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Correlation of aqueous solubility of salts of benzylamine with experimentally and theoretically derived parameters. A multivariate data analysis approach

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Abstract

Twenty two salts of benzylamine and *p*-substituted benzoic acids were prepared and characterized. The *p*-substituent was varied with regard to electronic, hydrophobic, and steric effects as well as hydrogen bonding potential. A multivariate data analysis was used to describe the relationship between the aqueous solubility of the salts and experimentally determined physicochemical parameters and theoretically derived molecular descriptors. The model, based on all descriptors, gave $R^2 = 0.86$ and $Q^2 = 0.72$. The most significant descriptors exhibiting VIP (variance of importance) values above 1.0 were intrinsic dissolution rate, intrinsic solubility of the unionized acids (S_0), Hansch's hydrophobic parameter, Charton's steric parameter and molecular weight (MW). Statistically good models for predicting solubility of a selected test set were obtained by using simple models consisting of a few descriptors only: (i) Charton, Hansch and MW ($R^2 = 0.73$; $Q^2 = 0.70$), and (ii) Charton and S_0 ($R^2 = 0.74$; $Q^2 = 0.72$).

Keywords: Salt; Solubility; Crystal lattice energy; Free energy of hydration; Hydrogen bonding; Hydrophobicity; Steric effects

1. Introduction

For a given weakly acidic or basic drug substance it is well known that the use of different counterions can result in salts differing significantly with respect to physicochemical and thus, biopharmaceutical properties. In recent years increasing efforts have been devoted to modify/optimize drug performance through salt formation as opposed to more complex molecular modifications (Bighley et al., 1996), and salt selection has become an important part of early drug development (Gould, 1986; Bastin et al., 2000; Panchagnula and Thomas, 2000). Although a vast number of drug salts have been employed (Berge et al.,

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1977; Gould, 1986; Bighley et al., 1996), the choice of salt forming agents most often has been made on a semi-empirical basis. The early pharmaceutical literature contains a large amount of information on drug salts designed for a variety of purposes including the use of poorly water-soluble salts to prolong the duration of drug action and to eliminate various undesirable drug properties (Berge et al., 1977). By modern medicinal chemistry a great number of compounds exhibiting desired receptor profiles emerges. Unfortunately, insufficient water solubility results in low and variable bioavailability after oral administration and thereby prevents many pharmacologically interesting chemical entities from further development (Lipinsky et al., 1997). Thus, the accomplishment of proper aqueous solubility constitutes an important objective in rational salt selection. Correlations have been observed between oil solubility and melting point in case of prodrugs of phenytoin (Yamaoka et al., 1983) and between aqueous solubility and melting point and distribution coefficients for a series of 5-fluorouracil prodrugs (Buur and Bundgaard, 1986). Whereas qualitative relationships between chemical structure and physicochemical properties are recognized (Berge et al., 1977; Gould, 1986; Rubino, 1989) quantitative relationships enabling prediction of aqueous solubility of salts are virtually non-existent (Anderson and Conradi, 1985; Shah and Chafetz, 1994; Pietiläinen et al., 1996).

Based on our interest in the design of poorly water-soluble salts for parenteral depot formulations the present study was undertaken to gain more insight into parameters influencing the aqueous solubility of salts. Twenty two salts of *p*-substituted benzoic acids and benzylamine were synthesized and characterized physicochemically. The highly water-soluble benzylamine was chosen as the model amine component, thus preventing salt solubility from being limited by the intrinsic solubility of the free base. By using *p*-substituted benzoic acids it was possible to preserve a common skeleton of the anionic counterions. High molecular diversity was achieved by the chemical nature of the selected *p*-substituents. The aim of the study was to use multivariate data analysis to establish models enabling a description of the correlation between aqueous solubility of the salts and experimentally determined physicochemical parameters and calculated molecular descriptors.

2. Materials and methods

2.1. Materials

The compounds used for salt formation were of highest purity commercially available. Benzoic acid, *p*-toluic acid, *p*-ethylbenzoic acid, *p*-propylbenzoic acid, *p*-isopropylbenzoic acid, *p*-butylbenzoic acid, *p*-tert-butylbenzoic acid, *p*-phenylbenzoic acid, *p*-chlorobenzoic acid, *p*-bromobenzoic acid, *p*-iodobenzoic acid, *p*-methoxybenzoic acid, *p*-cyanobenzoic acid, *p*-hydroxymethylbenzoic acid, *p*-nitrobenzoic acid, *p*-carboxybenzenesulfonamide, *p*-trifluoromethylbenzoic acid and benzylamine were purchased from Bie & Berntsen, Copenhagen, Denmark. *p*-Hydroxybenzoic acid, *p*-aminobenzoic acid, *p*-(*N*-methyl)aminobenzoic acid and *p*-(*N*,*N*-dimethyl)aminobenzoic acid were purchased from Fluka AG, Buchs, Switzerland. *p*-Carboxybenzamide was prepared from *p*-cyanobenzoic acid by acidic hydrolysis (Di Gangi and Gisvold, 1949). Chemicals for preparation of HPLC mobile phases were of analytical grade. Deionized water was used throughout the study.

2.2. Salt preparation

The salts were prepared by mixing a methanolic solution (50–60 °C) of the benzoic acid derivative, usually 0.1–0.4 mM, with an equivalent amount of benzylamine in methanol. After stirring at 50–60 °C for 3 h, methanol was partly removed in vacuo until precipitation occured. Recrystallization was done from methanol or in some cases from methanol and diethyl ether. After filtration the crystals were washed with diethyl ether and stored in vacuo over phosphorus(V)-oxide.

The salts were subjected to elemental analysis, and the formation of 1:1 salts was confirmed by HPLC analysis. The purity of the salts ($>97\%$) was also confirmed by HPLC. Melting points

Table 1
Characterization of the *para*-substituted benzoic acid salts of benzylamine

Compound no.	*Para*-subst.	S (mM)[a]	S_0 (mM)[b]	pH_{calc}[c]	pH_{meas}	IDR[d]	Mp salt[e]	Mp acid	pK_a[f]
1	H	839	34.6	6.8	7.1	n.d[h]	128	123	4.16 (4.20)
2	CH_3	145	3.66	6.9	6.8	7.68	162	179	4.37
3	C_2H_5	98.2	3.31	6.9	6.8	4.78	140	115	4.35
4	C_3H_7	59.0	0.471	6.9	6.7	3.49	142	143	4.35
5	i-C_3H_7	97.6	1.24	6.9	7.0	4.65	127	116	4.35
6	C_4H_9	38.9	0.229	6.9	6.9	2.30	117	102	4.36
7	tert C_4H_9	35.0	0.247	6.9	8.1	1.14	181	164	4.39
8	C_6H_5	12.8	0.059	6.8	6.9	1.15	176	226	4.21
9	Cl	120	0.546	6.7	6.8	7.11	161	241	3.96
10	Br	85.4	0.245	6.7	7.1	6.35	162	255	3.98
11	I	33.9	0.080	6.7	6.5	2.02	169	272	3.99
12	OCH_3	306	2.16	6.9	7.3	n.d	143	183	4.48
13	OH	138	82.6	6.9	6.7	11.9	218	215	4.41 (4.58)
14	NO_2	77.6	1.77	6.4	6.9	4.98	197	239	3.42
15	CH_2OH	964	37.1	6.8	5.9	n.d	154	184	4.16
16	CN	256	6.23	6.4	5.7	18.4	173	220	3.50
17	NH_2	272	58.4	7.1	7.1	n.d	194	187	4.77
18	$NH(CH_3)$	231	8.58	7.2	5.7	n.d	169	158	5.04
19	$N(CH_3)_2$	62.6	0.519	7.2	7.2	3.88	182	243	5.03
20	CF_3	29.3	0.426	6.5	6.4	2.89	197	220	3.67
21	SO_2NH_2	110	3.13	6.5	6.3	6.49	218	288	3.63
22	$CONH_2$	118	0.247	6.6	6.4	6.91	211	>300[g]	3.89

[a] Salt solubility, S (37 °C).
[b] Intrinsic solubility of benzoic acids S_0 (37 °C).
[c] Calculated according to Eq. (1).
[d] IDR is the intrinsic dissolution rate in $\mu M\ min^{-1}\ cm^{-2}$.
[e] The melting interval is below 5 °C at hot-stage microscopy. The melting point values for the benzoic acid derivatives are given by the manufacturer (Bie and Berntsen A/S).
[f] The pK_a of benzoic acid and *p*-hydroxybenzoic acid were determined experimentally ($pK_a \pm 0.06$). The calculated pK_a values of these compounds are given in brackets. The pK_a values for the remaining acids were calculated from Hammet constants (Perrin et al., 1981).
[g] Combustion occurred above 300 °C.
[h] n.d: not determined.

were determined by using hot stage microscopy (Leitz Wetzler 900, Germany) and from differential scanning calorimetry (DSC).

2.3. Characterization of salts

2.3.1. Solubility determination

About 200–800 mg of the salts were suspended in 5 ml deionized water. The screwcapped 10 ml vials were rotated at 37.0 ± 0.5 °C in an incubator hood (T-30, Holm & Halby, Denmark). Equilibrium was reached within 24–48 h as confirmed by HPLC. An aliquot of the supernatant was filtered through a 0.45 μm membrane filter (Chromacol) discarding the first 2 ml. The filtrate was diluted with deionized water prior to HPLC analysis. The filtration process was carried out in the incubator hood and the equipment used were all preheated to 37.0 ± 0.5 °C. The solubility of the salts was determined from experiments done in triplicate. For each salt, the equilibrium concentration of both the benzoic acid derivative and benzylamine was determined by HPLC. pH of the saturated solution was measured by using a Blueline 16 pH microelectrode, Metrohm. The intrinsic solubility of parent *p*-substituted benzoic acids (37.0 ± 0.5 °C) was determined in a similar manner where 20–200 mg of the substituted benzoic

acids were added to 20 ml 0.1 N HCl. The solvent used for the parent *p*-substituted benzoic acids of compound 17, 18 and 19 (Table 1) was deionized water.

2.3.2. Intrinsic dissolution rate measurements

Dissolution experiments were performed by the rotating disk method (Prakongpan et al., 1976). The rotating disk apparatus consisted of a rotor motor, a stainless steel stirrer shaft, centered in the vessel, connecting the motor to a stainless die (a tube) which encased the drug disk and a hydrodynamic vessel containing 1000.0 ml of deionized and degassed water. The rotation speed was set to 50 rpm.

About 200 mg of crystalline salt was compressed at a pressure of 2 tons into a flat disk having a diameter of 10 mm. The surface of the disk was placed 4 cm above the bottom of the vessel. At time zero the die containing the sample was lowered into the preheated dissolution medium (37.0 ± 0.5 °C). In case of low dissolution rates (< 6 μM min^{-1} cm^{-2}) the dissolution medium was circulated at a constant flow rate of 1.4 ml min^{-1} into flow-through cells (Helma). The dissolution medium was automatically assayed on a diode-array detector (Zeiss, Specord S 100) at appropriate intervals at λ_{max} for each salt. Concentrations were calculated from standard solutions of the individual salt assayed separately. At dissolution rates above 6 μM min^{-1} cm^{-2} 5 ml aliquots of the dissolution medium were manually withdrawn every second minute without replacement and assayed on a UV-spectrophotometer (Shimadzu 160-UV).

2.3.3. Thermal analyses

A Perkin–Elmer DSC 6 apparatus with Pyris software was used for the determination of the molar heat of fusion and the melting point of the salts. An accurately weighed amount of the sample (0.9–1.5 mg) was transferred to an aluminium pan in which a pinhole was punched in the pan lid, and the sides of the lid were crimped. A similar empty pan was used as reference. Samples were scanned from 50–260 °C at a rate of 10 °C min^{-1} under nitrogen gas flow (20 ml min^{-1}). The measurements were done in duplo. The instrument was calibrated using indium and zinc. Thermogravimetric analysis (TGA) was performed for each salt using a Universal V2.5H (TA Instruments) to study the loss of volatiles in the temperature range 25–250 °C at a scanning rate of 10 °C min^{-1} in nitrogen atmosphere.

2.3.4. Computational method

Semi-empirical calculations of the free energy of hydration, ΔG_{hyd}, for the *p*-substituted benzoate ions were carried out using the program Spartan 5.1.1[1]. The calculations for the aqueous phase were done using the AM1-SM2 method developed by Cramer and Truhlar (1992).

2.3.5. pK_a determination

The pK_a value of benzoic acid and pK_{a1} of *p*-hydroxybenzoic acid were determined as the mean of two independent potentiometric titrations at 37.0 ± 0.5 °C according to Albert and Serjeant (1971).

2.3.6. Statistical analysis

A principal component analysis (PCA) was performed on the data set consisting of the descriptors presented in Tables 1 and 2 (Joliffe, 1986; Jackson, 1991; Malinowski, 1991). In brief it is a method for summarizing the information residing in the descriptor matrix in so-called principal components (PCs). A large array of descriptors can be reduced to only a few orthogonal PCs, which represent the main variation in the data set. The descriptors were given an equal chance (weight) of influencing the analysis regardless of their respective scales by autoscaling, where the variance of each descriptor column was scaled to unit variance. The data set was mean-centered before any statistical operations was performed. Partial least squares (PLS) projection to latent structures is a modelling and computational method, which allows quantitative relations to be established between blocks of variables (Höskuldsson, 1996; Wold et al., 1993). In the present study PLS modelling was applied in order to identify relationships between the salt solubility

[1] SPARTAN SGI version 5.1.1. Wavefunction Inc. 18401 von Karman, suite 370 CA 92612, United States.

Table 2
Thermodynamic parameters of the benzylamine salts and substituent values of the *p*-substituted benzoic acid derivatives

Compound no.	*Para*-subst.	Charton[a]	MW	Hansch[b]	ΔG_{sol} (kJ/mole)	ΔG_{hyd}[c] (kJ/mole)	ΔH_{fus} (kJ/mole)
1	H	0	229	0	3.16	−298.1	34.5
2	CH_3	0.52	243	0.56	11.4	−296.9	51.9
3	C_2H_5	0.56	257	1.02	13.2	−298.5	43.4
4	C_3H_7	0.68	271	1.55	15.7	−296.0	43.1
5	i-C_3H_7	0.76	271	1.53	13.3	−297.1	37.3
6	C_4H_9	0.68	285	2.13	17.7	−294.9	39.2
7	tert C_4H_9	1.24	285	1.98	18.2	−296.0	39.3
8	C_6H_5	1.66	305	1.96	23.1	−294.3	35.6
9	Cl	0.55	264	0.71	12.3	−283.4	36.6
10	Br	0.65	308	0.86	13.9	−289.2	38.5
11	I	0.78	355	1.12	18.3	−302.1	44.2
12	OCH_3	0.36	259	−0.02	7.90	−304.8	51.1
13	OH	0.32	245	−0.67	11.6	−313.0	219
14	NO_2	0.59	274	−0.28	14.4	−260.2	51.4
15	CH_2OH	0.53	259	−1.03	2.51	−308.3	50.5
16	CN	0.40	254	−0.57	8.73	−281.2	43.7
17	NH_2	0.35	244	−1.23	8.45	−322.8	57.0
18	$NH(CH_3)$	0.39	258	−0.47	9.20	−321.6	54.6
19	$N(CH_3)_2$	0.43	272	0.18	15.4	−319.3	66.0
20	CF_3	0.91	297	0.88	19.0	−255.0	51.1
21	SO_2NH_2	–	308	−1.82	12.7	−355.8	61.3
22	$CONH_2$	–	272	−1.49	12.4	−319.2	70.0

[a] The steric parameter values (Charton) were taken from the literature (Charton, 1977).
[b] The Hansch hydrophobic values were taken from the literature of Hansch et al. (1973).
[c] The calculated free energy of hydration of the carboxylate anion.

and the descriptors used in the study. Initially, all descriptors were included in the model and evaluated on basis of their variable influence on projection (VIP) values. Descriptors with low values were excluded and the effect was judged by the cross-validated correlation coefficient (Q^2). If the value increased, the descriptor in question was deselected permanently from the model. This procedure resulted in a model with fewer descriptors. SIMCA v. 8.0 software[2] was used to perform PCA and PLS.

3. Results and discussion

3.1. Characterization of salts

All salts were crystalline and obtained from mixing a methanolic solution of benzylamine with an equivalent amount of the individual benzoic acid derivative dissolved in methanol. The possibility of precipitation of a physical mixture of the *p*-substituted benzoic acid and benzylamine can be ruled out as the amine is a liquid at ambient temperature. The absence of solvates or adhered solvent was established from TGA experiments. Formation of the 1:1 salt was confirmed by quantitative HPLC and elemental analysis (CHN). From the aqueous solutions of the salts the concentrations of the benzoic acid derivatives and benzylamine were determined and in most cases the cation to anion ratio was 1.00 ± 0.02, as determined by HPLC. In accordance with 1:1 salt formation, the experimentally determined pH values in saturated salt solution (Table 1) were found to be almost identical to those calculated from Eq. (1).

$$pH_{calc} = \frac{1}{2}(pK_a(\text{acid}) + pK_a(\text{base})) \quad (1)$$

[2] SIMCA, Umetrics AB, Box 7960: SE-90719 Umeå, Sweden, 1999.

For benzylamine a pK_a value of 9.32 has been used (Perrin, 1965). The ionization constants of the benzoic acids are presented in Table 1. In case of benzoic acid and *p*-hydroxybenzoic acid the pK_a values were obtained by potentiometric titration of 0.01 M solutions at 37 ± 0.5 °C whereas the values for the remaining acids were calculated by using tabulated Hammett substitution constants (Perrin et al., 1981). In case of *p*-hydroxybenzoic acid the calculated and experimentally determined pK_{a1} values were in favourable agreement. Since the aqueous solubility differs considerably, the pH data suggests that the pH in saturated salt solution is essentially independent on the actual concentrations of the respective cation and anion. Likewise upon a 10-fold dilution of the saturated solution of compound 7, constituting one of the least water-soluble salts in the present series, pH decreased only marginally. In contrast, addition of 2% (w/w) benzoic acid to the saturated solution of the benzoic acid salt lowered pH by approximately 0.5 pH units. So pH in the saturated solution is sensitive to minor excess of corresponding acid or base of the salt.

All solubility experiments were performed at 37.0 ± 0.5 °C using deionized water as the solvent. The aqueous solubilities of the salts presented in Table 1 were obtained from solubility experiments done in triplicate (SD's below 4%). The solubility of the salts varied by a factor 80. Polymorphic changes of the salts were not observed by DSC experiments performed over a period of 6 months implying that at least reasonable stable forms of the prepared salts had been isolated. By HPLC equimolar concentrations of acid and base were determined at saturation in the salt solubility experiments and thus no further analysis of the residual solid at equilibrium was performed (Anderson and Conradi, 1985).

In general, whenever one equivalent salt is dissolved, one equivalent cation and anion is formed

$$R_1COO^-,R_2NH_3^+(s) = R_1COO^-(aq) + R_2NH_3^+(aq) \quad (2)$$

In some cases, however, when the solubility of the salt is sufficiently high, precipitation of either the free base or acid may occur (Anderson and Conradi, 1985). In the present case benzylamine is freely soluble in water whereas the intrinsic solubility of the benzoic acid derivatives is limited. The influence of pH on the aqueous solubility of weak acidic electrolytes is given by:

$$pH = pK_a + \log\left(\frac{S - S_0}{S_0}\right) \quad (3)$$

where S_0 is the intrinsic solubility of the acid and S is the total solubility of the acid at a given pH value. For salts where precipitation of free acid occurs below the solubility of the 1:1 salt, the stoichiometric solubility ($S_{1:1}$) can be calculated by combining the antilogarithmic forms of Eq. (1) and Eq. (3).

$$S_{1:1} = S_0\left(1 + K_{a1}/10^{\frac{-(pK_{a1} + pK_{a2})}{2}}\right) \quad (4)$$

where pK_{a1} and pK_{a2} are the ionization constants of the benzoic acid derivative and benzylamine, respectively. At saturation the solution contains an excess of the base and thus pH exceeds that of pH_{calc}.

As seen from Table 1 only the pH value at saturation of compound 7 is clearly above the expected pH value, pH_{calc}, which might indicate that free acid precipitation has occurred. If free acid precipitation occurs before the attainment of a saturated salt solution, the pH can be calculated by the following equation (Anderson and Conradi, 1985):

$$[H^+] = \frac{K_{a1}S_0 + \sqrt{(K_{a1}S_0)^2 + 4C_TK_{a1}K_{a2}S_0}}{2C_T} \quad (5)$$

where S_0 is the intrinsic solubility of the benzoic acid derivative and C_T is the amount of salt added per liter of solution. However, in the present case HPLC analysis revealed that at saturation of compound 7 the ratio of base to acid was within 1.00 ± 0.04. Besides, the apparent solubility of compound 7 is well below the stoichiometric solubility calculated according to Eq. (4) further implying that precipitation of free acid had not occurred. Thus, the enhanced pH of the saturated solution possibly reflects the presence of some undetected basic impurities.

As apparent from Table 1 the intrinsic solubility of the parent benzoic acid derivatives varies by a factor 1400. The solubilities are determined from experiments done in triplicate and in all cases the relative standard deviations (RSDs) were below 4%.

The intrinsic dissolution rate (IDR) measurements gave linear profiles for the investigated salts ($R^2 > 0.99$) when plotting salt concentration in the dissolution medium against time. The RSD values were in all cases below 5%. For IDRs below approximately 6 μM min^{-1} cm^{-2} measurements were performed by using a continuous flow-through system. For salts exhibiting higher IDR values, measurements were done manually. In a few cases dissolution proceeded too fast (above 20 μM min^{-1} cm^{-2}) preventing IDR from being measured. After each experiment the surface of the disks was visually inspected. The disks with cracks or a disrupted surface were excluded and no IDR value could be assigned for these salts. A plot of IDR values of the salts against their respective solubilities was reasonably linear ($R = 0.97$) (Fig. 1). According to the Noyes–Whitney equation this plot should be linear if sink conditions prevail (Noyes and Whitney, 1897). Whereas such linear correlations between IDR and solubility have been established for both weak and non-electrolytes covering several chemical structures (Noyes and Whitney, 1897; Hamlin et al., 1965; Shah and Nelson, 1975; Nicklasson et al., 1981; Fini et al., 1985) only few correlations have been reported in case of salts. Fini et al. (1985) measured the dissolution rate of organic salts of 5 NSAID carboxylic acids, however the correlation to the solubilities of the salts was poorer ($R = 0.85$) than that observed in present case.

The heat of fusion was measured by DSC in volatile sample pans (Table 2) since preliminary experiments using vented and sealed aluminium pans indicated that sublimation of salt occurred resulting in a drifting baseline before and particularly after the endothermic peak. After scanning the aluminium pans were opened and it was observed that the samples had sublimated. TGA also confirmed that significant sample loss ($\approx 20\%$ in most cases) occurred at temperatures below the melting point. Use of volatile sample pans resulted in sharper endothermic peaks and more reproducible results (deviation between 2 runs below 5%). The melting point of the salts (Table 1) was determined as the onset temperature of the endotherm peak in the DSC scan. These temperatures were almost identical to those obtained by hot-stage microscopy (deviation below 2 °C). In a few experiments salts were spiked with 2–4% (w/w) of the corresponding acid. From DSC scans of these mixtures intense additional peaks emerged. Thus, in addition to HPLC analysis,

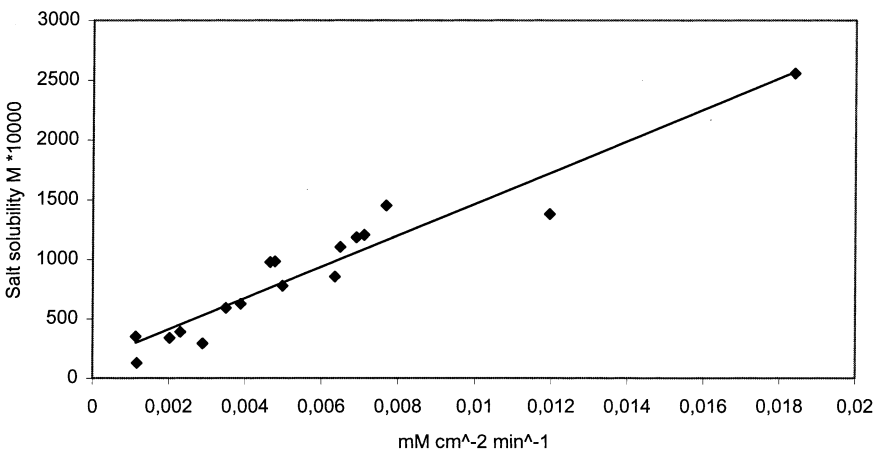

Fig. 1. IDR of the salts of benzylamine and *para*-substituted benzoic acid derivatives against their respective salt solubility in deionized water at 37 °C ($R = 0.97$).

DSC proved to be a reasonably sensitive method for the assessment of the purity of the salts.

3.2. Thermodynamic aspects of solubility

In previous publications aqueous solubility of organic weak and non-electrolytes has successfully been estimated on the basis of the octanol–water partition coefficient, K_{ow}, and the melting point of the solute (Chiou et al., 1977; Yalkowsky et al., 1983; Buur and Bundgaard, 1986). The applicability is, however, restricted to rigid molecules where the ideal solubility of the compound can be estimated by Waldens rule (Martin et al., 1979). In addition, the estimation relies on several other assumptions, which further restricts the applicability. Other approaches have been used to estimate the aqueous solubility of compounds of different chemical classes (for a review see Grant and Higuchi, 1990). More recently, Huyskens and co-workers (Huyskens, 1990) developed an equation for solubility prediction in pure solvents from the thermodynamics of mobile order. The equation was further developed and successfully applied to predict the aqueous solubility of solid aliphatic and aromatic hydrocarbons (Ruelle et al., 1994), alcohols (Ruelle and Kesselring, 1997a) and 232 proton-acceptor oxygen containing substances (Ruelle and Kesselring, 1997b). However, the selected compounds did not include any salts. In the present work a combined calorimetric and semi-empirical quantum chemical approach (AM1-SM2) is used in an attempt to describe the solution thermodynamics of organic salts and to establish a model for prediction of the aqueous solubility of the prepared salts.

The free energy of solution (ΔG_{sol}) for a salt is given by Hess' theorem

$$\Delta G_{sol} = \Delta G_{hyd\pm} + \Delta G_{lattice} \quad (6)$$

where $\Delta G_{hyd\pm}$ is the sum of free energy of hydration of the cation and anion and $\Delta G_{lattice}$ refers to the crystal lattice free energy (i.e. the free energy of sublimation of the ions). For a poorly soluble salt ΔG_{sol} is high (positive) resulting from the combination of low hydration energies and high crystal lattice energy.

ΔG_{sol} can be calculated from the experimental salt solubility data according to Eq. (7)

$$\Delta G_{sol} = -RT\ln K_{sp} \quad (7)$$

where K_{sp} is the thermodynamic solubility product. R and T refer to the gas constant and the absolute temperature, respectively. The activity coefficient, $\gamma_{\pm}$, used to obtain K_{sp}, is calculated from Debye–Hückel's extended equation

$$\log \gamma_{\pm} = \frac{-0.509\sqrt{I}}{1+\sqrt{I}} \quad (8)$$

where I is the ionic strength of the solution. The calculated Gibb's free energies of solution are presented in Table 2 revealing a 7–8-fold difference in ΔG_{sol} of the salts.

The relative variations of the two terms on the right side of Eq. (6) have been estimated. Since ΔG_{hyd+} (benzylammonium cation) is identical for all salts, it appears adequate in the present case only to consider variations in ΔG_{hyd-} (p-substituted benzoic acid anion). The free energy of hydration of the benzoic acid counterions was calculated by geometrical optimisation of the structures in the gas phase and the solvated phase and calculating the energy of formation in these two states (Reinwald and Zimmermann, 1998). Combining these values gave the free energy of hydration, ΔG_{hyd-}. From Table 2 it is apparent that the variation in ΔG_{hyd-} is modest. The maximum difference was less than a factor of 1.5. In the present study ΔH_{fusion} has been used as a measure of the crystal lattice energy due to difficulties in calculating or measuring $\Delta G_{lattice}$. It has been assumed that the entropy of fusion is similar for all the salts, which is a fairly good approximation, since investigations on 84 disubstituted benzenes, including p-substituted benzoic acids reflected that the entropy of fusion was fairly constant and independent of molecular size and shape, dipole moment and of inter- or intramolecular hydrogen bonding (Martin et al., 1979). Likewise, the entropy of vaporization can be assumed to be relatively similar for the salts as a first approximation, even though one might expect that the increase in entropy for the hydrogen bond containing compounds are somewhat higher (Chang, 2000). Theoretically, this leaves

only the heat of fusion, ΔH_{fusion} and heat of vaporization, ΔH_{vap} to account for the variation in ΔG_{sol}. The maximum difference in ΔG_{sol} for the salts is 7–8-fold, while both ΔG_{hyd-} and ΔH_{fusion} vary 2-fold at most when excluding compound 13. Apparently, the variation in ΔG_{sol} cannot only be accounted for in terms of ΔH_{fusion} and ΔG_{hyd-}. A closer look at the magnitude of the solubility reveals that the ionic strength in the saturated solutions for some salts exceeds 0.25 M. In such cases it is difficult to calculate the thermodynamic solubility product by using the extended Debye–Hückel equation because the interionic forces decrease and the activity coefficient decreases less than expected (James, 1986). If these 5 salts are excluded the maximum variation in ΔG_{sol} becomes less than 3-fold and thus in the range of variation obtainable from the sum of ΔH_{fusion} and ΔG_{hyd-}. The variation in heat of vaporization, ΔH_{vap}, of the salts could possibly also account for the variation in ΔG_{sol}. However, ΔH_{vap} is not easily obtainable due to combustion of the salts near the boiling point. Alternatively, the present results are not fully adequate for describing ΔG_{sol} in terms of Eq. (6).

3.3. Multivariate data analysis

In previous studies the influence of lipophilicity (Jones et al., 1969), molecular size (Chowhan, 1978) and ionic charge of the counterions (Anderson, 1985) on salt solubility has been recognized. Thus, the Hansch hydrophobic parameter, π, (Fujita et al., 1964; Leo et al., 1971), the Charton steric parameter, v, (Charton, 1975), molecular weight (MW), and the electronic effect (pK_a) of the p-substituents of the benzoic acid derivatives were used in the multivariate data analysis (Table 2). By proper selection of the chemical nature of the p-substituents significant variation in the latter parameters was obtained. In addition to the descriptors relating to the physicochemical properties of the counterions, solid state and solvation properties of the salts were included in the analyses since these are expected to influence the salt solubility as indicated by Eq. (6). Thus, all descriptors presented in Table 1 and Table 2 were initially included in the statistical analyses. ΔG_{sol} was left out since it was calculated from the salt solubility (Eq. (7)). All descriptors were log transformed except for the following: Hansch's hydrophobic parameter, Charton's steric parameter, mp. salt and acid and IDR. A PCA was performed for the 22 salts using all the descriptors in order to check for molecular diversity. Projection of latent structures by PLS analysis was subsequently performed to investigate the relationship between the physicochemical descriptors and the solubility data of the salts. The predictive power of the PLS model was checked by cross-validation and response permutation.

3.3.1. Principal component analysis

A PCA model with two PCs was chosen. The PCs account for 61% of the variation. The scatter plot (Fig. 2a) implies that the molecular diversity of the salts is reasonable since these are uniformly distributed in the four quadrants. It is noticed that the benzylamine salts of the p-alkylbenzoic acids tend to scatter in the second quadrant whereas those of p-N and p-O derivatives prevail in the first and forth quadrant. Likewise, the salts containing halogen benzoic acids prevail in the third quadrant.

3.3.2. Partial least squares analysis

The 22 salts were all used as training set to perform a PLS analysis, and as a starting point the solubility of the salts was described as a function of all 11 descriptors. A model containing two PCs resulted in $R^2 = 0.86$ and $Q^2 = 0.72$. Inclusion of an additional PC did not improve the predictability. Evidently, the compounds tend to form a linear profile in the PLS scatter plot (Fig. 2b). The corresponding loading plot shows that the IDR and the intrinsic solubility of the acids, S_0, correlate positively with the solubility of the salt, S (Fig. 2c). Similarly, the Charton steric parameter, v, and the MW seem to correlate negatively with S. Validation of the model by response permutation gives intercept values of R^2Y (interc.) $= 0.28$ and Q^2 (interc.) $= -0.12$. These terms are a measure of the 'background' R^2Y and Q^2 and the values indicate that the model is valid (Eriksson et al., 1999). To obtain a simpler model involving fewer descriptors, the

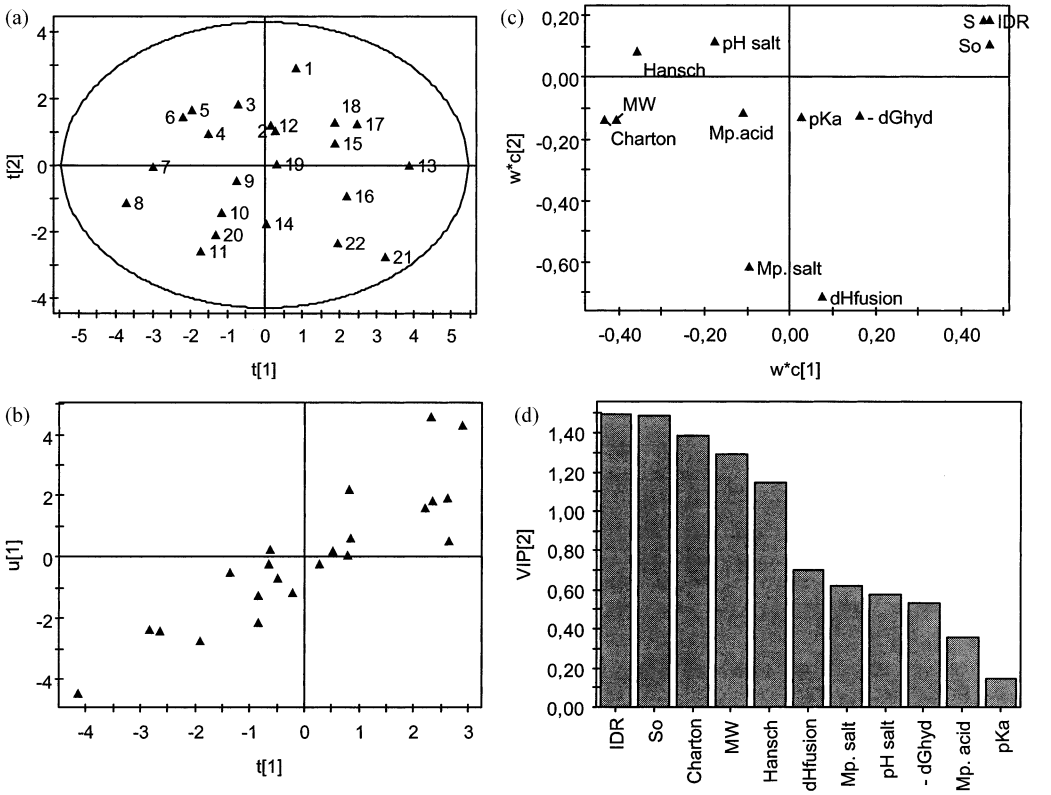

Fig. 2. (a) PCA scatter plot showing the two PCs, t[1] and t[2], plotted against each other. The ellipse corresponds to the confidence region (0.05). (b) PLS scatter plot of the first PC, t[1], against the aqueous salt solubility, u[1]. (c) Loading plot of w*c[1] against w*c[2] for the PLS analysis. PLS scatter plot of the first PC, t[1], against the aqueous salt solubility, u[1]. (d) Variable of importance in the projection (VIP) plot from the PLS analysis using all the compounds.

most significant descriptors were investigated according to variable importance in the projection (VIP) and the loading plot. From this analysis it appears that the IDR, v, S_0, MW, and Hansch are the most significant parameters, VIP > 1.0 (Fig. 2d). Terms having VIP > 0.8 are considered to be important (Eriksson et al., 1999). From the previous analysis (Fig. 1), it has already been shown that a significant positive correlation exists between IDR and salt solubility, S ($R = 0.97$), which is in accordance with that the two parameters are nearly superimposed on each other (Fig. 2b). The other correlations are less obvious, and are further dealt with in the following sections.

3.3.3. S versus S_0

An apparent bell-shaped profile was obtained by plotting salt solubility, S, against the intrinsic solubility, S_0, of the corresponding benzoic acid derivative (Fig. 3). The difference in salt solubility amounts to a factor of 75 whereas the benzoic acid intrinsic solubilities differ 1400-fold. From Table 1 it is apparent that most S_0 values are 'clustered' in the range 0.06–10 mM and that only 4 compounds exhibit intrinsic solubilities exceeding 10 mM. In confirmation of the shape of the curve it would have been desirable to include further data set for counterions having $S_0 > 10$ mM. However, we have not succeeded in obtaining additional benzylamine salts comprising p-substituted benzoic acids with S_0 values above 10 mM.

Interestingly, the salt solubilities of compound 13 and 17 are comparable to those observed for the salts derived from benzoic acid counterions characterized by $S_0 < 10$ mM, despite the fact that

the two former salts contain the most hydrophilic counterions. As indicated by Eq. (6) the unexpected low solubility of the two salts might reflect either a relatively low energy of hydration, ΔG_{hyd-}, (numerically) or a corresponding high crystal lattice energy, $\Delta G_{lattice}$, of the two compounds. It appears that the hydration energies of the two counterions are amongst the highest (numerically) ΔG_{hyd-} values for the series of *p*-substituted benzoic acid anions presented in Table 2. This is not surprising due to the hydrophilic nature of the OH and NH_2 substituents. Thus, the crystal lattice energy must be particularly high for these salts relative to that of the other salts in order to account for their solubility behaviour. Compound 13 does have a markedly high ΔH_{fusion} and melting point, which implies strong crystal lattice energies. Compared to the other salts, compound 17 does not have a significantly higher ΔH_{fusion} value, though the melting point is relatively high. Thus, the findings suggest that the salt solubility of compound 13 is limited by its relatively strong crystal lattice energy. However, the solubility behaviour of compound 17 seems to be more complex since the thermal results are ambiguous.

A linear correlation is observed between ΔG_{sol} of the salts of *p*-alkylbenzoic acids and the number of methylene units of the *p*-substituent (Fig. 4), supporting that the solubility of a salt tends to decrease as the counterion becomes more lipophilic. This is caused by either a decrease in the free energy of hydration, ΔG_{hyd-}, of the counterion or that the heat of fusion, ΔH_{fusion}, increases with increasing chain length. The thermal analyses show that ΔH_{fusion} and melting point for the salts actually decrease with increasing chain length (Table 1) suggesting that the crystal lattice energy of the salts tends to get weaker, reflecting inefficient crystal packing due to increasing flexibility (Yalkowsky, 1977). Thus, to account for the increasing free energy of solution, ΔG_{sol}, ΔG_{hyd-} has to decrease sufficiently (numerically) as the chain length increases in order to compensate for decreasing heat of fusion, ΔH_{fusion}. However, the calculated free energy of hydration is practically constant for the *p*-alkylbenzoic acids. This is somewhat surprising since there is a 1.5-2-fold difference in ΔG_{sol} between the salts of *p*-methyl- and *p*-butylbenzoic acid. Since the entropy of fusion, ΔS_{fusion}, calculated from ΔH_{fusion} and melting point, is almost constant for these salts, Eq. (6) seems to be inadequate in describing the apparent linearity between ΔG_{sol} and the number of methylene units.

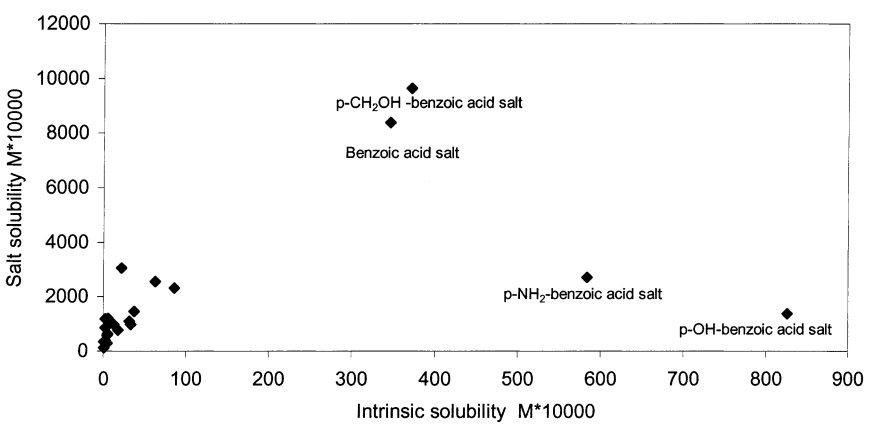

Fig. 3. Intrinsic solubility of the *para*-substituted benzoic acids (S_0) in 0.1 N HCl plotted against the aqueous salt solubility (S) using all the compounds (37 °C).

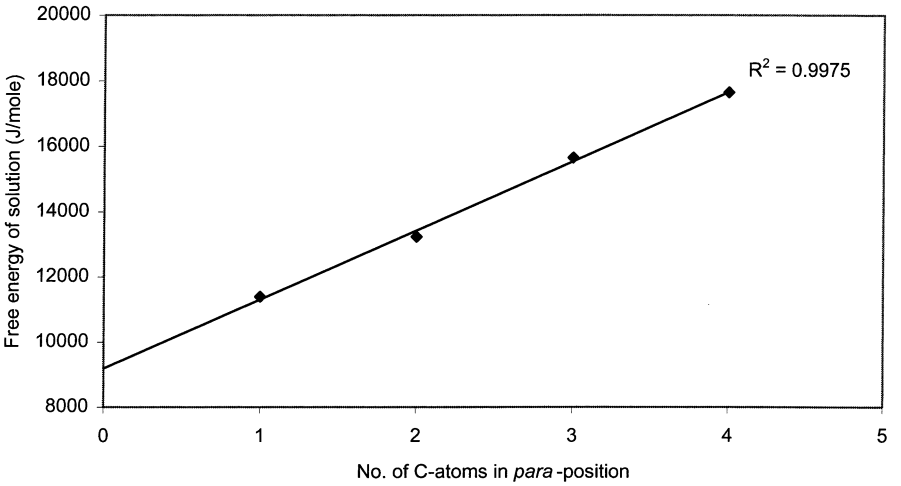

Fig. 4. Number of C-atoms of the counterion substituent plotted against the apparent free energy of solution of the corresponding benzylamine salt, ΔG_{sol}. (37 °C) ($R^2 = 0.998$).

3.4. PLS model development

It is desirable to obtain a model as simple as possible while maintaining a satisfactory validity. At the same time it would be preferable to have a model consisting of easily obtainable descriptors. On the basis of the VIP-values (Fig. 2d) simple and reasonable models can be derived which in some instances are better than the model comprising all descriptors (Table 3). Apparently, model 2 is the best model, as it exhibits the highest predictive ability (Q^2). Consequently, the combination of descriptors including the size and hydrophobic/hydrophilic character of the counterions together with the salt crystal lattice energy provides the best description and prediction of the solubility behaviour of the salts investigated. Furthermore, from the loading plot (Fig. 2c) it appears that the descriptors of model 2 contain different and significant information. So it appears adequate to combine these parameters in one model. The developed models shown in Table 3 indicate the importance of parameters which relate to the size and hydrophobic/hydrophilic character of the counterion, whereas the experimentally derived solid state properties of the salts (melting point and ΔH_{fus}) appears to be of less importance. Thus, reasonable predictions of salt solubility in the present series can be accomplished by using data for the counterion only. Apart from validation by response permutation and cross-validation (Q^2), the predictive power of the models has further been investigated by excluding a few representative compounds that were used as a test set. The rest of the compounds (the training set) were used to obtain a suitable model and afterwards the model was used to predict the solubility of the test set. The residual of the test set is a measure of the deviation between the experimental and calculated aqueous solubility of the compounds in the test set. In general, it is optimal to use a test set encompassing 6–7 compounds. However, in our case it was inappropriate to select more than three or four compounds in order not to weaken the parent structure of the model. To exclude clustering, the test set should be representative of the population and still contain some variation in solubility. On basis of the above considerations the following compounds were excluded: 2, 10, 17. The results of each model are presented in Table 3.

From Table 3 model 2 was expected to give the lowest residual value because of the highest Q^2 value. However, the predictive capability of the model is quite poor in comparison to the other more simple models. Apparently, model 6 has the best predictive power for the test set since RMSEP is the lowest. The parameters for model 6 can

Table 3
PLS models derived from the training set with and without test set

Model	Descriptors[a]	No. of components	R^{2c}	Q^{2c}	R^{2d} (test)	Q^{2d} (test)	RMSEP[b]
1	All	2	0.856	0.718	0.862	0.741	582
2	Hansch, ΔH_{fusion}, Charton, S_0	2	0.843	0.761	0.856	0.783	1418
3	Hansch, Charton, ΔH_{fusion}, MW	2	0.787	0.738	0.789	0.729	670
4	S_0, Hansch, ΔH_{fusion}	2	0.816	0.712	0.816	0.712	1947
5	Charton, ΔH_{fusion}, S_0	2	0.801	0.749	0.810	0.722	1207
6	Charton, Hansch, MW	2	0.732	0.698	0.736	0.695	576
7	Charton, S_0	1	0.737	0.720	0.733	0.683	922

[a] Descriptors used in PLS analysis.
[b] Root mean squared error of prediction set.
[c] All compounds were used as training set.
[d] Compound 2, 10 and 17 were used as test set.

easily be calculated by group contribution approaches, which make this model particularly attractive. However, one has to bear in mind that the results of RMSEP's are quite sensitive to the selected test set, and therefore it is not possible to generalise from these results that model 6 has the best predictive power. It can only be concluded that for the selected test set model 6 has the best predictive power. The general equations for model 6 and 7 (the most simple models) are given below:

Model 6

$$\log S = -0.58\ (\text{Charton}) + 0.24\ \log(S_0) + 3.10$$

Model 7

$$\log S = -0.50\ (\text{Charton}) - 4.47\ \log(\text{MW}) - 0.09\ (\text{Hansch}) + 14.2$$

4. Conclusion

Eleven descriptors, a priori expected to influence salt solubility, were initially used to construct a model for description and prediction of the aqueous solubility of 22 *p*-substituted benzoic acid benzylamine salts. Simple, predictive and moderately good models ($Q^2 = 0.70$ (model 6) and $Q^2 = 0.72$ (model 7)) consisting of two or three descriptors, respectively, were derived from the starting model by using multivariate data analysis. One of the derived models involves solubility prediction based on MW, the Hansch hydrophobic parameter and the Charton steric parameter (model 6). The greatest deviations from this model are observed for compound 13 and 17. The actual solubilities of the latter salts are lower than expected from the intrinsic solubility of their corresponding acid. The abnormal behaviour of these salts remains obscure. To this end, X-ray crystallographic analyses of the salts are presently carried out in order to gain a more in depth understanding of the solubility behaviour of the salts. Although, the solubility prediction of model 6 is fairly good it should be emphasized that the model has been derived using a series of *p*-substituted benzoic acid counterions endowed with a common skeleton in which significant molecular diversity was introduced by selection of *p*-substituents differing with respect to electronic, hydrophobic and steric effects as well as hydrogen bonding potential.

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