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# Biorelevant dissolution media as a predictive tool for glyburide a class II drug

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ABSTRACT

The purpose of this study was to predict the oral absorption of glyburide. Biorelevant dissolution methods, combined with permeability measurements and computational simulations, were used to predict the oral absorption of glyburide. The objective was to establish *in vitro/in vivo* correlations (IVIVCs) based on the biopharmaceutics drug classification system. The solubility of the glyburide powder was measured in different media. The dissolution behavior of two commercial tablet formulations was tested in different media. Two chemical grades of sodium taurocholate: low quality (LQ) = crude and high quality (HQ) = 97% purity, and egg-lecithin: LQ = 60% and HQ = 99.1% purity were used to prepare fasted state small intestinal fluid (FaSSIF). Simulated intestinal fluid (SIF) and blank FaSSIF without lecithin and taurocholate (BL-FaSSIF) were used as controls. The dissolution tests were performed under constant pH and dynamic pH conditions. The dynamic pH range from 5.0 to 7.5 simulated the biological pH range of gastrointestinal (GI) tract in the fasted state. The drug permeability was studied using Caco-2 cell line. The predictions of the fraction dose absorbed were performed using GastroPlus™. The results of the simulations were compared with actual clinical data taken from a bioequivalence study. The solubility of glyburide was highest in LQ-FaSSIF. The two tablet formulations had ~~significant~~ different dissolution behaviors in LQ-FaSSIF. The *in vitro* data ~~were~~ used as the input function into a simulation software. The dynamic LQ-FaSSIF dissolution data achieved the best prediction of the average AUC and $C_{max}$ of the clinically observed data. The present study shows that BCS based parameters combined with software simulations can be used to establish an IVIVC for glyburide. *In vitro/in silico* tools can potentially be used as surrogate for bioequivalence studies.



## 1. Introduction

Oral dosage forms are the most common formulations because of their convenient administration and their ~~economic way of manufacturing~~ (Goodman et al., 1999). In order to ~~develop~~ successfully an oral product, ~~the~~ formulation scientists have to investigate the physicochemical properties of all potential drug candidates. These include but are not limited to solubility, bulk density, $pK_a$, crystallinity, osmolality, pH, X-ray diffraction, IR spectra, density, particle size and surface area. High throughput *in vitro* technologies are commonly used for such screenings (Parrott and Lavé, 2002). These methodologies are optimized to characterize one ~~parameter~~ at a time. The disadvantage of such specialized tests is that they use artificial test conditions which might not reflect the drugs behavior in a biological environment. This is especially important for poorly

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soluble drugs. The *in vivo* performance and bioavailability of drugs ~~however, has to~~ be studied in patients. Due to the time consuming procedure and the high costs of clinical studies, *in vitro* and *in vivo* correlations (IVIVCs) are highly desirable to predict the *in vivo* performance of dosage forms (Vogelpoel et al., 2004). Therefore, the development of more universal *in vitro* methods ~~which~~ can be used to estimate the *in vivo* performance of a potential drug product in an early stage of the development process is highly desirable.

A mechanistic approach to the oral drug absorption was developed by Amidon et al. (1995) and is known as the biopharmaceutics drug classification system (BCS). It defined two fundamental parameters: solubility and permeability. Both are the key variables ~~to govern~~ rate and extent of oral drug absorption. Based on the theory of the BCS and the physiology of the gastrointestinal (GI) tract, a mathematical model was developed called Compartmental Absorption and Transit (CAT) model (Yu et al., 1996b). The CAT model can be used to predict the oral absorption of drugs. Compared to traditional models, such as the Single-Tank mixing model (Sinko et al., 1991) or the macroscopic mass balance (Oh et al., 1993), the CAT model adopted the physiological GI conditions much better (Yu et al., 1996a). However, the CAT model does not consider any absorption in the stomach or the colon. A new model called Advanced Compartmental Absorption and Transit model (ACAT) was developed by Simulations Plus Inc. and is available under the name GastroPlus™. The ACAT model includes more physicochemical and physiological factors, and accounts for the stomach and colon (GastroPlus™ Manual, 2004).

In order to predict the oral drug absorption the software requires certain input parameters. Such parameters should reflect the *in vivo* ~~situation~~ and include solubility and permeability. For poorly soluble drugs, the dissolution might be directly influenced by the solubility of the drug substances in the intestinal juices. If the permeability of a poorly soluble drug is high, its *in vivo* dissolution behavior might be the limiting/controlling factor of drug absorption (Galia et al., 1998). Therefore, for computer simulations it is important to develop *in vitro* dissolution methods that can simulate the *in vivo* dissolution behavior.

*In vitro* dissolution tests are standard methods accepted by regulatory agencies to assess the biopharmaceutical quality of drug products (Löbenberg et al., 2000). Drug release tests are routinely used in pharmaceutical industry for quality control and drug development (Costa and Lobo, 2001). Pharmacopoeias like the USP list several different dissolution apparatuses~~:~~ basket~~,~~ paddle, reciprocating cylinder or flow through cell ~~to name the most common ones.~~ The basket and paddle apparatus is routinely used because of its easy handling (Löbenberg et al., 2000). The simulation of the *in vivo* dissolution in such an apparatus is challenging because it may only simulate one condition at a time~~, e.g. gastric environment.~~ However, to be able to simulate the *in vivo* dissolution behavior changing environments are needed. The development of suitable dissolution media with changing environmental conditions is a critical issue, especially for the poorly soluble drugs. There are various dissolution media described in the national pharmacopoeias including simulated intestinal fluid (SIF) and simulated gastric fluid (SGF) ~~(Test Solutions, USP 28)~~.

~~The nature of t~~hese media ~~are~~ buffers that cover~~s~~ the physiological pH range from 1.2 to 6.8 (Löbenberg and Amidon, 2000). For many poorly soluble drugs, the *in vitro* dissolution in such media will not produce useful information because pH is not the only factor which influences solubility and drug release (Jinno et al., 2000). The modifications ~~of~~ dissolution media such as adding surfactants (Löbenberg and Amidon, 2000) or using emulsion or organic solvent were investigated in the past (El-Massik et al., 1996). But these modified media might not reflect ~~the~~ *in vivo* conditions ~~similarly~~. In order to improve *in vitro/in vivo* conditions, the dissolution media should mimic the physiological environment of the GI tract (Galia et al., 1998). New biorelevant dissolution media (BDM) were developed and published in the 1995 FIP guidance: fasted state simulated intestinal fluid (FaSSIF) and fed state simulated intestinal fluid (FeSSIF). They contain bile salts (sodium taurocholate) and lecithin to simulate the physiological environment in the GI tract (Dressman et al., 1998). The advantage of using these media is that they might simulate the *in vivo* dissolution. The *in vitro* dissolution can then be used to predict the oral drug absorption (Löbenberg et al., 2000).

Glyburide is a second-generation sulfonylurea. It is orally used as hypoglycemic agent to treat non-insulin-dependent (type II) diabetes mellitus (Pearson, 1985; Neuvonen and Kivisto, 1991). The aqueous solubility of the glyburide is low, and highly pH-dependent in the physiological range due to its $pK_a$ of 5.3 (Löbenberg et al., 2000). Previous studies have demonstrated that the oral absorption of the glyburide is formulation-dependent (Neugebauer et al., 1985). Blume et al. (1993) had shown that the dissolution behaviors of different formulations play an important role in the oral performance and the bioavailability of this drug.

In this study, we investigated two commercial glyburide formulations. Since the glyburide should be administered before a meal to obtain sufficient pharmacokinetic profiles, we only investigated the fasted state medium FaSSIF (Otoom et al., 2001; Euglucon N. Rote Liste, 2005). The research presented in this paper focusses on the dissolution behavior of glyburide formulations in the FaSSIF of different chemical ~~purity~~. The dissolution behaviors in other media including simulated intestinal fluid ~~(Test Solutions, USP 23)~~ and the blank-FaSSIF without bile salts and lethicin were also studied as controls. The obtained *in vitro* dissolution profiles were used as input function, in GastroPlus™ to predict the oral absorption. The establishment of *in vivo/in vitro* correlations (IVIVCs) is discussed.

## 2. Material and methods

### 2.1. *Chemicals*

Sodium taurocholate crude (low quality: LQ) and 97% pure (high quality: HQ) were purchased from Sigma–Aldrich (USA). Egg-lecithin 60% (LQ) was purchased from ICN (USA). Egg-phospharidylcholine, Lipoid E PC 99.1% pure (HQ) was a gift from Lipoid GmbH (Ludwigshafen, Germany). Potassium dihydrogen phosphate, potassium chloride, sodium chloride, sodium hydroxide, phosphoric acid and hydrochloride acid (analytical grade) were purchased from BDH (USA).

Two 3.5 mg glyburide tablets were used. Euglucon N® 3.5 mg tablets as reference product (Lot# 01N400, Boehringer Mannheim/Hoechs, Germany) and Glukovital® 3.5 mg tablet as test product (Lot# 09601, Dr. August Wolff Arzneimittel, Bielefeld, Germany).

Dulbecco's Modified Eagle's Medium (DMEM), L-glutamine, transferrin, trypsin-EDTA and HEPES were purchased from the GIBCO BRL Co. Fetal bovine serum (FBS), sodium pyruvate and Hank's solution were obtained from Sigma (MO, USA). PBS contains 140 mM NaCl, 260 mM KCl, 8.1 mM $Na_2HPO_4$, 1.47 mM $KH_2PO_4$, pH 7.2. The Hank's solution with 10 mM MES or HEPES adjusted the pH to 6.5 or 7.4 using 0.1N HCl or 0.2N NaOH, respectively. The resulting solution was used as transport medium in the permeability study. Transwell® inserts (24.5 mm, pore size 0.4 μm, 4.7 $cm^2$, Corning Costar) were used for the Caco-2 cell monolayer culture and transport experiments. Cell culture flasks (75 $cm^2$) were used for the normal cell culture experiments. Both were obtained from Corning Costar (USA).

### 2.2. Preparation of dissolution media

The composition of the simulated intestinal fluid was the same as USP 28 without pancreatin. Fasted state simulating intestinal fluids was made from two chemical grades (LQ and HQ) of sodium taurocholate and lecithin. The FaSSIF contains 3 mM sodium taurocholate and 0.75 mM lethicin (Galia et al., 1998). The blank of FaSSIF (BL-FaSSIF) had the same composition as FaSSIF but did not contain lecithin or sodium taurocholate.

### 2.3. Solubility of glyburide in different media

Twenty milligrams (excess) glyburide powder (Lot# N326, Hoechst AG, Frankfurt, Germany) was added into 10 mL of different dissolution media (two chemical grades of FaSSIF, SIF and BL-FaSSIF) at pH 1.7, 5.0, 6.5, 7.4 values and stirred overnight (12 h) at 37 ± 0.5 °C water bath. The pH of each sample was checked during the experiment time. The resulting solution was then filtered through a 0.22 μm Millipore membrane filter. The filter membrane was checked for adsorption and no adsorption was detected.

### 2.4. In vitro dissolution studies at pH 6.5

A USP dissolution apparatus II (DT 6 Erweka, Germany) was used for all dissolution studies. The dissolution test was carried out at 37 ± 0.5 °C in 900 mL dissolution media at 75 rpm. The samples were withdrawn using a 10 mL syringe (B-D, USA) assembled with the steel tube and 10 μm filter (Lot# 31119B, Varian, USA). At each sampling time, 5 mL sample was withdrawn and 5 mL blank medium (preheated at 37 ± 0.5 °C) was added back into vessels.

### 2.5. Dynamic dissolution studies

The dissolution apparatus and conditions were the same as previously described. The pH of the dissolution media was changed during the experiment. Five pH values were selected, 6.0, 6.5, 7.0, 7.5 and 5.0 corresponding to physiological environment in the duodenum, jejunum, ileum and colon, respectively. The pH change was adapted to the pH changes 5 used by GastroPlus™ software. Samples were taken at 30, 90, 150, 210 and 270 min. At the end of each time interval, the pH was changed using concentrated sodium hydroxide or phosphoric acid. A pH meter (Digital 109, Corning, USA) was used to monitor the adjustment to the desired pH value.

### 2.6. Permeability determination

Caco-2 cells (passages 36–45, ATCC, Rockville, MD, USA) were maintained at 37 °C in Dulbeco's Modified Eagle's Medium with 4.5 g/L glucose, 1 mM sodium pyruvate, 10% (v/v) fetal bovine serum, 10 μg/mL human transferrin and 4.8 mg/mL HEPES, in an atmosphere of 5% $CO_2$ and 90% relative humidity. A total of 50,000 cells/$cm^2$ in medium were seeded in each apical chamber of Transwell® insert. Three milliliters of medium was transferred in the basal receiving side.

The integrity and permeability of the cell monolayer was determined by electrical resistance measurements (VOHM, World Precision Inc., USA). The transepithelial electrical resistance values obtained in the absence of cells was considered as background measurements. The transport experiment was started based on the TEER of the monolayer when it reached 400 Ω $cm^2$ or higher. This is typically the case after 18–23 days after seeding cells on the transwell inserts. Lucifer yellow was used as a paracellular quality control marker, its effective permeability coefficient ($P_{eff}$) should be less than $2 \times 10^{-7}$ cm/s. Lucifer yellow was measured by 485 nm excitation and 530 nm emission using a spectrofluorometer (model: FLUOROMAX, SPEX Industries Inc., USA). Glyburide (20 μM) was dissolved in the transport medium (1.5 mL) and was carefully added to the apical surface. Three milliliters of blank transport medium was added to basal receiving side. The cells were incubated at 37 °C in an atmosphere of 95% humidity; the concentration of the glyburide in both chambers was analyzed by HPLC at predetermined time intervals. In order to maintain the sink condition, the inserts were moved to the pre-prepared wells that contained fresh transport medium at predetermined time intervals. After each experiment, the TEER values were measured in all inserts and the integrity of the cell monolayer was confirmed.

The effective permeability coefficient ($P_{eff}$) was calculated using the following Eq. (1):

$$P_{eff} = \frac{V}{A \times C_o} \times \frac{dc}{dt} \text{ (cm/s)} \quad (1)$$

where dc/dt, the flux across the monolayer (mM/s), is the initial slope of a plot of the cumulative receiver concentration versus time; *V* the volume of the receiver chamber (mL); *A* the surface area of the monolayer ($cm^2$), which is 4.7 $cm^2$ for the transwell insert in this experiment; and $C_o$ is the initial concentration (mM) in donor compartment.

### 2.7. HPLC analysis

Sample analysis was achieved by HPLC. The HPLC system consisted of an automatic sample injector (SIL-9A, Shimadzu, Japan), a pump (LC-60, Shimadzu, Japan), a UV detector (SPD-

6AV, Shimadzu, Japan) and an analytical column LiChoCART 125-4 LiChospher 60 Rp-select B (5 μm, Merck, Darmstadt, Germany) with a guard column. The samples were centrifuged at 12,000 rpm for 15 min using an Eppendorf centrifuge (Model 5415, Brinkmann, Germany). Thirty microliters of supernatant was directly injected into the HPLC system. The mobile phase consisted of a mixture of the acetonitrile and (25 mM, pH 4.5) sodium dihydrogen phosphate buffer. The percentage of the acetonitrile in the mobile phase was between 42 and 45% base on the separation of the impurities in the sample matrixes. The drug, glyburide was detected at a wavelength of 230 nm and the retention time was between 5 and 8 min depending on the organic ratio in the mobile phase. Samples were stable during the analytical time. An integrator (C-R3A, Shimadzu, Japan) was used for peak integration. Analysis of the dissolution and cell culture samples used the same HPLC condition.

### 2.8. Computer simulations

GastroPlus™ (Version 4.0.0005, Simulations Plus Inc., USA) was used to simulate the absorption and pharmacokinetics of the reference and test formulations. The program has three input pages: compound, physiology and pharmacokinetics, respectively. In the compound page, basic data of the drug's physical and chemical properties such as bulk density, solubility, dose, $pK_a$ and particle radius are entered. Our values were taken from the manufacturer's certificate of analysis, ~~the~~ literature or estimated using computer software (Reynolds, 1993; Budavari and O'Neil, 1996). The human permeability ($P_{eff}$) of glyburide was estimated using Caco-2 data (see Section 2.6). The solubility–pH profiles of glyburide were obtained as described in the previous section. The log *P* of glyburide was calculated by the KowWin software online-software on the Internet (~~http://www.syrres.com~~). The diffusion coefficient of glyburide was estimated by GastroPlus™.

The *in vitro* dissolution profiles of glyburide tablets were used as input functions into GastroPlus™ using the "tabulated *in vitro* data" function. The drug release profiles were used by the software to calculate the drug concentration in each compartment. The estimated human permeability data were computed using the human fasted log *D* absorption model to account for permeability. The *in silico* gut (GastroPlus™) then calculates the fraction dose absorbed based on the ACAT model using drug concentration, permeability, surface area and transit time in each compartment. Pharmacokinetic parameters, e.g. volume of distribution, clearance and microconstants can be added to the software in the pharmacokinetic page, which enables the software to calculate plasma concentration–time curves.

In the physiology page, the default values for transit time were selected for each compartment.

The clinical data for both formulations were obtained from bioequivalence study and all data were made available to us (Blume and Mutschler, 1989). The pharmacokinetic data were calculated using the software Kinetica 2000 (InnaPhase Corporation, USA). The Micro-Extravascular model fitting model was selected to calculate pharmacokinetic parameters. The mean values of the clinical data from 15 healthy volunteers for both formulations ~~were well fit~~ in a two-compartmental model (Rydberg et al., 1997). The pharmacokinetic parameters, such as clearance, volume of distribution, $K_{12}$, $K_{21}$, etc., were used for the simulations using GastroPlus™.

### 2.9. Statistics

Release profiles comparison: the difference factor ($f_1$, Eq. (2)) and similarity factor ($f_2$, Eq. (3)) were used to compare the drug release profiles. The equations are as below (Costa and Lobo, 2001):

$$f_1 = \frac{\sum_{j=1}^{n} |R_j - T_j|}{\sum_{j=1}^{n} R_j} \times 100 \quad (2)$$

$$f_2 = 50 \times \log \left\{ \left[ 1 + \left( \frac{1}{n} \right) \sum_{j=1}^{n} |R_j - T_j|^2 \right]^{-0.5} \times 100 \right\} \quad (3)$$

where $n$ is the sample number, and $R_j$ and $T_j$ are the percentages of the reference and test drug release, respectively, at different time intervals $j$. The $f_1$ value increases proportionally due to the dissimilarity between the two dissolution profiles. If $f_2$ of two dissolution drug release profiles is between 50 and 100, then these two drug release profiles are similar. Value under 50 indicates differences between the release profiles (Costa and Lobo, 2001).

Percent prediction error (%PE) was calculated using Eq. (4) (Guidance for Industry: FDA, 1997).

$$\%PE = \frac{\text{observed} - \text{predicted}}{\text{observed}} \times 100 \quad (4)$$

Liner regression: the linear regression for the observed and simulated data was performed using MS Office Excel (2000). The 95% confident interval was applied for analysis of linear regression.

Significance of differences between experiments was calculated by paired two-sample for means *t*-test in MS Office Excel (2000). In all cases, statistical significance was calculated at $p < 0.05$ level.

## 3. Results and discussion

### 3.1. Solubility of glyburide in different media

Glyburide ($pK_a$ 5.3) is a weak acid with poor aqueous solubility (El-Massik et al., 1996). The solubility of glyburide powder was measured in four different media including BL-FaSSIF, SIF, HQ-FaSSIF and LQ-FaSSIF at different pH values (pH 1.7, 5.0, 6.0, 6.5, 7.0 and 7.4; see Fig. 1). The pH of each sample did not change during the experimental period. The results showed that the solubility of the glyburide was highest in the LQ-FaSSIF (43.21 μg/mL) at pH 7.4, and decreased ~~following the order~~ from high quality HQ-FaSSIF, SIF to BL-FaSSIF. The solubility in all media decreased from high pH to low pH due to the drug's $pK_a$ of 5.3. As a weak acid, glyburide has a higher solubility in a basic aqueous environment. However,

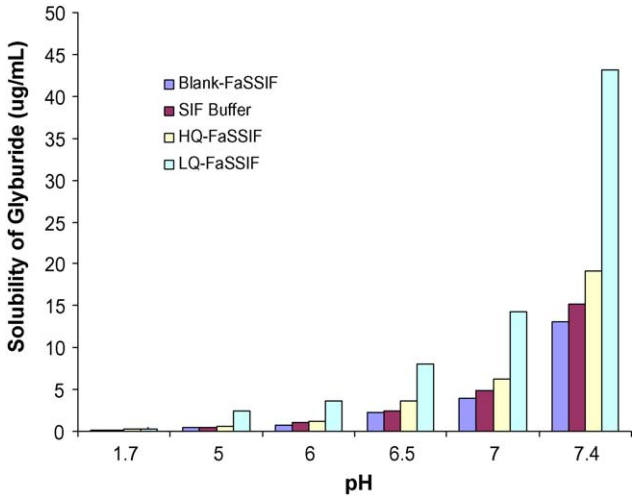


**Fig. 1 – Solubility of glyburide ~~powders~~ in different media ($n = 3$).**

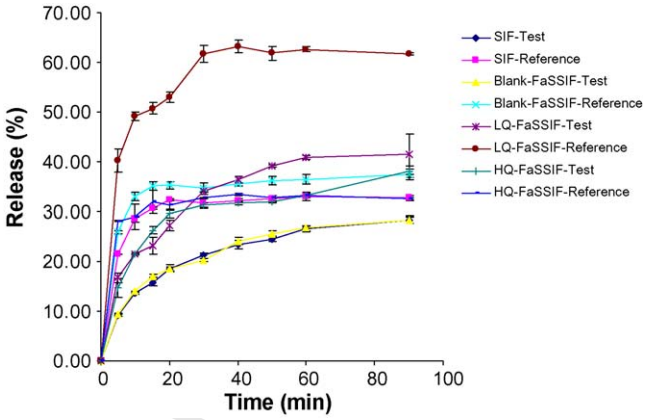


**Fig. 2 – Dissolution profiles of two formulations in different media at pH 6.5.**

it can be considered as a poorly soluble drug considering the entire physiological pH range. The results showed that glyburide had higher solubility in FaSSIF compared to BL-FaSSIF or SIF. FaSSIF contains lecithin and bile salts (sodium taurocholate). The concentrations of bile salts and lecithin in FaSSIF are adapted to physiological conditions (Dressman et al., 1998). Bile salts and lecithin can increase the wetting process for the lipophilic drugs and solubilize the drug into the micelles formed by bile salts and lecithin. Therefore, pH and micelles impact the solubility of glyburide. This is in accordance with results reported by Jinno et al. (2000) for piroxicam. Our solubility study showed that the micelles formed by LQ bile salt and LQ lecithin were able to solubilize glyburide better compared to the chemically purer HQ bile salt and HQ lecithin. The difference between HQ- and LQ-FaSSIF is the chemical grade of the bile salt and lecithin used to prepare the media. LQ-media contain other components like glycocholic, cholic, deoxycholic and other bile acids from crude ox bile to a higher extent while the HQ-media contain 97% pure sodium taurocholate. The different composition impacts the solubility of glyburide. Woodford (1969) reported that the addition of 1-monoolein to a taurocholate micelle system increased the solubility of cholesterol. He concluded that a three-component micelle system (monoolein–taurocholate–cholesterol) form different micelles compared to the pure taurocholate cholesterol system. The improved solubility of glyburide in LQ-media might be due to similar effects caused by the ~~presents~~ of the other bile components. BL-FaSSIF and SIF are plain buffers. They mainly influence the solubility of glyburide by means of pH. Such media might not reflect the physiological environment of GI tract due to the lack of micelle solubilization.

### 3.2. *In vitro dissolution studies at pH 6.5*

The common limiting factor for oral absorption of class II drug substances is their lack of dissolution due to limited solubility (Galia et al., 1998). For such drugs solubility might be the major factor ~~to influence~~ the dissolution behavior. In order to establish meaningful IVIVCs, the *in vitro* dissolution tests have to simulate the *in vivo* dissolution behaviors or need at least a relationship which can be established using a scaling factor (Löbenberg et al., 2000). Fig. 2 shows the dissolution of the reference and test formulations in four different media at pH 6.5. The drug release of the test formulation in pH 6.5 media was slower compared to the reference formulation during the first 30 min. This was observed in all four dissolution media. Both formulations had the highest release in LQ-FaSSIF. The graph shows that the drug release of the reference and test formulations in LQ-FaSSIF was over 60 and 40% within 90 min, ~~respectively~~. In the other three media, the drug releases were below 40% within 90 min which is due to limited drug solubility in these media as confirmed by results of the solubility study. Table 1 shows the values of two comparison factors: ($f_1$) is the difference factor and ($f_2$) is the similarity factor. Both can be used to assess dissolution profiles between formulations. The $f_1$ and $f_2$ factors were equal to 79.6 and 30.1, respectively, when the dissolution tests were performed in LQ-FaSSIF. A higher ~~the~~ $f_1$ value corresponds to dissimilarity while a $f_2$ value below 50 indicates differences between two dissolution profiles (Costa and Lobo, 2001). The $f_1$ obtained from LQ-FaSSIF is the highest among the four media and the $f_2$ factors obtained from LQ-FaSSIF is the lowest compared to the other three media. This indicates that the LQ-FaSSIF differentiated formulation differences better compare~~d~~ to the other media. The $f_2$ factors of dissolution profiles in SIF and BL-FaSSIF were 47.8 and 42, respectively. The $f_1$ factors are 51.9 and 67.9, respectively. Although in these media the $f_1$ and $f_2$ factors showed differences in the dissolution profiles, the values of the $f_2$ factors were close~~d~~ to the critical value 50 which divides between similarity and dissimilarity (Costa and Lobo, 2001). In contrast the comparison of the formulations in HQ-FaSSIF produced a $f_1$ value of 10.5 and a $f_2$ factor of 61.2. In this

**Table 1 – $f_1$ and $f_2$ factors ~~of~~ the dissolution profiles between reference and test formulation~~s~~ at ~~single~~ pH 6.5**

| | LQ-FaSSIF | HQ-FaSSIF | SIF | BL-FaSSIF |
|---|---|---|---|---|
| $f_1$ | 79.6 | 10.5 | 51.9 | 67.9 |
| $f_2$ | 30.1 | 61.2 | 47.8 | 42 |

medium, the dissolution profiles would be considered similar. The results above show that LQ-FaSSIF can best differentiate between the dissolution behaviors of both formulations. This might be due to a different interaction of the formulation components with the components of the dissolution media. Vertzoni et al. (2004) showed that the LQ-FeSSIF had substantially impact on the dissolution profiles of two highly lipophilic drugs. The study showed that the *in vitro* dissolution in LQ-FeSSIF were more suitable to describe the *in vivo* dissolution performance of those two drug products. In an earlier study, Löbenberg et al. (2000) reported that the drug releases of two glyburide formulations in a HQ-FaSSIF was able to differentiate between dissolution behaviors of two formulations. However, in the present study HQ-FaSSIF exhibited the lowest discriminative power between both tested formulations. The different results might be due to the different batches of lecithin and sodium taurocholate used to prepare the media and the volume used for the tests (Sznitowska et al., 2002). Leng et al. (2003) investigated the formation of vesicles and micelles using bile salts and lecithin. They identified different stages of the vesicle formation. Different vesicle shapes and mono and multi-laminar vesicles can be formed. This was shown to be highly sensitive to environmental and physicochemical factors of the used bile salts and lecithin. This effect might also explain the discriminative power of certain biorelevant media. The drug and excipients of pharmaceutical formulations might interact differently with media and either support or destruct the formation of certain vesicles and might solubilize the drug differently compared to other vesicles. However, this has to be investigated more. The characterization and investigation of the effect of vesicle structure on the dissolution behavior can help to further standardize biorelevant media.

### 3.3. *Dynamic dissolution studies*

The dynamic dissolution test of the two formulations was performed following the pH profile used by the ACAT model as discussed earlier. Fig. 3 shows the drug release under changing pH values in different dissolution media. The drug release of the test formulation was slower than that of the reference formulation. This was observed in all media. Compared to the single pH (Fig. 2) the drug release for both formulations was slower in all media within the first 30 min. However, after 4 h the drug release was higher in all media. This can be attributed to the solubility of glyburide at different pH values. The drug release increased when the pH increased. When the pH was changed

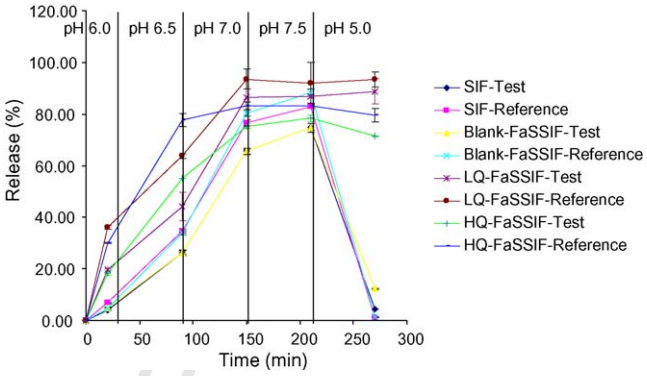


**Fig. 3 – Dissolution profiles of two formulations in different media at pH gradient.**

to 7.5, the drug releases reached a plateau for both formulations in all media. When the pH of the media was changed from 7.5 to 5.0, the drug concentration in LQ-FaSSIF had no change and kept on the plateau for 1 h. Our results show that the micelles formed keep the glyburide in solution without precipitation despite the unfavorable pH. The LQ lecithin and LQ bile salts enhanced either the stability of the micelles or increase the drug solubilization. However, in HQ-FaSSIF, the drug concentration dropped slightly from 83 to 79 and 78 to 71%. A *t*-test indicated that there are no statistically significant differences between the drug release changes. However, the observed decrease might be due to a precipitation of some glyburide due to the pH change and the unfavorable pH condition. A more pronounced precipitation was observed in the SIF and BL-FaSSIF. The concentrations dropped from above 75 to under 12% for both formulations. This can be explained by the nature of SIF and BL-FaSSIF which are plain buffers.

Comparing the 90 min drug release values of the fixed pH experiment and the dynamic dissolution experiment (Table 2) reveals that the pH change had an impact on the solubilization capacity of the HQ-FaSSIF. At 90 min the pH of both experiments was the same. While the drug release in the two buffers (SIF and BL-FaSSIF) and the LQ-FaSSIF were nearly the same for each formulation and media, a significant increase in drug release was observed in the HQ-FaSSIF. At all other pH values the HQ-FaSSIF had lower drug concentrations compared to the LQ-FaSSIF. This observation supports the earlier discussed formation of different types of vesicles and the impact of environmental factors on this process. However, such effects have to be studied in more detail.

**Table 2 – Comparison of the drug releases (%) at 90 min ($n = 3$) during the dissolution tests under single pH 6.5 and dynamic pH profiles for both reference and test formulations**

| | Reference formulation | | Test formulation | |
|---|---|---|---|---|
| | Single pH 6.5 | Dynamic pH | Single pH 6.5 | Dynamic pH |
| LQ-FaSSIF | 61.73 ± 0.18 | 63.75 ± 0.86 | 41.53 ± 4.00 | 44.02 ± 5.48 |
| HQ-FaSSIF[a] | 32.58 ± 0.28 | 77.75 ± 2.53 | 38.01 ± 1.12 | 54.99 ± 0.07 |
| SIF | 27.0 ± 0.60 | 34.74 ± 0.65 | 28.30 ± 0.58 | 26.11 ± 1.01 |
| BL-FaSSIF | 37.45 ± 1.10 | 33.91 ± 0.58 | 28.40 ± 0.82 | 26.43 ± 1.02 |

[a] *t*-Tests indicated that there is significant difference in the drug releases between the single pH and dynamic pH dissolution tests.

### 3.4. *Permeability studies*

The human permeability ($P_{eff}$) of the glyburide was estimated by GastroPlus™ as $3.5 \times 10^{-4}$ cm/s, using *in vitro* Caco-2 data. Vogelpoel et al. (2004) suggested that, if the human permeability ($P_{eff}$) of a drug is above $2 \times 10^{-4}$ cm/s or the bioavailability is over 90%, this drug can be considered as a highly permeable drug. Literature shows that glyburide's bioavailability can be up to 100% depending on the formulation (Neugebauer et al., 1985). Therefore, glyburide can be classified as a highly permeable drug as confirmed using the Caco-2 model. Based on the BCS (Amidon et al., 1995), glyburide is a typical class II drug, which has high permeability and low aqueous solubility.

### 3.5. *Computer simulations*

The computer simulations using the GastroPlus™ were performed by using dissolution profiles and pH–solubility profiles as major input functions. All the physical and chemical properties of the glyburide described previously were kept the same. The human permeability ($P_{eff}$) of the glyburide was estimated as $3.5 \times 10^{-4}$ cm/s. A parameter sensitivity analyses using GastroPlus™ showed that the predicted $C_{max}$ and AUC will not be significantly influenced between a permeability of $2 \times 10^{-4}$ and $10 \times 10^{-4}$ cm/s. This confirms that glyburide is a typical class II drug and dissolution and not permeability is the limiting factor in oral absorption (Löbenberg and Amidon, 2000). Using the single pH dissolution profiles, the simulated plasma concentration profiles did not match the clinical data. The predicted $C_{max}$ and AUC were half and one-third of observed data, respectively (Table 3). The prediction errors of the $C_{max}$ and AUC were ±38, 63, 59 and 67% for the reference and test formulations, respectively. The *in vitro* dissolutions at fixed pH condition were not able to simulate the *in vivo* plasma levels. Therefore, the *in vivo* dissolution seems to be different.

Using the dynamic pH dissolution profiles as input function for the simulations showed that only the dissolution profiles obtained from LQ-FaSSIF were able to predict the clinically observed data (Fig. 4). The prediction errors of $C_{max}$ and AUC were ±7, 14, 4 and 0.7% for the reference and test formulations, respectively (Table 3). The dynamic dissolution profiles obtained from LQ-FaSSIF showed the best simulation results compared to the results obtained from the other dissolution media (Table 3). The prediction errors of the AUC and $C_{max}$ obtained from the other three media such as HQ-FaSSIF, BL-FaSSIF and SIF are much higher (up to ±28%) compared to LQ-FaSSIF. The goodness of fit (linear regression) for the simulation obtained from LQ-FaSSIF, regression coefficient for the reference and test formulations were 0.94 and 0.93, respectively. The simulation results clearly showed that an *in vitro/in vivo* relationship between the dynamic dissolution in LQ-FaSSIF and the *in vivo* plasma curves exists. The *in vitro* dissolution following the dynamic pH profiles seems to mimic the *in vivo* dissolution. The USP 28 describes in Chapter 1088 different levels of IVIVC. A level A correlation is a point to point correlation and the strongest correlation possible (USP 28). The *in vitro* dissolution properties can serve as surrogate for *in vivo* performance. Our results in the different media show that LQ-media successfully predicted the oral performance of the two formulations. Applied *in vitro* dissolutions seem to predict the *in vivo* dissolution as required for a level A correlation.

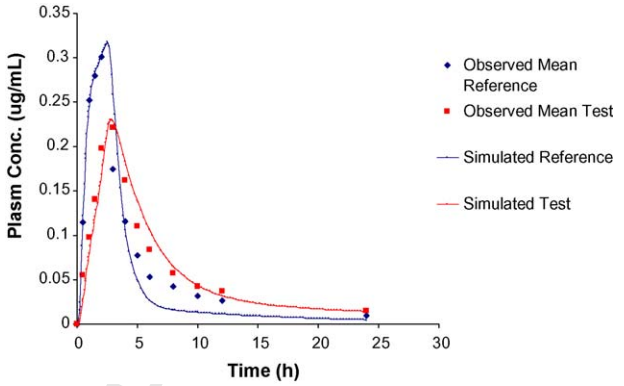


**Fig. 4 – Comparison of the simulated and observed data using dynamic dissolution ~~profiles.~~**

**Table 3 – Comparison of pharmacokinetic parameters of ~~the~~ bioequivalence study between observed and simulated data (observed reference—$C_{max}$: ~~0.~~301 ~~μ~~g/mL; $AUC_{0-24}$: 1359.6 ng/(mL h); observed test—$C_{max}$: ~~0.~~221 ~~μ~~g/mL; $AUC_{0-24}$: 1441.3 ng/(mL h))**

| | Simulated | | | | Prediction error (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | Reference | | Test | | Reference | | Test | |
| | $C_{max}$* | AUC* | $C_{max}$ | AUC | $C_{max}$ | AUC | $C_{max}$ | AUC |
| (a) | ~~0.~~187 | 499 | ~~0.~~092 | 477 | 38 | 63 | 59 | 67 |
| (b) | ~~0.~~385 | 1180 | ~~0.~~189 | 1230 | 28 | 13 | 14 | 15 |
| (c) | ~~0.~~355 | 1100 | ~~0.~~190 | 1240 | 18 | 19 | 14 | 14 |
| (d) | ~~0.~~384 | 1010 | ~~0.~~202 | 1270 | 28 | 26 | 8 | 12 |
| (e) | ~~0.~~318 | 1170 | ~~0.~~230 | 1452.1 | 7 | 14 | 4 | 0.7 |

(a) Single pH 6.5; (b) BL-FaSSIF; (c) SIF; (d) HQ-FaSSIF; (e) LQ-FaSSIF. $C_{max}$: ~~μ~~g/mL; AUC: ng/(mL h).

## 4. Conclusions

Biorelevant dissolution media are a complex mixture of bile salts and lecithin. The study showed that environmental changes which *in vivo* dynamically happen in the gastrointestinal tract have an impact on the solubilization of glyburide, as indicated by the LQ- and HQ-media. These effects have to be studied in more detail. Computer simulations using the ACAT model showed that the LQ-FaSSIF data were best able to predict plasma levels of two investigated glyburide formulations if a pH gradient was applied. The used *in vitro* and *in silico* methods were able to predict the oral performance of two glyburide formulations. An *in vitro/in vivo* correlation (IVIVC) could be established.

## ~~Uncited reference~~

~~Syracuse Research Corporation (1999–2004).~~

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## REFERENCES

Amidon, G.L., Lennernas, H., Shah, V.P., Crison, J.R., 1995. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm. Res. 12, 413–420.

Blume, H., Ali, S.L., Siewert, M., 1993. Pharmaceutical quality of glibenclamide products: a multinational postmarket comparative study. Drug Dev. Ind. Pharm. 19 (2), 713–2741.

Blume, H., Mutschler, E., 1989. Bioäquivalenz. Govi-Verlag, Eschborn.

Budavari, S., O'Neil, M.J., 1996. The Merck Index. Chapman & Hall.

Costa, P., Lobo, J.M.S., 2001. Modeling and comparison of dissolution profiles. Eur. J. Pharm. Sci. 13, 123–133.

Dressman, J.B., Amidon, G.L., Reppas, C., Shan, V.P., 1998. Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. Pharm. Res. 15 (1), 11–21.

El-Massik, M.A., Darwish, I.A., Hassan, E.E., El-Khordagui, L.K., 1996. Development of a dissolution medium for glibenclamide. Int. J. Pharm. 140, 69–76.

Euglucon N. Rote Liste, 2005. BPI, Frankfurt/Main.

Galia, E., Nicolaides, E., Hörter, D., Löbenberg, R., Reppas, C., Dressman, J.B., 1998. Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs. Pharm. Res. 15 (5), 698–705.

GastroPlus Manual, 2004. Simulation Plus Inc., Lancaster, USA.

Goodman, L.S.O.G. (orig), Hardman, J.G., Linbird, L.E., Molinoft, P.B., Ruddon, R.W., 1999. Goodman and Gilman's the Pharmacological Basis of Therapeutics.

Guidance for Industry: Extended Release Oral Dosage Forms: Development, Evaluation and Application of In Vitro/In Vivo Correlations, 1997. U.S. Department of Health Food and Drug Administration Center for Drug Evaluation and Research.

Jinno, J., Oh, D.M., Crison, J.R., Amidon, G.L., 2000. Dissolution of ionizable water-insoluble drugs: the combined effect of pH and surfactant. J. Pharm. Sci. 89 (2), 268–275.

Leng, J., Egelhaaf, S.U., Cates, M.E., 2003. Kineics of the micelle-to-vesicle transition: aqueous lecithin-bile salt mixtures. Biophys. J. 85, 1624–1646.

Löbenberg, R., Amidon, G.L., 2000. Modern bioavailability, bioequivalence and biopharmaceutics classification system. New scientific approaches to international regulatory standards. Eur. J. Pharm. Biopharm. 50, 3–12.

Löbenberg, R., Krämer, J., Shah, V.P., Amidon, G.L., Dressman, J.B., 2000. Dissolution testing as prognostic tool for oral drug absorption: dissolution behavior of glibenclamide. Pharm. Res. 17 (4), 439–444.

Neugebauer, G., Betzien, G., Hrstka, V., Kaufmann, B., Möllendorff Von, E., Abshagen, U., 1985. Absolute bioavailability and bioequivalence of glibenclimide (Semi-Euglucon®N). Int. J. Clin. Pharmacol. Ther. Toxicol. 23 (9), 453–460.

Neuvonen, P.J., Kivisto, K.T., 1991. The effects of magnesium hydroxide on the absorption and efficacy of two glibenclamide preparations. Br. J. Clin. Pharmacol. 32, 215–220.

Oh, D.M., Curl, R.L., Amidon, G.L., 1993. Estimating the fraction dose absorbed from suspensions of poorly soluble compounds in humans: a mathematical model. Pharm. Res. 10, 264–270.

Otoom, S., Hasan, M., Najib, N., 2001. The bioavailability of glyburide (glibenclamide) under fasting and feeding conditions: a comparative study. Int. J. Pharm. Med. 15, 117–120.

Parrott, N., Lavé, T., 2002. Predicting of intestinal absorption: comparative assessment of GASTROPLUS™ and IDEA™. Eur. J. Pharm. Sci. 17, 51–61.

Pearson, J.G., 1985. Pharmacokinetics of glyburide. Am. J. Med. 79 (Suppl. 3B), 67–71.

Reynolds, J.E.F., 1993. Martindale. The Extra Pharmacopeia, vol. 30. The Pharmaceutical Press, London.

Rydberg, T., Jönsson, A., Karlsson, M., Meldander, A., 1997. Concentration-effect relations of glibenclamide and its active metabolites in man: modeling of pharmacokinetics and pharmacodynamics. Br. J. Clin. Pharmacol. 43, 373–381.

Sinko, P.J., Leesman, G.D., Amidon, G.L., 1991. Predicting fraction dose absorbed in humans using a macroscopic mass balance approach. Pharm. Res. 8, 979–988.

Syracuse Research Corporation, 1999–2004. Interactive LogKow (KowWin) Demo, http://www.syrres.com/esc/est_kowdemo.htm.

Sznitowska, M., Dabrowska, E.A., Janicki, S., 2002. Solubilizing potential of submicron emulsions and aqueous dispersions of lecithin. Int. J. Pharm. 246, 203–206.

Test Solutions, Unites States Pharmacopeia, USP 23. U.S. Pharmacopeial Convention Inc., Rockville, MD, p. 2053.

Unites States Pharmacopeia, USP 28. U.S. Pharmacopeial Convention Inc., Rockville (Chapter 1088).

Vertzoni, M., fotaki, N., Kostewicz, E., Stippler, E., Leuner, C., Nicolaides, E., Dressman, J., Reppas, C., 2004. Dissolution media simulating the intralumenal composition of the small intestine: physiological issues and practical aspects. J. Pharm. Pharmacol. 56, 453–462.

Vogelpoel, H., Welink, J., Amidon, G.L., Junginger, H.E., Midha, K.K., Möller, H., Olling, M., Shah, V.P., Barends, D.M., 2004. Biowaiver monographs for immediate release solid oral dosage forms based on biopharmaceutics classification system (BCS) literature data: verapamil hydrochloride, propranolol hydrochloride and atenolol (Commentary). J. Pharm. Sci. 93 (8), 1945–1956.

Woodford, F.P., 1969. Enlargement of taurocholate micelles by added cholesterol and monoolein: self-diffusion measurements. J. Lipid Res. 10, 539–545.

Yu, L.X., Crison, J.R., Amidon, G.L., 1996a. Compartmental transit and dispersion model analysis of small intestinal transit flow in humans. Int. J. Pharm. 140, 111–118.

Yu, L.X., Lipka, E., Crison, J.R., Amidon, G.L., 1996b. Transport approaches to the biopharmaceutical design of oral drug delivery systems: prediction of intestinal absorption. Adv. Drug Deliv. Rev. 19, 359–376.