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Application of a ternary HP-β-CD-complex approach to improve the dissolution performance of a poorly soluble weak acid under biorelevant conditions

Thomas Zoeller[a,1], Jennifer B. Dressman[a], Sandra Klein[b,*]

[a] Johann Wolfgang Goethe University, Institute of Pharmaceutical Technology, 9 Max von Laue Street, Frankfurt am Main 60438, Germany
[b] Ernst Moritz Arndt University, Department of Pharmacy, Institute of Biopharmacy and Pharmaceutical Technology, 3 Felix Hausdorff Street, Greifswald 17487, Germany

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ABSTRACT

Over the last decades the poor solubility of new drugs has become an important issue, with one of the main challenges being to develop oral dosage forms with acceptable bioavailability for such compounds. The specific purpose of our study was to combine the advantages of cyclodextrins with those of solid dispersion approaches to improve the bioavailability of poorly soluble weak acids. Glyburide, an antidiabetic, was used as a model drug. First, binary drug inclusion complexes were prepared with 2-hydroxypropyl-β-cyclodextrin. Next, solid glyburide dispersions were prepared with polyvinylpyrrolidone (PVP) and a relatively new hydrophilic copolymer, Kollicoat® IR. Finally, to check for potential synergistic effects of the two types of excipients, ternary inclusion complexes were formulated by keeping the 1:2 drug:CD ratio constant but varying the polymer concentration (5–20%). The formulations were analyzed by differential scanning calorimetry and subjected to solubility and dissolution experiments in compendial and biorelevant media. The results of the study clearly indicate that all formulations result in better in vitro performance of the drug. Best results were obtained with the ternary inclusion complexes containing 10% Kollicoat® IR or 20% PVP K30. This formulation approach, particularly with the new polymer, appears to be promising in terms of enhancing the bioavailability of BCS class II drugs.

1. Introduction

In the early 1990s, a new era in drug discovery, characterized by the extensive use of combinatorial chemistry and high-throughput screening methodologies, was initiated. Since these methods make it possible to identify a huge number of potential new drugs within a short time frame, they have become standard methodologies in the field.

In order to classify marketed drug substances and new chemical entities (NCEs) according to their solubility and permeability through biological membranes, the Biopharmaceutics Classification System (BCS) was established in 1995 (Amidon et al., 1995). The BCS classifies drugs into four classes: BCS class I drugs have high solubility and high permeability, whereas drugs belonging to BCS class II have high permeability, but are burdened with poor solubility. Drugs with high solubility and poor permeability can be found in BCS class III and finally, drugs with poor solubility and poor permeability represent BCS class IV compounds. Unfortunately, as a result of the newer screening methods of drug discovery, NCEs often have high potency but, due to their high log *P* values, are characterized by poor solubility in aqueous fluids and often also have poor permeability due to high molecular weight (Lipinski, 2000). Thus, they tend to be classified as BCS class II or IV drugs. Whereas it is more difficult to make oral drug formulations of BCS class IV drugs, there is often a good chance of obtaining sufficient oral bioavailability of a BCS class II drug when the right formulation approach is used. For BCS class II drugs, the dissolution performance is the rate limiting step to drug absorption. Therefore, formulation strategies that improve dissolution properties can greatly enhance the bioavailability of these compounds.

Within the last decades, various strategies have been established to enhance the solubility of drugs (e.g. particle size reduction, salt formation, solid dispersions (SD), lipid based formulations and complex formation with cyclodextrins) (Leuner and Dressman, 2000; Li et al., 2001; Loftsson and Brewster, 1996). Solid dispersions are a well known method for improving solubility, with examples like griseofulvin (Saito et al., 2002), piroxicam (Wu et al., 2009) and tacrolimus (Yamashita et al., 2003) described in the open literature. As early as the middle of the last century, Sekiguchi and Obi had reported that formulations of eutectic mixtures lead to an increase in the solubility of poorly soluble drugs (Sekiguchi and Obi, 1961). Other research groups then also focused their activities on manufacturing solid dispersions (Joshi et al., 2004; Sethia and Squillante, 2004). Various formulation techniques intended to improve the efficiency in achieving glassy solid dispersions, i.e.

* Corresponding author. Tel.: +49 3834 86 4897; fax: +49 3834 86 4886.
E-mail address: Sandra.Klein@uni-greifswald.de (S. Klein).
[1] Present address: Gruenenthal GmbH, 52078 Aachen, Germany.

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formulations, where the poorly soluble drug is embedded in an amorphous state in a carrier, were investigated. These new technologies, such as solvent evaporation, spray drying, freeze-drying and hot melt extrusion (Leuner and Dressman, 2000; Vasconcelos et al., 2007), resulted in formulations that showed a significant increase in drug dissolution rate.

In addition to the preparation of solid dispersions, complexation with cyclodextrins (CDs) has become an important element of formulation development (Loftsson and Duchene, 2007; Stella and He, 2008). Natural CDs are α-1,4 linked oligosaccharides, consisting of 6, 7 or 8 glucose monomers which according to the number of glucose-units are called α-, β- or γ-CDs, respectively. The molecular structure of these glucose derivatives, which approximates a truncated cone or torus, generates a hydrophilic exterior surface and a nonpolar cavity interior. As such, CDs can interact with appropriately sized molecules to result in the formation of inclusion complexes (Loftsson and Brewster, 1996). Depending on the molecular capability (polarity, size and three-dimensional structure) of the guest molecule to form a non-covalent complex, CDs can either host the whole drug molecule or the non-polar part. By doing so, they can increase the aqueous solubility of sparingly soluble guest molecules by orders of magnitude in favorable cases. In addition, CD complexation is often accompanied by a variety of additional physicochemical advantages for the drug-molecule, most notably the stability of the drug or taste masking (Loftsson and Duchene, 2007; Szejtli, 1982). One of the few disadvantages of the natural CDs has been their limited water solubility (1.85 g/100 mL for β-CD) (Loftsson and Brewster, 1996). Despite its suboptimal aqueous solubility, β-CD is currently the most extensively used native CD in marketed products. The main reason for this is the dimensions of its inner cavity which appear to be optimal for many of the currently marketed drugs. However, nowadays the tendency is to modify the parent β-CD resulting in derivatives with increased aqueous solubility (Szejtli, 1998). To date only very few of these derivatives, i.e. methyl- (>50 g/100 mL), hydroxypropyl- (>60 g/100 mL), sulfobutylether- (>50 g/100 mL) derivates of β-CD and hydroxypropyl-γ-CD (>50 g/100 mL) can be found in pharmaceutical products (Brewster and Loftsson, 2007; Davis and Brewster, 2004; Loftsson et al., 2004; Loftsson and Duchene, 2007; Thompson, 1997).

Whereas the natural β-CD is generally recognized as safe (GRAS) by the FDA, approved as a food additive in Europe and Japan and listed in the European- (Ph.Eur.), the United States- (USP/NF) and the Japanese Pharmacopoeia (JP), several of the CD derivatives are cited in the FDA's list of Inactive Pharmaceutical Ingredients but do not enjoy GRAS status (Loftsson and Duchene, 2007). Currently only 2-hydroxypropyl-β-cyclodextrin (Hydroxypropyl Betadex, HP-β-CD) is listed in both Ph.Eur. and USP/NF. The available literature shows that the toxicity of HP-β-CD has been extensively studied. HP-β-CD is well tolerated in most species and humans, particularly if dosed orally. It shows limited toxicity, depending upon dose and route of administration. The main adverse effect observed in humans is diarrhoea. However, to date no effects on kidney have been reported (Gould and Scott, 2005; Irie and Uekama, 1997).

Despite its use in commercially available drug products such as intravenous voriconazole, sulfobutylether-β-cyclodextrin (SBE-β-CD) is not yet listed in any pharmacopoeial monograph. However, studies published recently indicate that in its toxicity is also limited and dependent upon dose and route of administration (Luke et al., 2010). In addition to HP-β-CD and SBE-β-CD, various chemically modified CDs have been described in the literature.

Some of the natural, non-modified CDs have either been associated with hepatotoxicity (Yong et al., 2007) or nephrotoxicity (Frijlink et al., 1991). By contrast, the chemical modifications applied to obtain the various CD derivatives have often greatly reduced the toxicity of the newer CD derivatives (Frijlink et al., 1991; Luke et al., 2010). It is thus likely that the regulatory status of CDs will evolve further, facilitating more frequent use of CD derivatives in pharmaceutical products in the future.

In order to act as a guest molecule in CDs, the drug compound has to fulfill some requirements such as a linear molecular structure and the ability to establish hydrophobic interactions with the CD molecule. Based on its molecular properties, its linear structure and its hydrophobic character, which appeared be ideal to interact with the cavity of CDs, glyburide was deemed to be an appropriate guest molecule. The drug has physicochemical properties typical of poorly water soluble drugs: a high molecular weight (M_W) (494 g/mol), a log P value of 4.8 and a high melting point of 172–174 °C (Lipinski, 2000; Yalkowsky, 1981). The aqueous solubility of glyburide has been reported as 0.06 μg/mL (Avdeef, 2007). Based on its poor solubility in aqueous fluids but high permeability through physiological membranes (Wei and Lobenberg, 2006) glyburide has been categorized as a BSC class II compound (Lindenberg et al., 2004). Since improving the rate and extent of in vivo dissolution typically results in an increased bioavailability of BCS class II compounds, glyburide represented a good candidate for our study. Complex formulation with natural β-CDs has been reported for glyburide previously. Literature data (Buchanan et al., 2002; Savolainen et al., 1998) suggest that glyburide forms 1:2 (mol:mol) drug:CD-complexes with β-CDs and its derivatives. The low single dose of glyburide (1.0–5.0 mg) widely used in the oral treatment of type-2 diabetes mellitus is very suitable for CD complexation since a 1:2 (drug:CD) complex with a CD derivate of high molecular weight (>1000 g/mol) can still be easily administered as an oral drug formulation.

In the present study glyburide was used as a model drug to evaluate the impact of different formulation technologies, including solid dispersions, binary CD inclusion complexes with 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) and the combination of both methods (ternary CD complexes), on its solubility and dissolution rate. Particular attention was given to the performance of ternary complexes. It has been reported that addition of water-soluble polymers can significantly increase the apparent stability constant of a drug:CD complex (Loftsson and Brewster, 1996). Thus, it was hypothesized that the combination of a CD-derivate and a hydrophilic polymer might increase the HP-β-CD complexation efficacy for glyburide and therefore result in an even better drug dissolution than a simple, binary system. Such a performance could then be beneficial in terms of achieving an even higher bioavailability after oral administration of the drug and could also result in reduction of the dose required to achieve the desired pharmacological effect. This effect could be of particular importance for higher dosed poorly soluble NCEs.

Two water-soluble polymers were selected to prepare solid dispersions and also to act as the ternary component in the glyburide:HP-β-CD:polymer complex. The first polymer was polyvinylpyrrolidone (PVP) K30 which, after addition to binary CD complexes, has already been shown to facilitate dissolution of poorly soluble compounds (Fouad et al., 2011; Mura et al., 2001). The second polymer was Kollicoat® IR, a relatively new commercial hydrophilic polyvinyl alcohol–polyethylene glycol graft copolymer, which has been developed to be used for instant release (IR) coatings (Bühler, 2007). The aim of using Kollicoat® IR was to study the feasibility of this novel polymer for preparing ternary CD complexes. In general, Kollicoat® IR is associated with several promising properties. It is a pharmaceutical grade polymer, dissolves rapidly and has an exceptional solubility in water. Aqueous solutions with a concentration of up to 50% can be easily obtained (Bühler, 2007). Moreover, its chemical structure is not ionizable and therefore provides distinct advantages for a drug release that is independent on the pH of the release medium (Fouad et al., 2011). Even though it was originally developed for coating purposes, Kollicoat® IR has

already been used for the manufacture of solid dispersions (Fouad et al., 2011; Janssens et al., 2007) and from our point of view also represented a promising excipient for preparing ternary CD complexes. The Kollicoat® IR polymer might thus represent a useful alternative to currently used polymers and therefore be of interest for industrial applications.

In the screening process of binary and ternary CD complexes physical methods like differential scanning calorimetry (DSC), FT-IR spectroscopy and X-ray diffractometry as well as solubility studies in water are frequently applied. Such experiments can provide excellent information on the physical properties and the aqueous solubility of the drug compound after complexation, but they might not always be helpful to predict the in vivo performance of the respective drug formulation after oral administration. A second objective of the present study was to obtain more detailed information about the potential in vivo behavior of the formulations, so in addition to physical characterization by the above-mentioned methods, solubility and dissolution experiments were performed in both compendial and biorelevant media. The main intention of these experiments was to screen the various formulations with respect to their ability to shift the solubility and dissolution performance of a BCS class II drug to those more like a BCS class I drug and, if possible, to achieve complete drug dissolution under biorelevant conditions.

2. Materials and methods

2.1. Materials

Glyburide (Gly) pure drug (lot # 024K0701) and HP-β-CD (average $M_W \sim 1390$ Da, lot # 130447461207059) were purchased from Sigma–Aldrich Co. (Steinheim, Germany). PVP K30 (average $M_W \sim 50{,}000$ Da, lot # 98988597V0) and Kollicoat® IR (average $M_W \sim 45{,}000$ Da, lot # 30771575L0) were a donation of BASF (Ludwigshafen, Germany). Size 1 white opaque gelatin capsules (Wepa, lot # 35756) were purchased from Wepa Apothekenbedarf GmbH & Co. KG (Hillscheid, Germany). Sodium taurocholate (PCA code 2012; lot # 2003040161) was purchased from Prodotti Chimici e Alimentari S.P.A. (Basaluzzo, Italy). Egg-phosphatidylcholine, Lipoid EPCS (99.1% pure, lot # 105036-1), was kindly donated by Lipoid GmbH (Ludwigshafen, Germany). All other chemicals were of analytical grade and were purchased commercially. Milli-Q-water and ethanol 96% (v/v) used to prepare the complexes were filtered through a 0.45 μm PTFE filter (Whatman-Rezist 30/0.45, Schleicher & Schuell, Dassel, Germany) before use. All equipment coming in direct contact with the formulations was first washed intensively with water, then with ethanol 96% (v/v) and subsequently dried at 70 °C for 24 h.

2.2. Preparation of solid dispersions (SDs) and inclusion complexes

First, solid dispersions of glyburide were prepared with PVP K30 (Leuner and Dressman, 2000). Then, Kollicoat® IR which, as already mentioned, has originally been developed as a coating polymer (Bühler, 2007) was investigated for the same purpose. The drug load of all solid dispersions was approximately 15 wt%.

Next, HP-β-CD was used to form an inclusion complex with glyburide. As indicated before, glyburide tends to form 1:2 (mol:mol) complexes with β-CD and its derivatives (Buchanan et al., 2002). Thus, corresponding amounts of drug and HP-β-CD were used for the complexation. The drug load of the 1:2 complexes was approximately 15 wt% in all cases, enabling direct comparison of the impact of different excipients on the performance of the formulation. It has been reported that by means of interacting with the outer surface of CDs and drug:CD complexes, hydrophilic polymers can form co-complexes or aggregates (ternary complexes) that show higher stability constants values than those for the binary drug:CD system (Asbahr et al., 2009). Therefore, in a final step the influence of adding a third component to an existing binary system of glyburide and HP-β-CD was investigated. For this purpose a range of ternary complexes was prepared by adding different amounts, i.e. 5 wt%, 10 wt% and 20 wt% of either PVP K30 or Kollicoat® IR to the binary glyburide:HP-β-CD 1:2 (mol:mol) complex. Subsequently, the impact of polymer concentration on the dissolution rate of glyburide was studied.

Binary and ternary complexes were prepared as follows: 5.628 g of HP-β-CD was exactly weighed and dissolved in 6.00 g Milli-Q-water. A clear solution (solution A) was obtained in all cases. For the ternary complexes, the required amount of PVP K30 or Kollicoat® IR (0.362 g, 0.724 g or 1.447 g corresponding to 5 wt%, 10 wt% or 20 wt% of the binary complex) was added to solution A.

A second solution was prepared by first dissolving ammonium carbonate (300 mg) in purified water (14.7 mL) and mixing this solution with ethanol 96% (25 mL). The resulting mixture which had a pH of about 9 (Klein et al., 2009) was added to a vial containing glyburide (1.00 g). Subsequently, if required, the mixture was sonicated for 30 min at a temperature of 50 °C (Transsonic Digital T700H, Elma, Singen, Germany) and then stirred on a magnetic stirrer (MR 2001 K, Heidolph Instruments, Schwabach, Germany), applying a stirring speed of 800 rpm until equilibration to room temperature to obtain a clear solution (solution B). Finally, solutions A and B were mixed and again stirred on a magnetic stirrer for an additional 24 h. The resulting mixture (clear solution) was filtered through a 0.45 μm PTFE filter into a round bottom flask and freeze-dried (Modulyo, B.O.C. Ltd., Crawley, England) for 24 h at 5 mbar. The resulting powder was then mixed with liquid nitrogen, homogenized using a mortar and a pestle and dried under vacuum at 30 °C for another 24 h.

Solid dispersions were prepared by dissolving 5.628 g of PVP K30 or Kollicoat® IR in 6.00 g Milli-Q-water to obtain solution A, filtering and freeze-drying as described above.

2.3. Preparation of the physical mixtures

To elucidate the impact of the preparation technique on the amorphous state of glyburide, its solubility and dissolution rate, an additional series of experiments was conducted on physical mixtures (PMs) of glyburide, CDs and/or the polymers. PMs were prepared in all drug:HP-β-CD, polymer or HP-β-CD:polymer combinations used to prepare complexes and solid dispersions. All PMs were prepared by simply blending drug and excipients with a mortar and pestle.

2.4. Differential scanning calorimetry (DSC)

As the presence of crystalline glyburide might have a significant impact on its solubility and dissolution rate, all glyburide formulations were screened for crystalline glyburide by DSC.

DSC thermograms of glyburide, its inclusion complexes and PMs were recorded directly after preparation using a Perkin Elmer DSC 7 (Waltham, USA). The samples (5–10 mg) were precisely weighed and heated in a perforated aluminum pan under nitrogen atmosphere. A heating rate of 10 °C/min over a temperature range of 50–220 °C was used in all experiments.

2.5. Solubility studies

Solubility studies were performed with the pure drug and selected formulations. Various compendial buffer media were chosen to represent the range of pH values in the upper human

gastrointestinal tract (GIT), i.e. stomach and upper small intestine. Biorelevant media were used to better mimic conditions in the upper small intestine, particularly the presence of physiological concentrations of bile salts and lecithin in the small intestinal lumen. In addition, the selection of media took into account media corresponding to BCS requirements (pH 1.2, 4.5 and 6.8) (World Health Organization, 2005) to determine if the test formulations could shift the solubility and dissolution performance of glyburide from BCS class II to BCS class I. The set of test media therefore comprised: Simulated Gastric Fluid *sine pepsin* USP 31 (SGFsp pH 1.2), acetate buffer pH 4.5, the bile salt and lecithin containing media Fed State Simulated Intestinal Fluid (FeSSIF) pH 5.0 and Fasted State Simulated Intestinal Fluid (FaSSIF) pH 6.5 (Klein, 2010; Marques, 2004), their corresponding buffers without bile compounds: Blank FeSSIF pH 5.0 and Blank FaSSIF pH 6.5 (Klein, 2010; Marques, 2004) and Simulated Intestinal Fluid *sine pancreatin* USP 31 (SIFsp pH 6.8).

All solubility studies were performed in triplicate using a miniaturized shake-flask method, i.e. Uniprep® filter systems (Whatman GmbH, Dassel, Germany) (Glomme et al., 2005), consisting of a polypropylene (PP) housing and a punch with an integrated 0.45 μm PTFE filter. 3 mL of the respective test medium was placed in the PP housing and an excess of substance was added. The Uniprep® filter systems were then closed without completely pushing down the punch, placed on a rotary shaking plate (Titramax 1000, Heidolph Instruments, Schwabach, Germany) and shaken at 800–1200 rpm at 37 ± 0.5 °C for 72 h to reach equilibrium. During the mixing period, the filter systems were inspected after 24, 48 and 72 h to ensure that each well still contained solid drug and to check the pH value. Drug was added if necessary to maintain an excess and the pH was adjusted to the original pH of the test medium if necessary. After 72 h of mixing, the punch of each Uniprep® filter was pushed down, which resulted in a filtration of the saturated drug solution. Subsequently, the filtrate was sampled and diluted immediately with HPLC mobile phase to prevent dissolved glyburide from precipitating as a result of temperature changes.

2.6. Dissolution studies

An USP apparatus II (Erweka DT 6, Heusenstamm, Germany) was used for all dissolution studies. Experiments were performed in triplicate using 500 mL of test medium at a temperature of 37 ± 0.5 °C and a stirring speed of 75 rpm (Buchanan et al., 2007). The dissolution media used for the dissolution experiments corresponded to those used in the solubility studies and, as already mentioned, were selected to represent the typical pH range in the upper human gastrointestinal tract and also to cover BCS conform conditions (see Table 1).

Glyburide (3.5 mg) or a corresponding amount of the respective test formulation was accurately weighed into a size 1 white opaque gelatin capsule. A helix wire sinker was then attached to keep the capsule at the bottom of the vessel until completely dissolved. Samples were periodically removed over 4 h using a 5 mL glass syringe, immediately filtered through a 0.45 μm PTFE filter and appropriately diluted prior to HPLC analysis. The results were expressed as mean % drug released (±SD) at the given sampling time.

In order to screen for the most effective formulation approach for potentially improving the in vivo performance of glyburide, we chose a simple dissolution approach: if a formulation could facilitate substantial dissolution in a buffer medium, it should be able to improve the in vivo performance as well. Thus, a physiologically based but still easy to handle and most important; discriminative dissolution medium needed to be identified. A set of preliminary experiments to compare drug release of a marketed glyburide formulation (Klein et al., 2009) and some glyburide:HP-β-CD formulations in the various test media shown in Table 1 was therefore conducted (data not shown here). The results from these experiments indicated that Blank FeSSIF pH 5.0 was the most discriminating medium for our purpose. In media having a pH value above the pK_a (5.4) of glyburide, the various formulations showed almost 100% drug release within 15–20 min, so these media were not sufficiently discriminating. In contrast, in media having a pH lower than the pK_a, dissolution was poor and it was therefore difficult to discriminate among formulations. Only in Blank FeSSIF pH 5.0 was a clear discrimination among the various formulations possible. Thus, this medium was subsequently used for further screening experiments.

Table 1
Compendial and biorelevant test media used in the solubility and dissolution experiments (Klein et al., 2009).

Test medium	pH	mOsmol/kg	Gastrointestinal segment	Type of medium
SGFsp	1.2	182	Stomach	Compendial and BCS-conform
Acetate buffer	4.5	291	Duodenum	Compendial and BCS-conform
Blank FaSSIF	6.5	266	Upper small intestine fasted	"Biorelevant" (pH)
FaSSIF[a]	6.5	273	Upper small intestine fasted	Biorelevant
Blank FeSSIF	5.0	622	Upper small intestine fed	"Biorelevant" (pH)
FeSSIF[a]	5.0	646	Upper small intestine fed	Biorelevant
SIFsp	6.8	100	Small intestine	Compendial and BCS-conform

[a] Contains physiological concentrations of bile components.

2.7. HPLC analysis

Samples from solubility and dissolution studies were analyzed by HPLC. The HPLC system consisted of a L6220 pump, an AS2000A auto sampler, a L4500 diode array detector and a D6000A interface (Merck Hitachi, Darmstadt, Germany). The analyses were performed on a 125/4 ET Nucleosil 120-^{5}C–^{18}column (Macherey-Nagel, Dueren, Germany), using a mixture of acetonitrile and purified water (65:35), adjusted to pH 3.0 with phosphoric acid, as mobile phase. The flow rate was set at 1.0 mL/min and the amount of released drug was determined at a wavelength of 230 nm (Klein et al., 2009). All samples were diluted with mobile phase in a ratio of 1:2 before injection. The linearity of the analytical method had previously been shown for all media over a concentration range of 0.288–5.76 μg/mL ($y = 85{,}796x + 1706.8$, $r^2 = 0.9999$). The limits of detection and quantification were 0.144 μg/mL and 0.288 μg/mL, respectively. The coefficient of variation was less than 1.5% for the intraday precision and less than 0.6% for the accuracy. The recovery of the method was 101.7–104.1% (Klein et al., 2009).

2.8. Statistical analysis

The amount of pure drug dissolved in biorelevant and BCS-conform dissolution media after 240 min was compared to the amount of glyburide released from the glyburide formulations at the same time point and tested with the same test conditions using an unpaired *t*-test (two-tailed *P* values). The results from the solubility studies of the pure drug and the glyburide formulations after 72 h were also compared using an unpaired *t*-test (two-tailed *P* values). All calculations were performed with Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA). A result of $P < 0.05$ denoted a significant difference in all cases.

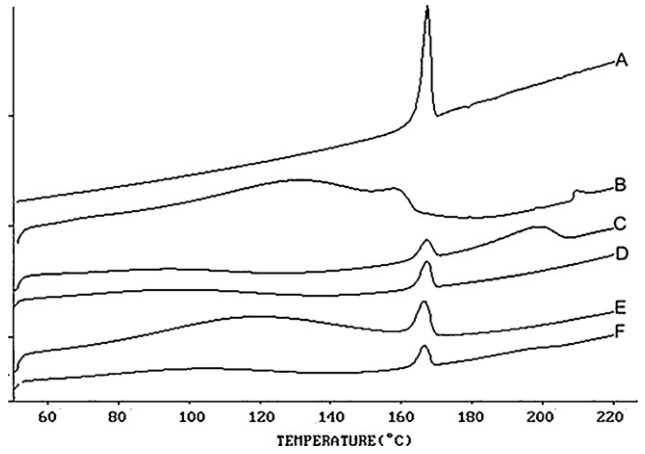

Fig. 1. DSC thermograms of Gly pure drug (A) and physical mixtures with various polymers and/or HP-β-CD; Gly:PVP K30 (B), Gly:Kollicoat® IR (C), Gly:HP-β-CD (D), Gly:HP-β-CD:PVP K30 (E), and Gly:HP-β-CD:Kollicoat® IR (F).

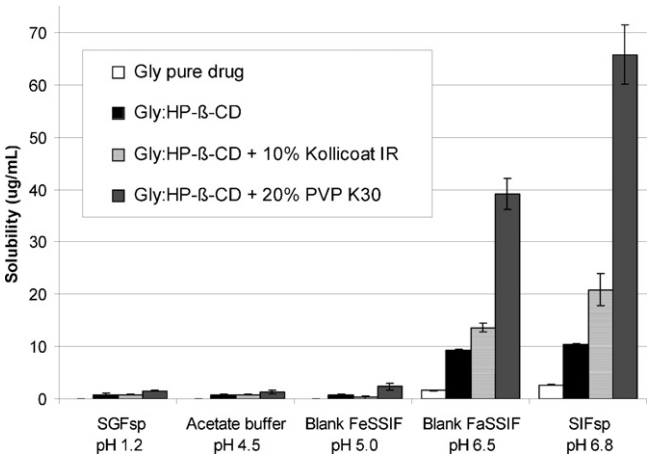

Fig. 3. Solubility of glyburide pure drug, a binary Gly:HP-β-CD complex and ternary Gly:HP-β-CD + Kollicoat® IR (10%) and Gly:HP-β-CD:PVP K30 (20%) complexes in selected test media ($n = 3 \pm SD$).

3. Results and discussion

3.1. Differential scanning calorimetry (DSC)

The DSC thermograms of the pure drug, the PMs, solid dispersions, the binary HP-β-CD complex and the ternary complexes obtained by adding 5% of PVP K30 or Kollicoat® IR during the preparation of the complex are shown in Figs. 1 and 2, respectively.

The thermogram of the pure drug (Fig. 1A) shows a sharp endothermic peak at 170 °C, which corresponds to the melting point of crystalline glyburide. DSC curves of the PMs (Fig. 1) show endothermic peaks in the temperature range of 170–175 °C, which indicate the presence of crystalline glyburide in these formulations. The thermogram of the PM of glyburide and PVP K30 (Fig. 2B) deviates somewhat from the others and shows a broad peak over a temperature range of 100–160 °C. There can be various reasons for the change in shape and location of this peak. For instance, it could be the result of a solid state interaction between glyburide and the hydrophilic polymer during heating or possibly due to elimination of residual water. For an exact interpretation of the thermal behavior of this PM additional techniques of physical characterization would be required. However, as overall the DSC results clearly indicate that by simple blending of drug and excipients it was not possible to obtain amorphous formulations, it was concluded that PMs do not represent the most favorable formulation approach.

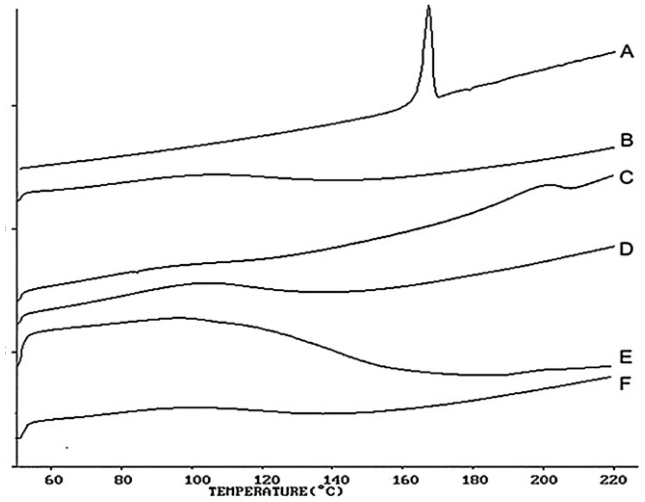

Fig. 2. DSC thermograms of Gly pure drug (A), Gly:PVP K30 (B), Gly:Kollicoat® IR (C) solid dispersions, Gly:HP-β:CD (D) binary complexes and Gly:HP-β:CD-PVP K30 (E), Gly:HP-β-CD:Kollicoat® IR (F) ternary complexes.

Therefore, we then focused on comparing the formulations in solubility and dissolution experiments. In Fig. 2, which shows the thermograms of glyburide solid dispersions, binary and ternary HP-β-CD-complexes, no peak can be observed in the melting range of glyburide (A). This indicates that the formulations, but not the PMs, consist primarily of amorphous material. Based on these observations, it is reasonable to expect that the dissolution performance of the latter formulations would be superior to that of the pure drug or the PMs.

3.2. Solubility studies

Solubility studies were performed with a subset of drug:excipient combinations, i.e. the binary glyburide:HP-β-CD complex and the two ternary complexes, glyburide:HP-β-CD + 10% Kollicoat® IR and glyburide:HP-β-CD + 20% PVP K30. The results were then compared with those obtained for with the pure drug in corresponding media (see Fig. 3).

As expected for a weakly acidic drug, a strong pH dependence of the solubility could be observed for all test formulations. The solubility was low in media having a pH lower than the pK_a value of glyburide and was not affected by formulation. However, in Blank FaSSIF pH 6.5 and SIFsp pH 6.8, the impact of excipients in the simple formulations was obvious. The complexation of glyburide with HP-β-CD resulted in a significantly higher solubility of glyburide compared to its crystalline form in Blank FaSSIF pH 6.5 ($P < 0.001$) and in SIFsp pH 6.8 ($P < 0.001$). The use of Kollicoat® IR as a ternary component in the glyburide complex resulted in a further enhancement of the solubility of the drug. However, the highest solubility values were observed using PVP K30 as the ternary component. Compared to the solubility of the pure drug, a 25-fold higher glyburide concentration (65.8 μg/mL) was observed in SIFsp pH 6.8 for the ternary glyburide:HP-β-CD + 20% PVP K30 complex.

To determine whether the formulations could achieve properties close to those of a BCS class I drug, the dose:solubility (D:S) (Amidon et al., 1995) ratio, representing the fluid volume that would be required to dissolve the highest single dose of a drug, was calculated. Assuming a maximum single dose of 5 mg, the D:S in SIFsp pH 6.8 was decreased from 1901 mL for the pure drug to 239 mL for the ternary complex using Kollicoat® IR and to 76 mL for PVP K30, respectively. These results suggest that, if administered by in form of one of these two complex formulations, the drug could be regarded as highly soluble in the small intestine (jejunum).

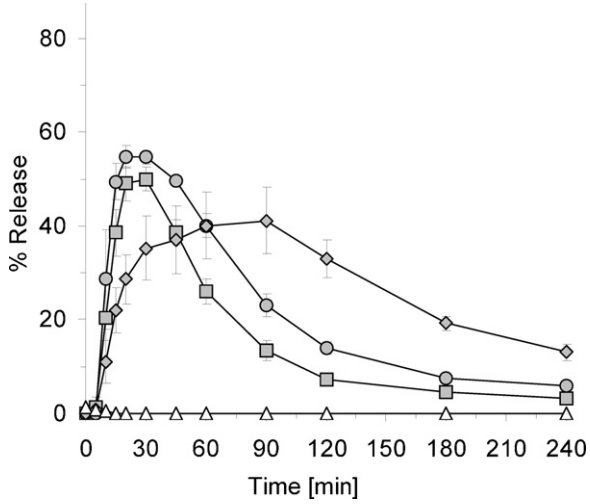

Fig. 4. Glyburide dissolution from pure drug (△), solid dispersions (SD) of glyburide and PVP K30 (□), Kollicoat® IR (○) and the binary HP-β-CD complex (◊) in Blank FeSSIF pH 5.0 ($n = 3 \pm SD$).

However, calculating the D:S ratio based on the solubility at pH 1.2 and 4.5 results in D:S ratios that were still higher than 250 mL indicating that in the stomach and duodenum BCS class I properties were not achieved with these formulations.

3.3. Dissolution studies

The first set of experiments was performed to study drug release from glyburide, the solid dispersions and a binary complex.

Fig. 4 shows the dissolution profiles of the pure drug, the SDs of glyburide with PVP K30 or Kollicoat® IR as polymeric carrier and the binary glyburide:HP-β-CD complex in Blank FeSSIF pH 5.0. The pure drug showed no dissolution within 4 h. In contrast, all formulations showed a rapid drug release within the first 20–30 min. This significant increase in the dissolution rate of products is most likely a result of the high-energy amorphous state of the products (Asbahr et al., 2009) and the excellent solubility of the chosen excipients. The maximum concentration of glyburide released in Blank FeSSIF and time of maximal glyburide concentration observed in the experiments differed depending on the test formulation. The dissolution profiles of the glyburide SDs with PVP K30 and Kollicoat® IR were characterized by a rapid release but also by pronounced precipitation of the drug after reaching the maximum of ~50–55% of the dose dissolved within 30 min. The binary glyburide:HP-β-CD complex also showed a relatively fast onset of drug release, but compared to the SDs, somewhat less drug was released and the maximum of ~40% drug released was reached after a longer test duration (90 min). However, the precipitation rate was lower than observed for the SDs. As a result, far more drug remained in solution after the 240 min test duration in the HP-β-CD complex experiments than in those performed with the SDs. Nevertheless, the amount of glyburide remaining dissolved after 240 min was significantly higher than for the pure drug for all binary formulations, with $P < 0.005$ for the binary HP-β-CD complex, $P < 0.001$ for the Kollicoat® IR and $P < 0.002$ for the PVP K30 solid dispersion.

The next set of experiments was performed to evaluate potential synergistic effects of polymers and HP-β-CD in terms of increasing the rate and extent of glyburide release, and in terms of possibly reducing the precipitation rate of the drug after dissolution. For this purpose, ternary complexes containing different amounts of PVP K30 or Kollicoat® IR as well as the HP-β-CD were subjected to dissolution experiments. The resulting profiles are shown in Fig. 5.

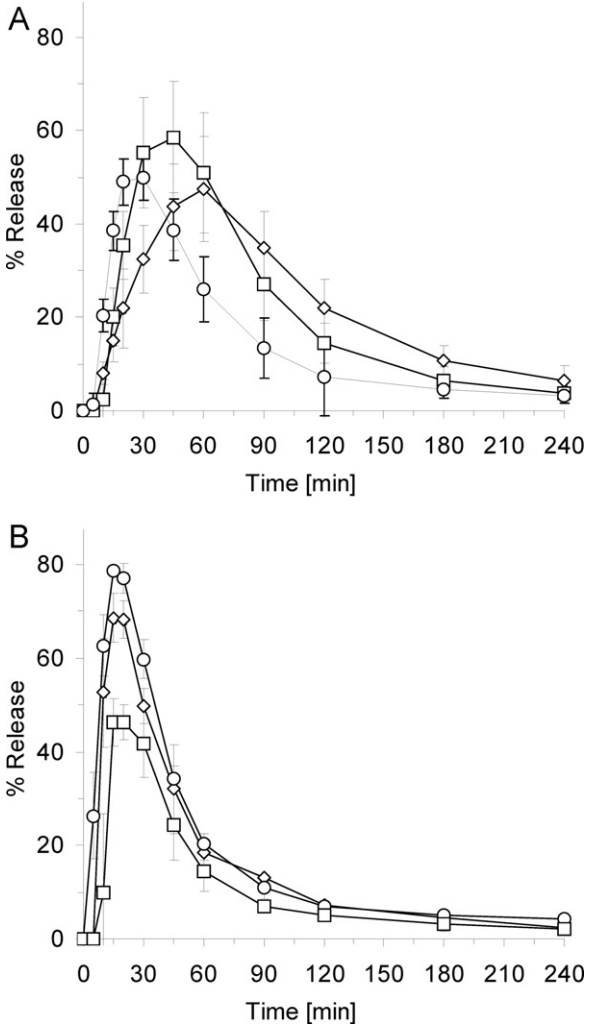

Fig. 5. Glyburide dissolution from ternary HP-β-CD:PVP K30 (left panel) and HP-β-CD:Kollicoat® IR (right panel) complexes obtained by addition of 5% (◊), 10% (○) and 20% (□) polymer during preparation of the complexes in Blank FeSSIF pH 5.0 ($n = 3 \pm SD$).

The results shown in Fig. 5 clearly indicate that a combination of hydrophilic polymers with HP-β-CD can further improve the dissolution performance of glyburide. Compared to the binary glyburide:HP-β-CD complex the extent of drug release was higher and also higher than from the solid dispersion made with the respective polymer. With a focus on the initial release rate and the maximum amount of drug released, ternary complexes obtained by adding 10% of Kollicoat® IR during the preparation of the complex were superior to all other formulations. However, as observed with the glyburide:Kollicoat® IR SDs, in vitro this improved dissolution behavior was associated with rapid precipitation. With respect to precipitation, the ternary complexes containing PVP K30 were superior to those containing Kollicoat® IR; although the initial drug release rate was somewhat slower, precipitation was delayed and also occurred more slowly. The improved dissolution behavior of the ternary complexes is most likely a result of the interplay of various phenomena, some of which are still unresolved, promoting both formation of soluble complexes and conversion of the drug to the amorphous form during the preparation of the complex (Mura et al., 2001). A potential mechanism for increasing the dissolution rate is the formation of a Kollicoat® IR or PVP K30 layer on the surface of the drug:HP-β-CD complex by interactions between the outer surface of the CD molecule and the hydrophilic polymer. As a

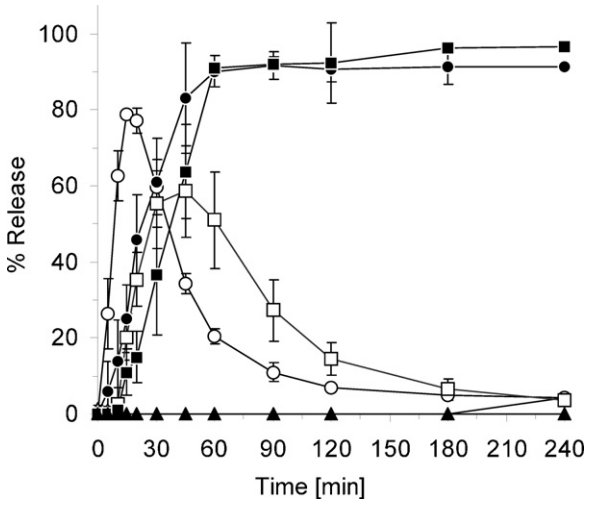

Fig. 6. Glyburide dissolution from pure drug (△), a ternary HP-β-CD:Kollicoat® IR 10% (○) and a ternary HP-β-CD:PVP K30 20% (□) complex in Blank FeSSIF (open symbols) and FeSSIF (closed symbols) ($n = 3 \pm SD$).

result of the hygroscopicity of the polymer this layer may increase the wettability of the formulation and, depending on the physicochemical properties of the polymer, may result in a faster or even instant drug release. From the dissolution profiles presented in Fig. 5, it is obvious that the optimal polymer concentration relates to the type of polymer. Concentrations higher and lower than the optimal concentration are often of no benefit, as can be seen especially in the release profile of the ternary complex obtained by addition of 20% Kollicoat® (see Fig. 5B).

Overall, comparing ternary complexes obtained by addition of 5, 10 or 20 wt% of polymer during complex preparation, the formulations with 10% Kollicoat® IR and 20% PVP K30 showed the highest maximum amount of drug released and were therefore selected for further investigation in the complete set of BCS-conform and biorelevant dissolution media (see Table 1). The results of the dissolution experiments in Blank FaSSIF pH 6.5/FaSSIF pH 6.5 and in Blank FeSSIF pH 5.0/FeSSIF pH 5.0 are shown in Figs. 6 and 7, respectively.

The dissolution performance of the ternary complexes showed a strong pH dependence, with precipitation observed in all compendial dissolution media having pH values lower than the pK_a of glyburide of 5.3 (data not shown here). In contrast, in Blank

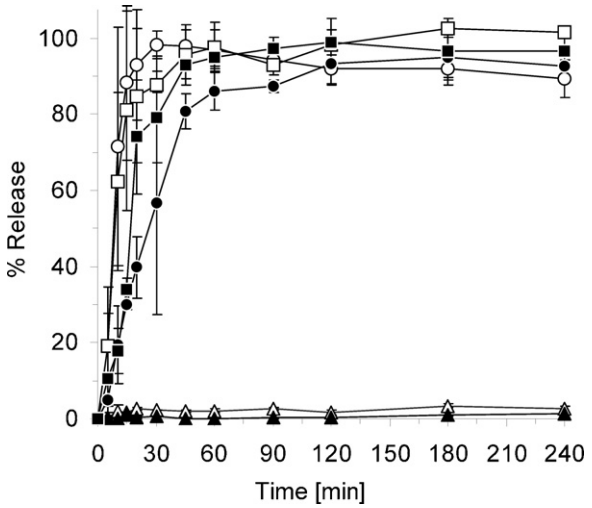

Fig. 7. Glyburide dissolution from pure drug (△), a ternary HP-β-CD:Kollicoat® IR 10% (○) and a ternary HP-β-CD:PVP K30 20% (□) complex in Blank FaSSIF (open symbols) and FaSSIF (closed symbols) ($n = 3 \pm SD$).

Table 2
P-values of the amount of drug dissolved from the ternary complexes compared with that of the pure drug in different dissolution media after 240 min test duration.

Test medium	pH	Kollicoat® IR	PVP K30
SGFsp	1.2	$P < 0.009$	$P < 0.001$
Acetate buffer	4.5	$P < 0.009$	$P < 0.003$
Blank FaSSIF	6.5	$P < 0.009$	$P < 0.002$
FaSSIF[a]	6.5	$P < 0.001$	$P < 0.001$
Blank FeSSIF	5.0	$P < 0.001$	$P < 0.001$
FeSSIF[a]	5.0	$P < 0.001$	$P < 0.002$
SIFsp	6.8	$P < 0.001$	$P < 0.001$

[a] Contains physiological concentrations of bile components.

FaSSIF having a pH of 6.5 complete release was observed within the first 30–60 min and no precipitation took place over the entire test duration of 240 min (see Fig. 6). The same performance could be observed in FaSSIF pH 6.5, the corresponding biorelevant medium, which contains bile salts at concentrations representative of the fasted small intestine. These results indicate that in the fasted small intestine, precipitation will most likely not occur and the entire dose of drug will be available for absorption.

As in most of the compendial media, precipitation in Blank FeSSIF pH 5.0 was observed for both formulations. However, in FeSSIF pH 5.0, a medium having the same unfavorable pH but covering additional relevant parameters of the small intestinal contents in the fed state, i.e. the presence of relatively high amounts of bile components, complete drug release was observed within 20 min. Moreover, in contrast to observations made in Blank FeSSIF, no precipitation was seen in FeSSIF. This was most likely a result of the solubilizing properties of the natural bile salts. Similar to the in vitro data representing the fasted state, it was also concluded that the entire amount of glyburide will be available after oral administration in the fed state.

Overall, these results agree well with the data from the solubility studies. It can be summarized that ternary complexes containing 10% Kollicoat® IR or 20% PVP K30 resulted in both a significantly higher dissolution rate and amount of drug released after 240 min when compared with the pure drug (see Table 2).

As the dissolution performance of both ternary complex formulations was much better than that of the pure drug in all media and, as both solubility and dissolution experiments indicate good dissolution performance under BCS conform and excellent performance under biorelevant conditions representing the small intestine, bioavailability problems are not expected for these formulations. It is also clear from the data that there are no unfavorable interactions between bile compounds and cyclodextrins with respect to solubility and dissolution properties. Moreover, the results obtained in the present study clearly show the importance of screening formulations with biorelevant media since the corresponding results will most probably better reflect the real in vivo performance of the drug formulation. For the ternary complexes containing 10% Kollicoat® IR or 20% PVP K30 this would mean 100% release and no drug precipitation in either the fasted or fed small intestine, indicating that the solubility/dissolution limitations to bioavailability are essentially removed with these formulations.

4. Conclusion

It can be concluded that SDs made from hydrophilic polymers Kollicoat® IR and PVP K 30 as well as binary HP-β-CD complexes are able to increase the solubility and the dissolution rate of the poorly soluble drug glyburide. Kollicoat® IR proved to be a new and useful alternative to be applied in such formulations. It was further shown that the combination of these two formulation strategies results in even better solubility and more reliable drug release of glyburide from the formulation. In SIFsp pH 6.8, one of the three

relevant media to classify drugs according to the BCS and reflecting average pH-conditions of the main site of glyburide absorption, i.e. the small intestine (Brockmeier et al., 1985), with ternary complexes it was possible to obtain a glyburide solubility that would be sufficient for classification as a BCS class I compound. Experiments performed in FaSSIF pH 6.5 and FeSSIF pH 5.0, the two biorelevant media simulating conditions in the upper fasted and fed state small intestine respectively indicate that no bioavailability problems would be expected with the ternary complexes. The ability of this formulation and screening approach to improve oral exposure of glyburide needs now to be confirmed with in vivo studies. It is most likely that this formulation approach can successfully be adopted for other (structurally related) weakly acidic drugs and this is also something to be investigated in future experiments.

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