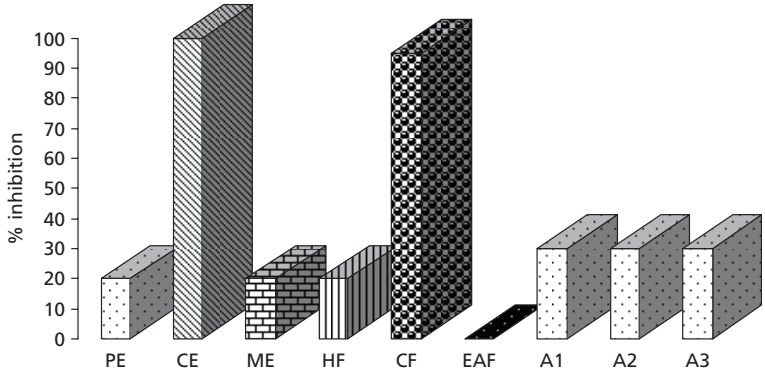

Figure 1 Comparison of antiangiogenic activity of different extracts of leaf of *Piper sarmentosum*, fractions of methanol extract and compounds A 1–3, each bar is mean of six replicates; PE, petroleum ether extract; CE, chloroform extract; ME, methanol extract; HF, *n*-hexane fraction; CF, chloroform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

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

Conclusion

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

Wednesday Poster Sessions

Analytical Chemistry

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

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Introduction and Objectives

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

Method

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

Results and Discussion

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

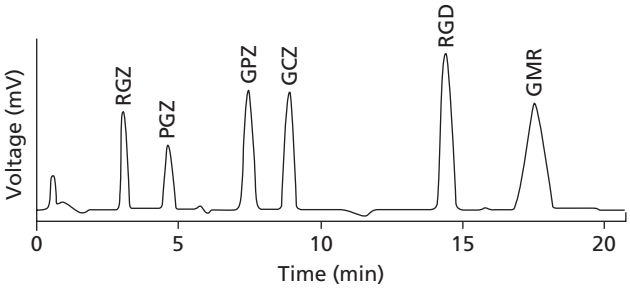

Figure 1 Chromatogram showing the elution of 10 μg samples of all the drugs extracted from human plasma.

Conclusion

A simple isocratic reverse-phase HPLC-UV method with single wavelength monitoring was developed and applied for the quantification of the six antidiabetic drugs. The mobile phase used was very simple to prepare and was economic and found no or little variation. Hence, the method can be easily and conveniently applied for the routine analysis of the drugs in plasma samples for pharmacokinetic investigations.

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The use of impedance analysis for the study of critical events in the frozen state, for formulation and lyo-cycle development

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Introduction and Objectives

There are several well-established techniques for characterizing a formulation prior to lyophilisation; however, formulations containing a mixture of crystallizing, reluctantly crystallizing and amorphous components may have several different phases within the frozen structure, these systems can undergo changes at low temperatures that, while not resulting in visible, gross morphological changes, may be significant for product stability. Impedance (Zsinϕ) analysis can provide detailed information, identifying subtle changes within the frozen solute. This study employed Lyotherm2 (Biopharma Technology LTD, Winchester, UK) to analyze simple and more complex formulations prior to freeze-drying, to establish whether Zsinϕ can provide meaningful information that other techniques may be unable to detect.

Method

Zsinϕ analysis and differential thermal analysis (DTA) was carried out simultaneously using the Lyotherm2 instrument. Aqueous samples were cooled to below −100°C and warmed at approximately 2–4°C/min to above 0°C. During cooling and warming, the level of Zsinϕ was monitored, for DTA the ΔT (temperature difference) upon warming was obtained by subtracting the sample temperature from the reference temperature, where the reference was analytical grade water or water for injection. Lyotherm2 data was compared with data obtained from freeze-drying microscopy (FDM) and modulated temperature differential scanning calorimetry (MTDSC), to determine whether correlations existed between these methods.

Results and Discussions

For a wide range of solutions tested, Zsinϕ analysis provided data on changes in mobility within the frozen solute phase that we have been able to relate to freeze-drying behaviour, many of which represented events related to glass transitions of (often low concentration) solute components that were not detectable using MTDSC. The temperature of TZonset observed with the Lyotherm2 instrument was often found to correlate with the expected glass-transition temperature in the frozen state (Tg'). A more complex multicomponent cell culture medium, which began to collapse at –43°C, was seen to exhibit mobility changes as low as −71°C during warming, with several events occurring prior to the TZonset of –45°C. This is indicative of some frozen solute mobility changes below the collapse temperature range, indicating that during the lyophilisation process, microscopic changes in the internal structure may result as the formulation is dried between these two temperatures. Indeed, for amorphous materials, the fact that some degree of mobility exists below the glass transition temperature has been well documented. These changes may, therefore, have an impact both on the potential for successful processing by lyophilization and the behaviour of the final dried product.

Conclusion

Zsinϕ analysis using Lyotherm2 successfully provided evidence of changes within the structure of a range of frozen solutions at temperatures less than that of gross changes observed using FDM and where no significant events were observed using MTDSC. Such changes may be important in understanding the potential for product defects occurring during the lyophilisation process and the stability of the final

lyophilized product itself, especially since the efficiency of the process can be markedly increased by employing higher temperatures, yet the need exists to balance processing efficiency against product defects, which may result from increased frozen state mobility.

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Development and validation of reverse-phase HPLC method for the determination of rosiglitazone in pharmaceutical formulations and human plasma using an experimental design

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Introduction and Objectives

The objective of the study was to develop a rapid and sensitive liquid chromatography method for determination of rosiglitazone in pharmaceutical formulations and human plasma. Pioglitazone was used as internal standard. The method was validated as per ICH guidelines for its linearity, accuracy, precision, specificity, selectivity, sensitivity and robustness. Experimental design was used for the validation of robustness and intermediate precision so that the method can be adopted for the routine quality control of pharmaceutical formulations and analysis of samples in therapeutic drug monitoring and pharmacokinetic studies.

Methods

Isocratic chromatographic method was done using Shimadzu class LC with dual pumps, and the detection was made by UV-Visible absorbance detector. A mixture of acetonitrile and water (pH = 3 adjusted with orthophosphoric acid) was used as mobile phase. The elution was performed on a reverse-phase C18 phenomenex (150 × 4.6 mm, 5 μ) column operated at ambient temperature and monitored by Spincotech software 1.7 version (Spinco Biotech services, Chennai, India). Central composite design with 2k factorial runs, 2k axial points, symmetrically spaced at $\pm\alpha$ along with each variable axis, and at least one center point, was used for robustness study, and software-based statistical analysis was done.

Results and Discussion

The method was also applied for the analysis of rosiglitazone in human plasma using spiked samples extracted by simple and single-step liquid–liquid extraction procedure using acetonitrile as solvent (protein precipitation). Organic layer evaporated was reconstituted with mobile phase and injected into HPLC system. Robustness and intermediate precision were validated using experimental design, with mobile phase flow rate and pH as variables for robustness, and analyst, equipment and number of days as variables for intermediate precision. The results were found to be satisfactory and can be successfully applied for analysis of the drug from clinical and pharmacokinetic samples.

Conclusion

The proposed HPLC method has been evaluated for the linearity, precision, accuracy and specificity and has been proved to be convenient and effective for the quality control of rosiglitazone in pharmaceutical formulations and can be applied for plasma sample analysis. The measured signal was shown to be precise, accurate and linear with a correlation coefficient greater than 0.9997. Moreover, the lower solvent consumption along with the short analytical runtime of 6 min leads to a cost-effective and environmentally friendly chromatographic procedure. The proposed methodology is rapid, selective, and requires a simple sample preparation and extraction procedures and thus is an effective procedure for determining rosiglitazone in pharmaceutical formulations.

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Sensitive and stereospecific high-performance liquid chromatography for flurbiprofen in human plasma

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Introduction and Objective

A method of analysis of enantiomers of flurbiprofen in biological fluids is necessary to study its stereospecific metabolism and tissue distribution.

Method

A simple high-performance liquid chromatography (HPLC) for the simultaneous determination of two enantiomers of flurbiprofen in human plasma has been described. The procedure involved extraction of flurbiprofen from plasma into isooctane-2-propanol (95 : 5, v/v); samples were mixed thoroughly using vortex mixer and then centrifuged at 3000 rpm for 15 min. The organic phase was separated and evaporated under reduced pressure in a vacuum oven. The residue was reconstituted in 50 ml of mobile phase, and 20 ml was injected into the HPLC column. Stereoselective separation was achieved with a CHIRALCEL OD-H analytical column (0.46 cm ID × 25 cm) without any derivatisation procedure. The detection wavelength

Table 1 Analytical recovery and interday and intraday precisions of R- and S-flurbiprofen

	Concentration found (mean ± SD; n = 5)					
Concentrations added	Day 1	Day 2	Day 3	Day 4	Day 5	Mean
R- flurbiprofen						
2 μg/ml	1.55 ± 0.05	1.40 ± 0.03	2.06 ± 0.04	2.12 ± 0.04	2.63 ± 0.05	1.95 ± 0.04
10 μg/ml	10.3 ± 0.06	11.0 ± 0.05	10.5 ± 0.04	10.8 ± 0.02	10.5 ± 0.01	10.6 ± 0.03
S- flurbiprofen						
2 μg/ml	2.28 ± 0.03	1.59 ± 0.02	2.32 ± 0.04	1.83 ± 0.02	2.37 ± 0.02	2.07 ± 0.02
10 μg/ml	9.89 ± 0.04	10.5 ± 0.05	9.89 ± 0.06	11.1 ± 0.04	9.88 ± 0.06	10.25 ± 0.05

Table 2 Absolute recovery values of R- and S-flurbiprofen

Concentration (μg/ml)	**Absolute recovery (mean ± SD; n = 6) S-flurbiprofen**	**Absolute recovery (mean ± SD; n = 6) R-flurbiprofen**	**Range (μg/ml)**
2.0	98.53 ± 1.68	98.88 ± 1.45	2.06–2.31
10.0	99.33 ± 2.22	99.59 ± 1.94	10.17–10.69

was set at 225 nm. Stereoselective chromatographic separation was completed within 15 min. The retention time for R-flurbiprofen was 10.80 min and S-flurbiprofen was 11.67 min. The limit of quantification was found to be 250 ng/ml. The precision of R-FLU and S-FLU was accurate.

Results and Discussion

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A new gas chromatographic method for limit test of isopropylamine in active pharmaceutical ingredient bisoprololi fumaras

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Introduction and Objectives

The aim of this study was to test all APIs and products for the presence of solvents. Isopropylamine is an unstable residual solvent and therefore it is not convenient to use headspace sampling. We developed a new gas chromatographic method for the limit test of isopropylamine (max. 50 μg/g of API) in bisoprololi fumaras using direct injection of bisoprololi fumaras diluted in pyridine with internal standard (diethylamine).

Method

An Agilent Technologies 6890 Series GC System gas chromatography with μ-FID detector, equipped with an Agilent Technologies 7683 Series Injector and 7683 Series Autosampler, was used together with 30- × 0.25-mm internal diameter column coated with 35% diphenyl and 65% dimethyl polysiloxane (Rtx-35 Amine) with film thickness of 1 μm (Restek, Bellefonte, PA). Carrier gas was helium; flow rate, 34 cm/min; inlet temperature, 140°C; split, 4 : 1; FID temperature, 260°C; oven, initial column temperature 40°C for 4 min; rate of the temperature increase, 4°C/min up to 50°C, and then raised at a rate of 40°C/min up to 250°C for 6 min; injection volume, 1 μl, internal standard method (peak height). Reference solution was 0.10 ml of diethylamine stock solution (10 mg/ml of diethylamine in pyridine), and 0.10 ml of isopropylamine stock solution (5 mg/ml of isopropylamine in pyridine) was introduced into the 100-ml volumetric flask and diluted to 100.0 with pyridine. Test solution was 100 mg of drug diluted with 1.0 ml of pyridine with the internal standard (10 μg/ml of diethylamine).

Results and Discussion

The specificity was satisfactory, Rt of isopropylamine was 3.5 min, Rt of diethylamine was 5.2 min (see Figures 1 and 2). The resolution of isopropylamine/diethylamine was 8.6.

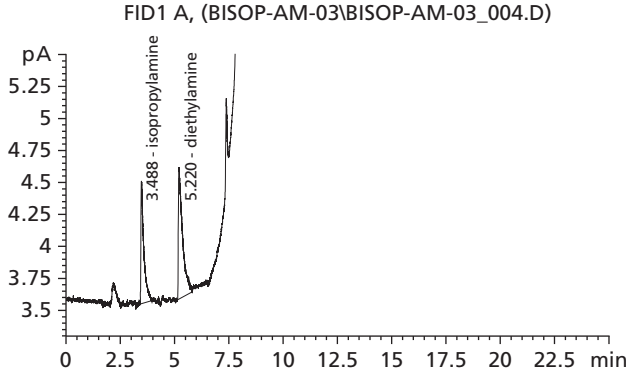

Figure 1 Chromatogram of the reference sample.

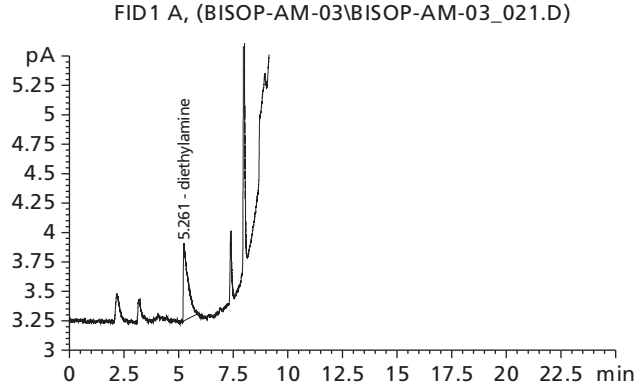

Figure 2 Chromatogram of the test sample.

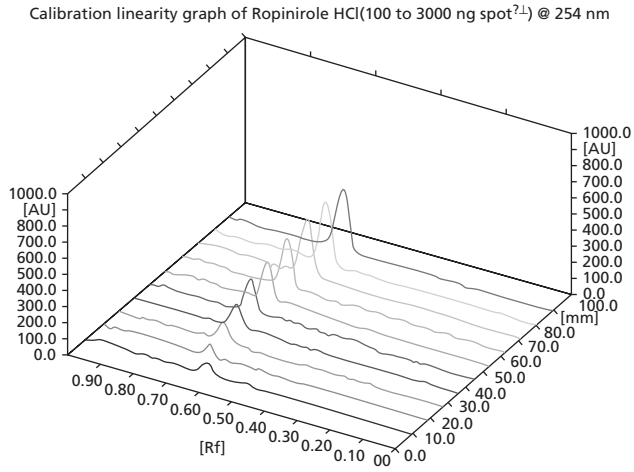

Figure 1 Linearity graph of ropinirole HCl.

The calibration graph was linear from 12.5 to 100 μg/g of isopropylamine (r = 0.99986) with a detection limit of 4.5 μg/g (S/N = 3) and lower limit of quantification of 15 μg/g (S/N = 10). The precision of the gas chromatographic method was 3.6% RSD for concentration level of 50 μg/g of isopropylamine.

Conclusions

The new gas chromatographic method is useful for quality control in pharmaceutical industry. The problem of solubility of API or incompatibility of isopropylamine with some other solvents (e.g. chloroform) was resolved using pyridine as the solvent. The evaluated validation parameters are in accordance with ICH guidelines for limit tests (specificity and detection limit).

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High-performance thin layer chromatographic stability-indicating method for quantitative determination of ropinirole HCl in the bulk drug and in marketed dosage forms

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Introduction and Objectives

High-performance liquid chromatography (HPLC)[1] and liquid chromatography-mass spectrometry (LC-MS)[2] have been reported for estimation of ropinirole HCl. High-performance thin layer chromatography (HPTLC) has advantages such as wide choice of stationary phases, less time consumption, ease of isolation of degradation products and less consumption of mobile phase. Therefore, study was designed to develop and validate HPTLC for the routine analysis of ropinirole.

Methods

Linomat-V HPTLC instrument (Camag, Muttenz, Switzerland) with Camag microlitre syringe on precoated silica gel aluminium Plates 60F-254 (10 × 10 cm, 200-μm thickness, Merck, Darmstadt, Germany) was used for the development of method. Design Expert was used to optimise variables.

Results and Discussion

Mobile phase was composed of toluene-ethyl acetate-6M ammonia solution (5 : 6 : 0.5, v/v/v). Chamber saturation was for 15 min, temperature was 25 ± 2°C and relative humidity was 55 ± 5%. Regression equations of the fitted quadratic model was $Y = 0.38 - 0.14\ X_1 + 0.23X_2 - 1.75\text{E-}003X_3 - 0.012X_1X_2 - 2.5\text{E-}003X_1X_3 + 7.5\text{E-}003X_2X_3 - 5.3\text{E-}003X_1^2 + 0.040X_2^2 + 0.030X_3$. Scanning was performed at λ_{max} = 250 nm, and linearity was 100–3000 ng/spot (Figure 1). The obtained R_f value was 0.58 ± 0.02, with r value of 0.9989 ± 0.0253, validated as per International Conference on Harmonisation (ICH) guidelines giving precision (% of regressed standard deviation (RSD) = 1.681), accuracy (%RSD = 0.472), robustness (%RSD = 0.605), recovery (99.979 ± 0.01527%), limit of detection (LOD) (5.045 ng/spot) and limit of quantification (LOQ) (25.186 ng/spot) in acceptable range. Stress studies with percentage recovery (acid (89.56%) and alkali (64.38%) hydrolysis, oxidation (99.58%), dry heat treatment (93.03%), wet heat treatment (89.05%) and photodegradation (101.22%)) of ropinirole were obtained. The validated method was used to estimate the ropinirole in tablet (Ropark; Sun pharmaceutical, Mumbai, India) giving 100.166% recovery (RSD = 0.187%).

Conclusion

Developed method can be used for routine estimation of ropinirole HCl in active pharmaceutical ingredients (API) and in marketed formulation. Stress testing showed that all degradation products were well separated from pure drug, confirming its stability-indicating capability. The method seems to be suitable for the quality control in the pharmaceutical industry and academia.

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The effect of polysorbate 80 on the stability of Xiang-Dan in injectable solutions

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Introduction and Objectives

Xiang-Dan, a traditional Chinese medicine prepared from Dalbergia odorifera and *Salvia miltiorrhiza*, has been widely used to treat cardiovascular diseases clinically in China. Its injectable solution is sometimes susceptible to physical instability such as turbidity and precipitation, and incompatible with phosphate-buffered saline and glucose solution when used for infusion. As a result, polysorbate 80 is incorporated into commercial Xiang-Dan injectable products. However, the mechanism by which polysorbate 80 *results* in an *increase* in the *stability* is unclear. Therefore, the aim of this study was to investigate the effect of polysorbate 80 on the stability of Xiang-Dan in solution.

Methods

Xiang-Dan injectable solutions with or without the addition of polysorbate 80 were prepared following a standard protocol. Four compounds, namely salvianolic acid B, protocatechuic aldehyde, vanillin and nerolidol, were used as chemical markers of Xiang-Dan. High-performance liquid chromatography (HPLC) assays were used for the quantification of the chemical markers. Polyethylene glycol 400 (PEG 400) and poloxamer 188 (P188) were used as control excipients for the stability study. Particle size analysis was carried out using a Mastersizer 2000 laser diffraction analyser.

Results and Discussion

PEG 400, P188 and polysorbate 80 were found to significantly increase the aqueous solubility of vanillin and nerolidol, and the solubilising effect of the latter was significantly better than those of PEG 400 and F188, while the three excipients could not increase the aqueous solubility of salvianolic acid B and protocatechuic aldehyde, which were soluble in the formulation (solubility > 50 mg/mL) in the absence of polysorbate 80. The solubility of both vanillin and nerolidol and the solubilising effect of polysorbate 80 appeared to be pH-dependent with the highest solubility and solubilising effect at pH 5–6. The results of particle size analysis showed that there existed microparticles/microemulsions in Xiang-Dan solutions, and the addition of polysorbate 80 dramatically decreased the mean particle size. The four chemical markers in Xiang-Dan solution in the absence of polysorbate 80 appeared to be susceptible to chemical degradation as a function of temperature and pH. With the addition of polysorbate 80, the stability of all chemical markers was found to be enhanced markedly in the pH range from 4 to 9.

Conclusion

Xiang-Dan comprises a variety of chemical ingredients which may form microemulsions in solution, leading to cosolubilise certain insoluble ingredients such as nerolidol. However, microemulsions are thermodynamically instable, and insoluble ingredients may precipitate. The addition of polysorbate 80 to Xiang-Dan solution could effectively decrease the particle size of microemulsions and solubilise insoluble ingredients as a result of micelle formation. The micelle formation may also lead to minimise the pH effect on solubility and chemical stability.

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Determination of tobramycin by HPLC following phenylisocyanate precolumn derivatisation in pharmaceutical preparations of approved tobramycin inhaled solution (TOBI)

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Objective

Tobramycin is a broad-range aminoglycosides antibiotic, produced by the fermentation of Streptomyces tenebrarius.[1] A simple, sensitive and specific high-performance liquid

chromatographic (HPLC) method has been developed for quantitative determination of the aminoglycoside, tobramycin, following precolumn derivatisation with phenylisocyanate (PIC).

Method

The PIC derivatisation procedure was performed by mixing 1 ml of an aqueous solution of tobramycin in a 4-ml screw-capped glass vial with 1 ml acetonitrile. The mixture was then derivatised with 300 μl of PIC 1% (v/v, in pyridine). The excess amount of PIC reagent was reacted with 300 ml of methanol and then the mixture was vortexed for 40 s and heated at 80°C. Chromatographic separation was performed on a Phenomenex Columbus C_{18} column (150 × 4.60 mm i.d.) at ambient temperature, using a constant flow rate of 1 ml/min. The mobile phase consisted of acetonitrile–glacial acetic acid–water (450 : 5 : 545, v/v/v) and ultraviolet detection at 240 nm.

Results and Discussion

The reaction time was 10 min at 80°C, and the resulting derivative was stable for 5 days at room temperature. The proposed method showed good validation data. The standard curve was linear (n = 5) at seven different concentrations, ranging from 20 to 140 μg/ml, and the correlation coefficient (R^2) of the regression line was 0.9995. The limit of detection (LOD) and the limit of quantitation (LOQ) were 0.86 μg/ml and 2.62 μg/ml, respectively. The relative standard deviation (RSD %) was less than 0.6% for intraday assay (n = 5) and 2.5% for interday assay (n = 5).

Conclusion

The method reported is simple, precise and accurate. It was used to *determine the amount of tobramycin in the commercial dosage form* TOBI *300 mg* per *5 ml* solution (*tobramycin/0.25 normal saline*).

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Pharmaceutical analysis of methyl and hexyl ester derivatives of 5-aminolevulinic acid

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Introduction and Objectives

Photodynamic therapy (PDT) using topical 5-aminolevulinic acid (ALA), a water-soluble precursor of the potent endogenous photosensitiser, protoporphyrin IX (PpIX), is a treatment and diagnostic tool for premalignant and malignant skin cancers. However, to improve drug delivery to deeper skin lesions, more lipophilic ALA esters have been proposed. Owing to the necessity in drug delivery research for efficient and validated assays for ALA esters in solution, this study aims to describe the optimised and validated protocols to quantify the methyl (m-ALA) and hexyl (h-ALA) esters of ALA.

Methods

The ALA and ALA esters were derivatised using acetyl acetone and formaldehyde reagents and analysed using high-performance liquid chromatography (HPLC) (Agilent, West Lothian, UK) with fluorescence detection (ex = 370 nm, em = 460 nm). Samples were injected onto a Spherisorb column (250 mm × 4.6 mm, C_{18} ODS2, 5 μm; Waters associates, Harrow, UK). The mobile phase consisted of 1% v/v acetic acid in water (solvent A) and 1% v/v acetic acid in methanol (solvent B). The m-ALA derivative was eluted using 40% A and 60% B. A gradient elution was performed for the hexyl ester: 0–10 min: 50–100% B, 10–10.5 min: 100–50% B, 10.5–20 min: 50% B. Least squares linear regression analysis and correlation analysis were performed on each calibration curve produced. Limits of detection, limits of quantification (LOQ) and intraday and interday variabilities were also determined for the analysis of each drug. The validated methods were used to evaluate the release profiles of ALA, m-ALA and h-ALA from an o/w cream across a model cellulose membrane.

Results and Discussion

Linear calibration curves were obtained for ALA (0.3–10 μg/ml, r^2 = 0.9995), m-ALA (30–500 μg/ml, r^2 = 0.9997) and h-ALA (15–300 μg/ml, r^2 = 0.9998). Greater sensitivity of the parent drug (LOQ = 0.70 mg/ml) was achieved compared to m-ALA (LOQ = 29.45 mg/ml) or h-ALA (14.88 mg/ml). Importantly, HPLC facilitated the adequate separation of ALA-impurities in ALA-ester samples. Intraday and interday variabilities were found to be acceptable for each method [coefficient of variation (CV) values < 5%]. Permeation studies showed that the percentage of drug loading released from cream preparations, across a model membrane after 5 h, was in the order of ALA (45.2%) > m-ALA (38.3%) > h-ALA (33.9%).

Conclusion

Validated assays have been developed for quantification of ALA esters in aqueous solution. For the first time, the potential of ALA fluorescent derivatives to interfere with the routine analysis of ALA esters has been highlighted. Hence, if the HPLC conditions are not optimised, you could potentially greatly overestimate ester release. The reduced release of ALA-esters from the cream may explain why, historically, some of the benefits seen with ALA-esters

using cell culture models[1] have not been demonstrated *in vivo*.[2]

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Quantitative determination of sulphasalazine and mesalazine by ^{1}H-NMR chemical shift variation

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Introduction and Objectives

The aim of this study was to quantitatively determine sulphasalazine and mesalazine (5-aminosalicylic acid), either in their authentic form or as pharmaceutical tablets, based on the fact that the chemical shift values of different protons of each drug change with aqueous concentration. These chemical shift changes have linear ranges and can therefore be used for the quantitative analysis of each drug in water, but not in typical organic solvents.

Method

Changes in chemical shift values of aromatic protons as a function of concentration were recorded in 4 M NaOD over a wide concentration range for sulphasalazine (1–200 mg/ml) and for mesalazine (5–500 mg/ml). The plots of proton chemical shift against concentration produced a linear range, and the proton with the best regression was selected for the quantitative determination of these gastrointestinal tract medicines.

Results and Discussion

This analysis revealed a strong concentration-dependent chemical shift variation. By increasing the concentration, the aromatic proton signals of sulphasalazine moved upfield; the peaks coalesced, crossed-over and then moved downfield (Figure 1). In the case of mesalazine, all the (aromatic) protons moved downfield. The linear ranges of 50–200 and 10–400 mg/ml were selected for the determination of sulphasalazine and mesalazine, respectively. Student's *t*-test and the variance ratio test (*F*-test) to compare the results with those obtained from the official British Pharmacopoeia method showed good accuracy and precision.

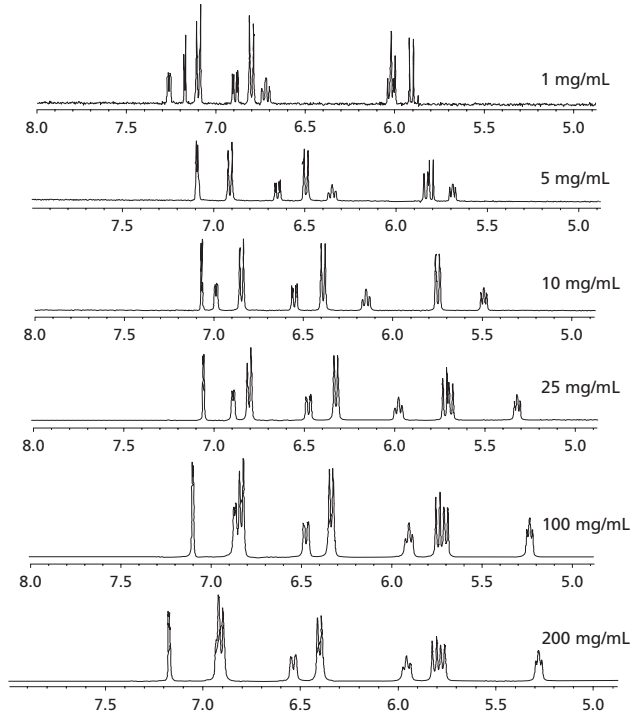

Figure 1 Partial ^{1}H-NMR spectra for sulphasalazine (1–200 mg/ml recorded in 4 M NaOD).

Conclusion

Self-association of the drug molecule changes the environment surrounding each proton and leads to significant chemical shift variations.[1] The molecules can be associated as a result of intermolecular hydrogen bonds, aromatic stacking or electrostatic interactions, which lead to the formation of aggregates of different number and orientation of molecules.[2,3]

Acknowledgement

We thank the Egyptian Government for financial support (a studentship to Manal S. Elmasry is gratefully acknowledged).

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The development and application of ultraviolet area imaging detection to the dissolution of pharmaceuticals

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Introduction and Objectives

The aim of this study was to develop and apply a novel ultraviolet (UV) area imaging technology and methodology to assist in understanding the dissolution behaviour of drug substances and products. Dissolution is the process whereby the drug substance within a solid drug product becomes available for absorption. While in-vitro dissolution testing using pharmacoepial methods is satisfactory for clearance and stability monitoring purposes, it gives little of the mechanistic information that is important in understanding any differences observed. We aim to develop and apply innovative UV area imaging technology to improve the understanding of the complex mass-transfer processes occurring at the dissolving surface.

Method

Substances including the diuretic furosemide and benzoic acid were packed into a sample holder which was placed within the imaging cell, and the dissolution buffer pumped across the surface. The imaging cell was placed within a 9×7 mm UV detection area, which is based upon an active pixel sensor (APS) system. The APS uses 1.3 million pixels and provides a maximum spatial resolution of 7 μm.

Results and Discussion

The UV imaging method allows us to examine the concentration gradients in solution near to the surface of a dissolving solid. This allows us to determine concentration profile as a function of flow rate or to determine diffusion processes. For example, Figure 1 shows the UV absorbance profiles for benzoic acid taken at 5-s intervals using a phosphate buffer solution at pH 7.8. As time progresses, the maximum concentration of benzoic acid increases as benzoic acid diffuses into the bulk phosphate solution.

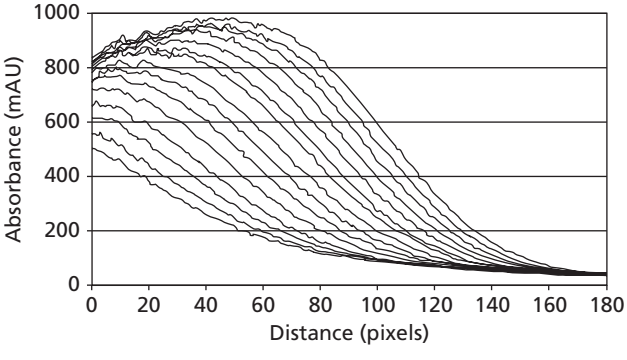

Figure 1 The evolving concentration gradient of benzoic acid in pH 7.8 phosphate.

Conclusion

The UV array imaging system can be used to provide important information on dynamic concentration gradients close to the surface of dissolving drug substances.

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Quantitative analysis of konjac glucomannan extracted from corms of *Amorphophallus konjac*

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Introduction and Objectives

Konjac glucomannan (KGM) extracted from corms of *Amorphophallus konjac* has a long history of use in China and Southeast Asia as a food source and recently has been used in the development of dietary supplements. Various methods have been used to process and assay KGM, resulting in inconsistency in quality of KGM-derived products. The objective of the current study is to develop a standard methodology to process and assay KGM by comparing existing protocols. The chosen methodology will be adopted for the extraction, purification and assay of KGM from corms grown in China in collaboration with Yunnan Agricultural University.

Method

KGM content in crude and nutraceutical grade konjac flour, namely LOT 1 and Megazyme powder, were determined by enzymatic assays using a glucomannan assay kit (Megazyme International Ireland Ltd), which is specific for KGM and its breakdown products. The results were compared with two nonenzymatic calorimetric assays using phenol[1] and 3,5 dinitrosalicylic acid[2] as colour developers, with reference to reliability, reproducibility and accuracy. The absorbance was measured at 490 and 550 nm, respectively. Glucose was used as a standard in the preparation of calibration curve for both calorimetric assays.

Results and Discussion

Enzymatic analysis gives much lower levels of KGM content in konjac flour compared with the levels using acid hydrolysis. Among these methods, C has the highest

Table 1 Mean percentage (±SD) of KGM in dried weight of crude konjac flour, LOT 1 and Megazyme powder determined using enzymatic and calorimetric assays (n = 3)

Sample	KGM content (%)		
	Glucomannan kit (A)	Phenol-sulphuric acid assay (B)	3,5-dinitrosalicylic acid assay (C)
Crude konjac flour	24.74 (±2.26)	31.24 (±0.31)	27.92 (±0.25)
LOT 1	41.45 (±0.89)	68.67 (±0.94)	94.5 (±2.70)
Megazyme powder	44.29 (±1.58)	66.75 (±6.67)	95.5 (±2.35)

reproducibility and the glucose recovery is 100.4 ± 1.67%. Linearity test of C showed that the correlation coefficient of test solutions (6.4–32 μg/ml) is 0.993 (Table 1).

Conclusion

Method C based on 3,5-dinitrosalicylic acid is inexpensive, reliable and reproducible compared with enzymatic assay, which is expensive, and phenol-sulphuric assay, which involved dangerous acid degradation of sample. It can be applied to determine the KGM content and hence the quality of commercial konjac products.

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Quantitative HPLC analysis of mebeverine HCl and other GIT medicines stored in a monitored dosage system for polypharmacy

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Introduction and Objectives

Monitored dosage-dispensing systems are designed for patients having difficulties in managing their medication; however, there is a lack of short-term stability data for the storing of medications in these packs.[1] We have developed a stability-indicating quantitative high performance liquid chromatography (HPLC) method for mebeverine HCl (Solvay Healthcare Limited, Southampton, UK), an over-the-counter (OTC) medicine for irritable bowel syndrome and mesalamine (5-amino-salicylic acid [5-ASA]) stored in Venalink blister packs along with other medications for up to 35 days. Patient and prescriber anonymized FP10s prescriptions were scrutinized in community pharmacies, noting prescriptions where mebeverine HCl or 5-ASA solid-dosage forms were prescribed alongside other solid-dosage forms on the same prescription, and from these four prescriptions were selected for the analysis.

Method

The medicines were packed in the blister packs using a cold-seal process, stored protected from light and heat. HPLC analysis was performed on days 0, 1, 7, 14, 21, 28 and 35. Each tablet was analysed by dissolving in mobile phase (100 ml for mebeverine, 4-aminobenzoic acid internal standard) or 0.1 M aq. HCl (100 ml for 5-ASA, veratric acid internal standard), filtered and analysed on a Synergi Max-RP-HPLC analytical column, mobile phase methanol : water : acetic acid (40 : 59 : 1 vol/vol/vol), flow rate 1.5 ml/min, sensitivity of 2.56 absorbance units full scale (AUFS) with detection at 263 and 300 nm for mebeverine HCl and 5-ASA, respectively.

Results and Discussion

Prescriptions analysed included mebeverine tablets (Generics Ltd, UK) stored with (1) cetrizine, simvador, lansoprazole, paracetamol and bendroflumethiazide; (2) aspirin EC, atenolol, Monomax SR and perphenazine; (3) Co-codamol 30/500 and (4) Asacol MR (Procter & Gamble Pharmaceuticals Ltd, UK), amitriptyline, buscopan and tramadol MR. On each day of the analysis, brief, routine visual inspection of the analysed tablet did not show any change in colour, or signs of degradation of the tablet or the enteric-coated film, especially in weeks 3, 4 and 5. The linear ranges were from 0.27–1.89 to 1–9 mg/ml (r^2 = 0.9998) and the limits of detection were 0.05 and 0.4 ng/ml for mebeverine HCl and 5-ASA, respectively. Precision, expressed as relative standard deviation (RSD, %), was 0.49% for mebeverine HCl and 0.59% for 5-ASA. Quantitative recoveries of both the drugs were observed. Robustness was proved by inducing small changes in the chromatographic conditions, which did not affect the analysis except for minor changes in the retention time, but still with good resolution.

Conclusion

The quantitative HPLC analytical method showed no degradation of either medicine. Mebeverine HCl and 5-ASA tablets stored in this package remained stable for at least 5 weeks (1 week of advanced packing and 4 weeks of supply). However, during the storage time, excessive handling can lead to rupture of the blister seals so that the remaining tablets are exposed to increased levels of air and humidity; therefore, the attention of the patient should be drawn to the storage of these blister packs away from light, air, heat and humidity.

Acknowledgement

We thank the Egyptian Government for financial support (a studentship to Manal S. Elmasry). We also thank Solvay Healthcare Limited, UK, for supplying mebeverine HCl.

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Applying dried blood spot analysis: the pathway to better paediatric care

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Introduction and Objectives

Chronic lung disease (CLD) of the newborn predominantly affects premature babies. The development of a dried blood spot (DBS) (heelstick), and thus the microvolume sampling methodology, for dexamethasone to facilitate pharmacokinetic (PK) studies is the focus of this investigation. Dexamethasone, a potent steroid, is used to oppose the proinflammatory state observed in babies with CLD. However, doses used in practice are empirical and accurately tailored dosage is confounded by a lack of PK data. A major barrier-preventing PK studies in premature babies is the limited circulating blood-volume. Existing methodologies for measuring dexamethasone require relatively large blood-volumes, rendering them unsuitable.

Method

Fifty microlitre sample volumes of venous blood spiked with dexamethasone were spotted onto specimen collection cards and allowed to air dry overnight at ambient temperature. DBSs were punched out and extracted using methanol : water (60 : 40% vol/vol)–containing internal standard. Extracts were cleaned via centrifugation and supernatant analyzed using a validated reversed-phase liquid chromatography-mass spectrometry (LC-MS) detection system. Chromatographic separation was obtained using a gradient with mobile phases of water containing 0.15% formic acid and acetonitrile. MS detection was by single-ion monitoring using ions m/z 393.1 and 435.1. The software on LC-MS designed to identify dexamethasone metabolites was investigated.

Results and Discussion

Factors during the collection and processing of specimens which may affect the quantification of dexamethasone were investigated. Specific concerns were the volume of sample collected and the size of the punch. Varying the volume of sample spotted onto the filter card did not significantly affect the quantification results, but the punch size was of importance in assuring accurate analysis. Increasing the punch size improved the detection sensitivity of the assay. Disc diameters in the range of 3–10 mm were investigated to determine the detection sensitivity. The selectivity of the DBS method was validated by analyzing control blood spot samples, which did not show any interference at the same retention time as dexamethasone. The recovery extraction efficiency for dexamethasone was >98%. The LC-MS method was optimized to give a current detection sensitivity of 0.25 ng/ml (25 μl injection). Results indicate that the DBS method coupled with mass spectrometry detection will support a paediatric PK study. The planned study has been ethically approved by the Leicestershire, Northamptonshire and Rutland NHS Research Ethics Committee and the De Montfort University Research Ethics Committee.

Conclusion

DBS analysis addresses a widespread drug-dosing problem inherent in current paediatric practice. The collection of blood onto Guthrie card is already a well-established method for screening various diseases in newborns,[1] and there are a number of reports of application to drug quantification.[2] This methodology enables simplified sample processing and storage conditions whilst maintaining paediatric applicability. In a parallel study, our research group has demonstrated proof of principle for captopril in a paediatric patient. Furthermore, captopril in DBS samples remains stable at room temperature for at least 3 months. This has important implications for more widespread paediatric PK studies.

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Qualitative comparison of Malaysian and UK paracetamol tablets by Near-Infra Red

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Introduction and Objectives

As part of the initial effort in developing a database on counterfeit medicines in Malaysia, the near-infra red (NIR) spectra of Malaysian' generic paracetamol (PCM) were compared to the UK samples.

Method

Sixteen brands of PCM tablets (active ingredient [AI]: 500 mg, except one batch of 650 mg) were purchased in Malaysian pharmacies. A further six brands (AI: PCM 500 mg) were bought in UK pharmacies. Three sets of negative controls (AIs: [NC1] ibuprofen 200 mg, [NC2]

PCM 500 mg, dihydrocodeine tartrate 7.46 mg, [NC3] PCM 200 mg; Aspirin 300 mg; caffeine 45 mg) were included in the study. Twenty tablets from each set were analysed in-tact using NIRSystems 6500 spectrophotometer, FOSS (USA). Measurements were made in reflectance mode over the wavelength 1100–2498 nm. Each spectrum was analysed in three forms: raw spectra, second derivatives and standard normal variate pretreated spectra. The acquired spectra were compared using principal component analysis (PCA) models and Soft Independent Modelling of Class Analogy (SIMCA) classifications (The Unscrambler 9.7).

Results and Discussion

NIR analyses indicated that all the samples produced a similar spectroscopic fingerprint of PCM. The PCA score plot showed that the 650 mg samples were well clustered with the other samples and all negative controls were excluded from the confidence interval ellipse (Figure 1). These results indicated the ingredients but not the amount of AI that influence the classifications. One batch from the Malaysian sample was excluded from the 95% confidence interval set, indicating significantly different quality of the tablet, which may indicate different excipients or manufacturing process (PCM-D). Further analysis by SIMCA demonstrated high interbatch variability among the Malaysian samples with the model distance up to 20 times higher than in UK samples. This might reflect the inconsistency of the quality of Malaysians samples. The pretreatment of spectra showed sample distributions in smaller score-plot scale, but the spectra interpretations were more complex – 5PCs were required compared to only 2PCs for the raw spectra.

Conclusion

Only one batch of PCM samples was significantly different from the tested set. Samples' comparison with the manufacturer-certified product is crucial to determine the product's authenticity. The analysis of friability, hardness and AI quantifications will provide further insight in the Malaysian tablets' quality.

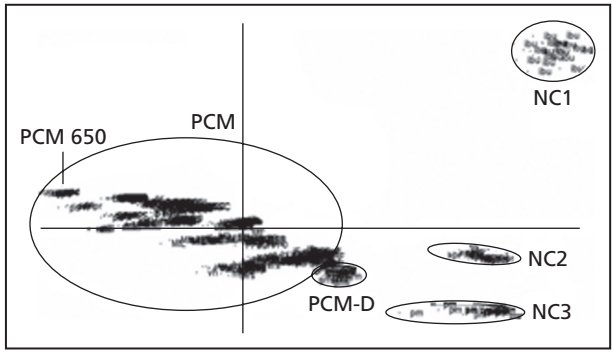

Figure 1 The score plot distribution of all PCM samples and negative controls.

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The use of ICP-OES and ICP-MS in the assessment of magnesium stearate levels on tablets

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Introduction and Objectives

Tablet presses have been developed to spray coat magnesium stearate lubricant onto the die and punch. This has significantly reduced the amount of lubricant on the tablet. This reduction in magnesium stearate levels on the tablet required the development of a new method of analysis for magnesium stearate. This study discusses the development of an analysis method capable of determination of magnesium stearate at levels below 0.02% w/w by using inductively coupled plasma mass spectrometry and inductively coupled plasma optical emission spectroscopy.

Method

Tablets were formed from lactose using a Manesty Accu-Spray tablet press. The tablet samples were placed in ceramic crucibles and were ashed at 800°C. The residue was then digested in 5 ml of nitric acid. The samples were then diluted to 100 ml. The solutions were analysed by ICP-MS (Thermo X series) using the 24, 25 and 26 mass/charge isotopes and ICP-OES (Thermo iCAP 6500 Duo) using the 285.213-, 280.270- and 285.213-nm lines. The ICP-MS was calibrated from 10 to 50 ng/ml using Mg standards (QMx) and Rh was used as an internal standard added in the digested solutions to improve reproducibility. The ICP-OES was calibrated from 100 to 500 ng/ml using Y as an internal standard added in the digested solutions.

Results and Discussion

Atomic absorption techniques are normally used to assess the magnesium stearate levels. However, the new application method used within the tablet press applied much less material (between 10- and 100-fold less). As a result, more sensitive methods such as ICP-OES and ICP-MS needed to be assessed. In each case, both the techniques assessed were capable of analysing magnesium stearate at levels below 0.02% w/w on each tablet. In each case, the ICP techniques were optimised for sensitivity by looking at plasma condition such as power, coolant flow, auxiliary flow and sample pressure. In ICP-OES, the axial view optical emission was optimised using the 279.553-, 280.270- and 285.213-nm line, with the yttrium 371.030-nm line being used as an internal standard. The ICP-MS used the m/z ratio of 24, 25 and 26 to monitor the Mg and the Rh 103 isotope as the internal standard. In this case, the quadrupole was optimised to give the optimum signal-to-noise ratio. In the case of ICP-OES, the optimum conditions were found to be coolant of 16 l/min,

Table 1 Optimum inductively coupled plasma-mass spectrometry conditions

Major	Settings	Minor	Settings	Add gases	Settings
Extraction	−145	Lens 2	−25.1	7% H_2-He	0.00
Lens 1	−1.5	Lens3	−200.0	CCT2	0.00
Focus	15.1	Forward Power	1404		
D1	−38.4	Horizontal	51		
Pole bias	−2.0	Vertical	500		
Hexapole bias	−0.5	D2	−140		
Nebuliser	0.95	DA	−44.7		
Sampling depth	110	Cool	13.0		
		Auxillary	1.00		

nebuliser gas flow of 0.5 l/min, radio-frequency power of 1350 W, auxiliary gas flow of 1.1 l/min and pump speed of 90 rpm. This resulted in an LoD of 2 ng/ml on the 285.213-nm line and a 7 ng/ml LoQ equivalent to a tablet level of 167 ng/g LoD and 583ng/g LoQ. The dual-axial radial detection system allowed linear calibration between 10 ng/ml and 1 g/ml. The recovery was tested using a powdered sample of lactose and magnesium stearate, which produced recoveries ranging from 95.15 to 102.7% with reproducibility of less than 3%. For the ICP-MS, the optimum conditions are given in Table 1. This produced an LoD of 0.18 ng/ml and an LoQ of 0.60 ng/ml, which amount to an LoD of 15 ng/g in the tablet and an LoQ of 50 ng/g in the tablet. The calibration was linear between 5 and 500 ng/ml. Reproducibility of the tablet analysis was less than 1% at the determination of 25 ng/ml level. Accuracy on the powdered sample after dilution was between 95 and 105%. The limitation of the technique was found not to be the ultimate sensitivity but the blank limitation of the lactose powder.

Conclusion

The ICP-MS and ICP-OES were shown to be suitable techniques for the assessment of magnesium stearate levels on tablets generated from the Manesty Accuspray tablet press.

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Extemporaneously prepared oral clobazam formulations for paediatrics: pharmaceutical characterisation and stability studies

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Introduction and Objectives

The purpose of this study was to assess the chemical stability and pharmaceutical quality of extemporaneously prepared liquid formulations of clobazam. Very few medicines are formulated specifically for children, which leads to extensive off label and unlicensed use of a wide variety of drugs.[1] One such drug is clobazam, an antiepileptic, which is only available as 10-mg tablets. Because of the lack of a licensed liquid formulation, its extemporaneous formulations are routinely used in the paediatric setting, despite the lack of evidence of pharmaceutical quality and clinical efficacy.[2]

Methods

Extemporaneous formulations of clobazam tablets were prepared using either Ora-Plus/Ora-Sweet (1 : 1) (Paddock Laboratories Inc, Minneapolis, USA) or Keltrol (xanthan gum) (S.Black Ltd, Hertfordshire, UK) as a vehicle and stored at both long-term (25°C/60% relative humidity (RH)) and accelerated (40°C/75% RH) storage conditions. At regular time intervals, the formulations were analysed by *high-performance liquid chromatography* (HPLC) for drug content and dose uniformity, while physical properties such as particle size, zeta potential, visual appearance, smell, viscosity and pH were also investigated. Formulations were also adjusted to several pH values to estimate the effect of pH on the stability of clobazam.

Results and Discussion

For both the Keltrol-based and Ora-Plus-based extemporaneous formulations, the concentration of clobazam remained above 90% of the initial concentration throughout the study, with no evidence of degradation peaks. All formulations were relatively stable in terms of physico-chemical characteristics – particularly particle size, zeta potential and viscosity – with no significant change at the long-term conditions throughout the study period ($P > 0.05$, student's *t*-test). However, the pH of the Keltrol-based formulation decreased significantly from an initial value of 6.4 to a value of 4 throughout the course of the study ($P < 0.05$, student's *t*-test). In addition, the odour of this formulation became unpleasant after around 5 days of the study – possibly as a consequence of microbial growth – which could affect patient compliance and safety. The Ora-Plus-based formulation, on the other hand – at its initial pH of 3.6 – was particularly stable throughout the study period for all parameters measured ($P > 0.05$, student's *t*-test). Nevertheless, when the pH was adjusted to 5, sedimentation was observed after 14 days, although this was easily redispersed on shaking. Furthermore, at an adjusted pH 9, there was a change in colour and odour of the formulation, which again would likely affect patient acceptability.

Conclusion

The results of this study show that extemporaneous formulations of clobazam may be prepared that exhibit good chemical stability, although formulation stability is dependent on appropriate choice of vehicle and pH. The Ora-Plus-based formulation was shown to be physically very stable throughout the 4-week study period at both long-term and accelerated stability conditions, suggesting an expiry date of up to 56 days at room temperature.

The Keltrol-based formulation, however, ought to be given a reduced shelf life or stored in refrigerated conditions as a consequence of the deterioration of organoleptic properties.

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Prediction of food effects in matrix controlled release formulations using in-vitro dissolution

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Introduction and Objectives

The influence of a meal on the drug release from a matrix-controlled release (CR) formulation can be significant and results in the dosage form having a different in-vivo performance when administered with or without food. A universal in-vitro method or technology to screen out these effects early before reaching the clinic would help with the development of a formulation with the greatest potential of successfully delivering the target profile to the patient.

Methods

Drug-release experiments using the USP apparatus III were performed with the Caleva Bio-Dis RRT9 (Caleva, Frankfurt, UK). Dip speed (or reciprocation rate) can be adjusted from 5 to 40 dips per minute (dpm). Screen mesh size used had a 420-μm pore size. Sampling was carried out manually using a 35-μm full flow filter or automated using an ASP 2000 (Icalis, Finchampstead, UK) robot with GD/X in-line filtration. Sample analysis was carried out by high-performance liquid chromatography. Experiments were carried out using compound A (BCS III) formulated as both matrix and multiparticulate dosage forms, Glucophage XR, Biaxin XL, Felodipine, Diclofenac sodium and Lescol XL. The dissolution profiles generated using the USP III were then compared to the human and animal pharmacokinetic data and also to the results obtained from conventional USP II fasted/fed condition experiments, which in most cases failed to predict known food effects of these CR formulations.

Results and Discussion

Figure 1 shows the human, animal and USP III data for compound A. Due to the poor predictive nature of the preclinical models and of the standard in-vitro techniques, a new approach using the USP III was developed where a two-stage test was designed to simulate the gastric conditions of the stomach and of the small intestine in the both fasted and fed conditions. The same conditions were able to predict the effects of food for most of the marketed matrix formulations chosen.

Conclusion

The use of USP III has been shown to successfully predict the in-vivo performance of the matrix and multiparticulate

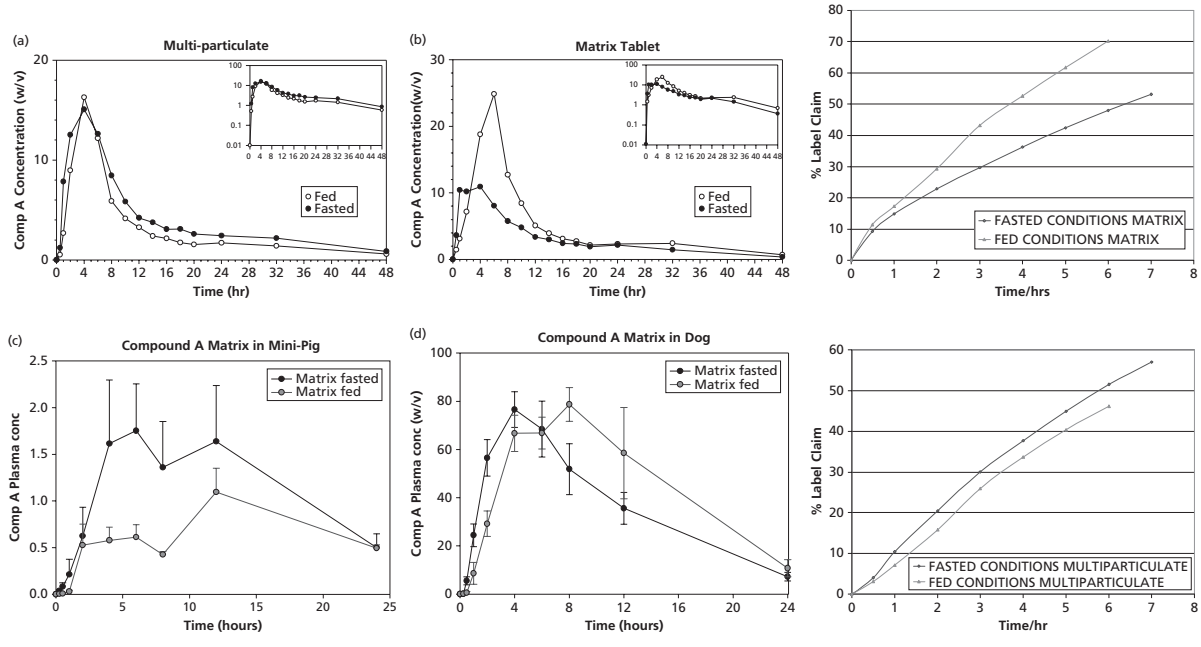

Figure 1 In-vivo pharmacokinetic profiles for compound A: a) multiparticulate human, b) matrix human, c) matrix mini pig, d) matrix dog and in-vitro USP III fed/fasted dissolution profiles for e) matrix and f) multiparticulate.

formulation of compound A by using a set of fixed conditions for the fed and fasted states. This level of prediction could not be achieved by using standard dissolution procedures or animal models. The USP III fasted/fed conditions could be used in future to screen out formulations that are more likely to demonstrate in-vivo food effects.

Drug Delivery

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Formulation of HIV-1 clade c envelope protein gp140 into directly compressed solid dosage forms as a potential delivery system for vaginal mucosal vaccination

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Introduction and Objectives

A fundamental strategy in the development of an HIV vaccine should include potent mucosal immunity to block HIV at mucosal epithelial barriers and prevent initial infection of target cells. There is considerable evidence indicating that local antigen delivery to the vagina is consistently effective for induction of a local mucosal immune response.[1] However, maintaining immunogenicity of antigens throughout formulation preparation is problematic because of their liable nature. The aim of this study was to develop solid dosage form formulation(s) for the sustained delivery of HIV-1 clade c envelope protein, gp140, as a potential vaginal mucosal vaccination strategy.

Method

gp140 stock solution was mixed with glycine, separated into four equal volumes and lyophilised. The individual dry cakes were mixed with (1) 100% Kollidon^{SR}, (2) 20:80 Kollidon/hydroxypropyl methyl cellulose (HPMC), (3) 50:50 Kollidon/HPMC and (4) 100% HPMC. Tablets were manufactured by direct compression on a Riva mini-press using a rounded and bevelled edge 6-mm punch and die set. Dissolution was carried out in PBST with NaN3 and placed in an orbital shaking incubator at 60 rpm and 37°C. Samples were taken and replaced with fresh dissolution media daily for 11 days. Gp140 was quantified by ELISA.

Results and Discussion

Positive concentrations of gp140 were detected from all the formulations studied; however, it is evident that the material used greatly influences the recovery and release of gp140. The highest percentage release of gp140 was achieved in those formulations composed of 100% Kollidon^{SR}, where more than 85% (46 μg) of the total gp140 was released after 48 h. The inclusion of HPMC in the solid dosage forms prolonged the release of gp140 for as much as 11 days; however, the fractional recovery of gp140 was considerably lower (40%). The total fraction of gp140 was below 10% when incorporated into 100% HPMC tablets. The results indicate that HPMC imposes a degradative effect on gp140. Protein–surface interactions with polymers are known to induce conformational changes that can result in their inactivation.[2] It is therefore no surprise that HPMC, which forms a sticky mucoadhesive gel upon hydration, is likely to adsorb to, and/or induce surface interactions with, gp140 thus reducing its activity. Conversely, Kollidon is composed of polyvinylacetate and povidone and will not form a mucoadhesive gel upon hydration. Instead release is governed by the uptake of water into the tablet matrix, which causes some swelling, and the slow dissolution/diffusion of gp140 into the aqueous medium, which is evidently favourable for gp140 formulations. Degradation of gp140 was characterised by SDS page and Western Blot analysis.

Conclusions

This study demonstrates that the gp140 has proven to be exceptionally stable, relative to other HIV-1 recombinant envelope glycoproteins. It was successfully formulated into Kollidon^{SR} directly compressed solid dosage forms with only minimal comprise to is antigenic activity (<15%), and the release sustained for up to 48 h. HPMC can be added to Kollidon solid dosage forms to prolong release of gp140 for 11 days, but it will compromise the immunogenicity of gp140. Formulations that prolong release of gp140 may induce a stronger response and immune memory and thus could potentially optimise a vaginal mucosal vaccination strategy against HIV.[3]

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Solubilisation of steroid drugs by simulated intestinal fluids containing lipids

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Introduction and Objectives

Simulated intestinal fluids (SIFs) have been developed to be used for dissolution and permeability screening of poorly water-soluble drugs. Most of these SIFs are composed only of a single bile salt (BS) and lecithin (L), as opposed to mixtures of BS containing the most common digestion products, namely monoglycerides and fatty acids. The objective of this study was to assess the solubility of two drugs: hydrocortisone (HC) and progesterone (P) in a media containing single BS, sodium taurocholate (STC) and mixed bile salts (MBS). SIFs containing STC, or MBS in combination with L, medium- and long-chain lipids were also investigated.

Method

MBS comprising STC, sodium glycodeoxycholate and sodium deoxycholate in a ratio of 60:39:1 were prepared. The solubilities of HC and P were determined in STC and MBS SIFs representing the fed state (pH 5.0). Solubilities of both drugs were also determined in STC and MBS SIFs containing L at a fixed ratio of BS:L of 4:1 and with different ratios and amounts of monoolein (MO) and oleic acid (OA) (long-chain lipids, LCL) as well as monocaprin and capric acid (medium-chain lipids, MCL). Increases in solubility compared to the solubility in acetate buffer were measured.

Results and Discussion

The solubility of P was increased 5-fold in media containing STC alone, but 15-fold in MBS solution, while the increase in HC was only approximately 2-fold in both media. Upon the incorporation of L, the solubility of P was increased further, both in STC (16-fold) and MBS (20-fold). However, there was no additional change in the solubility of HC in either system beyond the solubility in BS solution. The addition of LCL to the STC or MBS (final composition of BS:L:MO:OA of 4:1:3.75:1) produced a further increase in the solubility of P (to 27-fold greater than acetate buffer) in both BS systems. Again in the case of HC, there was no further increase in solubility above the original 2-fold enhancement observed using BS alone. The effect of incorporating MCL at the same ratio as LCL to the STC and MBS systems produced quantitatively similar results to the effects of LCL for P and HC. The increases in solubility upon the addition of lipids were possibly due to an increase in size of BS:L mixed micelles,[1] or solubilisation in other colloidal phases that are likely to be present within the SIFs used in this study.

Conclusions

Incorporation of lipid digestion products in the formulation of SIFs increases the solubility of lipophilic drugs and such complex media may provide more relevant fluids for dissolution and permeability screening.

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Improving the aerosolisation properties and lung deposition of spray-dried powders for inhalation

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Introduction and Objectives

Although spray-dried powders can be prepared to an appropriate particle diameter range for the pulmonary delivery of therapeutically active agents, the particles are prone to aggregation due to strong cohesive forces resulting in agglomerates with large aerodynamic diameters and reduced dispersibility during inhalation. The aim of this study was to investigate the influence of the addition of the dispersibility enhancer, L-leucine, and the permeation enhancer, dipalmitoyl phosphatidylcholine (DPPC), as an excipient of spray-dried formulations, for use in a dry powder inhaler, and to improve the delivery of the pharmaceutically active molecule salbutamol sulphate (SS).

Method

DPPC dissolved in ethanol and aqueous solution of SS; lactose (Lactohale 230) and L-leucine were mixed prior to spray-drying (Buchi 190; Buchi UK Ltd, Oldham, UK) with inlet temperature 160–170 °C, outlet temperature 80–82 °C, pump rate 600 l/h and aspirator setting 35 m^3/h. This produced dry powder formulations for inhalation of Lactohale 230/SS (control), Lactohale 230/SS + L-leucine 0–20% w/w and Lactohale 230/SS + L-Leucine:DPPC (20:20% w/w). All formulations contained 2% w/w SS. The aerosolisation performance of SS using a HandiHaler ($n = 3$) was analysed for fine particle fraction (FPF) and %dispersibility (%Disp) (emitted dose/recovered dose ×100) using a twin-stage liquid impinger at 60 l/min. The density of spray-dried formulations was measured using a pycnometer.

Results and Discussion

Dispersion and powder aerosolisation studies of the control sample indicated a FPF (27.41 ± 3.51%) and %Disp (73.23 ± 1.06%). Upon addition of L-leucine, the FPF (5–20% w/w = 36.73 ± 1.21 to 46.33 ± 1.18%) and %Disp (78.75 ± 0.28 to 81.62 ± 0.93%) were significantly elevated ($P < 0.05$) with increasing L-leucine concentration. The addition of DPPC to L-leucine further increased ($P < 0.05$) the FPF (50.19 ± 1.76%) and %Disp (84.47 ± 2.13) compared to control sample and only 20% of the leucine formulation. Incorporation of L-leucine enhanced the aerosol behaviour of spray-dried powders by reducing the surface tension during the spray-drying process.[1] Furthermore, the presence of DPPC at the particle surface decreased the surface energy and the hydrophobic character of DPPC also reduced moisture sorption and thereby cohesion.[1] DPPC also facilitated in creation of

porous particles, reducing the density from 1.22 ± 0.09 g/ml with L-leucine formulations to 0.85 ± 0.01 g/ml and thus enhancing the aerodynamic properties of the formulation, resulting in significant increase in FPF and dispersibility.

Conclusions

The results indicate that the addition of excipients can aid in the delivery of the pharmaceutically active molecules to the lower airways through changes in the physiochemical nature of the powder produced. Through the manufacturing process of spray-drying and analysis, the combination of the permeation (DPPC) and dispersibility enhancer (L-leucine) was shown to increase the aerosolisation and lung deposition of salbutamol to the lower airways as represented by FPF and dispersibility, thus potentially improving the therapeutic drug response.

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Factorial design of liposome-DNA complexes: influence of formulation and process parameters

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Introduction and Objectives

The aim of this study was to apply a design of experiment programme to obtain greater precision at estimating the overall main factors that may affect the characteristics of small unilamellar vesicles (SUV) and SUV-DNA complexes and subsequently influence the cytotoxicity and transfection efficiency of SUV-DNA complexes to macrophages *in vitro*. By considering all possible combinations, factorial design allows extensive exploration into the interactions between variables, i.e. type of cationic lipid, cationic lipid to helper lipid ratio, DNA concentration and the overall effect on the final outcome. The programme selectively generates a list of experimental parameters, significantly reducing the number of experimental preparations required for evaluation.

Methods

Factorial design and the evaluations were performed by the software MODDE 8.0 (Umetrics, Umea, Sweden). Transfection efficiency of SUV-DNA complexes consisting of a cationic lipid, either cholesterol 3b-N-(dimethylaminoethyl)carbamate (DC-Chol) or 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) and the helper lipid, L-alpha-dioleoylphosphatidylethanolamine (DOPE) to a BALB/c macrophage cell line was tested. Various lipid ratios and concentrations of gWIZ plasmid DNA, ranging from 1 to 4 mg, were tested. In addition, the cytotoxicity of each SUV-DNA formulation was measured and expressed as percentage of control. The z-average diameter (nm) and zeta potential (mV) of each liposome formulation were measured on a ZetaPlus (Brookhaven Instruments, New York, USA) in double distilled H_2O.

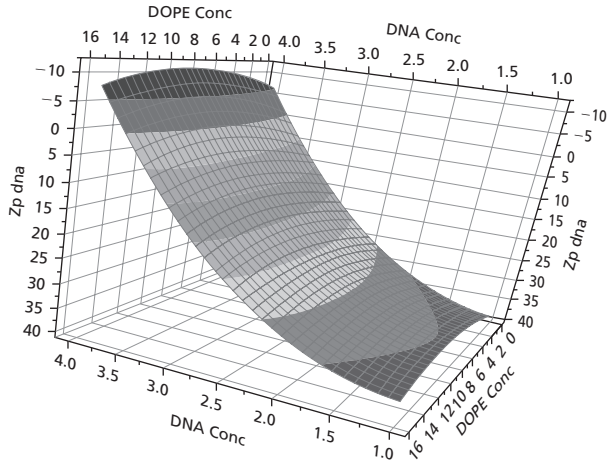

Figure 1 Response surface plot showing the effect of DNA concentration ($\times 10^1$ μg) and DOPE content (μmole) on the zeta potential (mV) of SUV liposomes.

Results and Discussion

The inclusion of plasmid DNA within all possible formulations increased the vesicle size and further decreased the positive charge of the cationic SUV, producing an overall negative surface charge at the highest plasmid DNA concentrations (Figure 1). All formulations were not shown to effectively transfect macrophages *in vitro*.

Conclusion

The outcomes obtained by the application of factorial design, for the screening of various factors, including; type of cationic lipid, the cationic lipid to helper lipid ratio and DNA concentration, clearly show that the most critical parameter influencing the mean size and zeta potential is the DNA concentration for both cationic lipids tested, DC-Chol and DOTAP. SUV-DNA complexes form spontaneously upon mixing DNA with positively charged liposomes. This is dependent on the charge ratio, whereby, as the DNA concentration increases, the SUV-DNA complex size increases due to the decrease in the positive zeta potential and the electrostatic repulsion between vesicles.

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Use of cyclodextrins to improve paediatric iron formulations

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Introduction and Objectives

It has been shown recently that infants are commonly exposed to potentially harmful excipients at levels greater than that recommended for maximum exposure in adults.[1] Moreover, these harmful excipients are extensively found in several commonly used drugs, for instance, iron preparations, which heavily feature in treatment regimes for neonates. One promising strategy to eliminate the use of potentially harmful excipients is the use of cyclodextrins, which have been shown to exhibit low toxicity, particularly via the oral route, due to a lack of absorption through the gastrointestinal tract.[2]

Method

The phase-solubility method[3] was used to determine the extent of solubility of sodium feredetate and hence the relative complexation efficiency of the various cyclodextrins. Briefly, an excess amount of sodium feredetate was added to citric buffer solutions (pH 5.0) with increasing concentration of either 2-hydroxylpropyl-β-cyclodextrin or sulfobutyl ether-7-β-cyclodextrin, and then shaken at room temperature. After equilibrium was obtained (3 days), the solution was centrifuged (3000 rpm, 10 min) and analysed for sodium feredetate by titration against ammonium and cerium nitrate, according to the British Pharmacopeia method.

Results and Discussion

Of the two cyclodextrins investigated, the negatively charged sulfobutyl ether-7-β-cyclodextrin showed the most significant influence on solubility of sodium feredetate, with an optimum concentration of cyclodextrin being achieved at approximately 0.05 mm/ml. However, this was preceded by an initial reduction in solubility at lower concentrations (<0.01 mm/ml) and a consequent drop in solubility at higher concentrations (>0.08 mm/ml), and the optimum concentration of cyclodextrin failed to improve significantly on the intrinsic solubility of sodium feredetate. The neutral 2-hydroxylpropyl-β-cyclodextrin, on the other hand, displayed a lack of ability to improve the solubility of sodium feredetate at all concentrations studied. Indeed, the use of 2-hydroxylpropyl-β-cyclodextrin, in this instance, actually reduced the solubility of the sodium feredetate compared to its intrinsic solubility. Although there are many forces involved in the process of complexation of drugs within cyclodextrins, including van der Waals interactions, hydrogen bonding, hydrophobic interactions, release of ring strain in the cyclodextrin molecule and changes in solvent surface tensions, it is likely to be the electrostatic interactions that have the most influence in this case. More details could be obtained by further studies utilising several analysis methods, such as electron paramagnetic resonance and complexometry.

Conclusion

In contrast to previous results (unpublished data), in this particular study the use of cyclodextrins, either neutral or positively charged, was unsuccessful in improving the solubility of sodium feredetate, the active ingredient of Sytron. However, of the two cyclodextrins studied, the negatively charged sulfobutyl ether-7-β-cyclodextrin showed the greatest effect on solubility of sodium feredetate, intimating the influence of electrostatic interactions, although there was no significant improvement over its intrinsic solubility, possibly due its relatively stable chelate complex.

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Influence of surfactant (pluronic l81) and cosolvent ethanol on their ability to modulate particle size distribution and lung deposition of pressurised metered dose inhalers

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Introduction and Objectives

There are two strategies that can be used to characterise and modulate aerosol output and deposition of pressurised metered dose inhalers (pMDIs); the first is altering the formulation parameters such as propellant, excipients and drugs, while the second resides in device manufacture, with specific emphasis on nozzle actuation.[1] In the current study, we aim to investigate the influence of formulation approaches on modulating particle size distribution using the propellant HFA-227, specifically examining the effect of varying a model surfactant, Pluronic L81 (PL81), and ethanol concentrations.

Method

A series of formulations were prepared containing constant fluorescein sodium (fluorescein Na) drug mass (% w/w) with varying ethanol (% w/w) or PL81 (% w/w) concentrations, and added to aluminium aerosol canisters. Metered dose valves of 25 μl were then crimped onto the canisters using a manual canister crimper and filled with the desired weight of HFA-227 propellant using a pressure burette. These pMDIs contained a 0.33-μm actuator. Particle size characteristics of aerosols were determined using laser diffraction and the aerosolisation properties determined using a Next Generation Impactor (NGI) at 30 L/min. The fine particle dose (FPD) and the mass median aerodynamic diameter (MMAD) were determined.

Results and Discussion

In effect of varying ethanol concentration on modulating particle size distribution and drug deposition in pMDI

formulations, an increasing trend in MMAD (5.05–5.79 μm) values as ethanol concentration increased from 5 to 15% w/w in HFA-227 propellant was observed, coinciding with a significant decrease ($P < 0.05$) in the mass of drug deposited in the respirable region (105.07 ± 2.31 to 61.28 ± 1.79 μg; i.e. FPD). It is proposed that the droplets from pMDIs with 5% w/w ethanol evaporated more rapidly than those from pMDIs with 10–15% w/w ethanol,[2] resulting in drug particles that were smaller when they reached the NGI, and hence greater drug deposition and FPD. This has also been associated with decreased energy available for atomisation due to a decrease in propellant HFA-227 concentration as ethanol concentrations increased.[3] In effect of varying PL81 concentration on modulating particle size distribution and drug deposition in pMDI formulations, 1% w/w compared to 5% w/w PL81 concentration exhibited significantly smaller MMAD (3.70–5.92 μm), which coincided with higher FPD (107.05 ± 3.53 to 71.16 ± 3.79) and deeper lung deposition. This was associated with reduced evaporative potential of droplets containing PL81 and may be attributed to stronger hydrogen bonds between PL81 and HFA-227, preventing atomisation and decreasing evaporation.

Conclusion

It was demonstrated that drug particle size increased with increasing ethanol and pluronic concentrations. Furthermore, increasing ethanol concentration was associated with a decrease in mass of drug deposited in the 1- to 5-μm respirable region, but only decreasing the pluronic concentration was able to shift the drug mode population to a more respirable fraction. These results demonstrate the importance of ethanol and surfactant concentrations in formulations of pMDI HFA-227 in modulating particle size and drug deposition in the respirable region.

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Observation of the uptake of TAT peptide at nanomolar concentrations

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Introduction and Objectives

Cell-penetrating peptides have been at the centre of controversy on the influence of endocytotic pathways to penetrate cells. The TAT transduction domain (TAT peptide) is the most commonly studied, and used at relatively high concentrations (micromolar), yet its mechanisms of uptake at these concentrations are still disputed. Single-particle techniques, such as fluorescent correlation spectroscopy (FCS), offer greater resolution at lower concentrations in determining uptake and subcellular distribution over previously published methods such as flow cytometry or confocal microscopy. Thus, a careful combination of confocal microscopy and FCS may help characterise TAT peptide's uptake at nanomolar concentrations.

Method

Uptake was studied in Caco-2 cells using the cell-impermeable fluorescent dye TAMRA (550/568) as a model drug. Cells were grown on an eight-well chamber slide and incubated for 1 h with the TAMRA-TAT. After incubation, medium was aspirated and a slide made up with OPTIMEM. Cells were imaged using confocal microscopy. Using the information, FCS measurements were taken in the cytoplasm, vesicles and areas of diffuse fluorescence ($n = 30$). Stacks of images were deconvolved. FCS data were fitted with a one-component anomalous diffusion model using a least squares fitting routine.

Results and Discussion

TAMRA-TAT peptide was shown to enter Caco-2 cells down to 50-nm bulk concentration. At 500 nm, confocal microscopy indicated localisation of TAMRA-TAT peptide in one large diffuse area of the cells or vesicles, while the cytoplasm appeared to remain mainly peptide free. Diffusion times in the diffuse areas and vesicles were very long and similar (300 ± 50 μs), while in the cytoplasm, diffusion time was much shorter (26 ± 8 μs), suggesting cleavage of TAMRA from peptide. At 500 nm the number of particles in the confocal volume is measured at 4.8 and 6.5 for the diffuse areas and vesicles, respectively.

Conclusion

While the distribution of the TAT peptide at nanomolar concentrations appears to be vesicular in nature, more work is required to elucidate the precise mechanism of entry. At higher concentrations, an increased number of particles is found in the cytoplasm compared to the vesicles and diffuse areas, suggesting a second mechanism of uptake.

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Use of oil-in-water self-microemulsifying system for oral drug delivery

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Introduction and Objectives

Self-microemulsifying system has received much attention due to its capability to improve solubility, absorption and bioavailability of lipophilic drugs.[1,2] This study explored the effect of single and combined nonionic surfactants on the production of self-emulsifying oil-in-water microemulsion of delivery of hydrophobic drug.

Methods

The surfactants used were Tween 80 (Sigma-Aldrich, Dorset, UK) and Solutol HS15 (BASF, Ludwigstrafen/D, Germany). Preconcentrates were prepared by mixing Capryol 90 (Gattefosse, France) as oil phase, with either a surfactant or a surfactant combinations at various ratios. The preconcentrates were then diluted stepwise with PBS pH 7.2, and the physical appearances were recorded visually to construct phase diagram. The droplet size distributions of the formulations were determined by using dynamic light scattering technique. Paracetamol was used as the model hydrophobic drug and was incorporated into the optimised systems to investigate the effect of self-emulsifying systems on the drug solubilisation capacity.

Results and Discussion

Solutol HS15, Tween 80 or combination of these two surfactants was shown to self-emulsify into transparent oil-in-water microemulsions on infinite dilution with PBS pH 7.2 when the ratio of oil to surfactant exceeds 3 : 7. As suggested in the phase diagrams, a high amount of surfactant or surfactant blend was required to maintain the microemulsion structures. The mean droplet diameters of these preparations were typically less than 200 nm, and the droplet size was found to increase with higher oil content in the preparation. The smallest droplet size distributions were found when the oil to surfactant ratio was 1 : 9 regardless of the type of surfactant or ratio of surfactants used in the combinations, with a typical droplet size less than 20 nm. Solutol HS15 led to formation of larger droplet size when used alone as surfactant in the preparation. All microemulsions showed an improvement in solubilisation of paracetamol compared to paracetamol, which was used alone in PBS pH 7.2 (control). The ratio of oil to surfactant of 1 : 9 achieved the highest solubilisation for paracetamol. At least a three-fold increase in drug concentration was observed for the all formulations prepared at this oil to surfactant ratio. Combinations of surfactants provided a better drug solubilisation capacity than lone surfactant except at the ratio of Solutol HS15 : Tween 80 of 2 : 3. When the ratio of the combined surfactants of Solutol HS15 : Tween 80 was 1 : 1, this formula offered the best drug solubilisation indicating the advantage of using blend over single surfactant due to formation of various forms including microemulsion and mixed micellar structures.

Conclusion

The use of combined surfactants with Capryol 90 as oil phase at the ratio at least 3 : 7 of oil to surfactant has been shown to produce the self-microemulsifying systems with small droplet size distributions and good solubilising capacity for hydrophobic drug.

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Enhancement of membrane permeation of diclofenac diethylamine using supersaturation

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Introduction and Objectives

Increasing the thermodynamic activity (α) of the drug in the delivery vehicle beyond unity, i.e. drug supersaturation, can theoretically have a large impact on drug mass transport since a rise in α produces a proportional increase in the delivery rate across a homogenous barrier.[1] However, most delivery systems deviate from this ideal behaviour due to possible thermodynamic instability of the formulation, vehicle–membrane interactions and drug–vehicle interactions. Therefore, the aim of this study was to investigate the feasibility of enhancing diclofenac diethylamine (DDEA) delivery using supersaturation, and examining the effect of vehicle–drug and vehicle–membrane interactions.

Methods

A cosolvent solubility curve was constructed by determining DDEA saturated solubility in mixtures of propylene glycol (PG)/phosphate buffered saline at pH 3 with varying PG content. Supersaturated systems were generated using a mixed cosolvent method.[2] Systems consisting of 50 : 50 and 70 : 30 PG : phosphate buffered saline (pH 3) with different degrees of supersaturation (DS) were produced by mixing appropriate amounts of DDEA in PG with 3% hydroxypropylmethylcellulose in PBS (pH 3). The final supersaturated solutions were examined by microscopy to detect any drug precipitation. The degree of enhancement was assessed by performing permeation studies using upright Franz diffusion cells and silicone membrane.

Results and Discussion

The saturated solubility of DDEA increased exponentially with increasing PG content of the vehicle. DDEA permeation

through silicone membrane from drug-saturated solutions was influenced by the ratio of PG in the vehicle; the steady-state flux of DDEA from drug-saturated 70% PG was significantly greater ($P < 0.05$, analysis of variance) than the equivalent flux obtained from drug-saturated 50% (v/v) PG at 2.52 ± 0.34 and 1.69 ± 0.13 $\mu g\ cm^{-2}\ h^{-1}$, respectively. However, the effect of the vehicle on the membrane was accounted for by using the appropriate controls for the supersaturated systems. All the supersaturated solutions produced proved physically stable over the course of the 6 hour study. A linear relationship was established between the enhancement in DDEA flux ratio and the theoretical DS. This linear relationship occurred for systems having DS ranging from 1 to 10 and 1 to 5 for systems containing 50% PG ($R^2 = 0.992$) and 70% PG ($R^2 = 0.974$), respectively. The highest flux achieved was 17.94 ± 4.00 and 12.41 ± 2.05 $\mu g\ cm^{-2}\ h^{-1}$, which corresponded to enhancement ratio of 10.63 ± 2.29 and 5.0 ± 0.91 for systems containing 50 and 70% PG.

Conclusions

DDEA membrane permeation enhancement using supersaturation was feasible, and the flux enhancement ratio was proportional to the theoretical DS. The enhancement capacity of the supersaturated systems was dependent on the formulation vehicle, with a vehicle containing 50% PG providing the greatest DDEA flux across silicone membrane.

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Drug Metabolism

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Effects of trichloroethylene at doses inducing formic aciduria on indices of thymic selection

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Introduction and Objectives

Our aim was to investigate the possibility that doses of trichloroethylene (TCE) considered low but sufficient to induce formic aciduria, which indicates disruption of B_{12}-dependent folate metabolism,[1] would disrupt thymic T-cell selection, offering an explanation of its autoimmune toxicity.[2]

Methods

A total of 18 male Fischer rats (~200 g) were housed individually in metabolic cages with access to food and water *ad libitum,* and acclimatised before treatment on 3 consecutive days by oral gavage with treatments and doses specified below. Using ^{1}H-NMR with trimethylsilyl propionic acid as reference, 24 hours' urine was sampled for formic acid. Two indices of thymic education were quantified: positive selection (production) of T-cells was determined by thymus to body weight ratio, and negative selection (deletion of potentially autoreactive clones) was determined by the abundance (count/section surface area) of diaphorase positive medullary cells, termed *nitrergic* because they expressed nitric oxide synthase, in 100 μm-thick vibratome sections of paraformaldehyde-fixed thymi.[3]

Results

Levels of formic acid in urine (mean ± SEM; mg/24 h; $n = 3$) compared to vehicle control (1.4 ± 0.1) were significantly induced by doses of TCE (16, 31 and 62.5 mg/kg), which were 17.7 ± 3.0, 11.3 ± 3.5 and 23.4 ± 8.0, respectively (analysis of variance, $P < 0.05$). Relative size of the thymus (positive selection) appeared to increase slightly over these doses, but changes were not significant. The mean nitrergic cell abundance (count/mm^2) was not dose dependently affected, although the 31 mg/kg dose produced a significant increase (4.44 ± 0.16) compared to control (2.82 ± 0.40) (unpaired t-test, $P < 0.05$). Nitrergic counts by a second observer were concordant. A single dose of 16 mg/kg TCE resulted in formic aciduria (11.7 ± 6.3) that was inhibited by L-methionine (300 mg/kg per 10 ml i.p.) supplementation (3.6 ± 0.3), indicating that the doses of TCE used were inhibiting the methionine salvage pathway. These preliminary experiments tested whether TCE at doses effective as an inhibitor of folate metabolism would also perturb thymic selection underlying immunological tolerance. Others have reported increase in positive selection indices following TCE[1] at doses not characterised for formic aciduria.

Conclusion

Our results suggest additional abnormalities in the nitrergic mechanism of the thymus at relatively low doses of TCE that might be relevant for its reported autoimmune toxicity.

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Material Science

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Modelling of a dosator: an analytical approach based on measured powder flow properties

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Introduction and Objectives

Dosators are widely used for dry inhaled powders that demand an accurate drug delivery to the patient. The aim of the project is to develop a mathematical model for the pickup–discharge of powders into and out of dosators to predict accuracy of dose weights based on measured powder flow properties. The model will be validated against a single-shot dosator 'test-rig' designed and manufactured at the University of Greenwich. Once validated it will be used to predict accuracy of dose weight for new blends and explore the effect of changes in flowability and permeability properties, as well as dosator geometry.

Method

A mathematical model was developed based on the method of differential slices.[1] Here the powder in the bed and powder into the dosator are assumed to be composed of a series of horizontal element slices at differential stresses. The model relies on flow, compaction and permeability properties of the powder, all of which are blend specific and must be measured experimentally. These properties include the flow function, effective angle of wall friction, wall cohesion, bulk density, stress ratio K and permeability. To simulate the real process and validate the model, the single-shot dosator 'test-rig' has been used to measure the forces acting between the powder and the body of the dosator during the filling and ejection stages.

Results and Discussion

Preliminary results are shown in Figure 1, where dose weight is strongly influenced by both high levels of compaction conditions of the powder bed and the proximity between the dosator and the bottom of the bed of powder at the end of the stroke. As the dosator comes closer to the bottom of the trough, the forces increase considerably in both stages. Also, dose weight increases with the level of precompaction in the bed.

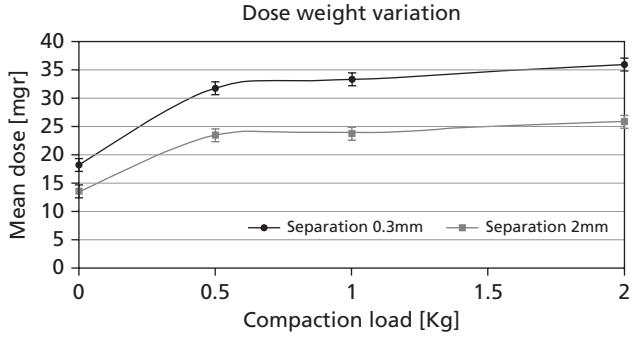

Figure 1 Dose weight variation resulting from separation between the dosator and the bottom of the powder bed at the end of the stroke, and level of compaction of the bed of powder.

Conclusion

The ongoing research aims to provide a comprehensive correlation between several powder properties and dose uniformity, also developing parameters for optimisation of the geometric design of dosators.

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Medicinal Chemistry

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Synthesis and evaluation of a novel range of non-steroidal inhibitors of 17β-hydroxysteroid dehydrogenase through the derivatisation of a C21 steroid backbone

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Introduction and Objectives

Type 3 17β-hydroxysteroid dehydrogenase (17β-HSD3) is responsible for the conversion of the C(17) carbonyl group of androgens to the hydroxy moiety (resulting in the biosynthesis of the more potent androgens). In the initial design of novel inhibitors of 17β-HSD3, we concluded that compounds which mimic the steroid structure would possess inhibitory activity and that these compounds would act as a substrate and would

therefore undergo the reduction step. Here, we report the initial results of the synthesis, biochemical evaluation and rationalisation of the observed inhibitory activity of a series of compounds based upon the backbone of progesterone.

Method

The proposed inhibitors were synthesised involving the initial oxidative ring cleavage of the A-ring of progesterone, leading to the progesterone 'keto-acid', which was subsequently derivatised to the ester functionality involving the reaction (in the absence of acid catalyst) with a range of alcohols so as to give the appropriate ester. The biochemical evaluation of the synthesised compounds was undertaken using literature procedure using microsomes from rat testes.[1] The assay was quenched using diethyl ether, and the substrate and products were separated using thin layer chromatography, each spot cut out and counted for tritium for 4 min.

Results and Discussion

The reactions proceeded in good yield and without any major problems (typically, the yield for the initial ring cleavage reaction was ~90%, while for the esterification reaction, the yield dropped to approximately 60%). Consideration of the initial screening data shows that the compounds are, in general, weak inhibitors of 17β-HSD3. The most potent was the butyl derivative that was found to possess ~57% inhibitory activity at [I] = 100 μM, while the weakest inhibitory activity was observed with the octyl derivative that was found to possess ~16% inhibitory activity under similar conditions. In comparison, the two standards used (namely 4-hydroxynonanophenone and 4-hydroxydecanophenone) were found to possess ~71% and ~62% inhibitory activity under similar conditions. Using the previously derived transition-state (TS) for 17β-HSD3, we propose that the weak inhibitory activity observed within the compounds (containing large alkyl chains) is due to steric hindrance between the alkyl chain within the inhibitor and the active site [corresponding to the C(3) area of the steroid backbone], and as a result there is a decrease in inhibitory activity. As such, the design of further potent novel inhibitors of 17β-HSD3 would be required to possess smaller alkyl chain lengths so as to reduce the steric interactions.

Conclusions

The results of our study show that in the inhibition of 17β-HSD3 and the mimicking of the steroid backbone, in particular, the C(17)=O group within the inhibitors is crucial for the inhibition process. We have therefore provided a novel range of inhibitors of this enzyme that are suitable for further development.

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Synthesis and biological evaluation of a range of thiosemicarbazone-based compounds as potential inhibitors of estrone sulfatase: a continued search for structure-activity relationship

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Introduction and Objectives

Thiosemicarbazone-based compounds have been reported as inhibitors of estrone sulfatase (ES)[1] and were found to possess allosteric inhibitory activity. Aminosulfonate-based compounds have been shown previously to be irreversible inhibitors of ES, and a number of physicochemical properties of sulfamate-based compounds have been rationalised[2,3]; however, no structure-activity relationship (SAR) has been fully rationalised. We report here our continued efforts into the synthesis and biochemical evaluation of a series of thiosemicarbazone-based compounds in an attempt to provide an insight into the SAR for these compounds.

Methods

In the synthesis of the target compounds, in particular, the cyclohexyl derivatives, we undertook a reaction between various benzaldehyde derivatives and cyclohexyl thiosemicarbazone in absolute ethanol (EtOH). The mixture was left to reflux until the formation of a solid and allowed to cool to room temperature. The resulting solid was filtered and recrystallised from aqueous EtOH to give the target compounds. For example, reactions with 4-, 3-, and 2-bromobenzaldehyde led to the synthesis of 4-bromobenzaldehyde *N*-cyclohexylthiosemicarbazone; 3-bromobenzaldehyde *N*-cyclohexyl thiosemicarbazone and 2-bromobenzaldehyde *N*-cyclohexyl thiosemicarbazone. In the biochemical evaluation, we used a modified literature procedure,[3] involving the use of rat liver microsomal enzyme (obtained from Sprague-Dawley exbreeder male rats) in place of human placental microsomal enzyme.

Results and Discussion

The target compounds were obtained in relatively moderate to excellent yield (ranging from ~30 to ~95%). Although no major problems were observed with the majority of derivatives of benzaldehyde, problems were, however, observed with the reaction time, in that the reaction time was required to be increased. Furthermore, the hydroxy derivatives proved to be troublesome since they were found to be more soluble in EtOH in comparison to the halogen-based derivatives, so water was added to crystallise the compound. In general, the thiosemicarbazone-based

compounds proved to be weaker inhibitors in comparison to the standard used, namely estrone-3-o-sulfamate (EMATE, which was found to possess ~84% inhibition at [I] = 100 μM). The most potent compound was found to be the hydroxy derivative (5-chloro-2-hydroxybenzaldehyde-*N*-cyclohexyl thiosemicarbazone), which was found to possess ~80% inhibition under similar conditions while the weakest was found to possess ~30% inhibition under similar conditions. With regards to SAR, from an initial consideration of the initial screening data, it would appear that the ortho-substitution of the phenyl ring (by an OH moiety) resulted in an increase in the inhibitory activity, whereas the substitution with a halogen moiety at the ortho-position did not show any impact on the inhibitory activity.

Conclusion

We have provided some novel, and in some cases, potent inhibitors of ES and have undertaken limited SAR, which has allowed the consideration of structural features, so as to allow us to design further derivatives of this novel range of compounds.

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Synthesis of highly potent sulfonate derivatives of estrone in the inhibition of the enzyme 17β-hydroxysteroid dehydrogenase and their activity against estrone sulfatase

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Introduction and Objectives

The enzymes estrone sulfatase (ES) and 17β-hydroxysteroid dehydrogenase (17β-HSD) are responsible for the transformation of estrone to estradiol (the more potent estrogen). As such, the inhibition of estradiol biosynthesis would greatly aid estrogen ablation and therefore allow regression of estrogen-dependent tumours. We have previously reported the derivation of a representation of the active site of 17β-HSD,[1] using which we have designed compounds based on the estrogen backbone. Here, we report our initial results for the synthesis and biochemical evaluation of 'dual-inhibitors' of ES and 17β-HSD.

Method

In the synthesis of the target compounds, we reacted estrone with various substituted derivatives of sulfonyl chloride in a solution of triethylamine (TEA) and anhydrous dichloromethane (DCM). The reactions were allowed to reflux for 4 days before the purification of the target compounds using recrystallisation. The biochemical evaluation of the synthesised compounds was undertaken using literature-based methodology and included the use of rat testes and liver homogenate for the 17β-HSD and ES assays, respectively. The substrate and products were then separated using thin layer chromatography (TLC) and each spot was cut and counted for tritium for 5-min per tube.

Results and Discussion

The synthesis of the target compounds was achieved in good to excellent yield (ranging from 55 to 80%). The reactions did not prove to be troublesome and purification was achieved *via* recrystallisation (from, in general, aqueous ethanol, 90%). The biochemical evaluation of the compounds shows that the target compounds are extremely potent inhibitors of 17β-HSD and moderate inhibitors of ES. For example, the most potent compound was shown to be estrone-3-O-(phenylsulfonate) that was found to possess ~90% inhibitory activity against 17β-HSD and ~24% inhibition against ES. Estrone-3-O-(trifluoromethanesulfonate) was found to possess ~80% inhibitory activity against 17β-HSD and ~50% inhibitory activity against ES. The latter compound would therefore appear to possess the appropriate characteristics for 'dual-inhibition' of these two important enzymes. The structure-activity relationship determination of these compounds shows that the use of bulky groups results in a decrease in inhibitory activity against both 17β-HSD and ES, as such, the trifluoromethane derivative (possessing a small functionality) allows the gem diol to attack the sulfonate moiety within ES, while decreasing steric hindrance between the enzyme active sites of 17β-HSD.

Conclusion

In conclusion, we have provided novel and highly potent dual inhibitors of both ES and 17β-HSD. The compounds possess good stability and therefore are good candidates for further development.

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A novel molecular modelling study into the binding of inhibitors of the enzyme estrone sulfatase

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Introduction and Objectives

To aid our efforts in the design, synthesis and rationalisation of inhibitory activity of novel inhibitors of estrone sulfatase (ES), we derived an approximate representation of the ES active site involving the determination of the transition state (TS) for the reaction catalysed by ES.[1] Although the TS proved to be highly successful, there were a number of deficiencies. We therefore undertook the development of a novel approach where we attempted to study the binding of inhibitors within a crystal structure of ES, so as to determine the binding conformer. Here we report the results of our study.

Method

The crystal structure was downloaded from the Protein Data Bank and read into the molecular modelling software CaChe. The active site within the crystal structure was highlighted [involving the location of the formylglycine moiety (FGL)] and an area about the FGL was highlighted and copied into another window. The atoms were locked and the file saved. The inhibitors under study were produced and then copied into the window containing the portion of the active site, weak bonds were created between the inhibitor and FGL and the whole structure allowed to minimise using MM3.

Results and Discussion

As an initial step, the use of the correct distance about the FGL moiety appears to be an important factor. That is, using a lower radius to choose parts of the active site results in a large proportion of the active site being 'lost'. We discovered that using a radius of 16 Å allows for the majority of the active site to be selected. We were then able to mimic the inhibition process closely by allowing the weak bonds to minimise (formed between the sulphur atom of the sulfonate group within the inhibitor and the FGL), this therefore allowed the inhibitors to take up a 'binding' conformer. From the modelling of known inhibitors of ES, we observed that a number of steroidal inhibitors containing the C(17)=O functionality were bound such that the C(17)=O group approached the active site within 3.2 Å. The C(17) moiety is then able to undergo hydrogen bonding, allowing greater binding leading to a subsequent increase in inhibitory activity. As such, the new approach allows us to consider the binding of inhibitors and to determine if they are able to undergo hydrogen bonding as well as the interaction between the S of the sulfonate moiety and the FGL.

Conclusions

In conclusion, we have provided a novel approach to modelling inhibitors of ES within the crystal structure of ES. Using the modelling approach, we have been able to add further support to our previous hypotheses regarding the ability of inhibitors to undergo hydrogen bonding with groups about the C(17) area of the substrate backbone.

Reference

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An investigation into the structural requirements in the inhibition of 3β-hydroxysteroid dehydrogenase

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Introduction and Objectives

3β-Hydroxysteroid dehydrogenase (3β-HSD) undertakes the biotransformation of 3β-OH-containing steroids into the C(3) = O-containing products. As such, this enzyme plays a role in the biosynthesis of steroids such as androstenedione (AD) from dehydroepiandrosterone (DHEA) – this biotransformation is important in the stimulation of androgen-dependent prostate cancer as AD can be converted to testosterone and subsequently to dihydrotestosterone (the most potent androgen). The inhibition of this enzyme may therefore lead to a loss of stimulation of prostate cancer cells leading to a reduction in tumour mass. We therefore report our initial efforts in the inhibition of this enzyme.

Method

In the synthesis of the target compounds, we reacted testosterone with various acid chlorides to give the target esters. In the biochemical evaluation of the synthesised compounds, the literature procedure was undertaken and involved the use of rat testicular microsomes and radiolabelled AD as the substrate.[1] The final volume was 1 ml and after incubation, the assay was quenched using diethyl ether. The substrate and products were extracted from the assay mixture and separated using thin layer chromatography. Each spot was cut out and scintillant added and the tubes counted for tritium for 3-min per tube.

Results and Discussion

The synthesis of the target compounds proved to be without any major difficulties. That is, the compounds were synthesised in good yield (ranging from 70 to 85%) and were purified using column chromatography. The biochemical evaluation of the target compounds shows that the compounds are good inhibitors of 3β-HSD. The most potent compound was the propionate derivative, which was found to possess ~52% inhibition at [I] = 100 μM. The weakest inhibitory activity was observed with the cyclohexane carboxylate derivative, which was found to possess ~5% inhibitory activity. The structure-activity relationship (SAR) determination shows that the compounds containing bulky substituents about the C(17) area of the steroid backbone leads to a lowering of inhibitory activity. This suggests that the area of the active site corresponding to the steroid C(17) position is restricted with regards to conformational space available to the substituents, as such, an increase in the bulk of the substituents results in an increase in steric hindrance and therefore a decrease in inhibitory activity.

Conclusion

In conclusion, we have provided a range of compounds that have proved to be good inhibitors of 3β-HSD. Furthermore, the consideration of the SAR suggests that the area of the active site that would be occupied by the C(17) moiety of the steroid backbone is restricted in terms of conformational space, as such, in the design of inhibitors of 3β-HSD, small and possibly flexible functional groups may result in increased inhibitory activity.

Reference

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Pharmaceutical Microbiology

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The role of complex formation between inhibitors and substrates of efflux pumps in bacteria

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Introduction and Objectives

Resistance to antibiotics is a growing problem and occurs via three major mechanisms: receptor alteration, antibiotic modification and drug efflux. Efflux pumps are membrane-bound proteins that assist in expulsion of a broad range of compounds that are structurally unrelated.[1] It is thought that inhibition of efflux pumps occurs via binding of the inhibitor to the hydrophobic regions of the pump, or via binding of the inhibitor to the substrate.[2] Here, modulators of P-glycoprotein (P-gp) and compounds from natural sources are investigated for their potentiating activity through complex formation between inhibitors and substrates of efflux pumps.

Method

Five molecules, L-theanine and verapamil hydrochloride (known P-gp inhibitors), plumbagin and resveratrol (naturally occurring compounds) and aspartame (used as a negative control), were analysed using minimum inhibitory concentration (MIC) and modulation assays to determine the antibacterial activity of the molecules and to verify whether any of these molecules possess any potentiating activity on the antibacterials (norfloxacin and tetracycline) by increasing the uptake of the antibacterial. Reserpine was used as a positive control. Checkerboard assays were used to establish the mode of interaction between any potentiating drugs and the antibacterial drug. MacroModel was used to perform an energy minimisation followed by LigPrep to set up correct protonation states of ionisable groups. Complexes of the molecules and antibacterials were generated using a 1000-step conformational search. VegaZZ was used to determine the complex molecular properties. NMR and mass spectrometry was then used to examine the possibility of complex formation between selected compounds and norfloxacin.

Results and Discussion

Plumbagin and resveratrol exhibited some antibacterial activity against all strains of bacteria tested. However, the modulation assays found that only resveratrol possessed some potentiating activity on the MIC of norfloxacin (8-fold) and tetracycline (2-fold). MacroModel established that in the majority of cases the dominating interactions between the molecules and the antibacterials were aromatic in nature, with the positive control (reserpine) exhibiting the strongest interactions. Spectroscopic analysis found some evidence of complex formation between resveratrol and norfloxacin. However, the checkerboard assay performed established that the interaction between resveratrol and norfloxacin resulting in the potentiation activity was additive in nature, rather than synergistic.

Conclusions

L-theanineand verapamil hydrochloride are known inhibitors of P-gp, however, lacked any inhibiting activity against norA and tetK. It is possible that complex formation of investigated molecules with antibacterial drugs does not induce adequate molecular properties to overcome norA and tetK efflux pumps. Further work is needed to establish which molecules can cause inhibition due to complex formation and which can cause inhibition through other mechanisms.

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Pharmaceutical Technology

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A novel laser-machining method for preparation of polymeric microneedle arrays

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Introduction and Objectives

An innovative strategy used to enhance the transdermal delivery of drugs is the use of devices known as microneedle (MN) arrays. To date, the majority of MN-based transdermal studies have used silicon MNs. However, because silicon is not a FDA-approved biomaterial, there has been a recent effort to develop MNs from biocompatible polymers.[1] Typically, this involves preparing inverse silicone micromoulds of silicon master arrays. The aim of the current study was to use laser-machining to directly manufacture moulds of various MN geometry and density. In addition, polymeric MNs prepared from the moulds were physically characterised.

Method

Silicone MN moulds were created by 2.5D laser-micro-machining, whereby the final shape of an inverted needle is directly machined into the mould using a diode-pumped solid-state laser operating at 355 nm and at a pulse length of 20 ns. Conical MN moulds were prepared with a depth of 600 μm, a diameter of 300 μm and of varying interspacings (50, 160, 330 and 600 μm). Polymeric arrays were prepared using poly(methylvinylether/maleic anhydride). The force required to fracture MNs and the force required to insert them into excised porcine skin were determined using a force-displacement station.

Results and Discussion

Laser-machining facilitated the manufacture of MN moulds within ±10% of the desired dimensions. Fracture force testing, whereby a given axial compressive load was applied to the array, revealed that MN spacing did not influence the amount of damage caused to needles. Indeed, compressive loads under 0.1 N/MN resulted in a reduction of needle height of less than 15% for all spacings. As the force was increased above 0.1 N/MN, there was a progressive increase in MN deformation. An increase in the percentage of needles that punctured excised porcine skin was observed as the insertion force was increased for all interspacings. Indeed, it was shown that when the insertion force was greater than 0.05N, over 80% of the needles successfully punctured the skin.

Conclusions

Conventional, micromoulding of silicon MN arrays is restricted to the geometry of the master structures. For example, the interspacing of wet-etched silicon MNs is usually at least three times the MN height.[2] For the first time, laser-machining has been used to directly prepare MN moulds in a silicone substrate. This provides much greater control over the mould dimensions. Indeed, this study has shown that arrays with an interspacing of 50 μm are mechanically strong and can successfully puncture the skin at relatively low forces.

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Assessment of the effect of drug solubility, drug loading and fluid volumes on the manufacture of pellets by extrusion spheronisation

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Introduction and Objectives

Multi-particulates have found application in the field of modified release drug delivery as they have significant advantages over single-unit dosage forms. Pellets can be prepared by the method of extrusion spheronisation. Although an accepted technique adopted by industry, extrusion-spheronisation is a complex, multi-step process, the outcomes of which depend on the interactions between the formulation and processing steps. Here, we investigate pellet manufacture using four drugs (solubility: 0.2, 1, 18 and 130 mg/ml) to elucidate information on the effects of formulation variables (drug loading, microcrystalline cellulose (MCC) content, fluid levels, lactose loading) and to determine optimal formulation properties suitable for all processing stages.

Method

Pellets were prepared by extrusion spheronisation. Dry ingredients (MCC, lactose and active pharmaceutical ingredient

(API)) were placed into a Bohle Mini-granulator and mixed. Fluid (1% polysorbate 80 in H_2O) was added gradually. Wet granules were placed into a ram-extruder (Lloyd Press MX50) and forced through a 1 mm diameter hole. Extrudate was spheronised in a Caleva 120 spheroniser, dried at 50°C overnight and characterised using G2 Morphologi image analyser. Dissolution was determined in USP II (water at 37°C). Drug loadings ranged from 15 to 50%, MCC content from 15 to 55% and MCC/fluid ratio varied from 0.6 to 1.4. Statistical modelling was carried out to ascertain trends (Design Expert).

Results and Discussion

Ideal pellets were characterised as having an aspect ratio of >0.9 and an RSD of <20%. The sphericity measure E_r was also used (E_r values of >0.6 were considered spherical).[1] Varying the formulation factors for pellet manufacture in some cases produced material which could not be extruded, or was too wet – these formulations lay outside the working formulation composition range. This working range tended to be larger at lower drug loadings, possibly owing to the functionality of the pellets being more dependent on the behaviour of the majority components (MCC and lactose, both well characterised for pellet use). The strongest trend was that as MCC proportion increased, the relative fluid level had to be decreased (i.e. the MCC: fluid ratio increased) to achieve suitable pellets. Furthermore, to ensure appropriate drug release, it is recommended that lower drug loadings and low MCC loadings should be used for low-solubility compounds. These formulation requirements become less critical as the drug solubility increases.

Conclusions

Several drug compounds with different solubility were used to manufacture pellets by extrusion spheronisation. The critical formulation and processing parameters were established. As drug solubility decreased, higher lactose levels were required to provide sufficient soluble component to form pellets with desired size and shape. The ease of formulation processing was dependent on drug solubility.

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Exciplex formation using a terminally located probes system and the effects of TFE concentration on the exciplex fluorescence emission intensity

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Introduction and Objectives

A nucleic acid detection method using an exciplex split-probes system[1] was recently showed to improve high background noise that usually occurs with other fluorescence techniques. The perfectly hybridised probes bring the exciplex partners (exci-partners) in close proximity and emit an exciplex fluorescence signal with large Stokes shift. The aim of this study is to investigate a parallel exciplex probes (exci-probes) system as a simplified detection model system (Figure 1). This method could potentially be applicable for the detection of single-nucleotide polymorphisms and the development of a solid-phase detection modality.

Method

The complementary 8-mer oligonucleotides from corresponding *Leishmania major* target DNA regions were prepared by conjugation with pyrenyl and napthalenyl moieties on the 5′- and 3′-phosphate terminal, respectively. The melting temperature (T_m) was determined based on the change in UV absorbance at 260 nm and fluorescence intensity at 340 nm. The fluorescence and T_m experiments were conducted in phosphate buffer containing different percentages of trifluoroethanol (TFE). The excitation wavelengths were 340 nm for the locally excited-state pyrene monomer and 350 nm for exciplex system.[2]

Results and Discussion

The excitation of fully hybridised parallel exci-probes resulted in the detection of a new emission band at 480 nm, characteristic of exciplex formation between pyrene and naphthalene exci-partners. The exciplex emission intensity at 480 nm for the parallel probes system in 80% TFE was found to be almost 15 times higher than that of the split-probes system.

Conclusion

The parallel exci-probes system offers a new way of detecting DNA mismatches. The exciplexes emit fluorescence signals with large Stokes shift and low background noise.

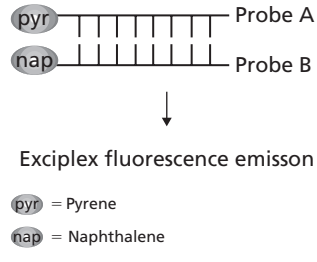

Figure 1 Parallel exci-probes with terminally located exci-partners in the parallel system.

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High-performance liquid chromatography method development for studying the degradation of freeze-dried adenosine triphosphate when stored at 4 and 40°C

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Introduction and Objectives

Methods for predicting the shelf life of freeze-dried amorphous formulations are being developed, which are based on an understanding of the relationship between the molecular relaxation behaviour and stability. The global aim of this work is to focus on the molecular dynamics of freeze-dried sugars and to examine the relationships with the chemical stability of a model co-freeze-dried drug. To achieve this aim, it is first necessary to develop a sensitive high-performance liquid chromatography (HPLC) assay for a model drug (in our case, adenosine triphosphate (ATP)) such that low levels of degradation may be quantified over a limited study period (i.e. up to 1 month).

Method

An ion pair HPLC method[1] was developed using an Agilent Eclipse C18 column and a two component mobile phase (A/B) at a constant flow rate of 1.5 ml/min (Mobile phase A: 50 mM potassium phosphate and 8-mM tetra butyl ammonium sulphate at pH 6.0; Mobile phase B: acetonitrile). Various gradients were investigated and the UV detector set at 260 nm.

Aqueous solutions of 0.1% w/v ATP with 10% w/v trehalose were freeze-dried in 3.0-ml aliquots in a Heto FD8 freeze dryer to moisture contents of ~1.5%. The dried samples were divided in two batches and stored at 4°C and 40°C.

Results and Discussion

Optimal separation of ATP, adenosine diphosphate (ADP) and adenosine monophosphate (AMP) was achieved with a gradient defined from 100 : 0 (A : B) at time 0–75 : 25 at 13 min and back to 100 : 0 at 16 min (total run time: 18 min). Calibration curves for ATP, ADP and AMP were constructed from the peak area, using ChemStation software, following baseline correction. The RMS error for each curve was used to define the limits of detection for ATP degradation. Based on a concentration of 1-mg/ml ATP, the RMS error estimates (from the calibration curves) equate to a detection limit of 2(±0.2)% degradation of ATP to ADP and/or AMP. This was considered sufficient to be able to differentiate between time points on the stability study. On freeze drying, 0.2% of ATP degraded to ADP (but not AMP). On further storage at elevated temperature (40°C), it was possible to induce a further 3.5(±0.3)% ATP degradation, whereas no degradation was detected at 4°C over same period. ATP degradation continued to increase over time to 6.5% at 40°C and 0.2% at 4°C (after 20 days).

Conclusion

The method developed was sufficiently sensitive to quantify low levels of ATP degradation, with some reasonable precision, such that it may be used as the basis for characterising the differential stability afforded by a range of disaccharides when co-freeze-dried with this model drug. Moreover, the extent of degradation occurred over a reasonable time frame, thereby creating the opportunity to develop short duration stability studies that can form the basis for investigating relationships between molecular dynamics and stability. These aspects will be the focus for continued study.

Reference

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178
The effect of xanthan gum on the in-vitro dissolution of clozapine

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Introduction and Objectives

The aim of the study was to use type-2 dissolution tests to examine the dissolution and diffusion of clozapine from extemporaneously dispensed suspensions of clozapine tablets in Keltrol (a xanthan gum based suspending agent) (S.Black Ltd., Hertfordshire, UK) and compare this to the dissolution of whole tablets. It has been observed that the bioavailability of clozapine from Keltrol suspensions is lower than the bioavailability of clozapine from the tablet form.[1] Raman, infrared spectroscopy and differential scanning calorimetry (DSC) will be used to analyse the suspension for evidence of

ionic and other noncovalent interactions occurring between clozapine and xanthan gum.

Methods

Clozapine suspension (extemporaneously dispensed) and tablets (Zaponex, IVAX Pharmaceuticals UK, London, UK) were kindly donated by St Georges Hospital. Infrared spectroscopy and DSC were performed on freeze-dried samples of the clozapine-Keltrol suspensions. Raman spectroscopy was performed on clozapine-Keltrol and clozapine-water suspensions. A number of differing type-2 dissolution studies were performed on the suspension. The suspension (5 ml) was added to the dissolution bath with either a syringe or a spoon or in a sealed pouch of muslin cloth. H*igh-performance liquid chromatography* (HPLC) was used to assay clozapine in the dissolution media.

Results and Discussion

The dissolution of clozapine was found to be retarded by the Keltrol suspension (Figure 1). When added using a spoon, the suspension was observed to form a large globule in dissolution bath. Only minimal evidence for interactions occurring between the xanthan gum of Keltrol and the clozapine was found by infrared and DSC. Changes were observed in the Raman spectra at frequency shifts 400–700 cm^{-1} and these may have been due to interactions between the two.

Conclusion

The reduction of bioavailability observed when extemporaneously dispensed clozapine-Keltrol solution is compared with clozapine tablets may well be due to changes in dissolution behaviour. There is evidence to suggest that this change in dissolution may be due to interactions that are occurring between clozapine and the xanthan gum within Keltrol, but the viscous nature of the material may also be an issue.

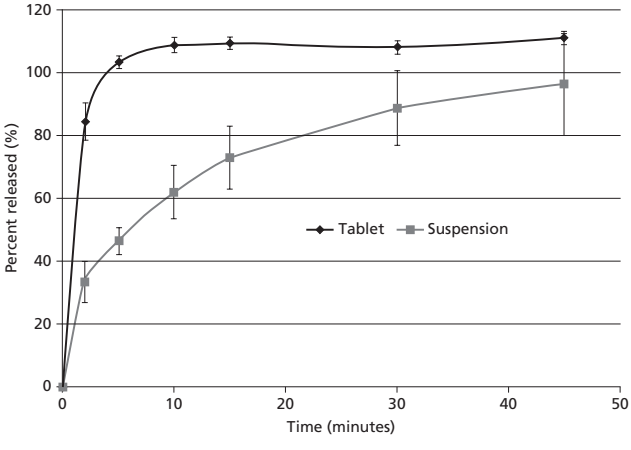

Figure 1 A comparison of the dissolution of clozapine from tablets or suspension expressed as a percentage of labelled content.

Reference

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179
In-vivo biodistribution of amphiphilic polymer quaternary ammonium palmitoyl glycol chitosan in mice

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Introduction and Objectives

The amphiphilic chitosan-based polymer GCPQ (quaternary ammonium palmitoyl glycol chitosan), capable of self-assembling into 100–300-nm clusters of 10–30-nm micelles, has been previously shown to form stable nanoparticles with lipophilic drugs with a high drug loading efficiency and significantly increases drug activity.[1] In this study, for the first time, the in-vivo biodistribution of radiolabelled GCPQ was investigated in mice. The biodistribution of these polymeric nanoparticles will allow their potential use as drug nanocarriers to be realised.

Methods

Radiolabelled GCPQ nanoparticles were prepared using a modification of the method previously reported,[2] where the Bolton-Hunter reagent was used to conjugate ^{125}I to the free amino groups of the polymer *via* an active ester reaction. Purified ^{125}I-labelled GCPQ was administered intravenously to the mice *via* the tail vein. Blood, major organs and parts of the mice were collected at various time points after polymer injection and samples were assayed for radioactivity using a WIZARD 2470 gamma counter (PerkinElmer LAS (UK) Ltd, UK). Results were expressed as % of the recovered dose. The level of GCPQ nanoparticles in the blood was calculated, based on an estimated total blood volume of the mice (7.5% of body weight).

Results and Discussion

Figure 1 shows that while higher amount of ^{125}I-labelled polymer was recovered in the blood (half life of GCPQ in blood was 20.3 min), kidney, bladder and skin, lower levels were recovered in the liver (peak levels ~ 4% of the dose) and insignificant levels were found in the other organs such as the spleen and lungs. The amount of radioactivity building up in the bladder through the kidney over time suggested that radiolabelled polymer was excreted in the urine. Interestingly, the total ^{125}I-labelled polymer recovered in the whole animal from all organs and parts reached a maximum at 10 min with only ~60% of the administered dose being recovered. Characterisation of the labelled GCPQ by thin layer chromatography (TLC) showed that a second

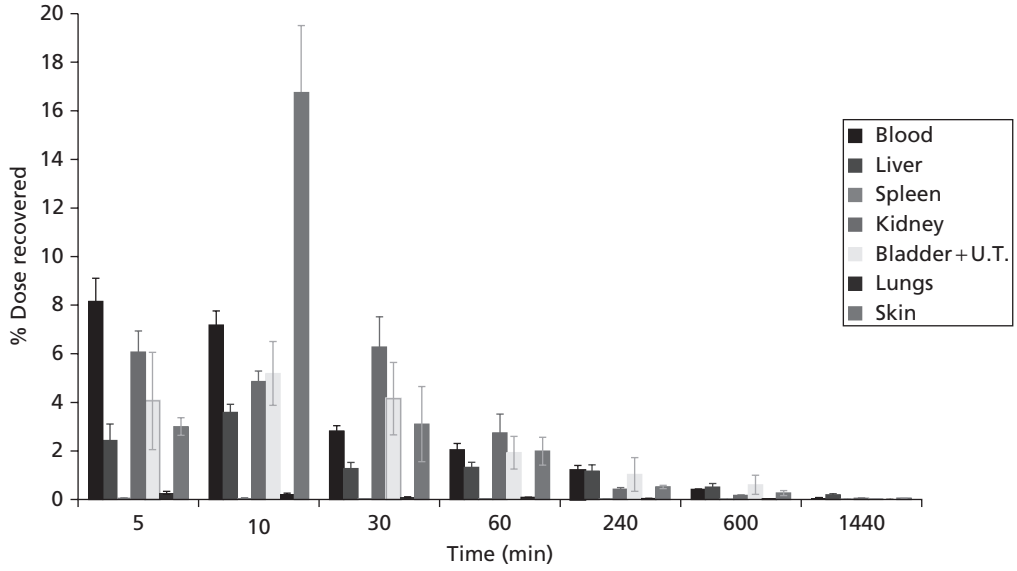

Figure 1 Pharmacokinetics of the radiolabelled GCPQ in the blood, kidney, spleen and bladder of the mice.

pool of labelled polymer (23%) was present in the sample which may account for the % dose that was passed out into the urine as low molecular weight labelled polymer and which could not be recovered in the animal.

Conclusion

Quaternary ammonium palmitoyl glycol chitosan is a promising drug delivery system that on intravenous administration does not target the reticuloendothelial system and is excreted *via* the urine.

References

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Addressing dose dumping issues in controlled release of a highly water soluble drug: a novel bull's eye tablet design

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Introduction and Objectives

Controlling the release of highly water soluble drugs has always been challenging for the scientists due to the initial burst effect. A novel noninfringing method to control the issue of dose dumping has been proposed.

Methods

This was achieved by partial compression coating the core (drug and an optimised combination of water swellable/erodible polymers) with an impermeable rate-controlling material, which surrounded the core from all sides except one resulting in a dot's or bull's eye tablet design (Figure 1a). In-vitro analysis was done by comparing the release profile of the in-house formulation in different dissolution conditions with the reference listed tablet containing drug core pan coated with ethyl cellulose with 5–10% weight build-up.

Reference Listed Drug (RLD) showed a release of less than 10% in the first 2 h (official method), which was not achieved by any conventional noninfringing methods such as simple matrix, matrix bilayered and trilayered tablets. An unconventional dot's/inlay/bull's eye tablet design was finally investigated.

Results and Discussion

Initial burst control (release less than 14% in the first 2 h) and similarity factor above 50 was achieved in all the tested

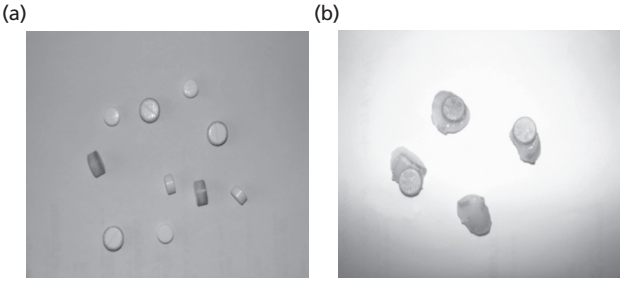

Figure 1 **(a)** Core and final tablet. **(b)** Tablet condition after dissolution (16 h).

dissolution conditions (official Office of Generic Drugs (OGD) approved method, change over, multimedia and multirotations per minute (RPM) studies). The pattern of release profile confirmed the technique for having potential to offset the initial burst effect yet offering complete release in the terminal phase. The partial polymer cushion (1.4 mm) laterally over the core was inadequate for the tablet to withstand swelling of the inside core matrix for long. The tablet and the ethyl cellulose polymer layer were intact in the first 1 h. The drug only released from the exposed surface as the impermeable polymer coating prevented the drug release from the lateral surface and the swelling of polymer combination in the core ruptured the coating to assure complete release in the end. Gelling and swelling of the polymer combination in the core caused the surrounding ethyl cellulose layer to rupture from the middle, and the off-white gelled portion of the core became visible in the centre of the tablet (Figure 1b).

Conclusion

The present study demonstrated a noninfringing method for successful preparation of controlled release matrix tablets of a highly water soluble drug with a controlled initial burst in the initial phase and a complete release in the terminal phase. The tablets prepared using this technique were able to provide the desired ascending release kinetics as that of the reference product.

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Evaluation of different scale-up approaches for wet granulation process

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Introduction and Objectives

Wet granulation is often used to produce agglomerates from primary particles. The goal of scale-up is to obtain similar granule and tablet attributes in both micro and macroscale granulation. It is typical in the pharmaceutical industry to use constant tip speed approach for scaling up this process. The aim of this study was to evaluate different scale-up approaches developed using chemical engineering principles [1,2] as defined in Table 1.

Method

Granulation trials were performed using 10 and 65 L granulators with a batch size of 2 and 12 kg, respectively. Experimental investigation was carried out using Design of Experiments (DoE; Stat Ease, Minneapolis, USA) at 10-L scale to understand the impact of granulation parameters, namely the impeller speed, water amount and addition rate on the size distribution, flow and compression properties of the granule.

Results and Discussion

The data generated at 10-L scale were evaluated using Design Expert (Stat Ease) and SIMCA P$^+$(Umetrics, Umea, Sweden) multivariate analysis softwares. The water amount was found to have a positive effect on the size distribution and flow properties of the granule. In contrast, increasing water levels resulted in decrease in the tablet tensile strength. The dimensionless numbers outlined in Table 1 were calculated using the 10-L granulation process data. Using SIMCA P$^+$, granule and tablet properties, namely size distribution, flowability, disintegration time and dissolution were evaluated as functions of dimensionless numbers. The power number was found to be suitable for predicting the effect of granulation parameters on the size distribution and flowability of the granule. The specific energy and the spray flux ratio were found to be suitable to characterise the tablet dissolution and disintegration time. Using the power

Table 1 Dimensionless numbers for scaling up the wet granulation process

Parameter	Formula	Approach
Tip speed (ms^{-1})	$v_t = \pi N D$	Maintaining tip speed can be important if the granulation process is sensitive to the maximum normal and shear forces.
Froude number	$Fr = \frac{DN^2}{g}$	Dimensionless group expressing the ratio between inertial and gravitational forces. Should be conserved to such an extent that at least the mode of powder flow is maintained (bumping or roping flow).
Dimensionless spray flux	$\psi = \frac{3\dot{V}}{1\dot{A}d_d}$	Relates the spray flux to the powder surface renewal rate. High values will lead to poor dispersion of liquid and generation of lumps.
Dimensionless power number	$N_p' = \frac{\Delta P}{N^3 R^2 M}$	Relates the drag force acting on a unit area of the impeller and the inertial stress. It should be noted that power number typically includes wet mass density. This is a modified expression that can be calculated without the need to sample the wet mass.
Specific energy	$\hat{E} = \frac{\int \Delta P(t) \mathrm{d}t}{M}$	Net specific energy is a measure of the transformation work done on a product during high shear granulation. Integration of the net power draw over the residence time gives the net energy consumed during the agglomeration process, which is normalised by the mass hold-up to give the net specific energy.

N is the impeller speed (s^{-1}), D is the bowl diameter (m), g is the acceleration due to gravity (9.81 ms^{-2}), ΔP is the overall variation of power draw: $P_{max} - P_{baseline}$ (W), R is the bowl radius (m), M is the powder mass (kg), $\dot{V}$ is the water volume flux (m^3 s^{-1}), $\dot{A}$ is the area flux of powder (m^2 s^{-1}) and d_d is the droplet size of the spray (m).

number and spray flux ratio approach, process conditions for 65-L-scale granulation were defined. Two confirmatory batches were run at the defined conditions to validate the approach. The properties of the granules and tablets produced were comparable with the 10-L product data.

Conclusion

Dimensionless numbers, namely the power number and spray flux, were successfully used to define process parameters for scale-up of the wet granulation. The granules and tablets produced at 10 L met the desired product quality and correlated well with those produced at the 65-L scale.

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Pharmacognosy

182 Uptake of nicotine from suspension cultures of *Nicotiana tabacum* by molecularly imprinted polymers

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Introduction and Objectives

The aim of the study was to use molecularly imprinted polymers (MIPs) for the selective recovery of nicotine in plant cell cultures. MIPs have been used efficiently in solid phase extraction of small-molecule compounds from real samples.[1] MIPs can selectively uptake nicotine from suspension cultures of *Nicotiana tabacum* and therefore may be useful for improving levels of secondary metabolites in plant cell cultures. Adsorption of products once it is released could decrease feed-back inhibition and enhance levels of secondary metabolites.

Methods

Suspension cultures of *N. tabacum* were initiated from callus and maintained in liquid Murashige and Skoog media[2] containing 3% w/v sucrose, 0.1-mg α-Naphthaleneacetic acid (NAA)/l and 0.25-mg kinetin/l. Tween 80 at 1% was used for permeabilisation of cell cultures. Preweighed XAD-2 (commercial polymer), and two types of synthesised polymers, MIPs (A & B) and nonimprinted polymers (NIPs, A & B), were introduced aseptically into the permeabilised suspension cultures of *N. tabacum*. After specific incubation time, the nicotine contents of polymers were determined by gas chromatography (Finnigan Focus) and the adsorption yield of polymers were determined.

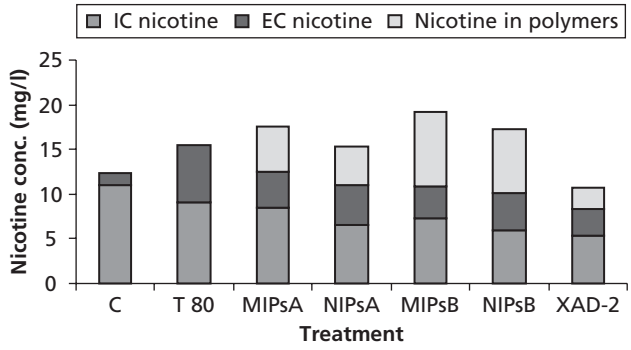

Figure 1 Effects of permeabilisation and different types of polymers on nicotine production in suspension cultures of *N. tabacum*; C, control; T 80, Tween 80 1%; EC, extracellular; IC, intracellular.

Results and Discussion

Cell cultures of *N. tabacum* accumulated nicotine alkaloid intracellularly in varying levels, 6.8–14.9-mg/l fresh weight. Permeabilisation of cell cultures was necessary to force excretion of nicotine into the medium, and different types of polymers were used to adsorb nicotine. Tween 80 1% enhanced both extracellular nicotine and total nicotine up to 7.3 and 17.5 mg/l, respectively, without effect on cell growth. MIPs were able to uptake 50–60% of released nicotine in suspension cultures of *N. tabacum*, whereas XAD-2 recovered only 30–40%. There was a significant difference between the extraction yields for MIPs B and NIPs B ($P < 0.05$). The total levels of accumulated nicotine were enhanced up to 20 mg/l by simultaneous use of Tween 80 and MIPs (Figure 1).

Conclusion

The synthesised MIPs were able to successfully uptake nicotine from suspension cultures of *N. tabacum* and increase productivity of secondary metabolites. This allows the possibility of using MIPs for improved alkaloid production and collection in plant cell cultures.

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Biochemical evaluation of extracts from *Caralluma tuberculata* against hormone-dependent breast cancer cells

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Introduction and Objectives

Compounds which inhibit oestrogen biosynthesis have been shown to lead to tumour regression, and therefore a reduction in tumour mass. It was hypothesized that the pregnane backbone may have an antiproliferative effect on hormone-dependent cancer cells through the disruption of the steroidal cascade, and therefore sex-steroid biosynthesis (in particular, oestrogen biosynthesis). Here, we report the initial results of our study into the use of extracts from *C. tuberculata* in the search for novel antitumour agents.

Methods

Extraction of plant crude extracts was carried out in ethanol and aqueous ethanol (50%) using both cold-extraction (maceration) and hot-extraction (Soxhlet) methods. Crude extracts were fractionated into four organic fractions using hexane, chloroform, ethyl acetate and methanol using gradient polarity system and aqueous fractions. Standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT)) assay was used for the evaluation of cell viability.[1] Crude extract dilution and organic fractions (at various concentrations) were added to 96 multiwell plates. Plates were incubated for 24 h. MTT solution was added and the plates were further incubated for 4 h, read and cell viability determined.

Results and Discussion

The *C. tuberculata* crude extract concentrations were evaluated (in the presence of 5000 cells/well) at concentrations of 10, 25, 50, 100, 200, 300, 400 and 500 μg/ml to give the following percentage inhibition of cell growth 25, 25, 25, 31, 56, 65, 75 and 82%, as such, the IC50 of growth inhibition is approximately 175 μg/ml for the crude extract. Furthermore organic fractions from *C. tuberculata* were obtained using hexane, chloroform, ethyl acetate, methanol and water. These fractions were observed to possess cell growth inhibition of 58, 97, 99, 97 and 58% at 200 μg/ml. As such, three fractions would appear to possess potent cell-inhibition properties, all of which are found to possess greater than 95% inhibition. This would, therefore, suggest that the cytotoxic compounds would appear to be hydrophobic in nature.

Conclusion

Some initial extractions of fractions from *C. tuberculata* have been undertaken, and they have shown that this plant contains fractions, which possess significant levels of antitumour activity. In particular, we have shown that these compounds possess good levels of inhibition of cell growth against MCF-7 cell line (not previously reported) and that the fractions require further investigations so as to discover the active components – an initial study into the nature of the active compounds suggests that the compound is of a steroidal nature.

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Antimicrobial activity of the essential oils of *Hygrophila auriculata (*Schumach.*)* Heine

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Introduction and Objectives

Aim of this study was to determine the effectiveness of the antibacterial activities of the essential oils of *Hygrophila auriculata* (Schumach.)Heine [family: Acanthaceae] against Candida species (*Candida stellatoidea*, *Candida albicans* and *Candida torulopsis*) and some selected bacteria species (*Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus*), which are often implicated in infectious diseases such as dysentery, eczema and putrefying wounds.[1] *Hygrophila auriculata* has also been reported to possess antitumour activity [2] and also used in local treatment of cough, eczema, urinogenital tract infection, diabetes, dysentery[1] and antibacterial.[3] Little information is available on the antibacterial, and antifungal activities of this plant.

Method

Essential oils obtained from *Hygrophila auriculata* (Schumach.)Heine [family: Acanthaceae] was assayed against *K. pneumoniae*, *B. subtilis, S. aureus* (ATCC 24213) and *P. aeruginosa* (ATCC 9027) and the yeast *C. albicans* (ATCC 10231), *C. stellatoidea* and *C. torulopsis* using the dilution methods of Vlientick *et al*.[3] Minimum inhibitory concentration (MIC) was determined. Standard antibiotics (Ampicillin and Ketoconazole) were used as positive control, whereas 75% methanol as negative control.

Results and Discussion

The percentage yield of essential oil obtained from hydrodistillation of both the aerial and the root parts of Hygrophila auriculata was 1.5% (w/w) (based on the dry weight of the plant).

Pseudomonas aeruginosa was only susceptible to the essential oil from the aerial part of the plant. *C. albicans, C. stellatoidea* and *C. torulopsis* were susceptible to the essential oils from both the root and the aerial parts at all concentrations. The MICs of *B. subtilis, K. pneumoniae, P. aeruginosa* and *S. aureus* to both the aerial and the root extracts were between 500 and 1500 μl/ml, respectively. However, the minimum inhibitory concentration of *C. albicans, C. stellatoidea* and *C. torulopsis* to the aerial and the root oil extracts were 500 μl/ml. In comparative terms, the MIC values of the essential oils compare moderately with the standards antibiotics.

Conclusion

This study demonstrated the antimicrobial activities of different concentration of *H. auriculata* essential oil and confirmed its antibiotic, bacteriostatic and fungistatic activities. There is a need for further studies on this plant as not much has been carried out on its antimicrobial activities.

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High-performance liquid chromatography comparison of eight beneficial secondary plant metabolites in the flesh and peel of 15 varieties of apples

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Introduction and Objectives

To characterise secondary metabolites (phytoalexins) present in apples, which are thought to be beneficial to health, and to establish any differences in chemical profiles between varieties and levels in organically grown crops and conventionally produced crops.

Method

Powdered samples (1 g each) were extracted with 10 ml of 30% methanol in water at room temperature for 24 h. Extracts were filtrated and transferred into high-performance liquid chromatography (HPLC) vials. A Hewlett-Packard (HP) 1100 series quaternary HPLC system with a diode-array ultraviolet–visible detector coupled to an HP chemstation was used. A Synergi Phenomenex polar-RP C18 column, 4.6 × 150 mm with guard cartilage, 4-μm particle size, was used, and the solvent system was methanol with 1% acetic acid (A), H_2O with 1% acetic acid (B) and acetonitrile (C). Solvent gradient was1–5% A for 8 min, 5–10% A for 15 min, 10–24% A for 37 min, 24–92% A for 1 min, 92–94% A for 9 min, with 1–5% C for 8 min and 5% C for 8–70 min. Injection volume was 100 μl, and the separation was performed at 35°C at a flow rate of 1 mL/min. Elute was monitored at 245, 280, 340, 420 and 530 nm.

Results and Discussion

Significant differences in the levels of beneficial phytoalexins were observed, both between varieties and crop husbandry systems. Cyanidin 3-galactoside, an important antioxidant, was the only anthocyanidin identified. Flavonoids, epicatechin and catechin, have high antioxidant activity and inhibit low-density lipoprotein oxidation.[1] Pendragon had highest amount of catechins in the whole apple, and Gala and Torr Hill had the lowest. Quercetin, a scavenger of highly reactive inflammatory peroxynitrite and hydroxyl radical species[2], appeared only in the flesh as quercetin 3-D-galactoside and quercetin glucoside. Highest levels of rutin were found in Bens Red. Phlorizin, which affects intestinal glucose transport, was found mainly in the peel of organic apples and not in nonorganic. If in flesh, it was present at 10-fold lower levels than in peel. Chlorogenic acid and p-coumaric acid are potent peroxyl-free radical scavengers. Nonorganic apples contained lowest amount of chlorogenic acid, but no p-coumaric acid.

Conclusion

There were significant differences in the levels of beneficial phytoalexins between different varieties of apples and apples grown organically and apples grown nonorganically. Organic apples consistently contained higher levels of all analysed components, and more healthy components were concentrated most in the peels. Pendragon-a 12th-century apple contained the highest levels of all components.

References

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Antistaphylococcal MRSA, antistreptococcal, anti-*Propionibacterium acnes*, anti-inflammatory, antiallergic properties of ghee and *Centratherum anthelminticum*

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Introduction and Objectives

The aim of this study is to provide an effective treatment method using evidenced based herbal medicine, non-steroidal, non-lanolin and without any potential skin sensitisers. Staphylococcal toxins are usually found in patients with psoriasis[1], atopic dermatitis, erythroderma, and millions of people suffer from some sort of dermatitis due to hypersensitivity to several chemicals present in conventional moisturisers. Some sort of potential skin sensitisers are present in most of the *over-the-counter* cosmetic creams. Acne patients suffer from chemical dermatitis due to presence of chlorocresol, cetosteryl alcohol, benzyl alcohol or propylene glycol, sorbic acid, wool fats, wool alcohols and their derivatives and also due *Propionibacterium acnes* infection. Streptococcal intertrigo is unrecognised condition in children.[2]

Method

The bacterial pathogens were tested using Health Protection Agency (HPA) stock cultures. Test bacterial pathogens were, Staphylococcus aureus NCTC 10654, NCTC 10655; Staphylococcus capitis NCTC 11046; group A betahaemolytic Streptococcus NCTC 8198; P. acnes NCTC 737. The extracts of the selected herbs were freeze-dried and the filtered concentration was expressed as the equivalent weight of original plant material per ml (i.e. 400 mg/ml). A dilution series was added to the sterile Tryptone Soy Broth, inoculated with 100 μl of overnight culture of the bacterial strains and was incubated at 37°C overnight. Crystal violet (0.002 mg/ml) was used as positive control and sterile water (100 μl) was used as negative control. A cream (5 g) spiked with 50 μl of culture (five different pathogens) was prepared in triplicate. Survival of the bacteria after 30 min was estimated using serial dilution method and plate counts were determined on Tryptone Soy agar and incubated overnight at 37°C. Oxy10 (benzoyl peroxide lotion) was used as a positive control.

Results and Discussion

The cream base material (ghee plus extracts of *Centratherum anthelminticum*, *Melia azadirachta* and *Cassia tora*) inhibited the bacterial pathogens after 30 min at room temperature as described in Figure 1 (*P. acnes* NCTC 737 6.0 ± –0.4 Log10 CFU/g; group A beta haemolytic streptococcus NCTC 8198 5.7 ± –1.0 Log10 CFU/g). The cream had some inhibitory effect on viable numbers of strains 10654, 11046 and 10655, with inhibition ranging from less than 10-fold to 1000-fold reduction in numbers. A more consistent 100-fold reduction in numbers of viable strains 737 and 8198 was observed after 30-min incubation in the skin cream. Antibacterial activity of the extract *C. anthelminticum* inhibited the growth of strain 10654 at 2 mg/ml and strain 737 at 4 mg/ml. *M. azadirachta* inhibited strain 737 at 4 mg/ml. *C. tora* gave inconsistent inhibition of strains 10654, 11046 and 737 (i.e. inhibition was observed in more than one growth experiment but not in all the three replicates). The mix-1, *C. anthelminthicum, M. Azadirachta* and *C. tora*, inhibited the strain 10654 at 4 mg/ml; strain 11046 at 2 mg/ml; strain 10655 at 4 mg/ml and strain 737 at 2 mg/ml. It did not inhibit strain 8198. The mix-2, *Picrorhiza kurroa* and *Andrographis paniculata*, inhibited strain 10654 at 4 mg/ml and strain 737 at 0.2 mg/ml. The topical ghee cream treatment for eczema brought great fold decrease in increased IgE levels.

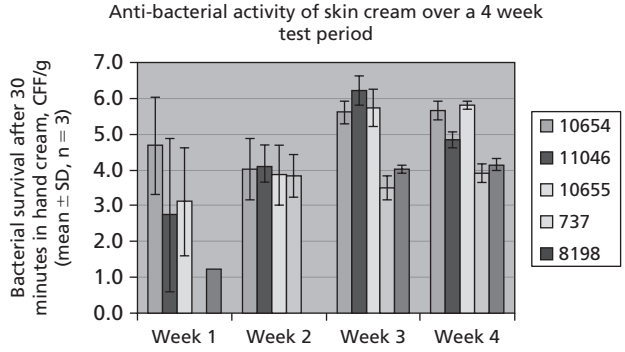

Figure 1 The mean bacterial counts present in the skin cream 30 minutes after inoculation with the five test bacterial strains (detailed below) is shown as determined upon serial dilution and by plate counting on Tryptone Soy agar. Data are expressed as mean Log_{10} CFU/g skin cream ± standard deviation (SD) and was done in triplicate. The mean bacterial inoculum added to the skin cream was: *Staphylococcus aureus* NCTC 10654 6.5 ± 0.3 Log_{10} CFU/g, *Staphylococcus capitis* NCTC 11046 6.9 ± 0.3 Log_{10} CFU/g, *Staphylococcus aureus* NCTC 10655 6.6 ± 0.1 Log_{10} CFU/g, *Propionibacterium acnes* NCTC 737 6.0 ± 0.4 Log_{10} CFU/g, A Beta-haemolytic *Streptococcus* NCTC 8198 5.7 ± 1.0 Log_{10} CFU/g.

Conclusion

Herbs are sensitive to Staphylococcus aureus, two strains of MRSA were difficult to treat (Staphylococcus aureus NCTC 10654, NCTC 10655) Staphylococcus capitis NCTC 11046 is also sensitive to Propionibacterium acnes. A greater sensitivity was recorded with the cream product. The cream product containing ghee and the three herbs showed effectiveness against Streptococcus pyogenes (group A beta haemolytic streptococcus), the herbs were effective both

separately and as a mixture. This proves that there is something effective in the cream preparation which is working against group A beta haemolytic streptococcus and we can confirmed it is the ghee. We also observed great fold decrease in levels of IgE in patients suffering from eczema, psoriasis.

References

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