

Acid–Base Equilibria of Cysteine in Artificial Sea Water: Effect of Ionic Strength on the Basis of Specific Interaction Theory†

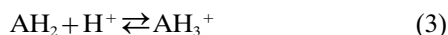
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Artificial sea water, containing NaCl, KCl, CaCl₂, MgCl₂ and Na₂SO₄, was used as a standard medium in potentiometric equilibrium studies of the sulfur-containing amino acid cysteine: the dependence of activity coefficients on ionic strength, and thus salinities, is discussed according to different models based on the Specific Interaction Theory.

Taking into account the relevance of protonation in biologically significant cysteine-rich ligands that take part in major metal-complexation processes, we thought it of interest to investigate the effect of ionic strength on the acid–base equilibria of cysteine in artificial sea water (ASW), reported as a good approximation in marine chemistry studies regarding the real situation (natural sea water).^{1,2}

Although the ionization equilibria of cysteine have been studied by several authors, all studies in this context have been carried out at a fixed ionic strength, as noted in a recent review.³ Lately, our group has started a systematic study of this amino acid and some results obtained in a simple electrolytic medium (KNO₃) have been already reported.⁴ The acid–base equilibria can be represented by



As is shown, the fully protonated form of cysteine contains three dissociable protons (CO₂H, NH₃⁺, SH). Clearly, the most acidic of these lies on the carboxylic group. By contrast, assigning the donating groups involved in its two most basic equilibria is less straightforward, since it is generally accepted that proton ionization occurs simultaneously from the SH and NH₃⁺ groups and the related constants result from intermingled microscopic processes.^{3,5,6}

Experimental

The L-cysteine used was supplied by Merck (for biochemistry, >99%). The potentiometric apparatus, procedure and conditions used have been described elsewhere.^{7–9} The synthetic sea water samples were prepared from NaCl, KCl, CaCl₂, MgCl₂ and Na₂SO₄ as electrolytes following the seawater recipe of Millero.^{9,10} The salinity, *S*, is related to the real ionic strength by the equation¹¹

$$I = \frac{19.9273S}{1000 - 1.00511S} \quad (4)$$

In Table 1 the stoichiometric equilibrium constants (molality scale) are compiled. No p*K*₁^{*} values were considered owing to the relatively high errors involved, consistent with previous findings of other authors.⁴

Results and Discussion

Even though a great number of interactions might be involved in such a complex medium as seawater, and taking into account the moderate values of the ionic strength in the working range studied, we considered the interactions of cysteine in artificial seawater to be given by a simple equation.

Fig. 1 shows the variation of p*K*₂^{*} and p*K*₃^{*} with the ionic strength as established from various models (Table 2) based on specific interaction theories (SIT).¹² The models result from empirical modifications of the Debye–Hückel equation by inclusion of different terms consisting of powers of the ionic strength. We tried different values for the parameter appearing in the Debye–Hückel term. Guggenheim and Turgeon¹³ proposed taking *c* = 1 for all solutes and letting the specific properties of each solute appear in the linear term, whilst later Scatchard¹⁴ showed that a value *a*₁ = 1.5 led to better results at ionic strength greater than 0.1 M.

As shown in Fig. 1, all the proposed equations fit the log *K*₂^{*} – *I* and log *K*₃^{*} – *I* plots for cysteine within experimental error and lead to extrapolated values (log *K*^T) at 25 °C that are consistent with those recently recommended by IUPAC³ (see Table 3). On the other hand, the plots exhibit a flat minimum over the ionic strength range 0.20–0.72 mol kg^{–1}. The presence of a minimum is typical of equilibria of the type AH₂ ⇌ AH[–] + H⁺ or similar, where a separation of electric charge occurs,¹⁵ but not, for example, for an isocoulombic equilibrium of the type BH⁺ ⇌ B + H⁺. The most simple way of accounting for the appearance of the minimum is provided by the Guggenheim equation,¹³ a model already proposed empirically by Brønsted in previous studies,¹⁶ which includes the electrostatic long-range term and a linear term proportional to the ionic strength whose coefficient is generally ascribed to the presence of specific interactions; competition between the two types of terms (specific and non-specific interactions) determines the position and amplitude of the minimum for equilibrium (3) above. In fact, a similar observation, viz. a virtually negligible effect of the ionic strength (a quasi-flat minimum) between 20 mM and 1 M on various derivatives of cysteine led Snyder¹⁷ to propose the following relation in studying the use of Brønsted equations for predicting inductive effects on rate constants for the thiol–disulfide exchange:

$$\log K^* = \log K^T + \frac{b\sqrt{I}}{1 + c\sqrt{I}} \quad (5)$$

The value of log *K*^{*} at infinite ionic strength would lead to a p*K*[∞] value (log *K*[∞] = log *K*^T + *b*/*c*) influenced by inductive

Table 1 Stoichiometric equilibrium constants of cysteine in ASW at 25 °C, molal scale, standard deviations in parentheses

<i>S</i> (%)	<i>I</i> /mol kg ^{–1}	–log(<i>K</i> ₂ /mol kg ^{–1})	–log(<i>K</i> ₃ /mol kg ^{–1})
0.50	0.10	8.149 (0.001)	10.168 (0.005)
1.05	0.21	8.080 (0.004)	9.945 (0.03)
1.45	0.29	8.073 (0.04)	9.876 (0.03)
2.00	0.40	8.045 (0.009)	9.844 (0.02)
2.50	0.51	8.042 (0.003)	9.842 (0.03)
2.90	0.60	8.086 (0.02)	9.831 (0.06)
3.50	0.72	8.07 (0.02)	9.80 (0.005)

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Table 2 Dependence of stoichiometric equilibrium constants on ionic strength according to different specific interaction models

	$\log K_i^* = \log K_i^\dagger +$		
	Guggenheim model	Scatchard model	Pitzer model ^a
Debye-Hückel term ^b	$z \frac{2}{\ln 10} \frac{-0.509\sqrt{I}}{1+c\sqrt{I}}$	$z \frac{2}{\ln 10} \frac{-0.509\sqrt{I}}{1+a_j\sqrt{I}}$	$z \frac{2}{\ln 10} f^{(v)}$
Linear term	$+ \varepsilon_i I$	$+ P_i I$	$+ A_i I$
Polynomial terms		$+ Q_i I^2 + \dots$	$+ B_i I^2$
Exponential terms			$+ C_i [1 - (1 + 2\sqrt{I})e^{-2\sqrt{I}}] + f(\text{med})$

^aRefs. 12 and 9; $f^{(v)} = -0.392 \left[\frac{\sqrt{I}}{1+1.2\sqrt{I}} + \frac{2}{1.2} \ln(1+1.2\sqrt{I}) \right]$; $f(\text{med} = \text{ASW}) = 0.2882[-1 + (1+2\sqrt{I}+2I)e^{-2\sqrt{I}}]$. ^b $z = i - 1$.

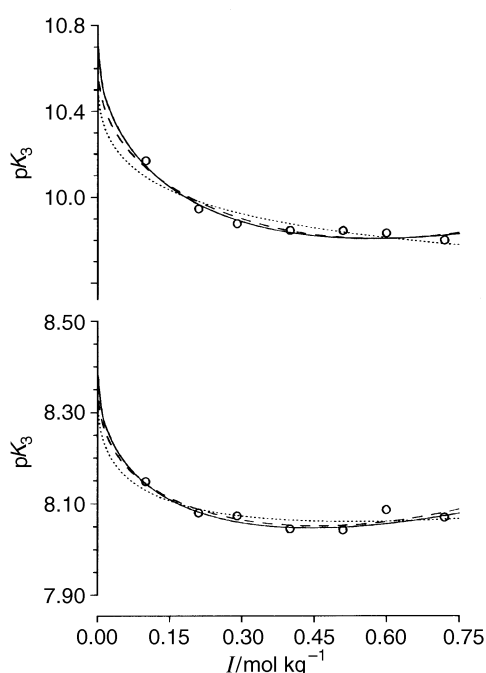


Fig. 1 Fitting curves based on specific interaction models appearing in Table 2: —, Pitzer; ---, Scatchard; ·····, Guggenheim

effects but not by purely electrostatic effects; this is equivalent to resolving pK into an electrostatic term, represented by the contribution $b/(1+c\sqrt{I})$ and other, non-electrostatic terms affected by the inductive effect. However, the assumption that electrostatic effects are represented exclusively by the electrostatic contribution up to $I \rightarrow \infty$ should only be taken as an approximation. Although extrapolating to infinite ionic strength from eqn. (5) might seem too speculative, since Snyder used it just in the range $20 \text{ mM} \leq I \leq 1 \text{ M}$, it should be noted that this equation leads to a finite value of pK^∞ and that any other equations such as those shown in Fig. 1 will lead to an infinite value of this parameter at $I \rightarrow \infty$. Whether or not an acid-base equilibrium constant (or its inverse logarithm) is finite when the solution ionic strength tends to infinity probably remains uncertain. The answer to this equation relies on the way different chemical-equilibrium models for a concentrated electrolyte solution are used.

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Table 3 Thermodynamic equilibrium constants obtained by applying specific interaction models appearing in Table 2

Fitting model	$-\log K_2^\dagger$	$-\log K_3^\dagger$
Guggenheim		
$c = 1.5$	8.33 ± 0.02	10.53 ± 0.04
$c = 1.2$	8.34 ± 0.01	10.55 ± 0.04
$c = 1.0$	8.35 ± 0.01	10.57 ± 0.03
Scatchard		
$c = 1.5$	8.37 ± 0.03	10.63 ± 0.06
$c = 1.2$	8.37 ± 0.03	10.64 ± 0.05
$c = 1.0$	8.38 ± 0.03	10.65 ± 0.05
Pitzer ^a	8.39 ± 0.04	10.71 ± 0.07
Berthon review ^b	8.36 ± 0.03	10.75 ± 0.05

^aQuadratic term not included. ^bRecommended values.

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