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RESEARCH PAPER

Multivariate Data Analysis of Factors Affecting the *In Vitro* Dissolution Rate and the Apparent Solubility for a Model Basic Drug Substance in Aqueous Media

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ABSTRACT

Purpose To evaluate the usefulness of a miniaturized rotating disk equipment for the determination of factors influencing the *in vitro* dissolution rate, G, of a model basic drug substance (terfenadine) in different aqueous media, using experimental design and multivariate data analysis. The apparent solubility, S, was included in the chemometric study.

Methods The dissolution rate was determined with a miniaturized rotating disk apparatus and the solubility by shake-flask methodology. Media were based on acetate, phosphate or maleate buffers—the latter used in fasted state simulated intestinal fluid (FaSSIF-V2). The chemometric analyses included fractional factorial design, principal component analysis (PCA) and orthogonal partial least squares (OPLS). Quantifications were made with a RP-HPLC-DAD system.

Results The most influential factor for both G and S of terfenadine in the different media was pH. Apart from the ionic strength and sodium chloride concentration in the acetate medium, the effects of the other variables were insignificant, implying no wetting effect of the surfactants.

Conclusions The miniaturized rotating disk equipment was suitable to use, in conjunction with the chemometric analyses, in the evaluation of the factors affecting the *in vitro* dissolution rate. The apparent solubility was found to be influenced by the same factors as G.

ELECTRONIC SUPPLEMENTARY MATERIAL The online version of this article (doi:10.1007/s11095-010-0111-0) contains supplementary material, which is available to authorized users.

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ABBREVIATIONS

ACN	acetonitrile
BCS	biopharmaceutical classification system
CMC	critical micelle concentration
DAD	diode array detector
DoE	design of experiments
G	*in vitro* dissolution rate
GI	gastrointestinal
I	ionic strength
IVIVC	*in vitro/in vivo* correlation
NaCl	sodium chloride
NaTC	sodium taurocholate
OPLS	orthogonal partial least squares
PCA	principal component analysis
RP-HPLC	reversed phase high-performance liquid chromatography
RSD	relative standard deviation
S	apparent solubility
TFA	trifluoroacetic acid

INTRODUCTION

To investigate the dissolution rate and the solubility of drug compounds in humans without studying this *in vivo*, different simulated media *e.g.* simulated intestinal fluids, SIFs (1–3), simulated gastric fluids, SGFs (1–3) and simulated colonic/cecal fluids, SCFs (4), have generally been developed and are used in *in vitro* systems. The primary objectives of these measurements are to evaluate oral absorption, since oral administration is the most convenient dosage route (5,6). Simulated media were initially based on existing intra-

lumenal data (7). Since then, more studies have been done. Buffer capacity and components, pH, bile salt composition, dietary lipids, surface tension, viscosity, osmolality, ionic strength, concentration of electrolytes, proteins, and volume of luminal contents have all been characterized and are important parameters in the media that affect drug solubility and dissolution in the GI tract (7–18). The usefulness of simulated media, though, are still very limited (2). It is apparently important to investigate which variables in the biorelevant media affect the dissolution rate and the solubility of a model drug the most, since the composition of GI fluids can shift widely (8,10,11,17,19). The standard media are complex in composition (10,11,17,20) and expensive to prepare (21), often with limited stability (22). Traditionally, FaSSIF (fasted state SIF) has been based on phosphate buffer, and FeSSIF (fed state SIF) on acetate buffer (23). However, these two media are now both revised to a second version based on a maleate buffer (SIF-V2) to better predict the *in vivo* performance of oral dosage forms (17), even though the principal physiological buffer in human GI tract is bicarbonate (14,15,24,25). This buffer is problematic to work with at a constant pH during, *e.g.*, dissolution studies.

The most frequently occurring bile acid/salt in human duodenum and jejunum is based on taurocholic acid (8). Bile acids/salts are also secreted together with phospholipids (6), such as lecithin. It has been reported that media containing non-physiologically relevant surfactant may overestimate dissolution rates (2). Enhanced solubilization of drug substances have been found in media with added surfactants above their CMC, but also before the CMC is reached (6,26,27). The latter is often explained as the surfactant's wetting ability (*i.e.* the ability to lower the surface tension) instead of a micellar capsulation of solubilized substances, as in the case of attaining the CMC (28–31). Nevertheless, multiple factors may play a role in the solubility effects of bile salts (8) and may vary from compound to compound (26). The CMC value of bile salts can be changed in biorelevant media compared to pure water as a result of differences in ionic strength and also in the presence of lecithin (8,18). CMC is not only dependent on the presence of other surfactants and/or lipids but also pH, temperature, and added salts (30,32). CMC for taurocholic acid is reported to be around 5 mM (6) but is decreased to approximately 0.25 mM in the presence of phospholipids (6). It has been established that mixed micelles of taurocholic acid and lecithin have the molar ratio of 4:1 (5).

Chemometric methods are commonly used within pharmaceutical applications (33), such as in drug design and formulation development, *e.g.*, to correlate structural features or physicochemical properties with the biological activity, *i.e.*, quantitative structure-activity relationships (QSAR) and quantitative structure-property relationships (QSPR) (34–36). Screening is the first stage of an investigation where the goal is just to identify the important factors (37). An important factor is a variable that causes substantial changes (effects) in the response when it is changed. In the screening stage, one uses simple models (linear or linear with interactions) and experimental designs that allow for the identification of the factors with the largest effects in the fewest possible number of experimental runs (38–41). A reduced form, as for example fractional factorial design, is an appropriate choice if dealing with many factors (33). Multivariate projection techniques are used to identify trends, clusters and outliers in data sets, *e.g.*, principal component analysis (PCA) (42,43), and to relate two data matrices to each other, *e.g.*, orthogonal partial least squares (OPLS) (44,45). Multivariate data analysis can determine significant factors affecting, *e.g.*, the *in vitro* dissolution rate and the apparent solubility for model drugs (or formulations) in different media (8,9,46,47) by changing more than one variable at a time in the experiments. The relative importance of these various factors can also be determined.

The histamine H_1 receptor antagonist terfenadine [1–(4-*tert*-butylphenyl)–4–[4–(α-hydroxybenzhydryl)piperidino] butan-1-ol] is widely used against hay fever (allergic rhinitis), skin rash (urticaria) and most allergic disesases (48). Terfenadine exists in three polymorphic forms (49). It has a relatively low bioavailability after oral administration due to its limited solubility in water (48,50), and it has a pK_a-value of 9.25 (51). Depending on the permeability values for the basic drug substance, it is classified as a class II substance (52) or a class IV substance (53) in the BCS (54). The model drug substance in this study was chosen so that it should be an amine, since the majority of drug-like compounds contain this functional group (55), with low solubility. It should preferably be a class II substance given that it is the class for which IVIVCs are most likely to be obtainable (20), and IVIVCs are usually developed in the fasted state (56). The limiting step to absorption for a class II drug is the dissolution, and this is dependent on the wide variety of factors in the media stated above (13).

Flow-through dissolution equipment is preferable to maintain sink conditions while performing the dissolution rate studies (57,58). It can also simulate *in vivo* hydrodynamics better than other apparatuses used for this purpose (57). The knowledge of the physicochemical composition of media united with the consideration of the motility in the GI tract during the design of a dissolution test can lead to a better IVIVC (15,59). A miniaturized apparatus that uses the flow-through technique combined with the rotating disk performance has been developed and characterized (60–62). The miniaturized equipment is used for simple screening of the dissolution rate—around 10 min for one run—and consumes small amounts of substance (approximately 5 mg) and volumes of dissolution media (roughly 20 ml per disk)

(61,62). It also has the advantage of being directly coupled to a separation system, RP-HPLC, which facilitates, *e.g.*, the ultraviolet detection, since the media components can be separated from the analyte of interest. The apparent solubility was measured by using the shake-flask method, which is a standard procedure for this purpose (21).

The present study aims to evaluate the usefulness of a novel miniaturized rotating disk equipment in combination with experimental design and multivariate data analysis to establish which factors in different aqueous media mainly affect the *in vitro* dissolution rate for the weak base terfenadine. The apparent solubility was also included in the study to investigate if the same patterns were found for these two physicochemical properties, since dissolution rate and solubility are being correlated at times (61–66), and these parameters are also proportional to each other according to the modified Noyes-Whitney equation (67–70).

MATERIALS AND METHODS

Chemicals

Terfenadine minimum 98%, Maleic acid ≥99% *Reagent-Plus*®, sodium chloride (NaCl) minimum 99.5% and acetonitrile (ACN) CHROMASOLV® were bought from Sigma-Aldrich (Chemie GmbH, Steinheim, Germany). Lecithin (Lipoid E PC S) minimum 96% M_w 775 g/mol came from Lipoid GmbH, Germany, and Sodium taurocholate (NaTC) minimum 98.0% M_w 537,69 g/mol was bought from Prodotti Chimici e Alimentari, Basaluzzo, Italy. Sodium di-hydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$) *p.a.* was bought from Acros Organics (Springfield, NJ, USA). Di-sodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$) *p.a.* and trifluoroacetic acid (TFA) ≥99.0% were both from Fluka Chemika (Chemie GmbH, Buchs, Switzerland). Sodium acetate *p.a.* and acetic acid minimum 99.8% came from Riedel-de Haën, Sigma-Aldrich (Laborchemikalien GmbH, Seelze, Germany). The water in this study was purified in a Milli-Q® Academic system (18.2 MΩ.cm/0.22 μm), Millipore, (Burlington, MA, USA).

Experimental

Instrumentation

The miniaturized equipment with the cell of Plexiglas integrated to a RP-HPLC system using a DAD for analysis, as well as an external HPLC pump connected to the cell, has been described previously (60–62). The disk of substance in the miniaturized apparatus had a diameter of 1.5 mm and was rotated by a magnetic stirrer with graded rotating speeds, obtained from Heidolph MR 3001 K (Steinheim, Germany). The chromatography was performed on an Agilent 1100 Series HPLC system with a binary pump, degasser, autosampler and DAD, Agilent Technologies Inc. (Palo Alto, CA, USA). A six-position switching valve with a 20 μl stainless steel loop attached to it was also purchased from Agilent Technologies Inc. The mobile phase contained ACN: Milli-Q® water:TFA (1:1:0.1, v/v/v), and detection was performed at 217 nm, the absorption maximum (λ_{max}) for terfenadine in the mobile phase determined by the DAD. The analytical column was a Zorbax SB-C8 (2.1×50 mm, 5 μm) from Agilent Technologies Inc. A guard column was also used, Micro-Guard™ column C8, 1.0×14 mm, (Grace Davison Discovery Science, IL, USA). The mobile phase flow was always 1.0 ml/min, and the temperature of the column compartment was measured to 25–28°C and was constant within approximately 2°C in one experiment. The external HPLC-pump was a Jasco PU-1585, Jasco Inc. (Tokyo, Japan). For the solubility determinations, microtubes MCT-200-C homo-polymer (2.0 ml) from Axygen scientific (Union City, CA, USA), a horizontal shaker from KABI AB (Stockholm, Sweden) and Spectrafuge 16 M micro-centrifuge from Labnet International Inc. (Woodbridge, NJ, USA) were used. The pH monitoring was carried out using a pH Meter 744, Metrohm (Herisau, Switzerland) with electrode Orion® 8220BNWP ROSS PerpHecT Micro Combination pH Electrode, ϕ 3 mm, (Thermo Scientific, MA, USA). Chromatographic data, *i.a.* peak area, were collected by ChemStation Rev.A.10.02 from Hewlett Packard, Agilent Technologies Inc.

The retention factor, k, for terfenadine was calculated to nearly three in the chromatographic system with an analysis of at maximum two minutes. A system suitability criterion was set for the chromatographic system to a precision within 1% in retention time and area of the main peak. This was tested by injecting a standard solution ($n=6$) with a concentration equivalent to the middle concentration in the standard curve. The linearity of the standard curves in Excel (71), determined by the coefficient of determination (R^2), was always at least 0.999 using ten different concentrations of terfenadine standard solutions. Standards were run in duplicate.

Preparation of Aqueous Media

Sodium phosphate buffer of pH 6–8 (6.55–65.5 mM) and sodium acetate buffer of pH 4–6 (6.55–65.5 mM) were prepared according to calculated acid/base ratios. The ratio calculations were made in a specially designed buffer recipe in Excel. The buffers had the ionic strengths of 7.8 mM to 290 mM and 1.7 mM to 100 mM, respectively, with the eventual addition of NaCl (0–100 mM) included.

The three pK_a-values, at 25°C, used for phosphoric acid in the Excel recipe for the phosphate buffer were changed depending on the final ionic strength in the medium (all additives included). Ionic strengths between 13 mM and 207 mM could be selected in six fixed levels for the different pK_a-values (72). This type of design was, however, not made in Excel for the acetate buffer, where the pK_a-value for acetic acid was fixed at 4.53 (I=0.15 M (73)) for all different ionic strengths in the acetate medium. In the maleate buffer-based medium, FaSSIF-V2, the pK_a-values were determined to 1.96 and 5.81 (sodium maleate, I=0.156 M, (74)). These values were changed by iterations in the Excel recipe using the different calculated ionic strengths in the final buffer composition (with all additives included). Two different equations for the calculation of the activity factors were used in the iteration depending on the ionic strength, Davies modification (I>0.1 M) and Güntelberg (I≤0.1 M) (75,76). FaSSIF-V2 was altered in the pH range of 6–8 (2–20 mM), NaCl 0–82 mM, lecithin 0–1 mM and NaTC 0–4 mM, generating ionic strengths of 2.9–126 mM (cf. Appendix). All variables in the media were at maximum differing ±3% from the calculated theoretical values in Excel, due to the weighing of the different buffer additives on the balance. The buffer components and additives were directly mixed together in volumetric flasks during the dilution in deairated Milli-Q® water, without any extra preparation steps.

Multivariate Data Analysis

By using DoE, the number of necessary experiments could be reduced to at maximum 16 in a fractional factorial design selected in the MODDE software, version 7 (77). The following factors were considered: pH, the concentrations of phosphate, NaCl, lecithin, acetate, maleate respective NaTC all in millimolar (mM) and I in molar (M). The quantities of these variables are given in the section "Preparation of Aqueous Media" above and tabelized in Appendix, with the mean value of the intervals as one center point. The run order of the experiments was randomized and given by the resulting fractional factorial design selected with the software, and these experiments were performed one time. PCA was performed to get an overview of the variables in the data set. OPLS was used to establish mathematical relationships between the factors (X) in the altered media and the two experimentally determined physicochemical responses G and S (Y). The PCA and OPLS calculations were performed using the software SIMCA-P+, version 12.0 (78). Prior to OPLS modeling, all factors were centered and scaled to unit variance. The number of significant components in the OPLS evaluation was determined by looking at the regression coefficient (R^2) and the goodness of prediction (Q^2) as determined by cross validation in SIMCA-P+. A 95% confidence interval was used.

In Vitro Dissolution Rate Measurements

Disks were compressed with a specially designed press for the apparatus (60) using 182 MPa for 15–30 s. Before any analysis, the smoothness of the surface of a pressed disk was evaluated microscopically using a microscope with an objective lens that gave a 100× (100 power) view. The flow of medium into the chamber of Plexiglas was always 1.0 ml/min, and the rotation speed of the miniaturized disk was set to 300 rpm. The dissolution media were used at room temperature (22.0°C±1.8°C for acetate and phosphate buffer experiments and 22.0°C±1.0°C for the study in FaSSIF-V2). Only the Milli-Q® water was deairated before the preparation of the different media. Three disks in total were used in all experiments ($n=3$).

Apparent Solubility Experiments

An excess amount of terfenadine powder (3–10 mg) was shaken for 24 h in capped microtubes containing 1.5 ml of media. The experiments were performed at room temperature (22.0°C±1.8°C for acetate and phosphate buffer experiments and 22.0°C±1.0°C for the study in FaSSIF-V2). Centrifugation was made instead of filtration before analysis (61). The pH after the defined shake-time was measured and used in the chemometric evaluation instead of the initial pH in the media (*i.e.* before the addition of terfenadine). Four samples in each media were used and diluted according to previously described routine (61,62). Each sample was thereafter analyzed in duplicate by the HPLC system ($n=8$).

RESULTS

The experimental values of G calculated from the rotating disk experiments had a maximum RSD of 10%. The RSD-values were for the solubility determinations, S, less than 25%. The relatively high RSD values of S in the solubility determination can be ascribed to the difficulties of having substance powder located at the medium surface after the centrifugation in some conducted tests.

Since no extra dilution steps were used in the media preparation, turbid solutions were achieved whenever lecithin was added to the aqueous media, even after some hours on a stirrer. This is due to the formation of a colloidal system. No opaque medium was, however, obtained if the sodium taurocholate (pK_a 1.4 (79)) was added in the different experiments, except when lecithin was present.

The OPLS models were used as tools to investigate the factors of importance for dissolution rate and solubility in the different aqueous media for the basic drug substance terfenadine. The results of the OPLS models are shown in Fig. 1A–C for the *in vitro* dissolution rate and 2A–C for the apparent solubility.

The loading plots are showing that pH, I (ionic strength) and the concentration of added NaCl in the acetate medium are negatively correlated to the response G in Fig. 1A. The relative importance of these various factors is approximately the same. In other words, if, *e.g.*, pH or the ionic strength is decreasing in the medium the *in vitro* dissolution rate (G) is increasing for the weak base terfenadine. In Fig. 1B and C, only pH is negatively correlated to G. Other variables are insignificant at the 95% confidence level used.

In Fig. 2A, it was found that pH, I (ionic strength) and the concentration of added NaCl in the acetate medium are negatively correlated to the response S. In Fig. 2B–C, only pH is negatively correlated to S. This is the same correlation result as in the loading plots for G (Fig. 1A–C).

DISCUSSION

A protonated basic drug substance might interact with anionic surfactants (80), *e.g.* taurocholate, and increase its solubility, but this was not a tendency found in this study, since this factor was insignificant. Ionic surfactants are otherwise thought to have higher surface activities and better solubilizing capabilities than non-ionic ones (81). The colloidal structures of the surfactants in the media were not investigated in this study. Rotating disk experiments for dissolution rate measurements have been reported to only be modestly affected by the increase of NaTC in the medium used for different drug substances (31). Moreover it is proposed that the solubility of most poorly soluble drugs increases linearly with increasing bile salt concentration (6), but this could noticeably not be confirmed for terfenadine in this study. A similar result was also obtained in a non-chemometric solubility study of different steroids in the presence of bile salts at the fasted state levels (26). It has been discussed if the solubility enhancement of poorly soluble drugs in biorelevant media is independent of the type of surfactant added and on the colloid structures formed (6). Nevertheless, no such verification could be done in this study since the surfactants were found to be insignificant in the multivariate data analysis in the concentration range and temperature used (Figs. 1A–C and 2A–C). The temperature is otherwise a major factor (47,82), but since the main scope of the study was to evaluate the miniaturized disk apparatus using different aqueous media combined with chemometric analyses, only room temperature was used. Further development of a thermostating mode of the equipment is needed to be able to use the preferred temperature of 37°C.

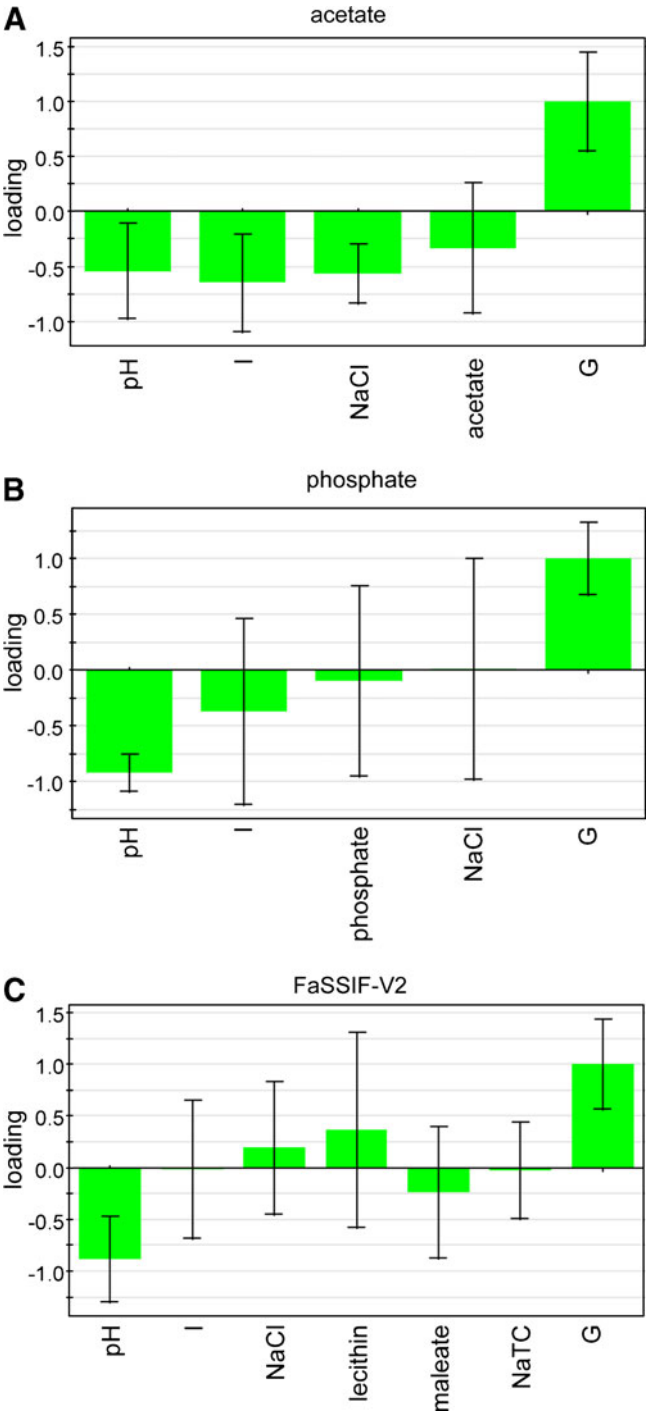

Fig. 1 A–C. Loading column plots of the OPLS models of the *in vitro* dissolution rate (G) in acetate, phosphate or maleate (fasted state simulated intestinal fluid 2nd version, FaSSIF-V2) buffer media with "biorelevant" additives (NaCl, lecithin and NaTC). The unit for G was $\mu g/s/cm^2$. The black error bars denote the 95% confidence level of the standard error.

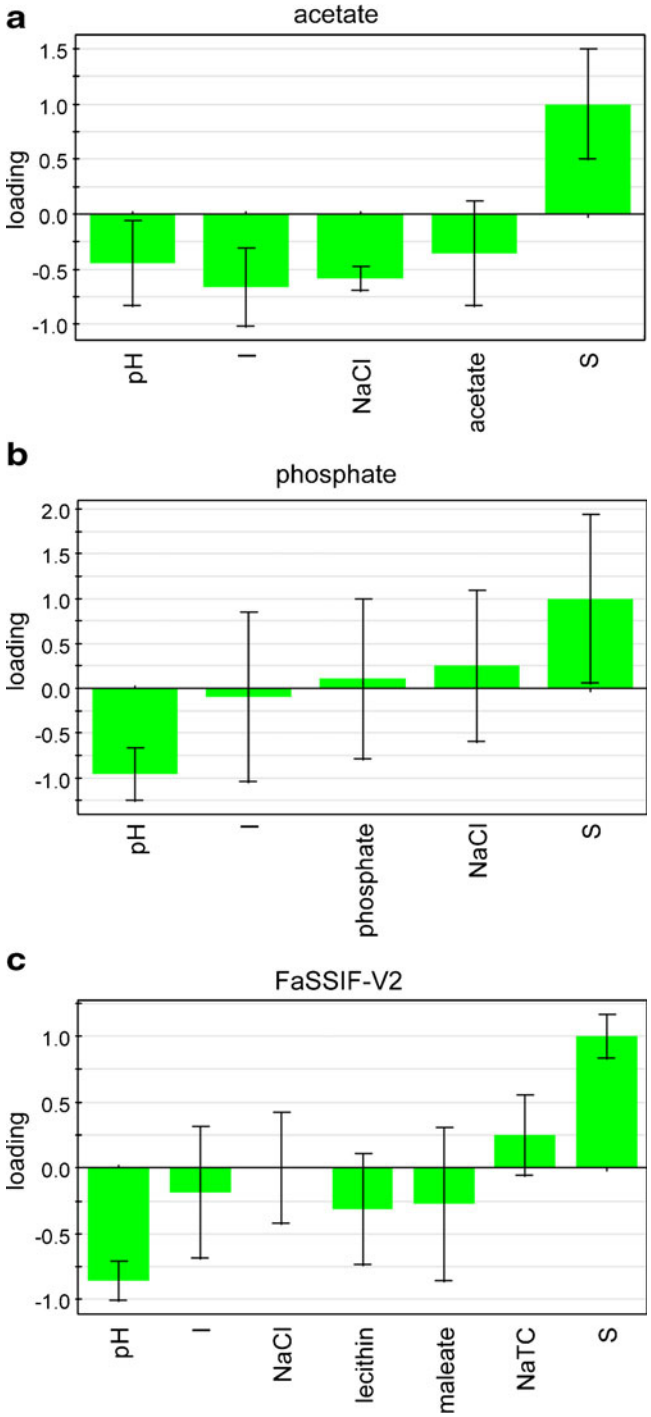

Fig. 2 A–C. Loading column plots of the OPLS model of the apparent solubility (S) in acetate, phosphate or maleate (fasted state simulated intestinal fluid 2nd version, FaSSIF-V2) buffer media with "biorelevant" additives (NaCl, lecithin and NaTC). The unit for S was mM. The black error bars denote the 95% confidence level of the standard error.

The ionic strength can influence drug release and should therefore be based on physiological values (17). This factor was therefore included in the multivariate data analysis since little information was found in the literature for the apparent *in vitro* dissolution rate experiments using pure drug substances, without focusing on the so-called common ion effect (83,84). It has been established by others that the ionic strength is a significant factor for dissolution testing of a felodipin formulation (aprotic drug substance) in phosphate buffer (47). In this study, however, it was only found that the ionic strength was statistically significant in the acetate medium (Figs. 1A and 2A). This buffer is the only one using monovalent buffer components (acetic acid/acetate), but otherwise the pH range 4–6 is suited for the pK_a of acetic acid. In the other two buffers, mostly divalent buffer components will be achieved in the pH range used, 6–8, due to the impact of the second pK_a-values. The phosphate and acetate buffers were grouped (clustered) together in the PCA loading plot, while the maleate buffer was found with the two surfactants in the opposite quadrant (plot not shown). It is important to stress that simulating intestinal surfactant composition alone with no attention to buffer components cannot predict the *in vivo* behavior of pH responsive drugs (24).

The buffer capacity, β (85), is essential for protolytic drug substances, since the pH should preferably remain constant during an *in vitro* determination (7,60–62,86,87). Buffer capacity has more prominent influence, compared to ionic strength, on dissolution (24). On the other hand, it has been shown that the buffer capacity of FaSSIF is higher than *in vivo* (6). The effects of pH on drug release are well demonstrated in the literature (8,17), and this variable could also be a benchmark of the buffer capacity in the used media. The value of β was estimated in the low buffer concentrations of the media, and compared to the solubility value of terfenadine, pH might be influenced by the dissolved base. However, the pH changed at maximum +0.5 in the acetate buffer, +0.2 in the phosphate buffer and ±0.5 in the maleate medium from the initial pH in the solubility study after 24 h in the shake-flask experiments. The main factor affecting G and S for terfenadine in all media was pH (Figs. 1A–C and 2A–C), which was expected, since the weak base becomes more protonated with decreasing pH in the solution.

Polymorphism (49) data for a drug substance should preferably be given as the solid state influences dissolution rate and apparent solubility (49,88,89). This was, however, not done, but one and the same batch of terfenadine was used throughout the experiments as well as the same pressure applied during disk preparation. Yet, eventual solvent-mediated phase transformations during the experiments can, therefore, not be confirmed.

Even though the miniaturized rotating disk apparatus is primarily thought of as a screening tool, it was found to be useful in this multivariate data analysis as well. It is not always the fact that the solubility is increased to the same extent as the dissolution rate in the presence of surfactants

in the medium (9,88). It has been proposed that the modified Noyes-Whitney equation might have to be refined even more when complex media is used in drug dissolution (5,9). This could not be confirmed in the present study, but it should also be stated that the obtained results may only be weighed against structural or functional analogs or certain homologues to the model drug substance used in the chemometric evaluations. The polarity, chain length and branching, molecular size/weight, shape and structure all affect solubilization of a drug substance in surfactant systems (26,30,88). Additives/surfactants which are limited in their solubility in aqueous media can form monomer/vesicular/micellar emulsions and complicate analysis (17), but in this rotating disk, equipment difficulties in the detection step are reduced by the use of a chromatographic separation system. If even higher selectivity and/or sensitivity are desired in the analysis, mass spectrometer (MS) with different types of mass analyzer techniques could be combined with the chromatographic system instead of using ultraviolet detection. As stated in reference (17), selection of appropriate drug separation and analytical methods is necessary to obtain meaningful results. Moreover, the miniaturized rotating disk apparatus does not consume large volumes of dissolution media (61) and can, therefore, be suited to dissolution experiments using expensive media (both simulated media and fluids attained from animals or humans) compared to the flow-through method called USP Apparatus 4 (57). The advantage of having a defined surface area of a disk instead of a powder particle size-surface area distribution is also achieved in the novel equipment.

CONCLUSIONS

The miniaturized rotating disk equipment, combined with multivariate data analyses, was established to be suitable to use in the investigation of factors affecting G for the weak base terfenadine. It was found that S was affected by the same variables as G. The main factor for G and S of terfenadine in all media was pH. In addition, I and NaCl were found to be significant factors in the acetate buffer solution. No wetting effect of the media surfactants at the concentrations and temperature used was distinguished, since the surfactants were insignificant in the OPLS evaluation (95% confidence level of the standard error). No attention of the polymorphic forms of terfenadine was made, since this study was focused on the evaluation of the novel miniaturized rotating disk equipment using different aqueous media in conjunction with chemometric analyses. The advantages of the miniaturized apparatus can be ascribed to fast quantification due to the integration with an online HPLC system and the need for only one data point to estimate the dissolution rate of a drug substance during the experiment, by the use of an external standard curve. Furthermore, the advantage of using a chromatographic separation system for the the analyte of interest and other eventual additives in the dissolution medium simplifies the determination. The consummation of relatively low volumes of media (approximately 20 ml per disk) is obviously also preferred if fluids from animals or humans are proposed for the experiments.

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REFERENCES

1. Dressman JB, Vertzoni M, Goumas K, Reppas C. Estimating drug solubility in the gastrointestinal tract. Adv Drug Deliv Rev. 2007;59:591–602.
2. Vertzoni M, Pastelli E, Psachoulias D, Kalantzi L, Reppas C. Estimation of intragastric solubility of drugs: in what medium? Pharm Res. 2007;24:909–17.
3. The United States Pharmacopeia. USP 29. Rockville: United States Pharmacopeial Convention Inc.; 2006.
4. Ravi V, Siddaramaiah, Kumar TMP. Influence of natural polymer coating on novel colon targeting drug delivery system. J Mater Sci: Mater Med. 2008;19:2131–6.
5. Sugano K, Okazaki A, Sugimoto S, Tavornvipas S, Omura A, Mano T. Solubility and dissolution profile assessment in drug discovery. Drug Metab Pharmacokinet. 2007;22:225–54.
6. Müllertz A. Biorelevant dissolution media. *Biotechnology; Pharmaceutical Aspects (Publisher: AAPS Press)* 2007;151–77.
7. Kalantzi L, Goumas K, Kalioras V, Abrahamsson B, Dressman JB, Reppas C. Characterization of the human upper gastrointestinal contents under conditions simulating bioavailability/bioequivalence studies. Pharm Res. 2006;23:165–76.
8. Moreno MPd1C, Oth M, Deferme S, FLammert, Tack J, Dressman J *et al.* Characterization of fasted-state human intestinal fluids collected from duodenum and jejunum. J Pharm Pharmacol. 2006;58:1079–89.
9. Persson EM, Gustafsson A-S, Carlsson AS, Nilsson RG, Knutson L, Forsell P *et al.* The effects of food on the dissolution of poorly soluble drugs in human and in model small intestinal fluids. Pharm Res. 2005;22:2141–51.

10. Kostewicz ES, Brauns U, Becker R, Dressman JB. Forecasting the oral absorption behaviour of poorly soluble weak bases using solubility and dissolution studies in biorelevant media. Pharm Res. 2002;19:345–9.
11. Lindahl A, Ungell A-L, Knutson L, Lennernäs H. Characterization of fluids from the stomach and proximal jejunum in men and women. Pharm Res. 1997;14:497–502.
12. Ibekwe VC, Fadda HM, Parsons GE, Basit AW. A comparative *in vitro* assessment of the drug release performance of pH-responsive polymers for ileo-colonic delivery. Int J Pharm. 2006;308.
13. Galia E, Nicolaides E, Hörter D, Löbenberg R, Reppas C, Dressman JB. Evaluation of various dissolution media for predicting *in vivo* performance of Class I and II drugs. Pharm Res. 1998;15:698–705.
14. Sheng JJ, MacNamara DP, Amidon GL. Towards an *in vivo* dissolution methodology: a comparison of phosphate and bicarbonate buffers. Mol Pharm. 2009;6:29–39.
15. Boni JE, Brickl RS, Dressman J. Is bicarbonate buffer suitable as a dissolution medium? J Pharm Pharmacol. 2007;59:1375–82.
16. Smidt JHd, Offringa JCA, Crommelin DJA. Dissolution kinetics of theofylline in aqueos polymer solutions. Int J Pharm. 1991;77:255–9.
17. Jantratid E, Janssen N, Reppas C, Dressman JB. Dissolution media simulating conditions in the proximal human gastrointestinal tract: an update. Pharm Res. 2008;25:1663–76.
18. Pedersen BL, Müllertz A, Brøndsted H, Kristensen HG. A comparison of the solubility of danazol in human and simulated gastrointestinal fluids. Pharm Res. 2000;17:891–4.
19. Lue B-M, Nielsen FS, Magnussen T, Schou HM, Kristensen K, Jacobsen LO *et al.* Using biorelevant dissolution to obtain IVIVC of solid dosage forms containing a poorly-soluble model compound. Eur J Pharm Biopharm. 2008;69:648–57.
20. Nicolaides E, Galia E, Efthymiopoulos C, Dressman JB, Reppas C. Forecasting the *in vivo* performance of four low solubility drugs from their *in vitro* dissolution data. Pharm Res. 1999;16:1876–82.
21. Bard B, Martel S, Carrupt P-A. High troughput UV method for the estimation of thermodynamic solubility and the determination of the solubility in biorelevant media. Eur J Pharm Sci. 2008;33:230–40.
22. Zoellerand T, Klein S. Simplified biorelevant media for screening dissolution performance of poorly soluble drugs. Dissolut Technol. 2007;8–13.
23. Dressman JB, Reppas C. *In vitro-in vivo* correlations for lipophilic, poorly water-soluble drugs. Eur J Pharm Sci. 2000;11:73–80.
24. Fadda HM, Basit AW. Dissolution of pH responsive formulations in media resembling intestinal fluids: bicarbonate *versus* phosphate buffers. J Drug Deliv Sci Technol. 2005;15:273–9.
25. MacNamara DP, Whitney KM, Goss SL. Use of a physiologic bicarbonate buffer system for dissolution characterization of ionizable drugs. Pharm Res. 2003;20:1641–6.
26. Mithani SD, Bakatselou V, TenHoor CN, Dressman JB. Estimation of the increase in solubility of drugs as a function of bile salt concentration. Pharm Res. 1996;13:163–7.
27. McNamara DP, Boehringer I. Dissolution of pharmaceuticals in simple and complex systems. Drugs and the pharmaceutical sciences. Transport Process Pharm Syst. 2000;102:109–46.
28. Efentakis M, Dressman JB. Gastric juice as a dissolution medium: surface tension and pH. Eur J Drug Metab Pharmacokinet. 1998;23:97–102.
29. Wei H, Löbenberg R. Biorelevant dissolution media as a predictive tool for glyburide a class II drug. Eur J Pharm Sci. 2006;29:45–52.
30. Charman WN, Porter CJH, Mithani S, Dressman JB. Physicochemical and physiological mechanism for the effects of food on drug absorption: the role of lipids and pH. J Pharm Sci. 1997;86:269–82.
31. Bakatselou V, Oppenheim RC, Dressman JB. Solubilization nad wetting effects of bile salts on the dissolution of steroids. Pharm Res. 1991;8:1461–9.
32. Leng J, Egelhaaf SU, Cates ME. Kinetics of the micelle-to-vesicle transition: aqueous lecithin-bile salt mixtures. Biophys J. 2003;85:1624–46.
33. Gabrielsson J, Lindberg N-O, Lundstedt T. Multivariate methods in pharmaceutical applications. J Chemometr. 2002;16:141–60.
34. Parshad H, Frydenvang K, Liljefors T, Larsen CS. Correlation of aqueous solubility of salts of benzylamine with experimentally and theoretically derived parameters. A multivariate data analysis approach. Int J Pharm. 2002;237:193–207.
35. Grover M, Singh B, Bakshi M, Singh S. Quantitative structure-property relationships in pharmaceutical research – Part 1. Pharm Sci Technol Today. 2000;3:28–35.
36. Du-Cuny L, Huwyler J, Wiese M, Kansy M. Computional aqueous solubility prediction for drug-like compounds in congeneric series. Eur J Med Chem. 2008;43:501–12.
37. Eriksson L, Johansson E, Kettaneh-Wold N, Wikström C, Wold S. Design of experiments, principles and application. Umeå: Umetrics Academy; 2000.
38. de Aguiar PF, Bourguignon B, Khots MS, Massart DL, Phan-Than-Luu R. D-optimal designs. Chemo Intell Lab Syst. 1995;30:199–210.
39. Olsson I-M, Gottfries J, Wold S. D-optimal onion designs in statistical molecular design. Chemo Intell Lab Syst. 2004;73:37–46.
40. Rosén J. ChemGPS-NP and the exploration of biologically relevant chemical space. U. Dissertation from the Faculty of Pharmacy (ed). Vol. 89, Acta Universitatis Upsaliensis Uppsala (2009).
41. Lundstedt T, Seifert E, Abramo L, Thelin B, Nyström Å, Pettersen J *et al.* Experimental design and optimization. Chemo Intell Lab Syst. 1998;42:3–40.
42. Wold S, Esbensen K, Geladi P. Principal component analysis. Chemo Intell Lab Syst. 1987;2:37–52.
43. Pearson K. On lines and planes of closest fit to systems of points in space. Phil Mag. 1901;2:559–72.
44. Gottfries J, Johansson E, Trygg J. On the impact of uncorrelated variation in regression mathematics. J Chemometr. 2008;16:119–28.
45. Trygg J, Wold S. Orthogonal projections to latent structures (O-PLS). J Chemometr. 2002;16:119–28.
46. Jinno J, Oh D-M, Crison JR, Amidon GL. Dissolution of ionizabl water-insoluble drugs: the combined effect of pH and surfactant. J Pharm Sci. 2000;89:268–74.
47. Gottfries J, Ahlbom J, Harang V, Johansson E, Josefson M, Morsing T *et al.* Validation of an extended release tablet dissolution testing system using design and multivariate analysis. Int J Pharm. 1994;106:141–8.
48. Redenti E, Pasini M, Ventura P, Spisni A, Vikmon M, Szejtli J. The terfenadine/β-cyclodextrin inclusion complex. J Inclusion Phenom Mol Recognit Chem. 1993;15:281–92.
49. Snider DA, Addicks W, Owens W. Polymorphism in generic drug product development. Adv Drug Deliv Rev. 2004;56:391–5.
50. Choi H-G, Lee B-J, Han J-H, Lee M-K, Park K-M, Young CS *et al.* Terfenadine-β-cyklodextrin inclusion complex with antihistaminic activity enhancement. Drug Dev Ind Pharm. 2001;27:857–62.
51. Skold C, Winiwarter S, Wernevik J, Bergstrom F, Engstrom L, Allen R *et al.* Presentation of a structurally diverse and commercially available drug data set for correlation and benchmarking studies. J Med Chem. 2006;49:6660–71.
52. Shugarts S, Benet LZ. The role of transporters in the pharmacokinetics of orally administred drugs. Pharm Res. 2009;26:2039–54.
53. Jain D, Pathak D, Pathak K. Pharmaceutical product development technologies based on the biopharmaceutical classification system. Pharmazie. 2009;64:483–90.
54. Amidon GL, Lennernas H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification: the correlation of

in vitro drug product dissolution and *in vivo* bioavailability. Pharm Res. 1995;12:413–20.
55. Bergström CAS, Luthman K, Artursson P. Accuracy of calculated pH-dependent aqueous drug solubility. Eur J Pharm Sci. 2004;22:387–98.
56. Emami J. *In vitro - in vivo* correlation: from theory to applications. J Pharm Sci. 2006;9:31–51.
57. Sunesen VH, Pedersen BL, Kristensen HG, Müllertz A. *In vivo in vitro* correlations for a poorly soluble drug, danazol, using the flow-through dissolution method with biorelevant dissolution media. Eur J Pharm Sci. 2005;24:305–13.
58. Ahrabi SF, Madsen G, Dyrstad K, Sande SA, Graffner C. Development of pectin matrix tablets for colonic delivery of model drug ropivacaine. Eur J Pharm Sci. 2000;10:43–52.
59. Nicolaides E, Symillides M, Dressman JB, Reppas C. Biorelevant dissolution testing to predict the plasma profile of lipophilic drugs after oral administration. Pharm Res. 2001;18:380–8.
60. Persson AM, Baumann K, Sundelof LO, Lindberg W, Sokolowski A, Pettersson C. Design and characterization of a new miniaturized rotating disk equipment for *in vitro* dissolution rate studies. J Pharm Sci. 2008;97:3344–55.
61. Persson AM, Sokolowski A, Pettersson C. Correlation of *in vitro* dissolution rate and apparent solubility in buffered media using a miniaturized rotating disk equipment: Part I. Comparison with a traditional USP rotating disk apparatus. Drug Discov Ther. 2009;3:104–13.
62. Persson AM, Pettersson C, Sokolowski A. Correlation of *in vitro* dissolution rate and apparent solubility in buffered media using a miniaturized rotating disk equipment: Part II: Comparing different buffer media. Drug Discov Ther. 2009;3:114–22.
63. Nelson KG, Shah AC. Convective diffusion model for a transport-controlled dissolution rate process. J Pharm Sci. 1975;64:610–4.
64. Nicklasson M, Brodin A, Nyqvist H. Studies on the relationship between solubility and intrinsic rate of dissolution as a function of pH. Acta Pharm Suecica. 1981;18:119–28.
65. Shah AC, Nelson KG. Evaluation of a convective diffusion drug dissolution rate model. J Pharm Sci. 1975;64:1518–20.
66. Hamlin WE, Northam JI, Wagner JG. Relationship between *in vitro* dissolution rates and solubilities of numerous compounds representative of various chemical species. J Pharm Sci. 1965;54:1651–3.
67. Noyes AA, Whitney WR. The rate of solution of solid substances in their own solutions. J Am Chem Soc. 1897;19:930–4.
68. Bruner L, Tolloczko S. On the velocity of solution of solid bodies. Z Phys Chem Stoechiomet Verwandts. 1900;35:283–90.
69. Brunner E. Reaction velocity in heterogenous systems. Z Phys Chem. 1904;47:56–102.
70. Nernst W. Theory of reaction velocity in heterogenous systems. Z Phys Chem. 1904;47:52–5.
71. Excel. Microsoft Office 2007, Microsoft Corporation, Europe.
72. Sirius. Sirius Technical Applications Notes (STAN). In S. A. I. L. (UK) (ed), Vol. 1 (S. A. I. L. (UK), ed), 1994.
73. Sirius. RefinementPro2 Sirius Analytical Instruments Ltd. (UK).
74. Gäfvert E. Personal communication. Uppsala: Medical Product Agency; 2009.
75. Davies CW. Ion association. London: Butterworths; 1962.
76. Güntelberg E. Untersuchungen über Ioneninteraktion. Z Phys Chem. 1926;123:199–247.
77. MODDE 7 software, Umetrics AB, http://www.umetrics.com/, Umeå, Sweden.
78. SIMCA-P+ 12.0 software, Umetrics AB, http://www.umetrics.com/, Umeå, Sweden.
79. Sales Office. Personal communication, Prodotti Chimici e Alimentari S.p.A. a Socio Unico Sede Legale, Basaluzzo (AL), Italy, 2009.
80. Bonferoni MC, Rossi S, Ferrari F, Stavik E, Pena-Romero A, Caramella C. Factorial analysis of the influence of dissolution medium on drug release from Carrageenan-Diltiazem complexes. *AAPS PharmSciTec* 1: Article 15; 2000.
81. Park S-H, Choi H-K. The effects of surfactants on the dissolution profiles of poorly water-soluble acidic drugs. Int J Pharm. 2006;321:35–41.
82. Kettaneh-Wold N. Use of experimental design in the pharmaceutical industry. J Pharm Biomed Anal. 1991;9:605–10.
83. Serajuddin AT, Sheen PC, Augustine MA. Common ion effect on solubility and dissolution rate of the sodium salt of an organic acid. J Pharm Pharmacol. 1987;39:587–91.
84. Li S, Doyle P, Metz S, Royce AE, Serajuddin AT. Effect of chloride ion on dissolution of different salt forms of haloperidol, a model basic drug. J Pharm Sci. 2005;94:2224–31.
85. Van Slyke DD. On the measurement of buffer values and on the relationship of buffer value to the dissociation constant of the buffer and the concentration and reaction of the buffer solution. J Biol Chem. 1922;52:525–70.
86. Mooney KG, Mintun MA, Himmelstein KJ, Stella VJ. Dissolution kinetics of carboxylic acids II: effect of buffers. J Pharm Sci. 1981;70:22–32.
87. Mooney KG, Mintun MA, Himmelstein KJ, Stella VJ. Dissolution kinetics of carboxylic acids I: effect of pH under unbuffered conditions. J Pharm Sci. 1981;70:13–22.
88. Hörter D, Dressman JB. Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. Adv Drug Deliv Rev. 1997;25:3–14.
89. Shah B, Kakumanu VK, Bansal AK. Analytical techniques for quantification of amorphous/crystalline phases in pharmaceutical solids. J Pharm Sci. 2006;95:1641–65.