

Acid–base chemistry of red wine: analytical multi-technique characterisation and equilibrium-based chemical modelling

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Abstract

In this paper an attempt is made to model the alkalimetric titration trend ($\text{pH} < 7$) of a series of red wines on the basis of an equilibrium-based calculation involving as input the chemical equilibria of the acid–base active substances, whose analytical concentration was determined in each wine under consideration. The chemical model (14 acid–base active substances and 22 protonation equilibria were considered) has been built in reliable conditions with respect to the chemical environment of the fluids under study, in order to describe the acid–base properties at a thermodynamic level using specific professional software. In this connection, protonation constant values of the substances involved were necessary, paying attention to: solvent composition, background salt, ionic strength and temperature. A series of synthetic solutions (based on mixed ethanol/water solvents at known ionic strength) was then pH-metrically titrated (25°C) in order to refine suitable protonation constant values.

Analytical measurements of carboxylic acids, amino acids, inorganic anions and metals were executed to know the concentration of a series of acid–base active reactants ($\text{pH} < 7$) and the ionic strength state of each wine. Finally, a comparison between the experimental trend of the alkalimetric titration of each wine and the calculated one via the chemical model tested by means of a computer-assisted simulation has been performed. As an overall check of our job, the charge balance principle was adopted. During the alkalimetric titrations of each wine conductometric detection was performed as well, with the aim of confirming the values of ionic strength calculated using both analytical and equilibrium concentrations. The agreement we found when comparing the calculated values of ionic strength with varying pH with the trend of the conductometric signal can be considered as a significant validation of the chemical model proposed. The jump from the analytical to the equilibrium composition may allow the prediction of the effect on red wines consequent to chemical actions (addition of substances) or natural phenomena.

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1. Introduction

It is well known that grape table wines are actually of great importance from technological merchandise and nutritional points of view [1]. Homogeneous and heterogeneous chemical equilibria are involved with several modifications occurring during the life of wines. Alcoholic, malo-lactic and acetic fermentation and potassium hydrogen tartrate precipitation are common chemical phenomena whose course gives rise to significant acid–base modifications of the products. On the other hand, the addition, even if illegal, of some

substances (salicylic acid, as an example) might induce alterations of the acid–base equilibria in solution. Hence, we can imagine it should be possible to reveal some chemical frauds, or other treatments forbidden by law, thanks to the careful knowledge of the acid–base related chemical equilibria. Moreover, the natural evolution of wines, to produce vintage and expensive wines appreciated all over the world, may be scientifically monitored in order to enhance the probability to obtain a product of very high quality. On the other hand, a lack of information on the stability of soluble complexes in the chemical environments corresponding to red wine characteristics has been underlined in the literature [2,3].

Many aspects of the chemistry of wine can be evidenced only on the basis of a chemical model (optimised by a

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computer-assisted procedure), built starting from in-depth knowledge of thermodynamic features of the substances involved in the fluids under investigation. In fact, the most of the species involved in the acid–base chemistry of wine cannot be regarded as static molecules. They are present in solution according to the network of multiple chemical equilibria, as a function of the pH value of the fluid.

In this paper an attempt is made to interpret the alkalimetric titration trend ($\text{pH} < 7$) of four red wines on the basis of an equilibrium calculation starting from the knowledge of the chemical equilibria involved with the acid–base active substances, whose analytical concentration was quantified in each wine under consideration. In order to build a chemical model, able to picture at a thermodynamic level the acid–base chemistry of red wine, what was lacking until now is:

- the knowledge of thermodynamic quantities coherent with the average chemical environment of red wine;
- the knowledge of the average ionic strength value of red wines, mainly due to metal ions, inorganic anions and carboxylate compounds;
- the treatment at the same time of all the acid–base equilibria hypothesised, in order to calculate the pH value as a function of the base added, to be compared with the experimental one.

A three-step work has consequently been planned to reach our objectives:

- analytical measurements to evaluate the concentration of metal ions, inorganic anions and carboxylate compounds;
- equilibrium measurements on wines (to record the alkalimetric titration curves) and on synthetic solutions (to evaluate protonation constants);
- comparison between experimental and calculated pH values of each wine under investigation (chemical modelling step).

pH-metric measurements were performed at 25°C in mixed water/ethanol solvents. By considering that in the range $12 \pm 1\%$ of ethanol we can find the most of the commercial table wines, in our experiments on synthetic solutions the percentage of ethanol was fixed at 8, 12 and 16. As far as the electrolytes employed as background medium is concerned, an inspection of the literature data on wines [4] suggests to add KCl in the range from 0.05 to 0.1 M, in order to reproduce the natural conditions of salinity during both the calibration process of the glass electrode and the alkalimetric titration of the acids.

Finally, the chemical model was further tested for each red wine: (a) by comparing the ionic strength trend calculated as a function of pH with that of the conductometric readings recorded during the alkalimetric titration; (b) by checking the charge balance.

2. Experimental

2.1. Chemicals

Carboxylic acids. 2-Ketoglutaric, skikimic, lactic, citramalic and gallic acids were from Sigma, tartaric and succinic acids were from Carlo Erba, malic, acetic, citric and ascorbic acids were from Merck, *cis*-aconitic, oxalacetic, glyoxilic monohydrate, *p*-hydroxybenzoic and fumaric acids were from Fluka.

Amino acids. L-Proline was obtained from Sigma.

Salts. KCl (Carlo Erba), KH_2PO_4 (Sigma) $(\text{NH}_4)_2\text{SO}_4$ (Carlo Erba), potassium hydrogenphthalate (Carlo Erba), Na_2CO_3 (Carlo Erba).

Solvents. Methanol was from Labochem, ethanol (96% solution) was from Merck.

Other reagents. H_2O_2 (30%, v/v) was from Fluka, phosphoric acid (85%, v/v) was from Carlo Erba, nitric acid (85%, v/v) was from Merck.

The purity of all chemicals employed was of analytical grade; standardisation process were performed all over it was imagined as necessary to achieve high-quality results.

Standard solutions (1000 ppm) for HPLC/IEC, F-AAS and AES-ICP measurements of metals were from Merck. Standard solutions of NaOH, KOH, HCl and HNO_3 were prepared by diluting concentrated Merck ampoules and were standardised against potassium hydrogenphthalate or sodium carbonate, respectively.

Grade A glassware and deionised and twice distilled water were used for all solutions.

2.2. Potentiometric apparatus

The potentiometric measurements were performed at $T = 25 \pm 0.1^\circ\text{C}$ and ionic strength $I = 0.05$ or 0.1 M (KCl) with $\text{C}_2\text{H}_5\text{OH}$ (EtOH) at 8, 12 or 16% level with a model 713 Metrohm potentiometer equipped with combined glass electrode. The titrant was dispensed with a model 765 Dosimat burette by Metrohm. The couple was calibrated in $-\log[\text{H}^+]$ units (pH) employing alkalimetric titrations of hydrochloric acid with standard, carbonate free, potassium hydroxide. Ionic strength, ionic medium and ethanol percentage of the calibrating solutions were the same as the solutions being examined. The alkalimetric titrations were carried out in a stream of purified nitrogen gently bubbled in the titration cell. Temperature control was achieved by means of a liquid circulation, in the outer chamber of the titration cell, of water from a model D1-G Haake thermocryostat. Each titration was at least twice repeated.

2.3. Chromatographic apparatus

As far as carboxylic acids and proline is concerned, the analysis was carried out with a Perkin Elmer chromatograph equipped with a series 200 LC pump. The injector was a reodyne valve with a $20\ \mu\text{l}$ sampling loop. The detector was

a model LC-290 Perkin Elmer UV-Vis photometer, connected with a PE Nelson computing integrator. The chromatographic separations were performed on a Merck Superspher 100 RP-18 end-capped (250 mm length and 4 mm ID) spherical phase column.

As for inorganic anions, analysis was carried with a Metrohm 690 Ion Chromatograph equipped with a Bischoff HPLC pump. The chromatographic separations were performed on a PRPTM-X100 column [poly(styrene-divinylbenzene)-trimethylammonium exchanger spherical phase column].

2.4. Chromatographic conditions

As for carboxylic acids and proline, an HPLC/RP separation was executed. The mobile phase, according to the method of Tousseau and Benoit [5], was composed of 70 g/l (0.52 M) potassium dihydrogen-phosphate and 14 g/l (0.10 M) ammonium sulphate adjusted to pH 2.1 with phosphoric acid, in order to have the highest protonation degree of the acids under examination. The flow rate was 0.8 ml/min at room temperature. Detection was effected by measurement of the UV absorption at $\lambda = 210$ nm.

As for inorganic anions, an HPLC/IEC separation was performed at pH ≈ 8 . The mobile phase was composed of 4 mM *p*-hydroxybenzoic acid and 1% methanol adjusted to pH 8.5 with NaOH (in order to have maximal deprotonation of the analytes). The flow rate was 1.5 ml/min at room temperature.

2.5. AES-ICP and Flame-AAS apparatus

A model Liberty 2 AES-ICP and a model Spectra 10 F-AAS Varian were used to determine metals in wine.

2.6. Conductometric apparatus

A model 160 Amel conductivity-meter, equipped with a model 196 Amel electrode, was used as indicating device during alkalimetric titration of wines ($T = 25^\circ\text{C}$).

2.7. Data analysis and calculations

The non-linear least squares computer program ESAB2M was used to evaluate the purity of the reagents (starting from acid–base titration data) and to refine all the parameters related to the calibration of the electrode system [6]. The protonation constant values were expressed by the general formula: $\beta_{pq} = [L_p H_q] / [L]^p [H]^q$ and refined (as $\log \beta^H$) by means of the BSTAC program, which minimises the error squares sum on electromotive force values and is able to take into account (if desired) eventual variations of ionic strength among and/or during titrations [7]. Distribution diagrams and simulated titration curves were obtained using the computer program ES4ECI [7]. The electrode response in the alkaline region was verified at each job by refin-

ing the pK_w value, that resulted, as an average value of at least five replicates, as follow: in KCl 0.05 M medium we have 13.806, 13.934, 14.000 and 14.069 at the percentage (v/v) of ethanol of 0, 8, 12 and 16, respectively; in 0.1 M KCl medium the corresponding values are 13.787, 13.915, 13.972 and 14.036. Uncertainty in pK_w value was estimated as ± 0.006 ($\pm 3s$, s : standard deviation).

Each substance determined via HPLC was identified by its retention time in comparison with the response of standard solution of pure compounds. Standard addition of some substance to the wine was performed in order to verify the attribution of the peak. Plot of the integrated peak area and/or height against concentration (ppm) of each molecule (or ion) was always linear (the correlation coefficient r ranged between 0.9997 and 0.9999) in the concentration range investigated. As for determination of metal ions, the same criterion of quantification based on external standard was followed.

3. Sample handling

3.1. Sample storage

Four red grape table wines were analysed in this paper. These wines are products from Piemonte (North-West of Italy) and are named Grignolino and Barbera. Two vintages were considered for each wine, so we have: Grignolino 1999 and 2000 (henceforth: G99 and G00, respectively) together with Barbera 1999 and 2000 (henceforth: B99 and B00, respectively). The wines were stored at room temperature, in dark place, and sub-divided in little bottles, to avoid air contact and other contaminations.

3.2. Preliminary treatments for HPLC measurements

The samples were filtered through a 0.45 μm Millipore filter and diluted 1:20 (v/v) with deionised and bidistilled water. Then, they were treated by means of Chromabond C18 (500 mg) MACHEREY-NAGEL cartridges, which allow to purify the matrix of those molecular compounds (polyphenols and saccharides, as an example) which can interfere in the chromatographic measurements. This treatment does not alter the carboxylic acid composition of the samples, as verified by means of a check on synthetic mixtures. Each cartridge was conditioned with little volume (few ml) of water and then with little volume (few ml) of ethanol, before sample purification procedure.

3.3. Preliminary treatments for titrations

First, each wine was filtered through a 0.45 μm Millipore filter; then, before the acid–base titration, CO_2 was removed by means of strong stirring under vacuum (few minutes), according to the indications of official methods [8].

3.4. Preliminary treatments for metal measurements

Potassium, sodium, calcium and magnesium were determined by F-AAS technique after suitable dilution of each sample, depending upon the nature of the analyte. Before the measure each solution was added of caesium and lanthanum salts to avoid interference due to ionisation or to refractory species formation, respectively. The other metals were determined by AES-ICP, after incineration (600 °C for one night) and dissolution of ashes in aqueous HCl (37%), except for volatile metals, for which a wet two-step treatment with HNO₃ (70%) and H₂O₂ (30%, v/v) reactants was employed to avoid loss of analytes.

3.5. Solution preparation

The solutions prepared by mixing water and ethanol, mainly used for titrations, show a little contraction in volume, experimentally evaluated (with an appreciable uncertainty, since the observed variations of volume are of very small entities) by means of a calibrated volumetric flask. The slight contraction in volume (around 0.3–0.4%) corresponding to the water/ethanol mixture formation has been considered in all measurements and calculations.

4. Results and discussion

4.1. Analytical determinations

4.1.1. AES-ICP and F-AAS determination of metals

Table 1 shows the results from atomic techniques for metal ions in wines.

Table 1
Concentration (mg/l) of the metal ions in each wine under investigation^a

Metals	G99 (mg/l)	G00 (mg/l)	B99 (mg/l)	B00 (mg/l)
Al	10.5 (2)	11.8 (3)	12.5 (3)	12.4 (3)
Ba	0.87 (3)	0.80 (5)	1.11 (4)	0.90 (4)
Ca	48.9 (2)	44.9 (2)	53.3 (2)	36.9 (1)
Cd	<d.l.	<d.l.	<d.l.	<d.l.
Co	<d.l.	<d.l.	<d.l.	<d.l.
Cr	11.0 (2)	16.2 (2)	16.6 (1)	14.5 (2)
Cu	0.09 (2)	0.11 (2)	0.55 (3)	0.26 (3)
Fe	3.03 (3)	2.96 (3)	2.95 (2)	3.72 (2)
K	477.0 (9)	766.4 (8)	375.4 (8)	778.1 (7)
Li	0.006 (2)	0.007 (3)	0.007 (3)	0.012 (3)
Mg	59.3 (3)	63.5 (3)	79.5 (2)	84.8 (3)
Mn	0.69 (5)	0.89 (4)	1.20 (6)	1.17 (4)
Na	2.85 (5)	2.86 (4)	4.11 (4)	3.93 (4)
Ni	0.002 (2)	0.025 (2)	0.046 (3)	0.035 (4)
Pb	0.38 (6)	0.06 (5)	0.13 (5)	0.15 (4)
Sr	0.965 (5)	0.513 (7)	0.903 (7)	0.812 (6)
Zn	0.11 (1)	0.37 (2)	1.06 (1)	0.61 (2)

^a The uncertainty (three replicates) is reported in parentheses as \pm (standard deviation) in the last significant digit.

4.1.2. Chromatographic determinations

To measure carboxylic acids we chose to adopt a method without derivatisation (avoiding contamination, loss of analytes and time-consuming steps of analysis) based of a classical RP separation (as in our previous paper [9]), fortified by an end-capped column with a spherical phase, and on the UV detection ($\lambda = 210$ nm). Moreover, the conditions adopted allowed us the simultaneous determination of the proline (see Fig. 1 for a standard run). Unfortunately, a co-elution of acetic and 2-ketoglutaric acids is observed under the adopted experimental conditions. A peak at $t_r = 7.17$ min appear in the chromatogram with a shoulder (see Fig. 2 for a run on a wine of ours). The superimposition was solved by means of a deconvolution technique. First, the non-resolution of the HPLC signal was successfully checked using the data of standard runs (both areas and heights of peaks). Then, starting from the known value of the concentration of acetic acid from titration of the volatile acidity, the concentration of the 2-ketoglutaric acid was easily calculated. Table 2 shows the results from HPLC/RP analysis of carboxylic acids and proline together with the results from HPLC/IEC analysis of both hydrogenphosphate and sulphate anions. As for nitrate ions, we found only 1.57 ppm of NO₃⁻ in G00.

4.1.3. Volume alcoholometric proof (alcoholic grade)

The composition of the hydro-alcoholic medium, namely the volume alcoholometric proof, was determined (results are in Table 3) according to the official method [8] by means of distillation and further density measurement (20 °C).

4.2. Equilibrium determinations

4.2.1. Volatile acidity

Volatile acidity was determined according to the official method by means of preliminary distillation and visual (phenolphthalein as indicator) acid–base titration [8]. CO₂ and SO₂ contribution were excluded, as indicated by the official definition of volatile acidity: CO₂ was previously removed while SO₂ (both free and combined) was measured (by titration with I₂) and subtracted (very little contribution). We checked by means of a blank analysis that lactic acid is not distilled under the adopted experimental conditions.

4.2.2. Alkalimetric titration of wines

CO₂ was preliminarily removed by means of strong stirring under vacuum. During the alkalimetric titration of each wine we found the first inflection point at about pH 7.5, as expected. The strong base used up to this flex allows the calculation of the total acidity parameter (C_H), usually expressed as g/l of tartaric acid (Table 3), fundamental in the chemical modelling step of this work. During the alkalimetric titration of each wine we found a second roughly detectable inflection point at about pH 10.5. It should be attributed to chemical compounds with high values of protonation constants, namely, biogenic amines, amino acids (amino group) and

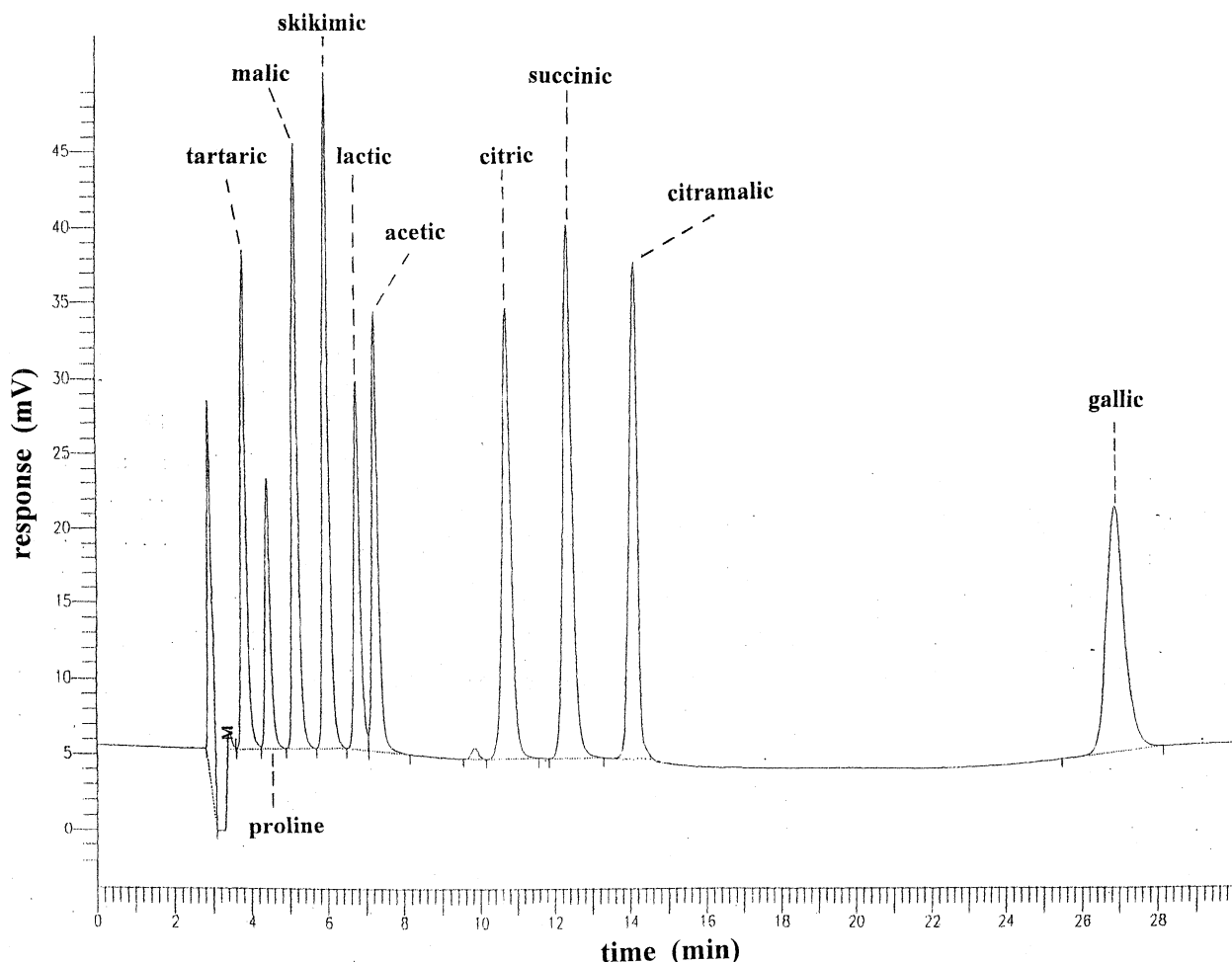


Fig. 1. HPLC/RP separation of standard carboxylic acids and proline. Concentrations in ppm are: tartaric acid 100, proline 250, malic acid 250, skikimic acid 5, lactic acid 250, acetic acid 300, citric acid 250, succinic acid 550, citramalic acid 300, gallic acid 2.5.

phenolic compounds. These compounds do not affect significantly the total acidity value, since they are fully titrated only when the second inflection point, at $\text{pH} \approx 10.5\text{--}11$, is reached. Moreover, the detection of this second inflection point is quite critical (particularly for B99 and B00, whose titrations were stopped at about $\text{pH} 9$). Redox and/or precipitation phenomena (oxidation of polyphenols and so on) are superimposed to acid–base reactions and do not allow this segment of the titration curve to be recorded with accuracy and reproducibility. On the other hand, this behaviour is a confirmation of the protective action of the acidity in wines: low total acidity values (far worse if combined with low alcoholic grade) do not allow to obtain long lasting wines.

4.2.3. Conductometric determinations

The chemical model-based simulation of each pH-metric titration allows the ionic strength trend with varying pH to be estimated. Conductometric data were recorded, during the alkalimetric titrations of each wine, to validate the results obtained by the simulation. The trend of the con-

ductometric signal (specific conductivity, mS cm^{-1}) for a wine of ours (namely, G99) is showed, as an example, in Fig. 3. We can observe how the conductometric trend strongly resembles to that of the calculated values of ionic strength with varying pH. As a consequence, we consider the conductometric evidences as a significant validation of the chemical model proposed. In addition, we found that conductometric measurements can be also employed to estimate the total acidity value (C_H). As in potentiometry, the derivative graph can be used to estimate the end point in the conductivity vs. V_{KOH} curve. We found an excellent agreement in the estimation of the C_H parameter, starting from both potentiometric or conductometric sources (Table 3). This second analytical evidence contributes to support and fortify our previous thermodynamic search of validation regarding the ionic strength values evaluated using the set of equilibrium concentrations (or analytical, where possible): we are now sure that conductometric and pH-metric outputs show fruitful convergence and comparable sensitivity with respect to our purposes of overall reliability and accuracy.

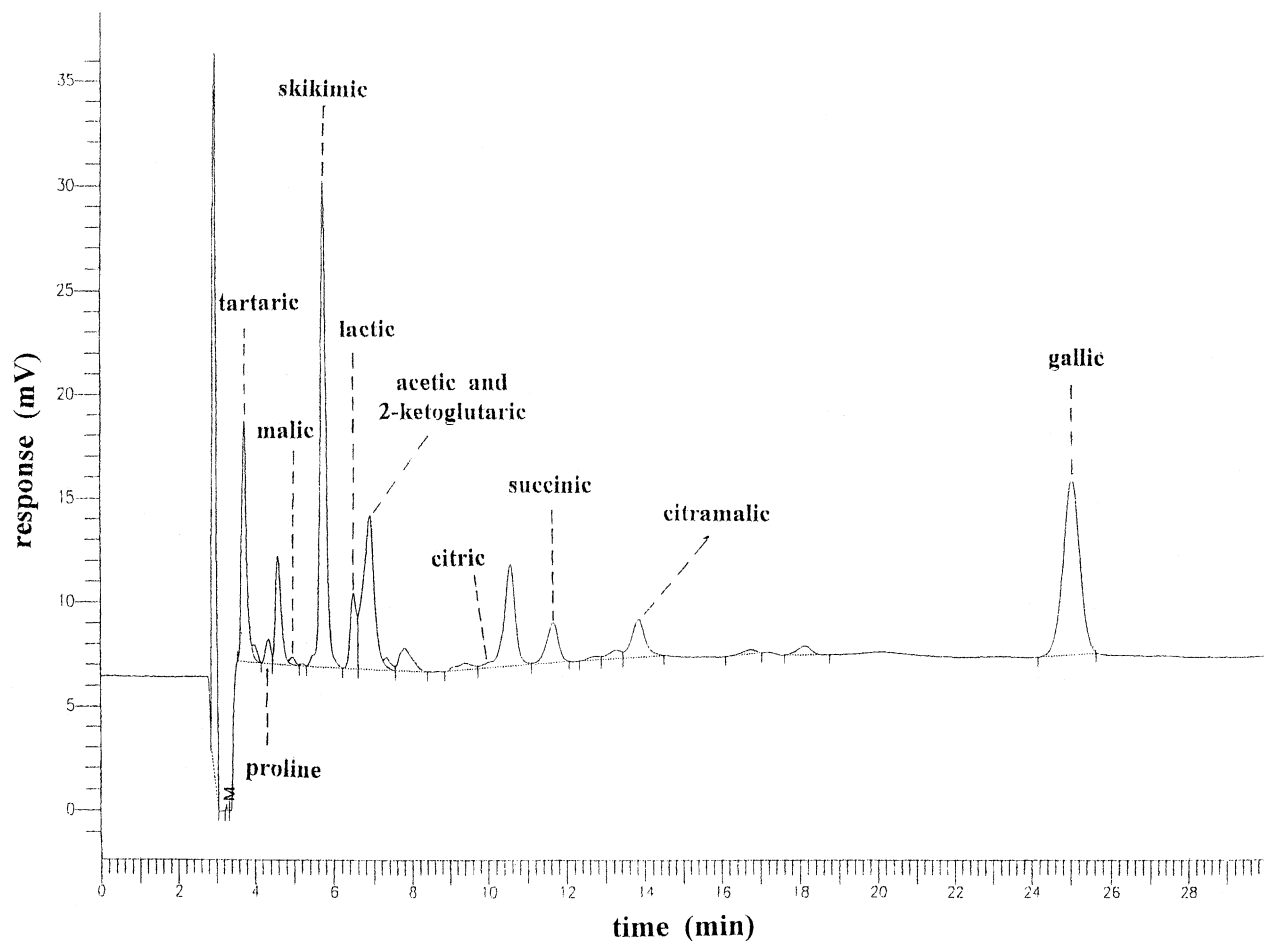


Fig. 2. HPLC/RP chromatogram of G99.

4.2.4. Acid–base titration of carboxylic acids in mixed solvent

Three carboxylic acids, chosen as model substances, were acidimetrically (sodium acetate) or alkalimetrically

(L-tartaric acid and citric acid) titrated in order to obtain the protonation constant values for mono-, di- and triprotic acids in water/ethanol media. As background salts we used KCl at two ionic strength values, $I = 0.05$ and 0.1 M, while

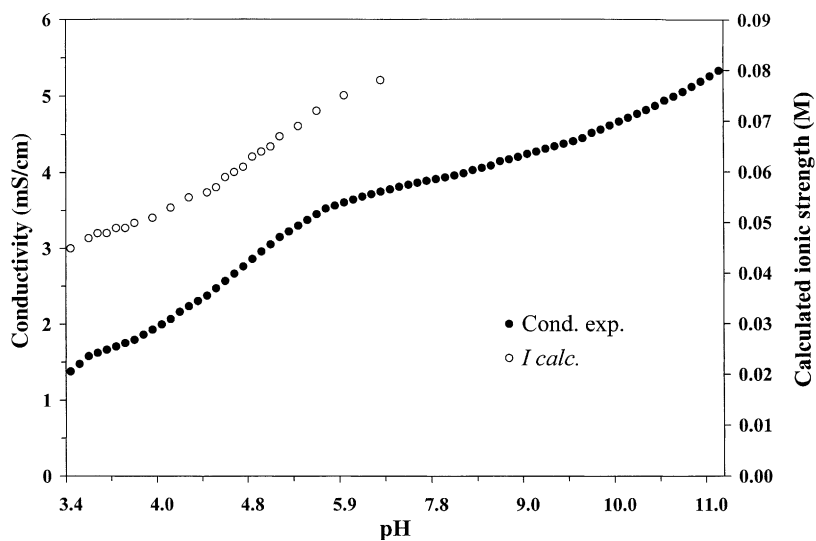


Fig. 3. Alkalimetric titration curve, recorded by conductometric way (●, left axis), of G99. Calculated trend of the ionic strength (○, right axis), with varying pH, of G99 by the simulation obtained on the basis of the chemical model (MODEL3, see Table 5).

Table 2

Analytical concentrations (*C*, by HPLC and hydrogen ion by pH-metric titration), species distribution calculated on the basis of MODEL3 (equilibrium concentrations indicated) for each wine under investigation and number of protonation equilibria of each acid–base active substance considered^a

Substance	G99 (mmol/l)	G00 (mmol/l)	B99 (mmol/l)	B00 (mmol/l)	No. of equilibria ^b
H ⁺ ion (titratable)	43.00	46.80	70.36	65.24	1
Acetic acid, <i>C</i>	8.29	9.89	10.86	7.58	1
[Ac ⁻]	0.35	0.46	0.22	0.37	
Gallic acid, <i>C</i>	0.15	0.16	0.11	0.17	1
[Gall ⁻]	0.01	0.01	0.004	0.01	
Lactic acid, <i>C</i>	8.96	9.05	24.06	36.67	1
[Lat ⁻]	2.35	2.55	3.45	10.57	
Skikimic acid, <i>C</i>	0.29	0.30	0.19	0.24	1
[Skik ⁻]	0.04	0.04	0.013	0.037	
Proline, <i>C</i>	3.18	3.43	3.92	5.11	1
[H ₂ Prol ⁺]	0.14	0.14	0.36	0.21	
Tartaric acid, <i>C</i>	4.58	6.87	8.05	8.06	2
[HTar ⁻]	2.79	4.25	3.83	5.01	
[Tar ²⁻]	0.44	0.76	0.29	0.92	
Citramalic acid, <i>C</i>	3.34	2.82	4.28	3.12	2
[Hcitmal ⁻]	1.53	1.36	1.24	1.53	
[Citmal ²⁻]	0.031	0.032	0.012	0.037	
Malic acid, <i>C</i>	0.6	0.5	0.70	0.72	2
[HMal ⁻]	0.25	0.24	0.19	0.34	
[Mal ²⁻]	0.008	0.008	0.003	0.01	
2-Ketoglutaric, <i>C</i>	1.26	1.29	0.55	0.66	2
[HKet ⁻]	1.14	1.17	0.48	0.59	
[Ket ²⁻]	0.06	0.07	0.01	0.03	
Succinic acid, <i>C</i>	5.57	6.23	5.11	5.53	2
[HSucc ⁻]	0.69	0.85	0.32	0.78	
[Succ ²⁻]	0.005	0.007	0.001	0.007	
Hydrogen phosphate, <i>C</i>	2.89	2.80	4.14	2.89	2
[H ₂ PO ₄ ⁻]	2.72	2.66	3.68	2.74	
[HPO ₄ ²⁻]	6 × 10 ⁻⁴	7 × 10 ⁻⁴	4 × 10 ⁻⁴	8 × 10 ⁻⁴	
Sulphate, <i>C</i>	8.96	9.50	12.05	9.85	1
[HSO ₄ ⁻]	0.19	0.18	0.52	0.18	
[SO ₄ ²⁻]	8.77	9.32	11.53	9.67	
Citric acid, <i>C</i>	0.46	0.42	0.67	0.81	3
[H ₂ Citr ⁻]	0.25	0.24	0.26	0.46	
[HCitr ²⁻]	0.03	0.03	0.01	0.06	
[Citr ³⁻]	6 × 10 ⁻⁵	7 × 10 ⁻⁵	1 × 10 ⁻⁵	1 × 10 ⁻⁴	

^a For each substance, a chemical form lacking in the table is easily calculable by the mass balance equation at the pH value of each wine. As for uncertainty (HPLC measurements), we can group carboxylic acids and amino acids examined according to their spectrophotometric features at ≈210 nm: (a) acetic, malic, lactic, succinic, citramalic and citric acids show low molar absorptivity values; (b) 2-ketoglutaric, tartaric acids and proline show intermediate molar absorptivity values; (c) gallic and skikimic acids show high molar absorptivity values. Moreover, based on their concentration in wines, we can have average values of uncertainty ranging between 1 and 10%.

^b For each substance (total = 14) is indicated the number of protonation equilibria (total = 22) which contributes to the chemical model.

Table 3

Volume alcoholmetric proof (vol.%) and equilibrium results obtained on each wine under investigation^a

Wine	Volume percent	pH exp.	V e.p. 1 M KOH (ml)		pH e.p.	C _H (Pot., mM)	Total acidity of tartaric acid (g/l)	Charge balance (mM)					
			Pot. detec.	Cond. detec.				∑ C ⁻ z ⁻	∑ C ⁺ z ⁺	∑ C ⁻ z ⁻ - ∑ C ⁺ z ⁺			
G99	12.2	3.38	1.075	1.100	7.53	43.00	3.23	30.99 ^b	73.40 ^c	22.67 ^b	65.09 ^c	8.32 ^b	8.31 ^c
G00	11.5	3.40	1.170	1.155	7.59	46.80	3.51	36.03	82.30	30.15	76.14	5.88	6.16
B99	12.0	3.07	1.759	1.760	7.43	70.36	5.28	37.94	108.27	22.77	91.96	15.17	16.31
B00	12.0	3.41	1.631	1.690	7.20	65.24	4.90	44.12	108.77	32.13	96.44	11.99	12.33

^a Results of the charge balance equation is applied at the pH of each wine and at the pH corresponding to the end point of their alkalimetric titration. exp., experimental; e.p., equivalent point (by the 2nd derivative of each titration curve); Pot., potentiometric; Cond., conductometric; detec., detection.

^b At the pH of wine.

^c At the pH of e.p.

Table 4

Thermodynamic data related to (a) the $\log \beta_i^H$ directly determined in this paper (measurements and computer-assisted refinement process) and (b) the chemical model building of red wines

Substance	<i>i</i>	<i>I</i> = 0	$\log \beta_i^H$ (0.05 M KCl)				$\log \beta_i^H$ (0.1 M KCl)			
		H ₂ O	H ₂ O	8% H ₂ O/ EtOH	12% H ₂ O/ EtOH	16% H ₂ O/ EtOH	H ₂ O	8% H ₂ O/ EtOH	12% H ₂ O/ EtOH	16% H ₂ O/ EtOH
(a) Thermodynamic data related to the $\log \beta_i^{Ha}$										
Acetate	1	4.750 [14]	4.598	4.656 (3)	4.710 (2)	4.776 (3)	4.565 (3) ^b	4.625 (3)	4.679 (1)	4.740 (1)
Tartrate	1	4.369	4.005	4.098 (3)	4.153 (3)	4.237 (2)	3.926	4.025 (2)	4.066 (4)	4.135 (3)
	2	7.400 [14]	6.874	7.018 (4)	7.178 (4)	7.302 (3)	6.757 [17]	6.930 (3)	7.053 (4)	7.118 (4)
Citrate	1	6.412	5.821	5.880 (2)	5.968 (4)	6.034 (5)	5.69	5.781 (2)	5.843 (3)	5.882 (2)
	2	11.189	10.250	10.378 (2)	10.528 (4)	10.643 (4)	10.08	10.218 (2)	10.323 (3)	10.402 (3)
	3	14.317 [14]	13.188	13.386 (4)	13.629 (5)	13.794 (4)	12.97 [23]	13.223 (3)	13.424 (3)	13.520 (4)
(b) Thermodynamic data for the chemical model building of red wines ^c										
Gallate	1	4.44 [15]	4.31	4.37	4.42	4.49	4.27 [15]	4.33	4.38	4.44
Lattate	1	3.86 [15]	3.70	3.76	3.81	3.88	3.66 [15]	3.72	3.77	3.84
Skikimate	1	4.207 [15]	4.048	4.106	4.158	4.226	4.01 [15]	4.07	4.12	4.18
Proline	1	1.952 [15]	1.96	2.02	2.07	2.14	1.96 [16]	2.02	2.07	2.14
Citramalate	1	5.374	5.051	5.144	5.199	5.283	4.972	5.071	5.112	5.181
	2	8.865 [15]	8.383	8.527	8.687	8.811	8.266 [15]	8.439	8.562	8.627
Malate	1	5.106	4.719	4.812	4.867	4.951	4.64	4.74	4.78	4.85
	2	8.570 [14]	7.997	8.141	8.301	8.425	7.88 [18]	8.05	8.18	8.24
2-Ketoglutarate	1	4.984	4.661	4.754	4.809	4.893	4.582	4.681	4.722	4.791
	2	7.135 [15]	6.653	6.797	6.957	7.081	6.536 [15]	6.709	6.832	6.897
Succinate	1	5.648	5.319	5.412	5.467	5.551	5.24	5.34	5.38	5.45
	2	9.857 [14]	9.347	9.491	9.651	9.775	9.23 [19]	9.40	9.53	9.60
Hydrogen phosphate	1	7.200	6.829	6.922	6.977	7.061	6.75	6.85	6.89	6.96
	2	9.347 [20]	8.787	8.931	9.091	9.215	8.67 [21]	8.84	8.97	9.03
Sulphate	1	1.998 [22]	1.619	1.712	1.767	1.851	1.54 [22]	1.64	1.68	1.75

^a The uncertainty is reported in parentheses as $\pm s$ (standard deviation) in the last significant digit. Conditions: two ionic strength values (0.05 and 0.1 M), four percentages of ethanol (namely: 0, 8, 12 and 16%), one background salt (K^+Cl^-), $T = 25^\circ C$. The aqueous values of $\log \beta_i^H$ are from the literature (references are reported) or are calculated by a Debye–Huckel type equation (see Section 4.3.1), except for acetate ion at $I = 0.1 M$.

^b This work.

^c The aqueous values of $\log \beta_i^H$ are from the literature (references are reported) or are calculated by a Debye–Huckel type equation (see Section 4.3.1). The values of $\log \beta_i^H$ in mixed solvents are calculated as explained in Section 4.2.4. Conditions: two ionic strength values (0.05 and 0.1 M), four percentages of ethanol (namely: 0, 8, 12 and 16%), one background salt (K^+Cl^-), $T = 25^\circ C$.

three percentages of ethanol were investigated, namely 8, 12 and 16%. Since K^+ ion is the most representative metal ion in wines [4], it seems correct the choice of KCl as background salt in order to obtain a set of protonation constant values ($\log \beta_i^H$) suitable to faithfully describe the acid–base chemistry of wines. These experimental values of $\log \beta_i^H$ (Table 4) were compared with that determined in water, at the same ionic strength (and temperature). We assumed that the differences experimentally observed ($\Delta \log \beta_i^H$) for our three model molecules are the same under the same conditions of electrical charges involved in a protonation reaction. With this criterion we have then calculated each value of protonation constants for the other acid–base active substances considered in this study (Table 4). This last series of protonation constant values must then be considered as a useful even if rough estimation which can be successfully employed to model the acid–base behaviour of a complex

mixture of electrolytes, within the overall uncertainty of a multi-step procedure related to natural fluids. In the future we will provide further experimental work to improve the thermodynamic accuracy of these equilibrium constants.

4.3. Chemical modelling

4.3.1. Protonation constants

As far as the aqueous values of the protonation constants is concerned, we found all the values requested for the aim of our project in the literature, mainly at $I = 0$ or 0.1 M (Table 4). Hence, adjustment of the tabulated values to take into consideration the correct value of the ionic strength was necessary. For this purpose we have used the Debye–Huckel type equation widely discussed in [10], which allows the calculation at different ionic strength values (in the range $0 \leq I \leq 1 M$) of $\log K_{eq}$ refined from data recorded on

solutions having Na⁺ or K⁺ salts as background electrolyte:

$$\log K_{\text{eq}}(I) = \log K_{\text{eq}}(I') - z^*A \left(\frac{\sqrt{I}}{1 + B\sqrt{I}} - \frac{\sqrt{I'}}{1 + B\sqrt{I'}} \right) + C(I - I') + D(I^{1.5} - I'^{1.5}),$$

$$A = 0.5, \quad B = 1.25, \quad C = 0.084p^* + 0.126z^*,$$

$$D = -0.062z^*, \quad p^* = \sum p_{\text{reagents}} - \sum p_{\text{products}},$$

$$z^* = \sum z_{\text{reagents}}^2 - \sum z_{\text{products}}^2$$

where (a) p and z are the stoichiometric coefficients and the electrical charges, respectively; (b) K_{eq} is the formation constant; (c) I and I' are the actual and the reference ionic strength values, respectively. For each substance of our interest, the value of $\log K_{\text{eq}}$ at $I = 0.05$ M was then calculated by means of the above equation [10].

The reliable values of $\log K_{\text{eq}}$ allow the real chemical form of each acid–base active substance to be correctly considered in order to model each fluid investigated. For this reason a series of titrations was performed in mixed solvent, to take into consideration the effect of the ethanol as well. Ionic strength value, ionic medium and mixed solvent status were considered for each wine in order to calibrate the electrode couple. The dependence of the E° value of the glass electrode on little variations of ethanol percentage has been found to be negligible.

In Table 4 gives all the values of the protonation constants corresponding to our pH-metric measurements and computer-assisted refinement process adopted in the development of this study. The values of $\log \beta^{\text{H}}$ follow a general trend with respect to both the ionic strength and the percentage of ethanol. As previously found for water/1-propanol solvents, the values of $\log K^{\text{H}}$ of carboxylate groups tend to increase with the increasing of the percentage of alcohol, as a consequence of dielectric constant decrease [11–13].

4.3.2. Ionic strength calculation of each wine

The ionic strength evaluation is performed by the software ES4ECI, by considering all the chemical equilibria involved in our model. It can be shown that the analytical concentration of ionic species (mainly metal ions and nitrates) is absolutely insufficient to account for the overall ionic strength value of each wine: big contribution, really

predominant, is from carboxylic acids, considering (thanks to the equilibrium calculation) all the various ionic chemical forms at the pH value of each wine. Since a difference between total anion and total cation charges was found during the calculation of the charge balance (see Section 4.4.2), we took into account this lack of balance by assuming the presence of a background mono-charged cation in the ionic strength calculation.

4.3.3. Chemical modelling

A chemical model can be built, for each wine, taking into account the analytical concentrations of the substances analysed and the refined values of the protonation constants in the suitable chemical medium. Fourteen acid–base active substances ($\text{pH} < 7$) were considered as reactants (see Table 2) during the input construction for the computer-assisted simulation of the alkalimetric titration of each wine, 16 metal ions were treated as background salt (as a contribution to the ionic strength of the fluid), 22 protonation equilibria were considered at the same time. We considered the hydrolysis of some cations, such as aluminium and iron [15] but the influence of these equilibria on calculation was near to zero. As for the number of protonation stages for each substance, we have considered the real conditions of the wine (and that the investigation has been stopped at $\text{pH} \approx 6$). Hence, as an example, in our specific context HPO_4^{2-} has been considered the fully deprotonated form for the reactant H_3PO_4 (Table 2).

The pH value of each wine was then calculated as a result of all the multiple chemical protonation equilibria set. Table 5 shows the results obtained ($\text{pH}_{\text{calc.}}$ and $I_{\text{calc.}}$) at the initial pH of each wine. Three chemical models were tested. They were built on the basis of: MODEL1, the aqueous values of each $\log \beta^{\text{H}}$ at $I = 0.05$ M (KCl); MODEL2, the values of each $\log \beta^{\text{H}}$ at $I = 0.1$ M (KCl) in 12% ethanol; MODEL3, the values of each $\log \beta^{\text{H}}$ at $I = 0.05$ M (KCl) in 12% ethanol. The best simulation of the acid–base chemistry of each red wine is obtained by the set of thermodynamic data corresponding to the MODEL3. An inspection of the results collected in Table 5 shows that the best accuracy is reached by a model that includes the ethanol contribution, while the role of the ionic strength seems to be of minor importance. We can easily test the relative importance of each reactant in the overall model. In fact, we can calculate

Table 5
Calculated pH value for each wine under investigation on the basis of various chemical models^a

Wine	$\text{pH}_{\text{exp.}}$	Model 1	Model 2 (0.1 M KCl,	Model 3 (0.05 M KCl,		ΔpH ($\text{pH}_{\text{exp.}} - \text{pH}_{\text{Model 3}}$)
		(0.05 M KCl, H ₂ O)	H ₂ O/EtOH 12%)	H ₂ O/EtOH 12%)	$I_{\text{calc.}}$ (M)	
		$\text{pH}_{\text{calc.}}$	$\text{pH}_{\text{calc.}}$	$\text{pH}_{\text{calc.}}$		
G99	3.38	3.24	3.35	3.37	0.045	+0.01
G00	3.40	3.27	3.39	3.41	0.051	−0.01
B99	3.07	2.90	3.02	3.04	0.057	+0.03
B00	3.41	3.28	3.40	3.42	0.057	−0.01

^a Calculated value of the ionic strength ($I_{\text{calc.}}$) is based on the equilibrium output from the MODEL3 (see the “Chemical modelling” paragraph of Section 4).

the pH value of a red wine after the selective exclusion of one substance by the protonation list. As an example, if we delete by G99 ($\text{pH}_{\text{exp.}} = 3.38$, $\text{pH}_{\text{calc.}} = 3.37$) tartaric, or succinic or acetic acid the pH value falls down to 2.88, 2.01 or 2.80, respectively. The model is obviously less sensitive to those substances with low analytical concentration and/or low values of $\log K_i^{\text{H}}$. For instance, if we delete malic acid (low C) we have pH 3.31 while if we neglect proline or sulphate ion (low values of $\log K_i^{\text{H}}$) we have pHs 3.36 or 3.37, respectively. Besides the first end-point, the ability of the chemical model to describe the acid–base behaviour of each wine fails.

In order to emphasise the potential technological usefulness of the equilibrium results on red wines, we have collected in Table 2 the species distribution of each substance (based on the thermodynamic findings from the MODEL3) for each wine under investigation. When hypotheses on potassium hydrogentartrate precipitation are necessary (paying attention to the temperature, whose value strongly influence the solubility product of this compound), as an example, the knowledge of the equilibrium value of the molar concentration of the species HTar^- in reliable conditions is fundamental. Moreover, we can observe by an inspection of Table 2, that the concentration of the chemical forms showing anionic characteristics, for the substances under consideration at $\text{pH} \approx 3$, is variable, according to $\log \beta^{\text{H}}$ values.

4.3.4. Calculation instruments for intermediate percentage of ethanol

Starting from the analytical results for each red wine, we used the set of $\log \beta^{\text{H}}$ at 8, 12 and 16% of ethanol (Table 4) to calculate three pH values. Then, a linear fitting was applied obtaining an equation pH vs. percentage of ethanol

for each wine. As an example, for G99 we found $Y = 0.0142X + 3.201$ ($Y = \text{pH}$, $X = \%$ ethanol, $r = 0.9999$). The refined slope values have been found to be practically the same for all the wines, indicating the correctness of our thermodynamic findings. At the actual state of the art, an attempt can be made to deal with red wines showing intermediate values of alcoholic grade, with respect to those directly investigated. First, a home-made equation must be built starting by each analytical concentrations and by the three chemical models at 8, 12 and 16% of ethanol (using our values of $\log \beta^{\text{H}}$). Then, the desired pH of the wine under consideration at an intermediate value of alcoholic grade can be calculated by the optimised equation.

4.4. Tools of check

4.4.1. Synthetic “wines”

A synthetic mixture reproducing the composition of each wine, with respect to the acid–base reactivity ($\text{pH} < 7$) was prepared. With this aim, a mixture of carboxylic acids, proline, inorganic anions and metal ions was prepared for each wine, thus faithfully reproducing the content of bases (organic and inorganic one) and the values of C_{H} , ionic strength and percentage of ethanol. Each synthetic mixture was alkalimetrically titrated, as the wines. Fig. 4 shows the overlap of the titration curves for natural and synthetic G99; in addition, the calculated trend pH vs. C_{KOH} (based on the chemical model) is reported. The very good agreement is clear. It is noteworthy to observe the difference of slope, for $\text{pH} > 6.5$, between the experimental titration curves recorded on both the wine and the synthetic mixture reproducing the wine. In fact, in the synthetic mixture are absent those compounds that give rise to the second buffer region. The presence of these compounds in the wines increases the buffer

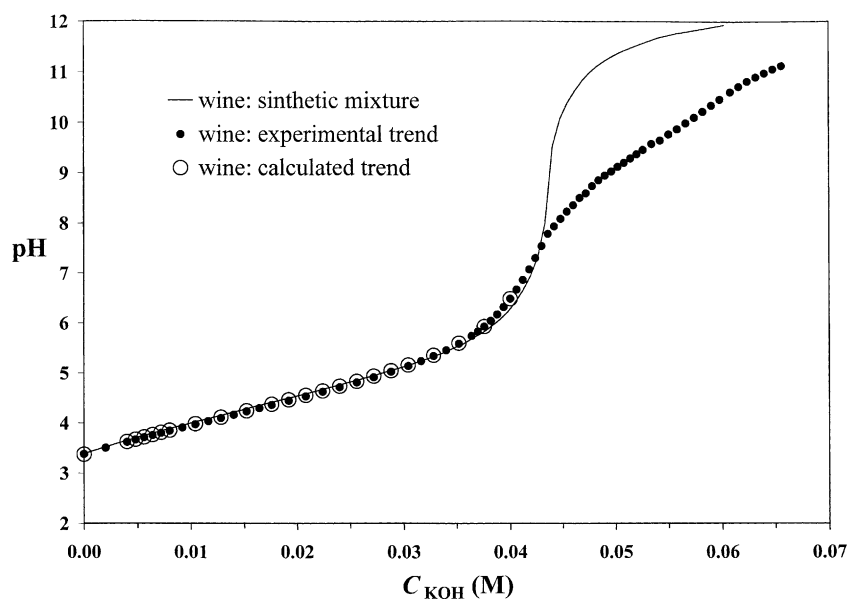


Fig. 4. Alkalimetric titration curves (as pH/C_{KOH}), recorded by potentiometric way, of natural and synthetic G99. Calculated trend based on the chemical model optimised by means of the computer-assisted simulation (MODEL3, see Table 5) is also reported until pH 6.5.

capacity of the fluid while the inflection at $\text{pH} \approx 7.5$ in the synthetic mixture resemble to that of a weak acid, without further substances able to neutralise the KOH added after $\text{pH} \approx 7.5$.

4.4.2. Charge balance

As an overall check of our job, the charge balance principle was adopted. For each wine, based on the distribution diagram obtained for each substance involved, a charge balance equation was written taking into account the state of protonation–deprotonation resulting on the basis of the thermodynamic findings of this paper. For all the wines investigated the lack of balance revealed a deficit of cations, as indicated in Table 3. We think this lack of cations should be attributed to chemical compounds protonated at $\text{pH} \approx 3.3$ (just as an indication the pH of wine) such as biogenic amines, oxonium cations by anthocyanines and the amino group of amino acids. This behaviour is well confirmed, qualitatively and quantitatively, by the same calculation performed at the equivalence point ($\text{pH} \approx 7.5$), thus indicating (Table 3) the reliability of the approach used towards both analytical and equilibrium aspects of this study. It is noteworthy that the lack of balance is practically identical for each wine and at the end point of their acid–base titration. Nevertheless, we have higher values of the product “ $C \cdot z$ ” at the end point of each wine, which is an evidence of the ionisation process occurred (the contribution of the titrant added has been considered). Moreover, we can observe higher values of the anion excess for B99 and B00 rather than for G99 and G00. This allow us to explain the behaviour of both B99 and B00 during the alkalimetric titration in the basic field: the higher the content of oxidable cations the higher the instability of the glass electrode potential caused by the redox and/or precipitation reactions.

5. Conclusions

In this paper the so-called analytical parameter “Total acidity”, usually employed in routine measurements on wines, was enriched of chemical meanings. In fact, it has been used, together with the results of the determination of the analytical concentration of metal ions, carboxylic acids, inorganic anions and amino acids, to build a general acid–base model based on an equilibrium analysis. Since precipitation and redox transformations, fundamental processes for the ageing of wines towards high-quality and high-priced products, are always acid–base dependent phenomena, the usefulness of our job can be also thought as an input for the investigation of many other aspects of the chemistry of wine. In fact, the jump from the analytical to the equilibrium composition may allow the prediction

of the effect on red wines consequent to chemical actions (addition of substances) or natural phenomena.

In this paper a general methodological approach is proposed, involving a multi-technique characterisation combined with a chemical modelling step. An accurate overall method to keep under chemical control many steps of the wine-making has been created, beyond the routine analytical measurements [24], considering that any effort on this topic is justified by the nutritional importance of red wine. Moreover, the paper shows how various experimental sources, together with an appropriate step of careful equilibrium analysis, may be joined to reach significant results in the modelling of natural products, even beyond the specific target of wine.

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