Impact of physiologically relevant viscosity on intrinsic dissolution rate of poorly soluble compounds in simulated gastric media.

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Abstract

In order to simulate the *in vivo* dissolution of drugs, biorelevant dissolution media (BDM), simulating the gastric or intestinal juices are often employed. For gastric BDM, pH, surface tension and osmolality is simulated, however, viscosity has, to our knowledge not been taken into account. The dissolution rate of a drug compound is influenced by the viscosity of the dissolution medium. In the present study it was desired to create BDM with viscosities similar to human gastric fluid. Human gastric fluid was collected from 7 volunteers and the apparent viscosities determined using cone and plate geometry. The apparent viscosity was determined to be 0.0017 - 0.012 Pa·s measured at a shear rate of 178 sec^{-1}. A fasted-state simulated gastric fluid (FaSSGF) was chosen as a starting point for the creation of viscous BDM. FaSSGF was prepared with addition of different amounts of a viscosity enhancer. Semi-synthetic neutral polymer hydroxypropyl methylcellulose (HPMC) was chosen as a natural viscosity enhancer due to the rheological behaviour. It was found that the addition of 0.2 – 0.6 % HPMC resulted in a medium with a viscosity range similar to the viscosity of human gastric aspirates. Dissolution of Griseofulvin and Cinnarizine in FaSSGF containing HPMC was investigated using the μDISS Profiler system. A decrease in intrinsic dissolution rate (IDR) for both drugs was observed as the viscosity of the media was increased. The IDR was lowered up to 70 % within the physiologically relevant viscosity range.

Keywords: Human gastric fluid, Biorelevant dissolution media, Viscosity, Dissolution, HPMC.

Introduction

Solubility and dissolution rate determines whether a drug dissolves fast enough and that the desired absorption is obtained. Both the physicochemical characteristics of the drug as well as the *in vivo* characteristics of the physiological environment impact the solubility and dissolution rate of a drug compound. Factors such as pH, surface tension, osmolality etc. are therefore tested in the physiological environments to enable mimicking of the physiological conditions (Dressman et al 1998).
Biorelevant dissolution media (BDM) are used for testing the solubility and dissolution rate. BDM has been optimized over the last decade. Early BDM only offered pH and salt concentration similar to physiological values whereas pepsin content was higher than physiological values (USP 2011). Later BDM also contained bile salt and phospholipids, where the content simulated in vivo conditions to a larger extent (Vertzoni et al 2005).
One factor never considered when designing BDM, is the viscosity (Vertzoni et al 2005). Fasted human gastric fluids have a higher viscosity than water or traditional BDM due to the presence of mucus components (Lai et al 2009). This would in theory produce a lower dissolution rate of drugs in said fluids (Kearney and Marriott 1986). In order to simulate the gastric fluid and thereby get a better understanding of the events taking place in connection to dissolution, one goal of the present study was to develop media with physiologically relevant viscosities. This could be a predictive tool in optimizing early drug development.
The second aim of the study was to determine the importance of the viscous BDM on IDR. An examination of changes in dissolution rate would clarify whether the physiologically relevant viscosity of the BDM is of significant importance.

Materials and Methods

Materials

Ammonium phosphate monobasic, cell culture tested, purity ≥ 99 %, Cinnarizine, purity by thin layer chromatography >99%, Griseofulvin, from penicillium griseofulvum 97.0 – 102.0 %, Hydroxypropyl methylcellulose**,** viscosity 2,600-5,600 cP, 2 % in H_2O(20 °C)(lit.),). Mucin, from porcine stomach, type II, bound sialic acids: ~1%, Pepsin, from porcine gastric mucosa, Sodium azide and Sodium taurocholate, purity thin layer chromatography ≥ 97 % was purchased from Sigma –Aldrich (Saint Louis, MO, USA). Chloroform, LiChrosolv, HPLC grade, Hydrochloric acid, fuming 37%, for analysis and Sodium chloride, of analytical grade, purity ≥ 99.5 % was purchased from Merck (Darmstadt, Germany). Lipoid S PC, purity ≥ 98 % phosphatidylcholine was purchased from Lipoid GmBH (Ludwigshafen, Germany). Purified water was obtained from a Millipore Milli-Q Ultrapure Water Purification System (Billeria, MA, USA). Human gastric aspirates were collected from 7 healthy volunteers (Copenhagen, Denmark).

Collection of human gastric fluid

Gastric aspirates were collected from 7 volunteers during gastroscopic examinations at Gentofte Hospital, Copenhagen. The aspirates were collected prior gastric examination and no fluid was used to rinse the gastroscope. The volunteers were not allowed to eat or drink respectively 6 and 2 hours prior the examination. The aspirates were stored on ice immediately after collection and viscosity measurements were performed on the same day as the aspiration.

Preparation of solutions:

FaSSGF:

Medium was prepared inspired by the method previously described by Vertzoni et al (Vertzoni et al 2005). The concentrations of excipients are given in table 1. In brief, for 1 litre of medium, phosphatidylcholine was dissolved in 10 ml chloroform in a 1000 ml volumetric flask and the solvent was evaporated under nitrogen. Sodium chloride and sodium azide was each dissolved in 100 ml purified water and the solutions were added to the volumetric flask. Sodium taurocholate and pepsin were added and the flask was filled with purified water to below 1000 ml. The solution was stirred over night at room temperature and on the following day the pH was adjusted to 1.6 with HCl and purified water was added to a final volume of 1000 ml. The pH of the solution was set to 1.6 and the solution was stored at 5 ºC until used.

Table 1: Composition of FaSSGF.

	Concentration
Sodium taurocholate	80 µM
Phosphatidylcholine	20 µM
Pepsin	0.1 mg/ml
Sodium chloride	34.2 mM
Sodium azide	3 µM
Hydrogen chloride q.s. pH=1.6	

FaSSGF containing hydroxypropyl methylcellulose (HPMC):

One third of the final volume of purified water was added to a volumetric flask. The temperature of the solution was increased by placing the flask in a water bath preheated to 80 ºC. Different amounts (2, 4, or 6 g) of HPMC were added under stirring until the HPMC was completely dispersed. The dispersion was cooled. Phosphatidylcholine was dissolved in 10 ml chloroform in a 500 ml beaker and the solvent was evaporated under nitrogen. Sodium chloride and sodium azide were each dissolved in 100 ml purified water and the solutions were added to the flask containing phosphatidylcholine. Sodium taurocholate and pepsin were added to the solution. The solution was transferred to the HPMC solution and purified water was added to below 1000 ml. The medium was stirred over night at room temperature to equilibrate. pH was adjusted to 1.6 with HCl and purified water was added to a final volume of 1000 ml. The solution was stored at 5 ºC until used.

Viscosity measurements

Viscosity characterisations of gastric aspirates and FaSSGF samples containing different amounts of viscosity enhancer were conducted using the cone and plate geometry on an AR-G2 rheometer, TA Instruments, Waters Corporation, (New Castle, USA). All measurements were performed at 37°C with a 40mm stainless steel cone with an angle of 1º To limit evaporation and damage of the plate a protective casing, custom fabricate at Department of Pharmacy, Faculty of Health and Medical Sciences (Copenhagen, Denmark), was attached onto the fixed plate and the sample-air interface on the edge of the cone was covered with 0.5mL of low viscosity silicone oil. A steady state flow tests were performed from 1 to 1000 1/s and all samples were measured in triplicate.

Surface tension measurements

Surface tension was determined by the pendant drop method on a KRÜSS DSA100 Drop Shape Analysis System, KRÜSS GmbH (Germany) connected to a Julabo ED-5 Open Bath Circulator, Julabo Labortechnik (Germany). The temperature was kept at 37 ºC. A syringe with a diameter of 1.825 mm was used and a drop of 10 ±0.5 µl was analysed each time.

Osmolality
Osmolality was measured by freezing point depression using an Osmomat 030-D Cryoscopic Osmometer, Gonotec (Berlin, Germany).

pH
pH was measured with a Metrohm electrode (6.0262.100) connected to a PHM 220 pH-meter, Radiometer (Copenhangen, Denmark).

Dissolution studies using µDISS Profiler system.
Intrinsic dissolution rate of Griseofulvin and Cinnarizine in FaSSGF with and without viscosity enhancer was measured on a µDISS Profiler System, pION inc. (Woburn, MA, USA) containing 6 dip probes connected to a UV-Vis spectrophotometer. Absorbance of Griseofulvin and Cinnarizine was measured in the wavelength ranges of 284 – 306 nm and 242 – 266, respectively.

A Mini-IDR™ compression system, Heath scientific (Buckinghamshire, United Kingdom) was used to make small miniaturized pellets with a constant surface area. Stainless steel dies containing a cylindrical hole with an area of 0.071 cm^2 was filled with pure drug powder and compressed to approximately 35 bar for 30 seconds (figure 1, a). Each die was then inserted into a teflon rotating disk carrier and placed on the bottom of the glass vials (figure 1, b and c).
The vial holder was preheated to 37 ºC with a Julabo Open Bath Circulator, Julabo Labortechnik GmbH (Seelbach, Germany). The probes were center-positioned in the vials and as the dissolution medium was transferred to each vial the magnetic stirring system was turned on. The absorbance was measured at least once every two minutes and at most once every five seconds for 24 (Cinnarizine) or 48 hours(Griseofulvin). Parafilm was placed over the opening of the vials to avoid evaporation of solvent.

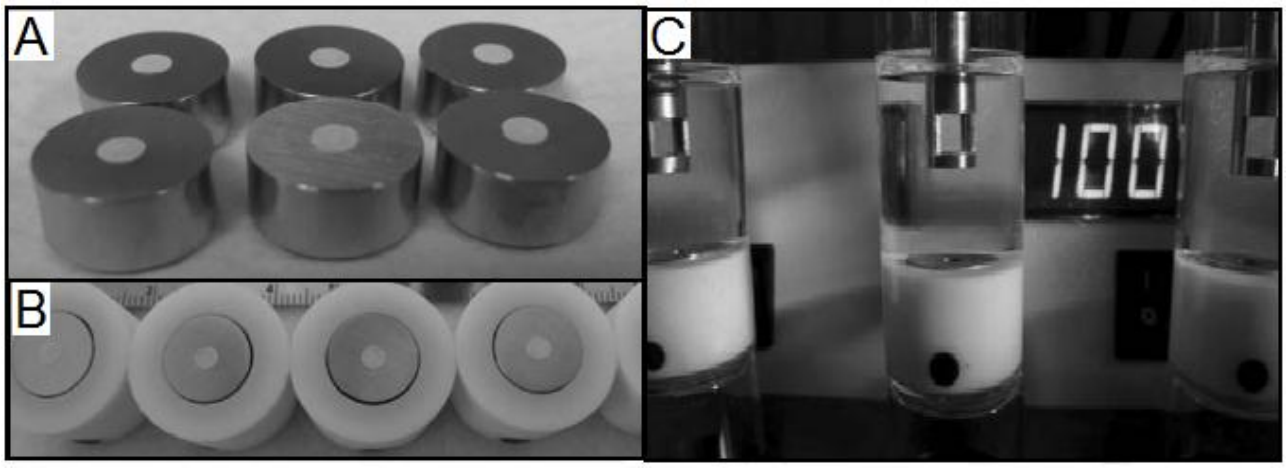


Figure 1: Parts of the µDISS profiler system. A) Steel dies with compressed powder. B) Dies inserted into teflon rotating disk carriers. C) Rotating disk carriers with dies placed on the bottom of the glass vials. Fibre optic probes with probe tips are immersed into dissolution medium (Picture from Avdeef and Tsinman, 2008).

Fitting of curves using µDISS profiler software

All dissolution curves were analysed using second derivative area-under-curve method. A biexponential function (equation 1) was selected to account for loosely packed powder on the tablet surface.

$$C(t) = C_{powder} \cdot \left(1 - e^{-\frac{A_{powder}}{V} \cdot P_{ABL} \cdot 60 \cdot (t - t_0)}\right) + C_{pellet} \cdot \left(1 - e^{-\frac{A_{pellet}}{V} \cdot P_{ABL} \cdot 60 \cdot (t - t_0)}\right) \quad [1]$$

where C_{powder} refers to the concentration in the final saturated solution due to the contribution of the powder burst . The area associated with the powder is A_{powder}. The second term refers to the pellet. P_{ABL} refers to the permeability across the aqueous boundary layer and t_0 is the lag time.

The five parameters, C_{pellet}, C_{powder}, A_{pellet}, A_{powder} and t_0 (lag time) have to be determined. In all analyses it was chosen to keep the area from pellet fixed at 0.071 cm^2 since this parameter, in theory, does not change during analyses. The remaining parameters were determined by the software as a curve was fitted to obtain a low R^2 value, low residuals and low standard deviations. In most cases all remaining 4 parameters were determined successfully by the software. In few cases only 3 remaining parameters could be determined by the software. If certain parameters could not be determined by the software a value was chosen manually within the range observed in similar experiments.

The intrinsic dissolution rate (IDR) was determined using equation 2.

$$IDR = DR_{max}/A_{eff} \quad [2]$$

Where DR_{max} is the maximum dissolution rate and A_{eff} is the effective area calculated by the µDISS Profiler software.

Results and discussion

Comparison of HGA and FaSSGF

Table 2 represents comparable characteristics of the human gastric aspirates (HGA) and the FaSSGF. 178 s^{-1} was chosen for the viscosity measurements as a point of comparison due to this was within the Newtonian plateau.

Table 2: Characterization of human gastric aspirates.

Property	HGA	FaSSGF
viscosity at 178 s^{-1} (mPas)	1.7-12 (n=7)	1.4±0.06 (n=3)
surface tension at 700 s (mN/m)	34.5 ± 4.7 (n=6)	42.72 ± 1.63 (n=6)
Osmolality (mOsm/kg)	240 ± 0.03 (n=6)	92.3 ± 4.11 (n=3)
pH	2.78 ± 1.93 (n=6)	1,6

It was observed that a high inter- and intra-variability exist in the human gastric fluid. The high intra-variability can be due to the gastric fluid being inhomogeneous and the high inter-variability is due to biological variation. The viscosity varied from 1.7-12 mPas. The surface tension was measured to be 34.5 ± 4.7 mN/m, pH 2.78 ± 1.93 and osmolality of 240 ± 0.03 mOsm/kg. The FaSSGF media was observed to have a lower viscosity than human gastric aspirates as expected. Furthermore the surface tension, osmolality and pH were different from the values measured in the human gastric aspirates in this study. Studies by Dressman et al. (1998) and Lindahl et al. (1997)

were used to develop the FaSSGF media. In these studies the viscosity of the human gastric fluid and FaSSGF was not evaluated. The observed differences in surface tension, osmolality and pH between the different studies can be explained by biological variations due to gastric aspirates are withdrawn from different volunteers.

Characterization of FaSSGF containing HPMC

Addition of 0.2 – 0.6 % HPMC to FaSSGF produced media with physiologically relevant viscosities as shown in figure 2.

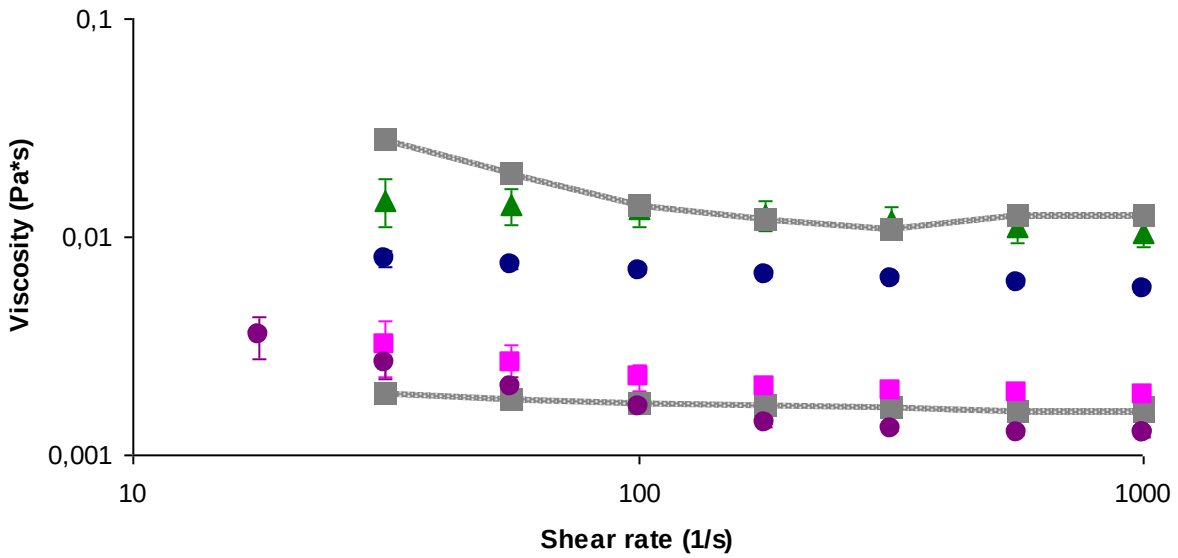


Figure 2: Steady-state flow test conducted on 2 human subjects (n=1) and 3 FaSSGF samples containing different amounts of HPMC (n=3; mean ± SD). Upper and lower ■ indicates the range of viscosities found in human gastric aspirates. ● FaSSGF, ■ 0.2 % HPMC, ● 0.4 % HPMC and ▲ 0.6 % HPMC. Viscosity (Pa·s) is shown as a function of the shear rate (1/s).

The pH of all media containing viscosity enhancer was adjusted to pH=1.6. The surface tension and osmolality of FaSSGF containing viscosity enhancer is given in table 3:

Table 3: Surface tension (mN/m) and osmolality (mOsm/kg) ± standard deviations for FaSSGF containing different amounts of viscosity enhancer. n=3 for all measurements unless otherwise stated.

Medium	Surface tension ± SD (mN/m)	Osmolality ± SD (mOsm/kg)
FaSSGF	42.72 ± 1.63 (n=6)	92.3 ± 4.11
FaSSGF 0.2% HPMC	33.16 ± 0.06	99.3 ± 4.78
FaSSGF 0.4% HPMC	36.51 ± 0.81	94.3 ± 2.06
FaSSGF 0.6% HPMC	36.20 ± 0.75	90.0 ± 3.56

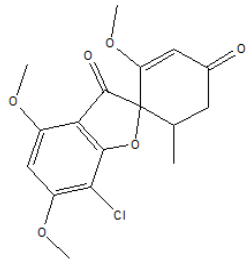
It was observed that the surface tension was lowered 10 – 15 % upon addition of HPMC and the values became similar to that of HGA. However, the surface tension lowering did not show a concentration dependent decrease. The osmolality remained fairly similar upon addition of HPMC as expected; due to HPMC is an uncharged molecule.

Dissolution studies

The model drugs, Griseofulvin and Cinnarizine, were chosen to observe whether an increase in viscosity of the BDM changed the IDR of the drugs. Both Griseofulvin and Cinnarizine are poorly soluble and were chosen due to the neutral and the positive charged, respectively, at pH 1.6. The physicochemical characteristics of Griseofulvin and Cinnarizine are illustrated in table 4 and 5, respectively.

Table 4: Structure and physico-chemical characteristics of Griseofulvin.

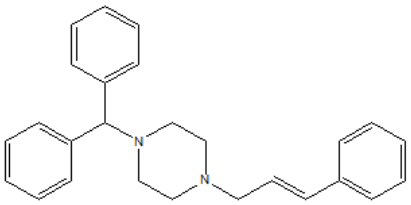
Griseofulvin	
Characteristics	**Structure**
logP	2.18[a]
Solubility (water)	0.01 mg/ml[b]
MW	352.766 g/mol
BCS	II



[a](Leo et al 1971), [b](De Carli and Larizza 1988).

Table 5: Structure and physico-chemical characteristics of Cinnarizine.

Cinnarizine	
Characteristics	**Structure**
logP	5.8[c]
pK_a 1	1.95
pK_a 2	7.5
Solubility (1 M HCl)	0.29 mg/ml[d]
Solubility (phosphate buffer, pH=7.2)	0.002 mg/ml[d]
MW	368.514 g/mol
BCS	II



[c](Kleberg et al 2010), [d](Gu et al 2005).

Dissolution studies with Griseofulvin and Cinnarizine in FaSSGF containing 0.2 – 0.6 % HPMC compared with the IDR measured of the drugs in traditional FaSSGF is illustrated in figure 3 and 4:

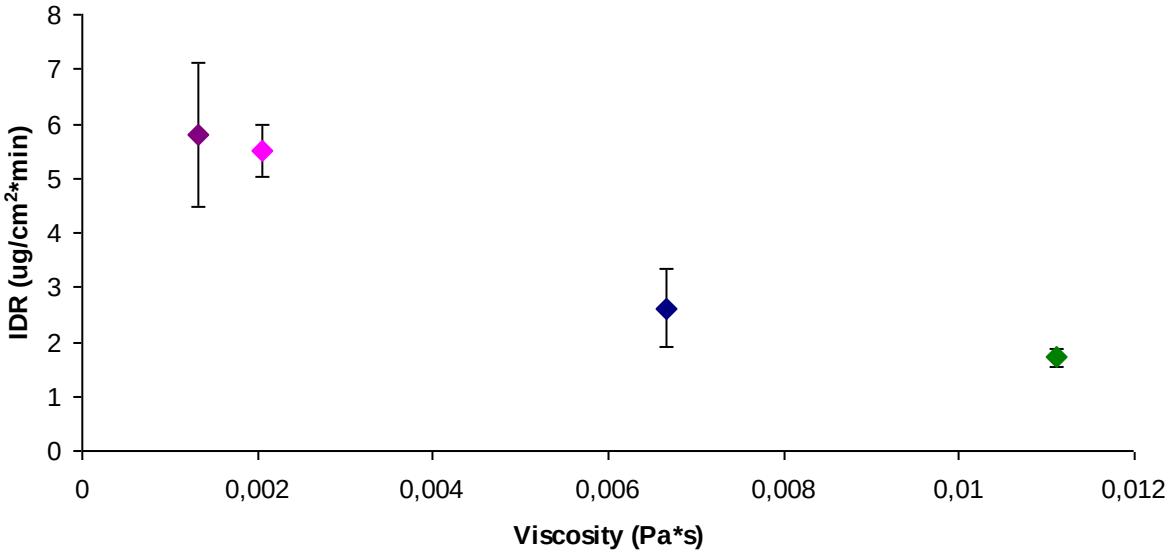


Figure 3: IDR's obtained from dissolution of Griseofulvin in FaSSGF of different viscosities. ◆ FaSSGF, ◆ 0.2 % HPMC, ◆ 0.4 % HPMC and ◆ 0.6 % HPMC. The viscosities are measured at 178 s^{-1}.

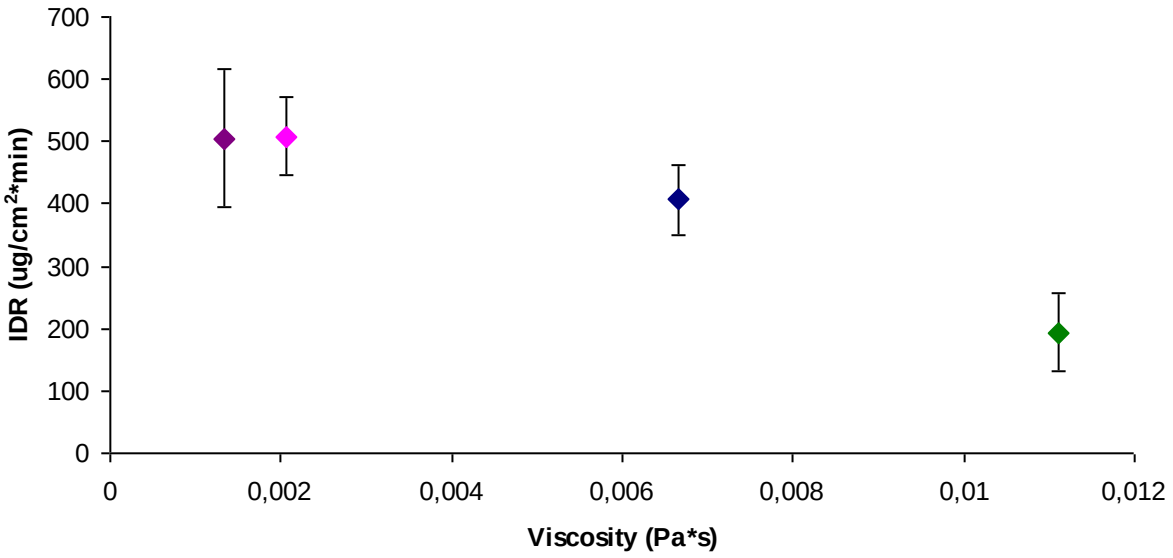


Figure 4: IDR's obtained from dissolution of Cinnarizine in FaSSGF of different viscosities. ◆ FaSSGF, ◆ 0.2 % HPMC, ◆ 0.4 % HPMC and ◆ 0.6 % HPMC. The viscosities are measured at 178 s^{-1}.

Figure 3 illustrates that the IDR of Griseofulvin decreased 70% in FaSSGF 0.6% HPMC compared to IDR in FaSSGF. This is due to the significant increase in the viscosity of the BDM.
Similar results are observed for Cinnarizine where the IDR decreased 62% in the FaSSGF 0.6% HPMC compared with IDR in FaSSGF as illustrated in Figure 4. These observations can be explained by the Noyes Whitney equation, according to which an increase in viscosity leads to decrease in diffusivity and thereby decreased dissolution rate (Horter and Dressman 2001).

It is observed that the IDR of Cinnarizine is significantly higher than the IDR of Griseofulvin, which could be explained by the significant different solubility of the drugs at pH 1.6. Cinnarizine

is positively charged and Griseofulvin uncharged under these circumstances which can explain some of these solubility differences.
The IDR of Griseofulvin and Cinnarizine could be influenced by different drug-polymer interactions or size of the model drug.

It is observed from figure 4 and 5 that the IDR does not change upon addition of 0.2% HPMC to FaSSGF. The 0.2% HPMC increases the viscosity of FaSSGF and significantly decreases the surface tension of the medium (table 3). The lowered surface tension causes an increased wetting effect which neutralizes the increased viscosity of the media and thereby does not change the IDR for neither of the drugs. Machiste and Buckton have also shown that even small concentrations of HPMC lower the surface tension of aqueous solutions (Machiste and Buckton 1996).

Conclusion

The overall aim of the study was to improve early drug development testing by optimizing biorelevant dissolution media to correspond to physiological values.
An apparent viscosity of 0.0017 - 0.012 Pa·s was found. In order to develop BDM with viscosities similar to the gastric fluid the use of a viscosity enhancer, HPMC, was tested. HPMC was chosen due to the transparent appearance and neutral charge. FaSSGF was chosen as a starting point for developing BDM with physiologically relevant properties. It was found that the addition of 0.2 – 0.6 % HPMC to FaSSGF resulted in a media with a viscosity similar to that of human gastric aspirates. The surface tension of the viscous BDM was approximately 10 – 15 % lower than the surface tension found for FaSSGF alone but similar to the value of human gastric fluid. The osmolality remained unchanged as HPMC was added to FaSSGF and somewhat lower than the values from the gastric aspirates.
Dissolution rates of Griseofulvin and Cinnarizine were determined using the physiologically relevant BDM containing HPMC and compared to the IDR for the FaSSGF alone. The IDR was found to decrease as the viscosity increased. The IDR of Griseofulvin and Cinnarizine were lowered up to 70 % and 62%, respectively, in FaSSGF containing up to 0.6 % HPMC. It becomes apparent that the molecular interactions and molecular size of the model drug might be important for the change in IDR.

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