app} data of different BCS class I compounds

Compound	A-B ($P_{app} \times 10^{-6}$ cms^{-1}) pH 7.4	B-A ($P_{app} \times 10^{-6}$ cms^{-1}) pH 7.4	A-B ($P_{app} \times 10^{-6}$ cms^{-1}) pH 6.5	Extent absorption in man (%)
Timolol	32.1 (1.7)	32.4 (2.8)	To be determined	>90
Pindolol	32.7 (2.7)	28.7 (4.6)	To be determined	92
Metoprolol	39.7 (1.14)	42 (3.4)	7.8 (0.9)	>90

Note: Standard deviation shown in brackets.

Introduction and Objectives

The aim of this study is to understand the transcriptomic changes that occur during permeability studies by bringing out the general indications of the significance of various transporter gene expressions for a poorly water-soluble drug candidate (indomethacin) when studied alone and as a solid dispersion.

Method

Caco-2 cells (passage 70) were obtained from American Type Culture Collection (ATCC) and used at passages 90–100. For the transport assay, cells were seeded into six-well transwell culture plate inserts (24 mm, 4.7 cm^2) at a density of 2×10^5 cells/cm^2. Total RNA was extracted and purified from Caco-2 cells using the Qiagen RNEASY Mini Kit (50) (GE Healthcare UK Limited, Buckinghamshire, UK) according to the manufacturer's protocol. Hybridization of the arrays was performed overnight, and the slides (comprising 38K genome) were washed sequentially in a series of buffer solutions.

Results and Discussions

The analysis of significant genes associated with drug transport network system (ATP-binding transporter (ABC) and solute carrier (SLC) transporter system) showed significant variations in gene expression profiles upon treatment with solid dispersion of indomethacin or drug alone when compared to untreated cells (control). Interestingly, analysis of ABC super family of genes showed that the downregulation was 10-fold for ABCB9 (gene encoding small-molecule transport) and 3-fold for ABCC6 (drug-resistance gene) when treated with indomethacin alone compared to control. However, formulation of indomethacin as a solid dispersion resulted in 6-fold downregulation of ABCB9 and 2-fold of ABCC6 when compared to control. Similarly, probing SLC transporter genes showed that the expression of SLC14A3 (symporter gene-encoding solute transport) was downregulated 10-fold and 4-fold upon treatment with drug alone or solid dispersion, when compared to control. Investigation of SLCA15 (gene controlling small-molecule transport into and out of the cell) showed no variations in expression profiling when treated with drug alone, whereas solid dispersion resulted in 4-fold downregulation. Results from our studies for the gene-encoding drug resistance (ABCC6) are in agreement with the earlier reported studies where indomethacin has been shown to inhibit its activity.[1] However, quantification of expression of ABCC6 has clearly shown that the downregulation was more pronounced upon treatment with drug alone when compared to solid dispersion. In contrast, these differences do not explain the higher permeability associated with solid dispersion when compared to drug alone (data not shown). A broader investigation of other transporter genes such as SLC17A3, SLC25A15 and ABCB9 can therefore explain the higher permeability associated with solid dispersion of indomethacin when compared to drug alone suggesting that synergism between other transporter networks enhances drug permeability.

Conclusion

Systems biology provides a holistic understanding of gene expression changes that occur during permeability of drug candidates. Our studies have shown that multiple transporter systems work in a dynamic environment to determine permeability profiling of drug moieties.

Reference

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Drug Delivery

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Ex-vivo dose emission from dry powder inhalers following one and two inhalations

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Introduction and Objectives

Dry powder inhalers (DPIs) rely on a patient's inhalation flow and inhalation volume for drug delivery to the lungs. Each inhaler due to its formulation and device design requires a specific inhalation technique. Previously, Al-Fadhl *et al.*[1] have reported that dose emission is influenced by inhalation flow and that two inhalations are required for each dose. In our study, we have designed an ex-vivo approach to investigate the effects of inhalation flow on dose emission from a variety of DPIs.

Method

A total of 12 adults, healthy volunteers, were trained to inhale through each DPI at slow (30 l/min) and fast (60 l/min) inhalation flows using the In-Check DialÒ (Clement Clark, International Limited, Harlow, UK). The DPIs studied were salbuatmol Accuhaler, Clickhaler, Easyhaler and terbutaline Turbuhaler. An electrostatic filter was placed between the mouthpiece of the DPI and the volunteer's mouth. The inhaled medicine was entrained on the filter. On each occasion, each volunteer first inhaled once from a dose. The filter assembly was then replaced by a new one and the volunteer performed two inhalations from a dose. The high-performance liquid chromatography (HPLC) was used to quantify the drug in emitted dose.

Table 1 Mean (SD) and statistical comparison of emitted dose (% nominal dose) from the 12 volunteers after one and two inhalations for each dose

Mean emitted dose (% nominal dose) for one inhalation				
Inhalation rate (L/min)	Accuhaler	Easyhaler	Clickhaler	Turbuhaler
30	80.5	78.6	57.4	58.71
60	93.1	95	73.7	81.3
Mean emitted dose (% nominal dose) for two inhalations				
Inhalation rate (L/min)	Accuhaler	Easyhaler	Clickhaler	Turbuhaler
30	90.6	84.8	67.1	67.1
60	100.8	104.0	81.6	81.6
Mean difference (95% confidence interval) one versus two inhalations				
Inhalation rate (L/min)	Accuhaler	Easyhaler	Clickhaler	Turbuhaler
30	−10.10* (−6.68,−3.51)	−6.25* (−10.22,−2.27)	−9.70* (−17.75,−.64)	−6.41* (−10.10,−.72)
60	−8.92* (−14.82,−3.02)	−9.01* (−13.54,−4.48)	−7.93 (−16.02,.15)	−10.08* (−14.44,−.72)

*$P < 0.05$ Two- way ANOVA (General Linear Model).

Results and Discussion

A two-way analysis of variance of the emitted dose was carried out using SPSS (Version 15.0) to determine any significant difference between one and two inhalations for each metered dose. A probability value of ($P < 0.05$) was considered as being significant.

Conclusion

The results from this study highlighted a flow-dependent dose emission property by all the DPIs to a varying degree, and that the total emitted dose from the inhalers following two inhalations was significantly ($P < 0.05$) greater than one inhalation.

Reference

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Effect of poloxamer 188 on lymphatic uptake of carvedilol-loaded solid lipid nanoparticles for bioavailability enhancement

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Introduction and Objectives

Poloxamer 188, commonly used as surfactant and stabiliser, has gained popularity in the field of solid lipid nanoparticles (SLNs). Therefore, our aim is to study the effect of poloxamer 188 concentrations on particle size, on entrapment efficiency as well as on lymphatic uptake of carvedilol-loaded SLNs.

Methods

Carvedilol-loaded SLNs were prepared using microemulsion technique by dispersing warm o/w microemulsion in an ice-cold aqueous medium under mechanical stirring. For the preparation of microemulsion, the following compounds were used: stearic acid (5%) as the oil phase, poloxamer 188 in different concentrations (5–10%) as surfactant, and a mixture of sodium taurocholate (5%) and ethanol (10%) as cosurfactant. A clear microemulsion was obtained by gentle stirring at 70°C. The prepared microemulsions were immediately dispersed in cold water (2–3°C), under mechanical stirring, in a ratio of 1:15 (v/v). The obtained nanosuspensions were filtered and lyophilised.

Results and Discussions

The surfactant (poloxamer 188) concentration is a critical parameter for the size as well as entrapment efficiency of carvedilol-loaded SLNs. The results showed that formulation containing 5% (w/w) poloxamer showed a monomodal size distribution with mean particle size of 120–200 nm, whereas SLNs formulated with more than 5% poloxamer 188 displayed a bimodal size distribution. It revealed that as the amount of poloxamer 188 increases, it induces thermodynamic instability of the nanoparticulate system causing adsorption of surfactant on the particle surface forming loops and tails, leading to the bridging between the nanoparticles which causes aggregation of particles. Moreover the excess amount of surfactant forms micelles, which cause an increase in the solubility of surface carvedilol in water phase, consequently reducing the entrapment efficiency of carvedilol from 96.5 to 72.8%. The area under

the curve (AUC) (0–t) of all three SLN formulations (6.27 ± 0.24 μgh/ml with FZ-1, 4.13 ± 0.11 μgh/ml with FZ-2 and 3.63 ± 0.10 μgh/ml with FZ-3) were significantly ($P < 0.05$) higher than that of carvedilol suspension (1.27 ± 0.23 μgh/ml). It is suggested that increase in AUC (0–t) of SLN is due to the avoidance of first pass metabolism by its lymphatic uptake and transport.[1] Lymphatic uptake is influenced by lipid nature, chain length and hydrophobicity of nanoparticles.

Conclusions

Poloxamer 188 was used in different concentrations as surfactant, and it was found that 5% of it was sufficient to cover as well as to produce stable nanoparticles. The studies indicated that on increasing the concentration of surfactant, an increase in particle size as well as a decrease in hydrophobicity of carvedilol encapsulated SLN resulted which reduced its lymphatic uptake.

Reference

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Peptide conjugated liposomal formulation for anticancer drug delivery: a novel approach towards efficient cancer treatment

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Introduction and Objectives

The aim of this study is to develop a novel liposomal drug delivery system which can target the 67-kDa high affinity laminin receptor which is over expressed on the tumour cells with help of pentapeptide YIGSR. Peptide-attached liposomes enabling its cytotoxic effect to cancerous cell with help of receptor-mediated uptake will be studied.

Methods

Etoposide-encapsulated liposomes were prepared using lipids egg yolk lecithin, soybean lecithin, hydrogenated soybean lecithin (HSPC) and cholesterol by thin film hydration method. Liposome of particle size below 200 nm was obtained by passing through polycarbonate membrane of various pore sizes using extruder (Emulsiflex, Avestin, Canada). Two linkers, i.e. NGPE-COOH and DSPE-PEG-COOH, were synthesised and incorporated to liposomes. A pentapeptide, YIGSR having antimetastatic potential, was attached to the distal ends of linkers by forming amide bonds between amine groups of YIGSR and carboxyl groups of linker. Liposomes were lyophilised with help of sucrose as a cryoprotectant. In-vitro chemosensitivity of liposomal formulation was performed on a highly metastatic B16F10 melanoma cell lines by MTT assay. Cell adhesion assay and cellular uptake study of liposomes were also conducted for assessment of binding efficiency and receptor-mediated internalisation, respectively.

Results and Discussion

Etoposide-loaded liposomes of average particle size 117.64 ± 12.2 nm were obtained with percent drug entrapment of 61.88 ± 2.17 with use of lipid HSPC and cholesterol. The results obtained showed higher drug loading with optimum particle size compared with other lipids tried.[1] Both the synthesised linkers were characterised for confirmation of final product formation with help of Fourier transform infrared. To the surface of linker incorporated liposomes, a peptide YIGSR was attached and observed that 1:1 mole ratio of linker to peptide showed maximum tagging efficiency. Sucrose used as cryoprotectant showed very good stability of liposomes over a longer period of time without any significant drug leakage and increase in particle size. In-vitro chemosensitivity assay showed higher cytotoxic effect of YIGSR-tagged liposomes compared with untagged liposomes with time of incubation. Cell adhesion assay performed using laminin coated 96 well plates showed potential of YIGSR conjugated liposomes for target site specific binding, and thus it can compete with laminin for binding to laminin receptor and hence help in prevention of tumour metastasis.[2] Cellular uptake studies on B16F10 melanoma cell line showed the higher uptake of YIGSR-tagged liposomes compared with nontagged liposomes indicating receptor-mediated uptake is possible with help of peptide YIGSR.

Conclusions

Present research studies showed the application of a pentapeptide YIGSR having antimetastatic potential for attachment to liposomes encapsulated etoposide for active tumour targeting. Etoposide-loaded liposomal system with surface attached peptide could be having a great benefit for cancer treatment with a synergistic effect of YIGSR and drug etoposide with reduction in toxic effects of drugs to normal cell with prevention of tumour metastasis.

References

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Receptor-appended long-circulating nanoparticles bearing temozolomide for brain targeting

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Introduction and Objectives

The aim of this study was to study the effect of receptor-appended PEGylated nanoparticles (NP) bearing anticancer drug temozolomide for effective management and targeting to brain tumor.

Methods

PLGA-PEG copolymers were synthesised by polymerisation under vacuum using stannous octoate as catalyst. PLGA-PEG NP were prepared by using precipitation solvent evaporation technique using synthesised copolymer and PVA. Transferrin (Tf)-coupled NP were prepared by coupling of transferrin to surface of PEGylated NP using periodate oxidation method and characterised for various attributes i.e. particle size, shape, zeta potential, percent drug entrapment, in-vitro release and in-vitro cytotoxicity. In-vitro stability and radiolabelling efficiency were assessed in normal saline and in serum. The qualitatively uptake of the formulations in albino rats was also assessed by fluorescent and confocal microscopy.

Results and Discussion

The size of the plain and Tf-coupled NP was found to be 112.93 ± 3.8 and 121.89 ± 3.11 nm, respectively, which was due to anchoring of Tf on the surface of the PEGylated NP. Nanoparticles prepared from PLGA-PEG copolymer have low negative x (zeta) potential values because the carboxylic acid end groups of PLGA are capped by the PEG segments. The percentage of drug entrapment of plain NP was found to be 76.02 ± 4.54, whereas it was decreased to 71.87 ± 2.81%, which is due to the residual drug leakage accounted during the incubation used for anchoring the transferrin to the PEG. Ex-vivo cytotoxicity against human cancer cell lines was assessed using sulforhodamine blue dye. The confocal laser scanning microscopy (CLSM) studies of brain tissue after the administration of nanoparticulate formulations clearly shows that the Tf-coupled PEGylated NP give maximum intensity of fluorescence as compared with non-PEGylated NP. The stability studies of the radiolabelled temozolomide (^{99m}TcTmz) showed a lesser reduction in the labelling efficiency i.e. 3–7% radioactivity was dissociated after 24 h. Results showed that ^{99m}TcTmz could be used for further in-vivo performance. Biodistribution study was performed using gamma scintillation technique. Tf-coupled PEGylated NP show decreased uptake by reticuloendothelial system (RES) while there is an increased radioactive accumulation in the brain, which was nearly 7–8 times higher than the plain NP. Results clearly show that Tf-coupled PEGylated NP accumulate in brain.

Conclusion

It could be concluded that Tf-coupled PEGylated NP were shown to have a breaching effect for targeting at the tumor site in the brain, and Tf-coupled PEGylated NP systems may prove particularly valuable to enable the use of a drug that seems to be ineffective or toxic if delivered systematically.

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Formulation and evaluation of cysteamine for ophthalmic delivery

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Introduction and Objectives

The ocular symptoms of cystinosis are treated by delivering a water-soluble cysteamine salt via eye drops. Despite excellent patient compliance, eye drops are rapidly drained from the ocular surface. In order to improve the bioavailability of ophthalmic delivery systems, it is desirable to prolong ocular residence time and encourage site-specific delivery through sustained drug release. Ophthalmic gels may provide a viable alternative to the current eye drop formulation.

Method

Carbomer 934 gels were formulated to include either a phenylalanine–cysteamine conjugate (chromophore present) or cysteamine hydrochloride. The rheological properties of each gel were evaluated using an advanced rheometer (AR1000, TA Instruments). Dissolution studies (1 L of media (water), stirred at 100 rpm, sampled every 5 min) were undertaken. Where the formulation lacked ultraviolet activity (cysteamine hydrochloride) a thiol-sensitive dye, Ellman's reagent, was utilized to measure release. Mucoadhesivity testing was performed on a texture analyser using bovine corneas as the mucosa model (contact force, 0.05 N; contact time, 60 s; probe speed 0.5 mm/s).

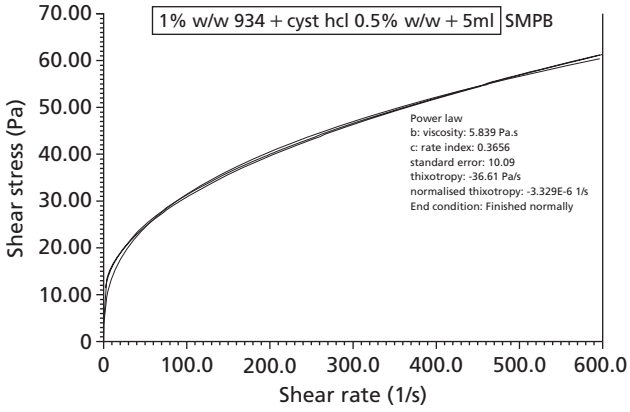

Figure 1 Shear stress versus shear rate for cysteamine hydrochloride gel.

Results and Discussion

All gels demonstrated pseudoplastic behaviour (Figure 1).

Both the actives were released from respective gels over an 8-h period with T_{75} = 180 min. The initial mucoadhesivity results indicated that there was significant adhesion (e.g. average AUC (g.s.): no gel 0.88, gel (no active) 8.72, cysteamine gel 8.21).

Conclusion

The carbomer gels released the actives over an 8-h period. All the formulations demonstrated pseudoplastic rheologies. The initial mucoadhesivity results indicated significant adhesion to bovine cornea. All these characteristics are desirable for ophthalmic gel preparations.

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The effects of surfactants on hydrophobic drug dissolution and their statistical analysis

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Introduction and Objectives

The aim of this research is to study the effects of surfactants on dissolution profiles of paracetamol and naproxen and also investigate the data using various statistical methods. It is likely that the intrinsic variability of the experiments is limited, which would give credence to using fewer samples. However, we would like to see an analysis of this variability i.e. is it constant for all dissolution experiments? How much variability is attributed to laboratory skill of the individual experimenter, equipment or formulation composition?

Methods

Naproxen and paracetamol are hydrophobic drugs. To enhance their release, dissolution media were formulated using Cremophor ELP, Cremophor RH40 and Solutol HS15 (BASF, Ludwigshafen, Germany), above and below their critical micelle concentration (CMC). Above CMC medium was obtained using 66% increases on the actual CMC; for below CMC 66% decreases in the CMC of the various surfactants were prepared. Dissolution profile for each formulation was conducted in triplicate using USP dissolution apparatus-II. In all, 10 ml of samples were withdrawn at 5, 10, 15, 20, 30, 40, 50 and 60 min. Statistical analysis of effects of surfactants and data size was investigated using ANOVA and model-independent methods.

Results and Discussion

Using the ANOVA method, the dissolution profile of paracetamol was significantly affected by Cremophor RH40 below CMC ($H(9) = 44.898$, $P < 0.001$). Therefore, evidence exists that data obtained from paracetamol dissolved in Cremophor RH40 below CMC differ significantly from the control.[1] The dissolution efficiency of paracetamol showed greatest improvement in Cremophor RH40 below CMC with 62.39%. Test statistics ($H(9) = 14.882$ and $P = 0.094$); for Naproxen shows a nonsignificant results; therefore, the null hypothesis was accepted that the data obtained from the dissolution of Naproxen in the mentioned surfactants may have come from the same distribution. Therefore, there are no significant differences between the control and Naproxen in the presence of Cremophor RH40, Solutol HS15 and Cremophor ELP. However, dissolution efficiency of Naproxen showed some improvement in Solutol HS15 at and below CMC giving values of 14.1 and 14.18%, respectively, though not statistically significant. The analysis found that dissolution profiles can be analysed with a combination.

Conclusion

Dissolution efficiency of Naproxen and paracetamol was obtained as model-independent method. Although variability in dissolution data is low, however, when variability in data set is high or the P value obtained is close to 0.05, then increase in sample size should be considered. Therefore, rather than standard triplicate results in dissolution experiment, there might be the need to increase sample sizes in experiments with high variability or significant result close to or bordering 0.05. This will give confidence to results from the statistical analyses. Therefore, a guideline has been proposed as a minimum standard in analysing drug dissolution profiles.

Reference

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Gatifloxacin-loaded solid lipid nanoparticles for topical ocular delivery

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Introduction and Objectives

The aim of this study was to evaluate solid lipid nanoparticles (SLNs) as carriers for topical ocular delivery of gatifloxacin (GTX) to enhance ocular bioavailability in a controlled manner for a desired time interval. SLNs represent an interesting alternative to traditional colloidal carriers as they consist of physiological and biocompatible lipids, which are suitable for the incorporation of lipophilic, hydrophilic and poorly water-soluble active ingredients. Their use might advantageously replace subconjunctival injections, which are necessary for treatment of resistant pseudomonal keratitis, or for prophylaxis against bacterial endophthalmitis, before cataract surgery.

Method

GTX-SLNs consisting of stearic acid alone (SLN-A) and a combination of stearic acid and Compritol (SLN-B), with Poloxamer 188, and a mixture of sodium taurocholate and transcutol and water, were prepared by microemulsion method.[1] The warm microemulsion was dispersed in cold water (5°C) under continuous stirring to obtain SLN. The SLN dispersions were filtered and lyophilised for quantitative determination of the incorporated drug by UV spectrophotometer. Crystallinity, particle size, polydispersity index and zeta potentials of SLNs were determined by differential scanning calorimetry (DSC) and Zetasizer. Permeation study was done using goat cornea in modified Franz diffusion cell.

Results and Discussion

Drug encapsulation efficiencies (%EE) about 77.5% for SLN-A and 89% for SLN-B were recorded. Higher %EE of SLNs showed that the lipid matrix, composed of stearic acid and Compritol possessed spaces where the drug was localised and drug expulsion was almost nonexistent. DSC was used to underline the high drug encapsulation potency of the SLN. The higher the enthalpy of the transitions, the more crystalline the SLN and consequently, the more difficult it will be for any drug to be encapsulated.[2] The enthalpies are 46.76 and 27.051 J/g for SLN-A and SLN-B, respectively. The result showed low crystalline state for the SA : Compritol (80 : 20, w/w) containing SLN, which helped the particles record high drug encapsulation efficiency compared with the SLN prepared with SA alone, which showed endothermic signal at about 59°C, confirming their higher crystallinity. The developed SLNs were in the size range less than 272 nm with PDI about 0.49, which indicated particle size uniformity. The zeta potential of the formulations was found to be −35.04 mV, which showed the stable dispersive nature of the SLNs. The permeated amount through goat cornea was found to be higher at higher drug entrapment (120.45 and 106.35 $\mu g/cm^2$ for SLN-B and SLN-A, respectively). The resulting permeation fluxes were found satisfactory.

Conclusion

The prepared SLNs were found in the colloidal size range with good particle size distribution, stability and high entrapment efficiency. The SLNs permeated the cornea and could be used in the delivery of GTX to the eye to offer a sustained pharmacological effect in the treatment of bacterial conjunctivitis, keratitis and other bacterial infections.

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The use of a dendrimer carrier to enhance paclitaxel delivery and bypass the P-glycoprotein efflux transporter

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Introduction and Objectives

The aim of this study was to evaluate the ability of a third-generation (G3) polyamidoamine (PAMAM) dendrimer-based drug-delivery system to enhance the permeability of paclitaxel (a substrate for the P-glycoprotein (P-gp) efflux transporter) across cellular barriers.

Method

Three and six lauryl (L) chains were covalently attached to the surface amine groups of G3 dendrimer *via* carbamate bonds. Paclitaxel was conjugated to the dendrimer conjugates through glutaric acid (glu) as a linker/spacer. The dendrimer-drug conjugates were labelled with fluorescein isothiocyanate (FITC) to enable quantitative transport studies. The resulting conjugates were characterised using thin layer chromatography (TLC), ^{1}H NMR, ^{13}C NMR and electrospray ionisation (ESI-MS). Cytotoxicity of the dendrimer conjugates was evaluated using the lactate dehydrogenase (LDH) assay. The transepithelial transport of the dendrimers conjugates was analysed in both apical-to-basolateral (A→B) and B→A directions, and the apparent permeability coefficient (P_{app}) was determined.

Results and Discussion

Paclitaxel was linked by its hydroxyl group at the C2 position with glutaric acid, forming an ester bond.[1] The successful formation of the pac-glu was shown by a downfield shift in the ^{1}H NMR spectrum of the methine group (CH-O) from 4.90 ppm in paclitaxel to 5.65 ppm in pac-glu. Pac-glu was conjugated to the surface amine group of G3/lauryl-G3 by the active ester NHS method. The 1 : 1 molar ratio of drug to dendrimer was confirmed by ^{1}H NMR. Lauryl chains were used as a surface modifier to enhance the permeability of the dendrimer-drug conjugates.[2] The measurements of the transport of dendrimer-drug conjugates showed significant increases of P_{app} in both A→B and B→A directions compared to that of free paclitaxel. The A→B P_{app} values of free paclitaxel were approximately 2.7×10^{-7} cm/s and was increased approximately 10 fold when conjugated to G3L6. G3 dendrimer-drug conjugate with six lauryl chains attached (G3L6-glu-pac) showed the highest permeability across the monolayers of Caco-2 cell. The enhanced permeation is possibly due to the positively charged surface of G3 dendrimer and the increased hydrophobicity by lauryl chains enhancing the interaction between the conjugates and the plasma membrane.

Conclusion

Novel dendrimer-based drug conjugates, G3L3-glu-pac and G3L6-glu-pac, labelled with FITC were successfully synthesised and characterised. Enhanced permeation of paclitaxel across the monolayers of Caco-2 cell was found when conjugated to surface-modified G3 dendrimer. This indicates that G3 dendrimer-based drug conjugates can bypass the P-gp efflux transporter and enhance the permeability of paclitaxel. Further study of the cytotoxicity and permeability of paclitaxel and dendrimer-drug conjugates across a blood–brain barrier cell model is ongoing.

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Characterisation of insoluble matrices of Eudragit RL PO prepared by hot-melt extrusion containing metformin hydrochloride as a model hydrophilic drug and quinine base as a model hydrophobic drug

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Introduction and Objectives

The aim of this study was to prepare films containing solid dispersions or solutions of quinine base and metformin hydrochloride in an Eudragit RLPO insoluble matrix using hot-melt extrusion technology, and to study the effect of drug type and loading on solid-state characteristics, polymer–drug interactions, rheological characteristics and in-vitro drug release.

Method

A Prism 16-mm twin screw extruder (Haake MiniLab) was used to prepare the matrices at 135°C, which were then characterised using modulated differential scanning calorimetry (MDSC), dynamic mechanical thermal analysis (DMTA), X-ray diffraction (XRD), attenuated total reflectance (ATR), Fourier transform infrared (FTIR) spectroscopy, Raman spectroscopy and capillary rheometry. The MDSC was run at a ramp rate of 5°C/min and at a modulation temperature amplitude of ±1°C for a modulation period of 60 s. DMTA was run at a ramp rate of 5°C/min with a force track of 125%, a preload force of 0.01N and an amplitude of 50 000 μm. The scanning range in XRD (2Θ) was from 5 to 65°. Drug loadings of 2, 5 and 30% (w/w) metformin hydrochloride and 5, 30 and 50% (w/w) quinine base were investigated. In-vitro drug release was assessed in PBS buffer pH 7.4 to mimic intestinal fluid using USP2 dissolution apparatus. The effect of drug type and concentration on the various physicochemical properties was statistically analysed using a two-way anova ($P < 0.05$).

Results and Discussion

This investigation highlighted marked differences in solubility of metformin and quinine base within the methacrylic acid copolymer. Using MDSC and XRD, it was shown that quinine base formed a one-phase solid solution at concentrations in excess of 25%, while metformin formed a two-phase solid dispersion at concentrations above 2%, attributing to the poorer solubility within the polymer matrix. This could be partially explained by the larger difference in solubility parameter (and hence poorer miscibility) between metformin and RLPO than by that between quinine base and RL PO. The DMTA results showed that the glass transition temperature of the polymer was influenced by drug type and loading. Both the model drugs lowered the glass transition temperature, hence acting as solid-state plasticizers. Quinine base was a more effective solid-state plasticizer than metformin and reduced the glass transition temperature by circa 18°C at a concentration of 5%. Increasing the concentration of quinine base to 30% afforded no further significant reduction in glass transition temperature. Addition of metformin did not cause a significant reduction in glass transition temperature. Capillary rheometry showed that Eudragit RL PO exhibited pseudoplastic flow (shear thinning) when subjected to shear stress within the extruder at the processing temperature, and addition of quinine base or

metformin altered the pseudoplastic behaviour of the polymer substantially. Drug-release results showed that metformin release was rapid compared with quinine base. Approximately 80% of metformin was released within the first 60 min compared with 40% for quinine base, and films with higher drug loading gave a more rapid release profile. These findings show that drug-polymer solubility and loading can strongly influence solid-state characteristics, polymer flow properties, release profile and processing attributes.

Conclusion

Hot-melt extrusion technology has emerged as a key technology for the production of solid solutions and dispersions in a single manufacturing process. These results show that quinine base acts as solid-state plasticizer significantly reducing the processing temperature, whereas metformin does not. Drug type and loading were found to affect drug-release profile. Eudragit RL PO exhibited shear thinning behaviour and addition of drug-polymer flow properties. This study links thermal and chemical characteristics of drug–polymer blends to develop our understanding of the physicochemical processes that occur during extrusion.

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The mechanical and drug-release properties of hydroxypropyl cellulose organogels

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Introduction and Objectives

Hydroxypropyl cellulose (HPC) is a nonionic water-soluble polymer widely used within pharmaceutical formulations, i.e. as a tablet binder, viscosity enhancer, etc. Despite its wide use, there is a notable lack of investigation into the use of HPC as an organogel with the potential for oral drug delivery. The purpose of this investigation was to address this issue by characterising gels formed by HPC in different solvents by means of oscillatory, steady shear and dilute solution measurements gaining an insight, at the macromolecular level, into the ability of HPC to form intermolecular entanglements and how this correlates to drug-release properties.

Method

The HPC gels were prepared by dissolving the required mass of HPC in the given solvent using mechanical stirring. Dynamic and continuous shear rheology was performed on a AR2000 rheometer (TA Instruments, Surrey, England). Dilute solution rheology was performed on a BS U-Tube viscometer. In-vitro drug-release studies were performed using a Caleva 7ST dissolution apparatus in conjunction with paddle stirrers. Gel formulations (5 g) were placed in dissolution tanks containing 900 ml of PBS with sample being removed at predefined intervals with drug release determined using UV spectroscopy. The viscoelastic properties of HPC gels formed in the different solvents were statistically investigated.

Results and Discussion

The elasticity of the HPC formulations in varying solvents increased in the following order: 1,5-pentanediol→1,2-pentanediol→ethylene glycol→ethanol→water. This response would suggest that as the size of the hydrophobic group contained on the solvent is increased polymer entanglements and therefore swelling are favoured. Furthermore, increasing the frequency across all formulations led to an increase in the storage modulus, loss modulus and dynamic viscosity, which was in good accordance with the Maxwell model for viscoelastic materials. At lower concentrations of HPC, the gels exhibited fluid-gel transitions across the frequency studied, whereas higher concentrations displayed gel-like behaviour across the frequency range studied. Dilute solution analysis agreed with dynamic mechanical results with the HPC formulations in 1,5-pentanediol yielding a lower C* than the other solvents with water having the highest C* suggesting that organic solvents are better than aqueous for the hydration of HPC formulations.

Conclusion

This study showed that the use of organic solvents with HPC gel systems offer the advantage of a system less prone to the mechanisms of erosion, and they would also favour the incorporation of both water soluble and insoluble drugs within them. The semisolid systems investigated may prove useful, not only for gel delivery systems but also for semisolid systems incorporated within a capsule for oral drug delivery.

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Drug release from ethyl cellulose: PVA-PEG graft copolymer-coated pellets under biorelevant conditions

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Introduction and Objectives

The aim of this study was to investigate the robustness of drug release from pellets coated with Aquacoat ECD-based films with respect to biorelevant changes during the release conditions (food effects).

Method

Diltiazem HCl and theophylline pellets were coated with an aqueous ethyl cellulose dispersion (Aquacoat-ECD, FMC BioPolymer; plasticization: 25% triethyl citrate) containing small amounts of poly(vinyl alcohol)-poly(ethylene glycol) graft copolymer (PVA-PEG graft copolymer) in a fluidised bed (curing: 1d; 60°C). Drug release was measured using the USP paddle apparatus (100 rpm) in 0.1N HCl, phosphate buffer pH 6.8 (USP) and release media containing different amounts of calcium ions, carbohydrates and fats. Drug release was also measured using the USP apparatus 3 to better simulate the pH changes along the gastrointestinal tract (GIT) with various buffer solutions (2 h:pH 1.2; 0.25 h:pH 5.8; 0.25 h:pH 6.0; 2 h:pH 6.8; 0.5 h:pH 7.2 and 1 h:pH 7.5; dipping speed: 20 dpm).[1] The drug was detected using UV spectroscopy or high-performance liquid chromatography (HPLC).

Results and Discussion

Importantly, the presence of up to 50 mmol/L calcium ions in the release medium did not significantly affect the resulting drug-release kinetics. Also changes in the pH within the physiological range had no major impact on drug release from the investigated systems. For instance, $51.8 \pm 5.4\%$ versus $44.6 \pm 0.6\%$ theophylline and $75.6 \pm 1.6\%$ versus $75.6 \pm 1.7\%$ diltiazem HCl were released after 2-h exposure to 0.1N HCl, followed by 4-h pH 6.8 buffer versus exposure to buffer solutions with the above-mentioned pH sequence better simulating the gradual changes along the GIT. Also when fats and carbohydrates were added to the release medium, drug release remained almost unaltered.

Conclusion

Drug release from pellets coated with ethyl cellulose containing small amounts of PVA-PEG graft copolymer is only little affected by the presence of biorelevant concentrations of calcium ions, fats and carbohydrates as well as by the pH changes along the GIT.

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Polymer-coated liposomal formulations for pH-responsive drug release

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Introduction and Objectives

The aim of this study was to produce and characterise a polymer-coated liposomal system that has the potential for site-specific drug delivery to the colon following oral administration. The coating will need to be gastro-resistant to protect the liposomes through the stomach and remain intact through the small intestine. The current work focuses on testing the formulations in pH conditions typical of various regions in the gastrointestinal (GI) tract.

Method

Cationic multilamellar liposomes containing a model drug (vitamin B_{12}) were prepared using a thin-film hydration method. The liposomes were then coated using the anionic polymer Eudragit S100 through electrostatic interactions. The presence of the coat was indirectly confirmed through zeta potential measurements and changes in vesicle size and size distribution. The formulations were also assessed using light microscopy and electron microscopy (EM). Drug-release trials were conducted for both uncoated and coated formulations at simulated gastric (pH 1.4), small intestine (pH 6.3) and large intestine (pH 7.8) conditions.

Results and Discussion

The zeta potential of the formulations at pH 7 shifted with the inclusion of Eudragit S100 (Evonik Industries, Essen, Germany) from +63 mV for the uncoated formulation to approximately −40 mV for the coated formulation. The presence of the polymer also caused an increase in the average size and size distribution of the formulations. Microscopy confirmed the presence of a coating layer around the liposomes. Drug release profiles from 0 to 140 h were investigated at all conditions. At pH 1.4, drug release at all time points was significantly lower for the coated formulations than the uncoated. Similar trends were observed at pH 6.3, with the coating significantly reducing

Table 1 Drug release (% of total encapsulated) for uncoated and coated liposomes at different pH conditions

Time	pH	% drug release	
		Uncoated	Coated
2 h	1.4	9.7 ± 0.9	2.7 ± 0.3
	6.3	14.8 ± 1.7	3.4 ± 0.4
	7.8	10.0 ± 3.5	11.0 ± 6.1
10 h	1.4	24.7 ± 1.9	7.1 ± 0.4
	6.3	34.1 ± 1.6	12.1 ± 0.4
	7.8	20.0 ± 2.4	20.4 ± 9.5
30 h	1.4	50.4 ± 3.6	18.2 ± 0.2
	6.3	52.3 ± 0.1	22.2 ± 2.6
	7.8	39.5 ± 11.7	33.4 ± 5
70 h	1.4	79.6 ± 3.4	42.8 ± 10.5
	6.3	81.3 ± 1.7	38.6 ± 2.8
	7.8	71.7 ± 8.5	64.0 ± 12.6

Each value represents the mean ± standard deviation of three independent experiments.

drug release. However, at pH 7.8, there was no significant difference between the release profiles of coated and uncoated formulations. Selected time points from the release studies are shown in Table 1.

Conclusion

A polymer-coated liposomal formulation has been prepared, which displays pH-dependent drug-release characteristics. Future work will concentrate on a variety of encapsulating strategies for liposomal formulations including the use of microspheres.

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Particle optimization for enhanced respiratory drug delivery

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Introduction and Objectives

Dry powder inhalers (DPIs) are becoming an increasingly important method of drug delivery to the lungs. DPIs are propellent free, with no excipient except carrier and do not require coordination by the patient. In developing DPIs, drugs are typically micronized to produce particles in the respirable size range of 1–6 μm.[1] This processing step, however, can lead to heterogeneous product containing mechanically activated surfaces with considerable amorphous content. The aim was to evaluate the effect of particle properties of a model compound and their interaction with process variables on the attributes of these materials postmicronization.

Method

Two sieved batches of lactose monohydrate, Respitose SV003 (D50 = 60 μm) and Respitose SV010 (D50 = 105 μm) (DMV Fonterra, Netherland), were evaluated by way of a statistical experimental design. A full-factorial design was used to evaluate the influence of the three process variables (GP = grinding pressure, IP = injector pressure and FR = feed rate) on the quality attributes of micronized material when set at two levels. After micronization, samples were characterized by a range of analytical methods, including laser-diffraction particle-size analysis, scanning electron microscopy (SEM), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), X-ray powder diffraction (XRPD) and inverse gas chromatography (IGC).

Results and Discussion

The results show that the size reduction to low-median size (3–6 μm) can be achieved using a combination of high GP, high IP and high FR. Although high FRs was shown to be the primary factor in generating the ultrafine material, the contribution from secondary factors including high GP or IP was found necessary on occasions. Low polydispersity (1.63) was also achieved using high GP, low IP and low FR. High GP and IP, however, when in combination with high FR showed marked effects on particle crystallinity with disruption of particle surfaces as shown by thermal analysis and IGC data. This could lead to poor formulation stability with associated difficulties in performance, particularly for inhaled medicines. Although the process variables have been shown to influence the quality of the micronized materials, these phenomena also appear to be dependent on the characteristics of the starting material with Respitose SV003 (D50 = 60 μm) showing less marked disruption of crystal form than Respitose SV010 (D50 = 105 μm). The particle-size distribution was also less polydispersed for Respitose SV003 compared to Respitose SV010.

Conclusion

Micronization process variables were shown to influence the particle-size distribution achieved following micronization, with each of the interacting factors conferring effects on particle-size distribution, crystallinity and surface energy. These effects were greatest for larger-sized starting material, which indicates that the quality of micronized materials can be manipulated through control of process variables in combination with starting material characteristics.

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Correlation of physicochemical properties of cationic liposome adjuvants with their in-vitro activation of macrophages

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Introduction and Objectives

Cationic liposomes prepared from dimethyldioctadecylammonium bromide (DDA) with α,α'-trehalose 6,6′-dibehenate (TDB) have been shown to be effective adjuvant delivery systems,[1] and this combination is known to successfully activate macrophages stimulating a Th1 response.[2] However, key characteristics of this formulation, which dictate their adjuvant properties are still not fully elucidated. The objective of this study was to

physicochemically characterise these liposome systems and correlate this with their cytotoxicity and phagocytosis of macrophages *in vitro*, particularly with reference to their cationic lipid content.

Methods

Liposomes were prepared *via* lipid hydration in Tris-buffer (10 mM, pH 7.4). Dynamic light scattering was used to measure particle size and zeta potential. Differential scanning calorimetry (DSC) was performed at 10°C/min to determine the gel-to-liquid crystalline phase. Nonadsorbed antigen was ascertained *via* the bicinchoninic acid (BCA) assay. In-vitro studies were conducted on a macrophage BALB/c cell line. A cell-proliferation (MTS) assay was applied with a sample concentration of 5 μg/ml, with the cell number 8×10^4 cells/ml, with incubation at 37°C, 5% CO_2. A 4-nitrophenyl-N-acetyl-β-D-glucosaminide (NAG) assay was conducted to ascertain levels of phagocytosis for the DDA/TDB-based systems.

Results and Discussion

Replacement of the cationic content within the DDA/TDB formulations with the lipids 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) or 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) influenced particle size with systems under a micrometer until complete cationic replacement occurred. The measured zeta potential was reduced as more DDA was replaced. On antigen loading of the systems, the particle size enhanced beyond a micrometer, yielding a net anionic surface charge after DDA was replaced with DPPC or DSPC. Nonadsorbed antigen was found to be increased upon an increased replacement of the cationic component, DDA. The DSC upon these systems showed that with increasing cationic replacement, the gel-to-liquid crystalline phase occurred at lower values until a thermal event was diminished within the tested temperature range. For cell proliferation, continual replacement of DDA increased cell viability from ~50% by up to 20–30%, displaying a reduction in cytotoxicity. Phagocytosis was detected and present in all the tested systems with phagocytic activity decreasing upon increased replacement of DDA with DPPC or DSPC.

Conclusion

The replacement of DDA within the DDA/TDB adjuvant shows the necessity of DDA in giving the liposome formulations a cationic charge, enhancing their potential efficacy as a vaccine delivery system. DSC of the systems allowed for the gel-to-liquid crystalline phase to be characterised. The necessity of DDA in providing the cationic charge for antigen adsorption was also recognised. In-vitro studies showed favourable cytotoxicity and levels of phagocytosis of macrophages in the tested systems with future activation studies to be completed, and the physicochemical properties of cationic liposome adjuvants can be correlated extensively with their in-vitro behaviour.

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Rheological characterization of novel high-viscosity semisolids for controlled vaginal delivery of HIV microbicides

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Introduction and Objectives

Novel freeze-dried formulations for vaginal delivery of HIV vaccines/microbicides have been developed. These solid formats are easier to handle than gels, yet reconstitute *in vivo* to retain the necessary mucoadhesive properties. In this study, rheological methods have been used to investigate the effect of freeze-drying on viscosity, viscoelastic and viscous flow properties on the reconstituted gels. The four gels tested are based on hydroxyethylcellulose (HEC) and polyvinylpyrrolidone and also contain another mucoadhesive polymer and either bovine serum albumin (BSA) or TMC120. The study was designed to determine whether freeze-drying affected the rheological performance of the reconstituted compared with the initial gels.

Method

The four formulations contained either Noveon AA1, Chitosan, sodium carboxymethycellulose (NaCMC) or Gantrez. Two gel types were analyzed; freeze-dried gels reconstituted with simulated vaginal fluid[1] (SVF) and gels were made up with SVF. Oscillatory rheometry was performed using an AR2000 rheometer (TA Instruments, UK) with 4-cm solvent-trap steel plate geometry at 37°C. The linear viscoelastic region (LVR) was investigated by application of an increasing oscillatory stress (0–40 Pa) at two frequencies. Oscillatory frequency sweep analysis was performed at 16 Pa over a range of 0.1–10 Hz (n = 5). The viscous flow properties of the gels were determined by creep-retardation within their LVR.

Results and Discussion

The gel formulations showed different viscosities, viscoelastic and viscous flow properties, with NoveonAA1

formulations, both freeze-dried reconstitute and nonfreeze-dried gel, showing the highest viscosity and storage modulus. Although, in all cases SVF-based gels showed higher viscosity and storage modulus than the freeze-dried reconstitute. For example, mean viscosity of SVF-based gel containing Noveon and TMC120 was 12094.4 ± 3768 Pa·s, whereas the freeze-dried reconstitute was 6163 ± 253 Pa·s. Addition of BSA to both types of gels greatly decreased the gels' viscosities (e.g. SVF-based gel containing Noveon and BSA was 6547 ± 663 Pa·s, whereas the freeze-dried reconstitute was 2612 ± 143 Pa·s). This effect was similar in all gel formulations except for NaCMC, where freeze-drying decreased the viscosity of the TMC120-loaded freeze-dried reconstitute from 640 ± 95.7 to 408 ± 52 Pa·s, but did not change the viscosities of the BSA-loaded freeze-dried reconstitute, e.g. SVF-based gel containing NaCMC and BSA was 559.3 ± 44.9 Pa·s, whereas the freeze-dried reconstitute was 542 ± 43.6 Pa·s. Also, addition of BSA to both NaCMC gel types did not greatly decrease their viscosities as was seen with the other formulations. The highest storage modulus was seen with formulations containing Noveon and TMC120 or BSA, indicating their high-elastic and low-viscous properties. This indicates the ability to maintain elastic properties longer *in vivo* prior to viscous flow under normal stress.

Conclusion

Freeze-drying the polymer gels had a profound effect on the rheological properties of the gels as shown in the results. Also, the addition of hydrophobic (TMC120) or hydrophilic (BSA) drug may increase or decrease the viscosity of the formulation, depending on the molecular structure of the drug and polymer compound. In this study, it has been shown that freeze-drying of polymeric gel formulations decreased their zero-rate viscosities but not necessarily their viscoelastic and viscous flow properties.

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109 Transdermal insulin delivery using self-dissolving microneedles *in vitro* and *in vivo*

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Introduction and Objectives

To address the limitations of oral delivery and hypodermic injections, microneedles (MNs) have been developed to deliver drugs in minimally invasive fashion with the efficacy of a needle and the convenience of a transdermal patch.[1] To date, the majority of MN-based transdermal studies have employed MNs made of non-Food and Drug Administration (FDA)-approved material – silicon, which has raised safety concerns in relation to the breakage of silicon MNs. In contrast, polymer MNs constitute an attractive alternative as an inexpensive and biocompatible drug-delivery device. This study was designed to assess the feasibility of transdermal insulin delivery using self-dissolving MNs *in vitro.*

Method

A micromoulding process was employed to prepare laser-drilled MNs. The MN matrix was formed from poly (methyvinylether/maleic acid) (PMVE/MA).[2] Bovine insulin was incorporated into matrix by hand mixing to solubilise the drug. To investigate the conformational changes in the insulin structure after loading into MNs, circular dichroism spectra were obtained. In-vitro drug-release studies from MNs were performed using Franz diffusion cells. In-vivo effect on MNs was assessed by their percutaneous administration to diabetic-induced rats and measurement of blood-glucose levels.

Results and Discussion

MNs prepared from PMVE/MA and loaded with insulin-constituted exact counterparts of mould dimensions and were approximately 600 μm high and 300 μm wide. Circular dichroism analysis of insulin solution compared with insulin incorporated into MN matrix and then released by dissolving in 0.01 M HCl showed no detectable change in protein secondary structure. In-vitro study revealed that the total amount of insulin loaded into the needles was released within 1.5 h. When intact silicone membrane, used as a control, was employed, no insulin was detected in the receiver compartment after 12 h. Blood-glucose levels after 3 h from percutaneous administration of MN arrays to rats decreased by approximately 70% compared with initially recorded blood-glucose levels.

Conclusion

In conclusion, the present study demonstrated the dissolvable MN design involving fabrication under mild conditions, without subjecting active substance to severe conditions, such as elevated temperature. Insulin incorporated into PMVE/MA matrices was proved to retain its secondary structure. After administration of insulin-loaded MNs, hypoglycaemic effect was obtained in rats. PMVE/MA MNs were shown to be useful drug-delivery system for the percutaneous administration of insulin.

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Encapsulation of epirubicin for the treatment of cancer

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Introduction and Objectives

Many synthetic and natural polymers have been used for drug delivery. Encapsulation of drugs by polymers can be used to achieve a sustained release, which could effectively lead to fewer side effects. The main objectives of this research were to investigate the possibility of encapsulating epirubicin within functionalised polyesters as a new method of controlled drug delivery. The particle preparation parameters were altered to optimise the encapsulation of the anticancer drug and its release *in vitro* and in cell culture were assessed.

Method

The multiple emulsion-solvent-evaporation method[1] was used to encapsulate epirubicin using two polyesters: poly (propandiol adipate)-co-ω-pentadecalactone (PPA-co-PDL) and poly(gycerol adipate)-co-ω-pentadecalactone (PGA-co-PDL). Particles were prepared employing a range of different variables, such as polymer type, speed of emulsification and solvent used. The prepared epirubicin particles were assessed for encapsulation efficiency and visualised using scanning electron microscopy. They were further subjected to *in-vitro* release studies over a period of 24 h at 37°C, pH 7.4. Exploiting the fluorescent nature of epirubicin (excitation 480 nm and emission 590 nm), the amount of released drug was assessed at regular time intervals. The particles with high-encapsulation efficiencies and adequate release profiles were subsequently tested in cell culture using EJ138 bladder cancer cells. Cell viability was quantified using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) toxicity assay.[2]

Results and Discussion

Analysis from the fluorescence assays suggested that using a higher emulsification speed, thus producing particles of approximately 1 μm in diameter and using the more hydrophilic polymer, PGA-co-PDL, increased the encapsulation efficiency from 35 to 95%. However, changing the oil phase from dichloromethane to ethyl acetate resulted in a lower encapsulation efficiency and drug load. The epirubicin *in-vitro* release profiles all showed that maximum release was achieved within 2 h. Particles made from PGA-co-PDL and PPA-co-PDL were found to release up to 30 and 55 ng epirubicin per milligram particles, respectively. From the cell culture assays, the PGA-co-PDL drug-containing particles resulted in the highest reduction in cell viability, up to 80%. This was comparable to the response that was achieved with the control, nonencapsulated, epirubicin at the same concentration of drug (500 ng/ml).

Conclusion

It was concluded that the encapsulation efficiency of epirubicin can be tailored by changing various parameters. Increasing the emulsification speed resulted in smaller particles and shorter preparation times, contributing to an increase in encapsulation. Changing the polymer to PGA-co-PDL, which has pendant hydroxyl groups, also resulted in increased encapsulation. Epirubicin delivered via the prepared particles was able to reduce the cell viability by up to 80%. Encapsulation of epirubicin by these polymers can be used for immediate release formulations and future work may explore the possibility of conjugating epirubicin along the polymer backbone to generate a more sustainable release profile.

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Spectroscopic and structural characterisation of a beclomethasone dipropionate clathrate pre- and post-micronisation for inclusion in a pMDI formulation

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Introduction and Objectives

Clathrates are crystalline inclusion compounds consisting of a lattice of a molecule that hosts a second type of guest molecule within its structure. Clathrates are thermodynamically unstable and tend to dissociate rapidly when removed from their stabilising medium, because of the presence of large empty cavities at the core of their structure. Guest molecules inhibit such a collapse and render the structure more thermodynamically stable. The aim of this study is to investigate and characterise the physicochemical properties of beclomethasone dipropionate (BDP) crystallised from tricholoromonofluoromethane (CFC-11) and determine the potential changes in surface energy after size reduction.

Method

BDP is a widely used corticosteroid for the treatment of asthma. It is formulated in metered dose inhalers (MDI) in the presence of propellant and other formulation ingredients. Crystal growth of anhydrous BDP in CFC-11 was examined. The crystals investigated in this study were grown in 1.67% w/w BDP in CFC-11 at room temperature. Under these conditions, BDP crystallises with a channel structure that allows the insertion of CFC-11 molecules. The structure is held together through hydrogen bonding.[1] An isopropyl alcohol clathrate of BDP and an ethanol solvate of BDP were also investigated. The effect of size reduction in these three different entities was investigated using atomic force microscopy (AFM) surface energy determination and adhesions measurements.

Results and Discussion

Anhydrous BDP suspended in CFC-11 resulted in spontaneous crystal growth. The structure of the BDP CFC-11 clathrate was characterised using X-ray powder diffraction, AFM topography imaging and Raman spectroscopy. Variable temperature Raman spectroscopy was also used to investigate the presence of any structural changes to the BDP CFC-11 clathrate. AFM was also employed for the determination of the dispersive surface free energy (SE) and the force of adhesion (F_{adh}) of both the crystalline and ball-milled BDP CFC-11 clathrate with different pMDI components in a model propellant (decafluoropentane). Variable temperature Raman spectroscopy shows a change in the structure of BDP CFC-11 clathrate at around 99°C, which corresponds to the release of CFC-11 from the clathrate structure due to a solid-state rearrangement of the crystal structure. The surface free energies for anhydrous BDP (micronised), BDP CFC-11 clathrate (ball milled for 2.5 h) and the CFC-11 clathrate (crystalline) are 47.5 ± 4.9, 15.24 ± 1.26 and 11.27 ± 4.05 mJ/m^2, respectively. F_{adh} results show that BDP CFC-11 clathrates have a lower F_{adh} as compared to anhydrous BDP with the different pMDI components studied.

Conclusions

We have characterised BDP CFC-11 clathrate pre- and post-micronisation and shown quite clearly the transitions involved during desolvation. In addition, to the authors' knowledge, this is also the first time that a propellant clathrate has been shown to be beneficial in terms of reduction in the F_{adh} with pMDI components. This could have implications for future HFA formulation development with APIs that are prone to the formation of propellant clathrates.

Reference

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Material Science

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Rheological characterisation of chitosan and poly (methyl vinyl ether-co-maleic acid) hydrogels

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Introduction and Objectives

Chitosan is a linear polysaccharide made by N-deacetylation of chitin. The increased solubility of chitosan with respect to that of chitin is related to its positively charged polyelectrolyte nature, due to the protonation of the free amine groups below a pH of 6.2. Poly (methyl vinyl ether-co-maeic acid) (gantrez S-97 BF) is a pharmaceutically acceptable copolymer with bioadhesive properties. However, the use of gantrez or chitosan as a primary component in drug delivery systems is restricted by its inappropriate rheological properties.[1] The aim of this study was to characterise the rheological and viscoelastic properties of chitosan and gantrez hydrogels.

Method

Chitosan solutions of 1 and 2% (w/w) were prepared using 1% (v/v) acetic acid. Gantrez gels of 7.5 and 15% (w/w) were prepared using different ratios of glycerol–water (80 : 20, 60 : 40 and 50 : 50). The gantrez gels (50 g) were then added to 30 g of chitosan solution. Viscoelastic properties of chitosan-gantrez hydrogel samples were applied to the lower stationery plate of the rheometer using stainless steel trap solvent plates of 4 cm diameter and a gap size of 1000 μm. The linear viscoelastic region was determined by torque sweep from 0.1 to 100 Pa at frequencies of 0.01 and 100 Hz at (25 ± 1°C), and was identified as the region where stress was directly proportional to strain and storage modulus (G'). The Fourier transform infrared spectroscopy (FTIR) was performed on samples of chitosan, gantrez powders and chitosan-gantrez hydrogel samples at a scanning range of 4000–400 cm^{-1}.

Results and Discussion

The rheological properties of chitosan and gantrez hydrogels enhanced significantly showing higher G' and G'', especially at higher concentrations of gantrez 15% (w/w) and chitosan 2% (w/w), indicating an increase in the viscosity in comparison to chitosan and gantrez gels each alone. A nonhomogeneous highly viscous gel was produced by adding an aqueous solution of gantrez to chitosan solution that becomes significantly more homogenous by using a combination of glycerol with water in dissolving gantrez.

Increasing the concentration of glycerol increased the storage and loss moduli and dynamic viscosity but decreased the loss tangent. The FTIR studies showed a significant shift in the carbonyl group of gantrez in chitosan-gantrez hydrogel samples compared with gantrez powder from 1701 to 1709 cm^{-1}, indicating the formation of hydrogen bonding of the carbonyl groups of gantrez with the amine groups of chitosan, which has a critical role in strong gel formation once gantrez solution was added to chitosan solution even that without using glycerol as a solvent.

Conclusion

It was observed that the rheological behaviour of chitosan and gantrez system changed as a function of polymer concentration forming a hydrogel of acceptable visocoelastic properties to develop a wide range of products for various pharmaceutical applications.

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The crystallisation of theophylline *via* a Langmuir–Blodgett approach

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Introduction and Objectives

This preliminary study describes a strategy to generate theophylline monohydrate and anhydrous crystals *via* the Langmuir–Blodgett (LB) approach. Theophylline is used routinely to manage respiratory disease and has previously been subject to investigation. The LB approach is well suited to perform crystal growth studies as the regular array of surfactant molecules provides nucleation and growth sites for crystallization. To our knowledge, this study represents the first time that crystals of a therapeutic agent have been generated *via* this route. It is envisaged that the methods applied will provide a platform on which to develop the understanding of crystal growth mechanisms.

Method

Crystallization was performed under surfactant monolayers at the air–water interface (pH 7.0/ambient conditions). The surfactants octadecylamine (ODA) and didodecyldimethylammonium bromide (DDAB) were individually dissolved in chloroform (1 mg/ml), and 10 μl spread across an aqueous and methanol subphase containing theophylline (5.7 mg/ml). Ten minutes were allowed for chloroform evaporation, and the monolayers were compressed to 20 mNm^{-1} and 30 mNm^{-1} and allowed to stand for 16 h to facilitate crystallization. Reference systems included batch crystallization and the absence of a LB monolayer. The crystalline material was analysed using a differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD) and scanning electron microscopy (SEM).

Results and Discussion

The LB isotherms of ODA and DDAB monolayers on pure water reflected plots described in literature. When theophylline was dissolved in the subphase, deviation in the isotherm was noted, signifying monolayer–theophylline interaction. Crystals were recovered and visualised using SEM. The theophylline monohydrate crystals were needle-like and exhibited evidence of fracture/fragmentation. The anhydrous theophylline samples were composed of smaller crystalline particles that were closely associated with each other, suggesting a cohesive nature. Crystalline material did present in the absence of a monolayer, however to a lesser extent. The DSC data confirmed the presence of water in the crystalline samples prepared in the aqueous environment, with the converse true for the material produced with the methanol subphase. The PXRD data show that theophylline monohydrate was obtained from the aqueous subphase in the presence of both the surfactants. Anhydrous theophylline was obtained using methanol as the subphase.

Conclusion

We have shown that compressed monolayers of ODA and DDAB support the crystallisation of theophylline at the LB air–water interface. The LB data indicate theophylline molecules within solution do interact with ODA and DDAB surfactant monolayers. We propose the principal mechanism of interaction is an ion-dipole association between the surfactant molecules and functionalities within the theophylline molecule. Future work shall involve the application of the atomic force microscope to probe pharmaceutically relevant parameters of theophylline crystals. It is anticipated the approaches applied here will provide a detailed understanding of crystal growth mechanisms and inform formulation practice.

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A novel application of amino acids as matrix-forming agents in lyophilised rapid disintegrating tablets

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Introduction and Objectives

The current study aims to investigate the feasibility of using cysteine, glutamine, glycine and serine as novel matrix-forming agents in the formulation of rapid disintegrating

tablets (RDT), by studying the effect of inclusion of these amino acids on the tablet properties.

Method

The RDTs were prepared by adding L-cysteine, glycine, L-glutamine and L-serine to 5% (w/w) low bloom strength gelatin (60) stock solutions at concentrations of 10, 30, 50 and 70% of total solid material. Approximately 1.5 g of the solution was poured into a tablet mould (13.5-mm diameter), frozen at −80°C and freeze-dried. The disintegration time of the lyophilised tablets was measured according to the United States Phamacopeia (USP) disintegration test. The hardness of the tablets was evaluated using texture analysis. The peak force (N) after 1 mm penetration of 5-mm diameter probe at a speed of 6 mm/min was determined.

Results and Discussion

Lyophilised tablet index (LTI) was calculated using the following equation: LTI = (H/DT) / (H°/DT°), where H: hardness of the tested tablet, DT: disintegration time of the tested tablet, H°: hardness of the control tablet (gelatin alone) and DT°: disintegration time of the control tablet. The LTI values provided a ratio indicative of whether the prepared amino acid formulation was better than the gelatine-only formulation (control). Values greater than 1 indicate improvements over the control, whereas values lower than 1 reflect compromise in the overall tablet properties (disintegration time and/or hardness).

The LTI values (Table 1) suggested that 30% glutamine formulation achieved the highest value (3.39) by displaying a hardness of 14.40 ± 1.26 N and disintegration time of 9 ± 3.61 s compared to 13.50 ± 0.74 N and 29 ± 2.16 s, respectively, for the control. Moreover, 10% glycine, 30% cysteine and 30% serine formulation improved the tablet properties, which were revealed by high LTI of 2.54, 1.35 and 1.26, respectively.

Conclusion

LTI allows simple assessment of the overall improvement in tablet properties and helps in obtaining the required balance between the disintegration time and hardness. Inclusion of the tested amino acids improved tablet properties significantly and exhibited concentration-dependent profiles.

Table 1 The lyophilised tablet index of the tablets

Amino acid	LTI			
	10%	30%	50%	70%
Cysteine	0.82	1.35	0.44	0.53
Glutamine	0.55	3.39	Not soluble	Not soluble
Glycine	2.54	1.01	0.59	Tablet collapse
Serine	0.72	1.26	0.67	0.30

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Characterisation of milled lactose particles using DSC, MTDSC, FTIR, X-ray diffraction and nanoscale thermal probe techniques

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Introduction and Objectives

Lactose is a commonly used carrier in dry powder inhaler formulations. Micronization of crystalline lactose often leads to disruption of its crystalline order and varying degrees of disorder might be possible. Any amorphous or disordered content has the potential to affect the behaviour of an inhaler formulation; hence, it is important to characterise this disorder and its distribution between the interior of the particles and their surfaces. The powder, after micronization, was analysed using various bulk techniques. We also describe the use of nanothermal analysis (nano-TA) and a novel use of force microscopy at different tip temperatures to interrogate surface properties.

Method

Anhydrous lactose powder was milled in a simple ceramic ball mill. The global polymorph and amorphous content was analysed using X-ray diffraction, FTIR, differential scanning calorimetry (DSC) and modulated differential scanning calorimetry (MTDSC). After depositing the particles on a glass slide coated with fast-setting araldite, the particle surface was imaged with scanning probe microscope using thermal nanoprobes. Selected regions on the particle surfaces were characterised using nano-TA and force microscopy at various tip temperatures (from room temperature to 120°C).

Results and Discussion

The DSC and FTIR results of crystalline lactose show that the starting material was mainly β-anomer lactose. Amorphous content of the milled lactose was measured to be 28% by X-ray diffraction and 23% by MTDSC. In earlier studies on model systems of mixtures of crystalline and amorphous lactose, it is proved straightforward to distinguish between crystalline and amorphous material by nano-TA.[1] In particular, the onset temperatures from local thermomechanical analysis measurements (L-TMA) for the glass transition and melting were highly reproducible with values of 130°C (SD 1.9°C) and 223°C (SD 3.3°C), respectively. The surfaces of the particles proved to be far more complex and heterogeneous. 105 measurements on 30 particles showed a wide range of onset temperatures distributed between 40 and 230°C. Force distance curves at progressively increasing tip temperature confirmed a distribution of onset temperatures for increased pull-off force (a measure of the glass transition temperature) supporting the L-TMA results. These data imply

that there is a broad range of states on the surface. Heated-tip pulsed force mode (PFM) images did not give clear domains, this also implies a complex surface morphology.

Conclusion

L-TMA, heated-tip force distance curve (FDC) and PFM imaging, all imply a very heterogeneous surface with a broad range of transition temperatures. The L-TMA experiments that showed an onset lower than 130°C most probably indicate the prescience of plasticised amorphous material; those with onsets between 130 and 240°C pose a more difficult interpretation problem. Further work is being undertaken to clarify the cause of this behaviour.

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Characterisation of thermal and rheological properties of zidovudine, lamivudine and ethyl cellulose blends to assess their suitability for hot-melt extrusion

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Introduction and Objectives

The aim of this study was to characterise the thermal and rheological properties of zidovudine, lamivudine (Anti HIV/AIDS drug) and ethylcellulose physical mixtures in different proportions and to assess their suitability for hot-melt extrusion (HME) for the production of sustained release monoliths. HME has been shown to control and modify drug release.[1] The objective was to determine the processing parameters that would be applied during HME to optimise process and formulation attributes.

Method

Polymer and drugs mixtures were prepared at ratios 80:20, 70:30 and 60:40% w/w, respectively. Tri ethyl citrate (TEC) and PEG 6000 were chosen as plasticisers. Physical mixtures of polymer and these plasticisers were prepared at ratios of 95:5, 90:10, 80:20, 70:30 and 60:40% w/w, respectively. Differential scanning calorimetric (DSC) analysis was carried out using a Q 2000 DSC (TA instruments, NJ). Samples (5 mg) were prepared in sealed aluminium pans and scanned at a heating rate of 5°C/min. Rheological parameters were obtained using the Anton Paar MCR 310 rheometer (Anton Paar GmbH, Austria) with parallel plates in oscillation mode. Frequency sweep was applied with torque range of 100–0.1 mN.

Results and Discussion

Viscosity was considerably lower for the physical mixtures containing zidovudine than for the polymer melt alone. This indicates that the drug may be acting as a plasticiser during extrusion, which was confirmed by lowering of glass transition temperature (T_g) of the physical mixtures (from 125 to 115°C) as measured using DSC. Lamivudine did not however significantly lower the glass transition temperature (T_g) of ethylcellulose, yet decreased the viscosity of the physical mixture with increase in concentration. The DSC data obtained for physical mixtures containing polymer and plasticisers showed that both TEC and PEG-6000 lowered the T_g of ethylcellulose. PEG-6000 however re-crystallised upon cooling, which makes it unsuitable for use in the controlled release formulation. Both plasticisers were shown to reduce the viscosity of ethylcellulose, although reductions in viscosity were greatest for TEC.

Conclusions

The results show that suitably low viscosity can be achieved to enable HME of ethylcellulose, zidovudine and lamivudine in the presence of TEC as a plasticiser. Zidovudine appears to have a plasticising effect, whereas the impact of lamivudine is not clear. TEC has also shown to be a more effective plasticiser than PEG-6000.

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Understanding miscibility within drug–polymer solid dispersions

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Introduction and Objectives

Seventy percent of new chemical entities are BCS class II and thus have a bioavailability that is limited by dissolution in gastrointestinal fluids.[1] Consequently, enhancing aqueous solubility is a significant challenge in formulating these compounds. Solid dispersions (SD) are one of the many different approaches that have been adopted to overcome this challenge. Lutrol F68 (BASF, Ludwigshafen, Germany) is a semicrystalline copolymer that forms amorphous solid

dispersed 'cages' that trap drugs and enhance solubility. In this study, we have investigated the miscibility of a class II drug, mefenamic acid (MA),[2] in Lutrol F68 and determined the effect of miscibility on in-vitro dissolution.

Methods

The SD with drug loadings ranging from 2 to 55% w/w were prepared by heating the Lutrol F68 to 65°C using a temperature-controlled stage, before adding the required quantity of MA and stirring continuously for 30 min. Mixtures were left at room temperature for 6 h and then milled to 90–125 μm. High-speed differential scanning calorimetry (hyper-DSC), Raman spectroscopy, PXRD and hot-stage/fluorescence microscopy were used to assess the miscibility of the MA in both the molten and the solid Lutrol F68. Drug-dissolution studies were conducted on the SD using phosphate buffer at pH 6.8 as dissolution media.

Results and Discussion

Drug miscibility examined using hyper-DSC, Raman spectroscopy and hot-stage microscopy suggested MA was soluble in molten Lutrol F68 up to a concentration of 35% w/w. Using Raman spectroscopy, PXRD and fluorescence microscopy, the solubility in the solid-state matrix, however, appears to be limited to <15% w/w. As expected the dissolution properties of MA are significantly influenced by the solubility of the drug in the polymer matrix. At a concentration of 10% w/w (i.e. a single-phase system), dissolution of MA in phosphate buffer at pH 6.8 was rapid, whereas at a concentration of 50% w/w (i.e. a biphasic system) dissolution of MA was significantly slower. Nevertheless, it is evident that all formulations significantly enhanced dissolution of MA compared with the pure drug.

Conclusion

This study has clearly demonstrated the complexity of drug–polymer binary blends and in particular assessing drug–polymer miscibility in the solid state. Moreover, it has demonstrated that the drug miscibility with a polymeric matrix has significant effect upon the in-vitro dissolution properties of solid drug–polymer binary blends.

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Effect of processing conditions on solid lipid matrices for drug delivery

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Introduction and Objectives

Solid lipid matrices possess a variety of physicochemical properties, providing an array of formulation possibilities, including enhanced solubility and prolonged release. Production generally involves a hot-melt method.[1] However, there are few examples examining the effects of processing conditions such as cooling rate. The Syncrowaxes are a range of synthetic wax esters and are mainly used in the cosmetics industry. There are currently no reports on their use for pharmaceutical applications. Therefore, our aim is to determine the release profile for a model drug, felodipine, from these materials and also investigate the effect of processing conditions.

Methods

The effect of supercooling was investigated by preparing samples *via* a hot-melt method, before cooling with liquid nitrogen. Samples were also prepared using a compression mould to achieve a controlled-cooling rate of 10°C/min. The differential scanning calorimetry (DSC) studies were conducted with samples of 5.0–7.0 mg being subjected to a thermal ramp of 10°C/min from 25 to 100°C (Table 1). Powder X-ray diffraction patterns were collected with samples packed onto graphite holders and scanned from 3° to 40° 2θ at a scan rate of 0.0423° 2θ per min. Dissolution studies were performed in triplicate using the paddle method in PBS (pH 6.5) containing 2% polysorbate 20 at 37°C.

Results and Discussion

Dissolution studies showed the wax inhibited release compared to drug alone, with a t_{50} of 8 h for the BB4 grade versus 2 h for the drug. PXRD traces showed there was no change in the polymorphic form of the samples following processing.

Conclusion

This study has shown that processing conditions can have a significant effect on the structure of the lipid matrix. Also, Syncrowax materials have a potential application in the development of a controlled-release formulation.

Reference

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Table 1 DSC enthalpies of samples under varied conditions

Syncrowax grade	As received (J/g)	Platen pressed (J/g)	Liquid nitrogen (J/g)
ERLC	203.64 ± 11.60	192.46 ± 9.96	180.58 ± 2.30
BB4	189.38 ± 3.55	153.58 ± 13.58	139.08 ± 5.18
HRC	148.38 ± 7.27	140.38 ± 5.15	122.8 ± 5.55
HGLC	149.4 ± 5.32	142.06 ± 7.85	113.1 ± 4.97

Medicinal Chemistry

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A quantitative structure-activity relationship analysis of the physicochemical determinants for organic anion transporting polypeptide 1A2 binding

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Introduction and Objectives

The superfamily of membrane transporters organic anion transporting polypeptides (OATPs) has a significant influence on the absorption, distribution, metabolism and elimination of xenobiotics.[1] The OATP1A2 subtype is localised in brain, liver, kidney and small intestine and has broad substrate specificity (including, e.g. fexofenadine and methotrexate). With respect to (quantitative) structure-activity relationships ((Q)SARs), little information is available for OATPs. The aim of this study was to develop a 2D-QSAR approach to identify specific physicochemical determinants of binding affinity between chemicals and OATP1A2.

Method

Human OATP1A2 apparent affinity (Km) values of 17 diverse chemicals were obtained from the studies of Hagenbuch and Gui.[2] These were used to derive partial least square (PLS) 2D-QSARs using the Molecular Operating Environment software (version 2009.03; Chemical Computing Group, Inc., Montreal, Quebec, Canada). The data set was subdivided into a training set of 13 compounds and a test set of 4 compounds. Five molecular descriptors were calculated: these were the logarithm of the octanol/water partition coefficient (log *P*), the number of hydrogen bond donor (a_don) and acceptor (a_acc) atoms and the total positive (q_vsa_pos) and negative (q_vsa_neg) van der Waals surface area.

Results and Discussion

Preliminary analysis of the full data set using all five descriptors led to a three parameter principal component model (model A) presenting a satisfactory predictive ability. The interpretation of PLS coefficients obtained for this model show the relationship between simple physicochemical features (e.g. hydrogen bonding capacities and lipophilicity) and binding potency between compounds and OATP1A2. For example, the positive contribution of log *P* indicates that affinity between ligands and OATP1A2 is favoured by higher lipophilicity of chemicals. To assess the external predictive ability, the 13 compound training set was used to derive a new model (model B). Good predictive capacity of model B was confirmed by the correct prediction obtained for three compounds of the training set. However, affinity was underestimated for one compound (rosuvastatin).

Conclusion

A predictive 2D-QSAR model for OATP1A2 has been developed using a set of 17 diverse chemicals. This model is based on simple descriptors in an attempt to understand the physicochemical features, which govern the molecular interactions between chemicals and OATP1A2. As few (Q)SARs are available for OATP affinity, these preliminary, positive results for OATP1A2 encourages future development of (Q)SARs in this area.

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Pharmaceutical Microbiology

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Investigation of the activity of triclosan on growth, morphology and cell integrity of bacteria

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Introduction and Objectives

Triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether) is a widely used broad-spectrum antibacterial agent. This agent has been shown to act upon enoyl-ACP-reductase as the primary target thereby inhibiting fatty acid synthesis.[1] This investigation was undertaken to further examine the antibacterial activity of Triclosan.

Methods

Effects of Triclosan were established by measuring viability of *Escherichia coli* NCTC 4174 and *Staphylococcus aureus* NCTC 6571 using standard microbroth assays. Impact of antimicrobial exposure on bacterial viability was also examined by flow cytometry, which measured fluorescence from cultures treated with propidium iodide (nonviable) or

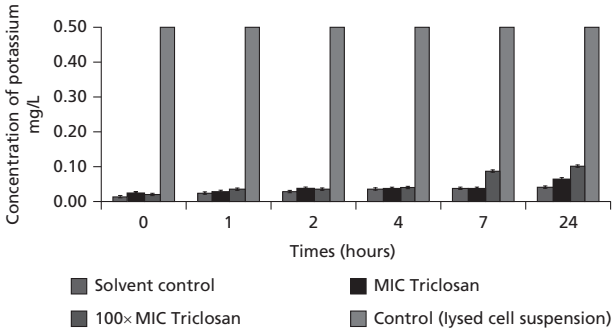

Figure 1 Loss of potassium from *E. coli* 4174 upon incubation with Triclosan; $N = 3$ SD, Maximum potassium loss = 3.2 mg/l in lysed cell suspension (MIC = 0.07 μg/ml).

SYTO 9 (viable). Scanning electron microscopy monitored bacterial morphology for changes in response to Triclosan. Damage to the cytoplasmic membrane was examined by measuring amount of internal potassium lost by populations of 1×10^6 cfu/ml cells using flame emission spectroscopy.

Results and Discussion

Analysis of bacterial viability established that Triclosan exerts a bacteriostatic effect at low concentrations, whereas high Triclosan concentrations are bactericidal. Triclosan inhibits onset of logarithmic growth phase in a concentration-dependent manner. Changes in dynamics of bacterial growth, dependant on length of exposure to/concentration of Triclosan, were identified *via* flow cytometry. Incubation of *E. coli* or *S. aureus* with either sub-minimum inhibitory concentration (MIC) or MIC of Triclosan revealed, *via* SEM, presence of cellular aggregates. Incubation of bacteria with Triclosan induced minimal potassium loss (Figure 1), suggesting that structural integrity of the bacterial membrane is not compromised by this agent. While previous investigations reported Triclosan to induce potassium loss, our investigation demonstrates potassium leakage does not occur, even at 100 × MIC.

Conclusion

Analysis *via* flow cytometry identified Triclosan induced changes in the dynamics of bacterial growth. Examination of bacterial populations revealed that Triclosan promotes cellular aggregation. Contrary to some previous reports, data presented here show this agent does not induce leakage of cytoplasmic potassium. This data confirm findings of Villalaín *et al.*[2] that structural integrity of the membrane is not compromised by Triclosan.

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Development of an in-vitro model for oral biofilm studies

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Introduction and Objectives

Oral biofilms generally exist in a benign state in healthy individuals; however, a shift in the microbial community exacerbated by factors such as poor oral hygiene can result in dental disease. The aim of this study was to develop reproducible biofilms of the cariogenic bacteria *Streptococcus sanguinis* and *Streptococcus mutans* on surface-characterised poly(methyl methacrylate) (PMMA), bovine enamel and bovine dentine using a modified Robbins device (MRD) that allows control over key parameters including flow rate, shear force and temperature.

Method

Surface energies of untreated substrates and substrates treated with mucin-containing artificial saliva (MAS) were calculated using contact angle goniometry; surface roughness was determined using atomic force microscopy (AFM). Substrates (500 μm thickness) were mounted on the MRD and exposed to MAS (30 min, 37°C, 0.45 ml/min), followed by monospecies bacterial suspensions (10^8 CFU/mL, 3 h, 37°C, 0.45 ml/min, 1 h static) and then to maintenance media (14 h). Substrates were imaged at 30 min and 18 h using confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM). Cell enumeration was achieved by sonication and vortexing of the substrates in phosphate-buffered saline containing Tween 80, followed by viable plate counts.

Results and Discussion

Surface energy data for MAS-treated substrates showed an increase in hydrophilicity for PMMA (55.5 mJ/m^2) and a decrease in hydrophilicity for bovine enamel (50.5 mJ/m^2) and dentine (54.0 mJ/m^2) versus native surfaces (42.6, 57.4 and 60.8 mJ/m^2, respectively). Following MAS treatment, contact mode AFM data for PMMA and enamel showed an increase in surface roughness while dentine showed a decrease, most likely due to the acquired pellicle occluding the tubules, which contribute to the roughness of this surface. One-way ANOVA indicated no significant difference ($P > 0.05$) between the formation of biofilms at various positions in the MRD for either species. Colonisation of PMMA, enamel and dentine by *S. sanguinis* and *S. mutans*

was apparent at 30 min; cell adhesion to dentine was typically associated with the tubules. Both organisms formed substantial biofilms after 18-h incubation, with *S. sanguinis* showing complete surface coverage while *S. mutans* showed more sparsely colonised areas, presumably attributing to its role as a secondary coloniser. However, the numbers of cells comprising 18-h biofilms of each species were not significantly different for PMMA and enamel (two-way ANOVA, $P > 0.05$). This could be due to the *S. mutans* biofilm being thicker in areas of colonisation despite an apparently lower surface coverage than *S. sanguinis*. The biofilms formed by each species on dentine resulted in significantly different viable counts ($P < 0.05$), with higher counts attributed to *S. mutans*.

Conclusion

MAS treatment of thin section of PMMA, enamel and dentine substrates within the flow cell causes surface modification due to acquisition of a salivary pellicle. Observations performed using CLSM and SEM combined with viable plate counts confirmed that it was possible to develop multiple, reproducible biofilms on MAS-treated surfaces using the MRD. This study has shown that the MRD is a suitable model for studying the development of biofilms on characterised test substrates of clear relevance to the oral cavity, and could be used to further investigate conditions and treatments pertinent to the oral environment and oral health care.

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Antimicrobial effect of tea tree oil and silver: potential enhancement with liposomal encapsulation

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Introduction and Objectives

Increasing antibiotic resistance of microbial pathogens has refocused scientific interest on preantibiotic antimicrobial compounds, such as silver ($AgNO_3$) and tea tree oil (TTO). Increased rate of kill is commonly observed with increased dose, but over-dosage could result in cytotoxicity and development of side effects. Thus, it would be useful if lower doses of each antimicrobial agent can be used in combination to achieve an effective treatment. Similarly, controlled release from appropriately formulated liposome-encapsulated agents could protect healthy tissues from overexposure.

Method

Minimum lethal concentration (MLC) of the agents (TTO and silver) needed to kill the microorganisms within 24 h was determined against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. Furthermore investigations measured the killing rate of decreasing concentrations of the agents in combination to determine an optimum lower combined dose. The reverse-phase evaporation vesicles method (REV) was used to synthesize liposomes composed of phosphatidylcholine and cholesterol (2:1 molar ratio). The lipids were hydrated with aqueous phase of 2.5% $AgNO_3$ solution to encapsulate the agent in the liposome. Time-kill analyses were conducted on broth cultures of *P. aeruginosa*, *S. aureus* and *C. albicans* treated with the liposomal silver at concentrations approximately equivalent to the MLC of free silver. A necessary step prior to the encapsulation of lipophilic TTO is to emulsify the oil with 1.0% wt/vol polyvinyl alcohol (PVA) (13–23 kDa) solution (40:60% vol/vol oil:aqueous). The response of the three microorganisms to this emulsified preparation was also assessed by MLC and time-kill studies.

Results and Discussion

Free TTO and silver ions used individually killed large numbers of microorganisms. When used at lower concentrations in combination, these agents showed an effect greater than the sum of the individual agents (synergy). Initial studies with TTO/PVA emulsions indicate greater action than TTO alone, and that the optimal molecular mass for this effect appears to be 30–70 kDa. Silver-encapsulated liposomes also killed large numbers of cells and demonstrated the feasibility of liposomes as a mechanism of transporting silver to microbial cells.

Conclusion

The present results demonstrate the possible development of combined treatments to increase the efficacy of wound treatment and infection control. Silver and TTO are effective antimicrobial agents, but both cause side effects when used inappropriately. Encapsulation also seems effective for silver. Emulsifying TTO in 1% PVA (a necessary step for TTO encapsulation) also improves its activity at doses below the MLC for free TTO. Minimising the quantity of antimicrobial agents in use has implications both for the environment (in terms of manufacturing), in addition to benefits for patients associated with reducing heavy-drug loading at infection sites.

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Manufacture and characterisation of pH-independent solubility formulation of Meloxicam by hot-melt extrusion process

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Introduction and Objectives

Hot-melt extrusion (HME) is a novel drug delivery technology that has been used effectively to produce solid dispersions.[1] Meloxicam (MX) is a nonsteroidal anti-inflammatory drug (NSAID) and has shown significant potential for the prevention and treatment of colorectal polyps. The MX is an acidic drug, practically insoluble in water and its solubility increases with increasing pH.[2] This pH-dependent aqueous solubility of MX would likely have a negative effect on the release pattern of the controlled drug delivery system such as colon targeting. In this study, HME was used to produce a pH-independent solubility formulation of MX.

Method

The MX of 10% (w/w) was melt extruded at a temperature of 140°C and a screw speed of 100 rpm with 90% (w/w) of Eudragit E100 alone or in combination with polyvinylpyrrolidone (PVP) K15 at four different polymer ratios (1 : 9, 1 : 3, 3 : 1 and 1 : 1 w/w). The produced extrudates were milled and passed through a mesh size of 250 μm. The milled extrudates were characterised using differential scanning calorimetry (DSC) and powder X-ray diffraction (PXRD). In-vitro drug release studies for the milled extrudates were performed at 0.1M HCl (pH 1.2, 6.8 and 7.4) using an equivalent weight of 7.5 mg MX.

Results and Discussion

The DSC results showed two separate glass transition (T_g) temperatures for Eudragit E100 and for PVP K15, indicating the immiscibility of PVP K15 with Eudragit E100. The PXRD confirmed the presence of crystalline MX in the extrudates containing Eudragit E100 alone, whereas MX crystallinity decreased significantly as the percentage of PVP K15 in the melt extrudates increased indicating the miscibility of MX with PVP K15. Drug-release properties of MX at pH 1.2 enhanced significantly as PVP concentration increased, which is mostly related to the low crystallinity of MX in the milled extrudates, whereas a significant decrease in the dissolution of MX occurred at pH 6.8 and 7.4 as Eudragit E100 concentration increased due to the low solubility of Eudragit E100 at pH above 5. Melt extrudates containing PVP-Eudragit (1 : 1 w/w ratio) showed the lowest difference in the rate and extent of MX release in different pHs used (around 5%).

Conclusion

The HME was efficient in producing a pH-independent solubility formulation for MX using a combination of PVP K15 and Eudragit E100 hydrophilic polymers. The miscibility of PVP with MX resulting in higher amorphous content of MX within the melt extrudates and the pH-dependent solubility of Eudragit E100 play a critical role in achieving such formulation that can be used for further studies for colon-targeting formulations.

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A study of the degradation of poly(lactic-co-glycolic acid) films by DRS, DSC and SEM

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Introduction and Objectives

Poly(lactic-co-glycolic acid) (PLGA) is one of the most important biodegradable polymers that have been used for implantable and drug delivery system. The objective of this study is to investigate the degradation of PLGA films by measuring changes in the thermal properties and dynamics of the primary and secondary relaxation during storage at elevated humidity. Dielectric relaxation spectroscopy (DRS) allows the monitoring of degradation induced changes in the dynamics of the sub-Tg b-process of PLGA, whereas differential scanning calorimetry (DSC) and scanning electron microscopy (SEM) enable the characterisation of the glass transition temperature and surface morphologies of films.

Method

Two types of PLGA polymers were sourced from Sigma (Poole, UK) and Boehringer Ingelheim (Bracknell, UK) (with a ratio of 50:50 lactic to glycolic acid). The molecular weights were 50 000 and 60 000, respectively. PLGA films were prepared by a solvent casting technique and stored in a desiccator with a relative humidity of 75.3% RH (25 °C) for 3 weeks. At regular intervals, the films were removed from the desiccator and measured by DRS (i.e. isothermal spectra between 1 Hz and 1 MHz at temperatures from −100 to +30 °C), DSC (i.e. −30–200 °C at a heating rate of 100 °C/min) and SEM.

Results and Discussion

Both PLGA films were shown to have amorphous domains with similar glass transition temperatures between 10 and 15 °C (before degradation). Over the first 4–5-day storage at 75% RH, the value of Tg increased to 20–25 °C owing to the consolidation of the remaining amorphous domains.[1] Tg did not change further up until the end of the study. SEM showed that extensive micropores had developed from the surface to inner of films during degradation. Sigma PLGA film with lower molecular weight showed

cracks on the surface at day 23. Three relaxation processes were found for both (Sigma and BI) samples: two sub-Tg processes (b_1 and b_2) and one above Tg process (a). Suggested mechanisms of relaxation were associated with the mobility of the pendant groups (b_1, high frequency), segmental motion (b_2, low frequency) of the polymer and glass transition (a). For fresh films (0 day), the activation energy for b_1 process was higher for the BI sample (45.5 kJ/mol) than for Sigma one (41 kJ/mol), that correlates with the molecular weight of polymers in film (50 000 and 60 000, respectively). After 8 days, the activation energy of the b-process increased, but at the day 24, it decreased. In the initial stages of degradation (before day 10), the faster degradation ester bond linkages of glycolic–glycolic acid or glycolic–lactic acid and slower bond linkage of lactic–lactic acid lead to the formation of more compact domains of poly lactic acid and therefore increase the activation energy. Further degradation destroys L–L bonds, which causes the decrease of activation energy of sub-Tg processes.[2]

Conclusions

PLGA films showed heterogeneous bulk degradation behaviour, which occurs in two stages. The first stage involves a consolidation of chains following initial breakdown of L–G or G–G links; the second stage involves the further breakdown of L–L domains and a collapse. The higher molecular weight of the sigma material results in a slower degradation and retention of its mechanical strength.

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Preliminary evaluation of the binding capacity of pullulan with ibuprofen granules

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Introduction and Objectives

A few novel formulations, e.g. tablets and granules, which contain pullulan in the sugar layer, have been shown to prevent brownish colour change of the composition and enhance the strength and shelf life of products.[1] The objective of this study is to determine the effective binding capacity of the pullulan-granulated ibuprofen, the distribution of drug within different granule sizes and thermal stability of these granules.

Methods

Wet granulation method was used to prepare ibuprofen granules using different concentrations of pullulan solutions from 0 to 20% (w/v). Preliminary studies have been done to measure the physical properties of granules, e.g. powder flow properties, granule size distribution, and to determine the amount of ibuprofen in different granule size distribution using UV spectroscopy. Thermal properties of the granules were measured with a Perkin-Elmer Diamond Hyper DSC (Beaconsfield, UK) scanned from −30 to 200°C at a heating rate of 100°C/min.

Results and Discussion

All batches of granules have an average powder flow time ranging between 1.41 and 2.15 s and Carr's Index values between 7.32 and 14.95%. All the batches studied have a unimodal granule size distribution of 180 μm except the batch without pullulan that has a bimodal distribution of 150 and 180 μm. Generally, the concentration of ibuprofen increased with granule size and the dissolution profiles also supported the results. DSC showed that there was a melting point around 75°C for ibuprofen for all batches; glass transition (Tg) of pullulan was around 60°C for the batches with pullulan concentrations >10%, which indicated that pullulan could have precipitated during the process.[2]

Conclusion

There was not a significant difference in average powder flow time with increasing concentration of pullulan among the batches. Carr's Index values ranged from 7.31 (1% of pullulan solution) to 14.95 (2.5% of pullulan solution) indicating good to excellent flow. Ibuprofen seemed to maintain its crystalline state as it gave a stable melting point in all batches. Pullulan appeared to remain in amorphous state as a thin film in the batches with concentrations <10%.

References

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A novel salt of flurbiprofen: influence of crystal structure on physicochemical properties

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Introduction and Objectives

The aim of this study is to examine the relationship between the crystal structure of flurbiprofen diethanolamine salt and its physicochemical properties to draw conclusions regarding the influence of the counter ion on the properties of the salt formed.

Methods

Flurbiprofen diethanolamine was prepared by dissolving flurbiprofen in acetonitrile and adding equimolar quantities of diethanolamine. Crystals, suitable for single crystal diffractometry, were obtained from the resultant gel on storage. The following techniques were used to confirm salt formation and characterise the product: DSC, TGA, 1H-NMR, solid-state NMR, FTIR, XRPD and true density determination. Saturated aqueous solubility was determined using an excess of flurbiprofen diethanolamine in distilled water stirred at ambient temperature for 24 h.

Results and Discussion

1 : 1 salt formation was confirmed by NMR and FTIR. DSC and XRPD confirmed crystallinity (Melting point = 63°C) with no evidence of solvent incorporation following TGA. Saturated solubility was >200 mg/ml, around 6000-fold higher than the parent compound (0.024 mg/ml).[1] Single crystal X-ray diffraction showed that two anions and two cations use their NH_2^+ and COO^- groups to form a discrete ring in contrast to the hydrogen-bonded carboxyl dimer structure of flurbiprofen.[2] Instead of linking adjacent rings, one ethanolamine hydroxyl uses the same carboxylate O atom already accepting from NH. The other ethanolamine OH can link to a carboxylate O atom across the large ring, but its disorder suggests limited importance. These 2 : 2 units are not H-bonded to other units.

Conclusion

Physicochemical characterisation of flurbiprofen diethanolamine revealed a 1 : 1 salt formation between the flurbiprofen and the diethanolamine counter ion with considerably greater solubility than flurbiprofen. Instability of the crystals due to disorder and the lack of a hydrogen-bonded spine are consistent with the low melting point of 63°C, density of 1.284 g/cm^3 and solubility of >200 mg/ml. Similar studies are being extended to other salts of flurbiprofen in an attempt to establish the relationship between the crystal structure and the physicochemical properties as well as the mechanical properties.

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The effect of hydroxypropylmethylcellulose particle size on drug release rate from hydrophilic matrix tablets

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Introduction and Objectives

Hypromellose, formerly known as hydroxypropylmethylcellulose (HPMC), is by far the most commonly used cellulose ether used to prepare hydrophilic matrix tablets. The basic parameters that affect its performance have been investigated and have been reviewed. The objective of this study was to further investigate the importance of HPMC particle size on drug release rate, using different tablet sizes and various viscosity grades of the HPMC.

Methods

Sieving was used to obtain different particle size fractions of various grades of HPMC, particle size was characterised using a Sympatec laser particle size analyser. Standard tablets (350 mg) were prepared using theophylline, 20% HPMC, lactose and magnesium stearate. Minitablets (30 mg) were also manufactured and the weight, thickness, diameter and hardness of all batches were measured. Dissolution testing was carried out using the basket method at 100 rpm in 900 ml of distilled water at 37 ± 1°C. Tablets prepared by addition of 12.5% of the smallest sieve fraction (20–45 μm) to the coarse fraction were also assessed.

Results and Discussion

For all batches of minitablets, the largest sieve fraction (180–425 μm) showed an uncontrolled burst release profile, whereas the other three size fractions showed similar controlled release profiles. For the standard tablets, the K4M grade provided four reproducible controlled release profiles from the various sieve fractions. However, both the higher viscosity grades K15M and K100M showed that the largest sieve fraction again produced a burst release profile compared with the other batches. Visual observations also showed that batches made of coarse particle sizes disintegrated very quickly. The effect of the HPMC particle size may be explained by the faster swelling of smaller particles resulting in a rapid formation of the gel barrier and production of robust tablets. However, higher viscosity grade particles may have slower swelling and gel times. The addition of 12.5% of the smallest sieve fraction (20–45 μm) to the coarse fraction was sufficient to produce a controlled release profile again in all cases.

Conclusion

HPMC particle size can have a dramatic impact on the dissolution rate from some matrix tablet formulations. In this

study, tablet size and HPMC viscosity grade have been shown to affect release profile. Mitchell and Balwinski[1] didn't show any impact of HPMC particle size on release rate from theophylline matrix tablets, which were prepared with K4M. This finding is consistent with our study; yet, we have seen that both K15M and K100M grades do show dramatic burst release with coarse particle size fractions. It is important to consider multiple factors in the design of robust HPMC matrix tablet formulations.

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Oral liquid nanomedicines for lansoprazole

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Introduction and Objectives

There is an increasing demand for a large number of licensed drug products to be reformulated into oral liquid formulations for use in the treatment of paediatric and geriatric patients. Lansoprazole (Sigma, UK) is currently available only as solid-dosage form. The objective of the current work was to study the suitability of two biodegradable polymers, poly caprolactone (PCL) and poly lactic-co-glycolic acid (PLGA-50:50) for the formulation of nanoparticulate system for the oral delivery of lansoprazole.

Method

Lansoprazole-loaded nanospheres were made by varying polymer-to-drug ratios, using the solvent-displacement technique originally described by Fessi *et al.*[1] Lansoprazole (10 mg) was added in every preparation. The ratios tested were 5:1, 6:1 and 7:1, with 0.2% wt/vol of pluronic F-127 (Sigma, UK) being used as a stabilizing agent. The formulations were characterized for particle size, zeta potential, polydispersity and percentage of drug entrapped.

Results and Discussion

Fabrication of nanoparticles using two different polymers revealed interesting trends. Investigation of drug loading for nanoparticulates prepared using PLGA (5:1) resulted in 80.5 ± 0.6% drug entrapment with particle-size distribution of 259.8 ± 17.4 nm. Measurement of zeta potential revealed the presence of slightly anionic surface (–25.9 ± 3.2 mV). Interestingly, increase of polymer concentration to higher ratios preserved the entrapment values (~80%) without any significant variations in particle-size measurements or zeta potential. However, preparation of nanoparticles using PCL (5:1) resulted in entrapment values of 85.5 ± 1.8%. Analysis of particle-size measurement showed nanoparticles in the range of 271.6 ± 4.8 nm. Zeta potential measurement revealed an anionic surface (–15.8 ± 4.0 mV). However, increase of polymer concentration to 6:1 and 7:1 resulted in the decrease of entrapment to 80 and 56%, respectively ($P < 0.05$). This was also followed by an increase in particle size with 6:1 ratio exhibiting 285.1 ± 13.4 nm and 7:1 showing particles of size 308.7 ± 19.5 nm. These interesting trends can be explained by two factors. Firstly, resistance to particle size, charge and entrapment upon increase of polymer concentration for PLGA could be possibly due to the higher degree of polymer–polymer interaction as well as higher macromolecular coiling of the polymer.[2] Consequently, increase in PLGA concentration possibly results in compaction and closer association of polymer chains, thus occupying same volume despite increase in concentration. However, PCL has relatively weaker coiling capabilities and exists as longer strands, thus resulting in weaker polymer–polymer interactions.[2] Secondly, the increase in size upon increase of polymer concentration (PCL) potentially explains reduction in drug entrapment. Increase in particle size occurs due to increase in viscosity of the organic phase upon increase of polymer concentration, thereby increasing the resistance for the drug to enter into the hydrophobic polymeric environment resulting in poor-loading efficiency.

Conclusion

The current study has shown that lansoprazole can be formulated as a nanoparticulate liquid-oral delivery system, with the drug-encapsulation efficiency being mainly dependent on the drug and polymer characteristics. Additionally, drug-to-polymer ratio requires optimisation for effective solubilisation.

References

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Investigation into the effects of blend energy input at different scales on alpha-lactose monohydrate

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Introduction and Objectives

The properties and behaviour of lactose play an important role in the delivery of drug to the lower airways in several dry powder inhaler (DPI) formulations. It is known that high-shear blending (commonly used in DPI manufacture) can affect lactose properties and that there is a relationship between blend energy input and fines content.[1] However, this earlier work was carried out at a single scale with one bowl size. The current study, therefore, focuses on establishing whether a similar relationship is seen at other larger scales.

Method

Experiments were carried out with a high-shear blender designed to allow real-time torque monitoring on the bowl during blending; this allowed the energy input (kilo Joules per kilogram of lactose) to be calculated. Inhalation grade lactose was blended at three different scales in bowls of 140, 168 and 220 mm diameter. The effects of blade speed (200–700 rpm) and bowl fill were also investigated. Initial experiments focused on establishing conditions that resulted in satisfactory mixing and stable torque readings. The effects of blending conditions on the particle-size characteristics of the lactose were then investigated. Samples of lactose were taken at regular intervals during blending and analyzed using laser diffraction (HELOS sensor with RODOS dispersion unit, Sympatec, Germany).

Results and Discussion

Blending regime maps could be constructed for each of the three bowls. These showed how the mixing patterns and torque fluctuations differed with bowl fill and impeller speed. For lactose blended in regimes where there was good bed turnover, cumulative energy input during blending was found to be the most important factor affecting lactose-particle-size distribution. Blade speed and bowl fill were not found to be as important at any given scale. The observed energy-dependent trends were similar at all of the investigated scales.

Conclusion

Energy input has a significant effect on the particle-size distribution of lactose during blending. It is likely that these effects will also impact on DPI performance. Hence, careful control and understanding of this process parameter during DPI manufacture is important. Furthermore work will concentrate on exploring the effects of blend energy input on the in-vitro behaviour of lactose and drug/lactose mixtures.

Reference

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Application of fluidised hot melt granulation as a novel granulation technique on preparing gastroretentive extended-release floating granules

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Introduction and Objectives

The aim of this study is to investigate the feasibility of using fluidised hot melt granulation (FHMG) as a novel granulation technique to prepare gastroretentive extended-release floating granules. Floating dosage forms are an example of gastroretentive drug delivery systems that are designed to improve the bioavailability of drugs with a narrow absorption window in the upper gastrointestinal tract or to enhance the local effects of drugs in the gastric region.

Method

Floating granules were manufactured using FHMG. FHMG is a solvent-free process in which granulation is achieved using low-melting point materials (Compritol 888 ATO and Gelucire 50/13 in this research) as meltable binder in place of conventional liquid binders. Sodium bicarbonate and citric acid were used to achieve improved buoyancy during in-vitro dissolution. Metronidazole was used as a model drug. The physical–chemical characteristics, morphology, floating properties and drug release of the granules in different formulations were investigated and compared. The floating properties were also evaluated using a resultant-weight method earlier described by Timmermans and Moës.[1]

Results and Discussion

The granules produced by FHMG were spherical in shape and had good flowability (Hausner ratio < 1.2; Carr's index < 5%). The floating granules exhibited controlled drug release properties extending beyond 10 h. Granule buoyancy (floating time and strength) and drug release properties were significantly influenced by formulation variables (excipients type and concentration) and the physical characteristics (particle size, hydrophilicity) of the excipients. Drug dissolution was increased by increasing the concentration of hydroxypropyl cellulose (HPC) and Gelucire 50/13 or by decreasing the particle size of HPC. Floating strength was obviously improved through the incorporation of sodium bicarbonate and citric acid and furthermore was influenced by the concentration of HPC within the formulation. Changing the drug loading from 5 to 30% had no significant effect on the drug release and floating properties of the granules.

Conclusions

Extended-release floating multiple-unit formulations (granules) were successfully developed as a new potential application for the FHMG process. The granules have shown good physical characteristics, floating capability and drug release properties in acidic media (HCl 0.1 N). Furthermore, the drug release and floating properties can be controlled and designed by modifying the ratio or physical characteristics of the excipients used in the formulation.

Reference

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An ATP-competitive fluorescent ligand as a probe for binding and inhibitor displacement studies on Mps1 kinase

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Introduction and Objectives

The typical methods for kinase assay involve single-point measurement based on [^{32}P]-labelling or antibody blotting, or fluorescence resonance energy transfer (FRET)-based analytical techniques.[1] These methods involve either radioactivity-based or expensive techniques. Moreover, obtaining binding constants from these methods is seldom straightforward. Here, we introduce a new approach for kinase assay to study the strength of binding interactions based on simple measurements. To develop this assay, the biophysical property of an intrinsic fluorescent ATP-competitive ligand SP600125 has been studied. Binding and displacement assays based on fluorescence spectroscopy have also been carried out to show the possible application of this approach for discovery of potential kinase inhibitors.

Methods

The UV-Visible and fluorescence spectra of a fluorescent kinase ligand SP600125 were studied in both aqueous media (pH range 1.3–12.7) and organic solvents. The pKa values from both ground and excited state of the ligand were calculated to understand its ionisation and biophysical properties. Fluorescence spectra were recorded with the addition of aliquots of wild-type (WT) phosphorylated Mps1 kinase to the SP600125 until saturation. The ATP (Mg^{2+}) was added to the complex of kinase and ligand to monitor the displacement assay. Several potential inhibitors of Mps1 were studied in the displacement assay to show their ability of displacing the original ligand. The K_d values were calculated for different inhibitors.

Results and Discussion

The λ_{max} of the SP600125 shifted with different polarities of solvents. The fluorescence spectra showed decrease in intensity and shift of λ_{max} to lower wavelength upon the addition of Mps1 kinase (Figure 1). The addition of ATP (Mg^{2+}) resulted in opposite effect of both the intensity change and the λ_{max} shift, which presumably indicated the occurrence of ligand displacement. Some potential inhibitors also show the effect of displacement with different K_d values.

Conclusion

The use of fluorescence spectroscopy to monitor the behaviour of an intrinsic fluorescent ligand enables to establish a nonradioactive kinase assay based on simple measurement. The study of ATP and various inhibitors displacing this nonspecific kinase ligand showed the possibility to apply this ligand-based assay to the screening of potential ATP-competitive inhibitors of different kinases.

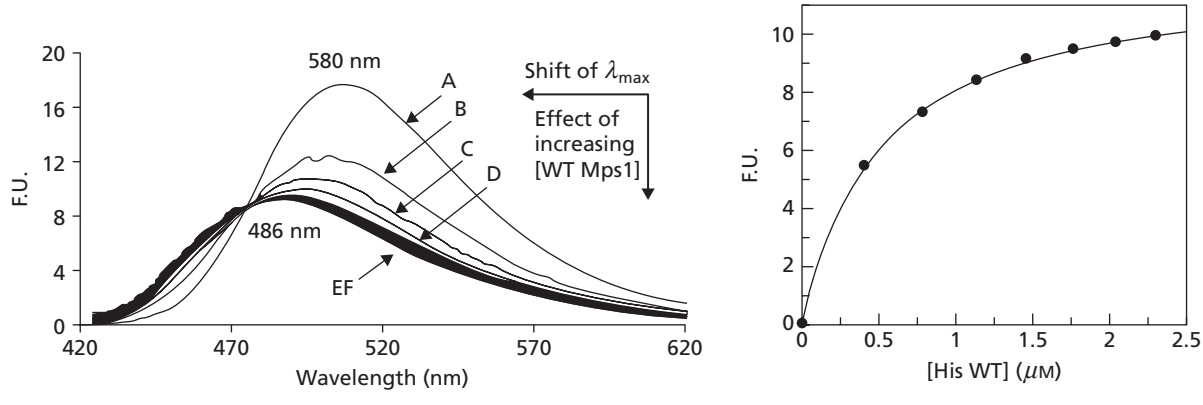

Figure 1 Fluorescence spectra of stepwise addition of WT Mps1 to SP600125 and the binding curve for the change (decrease) of F.U. against the concentration of Mps1.

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Studies on phytochemical and pharmacological evaluations of *Ocimum* species for antiarthritic activity

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Introduction and Objectives

Ocimum tenuiflorum Linn and *Ocimum americanum Linn* (commonly called *Tulsi*, family Labiateae), are widely used in the Ayurvedic system of medicine for bronchitis, bronchial asthma, skin diseases, arthritis, inflammation, fever and so forth. The study was carried out to corroborate the traditional claims for antiarthritic activity as well as quantification of two marker compounds ursolic acid and eugenol by high-performance thin-layer chromatography (HPTLC) and to check interspecies variation.

Methods

Method development and optimisation were done by HPTLC. Carrageenan induced rat paw oedema: the animals were given carrageenan (1%, 0.1 ml) by subplantar route. After 1 h, the extracts were administered orally, 100 mg/kg and 200 mg/kg body weight. The paw volume was noted from 0, 1, 2, 3, 4 and 24 h. Diclofenac sodium orally at a dose of 10 mg/kg was used as standard. Complete Freund's Adjuvant (CFA) for 21 days: in this model, diclofenac sodium was used as standard used (dose 5 mg/kg). Animals were given the extract as 100 mg/kg and 200 mg/kg body weight after 13th day of CFA. The paw volume was calculated with the help of plethysmometer.[1,2]

Results and Discussion

Reference standards used were ursolic acid and eugenol. Methanol extracts from the leaves of both the species were used. In HPTLC, the solvent system used was toluene : ethyl acetate : formic acid (8 : 2 : 0.2). Calibration curves were prepared using standard solution in the range of 80–480 ng/spot for ursolic acid and 200–700 ng/spot for eugenol, respectively.[3] Ursolic acid and eugenol were resolved at R_f 0.36 and 0.74, respectively. The validation parameters have shown good result. The correlation coefficients for ursolic acid and eugenol were $R_2 = 0.999$ and $R_2 = 0.998$, respectively. Percentage recovery at three different levels was in the range of 97.88–100.56%. Ursolic acid and eugenol contents were $0.41 \pm 0.03\%$ and $0.13 \pm 0.02\%$ in *O. tenuiflorum* and $1.15 \pm 0.02\%$ and $0.36 \pm 0.01\%$ in *O. americanum,* respectively. Injection of carrageenan results in increase in paw volume. The onset of inflammation was observed at 1 h. Both the extracts at 200 mg/kg showed significant result with $P < 0.001$ after 24th hour. The inhibition percentage at 100 and 200 mg/kg after 24th hour for *O. tenuiflorum* was 58.33% and 72.57%, respectively. Similarly, the inhibition percentages for *O. americanum* and standard were calculated. In CFA method, the standard drug and the extracts of both the plants showed significant results in all the doses with $P < 0.001$. Reduction in paw oedema was observed in all the doses for both the plants.

Conclusion

The developed HTPLC method is precise, specific and accurate for determination of both marker compounds. Findings show potential of both species for treatment of arthritis. This necessitates further detailed and systematic evaluation of the plants for the search of new lead molecule.

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Effect of low-temperature steam pasteurisation on chemical composition of Indian herbs

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Introduction and Objectives

The aim of this study is to assess the impact of steam treatment on the constituents of medicinal plants. Steam treatment is commonly used to bring the microbial load in dried plant materials, intended for human consumption to acceptable levels and is currently the only option of treatment for certified organic products in the UK. New EU legislation, effective from 2011, requires that registered herbal medicinal

products (Traditional Herbal Medicines) must be safe and of high quality. More information is required on the impact of steam pasteurisation on plant constituents, especially for Indian plants. Four species were selected to encompass a variety of types of chemical constituents.

Method

Andrographis paniculata (aerial parts), *Bacopa monnieri* (aerial parts), *Piper longum* (fruit) and *Curcuma longa* (rhizome) were examined. Dried powdered material was used. For each species, a methanolic extract of steam-pasteurised material and a control sample from the same batch prior to steam treatment were compared using high-performance thin layer chromatography (TLC) against appropriate reference standards. Water content was determined by loss on drying or by distillation. Quantitative assays were carried on *A. paniculata*, *B. monnieri* and *C. longa* – contents of Andrographolide, Bacoside A and curcumins, respectively, were determined. Microbial contamination was tested in a contract laboratory.

Results and Discussion

Microbial contamination of all samples was reduced after steam treatment.

A. paniculata Nees (Indian name: Kalmegh): The TLC profile of treated sample was very similar to control, including the diterpene lactones, neoandrographolide and andrographolide bands. The content of andrographolide was unchanged. The loss on drying of steamed sample was 9% and control sample was 8.8%.

B. monnieri (L.) Pennell (Indian name: Brahmi): The TLC revealed no qualitative change to TLC profile and no significant quantitative change to Bacoside A (glycoside) content in the treated sample. This was confirmed by the assay. Loss on drying of steamed sample was 10% and control sample was 9%.

P. longum L. (Indian name: Pippali): The TLC profiles of treated and control samples were the same. The concentration of piperine (alkaloid) in the sample was slightly smaller after steam pasteurisation. Loss on drying of treated sample was 14.1% and control sample was 12.4%.

C. longa L. (Indian name: Haridra): The TLC profiles of treated and control samples were the same. The volatile oil profile by gas-liquid chromatography showed a slight loss of more volatile components after steam treatment. The content of curcumins was unchanged, but moisture content increased significantly from 9.4 to 11.6% after steam treatment.

Conclusions

The marker compounds of *A. paniculata*, *B. monnieri* and *P. longum* were not affected significantly by steam pasteurisation. Minor changes to TLC profile were observed in *A. paniculata*. Although the curcuminoid content of *C. longa* was unchanged, more volatile components of the volatile oil were reduced. Moisture content increased after the treatment, especially in *C. longa* rhizome and *P. longum* fruit, perhaps because of their high starch content. Microbial contamination of all treated samples was lower than that of control samples of the same batch.

Acknowledgement

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Drug Delivery

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Rheological properties of Eudragit RL PO and Eudragit E100 containing Quinine Base, Quinine hydrochloride and Metformin hydrochloride prepared by hot-melt extrusion

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Introduction and Objectives

Capillary rheology is a well-established technique within the engineering sector. To date, however, it has attracted limited interest within the pharmaceutical field despite its unsurpassable potential to characterize drug-polymer systems. Using capillary rheometry, the purpose of the study was to characterize the melt-flow properties of a number of formulations containing either quinine base, quinine hydrochloride or metformin hydrochloride, and to study the effect of drug type, loading and temperature of the system on polymer viscosity.

Method

Blends of E100 or RL PO containing active pharmaceutical ingredient (API) in concentrations of 5–30% were compounded in a prism 16-mm twin-screw extruder (Haake minilab) and the melt rheology examined using a rosand RH22 (twin bore) advanced rheometer system. Tests were performed at a range of temperatures (between 115 and 160°C) and the effects of drug loading, shear rate and temperature on melt flow properties were investigated. DMTA was carried out using a three-point bending clamp, at a ramp rate of 5°C/min and a force track of 125%.

Results and Discussion

Addition of drug to the polymer matrix had a significant impact upon polymer flow properties. Eudragit RL PO and E100 exhibited pseudoplasticity where viscosity of the melt decreased when shear rate was increased from 100 to 1500/s.

Increasing drug concentration of quinine base caused reduction in melt viscosity for both polymers. The opposite effect was observed with quinine hydrochloride and metformin hydrochloride. Using the Arrhenius equation, the activation energies (E_a) at each drug loading and temperature were calculated. A decrease in E_a with increasing quinine base loading was observed, while the opposite was true for quinine hydrochloride and metformin hydrochloride. This showed that quinine base acted as a solid-state plasticizer for both polymers while metformin and quinine hydrochloride did not. DMA confirmed these findings and showed a depression of almost 20°C in the T_g when quinine base was added, while no substantial reduction was shown for metformin or quinine hydrochloride. E100 flow properties were more temperature dependant than shear dependant. Increasing temperature from 140 to 160°C decreased the viscosity by approximately 50–55% at 300/s. To achieve the same viscosity reduction by shear alone, shear rates had to be almost tripled. Similar findings were observed for RL PO.

Conclusion

This study has shown that capillary rheometry can be used to characterize drug-polymer systems of Eudragit RL PO and Eudragit E100 containing quinine base, quinine hydrochloride and metformin hydrochloride, and that the addition of such drugs changed the flow properties of the system significantly. This data was also in agreement with findings from DMA that quinine base acted as a solid-state plasticizer for both polymers while quinine hydrochloride and metformin did not.

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Physicochemical and mechanical characterisation of melt extruded hydroxypropyl cellulose films contain high metronidazole loadings

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Introduction and Objectives

The aims of this study were to design, manufacture and characterise the physicochemical and mechanical properties of melt extruded hydroxypropyl cellulose (HPC) films containing high metronidazole (MN) loadings. In so doing, we aim to assess these platforms as potential implantable oral delivery systems for the treatment of periodontal diseases.

Methods

HPC (Mwt ~140 K) films were melt extruded containing MN at loadings ranging from 5 to 80% w/w using a HAAKE MiniLab (Thermo Electron, Waltham, Germany). The thermal and mechanical properties of the extrudates were characterised using differential scanning calorimetry (DSC) (DSC Q100; TA Instruments, Newcastle, UK) and dynamic mechanical thermal analysis (DMTA) (Tritec2000 DMA; Gearing scientific, Hertfordshire, UK). In brief, DSC studies were conducted across temperature range of 25–220 °C using heating rate of degree Celsius per minute and sealed aluminium pans. DMTA was performed using a frequency of 1 Hz, displacement of 0.1 mm and a heating rate of 5 °C/min across temperature range of 25–220 °C. The tensile and mucoadhesive properties of the films were investigated using previously reported methods,[1,2] using a TA XT plus Texture Analyzer (Stable Micro Systems, Surrey, UK). Finally, drug dissolution studies were conducted under sink condition using phosphate buffered saline (pH 7.4). Samples were withdrawn at predefined time intervals, and the quantity of MN in solution was determined using Cary 50 UV-visible spectrophotometer (Varian, Palo Alto, Australia, λ_{max} = 326 nm).

Results and Discussion

HPC bioadhesive films have been reported in the literature[3]; however, no reports have been published about developing HPC mucoadhesive films with high MN loadings for treating periodontal diseases. DMTA results showed that MN acted as a plasticiser for HPC by significantly reducing Tg. Consequently films containing higher drug loadings could be extruded at lower barrel temperature. DSC results have shown that at MN concentration below 20% w/w, there was a single phase system; exceeding this concentration of MN resulted in a two-phase system whereby MN was solubilised and dispersed as discreet crystalline phase within the polymer; this was detected through the presence of a melting endotherm at 158 °C. Interestingly mucoadhesive films containing low concentration of MN showed significantly higher mucoadhesive strength than extrudates containing MN more than 20% w/w, and this may be attributed to the reducing concentration of HPC in the films and/or they increased HPC side chains mobility. The increased mobility may facilitate HPC interpenetration of the mucin polymer network thus enhancing mucin-HPC interaction and the mucoadhesive strength. Drug dissolution studies have also suggested that MN drug loading and molecular dispersion in the polymeric matrices have a significant role in the drug release.

Conclusions

HPC mucoadhesive platforms could be designed and produced by hot-melt extrusion with fewer processing steps and good drug content uniformity. MN, a model drug with low water solubility, was fully solubilised within HPC polymer below 20% loading with formation of single phase platform.

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Medicinal Chemistry

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DNA intercalators: effect of covalent binding sidechains on analogues of 9-chloroacridine-4-carboxylates

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Introduction and Objectives

The epoxide moiety in the azinomycins, potent antitumour antibiotics comparable to mitomycin C used clinically, is essential for their activity because it can alkylate the nucleophilic functional groups on DNA bases on opposite strands. Acridines have a planar aromatic ring system and its tricyclic chromophore has been shown to sandwich between base pairs generating local recoiling and distortion of the DNA in an intercalative manner. We aimed to synthesise acridine-4-carboxyesters possessing covalent binding side-chains, to explore their DNA binding affinity and specifically to couple the electrophilic epoxide moiety of azinomycin to a 9-chloroacridine chromophore.

Method

The epoxide was synthesised using the route developed by Hodgkinson and Shipman[1] and modified by Casely-Hayford *et al.*,[2] a Sharpless asymmetric dihydroxylation reaction giving (S) allylic alcohol by enantioselective attack of the alkene from its bottom face, followed by epoxidation to give (S,S) and (S,R) diastereoisomers. Acridone-4-carboxylic acid is available in two steps from 2-chlorobenzoic acid and anthranilic acid. It was converted to the acid chloride 9-chloroacridine-4-carboxyl chloride to effect a nucleophilic acyl substitution reaction. Further coupling reactions gave samples for biological testing. Drug-induced uncoiling of ΦX174 plasmid DNA was measured in terms of electrophoretic mobility on 1% agarose gel at 50 V for 3 h. The gel was stained with ethidium bromide and viewed by ultraviolet transillumination.

Results and Discussion

The epoxide unit was successfully characterised by infrared, nuclear magnetic resonance and mass spectrometry of each intermediate. Some of the conjugated structures included quinoxaline analogues because this is a known planar chromophore in some cytotoxic drugs, e.g. methotrexate. Unwinding assays (Figure 1) show that a quinoxaline epoxide analogue starts to unwind DNA at a drug/bp ratio of 10^{-1} (lanes 2 and 6) and total relaxation having occurred at a drug/bp ratio of 1 and higher (lanes 3, 4, 7 and 8).

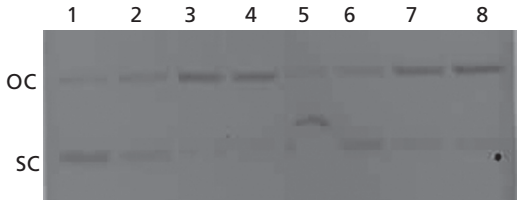

Figure 1 Structure of quinoxaline epoxide analogue and its effect on the electrophoretic mobility of ΦX174 plasmid DNA. Lane 1: DNA only. Lanes 2–4 and 6–8: 10^{-1}, 1 and 10 drug/bp ratio, respectively, and lane 5: 10^{-2} ratio. DNA, 3.84 μM; SC, supercoiled DNA; OC, open-circular DNA.

Conclusions

Cytotoxic activity of the quinoxaline analogue should be measured by cell culturing in tumour cell lines and by carrying out cell growth inhibition assays, i.e. 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide (MTT) colorimetric assays, which may show useful properties in the bid to develop leads for a novel class of compounds with improved affinity and anti-tumour activity.

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Dimethylsulfamate derivatives in the search for novel and potent inhibitors of estrone sulfatase

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Introduction and Objectives

Sulfamate-based inhibitors of estrone sulfatase (ES) have been shown to be chemically unstable due to the cleavage of the S-OAr bond. Modelling a range of sulfonate derivatives of phenol have suggested that the 4-O-dimethylsulfamate substituted compounds are able to undergo attack by the

active site of ES (i.e. the formylglycine moiety), whereas the CX3 group sterically protects the sulfur atom from attack in methanesulfonate- and trifluoromethanesulfonate-based compounds. In an effort to test our hypothesis, we undertook the synthesis and biochemical evaluation of a series of 4-O-dimethylsulfamate derivative of a range of N-(4-hydroxy-phenyl) alkyl amide-based compounds.

Methods

The synthesis of the target compounds involved an initial reaction between 4-aminophenol and an anhydride (ranging from acetic to dodecanoic) to give the appropriate amide, e.g. reaction between undecanoic anhydride and aminophenol gave undecanoic acid (4-hydroxy-phenyl)-amide. The synthesis of the dimethylsulfamate derivative involved literature-based methodology using dimethylacetamide as the solvent, e.g. dimethyl-sulfamic acid 4-undecanoylamino-phenyl ester.[1] The biochemical evaluation involved rat liver microsomal enzyme using radiolabelled estrone in tris-HCl buffer at pH 7.2.[2] After the incubation, the reaction was quenched using toluene and the samples counted for 5 min for tritium.

Results and Discussion

The synthesis of the target compounds did not prove to be troublesome. The synthesis of the intermediate gave the target compounds in a range from 60 to 90% yield, while the dimethylsulfamate derivatives were obtained from 40 to 80% yield. The results of our study into the biochemical evaluation of dimethylsulfamate-based compounds show that the compounds are, in general, weak inhibitors of ES in comparison to the standard, namely 3-O-estrone sulfamate (EMATE) which contains an unsubstituted derivative of the sulfamate moiety. EMATE was found to inhibit ES by ~87% (at [I] = 100 μM), while the most potent dimethylsulfamate-based compound (dimethyl-sulfamic acid 4-acetylamino-phenyl ester) was found to possess ~37% inhibitory activity. The methansulfonate derivative was shown to inhibit by ~7%. As such, the results of our study suggest that the dimethylsulfamate containing compounds, while being weak inhibitors in comparison to EMATE, possess sufficient level of inhibition to be derivatised in an effort to increase their inhibitory activity.

Conclusion

We have shown that dimethylsulfamate-based compounds are more potent inhibitors of ES than methanesulfonate-based compounds – these compounds may be further derivatised to increase their inhibitory activity.

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Pharmacognosy

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Piper sarmentosum extracts: antiobesity role through inhibition of vascularisation, standardisation and preclinical studies

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Introduction and Objectives

Obesity is not only a cosmetic problem but also associated with a number of comorbidities. The development of antiobesity drugs is difficult due to the involvement of different poorly defined pathways in obesity. Available antiobesity products with restricted diet and exercise may reduce 5–10% weight. Many natural products are being claimed to control obesity, which have to be tested scientifically to produce evidence-based botanicals. Since vascularisation is involved in adipogeneis, this study aimed to investigate different extracts of *Piper sarmentosum* for the inhibition of vascularisation and preclinical studies.

Method

Different types of extracts of the plant were evaluated for the inhibition of vascularisation using rat ring aorta model, which was evaluated by the Microscopic Angiogenesis Grading System (MAGS) score. The most potent extract, chloroform extract, possessing 100% activity was further investigated for dose response, cytotoxicity and in-vivo toxicological and pharmacokinetic studies. Additionally, the extract was standardised and evaluated for accelerated stability.

Results and Discussion

The results shown in Figure 1 indicated that leaf chloroform extract of the plant exhibited 100% antiangiogenic activity (IC^{50} 45 μg/ml). In cytotoxicity studies, the extract showed IC^{50} 76.24 μg/ml, which was higher compared to the IC^{50} of vascular inhibition. In high-performance liquid chromatography analysis, the extract was found to contain rutin (0.20 mg/g), flavonone (0.32 mg/g), pellitorine (0.043 mg/g), sarmentine (0.006 mg/g) and sarmentosine (0.005 mg/g). The extract also contained polyphenols (7%), amides (12%) and glycosaponins (3%). The LD50 of the extract was found to be more than 2000 mg/kg in rats. Moreover, the extract was found to be safe in rats with reference to antioxidant markers such as superoxide, catalase and peroxidase, and liver function markers such as aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and lactate dehydrogenase. In pharmacokinetic studies, pellitorine and sarmentine showed good oral bioavailability while sarmentosine was not absorbed orally and

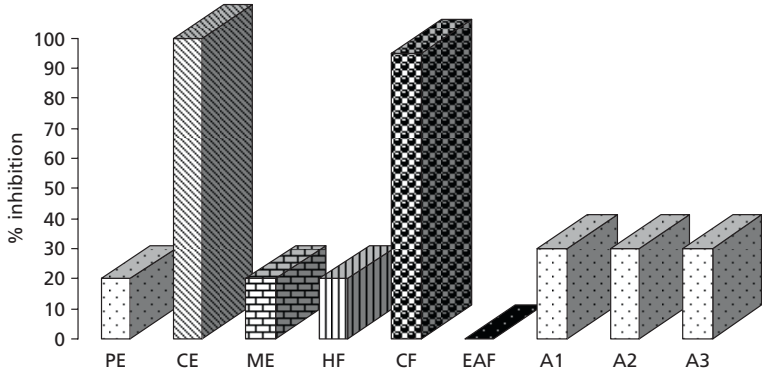

Figure 1 Comparison of antiangiogenic activity of different extracts of leaf of *Piper sarmentosum*, fractions of methanol extract and compounds A 1–3, each bar is mean of six replicates; PE, petroleum ether extract; CE, chloroform extract; ME, methanol extract; HF, *n*-hexane fraction; CF, chloroform fraction; EAF, ethyl acetate fraction; A1, pellitorine) A2, sarmentine; A3, sarmentosine.

excreted unchanged in faeces. The absorbed markers were excreted in urine as metabolites. Pellitorine and sarmentine exhibited different tissue affinities. Based on pellitorine, sarmentine and sarmentosine, the predicted shelf life (t_{90}) of the extract was 16 months at 25°C. The markers followed the zero-order reaction indicating that their degradation was independent of initial concentration.

Conclusion

The chloroform extract of the leaves of *Piper sarmentosum* has promising antiobesity activity by inhibiting vascularisation to adipocytes with good safety profile.

Wednesday Poster Sessions

Analytical Chemistry

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Quantification of gliclazide, glipizide, glimepiride, pioglitazone, repaglinide and rosiglitazone in human plasma using reverse-phase high-performance liquid chromatography

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Introduction and Objectives

The aim of this study was to develop a simultaneous quantification method for gliclazide (GCZ), glipizide (GPZ), glimepiride (GMR), pioglitazone (PGZ), repaglinide (RGD) and rosiglitazone (RGZ) in human plasma using reverse-phase high-performance liquid chromatography (HPLC) in isocratic mode. Separation of analytes was achieved in short analysis time. Validation of the method was carried out as per ICH guidelines for accuracy, precision, linearity, specificity, selectivity, robustness, lower limit of quantification and stability.

Method

Chromatographic method was developed on Shimadzu HPLC system (Shimadzu, Kyoto, Japan), dual pump (LC-10AT vp and LC-20AD) and SPD 10 A vp UV-Visible detector using a phenomenex C_{18} (150 × 4.6 mm i.d., 5 μm) at ambient temperature with a mobile phase containing a mixture of methanol and 0.05% (w/v) formic acid in water (67:33), fixed at a flow rate of 0.5 ml/min. The results were monitored at 240 nm using Spinchotech 1.7 version (Spinco Biotech services, Chennai, India). Drugs were extracted from the plasma using mobile phase, followed by centrifugation, evaporation and reconstitution of the drugs with mobile phase and were injected into HPLC for analysis.

Results and Discussion

The stock and working solutions of the drugs were prepared in methanol, the linearity was calculated in the range of 0.1–100 μg/ml ($r^2 = 0.9999 \pm 0.0003$) and the drugs were eluted at retention times of 3.21, 4.53, 7.45, 8.92, 14.37 and 17.48 min for RGZ, PGZ, GPZ, GCZ, RGD and GMR, respectively, and the peaks were found to be symmetric with minimum tailing. The drugs (50 μg/ml) were added to plasma vortexed (3 min) and extracted with mobile phase, centrifuged for 15 min and the organic layer was evaporated. Methanol was used for reconstitution of the drugs. The method was then validated for its accuracy (recovery was 95–105% from plasma), precision (intraday and interday for 3 days), specificity and selectivity (no interferences were found from the plasma at the retention times of the drugs as shown in Figure 1), robustness (change in formic acid concentration, flow rate and concentration of methanol were selected for testing where % RSD was found to be less than 2%) and stability of plasma samples (short-term at room temperature for 24 h (>98.2%), long-term for 30 days at −20°C (>94.3%) and three complete freeze thaw cycles (>93%)) and the obtained results were within the acceptable limits.