An *in vitro-in vivo* correlation study for nifedipine immediate release capsules administered with water, alcoholic and non-alcoholic beverages: impact of *in vitro* dissolution media and hydrodynamics

A. Mercuri[1], R. Fares[1,2], M. Bresciani[1], N. Fotaki[2,*]

[1] Research Center Pharmaceutical Engineering GmbH, Graz, Austria

[2]Department of Pharmacy and Pharmacology, University of Bath, Bath, United Kingdom

* Corresponding Author
Dr Nikoletta Fotaki
Department of Pharmacy and Pharmacology
University of Bath
Claverton Down
Bath, BA2 7AY
United Kingdom
Tel. +44 1225 386728
Fax: +44 1225 386114
E-mail: n.fotaki@bath.ac.uk

Abstract

The impact of hydrodynamics and media composition on nifedipine dissolution profile from IR (immediate release) soft capsules was investigated using dissolution apparatus USP1, USP2, USP3 and USP4 (United State Pharmacopoeia). Media composition was varied in terms of pH and content, to mimic the dosage form intake with water or non-alcoholic beverages (orange juice) and alcoholic beverages (orange juice/ethanol mixture (47% v/v)). Through construction of in vitro-in vivo correlations (IVIVC) with corresponding *in vivo* data from the literature, it was possible to evaluate the *in vitro* conditions that are likely to simulate the *in vivo* formulation behaviour. Both linear and nonlinear correlations were obtained depending on experimental set-ups. Testing of 20 mg nifedipine capsules in FaSSGFst (Fasted State Simulated Gastric Fluid pH 1.6; water administration) produced IVIVC with the USP3 (after time scaling) and USP4 apparatus. IVIVC were obtained for USP2, USP3 and USP4 in FaSSGFoj (Fasted State Simulated Gastric Fluid pH 3.4; orange juice administration). Linear and nonlinear correlations were obtained with the USP1, USP2 and USP3 apparatus when testing the capsules in FaSSGFoj/EtOH (orange juice/ethanol administration). This study highlighted that selection of physiologically relevant dissolution set-ups is critical for predicting the *in vivo* impact of formulations co-administration with water, non-alcoholic and alcoholic beverages.

Keywords: IVIVC, Biorelevant dissolution, capsule rupture time, Nifedipine, Immediate release, hydrodynamics, special dissolution media, non-alcoholic beverages, alcoholic beverages

Chemical compounds studied in this article

Nifedipine (PubChem CID: 4485)

1 Introduction

Oral dosage forms are usually taken with a glass of water (Fuchs, 2009), to aid the swallowing of the formulation. But in the clinic, as well as in everyday life, other beverages can also be used to aid the swallowing of the medicament, such as fruit juices and, in more extreme cases, alcoholic beverages. While metabolic interactions with fruit juice are well known (An et al., 2015; Bailey et al., 1998), the interaction between dosage forms and other type of beverages is still limited and mainly focused on tablets disintegration (Akinleye et al., 2007; Anwar et al., 2005; Chuong et al., 2010; Kalantzi et al., 2005; Wells and Losin, 2008; Zuo et al., 2013).

Regarding the intake of oral medications with alcoholic beverages, about ten years ago, serious concerns were raised by FDA for modified release (MR) formulations (FDA Alert for Healthcare Professionals, 2005; Meyer and Hussain, 2005). This led to the suggestion of specific experiments designed to verify, *in vitro*, the impact of ethanol on the dissolution profile from such formulations. These studies are requested by FDA depending on the product requiring registration (US FDA, n.d.). Consequently, extensive studies have been performed in order to investigate the interactions between MR formulations and ethanol (Jedinger et al., 2015; Johnson et al., 2008; Larsson et al., 2010; Lennernäs, 2009; Palmer et al., 2011; Roberts et al., 2007; Rosiaux et al., 2014, 2013a, 2013b; Roth et al., 2009; Sathyan et al., 2008; Smith et al., 2010; Traynor et al., 2008) and to develop alcohol resistant formulations (Jedinger et al., 2014; Keen et al., 2015). Recently, this interest has been extended also to immediate release (IR) formulations containing drugs with poor aqueous solubility (Fagerberg et al., 2015). It has been found that the solubility of neutral and acidic poorly soluble drugs is increased in media containing 20% v/v ethanol, compared to that in Fasted State Simulated Intestinal Fluid (FaSSIF), while bases have shown a substance specific solubility (Fagerberg et al., 2012).

Nifedipine is a calcium antagonist used clinically to treat hypertension and angina, and it is available in both IR and MR oral formulations. Nifedipine is a neutral compound classified as BCS Class II (Thelen et al., 2010), with a low solubility in water of 5 to 6 μg/mL over the pH range of 2 to 10 (Yang and de Villiers, 2004) and high permeability (Gajendran et al., 2015). Its pharmacokinetic parameters following oral dosing are dependent on the type of dosage form used (Toal, 2004). Generally, the peak plasma concentration (C_{max}) of nifedipine administered orally as IR capsules appears between 0.5 and 2.2 hours post-dose, for MR tablets it ranges from 1.6 to 4.2 hours (Sorkin et al., 1985) and for GITS (Gastro-Intestinal Therapeutic System) tablets, based on an osmotic pump system, the C_{max} is reached, following a lag phase of 1 to 2 hours, after 8 to 10 hours (Schug et al., 2002).

In the study of Qureshi and co-workers (Qureshi et al., 1992) it was observed that co-administration of nifedipine IR capsules with an orange juice drink containing 47% v/v ethanol resulted in a faster onset of action and an increased bioavailability of nifedipine, compared to the administration of the same dose with orange juice only. These effects were attributed to an increased absorption rate and a simultaneous inhibition of the metabolism of the drug due to the ethanol, as no differences in the elimination rate between nonalcoholic and alcoholic beverages were observed (Qureshi et al., 1992).

Studies investigating the increased absorption of drugs when taken with alcoholic beverage raise ethical issues, due to the high risks of toxicity and side effects that can expose the subject in a life threatening situation. In this contest, generation of *in silico* PK profiles of a drug, using solubility measurements, can be used to predict the *in vivo* absorption when co-administration with alcoholic beverages occurs (Fagerberg et al., 2015). However, the extent of drug dissolution from the IR formulation may be also affected by the presence of ethanol in the stomach. Furthermore, there is limited literature available regarding the possible interactions between dosage forms and beverages other than water. Therefore, in the present study the most

commonly used dissolution apparatus (namely USP1, USP2, USP3 and USP4 apparatus) were used, and their parameters were varied in such a way that different set-ups could be investigated. The aims of this study were to investigate the impact of the variation of the dissolution parameters on the drug dissolution and to evaluate which experimental conditions better simulate the *in vivo* scenario of taking an IR formulation with water, orange juice and an orange juice-alcoholic mixture. Based on the *in vitro* dissolution data and the *in vivo* absorption data, level A *in vitro-in vivo* correlations (IVIVC) were obtained. The drug dissolution from an IR capsule is dependent on the time at which the capsule ruptures and releases its content, and this value was also calculated to support the understanding of the dissolution data as well as the impact of water and alcoholic and non-alcoholic beverages on capsule rupture time.

2 Materials and Methods

2.1 Materials

Sodium chloride, pepsin from porcine gastric mucosa (Ph. Eur., lot BCBL9753V) and nifedipine powder (≥ 98% HPLC) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Egg- lecithin (Lipoid E PCS, Phosphatidylcholine from egg) was from Lipoid GmbH (Ludwigshafen, Germany). Sodium taurocholate was purchased or kindly donated by Prodotti Chimici e Alimentari S.p.A (Basaluzzo, AL, Italy). Ethanol 96% Ph.Eur. was from VWR BDH Prolabo Chemicals (Leuven, Belgium). Immediate release (IR) soft gelatine capsules of nifedipine (Adalat® 10 mg, 90 soft capsules, batch n°: ITA26UU, from Bayer Pharma AG, Leverkusen, Germany) were used in the studies. Water was of Milli-Q grade. Cellulose nitrate (CN) membrane syringe filters with a pore size of 0.45 µm were from Whatman® (GE Healthcare Life Sciences, UK), while regenerated cellulose (RC) membrane syringe filters with a pore size of 0.45 µm were from (Cronus, LabHut Ltd, UK). All other

reagents and chemicals were of analytical grade and were used as received, without further purification.

2.2 Dissolution media preparation

Dissolution experiments were performed in Simulated Gastric Fluid without pepsin (SGFsp) pH 1.2 (United States Pharmacopeia, 2015a), Fasted State Simulated Gastric Fluid at pH 1.6 (FaSSGFst) and pH 3.4 (FaSSGFoj). The FaSSGF media were freshly prepared for each experiment as described by Vertzoni et al. (Vertzoni et al., 2010), and `in the case of FaSSGFoj the pH of the buffer was adjusted with NaOH 1.0 N to obtain a pH value of 3.4. The adjustment of the FaSSGF pH from 1.6 to 3.4 was performed in order to mimic the gastric pH after administration of orange juice as in the *in vivo* study performed by Qureshi et al. (Qureshi et al., 1992), as the pH of orange juice was found to be 3.4. The experiments were not directly performed in orange juice as no difference in nifedipine's solubility was observed between FaSSGFoj and orange juice (data not shown) and therefore FaSSGFoj was chosen as the dissolution medium. The impact of orange juice components, which may affect capsule rupture time, was not taken into account in this study, as the type of orange juice used in the *in vivo* study was not indicated. The ethanol containing media were prepared by adding the required volume of ethanol to FaSSGFst or FaSSGFoj, in order to obtain a final ethanol concentration of 47% v/v, as the one used in the *in vivo* study from Qureshi et al (Qureshi et al., 1992).

2.3 Dissolution experiments

2.3.1 USP1 and USP2 Apparatus

Dissolution experiments were performed using USP1 and USP2 apparatus (Dissolution tester DT826 LH, Automatic Sampling Station, Syringe Pump SP840, Fraction Collector FRL800, all from Erweka). Each probe of the automatic sampling station was equipped with PTFE intake

148 liquid-filters (10 µm, Erweka). Dissolution of 10 mg nifedipine Adalat® IR capsules was
149 performed in the USP2 apparatus at 50 rpm, 900 mL SGFsp as described in the Nifedipine
150 Monograph (United States Pharmacopeia, 2015b). Dissolution of 20 mg (2 x 10 mg capsules)
151 nifedipine Adalat® IR capsules was performed in the USP2 apparatus at 50 rpm and 500 mL of
152 SGFsp.
153 The experimental combinations performed with the varying parameters are presented in Table
154 1. The parameters studied were: volume of media (500 and 900 mL), rotational speed (50 and
155 100 rpm), pH (1.6 and 3.4), and ethanol content (0 and 47% v/v). Two Adalat® 10 mg IR
156 capsules were used for each replicate in order to mimic the *in vivo* study from Qureshi et al
157 (Qureshi et al., 1992). In the case of USP2 apparatus each capsule was placed in a stainless steel
158 sinker (Copley, UK), in order to avoid floating of the capsule in the vessel. Run time for the
159 dissolution experiments was 2 h and the temperature was set to 37 ± 0.5 ^{0}C. One mL sample
160 was withdrawn at 5, 10, 15, 20, 25, 30, 40, 60, 90, and 120 min and collected in amber vials.
161 All experiments were performed in triplicate.
162
163 **2.3.2 USP3 apparatus**
164 Variables tested for experiments with the USP3 apparatus were: volume of media (100 and 200
165 mL), pH (1.6 and 3.4), dipping rate (5 and 15 dpm), and ethanol (0 and 47% v/v). The
166 experimental combinations performed with the varying parameters are presented in Table 1. In
167 the dissolution experiments (n = 3) performed with the USP3 apparatus (Bio-Dis Reciprocating
168 Cylinder Apparatus and 750 Heater, both from Agilent Technologies) two Adalat® 10 mg
169 capsules were inserted in the reciprocating cylinder. Run time for the dissolution experiments
170 was 18 minutes, as preliminary experiments showed that this was the optimal time required for
171 capturing the very fast dissolution of the capsules. The temperature was set to 37 ± 0.5°C.
172 Samples of 5 mL were collected at 3, 6, 9, 12, 15, and 18 minutes with a glass syringe (Fortuna

Optima, Poulten & Graf GmbH, Germany) and they were filtered discarding the first 1 mL. The remaining 4 mL were used for the drug analysis. CN filters were used while performing the dissolution in medium without ethanol; whereas RC filters were used when ethanol was present in the dissolution medium.

2.3.3 USP4 apparatus

Variables tested for the experiments with the USP4 apparatus were: flow rate (4 and 8 mL/min), pH (1.6 and 3.4), and mode of operation (open and closed mode). Experiments with ethanol were not performed with the USP4 apparatus, due to incompatibility of the tubing of this apparatus with this solvent. The experimental combinations performed with the varying parameters are presented in Table 1.

Dissolution experiments (n = 3) were performed on a USP4 apparatus - Flow-Through-cell Dissolution tester (type DFZ 720, Piston pump type HKP 720, and Heater DH 1520i, Erweka GmbH) equipped with large cells (22.6 mm diameter), a 5 mm glass bead at the bottom of the cell and small glass beads (1 mm diameter) filling the cone in the cell. In each cell, two Adalat® 10 mg capsules were placed on the top of the small beads and a tablet holder was placed in the reverse position in order to avoid floating of the capsules. On top of each cell, two filters were placed, namely a GF/D filter (Glass Microfibre Filters 24 mm, Whatman™) and a GF/F filter (Glass Microfibre Filters 24 mm, Whatman™). For each set-up, the run time was set to 2 hours and the temperature was set to 37 ± 0.5 °C. When the open mode was used, fresh medium flew through the cells and the samples were collected in glass cylinders. When the closed mode was used, 900 mL of medium were placed into a Duran bottle under continuous stirring and a sample of 5 mL was withdrawn and volume replacement with fresh medium was made after each sampling. For all the experiments, sampling times were 15, 30, 45, 60, 75, 90, 105 and 120 min.

2.4 HPLC analysis

Nifedipine quantification was performed with HPLC-UV (samples from USP1 and USP2 apparatus experiments: Waters 2695 Separation Module and 2996 Photodiode Array Detector; samples from USP3 and USP4 apparatus experiments: Agilent 1100) using a C18 column (250 X 4.6, 5μm, Kromasil, AkzoNobel, Sweden) and MeOH/H_2O 60/40 v/v as mobile phase. Injection volume was 50 μL, flow rate 1 mL/min, run time 15 min, detection at 238 nm and column temperature 22°C. Standard solutions for the calibration curves were freshly prepared in duplicate in the corresponding medium in the concentration range 0.1-54 μg/mL using a stock solution of nifedipine in methanol. All the experiments and sample preparation and analysis were performed in darkness to prevent nifedipine's photodegradation (O'Neil, 2006).

2.5 Data analysis and calculations

Capsules rupture times (T_R) were calculated as described by Vardakou et al. (Vardakou et al., 2011). Briefly, T_R was calculated as the mean time between the time at which nifedipine concentration was found to be greater than 1% ($t_{(c>1\%)}$) and the time at which nifedipine concentration was found to be 1% ($t_{(c=1\%)}$) (Eq. 1):

$$T_R = \frac{t_{(c=1\%)} + t_{(c>1\%)}}{2} \quad \text{(Eq.1)}$$

The times corresponding to 1% were obtained from interpolation of the dissolution data.

In order to be able to correlate the *in vivo* capsule rupture time with the *in vitro* capsule rupture time, it is necessary to take into account the gastric emptying. This is of high importance as the presence of orange juice or ethanol may alter the gastric emptying, and therefore influence the appearance of the drug into the bloodstream. Gastric emptying data were obtained from the literature for water, orange juice and various alcoholic mixtures (Bateman and Whittingham, 1982; Cooke, 1970; Kaufman and Kaye, 1979; Levitt et al., 1997; Sun et al., 1988). The *in vivo*

data were corrected using the method of Elashoff and co-workers (Elashoff et al., 1982), since some of the published data did not restrained the fitting through the maximum administered volume at time zero. Therefore, gastric emptying data were analyzed by fitting the volume remaining in the stomach over time with a power exponential equation (Solver tool in Excel, Office 2013, Microsoft) (Eq. 2) (Elashoff et al., 1982):

$$f = 2^{-(\frac{t}{t_{1/2}})^{\beta}} \quad \text{(Eq.2)}$$

where f is the fraction of volume in the stomach at the time t, $t_{1/2}$ is the time required to empty 50% of the meal (gastric emptying time half-life) and β is the shape of the curve.

In vivo absorption profiles of Adalat® nifedipine IR capsules administered with water, orange juice and orange juice/ethanol mixture were obtained after deconvolution of published oral data (Qureshi et al., 1992; Rämsch and Sommer, 1983) using the Loo-Riegelman two compartment deconvolution model (Loo and Riegelman, 1968) (Eq. 3) (Excel, Office 2013, Microsoft), since nifedipine follows two compartmental kinetics (Chung et al., 1987):

$$\left(\frac{A}{Vp}\right)_{t_n} = Cp_{t_n} + k_{el}\int_{t_0}^{t_n} Cpdt + Ct_{t_n} \quad \text{(Eq.3)}$$

The pharmacokinetic (PK) constants (k_{el}, k_{12} and k_{21}) used for the Loo-Riegelman deconvolution were calculated from published *in vivo* nifedipine intravenous data (Kleinbloesem et al., 1984) at the dose of 0.015 mg/kg body weight via the feathering method. Point to point *in vitro-in vivo* correlations (IVIVC) were obtained by correlating the *in vitro* dissolution and the *in vivo* absorption data for the same time point. When necessary, *in vitro* and *in vivo* data points were calculated using the linear interpolation method. Time scaling was applied only when the *in vitro* dissolution was much faster than *in vivo* absorption, i.e. the amount dissolved *in vitro* reached the plateau in 20 minutes. Levy plots were used to define the time scaling parameters, and were performed when a minimum of three time points could be used.

3 Results and discussion

3.1 Dissolution data

3.1.1 Simulated gastric fluid USP media (SGFsp) – Simulated fasted state stomach (acidic conditions - FaSSGFst)

The dissolution profile of nifedipine IR capsules under the different dissolution conditions showed to be affected by the parameters chosen for each experimental setting, as well as the type of apparatus.

The dissolution of a 10 mg nifedipine IR capsule (1 x 10 mg capsule), using the conditions described for the Nifedipine Monograph (USP 2 paddle apparatus, 50 rpm and 900 mL of SGFsp) (United States Pharmacopeia, 2015b), is shown in Figure 1, along with the dissolution of 20 mg (2 x 10 mg capsules) nifedipine IR capsules at 50 rpm and 500 mL of SGFsp. In the first experiment nifedipine dissolved completely within 20 minutes, while only about 30% of the drug dissolved in the second experiment. This indicates that increasing the dose of nifedipine and reducing the volume of the medium, induces a reduction of the amount of nifedipine dissolved, due to the lack of sink conditions and limited solubility of the drug.

Precipitation of nifedipine was observed in FaSSGFst, with a total % dissolved after 120 minutes of ~49%, 66%, 77% and 42% for the USP1, USP2, USP3 and USP4 apparatus, respectively (Figure 2). The theoretical maximum % of nifedipine dissolved, considering a solubility of 10.5 µg/mL in FaSSGFst (Thelen et al., 2010), would correspond to 26.25% in 500 mL and 47.25% in 900 mL for a 20 mg dose. These theoretical values based on solubility are in agreement with the values observed for the USP1 experiments, while for the USP2 experiments the amount of nifedipine dissolved after 2 h was found to be higher and it was not affected by the volume used in the experiment (~ 55% and 65% for 500 and 900 mL, respectively). In the USP2 apparatus the rotational speed showed to impact the rate of

nifedipine's dispersion from the capsules. At 100 rpm the dissolution of nifedipine was fast, and approximately 100% of nifedipine dissolved just after 5 minutes, and rapidly followed by precipitation. As this rapid dissolution and precipitation was not observed with the USP1 apparatus, it can be suggested that the different configuration of the two dissolution apparatus has an impact on the precipitation of nifedipine from the soft gelatin capsules. The different volume used in the USP3 apparatus did not seem to greatly impact the dissolution of nifedipine, as similar results were obtained in 100 and 200 mL after 18 minutes, while it showed to be influenced by the dipping rate, with higher dipping rate (15 dpm) leading to a higher % dissolved (74.50%) than the lower dipping rate (5 dpm) (56.87% - 61.48%). Similarly, the dissolution of nifedipine in the USP4 apparatus was not affected by the volume (open or closed system) but by the flow rate used, with higher dissolution (41.88%) to be observed at the higher flow rate (8 mL/min) compared to a 24.52-25.58% dissolved at the lower flow rate (4 mL/min).

3.1.2 Simulated stomach after administration of Orange Juice (FaSSGFoj)

Precipitation of nifedipine was observed in FaSSGFoj with a total % dissolved after 120 minutes of ~41, 66%, 77% and 55% for the USP1, USP2, USP3 and USP4 apparatus, respectively (Figure 2). In the case of the USP1 apparatus, the volume used showed an impact on the total amount of nifedipine dissolved, similarly to when FaSSGFst was used. However, the differences in amount dissolved between 500 and 900 mL FaSSGFoj were found to be slightly less pronounced (~ 30 and 41%) than in FaSSGFst. Bigger differences between the two volumes were observed for the USP2 apparatus, with values of nifedipine dissolved after 2 h of ~ 30% and 65% in 500 and 900 mL, respectively. Differences in nifedipine dissolution due to the volume used were also observed for the USP3 apparatus. In this case the % of nifedipine dissolved after 18 minutes were ~ 32% and 78% in 100 and 200 mL of FaSSGFoj, respectively.

Using the closed or open mode in the USP4 apparatus did not impact significantly the % of nifedipine dissolved at the end of the 2 h (55% and 49%, respectively).

3.1.3 Simulated fasted stomach after administration of Ethanol (FaSSGFst/EtOH)

Dissolution of nifedipine IR capsules in the fasted acid stomach in the presence of ethanol is shown in Figure 3 for the USP1, USP2 and USP 3 apparatus. For both USP1 and USP2 apparatus the % of nifedipine dissolved at the end of the 2 h dissolution was around 100%. However, differences in the rate of dissolution were observed between the two systems, as well as between the apparatus set-up. Specifically, the following were observed: *i*) in both apparatus the rate of dissolution was found to be faster at 100 rpm compared to 50 rpm; *ii*) overall the dissolution in the USP2 apparatus was faster than in the USP1 apparatus; and *iii*) the difference in dissolution rate at 50 and 100 rpm was larger for the USP1 apparatus compared to the USP2 apparatus.

For the USP3 apparatus, the dissolution of nifedipine IR capsules reached nearly 100 % for the dipping rate of 15 dpm, despite the lower volume (100 mL), compared to the experiment performed at 5 dpm (200 mL), where only around 72% of nifedipine was dissolved after 18 minutes. This suggests that the dipping rate plays a role in the dissolved amount of nifedipine, and a low dipping rate may not be sufficient to optimally dissolve the capsule shell and to disperse its content. This observation was supported by the fact that the capsule shell did not dissolve completely at the end of the dissolution experiment, especially when the dipping rate of 5 dpm was used.

3.1.4 Simulated stomach after administration of Orange Juice-Ethanol mixture (FaSSGFoj/EtOH)

The dissolution of nifedipine from the capsules was complete in nearly all the FaSSGFoj/EtOH experiments, (Figure 3). For the experiments performed with the USP1 apparatus, both rotation speed and pH showed to play a role in the dissolution, while the volume did not show to have any influence. The dissolution rate of nifedipine from IR capsules in FaSSGFoj/EtOH was lower than the one in FaSSGFst/EtOH. The same observations regarding the influence of rotation speed and pH can be made also for the experiments performed in the USP2 apparatus. In the USP3 apparatus, the dipping rate was not found to affect the dissolution as in the previous case (section 3.1.3). Dissolution was found to be influenced by the volume used and by the pH. These results suggest that the presence of ethanol and the pH change have a significant effect on the capsule shell dissolution, thus impacting the overall dissolution of nifedipine from IR soft gelatin capsules, and could give an insight on the *in vivo* impact of ethanol on the rupture of the capsule and delivery of the drug.

3.2 Absorption data

The % *in vivo* absorbed of nifedipine after administration of IR capsules under fasting conditions (at the strengths of 10 and 20 mg) as calculated from the Loo-Riegelman deconvolution of the plasma profiles are shown in Figure 4A (Rämsch and Sommer, 1983). Nifedipine's absorption after the administration of the 10 mg dose was faster than that of the 20 mg dose, as in the latter case nifedipine precipitates in the stomach (Thelen et al., 2010). The % *in vivo* absorbed obtained from the Loo-Riegelman deconvolution of the plasma profiles of two 10 mg nifedipine IR capsules administered with either orange juice or orange juice/ethanol (Qureshi et al., 1992) are shown in Figure 4 B. In this case, the onset of absorption occurs earlier

when the ethanolic mixture is co-administered with the drug, compared to the co-administration of the drug with the orange juice.

3.3 Capsules rupture time

The *in vitro* dissolution results (section 3.1) obtained in this study have shown that capsule rupture time T_R was affected by the dissolution conditions, and in particular, it was found to be faster in the alcohol free media compared to the alcoholic mixtures (Figure 5). The capsule content dissolved after few minutes in both FaSSGFst and FaSSGFoj in the experiments performed with the USP1, USP2 and USP3 apparatus (below 7 minutes), while the capsule rupture times observed with the USP4 apparatus were higher than with the other three apparatus (ranging from ~ 8 to 23 minutes, depending on the experimental set-up). The T_R was affected by the pH and the rotation speed/flow rate used, with a T_R increase as the pH increased and a T_R decrease as the rotation speed/flow rate increased. The pH effect was not observed in the case of the USP4 apparatus.

Comparing the T_R values obtained experimentally with *in vivo* data, it is possible to observe that the T_R value obtained from the USP 4 apparatus is within the 10-15 minutes rupture time observed *in vivo* for standard soft gelatin capsules (Teles et al., 2014) and within the 15 min *in vitro* requirement from the USP General Chapter <2040> on Disintegration and Dissolution of Dietary Supplements (United States Pharmacopeia, 2015c). On the contrary, the faster rupture time observed for the other three apparatus is likely to be due to the different hydrodynamics, which may accelerate the rupture of the capsule shell compared to the *in vivo* conditions. In the *in vivo* study from Qureshi et al. (Qureshi et al., 1992), the nifedipine plasma onset was found to be faster in the presence of ethanol, and this was related to an increased absorption rate and a simultaneous inhibition of the metabolism of the drug when ethanol was co-administered (Qureshi et al., 1992). However, when comparing the T_R values in FaSSGFoj obtained with all

363 the experimental setups with the *in vivo* calculated rupture time after the administration of
364 orange juice (T_R = 30.38 minutes), a lower value was observed *in vitro*. From the deconvoluted
365 nifedipine plasma data in the presence of the orange juice/ethanol mixture, the T_R was calculated
366 to be 12.06 minutes and similar values were obtained from the USP1, 2 and 3 apparatus in
367 FaSSGFoj/EtOH (between 12.72-19.48, 8.95-12.78 and 7.12-15.06 minutes, respectively).
368 Since the *in vivo* T_R originates from plasma deconvoluted data obtained from Qureshi et al.
369 (Qureshi et al., 1992), the values of T_R for the orange juice and the orange juice/ethanol mixture
370 (30.38 and 12.06 minutes, respectively) can be affected by the following factors: (*i*) interactions
371 occurring between the capsule shell and the beverage; (*ii*) gastric emptying rate of the beverage;
372 (*iii*) sampling times of the study; (*iv*) solubilisation/precipitation of the nifedipine due to the
373 composition and volume of the administered beverage; (*v*) nifedipine permeability.
374 Interactions can occur between the capsule shell and the beverage, and it is possible that the
375 presence of specific components in the orange juice may retard the capsule shell dissolution.
376 The capsule rupture times calculated from the *in vivo* deconvoluted data from Rämsch and
377 Sommer (Rämsch and Sommer, 1983) show that the T_R for a 10 mg capsule is 8.47 minutes,
378 while for 20 mg capsules is 12.84 minutes. Both values are within the expected *in vivo* times of
379 10-15 minutes observed by Teles et al for standard soft gelatin capsules (Teles et al., 2014) .
380 The different T_R calculated from the clinical experiments with water (12.84 minutes) and orange
381 juice (30.38 minutes) indicates the interaction between the orange juice and the capsules shell.
382 Gastric emptying is a process regulated by the calorific content of the meal and its volume(Hunt
383 and Stubbs, 1975). However, the impact of gastric emptying of orange juice on the *in vivo* T_R
384 calculation can be considered to be minimal and can be excluded by considering the gastric
385 emptying time data of these beverages (liquid meals). The half-emptying time ($t_{1/2}$) of 400 mL
386 orange juice has been found to be in the range of 14 to 18.7 minutes, depending on the
387 temperature (Sun et al., 1988), and of 16.37 minutes for 500 mL orange juice cordial (Bateman

388 and Whittingham, 1982) (a diluted orange juice drink). In comparison, a volume of 350 mL of
389 water has shown to have a $t_{1/2}$ of 9.66 minutes (Cooke, 1970). Similarly to the orange juice, the
390 influence of the gastric emptying on the calculated *in vivo* T_R can be excluded for the orange
391 juice/ethanol mixture, despite the ethanol inhibitory effect on gastric emptying (Franke et al.,
392 2004). Gastric emptying time studies performed *in vivo* for various ethanol mixtures have
393 shown that the value of $t_{1/2}$ is affected by the volume and the ethanol content. After reanalysis
394 of published data, a value of 11.05 minutes was calculated for 350 mL mixture of ethanol ~7%
395 v/v (Cooke, 1970), 3.38 minutes for a 380 mL mixture containing 0.15 g/Kg ethanol
396 (corresponding to ~ 3 to 4% v/v of ethanol) (Levitt et al., 1997), and 16.95 minutes for 750 mL
397 mixture of ethanol 11% v/v (Kaufman and Kaye, 1979). The latter value is within the range
398 observed by Franke et al. (Franke et al., 2004), which found that 500 mL of ethanol 10% v/v
399 were emptied after 22.7 minutes. For higher % of ethanol the only available study is that of
400 Franke et al (Franke et al., 2004), in which the $t_{1/2}$ of 125 mL of 40% v/v ethanol mixture and
401 125 mL of whisky 40% v/v have been found to be 27.8 and 26.4 minutes, respectively.
402 However, in this study, the alcoholic drinks were rapidly followed by the intake of 125 mL of
403 water, which will reduce the ethanol concentration in the stomach to about 20% v/v. The gastric
404 emptying times for these higher ethanol concentrations indicate that the gastric emptying of the
405 orange juice/ethanol mixture from the stomach does not affect the calculation of the *in vivo* T_R
406 for the nifedipine capsules.
407 The sampling times in the study of Qureshi et al. (Qureshi et al., 1992), with the first plasma
408 sample collected after 19.8 minutes, may affect the calculations of the T_R for the orange
409 juice/ethanol mixture, as any earlier rupture of the capsule and absorption of nifedipine was not
410 detected. The fact that in our experimental set up FaSSGFoj was used instead of orange juice
411 cannot exclude the possibility of interactions between the orange juice components with the
412 capsule shell.

It is likely that solubilisation (in the case of the orange juice/ethanol mixture) or precipitation (in the case of orange juice) of nifedipine, due to the administered liquid composition and volume, can affect the appearance of the drug in the plasma, and therefore the T_R. This is confirmed by the experiments performed by Thelen and coworkers (Thelen et al., 2010) in FaSSGF, for which precipitation of nifedipine was observed. Since nifedipine's permeability is rather high (Gajendran et al., 2015), the dissolved nifedipine will be absorbed as soon as it is released into the duodenum. Also, nifedipine's permeability is increased in the upper gastrointestinal tract due to the presence of ethanol (Lavo et al., 1992; Volpe et al., 2008). Therefore, the increased plasma onset observed *in vivo* could be due to the higher solubility of nifedipine in the alcoholic mixture, while the slightly lower plasma concentration observed in the presence of pure orange juice could be due to interactions between the capsules' shell and the orange juice, and the precipitation of nifedipine in the stomach.

3.4 In Vitro- In Vivo Correlations (IVIVC)

The development of IVIVC was based on the correlation of the absorption data from the deconvoluted plasma concentration time profiles in water, orange juice and orange juice/ethanol mixture with the dissolution data from the experiments in FaSSGFst, FaSSGFoj and FaSSGFoj/EtOH, respectively.

3.4.1 Fasted stomach (acidic conditions)

For the two Pharmacopoeial experiments performed in the USP2 apparatus in SGFsp, the *in vitro* dissolution was found to be faster than the *in vivo* absorption (calculated from the plasma concentration data from Rämsch et al. (Rämsch and Sommer, 1983), Figure 4), as it is shown in Figure 6. In the case of the test performed with a 10 mg dose under the Pharmacopoeial conditions (50 rpm and 900 mL), a linear correlation was obtained between the *in vitro* amount

dissolved and the *in vivo* amount absorbed after time scaling of the *in vitro* data (y = 1.0707x – 2.8809, R^2 = 0.9733) (Figure 6). In the case of the experiment performed with a reduced volume, lower rotation speed and higher dose, the dissolution *in vitro* was faster than the absorption *in vivo* at the beginning, but then the precipitation occurring *in vitro* prevented any further dissolution, while *in vivo* absorption was observed despite the precipitation (Figure 6). In the case of the dissolution experiments performed in FaSSGFst at varying conditions, generally it was not possible to obtain any correlation with the experiments performed with the USP1, and USP2 apparatus, as the *in vitro* dissolution was much slower than the *in vivo* absorption of nifedipine administered with a glass of water (Rämsch and Sommer, 1983) (Figure 4). Only in the case of the dissolution data obtained with the USP3 apparatus at 5 dpm and with 100 mL of FaSSGFst, a linear IVIVC was obtained after time scaling of the *in vitro* data (y = 0.7933x + 3.9437, R^2 = 0.9641), Figure 7A. The *in vitro* dissolution experiments performed with the USP4 apparatus resulted in two linear correlations, as shown in Figure 7B. The linear correlations were obtained for the experiment performed at 8 mL/min in the open mode (y = 2.4428x – 32.985, R^2 = 0.9319), and for the experiment performed at 4 mL/min in the open mode (y = 3.6093x - 12.294, R^2 = 0.9915).

3.4.2 Stomach after administration of Orange Juice

Time scaling of the *in vitro* data in FaSSGFoj was not possible for the dissolution data from the USP1 apparatus due to the fast and incomplete *in vitro* dissolution. The USP2 produced one linear correlation after time scaling for the experiment performed at 100 rpm and 900 mL (y = 1.0254x + 9.9612, R^2 = 0.9466), Figure 8A. For the dissolution data from the USP3 apparatus a linear correlation was obtained for the experiment performed at 5 dpm and 200 mL after time scaling (y = 0.8927 + 7.5831¸ R^2 = 0.9503), as shown in Figure 8B.

The dissolution data from the USP4 apparatus led, after time scaling of the *in vitro* data, to two linear IVIVC when simulating the intake of two nifedipine capsules with orange juice, Figure 8C. The *in vitro* dissolution data which correlated well with the *in vivo* data were from the experiments performed at 4 mL/min in the open mode ($y = 1.2274x – 1.944$, $R^2 = 0.9627$) and at 8 mL/min in the closed mode ($y = 1.0057 + 0.3331$, $R^2 = 0.9767$).

3.4.3 Stomach after administration of Orange Juice-Ethanol mixture

In the case of the dissolution experiments performed in FaSSGFoj/EtOH simulating the orange juice/ethanol mixture, one nonlinear and two linear IVIVCs were achieved for the in vitro data from the USP1 apparatus, Figure 9A.

The nonlinear correlation was obtained for the dissolution data from the experiment performed at 100 rpm and 500 mL ($y = 2.788\ e0.30309x$, $R^2 = 0.9908$), while the other two linear correlations were obtained for the dissolution data from the experiments performed at 50 rpm and 500 mL ($y = 0.7086x + 0.612$, $R^2 = 0.9978$) and 50 rpm and 900 mL ($y = 0.7595x + 2.5778$, $R^2 = 0.9813$).

Two time scaled linear and one non-linear correlations were obtained for the in vitro data from the USP2 apparatus, Figure 9B. The nonlinear correlation (without time scaling) was obtained for the data from the experiment performed at 50 rpm and 900 mL ($y = 2.4468\ e0.0356x$, $R^2 = 0.9972$). The in vitro data that showed correlations with the *in vivo* data after time scaling were from the experiments performed at 50 rpm and 500 mL ($y = 0.9793 + 2.8929$, $R^2 = 0.9820$), and 100 rpm and 500 mL ($y = 1.0382 + 0.024$, $R^2 = 0.9591$). After time scaling, a linear correlation was obtained also for the data from the USP3 apparatus at the experimental set up of 15 dpm and 200 mL ($y = 1.0327 + 3.7304$, $R^2 = 0.9300$). In the cases where time scaling of the data was required, *in vitro* dissolution was found to be faster than *in vivo* absorption.

4 Conclusion

The *in vitro* dissolution studies showed that the hydrodynamics, as well as the media composition, played a key role in the establishment of good IVIVC for nifedipine's IR formulations. With respect to the fluid dynamics, at 50 rpm the hydrodynamics in both USP1 and USP2 apparatus is much higher than the *in vivo* hydrodynamics. The fluid velocities generally produced by the dissolution apparatus are very high and have Reynolds numbers between 5000 and 10000 (Mudie et al., 2010), while *in vivo* the flow is non turbulent and the Reynolds number range between 1 and 30 (Mudie et al., 2010), with maximum values between 35 and 100-125 when considering spikes due to high flow (Diebold, 2005). At 50 rpm the USP2 apparatus produces maximum velocities between 0.049 and 0.067 m/s (D'Arcy et al., 2005), while the USP1 apparatus has shown to have maximum velocities generally lower than the USP2 apparatus at the same rotational speed and with a maximum value of 0.026 m/s (D'Arcy et al., 2009). The calculated velocities in the stomach due to retropulsive jets has been calculated to be around 0.0075 m/s (Pal et al., 2004), while the average transit time in the intestine ranges between 0.0002 and 0.0008 m/s (Diebold, 2005). So even at 50 rpm, the velocities experienced by the formulation *in vitro* are much higher than those *in vivo*, which is reflected by the observed IVIVCs. The hydrodynamics of the USP3 apparatus has been found to be influenced by the dip rate, with maximum velocities ranging between approximately 0.04 and 0.08 m/s for 5 and 10 dpm, respectively, and showed to have a Reynold number of 1870 (corresponding to a laminar flow) (Perivilli et al., 2015). Similarly to the case of the USP1 and USP2 apparatus, fluid velocity in the USP3 apparatus is much higher than that calculated *in vivo* (0.0002-0.0008 m/s (Diebold, 2005)).

The USP4 apparatus produces low Reynold numbers at flow rates between 4 and 50 mL/min, and the fluid velocities have been found to be, at 8 mL/min, between 0.0012 and 0.0014 m/s

(D'Arcy et al., 2011), which are closer to the *in vivo* values (0.0002-0.0008 m/s (Diebold, 2005)).

Regarding the impact of water, alcoholic and non-alcoholic beverages intake with a formulation, based on our study, when nifedipine capsules are administered with water at the dose of 10 mg, a good IVIVC was obtained with the standard dissolution set up required by the Pharmacopeia. The experiments in FaSSGFst using the four apparatus showed that no correlation could be obtained for the USP1 and USP2 apparatus, due to the fast precipitation of the administered 20 mg nifedipine capsules. For the USP3 an IVIVC was possible after time scaling of the *in vitro* data, due to the faster and incomplete dissolution *in vitro* compared to the *in vivo* absorption. For the USP4 apparatus the *in vitro* dissolution was found to be slower than the *in vivo* absorption.

Similarly to the FaSSGFst experiment, when 20 mg nifedipine capsules were tested in the media simulating the orange juice beverage (FaSSGFoj) correlations between *in vitro* data and *in vivo* data were obtained for the USP2, USP3 and USP4 apparatus. In all cases time scaling of the data was required to obtain IVIVC, due to the faster *in vitro* dissolution compared to the *in vivo* absorption. Mimicking the co-administration of orange juice/ethanol mixture showed that all three apparatus USP1, USP2 and USP3 were able to provide good IVIVC. Interestingly, the same experimental set ups for USP1 and USP2 generated the IVIVC, even though time scaling was required for two of the experimental set ups with the USP2 apparatus, while no time scaling was necessary for the USP1.

The co-administration of ethanol with nifedipine *in vivo* was found to impact the PK of the drug in terms of onset of action and increased bioavailability, due to faster absorption rate and metabolism inhibition (Qureshi et al., 1992). In our study, we observed that the faster absorption rate in the presence of ethanol, compared to the alcohol free water and orange juice, could be explained by several factors. The increased solubility of nifedipine in the presence of ethanol

47% v/v prevented precipitation of the drug, regardless of the liquid volume. Also, the presence of ethanol counteracted the effect of orange juice on the capsule rupture time. These two effects observed *in vitro* could contribute to the observed *in vivo* behaviour of the formulation.

Choosing the appropriate *in vitro* dissolution conditions in terms of media and hydrodynamics is critical in order to achieve a good correlation with *in vivo* data. The choice of a physiologically relevant dissolution set up is critical for investigating the formulation sensitivity to various beverages, and especially those containing ethanol, so that the risk associated with its co-administration can be predicted.

Acknowledgments

The authors acknowledge RCPE for funding support. Part of this work has been previously included in a poster at the AAPS Annual Meeting and Exposition in Orlando, October 2015.

References

Akinleye, M.O., Coker, H.A.B., Chukwuani, C.M., Adeoye, A.W., 2007. Effect of Five Alive fruit juice on the dissolution and absorption profiles of ciprofloxacin. Nig. Q. J. Hosp. Med. 17, 53–7.

An, G., Mukker, J.K., Derendorf, H., Frye, R.F., 2015. Enzyme- and transporter-mediated beverage-drug interactions: An update on fruit juices and green tea. J. Clin. Pharmacol. [Epub ahead of print].

Anwar, S., Fell, J.T., Dickinson, P.A., 2005. An investigation of the disintegration of tablets in biorelevant media. Int. J. Pharm. 290, 121–7.

Bailey, D.G., Malcolm, J., Arnold, O., Spence, J.D., 1998. Grapefruit juice-drug interactions. Br. J. Clin. Pharmacol. 46, 101–10.

Bateman, D.N., Whittingham, T. a, 1982. Measurement of gastric emptying by real-time ultrasound. Gut 23, 524–7.

Chung, M., Reitberg, D.P., Gaffney, M., Singleton, W., 1987. Clinical pharmacokinetics of nifedipine gastrointestinal therapeutic system. A controlled-release formulation of nifedipine. Am. J. Med. 83, 10–14.

Chuong, M.C., Taglieri, C.A., Crosby, S.J., Ferullo, J.W., Ng, P., 2010. Effect of beverages on the in vitro disintegration of immediate-release pain medications. Dissolution Technol. 17, 31–37.

Cooke, A.R., 1970. The stimultaneous emptying and absorption of ethanol from the human stomach. Am. J. Dig. Dis. 15, 449–454.

D'Arcy, D.M., Corrigan, O.I., Healy, A.M., 2005. Hydrodynamic simulation (computational fluid dynamics) of asymmetrically positioned tablets in the paddle dissolution apparatus: impact on dissolution rate and variability. J. Pharm. Pharmacol. 57, 1243–1250.

D'Arcy, D.M., Healy, A.M., Corrigan, O.I., 2009. Towards determining appropriate hydrodynamic conditions for in vitro in vivo correlations using computational fluid dynamics. Eur. J. Pharm. Sci. 37, 291–9.

D'Arcy, D.M., Liu, B., Corrigan, O.I., 2011. Investigating the effect of solubility and density gradients on local hydrodynamics and drug dissolution in the USP 4 dissolution apparatus. Int. J. Pharm. 419, 175–185.

Diebold, S.M., 2005. Physiological parameters relevant to dissolution testing: hydrodynamic considerations, in: Dressman, J., Krämer, J. (Eds.), Pharmaceutical Dissolution Testing.

Elashoff, J., Reedy, T., Meyer, J., 1982. Analysis of gastric emptying data. Gastroenterology 83, 1306–12.

584 Fagerberg, J.H., Al-Tikriti, Y., Ragnarsson, G., Bergström, C. a S., 2012. Ethanol effects on
585 apparent solubility of poorly soluble drugs in simulated intestinal fluid. Mol. Pharm. 9,
586 1942–52.

587 Fagerberg, J.H., Sjögren, E., Bergström, C. a. S., 2015. Concomitant intake of alcohol may
588 increase the absorption of poorly soluble drugs. Eur. J. Pharm. Sci. 67, 12–20.

589 FDA Alert for Healthcare Professionals, 2005. Hydromorphone Hydrochloride Extended-
590 Release Capsules (marketed as Palladone™).
591 http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsand
592 Providers/ucm129288.htm (accessed 8.10.15).

593 Franke, A., Teyssen, S., Harder, H., Singer, M. V., 2004. Effect of ethanol and some alcoholic
594 beverages on gastric emptying in humans. Scand. J. Gastroenterol. 39, 638–644.
595 doi:10.1080/00365520410005009

596 Fuchs, J., 2009. The amount of liquid patients use to take tablets or capsules. Pharm. Pract.
597 (Granada). 7, 170–174.

598 Gajendran, J., Krämer, J., Shah, V.P., Langguth, P., Polli, J., Mehta, M., Groot, D.W.,
599 Cristofoletti, R., Abrahamsson, B., Dressman, J.B., 2015. Biowaiver Monographs for
600 Immediate-Release Solid Oral Dosage Forms: Nifedipine. J. Pharm. Sci. 104, 3289–
601 3298.

602 Hunt, J.N., Stubbs, D.F., 1975. The volume and energy content of meals as determinants of
603 gastric emptying. J. Physiol. 245, 209–225.

604 Jedinger, N., Khinast, J., Roblegg, E., 2014. The design of controlled-release formulations
605 resistant to alcohol-induced dose dumping – A review. Eur. J. Pharm. Biopharm. 87,
606 217–226.

607 Jedinger, N., Schrank, S., Mohr, S., Feichtinger, A., Khinast, J., Roblegg, E., 2015. Alcohol
608 dose dumping: The influence of ethanol on hot-melt extruded pellets comprising solid
609 lipids. Eur. J. Pharm. Biopharm. 92, 83–95.

610 Johnson, F., Wagner, G., Sun, S., Stauffer, J., 2008. Effect of Concomitant Ingestion of
611 Alcohol on the In Vivo Pharmacokinetics of KADIAN (Morphine Sulfate Extended-
612 Release) Capsules. J. Pain 9, 330–336.

613 Kalantzi, L., Polentarutti, B., Albery, T., Laitmer, D., Abrahamsson, B., Dressman, J.,
614 Reppas, C., 2005. The delayed dissolution of paracetamol products in the canine fed
615 stomach can be predicted in vitro but it does not affect the onset of plasma levels. Int. J.
616 Pharm. 296, 87–93.

617 Kaufman, S.E., Kaye, M.D., 1979. Effect of ethanol upon gastric emptying. Gut 20, 688–692.

618 Keen, J.M., Foley, C.J., Hughey, J.R., Bennett, R.C., Jannin, V., Rosiaux, Y., Marchaud, D.,
619 McGinity, J.W., 2015. Continuous twin screw melt granulation of glyceryl behenate:
620 Development of controlled release tramadol hydrochloride tablets for improved safety.
621 Int. J. Pharm. 487, 72–80.

622 Kleinbloesem, C.H., van Brummelen, P., van de Linde, J.A., Voogd, P.J., Breimer, D.D.,
623 1984. Nifedipine: kinetics and dynamics in healthy subjects. Clin. Pharmacol. Ther. 35,
624 742–749.

625 Larsson, M., Hjärtstam, J., Berndtsson, J., Stading, M., Larsson, A., 2010. Effect of ethanol
626 on the water permeability of controlled release films composed of ethyl cellulose and
627 hydroxypropyl cellulose. Eur. J. Pharm. Biopharm. 76, 428–432.

628 Lavo, B., Colombel, J.F., Knutsson, L., Hallgren, R., 1992. Acute exposure of small intestine
629 to ethanol induces mucosal leakage and prostaglandin E2 synthesis. Gastroenterology
630 102, 468–473.

631 Lennernäs, H., 2009. Ethanol-drug absorption interaction: potential for a significant effect on
632 the plasma pharmacokinetics of ethanol vulnerable formulations. Mol. Pharm. 6, 1429–
633 1440.

634 Levitt, M.D., Li, R., DeMaster, E.G., Elson, M., Furne, J., Levitt, D.G., 1997. Use of
635 measurements of ethanol absorption from stomach and intestine to assess human ethanol
636 metabolism. Am. J. Physiol. 273, G951–G957.

637 Loo, J.C., Riegelman, S., 1968. New method for calculating the intrinsic absorption rate of
638 drugs. J. Pharm. Sci. 57, 918–928.

639 Meyer, R.J., Hussain, A.S., 2005. Awarenes Topic: Mitigating the Risks of Ethanol Induced
640 Dose Dumping from Oral Sustained/Controlled Release Dosage Forms - FDA's ACPS
641 Meeting. http://www.fda.gov/ohrms/dockets/ac/05/briefing/2005-4187B1_01_08-
642 Alcohol-Induced.pdf (accessed 8.10.15).

643 Mudie, D.M., Amidon, G.L., Amidon, G.E., 2010. Physiological parameters for oral delivery
644 and in vitro testing. Mol. Pharm. 7, 1388–405. doi:10.1021/mp100149j

645 O'Neil, M.J., 2006. The Merck Index- An Encyclopedia of Chemicals, Drugs, and
646 Biologicals. Merck & Co., Inc., Whitehouse station.

647 Pal, A., Indireshkumar, K., Schwizer, W., Abrahamsson, B., Fried, M., Brasseur, J.G., 2004.
648 Gastric flow and mixing studied using computer simulation. Proc. R. Soc. B Biol. Sci.
649 271, 2587–2594.

650 Palmer, P., Levina, M., Farrell, T.P., Rajabi-Siahboomi, A.R., 2011. The influence of hydro-
651 alcoholic media on drug release: From polyethylene oxide extended-release matrix
652 tablets. Pharm. Technol. 35, 50–58.

653 Perivilli, S., Kakhi, M., Stippler, E., 2015. Computational fluid dynamics simulation of
654 hydrodynamics in USP apparatus 3-the influence of dip rate. Pharm. Res. 32, 1304–15.

655 Qureshi, S., Laganière, S., Caillé, G., Gossard, D., Lacasse, Y., McGilveray, I., 1992. Effect
656 of an acute dose of alcohol on the pharmacokinetics of oral nifedipine in humans. Pharm.
657 Res. 9, 683–686.

Rämsch, K.D., Sommer, J., 1983. Pharmacokinetics and Metabolism of Nifedipine. Hypertension 5, II–18 – II–24.

Roberts, M., Cespi, M., Ford, J.L., Dyas, a. M., Downing, J., Martini, L.G., Crowley, P.J., 2007. Influence of ethanol on aspirin release from hypromellose matrices. Int. J. Pharm. 332, 31–37.

Rosiaux, Y., Muschert, S., Chokshi, R., Leclercq, B., Siepmann, F., Siepmann, J., 2013a. Ethanol-resistant polymeric film coatings for controlled drug delivery. J. Control. Release 169, 1–9.

Rosiaux, Y., Velghe, C., Muschert, S., Chokshi, R., Leclercq, B., Siepmann, F., Siepmann, J., 2014. Mechanisms Controlling Theophylline Release from Ethanol-Resistant Coated Pellets. Pharm. Res. 31, 731–741.

Rosiaux, Y., Velghe, C., Muschert, S., Chokshi, R., Leclercq, B., Siepmann, F., Siepmann, J., 2013b. Ethanol-resistant ethylcellulose/guar gum coatings – Importance of formulation parameters. Eur. J. Pharm. Biopharm. 85, 1250–1258.

Roth, W., Setnik, B., Zietsch, M., Burst, a., Breitenbach, J., Sellers, E., Brennan, D., 2009. Ethanol effects on drug release from Verapamil Meltrex®, an innovative melt extruded formulation. Int. J. Pharm. 368, 72–75.

Sathyan, G., Sivakumar, K., Thipphawong, J., 2008. Pharmacokinetic profile of a 24-hour controlled-release OROS formulation of hydromorphone in the presence of alcohol. Curr. Med. Res. Opin. 24, 297–305.

Schug, B., Brendel, E., Wonnemann, M., Wolf, D., Wargenau, M., Dingler, A., Blume, H., 2002. Dosage form-related food interaction observed in a marketed once-daily nifedipine formulation after a high-fat American breakfast. Eur. J. Clin. Pharmacol. 58, 119–125.

Smith, A.P., Moore, T.W., Westenberger, B.J., Doub, W.H., 2010. In vitro dissolution of oral modified-release tablets and capsules in ethanolic media. Int. J. Pharm. 398, 93–96.

Sorkin, E.M., Clissold, S.P., Brogden, R.N., 1985. Nifedipine: A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy, in ischaemic heart disease, hypertension and related cardiovascular disorders. Drugs 30, 182–274.

Sun, W.M., Houghton, L.A., Read, N.W., Grundy, D.G., Johnson, G.A., 1988. Effect of meal temperature on gastric emptying of liquids in man. Gut 29, 302–305.

Teles, H., Dries, K. Van Den, Chessa, S., Huatan, H., Sassene, P.J., Mullertz, A., 2014. Assessing Softgel Disintegration in the Gastric Compartment : Correlating the Dynamic Gastric Model with In Vivo Visualization by Camera-Capsules, in: Poster Presentation at the 2014 AAPS Annual Meeting and Exposition. San Diego, p. T3275.

Thelen, K., Jantratid, E., Dressman, J.B.., Lippert, J., Willmann, S., 2010. Analysis of nifedipine absorption from soft gelatin capsules using PBPK modeling and biorelevant dissolution testing. J. Pharm. Sci. 99, 2899–2904.

696 Toal, C.B., 2004. Formulation dependent pharmacokinetics - does the dosage form matter for
697 nifedipine? J. Cardiovasc. Pharmacol. 44, 82–86.

698 Traynor, M.J., Brown, M.B., Pannala, A., Beck, P., Martin, G.P., 2008. Influence of alcohol
699 on the release of tramadol from 24-h controlled-release formulations during in vitro
700 dissolution experiments. Drug Dev. Ind. Pharm. 34, 885–889.

701 United States Pharmacopeia, 2015a. Test solutions (TS), in: USP 38-NF 33. Rockville, MD,
702 USA.

703 United States Pharmacopeia, 2015b. Nifedipine capsules, in: USP 38-NF 33. Rockville, MD,
704 USA.

705 United States Pharmacopeia, 2015c. Disintegration <701>, Disintegration and Dissolution of
706 Dietary Supplements <2040>, in: USP 38-NF33. Rockville, MD, USA.

707 US FDA, Individual Product Bioequivalence Recommendations
708 http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm0
709 75207.htm (accessed 10.9.15).

710 Vardakou, M., Mercuri, A., Rizzo, D., Butler, J.M., Connolly, P.C., Wickham, M.S.J., Faulks,
711 R.M., 2011. Predicting the human in vivo performance of different oral capsule shell
712 types using a novel in vitro dynamic gastric model. Int. J. Pharm. 419, 192–199.

713 Vertzoni, M., Diakidou, A., Chatzilias, M., Söderlind, E., Abrahamsson, B., Dressman, J.B.,
714 Reppas, C., 2010. Biorelevant Media to Simulate Fluids in the Ascending Colon of
715 Humans and Their Usefulness in Predicting Intracolonic Drug Solubility. Pharm. Res.
716 27, 2187–2196.

717 Volpe, D.A., Asafu-Adjaye, Ebenezer B. Ellison, C.D., Doddapaneni, S., Uppoor, R.S., Khan,
718 M.A., 2008. Effect of ethanol on opioid drug permeability through caco-2 cell
719 monolayers. AAPS J. 10, 360–362.

720 Wells, K.A., Losin, W.G., 2008. In vitro stability, potency, and dissolution of duloxetine
721 enteric-coated pellets after exposure to applesauce, apple juice, and chocolate pudding.
722 Clin. Ther. 30, 1300–1308.

723 Yang, W., de Villiers, M.M., 2004. The solubilization of the poorly water soluble drug
724 nifedipine by water soluble 4-sulphonic calix[n]arenes. Eur. J. Pharm. adn Biopharm. 58,
725 629–636.

726 Zuo, J., Gao, Y., Almukainzi, M., Löbenberg*, R., 2013. Investigation of the Disintegration
727 Behavior of Dietary Supplements in Different Beverages. Dissolution Technol. 20, 6–9.

728

729

Table 1 Parameters used for the dissolution experiments in USP1, USP2, USP3 and USP4 apparatus. FaSSGFst = Fasted State Simulated Gastric Fluid at the standard pH of 1.6; FaSSGFoj = Fasted State Simulated Gastric Fluid at the pH of 3.4 as for orange juice; EtOH = ethanol; SGFsp = Simulated Gastric Fluid without pepsin.

Apparatus type	Exp n°	System Type	Media type	Rotational speed (rpm)	Dipping rate (dpm)	Flow rate (mL/min)	Volume (mL)	pH	Ethanol content (% v/v)
USP 1	1	-	FaSSGFoj	50	-	-	500	3.4	0
	2	-	FaSSGFst	100	-	-	500	1.6	0
	3	-	FaSSGFst	50	-	-	900	1.6	0
	4	-	FaSSGFoj	100	-	-	900	3.4	0
	5	-	FaSSGFst/EtOH	50	-	-	500	1.6	47
	6	-	FaSSGFoj/EtOH	50	-	-	500	3.4	47
	7	-	FaSSGFoj/EtOH	100	-	-	500	3.4	47
	8	-	FaSSGFoj/EtOH	50	-	-	900	3.4	47
	9	-	FaSSGFst/EtOH	100	-	-	900	1.6	47
USP 2	10	-	SGFsp	50	-	-	900	1.2	0
	11	-	SGFsp	50	-	-	500	1.2	0
	12	-	FaSSGFoj	50	-	-	500	3.4	0
	13	-	FaSSGFst	100	-	-	500	1.6	0
	14	-	FaSSGFst	50	-	-	900	1.6	0
	15	-	FaSSGFoj	100	-	-	900	3.4	0
	16	-	FaSSGFst/EtOH	50	-	-	500	1.6	47
	17	-	FaSSGFoj/EtOH	50	-	-	500	3.4	47
	18	-	FaSSGFoj/EtOH	100	-	-	500	3.4	47
	19	-	FaSSGFoj/EtOH	50	-	-	900	3.4	47
	20	-	FaSSGFst/EtOH	100	-	-	900	1.6	47
USP 3	21	-	FaSSGFst	-	5	-	200	1.6	0
	22	-	FaSSGFst	-	5	-	100	1.6	0
	23	-	FaSSGFoj	-	15	-	100	3.4	0
	24	-	FaSSGFoj	-	5	-	200	3.4	0
	25	-	FaSSGFst	-	15	-	200	1.6	0
	26	-	FaSSGFoj/EtOH	-	5	-	100	3.4	47
	27	-	FaSSGFst/EtOH	-	15	-	100	1.6	47
	28	-	FaSSGFst/EtOH	-	5	-	200	1.6	47
	29	-	FaSSGFoj/EtOH	-	15	-	200	3.4	47
USP 4	30	Open	FaSSGFoj	-	-	4	-	3.4	0
	31	Closed	FaSSGFst	-	-	4	-	1.6	0
	32	Open	FaSSGFst	-	-	8	-	1.6	0
	33	Closed	FaSSGFoj	-	-	8	-	3.4	0
	34	Open	FaSSGFst	-	-	4	-	1.6	0

735 Figure captions

736 Figure 1 Dissolution profiles (n = 3) of nifedipine IR capsules: (■) 1 capsule of 10 mg in 900
737 mL of SGFsp at 50 rpm and (●) 2 capsules of 10 mg in 500 mL of SGFsp at 50 rpm. Bars
738 represent standard deviation. SGFsp = Simulated Gastric Fluid without pepsin.

739 Figure 2 Dissolution profiles (n = 3) of nifedipine IR (2 x 10 mg) capsules in FaSSGF at varying
740 dissolution conditions using USP1, USP2, USP3 and USP4 apparatus. FaSSGFst = Fasted State
741 Simulated Gastric Fluid pH 1.6; FaSSGFoj = Fasted State Gastric Fluid pH 3.4.

742 Figure 3 Dissolution profiles (n = 3) of nifedipine IR (2 x 10 mg) capsules in FaSSGFst/EtOH
743 and FaSSGFoj/EtOH at varying dissolution conditions using USP1, USP2, and USP3
744 apparatus. FaSSGFst = Fasted State Simulated Gastric Fluid pH 1.6; FaSSGFoj = Fasted State
745 Gastric Fluid pH 3.4; EtOH = Ethanol.

746 Figure 4 A) % of nifedipine absorbed *in vivo* obtained from the deconvolution of the plasma
747 data of nifedipine capsules administered as (◆) 20 mg and (●) 10 mg (Rämsch and Sommer,
748 1983); B) % of nifedipine absorbed *in vivo* obtained from the deconvolution of the plasma data
749 of nifedipine capsules administered with orange juice (■) or a mixture of orange juice and
750 ethanol (□) (Qureshi et al., 1992). Loo-Riegelman two compartment model was used for the
751 deconvolution of the *in vivo* data.

752 Figure 5 Mean capsule rupture times (T_R) of nifedipine IR capsules in FaSSGFst, FaSSGFoj,
753 FaSSGFst/EtOH and FaSSGFoj/EtOH obtained with the four dissolution apparatus. Bars
754 represent the standard deviation (n = 3).

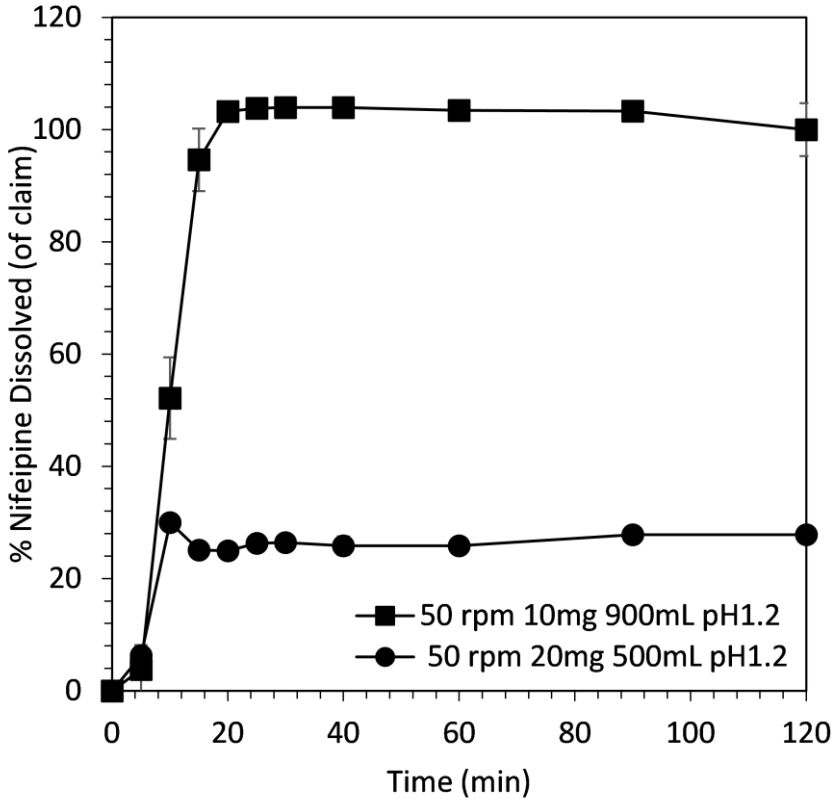
755 Figure 6 IVIVC for in vitro data from USP2 apparatus experiments simulating the intake of
756 nifedipine capsules with water and performed in SGFsp pH 1.2: (●) 10 mg in 900 mL at 50
757 rpm (after time scaling); (■) 2 x 10 mg in 500 mL at 50 rpm. *In vivo* amount absorbed were

obtained from the deconvolution of the *in vivo* plasma profiles of 10 and 20 mg nifedipine capsules published by Rämsch and coworkers (Rämsch and Sommer, 1983).

Figure 7 IVIVC for in vitro data from A) USP3 (after time scaling) and B) USP4 apparatus experiments simulating the intake of nifedipine capsules with water and performed in FaSSGFst (pH 1.6). *In vivo* amounts absorbed were obtained from the deconvolution of the *in vivo* plasma profiles of 20 mg nifedipine capsules published by Rämsch and coworkers (Rämsch and Sommer, 1983).

Figure 8 IVIVC for in vitro data from A) USP2, B) USP3 and C) USP4 apparatus experiments simulating the intake of nifedipine capsules with orange juice in FaSSGFoj (pH 3.4). *In vivo* amount absorbed were obtained from the deconvolution of the *in vivo* plasma profiles of 20 mg nifedipine capsules published by Qureshi and coworkers (Qureshi et al., 1992). Time scaling was applied in all cases.

Figure 9 IVIVC for in vitro data from A) USP1, B) USP2 and C) USP3 (after time scaling) apparatus experiments simulating the intake of nifedipine capsules with orange juice/ethanol mixture in FaSSGFoj/EtOH (pH 3.4). *In vivo* amount absorbed were obtained from the deconvolution of the *in vivo* plasma profiles of 20 mg nifedipine capsules published by Qureshi and coworkers (Qureshi et al., 1992).



778

779 Figure 1

780

781

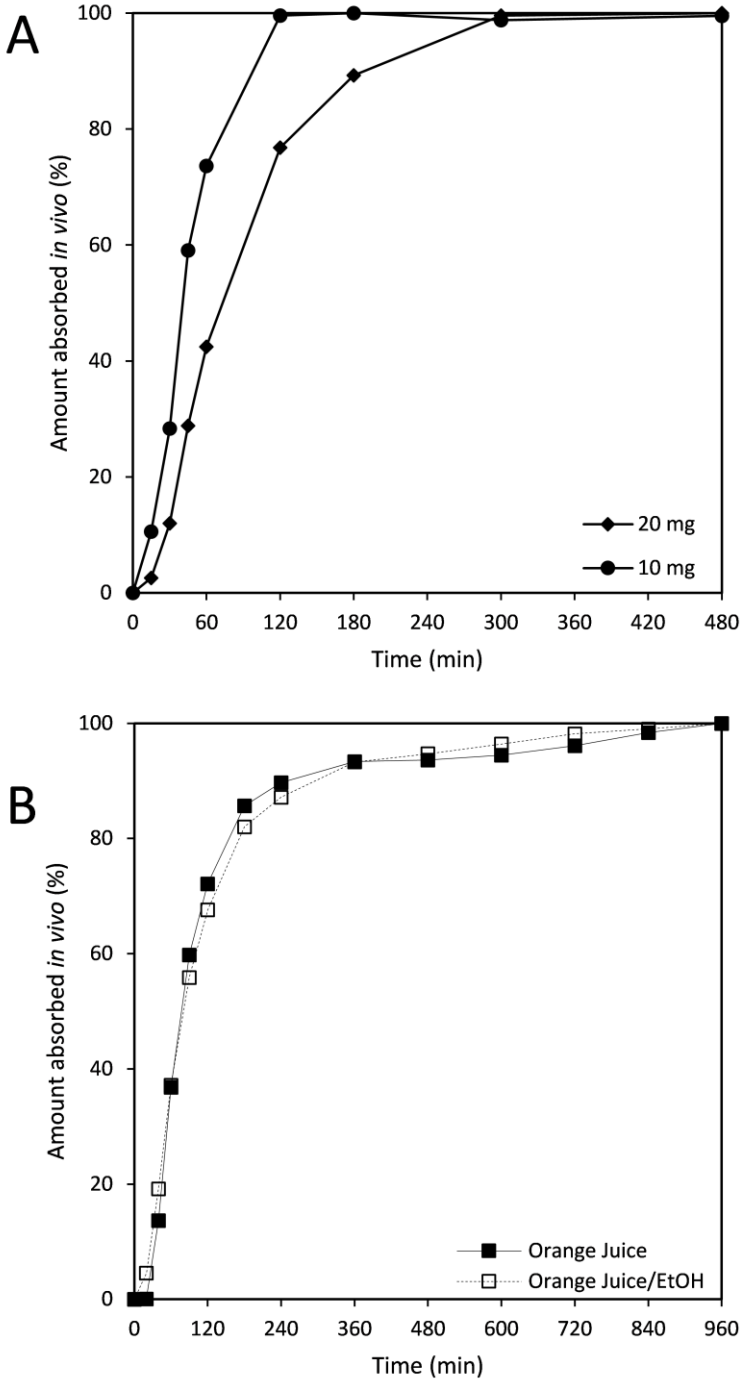
782 Figure 2

783

784

785 Figure 3

786

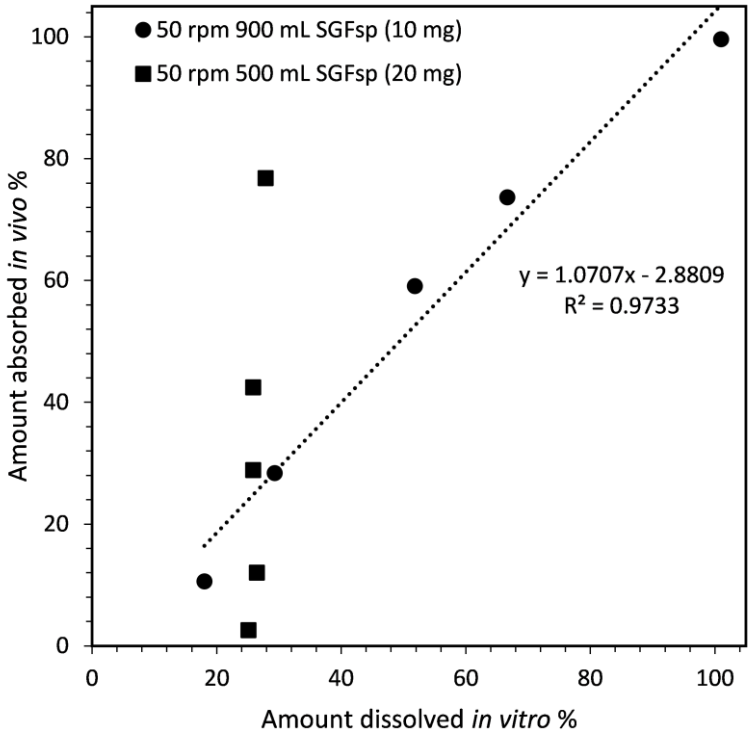


787

788 Figure 4

789

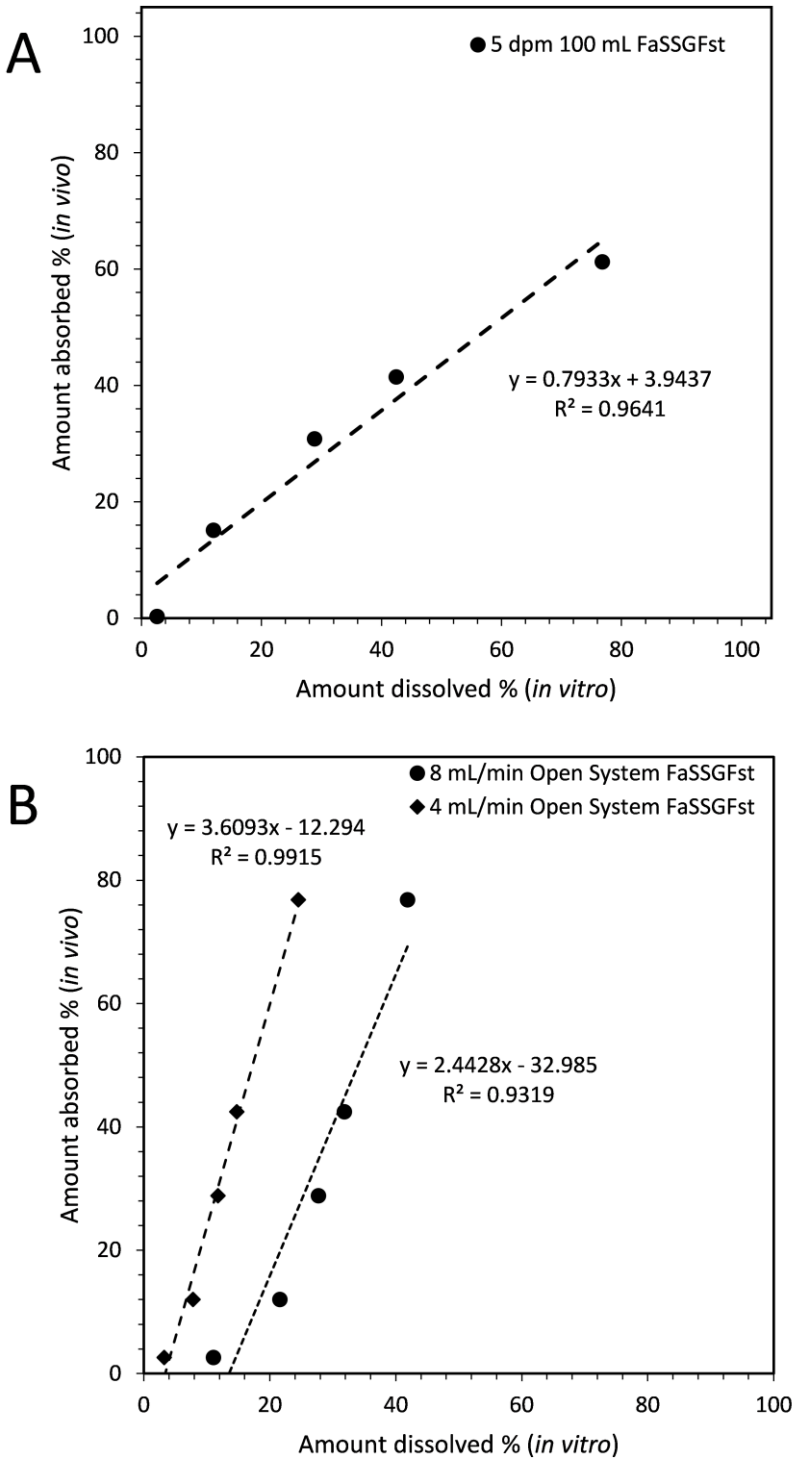
Figure 5



793

794 Figure 6

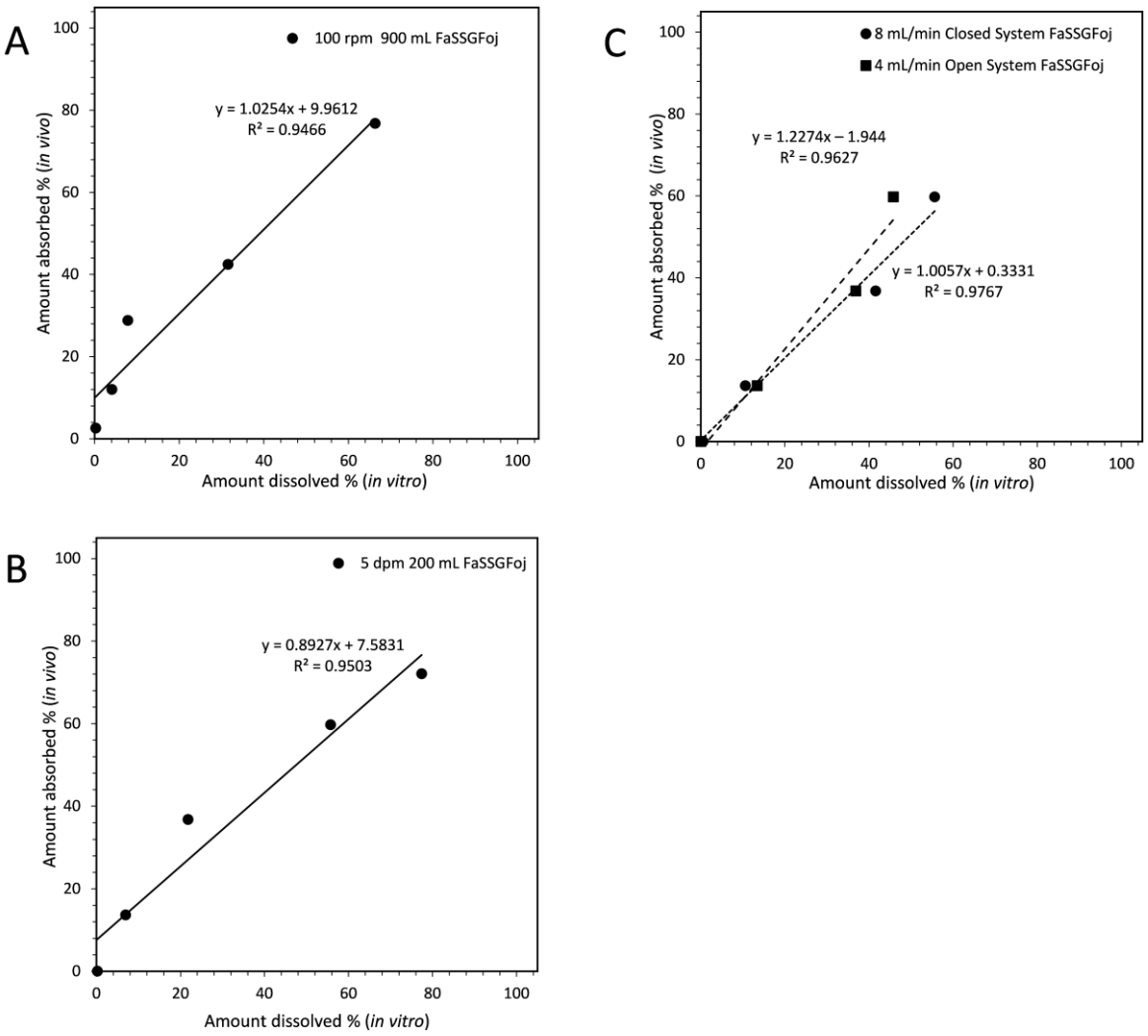
795



796

797 Figure 7

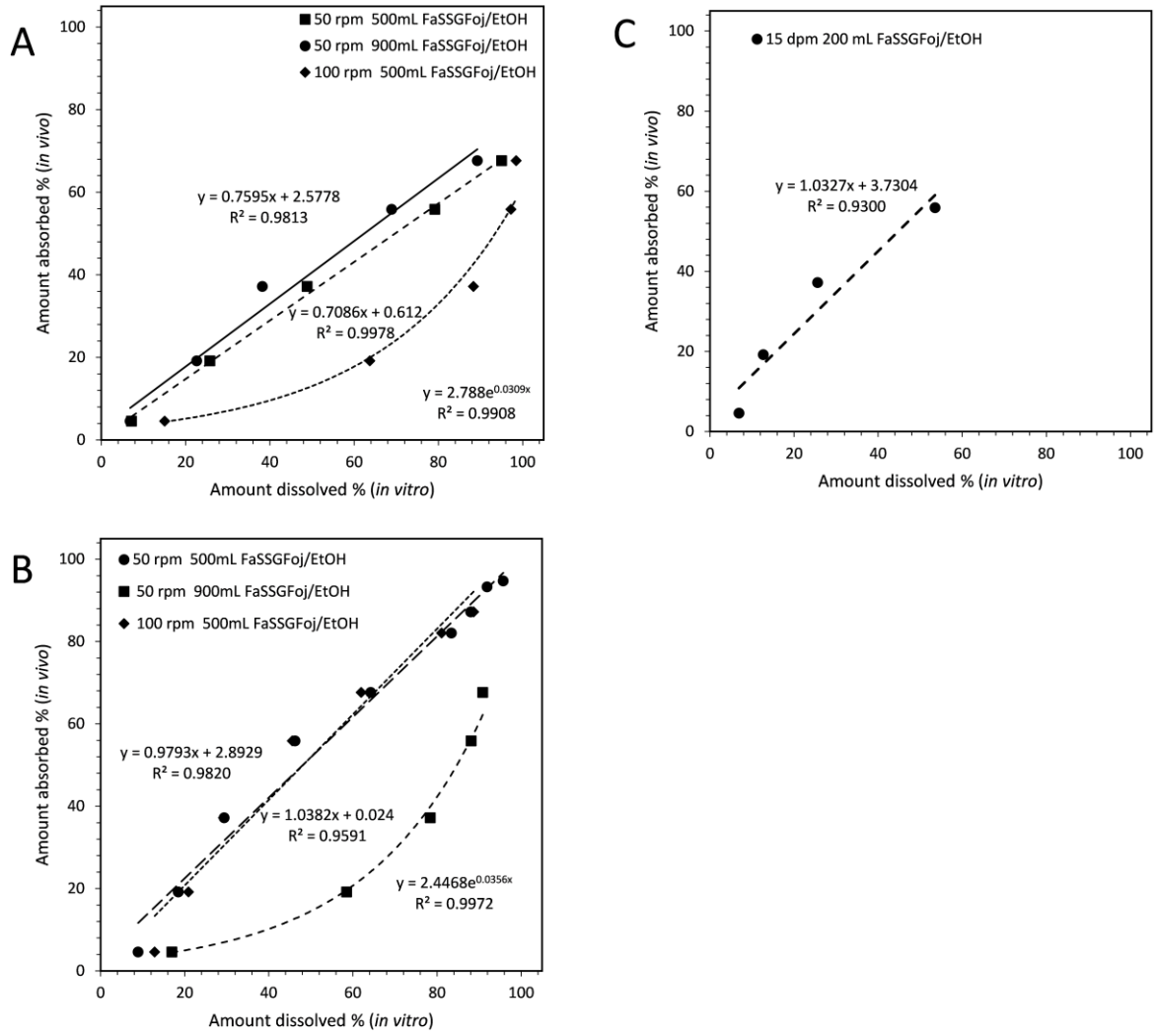
798



799

800 Figure 8

801



802

803 Figure 9