Original Article

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

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ABSTRACT: A correlation of the logarithmic values of the *in vitro* dissolution rate, *G*, and the apparent solubility, *S*, was evaluated in phosphate and ammonium acetate buffer at an initial pH of 7. The dissolution rates were determined with a newly designed and build miniaturized rotating disk equipment, as well as with a traditional rotating disk apparatus. The two apparatuses gave the same correlation pattern of log*G* and log*S*. Thirteen diverse drug substances from all of the classes in the Biopharmaceutics Classification System (BCS) were used for the correlation in the phosphate buffer system, with the results from the miniaturized apparatus only. A coefficient of determination, R^2, of 0.982 was found if bases formulated as hydrochloride salts were excluded in the correlation.

The miniaturized equipment is used for rapid screening of the dissolution rate, approximately 10 min for one run, and consumes small amounts of substance (about 5 mg) and dissolution media. All quantifications were performed by using reversed phase high-performance liquid chromatography (RP-HPLC) with a diode array detector (DAD), integrated with the miniaturized rotating disk equipment.

Keywords: Dissolution rate, solubility, *in vitro* models, correlation, HPLC (high-performance liquid chromatography)

1. Introduction

Solubility and dissolution rate are two important physicochemical properties during the lead selection and optimization process in drug discovery (*1*,*2*). Great effort in preformulation studies can sometimes circumvent insufficient solubility of a drug substance, but more cost- and time-effective strategies are preferred. Due to the large number of lead candidates in early development of drugs it would be desirable to develop a fast and adequate accurate method to measure the solubility, while consuming only minute amount of substances and media. Furthermore it should be advantageous to predict the solubility directly by simply studying the dissolution rate of a drug substance.

Good correlation between the solubility and the *in vitro* dissolution rate has previously been found for a number of compounds (*3*-*6*). However, it should always be stressed that this correlation will be dependent on the instrumental setup and the media used. The chemical properties of the dissolution medium (pH, buffer and additional components, *etc.*) play a decisive role for the dissolution rate studies (*7*-*10*). Furthermore the correlation can differ between free bases or acids and their respective salt forms (*11*,*12*). Additional studies of the correlation between solubility and dissolution rate are necessary in order to validate a predictive value of solubility from dissolution rate data due to the differences stated above.

Several different methods are used to determine the solubility of new substances (*13*,*14*), with the traditional shake-flask method as the most frequently used technique. Unfortunately, not all of the methods are adapted to the high throughput needs in modern drug discovery. Some methods that minimize the amount of substances while determining the solubility are *e.g.* the miniaturized shake-flask (*15*), pSol method (*16*), GLpKa/CheqSol method (*17*,*18*) and a multichanneled miniaturized device (*19*). The shake-flask method most often determines the solubility at a specific pH and buffer capacity (β) at a defined shake-time, which gives the apparent solubility. A problem during this solubility determination is the possibility of a degradation of the drug substance. The experimental determination of solubility can also be affected by buffer or additional components in the solution, *e.g.* due to the common ion effect, as well as the ionic strength (*20*-*22*).

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Many rotating disk theories of the dissolution rate are based on the diffusion layer model (*23*), where the dissolution rate is assumed to be controlled by the diffusion of the investigated drug substance through a stagnant layer near the drug surface. The hydrodynamics is thus a combination of laminar and convective fluids (*23*,*24*) and the disturbance of the diffusion layer will probably increase the dissolution rate. Even if media mimicking gastric and small intestinal environments are used, the simulation of the *in vivo* hydrodynamics is still difficult to achieve. Different techniques have been used in dissolution rate studies, *e.g.* USP rotating disk method (*25*), flow-through cell or channel flow methods (*5*,*26*,*27*), microcalorimetry (*28*) and a newly designed miniaturized equipment (*29*) as well as a miniaturized intrinsic dissolution rate apparatus (*30*,*31*).

In this study a comparison of the correlation between the logarithmic values of the *in vitro* dissolution rates (*G*) obtained with a newly designed miniaturized equipment and a traditional rotating disk apparatus, and the logarithmic values of the apparent solubilities (*S*) determined by a conventional shake-flask method at a volume of 1.5 mL was made. The evaluations were done with six different drug substances in an ammonium acetate and a phosphate buffer. In an extended study, thirteen substances in the correlation between the dissolution rate and the solubility was performed, using only the phosphate buffer and the miniaturized equipment. The initial pH was chosen to be biorelevant (*32*,*33*) and applied to the guidelines from the Federation International Pharmaceutic (FIP). FIP recommend USP buffer solutions in the pH range of 4.5 to 8.0 in dissolution testing (*34*,*35*).

The aim of the present study was to compare the possibility to predict the solubility based on the dissolution rate determined by a traditional rotating disk apparatus and a newly established miniaturized rotating disk equipment (*29*). The miniaturized system has the advantage of only consuming minute amount of substance, as well as dissolution medium. Furthermore, it also provides a value of the dissolution rate simply by one measurement. The comparison between the two dissolution systems is vital. If they give equal results, the newly developed instrument can be used in further studies of the correlation of log*G* and log*S*.

2. Theoretical

The stoichiometric apparent solubility (*S*) can theoretically be calculated by using the stoichiometric intrinsic solubility of a solute (S_0), pH of the solution and the stoichiometric pK_a-value of the solute at the actual ionic strength for both monoacidic (Eq. 1) and monobasic (Eq. 2) drugs. The stoichiomeric pK_a-value is dependent on the ionic strength, which consequently will affect the apparent solubility (*36*,*37*). The intrinsic solubility relates to the solubility of the completely unionized substance. These equations of ionizable substances are based on the Henderson-Hasselbalch relationship, cf. (*38-40*).

$$S = S_0 [1+10^{(pH-pK_a)}] \quad \text{Eq. 1}$$

$$S = S_0 [1+10^{(pK_a-pH)}] \quad \text{Eq. 2}$$

During solubility studies with shake-flask methodology the pH can differ from the start to the end of the experiments if protolytic drug substances are examined, as often is the case. It is therefore necessary to measure the pH in the medium before drug substance is added and particularly after the defined shake time.

At constant flow rate and rotation speed in the rotating disk experiments, the thickness of the diffusion layer above the rotating disk of drug substance will be constant. Assuming that no other side reactions, beside protolysis, exist in the experiments and that sink conditions is prevailed the modified Noyes-Whitney equation can be expressed as Eq. 3 (*41*):

$$G = \frac{D}{h} \cdot S = k \cdot S \quad \text{Eq. 3}$$

where *G* is the *in vitro* dissolution rate, *k* is a constant based on the thickness of the diffusion layer adjacent the disk surface, *h*, and the diffusion coefficient, *D*, of the substance in the dissolution medium at a certain temperature. *S* is the apparent solubility (as defined above) of the drug substance in the dissolution medium at a certain temperature. The logarithmated form of the equation will give a straight line when plotting log*G* versus log*S*, Eq. 4, cf. (*3*).

$$\log G = \log S + \log k \quad \text{Eq. 4}$$

3. Materials and Methods

3.1. *Chemicals*

Naproxen, ketoprofen and griseofulvin met USP specifications, enalapril maleate, nortriptyline hydrochloride, chlorprotixene hydrochloride, clomipramine hydrochloride, prednisone, bendroflumethiazide, furosemide and terfenadine were minimum 98% and carbamazepine 99%, were all purchased from Sigma-Aldrich (Chemie GmbH, Steinheim, Germany). Ciprofloxacin ≥ 98.0% was obtained from Fluka BioChemika, (Chemie GmbH, Buchs, Switzerland). Sodium di-hydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$) *p.a.*, Acros Organics (Springfield, NJ, USA), di-sodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$) *p.a.* and trifluoroacetic acid ≥ 99.0%, were both from Fluka Chemika (Chemie GmbH, Buchs, Switzerland). Ammonium acetate (NH_4Ac) *p.a.*, was bought from Riedel-de Haën, Sigma-Aldrich (Laborchemikalien GmbH, Seelze, Germany). Acetonitrile, HPLC grade was bought from Fisher

Scientific (UK Limited, Leicestershire, UK). The water used in this study was purified in a Milli-Q® Academic system (18.2 MΩ·cm/0.22 µm), Millipore, (Burlington, MA, USA).

3.2. *Experimental*

3.2.1. *Dissolution media and drug substances*

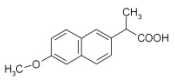
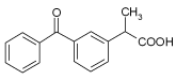
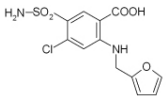
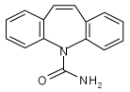
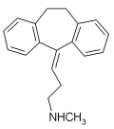
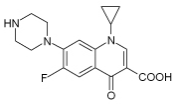
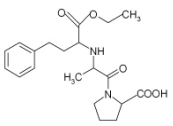
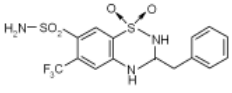
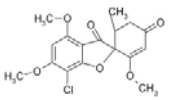
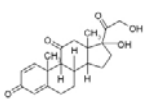
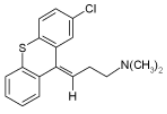
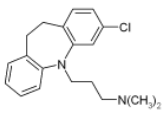
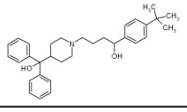
Sodium phosphate buffer pH 7.0 ± 0.1 (65.5 mM) and ammonium acetate buffer pH 6.8 ± 0.3 (10 mM) had the ionic strengths of 150 mM and 10 mM respectively. These buffers were prepared by dissolving 3.21 g $NaH_2PO_4 \cdot H_2O$ and 7.52 g $Na_2HPO_4 \cdot 2H_2O$ in 1000 mL Milli-Q® water and 0.757 g NH_4Ac in 1000 mL Milli-Q® water. The pK_a-values used for phosphoric acid are 1.89, 6.67, and 11.68 (I = 0.165 M, 25.0°C) (*42*), for ammonium 9.20 and for acetic acid 4.53 (both at I = 0.15 M) (*43*). The drug substances used in this study were from all of the classes in the BCS, and is an assortment of bases, acids, ampholytes and aprotic compounds. The structures of the substances and their pK_a-values are shown in Table 1. The pK_a-value from reference (*44*) was determined by using the Sirius PCA100 instrument, while the pK_a-values from reference (*45*) were determined by using the Sirius GLpKa instrument.

3.2.2. *Stability study of the drug solutions*

For the stability and solubility (section 1.2.3) 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 16M microcentrifuge 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 CMAW711 (Ø 4.5 mm) from Thermo Russell (Auchtermuchty Fife, Scotland).

Two different concentrations of the substances in phosphate buffer were used in the stability study. One was made by using saturated solutions of the drug substances in room temperature with the shake-flask methodology. The other concentration was approximately 15 µM, stored at different temperatures. In the saturated solutions each drug substance was added in excess (2-50 mg) to 1.5 mL of the phosphate buffer. The drug-buffer suspensions were shaken

Table 1. Structures and pK_a-values of the drug substances (N/A = not applicable)

Substance name [salt form] pK_a	Structure (not salt)	Substance name [salt form] pK_a	Structure (not salt)
Naproxen 4.18 (*16*)		Ketoprofen 3.95 (*45*)	
Furosemide 3.52 (*16*)		Carbamazepine N/A (*46*)	
Nortriptyline [HCl] 10.21 (*18*)		Ciprofloxacin 6.20 8.59 (*47*)	
Enalapril [maleate] 2.99 5.39 (*45*)		Bendroflumethiazide 8.77 (*48*)	
Griseofulvin N/A (*49*)		Prednisone N/A (*50*)	
Chlorprotixene [HCl] 8.80 (*51*)		Clomipramine [HCl] 8.83 (*44*)	
Terfenadine 9.25 (*50*)			

at room temperature (21.0 ± 1.5°C) for up to seven days, including the centrifugation at 14000 rpm for 10 min to find an optimal shake-time. The end of the experiments was chosen so that two following analyses of the supernatant gave the response (chromatographic peak area), ± 15%, and pH value, ± 0.15. Four samples of each drug substance were analyzed in duplicate at each time-point. Each drug substance of 15 µM were measured for eventual concentration changes at different time intervals at room temperature (21.0 ± 1.5°C), in refrigerator (+4°C) and in freezer (-20°C). The acceptance criterion was set to ≥ 85% of the initial drug substance concentration (at µM range) in room temperature.

3.2.3. *Apparent solubility determination by shake-flask methodology*

Each drug was added in excess (2-50 mg) to 1.5 mL of dissolution media, phosphate or ammonium acetate buffer, and was shaken at room temperature (21.0 ± 1.5°C) for 24 h. Centrifugation was used in separating the excess of substance from the dissolution medium after the defined shake-time. This was made instead of filtration to avoid problems with adsorption to filters, which had been noticed during initial dissolution studies of some of the substances. The pH was always measured before a substance was added into the buffer and after reaching the end of the study, but pH was not adjusted afterwards. The supernatant was diluted 600 times in mobile phase before HPLC analysis due to high absorbance and to avoid precipitation. Three samples of each drug substance were made for the shake-flask study. Double injections of each analyte were used for the apparent solubility determination and the average values with the relative standard deviations (RSD) were calculated.

3.2.4. *In vitro dissolution rate studies*

The conventional dissolution bath was a Dissolutest, Prolabo (Paris, France) and the thermometer a Testo 110 from Testo Inc. (Lenzkirch, Germany). The newly designed miniaturized equipment has been described previously (*29*). The disk in the miniaturized apparatus had a diameter of 1.5 mm. A magnetic stirrer with graded rotating speeds was obtained from Heidolph MR 3001K (Steinheim, Germany). The cell of Plexiglas was integrated with a HPLC (high-performance liquid chromatography) system using DAD (diode array detector) for analysis, see Figure 1. An external HPLC pump was connected to the cell for the distribution of dissolution medium, which was deaireated. The flow into the chamber of Plexiglas was always 1.0 mL/min (*29*). For the traditional rotating disk experiments a manual laboratory press from Perkin-Elmer (Waltham, MA, USA) with a stainless steel rotating disk die

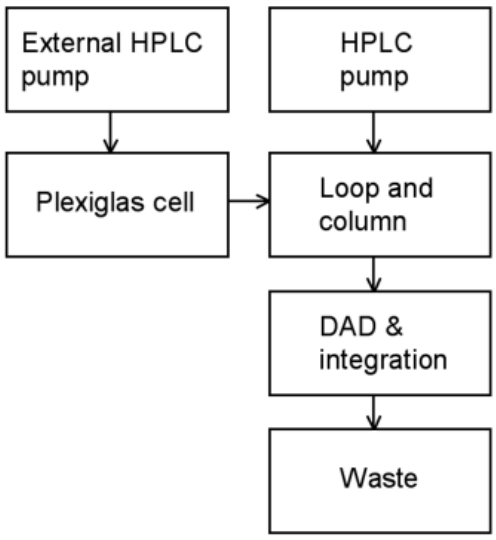


Figure 1. The setup for the miniaturized apparatus when measuring the *in vitro* dissolution rate, G. The sampling loop was positioned in the switching valve.

(Ø8 mm disk) similar to that proposed by Wood (*52*) was used. The making of the traditional, 195 MPa, and miniaturized, 182 MPa, disks were according to the previously described method in (*29*). Three disks in total of each drug substance were used in all experiments. The miniaturized disks of ciprofloxacin were always pre-wetted in the dissolution medium to remove any crust of substance above the disk surface that occurred by swelling. This was done to diminish the risk of breaking the Plexiglas cell due to obstruction of the peek tubing cf. (*29*). The effect on the dissolution rate value due to this pre-wetting of the ciprofloxacin disk was found to be negligible since the first run always was discarded during analysis to eliminate substance debris not firmly attached to the magnetic bar after compression. The magnetic bar with the attached ciprofloxacin disk was immediately placed in the cell after pre-wetting and the dissolution rate study was started.

For the traditional rotating disk equipment rotation speeds of 25 rpm, 50 rpm, 100 rpm and 150 rpm were used, whereas 100 rpm, 300 rpm, 500 rpm and 1000 rpm were applied for the miniaturized apparatus. When studying the correlation of log*G*, and logS, for all thirteen drug substances in the phosphate buffer, 300 rpm where used in the miniaturized apparatus. In order to improve the rotation robustness of the magnetic bar in the dissolution cell (with the flow of medium over the disk), 300 rpm was found to give more repeatable dissolution rate values compared to 100 rpm. The Plexiglas cell in this work was a newly constructed one and not the same as used in the previously study by (*29*), but with identical design and dimensions.

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 analysis by HPLC was described earlier in (*29*). The mobile phases were prepared so that the retention factor, k, was between 2.5 and 6.5. No analysis was longer than two min, which gives a total run time of less than three min. The analytical column was a Zorbax SB-C8 (2.1 × 50 mm, 5 µm) from Agilent Technologies Inc. The temperature of the column compartment was measured to 25-28°C and was constant within approximately 2°C in one experiment. The dissolution media were used at room temperature (21.0 ± 1.5°C). The external HPLC-pump was a Jasco PU-1585, Jasco Inc. (Tokyo, Japan). The data were collected by ChemStation Rev.A.10.02 from Hewlett Packard, Agilent Technologies Inc. A schematic view of the experimental setup can be found in Figure 1.

The system suitability criterion for the chromatographic system was set 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.

4. Results and Discussion

4.1. *Stability of drug substances and shake-time determination*

The stability of the drug substances (15 µM) was studied in different temperatures to optimize the shake-time and to confirm the repeatability of the solubility values, according to Figure 2. Shake-flask samples were also analyzed, but only in room temperature (see section 2.2 below).

As observed, 24 h can be used as shake-time in the phosphate buffer without noteworthy degradation of the drug substances at room temperature (ciprofloxacin at micro molar level is shown as an example in Figure 2). The percent remaining of the initial concentration was between 87% and 101% for all of the substances at room temperature at a maximum of 24 h, fulfilling the criterion set for stability.

The spectra in the diode array chromatograms during the stability study were investigated for extra peaks eluting prior to the main peak that could be related to degradation products. The spectrum of the main substance peak from an injected sample was also compared to the spectrum of the main peak from an injection of a newly prepared solution to confirm that no noteworthy chemical alterations had occurred. No changes of the main peak spectrum could be seen, thus it was assumed that the drug substance was stable during the study according to the set criterion, *i.e.* ± 15% in response area. Only a few small extra peaks could be detected in the chromatograms for some of the substances.

4.2. *Determination of apparent solubility using the shake-flask methodology*

The shake-flask studies were ended after 24 h, determined by the criterion set for degradation as discussed in section 2.1. The response peak areas were compared at each time-point to compare the percent remaining of the initial substance amount. The solubility of ciprofloxacin in the phosphate buffer at different shake times is presented in Figure 3. No difference in solubility was observed from 5 h to 2 days, whereas the degradation study showed a decreased stability beyond 24 h (Figure 2). Thus, 24 h was set as the shake-flask time in the determinations of the solubility.

The solubility results from the shake-flask experiments are summarized in Table 2. Six drug substances were tested in both ammonium acetate and phosphate buffer and further seven substances were tested in the phosphate buffer. The phosphate buffer had the best buffer capacity (β) (*53*), see Table 2, and it is also the buffer recommended as USP buffer by FIP. Acetate has been used to simulate fed state intestinal fluid (FeSSIF), while phosphate has been used in the fasted state simulated intestinal fluid (FaSSIF) (*54*) and acetate may have different impact on solubility of certain substances (*55*). ΔpH is the change in pH before addition of drug substance in the medium to the stop at 24 h in the shake-flask study.

As expected, the phosphate buffer showed the best buffer capacity at the chosen pH of approximately 7 since ΔpH in this study is consistently lower compared to in

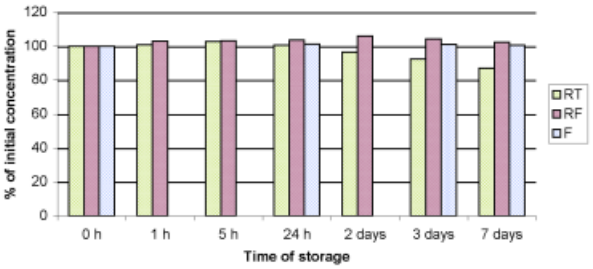


Figure 2. The stability study of ciprofloxacin (µM) in phosphate buffer. Stored at room temperature, RT, (21.0 ± 1.5°C), refrigerator, RF, (+4°C) and freezer, F, (-20°C).

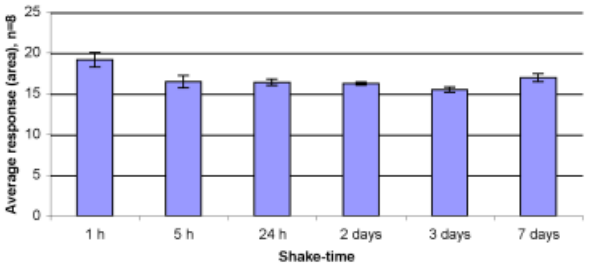


Figure 3. Determination of the shake-time of ciprofloxacin. The dissolution medium was phosphate buffer pH 7.0 at room temperature (21.0 ± 1.5°C). The main bars are the average area responses of four different samples using double injections (n = 8) at each time-point. The error bars show the standard deviation.

Table 2. The apparent solubility, *S*, measured after 24 h at room temperature (21.0 ± 1.5°C). *S*, is the average of eight values (four samples with double injections). RSD = relative standard deviation (n = 8). pH is the value after defined shake-time, 24 h, and ΔpH is the change in pH before addition of drug substance in the medium to the stop at 24 h. N/A = not applicable

Substance	Phosphate buffer pH 7.0 ± 0.1 (β = 0.0328 M)				Ammonium acetate buffer pH 6.8 ± 0.3 (β = 0.00021 M)			
	S (mM)	RSD (%)	pH 24 h	ΔpH	S (mM)	RSD (%)	pH 24 h	ΔpH
Naproxen	17	0.30	6.73	-0.41	1.6	7.5	5.30	-1.16
Ketoprofen	35	1.8	6.08	-1.02	4.5	3.9	4.80	-1.72
Furosemide	21	5.4	6.61	-0.53	1.6	1.8	5.24	-1.28
Carbamazepine	0.74	3.6	7.14	±0.00	0.79	3.2	6.40	-0.12
Nortriptyline HCl	2.8	11	7.10	-0.04	54	7.5	6.13	-0.42
Ciprofloxacin	0.22	9.2	7.16	+0.02	0.23	6.7	6.60	-0.13
Enalapril maleate	74	1.5	3.35	-3.75				
Bendroflumethiazide	0.34	5.2	7.15	+0.01				
Griseofulvin	0.13	4.5	7.14	+0.02				
Prednisone	0.76	16	7.10	+0.02		N/A		
Chlorprotixene HCl	0.92	32	6.80	-0.32				
Clomipramine HCl	2.4	2.2	7.10	-0.02				
Terfenadine	0.019	16	7.13	+0.05				

the ammonium acetate buffer. A significant difference in pH is achieved for enalapril maleate, which had a high solubility in the phosphate buffer. This high solubility significantly decreases the pH of the medium. A large excess was needed to reach equilibrium solubility, *i.e.* to maintain the excess of the drug substance throughout the experiment (24 h and beyond). The concentration of enalapril and maleate after 24 h is higher than the buffer capacity of the phosphate buffer. Since the lower acidic pK_a-value of enalapril is 2.99 and the pK_a-value of the protonated carboxylic group of maleate is 5.72 (*45*), this will generate the drastic decrease in pH as can be seen in Table 2. A noticeable decrease in pH is also observed for ketoprofen. The solubility of ketoprofen is 34.8 mM in the phosphate buffer, which is somewhat higher than the buffer capacity of the buffer. The acidic pK_a-value of 3.95 combined with the high concentration of ketoprofen will decrease the pH in the medium.

The apparent solubility, *S*, of the three monoprotic acids, *i.e.* naproxen, ketoprofen and furosemide, is approximately ten times lower in the ammonium acetate buffer compared to *S* in the phosphate buffer. From Table 2 it can be concluded that the ammonium acetate buffer has a much lower buffer capacity at a pH around 7. Thus a much larger decrease in pH after 24 h was found in the ammonium acetate buffer compared to in the phosphate buffer. By using Eq. 1 the solubility, *S*, can be calculated to be ten times lower when pH is decreased one unit provided that the ionic strength is constant.

4.3. *Dissolution rate determination with two types of rotating disk apparatuses*

Previous publications correlated the pH when determining the apparent solubility to the pH in the diffusion layer of a substance that dissolves in a dissolution medium (*30,56-59*). It can be assumed that pH of the medium in equilibrium with a solid drug, *i.e.* pH of a saturated solution, is an approximation of pH in the diffusion layer. This assumption was also used in this study, and no adjustment of pH was therefore made after the final shake-time of 24 h.

The average *in vitro* dissolution rates, *G*, for the six substances in the two buffers are presented in Table 3. for the traditional rotating disk studies and in Table 4 for the miniaturized rotating disk experiments. Four different rotation speeds were used for both apparatuses, 25-150 rpm for the traditional setup and 100-1000 rpm for the miniaturized setup. The RSD for the dissolution rate values in the studies were lower than 20% for all of the drug substances.

As has been found previously (*29*) a relative consistent ratio is found when dividing the dissolution rate values for the phosphate buffer with the values for the ammonium acetate buffer at the respective rotation speeds. The consistency of these ratios indicates that similar results of the two in-house constructed Plexiglas cells were achieved.

The high dissolution rate of nortriptyline HCl in both buffers is assumed to be a result of that the base is formulated as a salt. A difference between free bases or acids and their respective salt forms has been discussed by *e.g.* (*11,12*). The functional groups of drug substances might have different interactions with different buffer species (*9,60,61*). This may be seen for *e.g.* the drug substances containing weak carboxylic acids in Table 3 and 4. Problems associated with swelling of the disks of ciprofloxacin, cf. (*29*), were minimized by pre-wetting prior the miniaturized rotating disk study but this substance was still difficult to handle. In the traditional rotating disk equipment no extra preparation of the ciprofloxacin disk was performed preceding the dissolution rate study. The dissolution rate in the ammonium acetate buffer showed no clear trend at higher rotation speeds. The disks swelled in this apparatus as well and maybe this may explain the deviating trend of the influence of a change in flow pattern nearby the disk surface and the adjacent diffusion layer.

Table 3. Average *in vitro* dissolution rates, *G*, (*n* = 3). Six drug substances dissolved in two different buffer media using a traditional rotating disk apparatus

		Naproxen	Ketoprofen	Furosemide	Carbamazepine	Nortriptyline HCl	Ciprofloxacin
Phosphate buffer pH 7.0	G_25 (µg/s/cm^2)	2.7	6.7	4.5	0.26	24	0.042
	G_50 (µg/s/cm^2)	3.6	9.0	5.4	0.29	26	0.053
	G_100 (µg/s/cm^2)	5.4	12	8.4	0.35	31	0.081
	G_150 (µg/s/cm^2)	6.1	15	9.1	0.44	40	0.15
Ammonium acetate buffer pH 6.8	G_25 (µg/s/cm^2)	0.27	0.76	0.39	0.27	17	0.038
	G_50 (µg/s/cm^2)	0.35	1.1	0.62	0.35	22	0.039
	G_100 (µg/s/cm^2)	0.48	1.4	0.83	0.48	30	0.080
	G_150 (µg/s/cm^2)	0.59	1.7	0.86	0.49	35	0.080

Table 4. Average *in vitro* dissolution rates, *G*, (*n* = 3). Six drug substances dissolved in two different buffer media using the miniaturized rotating disk apparatus

		Naproxen	Ketoprofen	Furosemide	Carbamazepine	Nortriptyline HCl	Ciprofloxacin
Phosphate buffer pH 7.0	G_100 (µg/s/cm^2)	16	34	22	1.3	81	0.49
	G_300 (µg/s/cm^2)	21	40	26	1.8	106	0.63
	G_500 (µg/s/cm^2)	22	51	40	2.6	140	0.92
	G_1000 (µg/s/cm^2)	29	71	50	4.6	218	1.8
Ammonium acetate buffer pH 6.8	G_100 (µg/s/cm^2)	1.9	4.8	2.3	1.4	72	1.2
	G_300 (µg/s/cm^2)	2.5	6.0	3.2	1.8	95	1.7
	G_500 (µg/s/cm^2)	2.9	7.6	4.1	2.6	139	4.8
	G_1000 (µg/s/cm^2)	3.7	11	5.4	4.9	211	11

4.4. *Correlation of in vitro dissolution rate and apparent solubility*

To compare the new miniaturized rotating disk apparatus with traditional rotating disk equipment, six drug substances were tested in ammonium acetate and a phosphate buffer at four different rotation speeds, see Tables 3 and 4. The correlation of the logarithmic values of the *in vitro* dissolution rate, *G*, and the apparent solubility, *S*, (from Table 2) for both buffer media are presented in Figures 4 A-B and 5 A-B respectively. Triplicates of log*G* are shown in the figures, while average values are used for log*S*. The correlation of dissolution rate and solubility will for most substances be done in a pH interval of roughly 6.1 to 7.1 in the phosphate buffer and in the region of 4.8 to 6.8 in the ammonium acetate buffer, cf. Table 2.

The hydrochloride salt of nortriptyline is deviating from the more linear correlation pattern in the phosphate buffer for both rotating disk systems used. This can possibly be related to the fact that this is the only drug substance formulated as a salt during this study. The dissolution rates are found to be similar in the buffers, while the solubility is about twenty times higher in the ammonium acetate buffer compared to the phosphate buffer. This difference can partly be explained by the pH difference in the two buffers, which should result in a ten times higher solubility (cf. Eq. 2). Since the visual pattern of the correlations presented in Figures 4 and 5 are similar comparing the traditional and newly designed rotating disk equipments, only the miniaturized apparatus was used for further correlation studies in the phosphate buffer.

The correlation for all thirteen drug substances in the

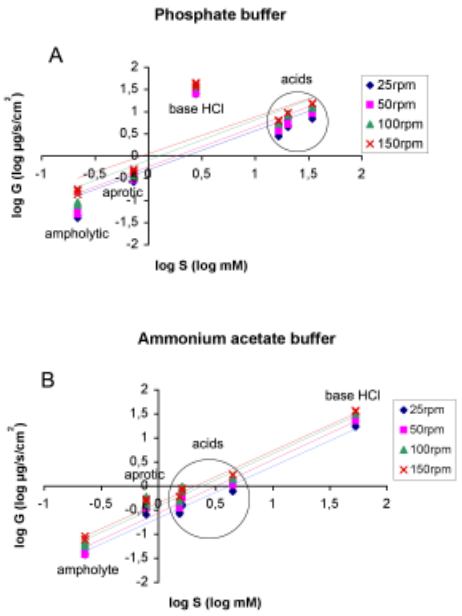


Figure 4. The correlation of the logarithmic average values of dissolution rate, *G*, and solubility, *S*, using the miniaturized rotating disk apparatus. The study was performed at 300 rpm (flow 1.0 mL/min) in phosphate buffer at the initial pH of 7.0 ± 0.1 at room temperature (21.0 ± 1.5°C) for thirteen different drug substances.

phosphate buffer (pH 7.0) at 300 rpm is shown in Figure 6. A good correlation (R^2 of 0.982, dashed line with the equation written in italic) was observed, provided that the substances formulated as hydrochloride salts were excluded from the series. All basic substances formulated as salts were found to depart from the more linear regression of the other substances tested. However, the free base terfenadine with a pK_a-value similar to chlomipramine is not deviating from the linear trend in Figure 6. Interestingly it seems that a linear correlation

is possible to achieve even though an assortment of different BCS substances are used in the correlation of *in vitro* dissolution rates and apparent solubility in aqueous buffer media. The ammonium acetate buffer gave a better correlation of the logarithmic dissolution rate and solubility values for the hydrochloride salt of nortriptyline, compared to the correlation found in the phosphate buffer. An optimization of the buffer composition might improve the possibility to obtaining a good correlation of dissolution rate and solubility for more substances, *e.g.* in early screening of solubility of drug candidates.

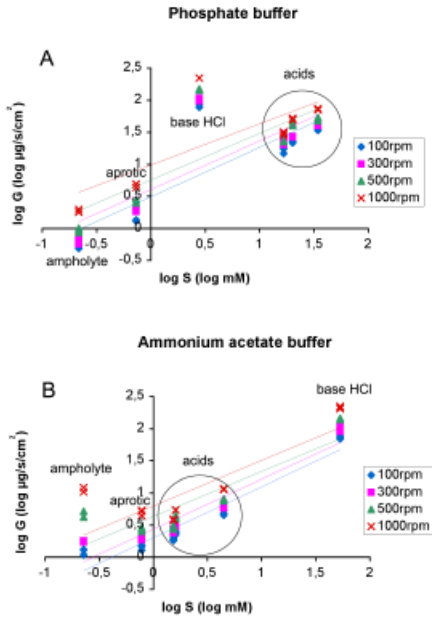


Figure 5. The correlation of the logarithmic values of the *in vitro* dissolution rate, *G*, and the solubility, *S*, using the miniaturized rotating disk apparatus. Four different rotation speeds and two dissolution media **A**. phosphate buffer pH 7.0 ± 0.1 and **B**. ammonium acetate buffer pH 6.8 ± 0.3 both at room temperature (21.0 ± 1.5°C).

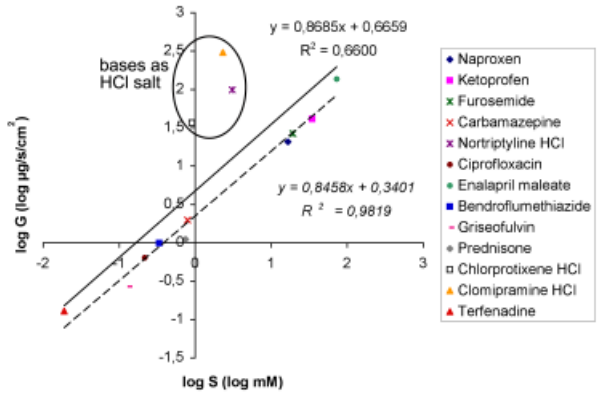


Figure 6. The correlation of the logarithmic average values of dissolution rate, *G*, and solubility, *S*, using the miniaturized rotating disk apparatus. The study was performed at 300 rpm (flow 1.0 mL/min) in phosphate buffer at the initial pH of 7.0 ± 0.1 at room temperature (21.0 ± 1.5°C) for thirteen different drug substances.

5. Comparison of the throughput for the two apparatuses

The applicability of the new miniaturized equipment is focused on low consumption of substance and dissolution media, while giving sufficient accurate values for screening studies of solubility in early drug development. The miniaturized equipment is also easy to use and it is straightforward to shift media during the experiments. The throughput of analyses for the two rotating disk apparatuses was compared during the dissolution rate studies, Table 5. Manual sampling was used in the traditional rotating disk determinations according to the previously described method (*29*).

By using the miniaturized equipment savings can be done in analysis time, amount of drug substances as well as dissolution media. If only one disk should be screened in one buffer using the miniaturized methodology, approximately 10 min is needed from making of the disk to finishing the chromatographic analysis. To speed up the screening further, more development work must be performed concerning the disk making. The reduction in volume of the dissolution medium can further be optimized by decreasing the void volume of the chromatographic system and by decreasing the total chromatographic analysis time (retention volume). This can *e.g.* be achieved by using ultra performance liquid chromatography (UPLC) or further miniaturization of the cell of Plexiglas.

6. Conclusions

The miniaturized apparatus for the *in vitro* dissolution rate is rapid compared to the traditional rotating disk method. Smaller amounts of samples and dissolution media are consumed by using the miniaturized system. The two rotating disk instruments were found to give similar logarithmic correlations of the *in vitro* dissolution rate versus the apparent solubility, as well as precision in the determinations. Consequently, the extended study of the correlation was only performed using the miniaturized apparatus. The logarithmic correlation of the

Table 5. Performance of the new miniaturized rotating disk equipment and the traditional rotating disk apparatus (using manual sampling)

Rotating disk apparatus	Total time for three disks at four different rotation speeds in both buffer systems per drug substance	Approximately amount of drug substance per disk	Approximately volume of dissolution medium per disk
Miniaturized	2 days	5 mg	35 mL
Traditional	8 days	100 mg	500 mL

dissolution rate and the solubility for ten drug substances in the phosphate buffer became as good as $R^2 = 0.982$, if the three substances formulated as hydrochloride salts were excluded. Interestingly, it seems that the correlation of dissolution rate and solubility for the hydrochloride salt of nortriptyline is not deviating from other drug substances in the ammonium acetate buffer. This correlation might be of interest to investigate, especially if the discrepancy is valid for other media and other salts formulations than hydrochlorides. A linear correlation by the use of an optimized medium for all types of substances might accordingly be used to estimate the apparent solubility from the *in vitro* dissolution rate in early screening.

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