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Dissolution and bioavailability enhancement of gliclazide using in situ micronization by solvent change method

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Objectives Gliclazide (GL) is a second-generation sulphonylurea, widely used for the treatment of non-insulin dependent diabetes mellitus. It has a low water-solubility, which determines a low dissolution rate and interindividual variability on its bioavailability. According to the biopharmaceutics classification system GL is a class II-drug, i.e., the dissolution rate is the limiting factor for this drug absorption rate (Arias-Blanco et al 1998). An enhancement in dissolution rate is important to attain suitable blood-levels of these drugs. The aim of this study was to enhance the dissolution of GL by preparation of micron-sized particles by solvent change method (Rasenack et al 2004).

Methods The *in situ* micronization process was carried out using solvent change method in the presence of HPMC or Brij 35 (0.05 or 0.1 g) as stabilizing agents. GL (0.5 or 1 g) was dissolved in acetone and the stabilizing agent in water (as non-solvent). By changing 3 procedure variables each at two levels, eight different formulations were prepared by a full factorial design. The non-solvent was poured rapidly into the drug solution under stirring at 26000 rpm by an ultrahomogenizer and freeze-dried. The untreated GL powder, its microcrystals and normal saline were administered orally in three groups of healthy normal rats (n = 6) and at predetermined time intervals blood samples were taken from their eyes. The samples were analysed for GL by HPLC and blood glucose levels by glucose kits.

Results The crystalline shape of GL changed from rod shape to diamond or cube-shape. The FTIR and DSC results showed no interaction between the drug and stabilizers. A significant reduction in enthalpy changes was seen in microcrystals of GL compared with the untreated drug, which may be related to the small amounts of stabilizers in addition to the change in crystalline habit of the microcrystals compared to untreated drug. XRD studies showed the sharp peaks were present in the diffractograms of microcrystals but with 10 times smaller height indicating no polymorphic but a crystalline habit modification occurred in the microcrystals. The particle size reduced to about 50 times (from 290 μm to about 6 μm) and the dissolution efficiency of GL up to 15 min (DE_{15}%) was increased to about 4 times. GL absorption was increased twice compared with the untreated drug (Figure 1).

Conclusions The microcrystallization has an effect on GL crystal habit modification. Micrystallization of GL in acetone resulted in cube or diamond shape crystals whereas the untreated crystals were rod like or columnar. Solvent change method, using ultrahomogenizer and stabilizing agents produced cubic or diamond crystals, with higher dissolution rate, compared to untreated sample. Changing the concentration of drug and stabilizing agent changed the size of crystals. However, dissolution efficiency was more affected by drug concentration and stabilizing agent type. Drug absorption was increased to about twice.

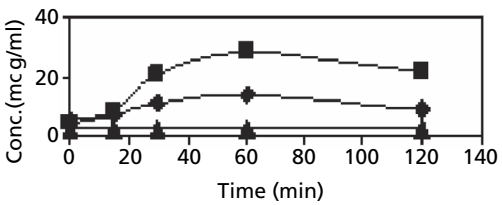

Figure 1 Gliclazide blood concentration as a function of time upon administration of suspensions of microcrystals of gliclazide (■) and untreated gliclazide (◆) in normal saline and the same volume of normal saline (▲) to 200 g Wistar male rats.

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Improved storage stability of controlled release pellets coated with aqueous ethylcellulose dispersion

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Objectives To improve long term stability of controlled release pellets consisting of drug-layered sugar cores coated with aqueous ethylcellulose dispersion. One of the major challenges when using aqueous polymer dispersions for controlled release film coatings is to provide long term stability: if the films are not completely formed, the permeability of the coatings can significantly decrease during long term storage, due to further gradual coalescence of the polymer particles. This results in decreasing drug release rates.

Methods Small amounts of a second polymer (poly(vinyl alcohol)-poly(ethylene glycol)-graft copolymer) (PVA-PEG-graft copolymer) were added to Aquacoat ECD (aqueous ethylcellulose dispersion), plasticized with 25 % triethyl citrate (TEC) or dibutyl sebacate (DBS). Diltiazem-HCl layered sugar beads were coated with these aqueous polymer dispersions in a fluidized bed coater (bottom spray). The coating level and film coating composition were varied. The pellets were cured for 1 day at 60 °C. Drug release before and after 3 and 6 months open storage under ambient and stress conditions (room temperature/ambient relative humidity (RH) and 40 °C/75% RH) was measured in 0.1 M HCl and phosphate puffer pH 7.4 using the USP paddle apparatus (n = 3; UV drug detection).

Results Interestingly, the presence of only small amounts of PVA-PEG-graft copolymer significantly improves long term stability of the investigated pellets, irrespective of the type of release medium and storage conditions (ambient or stress). For instance, 75 (±4)% and 74 (±1)% drug was released after 4 h exposure to phosphate buffer pH 7.4 from pellets coated with 90:10 Aquacoat ECD:PVA-PEG-graft copolymer at a coating level of 15% before and after 3 months storage under stress conditions. Importantly, there is a critical minimal PVA-PEG-graft copolymer content (between 5 and 10%), below which this stabilizing effect is not sufficient to prevent decreasing drug release rates (under the investigated coating conditions). For example, the percentage of diltiazem-HCl released from pellets coated with 95:5 Aquacoat ECD:PVA-PEG-graft copolymer at a coating level of 15% after 4 h exposure to phosphate buffer pH 7.4 decreased from 62 (±2)% to 46 (±0)% upon 3 months open storage under stress conditions. The stabilizing effect of PVA-PEG-graft copolymer might be attributable to improved film formation during coating and curing: the copolymer attracts water that acts as a plasticizer for the polymer particles. The resulting decrease in glass transition temperature of the system leads to an increased mobility of the macromolecules and, thus, facilitated polymer particle coalescence.

Conclusions Importantly, the addition of only small amounts of PVA-PEG-graft copolymer to Aquacoat ECD provides stable drug release patterns from coated pellets during open long term storage at ambient and stress conditions, even in the case of freely water-soluble drugs which are layered onto sugar starter cores.

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Dissolution properties and solid state characterization of spray dried and co-ground indometacin with hydrophilic carriers

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Objectives A poorly soluble drug, indometacin was made into amorphous by co-grinding and spray drying with hydrophilic carriers in order to improve its dissolution property and stability. Characterization study was done by X-ray powder diffractometry (XRPD) and differential scanning calorimetry (DSC), and the interaction of the drug with different carriers was studied by fourier transform infrared spectroscopy (FTIR).

Methods One part of drug and three parts of carrier (Neusilin US2, ProSolv SMCC 50, Emcocel MCC 50 M, PVP K30 and HPMCAS AS-LG) was co-ground using a Fritsch planetary mill. Samples were ground for 6 hours in a ZrO_2 jar (250 ml volume) with ZrO_2 balls (Ø = 15 mm) at 300 rpm. In spray drying, a certain amount of indometacin (0.75 g) and carrier (PVP, HPMCAS, 2.25 g) was dissolved in 200 ml of water-ethanol mixture (2:8) and then spray dried by a SD Niro spray dryer. Wettability of the samples was studied by measuring their contact angles. All samples were compressed into flat-faced discs with a hydraulic press, followed by applying 20 μl of water on the surface. Images were taken 1 second after wetting the samples by camera and analysed.

Results XRPD patterns showed that indometacin became amorphous in all the formulations after co-grinding and spray drying. DSC scans also confirmed this finding by showing the Tg of indometacin ranging from 60–125 °C for different carrier systems, and no melting peak of indometacin was shown. FTIR results

showed that after grinding with Neusilin, the dimer peak of indometacin at 1714 cm^{-1} disappeared with a shift of the carbonyl peak from 1691 to 1672 cm^{-1}, indicating hydrogen bonding occurred between these two materials. Hydrogen bonding was also found in SMCC-indometacin and MCC-indometacin systems as the dimer peak of indometacin was significantly decreased and shifted to 1707 cm^{-1} in addition to the shift of carbonyl peak to 1674 cm^{-1}. The co-ground and spray dried indometacin with PVP or HPMCAS showed similar FTIR spectrum with similar hydrogen bonding tendency. Dissolution studies were done in pH 6.8 phosphate buffer indicating that the dissolution rate of indometacin was greatly improved in the order of SMCC, MCC > PVP (spray dried, co-ground) > Neusilin> HPMCAS (spray dried, co-ground). These results were in good correlation with the wettability of indometacin in different formulations. Further studies had also shown that the apparent solubility of indometacin in different formulations was highly increased. Formulations with PVP showed the highest improved solubility profile while Neusilin showed the least. After grinding with SMCC and MCC, indometacin was stable for more than 4 months, while more than 6 months in the case of Neusilin on the storage condition of 30 °C and 75% RH.

Conclusions Indometacin became amorphous after co-grinding and spray drying with different carriers in this study, and hydrogen bonding between the drug and these carriers was confirmed by FTIR. The dissolution properties of indomethacin were efficiently improved by both co-grinding and spray drying preparations and this was in good correlation with the wettability results. The stability of indometacin was greatly improved.

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Gastrointestinal mucus and its effect on the transepithelial transport of PAMAM dendrimers

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Objectives As a class of highly branched polymers, dendrimers have been considered for numerous applications, including drug/DNA carriers, vaccine delivery and medical diagnostics. PAMAM dendrimers have been extensively studied due to their unique characteristics, i.e. large number of surface functional groups (carboxylic or amine), theoretically mono-disperse and a protected internal microenvironment (Tomalia et al 1990). PAMAM dendrimers have been shown to cross gastrointestinal epithelial cell models, e.g. Caco-2 monolayers (Jevprasesphant et al 2004). Caco-2 monolayers provide a good *in-vitro* model of the gut but lack the mucus layer found *in-vivo*. Mucus serves several functions in the gastrointestinal tract, one of which is to act as a physical barrier to the diffusion of solutes with respect to size and charge (Bhat et al 1995). The effect of mucus on the transepithelial transport of PAMAM dendrimer is described in the current study.

Methods Caco-2 and HT29-MTX cells were co-cultured and an optimum seeding density identified that produced mucus and gave cultures with acceptable TEER (transepithelial electrical resistance) values. Light and electron microscopy techniques were utilised to characterise the co-culture model. Co-culture integrity was assessed by determining the apparent permeability (P_{app}) values for paracellular and transcellular markers, FD4 (FITC dextran 4000) and testosterone respectively, after the co-cultures had been grown for 21 days on Transwell filters. PAMAM dendrimers labelled with FITC via a thio-urea bond were synthesised and their transport through the co-culture assessed. Further transport studies were performed across purified pig mucin (PPM) and crude pig mucus (CPM), and P_{app} values calculated. Rheological techniques were used to assess if any interactions occurred between mucin/mucus and G3 or G3.5 dendrimers.

Results An initial seeding ratio of 40:60 HT29-MTX-E12:Caco-2 cells was selected for the co-culture model. A significantly reduced P_{app} value was seen for testosterone across co-cultures with a mucus layer indicating a reduction in transcellular permeability which may be due to mucus. TEER values of 300–400 Ω cm^{-1} and low P_{app} values of FD4 suggest the presence of tight junctions in the co-culture model. No significant difference was seen in the P_{app} values for FITC-G3.5 in the presence or absence of mucus, indicating mucus has little effect on the transepithelial transport of G3.5 PAMAM dendrimers. In contrast, transport of FITC-G3 cationic dendrimers across the co-culture with mucus was significantly reduced compared to co-cultures absent of mucus. Transport of FITC-G3 PAMAM dendrimers across PPM and CPM was significantly reduced compared to FITC-G3.5 PAMAM dendrimers.

Conclusions An *in-vitro* cell culture model utilizing the cell lines HT29-MTX-E12 and Caco-2 has been developed which expresses a mucus layer. Histological techniques have been used to characterise the model and para-cellular and trans-cellular integrity assessed using permeability studies. Cationic FITC-G3 PAMAM dendrimer transport is retarded by mucin and mucus whilst this effect is not seen with FITC-G3.5 PAMAM dendrimers.

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Tomalia, D. A., et al (1990) *Angew. Chem.ie* **102**: 119–157