JPP Journal of Pharmacy and Pharmacology

JPP 2009, Supplement 1: A33–A138

POSTER SESSIONS

Monday Poster Sessions

Biopharmaceutics

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From laboratory to bedside and back again: the use of dried blood spot analysis in paediatric care

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Introduction and Objectives

A dried blood spot (DBS) sampling system for newborns was investigated as a means of using small-volume samples for measuring drug levels to obtain pharmacokinetic (PK) information and therefore to enable optimal paediatric patient care. We describe the development and validation of a microanalytical technique for the determination of captopril, used for the treatment of paediatric heart failure, from DBS collected on Guthrie card. The stability of this simple format for blood samples was also investigated.

Methods

A rapid sensitive LC/MS method, with selected ion monitoring, was developed to determine captopril levels in DBS. An 8.0-mm disc was punched from the DBS, cut into six pieces and extracted with 200 μl of methanol/water (60/40) containing 10% v/v 200 mM 1,4-dithiothreitol (DTT) as a captopril stabiliser. The sonicated (30 min) sample was centrifuged and the supernatant liquid analysed by LC/MS. LC separation was achieved using a Zorbax C8 column and a water-formic acid-acetonitrile-isopropanol mobile phase with gradient elution and a flow rate of 0.5 ml/min over a run time of 3.5 min. The mass detector was used with an electrospray interface and in positive ion mode. The use of DTT was necessary in both the Guthrie cards and the extraction medium to enable the determinations of low levels of captopril extracted from the DBS.

Results and Discussion

The extraction efficiency for the recovery of captopril from spiked blood spots was demonstrated to be 90 ± 10%. The pretreatment of the Guthrie card with DTT and formulating the stabiliser as part of the extraction medium were essential steps in demonstrating high and reproducible recovery of captopril from the DBS. Validation showed good specificity, accuracy, precision and linearity with a limit of detection of 10 pg in the DBS corresponding to 4 ng/ml in whole blood or 40-ng/kg body weight. This method was applied to blood spots on Guthrie card taken from a neonate patient previously administered 1-mg/kg captopril orally. The amount of captopril in the DBS was 1.8 ng, which equates to 88 ng/ml in whole blood or 7.04-μg/kg body weight. Captopril was stable in DBS stored at room temperature for at least 12 weeks. Ethical approval for this research project was obtained from the National Health Service-National Research Ethics Service (NHS-NRES) and De Montfort University ethics committees.

Conclusion

The DBS method offers a way to enable paediatric PK studies for captopril. The small volume (20 μl) of blood required combined with the simplicity of the extraction procedure and the sensitivity and selectivity of the mass spectrometric technique, together with the demonstrated stability makes this a feasible procedure for measuring captopril concentrations for paediatric PK studies. This method could also be adapted to determine the levels of other drugs and may facilitate assessing medication compliance and therapeutic monitoring in a routine clinical setting for paediatric patients for which large blood volume sampling is impractical and restricted by ethical guidelines.

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In vitro–in vivo correlation for the microsphere and in situ implant–based controlled-release depot injectable formulations

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Introduction and Objectives

The objective of this study was to find a correlation between in-vitro and in-vivo drug release data obtained from the developed microspheres and in situ implant–based depot injection of olanzapine and aripiprazole. Although the Food and Drug Adminstration in vitro–in vivo correlation (IVIVC) guidelines are only applicable for oral dosage forms, the same principles were used, with some modification, to develop IVIVC for nonoral products.

Method

IVIVC is established by comparing the in-vitro dissolution curve with the drug input rate curve, which may be obtained by various methods of mass balance model techniques, such as Wagner–Nelson procedure (in case the absorption curve adjusts to a model of one compartment) and Loo–Riegelman method (in case the adjustment is significant for a model of two compartments), or by model-independent evaluation using pharmacokinetic parameters. The simplest way of demonstrating IVIVC is to plot the fraction absorbed *in vivo* versus the fraction released *in vitro*. The results of in-vitro and in-vivo drug release studies were correlated, and their regression coefficient was calculated.

Results

A correlation of results of in-vitro and in-vivo drug release studies was established; the correlation coefficient was found to be 0.985 and 0.949 for olanzapine and aripiprazole microspheres, respectively, and 0.949 and 0.947 for olanzapine and aripiprazole in-situ implant formulations, respectively. The above values confirmed a good correlation between in-vitro and in-vivo drug release data.

Conclusion

The results showed that there is a good correlation between in-vitro and in-vivo data. Therefore, it is strongly recommended by the authors that this method should be critically examined and validated and can be included in the regulatory guidelines for the prediction of in-vivo pharmacokinetic data from the in-vitro data of the depot formulation with different drug release pattern. This will help in minimising the in-vivo studies and will be helpful during the development of such kind of formulation.

Reference

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Drug Delivery

44
Exploring the effect of aloe vera gel on the buccal permeability of didanosine: permeability and histomorphological studies

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Introduction and Objectives

The aim of the study was to determine the effect of aloe vera gel (AVgel) on the buccal permeability of didanosine (ddI) and to assess its histomorphological effects on the buccal mucosa. Buccal permeability can be improved by the use of penetration enhancers; thus, effective and safe enhancers need to be identified. AVgel has been reported as a potential enhancer for intestinal[1] and skin[2] permeability; however, data on its buccal permeability are yet to be reported.

Methods

Ethical approval was obtained from University of KwaZulu-Natal (UKZN) Ethics Committee (Ref: 006/09/Animal). In-vitro permeation of ddI was studied using Franz diffusion cells and porcine buccal mucosa with phosphate-buffered saline (PBS) pH 7.4 at 37°C. Varying concentrations of AVgel from 0.25 to 6.0% w/v were investigated. ddI was quantified by ultraviolet (UV) spectrophotometric analysis. Flux values were calculated using linear regression analysis. Histological investigations were undertaken using light microscopy. Data were analysed using one-way analysis of variance (ANOVA) with Bonferroni post-hoc tests.

Results and Discussion

The amount of ddI permeated with an increase in AVgel is shown in Figure 1. The initial flux of ddI was 293 $\mu g/cm^2h$ and was increased significantly ($P < 0.001$) with an increase in AVgel concentrations from 0.25 to 2% w/v. However, the flux values decreased to 84 and 62 $\mu g/cm^2h$ with further increases in AVgel concentrations of 4.0 and 6.0% w/v, respectively. Similar trends with other enhancers have been reported.[3] No major differences were observed in the thickness of the epithelium, cell architecture and cellular alignment of the mucosa in both the control (normal saline) and the treated (PBS/ddI or ddI/PBS/AVgel) mucosae.

Conclusion

The permeability of ddI is dependent on the concentration of AVgel. AVgel in combination with ddI does not adversely affect the epithelium and basal lamina of the buccal mucosa. AVgel can be considered as a potential buccal permeation enhancer.

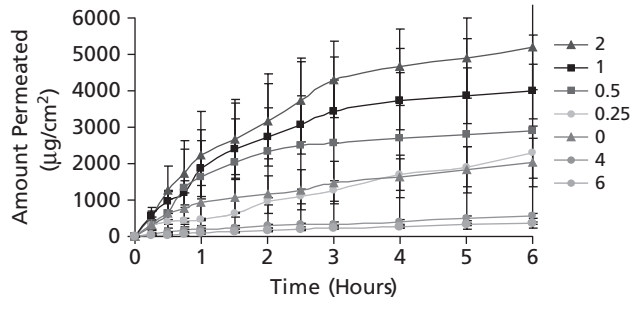

Figure 1 The effect of aloe vera gel concentrations on didanosine permeation.

References

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Preparation and evaluation of mucoadhesive polymeric films for buccal delivery of anti-HIV/AIDS drug (didanosine)

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Introduction and Objectives

Antiretroviral (ARV) drugs, such as didanosine (ddI), are available for the oral route of administration only. Its buccal administration may improve bioavailability by avoiding hepatic first-pass metabolism and gastrointestinal degradation. The incorporation of an ARV into a buccal delivery system has not been reported. This study aimed to prepare and evaluate ddI containing homopolymeric and monolayered multipolymeric films (MMFs) with polymers of similar and opposing solubilities for buccal delivery.

Method

ddI-loaded monopolymeric films with hydroxypropylmethylcellulose (HPMC) or Eudragit® RS 100 (Evonik Rohm GmbH, Darmstadt, Germany) were prepared in varying ratios using a silicone-moulded tray with individual wells. HPMC films were prepared by casting/solvent evaporation and EUD films by emulsification casting/solvent evaporation. MMFs comprising of ddI : HPMC : EUD in varying ratios were prepared by emulsification/casting/solvent evaporation. Films were characterised in terms of drug content (UV spectrophotometry) and drug release (shaking water bath). Film thickness was measured with an electronic digital micrometer and surface morphology assessed using scanning electron microscopy (SEM).

Results and Discussion

ddI : HPMC (1 : 0.5)-only films were homogenous and exhibited immediate release profiles. ddI : EUD (1 : 2.5)-only films were homogenous, elastic and flexible and showed controlled release profiles. The incorporation of ddI into MMFs with polymers and drug of opposing solubilities ddI : HPMC : EUD (1 : 0.5 : 2.5) resulted in homogenous, elastic and flexible films with immediate release profiles. Increasing the EUD concentrations led to controlled release profiles (Figure 1).

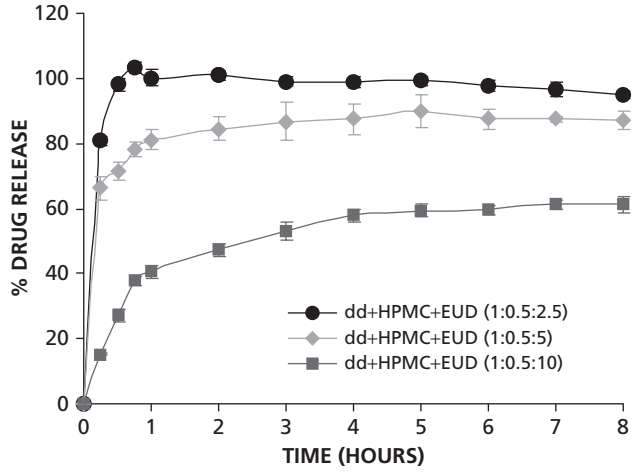

Figure 1 Effect of EUD on drug release profiles from MMFs.

Drug content, size, thickness and weight of the MMFs ddI : HPMC : EUD (1 : 0.5 : 2.5) were 97.65 ± 5.78%, 2×3 cm^2, 0.187 ± 0.023 mm and 108.65 ± 6.71 mg, respectively. SEM showed the films to have a smooth and homogenous compact surface before dissolution and changes in texture and pore formation after dissolution.

Conclusion

ddI can be incorporated into HPMC monopolymeric films for immediate ddI release applications. Homogeneous MMFs with drug and polymer (EUD) of opposing solubilities could also be prepared for controlled ddI release applications. MMFs with immediate and controlled ddI release profiles, with improved flexibility as compared with the monopolymeric films, can be prepared. The various films prepared in this study are potential candidates for optimisation of ddI films as a buccal delivery system.

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Specific swelling behaviour of bacterial cellulose composite as potential candidate for drug carriers

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Introduction and Objectives

The aim of this study was to produce a biocompatible hydrogel using bacterial cellulose as a natural filler in the composite. Intelligent hydrogels are being synthesised and

characterised as carriers and deliver the drugs to the target site in the body system. Cross-linking natural polymers such as polysaccharides will produce biodegradable device with improved mechanical strength.[1] Thus, bacterial cellulose, a potential natural biopolymer to produce biocompatible drug carrier was evaluated.

Method

Bacterial cellulose dispersion was mixed with acrylic acid and subjected to electron beam irradiation at 35 and 50 kGy. The hydrogels were extracted with distilled water at room temperature for 7 days. Gel fraction was determined by calculating the percentage of weight differences before and after the extraction of the hydrogels.[2] Swelling characterisation was further investigated by soaking the hydrogels in PBS with different pH. Changes in weight of the hydrogels were recorded at 1-h time interval for 24 h.

Results and Discussion

Gel fraction for hydrogels synthesised under 50 kGy electron beam irradiation was found to be 77.23% with respect to 60.21% for hydrogels under 35 kGy. It was also observed that hydrogels had very low swelling rate in the media pH 2 and pH 5 but greatly increased with the increasing pH where the highest swelling rate was reached at pH 7 as shown in Figure 1. However, when the pH was further increased to 10, the swelling rate decreased. Under the acidic condition, the carboxylic groups were in the nonionised form. However, as pH increases, the functional group ionises and enables hydrogen bonding with water molecules, thus providing a state of optimum swelling. But at pH 10, excessive presence of basic groups lowers the interaction.[3]

Conclusion

As renewable resources, bacterial cellulose enables production of more cost-effective hydrogels. The sensitivity and selectivity behaviours of the hydrogels to a range of pH protects the drug from strong acid, and the rapid swelling in the medium of pH 7 ensures the delivery of the drug to the intestine for optimal absorption.

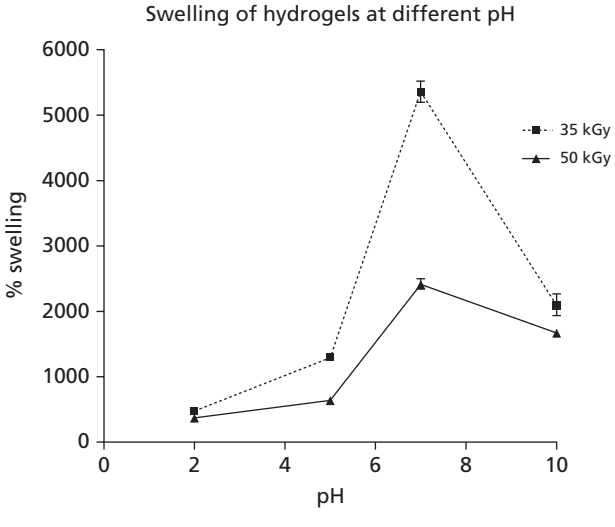

Figure 1 Swelling of the hydrogels at different pHs.

References

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The aerosol performance of spray-dried particles with different surface morphology from metered-dose inhalers

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Introduction and Objectives

'Raisin-like' (also called 'wrinkled') surfaces have been shown to lower the interparticulate interactions and increase the fine particle fractions (FPF) from dry-powder inhalers;[1] the feasibility of using such a strategy to improve aerosol performance of metered-dose inhalers (MDIs) has not been systematically studied. Therefore, the objective of this study was to determine the effects of this particle morphology on the aerosol performance of MDI-formulated spray-dried particles in the surfactant-free hydrofluoroalkane (HFA) propellants.

Methods

Three batches of rizatriptan particles with smooth (Figure 1a), 'dimpled' (Figure 1b) and raisin-like (Figure 1c) surfaces, respectively, were spray-dried. The surface morphology were qualitatively determined using scanning electron microscopy (SEM), whereas the aerodynamic properties of MDIs were evaluated using a next-generation pharmaceutical impactor (NGI).

Results and Discussion

The volume median diameters of the particles were found to be between 2.61 and 3.61 μm. After filling the cannisters with HFA 134a, smooth particles were observed visually to flocculate markedly and other particles with dimpled surface also flocculated but to a lesser degree. No apparent flocculation occurred when raisin-like particles were suspended. The dose uniformity results suggested that MDIs containing particles with smooth or dimpled surface afforded over 10% variations, whereas that with wrinkled particles afforded less than 6%. The deposition results showed that the drug fractions recovered from stage 3 down to the filter of the

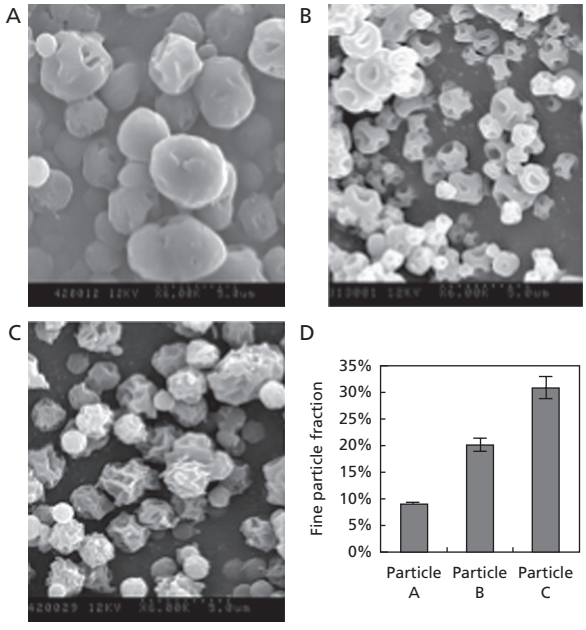

Figure 1 SEMs of spray-dried (a) smooth, (b) dimpled and (c) wrinkled particles and (d) fine particle fractions (FPF) of spray-dried rizatriptan particles dispersed in hydrofluoroalkane (HFA) metered-dose inhalers (MDIs) as measured using a next-generation pharmaceutical impactor (mean ± SD; $n = 4$).

NGI (FPF) for smooth, dimpled and wrinkled particle MDI formulations were about 9.2, 20.2 and 30.8% (Figure 1d) and the mass median aerodynamic diameters (MMAD) were 4.96 ± 0.12, 3.48 ± 0.10 and 2.63 ± 0.30 μm, respectively.

Conclusion

The present results demonstrated that wrinkled particles afforded a better aerosol performance over those with smooth or dimpled surface in terms of dose uniformity, FPF and MMAD when dispersed in a surfactant-free HFA 134a propellant.

Reference

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Curcuminoids-loaded solid lipid nanoparticles: development, optimisation and physicochemical evaluation

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Introduction and Objectives

The principle objective was to develop and optimise solid lipid nanoparticles (SLNs) capable of efficiently encapsulating curcuminoids, a photolabile drug, at the same time protecting it from photodegradation and to evaluate its physicochemical characteristics to support the development of stable and efficient curcuminoids-loaded SLNs. The other important objective of the work was to provide sustained release of curcuminoids over a prolonged period of time by formulating SLNs.

Method

The SLNs were prepared by hot high-pressure homogenisation.[1] 32 Factorial design was used for the optimisation of SLNs. The particle size and zeta potential measurement was carried out using ZetaSizer 3000 (Malvern Instruments, Malvern, UK). The encapsulation efficiency of the SLNs was determined by ultracentrifugation. Thermal analysis of SLNs was carried out using DSC 821e (Mettler Toledo, *Leicester*, UK). Transmission electron microscopy was done using Hitachi S-7500 (Hitachi, *Maidenhead*, UK). The stability study was carried out at 40°C/75% relative humidity in presence and absence of light for 3 months, by evaluating particle size, zeta potential and entrapment efficiency. In-vitro release study was carried out by diffusion using cellophane membrane.

Results and Discussion

The particle size, entrapment efficiency and zeta potential of SLNs were found to be 194 nm, 77.27% and –8.21 mV, respectively. The thermal analysis of curcuminoids and curcuminoids-loaded SLNs revealed that curcuminoids were encapsulated inside the SLNs in amorphous form. The stability study showed that the prepared SLNs were stable for 3 months, as no significant change in the particle size and zeta potential was observed. Also, no significant change in encapsulation efficiency showed that SLNs were capable of protecting curcuminoids from photodegradation. In-vitro release study showed that SLNs were capable of sustained release of curcuminoids for 24 h.

Conclusion

The developed SLNs were found to be efficient to encapsulate curcuminoids effectively. The physicochemical investigation supports the stable and efficient formation of SLNs by the proposed method. Also, the encapsulation provided good photostability to curcuminoids, which was evident from the stability study. The capability of prepared SLNs to sustain the release of curcuminoids provides reduced dosing frequency and hence, patient compliance.

Reference

1. Hou D *et al*. The production and characteristics of solid lipid nanoparticles (SLNs). *Biomaterials* 2003; 24: 1781–1785.

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Skin permeation mechanism of valsartan from transdermally applied gel formulation using 1, 8-cineole as permeation enhancer

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Introduction and Objectives

The purpose of this study was to investigate the skin permeation mechanism of valsartan by using 1,8-cineole (terpene) as permeation enhancer. The mechanism of permeation enhancement of valsartan by test terpene was elucidated with FTIR, DSC, thermodynamic study and histopathological study.

Methods

For determination of mechanism of enhancement of terpene, control and 1,8-cineole-treated stratum corneum samples were scanned on FTIR and DSC instruments. While in thermodynamic study, ex-vivo permeation studies were carried out across rat skin at 22, 32 and 42°C in neat vehicle and including 1% w/v cineole in donor phase. Activation energy of valsartan was then calculated from Arrhenius relationship. In histopathological study, albino rats were treated with valsartan gel formulation containing 1% cineole. Rats were sacrificed and the skin samples from the treated and untreated areas (control) were observed under light microscope.

Results and Discussion

FTIR spectrum of stratum corneum treated with cineole showed decrease in height and area of asymmetric (2920 cm^{-1}) and symmetric CH-stretching (2850 cm^{-1}), and shifting of amide peaks (1650 and 1550 cm^{-1}) to lower wave number compared to control spectra suggests the extraction of lipids and protein (keratin), respectively, from stratum corneum by the cineole. Whereas in the DSC thermogram of treated stratum corneum, it was observed that endotherm T_4 shifted to 122°C with broadening of the peak. Shift to higher transition temperature and peak broadening have been attributed to dehydration of stratum corneum as another mechanism of permeation enhancement of cineole.[1] The results of thermodynamic studies showed that the E_{act} values were found to be 3.17 and 2.76 kcal/mol in the presence of neat vehicle (control) and 1% w/v cineole in vehicle, respectively. The significant decrease in E_{act} for valsartan transport across rat skin by cineole (1% w/v) indicates that it disrupts the stratum corneum lipid bilayers. The photomicrograph of control skin showed well-defined epidermal and dermal layers while treated skin sample showed disruption of lipid bilayers as distinct and empty spaces were visible in the topmost layer (stratum corneum) due to the action of the penetration enhancer (cineole).

Conclusions

The results of this study indicated that 1, 8-cineole can be successfully used as a potential enhancer for enhancement of skin permeation of valsartan. The FT-IR, DSC and histopathological studies showed that 1,8-cineole enhanced the skin permeation of active medicament by disruption and extraction of lipid bilayers and keratin denaturation of stratum corneum; no apparent skin irritation (erythema, oedema) was observed on treatment of skin with enhancer.

Reference

1. Aqil M *et al*. Status of terpenes as skin penetration enhancers. *Drug Disc Today* 2007; 12: 1061–1067.

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The role of lyophilisation in the enhancement of glibenclamide release from hard gelatin capsules

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Introduction and Objectives

A significant number of drug products have low bioavailability and consequently low efficacy due to limited solubility in the gastrointestinal tract. The aim of this study was to enhance the release rate of the hypoglycaemic agent glibenclamide from hard gelatin capsules through co-lyophilisation with sodium lauryl sulphate (SLS) and perform physical characterisation of the lyophilised product.

Method

Glibenclamide or its mixture with SLS (2.5, 5, 7% w/v) was dissolved in a co-solvent system of 37.5% w/w tertiary butyl alcohol, 12.5% w/w ammonium hydroxide solution (30%) and 50% water, frozen and then lyophilised overnight. A corresponding drug/SLS physical mixture was prepared. Dissolution was conducted in a Copley dissolution apparatus (USP II, 37°C, 100 rpm, 1000 ml PBS (pH 7.4)). Glibenclamide release was determined spectrophotometrically at 240 nm. Physical characterisation was performed by XRPD (Bruker-AXS D8 X-ray powder diffractometer), DSC (Mettler Toledo differential scanning calorimetry 822e) and

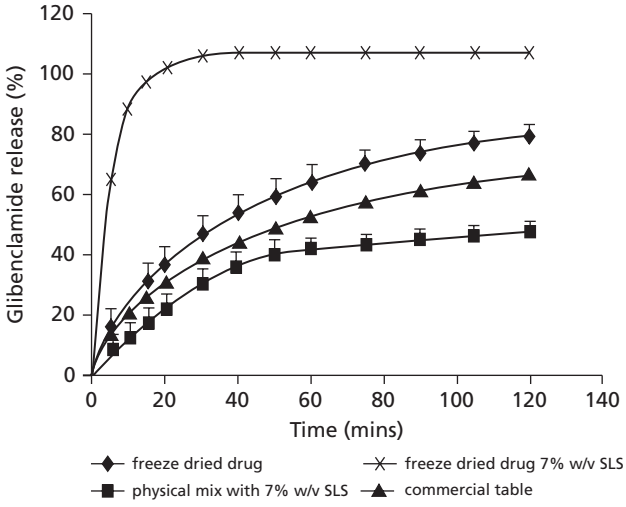

Figure 1 Mean dissolution profiles (±SD) of glibenclamide from hard gelatin capsules compared with commercial tablets (n = 6).

ATR-FTIR (Jasco FT/IR-4200 Fourier transform infrared spectrometer).

Results and Discussion

Lyophilised glibenclamide showed a marked dissolution enhancement over raw drug (F_2 = 30.5) and commercial tablets (F_2 = 39.8). Dramatic dissolution enhancement was exhibited on co-lyophilisation with 7% w/v SLS. The corresponding physical mixture exhibited a slower, more limited dissolution (Figure 1, F_2 = 8.30). Using DSC, the absence of a glibenclamide melting peak as well as a shift of the SLS endotherm to a lower temperature confirms an interaction between SLS and glibenclamide and may suggest solid dispersion formation.[1] FTIR demonstrated a shift and disappearance of some characteristic glibenclamide peaks in the lyophilisates, confirming a change of the physical form of the drug or intermolecular interactions. XRPD showed that the lyophilisates exhibited extensive differences in the position, number and intensity of peaks compared to the raw glibenclamide. This suggests that the majority of the drug converted to a more soluble amorphous form.

Conclusions

Combined physical characterisation data suggest that co-lyophilisation of glibenclamide with SLS enhances the drug release and dissolution by the formation of an amorphous solid dispersion.

Reference

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Penetration and wound-healing responses following application of microneedles and hypodermic needles to human volunteers

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Introduction and Objectives

Microneedles are currently being developed and tested for minimally invasive drug and vaccine delivery. A recent clinical study by Haq *et al.*[1] has shown that microneedles of length 180 and 280 μm are significantly less painful than 25-gauge hypodermic needles and elicit different sensations during application. To date, there has been little information published regarding the biological skin response to microneedle-mediated delivery. In this study, we analyse clinical biopsies of microneedle and hypodermic needle application sites to assess wound-healing response.

Methods

Following National Health Service (NHS) ethics approval, participants (n = 12) were randomised to receive on each buttock blinded applications of two types of microneedle array (36 pyramidal microneedles of either 180- or 280-μm height and a 25-gauge subcutaneous hypodermic needle). The creation and temporal retention of skin microchannels was assessed at time points 1, 4, 8 and 24 h by external topical staining, using 10% methylene blue to highlight microchannels. Measurement of transepidermal water loss (TEWL) was conducted under controlled environments to establish skin barrier disruption, by measuring water loss from the surface of the skin directly from each application site. At each time point, detection of proinflammatory and wound-healing markers, including keratins K16 and K17 and immune cell markers, e.g. CD68, was determined by immunohistochemistry of sectioned biopsy.

Results and Discussion

Both methylene blue staining and TEWL analysis clearly showed the formation of microconduits following microneedle application, with skin resealing evident within the 24-h study period. Topical staining of application sites showed that the 280-μm microneedle array punctured skin efficiently, with a mean of 96% of the 36 pyramidal needles creating puncture marks. The 180-μm microneedles showed less efficient penetration with a mean of 50% successful skin punctures. In both cases, the created microchannels appear to repair and reseal within 24 h as evidenced by skin staining

(22 and 31% puncture marks remaining open to stain at 24 h for the 180- and 280-μm microneedle arrays, respectively), TEWL (return to baseline at 24 h) and tissue sectioning (lack of visible microchannels at 24 h). Unlike hypodermic injection, no bleeding or erythema was evident at microneedle application sites. Immunohistochemical analysis of biopsy samples showed upregulation of wound healing and immunological markers around the hypodermic insertion site at 24 h. These markers were not upregulated in microneedle-treated biopsies.

Conclusion

This study demonstrates that microneedles represent a potentially efficient method for disrupting skin barrier properties for transcutaneous drug delivery applications. From a safety perspective, microneedle application, unlike hypodermic puncture, caused reduced cellular responses with physical microchannel repair and resealing appearing to occur within 24 h. In contrast with the hypodermic needle, there was no clear evidence of hyperproliferative or immunological wound response to microneedles over the time period.

Acknowledgement

This work was supported by The British Skin Foundation.

Reference

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The use of a quaternised amphiphilic polymer to modify insulin uptake in Caco-2 cells towards oral insulin

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Introduction and Objectives

This study extends previous work using a novel amphiphilic polymer.[1] The objectives of the study are (1) to demonstrate the ability of the polymer to facilitate insulin uptake into Caco-2 cells, (2) to determine the effect of incubation time on uptake of the polymer-insulin complexes and (3) to elucidate the mechanism of cellular uptake of the insulin-polymer complex.

Methods

Polyallylamine modified with 5% mole palmitoyl pendant groups and 70% mole quaternary ammonium moieties (QPa) was tagged with the fluorescent marker rhodamine (R), while insulin was labelled with fluorescein isothiocyanate (FITC). This allowed for the localisation of the polymer and the insulin to be distinguished when visualised with different filters using a fluorescent microscope. FITC-insulin (3 μg/ml), RQPa (48 μg/ml) or RQPa : insulin complexes (48 : 3 μg/ml) were incubated with Caco-2 cells for 0.5 or 1 h and images were recorded. Trypan blue was added to determine cell viability. The experiments were also carried out after preincubation in calcium-free Eagle's minimum essential media (EMEM) and in supraphysiological concentrations of insulin (3 μg/ml) for 1 h.

Results and Discussion

Polymer-insulin complexes were taken up by cells within 1 h while insulin alone was taken up into the cytoplasm within 0.5 h (Table 1). Trypan blue staining was not observed, indicating that the cells retained their viability. The polymer and insulin within the complex were colocalised within the cytoplasm and perinuclear area. Uptake of complexes took place in the absence of calcium or following down regulation of insulin receptors.

Conclusion

The polymer facilitated insulin uptake by cells *via* a calcium independent, nonreceptor-mediated pathway. Entry of insulin was slower than *via* receptor-mediated uptake of insulin alone.

Table 1 Location and uptake time for polymer/insulin samples

Sample	Preincubation	Time for maximal uptake, h	Intracellular location (+/–)	
			Polymer (R)	Insulin (FITC)
RQPa	None	1	+	n/a
	Calcium-free EMEM	1	+	n/a
	Insulin-supplemented EMEM	1	+	n/a
Insulin	EMEM	0.5	n/a	+
	Calcium-free EMEM	No uptake	n/a	–
	Insulin-supplemented EMEM	No uptake	n/a	–
Complex	None	1	+	+
	Calcium-free EMEM	1	+	+
	Insulin-supplemented EMEM	1	+	+

Abbreviations: R, rhodamine; FITC, fluorescein isothiocyanate; EMEM, Eagle's minimum essential media.

The polymer is able to facilitate the internalisation of the insulin and is a potential system for therapeutic oral protein delivery.

Reference

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The use of novel polyallylamine-based amphiphilic polymers to enhance the aqueous solubility of hydrophobic drugs

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Objectives

In the developmental stage 60% of drugs are hydrophobic; this presents a growing problem in today's pharmaceutical industry. Amphiphilic polymers spontaneously form self-assemblies in aqueous solution and have been explored in recent years as promising delivery systems for hydrophobic drugs. Here, we report the synthesis of two novel comb-shaped amphiphilic polymers based on polyallylamine (PAA) and their potential as hydrophobic drug solubilisers.

Methods

Two novel polymers were synthesised by grafting 15 kDa PAA with 5% mole of hydrophobic group X (X-PAA) and 10% of Y (Y-PAA). Polymer structure was characterised using elemental analysis, NMR and FTIR. Polymeric self-assemblies were formed by probe sonicating the polymers in water, and the particle size and surface tension measurements were carried out using a photon correlation spectrometer (PCS) and torsion balance, respectively. Three hydrophobic drugs, propofol, prednisolone and griseofulvin, were incorporated into aqueous polymer solutions by probe sonication, filtered with 0.45 μm filter and analysed by a reverse-phase HPLC. In-vitro drug release was carried out using dialysis method in PBS over 96 h, in sink conditions, and the biocompatibility studies were conducted using haemolysis and MTT assays.

Results and Discussion

Polymers were successfully synthesized. The results of elemental analysis correlated well with the expected hydrophobic modification based on initial molar feed ratios. The size of polymeric self-assemblies at 6 mg/ml was 296 nm and 126 nm, with the polydispersity index (PDI) of 0.285 and 0.312, respectively, indicating uniform size distribution. Critical association concentration (CAC) was measured on the surface-tension plot where no further decrease is observed, and the graph plateaus. This occurred at 0.093 mg/ml and 0.25 mg/ml for X-PAA and Y-PAA, respectively. Both polymers formed nanoparticulate formulations and showed high solubilising capacity. The Y-PAA consistently exhibited significantly higher capacity for all three hydrophobic drugs with excipient to drug ratio as low as 0.19 : 1 (Figure 1). The Y-PAA increased the aqueous solubility of propofol, prednisolone and griseofulvin up to 220-fold, 100-fold and 400-fold, respectively. Previous work showed that 5% mole Y-PAA exhibited similar solubilising capacity as the 5% mole X-PAA. Precipitation of 10% mole X-PAA occurred in water indicating high hydrophobic load was not desirable for this polymer and hence no further work was conducted. In-vitro drug release studies showed that both the polymers achieved sustained drug release over 48–96 h. Both the polymers exhibited good biocompatibility profile where no haemolytic activity was observed over the concentration range tested (0.005–1 mg/ml). The MTT assay using Caco-2 cells showed that both the polymers have similar IC50 values to unmodified PAA (0.023 mg/ml), indicating

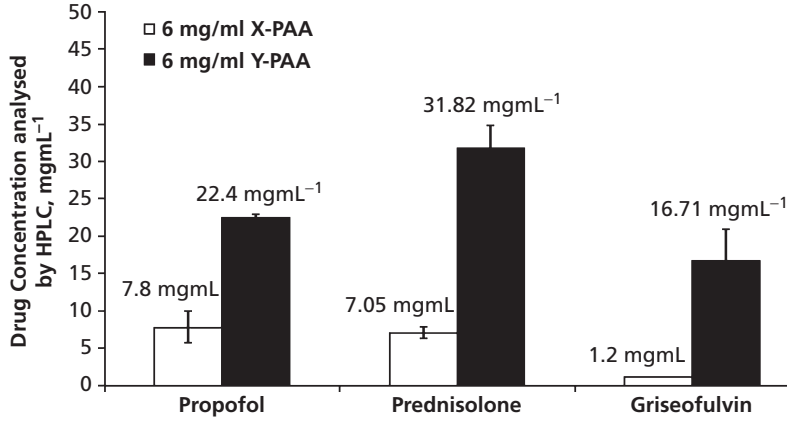

Figure 1 Optimal drug solubilisation of propofol, prednisolone and griseolfulvin using X-PAA and Y-PAA (n = 3; SD).

hydrophobic modification did not alter the cytotoxicity profile of PAA.

Conclusions

Our results demonstrated that the type of hydrophobic pendant groups has a major impact on the solubilising capacity. Compared to other conventional excipients such as low molecular weight surfactants that require typical excipient to drug ratios of 17 : 1, these novel amphiphilic polymers exhibited high solubilising capacity. In-vivo work will be conducted in the future to determine their suitability as new solubilisers for hydrophobic drugs.

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Tumour regression after systemic administration of a novel targeted vitamin E therapeutic system

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Introduction and Objectives

Tocotrienol, an extract of vitamin E, has been shown to exert antiproliferative and tumour suppressive effects on cancer cells.[1] However, its therapeutic potential is currently limited by its failure to reach tumours after intravenous administration, without secondary effects on normal tissues. The objectives of this study are (1) to prepare and characterise novel transferrin-targeted vesicles encapsulating tocotrienol, able to recognise transferrin receptors overexpressed on many cancer cell lines and (2) to evaluate *in vitro* and *in vivo* the therapeutic and targeting efficacies of this therapeutic system.

Methods

Tocotrienol was encapsulated in Span 60 vesicles upon heating and probe sonication, prior to transferrin conjugation by cross-linking. Upon purification by ultracentrifugation, vesicles were visualised by transmission electron microscopy and have their size and zeta potential measured by photon correlation spectroscopy. Transferrin conjugation was assessed by a Lowry protein quantification assay. Tocotrienol encapsulation was quantified by spectrofluorimetry. Tocotrienol uptake by cancer cells was assessed quantitatively by spectrofluorimetry and qualitatively by confocal microscopy. The therapeutic efficacy of this system was evaluated *in vitro* using a MTT assay and *in vivo* after intravenous administration to a murine xenograft model.

Results and Discussion

The sizes of tocotrienol-loaded vesicles were found to be 331 ± 0.615 nm for transferrin-bearing vesicles and 233 ± 0.833 nm for control vesicles, respectively. The zeta potential of these vesicles was found to be –2.18 mV and –4.19 mV, respectively, for transferrin-bearing and control vesicles. Transferrin was conjugated to the vesicles at a level of $89 \pm 5\%$ of the initial transferrin added. The tocotrienol loading within the vesicles was $44.1 \pm 0.6\%$ of the initial tocotrienol. *In vitro*, the therapeutic efficacy of tocotrienol when encapsulated in transferrin-bearing vesicles was improved by at least 100-fold compared to free tocotrienol and at least 2-fold compared to nontargeted vesicles in T98G, A431 and A2780 cancer cells (i.e. IC50 : 0.17 μg/ml, 0.97 μg/ml and 79.49 μg/ml, respectively, for Tf-vesicles, control vesicles and drug solution in T98G cells). *In vivo*, intravenous administration of tocotrienol encapsulated in transferrin-bearing vesicles led to the regression of well-established tumours, followed by a delayed progression. By contrast, treatment with tocotrienol in solution or encapsulated in control vesicles did not lead to any tumour regression. The treatment was well tolerated in mice, with no weight loss or visible signs of toxicity.

Conclusion

Transferrin-bearing vesicles encapsulating tocotrienol have been successfully prepared. This study corresponds to the first preparation of a tumour-targeted delivery system able to encapsulate tocotrienol. Our findings show that tocotrienol encapsulated in transferrin-bearing vesicles is a highly promising therapeutic system, leading to tumour regression after intravenous administration without visible toxicity.

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Design of a polymeric delivery system incorporating a model bacteriophage for wound dressings and biomaterial coatings

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Introduction and Objectives

The objective of this article was to develop a polymeric delivery system incorporating a model bacteriophage for use in wound dressings and/or biomaterial coatings. This type of delivery system could combat the growing problem of bacterial

infection by antibiotic resistant bacteria. The bacteriophage plaque assay method was optimised. The stability of the bacteriophage in a number of diluents and storage conditions and in a polymer system was also examined. The influence of phage incorporation on the physical properties of the polymeric material was characterised by differential scanning calorimetry, dynamic mechanical thermal analysis, thermal gravimetric analysis and dielectric spectrometry.

Method

The double layer plaque assay method[1] for bacteriophage viability was optimised by varying agar types, and diluents until uniform countable plaques were obtained. T4 bacteriophage, an *Escherichia coli*-specific virus, was purchased from ATCC (Manassas, USA) and reconstituted in Luria-Bertani (LB) broth. Stability of phage stock solutions in different diluents and under different storage conditions was examined using the plaque assay. Subsequently, 10^9 pfu/ml, 10^7 pfu/ml and 10^5 pfu/ml loadings of T4 phage were incorporated into poly(methyl vinyl ether-co-maleic anhydride) (PMVE/MA) polymeric films, dissolved in phosphate buffered saline (PBS) and their viabilities examined.

Results and Discussion

LB agar gave uniform, countable plaques and so was chosen for the plaque assay. T4 proved to be stable in solution. A phage titre of approximately 10^{12} pfu/ml was propagated. Samples were stored at 4 and 25°C, and 1000-fold drops in plaque titre were observed after 5 days, but then viability remained stable at 10^9 pfu/ml for 35 days. A similar titre drop occurred after 5 days with T4 samples stored at pH 3, 7 and 10 and at NaCl concentrations of 0.1, 1.0 and 5.0 M. Substantial decreases in plaque titre occurred at pH 3 and 10 after 7 days. However, the pH 7 sample showed prolonged stability. Similar results were noted for phage in PBS/Tween80 solutions and for samples stored in both glass and poly(propylene) containers. A 100–1000-fold drop in plaque titre occurred after 6 days, with stocks remaining stable after this time. T4 phage in PMVE/MA film formulations of modified pH (pH 4 and 7) showed improved survival of phage relative to unmodified formulations, which had a pH of around 2. Stability was greatest at pH 7. Bacteriophage loading had no significant effect on mechanical strength or thermal characteristics of PMVE/MA film formulations.

Conclusions

The plaque assay was optimised for use with T4 phage. T4 was stable in a series of diluents, with an average 10^9 pfu/ml titre remaining over prolonged times. The drop in titre after 5–6 days was shown to be concentration-dependent, with original stocks of 10^9 pfu/ml or lower not exhibiting this behaviour. Phage instability in films was likely to be due to the low pH of PMVE/MA. Neutralisation to pH 7 improved phage stability. We are currently investigating phage release from PMVE/MA films formulated at pH 4 and 7.

Reference

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Towards improved pulmonary delivery of budesonide using a nebulisable nanoparticulate hydrogel

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Introduction and Objectives

Pulmonary drug delivery forms the cornerstone of the therapeutic management and treatment of inflammatory airway conditions such as asthma and chronic obstructive pulmonary disease. Poor compliance with existing treatment regimes can lead to a loss of therapeutic efficacy with a corresponding reduction in disease management. Poor technique by patients when using pulmonary delivery devices is a major contributing factor to suboptimal efficacy of inhaled therapies, thereby demonstrating a need for improved delivery solutions. This abstract describes a nebulisable nanoparticulate hydrogel technology capable of delivering efficacious doses of budesonide to epithelial cell monolayers in culture.

Method

In-vitro epithelial cell-based 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and tetrazolium salt (WST-1) functional endpoint assays for corticosteroids were used to ascertain whether budesonide delivered by nanoparticulate hydrogel technology could actively suppress the production of superoxide anions (O_2^-), the main component of the so-called oxidative burst (OB), a recognised marker of inflammation in cells. Human keratinocyte (HaCaT) epithelial cells were preincubated for 24 h with budesonide delivered by RPMI fully supplemented media infused with Pulmicort (AstraZeneca, UK) or Nanagel (AGT Sciences LTD, West Yorkshire, UK) to a final drug concentration of 5 μg/ml. The cells were then stressed by priming with tumour necrosis factor (TNF)-α at 1 ng/ml for 30 min, followed by exposure to the formyl peptide (formyl-met-leu-phe – fMLP) at 10^{-7} M for further 120 min. Assay controls using superoxide dismutase at 250 U/ml were also used.

Results and Discussion

Through the sequential application of the inflammatory stimulants TNF-α and fMLP in the presence of Ca^{2+} ions, it has been demonstrated that HaCaT epithelial cells can be induced to elicit an inflammatory response, subsequent attenuation of which by budesonide delivered to cells by hydrogel vehicles can be studied *in vitro* using a functional MTT colorimetric-based assay, involving the reduction of tetrazolium salts by the cellular inflammatory product superoxide (O_2^-). No attenuation of the induced OB in cells pretreated with budesonide at 5 μg/ml delivered by Pulmicort was observed. In contrast, a *t*-test on data relating

to cells pretreated with budesonide at 5 μg/ml delivered by Nanagel confirmed significant ($P = 0.0337$) attenuation of the cellular inflammatory response. These findings were consistent independent of the assay methodology used with both MTT and WST-1 providing comparable data. In contrast to MTT, the latter technique is considered to exclusively assay extracellular superoxide production. Moreover, the MTT assay indicated no apparent toxicity to cultured cells following exposure to the Nanagel preparation.

Conclusion

This work describes a robust in-vitro cell-based functional assay for studying cellular OB and pharmacological approaches to its preclusion. The MTT technique represents an economical, rapid assay for studying OB activity. Combined, MTT and WST-1, data confirmed that budesonide delivered by nanoparticulate hydrogel technology (Nanagel) is functionally active and is able to suppress markers of inflammatory activity in stressed HaCaT cells. Given that Nanagel can deliver several-fold greater quantities of budesonide into cells than Pulmicort at an equivalent dose, and is nebulisable, this indicates a possibility of delivering inhaled budesonide more rapidly and less frequently while maintaining therapeutic benefit to patients in the clinical setting.

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Investigating the potential of gas-filled lipid-coated microbubbles as vaccine adjuvants

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Introduction and Objectives

Liposome systems are well reported for their activity as vaccine adjuvants; however, novel lipid-based microbubbles have also been reported to enhance the targeting of antigens into dendritic cells (DCs) in cancer immunotherapy.[1] The objective of this work was to investigate the formulation of gas-filled lipid-coated microbubbles and assess their potential activation of macrophages using in-vitro models.

Method

Neutral aqueous-filled liposomes comprising 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) with either cholesterol and/or polyethylene-glycol distearate (PEG-distearate), and cationic liposomes, 3β-[*N*-(*N*',*N*'-dimethylaminoethane)-carbamoyl]-cholesterol (DC-Chol), with cholesterol or 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine were prepared by film-hydration method. Microbubbles were prepared as described by Vangala *et al*.[2] by homogenisation with headspace filled with air or nitrogen (N_2). Alternatively, microbubbles were prepared by aqueous-filled liposomes being pressurised with perfluoropropane gas.[3] Macrophage Balb/c cell line was cultured in Dulbecco's Modified Eagle Medium containing 10% foetal bovine serum and 1% penicillin–streptomycin–glutamine. Liposome cytotoxicity was determined using CellTiter 96 Aqueous One Solution assay and phagocytosis by *N*-acetyl-ß-D-glucosaminidase assay.

Results and Discussion

In contrast to the liposomes possessing an aqueous core that are prepared at temperatures above their phase transition temperature (T_c), homogenisation of phospholipid such as DSPC in aqueous medium below their T_c was found to be crucial in formation of stable microbubbles. Of the three formulations, DSPC in the presence or absence of cholesterol and/or PEG-distearate, DSPC-based microbubbles filled with either air or N_2 were found to be the most stable in size over the 28 days. However, it was not possible to form cationic microbubbles by this method due to the low T_c (i.e. <25°C) of the DC-Chol lipid. These cationic microbubbles were, therefore, prepared by the Suzuki *et al*.[3] method. However, these systems had very limited stability for less than 30 min, demonstrating their lack of applicability as a realistic clinical application. The ability of the various lipid formulations incorporating an aqueous phase demonstrated a cell viability of above 90% for both neutral and cationic liposomes demonstrating no significant reduction of cell viability. These formulations also showed no phagocytotic activity suggesting the bilayer has limited adjuvant properties. However, the effect of incorporating a range of gases into these liposomes will also be presented to investigate their potential as vaccine adjuvants.

Conclusion

A range of lipid-based microbubbles can be prepared. For the delivery of subunit antigens, a cationic lipid coat is preferable; however, their lack of stability severely limits their application and therefore previous studies suggesting their application targeting of antigens into DCs and gene therapy cannot be realistically considered.

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In-vitro and in-vivo investigation of thermosensitive chitosan hydrogels containing liposomes or silica nanoparticles for vaccine delivery

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Introduction and Objectives

The objective of this study was to develop simple but effective vaccine formulations that deliver the vaccine antigen and adjuvant in a particulate form over a sustained period of time. The sustained release formulation used in this study was thermosensitive chitosan, which is a solution at room temperature but gels upon heating to body temperature. The solutions were loaded with liposomes or silica nanoparticles containing the model antigen ovalbumin (OVA) and the adjuvant Quil A (QA).

Methods

Thermosensitive chitosan solutions were made by dissolving chitosan in 0.1M hydrochloric acid, followed by the addition of glycerol 2-phosphate disodium hydrate. Liposomes containing OVA ± QA were manufactured by lipid film hydration. Silica nanoparticles containing OVA were manufactured using sol-gel emulsion chemistry. For in-vitro release studies, gels loaded with fluorescein isothiocyanate (FITC)-OVA entrapped within liposomes or silica particles were covered with buffer and incubated at 37°C. In-vivo release studies and assessment of immune responses were carried out in C57Bl/6 mice immunised with chitosan solutions containing OVA and QA either entrapped within particles or soluble form. As controls, immediate release particulate vaccines were administered. Mice were culled and immune responses were examined.

Results and Discussion

Thermosensitive chitosan gels, liposomes and silica nanoparticles were developed, optimised, and characterised. Release of OVA from both liposome- and silica nanoparticle-containing chitosan gels *in vitro* was sustained and incomplete (20–30% over a period of 10 days). Most of the antigen was released in a soluble form, that is, it was not associated with the particles. This is most likely due to the instability of the liposomes and the high porosity of the nanoparticles. The in-vivo release studies confirmed that antigen was released in a sustained manner. The immune responses generated by the sustained release formulations, as measured by determining antibody levels and the extent of both CD4 and CD8 T-cell expansion, was higher in mice immunised with the sustained release gels. However, the impact on immune responses of the antigen and adjuvant being in particulate, as opposed to soluble, form was variable. This is again most likely due to the characteristics of the particles used in these studies.

Conclusion

Chitosan thermosensitive gels are a promising sustained release delivery system for vaccines. However, more work needs to be done to optimise the formulation, in particular, the type of particles the antigen and adjuvant are loaded into before inclusion in the gel. The particles need to be stable in the gel and to retain loading of both antigen and adjuvant so that intact antigen- and adjuvant-loaded particles are released. This should increase the potency of any resulting immune response.

Material Science

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Characterisation of the drying kinetics and physicochemical properties of ethylcellulose films containing plasticizers and chlorhexidine

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Introduction and Objectives

The main objective of this work was to quantitatively examine and model the effects of drying conditions on the drying process of physicochemical properties of ethylcellulose (EC) films containing plasticizer and chlorhexidine. For this purpose, the drying kinetics of ethylcellulose films from organic solutions was studied. In addition, the thermal and mechanical properties, morphology, and Raman spectroscopy of EC films were also characterised.

Methods

Solutions of ethylcellulose containing triacetin and chlorhexidine were prepared using a range of solvents (dichloromethane, ethanol and mixtures therein). Following this, the polymeric solution was dispensed into the TGA aluminium pan and dried at constant temperature and airflow rate. Mass transfer during the drying process was continually monitored by thermogravimetric analysis (TGA) and modelled using MATLAB. In addition, the polymeric solution was dispensed into a mould and dried using a tray drier. The thermal and mechanical properties, morphology, and Raman spectroscopy of EC films were characterised using tensile analysis, differential scanning calorimetry (DSC), Raman, transmission electron microscopy (TEM), and modelled by MATLAB to describe the experimental data.

Results and Discussion

The mechanical properties of the films (5 × 1 cm sections) were determined using tensile analysis (1 mm/s crosshead

speed) from which the ultimate tensile strength, % elongation at break, and Young's modulus (YM) were derived. The glass transition temperature of the films was determined using modulated DSC under defined conditions (2°C/min heating rate), whereas the spectroscopic properties of the films were examined using Raman spectrometer (between 400 and 3200 cm^{-1} with a data collection time of 30 s). The morphological characteristics of the EC films were examined by using a high-resolution TEM at a 200 kV operation machine. The effects of plasticizer, drug, and solvent compositions on the drying rates of the drying process of EC films were determined using a three-way ANOVA ($P < 0.05$). Decreasing DCM content of the solvent and plasticizer significantly reduced the mechanical properties of EC films. Incorporation of chlorhexidine increased the drying rate and reduced the mechanical properties of EC film. The glass transition temperatures of EC films with more plasticizer content were the much lower than blank EC films. The spectroscopic properties of the films remained unaffected by different drying solvent. The phenomenon of chlorhexidine and triacetin effect on EC films also were confirmed by TEM. It was still found that the whole drying process of EC films with plasticizers and drugs have three distinct regions, which agreed with our earlier study on drying kinetics of EC blank films.

Conclusion

This study has highlighted and modelled the effects of the plasticizer and drug on the rate of drying and quality of EC films. The results have implications for convection drying process, including using hot air in tray dryers and also for tablet coating process.

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60 Characterisation of the drying kinetics and physicochemical properties of Eudragit films with different salts of chlorhexidine

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Introduction and Objectives

The aim of this study is to quantitatively examine the effects of drying conditions on the drying process of physicochemical properties of Eudragit E100 films containing different salts of chlorhexidine (CHX). For this purpose, the drying kinetics of Eudragit E100 films prepared from organic solvent casting was studied. And the thermal properties, mechanical properties and Raman spectroscopy of Eudragit E100 films with different CHX salts were also characterised.

Method

Solutions of Eudragit E100 (5, 10 and 15%) containing different salts of CHX were prepared using different organic solvents (dichloromethane (DCM) and ethanol (EtOH)). The varieties of CHX salts were prepared using CHX base with myristic acid and oleic acid with different molar ratios (2:1, 1:1 and 1:2). Following this, the polymeric solution (100 μl) was dispensed into the thermogravimetric analysis aluminium pan (100 μl volume) and dried at 25°C and under airflow rate at 60 ml/min. In addition, the polymeric solution (50 ml) was dispensed into a mould and dried using a tray drier to make films, operating at the same airflow rate and temperature.

Results and Discussion

The mechanical properties, thermal properties and Raman spectroscopy of Eudragit films were characterised using tensile analysis, modulated differential scanning calorimetry and Raman. The mechanical properties of the films (5×1-cm sections) were determined using tensile analysis (1 mm/s crosshead speed) from which the ultimate tensile strength, % elongation at break and Young's modulus were derived. The glass transition temperature of the films was determined using modulated differential scanning calorimetry under defined conditions (2°C/min heating rate), whereas the spectroscopic properties of the films were examined using Raman Spectrometer (between 400 and 3200 cm^{-1} with a data collection time of 30 s). The Eudragit E100 films prepared by EtOH had the greater mechanical properties than those prepared by DCM. Incorporation of CHX reduced the mechanical properties and increased the Tg of Eudragit E100 films. CHX also shows very limited solubility in EtOH than DCM even with high ratio of fatty acid. The Raman spectroscopy results showed the significant difference (from 1300 to 1600 cm^{-1}) when the molar ratios of CHX base and acid changed in both DCM and EtOH.

Conclusions

This study has highlighted the effects of different organic solvents and different CHX salts on the rate of drying process and quality of Eudragit E100 films.[1] These results have direct implications for the design of polymer protective coatings for formulation development and process technology.

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61
Physicochemical characterization of Grewia polysaccharide gum: effect of drying method

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Introduction and Objectives

Grewia gum is a natural polysaccharide obtained by extraction from the inner stem bark of *Grewia mollis* (family, Tiliaceae) under investigation as a potential excipient in pharmaceutical dosage forms. This study was designed to examine the effect of different drying processes on the physicochemical properties of the resultant gum.

Method

The polysaccharide was extracted from the pulverized inner stem bark of the plant by maceration in water and precipitation with absolute ethanol. Impurities were removed by treatment with 0.1 N HCl and NaOH, repeated redispersion, and precipitation with water or ethanol. The purified gum was dried either by air-drying, freeze-drying or spray-drying and the physicochemical properties of the dried polysaccharides were evaluated by Fourier transform-infrared (FT-IR), solid-state nuclear magnetic resonance (NMR), thermogravimetric analysis (TGA), X-ray photoelectron spectroscopy (XPS), gel permeation chromatography (GPC) and dilute solution viscometry.

Results and Discussion

Thermogravimetric parameters, such as oxidation temperature, weight loss onset and residual mass, showed variation among the samples. Intrinsic viscosities of 48.36 ± 0.37, 28.95 ± 0.52 and 24.03 ± 0.45 dl/g were obtained for the air-dried, freeze-dried and spray-dried polysaccharide samples, respectively. Molecular weight expressed as the 'pullulan polysaccharide equivalent' were 5925, 4555 and 3470 kDa for the air-dried, freeze-dried and spray-dried polysaccharide, respectively. Synthesized C1s spectra of the samples showed that no two samples were exactly the same. Solid state ^{13}C NMR of all the samples gave line widths typical of an amorphous natural polymer, with the air-dried giving slightly narrower lines indicating a higher degree of ordered structure than both the spray-dried and freeze-dried samples. The FT-IR spectroscopic scans of air-dried, freeze-dried and spray-dried samples of *G. mollis* polymer exhibited the typical bands and peaks characteristic of polysaccharides; however, the intensity of the peaks was variable depending on the drying method.[1]

Conclusion

The method used for the drying of *G. mollis* polysaccharide gum can have a profound effect on the intrinsic viscosity, molecular weight and relative elemental composition of polysaccharide gum. This will have an impact on the applicability of the gum as an excipient in different dosage forms. The viscosity grade of the polysaccharide could differ depending on the drying method employed and this must be considered when studying its suitability as an excipient in liquid and solid-dose formulations.

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Can animal tissues be replaced with synthetic materials to test mucoadhesive properties of dosage forms?

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Introduction and Objectives

Mucoadhesive polymers have received considerable attention as platforms for controlled delivery due to their ability to prolong the residence time of dosage forms on mucosal membranes and to consequently enhance drug bioavailability. One of the most commonly used *in-vitro* approaches to evaluate the performance of mucoadhesives is the determination of the force required to detach a dosage form from freshly-excised animal mucosal tissue and calculation of the work of adhesion. The objective of this study was to develop synthetic materials that can mimic animal mucosal tissues and be used as substrates for testing mucoadhesives.

Method

Model mucoadhesive tablets were prepared based on Carbopol 934P and hydroxypropyl methylcellulose by directly compressing the powder mixtures at different ratios. The adhesion of tablets towards mucosal tissues and synthetic substrates was studied using a TA XT.plus Texture Analyser (Stable Microsystems, UK) in a tensile mode. Porcine buccal mucosal tissues were taken from female Great White pigs and used for assessment of mucoadhesion. Polymeric hydrogels were synthesised by three-dimensional copolymerisation of 2-hydroxyethylmethacrylate (HEMA) with 2-hydroxyethylacrylate (HEA) and were purified by extracting unreacted monomers with deionised water.[1]

Results and Discussion

Previously, Choi *et al.*[2] reported the use of polypropylene-based substrate as a good substitute of animal tissues to

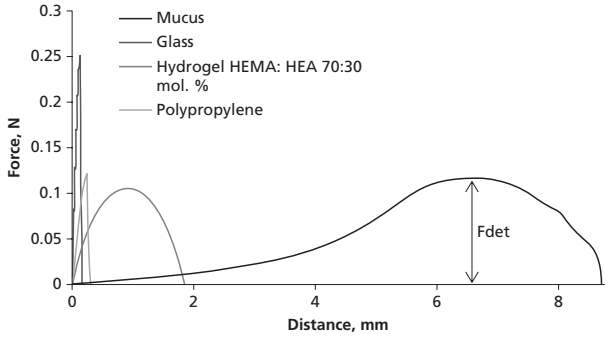

Figure 1 Detachment profiles of Carbopol R934P tablets from different artificial substrates and porcine buccal mucosa.

assess mucoadhesive properties of polymeric dosage forms. In this study, we have tested a range of synthetic materials as substrates to replace animal tissues for the assessment of the mucoadhesiveness of tablets. These included glass, polyethylene, polypropylene, polystyrene, polytetrafluoroethylene and HEMA-HEA hydrogels. Figure 1 shows the typical adhesiveness profiles, recorded for the detachment of Carbopol 934P tablets from different synthetic substrates in comparison with porcine buccal mucosa. Although some of the plastic materials such as polypropylene can give the detachment force (F_{det}) values similar to the animal tissue, the area under the curve, showing the work of adhesion, appears to be quite different. This difference is likely to be related to the lack of elasticity and porosity in plastic materials compared to mucosal tissues. More elastic materials such as HEMA-HEA copolymeric hydrogels exhibit better ability to mimic the adhesive properties of mucosal tissues compared to any other studied substrates. This ability depends greatly on the HEMA-HEA ratio in the hydrogels and the best mimicking characteristics were observed for samples having intermediate swelling capacity/mechanical characteristics.

Conclusions

Synthetic hydrogels composed of HEMA and HEA show better ability to mimic porcine mucosal tissues compared to plastics and glass in experiments on mucoadhesion. We believe that further development of these materials could offer a replacement for the use of animal tissues when testing mucoadhesive properties.

Acknowledgement

We are grateful to the Biotechnology and Biological Sciences Research Council UK for supporting our research (award [BB/E003370/1]).

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Overcoming dissolution effects: the use of hyper differential scanning calorimetry to detect drug melting in solid dispersion systems

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Introduction and Objectives

To investigate the potential advantages of hyper differential scanning calorimetry (DSC) over standard to determine the presence of crystalline indomethacin in solid dispersions using the polymer Gelucire 44/14. At slow heating rates, crystalline drug dispersed in a solid dispersion can further dissolve into the molten carrier, therefore eliminating the drug melting peak.[1] This may be interpreted incorrectly as the solid dispersion actually being a solid solution of drug in the carrier. This effect could potentially be overcome by increasing the rate of heating, as this is known to inhibit kinetically controlled events such as dissolution.[2]

Method

Physical mixes at concentrations of 5, 10, 15, 20 and 25% w/w indomethacin in Gelucire 44/14 were formulated in the DSC pan by weighing indomethacin on top of Gelucire 44/14. This allowed mixing of the solid indomethacin with molten Gelucire 44/14 during analysis. Solid dispersions at concentrations of 5, 10 and 50% w/w were formulated by adding indomethacin to molten Gelucire 44/14 with continuous stirring, then cooling at room temperature for 48 h. Samples were run at 10°C/min using a TA Q1000 DSC in aluminium standard crimped pans, and 500°C/min using a Perkin Elmer Diamond DSC in aluminium pin-hole pans.

Results and Discussion

(1) Physical mixes: Crystalline indomethacin melt enthalpies could be obtained for all physical mixes when heated at 500°C/min. These enthalpies (x) were plotted against physical mix initial crystalline indomethacin concentration (y) and extrapolated back to zero; the relationship $y = 1.468x + 3.3125$ was derived. This therefore allows us to calculate the % crystallinity in the solid dispersion systems. The 5 and 15% w/w physical mixes analysed at 10°C/min did not display melting peaks. An endothermic transition was present on the 25% w/w physical mix DSC trace; however, the peak max was found to be 139.70°C, approximately 20°C lower than that expected for indomethacin. (2) Solid dispersions: Solid dispersions at concentrations of 5, 10 and 50% w/w were heated at 500°C/min and analysed for crystalline indomethacin melt enthalpies. These enthalpies were used to calculate the

concentration of crystalline indomethacin remaining in the formulation by applying the physical mix calibration plot equation (mentioned earlier). The 5, 10 and 50% w/w solid dispersions were calculated to contain 3.9, 6.9 and 32.5% crystalline indomethacin, respectively. The study has therefore provided a new method of estimating crystalline drug concentration in polymers.

Conclusions

The fast heating rates employed by Hyper DSC appear to be a useful tool in determining a more accurate value for the true indomethacin solubility by significantly reducing its further dissolution in the polymer Gelucire 44/14 during analysis. The data obtained at slower rates has the disadvantage of giving misleading conclusions because of the elimination of crystalline drug melting peaks.

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Solute permeation across poly (ethylene glycol)-crosslinked poly (methyl vinyl ether-co-maleic acid) hydrogels

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Introduction and Objectives

Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of imbibing large amounts of water or biological fluids. In the present study, the swelling behaviour and network parameters of poly (ethylene glycol) (PEG)-crosslinked poly (methyl vinyl ether-co-maleic acid) (PMVE/MA) hydrogels were investigated. In addition, permeability studies of three solutes having different characteristics, such as hydrodynamic radius, molecular weight and pK_a values, were also performed. The goal of this research was to develop hydrogel membranes for drug delivery applications.

Methods

The required amount of PEG 10 000 was added to aqueous blends of PMVE/MA (at 10, 15 and 20%, w/w), in ratio of 2 : 1 (PMVE/MA : PEG) and films were cast. Films were cured at 80°C for 24 h to induce chemical crosslinking between PMVE/MA and PEG. Percentage swelling, equilibrium water content (%EWC), molecular weight between crosslinks (M_c) and crosslink density (V_e) were determined. Permeation behaviour of three solutes theophylline (3.5 Å), fluorescein sodium (5.0 Å) and vitamin B12 (8.5 Å) was investigated from equilibrium swollen hydrogels (pH 7.4) at a loading of 1 mg/ml, using side-by-side diffusion cells.

Table 1 Permeation studies of different solutes through the poly (ethylene glycol) (PEG)-crosslinked 10% poly (methyl vinyl ether-co-maleic acid) (PMVE/MA) hydrogels, $N = 3$

Solutes	$P \times 10^{-4}$, cm/s	K_d	$D \times 10^{-4}$, cm^2/s
Theophylline	12.87 ± 1.78	0.43	2.32 ± 0.32
Fluorescein Na	1.23 ± 0.10	0.26	0.36 ± 0.03
Vitamin B_{12}	1.33 ± 0.37	0.24	0.43 ± 0.12

Results and Discussion

Hydrogels cast from aqueous blends containing 10% (w/w) PMVE/MA showed lower crosslink density, V_e, and higher average molecular weight between crosslinks, M_c, followed by 15 and 20% PMVE/MA hydrogels. Table 1 shows the permeability, diffusion coefficient and partition coefficient values of hydrogels cast from aqueous blends containing 10% PMVE/MA : PEG (2 : 1). The P and D of solutes were decreased with increasing hydrodynamic radius; similar results were seen among other hydrogels. In addition, the permeation of solutes was higher in hydrogels cast from aqueous blends containing 10% PMVE/MA, followed by 15 and 20% PMVE/MA.

Conclusion

In conclusion, the present study showed the significance of polymer and crosslinker concentration on the swelling rates and crosslink densities of hydrogels. As a result, at lower polymer and crosslinker concentration, a higher rate of solute permeation was observed. Therefore, careful selection of these hydrogels is necessary if these are to be used as platforms for drug delivery systems in biomedical or pharmaceutical applications.

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Quaternary ammonium incorporation to yield antimicrobial polyvinyl chloride biomaterials

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Introduction and Objectives

Polyvinyl chloride (PVC) is one of the most widely used polymers in the manufacture of medical devices.[1] A major

issue with the use of PVC, as with most biomaterials, is bacterial colonisation, which contributes to poor patient outcomes, increased hospital stays and increased costs. This problem is typified by the role of the PVC endotracheal (ET) tube in the development of ventilator-associated pneumonia.[2] Bacteria colonise such surfaces forming biofilms, which render bacteria more resistant to antibiotic therapies.[3] This work explores the incorporation of quaternary ammonium compounds (QACs) into PVC as a means of improving resistance to infection.

Method

QACs were incorporated into PVC by one of two methods. In the first method, PVC resin was dissolved with increasing concentrations of QAC in tetrahydrofuran, and an homogenous film was formed by controlled evaporation of the solvent, and the second method involved hot-melt extrusion (HME) of physical mixtures of PVC resin and QAC powders in ratios similar to those used in method 1. These materials were characterised using attenuated total reflectance (ATR)–Fourier transform infrared (FTIR), and contact angle and tensile analyses. Microbiological studies of the biocidal efficacy of the new materials were performed using *Pseudomonas aeruginosa* and *Staphylococcus aureus* as models of in-vivo ET tube colonisation.

Results

ATR-FTIR confirmed the presence of the incorporated QACs on the PVC film surface, which is important mechanistically for biocidal action to be obtained. Importantly, films produced by HME have greater homogeneity than solvent cast films due to the reduced likelihood of recrystallisation of QAC in the HME films. Contact angle analysis shows significant reductions in contact angle at concentrations in excess of 2%, indicating increased hydrophilicity of films resulting from the presence of ammonium ions at the PVC surface. Early microbiological studies confirm a cidal action on adhering bacteria, reducing viable bacterial numbers by up to 90% that of native PVC. Mechanical testing of PVC films indicates low concentrations of QAC exert a plasticising effect on PVC, which may produce the additional benefit of reducing the need for addition of high levels of traditional plasticisers.

Conclusion

Two methods of producing PVC films incorporated with QACs have been demonstrated. Characterisation by ATR-FTIR and contact angle analysis has confirmed the presence of QACs on the surface of the films and demonstrated improved homogeneity in films produced by HME. Tensile analysis of samples indicated that the bulk effects of QAC incorporation are not deleterious to the mechanical properties of PVC at the levels being used, and may in fact reduce the need for addition of harmful plasticisers currently in use. Microbiological testing has confirmed the potential for such materials to exert a cidal action on potentially infective bacteria.

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Characterisation of prolamine-based polymeric nanocomposites

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Introduction and Objectives

The aim of this study is to determine the influence of varying prolamine-based polymeric blended compositions on the particle size and molecular structural transitions during nanocomposite formation. Fourier transform infrared (FTIR) spectroscopy was used to assess the extent of interactions between the prolamine-based polymeric blended structure to form composite nanoparticles, whereas temperature modulated differential scanning calorimeter elucidated the thermal properties and provided insight into the molecular dynamics of the prolamine-based polymeric blended nanocomposites.

Method

Prolamine-based polymeric blends were prepared in 50% v/v acetic acid in ratios of 1:1, 2:1, 1:2, 4:1, 1:4, 3:4 and 4.3 of prolamine:polymer. The blends comprised Tween 80 (1 ml) prior to the addition of 0.25% w/v of an aqueous sequestrator under agitation. The nanoparticulate suspensions were centrifuged (2750 rpm) and thereafter lyophilised. The particle size and zeta potential of the nanocomposites were measured using a ZetaSizer NanoZS. FTIR spectra were obtained for the native polymers and the prolamine-based nanocomposites using a PerkinElmer spectrometer over a range of 4000 to 650 cm^{-1} while the thermal behaviour was evaluated from 20 to 300°C.

Results and Discussion

The average particle size obtained was in the region of 146–527 nm, whereas the zeta potential values were between +1.23 and −8.11 mV. As the concentration of prolamine increased, the particle size increased due to agglomeration. However, at a ratio of 1:1, the average particle size was 206 nm that was indicative of miscibility between the prolamine and the polymer. FTIR analysis of the native polymers displayed the presence of weak bands due to less

bond vibrational frequencies. Spectral absorption bands were strengthened upon blending, and newly formed unsaturated bonds were noted at 1735.59 and 944.5 cm^{-1}. The FTIR spectra of the prolamine-based polymeric blends distinctly differed from the native polymer at 1639.79, 1349.42 and 1149.84 cm^{-1}, which may influence drug incorporation due to the presence of unsaturated bonds for potential polymer-drug interactions. The crystallisation temperatures of the native polymers increased to the range of 237–242°C after blending. The close crystallisation temperature range of the blends collaborated with the FTIR spectra revealing that the varying components of the nanocomposites did not significantly alter the spectra obtained.

Conclusions

The FTIR spectra of the prolamine-based polymeric blends distinctly differed from the native polymers. This indicated the presence of interactions between the prolamine-based polymeric blend during nanocomposite formation. The higher crystallisation temperature range also supported the interactive mechanism of the prolamine-based polymeric blends for nanocomposite formation and potential drug loading.

Medicinal Chemistry

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Synthesis and evaluation of substituted-2-phenyl-4*H*-1-benzopyran-4-ones as potential antifungal agents

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Introduction and Objectives

The objective of this study was to design and synthesise a novel series of substituted 2-phenyl-4*H*-1-benzopyran-4-ones and evaluate the title compounds and their intermediates for antifungal activity.

Methods

Title compounds were synthesised using Baker–Venkataraman rearrangement reaction. Structures of the synthesised compounds were confirmed by UV, IR, Mass, ^{1}H-NMR and ^{13}C-NMR spectroscopic analyses. All the compounds were evaluated for antifungal action using agar well (diffusion) method against standard griseofulvin and fluconazole.

Results and Discussion

Compounds 7-hydroxy-2-phenyl-4*H*-1-benzopyran-4-one (5a), 4'-chloro-7-hydroxy-2-phenyl-4*H*-1-benzopyran-4-one (5b) and 5,7-dihydroxy-2-phenyl-4*H*-1-benzopyran-4-one (10) were found to be the most potent among all the compounds. The zones of inhibition of compounds 5a, 5b and 10 in *Saccharomyces cerevisiae* were found to be 22, 22 and 24 mm, and for *Aspergillus niger*, they were found to be 17, 17.5 and 15 mm, respectively, at the concentration of 100 μg/ml. The zone of inhibition of compound 10 in *S. cerevisiae* was found to be more than in griseofulvin. The zone of inhibition of compound 5a in *A. niger* was found to be equal to that in griseofulvin.

Conclusion

These synthesised compounds were found to be more active than griseofulvin but less than fluconazole. This study opens new doors to future molecular modifications to the parent ring to explore some new leads possessing potent antifungal activity.

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A new QSAR model for human skin permeability

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Introduction and Objectives

The objective of the study was to develop a predictive skin permeability model using readily accessible quantitative structure–activity relationship (QSAR) descriptors. It has been widely assumed since the work of Potts and Guy[1] that skin permeability can be modelled by two descriptors, the octanol-water partition coefficient (log *P*) and the molecular size. In fact such models are not very accurate, and Abraham and Martins[2] have developed a better model using the Abraham descriptors. However, these descriptors are not readily accessible, and so we have developed a model based on more readily accessible descriptors.

Methods

Human skin permeability coefficients (Kp) for 107 compounds were taken from Abraham and Martins.[2] One hundred and forty-six descriptors for each compound were calculated using TSAR, HYBOT, ACD and VCCLABS software. Step-wise regression was used to find the descriptors that best modelled the Kp values.

Results and Discussion

The best QSAR model we obtained was as follows:

$$\log Kp = -0.118\ \Sigma Cad + 0.514 \log P - 0.293\,{}^{2}\chi^{v} + 0.00399\ VOct_{ZZX} - 0.189 \log S + 2.04\,{}^{4}\chi_{c} - 5.20$$

$n = 107$, $R^2 = 0.841$, $Q^2 = 0.779$, $s = 0.450$, $F = 88.2$

where ΣCad is the HYBOT hydrogen-bond acceptor and donor ability, $^{2}\chi^{v}$ is the 2nd order valence molecular connectivity, $VOct_{ZZX}$ is the VAMP octupole in ZZX plane, S is aqueous solubility, $^{4}\chi_{c}$ is the 4th order cluster molecular connectivity, n is the number of compounds used for the QSAR, R is the multiple correlation coefficient, Q is the cross-validated (leave-one-out) correlation coefficient, s is the standard error of estimate and F is the Fisher statistic. All descriptors had P values ≤ 0.01, indicating no higher than 1% probability that a descriptor was included by chance. There were no pair-wise correlations with $r^2 > 0.5$. The statistics of the QSAR are slightly better than those reported by Abraham and Martins.[2] In the QSAR, descriptors are given in order of their contribution to the correlation. It can be seen that hydrogen bonding ability is the most important factor controlling skin permeability, and that it reduces Kp. Partition coefficient is the second most important factor, with a positive coefficient, in agreement with the findings of Potts and Guy.[1] The $^{2}\chi^{v}$ term is strongly correlated with molecular size,[3] with a negative coefficient, again in agreement with Potts and Guy.[1] $VOct_{ZZX}$ is the VAMP octupole in the ZZX plane, and is a polarity term. The selection of aqueous solubility as a factor in controlling skin permeability has not been previously observed; its negative coefficient is to be expected. The $^{4}\chi_{c}$ term is a shape descriptor, in line with previous observations that molecular shape affects permeability.

Conclusions

A very good QSAR model of human skin permeability has been developed using readily available molecular descriptors. The high cross-validated correlation coefficient indicates that the QSAR can reliably be used for predictive purposes.

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Pharmaceutical Microbiology

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Investigation into the susceptibility of *Burkholderia cepacia* isolates to photodynamic antimicrobial chemotherapy

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Introduction and Objectives

The aim of the study was to determine the sensitivity of *Burkholderia cepacia* isolates LMG1222, LMG2161, LMG16665, LMG16659, LMG16656 and LMG18822 to methylene blue (MB) and meso-tetra (N-methyl-4-pyridyl) porphine tetra tosylate (TMP)-mediated lethal photosensitisation. Pulmonary colonisation with *B.cepacia,* a pathogen in the cystic fibrosis (CF) lung, can lead to an accelerated, often fatal, decline in lung function. *B.cepacia* is resistant to most antibiotics, with infected patients segregated from other CF patients and treatment limited to management of exacerbations with marginally effective antibiotic combinations.[1] Effective treatment of *B.cepacia* infection is therefore required to improve the prognosis and quality of life for infected CF patients.

Method

Planktonic cultures of *B.cepacia* were prepared from an inoculum incubated at 37°C overnight. Test samples of 10^7 organisms, approximated using culture optical density at 550 nm, were incubated for 30 min with solutions of photosensitisers of concentrations 50, 100, 250, 500 and 700 μg/ml and irradiated using a Paterson lamp (635 nm, 100 mW/cm^2, irradiation time: 10 min, distance from sample: 1.8 cm). Surviving bacteria were enumerated using the Miles and Misra technique for viable counts.[2]

Results and Discussion

The rate of kill was dependent on photosensitiser concentration for both photosensitisers, although inversely so for MB. For example, in the case of culture LMG1222, exposure to 50 μg/ml TMP dissolved in phosphate-buffered saline (PBS) pH 7.4 followed by a light dose of 200 J/cm^2, the percentage kill is 58.40(±12.24)%, in comparison with 99.90(±0.13)% for the 500 μg/ml sample ($P = 0.002$). However, in the case of MB, for the strain LMG1222, exposure to 50 μg/ml MB dissolved in PBS pH 7.4 and a light dose of 200 J/cm^2, the percentage kill is 98.90(±0.35)%, in comparison with 78.60 (±2.50)%, which is the percentage kill associated with the 500 μg/ml sample ($P = 0.0339$). This trend applied across all strains tested, where highest kill rates for photodynamic antimicrobial chemotherapy (i.e. exposure to a photosensitiser solution/light combination), were achieved with 50 μg/ml MB and 500 μg/ml TMP. Greater than 92.20% kill was achieved with all strains and photosensitiser–light combinations.

Conclusion

MB and TMP-mediated photosensitisation significantly reduced the bacterial load of *B.cepacia* cultures. Factors such as light penetration through photosensitiser solution may contribute to incomplete kill, especially for MB, which prevents light diffusion to a greater extent than TMP. Photodynamic antimicrobial chemotherapy, if photosensitiser and light can be delivered effectively, may be used as a method of reducing bacterial load in the CF lung.

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Pharmaceutical Technology

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Novel application of hydrotropic solubilisation in the preparation of solid dispersion of poorly water-soluble drug

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Introduction and Objectives

The dissolution rate and hence the rate of absorption and/or total bioavailability of a poorly water-soluble drug can be enhanced by solid dispersion technology.[1] The common solvent method involves the use of an organic solvent (volatile) to dissolve the poorly water-soluble drug as well as the carrier. Toxicity of residual organic solvent, cost of solvent and pollution are major drawbacks of this method. In the present study, newly developed hydrotropic solid dispersion (HSD) technology precludes the use of organic solvent. The proposed method is different from the common solvent method and is a novel application of hydrotropic solubilisation phenomenon. In this study, urea has been found to increase aqueous solubilities of poorly water-soluble drug, atenolol, significantly. Therefore, this drug has been selected as poorly water-soluble model drug and urea as a model hydrotropic agent (water-soluble carrier) to prepare HSDs.

Methods

There was a 6.1-fold enhancement in solubility of atenolol in 10 M urea (a hydrotropic solution) in comparison to distilled water solubility. For preparation of HSD containing atenolol and urea in 1 : 4 ratio, (AU 1 : 4 HSD), accurately weighed 20.0 g of urea and 5.0 g of atenolol were used. Minimum (possible) quantity of distilled water at 80–85°C contained in a 250-ml beaker was used to dissolve the urea. Then, atenolol was added to this beaker and a teflon-coated magnetic bar was dropped in it. Stirring of magnetic bar in beaker was started using a magnetic stirrer, maintaining the temperature at 80–85°C. Atenolol got completely solubilised. Stirring was continued till a semisolid mass was obtained in the beaker (after evaporation of a large quantity of water). Semisolid mass so obtained was spread on several watch glasses in thin layers for quick drying. The watch glasses were kept in oven (60–65°C) for drying. The dried mass was triturated and again kept in oven for drying. After almost complete drying, the powder of solid dispersion was passed through sieve # 100 and was kept for 6 days in a desiccator. Similarly HSDs containing atenolol and urea in ratios of 1 : 6 (AU 1 : 6 HSD) and 1 : 8 (AU 1 : 8 HSD) were made.

Results and Discussion

The prepared HSDs and physical mixtures (PMs) were characterised by DSC, IR and DR studies. The DSC study showed that the single endothermic peaks at 133.07°C for AU 1 : 8 PM and at 134.25°C for AU 1 : 8 HSD were very comparable indicating that there is no chemical interaction between atenolol and urea. Dissolution studies in distilled water showed that the rates of dissolution from HSDs were higher than PM and bulk drug. Initial rates of dissolution of drugs from HSDs were significantly higher compared to initial dissolution rates from bulk drug samples. The HSDs and PMs were subjected to stability studies at room temperature, 40°C/75% RH and 55°C for 6 months and the residual drug contents after storage for 6 months in all such cases were above 98% w/w, showing good chemical stabilities.

Conclusion

The simplicity of the proposed method and ease of scale-up bulk manufacturing render such HSDs economically viable preposition for enhancing the therapeutic efficacy of poorly water-soluble drugs from their solid oral dosage forms. This new method is cost-effective, environmentally friendly and safe (free from toxicity) compared to solvent evaporation methods, which involve use of an organic solvent. The proposed technique would be economical, convenient and safe.

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Synthesis and characterisation of a novel poly(amidoamine)s for use as a potential protein delivery system

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Introduction and Objectives

In recent years, gene, antisense and ribosyme therapies have been explored. All these systems share one common challenge, that of efficient delivery into the cytoplasm of the cell. Synthetic polymers have been developed as an alternative to viral gene delivery systems, which have brought some safety concerns in clinical trials. They may be tailored, through the application of rational design, to improve cytoplasmic access and modulate cell-specific targeting. Poly(amidoamine)s (PAA) are a family of synthetic functional polymers developed for use as polymer therapeutics. They were selected for this study as a potential protein delivery system.

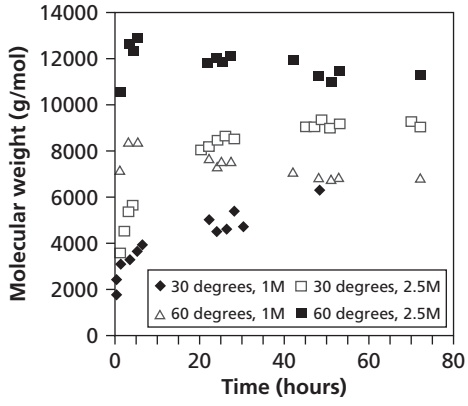

Figure 1 Evolution of PAA molecular weight (Mw) as a function of reaction time in water using different monomers' concentrations and temperatures.

Method

The general procedure for the polymerisation was as follows: equimolar amount of 6-amino-1-hexanol and 2, 2' bis(*N*-acrylamido)acetic acid was used. The kinetics of polymerisation was carried out at different temperatures (30 and 60°C) using different concentrations of monomers (1 and 2.5M) in different solvents (water, methanol and dimethyl sulfoxide (DMSO)). Structure of the polymers was identified by 1H NMR and Fourier transform infrared (FTIR) spectroscopy. Molecular weight and polydispersity were determined by gel permeation chromatography using poly(ethyleneglycol) as standards. Thermal analysis was carried out using differential scanning calorimetry.

Results and Discussion

The polymerisation mechanism of poly [2, 2' bis(*N*-acrylamido)acetic acid-*alt*-(6-amino-1-hexanol)] was studied under different reaction conditions by varying the concentration of the monomers, temperature and the solvent. The kinetics of the polymerisation was characterised in terms of percentage conversion and building up of the molecular weight of the polymer (Figure 1).

Best results were obtained when carrying out the polymerisation reaction using higher concentration of monomers and water. The yield of the polymerisation was 76% and that of the molecular weight of the polymer was 14 200 g/mol. However, degradation occurred at higher temperature. Structure of the synthesised polymer was confirmed by infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy. These results correlate well with that of previous studies.

Conclusion

Concentration of monomers and the temperature used in the polymerisation reaction were found to have a profound effect on the molecular weight and the percentage conversion. As this polymer is intended to be used as a protein delivery system, its cytotoxicity and delivery efficiency are currently under investigation.

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The application of microwaves to pharmaceutical formulation

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Introduction and Objectives

Pharmaceutical formulation methods often require a heating stage. An interesting alternative to conventional methods is controlled microwave heating, which offers a range of benefits including improved drug encapsulation. The potential of this method was investigated using ibuprofen (a well-characterised drug) formulated with cyclodextrin (β-CD) and polyvinylpirrolidone (PVP). The excipients were selected to allow comparison with the work of other researchers.[1,2] The resultant formulations were characterised using differential scanning calorimetry (DSC), Fourier transform infrared (FT-IR) and drug dissolution analysis.

Method

Formulations of 5 g (drug : excipient ratios of 1 : 9 or 1 : 1) were prepared using a modified microwave oven where the power was continually adjusted for 5 min to maintain a temperature of 85°C (±4°C), as measured using a fibre optic probe. For comparative purposes, formulations were also prepared using the same time and temperature conditions with conventional heating. To investigate the effect of a solvent on the properties of the products, formulations were also prepared as water slurries. All formulations were analysed using DSC (heating rate = 10°C/min, 25–130°C), FT-IR and dissolution analysis (37°C, pH 8, phosphate buffer, paddle speed = 50 rpm).

Results and Discussion

The three analytical methods demonstrate that all formulations produced using the microwave method are comparable with those produced using conventional heating. There was no indication of degradation products in either the FT-IR or DSC results, implying that the structures were unchanged after either method of heating. This was as expected based on the known thermal stability of the materials under investigation and suggests that the temperature in the microwave method had been accurately maintained for the duration of the experiment. The results of the dissolution studies are summarised in the table below, giving the total percentage drug release after 1 h for the different formulations. These

Formulation	Microwave	Conventional
Dry PVP	73% (±3%)	71% (±2%)
Dry β-CD	50% (±2%)	54% (±2%)
Wet PVP	51% (±2%)	54% (±2%)
Wet β-CD	95% (±4%)	99% (±3%)

results confirm that the method of heating had no significant effect on the nature of the products.

However, a clear difference was observed for both PVP and β-CD formulations with the inclusion of water. Interestingly, the presence of water during the preparation increases the percentage drug release for β-CD yet decreases it for PVP.

Conclusions

This work confirms the suitability of the application of microwave heating to pharmaceutical formulations, exemplified using PVP and β-CD. In particular, it has been shown that it is possible to accurately control the temperature using microwave heating without detrimental effects on the products. The results further show the critical effect that water can have on the formulation process for these materials.

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Application of a bench-top test with modified sampling method to determine the segregation behaviour of direct-compression and roller-compacted blends of ibipinabant

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Introduction and Objectives

This study assessed the refinement of a bench-top test described by Shah *et al.*[1] for the determination of blend segregation. A streamlined sampling method was developed to allow for improved test efficiency. A CB-1 antagonist, ibipinabant, was chosen as a model drug. This is a Biopharmaceutics Classification System Class II compound processed as an amorphous spray-dried intermediate of small particle size (D90 < 20 μm). Direct-compression and roller-compacted blends of the compound were tested for their segregation potential. In addition, the effect of using different particle size grades of microcrystalline cellulose on the segregation behaviour of a direct-compression blend was investigated.

Methods

The segregation test utilised a Jenike and Johanson apparatus in combination with flow through a polycarbonate tube to subject the blends under investigation (500-g batch size) to sifting and fluidisation segregation mechanisms. The method was modified at the sampling stage by taking powder samples using a slicer directly from the apparatus in the quantities needed for analysis. Samples were taken in triplicate from 10 locations in the blend. The potency of the samples was measured by ultraviolet analysis. In addition, direct-compression blends containing different grades of microcrystalline cellulose of different particle size were tested, and the effect on segregation potential was evaluated.

Results and Discussion

The roller-compacted blend results indicated a low segregation risk according to the criteria set by Shah *et al.*[1], with a relative standard deviation (RSD) of 2.65% between samples. The direct-compression blend indicated a high tendency to segregate (RSD = 9.81%). These results were confirmed by the behaviour of both blends during subsequent tablet manufacture, with the direct-compression blend giving more variable content uniformity than the dry granulated formulation. Increasing the particle size of the microcrystalline cellulose had a noticeable effect on the segregation risk of the direct-compression blend. Formulations containing the larger particle-sized grade Avicel PH200 had RSD values of 35% compared to 9–10% for Avicel PH101 and PH102 formulations.

Conclusions

The segregation test method showed good predictive ability for the segregation behaviour of ibipinabant blends during subsequent processing. Sampling directly from the vessel using a slicer apparatus removed the need for multiple powder riffling steps. Results identified that dry granulation was necessary to produce a robust formulation. They also indicated the most suitable grades of microcrystalline cellulose to use in order to minimise segregation within a direct-compression blend. This rapid and simple characterisation technique allowed for rational process and formulation selection at an early stage in formulation development conforming to quality-by-design principles.

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Spray-dried versus directly compressible mannitol grades for the development of orally disintegrating tablets

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Introduction and Objectives

Orally disintegrating tablets (ODTs) provide convenient means of administration for patient populations such as

geriatrics, paediatrics or patients with dysphagia as the tablets are easier to swallow than a conventional tablet. Mannitols such as Pearlitol 200 SD and 300 DC are commonly used as diluents in ODT formulations. Those grades are directly compressible and have an acceptable mouthfeel and a negative heat of solution that gives a cooling effect upon ingestion. The aim of this work was to evaluate and compare the overall usefulness of both mannitols in manufacturing placebo ODTs that possess good mouthfeel, rapid disintegration, acceptable physicomechanical properties and manufacturability.

Methods

Both mannitols were characterised with respect to particle size, flow properties and compressibility. Screening design-of-experiments were used to evaluate the effects of mannitol, microcrystalline cellulose (MCC) and superdisintegrant grades and concentrations on tablet properties. Formulation optimisation was subsequently conducted using the two most promising MCC and superdisintegrant grades. All tablets were manufactured at a 1- to 3-kg batch scale using an instrumented rotary press with target weights of 150 mg and hardness levels from 1.5 to 3.0 kp (screening) and 4–6 kp (optimisation) to improve the overall friability. Tablet evaluation included weight uniformity, hardness, thickness, friability, disintegration times and mouthfeel.

Results and Discussion

Four MCC grades (Avicel HFE102, CE15, PH105 and PH101) were compared, and PH101 was found to be the most suitable binder for both mannitols in achieving the desired hardness. For the disintegrants, Kollidon CL-SF performed better than CL-M, croscarmellose sodium and sodium starch glycolate in terms of performance. The overall process control and manufacturability of the DC grade were significantly poorer than the SD grade. In particular, it was difficult to achieve consistent weight control and hardness values greater than 4 kp, without the tablets starting to laminate. This was further supported by force–hardness profile data where the DC grade was significantly less compactible. In-vitro disintegration times for the DC-grade tablets also fell short of the 30-s criterion. On the contrary, the SD-grade tablets were manufactured with good process control and tight weight variations. Specifically, the best formulation containing the SD grade, PH101 and CL-SF achieved disintegration times of 26 and 29 s and friability of 0.14 and 0.06% for the 4 and 6 kp tablets, respectively. Due to the smaller particle size of the SD grade (180 μm), the overall mouthfeel of its tablets were better than the DC grade, which showed a slight grittiness.

Conclusion

This study identified the most suitable MCC and super-disintegrant grades for use in combination with SD mannitol, which has demonstrated good process control and compressibility. The ODTs fulfilling the desired physicomechanical properties (4–6 kp hardness, <0.2% friability), disintegration times < 30 s and good organoleptic properties could be readily manufactured. The next steps would be to include an active pharmaceutical ingredient in the formulation and to evaluate for taste acceptability in addition to the standard physical tests.

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Characterisation of spray-dried naproxen formulations

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Introduction and Objectives

An estimated 40% of newly developed drugs show poor aqueous solubility. Those poorly water-soluble drugs show poor dissolution and erratic absorption, which ultimately leads to therapeutic failure.[1] The enhancement of dissolution of hydrophobic drugs is, therefore, currently seen as one of the major challenges in the pharmaceutical industry. The aims of this study were (1) to improve the dissolution of a model hydrophobic drug, naproxen, by spray-drying in the presence and absence of hydrophilic surfactants (Pluronic-F127 and Caprol-PGE, 860) and (2) to assess the influences of these carriers on solubility and integrity of naproxen.

Method

A solution of naproxen (2% wt/vol) and surfactant (either Pluronic-F127 or Caprol-PGE, 860 in concentrations of 0.05, 0.1, 0.2 and 0.5% wt/vol) in ethanol/water (40% vol/vol) mixture was spray-dried using a Buchi B-290 mini spray-dryer at 95°C inlet temperature and 35°C outlet temperature. Spray-dried formulations were characterized by in-vitro dissolution. Additionally, preparations were evaluated for particle size analysis (using microscope); wetting properties (via surface tension determination); drug crystallinity (using differential scanning calorimetry [DSC]) and structural analysis (by Fourier transform-infrared [FT-IR]). All formulations were subjected to storage stability at 20°C/76% RH for 6 weeks to study the effects of storage on drug dissolution.

Results and Discussions

Spray-dried naproxen formulations produced a significant increase ($P < 0.05$) in drug dissolution compared to that of commercial drug. For example, after 10 min average percentages of the drug released were $68 \pm 1.4\%$ from spray-dried particles prepared with 0.2% pluronic-F127; $29 \pm 0.79\%$ for spray-dried naproxen with 0.2% Caprol-PGE, 860 and only $8 \pm 0.84\%$ from commercial drug. The higher drug release from Pluronic-F127 compared with Caprol-PGE, 860 may be due to the nature of the surfactant. The high drug dissolution from hydrophilic surfactant formulations may be explained by the large surface area as

indicated by particle size analysis and good wettability of spray-dried naproxen as demonstrated by surface tension measurements. Also, dissolution enhancement could be attributed to decreased crystallinity of hydrophobic crystalline drug as confirmed by DSC (in thermograms of spray-dried formulations, the endotherm for naproxen melting was shifted from 158°C to a temperature ranging from 157 to 143°C). Therefore, drug crystallinity played an important role in governing the drug dissolution. FT-IR analysis demonstrated that spray-dried naproxen with surfactants exhibited spectral changes, indicating a drug–surfactant interaction that led to the improvement of drug dissolution. From the stability results, it can be showed that the spray-drying process had no significant effect on the structure of naproxen, and the formulations produced are stable on storage.

Conclusion

Spray-drying process and the inclusion of hydrophilic surfactants enhanced the naproxen dissolution rate due to the (1) reducing drug-particle size, (2) decreasing degree of crystallinity of naproxen and (3) improving wettability of the drug particles. Pluronic-F127 was proved to be more effective in the enhancement of naproxen dissolution compared to Caprol-PGE, 860.

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Determination of the tensile strength of elongated tablets

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Introduction and Objectives

The tensile strength of a compressed tablet is an important attribute as the tablet needs to be mechanically strong to withstand handling such as film coating, packaging, transport and use by patients. The higher the tensile strength of a tablet, the greater its hardness and the longer its disintegration time. The objective of this work was to derive an equation to calculate the tensile strength by means of diametral compression of elongated tablets such as capsules and oval-shaped tablets. This work was compared to the mathematical solutions that are known for flat-face tablets[1] and convex-faced tablets.[2]

Methods

Two-dimensional (2D) and 3D finite element analysis software COMSOL was used to determine the stress distribution when tablets were loaded diametrically. The programme was first validated by comparing with the accepted stress solutions for flat-faced and convex-faced circular tablets prior to it being applied to elongated tablets. Analysis was undertaken along the X, Y and Z coordinates, where X is the width, Y is the length and Z is the thickness of the tablet. The solution was then checked by applying it to commercial paracetamol tablets of differing shapes to demonstrate its utility.

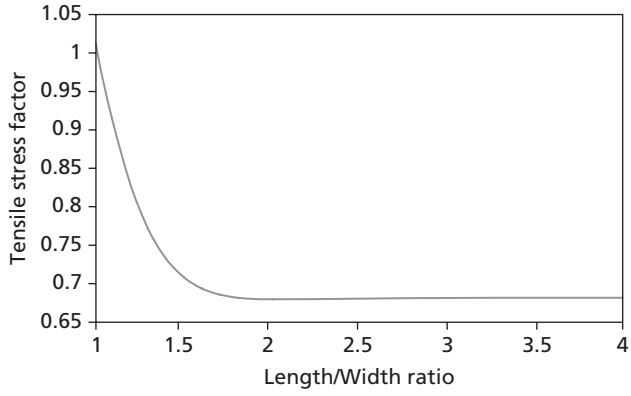

Figure 1 Peak principal tensile stress versus tablet length ratio.

Results

The stress analysis showed that as the Y axis of the tablet was elongated from a standard circular tablet to become that of an extended tablet shape, the peak principal tensile stress reached a limiting value (Figure 1). This limiting value was reached as the ratio of the X to Y dimensions exceeded 1 : 1.7, which encompasses most modern pharmaceutical tablets. In addition, the stress analysis showed that the limiting value reached approximately two-thirds that calculated for circular tablets. Hence, for a convex-faced, elongated tablet, the equation of tensile strength derived by Pitt *et al.*[2] would become

$$\sigma = 2/3\{(10P/\pi D^2)(2.84t/D - 0.126t/W + 3.15W/D + 0.01)^{-1}\},$$

where σ = tensile strength, P = fracture load, D = length of short axis, t =overall thickness and W = tablet wall height.

Conclusions

The tensile strength of capsules and oval-shaped tablets can now be readily estimated, facilitating the development and transfer of formulations and processes between tablets of different shapes.

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Dissolution enhancement of carbamazepine: solid dispersion formulations with glucosamine and the effect of different solvents

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Introduction and Objectives

The most effective method for improving dissolution rate of poorly water-soluble drugs is the use of a solid dispersion technique, but this is reliant on a suitable carrier and solvent. The aim of the present work is to explore D-glucosamine as a potential hydrophilic carrier to improve dissolution rate of a poorly water-soluble drug, carbamazepine, from physical mixtures and solid dispersion formulations. The effect of solvent used for the preparation of solid dispersion on the dissolution rate of carbamazepine–carrier dispersion formulations was also investigated.

Methods

Solid dispersions of the drug and D-glucosamine hydrochloride were prepared using different ratios by the conventional solvent evaporation method. Different solvents (ethanol, acetone and water) were used in the preparation of solid dispersions. Carbamazepine and carrier (glucosamine) were dissolved in acetone or ethanol. The solvents were then removed at 40°C for 24 h with stirring. The resultant solid dispersions were collected and pulverised using a mortar and pestle, and stored in desiccators. Physical mixtures of carbamazepine and glucosamine were also prepared for comparison. Properties of all solid dispersions and physical mixtures were studied using a dissolution tester, Fourier transform infrared spectroscopy, scanning electron microscopy and differential scanning calorimetry (DSC).

Results and Discussion

The results showed that the presence of glucosamine can increase dissolution rate of carbamazepine compared to pure carbamazepine. However, all solid dispersions of carbamazepine–glucosamine hydrochloride showed considerably higher dissolution rate for carbamazepine compared to the physical mixtures. The presence of water during preparation of the solid dispersions reduced the dissolution rate of carbamazepine (Figure 1) due to formation of carbamazepine dihydrate during the preparation of solid dispersion, as proved by DSC studies. To facilitate comparison, the dissolution efficiency was calculated, and the results showed that the dissolution efficiency of solid dispersions prepared with different solvents can be ranked as follows ethanol > acetone > ethanol–water > acetone–water.

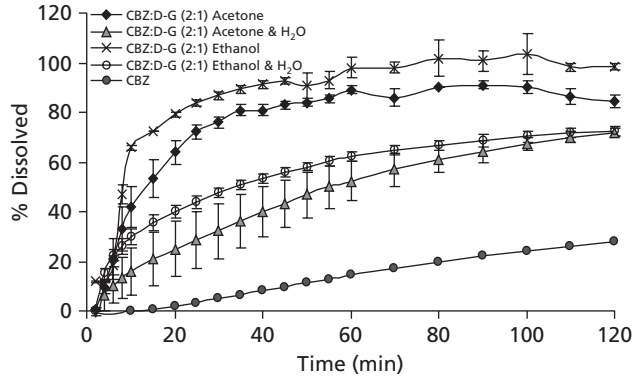

Figure 1 The effect of type of solvent on dissolution of carbamazepine-glucosamine solid dispersions.

Conclusions

It was shown that the use of D-glucosamine hydrochloride in solid dispersion formulations can enhance dissolution rate of poorly water-soluble drugs such as carbamazepine. The rate of this dissolution enhancement was dependent on the type of solvent used in the preparation of solid dispersion formulations.

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Liquisolid tablets and enhancement of naproxen dissolution

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Introduction and Objectives

Improving dissolution rate of a hydrophobic drug using liquisolid technique had been reported to be one of the most promising strategies.[1] The aim of this study was to investigate the dissolution profiles of naproxen (Roche-Syntex, S.A. de C.V., Mexico) by using different liquid vehicles, i.e. CremophorEL (BASF, Ludwigshafen, Germany), Synperonic PE/L61 (ICI surfactants, Everberg, Belgium) and polyethylene glycol 400 (PEG400; Sigma-Aldrich, Poole, UK) in formulating liquisolid tablets at two different drug concentrations, i.e. 20% wt/wt and 40% wt/wt.

Method

Model hydrophobic drug, naproxen, was dispersed in liquid vehicle (Cremophor EL, Synperonic PE/L61 and PEG400) and carrier (Avicel PH102 [FMC Corp., Philadelphia, USA]) was added under continuous mixing. Then, coating material (Cab-o-sil M-5 [Cabot Corporation, Rheinfelden, Germany]) was added to convert wet mixture into dry powder. Lastly, 5% wt/wt of maize-starch was mixed with the mixture for 10 min. Final powder was compressed using a single-punch tablet machine. Prepared liquisolid compacts were

characterized via uniformity of weight, content uniformity, tablet hardness, friability, disintegration, dissolution in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF), stability studies, differential scanning calorimetry (DSC) and Fourier-transform infrared (FT-IR) spectroscopy. All data were statistically analyzed.

Results and Discussion

Liquisolid tablets formulated with Cremophor EL and PEG400 vehicles complied with British-Pharmacopoeia weight-uniformity, content-uniformity and friability test. Tablet hardness for liquisolid naproxen tablets (20% wt/wt) with Cremophor EL or PEG400 were 39 or 179 N, respectively. Disintegration times for PEG400 containing formulations were longer compared with Cremophor EL liquisolid tablets. Dissolution results showed that liquisolid tablets with Cremophor EL at 20% wt/wt and 40% wt/wt have significantly higher dissolution rate ($P < 0.05$) compared to conventional tablets; e.g. after 15 min in SGF, 21.6% naproxen was released from Cremophor EL liquisolid tablets (20% wt/wt) versus 1% from conventional tablets. Also, drug dissolution of Cremophor EL liquisolid tablets has no significant difference ($P > 0.05$) in SGF and SIF. This implied that dissolution rates of Cremophor EL liquisolid tablets are superior and they have equally good dissolutions in both SGF and SIF. It was shown by stability studies that liquisolid tablets are not affected by aging. DSC of liquisolid powder mix showed complete amorphisation of naproxen in liquisolid systems, as melting peak of naproxen (at ~156°C) in liquisolid system had disappeared, this reflects higher solubility of liquisolid preparations. FT-IR spectra showed reduced intensity at C=O at 1600–1800/cm, which is attributed to hydrogen bonding between naproxen and liquid vehicle. The liquisolid formulation with Synperonic PE/L61 is not compressible.

Conclusion

Liquisolid technique changes the properties of naproxen by simply dispersing the drug particles in a hydrophilic nonvolatile vehicle, which in turn increases drug-wetting properties and surface area, and hence drug dissolution. Cremophor EL is a promising new liquid vehicle in formulating liquisolid tablets.

Reference

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Cogrinding technique as an approach to sustain the drug release

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Introduction and Objectives

Cogrinding is widely performed for reducing the particle size of powdered, poorly water-soluble drugs, with the aim of enhancing their dissolution rates and consequently their bioavailability.[1,2] However, there is little information in literature on the use of cogrinding technique for sustaining the drug release. The aim of the present study was to explore the cogrinding technique as a tool to slow down the drug release from capsule formulations containing theophylline and magnesium stearate.

Method

Theophylline powder and magnesium stearate with various concentrations (1, 3, 5, 10% wt/wt) were charged into the chamber of the vibration ball mill. Ten steel balls were added in the ball mill chamber and the powder mixture was then ground at 400 rpm for 1, 5, 15, 30, 60 and 120 min. Simple physical mixtures of theophylline–magnesium stearate were also prepared for comparison purposes. The dissolution rate of the drug from coground samples and physical mixtures from capsule formulations were determined at pH 6.5 according to USP. Morphology of samples before and after grinding was also investigated using a scanning electron microscope (SEM).

Results and Discussion

All coground formulations showed slower release rates than their physical mixture counterparts. This might be due to an increase in the temperature of milling balls around magnesium stearate particles as a result of friction between particles and balls/wall of the jar. The generation of heat, in turn, can melt some magnesium stearate particles (melting point around 110°C) and the molten hydrophobic particles can coat the drug particle surfaces and increase the hydrophobicity of the drug particles. The effect of milling time on the drug release was complex. Cogrinding time had no significant effect on drug release when the amount of magnesium stearate was 1% wt/wt. For samples containing 3% magnesium stearate, when milling time increased from 1 to 5 min, there was a significant reduction in drug release. Further milling caused an increase in drug release due to the increase in surface area of particles available for dissolution as proved by SEM results. Fourier transform-infrared (FT-IR) spectra revealed that as milling time increases the intensity of bands at 2850 and 2917/cm decreases. This was attributed to evaporation of water from the samples during the cogrinding process.

Conclusion

Cogrinding time and magnesium stearate concentration had significant effect on drug release from theophylline capsule formulations. It seems that sustained release action is amplified with coground approach. It was shown that cogrinding time is of crucial importance as additional cogrinding time could deteriorate retardation effect. FT-IR

and DSC results ruled out any significant interaction between theophylline and magnesium stearate in solid state.

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Bioavailability enhancement with humic substances and their comparative evaluation

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Introduction and Objectives

The objective of our study was to increase the bioavailability of a poorly water-soluble drug with the help of humic substances (fulvic acid [FA] and humic acid [HA]) isolated from rock shilajit. Here carbamazepine (CBZ, Novartis India Ltd, Mumbai, India) was taken as a model drug, and FA and HA were taken as natural complexing agents. Increase in solubility was to be confirmed by in-vitro analysis. Dissolution and drug release studies were performed with the help of high performance thin layer chromatography (HPTLC) and ultraviolet spectrophotometry. In-vivo assessment of bioavailability enhancement was carried out in Swiss albino mice.

Method

Inclusion complexes of CBZ and humic substances[1] were developed by the following four techniques in 1 : 1 and 1 : 2 molar ratios: (1) freeze drying, (2) solvent evaporation, (3) kneading and (4) physical mixture.

Developed complexes were characterized by techniques, such as differential scanning calorimetry (DSC), X-ray diffraction and Fourier transform-infrared (FT-IR). In-vitro solubility analysis and dissolution study was carried out to optimize the complexes. In-vivo studies by maximal electro shock (MES) method of the optimized complexes were carried out to assess the enhancement in bioavailability (approved by Institutional Ethical Committee).

Results and Discussion

Characterization of complexes by different techniques confirmed the complexation; DSC thermograms of CBZ and FA showed peaks at 189°C and 250–260°C, respectively, whereas HA was lacking any peak. Complexes developed were lacking the sharp peaks of the drug, indicating complex formation. FT-IR spectra of HA, FA and CBZ were in accordance with literature, like C=O stretching (1752/cm), N–H vibration (3460/cm), C=C vibration (1550/cm) peaks in CBZ. Interactions between intermolecular groups after complexation resulted in absence/reduction of peaks of drug, especially in the finger-print region, confirming the complexation interaction. X ray diffractions (XRD) of HA and FA do not show any sharp peak, whereas CBZ shows peaks at different angles, such as 15.41° (100%), 13.18° (83%), 27.84° (66%), 27.32° (60%) and 27.66° (56%), revealing the crystalline nature. Characteristic peaks of drug were absent/diminished in complexes, confirming the complexation. Solubility analysis showed that complexation greatly increased the solubility of CBZ (up to 2000%). Dissolution study of compressed tablets were carried out with paddle-type dissolution apparatus, 900 ml distilled water, RPM-75 and temperature 37.5 ± 1°C. Lyophilised 1 : 2 CBZ–FA complex showed the best release (99% in 1 h). MES activity indicated that complexation increased the potency of CBZ by three folds, whereas complexing agents did not exhibit inhibition to electroshock.

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Modulating hydroxypropyl methylcellulose matrix sensitivity to sucrose-rich dissolution environments using different polymer viscosity grades

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Introduction and Objectives

Our previous work showed how high concentrations of dissolved sucrose can accelerate drug release from a model hydroxypropyl methylcellulose (HPMC) matrix.[1] Here, we investigate the effect of different polymer viscosity grades on the sensitivity of HPMC matrices to sucrose-rich media.

Method

Tablets (8 mm, 250 mg) containing 10% wt/wt caffeine anhydrous, 30% HPMC (Methocel K100LV, K4M or

K100M [Colorcon Ltd, Dartford, UK], 63–90 μm) and a 2 : 1 mixture of lactose and microcrystalline cellulose were manufactured using an F3 Manesty tablet press (Manesty, Liverpool, UK) at 230 MPa. Drug-release kinetics was measured in 900 ml sucrose dissolution media (37 ± 0.5°C) using United States Pharmacopoeia (USP) apparatus I (Prolabo, France) at 100 rpm. Surface gel-layer development in sucrose solutions at 37 ± 1°C was imaged using a Bio-Rad MRC-600 confocal microscope (Bio-Rad, Hemel Hempstead, UK) using a method described earlier.[2]

Results and Discussion

In water, the drug release was progressively more extended as the polymer viscosity grade increased (K100LV T_{80}% = 3.6 h, K100M T_{80}% = 5.9 h). Increasing the sucrose concentration up to 0.6 M progressively elicited more extended release (ER) profile in all matrices, but at higher sucrose concentrations, different viscosity grades exhibited varying behaviour. K4M matrices were the most sensitive, showing a biphasic profile with accelerated drug release after 2 h in 0.7 M sucrose (T_{80}% = 3.4 h). K100LV matrices exhibited similar behaviour in 0.8 M sucrose. In contrast, K100M matrices provided ER in sucrose-rich environments. Confocal studies attributed the accelerated drug release of K4M matrices to suppression of polymer particle swelling and the formation of fragile, particulate gel layers, which will be rapidly eroded in the dissolution test. Interestingly, K4M and K100M matrices developed similar gel morphologies in sucrose-rich media. The continued ER of K100M matrices suggests that the high gel strength of this HPMC offers greater protection against the erosional forces in the dissolution test. In comparison, confocal studies suggested that the resistance of K100LV matrices to 0.7 M sucrose was a result of more rapid particle hydration and swelling, which permitted the formation of a more coherent gel-layer, although particle swelling was significantly reduced in 0.8 M sucrose.

Conclusion

The study shows how HPMC matrix sensitivity to high concentrations of sucrose can be modulated by polymer viscosity grade in the following rank order: K100M > K100LV > K4M. Faster particle hydration appeared to confer K100LV matrices with greater resistance to sucrose than K4M matrices, whereas K100M HPMC may enhance the resistance to gel erosion in the dissolution test.

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Nanoemulsion vehicle for enhanced transdermal delivery of carvedilol

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Introduction and Objectives

Carvedilol (CVD) is a nonselective β receptor blocker, widely used in management of essential hypertension and congestive heart failure. It provides the advantages of α and β blocking, antioxidant and antiproliferative properties.[1] On oral administration, bioavailability remains low (23%) because of significant first-pass hepatic metabolism, and plasma half-life is short (4–7 h). Thus, to improve the bioavailability and patients' compliance, and to surmount the problem related to oral administration, an alternative route is always desirable. Nanoemulsion-based transdermal delivery is a viable alternative to administration of such drugs.

Method

Solubility of CVD in various nanoemulsion components was determined. The surfactants were screened for their nanoemulsifying ability of oil + water (1 : 1) mixture. Phase diagram study was performed by aqueous titration using oil (Miglyol 810) and surfactant/cosurfactant mixture, S_{mix} (Acconon CC6 and CO 20TX), to delineate the nanoemulsion region. Nanoemulsions selected from phase diagrams of S_{mix} ratio 1 : 1 were characterised by thermodynamic stability, globule size analysis, transmission electron microscopy study, refractive index and pH measurement. Ex-vivo skin permeation studies were performed through rat skin at 32 ± 0.5°C using Franz diffusion cell (Logan SFDC6, LOGAN instruments Co., NJ, USA).

Results and Discussion

Miglyol 810 showed the highest solubility of CVD (31.46 ± 4.27 mg/ml), and an extremely low concentration of surfactant (Acconon CC6, 39.71% w/w) was able to solubilise an equal mixture of oil + water.[2] Thus, Miglyol 810 and Acconon CC6 were selected as oil and surfactant, respectively. Phase diagram study reveals that the nanoemulsion region decreases with increasing surfactant or cosurfactant fraction. All the selected nanoemulsions of S_{mix} ratio 1 : 1 were thermodynamically stable. Mean globule size (9.28–95.47 nm) increased with increase in oil percentage (10–20% w/w), in contrast to viscosity (34.83–105.76 cp.). All the nanoemulsions showed refractive index and pH well within acceptable range (1.339–1.410 and 5.7–6.5, respectively). The optimised nanoemulsion consisted of 12.5% w/w Miglyol 810, 50% w/w S_{mix} (1 : 1) and 37.5% w/w water, and exhibited highest skin permeation rate (flux) of 161.53 ± 11.08 μg/cm^2 per hour and cumulative percentage of drug permeated (89.45%) in comparison to control (13.08 ± 2.31 μg/cm^2 per hour).

Conclusion

The CVD-loaded nanoemulsion transdermal vehicle studied showed sustained and prolonged delivery with high flux (161.53 ± 11.08 $\mu g/cm^2$ per hour) over control. High flux achieved through rat skin could be due to small globule size and high thermodynamic activity. The flux obtained is sufficiently high for therapeutic effects of CVD.

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Aqueous wet granulation to alter the physical characteristics of an active pharmaceutical ingredient

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Introduction and Objectives

The active pharmaceutical ingredient (API) under investigation has poorly compressible needle-shaped crystals; $d_{10} \sim 4$ μm, $d_{50} \sim 20$ μm and $d_{90} \sim 50$ μm. It is electrostatic and has physically cohesive properties that permit adhesion to steel and polytetrafluoroethylene (PTFE) surfaces. Consequently, API has been preferentially lost during roller compaction resulting in a loss of drug product potency. This work aimed to quantify the tribo-electrification of the API and increase the particle size distribution (PSD) of the API by high-shear wet granulation (HSWG) using hypromellose as the binder. The effect of impeller speed, binder addition rate and wet massing time were then analysed for statistically detectable effects.

Methods

Charge build-up of API grades was quantified using a Faraday cup to assess tribo-electrification. Charge on samples and API adhesion were measured following vibration at fixed frequency in stainless steel and PTFE vessels. Test conditions were as follows: ambient temperature and humidity. HSWG experiments were conducted in the Fukae (250 g) and Aeromatic Fielder GP1SP (1500 g). Binder concentration and amount were varied between 5 and 20% (w/w) and 15 and 35% (w/w), respectively. Impeller speed was between 200 and 600 rpm. API granules were characterised using Sympatec Particle Size Analyzer (Sympatec GmbH, System-Partikel-Technik, Clausthal-Zellerfeld, Germany). Final formulations were roller-compacted (RC) on a Gerteis minipactor and resultant granules were assessed by high-performance liquid chromatography. Data were analysed using statistical software, Design Expert version 7 (Stat-Ease Inc, Minneapolis, USA) and summarised in pareto charts.

Results and Discussion

Hypromellose solution of 10% (w/w) was defined as the optimum concentration, with 30% (w/w) added as binder during granulation. PSD analysis revealed an increase in d_{10} from 50 to 110 μm, where optimised granulation settings were used. Statistical analyses confirmed a positive (increasing the factor increases the response) effect on mean d_{10}, d_{50}, d_{90} and granule potency with respect to binder addition rate. Pareto charts show a positive effect between impeller speed and d_{90} relative standard deviation (RSD) results. An interaction between binder addition rate and wet-massing time results in increased variability of d_{10} and d_{50} RSD values. Electrostatic testing confirmed HSWG API possessed a mass-to-charge ratio significantly lower than those of the other API grades on stainless steel. Significantly more material adhered to PTFE surfaces than stainless steel with unmilled API most susceptible. Analyses conducted on

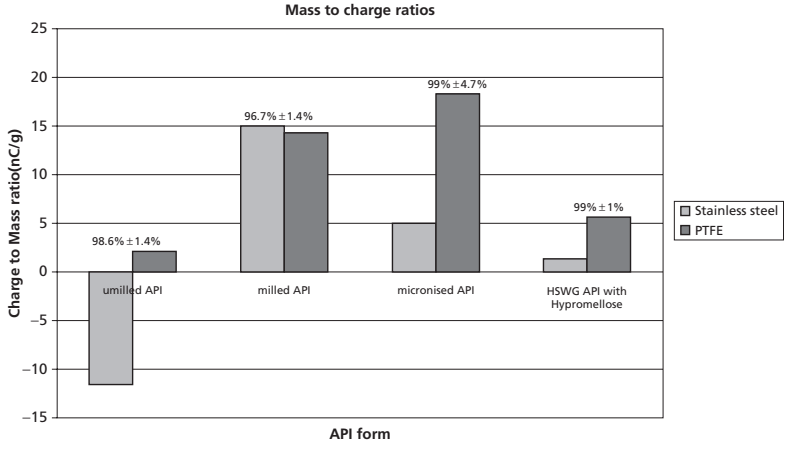

Figure 1 Mass-to-charge ratios against stainless steel and poly-tetrafluoroethyene.

post-RC granules demonstrated improved potency compared with formulations using milled API (Figure 1).

Conclusion

- HSWG of API successfully increased the PSD.
- HSWG of API reduced its charge to mass ratio.

Aqueous wet granulation reduced the electrostatic potential/physical cohesion of the API, thereby reducing potency losses in the final granule (mass-to-charge ratio: 1.345 nC/g and potency: 99% ± 1%).

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Application of low-substituted hydroxypropyl cellulose to dry granulation

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Introduction and Objectives

Low-substituted hydroxypropyl cellulose (L-HPC) is a pharmaceutical excipient that has been widely used as a disintegrant for tablets and granules. Because L-HPC has a good compressibility, it also works as a dry binder in tablets. Currently, the most common application of this excipient is to produce tablets through wet granulation or direct compression process.[1] In the present study, performance of L-HPC in dry granulation was investigated.

Methods

Powder mixture consisting of paracetamol (10%), L-HPC or other binder/disintegrant (5–20%) and lactose (70–85%) was applied to a roller compactor (model TF-MINI, Freund Industry, Japan) to prepare flakes. Various grades of L-HPC having different particle sizes and particle shapes (fibrous or nonfibrous) were tested to compare performance. The flakes were further milled using a low-shear mill (model M-10, Yamato-Kakou, Japan) to adjust particle size. The obtained granules were evaluated by physical testing such as bulk density and flowability, and tablets were prepared from the granules using a rotary tableting machine (model Vergo, Kikusui, Japan) to evaluate hardness and disintegration time.

Results and Discussion

When the roll pressure was 4 MPa or more, yield of granules was sufficient (i.e. more than 70%). However, tablet hardness was decreased when the roll pressure was over 6 MPa. Therefore, the pressure between 4 and 6 MPa would be considered to be the normal range for this formulation. Mean particle size of granules was increased as the roll pressure increased. Disintegration time of tablets was independent on the roll pressure, as long as L-HPC is used in the tablets. Among grades of L-HPC, LH-31 (a commercial grade with small particle size) and NB-02 (a trial grade having better flowability) were found to have better performance, such as reasonable tablet hardness and shorter disintegration time at the same time. Flowability, as expressed as Carr's index, of the granules was also better when using these two grades. Relationship between the performance of tablets and physical characteristics of L-HPC was also studied. The results indicated that grades of L-HPC that have larger surface area or greater plastic deformation showed higher binding strength with reasonable disintegration time.

Conclusion

By choosing a proper grade, the formulation using L-HPC showed both a sufficient binding strength and disintegration time in dry granulation process without using additional binder or super disintegrants.

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Improving dissolution properties of griseofulvin using solid dispersion systems with Solutol HS15 and Acconon CO-7

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Introduction and Objectives

The formulation of hydrophobic drugs is challenging due to poor aqueous solubility and poor dissolution of those drugs. The aims of this study were to enhance the dissolution of griseofulvin, a model hydrophobic drug, *via* solid dispersions with hydrophilic carriers (Solutol HS15 and Acconon CO-7) and to evaluate the influences of carriers and solid dispersion preparation method (either melting or solvent method) on griseofulvin dissolution.

Methods

Drug solid dispersions were prepared by two methods: (1) melting method in which the drug was dispersed in the molten carrier (either Solutol HS15 or Acconon CO-7); drug : carrier weight ratios were 10 : 90, 20 : 80 and 30 : 70% w/w; (2) solvent method in which the drug and the carrier (in the same above mentioned ratios) were dissolved in acetone. Solid dispersion formulations were characterised *via* dissolution testing in hard gelatine capsules (each capsule contained weight of solid dispersion equivalent to 5 mg griseofulvin), solubility study, drug content uniformity, Fourier-transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC).

Results and Discussions

The incorporation of griseofulvin in either Solutol HS15 (BSAF, Ludwigshafen, Germany) or Acconon CO-7 (Abitec Corporation, Columbus, Ohio, USA). *via* solid dispersion, by melting method, led to 4- to 5-fold increase in the drug solubility. There was an increase in the drug's solubility with increasing carrier concentrations, revealing the solvent properties of these carriers for griseofulvin. On the other hand, solid dispersions prepared by solvent method did not improve the drug's solubility. In addition, melting method resulted in uniformity of drug's distribution, suggesting intimate contact between the drug and the carrier. Solid dispersions, prepared by melting method (with either Solutol HS15 or Acconon CO-7), significantly ($P < 0.05$) enhanced griseofulvin release. Formulations containing 10 : 90 and 30 : 70% w/w griseofulvin : carrier produced burst drug release. Drug release rate (mg/l/min) was calculated as the amount of the drug dissolved after 5 min divided by five.[1] The initial griseofulvin release rate results for solid dispersions prepared by melting method were 0.49, 0.58 and 0.40 mg/l/min for 10 : 90% w/w griseofulvin/Solutol HS15, 30 : 70% w/w griseofulvin/Solutol HS15 and 30 : 70% w/w griseofulvin/Acconon CO-7, respectively, versus only 0.15 mg/l/min for pure drug. The FTIR spectroscopy data for formulations containing Solutol HS15 indicated shift in the peak around 1580 cm^{-1}, suggesting formation of hydrogen bonds between Solutol HS15 and griseofulvin. The DSC data for solid dispersions prepared by both the methods revealed that presence of Solutol HS15 and Acconon CO-7 and this led to reducing crystallinity of griseofulvin (as indicated by decrease in drug calorimetric enthalpy). The degree of crystallinity decreased with increasing carrier concentrations, explaining the enhancement of griseofulvin dissolution.

Conclusion

Solutol HS15 and Acconon CO-7 show promise for dissolution enhancement of griseofulvin.

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Medicinal Chemistry

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Synthesis and immunomodulation of human lymphocyte proliferation and cytokine (INF-g) production of four novel malonitrilamides

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Introduction and Objectives

Leflunomide is an immunomodulator drug with applications in the management of arthritis rheumatoid.[1] In this study, four novel analogues (4a–d) of A771726, the active metabolite of leflunomide, were synthesised and examined *in vitro* for their immunomodulatory activity through MTT assay on human lymphocytes and the cytokine interferon-g (INF-g) concentrations in human lymphocyte cell culture were determined.[2]

Method

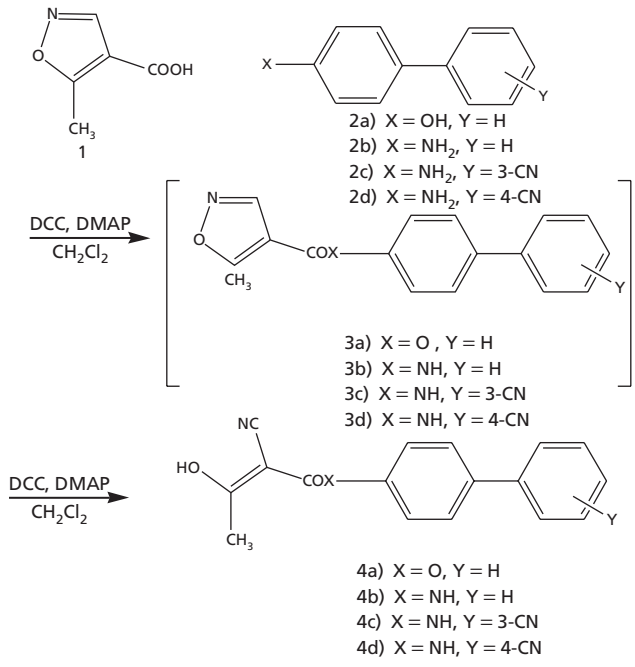
Four novel analogues were synthesised (Scheme 1), then 50 000 human lymphocyte cells, culture medium, phytohemagglutinin and different concentrations of the analogues or controls were added to each well of a 96-well tray. Effects of compounds on lymphocyte proliferation were determined by MTT assay. Supernatant was removed, and absorbance was measured by using a human IFN-g ELISA kit.

Results and Discussion

Our results showed that the most potent analogue was 4b with an amide linkage (X = NH) and the weakest analogue was 1a with an ester linkage (X = O). Compound 1a has little similarity to the leflunomide active metabolite, which has an amide linkage. Therefore, some of these analogues have immunosuppressive effects ($P \leq 0.05$).

Conclusions

In this study, four analogues of A771726, the active metabolite of leflunomide, showed some promising activities



Scheme 1

as inhibitors of lymphocyte proliferation and IFN-γ production. The effects of these four compounds in clinical settings deserve further studies *in vivo*.

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Toxicity modelling of benzodiazepine drugs by partial least square analysis

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Introduction and Objectives

Toxicological endpoints can be modelled by multiparameter approaches using multiple linear regression or multivariate techniques, such as partial least square (PLS) analysis. The PLS approach has the following advantages: it allows the application of several descriptors and leads to stable, correct and highly predictive models even for correlated descriptors.[1] This study presents a structure–toxicity study by PLS applied to a series of 54 benzodiazepine derivatives and compares the obtained results with the previously reported ones achieved by other statistical methods.[2]

Methods

The lethal oral dose for mouse LD50 (expressed in mol/kg), retrieved from the RTECS database (RTECS Database, MDL Information Systems, Inc., San Leandro, California, USA) was used as dependent variable. Structural descriptors were computed by several methods: quantum chemical descriptors by the MOPAC 2007 (http://openmopac.net/home.html) program (RM1 approach); volumes and accessible surface area by the Winmostar (Winmostar v.3.59c, Winmostar by Delphi, Norio Senda) and Chem3D (Chem3D Ultra 6.0, CambridgeSoft.Com, Cambridge, Massachusetts, USA); hydrophobicities by the ALOGPS 2.1 (http://www.vcclab.org/lab/alogps/start.html) and 22 types of descriptors by the Dragon (Dragon 5.5-2007, Talete SRL, Milan, Italy) packages.

Results and Discussion

A training set of 48 compounds and a test set of 6 compounds were considered. All the statistical tests were performed at a significance level of 5% or less. The goodness of prediction was checked by the leave-7-out cross-validation procedure and by the criterions stated by Golbraikh *et al.*[3] The PLS calculations were performed by the SIMCA package (SIMCA P+, v. 12.0, Umetrics AB: Umea, Sweden). From the entire descriptor matrix the following most significant descriptor types were included in the final PLS model: constitutional, edge adjacency indices, Randic molecular profile, 3D-Morse descriptors, functional group counts (Dragon descriptors), Winmostar, AlogPS2.1, Chem3D Ultra 6.0 and quantum chemical descriptors. The importance of descriptors was evaluated by the variable influence on projection (VIP) values. The VIP values higher than 1.0 were considered. An acceptable PLS model with three principal components was obtained: $R^2X(Cum) = 0.5$, $R^2Y(cum) = 0.828$ and $Q^2(cum) = 0.558$. Four outliers were found based on the Hotelling's T^2 criteria and were omitted from the final PLS model. The calculated criteria stated by Golbraikh *et al.*[3] indicated a model with predictive power.

Conclusion

The PLS model gave a new insight into structural parameters that influence the benzodiazepine toxicity and a better goodness of fit and prediction in comparison to the previous models by multiple linear regression, support vector machines and artificial neural networks. The present study revealed that an increased number of aromatic amino groups, electron donor capacity and number of aliphatic and aromatic ether groups increased the benzodiazepine toxicity. Increased hardness and more flexible molecules had lower toxicity. The presence of halogen atoms and of three-membered rings in the molecule and higher hydrophobicity (opposed to solvent-accessible area and volume) reduced their toxicity.

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Carcinogenicity modelling of diverse chemicals based on substructure grouping and support vector machines

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Introduction and Objectives

The prediction of carcinogenicity has become a subject of great significance for regulatory perspectives and ecotoxicity assessments. Several quantitative structure–activity relationship (QSAR) systems for predicting carcinogenicity were submitted to the Predictive Toxicology Challenge 2000–2001 Workshop; however, the exercise revealed that performances of all proposed models were not sufficient in predicting carcinogenicities of test chemicals.[1] In this study, an attempt was performed to construct a reliable QSAR model for predicting carcinogenicity of non-congeneric chemicals with a satisfactory performance. The support vector machines (SVM) approach[2] was applied to develop QSAR models.

Method

Experimental carcinogenicity data on about 1500 chemicals was collected from six sources including International Agency for Research on Cancer (IARC) and National Toxicology Program (NTP) databases, and their reliabilities were evaluated to rank into six categories. Negative chemicals were collected from NTP, where experimental data of animal tests using male and female rats and mice were accumulated. To develop a QSAR model for predicting carcinogenicities of diverse chemicals, 880 different molecular descriptors for 935 organic chemicals were calculated from their 3D geometries using Dragon professional software version 5.4.[3] For selecting effective descriptors, correlation coefficients between carcinogenicities and descriptors were calculated, and descriptors with higher correlation coefficients were adopted. The relationship between carcinogenic rank and selected descriptors was analysed with the SVM software LIBSVM version 2.85. Statistical quality and stability of presented models were tested by performing five-fold cross-validation (FFCV).

Results and Discussion

By using atom and functional group count descriptors in Dragon, positive (P) and negative (N) chemicals containing those substructures were counted. It was found that several substructures were judged as statistically significant for P/N ratio, but most of them were few and negative rich. Contrary to our typical rules of thumb for carcinogenicity of organic compounds, very limited substructures were strongly related with carcinogenicity. In the first step, a global SVM model was developed for all 935 compounds using 257 selected descriptors. The model gave overall accuracy, i.e. positive and negative correct estimate, of about 67%. To improve the performance of the SVM model, 935 chemicals were serially divided into 18 mutually overlapping subgroups according to their substructures, and a specific SVM model was selected and optimised for each subgroup. This serial model, developed as the combination of predictions obtained by 18 substructure-based models, predicts the carcinogenicity of diverse chemicals with a satisfactory overall accuracy of 83%.

Conclusions

The SVM, a novel type of learning machine, was applied to develop QSAR models for predicting the carcinogenic activity of diverse chemicals based on molecular descriptors calculated from the molecular structure alone. The prediction results were in reasonable agreement with the experimental data. It was shown that the combination of the ensemble learning technique and SVM can be a powerful, efficient tool in the structure–activity studies of carcinogenicity. The results reveal the superiority of the SVM models over other techniques such as multiple linear regression and artificial neural networks.

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Drug Metabolism

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Predicting the environmental toxicity of pharmaceuticals

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Introduction and Objectives

Recent reports have highlighted the ubiquitous detection of pharmaceuticals and their metabolites in the environment (rivers, lakes, surface and ground waters etc.). This has led to growing concern of their potential toxic effect on nontarget organisms. A recent publication by Sanderson *et al.*[1] provides a compilation of publicly available ecotoxicology data for pharmaceuticals. The objective of this study was to assess whether or not ecotoxicity of pharmaceuticals could be reliably predicted using hydrophobicity-based structure-activity relationship models or whether their effects may be related to the reactivity of the molecules.

Methods

The 96-h LD50 values for 90 pharmaceuticals, measured in fish, were obtained from Sanderson *et al.*[1] These values were compared with those predicted using the US EPA's ECOSAR program, which predicts ecotoxicity using linear

regression models based on hydrophobicity. The method of Madden *et al.*[2] was used to determine whether or not the pharmaceuticals were likely to fall within the applicability domain of the ECOSAR models and hence establish the reliability of the predictions. The method of Enoch *et al.*[3] was used to determine the potential of the compounds to elicit toxicity *via* electrophilic mechanisms.

Results and Discussion

Of the 90 pharmaceuticals investigated, 25 (28%) were deemed to fall within the domain of the ECOSAR models. The 96-h LD50 values in fish, predicted using the ECOSAR program, for all 25 of these compounds was within two log units of the measured 96-h LD50 values (measured values taken from the publication of Sanderson *et al.*[1]). For the 65 compounds (72%), deemed to be outside the domain of the ECOSAR models, LD50 values predicted by ECOSAR for 42 of these (65%) fell within two log units of the measured values, but for 23 compounds, (35%) toxicity values fell outside this range with measured LD50 values being up to six log units higher than the predicted value. This indicates that existing models for ecotoxicity may perform poorly in predicting the effects of pharmaceuticals. In addition, 50 of the 90 compounds (56%) were found to possess alerts for molecular features associated with potential electrophilic reactivity. This provides further evidence that hydrophobicity-based models may be inadequate for accurately predicting ecotoxic effects unless they are used within carefully identified applicability domains.

Conclusion

Development of models to predict the environmental toxicity of pharmaceuticals is hampered by the lack of available data, particularly for chronic effects. While pharmaceuticals are not designed to be acutely toxic, continual replenishment in the environment may lead to chronic toxicity. Current models to predict such effects require further development based on an understanding of potential toxic mechanisms to improve the reliability of the models.

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Pharmacognosy

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Investigation of the absorption and vascular effects of the indoloquinoline alkaloid, cryptolepine

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Introduction and Objectives

Cryptolepine, extracted from the West African shrub *Cryptolepis sanguinolenta* Lindl. Schltr. (Periplocaceae), possesses antiparasitic activity. Decoctions are used in traditional African medicine to treat malaria, hypertension and intestinal disorders. Previous experiments showed that cryptolepine (CRYP) relaxes intestinal muscle by antimuscarinic actions, but low CRYP concentrations act like alpha1 adrenoceptor antagonists, potentiating contraction.[1,2] We aimed to estimate the serum cryptolepine concentration achievable following oral dosage and to investigate its vascular actions, using rat portal vein.

Methods

Thirty-three rats received oral cryptolepine, 10 mg/kg. Three animals were sacrificed after 30 min followed by sacrifice of three animals/h for blood sampling. Administration of CRYP and blood sampling was performed by Mr Kuntworbe, KNUST, Ghana; frozen serum was sent to Bradford School of Pharmacy for analysis. To study vascular effects, contractile activity of rat portal veins was investigated in Krebs' solution in the absence or presence of cryptolepine (0.1–100 μM). Effects of CRYP (0.1–30 μM) on responses to the alpha1 adrenoceptor agonist, phenylephrine (PHENY 1–100 μM) were compared with phentolamine (PHENT, nonselective alpha antagonist, 1 μM) or prazosin (PRAZ, alpha1 antagonist, 0.1 μM).

Results and Discussion

Mean serum CRYP concentration for each group of three rats was calculated. Cryptolepine was detected in serum 30 min after oral administration, and the EC50 concentration of CRYP achieved was 0.21 ± 0.05 μM at time 1.67 ± 0.29 h. Maximum concentration was attained after 5 h (0.42 ± 0.08 μM) and later declined to 0.23 μM. In spite of some variation in CRYP concentration between samples, CRYP was detectable in serum 10 h after oral administration. No CRYP metabolites were detected in the samples. Cryptolepine (0.1–100 μM) did not affect the magnitude of spontaneous contractile activity in portal vein but reduced the frequency of myogenic activity by up to 50%. CRYP (10 μM)

reduced contraction induced by low concentrations of PHENY (0.1–3 μM) but, unexpectedly, did not affect the magnitude of contraction produced by higher concentrations of this alpha1 agonist. In contrast to the effects of CRYP, neither the selective nor the nonselective alpha1 adrenoceptor antagonists, prazosin and phentolamine, affected spontaneous myogenic activity of portal vein, but both drugs antagonised the contractile effects of PHENY throughout the dose range employed. In portal vein, the actions of CRYP clearly differed from those of the established alpha adrenoceptor antagonists, whereas in the intestinal muscle the actions of CRYP resembled the actions of these antagonists.

Conclusion

We conclude that CRYP can be absorbed from the rat intestine following oral administration. A nanomolar blood CRYP concentration is maintained for many hours following oral dosage and metabolism appears to be negligible. Although CRYP shows typical alpha1 adrenoceptor antagonist activity in intestinal muscle, alpha1 antagonist activity was not confirmed in the blood vessel: this may be due to the known differences in distribution of alpha1 adrenoceptor subtypes in vascular and intestinal muscle.

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Pharmaceutical Technology

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Dielectric relaxation in freeze-dried disaccharides: developments in our understanding of the potential significance to moisture buffering of freeze-dried products

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Introduction and Objectives

A variety of excipients (including various disaccharides) are used routinely in lyophilised product formulation to provide a moisture buffering environment and thereby sustain shelf life. Our earlier work[1] on lactose, sucrose, trehalose and maltose suggested that the temperature dependency of the Fröhlich parameter (B.T) is a key parameter to our understanding of the degree of molecular mobility of the sugar. Here, we examine, in further detail, the impact of moisture on the Fröhlich parameter and further speculate on the importance of the sub-T_g molecular dynamics to the moisture buffering capacity of these disaccharides.

Method

Lactose, trehalose, sucrose and maltose were individually freeze-dried to moisture contents in the region of 1–4% and measured using a Solartron 1296/1255 dielectric analyser, in the frequency and temperature ranges 0.1 Hz–1 MHz and −100 to 0°C, respectively.

Results and Discussion

All the four disaccharides showed two sub-T_g processes. The faster of the two relaxation processes was singled out for further analysis by fitting the Havrialiak–Negami (HN) function to each spectrum. Applying the HN function to the data represents an improvement over our earlier work,[1] which had assumed that the spectra were symmetrical, and hence a Cole-Cole function was considered appropriate for data fitting. The temperature dependency of the fit parameters ($\Delta\varepsilon$, tau, ε_∞) were used to calculate values for the Fröhlich parameter B.T (derived from $\Delta\varepsilon$, ε_∞) along with values of activation energy ΔH (from Arrhenius plots of tau against 1000/T). As mentioned before, the mechanism of the faster relaxation process was ascribed to the rotation of the pendant hydroxymethyl group on each sugar ring.[2] The magnitude of B.T for the fast relaxation process was plotted as a function of temperature and a simple exponential function fitted to the trend for each sugars. The trend line then permitted the calculation (prediction) of values for B.T at 20°C. The moisture sensitivity of B.T was also characterised by plotting values for the Fröhlich parameter at 20°C against moisture content for each sugar. The degrees of freedom within each sugar (as evidence by the magnitude of B.T) were seen to increase (with either temperature or moisture) to a greater extent for trehalose than for maltose or sucrose. The response of lactose, however, was quite different, in that B.T decreased with increased moisture. This was ascribed to the formation of lactose monohydrate and the associated reduction in the number concentration of polarisable dipole vectors. In some ways, the formation of the monohydrate and the 'locking up' of moisture could be viewed as the ultimate form of moisture buffering.

Conclusion

It is proposed that the temperature dependency of B.T is a key parameter, which reflects degrees of intermolecular and intramolecular freedom. In turn, we expect these degrees of freedom to underpin (to a certain extent) the diffusion rate of the trace amounts of water within the material, and thereby define a relative moisture buffering capability of a range of excipients. This work further supports the argument that moisture sensitive drugs will be more stable when freeze-dried with lactose, followed by sucrose, maltose and finally trehalose (with sucrose being more effective than maltose at the higher moisture contents).

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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.