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Chelation of aqueous iron(III) by 2-acetyl-1,3-cyclohexanedione and beer ageing

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Abstract

Fe(III) is present in alcoholic drinks, such as wine and beer, and it is well known that this metal ion may affect beer fermentation, stability and quality. From a study of the complexation of 2-acetyl-1,3-cyclohexanedione with Fe(III)in aqueous solution, thermodynamic and kinetic parameters were obtained. Kinetics and equilibria in aqueous 1:1 chelation were investigated spectrophotometrically in aqueous solution at 25 °C and ionic strength of 0.5 mol dm^{-3} $NaClO_4$. The mechanism proposed to account for the kinetic data involves a double reversible pathway, where both Fe^{3+} and $Fe(OH)^{2+}$ react with the enol tautomer of the ligand. Some comparative analyses with the homologous iron(III) 2-acetylcyclohexanonate were established and the effect of previously cited changes during beer ageing considered.

Keywords: Brewing; 2-Acetyl-1,3-cycloalkanedione; Iron; Kinetics; Chelation

1. Introduction

Reagents containing multiple functionalities are very important, in their mechanistic behaviour as well as in organic synthesis since they are versatile and effective in the efficient construction of complex structures from relatively simple starting materials.

Over the past 40 years there has been a rapid proliferation of different types of processed hops, or hop products, which offer the brewer several advantages in terms of economy and quality. In addition, the contribution of hops to beer aroma, bitterness, foam, and light stability can now be controlled more effectively than ever before (Moir, 2000). The species that primarily determine the flavour of fresh beer, include sulphur-containing substances, esters, higher alcohols, vicinal diketones and the bitter resin and oil fractions from hops (Bamforth, 2000). Of all the herbs that have been used to flavour and preserve beer over the ages, only the hop (*Humulus lupulus* L.) is now regarded as an essential raw material in brewing throughout the world (Moir, 2000).

It has become clear that the value of hops is due, not only to their effect on taste, but also their antibiotic action, by which yeast grows freely and the development of putrefying bacteria is suppressed (Hellyer, 1968). The antibiotic properties of hops are connected with the so-called "soft resin" fraction in hop extracts, the main components of which are cyclic β,β′-triketones (Lounasma, Widen, & Huhtikangas, 1973). The oxidation of β,β′-triketones produces iso-alpha-acid compounds that retain a β,β′-triketone nature. These iso-alpha-acids impart specific bitter nuances to the beer (De Cooman, Aerts, Overmeire, & De Keukeleire, 2000), and therefore the problem of controlling the quality of the beer depends on rapidly evaluating the depth of the chemical transformations of the hops acids (Sandra, Steenbeke, Ghys, & Schomburg, 1990). The fate of 2-acetyl-1,3-cyclohexanedione derivatives, such as the *trans*-iso-alpha-acids, in particular may, adversely affect beer bitterness consistency. In addition, by using hop products containing low concentrations of *trans*-iso-alpha-acids, brewers may profit from the remarkable stability of tetrahydroiso-alpha-acids, even in prolonged storage, for the production of consistently bitter beers (De Cooman et al., 2000).

Many 2-acylcyclohexane-1,3-diones with simple structures have been found in the essential oils of plants

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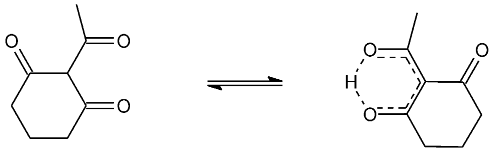
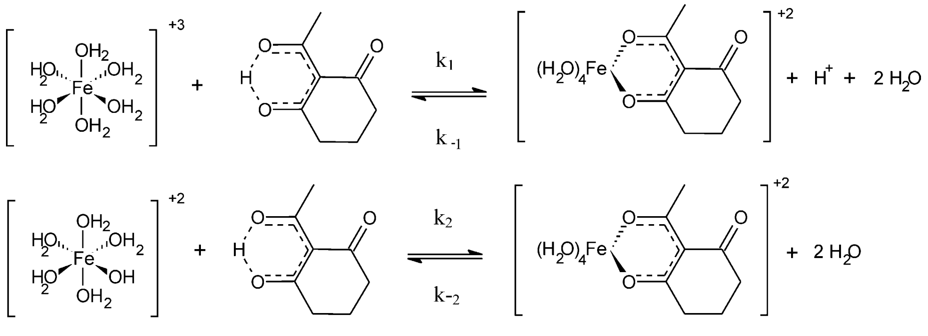




of the genera Eucalyptus, Leptospermum and others in the Myrtaceae family growing in Australia and New Zealand and used in folk medicine (Hellyer, 1968). Most of these compounds are used in the treatment of dysentery, diarrhea, and skin diseases, and, recently, in treatment of some forms of cancer (Mayer, 1993).

In 1978, 2-acylcyclohexane-1,3-diones were first identified as the main components of secretions of the mandibular glands of larvae of the Mediterranean flour moth, Lepidoptera (Mudd, 1978). Later, cyclohexane β,β′-triketones (Mudd, 1981, 1983), containing long unbranched mono- and diunsaturated 2-acyl substituents, were also detected in secretions of the larvae of this species. It has been shown that these triketones possess pheromonal properties, affecting the egg-laying behaviour of butterflies and the distribution of pyralid larvae (Corbet, 1971, 1973; Mudd, 1983), and also exhibit kairomonal activity, attracting their parasites; thus, they are natural regulators of the pollution densities of the above-mentioned Lepidoptera species.

The β-tricarbonyl polyfunctional group forms part of the skeleton of many biologically active natural products with both simple and rather complex structures. Many of these biologically active compounds are used in modern and traditional medicine. They exhibit antibiotic (Ishiguro, Yamataki, Tomita & Yamagata, 1985; Lounasma et al., 1973), antimalarial (Gu, Feng, & Wang, 1988), antidiabetic (Japanase Patent, 1984), anticancer (Sato, Obara, Endo, Onodera, & Matsuba, 1992) and other useful therapeutic properties.

Details of chemical structure and biological activity, as well as the syntheses of 2-acylcycloalkane-1,3-diones have been summarized in earlier reviews (Hassall, 1958; Stevens, 1967) and also in a more recent review (Rubinov, Rubinova, & Akhrem, 1995).

These ligands also exhibit the phenomenon of keto-enol tautomerism and are able to form complex compounds similar to those of the β-diketones.

β,δ-Tricarbonyl compounds may include triketo-, monoenol- and bisenol- forms in their tautomeric equilibrium and, accordingly they can behave as bidentate or tridentate ligands producing polynuclear metal chelate complexes (Casellato, Vigato, & Vidali, 1977). Cyclic β,β′-triketones present only one acidic proton and subsequently cannot form polynuclear coordination compounds with metal ions.

Iron and zinc are trace minerals of critical importance for normal growth and development (Krebs, 2000). As iron can apparentely produce a negative effect on zinc absorption (Lonnerdal, 2000), yeast fermentation has been tested as a strategy for improving iron/zinc bioavailability in cereals (Agte, Gokhale, & Paknikar, 1999). The prevalence of anemia is high in adolescent girls. Iron-folic acid (IFA) supplements have been shown to enhance adolescent growth in some parts of the world (Kanani & Poojara, 2000). Folic acid is present in beer (Sanchis, Orive, & Ramos, 2000).

Iron(III) is present in foods such as meat, fish and milk among others (Bocca et al., 2000; Hashem & Abubakr, 2000; Jorhem & Engman, 2000). It is also present in alcoholic drinks such as wine and beer (Svendsen & Lund, 2000; Zeng & Jewsbury, 2000), and it is well known that many metallic cations markedly affect beer fermentation, stability and quality (Elliot, Janes, & Jeffs, 1969). Speciation analysis indicated that Mn was probably present as Mn^{2+} in beer, whereas Fe was found as negatively charged complexes (Svendsen & Lund, 2000; Zeng & Jewsbury, 2000). When the beer was spiked with Fe(III), the added metal ion was recovered as negatively charged species, indicating that the beer contained free metal-complexing ligands(Svendsen & Lund, 2000; Zeng & Jewsbury, 2000).

One of the most conspicuous features of iron(III) in aqueous solution is its tendency to hydrolyze. The hydrolysis of the pale purple ion $[Fe(H_2O)6]^{3+}$ in noncomplexing media is complicated and condition-dependent (Khoe, Brown, Sylva, & Robins, 1986). At pH < 1 the sole species is the aqua ion but above pH 1 stepwise hydrolysis occurs. In the 1–2 pH range other types of oxo species may be formed. At pH > 2 more condensed species and colloidal gels are formed, leading to precipitation of the red brown gelatinous hydrous oxide. The hydroxo species are yellow because of charge-transfer bands in the ultraviolet region that have tails in the visible spectrum.

As part of a continuing interest in complexation reactions (Blanco, 1998; Blanco & Hynes, 1992; Romero, Verdú, & Blanco, 1998) in aqueous solutions we now report a kinetic study of complexation of a bidentate ligand with multiple functionality. In order to study chelation abilities of cyclic β,β′-triketones in aqueous solutions, iron(III) has been used as a metal centre and 2-acetyl-1,3-cyclohexanedione as a ligand, for the first time.

To our knowledge, this is the first reported kinetic work where a complex of stoichiometry 1:1 between a cyclic β,β′-triketone and iron(III) in aqueous solution has been studied.

2. Materials and methods

Reagent grade 2-acetyl-1,3-cyclohexanedione(Aldrich), abreviated as Hachd, H being the enolic hydrogen atom, was freshly distilled under reduced pressure. Stock solutions were standardised by titration with standard sodium hydroxide.

Solutions of Fe(III) were prepared from reagent grade $Fe(NO_3)_3{\cdot}9H_2O$ (Aldrich) and were standardised by gravimetric analysis.

The source of hydrogen ions was perchloric acid (Merck), and the ionic strength of reactant solutions was 0.5 mol dm^{-3}, adjusted using $NaClO_4{\cdot}H_2O$ (Merck). Unless otherwise indicated, all materials were of analytical grade, and were used without further purification.

pH measurements were made using a CRISON 2002 pH-meter, calibrated to read hydrogen ion concentration directly by titrating solutions of perchloric acid with standard sodium hydroxide solutions. Endpoints were determined using the method of Johansson (1970).

UV–vis spectra and kinetic experiments were recorded on a Hitachi U-3OOO spectrophotometer.

As ligands of this class usually undergo hydrolysis in alkaline solution, it was thought desirable to determine their ionisation constant by a spectrophotometric method such as the parallel straight lines method (Maroni & Calmon, 1964), allowing comparisons to be made between different deprotonation degrees of the ligand without using high concentrations of alkaline solutions. The wavelengths were selected at regular intervals around the absorption maximum of the ligand (250–260 nm). The concentration of ligand used was 2.0×10^{-5} mol dm^{-3}. Standard buffer solutions were prepared in a range of pH close to the pKa of the ligand.

The complex stoichiometry was characterised according to the method of continuous variations (Job, 1928; Rose, 1961) and also by the Yoe and Jones method (1944); the actual concentrations of the products were monitored spectrophotometrically near the absorption maxima of the complex (500 nm). The proton concentration range (pH = 2.2–2)was low enough to prevent hydrolysis products other than $Fe(H_2O)_5OH^{2+}$ and the ionic strength was kept at 0.5 mol dm^{-3}.

The complex formation constant of the complex was determined by a spectrophotometric method. Series of solutions were prepared, where Fe(III) concentration ranged from 112 to 279 ppm, $[Hachd]_o = 30.8$ ppm and pH = 2. Measurements were monitored by recording absorbance changes at 500 nm.

In the kinetic study of the reaction, each observed rate constant, k_{obs}, subsequently used for further calculation, was the average of at least three determinations. The standard deviation in individual runs was less than two percent.

Several series of experiments were carried out to study the influence of the metal and proton concentrations on the observed rate constants, while the ligand concentration, ionic strength and temperature remained constant (Table 1, Fig. 2).

To allow a check of the accuracy of the measurement and the correctness of the evaluation, in addition to NAG fitting, SOLVER routines were used for refitting data.

Table 1
Kinetic data for reaction of iron(III) with 2-acetyl-1,3-cyclohexanedione in aqueous solution at 25 °C and ionic strength 0.5 mol dm^{-3}, with [Hachd] = 12.3 ppm

[Fe(III)]/ppm	pH	k_{obs}/s^{-1}	k_{cal}/s^{-1}	$k_{obs}-k_{cal}$
44.7	2.4	1.21	1.28	−0.07
55.8	2.4	1.48	1.52	−0.04
67.0	2.4	1.69	1.77	−0.08
78.2	2.4	1.99	2.01	−0.02
89.3	2.4	2.33	2.26	0.07
44.7	2.2	1.08	1.06	0.02
55.8	2.2	1.25	1.25	0.00
67.0	2.2	1.48	1.44	0.04
78.2	2.2	1.71	1.62	0.09
89.3	2.2	1.83	1.81	0.02
44.7	2.1	0.88	0.93	−0.05
55.8	2.1	1.11	1.09	0.02
67.0	2.1	1.22	1.24	−0.02
78.2	2.1	1.39	1.39	0.00
89.3	2.1	1.62	1.55	0.07
44.7	2.0	0.81	0.85	−0.04
55.8	2.0	0.93	0.98	−0.05
67.0	2.0	1.10	1.11	−0.01
78.2	2.0	1.22	1.25	−0.03
89.3	2.0	1.38	1.38	0.00

$k_1 = (1.02\pm0.04)\cdot10^2$ mol^{-1} dm^3 s^{-1}; $k_2 = (3.64\pm0.13)\cdot10^3$ mol^{-1} dm^3 s^{-1}; RSS = 3.27.

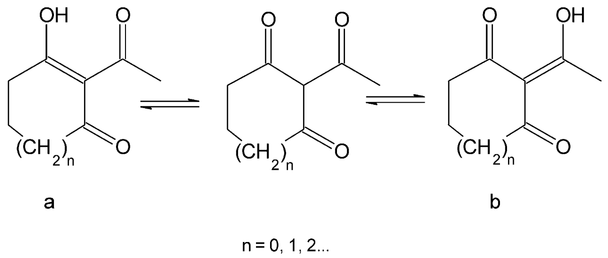


Fig. 1. Keto and enol forms of 2-acetyl-1,3-cycloalkanediones.

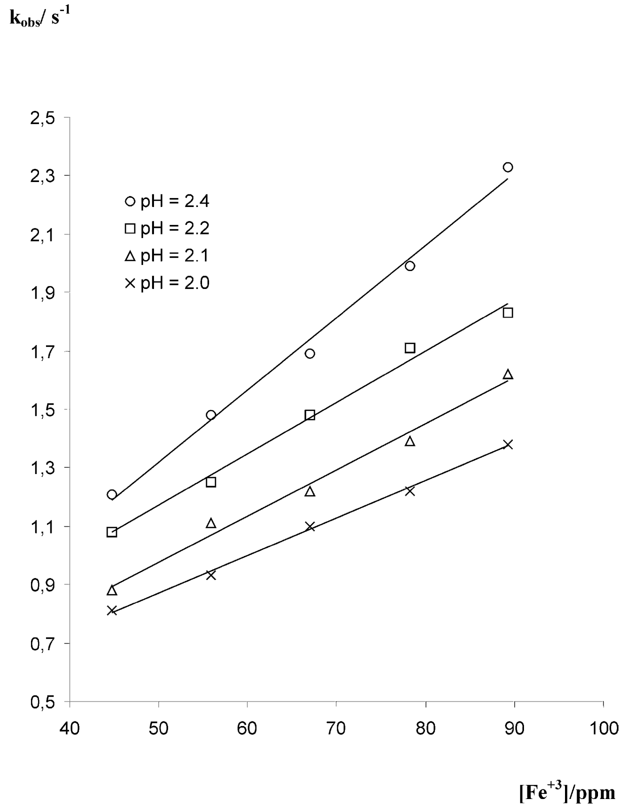


Fig. 2. Observed rate constant as a function of the total concentration of iron(III) at 25 °C and ionic strength 0.5 mol dm^{-3}. [Hachd] = 12.3 ppm.

3. Results and discussion

3.1. Structures and equilibria

Since the structure of molecules can indeed affect their equilibria and rates of reactions in a large number of ways, previous to the kinetic study, ligand equilibria (tautomerization and protonation) were studied.

3.2. Keno-enol equilibrium

In general, all cyclic β,β′ triketones easily undergo two different modes of enolization (Adembri, Scotton, & Sega, 1988). The first is enolization into the ring with the formation of the enol form *a* (Fig. 1). For unsymmetrical β,β′-triketones, where the cyclic carbonyl groups have different attached substituents, additional regioisomeric forms must be considered. The second enolization occurs in the side chain with the formation of the enol form *b*.

The keto-enol interconversion in this ligand has also been investigated in aqueous solutions by using a spectrophotometric method (Blanco & Sumillera, 1994) to measure the rate at which bromine reacts. Bromine first reacts very rapidly with any enol already present in solution. Thereafter, the bromination rate is governed by the rate of enol formation. In this situation, the concentration of the keto form remaining is given by (Blanco & Sumillera, 1994), $A/\varepsilon + [\mathrm{Hachd}]_o - [\mathrm{Br}_2]_o$, where A is the absorbance due to bromine at any time t at the observation wavelength (452 nm), $\varepsilon = 103$ is the absorption coefficient of bromine at 452 nm, $[\mathrm{Hachd}]_o$ and $[\mathrm{Br}_2]_o$, are the total concentrations of 2-acetyl-1,3-cyclohexanedione and bromine, respectively, at time zero. In this case, where a β,β′-triketone is involved, a particular kinetic behaviour was observed since, when bromine and triketone are in equimolar proportions, the whole bromine concentration reacts instantaneously. These results suggest that the mentioned ligand is present only in monoenol form. The results also show that neither the acidity nor temperature (7–25 °C) produce any appreciable amount of triketo form.

3.3. Ionisation constant

The average K_{HL} at 25 °C is $(2.0 \pm 0.1) \times 10^{-6}$ and the influence of the temperature over the equilibrium constant is practically negligible in the range of temperatures studied.

3.4. Complex stoichiometry

Results obtained from both methods are in good agreement and they indicate that the complex stoichiomery was indeed 1:1, as expected. Finally, a COMIC plot was used. COMIC is a computer program (Ginzburg, 1976) which can calculate the equilibrium concentrations of each entity (both free and complexed species) in a multicomponent system of metal ions and ligands.

3.5. Complex formation

The overall equilibrium of complex formation, which may be expressed as:

$$K_{\mathrm{ML}} = \left[\mathrm{Fe(achd)}^{2+}\right]\left[\mathrm{H}^{+}\right] / \left[\mathrm{Fe}^{3+}\right][\mathrm{Hachd}] \qquad (1)$$

was obtained spectrophotometrically. The solutions contained such an excess of metal ion that only the 1:1 complex was formed.

It should be taken into account that,

$$[\text{Hachd}] = [\text{Hachd}]_o - \left[\text{Fe(achd)}^{2+}\right] \tag{2}$$

and

$$\left[\text{Fe(achd)}^{2+}\right] = \text{A}/\varepsilon \tag{3}$$

where A is the metal complex absorption and ε is the metal complex absorption coefficient. Thus, the absorption change, at a fixed wavelength where the monochelated complex absorbs strongly, is a function of the equilibrium constant, and the metal, ligand and proton concentrations used:

$$[\text{Hachd}]_o/A = \left[\text{H}^+\right]/\left(\left[\text{Fe}^{3+}\right]\varepsilon K_{\text{ML}}\right) + 1/\varepsilon \tag{4}$$

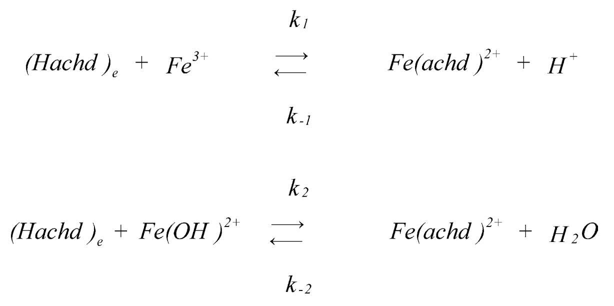
From an appropriate analysis of the data, using a correlation of the ratio $[\text{Hachd}]_o/A$ vs. the ratio $[\text{H}^+]/[\text{Fe}^{3+}]$, K_{ML} may be readily determined. At 0.5 mol dm^{-3} ionic strength a value of 23,9±0.1 was obtained for K_{ML}. The results show that K_{ML} may be considered to be approximately constant in a wide range of temperatures (7–25 °C).

3.6. Kinetic study

The complex formation was investigated with the metal ion concentration in great excess over the ligand to ensure the pseudo-first order conditions. A first order rate constant was obtained by fitting absorbance data to the general first order kinetic equation.

Although most iron(III) substitution reactions are carried out under sufficiently acidic conditions for the monohydroxy-species to be ignored, under the range of hydrogen ion concentration (pH = 2.4–2) the conjugate base, $\text{Fe(H}_2\text{O)}_5\text{OH}^{2+}$ is in equilibrium with the hexaaqua ion, offering an alternative pathway for complexation, which must be accounted for. The final product is invariably the metal-enolate complex, irrespective of which metallic species predominates. This process obviously involves the loss of a proton from the ligand (Scheme 1), where coordinated water molecules have been omitted for clarity.

In Scheme 1, k_1 and k_2 are the rate constants for reaction of the enol tautomer with the hexahydrated and hydrolysed metallic species, respectively, and k_{-1} and k_{-2} are the rate constants for complex dissociation.



Scheme. 1. Complexation.

The Fe(achd)^{2+} formation rate can be expressed as:

$$\frac{\text{d}\left[\text{Fe(achd)}^{2+}\right]}{\text{d}t} = -\left(k_{-1}\left[\text{H}^+\right] + k_{-2}\right)\left[\text{Fe(achd)}^{2+}\right] + \left(k_1 + \frac{k_2 \cdot K_\text{h}}{[\text{H}^+]}\right)\left[\text{Fe}^{3+}\right][\text{Hachd}] \tag{5}$$

since $[\text{Fe(achd)}^{+2}] = [\text{Hachd}]_0 - [\text{Hachd}]$ and, in addition, $\text{d}[\text{Fe(achd)}^{2+}]/\text{d}t = -\,\text{d}[\text{Hachd}]/\text{d}t$, therefore

$$\begin{aligned} -\frac{\text{d}[\text{Hachd}]}{\text{d}t} = &-\left(k_{-1}\left[\text{H}^+\right] + k_{-2}\right)[\text{Hachd}]_0 \\ &+ \left(k_{-1}\left[\text{H}^+\right] + k_{-2}\right)[\text{Hachd}] \\ &+ \left(k_1 + \frac{k_2 \cdot K_h}{[\text{H}^+]}\right)\left[\text{Fe}^{3+}\right][\text{Hachd}] \end{aligned} \tag{6}$$

where K_h is the hydrolysis constant of Fe^{3+} to form Fe(OH)^{2+}, which, at 25 °C, was found (Khoe et al., 1986) to be 1.86×10^{-3}.

Integrating Eq. (6) and taking into account that since $[\text{Fe(III)}]_o = [\text{Fe}^{3+}] + [\text{Fe(OH)}^{2+}]$, the concentrations of $[\text{Fe}^{3+}]$ can be connected with the entire metal concentration through the relationship,

$$\left[\text{Fe}^{3+}\right] = \left[\text{Fe(III)}_o\right]/\left(1 + \left[\text{H}^+\right]/K_\text{h}\right) \tag{7}$$

and noting that $k_1/k_{-1} = K_{\text{ML}}$ and that $k_2/k_{-2} = K_{\text{ML}}/K_\text{h}$, the pseudo-first order rate constant derived, may be expressed as:

$$k_{\text{obs}} = \left[\frac{1}{K_{\text{ML}}} + \frac{[\text{Fe(III)}]_0}{[\text{H}^+] + K_h}\right] y\left(k_1\left[\text{H}^+\right] + k_2 K_\text{h}\right) \tag{8}$$

Eq. (8) indicates that k_{obs} values are independent of the initial concentration of Hachd, as was found experimentally. The observed rate constant represented in Eq. (8) now only contains two unknown parameters, k_1 and k_2. In order to obtain them, the equilibrium and kinetic data obtained were fitted to the above equations. Using a NAG Fortran Library routine, values of k_1 and k_2 were determined at 25 °C (Table 1). The agreement between k_{obs} and k_{calc} values is quite satisfactory over the range of ligand and hydrogen ion concentrations studied, as can be seen in Table 1.

Good agreement was seen in all cases with excellent correlation coefficients. Thus, taking into account that the reaction is first order, and the nature of the initial and final products, the tentative mechanism depicted in Scheme 1 can be assumed.

In order to confirm the proposed mechanism and calculate the activation parameters associated with k_1 and k_2 pathways, a full kinetic and thermodynamic study was carried out in the range of temperatures between 7 and 25 °C (Table 2).

Consequently, the kinetic and equilibrium data obtained at every temperature were fitted to Eq. (8).

Although it is not a goal of this work, it is interesting to compare the results of 2-acetyl-1,3-cyclohexanedionate

Table 2
Rate constants and activation parameters in the formation of the iron(III) monochelate of 2-acetyl-1,3-cyclohexanedione

T (K)	k_1 (mol^{-1} dm^{-3} s^{-1})	$k_2/10^{-2}$ (mol^{-1} dm^{-3} s^{-1})	RSS
280	9.56	4.60	3.15
283	12.9	8.02	3.60
288	33.5	12.3	4.22
293	69.4	24.1	3.45
298	102	36.4	3.27
$\Delta H_1{}^{\#} = 99 \pm 20$ J mol^{-1}		$\Delta H_2{}^{\#} = 75 \pm 15$ J mol^{-1}	
$\Delta S_1{}^{\#} = 130 \pm 25$ J mol^{-1} K^{-1}		$\Delta S_2{}^{\#} = 76 \pm 10$ J mol^{-1} K^{-1}	

iron(III) with the homologous 2-acetylcyclohexanonate iron(III) in order to analyse particular behaviour. The K_{HL} of 2-acetyl-1,3-cyclohexanedione is larger than the K_{HL} of 2-acetylcyclohexanone (Hachx), but is also larger than any other alkyl-substituted 1,3-dicarbonylic compound studied (Blanco, 1998). The two most important effects that can influence a compound's acidity are considered to be the inductive effect and the resonance effect. In the case of bulky molecules, the steric effect may also be considered. Unfortunately, in most molecules, several effects are working simultaneously and it becomes extremely difficult to interpret the observed differences in acidity or basicity in terms of a single structural feature.

It is also noteworthy that the reaction rate, via the hydrolysed species and the hexahydrated metallic species, is three orders of magnitude smaller for 2-acetylcyclohexanone (Blanco & Rojas, 1998) complexation. This may be because the 2-acetyl-1,3-cyclohexanedione is present only in the enol form in aqueous solution while the 2-acetylcyclohexanone is found in enol form as well as keto form.

The reaction rates of both pathways, are considerably larger than would be predicted on the basis of the outer-sphere association constant and the rates of solvent exchange (k_s).

In this case, the overall water exchange rate constant, k_s, will therefore be the sum of contributions from the two reaction paths available at equilibrium, the water exchange on the hexaaqua species, with rate constant k_o and on its hydrolysed form, with rate constant k_{OH}. Thus, a two-term rate equation of the type

$$k_s = k_o + k_{OH} K_h \left[H^+\right]^{-1} \tag{9}$$

has to be considered (Blanco, Hernando, & Mateo, 1992). By interpolation of the data points the values of k_o and k_{OH} obtained under our particular conditions are 1.6×10^2 s^{-1} and 1.2×10^5 s^{-1}, respectively. From these data the value of k_s at 25 °C and pH = 2 is calculated to be 2.23×10^{-4} s^{-1}, implying that, in Eq. (9), the term which involves the rate coefficient for exchange of an aqua ligand on $Fe(H_2O)_5OH^{2+}$ is the predominant one.

The variation of the activation entropy may give a guide to the mechanism of water–ligand interchange. This activation parameter suggests that $\Delta S^{\#}$ tends to be positive for I_d activated substitutions. Thus, $\Delta S_1^{\#} = 130 \pm 25$ J mol^{-1} K^{-1} and $\Delta S_2^{\#} = 76 \pm 10$ J mol^{-1} K^{-1} suggest a dissociative interchange although, under our conditions, the presence of ClO_4^- ion and solvation effects may mask the meaning of the $\Delta S^{\#}$ parameter (Swaddle, 1974) due to secondary effects.

Certain methods have been developed for studying the speciation of iron in beer (Svendsen & Lund, 2000). It has been shown that some metallic cations markedly affect alcoholic fermentation (Mozaz S. R., Sotro A. G. Segovia J. G. and Azpilicueta C. A., 1999). Zinc ion is a major cofactor in yeast development when the concentration is 0.3 ppm, but iron inhibits yeast development during beer production and affects its organoleptic properties (Sanchis et al., 2000). On the other hand, iron chelates are important because they present an enhanced intestinal availability; thus, iron absorption from infant cereals and bread rolls fortified with iron-chelates was 1.9–3.9 times greater than when the same product was fortified with $FeSO_4$ (Hurrell, Reddy, Burri, & Cook, 2000).

2-Acylcycloalkane-1,3-diones are usually completely enolized and have acidities close to those of aliphatic fatty acids. The low volatility of triketones results from their polarity and relatively large molecular mass; this suggests that volatility is not the cause of the loss of the properties. However, solutions of 2-acetylcycloalkane-1,3-diones in water or dilute acids are stable at moderate temperatures and, practically, hydrolysis only occurs above 90 °C (Nilsson, 1981). During the brewing process the α-acids are converted into iso-α-acids, and it is well known that the iso-α-acids survive wort boiling to a large extent (De Cooman et al., 2000); the β-acids, however, decompose and do not add anything to the final product (Hoek, Hermans-Lokkerbol, & Verpoorte, 2001).

These iso-alpha-acids and their chemically modified variants play a disproportionately large role in the final quality of beer (Hughes, 2000). Ageing also has a significant influence on the consistency of beer bitterness (De Cooman et al., 2000) and can be attributed to the decomposition or to the volatility of its components.

Beer foam quality is characterized by its stability, adherence to glass, and texture, which are all inherently determined by the quality of the barley and hop raw materials used for beer production (Evans, Sheehan, & Nathan, 2002). The most widely accepted model for beer foam structure is that of Asano and Hashimoto (1976). They proposed that enhancement of foam stability results from ionic attractions between negatively-charged iso-α-acids and positively-charged ammonium ions on the foam polypeptides. The hop-derived bitter acids do not have to be ionized in order to enhance beer foam stability, since metal cations can bind both ionized and un-ionized forms of the hop, and the binding of hop acids to trivalent cations such as iron(III) increases the strength of interaction with foam polypeptides (Simpson & Hughes, 1994). Experimental results show that foam stability is affected by iron-complexes but these complexes also affect foam colour.

At $pH = 2$, iso-α-acids and α-acids are present in very stable un-ionized form. The chelation mechanism proposed can be applied the beer brewing process ($pH = 4.2$). In fact, previous studies (Blanco & Hynes, 1992) have shown that mechanisms and complexation behaviour may be explained over a wide range of pH values.

According to the present results, the chelation of iron by 2-acetyl-1,3-cyclohexanedione involves a double reversible pathway, where both Fe^{+3} and $Fe(OH)^{+2}$ react with the enol tautomer of the ligand. This reaction is enhanced by increases in temperature or acidity. Furthermore, it seems that iron(III) induces deprotonation of certain triketones, the main bittering principles of beer. Thus, all these reactions might contribute to changes during beer ageing and consequently contribute to final beer quality.

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