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LIPID BASED FORMULATIONS OF BIOPHARMACEUTICS CLASSIFICATION SYSTEM (BCS) CLASS II DRUGS: STRATEGY, FORMULATIONS, METHODS AND SATURATION

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ABSTRACT

Active ingredients in pharmaceuticals differ by their physico-chemical properties and their bioavailability therefore varies. The most frequently used and most convenient way of administration of medicines is oral, however many drugs are little soluble in water. Thus they are not sufficiently effective and suitable for such administration. For this reason a system of lipid based formulations (LBF) was developed. Series of formulations were prepared and tested in water and biorelevant media. On the basis of selection criteria, there were selected formulations with the best emulsification potential, good dispersion in the environment and physical stability. Samples of structurally different drugs included in the Class II of the Biopharmaceutics classification system (BCS) were obtained, namely Griseofulvin, Glibenclamide, Carbamazepine, Haloperidol, Itraconazol, Triclosan, Praziquantel and Rifaximin, for testing of maximal saturation in formulations prepared from commercially available excipients. Methods were developed for preparation of formulations, observation of emulsification and its description, determination of maximum solubility of drug samples in the respective formulation and subsequent analysis. Saturation of formulations with drugs showed that formulations 80 % XA and 20 % Xh, 35 % XF and 65 % Xh were best able to dissolve the drugs which supports the hypothesis that it is desirable to identify limited series of formulations which could be generally applied for this purpose.

Key words: biopharmaceutics classification system – class II drugs; lipid formulations; maximum saturation; spontaneous emulsification

INTRODUCTION

Biopharmaceutics classification system (BCS)

Biopharmaceutics classification system classifies drugs according to their solubility in water and membrane per-

Note: *Due to confidentiality of information obtained from Astellas Pharma Europe BV we cannot disclose identity of the tested excipients.*

meability into four classes described in Table 1. Drugs included in the BCS II class are generally classified as drugs with low solubility but high permeability. These drug little dissolve during their passage through the gastrointestinal (GI) tract [13] and therefore are not completely absorbed. Their bioavailability is therefore limited by their low solubility in water which means that even a small increase in solubility may result in considerable increase in bioavailability [6]. With preliminary dissolution of such drugs in lipids, surfactants or their mixtures we can avoid to the dissolution step which limits absorption of these drugs from the GI tract [2]. It is important to achieve adequate absorption from the water environment. This is the reason why stress is laid on the potential of excipients to produce spontaneous emulsions and form micro- or nano-droplets.

Table 1. Biopharmaceutics classification system (BCS)

Class	Permeability	Solubility
I	High	High
II	High	Low
III	Low	High
IV	Low	Low

Formulations

Lipid-Based delivery systems range from simple oil solutions to complex mixtures of oils, surfactants and co-solvents [8]. They have a high potential for increasing solu-

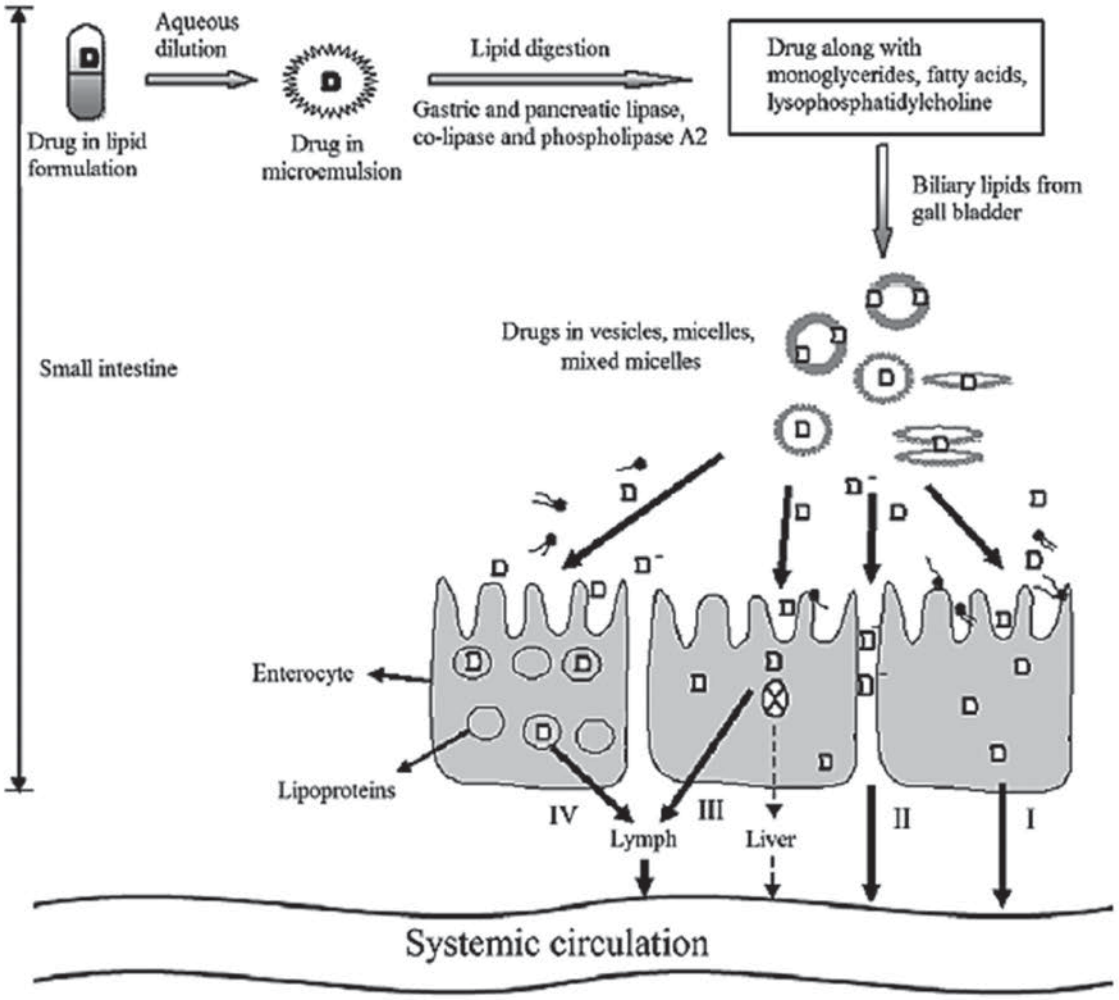


Fig. 1. Scheme of intestinal transport of drug from LBF [5].

bility of BCS class II drugs. Drugs in LBF are already in a soluble form and thus the dissolution step is omitted in the GI tract. Moreover, these formulations should be able to form emulsions in the water environment and maintain the drug in the solubilized state without its precipitation. In the GI tract, the LBF systems increase absorption of drugs by accelerating the dissolution process facilitating the formation of solubilised phases by reduction the particle size to the molecular level [3, 9] changing drug uptake, efflux and disposition by altering enterocyte-based transport, [11, 14] and enhancing drug transport to the systemic circulation via intestinal lymphatic system [1, 4, 10].

The aim of the study was to identify a limited series (mixtures of) excipients that can be used for oral administration of a range of structurally different poorly water soluble drugs and study their potential as simplified strategy formulation.

Toxicity of excipients

Excipients are essential components of drug products. They may be also potential toxicants. Examples of known excipients-induced toxicities include renal failure and death caused by diethylene glycol, or cardiotoxicity induced by propylene glycol [7]. Safety of excipients used in this project was supported by extensive toxicological evaluations and precedence of use in approved pharmaceutical products. Their identification as GRAS (Generally Recognized As Safe), or inclusion in FDA (Food and Drug Administration) database of non-active substance should provide the guarantee of their status [12].

MATERIAL AND METHODS

The following additives were tested: XA, Xf, Xg, Xh, XB, Xi, Xj, XD, XE, XC, Xk, Xo, Xm, XF. The biorelevant medium was prepared using the following: SIF powder original, 36 % HCl, sodium chloride, sodium dihydrogen phosphate dehydrate, sodium hydroxide. Methanol (MeOH), tetrahydrofuran (THF), acetonitrile (AcN) and ammonium formate were used for preparation of solutions for UPLC (Ultra Performance Liquid Chromatography). Additional material was used for preparation of formulation, manipulation with samples and analysis as follows: stirring bars, serum and injection bottles (10 and 20 ml), BD Plastipak syringes 1 ml, 2 ml, 5 ml, 20 ml, Transferpipette®, Plastibrand® pipette tips, Eppendorf combi-tips, HPLC (High Pressure Liquid Chromatography) bottles, GHP Acrodisc® 13mm syringe filter with 0.2 µm GHP membrane, BEH C18 chromatographic column (50 × 2.1 mm; 1.7 mm particles), Waters Acquity UPLC® System, and model drugs: Griseofulvine, Praziquantel, Rifaximin, Itraconazol, Haloperidol, Carbamazepine, Glibenclamide and Triclosan.

Preparation of formulations

The selected excipients were warmed up in a water bath at 55 °C for 5—10 min and homogenised. Required quantities of the relevant excipients were weighed into serum bottles (20 ml) and mixed (magnetic mixer, 300 rpm) for 15 min in a water bath. After mixing the stirring bar was removed, nitrogen was introduced into bottles and the bottles were closed.

Emulsification potential — water, FaSSGF and FaSSIF (Fasted State Simulated Gastric and Intestinal Fluid)

Behaviour was observed at laboratory temperature. 5 ml of medium was transferred into injection bottles (10 ml) and added 50 µl of formulation by means of 1 ml injection syringe. Observed was the content of bottles during the first contact with the medium for the presence of spontaneous emulsification. Subsequently, was added a stirring bar and the bottle was closed. Rotation started at 100 rpm and increased every minute by 100 rpm until emulsion formed. Rotation speed was one of main criteria for selection of formulation. Observations were recorded in protocols.

The simulated gastric fluid contained 30 mg SIF powder original in 0.5l (17 mmol.l^{-1} solution, pH = 1.6). The simulated intestinal fluid was prepared by dissolving 1.12 g SIF powder original in 0.5l FaSSIF buffer solution (pH = 6.5) containing NaOH (5 mmol.l^{-1}), $NaH_2PO_4 . 2H_2O$ (14 mmol.l^{-1}) and NaCl (52 mmol.l^{-1}1).

UPLC screening

For the screening were prepared 100 ml solutions of formulations (7 µl.ml^{-1}) and drugs (0.1 mg.ml^{-1}) in tetrahydrofuran.

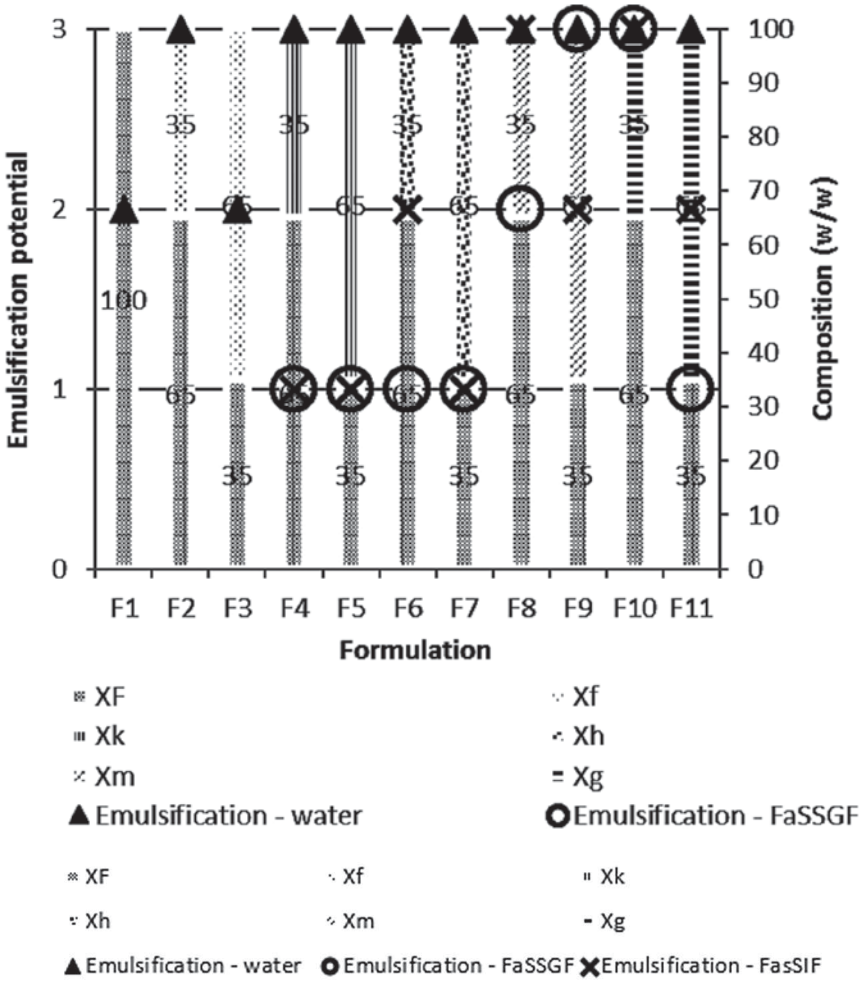
Instrumental methods: gradient analysis. Column temperature: 40 °C. UV spectrum was observed in the range 200—400 nm using PDA (Photodiode Array Detector) detector.

Table 2. Description of instrumental gradient method

No.	pH buffer	Modifier	Column	Runtime [min]	Flow rate [ml.min^{-1}]	Inj. volume [µl]
1	3	AcN	BEH C18	1.5	0.8	4
13	3	AcN	BEH C18	5.5	0.8	4

Time [min]	% A (buffer)	% B Modifier
0	95	5
1.0/5.0	10	90
1.01/5.01	95	5
1.50/5.50	95	5

Week needle wash: 10 % ACN
Strong needle wash: 70 % CAN
Seal wash: 10 % MeOH
pH buffer: ammonium formate 20 mmol.l^{-1}



0 = more than 500 rpm — no potential; 1 = 400–500 rpm — unsatisfactory potential;
2 = 200—300 rpm — satisfactory potential; 3 = 100 rpm — the best emulsification potential

Fig. 2. Example of composition (w/w) of formulations and of their emulsification potential

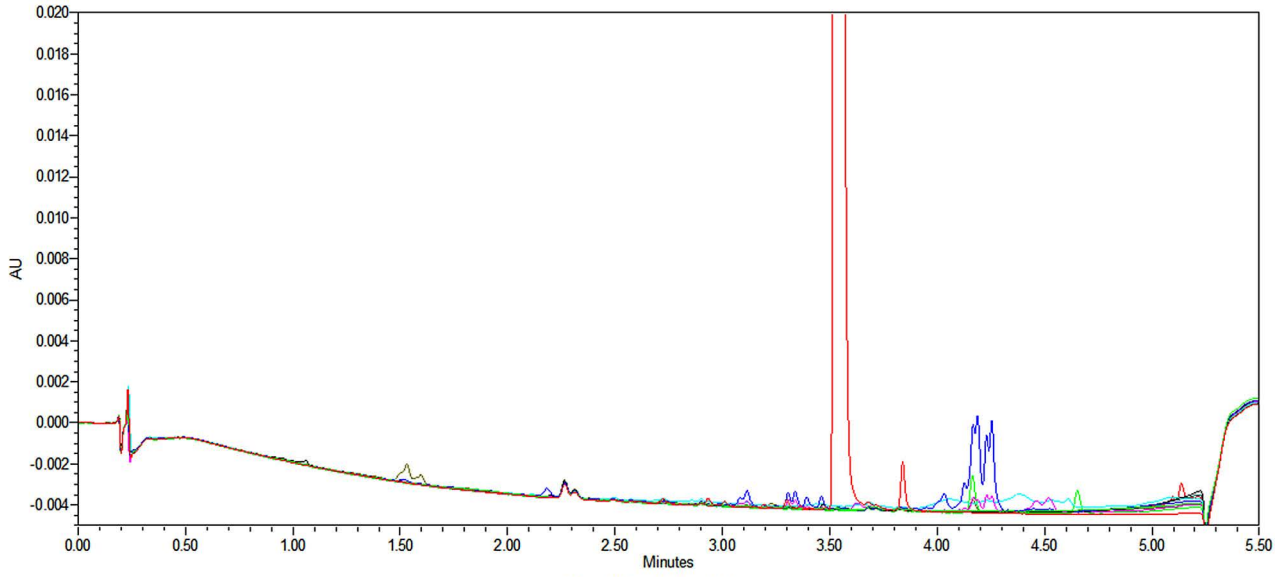


Fig. 3. Example of obtained chromatograms: screening of excipients and Itraconazol using Method 13

Itraconazol (the highest peak — red); XA — green; XCf — blue; XCh — blue; XB — red
Xi — green; XF — blue; Xk — brown; Xg — red; Xm — green

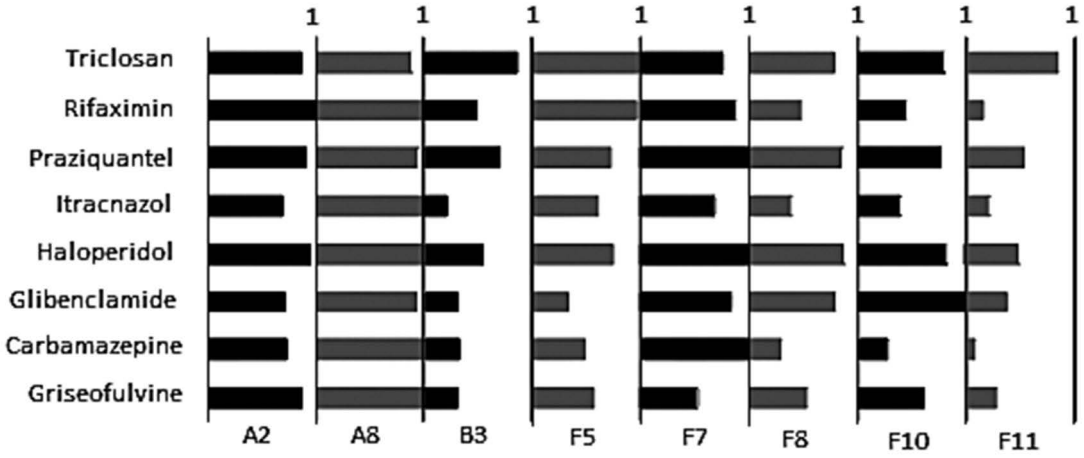


Fig. 4. Relative solubility of drugs in the tested formulations

Maximum saturation

The experiment was carried out in duplicate. Five ml of formulation was added into a glass 10 ml bottle containing 125 mg of the API and the stirring bar. The content was mixed at 350 rpm for 20 hours at 50 °C. Subsequently, the mixing was continued at laboratory temperature at 100 rpm for 20 hours. If the drug dissolved completely, additional quantity of drug was added and the procedure was repeated. Analysis was carried out using 1 ml of sample withdrawn by means of a 2 ml syringe.

The sample was filtered into a HPLC bottle through GHP Acrodisc® 13 mm syringe filter equipped with 0.2 µm GHP membrane and was diluted with tetrahydrofuran to a required concentration. Fresh reference standards were prepared by weighing accurate amounts of drugs and dissolving it in THF. THF was used as a blank. Then, 1 µl of sample was injected to Waters Acquity UPLC ® System using Method 13.

RESULTS AND DISCUSSION

Formulations

Series of formulations were prepared (A, B, C, E, F) and their physical properties (viscosity, phase, homogeneity, colour) were described. To each of the formulations a factor was assigned according to rotational speed which resulted in formation of emulsion and this factor together with the stability and degree of dispersity were the main criteria for selection of formulations for subsequent study. Several formulations were unstable, e.g. the phases separated in water environment. Also some of them exhibited inadequate emulsification potential and degree of dispersity. In this way we were able to reduce the number of prospective formulations.

The following formulations were selected for studies in FaSSGF and FaSSIF (Fasted State Simulated Gastric and Intestinal Fluid): A1; A2; A3; A8; A9; B3; B4; C2; C3; C9; C13; E7; F4–F11.

The following formulations were selected for studies of maximum saturation: A2; A8; B3; F5; F7; F8; F10; F11.

Waters Acquity UPLC screening method

All drugs and excipients were analysed using Method 1 and Method 13 (Waters Acquity UPLC ® System). These methods differ in the length of analysis. The longer Method 13 appeared more suitable for all drugs and excipients. On the basis of UV spectra of individual drugs (Fig. 3) we selected optimum wavelength for analysis of additional samples at their saturation.

Maximum saturation

Fig. 4 illustrates relative solubility of drugs in tested formulations. As the maximum solubility of drugs in individual formulations differed (see Table 3), a factor 1 was used to indicate 100 % solubility. Thus the drug with factor 1 means the best solubility. Maximum saturation may be related to the composition of formulation (e.g. 12.7-fold difference between solubility of Carbamazepine in F7 and F11). It should be noted that relative solubility of all drugs in formulation A8 was relatively high.

Haloperidol, Carbamazepine and Praziquantel reached the highest concentration in F7: 14.1 mg.ml^{-1}, 70.0 mg.ml^{-1} and 56.4 mg.ml^{-1}, respectively. Griseofulvine and Itraconazol in A8: 10.2 mg.ml^{-1} and 3.3 mg.ml^{-1}, respectively. The highest concentration of Triclosan was reached in F5:

Table 3. Solubility of drugs in water and in suitable formulations

Molecule	Concentration		Molecule	Concentration	
	Water [mg.l^{-1}]	Formulation [mg.ml^{-1}]		Water [mg.l^{-1}]	Formulation [mg.ml^{-1}]
Griseofulvine	8.6	A8: 10.2	Glibenclamide	4.0	G4: 15.8
Carbamazepine	17.7	F7: 70.0	Praziquantel	400	F7: 56.4
Haloperidol	14.0	F7: 14.1	Rifaximin	7.4	A2: 109.1
Itraconazol	insoluble	A8: 3.3	Triclosan	6.05	F5: 533.4

533.4 mg.ml^{-1}; of Rifaximin in A2: 109.1 mg.ml^{-1}; of Glibenclamide in G4: 15.8 mg.ml^{-1}. Formulation G4 was experimental and was not mentioned in materials and methods above.

The solubility of drugs in the selected formulations was increased considerably and with some formulations was higher by several orders of magnitude in comparison with their solubility in water (Table 3).

CONCLUSION

For improvement of the oral bio-availability of poorly water soluble drug substances, adjustment of the Lipid based formulations, prepared from mixtures of commercially available excipients with self-emulsifying properties in aqueous environment was investigated. Observed was emulsification potential of various formulations in water, FaSSGF and FaSSIF with respect to solubility of eight structurally different drugs. On the basis of selection criteria and relevant properties, eight formulations were selected for study of their ability to dissolve drugs.

Maximum saturation of drugs was determined by means of UPLC and previous treatment and dilutions of samples. Formulations A8 and F7 showed generally the best ability to dissolve almost all tested drugs which supports the hypothesis to identify a limited series of formulations generally suitable for this purpose.

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