Physico-Chemical Characterization of Drugs: Acidity and Solubility

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CHAPTER 3

Solubility-*pH* profiles of some acidic, basic and amphoteric drugs

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3.1 INTRODUCTION

Solubility is one of the most important parameters in drug development processes because, to be orally well absorbed, a compound should be soluble in aqueous medium [1-3], or be prepared in an appropriate formulation to overcome solubility limitations. Solubility and dissolution studies under variation of *pH* are essential *in-vitro* experiments to mimic behavior under physiological conditions. Most drugs exhibit strong *pH*-dependence on these parameters if drug ionisation constants (pK_a) are in the physiological relevant *pH*-range, 1-7. This may also be accompanied by a change in the respective solid-state form. Whether certain acidic or basic drugs would form salts and, if salts are formed, how easily they would dissociate back into their free acid or base forms depends on interrelationships of several factors, such as S_o (intrinsic solubility), *pH*, pK_a, K_{sp} (solubility product) and pH_{max} (*pH* of maximum solubility) [4]. This explains at which *pH* solubility will be governed by solubility of the parent (free acid or base) and at which *pH* solubility of a salt form becomes crucial. The concept of *pH*-dependent solubility and dissolution behavior was further progressed to mimic biorelevant media, with media like FaSSIF (Fasted State Simulated Intestinal Fluid) and FeSSIF (Fed State Simulated Intestinal Fluid) being introduced [5-7]. The scope of the biorelevant media approach is to also reflect the solubilization effect of *in-vivo* relevant surfactants such as bile salts and lecithine, as well as prediction of potential food-effects resulting from this.

There is a large list of methods to measure the solubility [8-14], being the classical one the shake-flask (S-F) method [8]. This method is based on the measurement of the concentration of the compound of interest in a saturated solution. To reach the equilibrium, there is a shaking step that can last from few hours to 72 hours or more, followed by an equilibration step, normally of 24 hours. Then the amount of drug in solution is measured and that concentration provides the solubility of the compound. A particularity of ionisable substances is that they show different solubility values depending on their acidity constants (pK_a in its logarithmic form) and the *pH* of the medium. Therefore, solubility-*pH* profiles are essential to predict the behaviour and bioavailability of these compounds. To obtain such profiles experimentally by S-F

method, several buffered solutions must be prepared at different *pH* values. Then the drug is dissolved until saturation in the buffered aqueous solution, and after a period of shaking and equilibration with the solid, the concentration of drug in the saturated solution is determined. The *pH* of the saturated solution is measured, and the solubility-*pH* plots can be achieved.

Another relevant approach, the potentiometric method developed by Sirius Analytical Ltd., was described in chapter 2 (section 2.1.1.2) and it offers an alternative to the S-F method to measure the solubility of ionizable compounds [13, 15]. It requires much less time than the S-F method to obtain a reliable intrinsic solubility value. It should be recalled here that the term intrinsic solubility refers to the equilibrium solubility of the free acid or base form of an ionizable compound at a *pH* where it is fully unionized, S_o [13] as it is already defined in section 2.1.1.1 (Chapter 2). Solubility-*pH* profiles can be also determined potentiometrically [16, 17], however the CheqSol method provides only a calculated profile based on the direct application of Henderson-Hasselbalch (H-H) equations.

Henderson-Hasselbalch equations are easily derived from the equilibrium processes for a given system, and for a monoprotic compound, allow the calculation of the profile by means of only two experimental determinations: the pK_a of the compound and its intrinsic solubility, S_o. Polyprotic systems require more elaborated equations, so that different modalities of the H-H equation must be used depending on the nature and complexity of the compounds. H-H equations have been clearly summarized by Avdeef in a comprehensive review [18]. However, these equations do not stand for complex processes that often can occur in the frame of the precipitation, as for example aggregation or salt co-precipitation.

The purpose of the work described in this chapter is the critical discussion of several aspects of the determination of solubility-*pH* profiles by means of S-F and potentiometric methods. To this end, five compounds of different nature (a monoprotic acid, a monoprotic base, a diprotic base, and two amphiprotic compounds showing each one a zwitterionic species) have been selected. Their solubility-*pH*

profiles have been determined experimentally through the S-F method using buffered solutions at different *pH* values and constant ionic strength. Some practical aspects of the S-F method have been evaluated, and also the effect of the different buffer components on the solubility values obtained. In addition, solubility-*pH* profiles achieved from the data obtained by both experimental methods (S-F and potentiometric) are discussed. Although the two methods should be equivalent, the experimental conditions (*pH* and pK_a scales and ionic strength) in which measurements are performed are slightly different, and, then, these factors have been also evaluated.

3.2 EXPERIMENTAL

3.2.1 Instruments

3.2.1.1 Shake-Flask method for solubility determination

All *pH* measurements were taken with a Ross Combination electrode Orion 8102 from Thermo Fisher Scientific (Waltham, MA, USA) in a Crison micro-*pH* 2002 potentiometer with a precision of ±0.1 mV (±0.002 *pH* units) from Crison Instruments S.A. (Alella, Spain). A Movil-RoD from Selecta (Abrera, Spain) rotational stirrer was used to shake the samples. The concentration in the supernatant was quantified by liquid chromatography, using a Shimadzu (Duisburg, Germany) liquid chromatograph, equipped with two Shimadzu LC-10AD pumps, and a Shimadzu SPD-10AV detector. Temperature was controlled at 25.0 ± 0.1°C with a Shimadzu CTO-10AS column oven. The reversed phase HPLC measurements were carried out on a 5 µm XTerra RP C_{18} column, with dimensions of 50 x 4.6 mm from Waters (Milford, MA, USA) [4].

3.2.1.2 CheqSol method for pK_a and solubility determination

The apparatus used to perform the solubility determinations was a PCA200 from Sirius Analytical Instruments Ltd. (Forest Row, UK), equipped with a Sirius *D-PAS* spectrometer, described in Chapter 2 (section 2.1.1.3).

3.2.1.3. MS and MS/MS measurements

An ESI-Q-TOF-MS/MS instrument (Q-Star) from Applied Biosystems (Foster City, CA, USA) was used for the MS and MS/MS experiments. The instrument was calibrated for exact mass calculation in positive mode with reserpine (*m/z* 195.0651 and 609.2812). The settings were: ion spray voltage 4500 V, ion source gas (N_2) 40 arbitrary units

(a.u.), curtain gas (N_2) 40 a.u., declustering potential (DP) 10 V, and focusing potential 380 V. The settings for MS/MS experiments were: collision gas (N_2) 10 a.u., and collision energy (CE) 13 V (monomer), 27 V (dimer), and 22 V (trimer). Direct infusion of the sample was performed. Analyst QS 2.0 software from Applied Biosystems was used for data acquisition and processing.

3.2.2 Reagents and solvents

- Hydrochloric acid 0.5 M, Merck, Titrisol®, 99.5%
- Potassium hydroxide 0.5 M, Merck, Titrisol®, 99.5%
- Sodium hydroxide 0.5 M, Merck, Titrisol®, 99.5%
- Potassium chloride, Merck, > 99.5%
- Potassium biphtalate, Merck, > 99.8%
- Buffer solution *pH* 7.00 (KH_2PO_4 and $Na_2HPO_4.2H_2O$), Crison
- Sodium hydrogenphosphate monohydrate, 99.5%
- Lactic acid, J. T. Baker (Deventer, Holland), 90%
- Sodium dihydrogenphosphate monohydrate, Merck, 99%
- Sodium citrate dehydrate, Merck, 99%
- Sodium acetate, Merck, >99%
- Ammonium chloride, Merck, 99.8%
- Sodium phosphate dodecahydrate, Aldrich (Milwaukee, WI, USA), >98%
- Sodium phosphate dodecahydrate, Aldrich (Milwaukee, WI, USA), >98%
- Potassium dihydrogencitrate, Fluka, 99%
- Citric acid, Fluka, 99.5%
- Sodium tetraborate decahydrate, Sigma St Louis, MO, USA), 99.5%
- Methanol, Merck, HPLC grade
- Water purified by a Milli-Q plus system from Millipore (Bedford, MA, USA), with resistance higher than 18 MΩ.

3.2.3 Studied substances

- Propylparaben, Fluka (Buchs, Switzerland) > 99.5%
- Acebutolol hydrochloride (analytical grade), Fluka (Buchs, Switzerland) > 99.5%
- Sulfadimethoxine, Fluka (Buchs, Switzerland) > 98.5%
- Cefadroxil (analytical grade), Fluka (Buchs, Switzerland) > 99.5%
- Quetiapine hydrogenfumarate was from AstraZeneca (Madrid, Spain)

3.2.4 Procedures

3.2.4.1 Determination of pK_a by potentiometry

Experiments were carried out with the PCA200 apparatus. For acebutolol and cefadroxil, a given amount of compound (5 mg) was dissolved in 15 mL of a 0.15 M KCl aqueous solution. The sample was preacidified to *pH* 1.8 with 0.5 M standardized HCl. Then it was titrated with standardized 0.5 M KOH. All titrations were carried out at 0.16 M ionic strength and a temperature of 25°C, under nitrogen atmosphere. A minimum of three measurements for each compound were carried out, and the pK_a values were calculated through the RefinementPro software.

3.2.4.2. Determination of pK_a by spectrophotometry

The determination of the pK_a values of sulfadimethoxine was carried out by spectrophotometry. These experiments were also carried out with the PCA200 apparatus as described in Chapter 2 (2.2.1.4.4).

3.2.4.3. Determination of solubility by the Shake-Flask method

In order to obtain the solubility-*pH* profiles and determine S_o for each compound, different buffer solutions of 0.1 M ionic strength covering a wide *pH* range were prepared. For monoprotic buffering electrolytes and phosphoric acid/dihydrogen phosphate buffer, the desired *pH* and ionic strength were obtained as follows: a given amount of a stock solution of the basic or acidic form of the electrolyte was added to a volumetric flask and neutralized with 0.5 M HCl or 0.5 M NaOH, respectively, up to the desired *pH*, before diluting to the volumetric mark. In case of di- or triprotic electrolytes, the buffers were prepared mixing stock solutions of the acidic and the basic form of the electrolyte in the adequate proportion in a volumetric flask, and then, adding water up to the volumetric mark. Table 3.2.1 shows the constituents, working *pH* range, stock solutions, and buffering capacity of the different buffer solutions employed.

Table 3.2.1: Buffer solutions used in the Shake-Flask method.

Buffer constituents	pK_a	Working *pH* range	Stock solutions[a]	β_{min}-β_{max}
$H_3PO_4/H_2PO_4^-$	2.15	2.00 – 3.00	0.25 M NaH_2PO_4 + 0.5 M HCl	0.02-0.08
H_3Cit/H_2Cit^-	3.13	2.50 – 3.50	0.5 M H_3Cit + 0.5 M KH_2Cit	0.07-0.20
HFor/For$^-$	3.75	2.75 – 3.75	0.2 M HCOONa + 0.5 M HCl	0.02-0.06
HLac/Lac$^-$	3.86	2.86 – 4.86	0.2 M $CH_3CHOHCOONa$ + 0.5 M HCl	0.02-0.06
HAc/Ac$^-$	4.76	3.70 - 5.80	0.2 M CH_3COONa + 0.5 M HCl	0.02-0.06
$H_2Cit^-/HCit^{2-}$	4.76	3.80 – 5.60	0.5 M KH_2Cit + 0.2 M Na_3Cit	0.02-0.07
$HCit^{2-}/Cit^{3-}$	6.4	5.40 - 7.00	0.5 M KH_2Cit + 0.2 M Na_3Cit	0.005-0.02
$H_2PO_4^-/HPO_4^{2-}$	7.21	6.00 - 8.00	0.2 M $NaH_2PO_4 \cdot H_2O$ + 0.2 M Na_2HPO_4	0.005-0.03
NH_4^+/NH_3	9.25	8.20 - 10.20	0.2 M NH_4Cl + 0.5 M NaOH	0.02-0.06
$H_3BO_3/H_2BO_3^-$	9.5	8.50 - 10.10	0.2 M H_2BO_3Na + 0.5 M HCl	0.02-0.06
HPO_4^{2-}/PO_4^{3-}	12.32	10.80 - 12.00	0.2 M $Na_3PO_4 \cdot 12H_2O$ + 0.2 M Na_2HPO_4	0.02-0.05

[a]Different amounts of the given stock solutions are mixed and diluted to obtain the desired *pH* (within the working *pH* range) and 100 mM ionic strength.

For each compound, three aliquots were prepared at each *pH* value adding a given amount of solid compound (enough to obtain saturated solutions) to 3 mL of the buffered solution. Then the three aliquots were stirred at controlled temperature (25 ± 0.2)°C for 24, 48 and 72 hours respectively. In this way it was possible to check whether equilibrium was reached or not. After the corresponding stirring time, samples were equilibrated for 24 hours, and then the supernatant was filtered through

Millex–LH membrane 0.45 μm porous size filters from Millipore. The *pH* of the supernatant solutions was then measured. The concentration of substance in the supernatant was determined by liquid chromatography. To obtain reasonable retention times for all compounds, two different mobile phases in isocratic mode were used: acetonitrile/0.01 M phosphate buffer (*pH*=7) (30/70) for propylparaben, acebutolol and cefadroxil, and acetonitrile/0.01 M acetate buffer (*pH* 4) (30/70) for quetiapine and sulfadimethoxine. Flow rate was 1 mL/min, the injection volume was 10 μL, and the detection wavelengths 190 nm for propylparaben, acebutolol and quetiapine, and 197 nm for sulfadimethoxine and cefadroxil. In order to quantify, five standard solutions were prepared in the linear range in an acetonitrile/water (30/70) mixture for each compound. Linearity of the calibration curve was checked and the limit of quantification determined. In a first attempt, supernatants were injected directly, and when necessary, they were appropriately diluted in water in order to make them fit in the calibration curve.

3.2.4.4. Determination of the intrinsic solubility by the potentiometric method

Suitable amounts of samples were accurately weighted into the titration vessel and 10 mL of 0.15 M KCl solution were added. The procedure was explained in Chapter 2 (2.1.1.2).

3.3 RESULTS & DISCUSSION

3.3.1. Some considerations on Shake-Flask solubility determination

There are some practical aspects that must be remarked when the S-F method is used to obtain solubility-*pH* profiles. Thus, it is illustrative to consider the solubility results obtained for cefadroxil by means of the S-F method, which are given in Table 3.3.1. It shows the electrolyte used to prepare the buffer solution, the initial *pH* of the buffer, the stirring time of the aliquot, the *pH* of the supernatant after the equilibration step (equilibrium *pH*), the *pH* difference between the initial buffer and the supernatant (ΔpH) and also the concentration of compound in the supernatant (solubility) with the corresponding statistics. The first point to remark is the significant change in *pH* for several buffers. Note that in some occasions, such as for boric acid or ammonium buffers, there are up to 2 *pH* units of difference between the initial *pH* and the equilibrium *pH*. The reason for the *pH* shifts is related to the pK_a values of the compound and the solubility of its charged species. Because of the ionic species are, usually, more soluble than the neutral or zwitterionic ones, at the *pH* values where the compound is highly soluble it can react with the proton showing its own buffering ability. The more compound in the solution the more significant its buffering effect is. Cefadroxil shows three pK_a values (2.52, 7.65, and 10.05) corresponding to a carboxylic acid, a protonated primary amine and a phenol, respectively, and, according to them, its zwitterionic form should predominate in the 3.5-6.6 equilibrium *pH* range. Since the zwitterionic species shows the lower solubility, the difference between the initial and equilibrium *pH* should be almost negligible in this *pH* range, but, unexpectedly, it increases from equilibrium *pH* about 5. This is due to the incipient aggregation of the monoanionic species increasing in this way the compound solubility. The presence of these very soluble aggregate species will be discussed in detail below. Out of this *pH* range, at lower *pH* values the cationic species increases and at higher *pH* cefadroxil shows its anionic forms. In both instances, the solubility increases. Thus, as more cefadroxil is solubilized, its buffering effect is more evident, shifting the initial *pH*

according to the amount of solved compound. As expected, when buffers with initial *pH* lower than 3 are used positive *pH* variations are observed whereas buffers with initial *pH* higher than 5 show the opposite behaviour. Similar *pH* shifts were already reported for a wide series of drugs containing an amino group and buffered by phosphate [19].

Another key point is to know whether the equilibrium has been achieved or not. In order to know that, three aliquots were prepared for each initial *pH* value, and different stirring times were applied to each aliquot (24, 48, and 72 hours, respectively). In case equilibrium is achieved in 24 hours of stirring, it is expected that the final *pH* would be the same for the three aliquots. However, small *pH* variations are observed, especially where the difference between the initial and the final *pH* is higher (initial *pH* from 6.35 to 9.31). In these cases there is a clear trend, being the difference higher when the stirring time increases, showing the equilibrium has not been reached neither after 24h nor 48h of stirring and some cefadroxil is still being solubilized after this time. In this work the maximum stirring time was 72 hours. It cannot be said that after this time equilibrium is achieved in all instances, since the *pH* difference does not always converge to a constant value. However, when solubility values determined through the three aliquots are averaged, the variance coefficient obtained is low, always below 5%. This means that even when equilibrium has not been totally achieved after 24h shaking, this period of time could be taken as an appropriate shaking time, without making an important error in the determination of solubility. The measurement of the *pH* after the equilibration time is of main importance, since this *pH* value is the one that must be correlated in the profiles, and can be significantly different from the initial *pH*. In addition, the difference between the initial and the final *pH* can act as indicator to know whether equilibrium has been reached or not, if different stirring times are tried in the experiment. These two factors must be taken into account when using the S-F method, especially to obtain solubility–*pH* profiles. It should be noticed that the results achieved from measurements with buffers prepared with citric and lactic acids are omitted in Table 3.3.1 because of the resulting anomalous results as explained below.

Table 3.3.1 Experimental determination of the solubility - pH profile of cefadroxil by the Shake-Flask method.

Buffer	Initial pH	Stirring time (h)	Equilibrium pH	ΔpH	Solubility (mg/mL)	Average (mg/mL)	SD (mg/mL)	Coeff. of variation (%)
$H_3PO_4/H_2PO_4^-$	2.61	24	3.14	0.53	10.13			
$H_3PO_4/H_2PO_4^-$	2.61	48	3.03	0.42	17.02			
$H_3PO_4/H_2PO_4^-$	2.61	72	3.11	0.50	16.03	16.40	0.55	3.34
$HFor/For^-$	2.77	24	3.01	0.24	18.47			
$HFor/For^-$	2.77	48	2.95	0.19	18.16			
$HFor/For^-$	2.77	72	3.00	0.23	17.56	18.06	0.46	2.56
$HFor/For^-$	3.39	24	3.40	0.01	15.18			
$HFor/For^-$	3.39	48	3.36	-0.03	15.08			
$HFor/For^-$	3.39	72	3.38	-0.01	14.85	15.03	0.17	1.13
HAc/Ac^-	3.73	24	3.70	-0.02	14.34			
HAc/Ac^-	3.73	48	3.67	-0.06	15.20			
HAc/Ac^-	3.73	72	3.67	-0.05	14.42	14.65	0.48	3.26
HAc/Ac^-	4.87	24	4.74	-0.13	13.59			
HAc/Ac^-	4.87	48	4.64	-0.23	12.80			
HAc/Ac^-	4.87	72	4.70	-0.17	13.08	13.16	0.40	3.04
$H_2PO_4^-/HPO_4^{2-}$	6.35	24	5.34	-1.02	13.00			
$H_2PO_4^-/HPO_4^{2-}$	6.35	48	5.14	-1.22	12.43			
$H_2PO_4^-/HPO_4^{2-}$	6.35	72	4.96	-1.40	11.88	12.44	0.56	4.50
$H_2PO_4^-/HPO_4^{2-}$	6.87	24	5.92	-0.95	13.56			
$H_2PO_4^-/HPO_4^{2-}$	6.87	48	5.83	-1.04	13.76			
$H_2PO_4^-/HPO_4^{2-}$	6.87	72	5.57	-1.31	13.16	13.49	0.31	2.27
$H_2PO_4^-/HPO_4^{2-}$	7.86	24	6.23	-1.63	14.42			
$H_2PO_4^-/HPO_4^{2-}$	7.86	48	6.12	-1.74	14.79			
$H_2PO_4^-/HPO_4^{2-}$	7.86	72	5.84	-2.02	14.07	14.43	0.36	2.48
$H_3BO_3/H_2BO_3^-$	9.07	24	7.08	-1.99	35.88			
$H_3BO_3/H_2BO_3^-$	9.07	48	6.86	-2.21	34.45			
$H_3BO_3/H_2BO_3^-$	9.07	72	6.74	-2.33	35.51	35.28	0.74	2.10
NH_4^+/NH_3	9.31	24	7.05	-2.26	29.39			
NH_4^+/NH_3	9.31	48	6.96	-2.35	30.06			
NH_4^+/NH_3	9.31	72	6.63	-2.68	29.47	29.64	0.37	1.23

3.3.2. Solubility–*pH* profiles

Five compounds belonging to different chemical and therapeutical families and with different ionization patterns have been chosen as model compounds. For each compound, the suitable Henderson-Hasselbalch equation or a derived expression has been fitted to plot solubility vs. *pH* by using the S-F data. CheqSol profiles are also directly obtained from the RefinementPro software. The two resulting profiles have been compared: the ones obtained fitting the solubility data determined from the S-F method to the corresponding equation, and those derived from the intrinsic solubility, S_o, and pK_a values obtained, independently, by potentiometry.

At this point it is important to remark that experimental conditions are not the same in both approaches. Whereas all S-F experiments have been carried out at a constant ionic strength of 0.1 M achieved from the buffer concentration, potentiometric measurements have been performed at 0.15 M ionic strength due to the addition of KCl. Moreover, different *pH* and pK_a scales are used for each method. Thus, in the S-F method, *pH* is measured in the activity scale (activity of the proton), and the pK_a' quantity (the acidity constant which includes the contribution of hydrogen ion expressed in activity but those of the other species in concentration) is currently involved in the calculations. However, in the profiles obtained by the RefinementPro software (CheqSol method) both parameters are in the concentration scale (p_cH and pK_a^c) due to operational requirements, which is something users must be aware of. Table 3.3.2 shows the solubility data as determined by the S-F method for the five studied drugs. More complete data, i.e buffer used, the initial and equilibrium *pH*, standard deviation and coefficient of variation are given in the Appendix (Tables (3-1A)-(3-5A)).

Table 3.3.2: Solubility–*pH* profiles data of the studied compounds in the Shake-Flask method.

Propylparaben		Acebutolol		Quetiapine		Sulfadimethoxine		Cefadroxil	
pH	**log S (mol/L)**	***pH***	**log S (mol/L)**	***pH***	**log S (mol/L)**	***pH***	**log S (mol/L)**	***pH***	**log S (mol/L)**
2.53	-2.66	9.15	-2.08	3.67	-1.55	1.89	-3.92	2.81	-1.0
2.55	-2.67	9.29	-2.27	3.69	-1.57	2.08	-3.99	2.98	-1.1
2.55	-2.72	9.35	-2.37	3.82	-1.62	2.37	-4.09	3.00	-1.3
2.56	-2.82	9.37	-2.34	3.88	-1.70	2.50	-4.12	3.07	-1.2
2.67	-2.79	9.60	-2.44	4.02	-1.73	2.54	-4.10	3.11	-1.3
2.70	-2.72	9.63	-2.47	4.03	-1.70	2.54	-3.97	3.19	-1.2
2.76	-2.68	9.68	-2.52	4.87	-1.94	2.70	-4.15	3.36	-1.2
3.01	-2.73	9.72	-2.54	5.77	-2.04	2.73	-4.07	3.38	-1.4
3.21	-2.72	9.76	-2.61	6.31	-2.13	2.87	-4.20	3.47	-1.3
3.43	-2.73	9.86	-2.62	7.04	-2.56	3.03	-4.21	3.63	-1.3
3.58	-2.78	9.972	-2.65	7.16	-2.64	3.16	-4.19	3.67	-1.4
4.01	-2.75	10.26	-2.69	8.76	-2.91	3.27	-4.20	4.13	-1.4
4.03	-2.74	10.94	-2.77	9.06	-2.96	3.49	-4.24	4.59	-1.4
4.55	-2.75	11.46	-2.78	9.79	-2.88	3.75	-4.22	4.70	-1.4
4.73	-2.78	11.86	-2.77	9.98	-2.89	4.00	-4.24	4.81	-1.4
4.92	-2.75			11.30	-2.82	4.30	-4.22	4.96	-1.5
5.00	-2.74					4.51	-4.23	5.43	-1.4
5.48	-2.78					4.75	-4.19	5.57	-1.4
5.52	-2.78					5.21	-4.20	5.84	-1.4
6.04	-2.77					5.23	-4.16	5.95	-1.4
6.65	-2.77					5.23	-4.15	6.31	-1.4
6.69	-2.77					5.79	-4.07	6.38	-1.3
6.99	-2.76					5.96	-3.96	6.50	-1.2
7.57	-2.70					6.02	-3.97	6.52	-1.2
8.70	-2.15					6.37	-3.74	6.59	-1.2
8.81	-1.98					6.55	-3.60	6.63	-1.1
9.27	-1.70					6.64	-3.53	6.74	-1.0
9.36	-1.54					6.74	-3.44		
						6.87	-3.28		
						6.93	-3.30		
						7.28	-2.98		
						7.37	-2.88		
						7.99	-2.26		
						8.15	-2.08		
						8.56	-1.65		
						8.74	-1.46		

3.3.2.1 Propylparaben

Propylparaben is a monoprotic neutral acid (HA) with a pK_a value of 8.41. Therefore, the solubility should not change with *pH* until *pH* value close to pK_a, when ($pH = pK_a$-1). In this point the ionic species begins to be significant and the solubility increases. This behaviour can be observed in Fig. 3.3.1 where the experimental log S value has been plotted *versus pH* values. The solubility obtained at *pH* values lower than (pK_a-1) corresponds to the intrinsic solubility. The experimental points have been fitting to the H-H relationship derived for a monoprotic neutral acid [18] (Eq. 3.3.1):

$$\log S = \log S_o + \log(10^{pH-pK_a'} + 1) \qquad [3.3.1]$$

The obtained profile is shown in Fig. 3.3.1 as a solid line. From this fit, the pK_a' and $\log S_o$ have been calculated and given in Table 3.3.3, which also shows the values obtained by the potentiometric method (pK_a^c and $\log S_o$, Chapter 2). The $\log S_o$ and thermodynamic pK_a values obtained from each approach agree. A solubility profile can also be obtained from the potentiometric data (pK_a^c and $\log S_o$) if a suitable H-H equation is used, Eq 3.3.2:

$$\log S = \log S_o + \log(10^{p_cH-pK_a^c} + 1) \qquad [3.3.2]$$

The obtained solubility profile from potentiometric values and Eq. 3.3.2 is also shown in Fig. 3.3.1 as a dashed line. Both lines of S-F and CheqSol profiles, have been plotted vs. an unique *pH* axis to facilitate their comparison. Note that the difference between Eq. 3.3.1 and Eq. 3.3.2 is the scale of *pH* and pK_a used.

Since (pH-pK_a') = (p_cH-pK_a^c), both solubility-*pH* profiles should overlap when the appropriate *pH* scale is taken in the *pH* axis. The difference between both *pH* scales is the logarithm of the activity coefficient of the hydrogen ion, which in aqueous solution

at 25°C and ionic strength 0.15 M is -0.13, as calculated by the Debye-Hückel approach. The eventual differences between the solubility-*pH* profiles come from the difference in S_o values evaluated by each method and also the difference between the potentiometric and fitting pK_a values. When the *pH* is higher than the pK_a, solubility increases sharply with slope equal to 1 showing the higher solubility of the ionized species.

As regards to the fit of the experimental S-F points to Eq. 3.3.1 (solid line), the solubility values obtained do not depend on the buffer used for their determination. However, small deviations showing enhanced solubility are observed for some points at *pH* values lower than 3.5, which are buffered by citric or lactic acids. It is well known that these acids show hydrotrophic properties and they are able, in acidic solution, to interact with some drugs enhancing in this way the apparent drug solubility. Similar behaviour for hydrochlorotiazide [20] and for morphine [21] was observed when citric acid was used as the buffer, and for terfenadine buffered with lactic acid [9]. For solutions buffered by phosphoric acid, the opposite effect occurs, and the solubility is lower than the expected one as described also for celecoxib [22] and for hydrochlorothiazide which show lower solubility when the acidic solution is buffered with phosphate (Briton-Robinson or Sörensen I buffers) than when a citrate based buffer (Sörensen II) is used [20]. Due to the anomalous effect on solubility of citric, lactic and phosphoric buffers at *pH* lower than 3, the corresponding experimental points have been deleted to fit Eq. 3.3.1 as it can be seen in Table 3.3.3, when these points are removed, the fitting parameters obtained are very good, and the thermodynamic pK_a is consistent with the one obtained by the potentiometric method or the literature one. These results suggest again the solubility enhancing effect of the citric and lactic buffers and the solubility lowering effect of phosphate buffer on propylparaben.

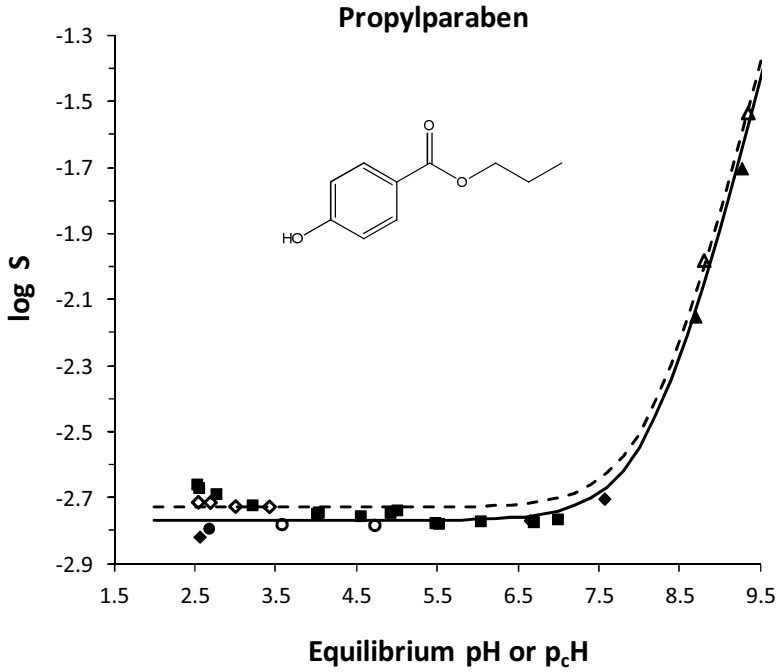


Figure 3.3.1 Solubility–*pH* profiles for propylparaben. Buffers used: ◆ phosphate, ● formate, ■ citrate, ○ acetate, ◇ lactate, △ ammonium, ▲ borate. Solid line: fit of S-F data to H-H equation. Dashed line: profile obtained through the CheqSol method.

Table 3.3.3 pK_a and intrinsic solubility, log S_O values, of the studied compounds determined by the Shake-Flask and the Potentiometric methods.

	Shake-Flask method							Potentiometric method			
Compound	pK_a' **(I = 0.1M)**	pK_a **(I = 0)**	**log S_o**	**r^2**	**s**	**F**	**Fitting Equation**	pK_a^c **(I = 0.15M)**	pK_a **(I = 0)**	**log S_o**	pK_a **(Lit.)**[a]
Propylparaben	8.18 ± 0.02	8.29	-2.77 ± 0.01	0.995	0.03	3205	3.3.1	8.16[b]	8.41	-2.73[b]	8.14
Acebutolol	9.59 ± 0.02	9.48	-2.78 ± 0.01	0.991	0.02	968	3.3.3	9.48 ± 0.01	9.48	-2.70 ± 0.04	9.41
Quetiapine	3.89 ± 0.04 7.01 ± 0.06	3.56 6.90	-1.96 ± 0.02[e] -2.89 ± 0.02	0.965 0.975	0.03 0.05	279 238	3.3.7 3.3.6	3.57[c] 6.97[c]	3.31[c] 6.97[c]	-3.03	3.56[d] 6.83[d]
Sulfadimethoxine	1.95 ± 0.04 6.01 ± 0.01	1.84 6.12	-4.24 ± 0.01	0.999	0.02	18041	3.3.8	1.73 ± 0.01 5.88 ± 0.04	1.73 6.21	-4.20 ± 0.04	-- 6.10
Cefadroxil	2.60 ± 0.13 6.63 ± 0.05	2.49 6.74	-1.45 ± 0.02	0.892	0.04	70	3.3.8	2.52 ± 0.04 7.40 ± 0.01 9.55 ± 0.01	2.52 7.65 10.05	-1.35 ± 0.06	2.70 7.22 ---

[a] (Bio-loom) [23]; [b][24]; [c][25]; [d][26] at 37°C; [e]log S value.

3.3.2.2 Acebutolol hydrochloride

Fig. 3.3.2 shows the plot of experimental log S values *versus pH* for acebutolol hydrochloride. Because this compound is a monoprotic base (BH^+) with an amino group of pK_a 9.48 (see Table 3.3.3) the logS decreases with the *pH* until neutral species is predominant. From this moment the logS remains constant with the increase of *pH* and the intrinsic solubility value is obtained. The experimental points have been fitting to the H-H equation corresponding to a monoprotic base [18] (Eq. 3.3.3).

$$\log S = \log S_o + \log(10^{pK_a' - pH} + 1) \qquad [3.3.3]$$

From this fit, represented as the solid line in Fig. 3.3.2, pK_a' and $\log S_o$ are obtained. They are shown in Table 3.3.3 as well as those obtained by potentiometry. The thermodynamic pK_a values obtained by the two methods agree. However, the difference between the intrinsic solubility determined by both methods is slightly higher than the one for propylparaben, although it is still very small. Fig. 3.3.2 also shows (dashed line) the solubility profile obtained from the pK_a' and $\log S_o$ values obtained from the potentiometric method and Eq. 3.3.4.

$$\log S = \log S_o + \log(10^{pK_a^c - p_cH} + 1) \qquad [3.3.4]$$

As explained for propylparaben, the two solubility profiles should overlap if each curve is referred to the suitable *pH* scale. The observed difference in the flat region (area where the neutral form of the base predominates) is due to the difference in S_o values determined by both methods. Very nice consistency is observed between the H-H equations and the experimental points in the *pH* range where the ionic form of acebutolol is significant, so it can be stated again that solubility values obtained do not depend on the buffer used for their determination.

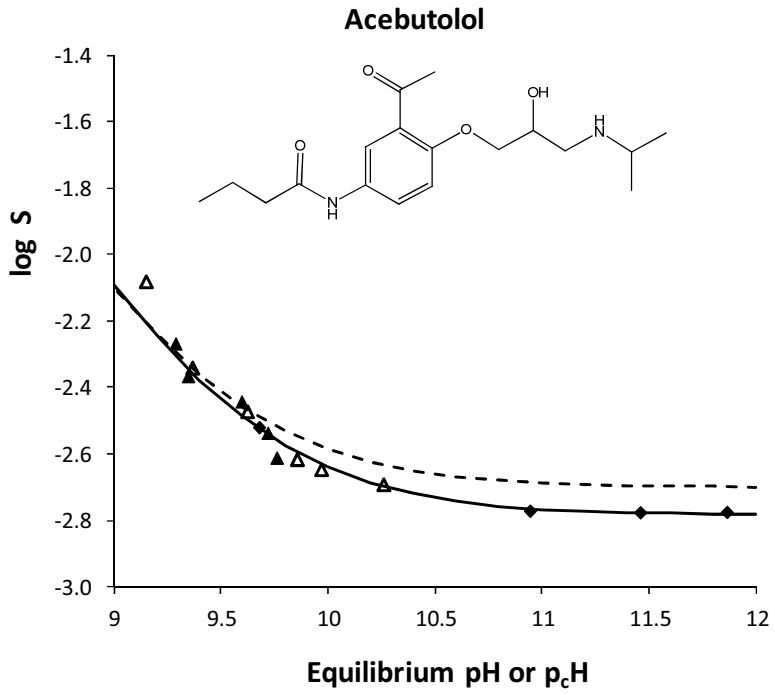


Figure 3.3.2 Solubility–*pH* profiles for acebutolol. Identification of buffers and profiles as in Figure 3.3.1.

3.3.2.3 Quetiapine hemifumarate

Quetiapine is a diprotic base (BH_2^{2+}) commercialized as hemifumarate salt. Its pK_a values, 3.31 and 6.97 [25], are given in Table 3.3.3. and, according to CheqSol measurements, its intrinsic solubility is 0.93 mM ($\log S_o$ = -3.03). This value is in concordance with the one obtained by Völgyi et al. using the same method [26]. Fig. 3 shows the solubility–*pH* profile for this compound. In this case the S_o value obtained by the CheqSol method has been used in the H-H equation developed for a diprotic base (Eq. 3.3.5) [18].

$$\log S = \log S_o + \log(10^{pK_{a1}^c + pK_{a2}^c - 2p_cH} + 10^{pK_{a2}^c - p_cH} + 1) \qquad [3.3.5]$$

However, the model (dashed line) does not fit the experimental points since they show two different solubility plateau where compound precipitates. The first plateau is between *pH* 4 and 6, and the second at *pH* values higher than 8. However, with the CheqSol method only the free base solubility is determined (equivalent to that of the second plateau) and this is the reason of the lack of fit with the experimental points below *pH* 6. At *pH* values higher than 8, quetiapine is in its neutral form and precipitates as a neutral free base (B). When *pH* decreases, the base starts to protonate, and a new precipitate appears. Thus, it should be noticed that pK_a values of fumaric acid are 3.02 and 4.38 [27] and then, in the 4.5-6.0 *pH* range the monoprotonated base precipitates with the fumarate ion being $(BH)_2Fum$ the solid species. At *pH* values lower than 4, quetiapine starts to be totally protonated (BH_2^{2+}) and solubility increases sharply. Therefore, to model the solubility behavior of quetiapine through the S-F experimental data, two independent and consecutive hybrid-type H-H equations for monoprotic bases, one for each species, have been used:

$$\log S_B = \log S_{o,B} + \log(10^{pK'_{a2}-pH} + 1) \qquad [3.3.6]$$

$$\log S_{(BH)_2Fu} = \log S_{o,(BH)_2Fu} + \log(10^{pK'_{a1}-pH} + 1) \qquad [3.3.7]$$

The solid line in Fig. 3.3.3 is the result of the combination of these two equations, the first used at *pH* higher than 6 and the second one at *pH* lower than 6. In this instance the symbol log $S_{o,(BH)2Fu}$ stands for the solubility of the non charged salt formulated in the subscript. Then, two different solubility values as determined by the S-F method are $\log S_{o,B}$ (M)=-2.89 for the free base, B, and $\log S_{o,(BH)2Fu}$ (M)= -1.96 for the salt, $(BH)_2Fu$. The first value is consistent with the one encountered by Völgyi et al. [26] ($\log S_o$ (M) = -2.84 for B), whose experimental data are included in our solubility profile matching very well with our results. Thus, treating both equilibria separately, a good model of the solubility behavior of quetiapine hemifumarate is obtained.

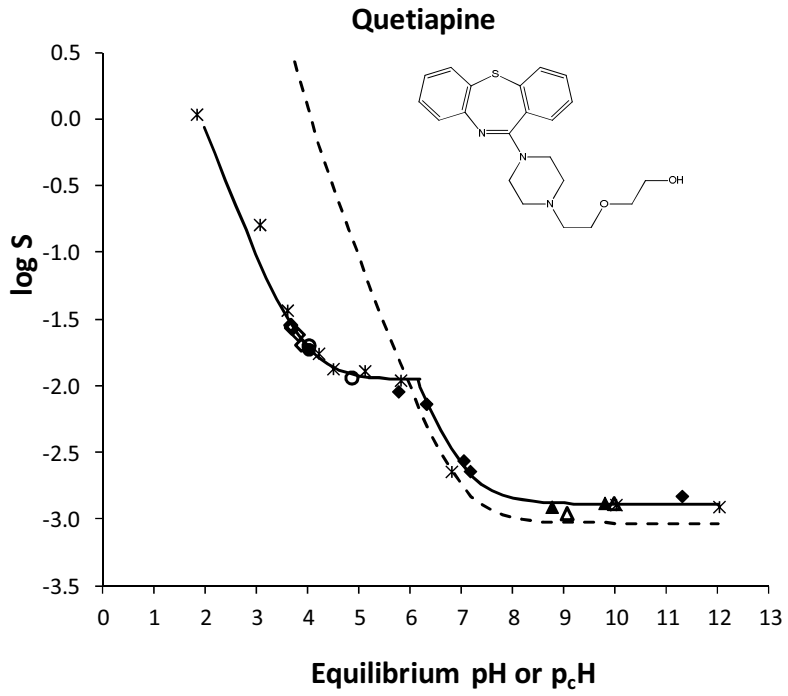


Figure 3.3.3 Solubility–*pH* profiles for quatiapine hemifumarate. Buffers used: ◆ phosphate, ● formate, ■ citrate, ○ acetate, ◇ lactate, △ ammonium, ▲ borate, * data from reference [26]. Identification of profiles as in Fig. 3.3.1.

3.3.2.4 Sulfadimethoxine

Sulfadimethoxine, presents two acid-base equilibria (Table 3.3.3). According to the assignation of acidity sites by the Sparc calculator [28] (see Figure 3.3.4), the first pK_a of sulfadimethoxine corresponds to the anilinium and the second to the pyrimidinium deprotonation. Then, the compound is in its zwitterionic form in the *pH* region comprised between these two pK_a values. The S_o values determined for this compound by both methods agree (Table 3.3.3) showing that sulfadimethoxine is the most insoluble of the studied drugs. In Fig. 3.3.5 the experimental solubility–*pH* profile (data in Table 3.3.2) and the curves of the corresponding H-H equations (Eqs. 3.3.8 and 3.3.9 for S-F and CheqSol methods, respectively) are plotted:

$$\log S = \log S_o + \log(10^{pK'_{a1}-pH} + 1 + 10^{pH-pK'_{a2}}) \qquad [3.3.8]$$

$$\log S = \log S_o + \log(10^{pK_{a1}^c - p_cH} + 1 + 10^{p_cH - pK_{a2}^c}) \quad [3.3.9]$$

H-H equations match well the experimental points. Only slight deviations to higher solubilities are observed for citric acid buffered solutions at *pH* < 3, accordingly with the behaviour already explained for propylparaben. Again these points have been omitted in the fitting the experimental solubility values to Eq. (3.3.8) (solid line). Thermodynamic pK_a values calculated from potentiometric experiments agree with those derived from the fit of experimental S-F points to the suitable H-H equation (Table 3.3.3). The agreement between them and, also, with the literature value confirms again the anomalous effect on solubility of citric acid.

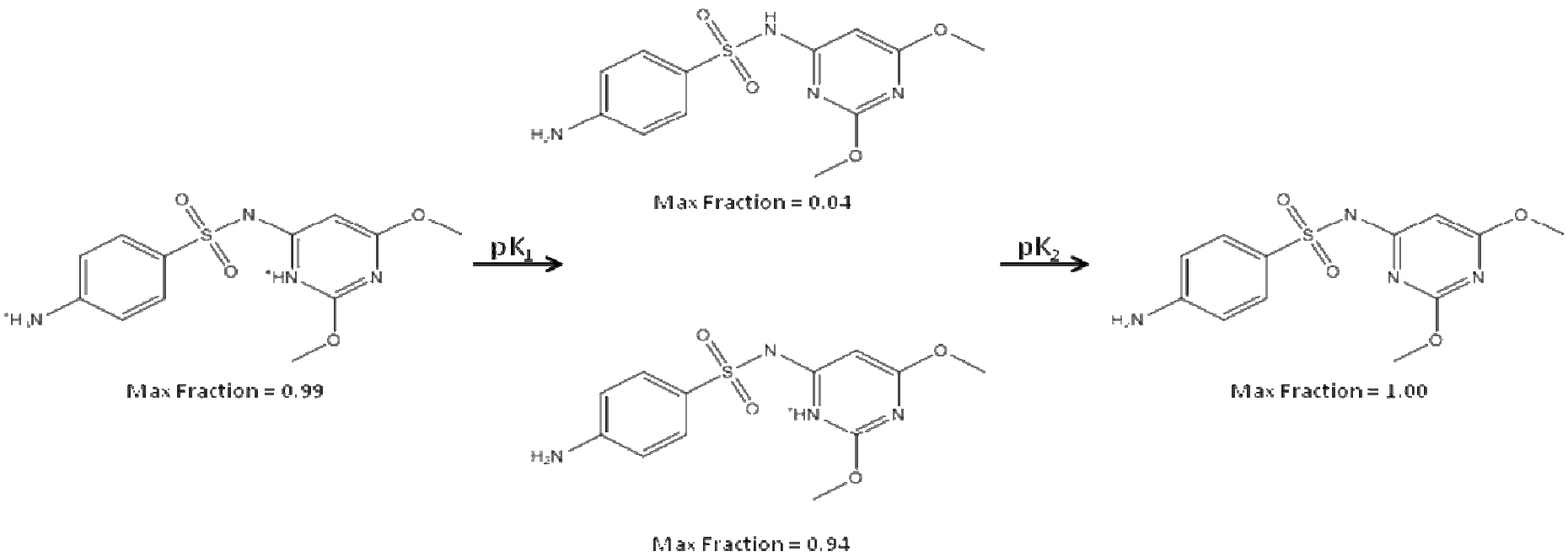


Figure 3.3.4: Scheme of sulfadimethoxine acid-base equilibria

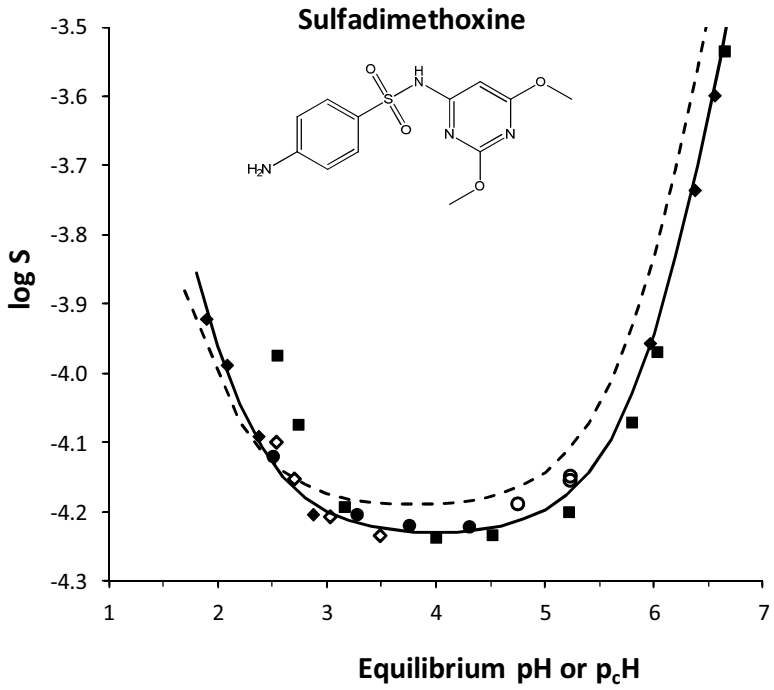


Figure 3.3.5: Solubility–pH profiles for sulfadimethoxine. Buffers used: ◆ phosphate, ● formate, ■ citrate, ○ acetate, ◇ lactate, △ ammonium, ▲ borate. Solid line: fit of S-F data to H-H equation. Dashed line: profile obtained through the CheqSol method.

3.3.2.5 Cefadroxil

Cefadroxil (XH_3^+) shows three pK_a values (Table 3.3.3). As explained, the zwitterionic species predominates in the *pH* range between the first and the second pK_a and this is the most insoluble species. Solubility of zwitterionic cefadroxil has been determined by both methods, but the results obtained are not as consistent as for the other compounds, as given in Table 3.3.3 and in Fig. 3.3.6A. Significant deviations are observed in some instances when H-H curves (Eqs. 3.3.8 and 3.3.9) are compared to the experimental points. Again, higher solubility than that shown in H-H curve is obtained when citric or lactic acids are used as buffers at low *pH* values, pointing out any interaction between the acidic form of these acids and the drug. Then, these points have been deleted in the fitting of Eq. 3.3.8 (solid line). In addition, both curves follow different trends at *pH* values higher than 6. This fact mainly occurs because of the difference in the pK_{a2} value of the fits. Whereas the pK_a values used in CheqSol

profile (dashed line) are the potentiometric ones, the pK_a values derived from the S-F experiments are calculated through the fit of experimental solubility points to the H-H equation. The CheqSol profile (with the right pK_{a2} value) does not fit the experimental points, and the fit of the S-F experiments (solid line) provides a pK_{a2} quite lower than the expected one. In any case, experimental S-F solubility values show a significant enhancement of solubility with respect to those calculated by CheqSol for *pH* values higher than 6. It should be noticed that the experiments were performed in dihydrogenphosphate, ammonium, and boric acid buffers, and it would not be realistic to attribute this unexpected solubility increase to specific interactions of the drug with three buffers of such different nature. For this reason the hypothesis of the formation of compound aggregates, a quite common fact in precipitation processes [18, 29], was tested.

In order to investigate the aggregation process, the supernatant of one of the aliquots prepared in ammonia buffer was analysed by high resolution mass spectrometry, using an ESI-Q-TOF-MS/MS instrument. Fig. 3.3.7A shows the obtained spectra, in positive mode. The most intense peak is the one corresponding to $[2M+H]^+$= 727.19 Da, being M the molecular weight of a cefadroxil unit (363.09 g/mol) in its zwitterionic form. Also the monomeric form of the compound was observed ($[M+H]^+$= 364.10 Da), and with lower intensity, a peak corresponding to a trimeric structure ($[3M+H]^+$ = 1090.27 Da) was also detected. In order to discard the possible formation of the aggregates in the ionization source, the declustering potential (DP) rose to 200 V, and even in this conditions the dimeric and trimeric structures were observed, confirming that they came from the sample itself. The correct identification of the peaks was done by the calculation of the exact mass of the three species and also through the fragmentation patterns in the MS/MS mode. Table 3.3.4 shows the results obtained, and Fig. 3.3.7B the MS/MS spectra of the dimeric form. It can be seen that the fragmentation pattern of the dimer is the same as the one of the monomer.

As the presence of aggregates at *pH* values about 7 is demonstrated, a different equation must be proposed. At *pH* > 6.5, cefadroxil is partially in its anionic form, and

according the MS evidence, a model where the monoanionic species of the compound forms an aggregate of *n* stoichiometry should be proposed:

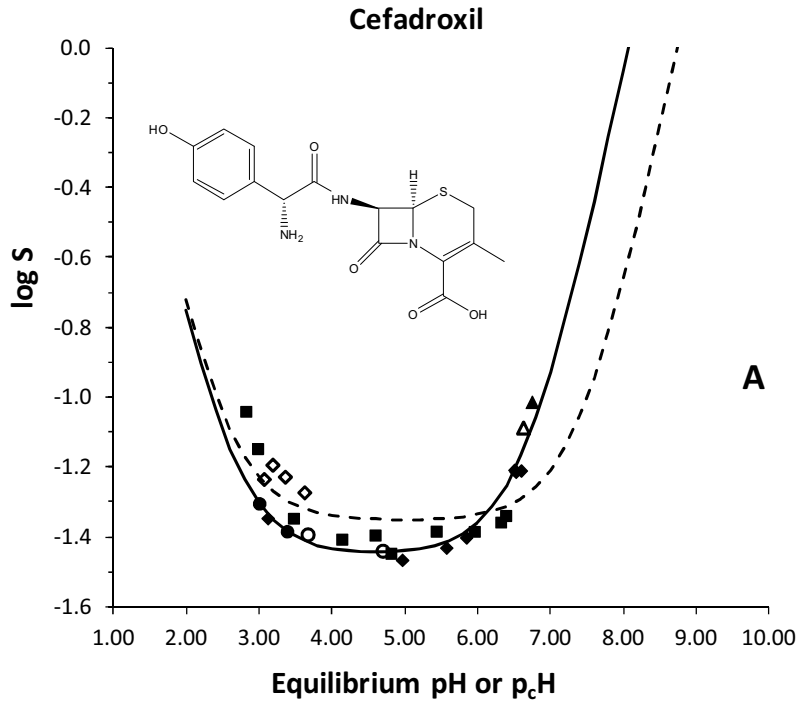
$$nXH^- \rightleftharpoons (XH^-)_n \qquad [3.3.10]$$

Then, a specific solubility-*pH* equation that accounts for this type of aggregation was derived [18]:

$$\log S = \log S_o + \log(1 + 10^{pK'_{a1}-pH} + 10^{pH-pK'_{a2}} + n10^{\log K_n + (n-1)\log S_o - npK'_{a2} + npH}) \qquad [3.3.11]$$

Where K_n is the aggregation constant and n is the aggregation number. The whole set of S-F solubility data have been fitted to Eq. 3.3.11 for n = 2 despite the presence of trimers has been demonstrated. This is because, on one hand the main aggregate is the dimeric species, and on the other hand, the number of parameters to estimate would be too high taking into account the limited number of experimental points in this part of the solubility profile. Fig. 3.3.6B shows the obtained fit showing the suitability of the selected model to describe the solubility-*pH* profile of cefadroxil.

The fit to Eq. 11 instead of Eq. 8 of the S-F experimental points for cefadroxil allows the determination of new values for pK'_{a1} and pK'_{a2} (2.51 and 7.18, respectively) and the derived thermodynamic ones pK_{a1} and pK_{a2} (2.40 and 7.29, respectively). These values are consistent with those obtained from the potentiometric method and also from the literature proving again the suitability of the model used, which embody the aggregation of cefadroxil at *pH* higher than 6.5. However, it should be pointed out that, as expected, the statistics associated to the fit to Eq. 3.3.11 are not satisfactory because of the number of available experimental points is not enough to account for an equation that involves four adjustable parameters. For this reason the derived pK_a values, as well as the aggregation constant, are not included in Table 3.3.3.



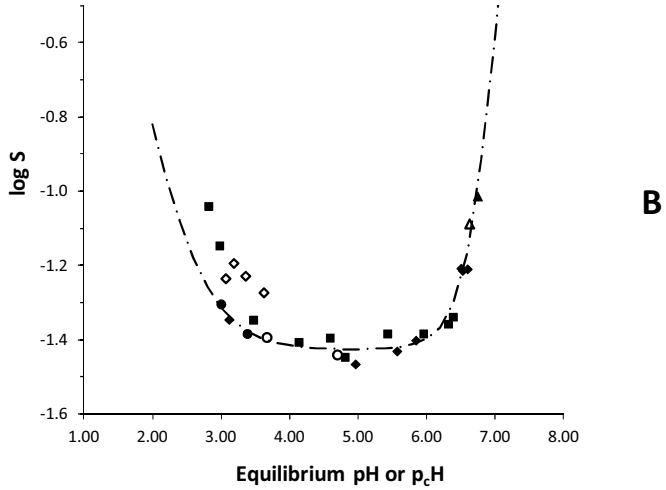


Figure 3.3.6A: Solubility–*pH* profiles for cefadroxil. Figure3.3.6B: The obtained fit showing the suitability of the selected model to describe the solubility-*pH* profile of cefadroxil. Buffers used: ◆ phosphate, ● formate, ■ citrate, ○ acetate, ◇ lactate, △ ammonium, ▲ borate. Solid line: fit of S-F data to H-H equation. Dashed line: profile obtained through the CheqSol method.

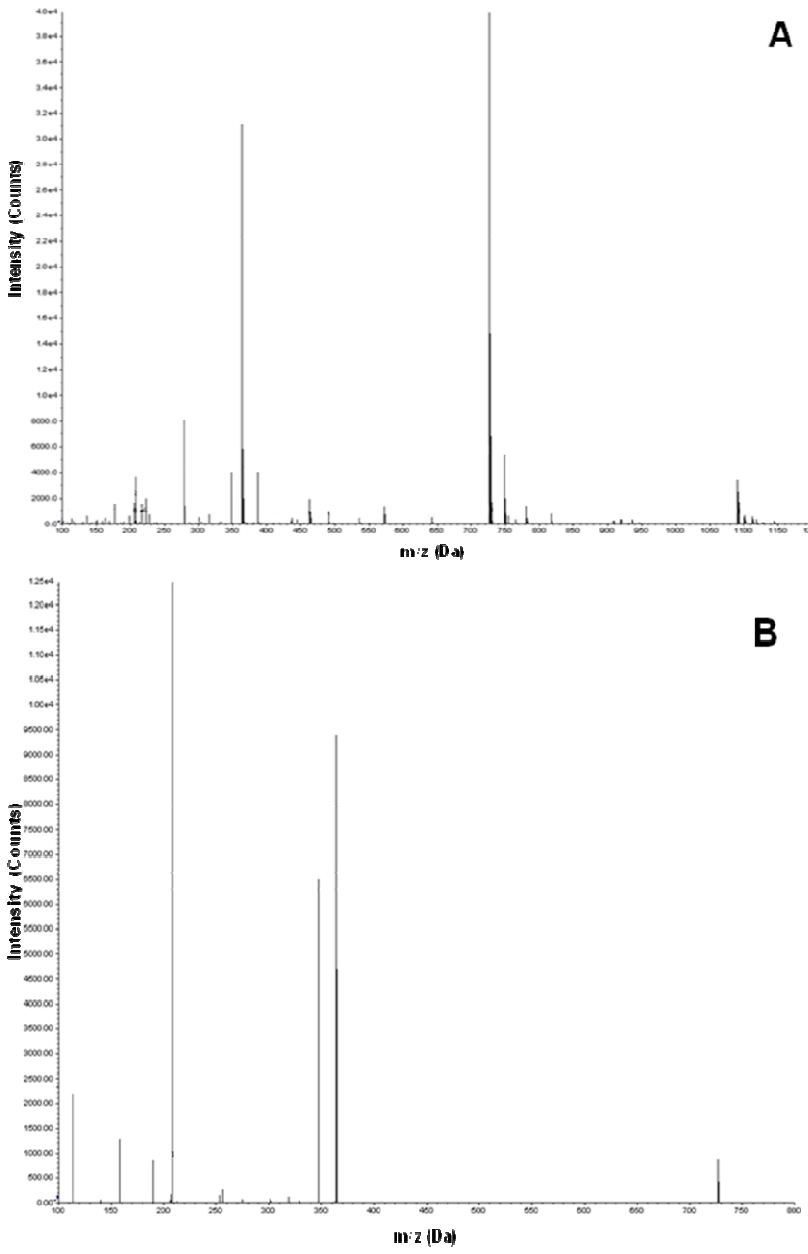


Figure 3.3.7: (A) MS spectrum of cefadroxil supernatant solution in ammonium/ammonia buffer. (B) MS/MS spectrum of the dimeric structure of cefadroxil ($[M+H]^+$ = 727 Da).

Table 3.3.4 Confirmation of the identity of cefadroxil aggregates through ESI-Q-TOF-MS/MS experiments.

	Theoretical Mass (Da) $[M+H]^+$	Exact Mass (Da) $[M+H]^+$	Error (ppm)	MS/MS main fragments (Da)
Monomer	364.0962	364.0967	1.0	347, 208, 190, 158, 113
Dimer	727.1851	727.1858	1.9	364, 347, 208, 190, 158, 113
Trimer	1090.2740	1090.2723	1.6	727, 364

In summary, for the studied drugs, the solubility values obtained by the S-F method whenever the buffer agent used, and the potentiometric CheqSol method are consistent. The exceptions are the hydrotrophic lactic and citric acids which, at low *pH*, enhance the solubility of propylparaben, sulphadimethoxine and cefadroxil. Then, these buffers are not advisable for solubility measurements of these drugs in acidic media as already advised in literature. The achieved results prove the suitability of the potentiometric approach to get reliable values of intrinsic solubility and solubility-*pH* profiles. However, when side reactions, such as drug aggregation, occur in the supernatant solution, the potentiometric approach is unable to detect them and derived solubility profile does not match the true solubility variation with the solution *pH* as clearly demonstrated in the case of cefadroxil. In the same way, when the drug precipitates in any salt form, the solubility profile achieved from both approaches differs, as shown in the study about the quetiapine. Then, the chemistry associated to the studied compound should be properly known for the right application of the very useful potentiometric methodology.

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