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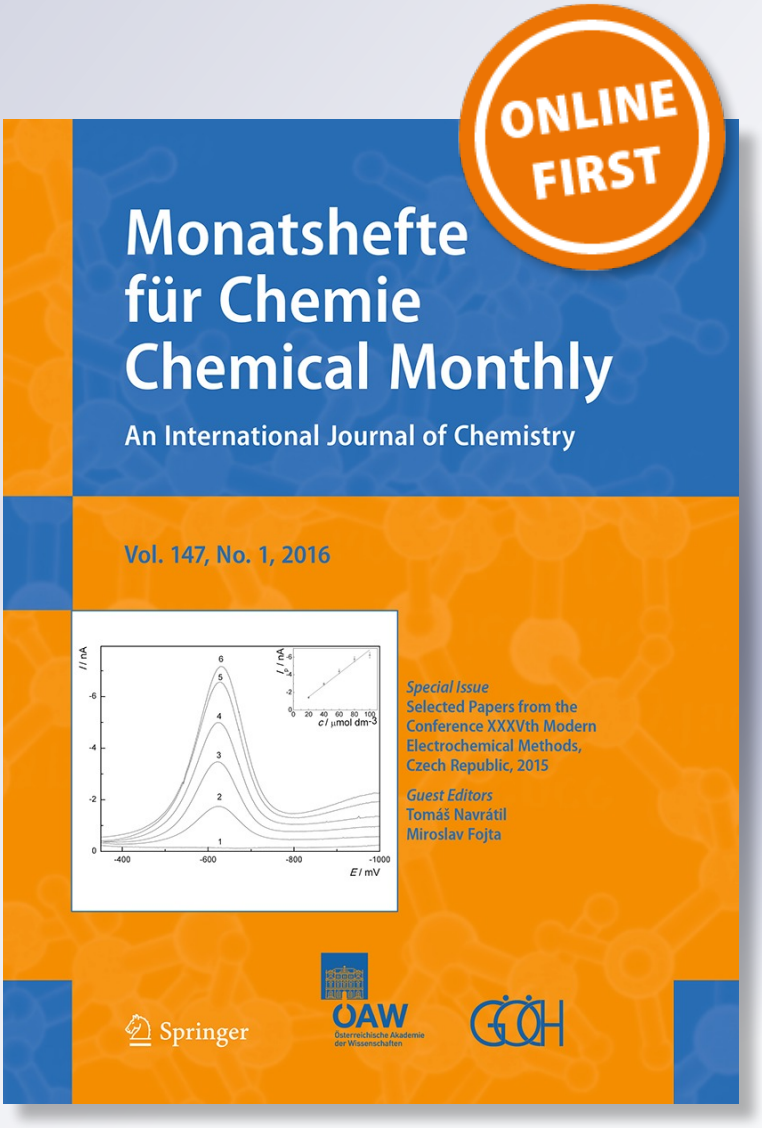

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ORIGINAL PAPER

Substituent effects on ionization constants as a predictive tool of coordinating ability

Valeria M. Nurchi[1] · Guido Crisponi[1] · Joanna I. Lachowicz[1] · Gavino Sanna[2] · Massimiliano Peana[2] · M. Antonietta Zoroddu[2]

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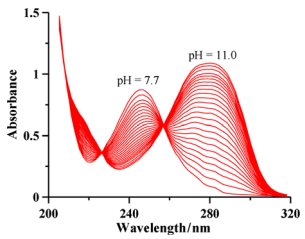
Abstract The ionization equilibria of a set of *ortho*, *meta*, and *para* substituted benzoic acids have been studied by spectrophotometric and potentiometric methods. A dual substituent analysis of the obtained ionization constants is presented, according to the Swain and Lupton procedure. This analysis allows to assign the weight of field and resonance contributions to equilibrium constants and, furthermore, it greatly contributes to forecast the effect of substituents on other correlated properties.

Graphical abstract



Keywords Benzoic acid · Potentiometry · Ionization · Substituent effect · UV–Vis spectroscopy

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Introduction

The use of chelating agents for iron and aluminium has found increasing attention in the last 30 years in different clinical applications [1–3]. To design and synthesize efficient chelators, the knowledge of proper coordinating groups and of the effect of substituents on chelating and pharmacokinetic properties is of paramount importance. Some simple moieties formed by two coordinating groups based on oxygen atoms have been taken into consideration. Among them the salicylic and catecholic moieties, largely studied for iron(III) and aluminium(III) coordination, attracted out research interest [4]. The coordinating ability of ligands toward iron and aluminium are strongly correlated with the ionization constants. A plot showing the linear correlation between ligand ionization constants and 1:1 iron complex formation constants was previously presented [3]. This correlation can be the basis for evaluating the coordinating properties of a ligand toward Fe(III) from its ionization constants. The knowledge of the substituent effects on ionization constants, obtainable in a simple and reliable way, allows thus to extend the gained information to coordinating ability. This permits a molecular design of new ligands, which take into consideration the effects of a given substituent, in a given ring position, not only on the chelating properties but also on the physical properties that determine the bioavailability of the drug and the pharmacokinetic properties, such as water solubility, octanol/water partition coefficient, and membrane permeability. A good approach to gain these information on the salicylic moieties consists in the separate evaluation of substituent effects on the constituent phenolic and carboxylic groups. The effects of nine different substituents in *ortho*, *meta*, and *para* on phenolic group were previously presented [5], and the trend of calculated p*K*s was explained in terms of Swain–Lupton

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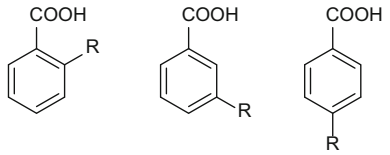
Scheme 1



R = H, Cl, Br, NO_2, CH_3, OCH_3, OH, $OCOCH_3$, $COCH_3$, NH_3^+, COO^-, COOH

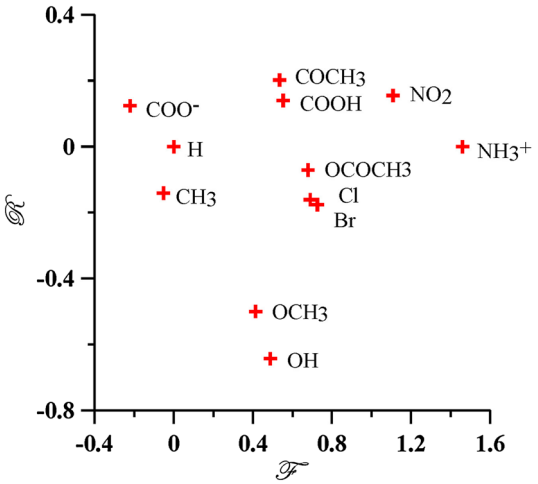


Fig. 1 Distribution of the substituents in a F–R plane

parameters [6]. The Swain–Lupton method, an extension of Hammett treatment, is a dual parameter approach to the analysis of the effect of substituents on the physico-chemical properties of molecules. It is based on two empiric parameters to which the significance of resonance (R) and field (F) parameters is applied. This procedure has been applied successfully in a number of fields [7, 8], as correlation analysis of NMR data [9–11], biological activity data [12], chemical reactivity [13], and acid–base strength [14]. In previous works, we used this method to correlate the pK values of a family of sulfonephthalein indicators [15], and the ionization properties of mercapto-carboxylic acids [16] with field and resonance variables. In the present study, we analyze the effects of substituents in *ortho*, *meta*, and *para* to a carboxylic group on its ionization. The steric hindrance and the possibility of intramolecular hydrogen bonding could perturb the results related to the *ortho* position (Scheme 1). The same nine substituents previously considered in the work on phenol ionization [5] were taken into account (Fig. 1).

The pK values of benzoic acids lie between 2 and 5. For this reason the use of potentiometry was not possible in some cases, being this technique ineffective to accurately measure ionisation constants with p$K < 3$. The high difference between spectra of acidic and basic forms of all the studied compounds made UV–Vis spectrophotometry a convenient procedure [17].

Results and discussion

With the use of fibre optic probe, it is possible to carry out spectrophotometric measurements directly into the titration vessel. This allows to obtain potentiometric and spectrophotometric data from the same titration of 20 cm^3 of ca. 1×10^{-3} M benzoic acids using a dip probe of 0.2 cm optical path length, provided that the absorbance values are not higher than 1.5 [20]. The spectrophotometric measurements were preliminarily evaluated by evolving factor analysis (EFA) [21] taking into account 50 experimental values equally spaced in the range p$K \pm 1$ [21]. The protonated and deprotonated forms of all ligands present different spectra. The principal band of the acidic forms at about 250 nm shifts to lower wavelengths due to ionization. Selected spectra among those collected during the titration of 4-hydroxybenzoic acid are reported as an example in Fig. 2a. The fully protonated molecule LH_2 exhibits a band centred at 252 nm; the ionization of carboxylic group induces a blue-shift of the band to 246 nm (Fig. 2a), whereas the successive deprotonation of the phenolic group produces a shift to higher wavelengths, giving a new band centred at 288 nm (Fig. 2b). Both equilibria are characterized by two sharp isosbestic points (221 and 247 nm the first, 227 and 258 nm the second), which remark the goodness of experimental data and the existence of simple two species equilibria.

These spectra were preliminarily processed by EFA (Fig. 2c). A least-squares procedure was then applied using the program HypSpec [22], which allowed to obtain the values pK_1 4.70, attributable to the carboxylic group, and pK_2 9.56, to phenolic group. The resulting speciation plot by HypSpec calculation (lines) and that obtained by EFA (symbols) are reported in Fig. 3.

Table 1 reports the ionization values of the carboxylic function of the 31 variously substituted benzoic acids. In the case of hydroxy and amino carboxylic acids, also the pK values of NH_3^+ and OH groups were evaluated (i.e., 4.77 (1), 4.55 (2), and 4.72 (1) for *ortho*, *meta*, and *para* aminocarboxylic acids, respectively, and 13.5 (1), 9.70 (3), and 9.25 (2) for *ortho*, *meta*, and *para* positions in hydroxycarboxylic acids, respectively. The pK value of 13.5, reported for salicylic acid, has to be cautiously considered: actually, it was not evaluated at ionic strength 0.1 M since we had inevitably to add KOH 1 M; moreover proton concentration was evaluated from added base using the K_w estimated at 0.1 M ionic strength.

A comparison between our results and literature values from IUPAC Stability Constant database [23] shows a

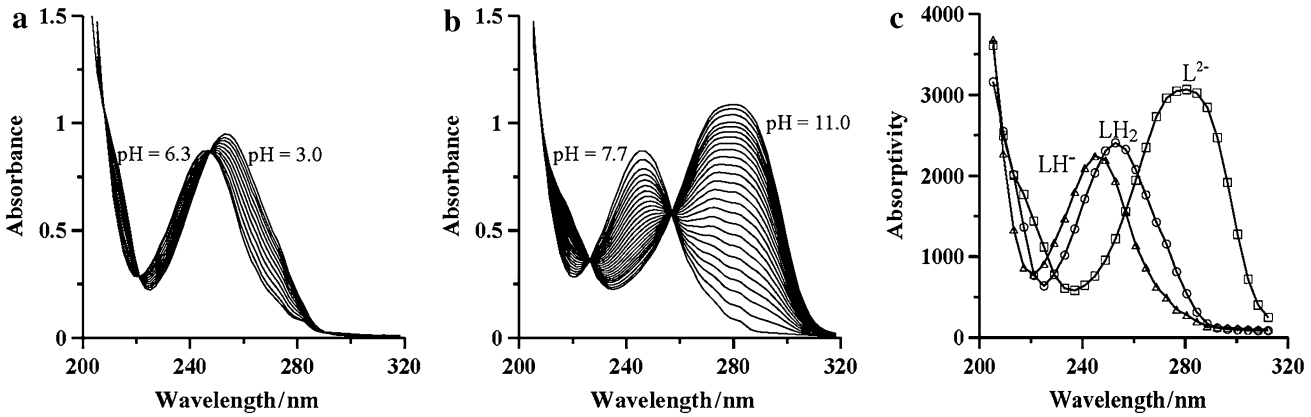


Fig. 2 **a** UV spectra of 4-hydroxybenzoic acid 4.1×10^{-4} M measured with a 0.2 cm fiber optics probe in the pH range 4.1–5.7; **b** spectra relative to the ionization of phenolic group in the pH range 8.2–10.4; **c** absorptivity spectra of the differently protonated forms of 4-hydroxybenzoic acid obtained by EFA

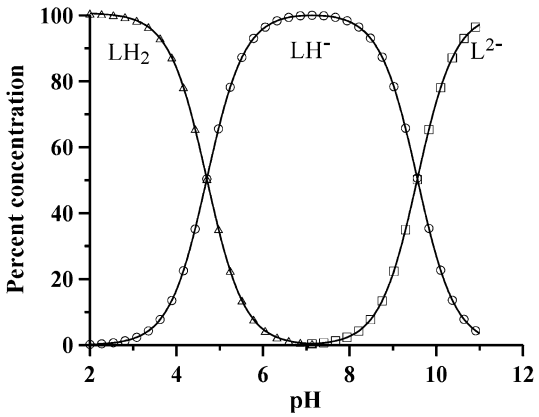


Fig. 3 The distribution curves for the first and second ionisation of 4-hydroxybenzoic acid resulting from HypSpec (*lines*) and from EFA (*symbols*) calculations

Table 1 Ionization p*K*s of the carboxylic groups for the different substituted benzoic acids at 25 °C and 0.1 M KCl ionic strength

Substituent	*ortho*	*meta*	*para*
H	4.07 (1)	4.07 (1)	4.07 (1)
Cl	2.96 (4)	3.70 (1)	3.97 (2)
Br	2.69 (7)	3.77 (1)	3.86 (3)
NO_2	2.17 (7)	3.24 (2)	3.38 (3)
CH_3	3.89 (1)	4.25 (1)	4.41 (2)
OH	2.94 (1)	4.06 (2)	4.46 (3)
OCH_3	3.86 (1)	3.87 (1)	4.26 (1)
$OCOCH_3$	3.59 (3)	3.83 (1)	3.88 (1)
$COCH_3$	3.43 (2)	3.69 (3)	3.61 (3)
NH_3^+	2.14 (2)	3.26 (3)	2.43 (3)
COOH	2.82 (3)	3.52 (5)	3.58 (3)
COO^-	5.14 (2)	4.37 (3)	4.58 (5)

general agreement, even if in some cases literature data are rather scattered, or are lacking.

The influence of the substituents on p*K* values was evaluated by the dual substituent analysis of Swain and Lupton [6]; these authors redefined the substituent parameter, σ, in the Hammett treatment. This was done considering that two Resonance (R) and Field (F) variables are necessary to describe the effects of any given substituent. The resonance parameter R takes into account both the electron-donating and the electron-accepting ability, and the field variable F is defined to take into account all the inductive and pure field effects. Since these two effects are assumed to act in an independent way, the Swain–Lupton treatment assumes the form $Y = Y^\circ + a\mathrm{F} + b\mathrm{R}$; where Y is the described physical property (in our study the p*K*), Y° is the physical property in absence of substituents, and a and b are the intensive factors that quantify the field and resonance contributions to the physical property.

The results relative to the three positions (*ortho, meta*, and *para)* with respect to the carboxylic group are presented in Table 2. These values a and b allow to quantify the field and resonance contributions to ionization constants for a given substituent position according to the relations

$$C\mathrm{F} = \Sigma|a\mathrm{F}|/(\Sigma|a\mathrm{F}| + \Sigma|b\mathrm{R}|)$$

$$C\mathrm{R} = \Sigma|b\mathrm{R}|/(\Sigma|a\mathrm{F}| + \Sigma|b\mathrm{R}|)$$

The p*K* values calculated from the Swain–Lupton parameters reported in Table 2 have been reported vs. the experimental p*K* values (Table 1) in Fig. 4.

This presentation visualizes the effectiveness of dual substituent analysis. It is evident that, while the results for *meta* and *para* positions are in good agreement with experimental data, those relative to the *ortho* position are quite scattered on the line $\mathrm{p}K_{\mathrm{calc}} = \mathrm{p}K_{\mathrm{exp}}$. The lack of effectiveness in reproducing experimental results for *ortho*

Table 2 Results ($Y°$, a, and b) of the dual substituent Swain–Lupton analysis applied on the pK values in Table 1

Position	$Y°$	a	b	$C_F^{\%}$	$C_R^{\%}$
ortho	4.204	−1.642	0.259	93.6	6.4
meta	4.128	−0.632	−0.206	87.6	12.4
para	4.431	−1.065	−0.446	84.7	15.3

The last two columns report the field and resonance percent contributions $CF^{\%}$ and $CR^{\%}$

Corresponding field and resonance percent contributions for phenols were 79.8−20.2 for *ortho*, 82.2−17.8 for *meta*, and 50.5−49.5 for *para* position

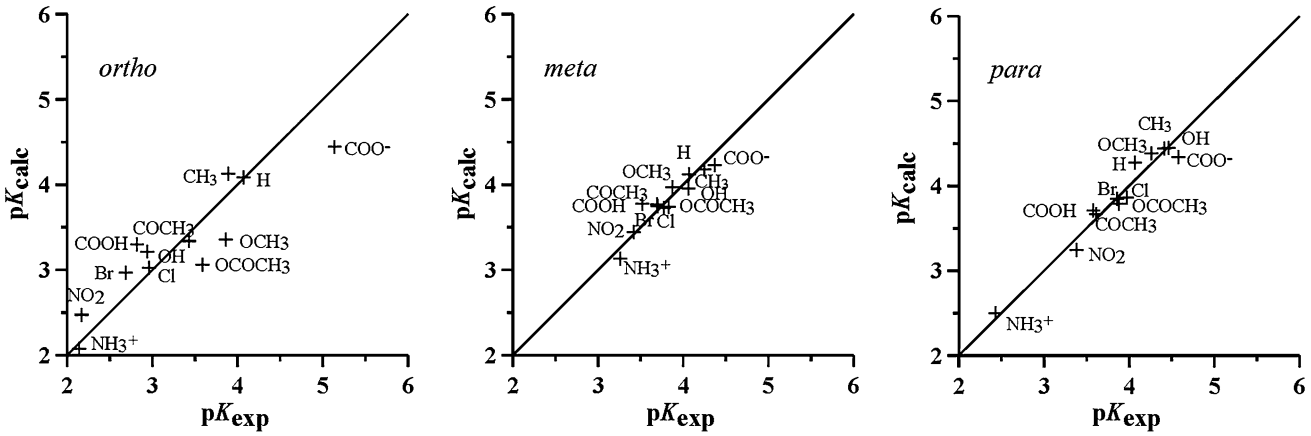
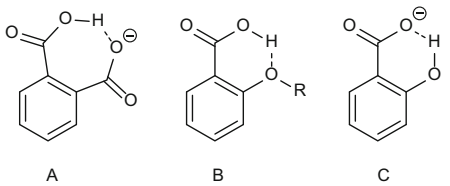


Fig. 4 Calculated pK values (with the Swain–Lupton parameters in Table 2) vs. experimental pK values (Table 1) for *ortho*, *meta*, and *para* substituents

Scheme 2



A) Phthalic acid; B) *o*-Anisic acid (R=CH_3), acetylsalicylic acid (R=$COCH_3$); C) Salicylic acid

position presumibly depends, for a number of substituents, on an additional effect connected to the formation of intramolecular hydrogen bonding (Scheme 2).

The first deprotonation of benzene-1,2-dicarboxylic acid (phthalic acid) takes place with a pK value of ca. 0.4 units lower than that expected because of the hydrogen bond stabilization of LH^- form in Scheme 2A. This stabilizing effect further contributes in the second deprotonation step to increase the pK value of ca. 0.7 units. As far as regards 2-methoxybenzoic acid (*o*-anisic acid) and 2-acetoxybenzoic acid (acetylsalicylic acid) in their fully protonated form LH, they also are stabilized by the intramolecular hydrogen bonding depicted in Scheme 2B, resulting in a pK increase of ca. 0.5 units. On the contrary, in 2-hydroxybenzoic acid (salicylic acid) the hydrogen bonding between benzoate and phenolic groups makes easier the carboxylate deprotonation, lowering its pK of ca. 0.3 units.

Taking into account that hydrogen bonding acts by both lowering and increasing the expected pK values, the obtained regression coefficients a and b can, at any rate, be considered reliable, and, as a consequence, also the estimates of field and resonance contributions. The field contribution is always prevalent, and decreases with the distance of the substituent, passing from 93.6 % in *ortho* position to 87.6 % in *meta* and 84.7 % in *para*. The prevalent dependence on the field contribution is well

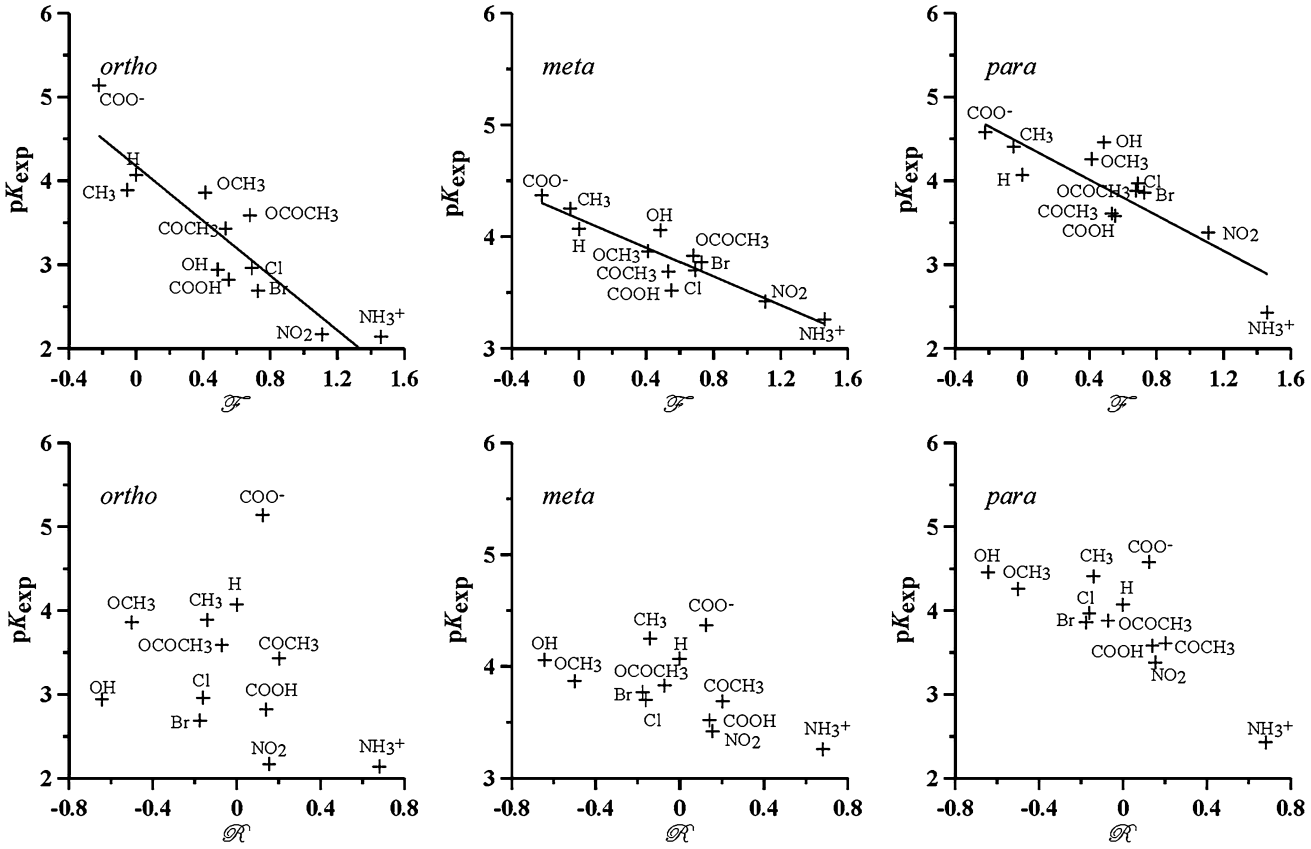


Fig. 5 Experimental p*K* values (Table 1) for *ortho*, *meta*, and *para* substituents are reported vs. the F Swain–Lupton parameters (*upper plots*), and vs. the R Swain–Lupton parameters (*lower plots*)

observable in Fig. 5, where the measured p*K* values are reported as a function of Swain–Lupton F and R parameters in the upper and lower plots, respectively; in fact, while a negative linear correlation between the p*K* values and the F parameter is clearly visible for all the three *ortho, meta*, and *para* positions, no correlations between the p*K* values and the R parameter are observed, except for *para* position.

The evaluation of F and R contributes in benzoic acids can be compared with the analogous evaluation in substituted phenols presented in an our previous study [5]. While the field contributions in *meta* position are comparable (87.6 for benzoic acids and 82.2 for phenols), a marked decrease is observed in *ortho* and *para* positions passing from benzoic acids to phenols (93.6–79.8; 84.7–50.5), with the corresponding increase of resonance contribution. The further –C–C– bond connecting the aromatic ring to the oxygen atom linked to the acidic proton damps the resonance contribute of the substituents to the oxygen basicity.

As a concluding remark, we can observe that the presence of substituents leads to a general decrease of the carboxylic group basicity (lower p*K*), except for CH_3 and COO^- in all the positions, and OH and OCH_3 in *para* due to the resonance effect. The increase of basicity surely will act positively on iron(III) binding properties. As a general rule, the increase of iron(III) complex formation constants does not necessarily imply better iron chelating ability at physiological pH, since this depends on the proton competition for the same coordination sites. To take into account this competition, as well as different possible stoichiometry of the complexes, Harris et al. [23] introduced the concept of pFe, defined as "the concentration, expressed as negative logarithm, of free iron(III) in a solution at pH 7.4 that is 10 μM in ligand and 1 μM in metal".

In benzoic acids, which are totally dissociated at pH 7.4, any increase of basicity lead to an increase to iron coordination expressed as pFe. The case of chelating agents constituted by at least two different coordination groups shows a more complex trend: in an acid as 1,2-benzenedicarboxylic acid the proximity of the two carboxylate groups at pH 7.4 strengths the chelating efficiency for their mutual positive influence. On the contrary, in the case of salicylic acid, the increase of basicity of both O^- and COO^- groups is contrasted by the high p*K* value of the phenolic group that favours proton competition. In such a situation, substituents as nitro group that strongly decreases

the basicity of phenolate can favour an increase of pFe [25].

To conclude, we want remark here that in this work we obtained a good reproduction of the data by the Swain and Lupton treatment thanks to the number and to the variety of substituents. On the basis of the results of the applied dual substituent analysis ($Y°$, a, and b), the evaluation of the effect of any substituent on the ionization constant of carboxylic group (as well as on iron(III) and aluminium(III) complex formation constants) results possible and advantageous before the synthesis and the experimental studies on new ligands.

Experimental

Benzoic acids (Scheme 1), KCl, and KOH were purchased from Sigma–Aldrich. Hydrochloric acid was purchased from Fluka. All the products were used without any further purification. Carbonate free KOH solution 0.1 M was prepared using the method described in literature [18].

Spectrophotometric–potentiometric measurements

Ionization equilibrium studies were carried out as described in a previous publication, at 25 °C and 0.1 M KCl ionic strength, using a 0.2 path length dip probe [10]. The glass electrode was daily calibrated by titrating HCl 10^{-3} M with KOH 10^{-1} M at 0.1 M KCl ionic strength. Ligand concentration ranged from 3×10^{-4} to 3×10^{-3} M. UV–vis spectrophotometric measurements were performed in the 200–400 nm spectral range. Ionization data were analyzed using the HypSpec and Hyperquad programs [19].

Choice of the substituents

The same set of substituents used in a previous our study (i.e., the phenol ionization equilibria [5]) were used also for this research, with the sole exception of the group $–COOCH_3$ that was replaced by the acetoxy group, $–OCOCH_3$. In the previous study on phenols, the amino group was in the NH_2 form during OH deprotonation, while in the present case it is in the NH_3^+ form during –COOH deprotonation. In fact, the deprotonation of NH_3^+ group is characterized by a pK of ca. 5, higher than that of carboxylic group and lower than that of phenols. Furthermore, the three 1,2-dicarboxylic acids, i.e. *o*-, *m*-, and *p*-phthalic acids have been studied. This implies that two different ionization constants are determined for each phthalic acid. In the case of the first ionization the substituent is the fully protonated carboxylic group –COOH, while in the second ionization the substituent is the carboxylate group $–COO^-$.

These functional groups are characterized by a large variability of inductive and resonance Swain–Lupton parameters (Fig. 1), which allows a statistically reliable analysis of substituent effects.

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