

Instant FaSSIF and FeSSIF—Biorelevance Meets Practicality

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ABSTRACT

The objective of this study was to prepare an “instant” form of FaSSIF and FeSSIF that could be implemented easily and reproducibly in the laboratory setting. Concentrated solutions containing bile salts and lecithin were freeze-dried and further processed into bulk packs, blisters, or tablets, or sealed in powder form under nitrogen in vials. Physical properties as well as solubility and dissolution data for two poorly soluble compounds, danazol and a Boehringer-Ingelheim development compound (BIXX), were compared with data for freshly prepared FaSSIF and FeSSIF and a commercially available “instant” product. After the freeze-dried FaSSIF and FeSSIF media were reconstituted with appropriate buffers, their properties were close to those of freshly prepared media and a commercially available product for reconstitution, SIF Powder. Additionally, the solubility and dissolution profiles of danazol and BIXX in media prepared by all three methods were similar. Reconstitution of freeze-dried “instant” FaSSIF and FeSSIF appears to be an accurate, reproducible, and efficient way of preparing biorelevant dissolution media.

INTRODUCTION

Biorelevant dissolution testing (1) has made it possible to predict, at least on a rank-order basis, in vivo formulation behavior. Although biorelevant dissolution media like FaSSIF and FeSSIF (Fasted- and Fed-State Simulated Intestinal Fluid) are not a one-to-one copy of human intestinal fluids, they attempt to simulate the most relevant parameters including pH, osmolality, surface tension, and the solubilization capacity for drugs (2). As a result, utilization of biorelevant media in dissolution testing has increased at least tenfold (as judged by the number of publications) over the last ten years.

The conventional preparation method of these fluids (3) is time-consuming, requires organic solvents, makes daily preparation necessary, and sometimes does not result in a usable medium. Additionally, in conventionally prepared media, residual organic solvents may impact physicochemical properties and dissolution behavior. Elaborate preparation and the high direct costs of the solubilizing agents are reasons why biorelevant media are not used for routine quality control. In earlier studies (4), replacement of the solubilizing excipients with less expensive substitutes was attempted. However, differences in dissolution led to these attempts being abandoned.

Easier and faster preparation combined with high reproducibility and indirect cost savings would make biorelevant media more practical for routine laboratory use. The freeze-drying method enables bulk preparation of storable, instant media. Like the novel, commercially

available SIF Powder for preparation of FaSSIF and FeSSIF, it can be easily reconstituted before use (5). Media containing the different bile salts were prepared by the conventional method, the freeze-drying method, and the commercially available media. The physicochemical properties, compound solubility, and dissolution behavior of these media were compared.

MATERIALS AND METHODS

To prepare media by the conventional and the freeze-drying methods, pure phosphatidylcholine (PC) from egg and pure sodium taurocholate (NaTC) were used. PC was donated by Lipoid, Germany. NaTC was purchased from PCA, Italy and CPC, New Zealand. SIF Powder instant biorelevant media was purchased from Phares AG, Switzerland. Analytical grade acetonitrile, ammonium acetate, ethanol, isopropanol, methylene chloride, mono-sodium phosphate, sodium acetate, and sodium hydroxide and HPLC grade methanol were used to prepare the media by different methods or to run analytical methods.

Drugs and Dosage Forms

A multiparticulate BIXX formulation was provided by Boehringer-Ingelheim Pharma GmbH & Co. KG (Germany). It is a weakly basic BCS Class II drug with solubility decreasing from 0.082 mg/mL at pH 4.0 to 0.001 mg/mL at pH 6.5. The formulation is not yet commercially available and is therefore referred to as BIXX-F (6).

Danatrol capsules (100 µg) were purchased from Sanofi-Synthelabo, Switzerland. This steroidal BCS Class II drug is a neutral and lipophilic compound (log *P* = 4.2)

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that is practically insoluble in water (1 µg/mL). Because danazol is not ionizable, solubility is not pH dependent (7).

Media

FaSSIF and FeSSIF blank buffers were used for the preparation of the various media. The pH of FaSSIF blank (0.029 M phosphate, 0.106 M NaCl) was adjusted to 6.5 and FeSSIF blank (0.144 M acetate, 0.203 M NaCl) was adjusted to pH 5.0. The method introduced by Galia et al. (2) was used to prepare FaSSIF-CM (CM: conventional method) and FeSSIF-CM. To prepare FaSSIF-FD (FD: freeze-dried method) and FeSSIF-FD, concentrated solutions of NaTC (from PCA or CPC) and PC in water were freeze-dried, and 2.24 g/L or 11.2 g/L of lyophilizate were reconstituted in the corresponding blank buffers to prepare the media. Likewise, 2.24 g/L and 11.2 g/L of instant SIF Powder were dissolved in the corresponding blank buffers to prepare FaSSIF-SIF and FeSSIF-SIF, respectively.

Manufacture of FD Media

The FD media were prepared by freeze-drying concentrated solutions of biorelevant media, omitting the buffer salts and NaCl. The composition of the media is given in Table 1.

For the preparation of the media, concentrated solutions of all solutes in water were prepared at room temperature for subsequent freeze-drying. To prepare FaSSIF and FeSSIF instant media, a solution containing 247.5 g/L sodium taurocholate and 88.5 g/L phosphatidylcholine in water was used. The water-soluble taurocholate was dissolved first, followed by the phosphatidylcholine, which was dispersed and subsequently solubilized by the taurocholate, and a clear solution was obtained.

Materials can be dried in bulk or in predosed packages. For bulk drying, a thin layer of solution (1–2 cm) was poured on a tray. For predosed packaging, the density of the solution was measured to dispense the solution volumetrically into glass vials. The solutions were cooled to –40 °C at 1 K/min. Primary and secondary drying strongly depended on the degree to which the trays or vials were filled and on the freeze-dryer capacity. After freeze-drying, the highly hygroscopic powder had to be handled under dry room conditions or sealed in vials

Table 1. Composition of Concentrates and Freeze-Dried Media per 1-L Vial

Substance	FaSSIF g in one liter of final media	FeSSIF g in one liter of final media
Sodium taurocholate	1.65	8.25
H ₂ O	10	35
Phosphatidylcholine	0.59	2.954
Total (dried)	2.24	11.204

directly under a nitrogen atmosphere. The shelf life of the instant media under these conditions is expected to be 2–3 years at room temperature.

Instant biorelevant media prepared by freeze-drying were white to slightly yellow dry powders that dissolved rapidly in water or aqueous buffer solutions. Reconstitution of the media was performed with the corresponding “blank” buffers immediately before use.

Characterization of Physicochemical Properties

The physicochemical properties of the various reconstituted dissolution media were characterized. Surface tension was determined using a bubble-pressure tensiometer (DynaTester, SITA Messtechnik GmbH, Dresden, Germany). Particle-size distribution (PCS) was performed using a Zetasizer Nano_S, Malvern Instruments Limited, Malvern, UK. Density was determined at 20 ± 2 °C using a harmonic-oscillation densitometer (DMA 4500, Anton Paar, Graz, Switzerland), and osmolality using a freezing-point osmometer (Osmomat 030-D, Gonotec GmbH, Berlin, Germany). The solubility of danazol (via HPLC–UV-vis) was measured to compare the solubilization capacity of the various media. Danazol (200 µg) was weighed into 2-mL Eppendorf tubes and shaken at 400 rpm, 37 °C for 24 h. The solutions were filtered using 0.2-µm membrane filters (Acrodisc, Pall Life Science, Ann Arbor, USA). The filtrate was diluted 1:1 with MeOH and measured via HPLC.

Dissolution Tests

Dissolution was conducted in all biorelevant media and their corresponding blank buffers. In vitro dissolution data were generated at 37 ± 0.5 °C in 500 mL dissolution medium using USP Apparatus 2 (AT 7, Sotax, Allschwill, Switzerland) with the paddle rotating at 75 rpm. Samples were removed automatically using a CY-7-50 piston pump (Sotax, Allschwill, Switzerland), filtered online using a GF/D 2.7-µm filter (Whatman, Kent, UK), and then measured online using a UV-vis spectrophotometer (Agilent 8453, Bensheim, Germany). Standard curves for danazol and BIXX were run every analytical day. The pH was measured after dissolution of BIXX using a standard pH meter (CG840, Schott, Hofheim/Ts., Germany) fitted with Inolab electrodes from Mettler Toledo (Schwerzenbach, Switzerland). All dissolution experiments were run in triplicate.

To check the accuracy of the online UV-vis measurements, additional samples were removed manually at predetermined time points and analyzed by HPLC, and the results were compared with the online UV-vis results. These verification samples were filtered through 0.45-µm Sartorius membrane filters (Minisart, Sartorius, Goettingen, Germany) and subsequently diluted 1:1 with methanol. Analyses were carried out by HPLC with UV-vis measurement (Agilent HPLC1100, Bensheim, Germany) using a reversed phase column (Zorbax C₁₈ Extended Release, Agilent, Bensheim, Germany) and pure water/acetonitrile

(68/32) as mobile phase for danazol and 0.05 M ammonium acetate pH 4.4 and acetonitrile as mobile phase for BIXX.

RESULTS

All solutions prepared for freeze-drying appeared optically clear due to the formation of stable, isotropic mixed micelles (8).

Physicochemical Characterization

Physicochemical properties of media prepared by either reconstitution of freeze-dried media or the conventional method showed no significant differences in surface tension, osmolality, or density (Table 2).

Results obtained from particle-size measurements are given in Table 3. Average diameter (Z_{ave}) values of FeSSIF did not show significant differences, with a mean value of 6.4 nm. However, for conventionally prepared FeSSIF, a broad and bimodal distribution with a second peak at 200 nm was observed, leading to an increased Z_{ave} of 8 nm. In contrast, a narrow and unimodal distribution was obtained for instant FeSSIF-SIF and freeze-dried FeSSIF-FD.

The particle size of FaSSIF showed higher variance. A stable particle size of about 90 nm was obtained for freeze-dried FaSSIF. A polydispersity index (Pdl) less than 0.05 indicated a narrow, unimodal distribution. The particle size in FaSSIF-SIF is only half that of reconstituted FaSSIF-FD but also shows a narrow, monomodal distribution. We speculate that the smaller particle size might be due to use of soy (S-PC) rather than egg (E-PC) phosphatidylcholine; the different fatty acid composition and higher concentration of unsaturated acids would allow a more compact alignment within the mixed micelles. FaSSIF prepared by the conventional method shows a bimodal distribution with a Z_{ave} of 88 nm; however, the particles are not stable. Directly after preparation, a Z_{ave} of about 60 nm was calculated with a second peak at about 200 nm. Particle size increased to a Z_{ave} of 90 nm within one day with a reduction in the intensity of the 200 nm peak. It can be assumed that equilibrium was achieved at a particle size of 90 nm in micelles using E-PC and 45 nm using S-PC. The slow approach to equilibrium particle size makes conventional FaSSIF less reproducible. At least in part, the

Table 2. Physicochemical Properties of FaSSIF and FeSSIF Prepared by Reconstitution of Freeze-Dried Media or by the Conventional Method

Property	FaSSIF	FeSSIF
Surface tension (mN/m)	53 ± 0.4	47.4 ± 0.3
Osmolality (mosmol/kg)	1.00693 ± 0.00004	1.01484 ± 0.00015
Density (mg/cm ³)	296 ± 3	706 ± 9

Table 3. Mean Values and Standard Deviation of D_{90} and Polydispersity Indices (Pdl) for Various FaSSIF and FeSSIF Media

Medium	mean Z_{ave} (nm)	SD Z_{ave}	mean Pdl	SD Pdl
FaSSIF FD CPC ^a	89	2	0.046	0.015
FaSSIF FD PCA ^b	89	1	0.038	0.012
FaSSIF CM CPC ^c	88	11	0.124	0.019
FaSSIF CM PCA ^d	88	15	0.038	0.012
FaSSIF-SIF	45	2	0.025	0.008
FeSSIF FD CPC	6.4	0.1	0.071	0.013
FeSSIF FD PCA	6.4	0.3	0.130	0.066
FeSSIF CM CPC	8 (6 and 200)	0.4	0.404	0.071
FeSSIF-SIF	6.4	0.1	0.090	0.015

^a Bile salts from CPC, New Zealand

^b Bile salts from PCA, Italy

^c 2 h after preparation

^d 24 h after preparation

high standard deviations in solubility and physicochemical parameters with conventional FaSSIF can be explained by the variation in the rate of approach to equilibrium in the test solutions. Using instant SIF Powder or freeze-dried media, equilibrium was reached immediately after reconstitution.

The solubility of danazol in FaSSIF and FeSSIF was 15 and 49 µg/mL, respectively, in conventionally prepared media. In the freeze-dried media, 14 and 48 µg/mL could be dissolved. Use of bile salts from different suppliers (PCA vs CPC) had no impact on the results. The very slightly higher concentrations in conventionally prepared media may have resulted from residual organic solvent, which could cause increased solubility of danazol. In the SIF Powder media, the solubility of danazol was marginally lower, with values of 13 and 47 µg/mL. If soy lecithin was indeed used to manufacture this preparation, the tighter mixed micelles formed might show a slightly reduced capacity for solubilizing additional molecules. However, for all solubility measurements, no significant differences were calculated with either the *F* or *t*-tests.

Dissolution Results

Danazol

Figure 1 shows the mean dissolution of danazol from Danatrol capsules. The profile obtained in blank buffers is not included because the solubility of danazol is less than 1% in the blank solutions.

Dissolution of danazol from Danatrol capsules in FeSSIF was about threefold higher than in FaSSIF. In blank buffers, no difference could be observed, indicating that the effect on solubility is dependent on surfactant levels

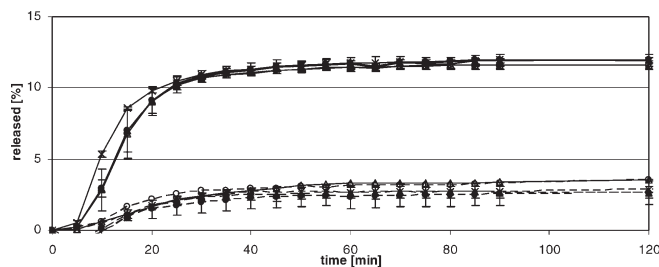


Figure 1. Mean dissolution values and standard deviations for danazol in FaSSIF (---) and FeSSIF (—): SIF (x), FD CPC (▲), FD PCA (Δ), CM CPC (●), and CM PCA (○).

and not on pH. For FaSSIF, no significant difference in dissolution profiles could be calculated using two-way ANOVA for repeated measurements among the various media-preparation methods. The dissolution in FeSSIF showed no significant difference from that in the freeze-dried and conventionally prepared media. FeSSIF prepared using instant SIF Powder showed a significantly higher percentage release at time points between 10 and 20 min using the post hoc Bonferroni test.

BIXX

Figure 2 shows the mean dissolution of BIXX from the formulation in FaSSIF. The source (supplier) of bile salt had little effect on the results. The maximum concentration in conventionally prepared media was reached in 35 min, whereas in the FD and SIF FaSSIF, the maximum concentration was reached after 40 min. The mean maximum concentrations were 15.3% released in conventionally prepared media, 16% in FD, and 18% in FaSSIF-SIF. None of these differences were statistically significant. In all FaSSIF media, an initially higher percentage released reverted to a final value of $7.5 \pm 0.5\%$ released by 120 min. The high initial solubility is driven by excipients, like supersaturating agents or pH modifiers, that effect supersaturation and cause a delay in re-precipitation of the API, whereas the final percentage released is defined by the saturation solubility.

Figure 3 shows the mean dissolution data for the BIXX formulations in the various FeSSIF media. The stable pH generated by the FeSSIF buffers led to a final release of just 2% in the blank media. The strong solubilizing effect in FeSSIF led to a threefold higher percentage released with dissolution continuing over an extended period. A final percentage release of 40% was reached after 4 h (data not shown).

Acidifying excipients in the formulation can cause a decrease in pH, depending on the buffer capacity of the medium, which in turn affects the solubility of the weakly basic BIXX compound. However, such effects depend on the buffer capacity of the dissolution medium. A pH drift from 6.5 to 6.14 was observed in FaSSIF, which has a low buffer capacity, whereas in FeSSIF, the pH remained stable at 5.0.

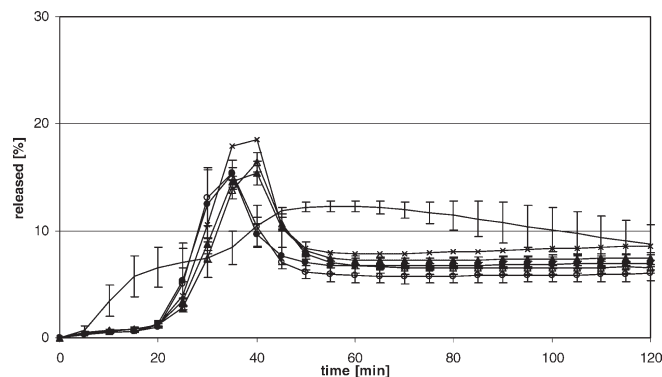


Figure 2. Mean dissolution values and standard deviations for BIXX in FaSSIF: SIF (x), FD CPC (▲), FD PCA (Δ), CM CPC (●), CM PCA (○), and buffer blank (without symbol).

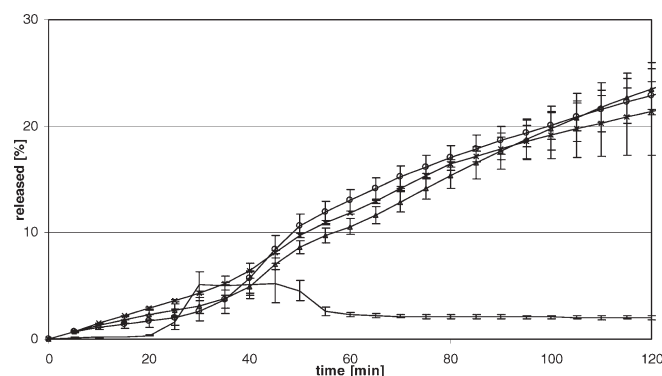


Figure 3. Mean dissolution values and standard deviations for BIXX in FeSSIF: SIF (x), FD CPC (▲), FD PCA (Δ), CM CPC (●), and buffer blank (without symbol).

CONCLUSIONS

Effect of Sodium Taurocholate (NaTC) Source on Media Performance

Results with the sodium taurocholate from CPC with respect to the physicochemical properties and solubilizing effects of the biorelevant media as well as the dissolution behavior of danazol and BIXX were equivalent to those obtained with the product from PCA. Therefore, substitution of NaTC from CPC for the more expensive NaTC from PCA can be recommended.

Comparison of Freeze-Dried Media versus Conventional Preparation Method

Media reconstituted from the freeze-dried product showed very similar properties but higher reproducibility than freshly prepared media. The freeze-dried FaSSIF and FeSSIF can be manufactured on a larger scale to produce material to supply the media for several weeks or even months, depending on the equipment used. Furthermore, reconstitution of the freeze-dried FaSSIF and FeSSIF is facile, so the problems sometimes encountered with manufacture of the freshly prepared media are

circumvented. For both reasons, savings in costs of the taurocholate and lecithin and in the time required for preparation in the laboratory can be realized. The freeze-dried media, therefore, have substantial advantages over the freshly prepared media.

SIF Powder versus Freeze-Dried FaSSIF and FeSSIF

The SIF Powder media, which are speculated to contain soy phosphatidylcholine instead of egg phosphatidylcholine, lead to a slightly different particle size of the mixed micelles. However, the solubilization capacity of SIF Powder-based media for danazol and the dissolution of BIXX were similar for all media. Even though the dissolution of danazol in FeSSIF was initially slightly faster in media prepared from SIF Powder, the maximum concentration and the time at which this was reached were identical. Therefore, either of the "instant" media (freeze-dried or SIF Powder) would be suitable for substitution of the freshly prepared media.

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