



# Stability and Structure of Silver–L-methionine Complexes

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## Abstract

Stability and structure of complexes between silver (I) and L-methionine (L) deduced from potentiometric and <sup>1</sup>H NMR measurements allow to assume the prevailing of several protonated species. The experimental data are compatible with the formation of the following complexes: AgL, AgL<sub>2</sub>, AgH<sub>2</sub>L, AgH<sub>1</sub>L<sub>2</sub>, AgH<sub>2</sub>L<sub>2</sub>, AgH<sub>3</sub>L<sub>2</sub> and AgH<sub>4</sub>L<sub>2</sub>. The coordination sites are obtained by <sup>1</sup>H NMR spectra, showing that only the bond between the methylthioether sulfur atom and silver (I) is responsible of the complex stability. The system is studied potentiometrically with silver and glass electrodes at 25 °C and 1.00 mol·dm<sup>-3</sup> NaClO<sub>4</sub> as ionic medium. Amino acids containing sulfur are few and not extensively studied. In particular, L-methionine, even if it is the most important, enantiomer and their complexes with silver (I) present anticarcinogenic properties, is quite not investigated. In the same experimental conditions, L-methionine protonation constants are determined. The <sup>1</sup>H NMR data allow one to assume that, in moderately alkaline solution, silver (I) is bond with six membered chelate rings with sulfur and amino nitrogen, while carboxylic groups are not involved. No polynuclear species are present. The high stability of the complex with ratio 1:2 (silver (I)/L-methionine), involving also two hydrogen ions, predominating in a wide range of hydrogen ion concentration suggests to propose a study for the preparation of an electrode to measure the deprotonated L-methionine concentration.

**Keywords** Coordination chemistry · Electrochemistry · Equilibrium constants · Silver-L-methionine Complexes

## 1 Introduction

Amino acids containing sulfur are less investigated as ligands of cations [1]. Most of the studied sulfur containing amino acids contain the group –SH, like cysteine [2], but few results exist about methylthioether reagents acting as ligands [1]. Methionine (2(S)

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amino-4-methylmercaptobutanoic acid), as shown in Fig. 1, is an  $\alpha$ -amino acid containing sulfur as methylthioether and a chiral carbon so that the DL-, D-, and L-enantiomeric forms exist.

Only the form L- is present in the proteins. It has important biological properties because it occupies the N-terminal of all proteins of eukaryotes and archaea and in the activated form, S-adenosylmethionine (SAM) [3] is also involved in lecithin and other phospholipids trans-sulfurization processes as in the synthesis of cysteine, carnitine and taurine.

The SAM is often present in food supplements, even for depression [4], osteo arthritis [5], and liver pathology therapies [6]. L-methionine is present in most of the food consumed daily.

On the other hand, the silver (I) complex formation is also not extensively studied [1], even if the complex formation of silver (I) deserves attention for possible application in biology and medicine.

Shukly and Mishura [7], supposed that compounds of methionine and silver (I) could have anticarcinogenic properties. Banti and Hadjikakou [8] studied the antitumor activity of a few compounds of silver (I). Recently, Hecel et al. [9] considered silver (I) complexes as potential therapeutical reagents in pharmacy and medicine.

These references suggest studying the reactions taking place between silver (I) and L-methionine.

Only three papers are reported on the complex formation between methionine and silver (I) in the dedicated literature [1]. Lenz and Martell [10], explaining their electromotive force (emf) measures carried out at 25 °C with a glass electrode in 0.1 mol·dm<sup>-3</sup> KNO<sub>3</sub>, assumed the formation of only one complex with the constant  $\log_{10} K_1 = 3.17$ .

Pettit et al. [11] explained their data obtained at 25 °C in 0.1 mol·dm<sup>-3</sup> KNO<sub>3</sub> with an ion-selective electrode and NMR measurements, assuming beyond the constant  $\log_{10} K_1 = 5.22$ , the presence of two complexes with participation of protons having  $\log_{10} \beta (\text{AgHL}) = 12.28$  and  $\log_{10} \beta (\text{AgH}_2\text{L}_2) = 24.2$ , but to achieve the constants of the mixed complexes, two experimental methods were applied, which, however, gave different results.

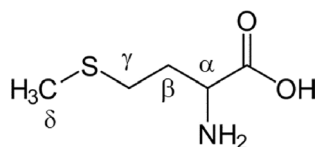
Finally, Tombeux et al. [12], from emf measurements at 25 °C in 0.5 mol·dm<sup>-3</sup> KNO<sub>3</sub> with glass and an ion-selective electrode, assumed also the formation of two polynuclear in silver (I) complexes:  $\log_{10} \beta (\text{Ag}_2\text{L}) = 7.46$  and  $\log_{10} \beta (\text{Ag}_2\text{L}_2) = 13.41$ .

The above references show that few and data, with poor agreement, exist on the complex formation between silver (I) and methionine and in particular, the disagreement is relevant concerning the hydrogen ions participation and the eventual presence of polynuclear species in silver (I). Furthermore, it cannot be deduced which enantiomeric form of methionine was employed.

Pettit and Swansk [13], in their study on complex formation of D-, L-, DL- and meso-tartaric acid, found a little difference between the protonation constants of the different enantiomeric forms.

The aim of our research is to study the behavior of L-methionine as ligand of silver (I) in a large range of concentrations of Ag (I), L-methionine, and hydrogen ions, to find the type of interaction taking place in terms of equilibrium constants and structure. This information and the biological importance of L-methionine suggest to focus the subject of this paper on

Fig. 1 Structure of methionine

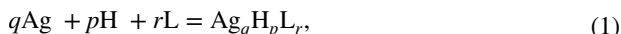


the L-enantiomeric form as well as to deduce the stability and the structure of the formed species.

A complete and exhaustive investigation has to be performed in a large range of reagents concentrations that would provoke a variation of activity coefficients of the reagents. To minimize this effect, the method of the constant ionic medium, proposed by Biedermann and Sillèn [14], was adopted. This means that by substituting up to 15% of the ionic medium with the reagents, the activity coefficients do not change appreciably, in spite of the variation of concentrations, so that in the constant ionic medium, concentrations can substitute activities.

All measurements are carried out in  $1.00 \text{ mol}\cdot\text{dm}^{-3} \text{ NaClO}_4$  as ionic medium,  $25 \text{ }^\circ\text{C}$  as well as the enantiomeric form L-methionine was employed. Solutions were prepared and kept in red light.

The equilibrium for the formation of a complex  $\text{Ag}_q\text{H}_p\text{L}_r$  (where L is methionine in completely deprotonated form) from  $\text{Ag}^+$  and methionine (L), is written as follows, without any preliminary hypothesis:



with the constant  $\beta_{p,q,r} = c_{\text{Ag}_q\text{H}_p\text{L}_r} c_{\text{Ag}}^{-q} c_{\text{H}}^{-p} c_{\text{L}}^{-r}$ .

In Eq. 1, charges are omitted, for simplicity and  $q \geq 1$ ,  $p \geq 0$  and  $r \geq 1$ . When  $q > 1$  polynuclear complexes in silver (I) are present,  $p \neq 0$  refers to the presence of complexes with the participation of protons. Protonated complexes correspond to  $p > 0$ , while  $p < 0$  could be referred to eventual hydroxylated species (without preliminary hypothesis a specie like  $\text{Ag}(\text{OH})\text{L}$  could exist).

In Eq. 1 and in the following,  $c$  indicates the free concentration of the reagents, while  $C$  represents their total concentration. For example, the free concentration of deprotonated L-methionine ( $\text{L} = \text{C}_5\text{H}_{10}\text{SNO}_2^-$ ) will be indicated as  $c_{\text{L}}$ .

The aim of this paper is the determination of  $q$ ,  $p$ , and  $r$  as well as the corresponding  $\beta_{p,q,r}$ .

To study the equilibria taking place between silver (I) and methionine, it is necessary to determine the protonation constants of the ligand in the same experimental conditions.

The main literature investigations on the protonation of methionine are collected by Powell [1], but they are incomplete and show poor agreement. Two authors [12, 15] studied the DL-methionine, other researchers did not declare the enantiomeric form. In our paper, the reagent L-methionine, simply reported as methionine is studied.

Many papers report only the first protonation constant. Only Stypinski-Mis and Andereg [15] worked in  $1.00 \text{ mol}\cdot\text{dm}^{-3} \text{ NaClO}_4$ , while others used diluted to  $0.01 \text{ mol}\cdot\text{dm}^{-3} \text{ LiClO}_4\text{-NaClO}_4$  mixtures. Other studies are performed in different ionic media.

## 2 Experimental

### 2.1 Materials and Analysis

L-Methionine p.a. Sigma reagent was crystallized from bi distilled water and dried at  $80 \text{ }^\circ\text{C}$ . The absence of water or of other impurities was verified by thermogravimetric analysis. The dissolution in water in preparing concentrated solutions was slow but checked.

A stock solution of  $\text{AgClO}_4$  was prepared by adding a moderate excess of  $\text{Ag}_2\text{O}$  (p.a. Sigma) to about  $2 \text{ mol}\cdot\text{dm}^{-3}$   $\text{HClO}_4$  and kept at about  $70\text{--}80^\circ\text{C}$  for two hours to allow the formation of  $\text{AgClO}_4$  with little residual acidity. The absence of chloride was verified. The residual acidity was determined according to Gran [16]. The concentration of silver (I) was determined by Volhard titration (with  $\text{Fe(III)}$  as visual indicator). Results of analyses agreed within  $\pm 0.1\%$ .

$\text{NaClO}_4$ ,  $\text{HClO}_4$  and  $\text{NaOH}$  stock solutions were prepared and analyzed as described previously [17].

All solutions were freed from  $\text{O}_2$  and  $\text{CO}_2$  by bubbling nitrogen, (purity 99.999%) which was further purified by passing it through 10%  $\text{NaOH}$ , 10%  $\text{H}_2\text{SO}_4$ , distilled water and  $1.00 \text{ mol}\cdot\text{dm}^{-3}$   $\text{NaClO}_4$ .

## 2.2 Apparatus

The emf measurements, the cell arrangement and the reference electrode R.E. were described previously [18].

$\text{Ag}_{(s)}$  and  $\text{Ag}_{(s)}/\text{AgCl}_{(s)}$  electrodes are prepared according to Brown [19]. To prepare the  $\text{Ag}_{(s)}$  and  $\text{Ag}_{(s)}/\text{AgCl}_{(s)}$  electrodes a constant current electricity generator (HP mod. 6186 C) was used.

The glass electrode potential ( $E_G$ ) was measured using a Radiometer potentiometer (PHM 64) and glass electrode from the same firm. Constant values were obtained within a few minutes after each addition and remained constant for several hours. The values  $E_G - E_G^\circ$  was reproducible within  $\pm 0.2 \text{ mV}$ . In the range  $-\log_{10} c_H \geq 9$ , the response of the glass electrode was corrected on the basis of that of a  $\text{H}_2$  electrode.

$\text{Ag}_{(s)}$  electrode potential ( $E_{\text{Ag}}$ ), measured with a Keithley 2000 Multimeter, provided constant values within half an hour after each addition, remaining constant for several hours.  $E_{\text{Ag}} - E_{\text{Ag}}^\circ$  values were reproducible within  $\pm 0.15 \text{ mV}$ .

NMR measurements were conducted at  $25^\circ\text{C}$  on a Bruker Unity spectrometer operating at a proton frequency of 400 MHz. The  $^1\text{H}$  NMR spectra were collected using a  $30^\circ$  pulse, a sweep width of 3.7 kHz, 16 K data points, and a 0.5 s recycle delay between scans. A total of 16 scans were integrated. Spectra were referenced internally using the residual  $\text{D}_2\text{O}/\text{H}_2\text{O}$  peak at 4.80 ppm.

## 2.3 Method of Investigation

### 2.3.1 L-Methionine Protonation

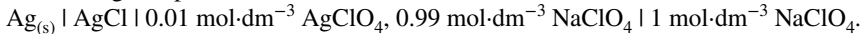
The protonation constants of methionine in its completely deprotonated form indicated with L ( $\text{L} = \text{C}_5\text{H}_{10}\text{SNO}_2^-$ ), defined as follows:

$$c_{\text{H}_n \text{L}} = c_{\text{H}_{n-1} \text{L}} c_{\text{H}} K_n, \quad (2)$$

where  $n$  can be 1, 2, were determined in  $1.00 \text{ mol}\cdot\text{dm}^{-3}$   $\text{NaClO}_4$  and at  $25^\circ\text{C}$ , by performing electromotive force (emf) measurements of the cell:



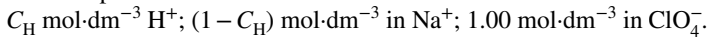
where  $TS_1$  represents test solution:  $C_H$  mol·dm<sup>-3</sup> in H<sup>+</sup>;  $(1 - C_H)$  mol·dm<sup>-3</sup> in Na<sup>+</sup>; 1.00 mol·dm<sup>-3</sup> in ClO<sub>4</sub><sup>-</sup>. GE indicates a glass electrode while RE is a reference electrode with following composition:



At 25 °C, in millivolts and in constant ionic medium, the emf of cell Eq. 3 can be expressed as follows:

$$E_G = E_G^{\circ} - 59.16 \log_{10} c_H + E_j. \quad (3)$$

In Eq. 3, the constant  $E_G^{\circ}$  and  $E_j = -j c_H$ , according to Biedermann and Sillèn [14] were determined in the first part of each measurement, where solution  $TS_1$  had the following general composition:



In the second part of the measurement, addition of an alkaline solution of methionine to the selected ionic medium gradually increased  $-\log_{10} c_H$  while keeping  $C_L$  constant (by addition of an adequate volume of solution having a  $2C_L$  concentration). The results are reported below.

### 2.3.2 Complex Formation

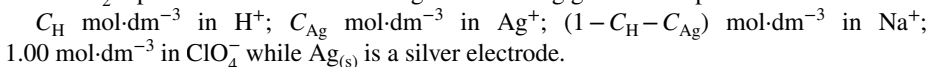
The complex formation equilibrium (Eq. 1) has been little studied. The literature reports only four papers concerning the behavior of methionine as a ligand of silver (I). Furthermore, papers treating the behavior of the enantiomer, L-methionine are not available.

The disagreement among the literature data and the lack of information about the kind of bond between silver (I) and L-methionine sulfur or amino or carboxyl groups, suggested our investigation on the equilibria taking place between methionine and silver (I) in a wide range of reagent concentration to find the existing species, with particular attention to the participation of protons and the presence of polynuclear complexes in silver (I). On the other hand, as treated below, the bond between silver (I) and methionine, is investigated.

The complex formation according to Eq. 1 is studied at 25 °C in 1.00 mol·dm<sup>-3</sup> NaClO<sub>4</sub> by measuring the emf of the cells:



where  $TS_2$  represents test solution having the following general composition:



At 25 °C, in millivolts and in constant ionic medium, the emf of cells (II) and (III) can be expressed respectively by Eqs. 3 and 4,

$$E_{Ag} = E_{Ag}^{\circ} + 59.15 \log_{10} c_{Ag} + E_j. \quad (4)$$

Cell constants,  $E_G^{\circ}$  and  $E_{Ag}^{\circ}$  were determined in the first part of each experiment in the absence of ligand.

The junction potential,  $E_j$ , was evaluated as  $E_j = (-490 \pm 10) c_H$  mV·L·mol<sup>-1</sup>, according to Biedermann and Sillèn [14]. In the second part of the titration, two solutions were used: an alkaline solution (1) of methionine, in the constant ionic medium and a solution

(2) with  $\text{Ag}^+$  concentration equal to  $2C_{\text{Ag}}$ , with the same  $C_{\text{H}}$  concentration. In this way the concentration of methionine and  $-\log_{10} c_{\text{H}}$  gradually increased, whereas, for each measurement,  $C_{\text{Ag}}$  and  $C_{\text{H}}$  were kept constant.

The free hydrogen ion concentration,  $-\log_{10} c_{\text{H}}$ , was provided by the emf measurements of cell (II), whereas, from the emf measurements of cell (III), the free silver (I) concentration  $-\log_{10} c_{\text{Ag}}$  was obtained.

Together with the emf measurements, in the same ionic medium, a parallel investigation with proton Nuclear Magnetic Resonance ( $^1\text{H}$  NMR) was performed looking to obtain the possible structure of the formed complexes.

### 3 Results and Discussion

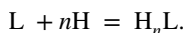
#### 3.1 Protonation Constants of L-Methionine

Measurements were performed at 0.010 and 0.020 mol·dm<sup>-3</sup> methionine concentrations. From the material balance of the methionine,  $C_{\text{L}}$ , and the analytical excess of hydrogen ion,  $C_{\text{H}}$ , and taking into account the law of mass action, it can be written:

$$C_{\text{L}} = c_{\text{L}} + \sum K_1 \cdots K_n c_{\text{H}}^n c_{\text{L}}, \quad (5)$$

$$C_{\text{H}} = c_{\text{H}} + \sum nK_1 \cdots K_n c_{\text{H}}^n c_{\text{L}}, \quad (6)$$

where  $K_1 \dots K_n$  represent the protonation equilibria (charges are omitted, for simplicity):



Arranging  $C_{\text{L}}$  and  $C_{\text{H}}$  with  $c_{\text{H}}$ , experimentally measured by cell (I), the protonation function  $p^*$ , defined as the average number of protons per methionine, can be calculated as:

$$p^* = \frac{C_{\text{H}} - c_{\text{H}}}{C_{\text{L}}} = \frac{\sum nK_1 \cdots K_n c_{\text{H}}^n}{(1 + \sum K_1 \cdots K_n c_{\text{H}}^n)}. \quad (7)$$

The values of the constants  $\log_{10} K_1$  and  $\log_{10} K_2$  are obtained both applying the graphic method proposed by Sillèn [20] and by elaborating the primary data by the PC program HYPERQUAD [21]. The results show excellent agreement as:

$$\log_{10} K_1 = 9.32 \pm 0.02 \text{ and } \log_{10} K_1 K_2 = 11.60 \pm 0.05. \quad (8)$$

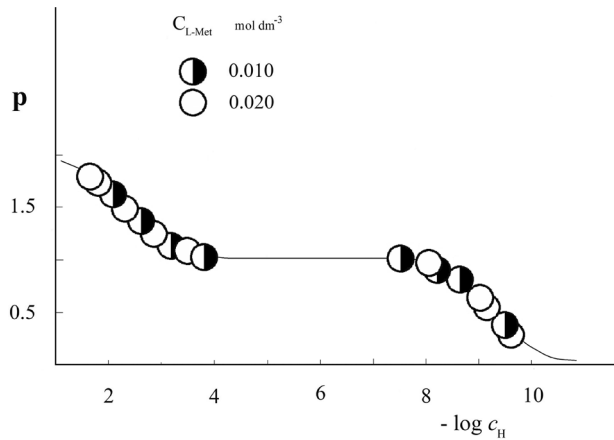
The limits of error were estimated from the maximum shift possible for the calculated curve and experimental points for which agreement was still acceptable.

The values in (Eq. 8) are applied to calculate the theoretic curve drawn on Fig. 2.

#### 3.2 Complex Formation

Experimental data were obtained for  $C_{\text{H}} = 0.025; 0.035; 0.050; 0.070$  and  $0.100$  mol·dm<sup>-3</sup> and, at the same time,  $C_{\text{Ag}}$  varied in the range  $0.25\text{--}2.00 \times 10^{-3}$  mol·dm<sup>-3</sup>. The methionine concentration increased to about  $0.10$  mol·dm<sup>-3</sup>. From emf measurements of cells (II and III)  $-\log_{10} c_{\text{H}}$  and  $-\log_{10} c_{\text{Ag}}$  are obtained.

**Fig. 2** Protonation function of L-methionine. Curve is calculated with the constants  $\log_{10} K_1=9.32$  and  $\log_{10} K_1 K_2=11.60$



To study reaction 1, material balance of silver (I), taking into account the law of mass action in the constant ionic medium, without preliminary hypotheses,  $C_{Ag}$  can be written as:

$$C_{Ag} = c_{Ag} + \sum \sum \sum q\beta_{q,p,r}c_{Ag}^q c_H^p c_L^r \quad (9)$$

In Eq. 9, hydrolytic species of silver (I) are neglected. This is permissible for the investigated  $c_{Ag}$  and  $c_H$  ranges as estimated from results reported by Brown and Ekberg [22].

Elaborating Eq. 9, the following equation can be obtained:

$$\eta = \log_{10} \left( \frac{C_{Ag}}{c_{Ag}} \right) = \log_{10} \left( 1 + \sum \sum \sum q\beta_{q,p,r}c_{Ag}^q c_H^p c_L^r \right) \quad (10)$$

The function  $\eta$  is plotted versus  $-\log_{10} c_H$  in Fig. 3.

The plot of Fig. 3 shows that points obtained at the same  $C_H$ , but at different  $C_{Ag}$ , fall on the same curve. The function  $\eta$  is independent of  $C_{Ag}$  and so  $q=1$ . Thus, as only mononuclear silver (I) species, like  $AgH_pL_r$ , are present and Eq. 10 becomes:

$$\eta = \log_{10} \left( \frac{C_{Ag}}{c_{Ag}} \right) = \log_{10} \left( 1 + \sum \sum \beta_{1,p,r}c_{Ag}^p c_H^r \right) \quad (11)$$

To study the complex formation between silver (I) and methionine, it is necessary to know the free.

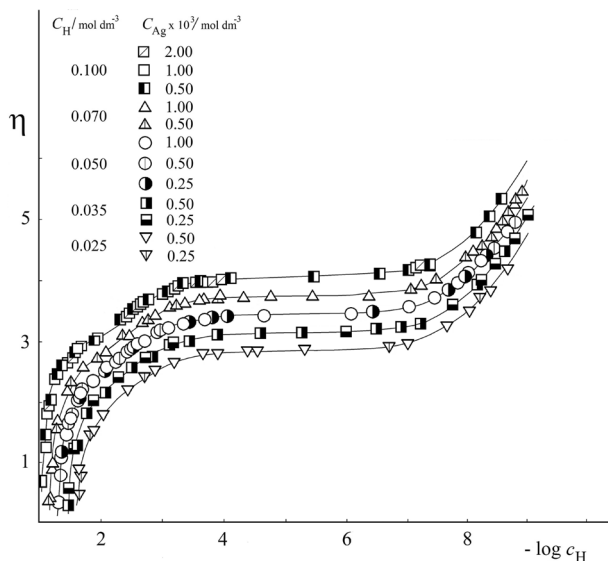
concentration of methionine,  $c_L$ , which can be calculated from the material balance of  $C_H$ , in the constant ionic medium, by taking account of the law of mass action:

$$C_H = c_H + K_1c_Hc_L + 2K_1K_2c_H^2c_L + \sum \sum p\beta_{1,p,r}c_{Ag}^p c_H^r c_L^r \quad (12)$$

where  $K_1$  and  $K_2$  are the protonation constants of methionine previously determined in the same experimental conditions and  $q=1$ . The last term of Eq. 12 can be neglected, because in all investigated solutions, there are  $C_{Ag} \leq 0.02 C_H$ .

Figure 4 shows the plot  $\eta$  versus  $-\log_{10} c_L$  for  $C_H$  0.100; 0.050 and 0.025 mol-dm<sup>-3</sup>. It can be observed that points obtained at different  $C_H$  fall on different curves and that,

**Fig. 3** The experimental data in the form  $\eta$  versus  $-\log_{10} c_H$ . Curves are calculated with the constants reported in Table 1



as  $\eta$  is an increasing function of  $C_H$ , mixed complexes with assumption of protons are formed,  $p \geq 0$ .

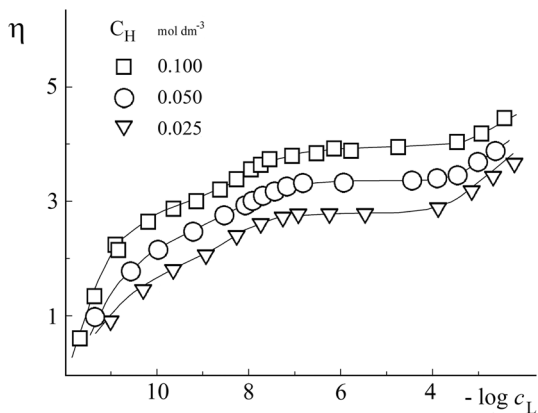
To obtain the  $p$  and  $r$  values and the relative  $\beta_{1,p,r}$  constants, the experimental data are treated both graphical methods and elaborated with a PC program.

The graphical procedure is divided in two parts.

First, values of  $c_L$  are calculated from the data of Fig. 3. After that, the values of  $p$  and  $r$  and the constants  $\beta_{1,p,r}$  of  $\text{AgH}_p\text{L}_r$ , are obtained from the set  $\eta(c_H, c_L)_{c_{\text{Ag}}, c_H}$  according to the procedure suggested by Sillèn [23], which entails deducing from a three variable function such as  $\eta$ , relationships between two of the variable concentrations by keeping the third constant [20].

By applying the proposed graphic procedure, keeping  $c_H$  constant, Eq. 11 can be written as:

**Fig. 4** Main of experimental data obtained at different  $C_H$ , in the form  $\eta$  versus  $-\log_{10} c_L$ . Curves are calculated with the constants reported in Table 1





$$\eta = \log_{10} \left( 1 + \sum \gamma_r c_L^r \right) \text{ where } \sum \gamma_r = \sum \sum \beta_{1,p,r} c_H^p \quad (13)$$

For each curve of Fig. 3,  $\eta_{c_H}$  values are selected for corresponding successive  $-\log_{10} c_H$ . From the  $C_H$  and  $c_H$  values, the corresponding  $c_L$  can be calculated from Eq. 12, by neglecting its last term. In this procedure, no appreciable error is involved, because of the presence of many points. Introducing those  $c_L$  values in Eq. 13, the following expression can be obtained:

$$\phi = \log_{10} \left[ (10^{\eta_{c_H}} - 1) c_L^{-1} \right] = \log_{10} \left( \sum \gamma_r c_L^{r-1} \right). \quad (14)$$

By plotting the function  $\Phi = 10^\phi$  versus  $c_L$ , for every considered  $-\log c_H$ , a straight line is always obtained, then  $r$  can be only 1 or 2. For each plot,  $\gamma_1$  and  $\gamma_2$  respectively from extrapolation and slope can be obtained.

In Fig. 5, an example obtained for  $-\log_{10} c_H = 5$  is plotted.

By taking in to account that each methionine can assume not more than two protons, Eq. 13 can be written, without preliminary hypothesis, as:

$$\log_{10} \gamma_1 = \log_{10} \beta_{1,0,1} + \log_{10} \left( 1 + \beta_{1,1,1} \beta_{1,0,1}^{-1} c_H + \beta_{1,2,1} \beta_{1,0,1}^{-1} c_H^2 \right). \quad (15)$$

By applying the graphical procedure proposed by Sill en [20], it can be deduced that Eq. 18 cannot be normalized with three parameters, but it can be fitted with the following theoretical equation:

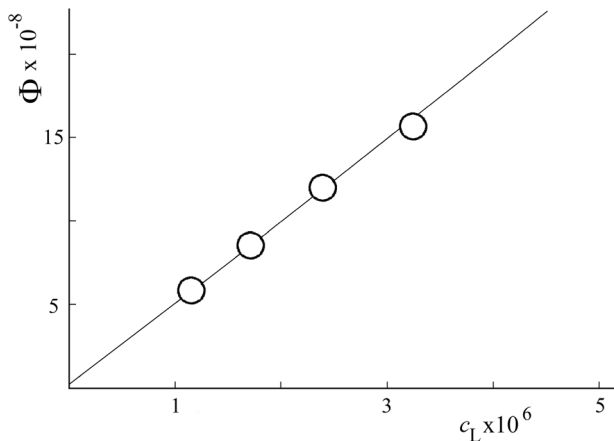
$$G = \log_{10} (1 + u^2). \quad (16)$$

This means that the 1,1,1 complex is not present in appreciable concentration, so that Eq. 15 can be simplified, as follows:

$$\log_{10} \gamma_1 = \log_{10} \beta_{1,0,1} + \log_{10} \left( 1 + c_H + \beta_{1,2,1} \beta_{1,0,1}^{-1} c_H^2 \right). \quad (17)$$

By superimposing the theoretical (Eq. 16) to the points of  $\log_{10} \gamma_1$ , a good fit can be obtained and from the positions:

**Fig. 5** The straight dependence of  $\Phi$  on  $-\log_{10} c_L$  at  $-\log_{10} c_H = 5$



$$\log_{10}\gamma_1 - G = \log_{10}\beta_{1,0,1} \text{ and } \log_{10}u = \log_{10}c_H + 1/2 \log_{10}\beta_{1,2,1} - 1/2 \log_{10}\beta_{1,0,1},$$

at the best fit, the values of  $\log_{10}\beta_{1,0,1}$  and  $\log_{10}\beta_{1,2,1}$  collected in Table 1 are obtained.

The elaboration of  $\log_{10}\gamma_2$  is more complicated because the possibility of four protons must be considered, so that the following equation can be written:

$$\log_{10}\gamma_2 = \log_{10}(\beta_{1,0,2} + \beta_{1,1,2}c_H + \beta_{1,2,2}c_H^2 + \beta_{1,3,2}c_H^3 + \beta_{1,4,2}c_H^4). \quad (18)$$

By taking into account that, in the range  $5 \leq -\log_{10}c_H \leq 9$ , to a first approximation the terms  $\beta_{1,3,2}c_H^3$  and  $\beta_{1,4,2}c_H^4$  can be neglected, Eq. 18 can be reduced to:

$$\log_{10}\gamma_2 = \log_{10}\beta_{1,0,2} + \log_{10}\left(1 + \beta_{1,1,2}\beta_{1,0,2}^{-1}c_H + \beta_{1,2,2}\beta_{1,0,2}^{-1}c_H^2\right), \quad (19)$$

comparable with the normalized equation:

$$Y = \log_{10}(1 + R^*u + u^2), \quad (20)$$

where

$$\log_{10}\gamma_2 - Y = \log_{10}\beta_{1,0,2} \text{ and } \log_{10}u = \log_{10}c_H + 1/2 \log_{10}\beta_{1,2,2} - 1/2 \log_{10}\beta_{1,0,2},$$

and

$$\log_{10}\beta_{1,1,2} = \log_{10}R^* + 1/2 \log_{10}\beta_{1,2,2} + 1/2 \log_{10}\beta_{1,0,2}.$$

By superimposing the family of theoretical curves of Eq. 20 to the  $\log_{10}\gamma_2$  points of Eq. 19 and shifting the two plots parallel to both axes, first approximations of  $\beta_{1,0,2}$ ,  $\beta_{1,1,2}$  and  $\beta_{1,2,2}$  are obtained from the position of best fit.

Equation 18, rearranged with the introduction these values, becomes:

$$Fc_H^{-3} = [\gamma_2 - (\beta_{1,0,2} + \beta_{1,1,2}c_H + \beta_{1,2,2}c_H^2)] + \beta_{1,3,2} + \beta_{1,4,2}c_H. \quad (21)$$

Figure 6 shows the straight dependence of  $Fc_H^{-3}$  on  $c_H$  and then  $\beta_{1,3,2}$  is obtained from the intercept and  $\beta_{1,4,2}$  from the slope. By repeating the procedure, accurate values of all the four constants are obtained.

**Table 1** Logarithms of  $\beta_{pqr}$  constant, at 25 °C and in ionic medium 1.00 mol·dm<sup>-3</sup> NaClO<sub>4</sub>, relative to generic equilibrium:  $qAg + pH + rL = Ag_qH_pL_r$  (charges are omitted, for simplicity) obtained with different equilibrium models

Species (q,p,r)	Graphical Method	HYPERQUAD	
		M1	M2
1,0,1	5.3 ± 0.2	5.30 ± 0.05	5.30 ± 0.05
1,0,2	8.4 ± 0.18	8.16 ± 0.10	8.30 ± 0.10
1,2,1			15.10 ± 0.10
1,1,2	17.1 ± 0.1	17.00 ± 0.08	17.00 ± 0.05
1,2,2	24.7 ± 0.15	24.76 ± 0.05	24.72 ± 0.03
1,3,2	26.9 ± 0.2	26.93 ± 0.2	26.80 ± 0.15
1,4,2			28.76 ± 0.05
$\sigma^2$		6.4	4.5
$\chi^2$		21.7	13.5

Next to the graphic procedure, the experimental data: total concentration of silver (I)  $C_{Ag}$ , of methionine  $C_L$ , the analytic excess of hydrogen ions  $C_H$ , and the free concentrations  $c_{Ag}$  and  $c_H$ , obtained from the emf measurements, are elaborated by applying the PC program HYPERQUAD [24].

After a lot of different trials, such as the elimination of some species or the introduction of others, the program interpreted the experimental data by assuming the same species found with the graphic method (see Table 1). In particular, a model with  $\sigma^2$  lower than 9 is considered satisfactory. If more than one model given acceptable  $\sigma^2$  values a  $\chi^2$ -test is considered next. For  $\chi^2$  values inferior to 12.6 the weighted residuals follow a normal distribution.

Systematic testing of different  $(q,p,r)$  complexes leads to the conclusion that acceptable agreement factors are only found with models including 7 complexes. The most relevant results of analysis of the whole set of data, comprising 345 data points (with 165 negative deviations and 180 positive deviations) of 16 titrations are summarized in Table 1. The model M2 with lowest  $\chi^2$  and  $\sigma^2$  is the most reliable. All formation constants are well defined even though the model is fairly complicated consisting of 7 complexes.

The limits of error were estimated from the maximum shift possible for the calculated curve and experimental points for which agreement was still acceptable.

The constants of Table 1 are used to calculate the theoretical curves of Figs. 3 and 4.

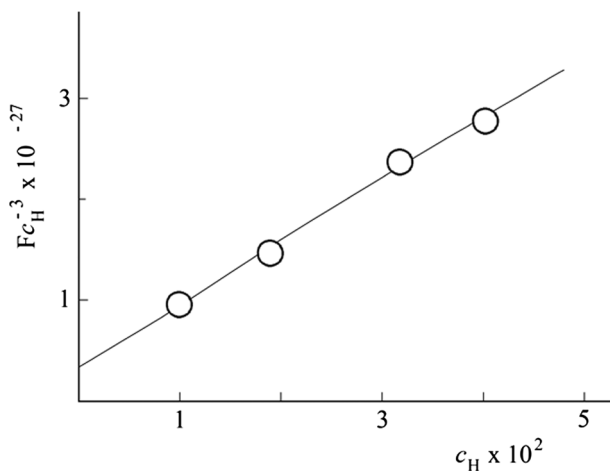
The same constants are also used to calculate the percentage distribution of silver (I) versus  $-\log c_H$ , plotted in Fig. 7.

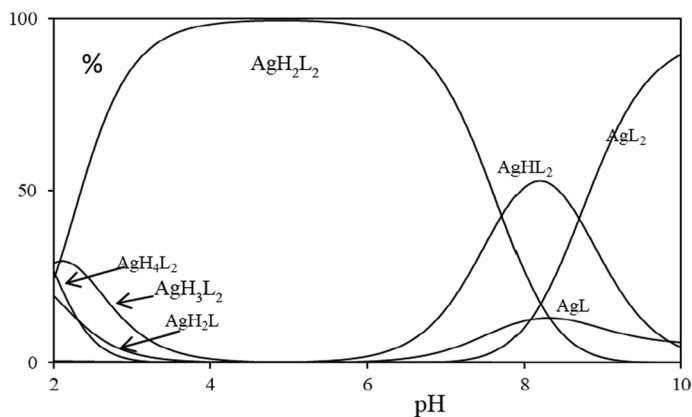
It can be observed from Fig. 7 that the complex  $AgH_2L_2$  is predominant in a wide range  $3.5 \leq -\log c_H \leq 7$ , while the species  $AgHL$  is absent because it was not found at appreciable concentration ( $\leq 0.1\%$ ) nor by the graphic nor by the program HYPERQUAD procedures.

### 3.3 $^1H$ NMR Measurements

To confirm the results obtained by potentiometric measurements as well as to investigate on the type of bond involved between  $Ag^+$  and methionine,  $^1H$  NMR spectra at different pH values and as a function of the  $Ag(I)$ /methionine ratio were collected in  $1.00 \text{ mol}\cdot\text{dm}^{-3}$

**Fig. 6** Straight dependence of  $Fc_H^{-3}$  on  $c_H$ , according to Eq. 21





**Fig. 7** Distribution diagram of Ag(I)–L-methionine system in  $0.1 \text{ mol}\cdot\text{dm}^{-3}$   $\text{NaClO}_4$  with  $1.0 \times 10^{-3} \text{ mol}\cdot\text{dm}^{-3}$  Ag(I) and  $2.0 \times 10^{-2} \text{ mol}\cdot\text{dm}^{-3}$  of L-methionine

$\text{NaClO}_4$  (10%  $\text{D}_2\text{O}$ ). In particular, spectra from  $-\log_{10} c_{\text{H}} \sim 0$  to  $-\log_{10} c_{\text{H}} \leq 8$  and from Ag(I)/methionine ratio from 0 to 2 were recorded. The chemical shifts of the protons of methionine as a function of Ag(I)/methionine ratio are reported in Fig. 8.

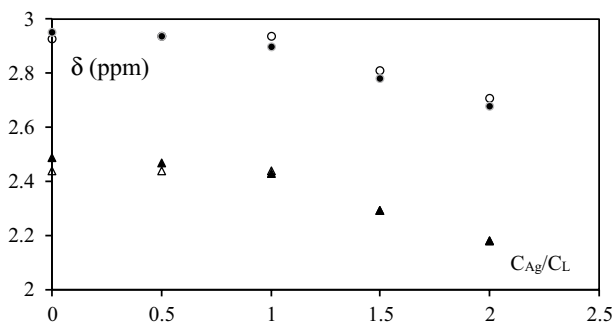
The proton chemical shifts of methionine for all pH values investigated are reported in the Supplementary Material (Table S1).

$^1\text{H}$  NMR spectra show significant variations in chemical shifts until to a  $C_{\text{Ag}}/C_{\text{L}}$  ratio equal to 0.5 (see Table S2). This is in agreement with the formation of complexes with 1:2 stoichiometric ratio. Measurements carried out at  $-\log_{10} c_{\text{H}} = 0$  show that the signal of the proton H( $\alpha$ ) does not undergo appreciable variations, unlike the signals of the protons H( $\gamma$ ) and H( $\delta$ ), which show variations of 0.25–0.3 ppm. This indicates the involvement of the thioether group in the formation of the  $\text{AgH}_2\text{L}_2$  and  $\text{AgH}_4\text{L}_2$  complexes (as can be seen from the distribution diagram in Fig. 7). The interaction between  $\text{Ag}^+$  ions and the carboxyl and amino groups is excluded.

Spectra recorded in solution  $3 \leq -\log_{10} c_{\text{H}} \leq 7$  show variations in the signals similar to those obtained at  $-\log_{10} c_{\text{H}} = 0$ . In this pH range, the signal of the proton H( $\alpha$ ) is almost constant, the coordination between the carboxyl group and the silver ion can be excluded.

Investigations on solutions at  $-\log_{10} c_{\text{H}} = 8$ , a shift of the signal of the proton H( $\alpha$ ) of about 0.2 ppm is observed, as well as variations of the signals H( $\gamma$ ) and H( $\delta$ ) (about 0.3 ppm). This is in agreement with the formation of the predominant species  $\text{AgHL}_2$  and

**Fig. 8** Chemical shift,  $\delta$  (ppm), of H( $\gamma$ ) (circles) and H( $\delta$ ) (triangles) protons of methionine (v. Fig. 1) as a function of  $C_{\text{Ag}}/C_{\text{L}}$  ratio at pH 3.0 (full) and pH 7.0 (empty)



$\text{AgL}_2$ , where  $\text{Ag}^+$  ion coordinates to both the thioether group and the amino group. In all measurements carried out, no significant changes in the chemical shifts of the various protons are found for  $C_{\text{Ag}}/C_{\text{L}}$  ratio greater than 1.0. This agrees with the absence of the formation of dinuclear species that involving two silver (I) ions.

## 4 Conclusions

This paper shows the investigation performed on solutions silver (I) L-methionine at 25 °C and in ionic medium  $1.00 \text{ mol}\cdot\text{dm}^{-3} \text{ NaClO}_4$ , in a wide range of reagent concentration. In particular, the cation concentration varied in the range (from 0.25 to  $2.00$ )  $\times 10^{-3} \text{ mol}\cdot\text{dm}^{-3}$  and the methionine concentration increased from 0 to about  $0.1 \text{ mol}\cdot\text{dm}^{-3}$ , so that the concentration of silver (I) varied eight times and the ratio between cation and ligand arrived to about 100 times.

The protonation constants of L-methionine were also determined in the same experimental conditions.

In the studied concentration range, complexes with ratio 1:1 and 1:2 and with assumption of 1, 2, 3 and 4 protons are present, while polynuclear complexes are absent. As expected, no species with assumption of  $\text{OH}^-$  are present.

It seems interesting to compare the different behavior of sulfur contained in methionine with that present in cysteine.

In a previous paper [2] equilibria between cysteine and cadmium(II) were studied. Even if the ionic medium was different, the complex formation of cysteine begun at  $-\log_{10} c_{\text{H}} \geq 4$ . In that case the participation of sulfur could be considered with the formation of 5 and 6 membered rings, because it was calculated that the cadmium (II) complexes with cysteine were more stable than those of serine, containing oxygen instead of sulfur. The contribute of sulfur becomes determinant at  $-\log c_{\text{H}} \geq 11$ . From the conclusions of paper [2] the single participation of sulfur without that of the amino acid group was excluded.

The contribute of sulfur contained in methionine is very different. From the distribution plot and from the data of Fig. 3, it is evident that the contribute of sulfur is determining, because carboxyl- and nitrogen amine in the range  $1 \leq -\log_{10} c_{\text{H}} \leq 2$  are protonated and silver (I) is bound only with sulfur. This sentence is supported by the  $^1\text{H}$  NMR spectrum of Fig. 9.

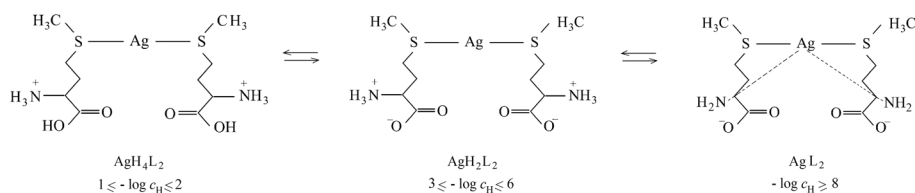
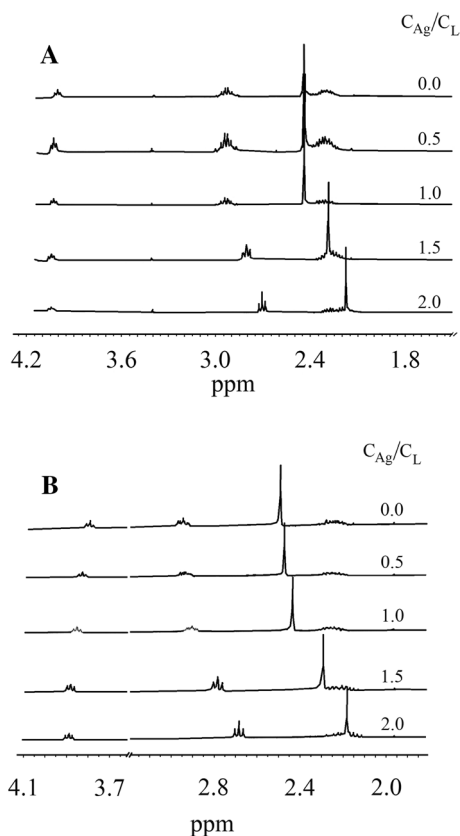
The participation of sulfur to this complex can be explained observing that in methionine it is present as thioether and can expand its valence layer through their free  $3d$  orbital.

In the range  $4 \leq -\log_{10} c_{\text{H}} \leq 6$ , where the complex  $\text{AgH}_2\text{L}_2$  predominates, the direct participation of sulfur is still determining. In this range the amino donor group is still protonated and the eventual formation of 7 membered rings, involving sulfur with carboxylic oxygen is not probable (as confirmed by  $^1\text{H}$  NMR data), because of a weak stability. In the range  $6 \leq -\log c_{\text{H}} \leq 9$ , where the deprotonation of amino group occurs, the formation of six membered ring chelates with the participation of sulfur and the two amino nitrogens can be assumed.

$^1\text{H}$  NMR spectra reported in Fig. 9, support this hypothesis, as the formation of complexes with 1: 2 stoichiometric ratio is confirmed.

In conclusion, the following scheme could represent the structure of the silver (I)—L-methionine complexes from acid solution ( $-\log_{10} c_{\text{H}} \leq 2$ ), in neutral solution ( $3 \leq -\log_{10} c_{\text{H}} \leq 6$ ) and in alkaline solution ( $-\log c_{\text{H}} \geq 8$ ):

**Fig. 9.**  $^1\text{H}$  NMR spectra for Ag(I)–Methionine system in  $1 \text{ mol dm}^{-3} \text{ NaClO}_4$  (10%  $\text{D}_2\text{O}$ ) at  $-\log_{10} c_{\text{H}} = 3$  (A) and  $-\log_{10} c_{\text{H}} = 7$  (B)



Scheme illustrates the fundamental role of sulfur and the 6 membered rings among sulfur, silver (I) and amino nitrogen in alkaline solution.

In addition, the formation of dinuclear species, that involving two silver (I) ions, is excluded (as confirmed by  $^1\text{H}$  NMR spectra recorded at  $C_{\text{Ag}}/C_{\text{L}} = 2$ ).

The inspection of Fig. 7, showing the presence of the only complex  $\text{AgH}_2\text{L}_2$  in a large range of pH, could suggest the possibility to measure the free concentration of methionine. It seems possible to prepare a galvanic cell with the following composition:



At 25 °C, in millivolts and in constant ionic medium, the emf of cell (IV) could be expressed as follows:

$$E_{IV} = E_{IV}^{\circ} - 59.16 \log_{10} c_L. \quad (22)$$

In Eq. 22,  $E_{IV}^{\circ}$ , a constant, can be determined in the first part of each measurement, where solution  $S_3$  has the following general composition:

$C_{AgH2L2}$  constant,  $C_L$  mol·dm<sup>-3</sup> methionine; in the range  $4 \leq -\log_{10} c_H \leq 6$ ; 1.00 mol·dm<sup>-3</sup> in Na ClO<sub>4</sub>.

In the second part of the measurement, the concentration of a methionine solution could be measured.

The eventual preparation of a galvanic cell like (IV) can be the subject of a future approach, in order to verify the thermodynamic behavior, the possibility of interferences and any other arising difficulties. However, it can be calculated that, from the value  $\log \beta_{1,2,2}$ , the presence of chloride ions  $\leq 0.1$  mol·dm<sup>-3</sup> should not influence the response of the eventual electrode.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s10953-022-01191-x>.

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**Data Availability** The following material is available online. Table S2. Chemical shift protons belonging to methionine in methionine/Ag(I) system.

## Declarations

**Conflict of interest** The authors declare no competing financial interest. Are no conflict of interest.

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