the level of estrogens (in particular, estradiol) through the inhibition of 17β-HSD1. We also hypothesise that the mode of action of these compounds involves hydrogen bonding to the active site of 17β-HSD1*via* the N atom within the imidazole moiety.

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Continued search for potent inhibitors of the cytochrome P-450 enzyme 17α-hydroxylase/17,20-lyase in the treatment of androgen-dependant prostate cancer

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Introduction and Objectives

17α-Hydroxylase/17,20-lyase (P45017α) is under investigation in the treatment of androgen-dependant prostate cancer. We have previously evaluated a series of compounds based upon the benzyl imidazole backbone;[1] however, only a small number of compounds were found to be equipotent to the standard, ketoconazole (KTZ). To increase our library of potent inhibitors of P45017α, we have undertaken the synthesis of a range of (4-alkylphenyl)sulfonate derivatives of 4-hydroxybenzyl imidazole. Here, we report the initial results of our study into the biochemical evaluation of the synthesised compounds as potential inhibitors of P45017α.

Method

In the synthesis of the target compounds, we initially synthesised 4-hydroxybenzyl imidazole (1) using the methodology previously reported by us.[1] The 4-hydroxyphenyl moiety was then derivatised using various (4-alkylphenyl) sulfonyl chlorides and anhydrous dichloromethane as solvent to give the target compounds. The biochemical evaluation of the synthesised compounds (against both the lyase and 17α-hydroxylase components) was undertaken using literature assay procedure using rat testicular homogenate and radiolabelled progesterone and 17α-hydroxyprogesterone for the 17α-hydroxylase and lyase components, respectively.[1] The inhibitors were evaluated at [I] = 100 μM with a substrate concentration of 1.5 μM.

Results and Discussion

The reactions proceeded in good yield (ranging from 40 to 85%), and no major problems were encountered. However, the purification of the potential inhibitors did prove to be troublesome, and a number of purification steps were required. The results of the biochemical evaluation of the compounds against both the hydroxylase and lyase components suggest that the compounds are highly potent inhibitors of P45017α. For example, KTZ was found to possess IC50 values of 206 nm and 2660 nm against lyase and 17α-hydroxylase components, respectively. The weakest inhibitor within the range was found to be the 4-(4-pentylphenyl) sulfonyl benzyl imidazole, with IC50 values of 34 nm and 1040 nm, respectively. Indeed, the most potent compound was found to be approximately 6.8 and 66.5 times more potent than KTZ against lyase and 17α-hydroxylase, respectively. Molecular modelling of these compounds suggests that the sulfonyl moiety is able to undergo interaction with the hydrogen bonding groups at the active site and that there is limited conformational space within the active site, as such, large groups at the 4-position of the phenylsulfonate moiety cannot be accommodated, resulting in a decrease in inhibitory activity.

Conclusion

We have provided novel compounds that have been shown to possess high potent inhibitory activity, which is in the nm range. These compounds show selectivity against the 17α-hydroxylase component, which is therefore beneficial since this would limit side effects of these highly potent inhibitors.

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Short Papers in Material Science

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Sustained release of triclosan from silicone elastomers modified with allyl monomethoxy poly(ethylene glycol)

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Introduction and Objectives

Silicone elastomer, extensively used in the manufacture of medical devices (e.g. catheters and drug delivery systems), is prone to surface biofilm formation, which may lead to infection. There is interest in developing silicone elastomer biomaterials that are better able to resist microbial adhesion. A possible solution in preventing biofilm formation is the incorporation of a broad-spectrum antimicrobial agent into the biomaterial.[1] In this study, condensation-cured silicone elastomer materials were pegylated in a bid to achieve sustained release of the broad-spectrum antimicrobial agent triclosan. Resulting materials were evaluated for in-vitro triclosan release and microbial activity.

Method

A monopegylated trialkoxysilane cross-linking agent was synthesised by reacting triethoxysilane with allyl monomethoxy poly(ethylene glycol) (AMPEG). The crosslinker was subsequently reacted with hydroxy-terminated polydimethylsiloxane in the presence of the tin catalyst to produce the pegylated silicone elastomer. Triclosan-loaded elastomers (1% w/w) were prepared in a similar fashion by dispersing triclosan in the elastomer mix prior to curing. The in-vitro release of triclosan was quantified in a sink-condition model. Adherence of *Escherichia coli* to the silicone elastomers was evaluated. Microbial persistence on the surface of silicone elastomer discs modified with AMPEG and triclosan was also evaluated using zones of inhibition analysis.

Results and Discussion

Compared to the nonpegylated control that contained no triclosan, the greatest reduction in adherence was in the nonpegylated triclosan-loaded silicone elastomer, where no *E. coli* was adhered to the material after 4 h, and a reduction in adherence by over 99% was observed after 24 h. At 4 and 24 h, the amount of adherence to the triclosan-loaded formulations increased: nonpegylated triclosan-loaded silicone elastomer <2% w/w pegylated triclosan-loaded silicone elastomer <4% w/w pegylated triclosan-loaded silicone elastomer. Microbial persistence also followed this trend, with 4% pegylated silicone elastomers exhibiting zones of inhibition for 91 days compared to 49 days for the nonpegylated control. Ultraviolet detection (282 nm) of triclosan over 14 days demonstrated that modification of silicone with AMPEG in this manner inhibited release of triclosan. On day 14, nonpegylated triclosan-loaded silicone had released 63% triclosan, 2% w/w pegylated silicone loaded with triclosan had released 24% and 4% w/w pegylated silicone loaded with triclosan had released 13% triclosan.

Conclusion

Understanding how pegylation affects the release of triclosan from silicone elastomer may prove useful in the development of a medical device material that has prolonged antimicrobial effect. The results of this study suggest that for short-term catheterisation (<30 days), the nonpegylated silicone elastomers containing 1% w/w triclosan could be useful in preventing biofilm formation, with the potential to reduce catheter-associated urinary tract infections. For long-term catheterisation (≥30 days), a pegylated silicone elastomer containing 1% w/w triclosan may prove effective in providing a prolonged antimicrobial effect.

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A study of the miscibility of ethylcellulose (Ethocel™ 20) with different concentrations of plasticiser in cast film coats

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Introduction and Objectives

Polymer and plasticiser phase separation and saturation are critical in functionality performance and stability of pharmaceutical film coating systems. Here we explore the relationship between the composition and the associated thermal events of ethylcellulose (EC; Ethocel™ 20) films loaded with fractionated coconut oil (FCO). In particular, we compare the applicability of two techniques, modulated temperature differential scanning calorimetry (MTDSC) and dynamic mechanical analysis (DMA), to measure the glass transition temperature (T_g) and detect potential phase separation processes.

Method

EC solutions were prepared by dissolving 2 g Ethocel™ 20 (Colorcon, West Point, USA) in 20 ml ethanol/acetone (60/40 v/v) with 5, 10, 15, 20, 25 and 30% (w/w) FCO (Colorcon) added. Solutions were cast by using an REF 1117 film applicator (Sheen Instruments, Surray, UK) and dried at 45°C for 3 h. MTDSC experiments were conducted using a DSC Q1000 (TA Instruments, New Castle, USA) from 20 to 220°C with a modulation amplitude of ±0.5°C/40 s at 2°C/min in pinholed crimped pans. A DMA 2980 (TA Instruments; tensile film mode) was used, ramping from 30 to 180°C at 3°C/min.

Results and Discussion

MTDSC gave a value of T_g for the EC film of 129.2 ± <0.1°C. An endothermic event was observed around 180°C, which was ascribed to melting of microcrystallites in the EC.[1] The T_g decreased when FCO was added. However, on

adding FCO to 20% and beyond, the T_g remained constant at approximately 97°C, while a lower temperature peak was observed at approximately 53°C. Such phenomena have been associated with phase separation. This event may indicate multiple mixed phases. The composition of these phases was estimated by considering the Gordon Taylor–Simba Boyer equations in the context of the T_g of the 5% FCO films (allowing K and the T_g of FCO to be estimated), and then estimating the compositions of the two phases in the 20% systems as being 23.5 and 8.3% FCO. It is noted that between 20 and 30% FCO, the T_g values do not change, implying further phase separation. DMA data show a similar trend of decreasing T_g with increasing FCO concentrations. Two glass transitions were observed in the tan delta for 20% FCO film, supporting the suggestion of phase separation. However, the storage modulus decreased dramatically above T_g, thus rendering it difficult to observe any higher temperature transitions.

Conclusion

MTDSC and DMA results suggest that phase separation can occur in EC/FCO films. We suggest the formation of binary mixed systems rather than pure component separation and outline a novel method for estimating the associated composition.

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Preparation of poorly water-soluble drug nanoparticles and their release into aqueous solutions

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Introduction and Objectives

The aim of this study was to prepare drug nanoparticles using an emulsion freeze–drying technique.[1] These nanoparticles are produced *in situ* within highly interconnected porous polymeric structures.[2] The drug nanoparticles are released into water to form stable aqueous nanoparticle dispersions by dissolving the nanocomposites in water.

Method

Indomethacin (IMC) was used as a model drug. Firstly, an aqueous solution of poly(vinyl alcohol) (PVA)/sodium dodecyl sulphate was prepared. Triton X-405 surfactant was added to this solution, and the IMC solution in xylene was added dropwise while stirring. The emulsion formed was rapidly frozen in liquid nitrogen and freeze dried at –30°C for 2 days. The dried monoliths could be dissolved in water to produce aqueous IMC nanoparticle dispersions. Scanning electron microscopy (SEM), dynamic laser scattering (DLS) and X-ray diffraction (XRD) were used to characterise the materials and the nanoparticles.

Results and Discussion

The SEM images showed that a highly interconnected porous structure was produced. IMC is poorly soluble in water. The dissolution of PVA monolith led to the formation of a clear aqueous phase. This indicated the small size of IMC particles in water, without diffracting light. The ultraviolet (UV) measurement further demonstrated the presence of IMC in water. The UV absorption increased with the concentration of the starting drug and internal phase volume in the emulsions, which were used to prepare the monolith. DLS measurements of the nanoparticles released from the polymeric scaffold into water showed a narrow distribution of particles, with a mean particle size of 130 nm, while another small peak could be responsible to the micellisation of the surfactants and/or polymer. SEM images further confirmed the formation of IMC nanoparticles. The average size agreed with the DLS measurement. XRD data showed that IMC nanoparticles produced using this technique gave amorphous structures, different from its as-purchased crystalline state.

Conclusion

IMC, a poorly water-soluble drug, has been processed to form aqueous nanoparticle dispersion. The nanoparticles were formed *in situ* within water-soluble porous polymer matrix by the emulsion freeze-drying technique. Stable aqueous nanoparticle dispersions were successfully prepared by dissolving the nanocomposites in water.

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Influence of polymer concentration, plasticiser molecular weight and swelling conditions upon the dielectric behaviour of polyethylene glycol–plasticised poly (methyl vinyl ether-co-maleic acid) films intended for transdermal iontophoretic applications

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Introduction and Objectives

The aim of the study was to investigate the dielectric properties of poly(ethylene glycol) (PEG) crosslinked poly (methyl vinyl ether-co-maleic acid) (PMVE/MA) hydrogels as a function of polymer concentration, PEG molecular weight and swelling. The performance of hydrogels as transdermal delivery systems can be influenced by their mechanical and swelling properties.[1] In addition, when such systems are intended to allow the permeation of charged carriers following the application of an electric field, it may be useful to characterise the hydrogel in terms of conductivity. Ultimately, this could aid in the optimisation of a formulation for use in controlled transdermal iontophoretic drug delivery.

Method

Aqueous films were cast by adding the required amount of PEG 10 000, PEG 1000, or PEG 200 to PMVE/MA (at 10, 15 and 20% w/w), in a ratio of 2 : 1 (PMVE/MA : PEG). After drying, the films were cured at 80°C for 24 h. The films were then left in the dried state or swollen to equilibrium in solutions containing phosphate buffered saline (PBS) pH 7.4, 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) pH 7.4 or theophylline-loaded HEPES solution pH 7.4. The dielectric properties over a temperature range of 20–40°C were evaluated using a DEA 2970 Dielectric Analyser (TA Instruments, New Castle, USA) with ceramic parallel plates.

Results and Discussion

It was found that the polymer content and molecular weight of PEG had an effect on the ionic conductivity of the hydrogel films. While increasing polymer content led to a slight increase in the ionic conductivity, the most dramatic effect was achieved through an increase in the PEG molecular weight. Indeed, increasing the PEG molecular weight from 200 to 10 000 led to at least a sixfold increase in the conductivity of the swollen hydrogel. It was also found that the medium used to swell the hydrogels to equilibrium had an effect on their resultant dielectric properties. In particular, it was found that hydrogels swollen in a 25 mM HEPES solution (pH 7.4) showed a pronounced decrease in ionic conductivity in comparison to films that had been swollen in PBS solution. In particular, the conductivity of films consisting of 15% PMVE/MA–7.5% PEG (MW 200) swollen in 25 mM HEPES dramatically decreased to 25 pmho/cm in comparison to 12×106 pmho/cm when swollen in PBS. In addition, the ionic conductance of the hydrogels swollen in a 25 mM HEPES solution was found to be enhanced in the presence of anhydrous theophylline, with the increase related to an increase in the concentration of theophylline.

Conclusion

The present study demonstrated the significance, for the first time, of the effect of polymer and crosslinking concentrations, crosslinker molecular weight and swelling conditions on the dielectric behaviour of PMVE/MA–PEG hydrogels. This study is seen to represent proof of concept that these systems can be modified to serve as an electrically conducting matrix, with further work aiming to investigate the drug release profile of these films following the application of an electric field.

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Therapeutic ion release from modified-titanium surfaces

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Introduction and Objectives

Titanium is currently the favoured biomaterial for load-bearing applications. Several methods have been applied to increase the osseointegration capabilities of titanium surfaces. Among these methods, hydrothermal treatment[1] and calcium ion implantation[2,3] have been shown to offer improvement in the bone bonding ability in comparison to untreated titanium. In this study, we demonstrate that it is possible to load titanium surfaces with a range of ions, which can then be released into solution at a controlled rate. Both antibacterial ions and those associated with bone growth were investigated.

Method

Titanium discs (grade 1, commercial purity) were hydrothermally treated in a range of solutions containing 10 M NaOH with varying amounts of $CaCl_2$, $ZnCl_2$, MgOH, KOH and $AgNO_3$. Following rinsing in pure water, the samples were heat treated in an air furnace at 200°C for 24 h. Surface examination was performed using scanning electron microscopy, whereas energy dispersive X-ray (EDX) analysis was used to measure the levels of ions incorporated. Dissolution testing was performed in pure water, and ion concentrations were analysed by either atomic absorption spectroscopy or flame spectrophotometry.

Results and Discussion

The results showed that it is possible to incorporate all of the ions into the titanium surface at significant concentrations. Slight differences in the surface morphologies were found for each treatment type. The dissolution tests showed that the rate of ion release was highly dependent on the ion that had

been added. The dissolution rates found were likely to have physiological significance. EDX analysis following immersion reveals that although ions are released, the concentration remains largely unchanged, suggesting that there is much potential for release over significant periods from these surfaces.

Conclusions

Modified-titanium surfaces were produced with a range of different ions incorporated into the surface structure. The process is far more commercially viable than ion-implantation techniques and warrants further investigation.

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A nanoscale study of the mechanical properties, micronisation and surface energy change of carbamazepine polymorphs

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Introduction and Objectives

Particle size reduction is commonly applied to active pharmaceutical ingredients during formulation development to enhance solubility. A common method used to reduce particle size is micronisation in a jet air mill. During this process, a large amount of energy is applied to a material that can lead to changes in surface properties in an uncontrolled manner. A common change that is observed is an increase in surface energy. The aim of this work is to investigate the link between the mechanical properties, micronisation behaviour and surface energy of carbamazepine polymorphs using atomic force microscope (AFM).

Method

Carbamazepine Forms I, II and III were prepared and confirmed using X-ray powder diffraction (XRPD). AFM measurements of indentation hardness, Young's modulus[1] and surface energy were made on the starting material. In addition, the surface energy was measured immediately after micronisation and after storage for 4 weeks.

Results and Discussion

Carbamazepine polymorphs could be ranked by Young's modulus and indentation hardness. Surface energy measurements showed an increase in surface energy after micronisation in all forms, with the most significant change observed for Form II (62.9 mJ/m^2). All forms underwent a relaxation in surface energy following storage for 4 weeks. Form I demonstrated the greatest change returning to a value close to its starting surface energy. A comparison was made between the mechanical properties (hardness and Young's modulus ratio) of the polymorphs and the surface energy change upon micronisation. A correlation was observed for this material that indicated that low hardness and Young's modulus ratio was associated with a small surface energy change upon micronisation.

Conclusion

AFM measurements have been used to explore the links between the nano-mechanical properties of materials and the outcome of the micronisation process with respect to surface property changes. The results show potential for the predictive capacity of this approach and provide a greater understanding of material behaviour and properties during micronisation.

Reference

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23
Site-selective covalent protein immobilisation on nanofabricated surfaces mediated by a phosphopantetheinyl transferase towards nanomedical arrays and biosensors

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Introduction and Objectives

A major area in bionanotechnology is the attachment of proteins onto nanofabricated surfaces in the drive towards the development of diagnostic 'nanoarrays' and highly miniaturised biosensors. However, one major limitation has been the reliance on methods that attach proteins with random orientations resulting in a large population of inactivated proteins, potentially a major issue when only a

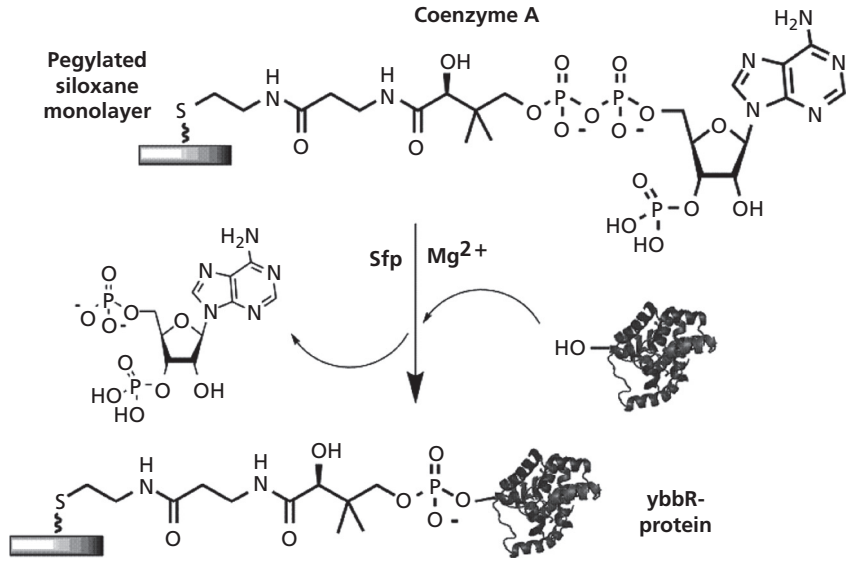

Figure 1 General scheme of the attachment of proteins to immobilised CoA.

relatively small number of proteins are immobilised on nanoscale features. Here, the phosphopantetheinyl transferase Sfp was used to site specifically and covalently immobilise proteins bearing the small ybbR-tag[1] onto nanopatterned siloxane monolayer surfaces bearing coenzyme A (CoA).

Method

The gene for a model protein thioredoxin (Trx) was produced as a ybbR-Trx fusion protein using standard molecular biology techniques. Bioresistant photoreactive siloxane monolayers were prepared and patterned by exposure to 325-nm laser light.[2] CoA was subsequently attached to the exposed areas *via* a suitable maleimide linker molecule. The ybbR-protein was immobilised by attachment to the surface CoA under Sfp as catalyst (Figure 1). The surfaces were then imaged by atomic force microscopy (AFM).

Results and Discussion

The AFM images show an increase in height in the patterned areas corresponding to the immobilisation of the ybbR-Trx. This was further confirmed by binding with anti-Trx antibodies, which gave a further increase in height. In contrast, in control experiments where the catalysing enzyme Sfp was omitted, no height increase was observed, confirming that Sfp was required for immobilisation. In areas that were not previously exposed to light, no proteins were bound, indicating bioresistance was maintained. Using scanning near-field photolithography, submicro patterns as small as 150–200 nm could be obtained.

Conclusion

The use of Sfp-mediated protein immobilisation onto a nanofabricated siloxane surface is demonstrated. This mild, facile and covalent method is site-specific in terms of the site of attachment on the protein, as well as with respect to the location on the nanofabricated monolayers. This is thus a significant step towards the development of a variety of highly miniaturised biomedical instruments.

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Short Papers in Pharmaceutical Science

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In-silico identification of the skin sensitisation potential of active pharmaceutical ingredients

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Introduction and Objectives

The aim of this study was to assess the potential of an in-silico method to identify the skin sensitisation potential of active pharmaceutical ingredients. Recent efforts have been focussed on the identification of the skin sensitisation potential of (nonpharmaceutical) substances (e.g. for the Registration, Evaluation Authorization, and restriction of