Polymer Selection for Simulation of Rheological Properties of Human Gastric Fluid

Pernille Barbré Pedersen[1], Peter Vilmann[3], Daniel Bar-Shalom[1,2], Anette Müllertz[1,2] , and Stefania G. Baldursdottir[1].

[1] Department Pharmacy, Faculty of Health and Medical Sciences,
[2]Bioneer:FARMA, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark.
[3] Copenhagen University hospital Herlev, DK-2730 Herlev, Denmark

ABSTRACT

The purpose of this study was to select a polymer for a simulated gastric media (simHGF) to obtain rheological properties similar to that of aspirated human gastric fluid (aspHGF). Rheological characterization showed that addition of 0.2% (w/v) MC 20.000 mPa·s to simHGF provided a simulated media with rheological properties similar to that of aspHGF.

INTRODUCTION

Many efforts have been made towards simulating the conditions in the human stomach. In current simulated gastric media physiological characteristics, such as presence of gastric lipase, mucin, and viscosity differences have not been considered. Parameters that possibly impact drug dissolution characteristics[1]. The dissolution characteristics can be described by the Stoke Einstein (Eq 1) and Noyes Whitney equation (Eq 2)

$$D = \frac{K_B \cdot T}{6 \cdot \pi \cdot r \cdot n} \quad (1)$$

Where D is the diffusion coefficient, K_B is Boltzman constant, T is absolute temperature, n is viscosity, and r is radius of sphere.

$$\frac{dM}{dt} = -D \cdot A \cdot \frac{C_S - C}{h} \quad (2)$$

Where dM/dt is the rate of dissolution, D is the diffusion coefficient, A is the surface area, C_S is the saturation solubility, C is the apparent concentration of drug, and h is the thickness of the boundary layer. From the Stoke Einstein equation (Eq 1) it is known that an increased viscosity decreases the diffusions coefficient. The Noyes Whitney equation (Eq 2) predicts that a decreased diffusion coefficient decreases the intrinsic dissolution rate of drugs. Human gastric fluid (HGF) consists mainly of water (98%) and the other components are hydrochloride acid, salts, mucus, proteins, phospholipids and enzymes. Bile

salts refluxed from the intestine can also be present in HGF[2]. The existing simulated gastric fluid, fasted state simulated gastric fluid (FaSSGF) and fasted state simulated gastric fluid version 2 ((FaSSGF-V2) contains salts, pepsin and low amounts of bile salts and phospholipids[1,3]. FaSSGF-V2 was developed with *in vivo* relevant osmolality[3,4].

HGF contain mucin, proteins and lipids that have shown to impact the rheological properties of human gastric fluid and therefore differ from the rheological properties of the existing simulated gastric media (FaSSGF and HCL pH 1.2)[5]. The mucin molecules are extracellular glycoproteins with a high molecular weight, 0.5-20 MDa. Further mucus consists of DNA, lipids, ions, other proteins, cells, cellular debris, and water[6]. The mucins present in the human gastrointestinal mucus are either membrane bound (MUC 1 and MUC 4) or secreted (MUC2, MUC5AC, MUC5B, and MUC7)[5,7].

The pKa-value of mucin is reported to be 2.688 due to the presence of sialic acid groups and is therefore partly ionized at gastric pH[8]. The glycoproteins are to a large extent responsible for the viscosity and gel-forming properties of the mucus[7,9,10].

The purpose of this study was to select a polymer for a simulated gastric media (simHGF) to obtain rheological properties similar to that of aspirated human gastric fluid (aspHGF).

METHODS

Volunteers

The study was approved by the Ethical Committee of Denmark, Copenhagen, Denmark and followed the convictions of the Declaration of Helsinki (H-2-2011-073). Fasted aspHGF were collected from volunteers during gastroscopy at Herlev and Gentofte Hospital, Copenhagen. Nineteen volunteers with normal body weight, aged 20 – 79 years old were included in the study. The volunteers all gave their written informed consent to the experimental procedure. The volunteers were not allowed to eat or drink, 6 and 2 hours prior to the examination, respectively. Only volunteers that did not have any upper gastrointestinal diseases were included. Smokers, pregnant or breastfeeding women, and volunteers that ingested any medication, food or water on the day of gastric fluid aspiration were excluded. A few volunteers were excluded due to lack of compliance with the protocol either due to, diseases in the upper gastrointestinal tract, or because it was not possible to collect a sufficient amount of aspHGF for analysis.

The aspHGF were collected immediately after introduction of the endoscope into the stomach. A clear content in the stomach was seen in most individuals, and this content was aspirated via the suction channel of the endoscope into a closed container.

Preparation of media, FaSSGF

FaSSGF was prepared according to Vertzoni et al. 20051. The concentrations of the components are given in table 1. The solution was stirred overnight and pH was adjusted to 1.6 with HCL. Purified water was added to a final volume of 1000 ml and the FaSSGF media was stored at 5○ until used.

Development of simulated gastric fluid (simHGF)

SimHGF containing pepsin, Taurocholic acid sodium salt hydrate (NaTC), phosphatidyl choline (Lipoid S PC), and sodium chloride (NaCl) was prepared and different types and concentrations of viscosity enhancers were added in order to obtain *in vivo* relevant viscosities. The solution was stirred overnight and pH was adjusted to 2.5 with 1 M HCL. Purified water was added to a final volume of 1000 ml and the simHGF media was stored at 5 °C until used.

The viscosity enhancing polymers tested were porcine gastric mucus (PGM), Alginate, Xanthan gum, Hydroxypropyl methylcellulose (HPMC), and methylcellulose (MC). Some polymers were tested in different viscosity grade and concentration. The composition of simHGF is illustrated in Table 1.

Table 1. The Composition of FaSSGF and simHGF

Components	FaSSGF	simHGF
Pepsin	**0.1 mg/ml**	**0.1 mg/ml**
NaTC	**80 µM**	**330 µM**
Phospholipids	**20 µM**	**82.5 µM**
NaCl	**34.2 mM**	**120.7 mM**
Polymer X	**-**	**X**

Rheological characterization

Rheological characterizations of FaSSGF, HCl (pH 1.2), simHGF, and aspHGF samples were conducted using the cone and plate geometry technique on an AR-G2 rheometer, TA Instruments, Waters Corporation, (New Castle, USA). All measurements were performed at 37 °C with a 40 mm aluminum steel cone, at a gap of 33 µm. To limit evaporation a protective casing, custom fabricate at Department of Pharmacy, Faculty of Health and Medical Science (Copenhagen, Denmark), was attached onto the fixed plate and the applied sample edge was covered with 0.5 mL of low-viscous poly(dimethylsilcoxane) oil after lowering of the cone to the measurement gap.

The sample container was turned to prevent a possible sedimentation prior to the sampling of approximately 350 µL. The sample was allowed to equilibrate for 10 minutes at 37 °C before measurements were conducted.

Three consecutive tests were conducted to determine the rheological properties of the aspHGF samples, simHGF, FaSSGF, and HCL (pH 1.2). Inertia dominated measurements were excluded in the data evaluation.

Stress sweep test

The oscillation torque was measured from 0.01 to 100µNm with an angular frequency of 0.5 rad/s. The test was conducted once on each sample to determine the linear viscoelastic region (LVR).

Frequency sweep test

The angular frequency was measured from 0.1 to 10 rad/s with an oscillation torque of 0.1µNm for aspHGF samples, simHGF, FaSSGF, and HCL (pH 1.2).

Steady state flow test

The apparent viscosity of the HGAs samples and simHGF were measured as a function of the shear rate from 0.001 to 1000 s^{-1}. The tolerance was set to 5%, which indicated what the consecutive mean rates for three measurements had to be within. The maximum measuring time for each shear rate was set to 2 minutes. Measurements not reaching equilibrium

within the 2 minutes were not taken into account. The test was also conducted on FaSSGF and HCl (pH 1.2).

RESULTS

In an earlier study an assessment of the traditional physico-chemical parameters, rheological properties as well as gastric lipase activity in aspHGF were performed[2]. Based on these human characterization results simHGF was developed (Table 2).

Table 2. Human characterization and FaSSGF data[1,2]

Parameter	Human data[2]	FaSSGF[1]
pH	**2.5 ±1.4**	**1.6**
Osmolality (mOsm/kg)	**220 ±58**	**120.7**
Surface tension (mN/m)	**34.8 ±5.8**	**42.6**
Buffer Capacity (mmol L^{-1} ΔpH^{-1})	**14.3 ±9.5**	**-**

Apparent viscosity

In an earlier study rheological examination was performed of aspHGF in order to develop a simulated gastric medium (simHGF). AspHGF showed non-Newtonian shear-thinning behaviour as illustrated in Fig.1[2]. It was shown that at a shear rate of 0.01 s^{-1} and 50 s^{-1} corresponding the hydrodynamics of the respectively fundus and antrum, the shear viscosity range was variable for the included aspHGF. At a shear rate of 0.01 s^{-1} and 50 s^{-1} the apparent viscosity range were 0.6 – 45.5 Pa·s and 1.7 - 9.3 mPa·s, respectively. At high shear stresses a plateau was observed and the apparent viscosity was measured to be in the range of 1.6 – 6.4mPa·s at a shear rate of 100 s^{-1} (Fig. 1)[2]. Fig. 1 illustrates this apparent viscosity interval for aspHGF (grey area). The aspHGF having the minimum apparent viscosity was not measurable at low shear rates (below 3.16 s^{-1}). Thus the measurable viscosity points are used as the maximum and minimum range of the aspHGF collected shown as the grey area in Fig.1, 2, and 3.

In order to simulate the rheological properties of aspHGF, a simHGF was developed and different polymers were tested to obtain simHGF with average rheological properties similar to that of aspHGF.

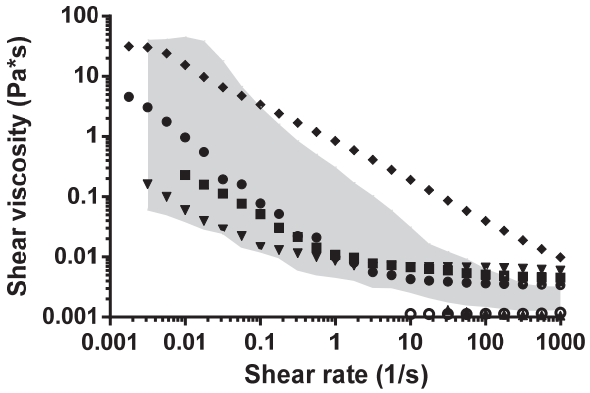

Figure 1. The apparent viscosity of simulated gastric media containing different polymers. The apparent viscosity interval of aspHGF (n=17) (grey area) and simHGF containing PGM (6% (w/v)) ■, Xanthan gum (0.8 % (w/v)) ♦, HPMC 15.000 mPa·s (0.3% (w/v))▼, MC 1500 mPa·s (0.4% (w/v)) ●. And the apparent viscosity of HCL (pH 1.2)▲, and (FaSSGF pH 1.6) ○(n=3).

PGM was added as viscosity enhancing agent due to the charge and structure similarity to mucus present in the aspHGF but did not show shear viscosity profile similar to that of aspHGF (Fig. 1).

Additionally, PGM induced analytical problems during the dissolution testing on a µdiss profiler. Alginate and Xanthan gum were tested because both polymers are negatively charged (pKa = 3.38 (mannuric acid) and pKa = 3.65 (guluronic acid)) at the relevant gastric pH of, 2.5. However, Alginate precipitated at pH 2.5 and the simHGF containing xanthan gum did not have the desired rheological properties (Fig. 1). At gastric pH the mucin molecules will be partly uncharged due to the pKa of 2.688 and therefore uncharged polymers should be equally suitable. Two cellulose derivatives, which are neutral at gastric pH, HPMC and MC were tested.

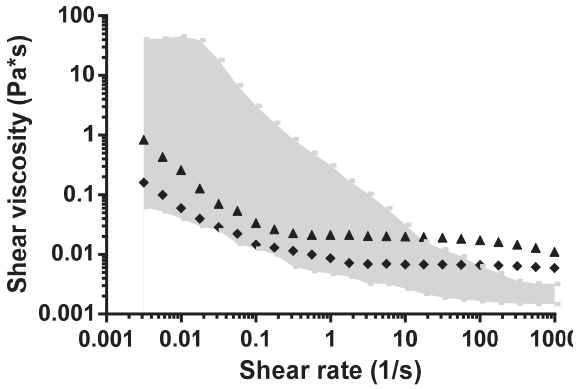

Figure 2. The apparent viscosity interval of aspHGF (n=17) (grey area) and simHGF containing HPMC 15.000 mPa·s (0.3% (w/v)) ♦, HPMC 100.000 mPa·s (0.3% (w/v)) ▲ (n=3).

Fig. 1 and fig. 2 illustrate that simHGF containing HPMC showed Newtonian behaviour at shear rates above 0.1 s^{-1}. Further it was observed that replacing the HPMC in the simHGF to a HPMC with a higher viscosity grade (15.000 mPa·s or 100.000 mPa·s) did not change the rheological profile but increased the viscosity of simHGF (Fig. 2).

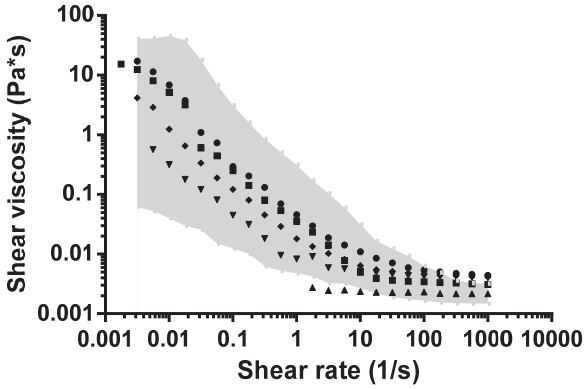

Figure 3. The apparent viscosity interval of aspHGF (n=17) (grey area) and simHGF containing MC 15 mPa·s (0.6% (w/v)) ▲, MC 1500 mPa·s (0.4% (w/v)) ▼, MC 4000 mPa·s (0.35% (w/v)) ♦, MC 20.000 mPa·s (0.20% (w/v)) ■, MC 40.000 mPa·s (0.20% (w/v)) ● (n=3).

MC polymers were selected as the polymer suitable for the simHGF because simHGF containing MC showed shear thinning properties similar to that of aspHGF (Fig. 3). Figure 3 illustrates that addition of MC with increasing viscosity grade increased the shear thinning properties of simHGF. As expected, the concentration of MC had an impact on the viscosity. MC with a viscosity grade of 20.000 mPa·s and a concentration of 0.20% showed to have a shear viscosity in the range of aspHGF at all measured shear rates (0.001-1000 s^{-1}) and was chosen as the polymer candidate for simHGF.

Storage and loss modulus

aspHGF has been shown to have predominant elastic behaviour in the linear

range[2].

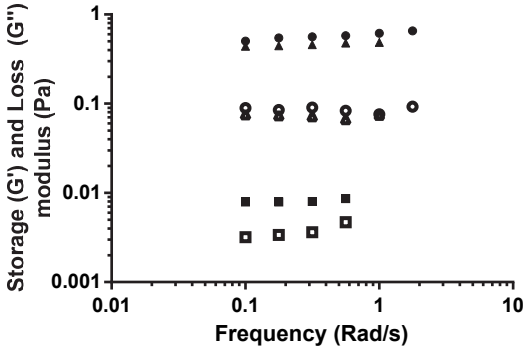

Figure 4. The storage modulus (G') and loss modulus (G'') for maximum aspHGF ▲(G') and Δ (G''), minimum aspHGF ■ (G') and □ (G''), and simHGF (MC 20.000 mPa·s 0.2%) ● (G') and ○ (G'').

Fig. 4 illustrates that the relation between the elastic and storage modulus of simHGF is similar to that of the maximum moduli from the aspHGFs. Pedersen et al. (2013) showed that the elastic modulus, G' was highly variable for the aspHGF, and that the storage modulus interval was determined to be 0.008-0.439 Pa at an oscillation torque of 0.1 mN/m indicating different amounts of elastic components are present in aspHGF (see Fig. 5)[2]. Additionally, the G' of simHGF was similar to the sample showing the highest modulus of the aspHGFs, with a storage modulus of 0.570 Pa at an oscillation torque of 0.1 mN/m. In order to decrease the storage modulus or elastic components the viscosity grade and molecular weight or the concentration of the polymer should be reduced.

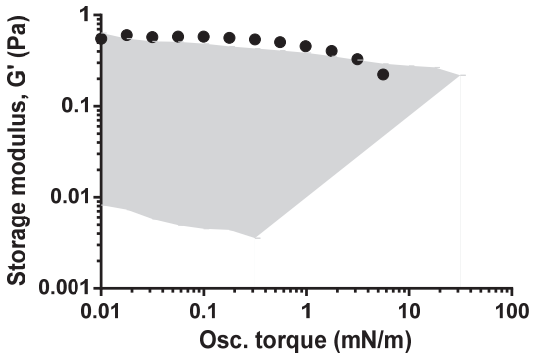

Figure 5. The elastic or storage modulus obtained from an oscillatory sweep test of aspHGF (n=17) (grey area) and for simHGF (MC 20.000 mPa·s 0.2% (w/v)) ● (n=3).

CONCLUSIONS

The type, viscosity grade and the concentration of the viscosity enhancing polymer chosen has an impact on the shear thinning properties of simHGF. MC was found to be a suitable polymer to obtain a simulated gastric media with similar rheological properties to that of aspHGF. The addition of MC 20.000 mPa·s in a concentration of 0.2% to simHGF provided a simulated media with shear thinning properties similar to the aspHGF, where the simHGF at high shear rates showed viscosity in the higher range of the aspHGFs. Additionally G' and G'' where measured to be in the similar range as the highest detected for the aspHGFs. Thus, further development is needed in order to optimize the rheological profile.

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